Proteomic study of beta-catenin protein interactions in colon cancer

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Declaration

I certify that the work presented in this thesis entitled "Proteomic study of beta-catenin protein interactions in colon cancer" has not been submitted for the purpose of obtaining a degree to any other university or institution. Unless otherwise acknowledged, the work presented in this thesis was carried out by the author.

The research involving genetically modified cell lines was performed at Westmead Millennium Institute, under the approval of the Western Sydney Local Health District Institutional Biosafety Committee (WSLHD-IBC) (IBC Ref 04/08).

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Abstract

β-catenin is a protein well-known for its association with several human cancers and is known to accumulate in the nucleus with cancer progression. It is a multi-functional protein that can be found at different subcellular localisations, regulated in part by protein interactions and phosphorylation. The aim of this thesis was to use proteomics to investigate β-catenin protein interactions in colon cancer and explore the role of phosphorylation at amino acid Y654 in regulating these interactions. It was hypothesised that determining β-catenin protein interactions could reveal new functional roles for it.

Recombinant human full-length β-catenin constructs were used as affinity baits to isolate protein binding partners from stable isotope labelled SW480 and HT29 colon cancer cell lines while high-resolution mass spectrometry (Orbitrap ELITE) was used for protein identification and quantitation. For wildtype β-catenin (Y654), 379 and 123 putative protein interactors were detected in SW480 and HT29 respectively. For the phosphomimetic (Y654E) construct, 92 and 224 putative protein interactors were detected in SW480 and HT29 respectively. For the non-phosphorylatable (Y654F) construct, 129 and 48 putative proteins interactors were detected in SW480 and HT29 respectively. A number of these candidate binding proteins were further validated by immunoprecipitation and immunofluorescence and strongest evidence was shown for golgi protein COPB and mitochondrial HSP70 – providing both a novel interaction and localisation for β-catenin. These observations confirm the multi-functional roles of β-catenin in colon cancer confirming dependence on protein interactions to regulate its subcellular localisation and function.

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Publications

- Jamieson C, Mills K, Lui C, **Semaan C**, Molloy MP, Sharma M, Forwood K, Henderson BR. Characterization of a beta-catenin nuclear localization defect in MCF-7 breast cancer cells. Exp Cell Res. 2016. 341:196-206.

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Poster presentations

- C. Semaan, M. Sharma, B. Henderson and M. P. Molloy. Identification of new beta-catenin protein interactors in colon cancers by mass spectrometry. US HUPO conference, Arizona, USA, 2015

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Awards

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Abbreviations

%	Percent
°C	Degrees Celsius
2YT	Yeast extract tryptone
ACN	Acetonitrile
APC	Adenomatous polyposis coli
Arg	Arginine
Arm	Armadillo
AUC	Area under the curve
BCA	Bicinchoninic acid assay
Bis-Tris	1,3-bis**(**tris(hydroxymethyl)methylamino)propane
BSA	Bovine serum albumin
Ca^{++}/Ca^{2+}	Calcium/calcium ion
$CaCl_2$	Calcium chloride
CaMKII	Calmodulin-dependent kinase II
CID	Collision-induced dissociation
CMX-Ros	Chloromethyl-x-rosamine
CO_2	Carbon dioxide
CSK	C-Src kinase
CSK buffer	Cytoplasmic extraction buffer
CTNNB1	Beta-catenin gene
CV	Column Volume

DAPI	4',6-diamidino-2-phenylindole
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
Dvl	Dishevelled
E	Glutamic acid
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethylene glycol tetraacetic acid
EMT	Epithelial-mesenchymal transition
ER	Endoplasmic reticulum
F	Phenylalanine
FA	Formic acid
FAP	Familial adenomatous polyposis
FBS	Fetal bovine serum
FDR	False discovery rate
FITC	Fluorescein isothiocyanate
g	G-force
GSK3β	Glycogen synthase kinase 3 beta
GST	Glutathione S-transferase
GTP	Guanosine triphosphate
h	Hour(s)

HCC	Hepatocellular carcinoma
HCD	Higher-energy collisional dissociation
HCl	Hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid
IAA	Iodoacetamide
IP	Immunoprecipitation
IPTG	Isopropyl β-D-1-thiogalactopyranoside
iTRAQ	Isobaric tag for relative and absolute quantitation
JNK	c-Jun N-terminal protein kinase
kDa	Kilodalton
L	Litre
LC/MS	Liquid chromatography mass spectrometry
LDS	Lithium dodecyl sulphate
LEF	Lymphoid enhancer-binding factor
LFQ	Label-free quantitation
LRP	Lipoprotein receptor-related protein
M	Molar
m/z	Mass-to-charge ratio
mAb	Monoclonal
MBP	Maltose binding protein
mg	Milligram
$MgSO_4$	Magnesium sulphate
min	Minutes
ml	Millilitre

mM	Millimolar
MMP	Matrix metalloproteinase
$MnCl_2$	Manganese chloride
MOPS	3-(N-morpholino)propane sulfonic acid
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
Na_3VO_4	Sodium vanadate
NaCl	Sodium chloride
NF-AT	Nuclear factor of activated T-cells
NFκβ	Nuclear factor kappa beta
ng	Nanogram
NH_4HCO_3	Ammonium bicarbonate
NHS	N-hydroxysuccinimide
NLS	Nuclear localisation sequence
nM	Nanomolar
NPC	Nuclear pore complex
Nups	Nucleoporins
O/N	Overnight
OD	Optical density
pAb	Polyclonal
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PKC	Protein kinase C
PKD	Protein kinase D

PMSF	Phenylmethanesulfonylfluoride
PO_4	Phosphate
POL	Polarised light
RbCl	Rubidium chloride
RIPA	Radioimmunoprecipitation assay
ROCK	RhoA-Rho-associated kinase
rpm	Revolutions per minute
RT	Room temperature
s	Second
S/N	Supernatant
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SILAC	Stable isotope labelling by amino acids in cell culture
SWATH	Sequential window acquisition of all theoretical mass spectra
SYBR	N',N'-dimethyl-N-[4-[(E)-(3-methyl-1,3-benzothiazol-2-ylidene)methyl]-1-phenylquinolin-1-ium-2-yl]-N-propylpropane-1,3-diamine
TBS	Tris buffered saline
TBS-T	Tris buffered saline with Tween
TCF	T-cell factor
TFA	Trifluoroacetic acid
TMT	Tandem mass tags
Tris	Tris(hydroxymethyl)aminomethane

TRITC	Tetramethylrhodamine
µg	Microgram
µL	Microliter
uPA	Urokinase receptor
V	Volts
WT	Wildtype
WTX	Wilms tumour gene on X chromosome
XIC	Extracted ion chromatogram
Y	Tyrosine

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1 Introduction

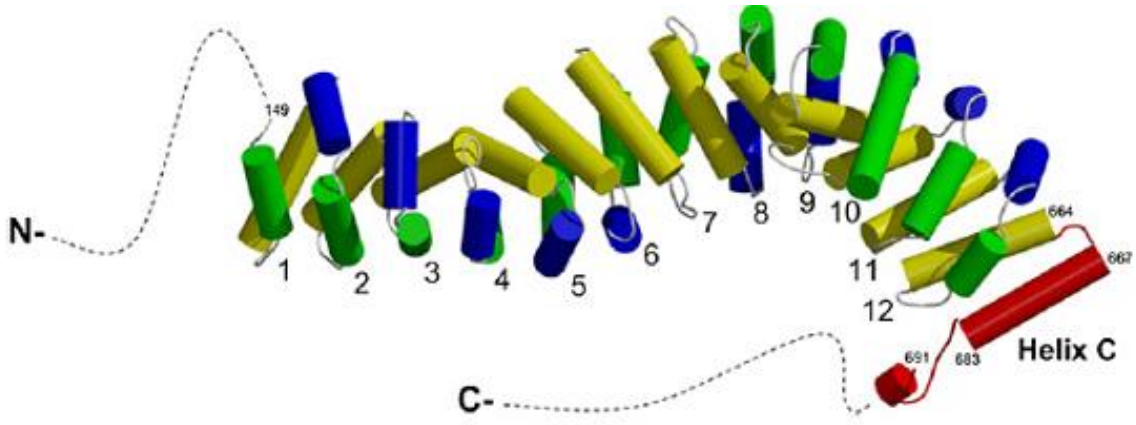
1.1 The catenin family

1.1.1 Structure of the catenins

The catenins are a family of 80-110 kDa proteins comprising of α-catenin, β-catenin, δ-catenin and γ-catenin [1]. Between members of the catenin family there is a range of only 10-60 % sequence homology and so what classifies the catenins as a family is their interactions, functions, and with the exception of α-catenin, they all they contain an Armadillo (Arm) domain within their protein structure [2-4].

The Arm domain was first discovered in the common fruit fly *Drosophila* and consists of repeating amino acid sequences of about 40 residues, which are termed Arm repeats [5, 6]. Each Arm repeat is composed of three α-helices that form a hairpin structure [6]. These repeats fold to produce a superhelix of helices containing a positively charged groove which contains crucial binding interfaces for protein interactors of the catenin family [6]. Protein interactors include the cadherins [7, 8] and components of the Wnt pathway such as adenomatous polyposis coli (APC) and axin [9] or TCF/LEF-1 [10]. Figure 1 demonstrates the protein structure of one catenin family member, β-catenin.

β-catenin is comprised of an N-terminus (130 amino acids), Arm domain (550 amino acids) and C-terminus (100 amino acids) [11-14]. The N-terminal regulatory sequence contains phosphorylation sites for casein kinase I (CKI) and glycogen synthase kinase 3 beta (GSK3β) while the C-terminal transactivation sequence is the domain required for activation of target genes [15-17]. The N-terminal and C-terminal domains are unstructured and have the potential to interact with the Arm domain which consists of 12 Arm repeats [18, 19].



<u>Figure 1</u> Crystal structure of β-catenin demonstrating the N-terminus, core Arm domain and C-terminus (C-terminal α-helix is named helix C).

The blue (helix 1), green (helix 2) and yellow (helix 3) represent the three α-helices that make up each individual Arm repeat (with the exception of repeat 7, composed of only two α-helices). Here, β-catenin contains twelve Arm repeats. Figure from Xing *et al.* (2008) [18].

1.1.2 Functions and interactions of the catenins

The catenin family members are considered multi-functional proteins, relying on interactions with other proteins to provide specificity and functional consequences. The word catenin stems from the latin word for chain, *catena* [20], reflecting the function of catenins in linking cadherin to the actin cytoskeleton at adherens junctions [21, 22]. Catenin family members were originally isolated while complexed with cadherins such as E-cadherin or N-cadherin at cell-cell junctions, where their functions are multifaceted and under much current investigation [4, 23].

Interactions of the catenins at the cytoskeleton involve cadherin-dependent morphogenic movements that promote contractile forces at cell-cell contact zones [24]. One type of cell-cell contact zone is the microfilament-associated (actin-associated) adherens junctions, which facilitates the attachment of epithelial cells to one another [25]. Another type of major adhesive junction is the intermediate filament-associated desmosomes. The catenins also function here, linking to the intermediate filament system as opposed to the actin cytoskeleton [26-28]. At the desmosome, the catenins bind to the cytoplasmic domain of desmosomal cadherins (the desmogleins and desmocollins) [29]. Tight junctions are another type of cell-cell adhesion but instead of initiating and mediating cell-cell contacts, tight junctions regulate the paracellular pathway for ions and solutes in between cells [30]. The catenins do not function in this type of cell-cell adhesion.

Family member β-catenin was originally identified at the adherens junctions [31] where it binds the cytoplasmic tails of cadherins such as E-cadherin, and anchors the adhesion complex to the actin cytoskeleton by interacting with α-catenin [32-35]. It was previously found that α-catenin exists as a monomer or a homodimer with particular binding properties [36]. The interaction between monomeric α-catenin and E-cadherin/β-catenin was strong, whereas the dimer conversely bound actin filaments. It was widely accepted that α-catenin binds to E-cadherin/β-catenin and bridges these components to actin [36] but later it was discovered that α-catenin was unable to bind simultaneously to E-cadherin/β-catenin and actin, indicating its functioning as a molecular switch, where binding to one partner changes the ability to interact with the other [37].

The catenin family, in particular, β-catenin, also functions in the nucleus [38-40]. Although the adherens junction pool of β-catenin is highly stable, non-junctional, cytoplasmic β-catenin is rapidly degraded [41-43]. In response to Wnt signalling (discussed later in section 1.2.2), the β-catenin destruction complex (also discussed in section 1.2.2) is inhibited, allowing stabilisation of β-catenin [44]. Ordinarily this destruction complex (comprised of proteins including APC and axin) results in the phosphorylation and consequential degradation of β-catenin by the proteasome [45, 46]. The stabilisation of β-catenin allows it accumulate to a sufficiently high enough level that it is able to translocate into the nucleus and alter gene activity by interacting with transcription factors such as T-cell factor (TCF) and lymphoid enhancer-binding factor (LEF) [47, 48]. Downstream outcomes of β-catenin interactions with transcription factors include stem-cell maintenance, stem-cell differentiation, proliferation and apoptosis [49-52].

The catenins can also be regulated or modified by various kinases and phosphatases that are enriched at cell-cell contacts [53, 54]. Phosphorylation of the catenins promotes their release from the adherens junction, allowing for their re-localisation to the cytoplasm or nucleus where they can perform a range of site-specific functions [4]. For example, receptor tyrosine kinase stimulation can lead to the dissociation of β-catenin and E-cadherin, resulting in the ability of β-catenin to move freely to the cytoplasm or nucleus [55, 56].

The interactions of the catenins, in particular, β-catenin, implicate this family of proteins in influencing cadherin-dependent cell adhesion, motility, and cell polarity, consequentially impacting larger processes including development, morphogenesis, tissue homeostasis and progression of diseases including cancer [4]. Figure 2 demonstrates some protein interactors of β-catenin, and the particular domains of β-catenin that they bind [18].

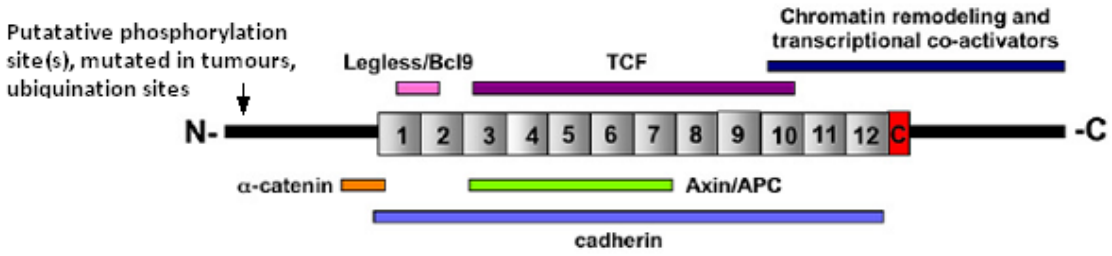


Figure 2 Linear schematic of β-catenin.

Various binding partners bind to the different domains of β-catenin. For example, TCF binds Arm 3-8 β-catenin, while the cadherins bind Arm 1-12 β-catenin. Figure from Gottardi *et al.* (2008) [57].

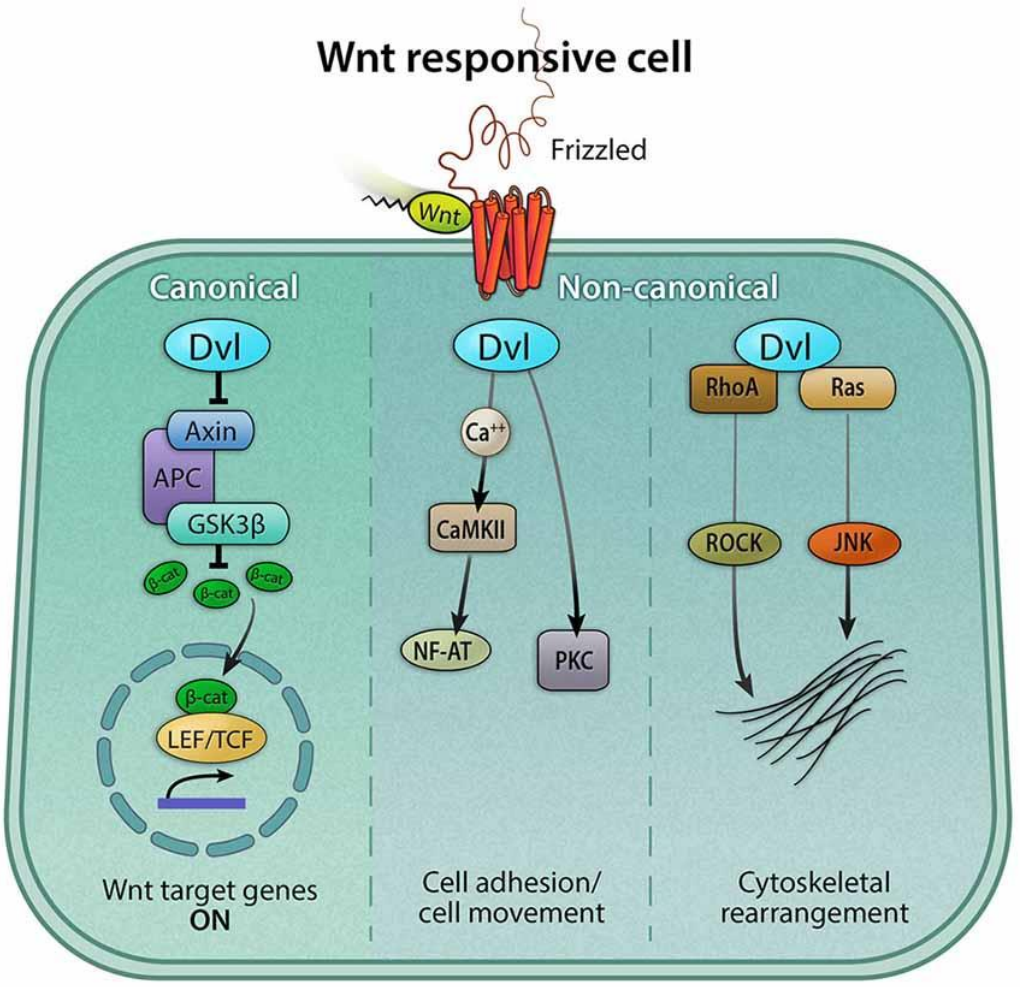
1.2 The Wnt signalling pathway

1.2.1 Discovery and roles of the Wnt signalling pathway

The Wnt signalling pathway is a highly evolutionarily conserved pathway in both vertebrates and invertebrates [58, 59]. The pathway consists of a complex network of proteins, passing signals from cell surface receptors to the nucleus to regulate transcription of target genes [60].

Almost 40 years ago, the Wnt gene was discovered in *Drosophila*, where mutation of the gene resulted in the absence of wing and haltere and was thus named Wingless (Wg) [61]. Another study identified integration-1 (Int-1) as a gene that could induce tumorigenesis in mice [62]. Upon findings that the Wg and Int-1 genes were homologues, the gene was renamed Wnt by fusion of the two names [63]. Since the discovery of dual roles in development and carcinogenesis, the Wnt signalling pathway has been an area of interest for many researchers.

The role of the Wnt pathway in embryogenesis and early development consists of regulating a number of cellular processes including pattern formation (spatial orientation of cells), cell proliferation, differentiation and migration, induction of polarity and gene expression [64]. This is achieved by two of the three Wnt signalling pathways, the non-canonical planar cell polarity Wnt pathway and the non-canonical Wnt/Ca^{2+} pathway (Figure 3). These non-canonical pathways lead to regulation of cytoskeleton or calcium inside the cell [65]. The third pathway, the canonical pathway, comprises a series of events that lead to the regulation of transcription by β-catenin [66].



<u>Figure 3</u> The canonical and non-canonical Wnt signalling pathways. Figure from Jansson *et al.* (2015) [67].

All Wnt pathways are activated by Wnt ligand binding to the Frizzled receptor. The canonical pathway (left) is mediated by β-catenin which translocates into the nucleus and activates transcription factors (eg. LEF/TCF). For the non-canonical pathways, Frizzled activates Dishevelled (Dvl) resulting in calcium (Ca^{++}) release from the endoplasmic reticulum and activation of calcium-binding proteins such as protein kinase C (PKC), calmodulin-dependent kinase II (CaMKII) and transcription factor NF-AT. The Wnt/Ca^{++} pathway has roles in embryogenesis by regulating cell movement and axis formation (middle), while the Wnt/planar cell polarity pathway (right) is mediated by GTPases RhoA and Ras, which affect the cytoskeleton by activation of RhoA-Rho-associated kinase (ROCK) or c-Jun N-terminal protein kinase (JNK).

1.2.2 The canonical Wnt signalling pathway - β-catenin

The focus of this thesis is the canonical pathway, where β-catenin is the primary effector. β-catenin is a 90 kDa multifunctional and oncogenic protein [68], first identified at the plasma membrane [31] linking E-cadherin to the cytoskeleton and aiding cell adhesion (Figure 4) [69]. Later research in *Drosophila* [70] and colorectal tumours [71-73] revealed that β-catenin was not just at the plasma membrane and that other pools of β-catenin existed in the nucleus and cytoplasm, regulated by Wnt signalling.

The Wnt pathway is activated by binding of Wnt ligand to cell surface receptor Frizzled which then interacts with Dishevelled [66]. This inhibits the formation of a destruction complex (consisting of proteins including axin, APC, GSK3β) that would otherwise lead to the degradation of β-catenin [45, 46]. Thus, when the Wnt pathway is activated, β-catenin accumulates in the cytoplasm and translocates into the nucleus, where it transcribes its target genes [66] involved in cell cycle regulation and transmigration [74].

In the absence of Wnt signalling β-catenin is recruited into the destruction complex, phosphorylated, tagged for ubiquitination and subsequently degraded by the proteasome [45, 46]. Each protein in the destruction complex serves a purpose. Axin assists as a scaffolding protein, holding the complex together [32, 41]. GSK3β (a serine/threonine kinase) phosphorylates β-catenin (along with APC and axin) [75, 76]. CKI phosphorylates β-catenin at serine residues [46] and phosphorylation sites constitute a recognition motif for β-TrCP, a ubiquitin ligase [41].

However, not all β-catenin that has been phosphorylated will be degraded. For example, N-terminally phosphorylated β-catenin has been detected at centrosomes, where it may contribute to centrosomal cohesion, separation at the formation of the mitotic spindle and segregation of chromosomes [77, 78]. Other components of the destruction complex have been reported at the centrosome, including APC and axin, suggesting a role of these components in centrosomal separation and maintenance [79-81]. For example, both APC and axin have been shown to regulate microtubule nucleation and growth from the centrosome [82, 83].

An illustration depicting the inactive and active Wnt pathway is shown in Figure 5 [84]. The following section covers the stabilisation of β-catenin through inhibition of the destruction complex which mimics activation of the Wnt pathway.

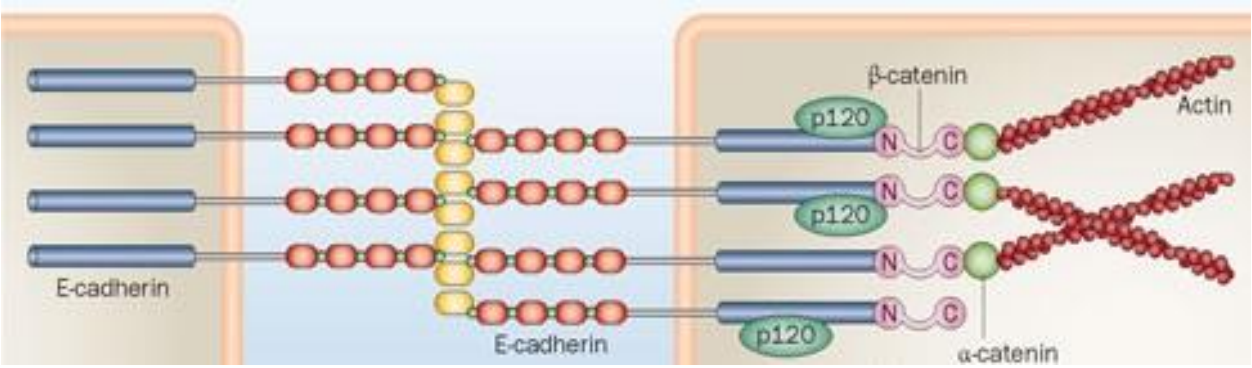
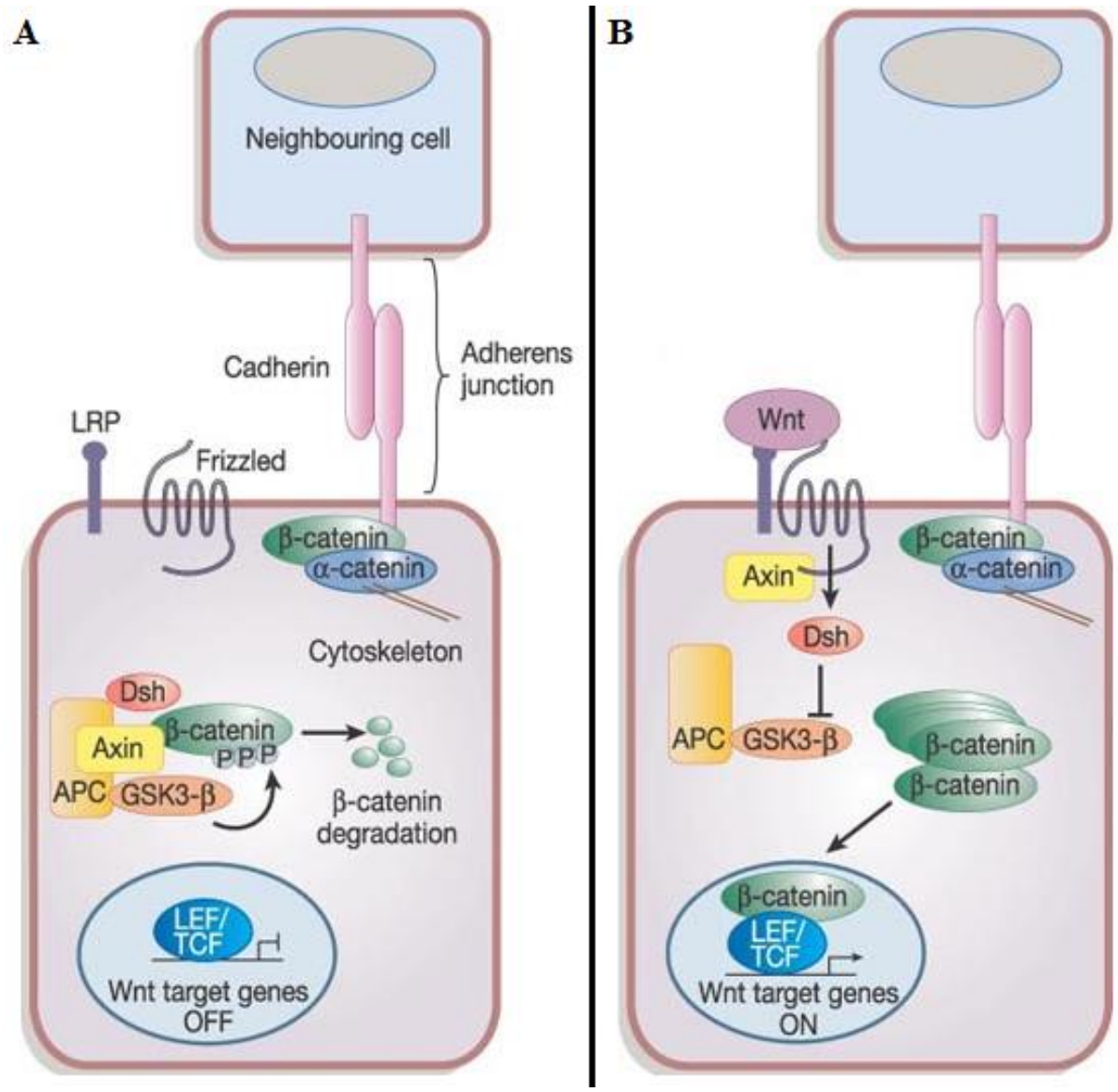


Figure 4 The role of β-catenin at the plasma membrane in cell-cell adhesion. Figure from Perry *et al.* (2010) [85].

E-cadherin is a transmembrane protein and its extracellular domains (yellow and orange) bind each other in a calcium-dependent manner. Its intracellular domains (blue) interact with the actin cytoskeleton (red) by interactions with α-catenin (light green), β-catenin (pink) and δ-catenin (dark green). This interaction constitutes the adherens junction.



<u>Figure 5</u> The Wnt signalling pathway. Figure from Reya *et al.* (2005) [84].

A) The inactive Wnt pathway: In the absence of Wnt signalling, β-catenin remains at the plasma membrane or interacts with a degradation complex consisting of proteins including axin, APC and GSK3β. β-catenin is phosphorylated and degraded.

B) The active Wnt pathway: In the presence of Wnt signalling the destruction complex is disrupted when axin is recruited to the plasma membrane. β-catenin is uncoupled from the degradation complex and translocates to the nucleus where it binds transcription factors such as TCF/LEF and activates target genes.

1.2.3 Inhibition of the destruction complex and stabilisation of β-catenin

Inhibition of the destruction complex stabilises β-catenin allowing it to accumulate in the cytoplasm and translocate into the nucleus. The precise mechanism driving the inhibition of the destruction complex is still largely unknown although there are a few theories relating to the inhibition of GSK3β.

The most popular model of Wnt-induced stabilisation of β-catenin proposes that phosphorylation of GSK3β inhibits its kinase activity. It states that GSK3β is phosphorylated by growth factor activated protein kinase B/Akt which inactivates it [86-88]. It has also been speculated that Erk associates with GSK3β and primes it for inactivation [89].

Another model suggests that GSK3β is inhibited by low-density lipoprotein receptor-related protein 6 (LRP6). The destruction complex is recruited to the plasma membrane upon stimulation of the Wnt pathway [90-94] and a cascade of events leads to the phosphorylation of LRP6 by GSK3β [95-97]. Phosphorylated LRP6 recruits axin-bound GSK3β to the plasma membrane [98] while also binding to the catalytic pocket of GSK3β [99]. The theory asserts that these two events prevent the activity of GSK3β towards β-catenin.

Briefly, other theories concerning inhibition of the destruction complex include inhibition of GSK3β when Wnt signalling promotes the internalisation of GSK3β into multivesicular bodies [100] (Figure 6), degradation and, therefore, limitation of axin [101-103] and dissociation of β-TrCP from the destruction complex which affects ubiquitination of β-catenin [45].

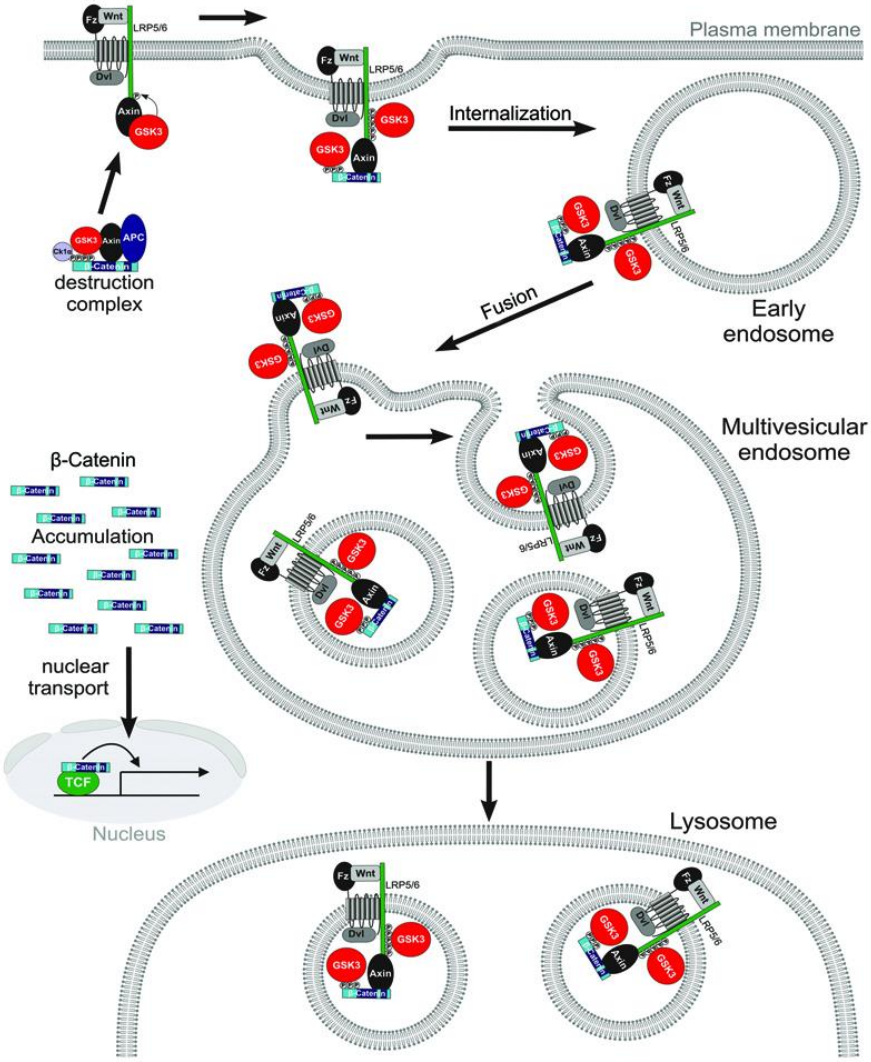


Figure 6 Sequestration of GSK3β into multivesicular bodies. Figure from Taelman *et al.* (2010) [100].

Binding of GSK3β (red) to the Wnt receptor complex (includes axin, Frizzled, Dishevelled) sequesters it into multivesicular bodies, resulting in stabilisation of its cytosolic substrates such as β-catenin (blue).

1.3 Dysregulation of the canonical Wnt signalling pathway and disease

Dysregulation of the Wnt pathway has been associated with a number of diseases including cancer and type II diabetes. Given the role of Wnt signalling in development and homeostasis it is not surprising that impairment of the pathway is linked to several human diseases. Table 1 demonstrates various proteins involved in Wnt signalling and their associated diseases upon the mutation or alteration of their gene. Discussed further in this section are mutations in APC and β-catenin.

Table 1 Associated diseases of mutated Wnt pathway components

Wnt component	Function	Mutation/ Alteration	Associated disease	Reference
APC	β-catenin degradation	Loss of function	Familial adenomatous polyposis, lung cancer, medulloblastoma, gastric cancer, colorectal cancer	[104-109]
Axin	β-catenin degradation	Loss of function	Caudal duplication, tooth agenesis, hepatocellular carcinoma, colorectal cancer, medulloblastoma	[110-116]
β-catenin	Primary Wnt effector	Gain of function	Pilomatrixoma (hair follicle tumour), pancreatic cancer, colorectal cancer, ovarian cancer, prostate cancer, hepatocellular carcinoma, medulloblastoma, melanoma	[71, 117-120]
LEF1	β-catenin transcriptional partner	Loss of function	Sebaceous skin tumour	[121]
LRP5 & LRP6	Wnt co-receptors	Loss/gain of function	Hyperparathyroid tumours, osteoporosis, high bone mass, osteoporosis-pseudoglioma, vascular eye defects, early coronary disease, breast and thyroid cancer	[122-128]
TCF4	β-catenin transcriptional partner	Gain of function	Type II diabetes, colorectal cancer	[129-131]
Wnt ligands (Wnt-3, 4, 7a, 10a, 10b)	Ligands for Wnt pathway	Loss of function	Tetra-amelia, Mullerian-duct regression and virilisation, Fuhrmann syndrome, dermal hypoplasia, obesity	[132-136]
WTX	β-catenin degradation	Loss of function	Wilms tumour	[137, 138]

1.3.1 Cancer

A well-known link between the Wnt pathway and human disease is a genetic lesion that occurs early in the onset of colon cancer. Patients with familial adenomatous polyposis (FAP) develop hundreds or even thousands of polyps in the colon, and without early detection and removal these polyps can develop into malignant carcinomas [139]. Extracolonic lesions may also develop and include desmoid fibromas, epidermoid cysts and osteomas [140].

The polyps that arise are due to heterozygous mutations in the APC gene that lead to truncated APC and have been defined in patients with FAP and patients with frequent sporadical colorectal cancers, the latter representing 85 % of human colorectal cancers [141-143]. A single mutation in the APC gene is usually insufficient for the initiation of colon cancer but a mutation in the second allele predisposes to susceptibility of colon cancer.

For patients with sporadical colorectal cancer, it has been reported that a portion of patients have a gain of function mutation in the β-catenin gene which prevents degradation of β-catenin [72]. Although some mutated forms of APC can still bind β-catenin, most lack the SAMP motif which is required to recruit axin into a stable destruction complex [144, 145]. Therefore, it is mutations in APC and β-catenin that generally lead to stabilisation of β-catenin.

Mutations of other Wnt pathway components have also been implicated in cancer, such as β-catenin (discussed later in 1.4). Briefly, increased β-catenin nuclear levels are associated with malignant tumours by consequential constitutive transcriptional activation of Wnt target genes including proto-oncogenes.

Other components of the pathway involved in cancer include Wnt ligands such as Wnt1. Wnt1 was first discovered as breast cancer a proto-oncogene [146] and due to its involvement in development, a process requiring rapid cell division and migration, dysregulation of Wnt1 can result in unwanted cell growth and movement, and potentially development of tumours [146].

The Wnt pathway has also been associated with metastasis of cancers due to its involvement in epithelial-mesenchymal transition (EMT) (Figure 7) [147, 148]. Several oncogenic pathways, including the Wnt/β-catenin can induce EMT and the repression of E-cadherin has been found to be a critical event in this [147, 149, 150]. Because β-catenin is released from E-cadherin into the cytoplasm during EMT, it was thought that Wnt/β-catenin contributed to EMT [151]. Upon investigation of this, it was discovered that in EMT induced by hypoxia-inducible factor 1a (HIF-1a) in prostate cancer, there was an upregulation of vimentin, N-cadherin and fibronectin, and downregulation of E-cadherin and cytokeratin-18. This was consistent with the invasive behaviour switch of prostate cancer cells by EMT [151].

Wnt-1 and β-catenin were also found to be upregulated in prostate cancer cells, and increase in β-catenin and phosphorylated GSK3β (inactive GSK3β) expression was found to be associated with EMT [151]. Furthermore, inhibition of GSK3β has been found to lead to transcription of E-cadherin transrepressor, Snail [152]. Transcription and stabilisation of Snail resulted in the repression of E-cadherin and EMT [152, 153]. These results suggest that Wnt/β-catenin may be involved in EMT in prostate cancer.

Wnt/β-catenin has also been implicated in metastasis of breast cancer. DiMeo *et al.* (2009) observed that metastasis of basal-like breast cancer to the lung was inhibited by repression of the canonical Wnt pathway [154]. Inhibition of Wnt signalling (through LRP6, a signal transducer of the Wnt pathway) was found to reduce the ability of cancer cells to self-renew and seed tumours *in vivo* and resulted in the repression of EMT transcription factors [154].

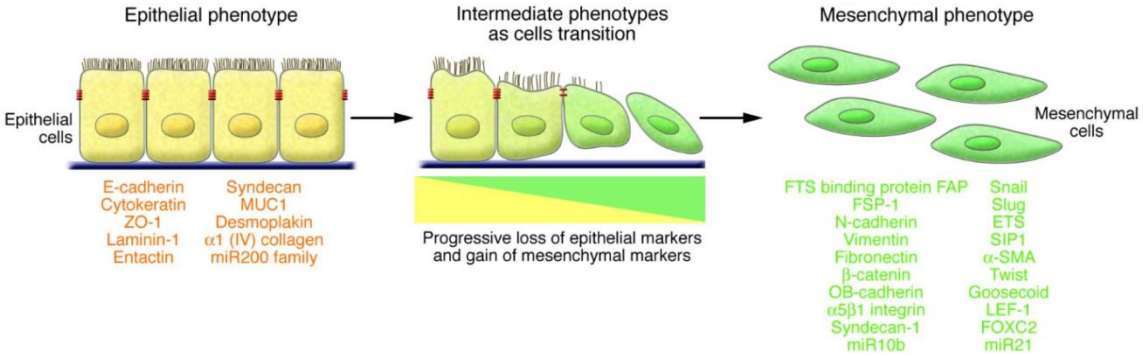


Figure 7 Basic and most characteristic features of epithelial-mesenchymal transition. Figure from Kalluri *et al.* (2009) [155].

The transition from the epithelial phenotype to the mesenchymal phenotype involves loss of epithelial markers (eg. E-cadherin) and an increase of mesenchymal markers (eg. β-catenin). Loss of epithelial markers results in events such as adherens and tight junction dissociation while the increase of mesenchymal markers results in events such as cytoskeletal organisation, cell migration, and invasion.

1.3.2 Type II Diabetes

The Wnt pathway is also involved in insulin sensitivity and dysregulation of the pathway could be involved with the development type II diabetes [156]. Insulin functions in increasing uptake of glucose from the blood, a process mediated by activation of the canonical Wnt pathway, which can lead to an increase in the cells sensitivity to insulin [157]. Overexpression of Wnt ligands may increase susceptibility to diabetes due to its role in fat production [158]. Wnt can also activate mitochondrial biogenesis, generating reactive oxygen species (ROS), which can cause acute hepatic insulin resistance [159]. Mutations in Wnt-associated transcription factors such as TCF7L2 have also been linked to the development of type II diabetes [130].

1.3.3 Pleiotropic effects

There are other, pleiotropic effects of APC gene mutations, impacting more than β-catenin. APC has been found to shape the cytoskeleton by promoting actin assembly [160], attaching onto growing microtubule ends [161], stabilising them and connecting them to actin [162, 163]. An earlier study found that staining of wildtype (WT) APC in mammalian cells revealed a filamentous network, extending through the cytoplasm and co-localising with microtubules [164]. Staining of mutant APC revealed a diffuse cytoplasmic pattern and this contrast in staining suggested that only WT APC associates with the actin cytoskeleton [164].

APC also interacts with IQGAP1 and APC-stimulated guanine nucleotide exchange factor (Asef) [161]. IQGAP1 moderates the association of APC with cortical actin in the leading edge of migrating cells and both proteins are necessary for directional migration and cell polarisation. APC also stimulates the activity of Asef, regulating the actin cytoskeleton and, therefore, cell adhesion, migration and morphology. Thus, when APC is truncated in colorectal cancer, the mutant APC activates Asef constitutively, inducing abnormal migratory properties which may be an important factor in the formation of adenomas and tumour progression [161].

APC mutations have also been found to have an effect on mitochondria in colon cancer cells [165]. Truncating mutations in APC eliminated its ability to bind Miro/Milton (a mitochondrial kinesin-motor complex), reducing formation of the complex. This resulted in disruption of mitochondrial distribution suggesting that APC is involved with driving mitochondria to the membrane in order to supply energy for processes such as cell migration [165].

1.4 Roles of the catenin family in disease

The roles that the catenin family play in the normal functioning of the cell can conversely be implicated in disease if their expression is dysregulated or they are localised aberrantly.

1.4.1 Aberrant expression in development

Roles of the catenins in development, in particular, β-catenin, include the modulation of cadherin function and the transduction of canonical Wnt signals in the Wnt pathway [14]. In vertebrates and invertebrates, a number of studies have shown that whole-animal knock-outs or knock-downs of β-catenin result in embryonic lethality [52, 166-169]. Other studies have provided evidence for the necessity of β-catenin and its role in maintaining stem cell compartments, differentiation, apoptosis and proliferation [40, 49-52].

Removal of another catenin, δ-catenin, was shown to impact vertebrate development, where its absence was embryonic lethal [170]. Other studies in mice implicated δ-catenin in cell hyper-proliferation and the development of neoplasia, due to the relationship of δ-catenin and nuclear factor-κβ (NF-κβ) signalling [171-173].

1.4.2 Aberrant localisation of β-catenin in cancer

Pathological contributions of β-catenin to the progression of cancer have also been clarified. In the presence of Wnt ligand, and in many cancers where there are mutations in the β-catenin gene, cytoplasmic β-catenin is stabilised, translocates into the nucleus [45, 174, 175] and increases transcription of genes involved in cancer development [4] (covered in 1.1.2 and 1.2.3). Briefly, nuclear β-catenin activates the LEF1/TCF family of transcription factors [45] resulting in upregulation of several genes linked to key cellular processes [176, 177]. This activates tumour promoting genes involved in cell proliferation (c-myc [178] and cyclin D [179]) and invasion (MMPs [180], uPA receptor [181]) and is a key contributor to cancer progression [49].

Recent research has found an association of β-catenin with colorectal cancer [182], melanoma [183], hepatocellular cancer [184], prostate cancer [184], thyroid cancer [185], ovarian cancer [186], breast cancer [187], medulloblastoma [188], pancreatic cancer [189] and gastric cancer [190]. In particular, it is the increased protein levels of β-catenin that have been linked to cancer [191-193]. Gene mutations of β-catenin, particularly N-terminal mutations [119] resulting in its stabilisation [194] have been found in several types cancers including colorectal cancer [71, 72, 195], hepatocellular carcinoma [196, 197] and melanoma [198].

Stabilisation of β-catenin (covered in section 1.2.3) can occur due to mutations in components of the degradation complex such as axin [111] or APC [72] or by mutations in β-catenin itself, such as the loss or mutation of the N-terminus which alters GSK-phosphorylation sites critical for degradation [14].

1.4.3 Mutations of the β-catenin gene (CTNNB1) and accumulation of β-catenin

Mutations in CTNNB1 result in the accumulation of β-catenin in many cancers. The majority of colorectal cancers (80 %) acquire mutations in both copies of the APC gene resulting in loss of function of the protein, which subsequently causes inefficient breakdown of β-catenin [199]. In a subset of tumours, oncogenic β-catenin mutations are detected at the N-terminal phosphorylation sites [84, 200, 201]. Both of these mutations result in stabilised β-catenin which activates downstream target genes and initiates tumour formation [202]. However, human colorectal tumours with β-catenin mutations are typically less aggressive and smaller in size than those with APC mutations [203]. In the colon cancer cell lines used in the research presented in this thesis (SW480 and HT29) β-catenin is WT. However, APC is mutated in both cell lines. In SW480 APC is truncated at aa1337 [204], while in HT29 two truncated forms of APC are observed at aa853 and aa1555 [204].

Several mutations in CTNNB1 have been reported in gastric cancers, which occur with increased incidence in patients with FAP [205, 206]. Gene mutations of β-catenin have also been implicated in hepatoblastoma and hepatocellular carcinoma (HCC) where missense mutations and internal deletions of β-catenin have been reported [207, 208]. In the case of HCC, the frequency of CTNNB1 mutations is 20 %, and even higher for HCCs associated with the hepatitis C virus [196, 209, 210]. Poor prognosis was shown to be associated with nuclear accumulation of β-catenin in HCC where there was enhanced nuclear staining in the invasive compartments of the tumours [211].

Other cancers that have been associated with mutations in CTNNB1 include melanoma [212], ovarian cancer [213], lung adenocarcinoma [214] and prostate cancer [215]. In prostate cancer, in addition to a CTNNB1 mutation, decreased levels of E-cadherin were reported [216]. This resulted in an increase of β-catenin which was free to bind and activate androgen receptors, facilitating tumour invasion and progression [217]. A high frequency of CTNNB1 mutations have also been found in anaplastic thyroid cancers [218, 219], while pilomatrixoma were found to have the highest percentage of CTNNB1 mutations of all human tumours examined so far, with at least 75 % possessing a β-catenin mutation [118, 220].

1.4.4 Nuclear accumulation of β-catenin in human tumours

Increased β-catenin signalling has also been associated with tumour progression, where heterogeneous patterns of nuclear, cytoplasmic and plasma membrane-bound β-catenin are observed in colorectal tumours [221].

Colorectal adenomas were the first tumours to be discovered to have nuclear localisation of β-catenin [222, 223] and increased nuclear β-catenin has since been found to be associated with invasion and EMT [224]. Levels of nuclear β-catenin have been shown to be at their highest at the invasion front of colorectal adenocarcinomas (Figure 8) [225-227] and have also been found to be associated with tumour size and grade of dysplasia [228-230]. These findings have led researchers to believe that nuclear β-catenin is important for the induction of invasive and metastatic colorectal tumours [231, 232].

Although CTNNB1 mutations have been observed in malignant melanoma, nuclear accumulation of β-catenin is far more common, with a third of tumours displaying nuclear accumulation of β-catenin [212, 233]. Similarly, CTNNB1 mutations were reported in only a small subset of endometrial and ovarian carcinomas, yet levels of nuclear β-catenin were increased in many more of the tumours [234]. Another study found that in 60 % of endometrial cancers there was an elevated level of β-catenin in the nucleus while only 10 % had mutations in CTNNB1 [235].

These findings suggest that there are other mechanisms at play that are associated with the stabilisation and accumulation of β-catenin, other than mutations in CTNNB1, and that high levels of nuclear β-catenin are implicated in the progression of a number of cancers.

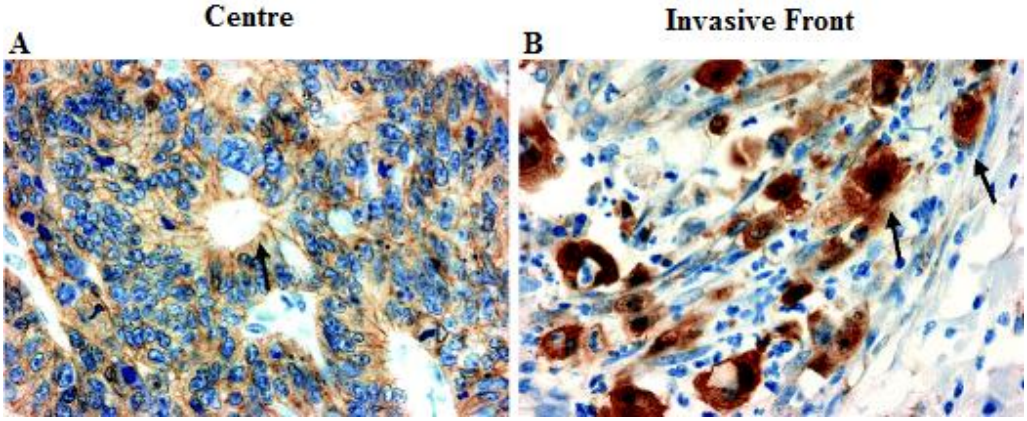


Figure 8 Serial section of a tumour showing nuclear accumulation of β-catenin at the tumour invasion front of human colorectal adenocarcinoma. Figure from Schmalhofer *et al.* (2009) [227].

A) Membranous staining of β-catenin (brown) and little nuclear β-catenin (nuclear counterstain in blue) in cells from the central area of a tumour.

B) Loss of membranous β-catenin and increased cytoplasmic/nuclear β-catenin (brown) in cells from the invasion front of a tumour (nuclear counterstain in blue).

1.4.5 Nuclear accumulation of β-catenin in mouse models

Studies using experimental mouse models found that loss of APC [236] or overexpression of active β-catenin [237] was sufficient for the formation of polyps. Transgenic mice overexpressing active β-catenin developed multifocal dysplastic lesions in the small intestine [238] while deletion of a β-catenin N-terminal fragment led to adenomatous polyposis [237].

Mutations in CTNNB1 were also identified in half of the hepatocellular carcinomas generated by transgenic expression of c-myc in murine liver [209], suggesting that CTNNB1 mutations occur during hepatocellular carcinoma tumour progression, in association with a cancer pathway facilitated by c-myc. Another study found an association between the activated mutant of β-catenin and development of skin tumours in mice. Transgenic mice expressing this activated mutant form of β-catenin in the skin showed increased hair follicle morphogenesis and were predisposed to developing pilomatrixoma-like skin tumours [239].

1.5 Nuclear Import of β-catenin

Researchers are endeavouring to shed light on the nuclear translocation mechanism of β-catenin because there is limited empirical evidence on how it shuttles in and out of the nucleus without possessing a nuclear localisation sequence (NLS), which is usually required for the nuclear import of proteins [240]. The principle nuclear entry pathway for proteins is through a chaperone system mediated by the importin receptors. Proteins that possess an NLS bind to importin-α, which binds importin-β which docks the complex to the nuclear pore by binding to nucleoporins (Nups) [241]. This protein-NLS-importin complex translocates through the nuclear pore by means of an energy-dependent pathway [241]. β-catenin lacks a distinct NLS sequence and its accumulation in the nucleus is independent of the importin system [241].

Orsulic *et al.* (1999) investigated β-catenin mediated nuclear transcription. It was found that cadherin overexpression caused the recruitment of β-catenin to the adherens junction, which meant that there was less β-catenin available to bind interactors in the nucleus and so β-catenin mediated nuclear transcription was reduced. Here, overexpression of β-catenin interactors (cadherin) effected β-catenin nuclear localisation. In contrast, overexpression of a nuclear interactor such as LEF-1 resulted in the translocation of β-catenin into the nucleus [242]. Furthermore, reduction in cell-cell adhesion during EMT may result in the release of β-catenin, contributing to increased nuclear transcription [243, 244].

The importins and β-catenin share similarities in their central cores, the Arm repeats. These Arm repeats have been implicated in many protein interactions and are required and sufficient for the nuclear localisation of β-catenin [245]. Our collaborators at the Westmead Millennium Institute (led by Dr Beric Henderson) have evidence behind the Arm 10-12 domain mediating the strongest nuclear import and export activity of β-catenin. They found that direct interaction of β-catenin with specific Nups allowed for translocation and that β-catenin functioned like a nuclear transport receptor in its ability to independently translocate across the nuclear pore complex (NPC). Furthermore, they found that activity was stimulated by phosphorylation at tyrosine-654 (Y654) (source: personal communication).

Another factor regulating protein interactions of β-catenin is phosphorylation, which can cause changes in the affinity of protein binding partners [246]. Our collaborators at the Westmead Millennium Institute found that mutations of Y654 altered transport activity. Mutagenesis of Y654 to a phosphomimetic (Y654E) or non-phosphorylatable (Y654F) mutant showed that both mutations resulted in increased import rate when compared to the WT. Regarding export rates, the phosphomimetic and WT showed comparable rates while the non-phosphorylatable mutant demonstrated a significantly slower rate.

1.6 The effect of phosphorylation at tyrosine-654 (Y654) on β-catenin

The phosphorylation of proteins is a post-translational modification (PTM) of particular amino acids (serine, threonine and tyrosine) that involves sequence-specific kinases and phosphatases [247]. Phosphorylation modulates protein activity (acting like a switch), altering protein folding, and hindering or enhancing interactions of other proteins [247].

Phosphorylation has been evidenced as central to cancer biology due to its roles in cell proliferation, oncogenic kinase signalling [248], transcriptional regulation [72], and the fact that phosphorylation is frequently altered by mutations in cancer, providing selective advantages to tumour cells [247]. A study investigating somatic cancer mutation datasets found that there was significant enrichment for mutations which resulted in gain/loss of phosphorylation when compared to other mutations (eg. random amino acid substitutions) [249]. Mutations in kinase genes in particular represented the highest number of mutations that disrupted phosphorylation, suggesting that phosphorylation target site mutations are associated with aberrant phosphorylation [249].

Phosphorylation has also been shown to be pivotal in treatment, as it is a pharmacologically targetable mechanism [250, 251]. Chapman *et al*. (2011) found that BRAF kinase inhibitor vemurafenib (PLX4032) showed promise for patients with metastatic melanoma carrying the BRAF V600E mutation [250].

Relevant to this thesis is that in a variety of tumours, β-catenin is stabilised by point mutations of N-terminus phosphorylation sites [192, 193, 252]. For example, phosphorylation of serine residues in the N-terminus of β-catenin is required for its degradation [14] and suppresses its nuclear transcriptional role [253-255]. β-catenin is also phosphorylated on its tyrosine residues and this is thought to increase β-catenin nuclear accumulation and tumour invasiveness [256]. Furthermore, a link has been found between Y654 phosphorylation and intestinal tumorigenesis in mice. Intestinal tumours that presented with nuclear β-catenin (highest nuclear levels at the invasive front of the tumours) and overexpression of β-catenin phosphorylated at Y654 predisposed mice to intestinal tumour development [256].

Other research provides a link between phosphorylation at Y654 and a change in binding. Roura *et al*. (1999) found that phosphorylation at Y654 resulted in a decreased binding of β-catenin to E-cadherin, suggesting this interaction is controlled by phosphorylation at this amino acid. Piedra *et al.* (2001) provided further evidence for changes in structure and binding partners of β-catenin upon phosphorylation at Y654. It was discovered that Y654 phosphorylation decreased binding to E-cadherin due to the negative charge that a phosphorylation presents. That is, the interaction is dependent on the absence of a negative charge at Y654 [257]. In contrast, it was found that binding of TATA-binding protein to β-catenin was enhanced by Y654 phosphorylation due to phosphorylation resulting in decreased association of the C-terminal to the Arm domain, allowing for binding (absence of steric hindrance of C-terminus) [257].

1.7 Therapeutic strategies for targeting β-catenin in cancer

A well-established base of empirical evidence in the literature has provided a link between nuclear β-catenin and cancer and this has generated significant interest among researchers to explore blocking the Wnt/β-catenin pathway in cancer treatment. Along with the research presented in this thesis that has investigated novel protein binding partners of β-catenin, it is conceivable that these same novel interactors could also become new therapeutic targets. Currently, there are alternative ways in which the Wnt/β-catenin pathway can be targeted for treatment.

Protein inhibitors of the Wnt/β-catenin pathway have been identified and prevent the interaction of β-catenin and TCF and reduce transcriptional output. However, these inhibitors are unsuccessful at controlling the high levels of stabilised β-catenin observed in cancer. Examples include Chibby which works with 14-3-3 to regulate subcellular distribution of β-catenin [258] or Duplin which binds β-catenin to inhibit β-catenin-dependent TCF activation [259]. Fragile histidine triad (Fhit) acts as a tumour suppressor by repressing transcriptional activity of β-catenin [260] as does protein inhibitor tax-interacting protein 1 [261]. ICAT prevents the interaction of β-catenin and the TCF/LEF family of transcription factors [262] while hydrogen peroxide-induced clone 5 (HIC5) represses TCF/LEF-driven transcription [263].

There is an interest in molecular inhibitors that target upstream events such as Wnt and Frizzled receptors [41, 139, 264]. Antibodies against Wnt ligand Wnt-1 have been shown to induce apoptosis in a number of human cancer cells including non-small cell lung cancer (NSCLC), breast cancer, mesothelioma and sarcoma [265]. Other studies have shown that small molecule and peptide inhibitors that interfere with Frizzled/Dishevelled interactions inhibit Wnt signalling [266, 267]. Inhibition of Wnt lipidation and secretion has also been achieved by studies using small molecules to disrupt Wnt signalling [268].

Other therapeutic strategies include non-steroidal anti-inflammatory drugs (NSAIDs) such as COX-2 inhibitor celecoxib which reduces nuclear β-catenin localisation and transcription [269, 270] or small molecules that disrupt β-catenin/TCF interactions [271]. Targeting β-catenin-coactivator interactions has had mixed success [272] due to complications in targeting interactions of β-catenin/TCF without interfering with other critical bindings partners such as APC, axin and E-cadherin, which bind the same region of β-catenin [273].

Tankyrase inhibitors (TNKSi) are emerging as potential targets in therapy, however, the functional consequence of this inhibition remains to be resolved because tankyrase binds an array of proteins [274]. The inhibitors stabilise axin (a key component in β-catenin degradation) and induce mobile degradosomes (which consist of destruction complex components) to rapidly turn over β-catenin, even in the presence of truncated APC [274].

De La Roche *et al.* (2014) found that β-catenin could be shielded from inactivation by axin in colorectal carcinomas, resulting in the desensitisation of colon cancer cells to TNKSi. After prolonged Wnt stimulation, β-catenin became unresponsive to TNKSi by its association with LEF1 and BCL9-2/B9L, which accumulate during Wnt stimulation and are upregulated in colorectal carcinomas [275]. Results suggest that for successful inhibition, interactions of β-catenin and LEF1/BCL9/B9L need to be targeted.

Research presented in this thesis may lead to the discovery of new, specific targets, in the form of protein interactors of β-catenin that are involved/aid in its role in the progression of cancer.

1.8 Protein interactions of β-catenin

β-catenin interacts with a number of intracellular proteins, and these interactions can occur simultaneously [14]. Interactions are regulated by the abundance of β-catenin and phosphorylation at specific serine and tyrosine residues within various domains [246]. When levels of β-catenin are relatively low, due to an absence of Wnt signalling, there is strong competition between protein binders for the limited amount of β-catenin [14]. When β-catenin is abundant, due to an activation of Wnt signalling or overexpression in some cancers, competition of protein binders is alleviated, allowing β-catenin to function in cell adhesion and Wnt signalling [276, 277]. Other studies have investigated the protein binders of β-catenin and Table 2 lists some reported in the literature.

One of the most well-studied roles of β-catenin is at adherens junctions where it plays an important structural role by interacting with E-cadherin [7, 278, 279], N-cadherin [280] and VE-cadherin [281, 282]. The cadherins bind Arm 1-12 of β-catenin, linking it to the actin cytoskeleton [246]. While the majority of cellular β-catenin is engaged in this structural role, the multifunctional activities of β-catenin are due to other protein interactions at other cellular locations [283, 284].

As described in 1.1.1 the Arm domain of β-catenin is where the majority of protein interactions occur [14], making it a domain of interest for those studying protein interactions of β-catenin. Section 1.5 outlined how Arm 10-12 has been implicated in nuclear transport and that phosphorylation of an amino acid within this domain, Y654 effects binding partners [246, 256, 257, 285].

While the literature has explored β-catenin at the nucleus, only a few studies concern the roles of β-catenin at other subcellular locations such as the mitochondria. Mitochondria, the powerhouse of the cell is essential for the health of cells and dysfunction of the mitochondria can result in a number of diseases, including cancer [286]. Some research has shown that β-catenin can have a negative effect on homeostasis of mitochondria which is significant because alteration in respiratory activity and mitochondrial DNA (mtDNA) transcription is a key feature of cancer cells [287, 288]. Mezhybovska *et al.* (2006) also found that β-catenin was capable of translocating to the mitochondria.

The golgi is another organelle of interest. The golgi receives proteins from the endoplasmic reticulum (ER) and prepares proteins for their role in the cell, or secretion out of the cell, by packing them into membrane-bound vesicles [289]. The golgi is also involved in lipid transport and lysosome formation [289]. Brunner *et al.* (2006) located β-catenin at golgi vesicles in tumours of the meningeal brain tissue (meningiomas) [290] while another study provided evidence that the interaction of β-catenin with presenilin occurred at the golgi [291].

Table 2 Known interacting partners of β-catenin in the literature.

*Subcellular location listed is the most commonly reported subcellular location. Note: this is also dependent on cell type

Known interactor	Site of interaction	Function	Subcellular location*	Reference
α-catenin	N-terminus and Arm 1	Adhesion	Cytoplasmic	[292]
Adenomatous polyposis coli (APC)	Arm 3-7	Degradation	Cytoplasmic/nuclear	[293]
Axin	Arm3-7	Degradation	Cytoplasmic/nuclear	[294]
Cadherins	Arm 1-12	Adhesion	Cell membrane	[31]
Caveolin-1	Not validated	Membrane localisation	Cell membrane	[295]
Epidermal growth factor receptor (EGFR)	Arm repeats	Catenin phosphorylation	Cell membrane	[296]
Fascin	Arm repeats	Adhesion	Cytoplasmic	[297]
F-box/WD repeat-containing protein 1A	N-terminus	Degradation	Cytoplasmic/nuclear	[298]
LEF-1	Arm repeats	Transactivation	Nuclear	[284]
Paxillin	Not validated	Adhesion	Cytoplasmic	[299]
Presenilin	Not validated	Degradation	Cell membrane, ER-membrane, golgi-apparatus membrane	[300]
RasGTPase-activating-like protein IQGAP1	N-terminus and Arm 1	Adhesion	Cytoplasmic	[301]
WW and PDZ domain-containing protein 1	C-terminus	Adhesion	Cell membrane	[302]

1.9 Quantitative Mass Spectrometry

Mass spectrometry (MS) has emerged as a powerful tool in proteomics over the past decade, useful for both peptide identification and quantitation. [303-305]. In fact, a draft of the human proteome equivalent to the human genome sequence did not exist prior to 2014 until mass spectrometry made a mass spectrometry-based draft map possible [306, 307].

Quantitative MS allows for comparisons between biological samples to determine molecular changes at the expressed protein level. The most widespread form of proteomic MS experiments are ‘bottom-up’, where comparisons are made on the peptide level and data extrapolated to the protein level. Quantitation can be achieved through labelling (metabolic or chemical) or in a label-free manner. SILAC is the best applied metabolic labelling (discussed 1.9.1) utilised for a number of studies including investigation of protein complexes [308], identification of cancer biomarkers [309], temporal dynamics of signalling pathways [310, 311] and research into enzyme substrate [312].

1.9.1 Metabolic labelling: stable-isotope labelling by amino acids in cell culture (SILAC)

SILAC is a reliable strategy for simultaneous identification and quantitation of two or more samples of complex protein mixtures, such as cell lysates [313]. It is a simple and efficient method enabling *in vivo* incorporation of specific stable isotope labelled amino acids into newly synthesised proteins [313]. Figure 9 demonstrates the SILAC methodology. In a two-plex experiment, it involves the culturing of two populations of cells – one grown in medium containing 'light' amino acids, and the other grown in medium containing 'heavy' amino acids incorporating ^{13}C and ^{15}N [314]. Therefore, when the 'heavy' amino acid is incorporated into peptides in growing cells, a known mass shift occurs when compared with the peptide that contains the 'light' amino acid, with no other chemical changes [314]. For example, for the amino acid arginine (Arg), which contains six carbons, there will be a difference of +6 Da (3 *m/z* for a doubly charged tryptic peptide) from 'light' to 'heavy' Arg when ^{13}C Arg replaces light ^{12}C Arg in the culture media.

The advantages of SILAC (a metabolic labelling strategy) include the exclusion of by-products from incomplete chemical labelling and mixing of samples occurs at the earliest possible step prior to fractionation and digestion, thereby minimising bias [314, 315].

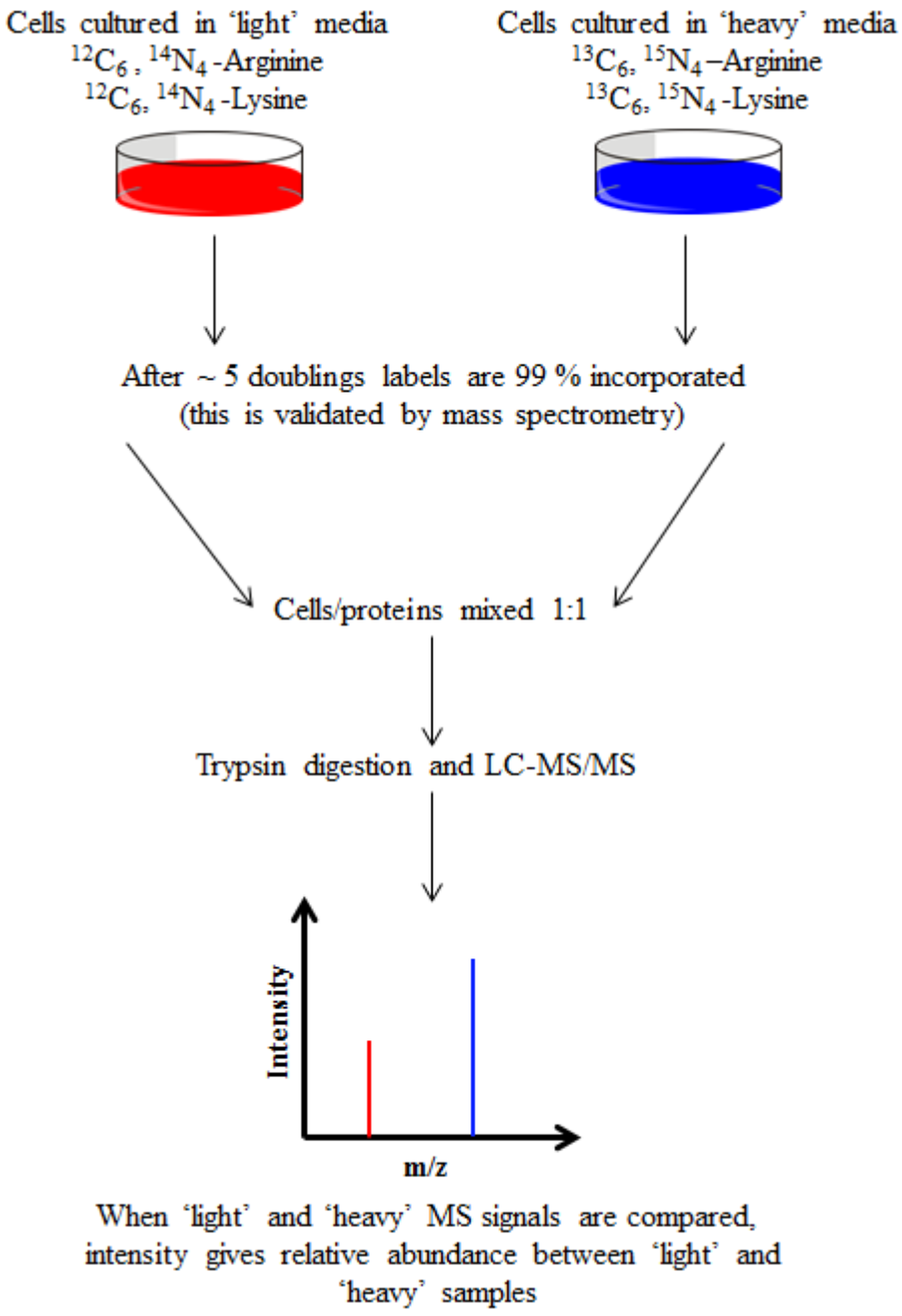


Figure 9 Schematic of the SILAC methodology.

'Light' and 'heavy' amino acids are incorporated into cells while they are being cultured. Mixing of the cells or proteins after lysis and consequential MS allows for quantitation by comparing intensity of signals between the samples.

1.9.2 Isobaric labelling: isobaric tag for relative and absolute quantitation (iTRAQ) and tandem mass tag (TMT)

iTRAQ or TMT tagging are two chemical labelling methods that use N-hydroxysuccinimide (NHS) based reagents for derivatization of peptide free amines [316-318]. The procedure requires peptides from samples for comparison to be individually labelled with isobaric tags that react with the primary amines of peptides to generate unique 'reporter ions' upon peptide fragmentation that are useful for peptide quantitation [319]. iTRAQ and TMT tags chemically consist of a charged reporter group, a peptide reactive group, and a neutral balance group to maintain an overall isobaric mass (Figure 10). A combination of $^{12/13}C$ and $^{14/15}N$ is used to produce isobaric reagents. The workflow consists of reducing, alkylating and digesting peptides, then labelling them separately before pooling at equal ratios and MS analysis [319].

When a peptide is fragmented by CID or HCD, the reporter groups are liberated, yielding specific product ions at low mass m/z values (reporter values). For example, iTRAQ 8-plex reagents yield the following reporter ions m/z: 114, 115, 115, 117, 118, 119, 121, 122 [319, 320]. Reporter ion intensities are proportional to the abundance of each peptide in the samples being compared and, therefore, used to quantitate changes in peptide abundance [320, 321]. In addition to these reporter ion signals used for quantitation, fragmentation also produces strong y- and b-ion signals to assist in confident peptide identification [319, 320].

A significant advantage of using isobaric tagging reagents is that they enable sample multiplexing, which is ideal for examining replicates and conducting temporal studies in a single MS experiment [320]. These chemical labelling strategies are an alternative when other methods such as metabolic labelling are not optional. For example, metabolic labelling requires dividing cells for label incorporation, so is unsuitable for plasma samples [322]. Cons of chemical labelling are that it involves chemical reactions that may not be completed and side-products may be produced [323, 324]. The labelling step is also introduced after the proteomes being compared have been digested, somewhat increasing sample error and variance between replicates. Chemical labelling reagents are also rather expensive so this needs to be considered in any experimental plan.

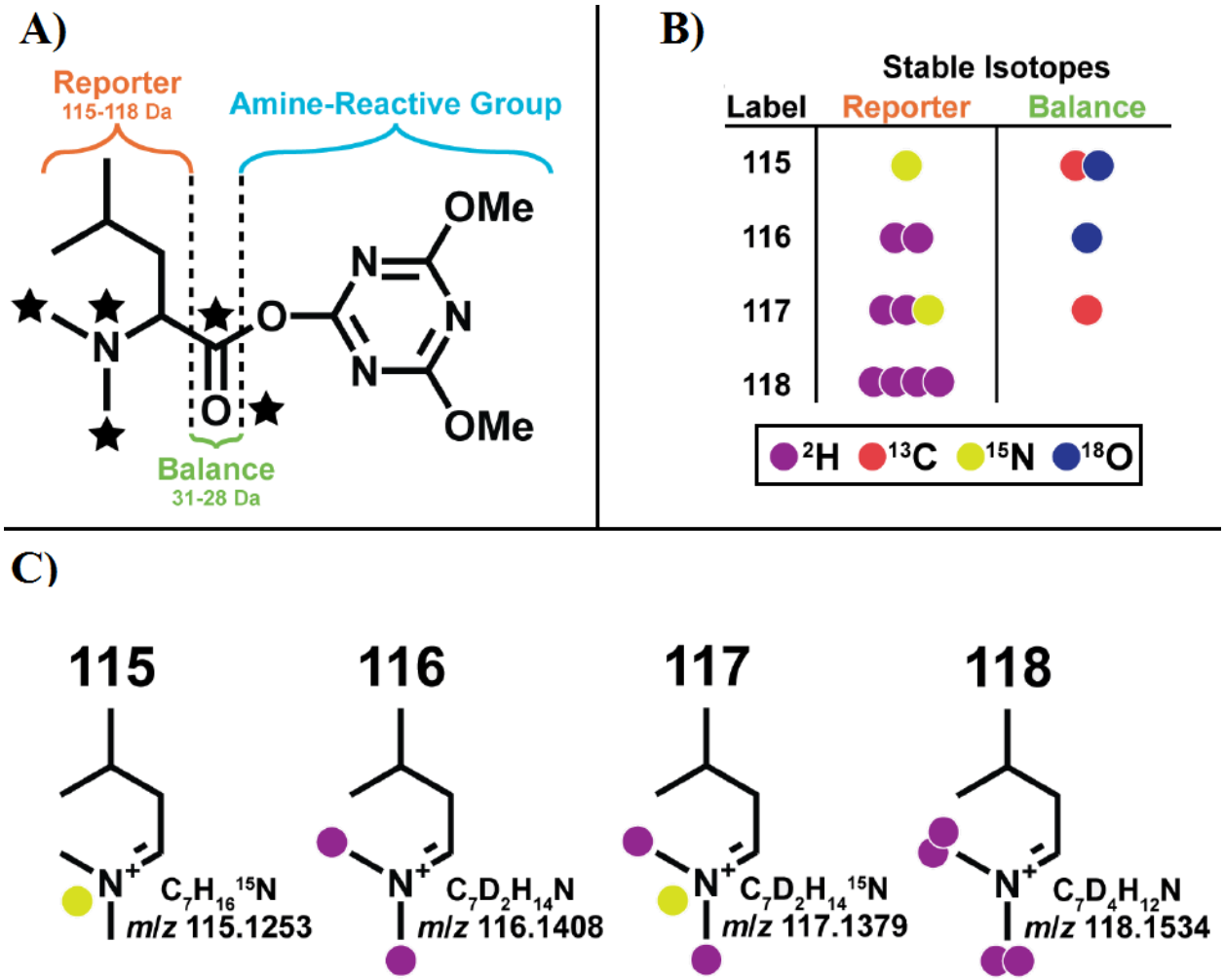


Figure 10 Structure of an iTRAQ 4-plex isobaric tag reagent. Figure from Greer *et al.* (2015) [325].

A) Structure the tag which is comprised of a uniquely charged reporter group (114-117 Da), an amine-reactive group and a neutral balance group (31-28 Da).

B) Isobaric incorporations. For example, the 115-label has incorporated a ^{15}N atom into the reporter group and a ^{13}C/ ^{18}O into the neutral balance group.

C) Reporter ion groups generated in 4-plex iTRAQ.

1.9.3 Label-free quantitative (LFQ) MS

Label-free approaches offer the advantage of compatibility with any biological sample type, with quantitation being derived from either precursor ion detection or product ion spectra. Label-free quantitation uses extracted ion chromatograms (XIC) from MS1 or MS2 spectra for peak integration [320]. Provided sufficient duty cycle has been set to enable 8-10 measurements across the eluting peak, area under the curve (AUC) quantitation is highly quantitative and reliable. More recently data-independent acquisition methods such as sequential window acquisition of all theoretical mass spectra (SWATH) have become topical [326-328]. While the acquisition method does not rely on precursor intensity to trigger MS/MS scans, the resultant quantitation is simply determined from AUC measurements of MS2 fragment ions. A simpler approach for LFQ that provides semi-quantitative data is to simply count the number of peptide spectra observed in a data-dependent acquisition experiment [329]. The number of MS/MS spectra is proportionate to protein abundance [329]. To be quantitatively reliable sufficient peptide counts per protein must be observed. Label-free strategies are beneficial when labelling methods (eg. metabolic labelling) are not compatible with the samples to be analysed (eg. plasma samples) or when labelling methods are not available or economical.

1.10 Investigation of β-catenin protein interactions in cancer studied by MS

Mass spectrometry has become a significant approach for research in proteomics, allowing qualitative and quantitative analysis of various samples. The research presented in this thesis utilised labelled MS and other studies have also used MS to investigate protein binding partners of β-catenin in cancer. Examples are listed below in Table 3.

Table 3 Other studies using MS to investigate binding partners of β-catenin.

Mass spectrometry	Method description	Interactors identified	Cell line(s)	Validation	Ref
Immunoprecipitation-HPLC tandem MS (IP-LC-MS/MS)	IP using β-catenin antibody in HEK293T overexpressing β-catenin and samples analysed by LC-MS/MS	Twelve. Two were known. Focused on 14-3-3zeta (1 peptide)	HEK293T (non-cancer)	IP, reverse-IP	[330]
Affinity purification mass spectrometry (AP-MS)	Introduced 3xFLAG tag into the DNMT1 locus in HCT116. MS identified 2 peptides of β-catenin. Interaction detected in RKO only when Wnt pathway was activated (increased levels of β-catenin as RKO has low endogenous levels unlike HCT116)	Dnmt1 (they were studying Dnmt1 and identified 2 peptides of β-catenin by AP-MS	HCT116 and RKO (colon cancer)	IP, reverse- IP and IF	[331]

Mass spectrometry	**Method description**	**Interactors identified**	**Cell line(s)**	**Validation**	**Ref**
Multidimensional protein identification technology (MudPIT)	HEK293 cells expressing Flag-tagged α-catenin, β-catenin, LEF1 and TCF4 were used for affinity purification and MudPIT analysis. Cells treated/non-treated to stabilise β-catenin, allowing for nuclear accumulation. Assessed effect of phospho-Y177-α-catenin on binding to β-catenin using IP with Flag:α-catenin-Y177 and Flag:α-catenin-Y177F. No effect was found	α-catenin, LEF1, TCF	HEK293 (non-cancer)	Western blot of Flag-tagged α-catenin, LEF1 and TCD	[34]

1.11 Investigation of the effect of tyrosine phosphorylation at Y654 on β-catenin

Other studies have investigated the effect of phosphorylation at Y654 on protein binders or function of β-catenin. Other research performed utilising recombinant β-catenin with the introduction of a point mutation at amino acid Y654 in order to produce a phosphomimetic (Y > E) or non-phosphorylatable (Y > F) are listed below in Table 4.

Table 4 Other studies that have utilised Y654E/F mutant constructs to investigate effect of phosphorylation at Y654.

Construct	Focus	Cell line(s)	Notes	Ref
GST-tagged Y654E/F β-catenin	Effect of phosphorylation on β-catenin/E-cadherin using binding assays, GST- and polyhistidine-tagged constructs	Caco-2 (colon cancer)	• First study to assess effect of tyrosine phosphorylation at Y654 • Phosphorylation at this amino acid in particular (when compared to other tyrosine residues) affected binding to E-cadherin • Binding of E-cadherin was reduced by phosphorylation at Y654 • For reasons of validation demonstrated that only the WT β-catenin construct could be phosphorylated and not the mutants	[285], section 1.5

Construct	**Focus**	**Cell line(s)**	**Notes**	**Ref**
GST-tagged Y654E/F β-catenin	Effect of phosphorylation on β-catenin/TATA-binding protein (TBP) using GST-tagged constructs and polyhistidine- and X-Press®- tagged constructs	SW480 (colon cancer)	• Phosphorylation at Y654 increased binding to TBP • Investigated structural basis for differences in binding and described phosphorylation at Y654 as decreasing association of the Arm domain with the C-terminus, opening the region up for interaction • Found that for β-catenin/TBP steric hindrance of the C-terminus is an issue but for β-catenin/E-cadherin it is the negative charge of the phosphorylation that is the issue • Studied the interaction *in vivo* and measured the amount of associated TBP with the constructs to find binding was increased when Y654 was phosphorylated	[257], section 1.5

Construct	Focus	Cell line(s)	Notes	Ref
GFP-tagged Y654E/F β-catenin	Effect of phosphorylation on Wnt signalling and intestinal tumorigenesis *in vivo* using GFP-tagged constructs and a conditional knock-in mouse model for Y654E	Cos-1 (non-cancer)	• Provided *in vivo* evidence that phosphorylation at Y654 decreases affinity to E-cadherin using a conditional knock-in mouse model into which a Y654E modification was introduced into CTNNB1 • Transfected cells with GFP-tagged constructs and found phosphorylation at Y654 facilitated phosphorylation at S675 • Results contrast the current belief that phosphorylation of Y654 aids tumour progression to a more invasive phenotype, showing that it increases tumour initiation by enhancing Wnt signalling • This enhanced Wnt signalling was attributed to facilitated phosphorylation of S675 which increases Wnt signalling by recruiting transcriptional co-activators [332-335], and reduced affinity to E-cadherin leading to an increased cytoplasmic pool of which the destruction complex has to control [285, 336]	[256], section 1.5

1.12 Thesis aims and scope

This thesis aims to identify new protein interacting partners of β-catenin using a discovery oriented approach. This is significant because β-catenin exerts its functional effects through interactions with binding partners. An unbiased proteomic approach using MS will enable hitherto unknown interactors to be detected.

Therefore, the aims of this thesis are to:

1. Use MS to identify putative β-catenin binding proteins in SW480 and HT29 colon cancer cells.
2. Examine the effect of β-catenin-Y654 phosphorylation on protein interactors.
3. Use orthogonal approaches including immunoprecipitation and immunofluorescence to validate putative novel β-catenin protein interactors.

This research in this thesis was conducted within the scope of a 4 year timeframe and restricted to human colon cancer cells lines. The study was approached from a proteomic perspective for candidate discovery and concluded with cell biology techniques for validation of binders. It is hypothesised that novel protein binders of β-catenin will be detected and that phosphorylation will have an effect on this interactome. These novel protein binders may shed light on the activity of β-catenin in the cell.

2 Methods and materials

2.1 Expression and purification of MBP-β-catenin fusion-proteins

2.1.1 Sequencing of β-catenin DNA constructs

Full-length, WT human β-catenin fused with N-terminal MBP in a pMALC2 vector (Figure 11) was obtained from GenScript. Point mutagenesis was performed by GenScript to produce phosphomimetic-Y654E and non-phosphorylatable-Y654F β-catenin. DNA sequencing conducted at the Australian Genome Research Facility confirmed the mutated DNA sequences were in-frame. Primers used are listed in Table 5.

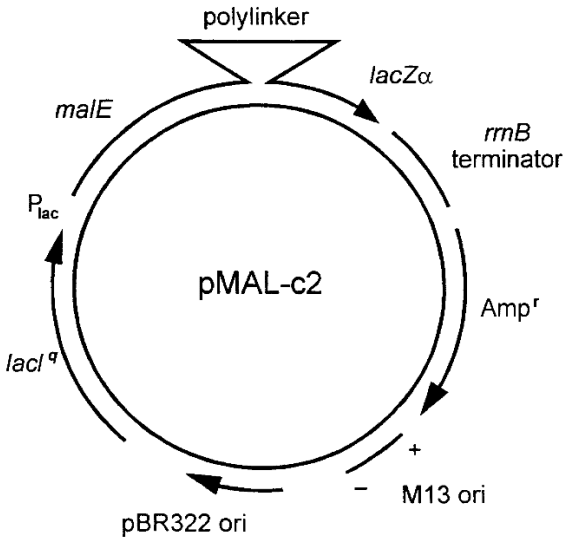


Figure 11 Diagram of expression vector pMALC2 (New England BioLabs pMAL Protein Fusion and Purification System Manual, #E8000S)

Table 5 Primer sequences used to sequence β-catenin-MBP DNA constructs.

Name	Sequence
pMALC2 forward	GGTCGTCAGACTGTCGATGAAGCC
pMALC2 reverse	TGTCCTACTCAGGAGAGCGTTCAC

2.1.2 Preparation of competent *E. coli* (DH5α) cells using $RbCl_2$

A loop of DH5α cells was used to inoculate 2YT broth which was incubated overnight (O/N) at 37 °C at 220 rpm. This culture was used to inoculate 50 ml 2YT broth/1 ml 1 M $MgSO_4$ and was incubated at 37 °C at 220 rpm until OD_{600}= 0.8-1. Cells were pelleted at 4500 rpm for 5 min at 4 °C. Supernatant (S/N) was discarded and cells resuspended in transformation buffer (TB) 1 (30 mM potassium acetate, 10 mM $CaCl_2$, 50 mM MnCl, 100 mM RbCl and 15 % glycerol) and incubated on ice for 5 min. Cells were pelleted at 4500 rpm for 5 min at 4 °C and resuspended in TB 2 (MOPS, 75 mM $CaCl_2$, 10 mM RbCl, 15 % glycerol) and incubated on ice for 30 min. Aliquots were stored at -80 °C.

2.1.3 Transformation of competent DH5α cells

Competent cells were thawed on ice and once thawed were incubated on ice for a further 10 min. DNA was added to the competent cells and incubated on ice for 30 min. Cells were heat pulsed in a 42 °C water bath for 2 min followed by an incubation on ice for 5 min and an incubation at RT for 5 min. 2YT broth was added to the cells and incubated at 37 °C for 1 h at 200 rpm. Cells were pelleted at 3000 rpm for 3 min and S/N discarded. Cells were resuspended in 2YT and plated onto 2YT/ampicillin agar. Plates were incubated at 37 °C O/N.

2.1.4 Colony screening

Colonies grown O/N were screened for the DNA of interest. PCR reactions were made up using Taq polymerase, 10x Taq buffer, 25 mM $MgCl_2$, 10 mM dNTP, water and relevant primers. Colonies were transferred into the PCR reaction and prior to cycling the reactions were incubated for 2 min at 50 °C and then 2 min at 95 °C. The reactions were cycled using a two-step protocol consisting of 40 cycles of 1 min at 95 °C and 1 min at 60 °C. After cycling, reactions were subjected to melting curve analysis, where the temperature was raised from 60 °C to 95 °C over a period of 20 min and SYBR green fluorescence was continually monitored.

Fragments were validated by purification of the PCR product (Wizard SV Gel and PCR Clean-Up System Kit, Promega) according to manufacturer's instructions and running them on an agarose gel where correct size could be checked against a molecular DNA ladder.

2.1.5 Preparation of glycerol stocks

Transformed colonies were grown in O/N cultures of 2YT/ampicillin broth. 700 µl of O/N culture was combined with 300 µl of 50 % glycerol and stored at -80 °C.

2.1.6 Expression of MBP-β-catenin fusion-proteins

A loop of glycerol stock was used to inoculate 10 ml 2YT/ampicillin broth and incubated O/N at 37 °C, 220 rpm. This culture was used to inoculate 200 ml 2YT/ ampicillin broth at 37 °C, 220 rpm until OD_{595}=1.0. IPTG was added (final concentration of 0.3 mM) and incubated at 25 °C for 4 h. Cells were pelleted at 5000 rpm for 5 min and S/N discarded. Cells were washed in TBS and pelleted at 5000 rpm for 5 min and S/N discarded. Cells were resuspended in H-buffer (TEDM, 0.5 M NaCl, protease inhibitor) (TEDM = 20 mM Tris (pH 8), 1 mM EDTA, 1 mM DTT, 5 mM $MgCl_2$) and lysate sonicated for 10 s at 10 % output (x10) and spun at 20,000 x g for 30 min at 4 °C. S/N was stored at 4 °C.

2.1.7 Purification of MBP-β-catenin fusion-proteins

Amylose columns were sanitised with 1 CV water, 3 CV 0.1 % SDS, 1 CV water, 2 CV 1 M NaCl and 1 CV water and pre-equilibrated with 2 CV TEDM followed by 5 CV H-buffer. Lysate was loaded onto the column followed by washes with 2 CV H-buffer and 10 CV TEDM. Protein was eluted with E-buffer (TEDM + 36 mg/ml maltose + protease inhibitor). The column was regenerated with 1 CV water, 3 CV 0.1 % SDS, 1 CV water, 2 CV 1 M NaCl, 1 CV water and 10 CV TBS and stored in fresh TBS.

2.1.8 Dialysis of MBP-β-catenin fusion-proteins

Dialysis was conducted according to manufacturer instructions using Slide-A-Lyzer dialysis cassettes (Thermo Scientific). Briefly, fusion-proteins were dialysed against TEDM for 2 h at 4 °C followed by a change of buffer and further dialysis for 2 h at 4 °C. Dialysis buffer was changed and the protein dialysed O/N at 4 °C.

A flow-chart which illustrates the methodology of expression and purification of proteins from transformation of cells to dialysis of purified proteins is shown in Figure 12.

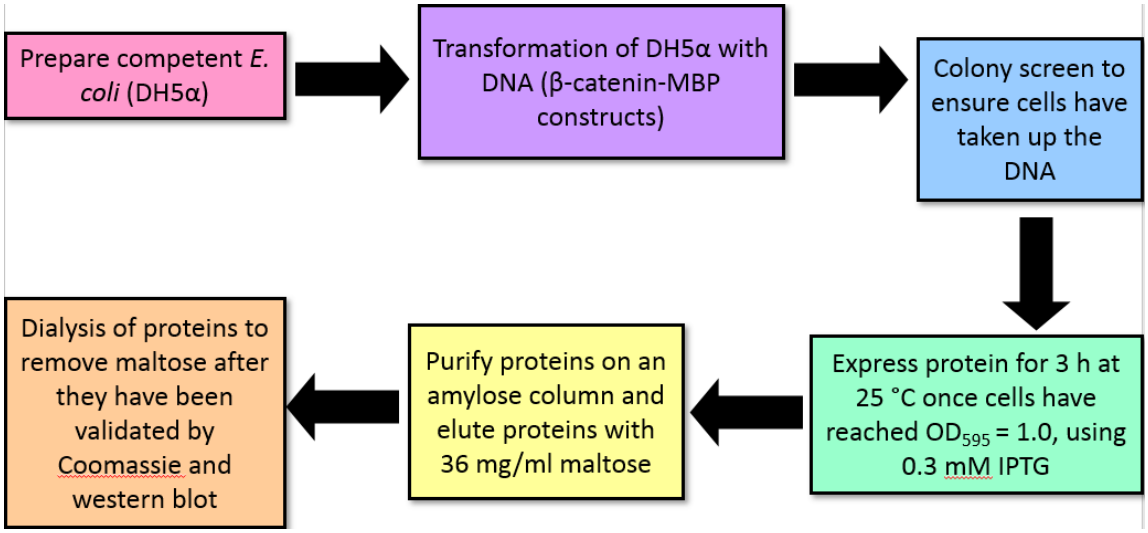


Figure 12 Flow-chart depicting expression and purification of proteins.

2.2 Cell culture

2.2.1 Cell line propagation

Human cancer cell lines were cultured. Cell lines are shown in Table 6.

Table 6 Human cancer cell lines.

Cell Line	Cancer Type
SW480	Colon
HT29	Colon
HEK293T	Non-cancer (human embryonic kidney)

Cells grown in non-SILAC media were cultured in Dulbecco's Modified Eagle Medium (DMEM) + Glutamax media (Invitrogen) supplemented with 10 % fetal bovine serum (FBS) (Invitrogen), 1 % Penicillin/Streptomycin (Invitrogen) and 10 mM HEPES (Invitrogen). Incubator conditions were 37 °C, 5 % CO_2. When confluency reached 70-80 %, cells were washed with phosphate-buffered saline (PBS) (Astral Scientific) and detached with trypsin (Invitrogen). Cells were then seeded into flasks (Corning Incorporated) or dishes (Greiner-BioOne).

Cells grown in SILAC media were cultured in DMEM minus L-Lysine and L-Arginine (Thermo Scientific) supplemented with 10 % dialysed fetal bovine serum (FBS) (Invitrogen), 1 % Penicillin/Streptomycin (Invitrogen).

Heavy labelled media was supplemented with 84 mg/L L-Arginine ($^{13}C_6$, $^{15}N_4$) (Cambridge Isotopes) and 146.2 mg/L L-Lysine ($^{13}C_6$, $^{15}N_4$) (Cambridge Isotopes). Light labelled media was supplemented with 84 mg/L L-Arginine ($^{12}C_6$, $^{14}N_4$) (Cambridge Isotopes) and 146.2 mg/L L-Lysine ($^{12}C_6$, $^{14}N_4$) (Cambridge Isotopes). Incorporation of heavy isotope labels was confirmed by MS after at least six doublings.

Incubator conditions were 37 °C, 5 % CO_2. When confluency reached 70-90 %, cells were washed with phosphate-buffered saline (PBS) (Astral Scientific) and detached with trypsin (Invitrogen). Cells were then seeded into flasks (Corning Incorporated) or dishes (Greiner-BioOne).

2.2.2 Whole cell lysis

Cells in dishes were washed in PBS and lysed directly with RIPA buffer (pH 7.5) (20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 % Triton X-100) supplemented with protease inhibitor (2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 µg/ml leupeptin) (Roche Diagnostics). Cell lysate was collected using cell scrapers (Corning Incorporated) and sonicated for 10 s. Samples were centrifuged at 10,000 rpm and the S/N retained. Samples were stored at -20 °C.

2.2.3 Cell thawing and freezing

Cells were thawed from -80 °C freezer aliquots in a 37 °C water bath and placed into media and media changed the next day. Cells were also frozen for -80 °C stocks. Freezing media consisted of 10 % DMSO cooled to 4 °C before being added to cell pellets and transferred into cryo-vials. Cryo-vials were placed into an isopropanol freezing container for 24 h before being transferred to -80 °C storage.

2.3 Protein quantitation

2.3.1 Bicinchoninic acid (BCA) protein assay

BCA assays were conducted according to manufacturer's instructions (BCA Protein Assay Kit, Thermo Scientific). Briefly, standard curves were prepared using bovine serum albumin (BSA) (Sigma). BCA reagent was added to the standards and samples of unknown concentration. After incubation at 37 °C for 30 min, absorbance was measured at 595 nm against a standard curve using the Fluorostar Optima (BMG Labtech).

2.3.2 Bradford assay

Bradford assays were conducted according to manufacturer's instructions (Bradford reagent, Biorad). Briefly, Bradford reagent was diluted with water (1:5). Known concentrations of BSA or 1 µl of unknown sample was added to 1 ml of the Bradford dilution. Samples were incubated for 5 min at RT and absorbance measured at 595 nm against a standard curve using a LambdaBio spectrophotometer (Perkin-Elmer).

2.4 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

2.4.1 SDS-PAGE

Samples comprised of protein, LDS (Invitrogen), reducing agent (Invitrogen) and water were heated at 95 °C for 5 min with gentle shaking and loaded into 4-12 % Bis-Tris gels (Invitrogen). Gels were run in 1× MOPS Running Buffer (Invitrogen) at 200 V for 40 min.

2.4.2 Coomassie Blue gel staining

Gels were fixed in 40 % methanol, 7 % acetic acid for 20 min and stained with Coomassie G250 (Sigma) for 10 min. Gels were destained with 25 % methanol.

2.4.3 Western blotting

The gel was transferred onto a nitrocellulose membrane by a transfer performed at 30 V for 90 min. Membranes were washed with tris-buffered saline (TBS) (1 M Tris-HCl, 3 M NaCl) and blocked using 5 % skim milk (Diploma Skim Milk Powder) in TBS-T (TBS with 0.1 % Tween 20). Membranes were washed with TBS-T and incubated with primary antibody (diluted in 5 % BSA/TBS-T) for 1 h at RT. Primary antibodies employed are listed in Table 7. Membranes were washed in TBS-T and incubated with secondary antibody for 30 min at RT. Secondary antibodies employed are also listed in Table 7. Membranes were rinsed in water and imaged using the Odyssey Licor 3.0 (Millennium Sciences) at 700 nm or 800 nm.

Table 7 Antibodies and dyes.

Primary antibodies			
Name	**Company**	**Catalog number**	**Species**
APC Ab1	Calbiochem	OP44	Mouse
APC H290	Santa Cruz	sc-7930	Rabbit
β-catenin	BD	610154	Mouse
β-catenin	Santa Cruz	sc-7199	Rabbit
β-catenin-pY654	Metagene	sc-57599	Mouse
COPB	Thermo Scientific	Pa1-061	Rabbit
Cdk1	BD	610037	Mouse
COPS5	Cell Signalling	6895	Rabbit
hnRNPK	Santa Cruz	sc-25373	Rabbit
HSP70 (mito)	Thermo Scientific	MA3-028	Mouse
HSP90	Cell Signalling	4874	Rabbit
KCL4	Metagene	sc-376702	Mouse
KPNA1	Thermo Scientific	PA5-21032	Rabbit
Opa1	BD	612606	Mouse
Rab11	Cell Signalling	5589	Rabbit
Rab5	Cell Signalling	3547	Rabbit
Rab7	Cell Signalling	9367	Rabbit
Tab182	Thermo Scientific	PA5-28728	Rabbit
Secondary antibodies			
IRDye 800CW goat α-rabbit	Millennium Science	926-32211	Rabbit
IRDye 800CW goat α-mouse	Millennium Science	926-32210	Mouse
IRDye 680CW goat α-rabbit	Millennium Science	926-32221	Rabbit
IRDye 680CW goat α-mouse	Millennium Science	926-32220	Mouse
AlexaFluor® 405 α-mouse	Invitrogen	RM2626	Mouse
AlexaFluor® 405 α-rabbit	Invitrogen	A31556	Rabbit
AlexaFluor® 488 α-mouse	Invitrogen	A11029	Mouse
AlexaFluor® 488 α-rabbit	Invitrogen	A11034	Rabbit
AlexaFluor® 594 α-mouse	Invitrogen	A11032	Mouse
AlexaFluor® 594 α-rabbit	Invitrogen	A11037	Rabbit
Dyes			
CMX-Ros	Invitrogen	M7512	n/a
Hoechst 33254 (nuclear stain)	Sigma-Aldrich	H6024	n/a

2.5 Pull-downs using fusion-protein and MS

2.5.1 Pull-down using MBP-constructs and amylose resin

Each pull-down was performed in triplicate, using independent cell lysates for each replicate. Furthermore, the β-catenin-MBP used for each replicate was purified in a single experiment (single cell extracts).

Amylose beads were centrifuged at 2000 rpm for 2 min and S/N discarded. Beads were incubated in TEDM at RT for 5 min with gentle shaking. Beads were centrifuged at 2000 rpm for 1 min and S/N discarded. Beads were resuspended in TEDM (1:1) and 120 µg used for the pull-down. Excess fusion-protein or MBP-tag (200 µg) was incubated with the beads for 1 h at 4°C with gentle shaking.

Beads (now with the tag bound) were centrifuged at 2000 rpm for 30 s and S/N discarded. Beads were washed in TEDM to remove unbound protein and incubated in excess cell lysate (1 mg) for 1 h 10 min at 4°C with gentle shaking. Samples were centrifuged at 2000 rpm for 30 s and pellets washed in PBS by gentle pipetting to remove any unbound proteins.

Samples were centrifuged at 2000 rpm for 30 s and S/N discarded. Bound proteins were eluted by a 5 min incubation at RT with maltose (100 mM maltose in TEDM). Eluates were loaded onto gels for SDS-PAGE.

2.5.2 In-gel trypsin digestion and peptide extraction

Following SDS-PAGE and gel staining/destaining, protein bands of interest were cut out and washed in 100 mM ammonium bicarbonate (NH_4HCO_3). They were dehydrated with 50 % acetonitrile (ACN)/100 mM bicarbonate for 10 min and washed for 5 min in 100 % ACN. Samples were reduced with 10 mM dithiothreitol (DTT) (Biorad) in 100 mM NH_4HCO_3 for 30 min at 56 ˚C and alkylated with 25 mM iodoacetamide (IAA) (Sigma) in 100 mM NH_4HCO_3 for 30 min at RT in the dark. Pieces were washed in 100 µl 100 mM NH_4HCO_3 for 5 min and 50 % ACN/50 mM NH_4HCO_3 for 5 min and dehydrated with 100 % ACN. 13 ng/µl trypsin solution was used to cover gel pieces and the digest occurred at 37 ˚C for 16-18 h.

Peptides were extracted using sonication and 0.1 % trifluoroacetic acid) (TFA) (Sigma) followed by drying down of the peptides by vacuum centrifugation (SpeedVac SC110A, Thermo Scientific). Peptides were stored at -20 °C and resuspended in 0.1 % FA for MS.

2.5.3 Mass spectrometry (LC/MS/MS)

Samples were analysed using LC-MS instrumentation consisting of an Easy nano-flow HPLC system (Thermo Scientific) coupled via a nanoelectrospray ion source (Thermo Scientific) to an Orbitrap Elite mass spectrometer (Thermo Scientific). Peptide separation was performed on a 20 cm x 100 µm column packed in-house with Halo® 2.7 µm 160 Å ES-C18 (Advanced Materials Technology). Peptides were loaded in buffer A (0.1 % (v/v) FA) and eluted with a 97 min linear gradient of buffer B (100 % (v/v) ACN/0.1 % (v/v) FA) at 300 nL/min (2-85 % buffer B in 97 min). Mass spectra were acquired in a data-dependent manner, with an automatic switch between MS and MS/MS using a top 15 method. MS spectra were acquired in the Orbitrap analyser with a mass range of 350-1800 *m/z* and 120000 resolution at *m/z* 400 (Orbitrap Elite) and AGC target of 1e6 ions. HCD peptide fragments, acquired at 35 normalized collision energy, were analysed at high resolution in the Orbitrap with dynamic exclusion of ions for 10 sec.

2.5.4 Data acquisition and statistical analysis

Raw files were processed for through Proteome Discoverer 1.3 (Thermo Scientific). In the spectrum selector nodule, minimum-maximum precursor mass was set to 200-2000 Da. Peptide identification was performed with Mascot (Matrix Science) against the SwissProt human database and a reversed human decoy database. Searches specified 10 ppm precursor mass tolerance, 0.02 Da fragment mass tolerance, maximum 2 missed cleavages, fixed modifications of carbamidomethylation (C) and variable modifications of oxidation (M), acetylation (N-terminal), $^{13}C_6$, $^{15}N_2$ on lysine (K), and $^{13}C_6$, $^{15}N_4$ on arginine (R) and peptides filtered at 0.01 % FDR. SILAC pairs (heavy/light ratio calculation) were identified and quantitated using the built-in SILAC 2-plex quantification method (Proteome Discoverer 1.3) using Arg-10 and Lys-8 labels and mass precision set to 2 ppm for event detection.

Utilising the data from Proteome Discoverer 1.3, candidate lists were produced. This list excluded proteins not present in all 3 replicates at a fold-change of > 2 for light (β-catenin-MBP):heavy (MBP). A t-statistic was generated using the equivalent of a one-sample t-test to test deviance from a fold-change of > 2. Based on this t-statistic a p-value was generated by using a one-tailed t-distribution with two-degrees of freedom. Proteins with a p-value of > 0.05 were excluded from the candidate list.

A flow-chart which illustrates the methodology from pull-down through to data analysis is shown in Figure 13.

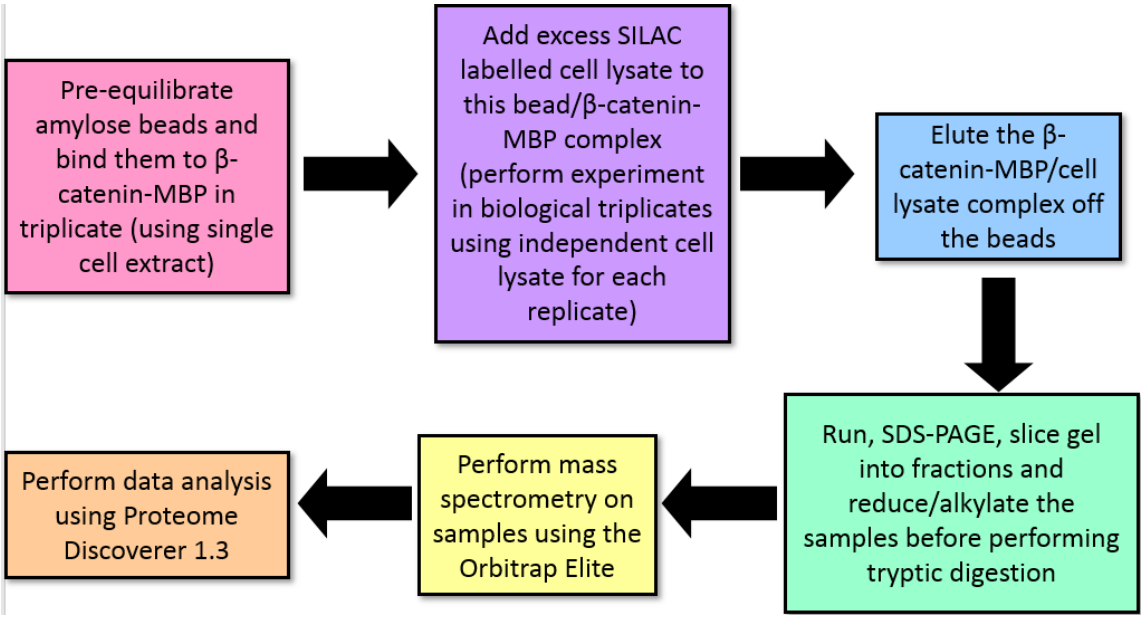


Figure 13 Flow-chart depicting pull-down, mass spectrometry and data analysis methodology.

2.6 Immunoprecipitation (IP)

2.6.1 Protocol for sepharose A beads (for polyclonal rabbit antibodies) and protein G agarose beads (for monoclonal mouse antibodies)

Sepharose A beads were prepared by swelling 150 mg of beads in 5 ml water and allowing them to settle. S/N was removed and 2 ml of water added. Upon beads settling, S/N was removed and 5 ml of lysis buffer added. Upon beads settling, S/N was removed and 2 ml of lysis buffer added. S/N was removed and final bead volume in lysis buffer made up to 1 ml (3:1 slurry of beads:buffer).

Cells were lysed in RIPA buffer (with protease inhibitor) and incubated on ice for 10 min, spun at 13,000 rpm for 10 min and S/N collected. 1 mg of lysate was used for 2 µg of polyclonal rabbit antibody (

) or 2 mg of lysate for 1 µg of the monoclonal mouse antibody (

). Samples were placed on a rotating wheel O/N at 4 °C. 30 µl sepharose A or protein G agarose was added to the lysate/antibody complex and rotated on a wheel for 2 h at 4 °C. Samples were centrifuged at 2000 rpm for 3 min at 4 °C and S/N removed. Pellets were washed in 700 µl RIPA, spun on a rotating wheel for 5 min at 4 °C and centrifuged at 2000 rpm for 3 min at 4 °C. S/N was removed, pellets washed in 700 µl TBS, spun on a rotating wheel for 5 min at 4 °C and centrifuged at 2000 rpm for 3 min at 4 °C. S/N was removed and the lysate/antibody complex eluted from beads using Laemmli buffer and incubation at 95 °C for 7 min. Samples were spun at 13,000 rpm for 1 min and loaded onto a gel.

2.7 Immunofluorescence (IF) and co-localisation

Cells were seeded into 6-well plates with a microscopy slide in each well. At the conclusion of the experiment, the slides were removed from the wells and mounted in order to be viewed under the microscope.

Upon cells reaching ~ 80 % confluency, wells were washed three times in PBS. Cells were fixed in methanol-acetone (1:1) for 10 min at -20 °C and washed in PBS three times. Cells were blocked with 3% BSA/PBS for 1 h, washed three times in PBS and incubated with primary antibody for 1 h at RT. Antibodies (

) were diluted in 3 % BSA/PBS and added to each well. Cells were washed in PBS three times and secondary antibody/Hoechst nuclear stain (

) added for 1 h at RT. Cells were washed three times in PBS and slides mounted using Vectashield and stored at 4 °C until ready for imaging on the DeltaVision (GE Healthcare).

2.7.1 CSK wash

Cell lines with a high amount of cytoplasmic β-catenin required a CSK/0.1 % triton X-100 wash. This wash, performed on ice, washes out some cytoplasmic content, reducing cytoplasmic β-catenin signal intensity. CSK buffer consists of 100 mM NaCl, 300 mM sucrose, 1 mM $MgCl_2$, 10 mM PIPES, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, protease inhibitor and 0.1 % triton X-100. The CSK wash is performed before cell fixation in the IF protocol.

2.7.2 Mitochondrial staining

CMX-Ros dye (

) was used to stain the mitochondria. At the beginning of the IF protocol (before the cells are first washed in PBS three times after media is removed), CMX-Ros dye is added to wells containing media at 1:10,000. Cells are incubated for 10 min at 37 °C in the dark.

2.8 Imaging on the DeltaVision (GE Healthcare)

The DeltaVision is a high-resolution fluorescence microscope used to image IF experiments. This microscope is capable of deconvolution of images and z-stacking. IF slides were viewed by the microscope which detects DAPI probes (blue dye), FITC probes (green dye) and TRITC probes (red dye). SoftWoRx Explorer 1.3 software was used to view and analyse images acquired off the DeltaVision.

3 Novel protein interactions of β-catenin in SW480

3.1 Introduction

In this chapter, the colon cancer cell line SW480 was used to investigate protein interactors of β-catenin. The Wnt pathway in SW480 is constitutively active and β-catenin is wildtype (WT). β-catenin is expressed in high levels in this cell line, and its localisation is both cytoplasmic and nuclear. All these characteristics make it an appropriate cell line to use when investigating protein interacting partners.

Recombinant β-catenin-MBP was used to pull-down protein binding partners in SW480 and with the use of amylose resin, this complex was captured and eluted. Eluates were run on SDS-PAGE and gel bands excised and peptides prepared for MS. The approach utilised in this thesis uses the advantages of SILAC labelling, in order to reduce variation between replicates. Therefore, all cells in cell culture (consequently used for pull-downs) had been grown in heavy or light SILAC media, and validation of incorporation of the labels was performed before any pull-down experiments were conducted. High-resolution LC/MS (Orbitrap Elite, Thermo Scientific) was used in order to best identify novel interacting partners of β-catenin.

3.2 Results

3.2.1 β-catenin-MBP DNA constructs

Full-length, WT human β-catenin fused with N-terminal MBP in a pMALC2 vector was obtained from GenScript. Point mutagenesis was performed by GenScript to produce phosphorylation mutants of full-length β-catenin at Y654 to obtain Y654E (phosphomimetic) and Y654F (non-phosphorylatable). DNA sequencing conducted at the Australian Genome Research Facility confirmed the mutated DNA sequences were in-frame.

3.2.2 Purification of fusion-proteins

Recombinant protein was produced by expression in *E.coli* (DH5α) and purification on an amylose resin column. A number of checks were performed on the purified protein to ensure they were fit for experimental use and were performed by Coomassie Blue staining, western blot and MS. Checks include ensuring the purified proteins were β-catenin, the MBP tag had not been cleaved, there was little or no bacterial protein contamination, there was little or no degradation of the purified protein and that the point mutations were present in the expressed gene products.

Figure 14 shows a Coomassie Blue stained SDS-PAGE gel of the MBP-β-catenin fusion-protein. From these images, it is clear that most of the signal was at the expected molecular mass (130 kDa) and that there was only minor degradation of the protein and little background contamination due to other bacterial proteins. Figure 15 shows immunodetection of β-catenin further confirming the identity of the recombinant protein.

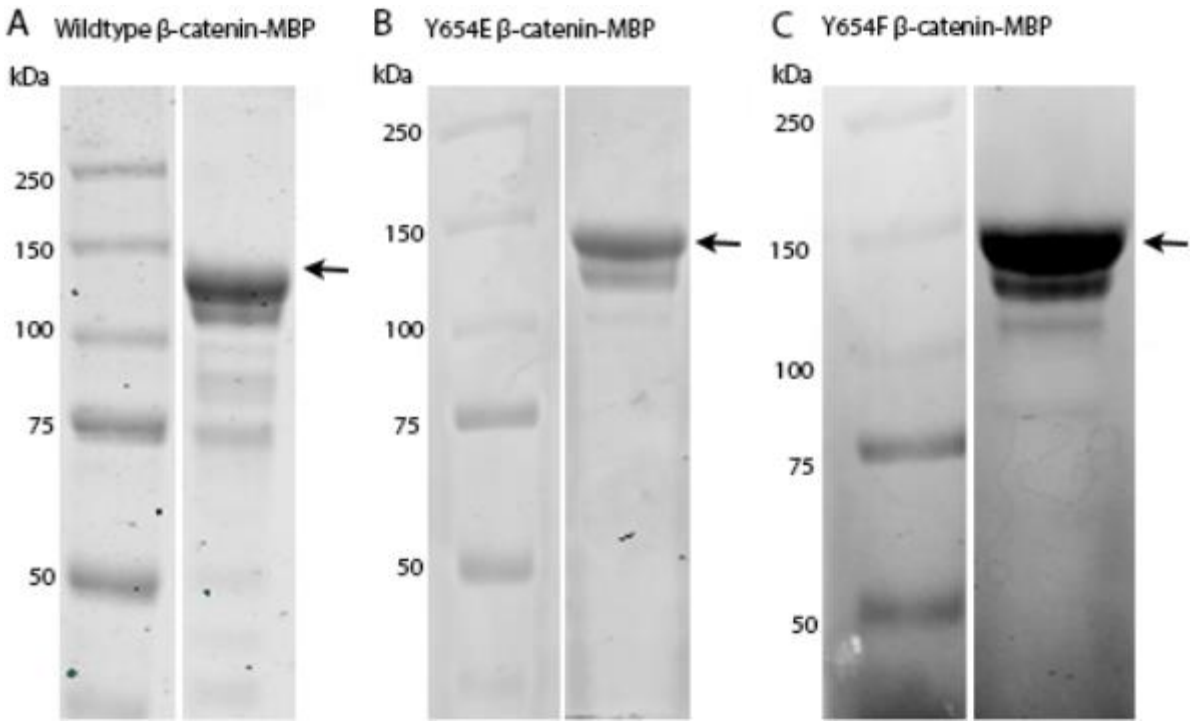


Figure 14 Coomassie Blue staining of purified β-catenin-MBP.

Coomassie Blue staining shows minimal degradation of the protein and the molecular weight of the protein (130 kDa) provides evidence that the MBP tag has not been cleaved off.

A) WT β-catenin-MBP purified protein.

B) Phosphomimetic β-catenin-MBP purified protein.

C) Non-phosphorylatable β-catenin-MBP purified protein.

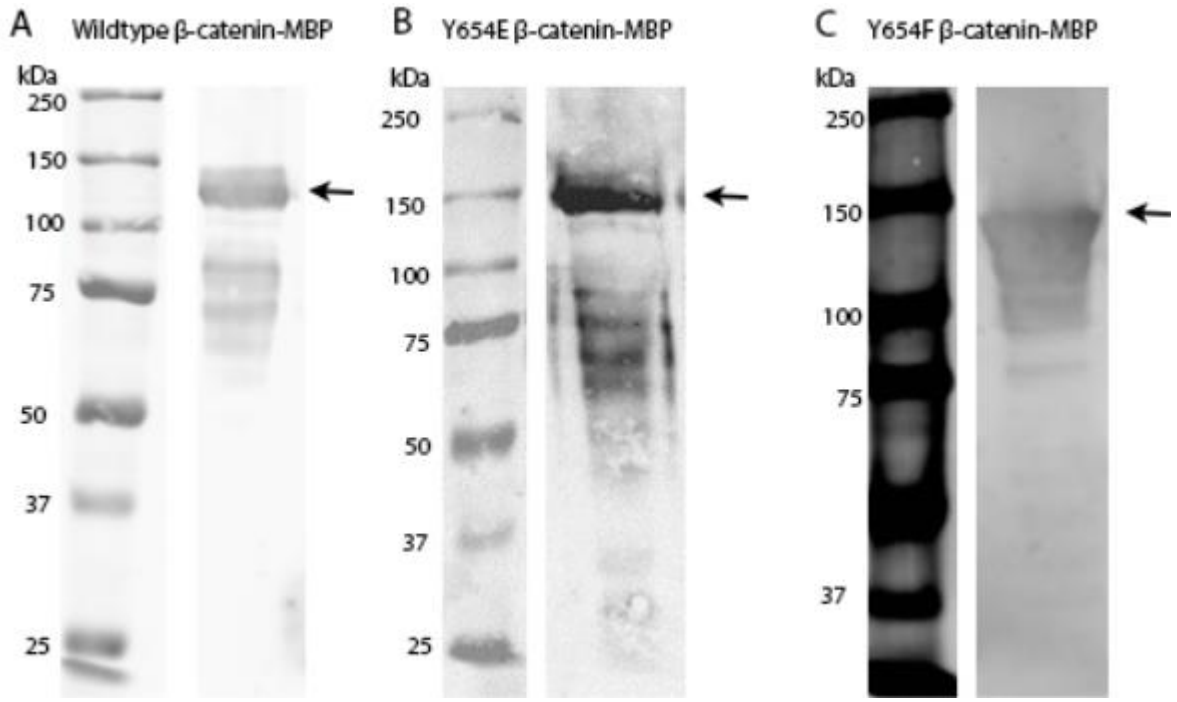


Figure 15 Western blot of purified β-catenin-MBP using α-β-catenin antibody.

Western blot with α-β-catenin antibody shows that the protein produced was β-catenin, and that there is minimal degradation of the protein. Furthermore, the molecular weight (130 kDa) of the protein provides evidence that the MBP tag has not been cleaved off.

A) WT β-catenin-MBP purified protein.

B) Phosphomimetic β-catenin-MBP purified protein.

C) Non-phosphorylatable β-catenin-MBP purified protein.

3.2.3 Mass spectrometric analysis of β-catenin phospho-mutants

The purified protein was separated by SDS-PAGE and bands of interest were excised and underwent trypsin digest in preparation for MS. Table 8 shows the number of β-catenin peptides detected at > 95 % confidence using Protein Pilot 4.2 and the percentage of β-catenin sequence covered. Table 9 shows the detection of the actual mutations. Each peptide was detected at > 99 % confidence.

Furthermore, fragmentation evidence for amino acid mutations is illustrated in Figure 16. A comparison of peptide sequence NEGVATYAAAVLFR is shown between the Y654E, Y654F and Y654 in order to validate the amino acid mutation at Y654.

By Coomassie Blue staining, western blot and MS, it has been demonstrated that high-yield and high-purity fusion-proteins were produced, fit for use in protein interaction pull-down experiments.

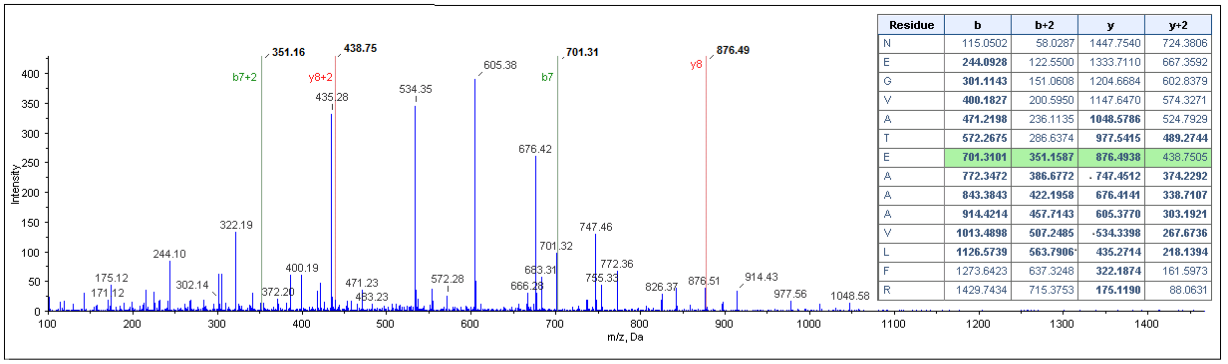
Table 8 Peptides of β-catenin from purified β-catenin-MBP detected by MS.

Construct	B-catenin peptides	Sequence coverage (%)
Y654	305	71.7
Y654E	200	60.8
Y654F	379	84.3

Table 9 Point mutations in purified β-catenin-MBP detected by MS.

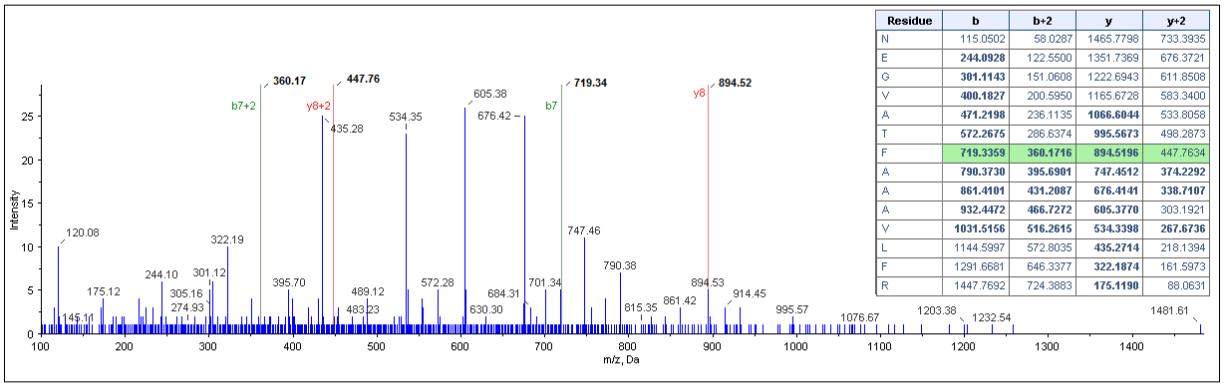
Construct	Peptide Sequence Detected	Modification	No. of Peptides with Modification
Y654	NEGVATYAAAVLFR	None	30
Y654E	NEGVATEAAAVLFR	Tyr->Glu@7	24
Y654F	NEGVATFAAAVLFR	Tyr->Phe@7	33

A MS/MS spectra of peptide NEGVATEAAAVLFR for Y654E



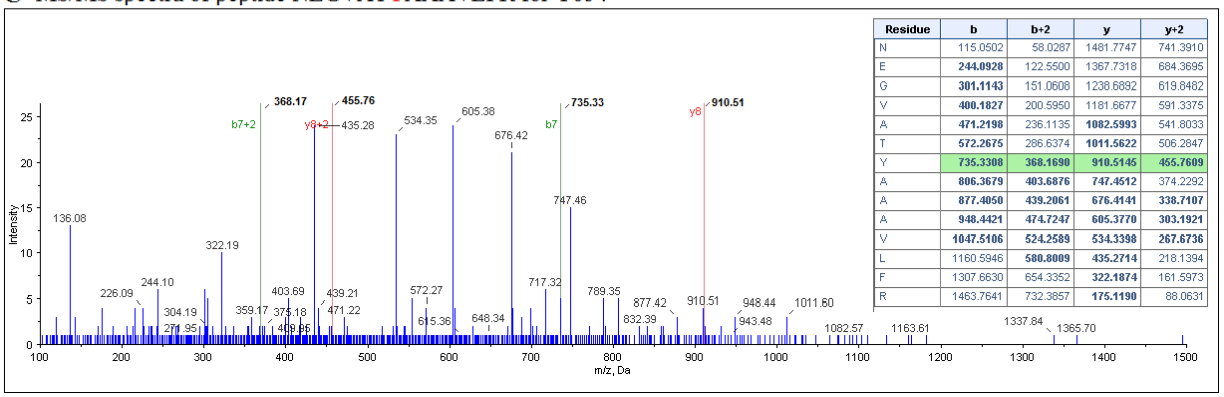
Residue	b	b+2	y	y+2
N	115.0502	58.0287	1447.7540	724.3806
E	**244.0928**	122.5500	1333.7110	667.3592
G	**301.1143**	151.0608	1204.6684	602.8379
V	**400.1827**	200.5950	1147.6470	574.3271
A	**471.2198**	236.1135	**1048.5786**	524.7929
T	**572.2675**	286.6374	**977.5415**	**489.2744**
E	**701.3101**	**351.1587**	**876.4938**	438.7505
A	**772.3472**	**386.6772**	**747.4512**	**374.2292**
A	**843.3843**	**422.1958**	**676.4141**	**338.7107**
A	**914.4214**	**457.7143**	**605.3770**	**303.1921**
V	**1013.4898**	**507.2485**	**534.3398**	**267.6736**
L	**1126.5739**	**563.7906**	**435.2714**	**218.1394**
F	1273.6423	637.3248	**322.1874**	161.5973
R	1429.7434	715.3753	**175.1190**	88.0631

B MS/MS spectra of peptide NEGVATFAAAVLFR for Y654F



Residue	b	b+2	y	y+2
N	115.0502	58.0287	1465.7798	733.3935
E	**244.0928**	122.5500	1351.7369	676.3721
G	**301.1143**	151.0608	1222.6943	611.8508
V	**400.1827**	200.5950	1165.6728	583.3400
A	**471.2198**	236.1135	**1066.6044**	533.8058
T	**572.2675**	286.6374	**995.5673**	498.2873
F	**719.3359**	**360.1716**	**894.5196**	447.7634
A	**790.3730**	**395.6901**	**747.4512**	**374.2292**
A	**861.4101**	**431.2087**	**676.4141**	**338.7107**
A	**932.4472**	**466.7272**	**605.3770**	303.1921
V	**1031.5156**	**516.2615**	**534.3398**	**267.6736**
L	1144.5997	572.8035	**435.2714**	218.1394
F	1291.6681	646.3377	**322.1874**	161.5973
R	1447.7692	724.3883	**175.1190**	88.0631

C MS/MS spectra of peptide NEGVATYAAAVLFR for Y654



Residue	b	b+2	y	y+2
N	115.0502	58.0287	1481.7747	741.3910
E	**244.0928**	122.5500	1367.7318	684.3695
G	**301.1143**	151.0608	1238.6892	619.8482
V	**400.1827**	200.5950	1181.6677	591.3375
A	**471.2198**	236.1135	**1082.5993**	541.8033
T	**572.2675**	286.6374	**1011.5622**	506.2847
Y	**735.3308**	**368.1690**	**910.5145**	**455.7609**
A	**806.3679**	**403.6876**	**747.4512**	374.2292
A	**877.4050**	**439.2061**	**676.4141**	**338.7107**
A	**948.4421**	**474.7247**	**605.3770**	**303.1921**
V	**1047.5106**	**524.2589**	**534.3398**	**267.6736**
L	1160.5946	**580.8009**	**435.2714**	218.1394
F	1307.6630	654.3352	**322.1874**	161.5973
R	1463.7641	732.3857	**175.1190**	88.0631

Figure 16 MS/MS spectra of β-catenin Y654, Y654E and Y654F.

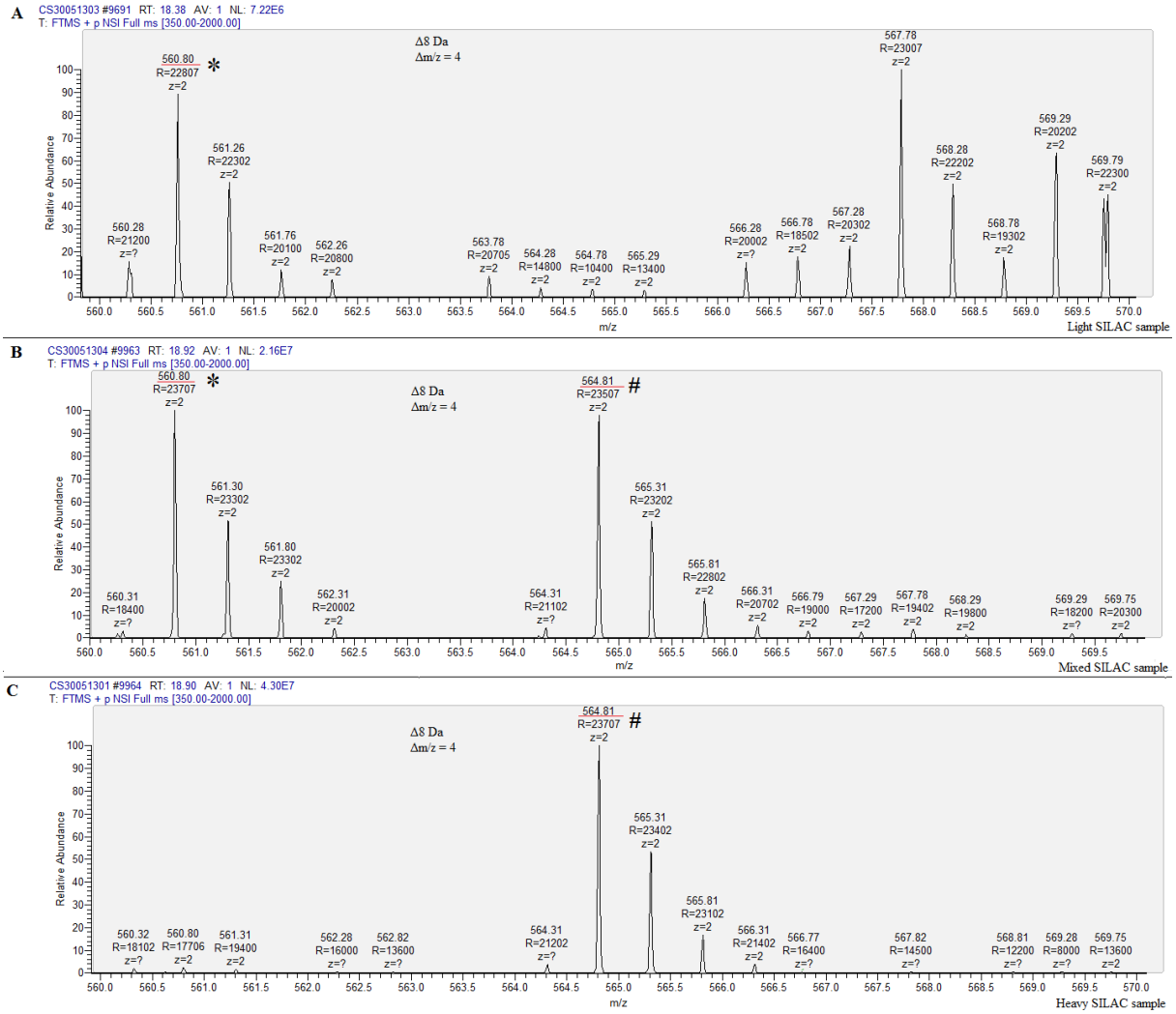
A) MS/MS spectra showing the tyrosine to glutamic acid residue mutation at amino acid 7 in the peptide NEGVATEAAAVLFR.

B) MS/MS spectra showing the tyrosine to phenylalanine residue mutation at amino acid 7 in the peptide NEGVATFAAAVLFR.

C) MS/MS spectra of the WT peptide NEGVATYAAAVLFR.

3.2.4 SILAC incorporation

For the quantitative proteomics in this thesis, SILAC was utilised for *in vivo* incorporation of 'heavy' and 'light' lysine and arginine labels into proteins for MS. After a number of cell divisions, there was a check for full incorporation of these labels into the cell lines. This was achieved by collecting lysate from the 'heavy' and 'light' labelled cells. A known and consistent amount of 'heavy', 'light' and mixed (1:1 of heavy:light) lysate was run on the mass spectrometer (Orbitrap Elite, Thermo Scientific). Analysis of the spectra showed full incorporation of the labels (Figure 17 and Figure 18).

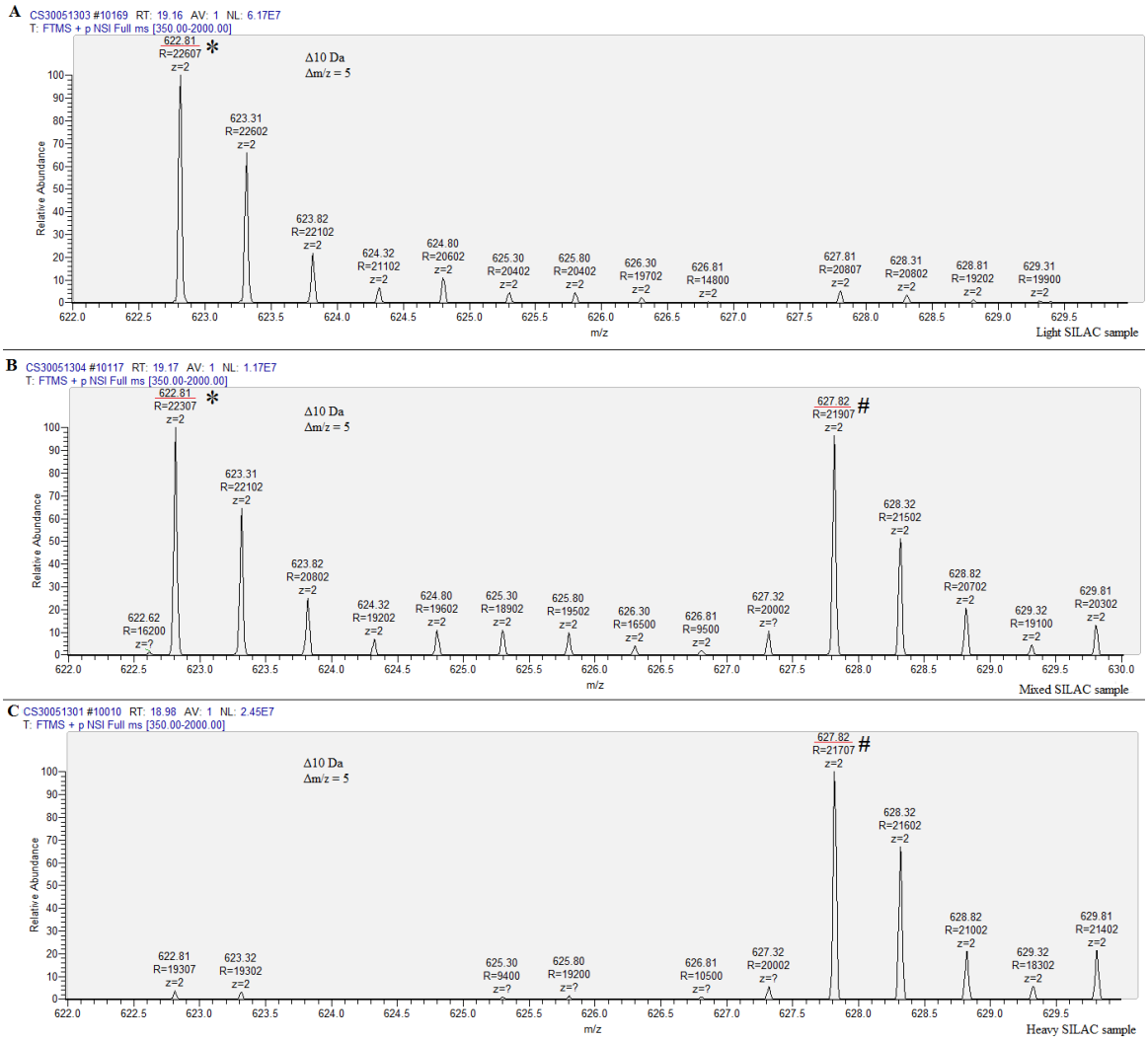


<u>Figure 17</u> Incorporation of heavy lysine into amino acids in cell culture.

A) Light peptide <u>m/z = 560.80</u> is underlined in the light sample (denoted by an asterisk to its right).

B) Light peptide <u>m/z = 560.80</u> is underlined in the mixed sample (denoted by an asterisk to its right) along with its heavy counterpart at <u>m/z = 564.81</u> (denoted by a hashtag to its right).

C) Heavy peptide <u>m/z = 564.81</u> is underlined in the heavy sample (denoted by a hashtag to its right) as the heavy lysine has been fully incorporated.



<u>Figure 18</u> Incorporation of heavy arginine into amino acids in cell culture.

A) Light peptide <u>m/z = 622.81</u> is underlined in the light sample (denoted by an asterisk to its right).

B) Light peptide <u>m/z = 622.81</u> is underlined in the mixed sample (denoted by an asterisk to its right) along with its heavy counterpart at <u>m/z = 627.82</u> (denoted by a hashtag to its right).

C) Heavy peptide <u>m/z = 627.82</u> is underlined in the heavy sample (denoted by an asterisk to its right) as the heavy arginine has been fully incorporated.

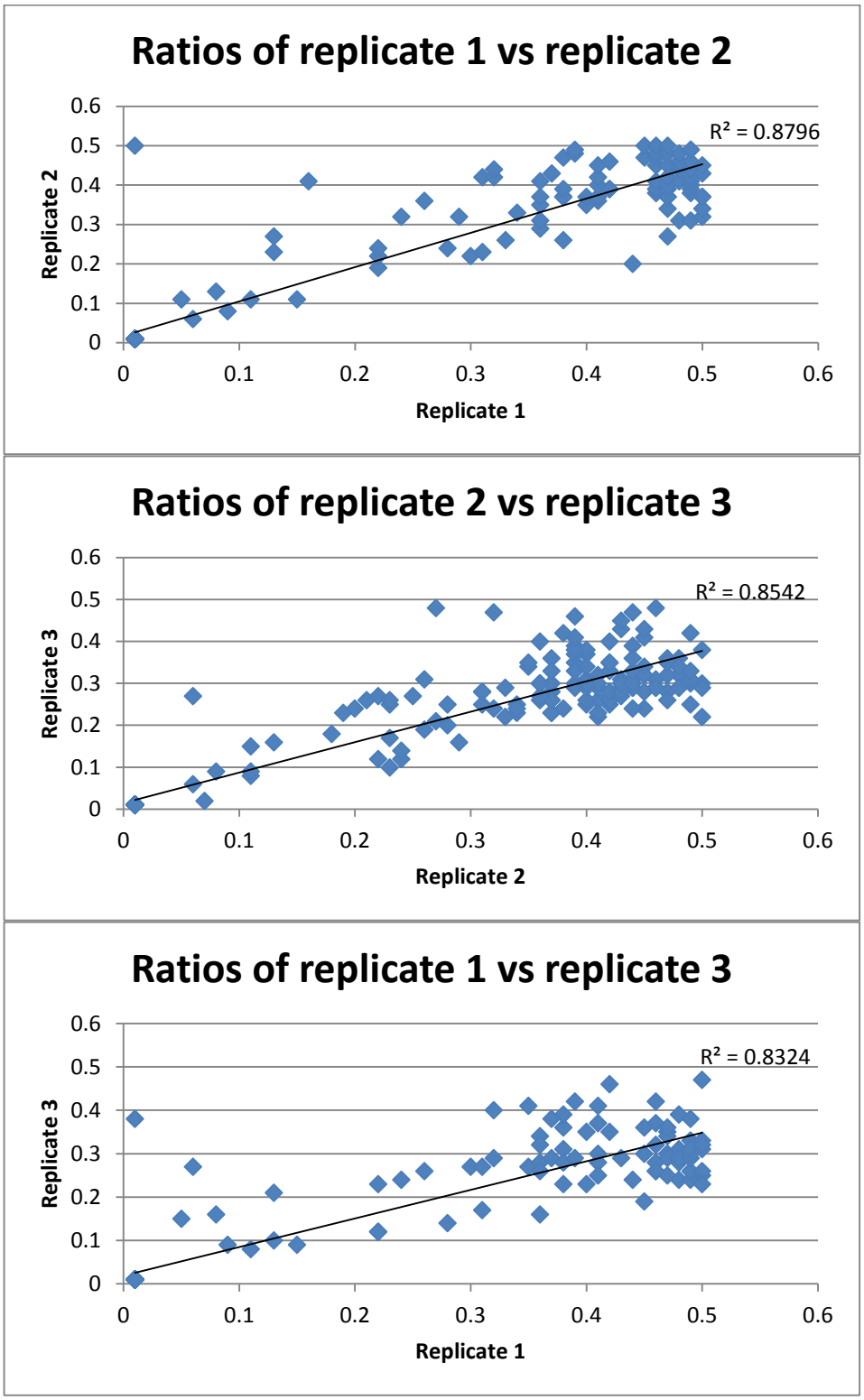
3.2.5 Protein interactions of β-catenin in SW480

The present research investigates protein interactors of human full-length WT β-catenin by pull-downs. Experiments were performed in triplicate using SILAC labelled SW480 cell lysate. Purified β-catenin-MBP was used as protein bait for pulling out binding partners from the lysate and appropriate reproducibility was demonstrated between replicate analyses (average $r^2 > 0.85$) (

Figure 19). 379 proteins were pulled-down that fulfilled candidate list parameters. These parameters include that the protein had to be present in all three replicates, have a p-value of < 0.05 and a fold-change of > 2 for light (β-catenin-MBP):heavy (MBP control) (Figure 20). This list included well-known protein interactors of β-catenin.

i. Known interactors of β-catenin pulled-down in SW480

Table 10 displays known interactors of β-catenin which serves as evidence for the utility of the experiment. Only known interactors that could have an average fold-change generated are listed. That is, their fold-change had a value assigned rather than being listed as an infinite fold-change. Well-known interactors detected with infinite ratios (and therefore could have no average generated) include junction plakoglobin [337], KN motif and ankyrin repeat domain-containing protein 1 [338], membrane-associated guanylate kinase WW and PDZ domain-containing protein 1 [302] and S-phase kinase-associated protein 1 [339]. Known interactors in Table 10 were present in all three replicates, have a p-value of < 0.05 and a fold-change of > 2 for light (β-catenin-MBP):heavy (MBP control). The validity of the pull-down method was confirmed by immunoblot for known interactor APC (Figure 21).



<u>Figure 19</u> Regression plots of heavy:light ratios across replicates in the SW480 wildtype dataset

The linearity of the graph (average r^2-value of > 0.85) demonstrates the reproducibility of β-catenin protein interactors between the three replicates.

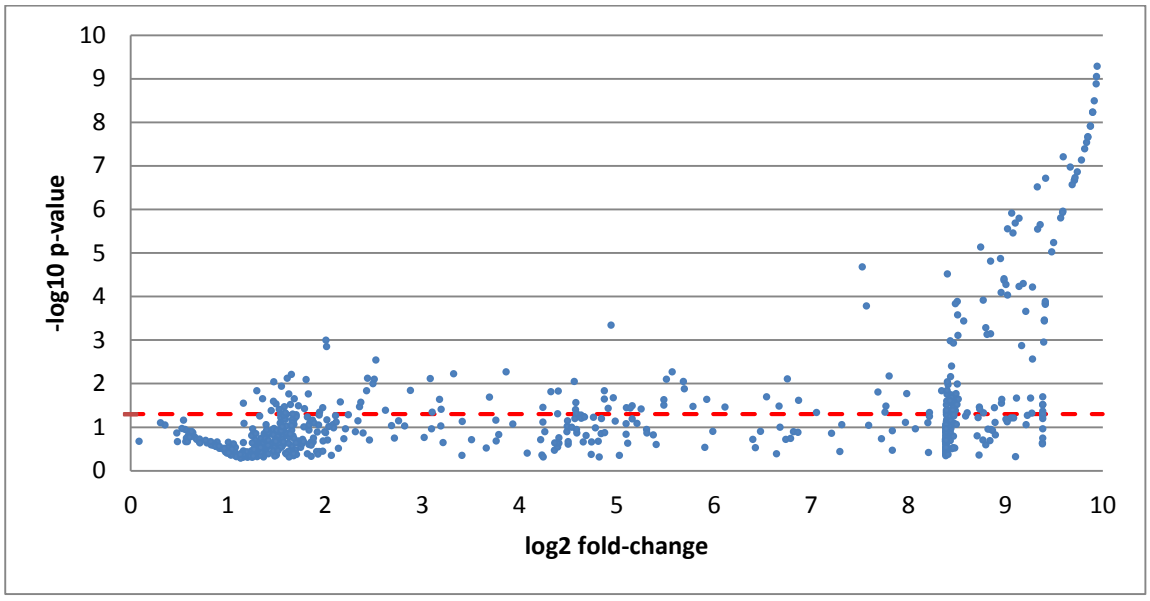


Figure 20 Plot of differentially detected wildtype β-catenin protein interactors in SW480

Log2 fold-change of proteins is plotted against the -log10 p-value reporting threshold (1.3) (red-dashed line).

Table 10 Known interactors of β-catenin in SW480.

Accession number	Known interactor	Unique peptides	Average fold-change (β-catenin:control)
P25054	Adenomatous polyposis coli protein [293]	5	749
P35221	Catenin alpha-1 [340]	33	534
O60716	Catenin delta-1 [337]	4	680
Q14192	Four and a half LIM domains protein 2 [341]	13	976
O14745	Na(+)/H(+) exchange regulatory cofactor NHE-RF1 [342]	5	680
P49023	Paxillin [299]	3	486
Q9Y265	RuvB-like 1 [343]	19	583

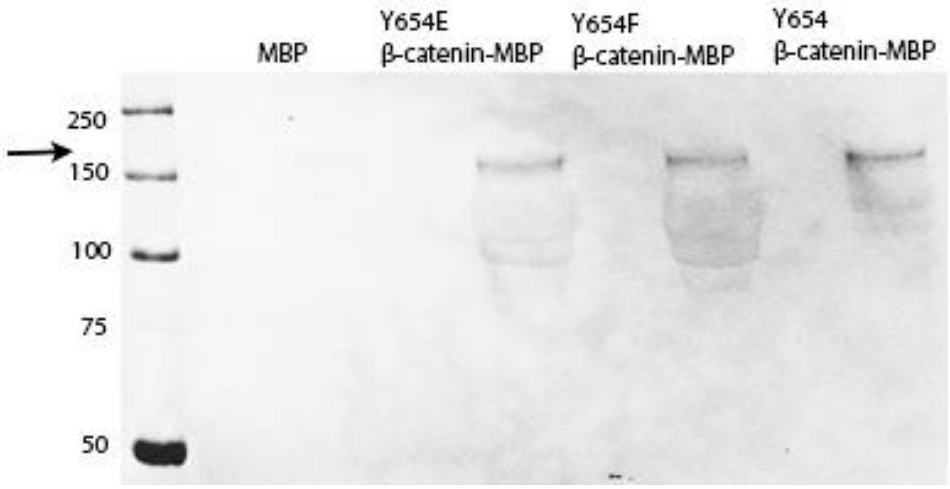


Figure 21 Pull-down performed using β-catenin-MBP constructs and probed for known interactor, APC.

Figure shows that known interactor of β-catenin, APC (160 kDa) binds to the various β-catenin-MBP constructs but not the control (MBP). This further validates the pull-down method.

ii. Candidate novel interacting proteins of β-catenin in SW480

Novel candidate interacting proteins of β-catenin were pulled-out during a SILAC experiment performed in triplicate using full-length WT β-catenin-MBP, using MBP binders as control. Candidates had to be present in all 3 replicates, have a p-value of < 0.05 and a fold-change of > 2 for light (β-catenin-MBP):heavy (MBP). The full candidate list (378 proteins) can be found in appendix 8.1. A shorter and highly stringent reporting threshold analysis of candidate interactors is shown in Table 11, where 64 candidates are reported. This table has candidates where an average fold-change could be generated based on all three replicates. The fold-change needed to range between 350-1000 due to the distribution of known interactors and their average fold-change of ~ 700. Furthermore, ≥ 5 unique peptides in total were detected.

Gene accession numbers were converted into Uniprot IDs using the Uniprot ID mapping tool (http://www.uniprot.org/mapping) where gene accession numbers were entered (under UniProtKB AC/ID) and converted into Uniprot IDs (under UniprotKB). A GO annotation was then downloaded for each Uniprot ID (using Uniprot Biomart). Following the GO annotation report, candidates were assigned a main process of function based on this GO annotation. Distribution of the various processes/functions is shown in Figure 22. Of particular interest were golgi-proteins, mitochondrial-proteins and proteins involved in nuclear import and export. An example of a group belonging to the 'other' category is cytoskeletal proteins. Proteins grouped as golgi-, mitochondrial-, or nuclear import and export- related are displayed in Table 12, Table 13, Table 14 respectively.

Furthermore, the candidate list was compared to interactors of β-catenin provided by BioGRID 3.3. BioGRID 3.3 is an interaction repository of data compiled based on published empirical evidence. Upon entering β-catenin's gene name (CTTNB1) into BioGRID 3.3's gene/identifier search bar, 272 protein interactors of β-catenin were generated, and these were compared to the SW480 interactor candidate list reported in this thesis. Table 15 shows 28 proteins shared with BioGRID 3.3 and the SW480 dataset presented in this thesis. A literature search was performed on each protein in order to understand the sample type where the interaction was observed.

It is of note that one of the highest fold-changes detected was for β-catenin (in all experiments, but an example is listed in Table 11). This is most likely due to the large excess of β-catenin-MBP present in the eluate rather than β-catenin dimerisation. Although α-catenin is known to form homodimers, β-catenin is not known to dimerise [36]. The higher level of β-catenin-MBP was detected from analysis of a gel band ~ 130 kDa. This gel band was analysed in attempt to detect other proteins of similar molecular mass, a strategy that was successful and a number of proteins of ~ 130 kDa were detected including vinculin, reticulin and exportin-5.

Table 11 Candidate novel interacting proteins of full-length WT β-catenin in SW480.

Accession number	Protein	Unique peptides	Average fold-change (β-catenin:control)
P61160	Actin-related protein 2	7	553
P25054	Adenomatous polyposis coli protein	5	749
O00170	AH receptor-interacting protein	6	618
P01009	Alpha-1-antitrypsin	24	937
P01023	Alpha-2-macroglobulin	5	901
Q5T9A4	ATPase family AAA domain-containing protein 3B	6	680
P53004	Biliverdin reductase A	5	356
Q99439	Calponin-2	6	599
P35221	Catenin alpha-1	33	535
P35222	Catenin beta-1	32	912
Q13185	Chromobox protein homolog 3	5	363
P53621	Coatomer subunit alpha	19	498
Q86VP6	Cullin-associated NEDD8-dissociated protein 1	12	351
Q7L576	Cytoplasmic FMR1-interacting protein 1	5	365
P33992	DNA replication licensing factor MCM5	22	621
P04843	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	13	351
Q99613	Eukaryotic translation initiation factor 3 subunit C	5	679
Q9BSJ8	Extended synaptotagmin-1	7	438
Q14192	Four and a half LIM domains protein 2	13	976
P07954	Fumarate hydratase, mitochondrial	5	494
P78347	General transcription factor II-I	7	519
P11413	Glucose-6-phosphate 1-dehydrogenase	9	365
P06737	Glycogen phosphorylase, liver form	9	921
P52292	Importin subunit alpha-2	5	449
Q13418	Integrin-linked protein kinase	7	675
P41252	Isoleucine--tRNA ligase, cytoplasmic	15	437
Q9NSK0	Kinesin light chain 4	7	920
P20700	Lamin-B1	5	675
P09960	Leukotriene A-4 hydrolase	11	498
P10619	Lysosomal protective protein	8	592
Q3ZCQ8	Mitochondrial import inner membrane	6	350

Accession number	Protein	Unique peptides	Average fold-change (β-catenin:control)
	translocase subunit TIM50		
O14745	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	5	680
Q9Y314	Nitric oxide synthase-interacting protein	5	363
P50479	PDZ and LIM domain protein 4	5	854
Q9NR12	PDZ and LIM domain protein 7	10	1000
O75420	PERQ amino acid-rich with GYF domain-containing protein 1	7	507
Q99959	Plakophilin-2	7	549
O15355	Protein phosphatase 1G	5	520
Q9Y570	Protein phosphatase methylesterase 1	5	473
Q96C36	Pyrroline-5-carboxylate reductase 2	7	445
Q92900	Regulator of nonsense transcripts 1	12	574
Q8WUF5	RelA-associated inhibitor	12	823
Q15293	Reticulocalbin-1	7	426
Q9Y265	RuvB-like 1	19	564
Q9Y230	RuvB-like 2	25	621
P49591	Serine--tRNA ligase, cytoplasmic	9	461
P42224	Signal transducer and activator of transcription 1-alpha/beta	7	668
O75533	Splicing factor 3B subunit 1	15	429
Q9UBT2	SUMO-activating enzyme subunit 2	10	668
P78371	T-complex protein 1 subunit beta	15	354
P02766	Transthyretin	5	879
P40939	Trifunctional enzyme subunit alpha, mitochondrial	11	351
Q14134	Tripartite motif-containing protein 29	6	812
Q9BUF5	Tubulin beta-6 chain	7	385
P18031	Tyrosine-protein phosphatase non-receptor type 1	6	668
P45974	Ubiquitin carboxyl-terminal hydrolase 5	7	672
P22314	Ubiquitin-like modifier-activating enzyme 1	15	354
Q9Y224	UPF0568 protein C14orf166	6	722
Q96QK1	Vacuolar protein sorting-associated protein 35	8	668
P18206	Vinculin	18	389
Q9Y5K8	V-type proton ATPase subunit D	6	539

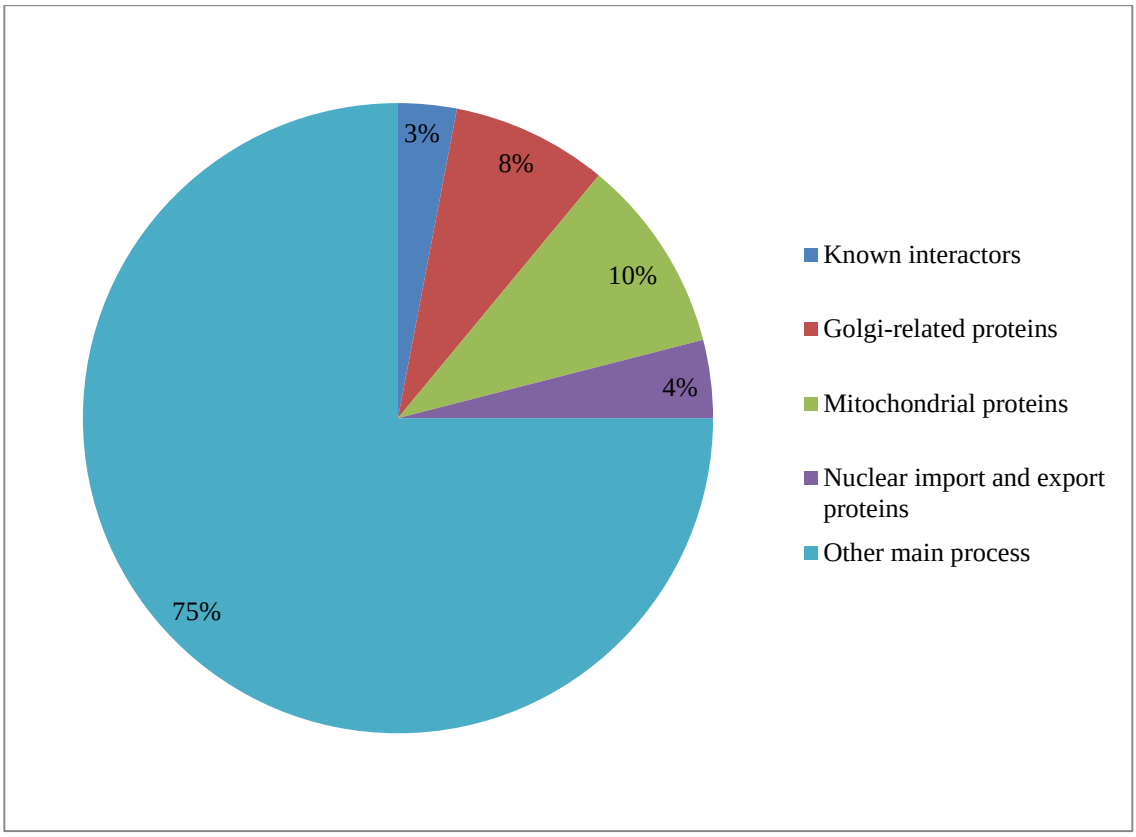


Figure 22 Distribution of processes and functions of candidate novel binding partners of β-catenin in SW480.

Table 12 Golgi-related candidate novel interacting proteins of full-length WT β-catenin in SW480.

Accession number	Protein	Unique peptides
P84085	ADP-ribosylation factor 5	1
P62330	ADP-ribosylation factor 6	2
P01009	Alpha-1-antitrypsin	24
P04114	Apolipoprotein B-100	52
P16278	Beta-galactosidase	4
P11717	Cation-independent mannose-6-phosphate receptor	11
P09496	Clathrin light chain A	4
P53621	Coatomer subunit alpha	19
P35606	Coatomer subunit beta'	7
O14579	Coatomer subunit epsilon	3
P61923	Coatomer subunit zeta-1	3
Q02750	Dual specificity mitogen-activated protein kinase kinase 1	1
P36507	Dual specificity mitogen-activated protein kinase kinase 2	1
Q92538	Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1	1
Q15382	GTP-binding protein Rheb	2
Q9Y6B6	GTP-binding protein SAR1b	1
P50851	Lipopolysaccharide-responsive and beige-like anchor protein	5
Q14697	Neutral alpha-glucosidase AB	15
O00443	Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit alpha	5
P61026	Ras-related protein Rab-10	4
P62820	Ras-related protein Rab-1A	4
Q9H0U4	Ras-related protein Rab-1B	2
Q9UL25	Ras-related protein Rab-21	2
P50454	Serpin H1	12
Q9Y5X3	Sorting nexin-5	4
Q9UBQ0	Vacuolar protein sorting-associated protein 29	5
Q96QK1	Vacuolar protein sorting-associated protein 35	8
Q15836	Vesicle-associated membrane protein 3	3
O95292	Vesicle-associated membrane protein-associated protein B/C	6
Q12907	Vesicular integral-membrane protein VIP36	3

Table 13 Mitochondrial-related candidate novel interacting proteins of full-length WT β-catenin in SW480.

Accession number	Protein	Unique peptides
Q02218	2-oxoglutarate dehydrogenase, mitochondrial	3
Q9BZE1	39S ribosomal protein L37, mitochondrial	6
Q99714	3-hydroxyacyl-CoA dehydrogenase type-2	6
P23396	40S ribosomal protein S3	19
Q99798	Aconitate hydratase, mitochondrial	3
P12235	ADP/ATP translocase 1	2
P05141	ADP/ATP translocase 2	5
P78540	Arginase-2, mitochondrial	6
P25705	ATP synthase subunit alpha, mitochondrial	14
P48047	ATP synthase subunit O, mitochondrial	9
Q5T9A4	ATPase family AAA domain-containing protein 3B	6
P61221	ATP-binding cassette sub-family E member 1	10
Q9NX63	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial	4
P00403	Cytochrome c oxidase subunit 2	4
P54886	Delta-1-pyrroline-5-carboxylate synthase	4
P36957	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	4
P38117	Electron transfer flavoprotein subunit beta	9
P43897	Elongation factor Ts, mitochondrial	4
P49411	Elongation factor Tu, mitochondrial	15
P07954	Fumarate hydratase, mitochondrial	5
Q9UIJ7	GTP:AMP phosphotransferase, mitochondrial	3
P52789	Hexokinase-2	1
Q9H2U2	Inorganic pyrophosphatase 2, mitochondrial	3
O43837	Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial	4
P48735	Isocitrate dehydrogenase [NADP], mitochondrial	4
P36776	Lon protease homolog, mitochondrial	2
O95573	Long-chain-fatty-acid--CoA ligase 3	2
O60488	Long-chain-fatty-acid--CoA ligase 4	3
Q9Y3D6	Mitochondrial fission 1 protein	3
Q3ZCQ8	Mitochondrial import inner membrane translocase subunit TIM50	6
O75439	Mitochondrial-processing peptidase subunit beta	2
O75489	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial	2
P30044	Peroxiredoxin-5, mitochondrial	6

Accession number	Protein	Unique peptides
Q9BPW8	Protein NipSnap homolog 1	6
P32322	Pyrroline-5-carboxylate reductase 1, mitochondrial	5
Q00796	Sorbitol dehydrogenase	5
Q9UJZ1	Stomatin-like protein 2	6
P53597	Succinyl-CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial	5
P40939	Trifunctional enzyme subunit alpha, mitochondrial	11
Q9HB07	UPF0160 protein MYG1, mitochondrial	5
P21796	Voltage-dependent anion-selective channel protein 1	6
P45880	Voltage-dependent anion-selective channel protein 2	6

Table 14 Nuclear import and export related candidate novel interacting proteins of full-length WT β-catenin in SW480.

Accession number	Protein	Type	Unique Peptides
P06493	Cyclin-dependent kinase 1	Nuclear import	11
Q9HAV4	Exportin-5	Nuclear export	2
Q9UIA9	Exportin-7	Nuclear export	4
P52292	Importin subunit alpha-2	Nuclear import	5
P78406	mRNA export factor	Nuclear export	4
P12270	Nucleoprotein TPR	Nuclear import and export	8
P61326	Protein mago nashi homolog	Nuclear export	3
P60468	Protein transport protein Sec61 subunit beta	Nuclear import	3
Q92900	Regulator of nonsense transcripts 1	Nuclear export	12
Q01130	Serine/arginine-rich splicing factor 2	Nuclear export	5
Q13838	Spliceosome RNA helicase DDX39B	Nuclear export	2
Q13263	Transcription intermediary factor 1-beta	Nuclear import	8

Table 15 BioGRID 3.3 protein interactors of β-catenin and relevance to SW480 dataset.

* Cell lines denoted in bold text.

Name	Protein	Cancer; cell line	Relevance
APC	Adenomatous polyposis coli	*Colon*; **HT29**, **SW480**, **HCT116, KMS-4, KMS-8, LS174T**. *Prostate*; **LNCapFGC, PC-3**. *Breast*; **MCF-7**. *Cervical*; **HeLa**. *Osteosarcoma*; **U2OS**. *Human embryonic kidney (non-cancer)*; **HEK293**	Known interactor
CDK2	Cyclin-dependent kinase 2 (p33)	*Rat fibroblast*; **Rat1**. *Human embryonic kidney (non-cancer)*; **HEK293**	CDK2 and CDK1 pulled-down using WT-β-catenin
CDK5R1	Cyclin-dependent kinase 5, regulatory subunit 1 (p35)	Yeast two-hybrid system	
COPS3	COP9 signalosome subunit 3	*Colon*; **HT29, HCT116.** *Cervical*; **HeLa**. **Mouse B8 fibroblast**.	COP9 signalosome complex subunit 5 pulled-down using WT-β-catenin
COPS5	COP9 signalosome subunit 5		
COPS8	COP9 signalosome subunit 8		
CTNNA1	Catenin (cadherin-associated protein), alpha 1, 102kDa	*Colon*; **LS174T**. *Human embryonic kidney (non-cancer);* **HEK293**. *Gastric*; **HSC-39, HSC-404.** *Epidermoid*; **A431.** *Intestinal epithelial (non-cancer)*; **IEC18, IEC18-K-ras** (mutant). *Pancreatic*; **RWP1**	Known interactor
CTNND1	Catenin (cadherin-associated	*Breast*; **MDA-**	Known interactor

Name	Protein	Cancer; cell line	Relevance
	protein), delta 1	**MB-468, MDA-MB-361, BT549, MCF-104.** *Epidermoid*; **A431**	
CUL1	Cullin 1	*Human embryonic kidney (non-cancer)*; HEK293	Cullin-associated NEDD8-dissociated protein 1 pulled-down using WT-β-catenin
CUL4B	Cullin 4B	*Embryonal*; **PCC4.** *Mouse embryonic*; **C3HT10.** *Cervical*; **HeLa.** WT *Drosophila* embryo.	
DDX1	DEAD box helicase 1	*Colon*; **DLD1**	A number of RNA helicases pulled-down across datasets
DDX5	DEAD box helicase 5		
FHL2	Four and a half LIM domains 2	*Cervical*; **HeLa.** Yeast-two hybrid system	Known interactor
FUS	FUS RNA binding protein (HNRNPP2)	*Colon*; **DLD-1**	A number of HNRNPs pulled-down across datasets (H, F, K, Q, A2/B1)
HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1	*Human embryonic kidney (non-cancer)*; **HEK239**	
HNRNPA2B1	Heterogeneous nuclear ribonucleoprotein A2/B1	*Colon*; **DLD-1.** *Human embryonic kidney (non-cancer)*; **HEK293**	
HNRNPK	Heterogeneous nuclear ribonucleoprotein K		
HNRNPM	Heterogeneous nuclear ribonucleoprotein M		
HSP90AB1	Heat shock protein 90kDa alpha (cytosolic), class B member 1	*Human embryonic kidney (non-cancer);* **HEK293**	Heat shock protein HSP 90-alpha (HSP90AA1) pulled-down using phosphomimetic β-catenin
HSPA1B	Heat shock protein 70kDa protein 1B		Heat shock 70 kDa protein 1A/1B (HSPA1A) pulled down using phosphomimetic- and non-phosphorylatable β-catenin
HSPA8	Heat shock protein 70kDa protein 8		

Name	Protein	Cancer; cell line	Relevance
ILK	Integrin-linked kinase (p59)	*Colon;* **DLD1**	Pulled-down using WT-β-catenin
RPSA	Ribosomal protein SA	*Human embryonic kidney (non-cancer)*; **HEK293**	Pulled-down using non-phosphorylatable β-catenin
RUVBL1	RuvB-like AAA ATPase 1	*Colon*; **SW480.** *Human embryonic kidney (non-cancer)*; **HEK293**	RUVBL1 known interactor. RUVBL1 and RUVBL2 pulled-down.
RUVBL2	RuvB-like AAA ATPase 2	*Prostate*; **LNCaP**. *Human embryonic kidney (non-cancer)*; **HEK293**	
SNRPD1	Small nuclear ribonucleoprotein D1 polypeptide 16 kDa	*Human embryonic kidney (non-cancer)*; **HEK293**	SNRPs pulled-down using WT-β-catenin. Include E, Sm D2 and Sm D3
TAX1BP3	Tax1 (human T-cell leukemia virus type I) binding protein 3	Crystallisation of purified TAX1BP3 (*E. coli*) and purchased C-terminal β-catenin peptide (NQLAWFDTDL)	Pulled-down using non-phosphorylatable -β-catenin
TUBA1A	Tubulin, alpha 1a	*Human embryonic kidney (non-cancer)*; **HEK293**	Pulled-down using phosphomimetic β-catenin

3.2.6 The effect of phosphorylation at tyrosine-654 on the protein interactions of β-catenin in SW480

Full-length β-catenin-MBP containing Y654 mutants were produced to explore the potential role of phosphorylation in mediating β-catenin protein interactions. The experiment was performed in triplicate using SW480 cells.

i. Candidate novel interacting proteins of phosphorylated-Y654-β-catenin in SW480

To mimic the phosphorylation of Y654, this residue was replaced with glutamic acid (Glu), to provide the negative charge associated with amino acid phosphorylation. This construct was used to isolate binding proteins and compared with MBP control using a SILAC strategy. 92 putative interacting proteins were detected by MS and are shown in appendix 8.2 and Figure 23. Table 16 reports a shorter and highly stringent reporting threshold analysis of candidate protein interactors. This table has candidates where an average fold-change could be generated based on 3 replicates. Furthermore, ≥ 5 unique peptides needed to be detected.

Proteins annotated as being localised as golgi-, mitochondrial-, or nuclear import and export- related based on GO cellular component criteria are displayed in Table 17, Table 18 and Table 19 respectively.

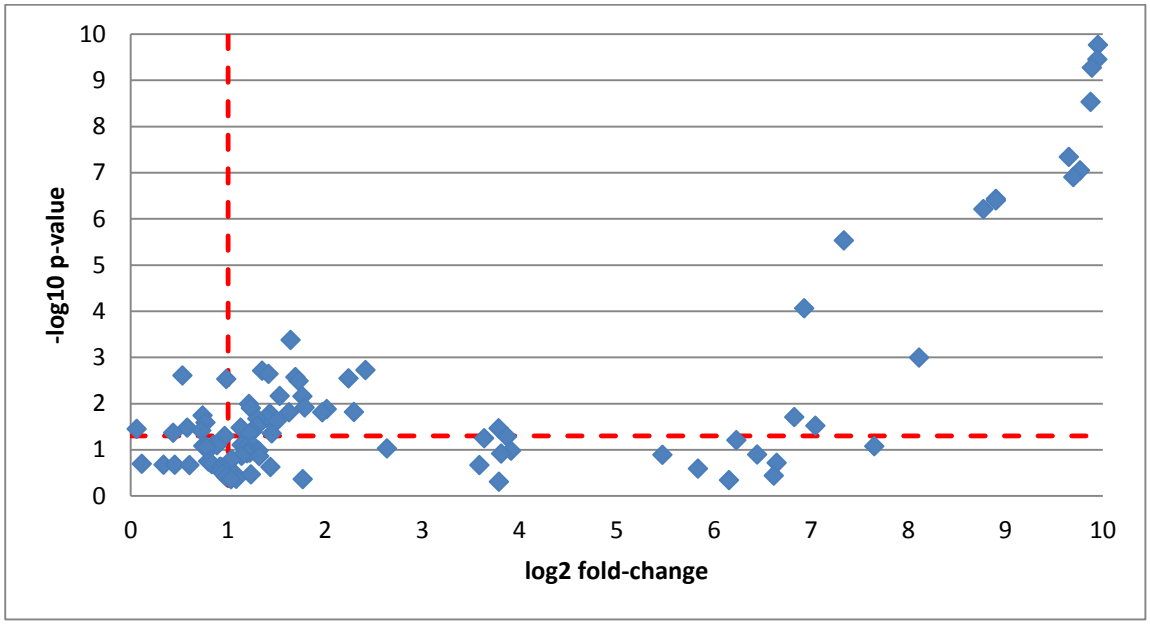


Figure 23 Plot of differentially detected phosphomimetic-Y654-β-catenin protein interactors in SW480

Log2 fold-change of proteins (reporting threshold = 1)(vertical red-dashed line) is plotted against the -log10 p-value reporting threshold (1.3) (horizontal red-dashed line).

Table 16 Candidate novel interacting proteins of the phosphomimetic mutant of β-catenin at tyrosine-654 in SW480.

* This table has candidates where an average fold-change could be generated based on 3 replicates. Furthermore, ≥ 5 unique peptides needed to be detected

Accession number	Protein	Unique peptides	Average fold-change (β-catenin:control)
P61247	40S ribosomal protein S3a	7	2
P10809	60 kDa heat shock protein, mitochondrial	10	5
P62913	60S ribosomal protein L11	5	3
P11021	78 kDa glucose-regulated protein	18	5
O43707	Alpha-actinin-4	7	14
P06733	Alpha-enolase	6	3
P31939	Bifunctional purine biosynthesis protein PURH	6	3
P35221	Catenin alpha-1	12	804
P35222	Catenin beta-1	32	1000
P15924	Desmoplakin	8	162
Q14258	E3 ubiquitin/ISG15 ligase TRIM25	6	2
P49915	GMP synthase [glutamine-hydrolyzing]	5	4
P08107	Heat shock 70 kDa protein 1A/1B	5	3
P07900	Heat shock protein HSP 90-alpha	7	3
Q86YZ3	Hornerin	5	122
Q9Y6M1	Insulin-like growth factor 2 mRNA-binding protein 2	7	4
P00338	L-lactate dehydrogenase A chain	11	2
Q06830	Peroxiredoxin-1	6	3
P21980	Protein-glutamine gamma-glutamyltransferase 2	9	114
P14618	Pyruvate kinase isozymes M1/M2	12	2
Q9Y230	RuvB-like 2	13	761
P38646	Stress-70 protein, mitochondrial	8	277
P29401	Transketolase	8	15
P60174	Triosephosphate isomerase	5	3

Table 17 Golgi-related candidate novel interacting proteins of phosphomimetic mutant of β-catenin at tyrosine-654 in SW480.

Accession number	Protein	Unique Peptides
P11021	78 kDa glucose-regulated protein	18
P16278	Beta-galactosidase	4
P17655	Calpain-2 catalytic subunit	4
Q00610	Clathrin heavy chain 1	20
Q9Y5X1	Sorting nexin-9	3
P55072	Transitional endoplasmic reticulum ATPase	1

Table 18 Mitochondrial-related candidate novel interacting proteins of phosphomimetic mutant of β-catenin at tyrosine-654 in SW480.

Accession number	Protein	Unique Peptides
P10809	60 kDa heat shock protein, mitochondrial	10
P61221	ATP-binding cassette sub-family E member 1	6
P41250	Glycine--tRNA ligase	4
P08107	Heat shock 70 kDa protein 1A/1B	5
Q12931	Heat shock protein 75 kDa, mitochondrial	2
P38646	Stress-70 protein, mitochondrial	8

Table 19 Nuclear import related candidate novel interacting proteins of phosphomimetic mutant of β-catenin at tyrosine-654 in SW480.

Accession number	Protein	Type	Unique Peptides
P62829	60S ribosomal protein L23	Nuclear import	4
P21333	Filamin-A	Nuclear import	7
P07900	Heat shock protein HSP 90-alpha	Nuclear import	7
Q06830	Peroxiredoxin-1	Nuclear import	6
P02545	Prelamin-A/C	Nuclear import	10

ii. Candidate novel interacting proteins of non-phosphorlatable-Y654-β-catenin in SW480

To mimic the un-phosphorylatable amino acid of Y654, this residue was replaced with phenylalanine (Phe), providing an amino acid identical to the tyrosine, but missing the –OH group, making it un-phosphorylatable. This construct was used to isolate binding proteins and compared with an MBP control using a SILAC strategy. 129 putative interacting proteins were detected by MS and shown in appendix 8.3 and Figure 24. Table 20 reports a subset of these in a highly stringent list of candidate protein interactors. This table has candidates where an average fold-change could be generated based on 3 replicates. Furthermore, ≥ 4 unique peptides needed to be detected.

Proteins annotated as being localised as golgi-, mitochondrial-, or nuclear import and export- related based on GO cellular component criteria are displayed in Table 21, Table 22 and Table 23 respectively.

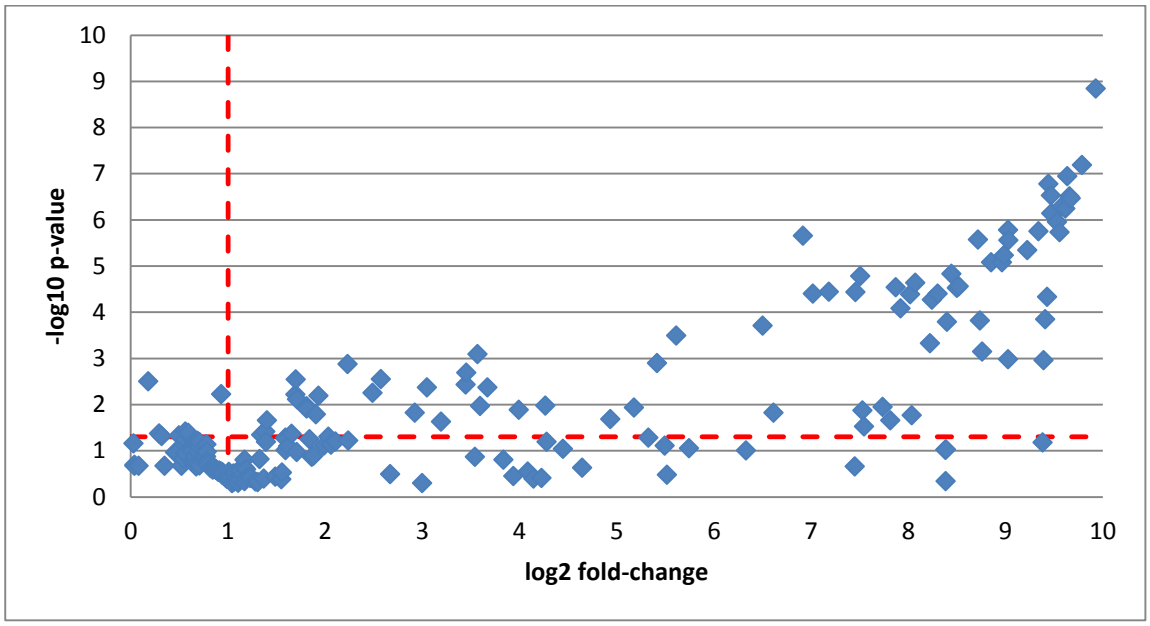


Figure 24 Plot of differentially detected non-phosphorylatable-Y654-β-catenin protein interactors in SW480

Log2 fold-change of proteins (reporting threshold = 1)(vertical red-dashed line) is plotted against the -log10 p-value reporting threshold (1.3) (horizontal red-dashed line).

Table 20 Candidate novel interacting proteins of the non-phosphorylatable mutant of β-catenin at tyrosine-654 in SW480.

* This table has candidates where an average fold-change could be generated based on 3 replicates. Furthermore, ≥ 4 unique peptides needed to be detected

Accession number	Protein	Unique peptides	Average fold-change (β-catenin:control)
P62913	60S ribosomal protein L11	4	6
P11021	78 kDa glucose-regulated protein	7	4
Q8N6T3	ADP-ribosylation factor GTPase-activating protein 1	4	421
O00571	ATP-dependent RNA helicase DDX3X	14	522
P11586	C-1-tetrahydrofolate synthase, cytoplasmic	7	433
P35221	Catenin alpha-1	8	796
P35222	Catenin beta-1	31	975
P62633	Cellular nucleic acid-binding protein	4	213
O60313	Dynamin-like 120 kDa protein, mitochondrial	4	269
P49411	Elongation factor Tu, mitochondrial	4	36
P23588	Eukaryotic translation initiation factor 4B	5	19
P49327	Fatty acid synthase	27	4
Q14192	Four and a half LIM domains protein 2	4	234
P04406	Glyceraldehyde-3-phosphate dehydrogenase	8	3
P06737	Glycogen phosphorylase, liver form	5	3
P08107	Heat shock 70 kDa protein 1A/1B	6	3
P31943	Heterogeneous nuclear ribonucleoprotein H	4	672
P61978	Heterogeneous nuclear ribonucleoprotein K	10	8
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1	4	9
Q9Y6M1	Insulin-like growth factor 2 mRNA-binding protein 2	6	49
Q9NSK0	Kinesin light chain 4	4	649

Accession number	Protein	Unique peptides	Average fold-change (β-catenin:control)
Q14847	LIM and SH3 domain protein 1	11	11
P00338	L-lactate dehydrogenase A chain	6	40
P50479	PDZ and LIM domain protein 4	7	753
O75420	PERQ amino acid-rich with GYF domain-containing protein 1	7	710
Q6Y7W6	PERQ amino acid-rich with GYF domain-containing protein 2	10	182
Q86UU1	Pleckstrin homology-like domain family B member 1	7	242
P21980	Protein-glutamine gamma-glutamyltransferase 2	6	5
Q8WUF5	RelA-associated inhibitor	12	784
Q9Y265	RuvB-like 1	8	522
Q9Y230	RuvB-like 2	17	766
P38646	Stress-70 protein, mitochondrial	4	185
Q9UGI8	Testin	5	91
P68363	Tubulin alpha-1B chain	8	3
P62979	Ubiquitin-40S ribosomal protein S27a	4	3

Table 21 Golgi-related candidate novel interacting proteins of non-phosphorylatable mutant of β-catenin at tyrosine-654 in SW480.

Accession number	Protein	Unique peptides
P11021	78 kDa glucose-regulated protein	7
Q8N6T3	ADP-ribosylation factor GTPase-activating protein 1	4
P48444	Coatomer subunit delta	3
Q567U6	Coiled-coil domain-containing protein 93	2
Q14697	Neutral alpha-glucosidase AB	3
4P07237	Protein disulfide-isomerase	4
Q9Y5X1	Sorting nexin-9	3

Table 22 Mitochondrial-related candidate novel interacting proteins of non-phosphorylatable mutant of β-catenin at tyrosine-654 in SW480.

Accession number	Protein	Unique peptides
O00571	ATP-dependent RNA helicase DDX3X	14
P33316	Deoxyuridine 5'-triphosphate nucleotidohydrolase, mitochondrial	1
O60313	Dynamin-like 120 kDa protein, mitochondrial	4
P38117	Electron transfer flavoprotein subunit beta	2
P49411	Elongation factor Tu, mitochondrial	4
P08107	Heat shock 70 kDa protein 1A/1B	6
P48735	Isocitrate dehydrogenase [NADP], mitochondrial	1
Q04837	Single-stranded DNA-binding protein, mitochondrial	1
P38646	Stress-70 protein, mitochondrial	4
P53597	Succinyl-CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial	2
P40939	Trifunctional enzyme subunit alpha, mitochondrial	2

Table 23 Nuclear import and export related candidate novel interacting proteins of non-phosphorylatable mutant of β-catenin at tyrosine-654 in SW480.

Accession number	Protein	Type	Unique peptides
P08865	40S ribosomal protein SA	Nuclear export	3
P52294	Importin subunit alpha-5	Nuclear import	2
P60468	Protein transport protein Sec61 subunit beta	Nuclear import	1

iii. A comparison of binding-partners of WT, phosphomimetic, and non-phosphorylatable β-catenin in SW480

β-catenin protein interactors based on Y654 phosphorylation status were compared from data obtained using WT (Y654), phosphomimetic (E654) and non-phosphorylatable (F654) constructs. The full list (512 proteins) can be found in appendix 8.4. This table has their presence or absence across all three datasets. A Venn diagram represents the overlap of potential candidates in Figure 25.

A shorter and highly stringent reporting threshold analysis of candidate interactors is reported in Table 24, where 49 candidates are reported. This table has candidates which were affected by phosphorylation. That is, the proteins were present in the WT dataset and unique to either the phosphomimetic or non-phosphorylatable dataset. For example, the data shown in Table 24 for alpha-actinin-4 (O43707) demonstrates it only binds to β-catenin when tyrosine-654 is phosphorylated, as determined by using phosphomimetic Y654E. On the other hand, kinase light chain 4 (Q9NSK0) only bound to β-catenin in the absence of phosphorylation at tyrosine-654. Furthermore, a known interactor such as RuvB-like 1 (Q9Y265) may only bind to β-catenin in the absence of phosphorylation at tyrosine-654.

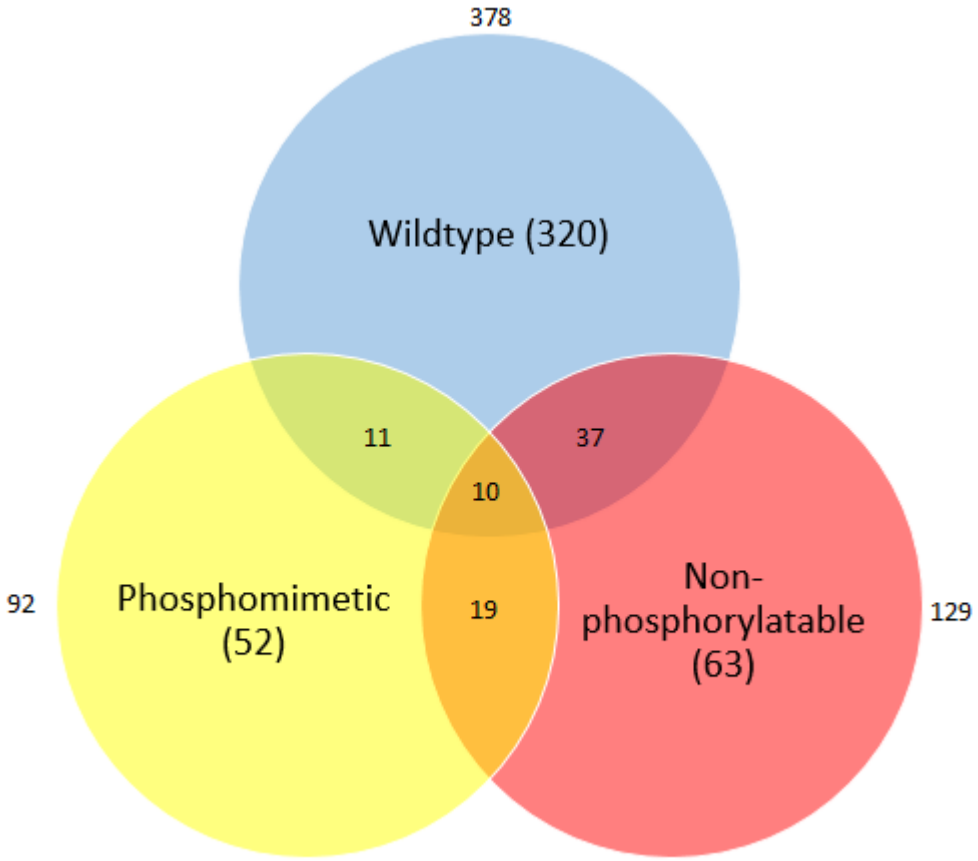


Figure 25 A Venn diagram representing overlap of candidate protein binders of β-catenin across wildtype, phosphomimetic and non-phosphorylatable datasets in SW480.

Table 24 Candidate novel interacting proteins of β-catenin that may be affected by phosphorylation.
*PM denotes phosphomimetic and NP denotes non-phosphorylatable

**White denotes absence of that protein in the dataset

***Grey denotes presence of that protein in the dataset

Accession number	Protein	Wildtype	PM	NP
P68032	Actin, alpha cardiac muscle 1			
O43707	Alpha-actinin-4			
P61221	ATP-binding cassette sub-family E member 1			
P16278	Beta-galactosidase			
P30711	Glutathione S-transferase theta-1			
Q86YZ3	Hornerin			
Q9NZI8	Insulin-like growth factor 2 mRNA-binding protein 1			
O00425	Insulin-like growth factor 2 mRNA-binding protein 3			
Q9BYZ2	L-lactate dehydrogenase A-like 6B			
O14744	Protein arginine N-methyltransferase 5			
P26640	Valine--tRNA ligase			
P62906	60S ribosomal protein L10a			
Q01813	6-phosphofructokinase type C			
P17858	6-phosphofructokinase, liver type			
P11586	C-1-tetrahydrofolate synthase, cytoplasmic			
Q99439	Calponin-2			
O60716	Catenin delta-1			
P33992	DNA replication licensing factor MCM5			
P62877	E3 ubiquitin-protein ligase RBX1			
P38117	Electron transfer flavoprotein subunit beta			
P49411	Elongation factor Tu, mitochondrial			
P23588	Eukaryotic translation initiation factor 4B			
Q14192	Four and a half LIM domains protein 2			
P04406	Glyceraldehyde-3-phosphate dehydrogenase			
P06737	Glycogen phosphorylase, liver form			
Q16576	Histone-binding protein RBBP7			
P48735	Isocitrate dehydrogenase [NADP], mitochondrial			

Accession number	Protein	Wildtype	PM	NP
Q9NSK0	Kinesin light chain 4			
A6NHZ5	Leucine-rich repeat-containing protein 14B			
P09960	Leukotriene A-4 hydrolase			
P48059	LIM and senescent cell antigen-like-containing domain protein 1			
P0CW20	LIM and senescent cell antigen-like-containing domain protein 3-like			
Q14847	LIM and SH3 domain protein 1			
O43684	Mitotic checkpoint protein BUB3			
O76041	Nebulette			
Q14697	Neutral alpha-glucosidase AB			
P50479	PDZ and LIM domain protein 4			
O75420	PERQ amino acid-rich with GYF domain-containing protein 1			
Q8WW12	PEST proteolytic signal-containing nuclear protein			
O15355	Protein phosphatase 1G			
P60468	Protein transport protein Sec61 subunit beta			
Q8WUF5	RelA-associated inhibitor			
Q9Y265	RuvB-like 1			
Q9Y5M8	Signal recognition particle receptor subunit beta			
P53597	Succinyl-CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial			
Q9UGI8	Testin			
P04183	Thymidine kinase, cytosolic			
P40939	Trifunctional enzyme subunit alpha, mitochondrial			

3.3 Discussion

β-catenin is a multi-faceted protein that elicits its varied functions through numerous protein-protein interactions. While there are several well described and functionally explained consequences of β-catenin interactions (for example with cadherins at the cell membrane), the functional significance of other interactions remains to be elucidated [14]. By applying a discovery-based proteomics approach the aim here was to identify putative new β-catenin binding partners as a first step towards understanding the multi-layered activities of this well-known protein in the context of colon cancer.

In this chapter, hundreds of proteins were shown to bind to β-catenin based on affinity purification and MS analysis. Many of the known interactors that have been validated in the literature were also detected here, confirming the reliability of the described experiments. Results showed that known β-catenin interactors accounted for just 3 % of targets, suggesting that most have been reported for the first time, representing putative novel interactors. As shown in Figure 22, a considerable number of β-catenin protein interactors were proteins annotated to reside at subcellular locations associated with mitochondria, golgi and nuclear import/export.

3.3.1 β-catenin at the mitochondria

10 % of the novel candidate protein interactor list is comprised of proteins with reported roles at the mitochondria such as mitochondrial HSP70 (mtHSP70) and dynamin-like 120 kDa protein (Opa1). Not only the interaction of these proteins with β-catenin themselves is novel, but also the localisation of β-catenin at the mitochondria is novel, making these findings significant in two ways. It is important to note that pull-downs were performed in cells lysed in RIPA buffer (with Triton X-100), and it is expected that organelle membranes such as the mitochondrial membrane will rupture in this buffer [344-348], resulting in leaching of protein content into the soluble cytoplasmic fraction. Binding of proteins thereupon may be real but otherwise, would not occur *in vivo* where membrane partitioning would prevent these interactions from occurring.

i. Mitochondrial HSP70 - mtHSP70

mtHSP70 is involved in the folding of proteins outside the mitochondria and unidirectional translocation across the mitochondrial membrane [349, 350]. It also degrades misfolded proteins and assists the movement of tumour suppressor p53, into the outer mitochondrial membrane [350]. In yeast, mtHSP70 is required for cell viability [351] where it interacts with inner mitochondrial membrane protein TIM44, and is an essential part of mitochondrial import machinery [352]. In mammalian cells, it is involved in mitochondrial activity and biogenesis [353] and is induced by glucose deprivation [354]. A number of studies have also found that mtHSP70 is upregulated in human cancer cell lines and tumours [350, 355-357]. Its association with β-catenin will be discussed later in 5.3.2.

ii. Dynamin-like 120 kDa protein - Opa1

Opa1 is a dynamin-related GTPase, localised to the inner mitochondrial membrane [358]. It is a protein essential for the fusion of the inner mitochondrial membrane [359], maintains the structure and integrity of mitochondrial cristae, acts a diffusion barrier for proteins across the cristae [360], and regulates apoptosis which involves the reshaping of mitochondrial cristae [361].

Overexpression of β-catenin has been associated with increased mtDNA and consequential altered respiratory activity, both of these being important features of cancer cells [342] and so it is possible that the interaction of β-catenin with Opa1 inhibits Opa1 from performing its roles which are beneficial to mitochondrial health. That is, overexpression of β-catenin and its association with cancer by dysregulation of the mitochondria could be attributed to it inhibiting the functional role of Opa1 and keeping healthy mitochondria.

3.3.2 β-catenin at the golgi

There is not much documented in the literature about β-catenin and the golgi. Pull-down experiments in this thesis identified a number golgi-related proteins (8 % of the candidate interactor list), and for many of these proteins multiple members of the family were detected. These include 4xcoatomer subunits, 2xdual specificity mitogen-activated protein kinases, 4xRas-related proteins, 2xvacuolar protein sorting-associated proteins and 2xvesicle-associated membrane proteins. Due to empirical evidence found by our collaborators, the coatomer complex was of particular interest.

i. Coat protein (COP) complex I – COPI

During protein synthesis, membrane proteins and proteins destined for secretion are targeted to the endoplasmic reticulum (ER), where synthesis, folding and modification of proteins occurs [362]. The proteins are then transported to the golgi in membrane vesicles encased by coat protein (COP) complexes, for further processing [362]. COPI-coated vesicles are 1/3 types protein coated vesicles (the other two are clathrin-coated and COPII-coated vesicles) [363]. COPI-coated vesicles are involved in vesicular trafficking (retrograde and anterograde trafficking from ER-golgi and golgi-ER) and are composed of seven subunits; COPA, COPB, COPB', COPG, COPD, COPE and COPZ [363]. Here, pull-downs identified 4/7 of these subunits in SW480 cells: COPB', COPA, COPE, COPZ. A fifth subunit, COPB was found by our collaborators to interact with β-catenin by IF in breast cancer cell line MCF-7 [364]. Upon discussion, it was decided to further the investigation of β-catenin and the coatomer complex by use of COPB as there was a well-characterised antibody against it.

3.3.3 β-catenin and nuclear import and export

i. Heterogeneous nuclear ribonucleoprotein K - hnRNPK

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a family of RNA-binding proteins [365, 366]. They are multifunctional proteins with roles in processing heterogeneous nuclear RNAs (hnRNAs) into mature mRNAs and acting as *trans*-factors in regulating gene expression [367]. Other roles include pre-mRNA processing and splicing and mRNA export, localisation, translation and stability [368]. Many of the hnRNPs associate with mRNA as it is being transported through nuclear pores to ribosomes, constantly shuttling between the nucleus and the cytoplasm [368, 369]. A number of hnRNPs bound to the non-phosphorylatable mutant of β-catenin in SW480 by pull-down and include hnRNPF, hnRNPH, hnRNPK, hnNRPU and hnRNP A2/B1.

The shuttling of the hnRNP proteins to and from nucleus to cytoplasm may provide a mechanism for β-catenin shuttling. hnRNP A1 contains a glycine-rich nucleo-cytoplasmic shuttling (NS) domain called M9, necessary for nuclear localisation [370]. M9 is a nuclear localisation domain and does not bear a resemblance to the classical NLS [367] and also acts like a nuclear export signal, allowing the protein to shuttle between nucleus and cytoplasm. hnRNPK possesses both an NLS and an NS domain [371] and can also shuttle between nucleus and cytoplasm [372]. hnRNPK has several cellular roles including transcription, mRNA shuttling, RNA editing and translation [373]. This protein has also been implicated in tumorigenesis. Its expression has been seen to be regulated by the p53/MDM2 pathway and it has been found to increase transcription of oncogene c-myc [373].

Ciarlo *et al.* (2012) looked at the complexing of β-catenin with hnRNPK in prostate cancer cells where hnRNPK is overexpressed and found that this interaction may play a role in prostate cancer progression and resistance to treatment [374]. β-catenin signalling was activated during progression to the treatment-resistant phenotype. It was proposed that protein kinase Akt controls hnRNPK/β-catenin phosphorylation and their transcriptional activities leading to the treatment-resistant phenotype.

Other studies have also identified hnRNPs to interact with β-catenin. In colon cancer cell line DLD-1 it was found that hnRNPK, hnRNP A2/B1 [375] and hnRNP A1 [330] interact with β-catenin.

3.4 Conclusion

In conclusion, hundreds of novel candidate protein interactors of β-catenin were pulled-down in SW480 by use of β-catenin-MBP constructs. Along with pull-down of known interactors which validated the pull-down method, novel interactors were detected by MS and also provided potential novel localisations for β-catenin. Of particular interest were a number of mitochondrial- and golgi-related proteins because β-catenin has not previously been found at either of these organelles. Following this chapter, the same experiments were performed in another cell line, HT29, which has the same mutational status of β-catenin (WT). By doing so, the aim is to make the data in this current chapter stronger by reiterating particular protein interactors detected in SW480, providing further evidence for these proteins to interact with β-catenin in colon cancer.

4 Novel protein interactions of β-catenin in HT29

4.1 Introduction

In chapter 3, colon cancer SW480 was used to identify novel β-catenin binding proteins. SW480 cells are characterised by a constitutively active Wnt pathway, WT β-catenin which is highly and ubiquitously expressed and mutated APC. In this chapter, the same SILAC MS strategy was used but in colon cancer cell line HT29. Reasons for why this cell line was selected for comparison against the SW480 dataset are discussed in 4.3. Essentially, both cell lines have a constitutively active Wnt pathway, with WT β-catenin and mutated APC. However, it was found that the localisation and abundance of β-catenin is different between the two cell lines.

Recombinant β-catenin-MBP was used to pull-down protein binding partners and with the use of amylose resin, this complex was captured and eluted. Eluates were run on SDS-PAGE and gel bands excised and prepared for MS. The approach used in this thesis utilised the advantages of SILAC labelling, in order to reduce variation between replicates. Therefore, all cells in cell culture (consequently used for pull-downs) had been grown in heavy or light SILAC media, and validation of incorporation of the labels was performed before any pull-down experiments were conducted. High-resolution LC/MS (Orbitrap Elite, Thermo Scientific) was used in order to best identify novel interacting partners of β-catenin, which may aid the shuttling of β-catenin into the nucleus and support its nuclear accumulation.

4.2 Results

4.2.1 Candidate novel interacting proteins of β-catenin in HT29

Candidate interacting proteins of β-catenin were pulled-out in HT29 cell lysate during a SILAC experiment performed in triplicate using full-length WT β-catenin-MBP, and comparing to an MBP control. Candidates had to be present in all 3 replicates, have a p-value of < 0.05 and a fold-change of > 2 for light (β-catenin-MBP):heavy (MBP) (Figure 26). The full candidate list (123) can be found in appendix 8.5.

A shorter version of this candidate list can be found in Table 25. This table has candidates where an average fold-change could be generated based on 3 replicates. Furthermore, ≥ 5 unique peptides needed to be detected.

A GO annotation of all candidates was performed and each protein assigned a main process or function. Distribution of the various processes/functions that all candidates are involved is shown in Figure 27. Of particular interest were golgi-related proteins, mitochondrial proteins and proteins involved in nuclear import and export. An example of a group belonging to the 'other' category is cytoskeletal proteins. Proteins grouped as golgi-, mitochondrial-, or nuclear import and export- related are displayed in Table 26.

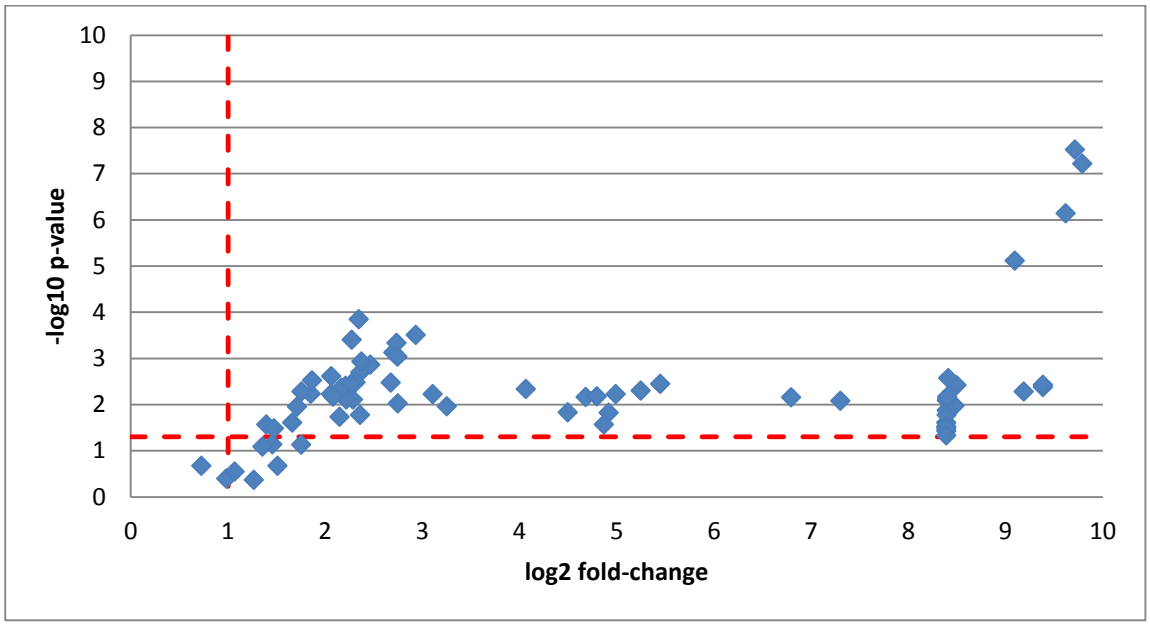


Figure 26 Plot of differentially detected wildtype β-catenin protein interactors in HT29

Log2 fold-change of proteins is plotted against the -log10 p-value reporting threshold (1.3) (horizontal red-dashed line) and log2 fold-change threshold (1) (vertical red-dashed line).

Table 25 Candidate novel interacting proteins of full-length WT β-catenin in HT29.

Accession number	Protein	Unique peptides	Average fold-change (β-catenin:control)
Q9C0C2	182 kDa tankyrase-1-binding protein	11	453
P62269	40S ribosomal protein S18	6	3
P15880	40S ribosomal protein S2	6	4
P23396	40S ribosomal protein S3	13	2
P61247	40S ribosomal protein S3a	7	4
P62241	40S ribosomal protein S8	7	3
P46781	40S ribosomal protein S9	7	4
P46777	60S ribosomal protein L5	5	4
P18124	60S ribosomal protein L7	5	2
P06733	Alpha-enolase	8	3
P07355	Annexin A2	12	4
P27708	CAD protein	31	336
P35222	Catenin beta-1	28	667
Q00610	Clathrin heavy chain 1	20	214
P68104	Elongation factor 1-alpha 1	5	4
P49327	Fatty acid synthase	46	667
P04406	Glyceraldehyde-3-phosphate dehydrogenase	10	5
P63244	Guanine nucleotide-binding protein subunit beta-2-like 1	9	4
P07900	Heat shock protein HSP 90-alpha	6	3
P08238	Heat shock protein HSP 90-beta	6	16
P52272	Heterogeneous nuclear ribonucleoprotein M	10	61
Q14847	LIM and SH3 domain protein 1	5	7
P00338	L-lactate dehydrogenase A chain	9	3
P07195	L-lactate dehydrogenase B chain	6	5
P62937	Peptidyl-prolyl cis-trans isomerase A	8	5
Q06830	Peroxiredoxin-1	7	3
Q15149	Plectin	164	3
P09874	Poly [ADP-ribose] polymerase 1	5	581
P11940	Polyadenylate-binding protein 1	7	8
P02545	Prelamin-A/C	23	87
P14618	Pyruvate kinase isozymes M1/M2	11	5
Q9Y230	RuvB-like 2	16	667
P08670	Vimentin	17	2

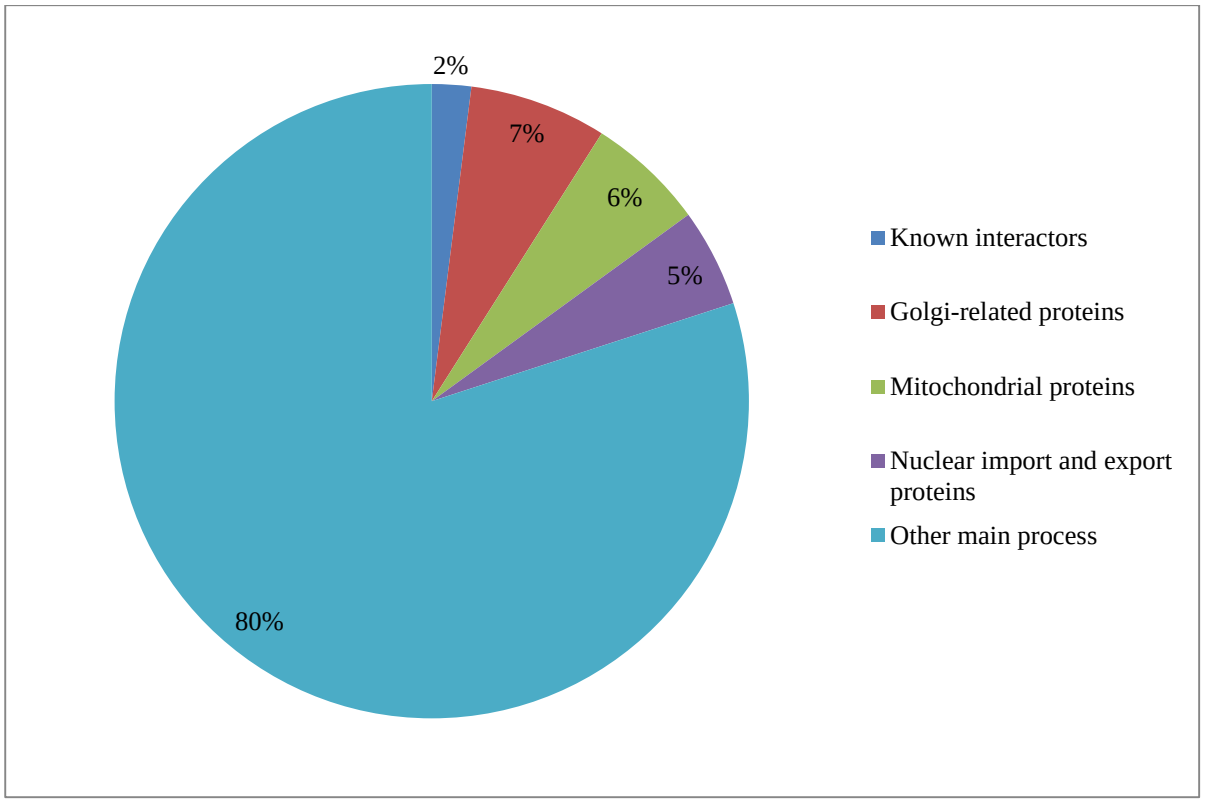


Figure 27 Distribution of processes and functions of candidate novel binding partners of β-catenin in HT29.

Table 26 Golgi-, mitochondrial- and nuclear import and export related candidate novel interacting proteins of WT β-catenin in HT29.

Accession number	Protein	Process/function	Unique peptides
Q00610	Clathrin heavy chain 1	Golgi	20
P61106	Ras-related protein Rab-14	Golgi	1
P49327	Fatty acid synthase	Golgi	46
P10619	Lysosomal protective protein	Golgi	3
P25705	ATP synthase subunit alpha, mitochondrial	Mitochondrial	3
P21796	Voltage-dependent anion-selective channel protein 1	Mitochondrial	4
P08107	Heat shock 70 kDa protein 1A/1B	Mitochondrial	6
P08238	Heat shock protein HSP 90-beta	Mitochondrial	6
P07900	Heat shock protein HSP 90-alpha	Mitochondrial / Nuclear import	6
P09874	Poly [ADP-ribose] polymerase 1	Mitochondrial / Nuclear import	5
P30101	Protein disulfide-isomerase A3	Nuclear import	3
Q06830	Peroxiredoxin-1	Nuclear import	7
P02545	Prelamin-A/C	Nuclear import	23
P62826	GTP-binding nuclear protein Ran	Nuclear import and export	4

4.2.2 The effect of phosphorylation at tyrosine-654 on the protein interactions of β-catenin in HT29

Here, the effect of phosphorylation at tyrosine-654 on the interactor network of β-catenin was assessed. Mutants of full-length β-catenin-MBP were produced, to mimic a phosphorylation at tyrosine-654 or a non-phosphorylatable amino acid at tyrosine-654. These mutants were used for pull-downs in SILAC labelled HT29 which were performed in triplicate.

i. Candidate novel interacting proteins of phosphorylated-Y654-β-catenin in HT29

Candidate interacting proteins of β-catenin were pulled-out during a SILAC experiment performed in triplicate using full-length phosphomimetic β-catenin-Y654E-MBP, and comparing to an MBP control. Candidates had to be present in all 3 replicates, have a p-value of < 0.05 and a fold-change of > 2 for light (β-catenin-MBP):heavy (MBP) (Figure 28). The full candidate list (224 proteins) can be found in appendix 8.6.

A shorter version of this candidate list can be found in Table 27. This table has candidates where an average fold-change could be generated based on 3 replicates. Furthermore, ≥ 6 unique peptides needed to be detected.

Proteins grouped as golgi-, mitochondrial-, or nuclear import and export- related are displayed in Table 28.

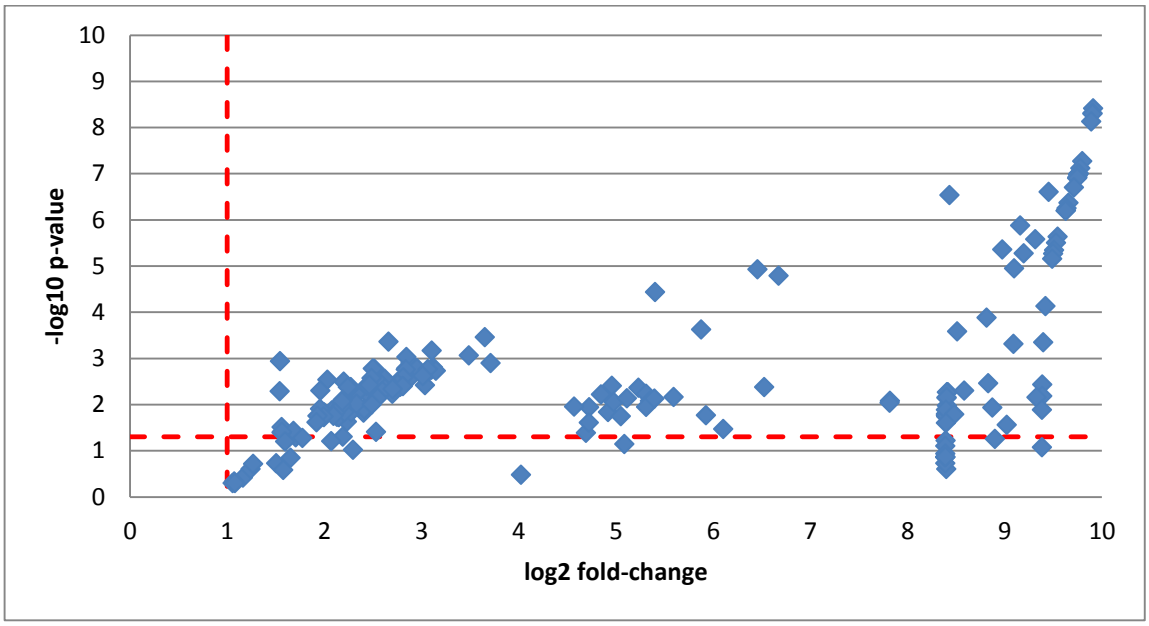


Figure 28 Plot of differentially detected phosphomimetic-Y654-β-catenin protein interactors in HT29

Log2 fold-change of proteins is plotted against the -log10 p-value reporting threshold (1.3) (horizontal red-dashed line) and log2 fold-change threshold (1) (vertical red-dashed line).

Table 27 Candidate novel interacting proteins of the phosphomimetic mutant of β-catenin at tyrosine-654 in HT29.

Accession number	Protein	Unique peptides	Average fold-change (β-catenin:control)
P62280	40S ribosomal protein S11	7	337
P39019	40S ribosomal protein S19	8	5
P15880	40S ribosomal protein S2	8	9
P23396	40S ribosomal protein S3	14	4
P61247	40S ribosomal protein S3a	8	6
P62701	40S ribosomal protein S4, X isoform	7	6
P46781	40S ribosomal protein S9	7	7
P26373	60S ribosomal protein L13	6	9
P50914	60S ribosomal protein L14	6	225
P36578	60S ribosomal protein L4	7	11
P18124	60S ribosomal protein L7	7	5
P62424	60S ribosomal protein L7a	6	6
P11021	78 kDa glucose-regulated protein	7	5
P60709	Actin, cytoplasmic 1	10	4
P07355	Annexin A2	16	4
O00571	ATP-dependent RNA helicase DDX3X	7	384
P11586	C-1-tetrahydrofolate synthase, cytoplasmic	7	469
P27708	CAD protein	15	503
P35221	Catenin alpha-1	16	865
P35222	Catenin beta-1	28	1000
P33993	DNA replication licensing factor MCM7	7	957
P68104	Elongation factor 1-alpha 1	7	5
P13639	Elongation factor 2	12	4
P20042	Eukaryotic translation initiation factor 2 subunit 2	6	3
P49327	Fatty acid synthase	21	858
P04406	Glyceraldehyde-3-phosphate dehydrogenase	9	7
P11216	Glycogen phosphorylase, brain form	11	587
P63244	Guanine nucleotide-binding protein subunit beta-2-like 1	12	8
P08107	Heat shock 70 kDa protein 1A/1B	7	9

Accession number	**Protein**	**Unique peptides**	**Average fold-change (β-catenin:control)**
P11142	Heat shock cognate 71 kDa protein	11	4
P08238	Heat shock protein HSP 90-beta	7	61
O60506	Heterogeneous nuclear ribonucleoprotein Q	8	69
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1	6	684
Q14847	LIM and SH3 domain protein 1	6	339
P00338	L-lactate dehydrogenase A chain	8	5
P07195	L-lactate dehydrogenase B chain	7	6
Q14697	Neutral alpha-glucosidase AB	7	860
P62937	Peptidyl-prolyl cis-trans isomerase A	8	6
P23284	Peptidyl-prolyl cis-trans isomerase B	6	337
Q06830	Peroxiredoxin-1	7	3
P09874	Poly [ADP-ribose] polymerase 1	8	7
P11940	Polyadenylate-binding protein 1	7	8
P02545	Prelamin-A/C	19	7
P17844	Probable ATP-dependent RNA helicase DDX5	6	547
P35232	Prohibitin	8	5
P14618	Pyruvate kinase isozymes M1/M2	14	5
P13489	Ribonuclease inhibitor	6	92
Q9Y265	RuvB-like 1	8	674
Q9Y230	RuvB-like 2	12	723
Q9UHD2	Serine/threonine-protein kinase TBK1	6	450
Q14247	Src substrate cortactin	6	839
Q14134	Tripartite motif-containing protein 29	7	643
O00159	Unconventional myosin-Ic	6	728
P08670	Vimentin	14	5
P12956	X-ray repair cross-complementing protein 6	6	5

Table 28 Golgi-, mitochondrial- and nuclear import and export related candidate novel interacting proteins of phosphomimetic mutant β-catenin at tyrosine-654 in HT29.

Accession number	Protein	Process/function	Unique peptides
P11021	78 kDa glucose-regulated protein	Golgi	7
P60953	Cell division control protein 42 homolog	Golgi	2
P35606	Coatomer subunit beta'	Golgi	1
P11142	Heat shock cognate 71 kDa protein	Golgi	11
Q15084	Protein disulfide-isomerase A6	Golgi	5
Q15436	Protein transport protein Sec23A	Golgi	1
Q15437	Protein transport protein Sec23B	Golgi	1
P63000	Ras-related C3 botulinum toxin substrate 1	Golgi	3
P61026	Ras-related protein Rab-10	Golgi	1
O95292	Vesicle-associated membrane protein-associated protein B/C	Golgi	2
P18085	ADP-ribosylation factor 4	Golgi	3
Q14697	Neutral alpha-glucosidase AB	Golgi	7
P61106	Ras-related protein Rab-14	Golgi	1
P49327	Fatty acid synthase	Golgi	21
P10619	Lysosomal protective protein	Golgi	3
P00505	Aspartate aminotransferase, mitochondrial	Mitochondrial	1
Q9NVI7	ATPase family AAA domain-containing protein 3A	Mitochondrial	4
P61221	ATP-binding cassette sub-family E member 1	Mitochondrial	4
Q9UG63	ATP-binding cassette sub-family F member 2	Mitochondrial	2
Q9NX63	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial	Mitochondrial	4
P00403	Cytochrome c oxidase subunit 2	Mitochondrial	1
P49411	Elongation factor Tu, mitochondrial	Mitochondrial	4
Q12931	Heat shock protein 75 kDa, mitochondrial	Mitochondrial	1
Q16891	Mitochondrial inner membrane protein	Mitochondrial	1
P45880	Voltage-dependent anion-selective channel protein 2	Mitochondrial	5
P38646	Stress-70 protein, mitochondrial	Mitochondrial	4
P21796	Voltage-dependent anion-selective	Mitochondrial	4

Accession number	Protein	Process/function	Unique peptides
	channel protein 1		
P08107	Heat shock 70 kDa protein 1A/1B	Mitochondrial	7
P08238	Heat shock protein HSP 90-beta	Mitochondrial	7
P09874	Poly [ADP-ribose] polymerase 1	Mitochondrial / Nuclear import	8
P62829	60S ribosomal protein L23	Nuclear import	4
Q14974	Importin subunit beta-1	Nuclear import	2
Q99623	Prohibitin-2	Nuclear import	3
Q13263	Transcription intermediary factor 1-beta	Nuclear import	4
Q06830	Peroxiredoxin-1	Nuclear import	7
P02545	Prelamin-A/C	Nuclear import	19
P09651	Heterogeneous nuclear ribonucleoprotein A1	Nuclear import and export	3
P50402	Emerin	Nuclear import and export	2
P62826	GTP-binding nuclear protein Ran	Nuclear import and export	5

ii. Candidate novel interacting proteins of non-phosphorylatable-Y654-β-catenin in HT29

Candidate interacting proteins of β-catenin were pulled-out during a SILAC experiment performed in triplicate using full-length non-phosphorylatable β-catenin-Y654F-MBP, and comparing to an MBP control. Candidates had to be present in all 3 replicates, have a p-value of < 0.05 and a fold-change of > 2 for light (β-catenin-MBP):heavy (MBP) (Figure 29). The full candidate list (48 proteins) can be found in appendix 8.7.

A shorter version of this candidate list can be found in Table 29. This table has candidates where an average fold-change could be generated based on 3 replicates. Proteins grouped as golgi-, mitochondrial-, or nuclear import and export- related are displayed in Table 30.

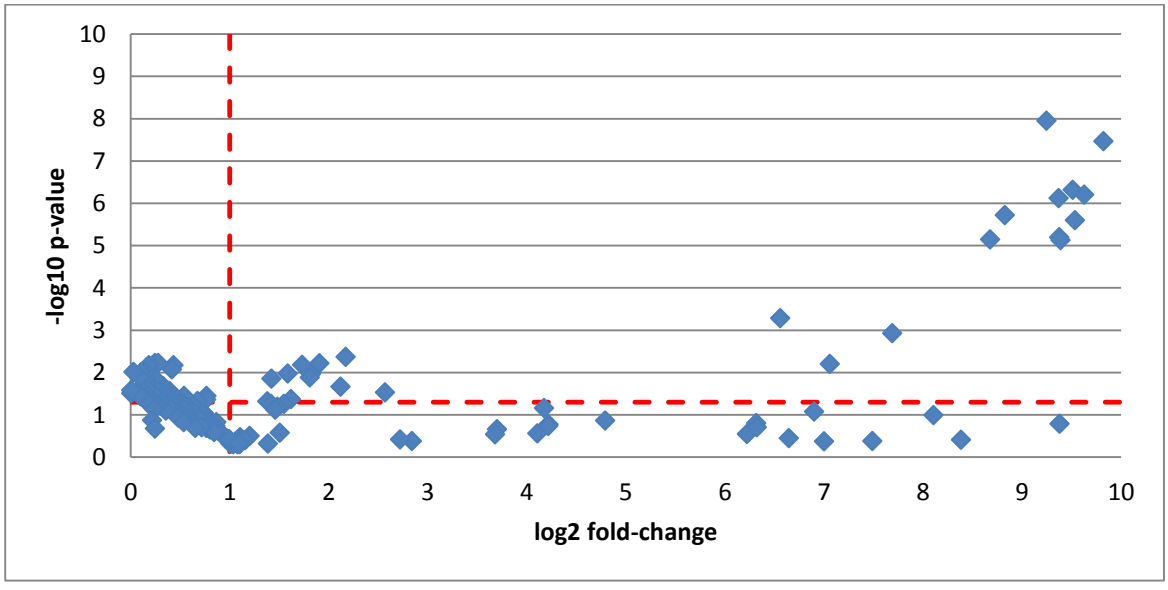


Figure 29 Plot of differentially detected non-phosphorylatable-Y654-β-catenin protein interactors in SW480

Log2 fold-change of proteins is plotted against the -log10 p-value reporting threshold (1.3) (horizontal red-dashed line) and log2 fold-change threshold (1) (vertical red-dashed line).

Table 29 Candidate novel interacting proteins of the non-phosphorylatable mutant of β-catenin at tyrosine-654 in HT29.

Accession number	Protein	Unique peptides	Average fold-change (β-catenin:control)
P04114	Apolipoprotein B-100	3	666
P35221	Catenin alpha-1	13	207
P35222	Catenin beta-1	25	1000
P13804	Electron transfer flavoprotein subunit alpha, mitochondrial	1	4
P58107	Epiplakin	38	671
P49327	Fatty acid synthase	49	3
P56470	Galectin-4	3	608
P30711	Glutathione S-transferase theta-1	1	1000
P11216	Glycogen phosphorylase, brain form	13	133
P08107	Heat shock 70 kDa protein 1A/1B	5	4
P08238	Heat shock protein HSP 90-beta	6	3
P02042	Hemoglobin subunit delta	1	1000
Q02241	Kinesin-like protein KIF23	6	4
Q13765	Nascent polypeptide-associated complex subunit alpha	3	6
Q14697	Neutral alpha-glucosidase AB	6	3
Q9Y446	Plakophilin-3	11	3
Q15149	Plectin	157	3
Q01658	Protein Dr1	1	4
Q8WVV4	Protein POF1B	5	454
Q9Y230	RuvB-like 2	14	662
Q14247	Src substrate cortactin	8	4
P38646	Stress-70 protein, mitochondrial	8	3
P29401	Transketolase	4	3

Table 30 Golgi-, mitochondrial- and nuclear import and export related candidate novel interacting proteins of non-phosphorylatable mutant of β-catenin at tyrosine-654 in HT29.

Accession number	Protein	Process/function	Unique peptides
P61204	ADP-ribosylation factor 3	Golgi	1
P04114	Apolipoprotein B-100	Golgi	3
P18085	ADP-ribosylation factor 4	Golgi	1
Q14697	Neutral alpha-glucosidase AB	Golgi	6
P49327	Fatty acid synthase	Golgi	4
P10619	Lysosomal protective protein	Golgi	3
P13804	Electron transfer flavoprotein subunit alpha, mitochondrial	Mitochondrial	1
P38646	Stress-70 protein, mitochondrial	Mitochondrial	8
P08107	Heat shock 70 kDa protein 1A/1B	Mitochondrial	5
P08238	Heat shock protein HSP 90-beta	Mitochondrial	6
P40926	Malate dehydrogenase, mitochondrial	Mitochondrial	3
P55060	Exportin-2	Nuclear export	1

iii. A comparison of binding-partners of WT, phosphomimetic, and non-phosphorylatable β-catenin in HT29

Candidate proteins were compared across the WT, phosphomimetic and non-phosphorylatable experiments. The full list (270 proteins) can be found in appendix 8.8. This table has their presence or absence across all three datasets. A Venn diagram represents the overlap of potential candidates in Figure 30.

A shorter version of this candidate list can be found in Table 31. This table has candidates which were affected by phosphorylation. Proteins were present in the WT dataset and unique to either the phosphomimetic or non-phosphorylatable dataset.

For example based on the data one may assume that 182 kDa tankyrase-1-binding-protein (Q9C0C2) only binds to β-catenin when tyrosine-654 is phosphorylated. On the other hand, heterogeneous nuclear ribonucleoprotein D0 (Q14103) may only bind to β-catenin in the absence of phosphorylation at tyrosine-654. Furthermore, a known interactor such as RuvB-like 1 (Q9Y265) may only bind to β-catenin in the presence of phosphorylation at tyrosine-654.

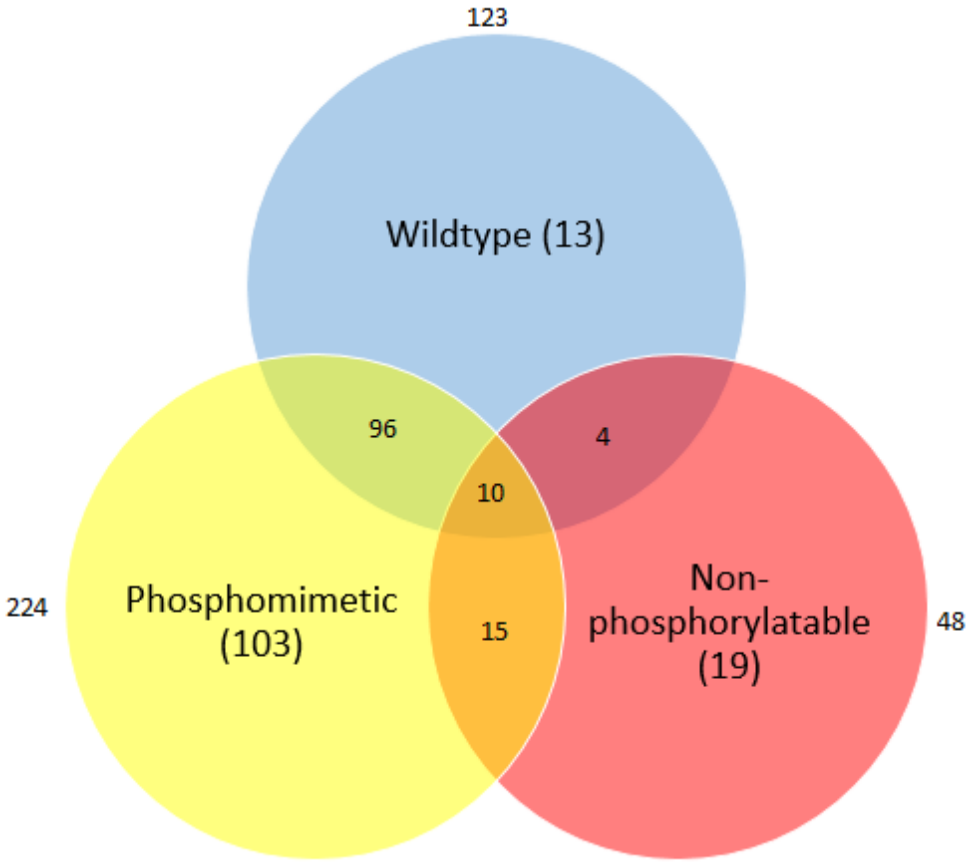


Figure 30 A Venn diagram representing overlap of candidate protein binders of β-catenin across wildtype, phosphomimetic and non-phosphorylatable datasets in HT29.

Table 31 Candidate novel interacting proteins of β-catenin that may be effect by phosphorylation.

*PM denotes phosphomimetic and NP denotes non-phosphorylatable

**White denotes absence of that protein in the dataset

***Grey denotes presence of that protein in the dataset

Accession number	Protein	Wildtype	PM	NP
Q9C0C2	182 kDa tankyrase-1-binding protein			
P62280	40S ribosomal protein S11			
P62277	40S ribosomal protein S13			
P62263	40S ribosomal protein S14			
P62244	40S ribosomal protein S15a			
P62249	40S ribosomal protein S16			
P62269	40S ribosomal protein S18			
P39019	40S ribosomal protein S19			
P15880	40S ribosomal protein S2			
P62851	40S ribosomal protein S25			
P42677	40S ribosomal protein S27			
P23396	40S ribosomal protein S3			
P61247	40S ribosomal protein S3a			
P62701	40S ribosomal protein S4, X isoform			
P62753	40S ribosomal protein S6			
P62241	40S ribosomal protein S8			
P46781	40S ribosomal protein S9			
P05388	60S acidic ribosomal protein P0			
P62913	60S ribosomal protein L11			
P26373	60S ribosomal protein L13			
P40429	60S ribosomal protein L13a			
P50914	60S ribosomal protein L14			
P61313	60S ribosomal protein L15			
Q07020	60S ribosomal protein L18			
P84098	60S ribosomal protein L19			
P35268	60S ribosomal protein L22			
P62750	60S ribosomal protein L23a			
P83731	60S ribosomal protein L24			
P61353	60S ribosomal protein L27			
P46776	60S ribosomal protein L27a			

Accession number	Protein	Wildtype	PM	NP
P46779	60S ribosomal protein L28			
P62888	60S ribosomal protein L30			
P18077	60S ribosomal protein L35a			
Q9Y3U8	60S ribosomal protein L36			
P46777	60S ribosomal protein L5			
Q02878	60S ribosomal protein L6			
P18124	60S ribosomal protein L7			
P53999	Activated RNA polymerase II transcriptional coactivator p15			
P02765	Alpha-2-HS-glycoprotein			
P07355	Annexin A2			
P09525	Annexin A4			
P27708	CAD protein			
Q9HB71	Calcyclin-binding protein			
P68104	Elongation factor 1-alpha 1			
P60842	Eukaryotic initiation factor 4A-I			
P47756	F-actin-capping protein subunit beta			
P04406	Glyceraldehyde-3-phosphate dehydrogenase			
P62826	GTP-binding nuclear protein Ran			
P62879	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2			
P63244	Guanine nucleotide-binding protein subunit beta-2-like 1			
P04792	Heat shock protein beta-1			
P52272	Heterogeneous nuclear ribonucleoprotein M			
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1			
O15347	High mobility group protein B3			
P16403	Histone H1.2			
P16401	Histone H1.5			
Q96KK5	Histone H2A type 1-H			
O60814	Histone H2B type 1-K			
P12268	Inosine-5'-monophosphate dehydrogenase 2			
P14923	Junction plakoglobin			
Q14847	LIM and SH3 domain protein 1			
P00338	L-lactate dehydrogenase A chain			
P07195	L-lactate dehydrogenase B chain			
P60660	Myosin light polypeptide 6			

Accession number	Protein	Wildtype	PM	NP
P19105	Myosin regulatory light chain 12A			
P49116	Nuclear receptor subfamily 2 group C member 2			
Q9NR12	PDZ and LIM domain protein 7			
P62937	Peptidyl-prolyl cis-trans isomerase A			
Q06830	Peroxiredoxin-1			
P09874	Poly [ADP-ribose] polymerase 1			
P11940	Polyadenylate-binding protein 1			
Q13310	Polyadenylate-binding protein 4			
P02545	Prelamin-A/C			
P17844	Probable ATP-dependent RNA helicase DDX5			
P35232	Prohibitin			
P14618	Pyruvate kinase isozymes M1/M2			
P61106	Ras-related protein Rab-14			
Q9Y265	RuvB-like 1			
P62136	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit			
O14907	Tax1-binding protein 3			
P49368	T-complex protein 1 subunit gamma			
P07437	Tubulin beta chain			
P68371	Tubulin beta-4B chain			
P61088	Ubiquitin-conjugating enzyme E2 N			
P08670	Vimentin			
P21796	Voltage-dependent anion-selective channel protein 1			
P61981	14-3-3 protein gamma			
P63261	Actin, cytoplasmic 2			
Q14919	Dr1-associated corepressor			
Q14103	Heterogeneous nuclear ribonucleoprotein D0			

4.3 Discussion

4.3.1 SW480 versus HT29

Colon cancer cell line HT29 was chosen for experiments in this chapter as it would complement the data collected from colon cancer cell line SW480. As mentioned previously, SW480 has a constitutively active Wnt pathway and the β-catenin is WT. These two properties are shared between the two chosen cell lines, further validating any interacting partners pulled-down, as they would, therefore, be present across two cell lines that shared the same mutational properties.

However, although β-catenin is abundant in SW480, in HT29 its levels are reduced (Figure 31). The localisation of β-catenin in HT29 is also different, where in SW480 localisation is both cytoplasmic and nuclear and in HT29 β-catenin is strictly localised to the cell membrane (Figure 32). These differences in expression and localisation may impact the findings of β-catenin interactions using our β-catenin-MBP bait approach. For example in SW480 cells, high endogenous cytoplasmic/nuclear levels may compete with β-catenin-MBP to bind interacting proteins, while in HT29 (β-catenin at the cell membrane) different protein interactors may be expected. In this regard the data shown here is comprehensive, investigating the potential of both membrane-bound and free cytosolic β-catenin to bind interacting partner proteins.

There were marked differences between the two cell lines where only 111 proteins were shared in common between the two cell lines. However it is known that SW480 and HT29 differ in morphology, migratory characteristics, EMT markers, metastasis potential, and most relevant to our study, Wnt activity [376].

Utilising data from both cell lines and looking at protein binders shared between the two datasets really narrows down the list of potential candidates to the strongest and strictest partners. This will provide insight on the importance and effect of β-catenin localisation and its interactome.

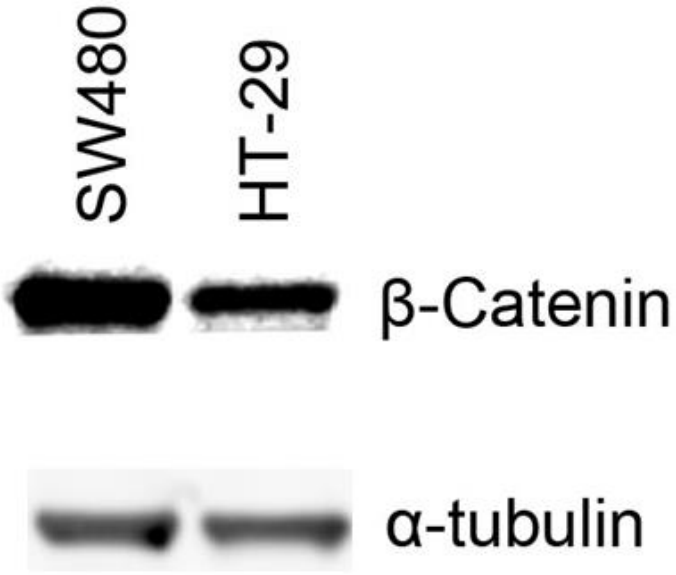


Figure 31 Endogenous expression of β-catenin in SW480 compared to HT29. Image provided by Kate Mills, WMI.

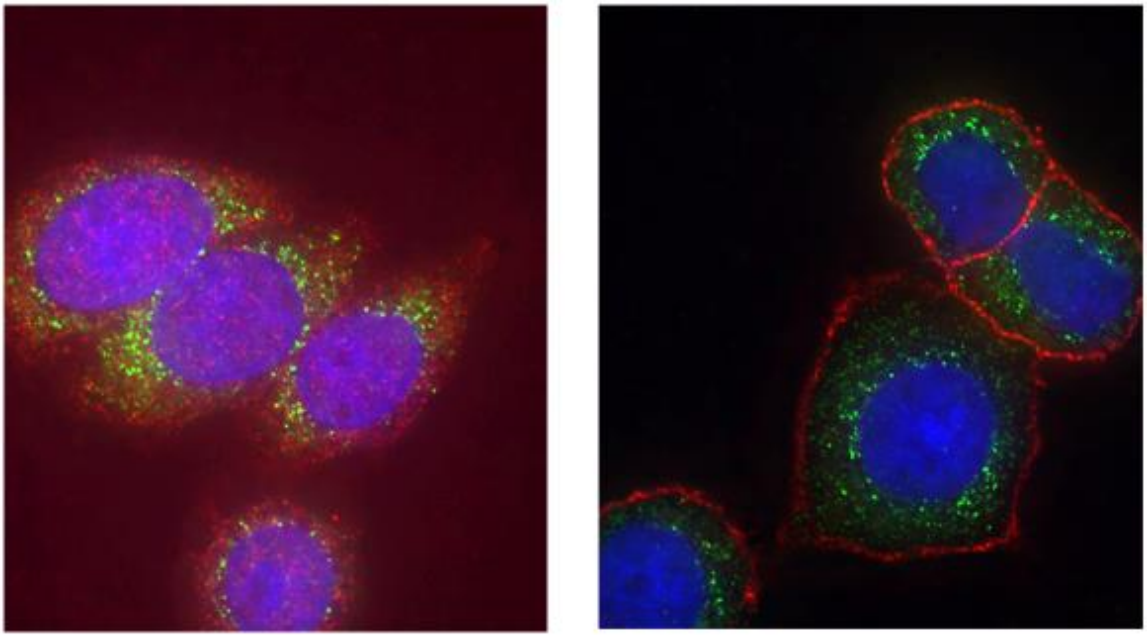


Figure 32 Immunofluorescence presenting localisation of β-catenin (red) in SW480 (left) and HT29 (right). Nuclei stained with Hoechst (blue) while another protein (Drp) is depicted by green.

4.3.2 Common protein interactors of β-catenin between SW480 and HT29

Across SW480 and HT29 datasets, 672 proteins were identified as potential candidate interacting partners of β-catenin. Of these proteins, only 111 proteins were common to both cell lines in at least one aspect. That is, the protein was detected in either the WT, phosphomimetic or non-phosphorylatable dataset for either cell line (Table 32). Figure 33 depicts this in Venn diagram.

Importantly, candidate proteins of interest in Chapter 3 (SW480) were also identified as candidates in Chapter 4 (HT29). These include COPB2, mtHSP70 and a number of hnRNPs. COPB2 bound to phosphomimetic β-catenin in HT29 and WT β-catenin in SW480. mtHSP70 bound all three β-catenin constructs in HT29 (WT, phosphomimetic and non-phosphorylatable), while in SW480 it bound to the phosphomimetic and non-phosphorylatable β-catenin. Regarding the hnRNPs, hnRNPH and hnRNPQ bound to the non-phosphorylatable β-catenin in HT29 but bound to the phosphomimetic β-catenin in SW480. hnRNP A2/B1 bound to the WT and phosphomimetic β-catenin in HT29, while binding the non-phosphorylatable β-catenin in SW480. The differences in preference for the various β-catenin constructs across cell lines needs to be further investigated so the reason why is clear.

Other studies have also utilised the differences and commonalities between SW480 and HT29 for their research. Yang *et al.* (2006) looked at the regulation of β-catenin phosphorylation and degradation by APC mutations in colon cancer. They utilised three colon cancer cell lines in particular – SW480, HT29 and DLD-1 - each selected by reason that each cell line carries mutated APC (which stabilises β-catenin as discussed in 1.3.1).

Inhibition of phosphorylation by APC in the cell lines was expected yet a high level of β-catenin phosphorylation was found [377]. However, ubiquitination and degradation of β-catenin was inhibited in SW480 but not HT29 and DLD-1. This inhibition of ubiquitination in SW480 was rescued by exogenous expression of APC, and domains of APC necessary for ubiquitination of β-catenin were analysed. Results indicate that phosphorylation and ubiquitination of β-catenin is regulated by distinct domains of APC and separate molecular mechanisms. Here, the cell lines chosen were appropriate for the study because they were all colon cancer cell lines with WT β-catenin but possessed different APC truncations.

Bahrawy *et al*. (2004) also utilised SW480 and HT29 in order to determine if there was a correlation between β-catenin and APC mutations and growth pattern/distribution of the E-cadherin/catenin complex. These factors did not appear to be affected by the mutations but rather were influenced by presence or absence of cell-cell contact. These cell lines, in particular, were significance to the study because both cell lines express mutant APC and possess different patterns of E-cadherin [378].

Dvory-Sobol *et al.* (2006) used HT29 and SW480 in their study as both cell lines display hyperactive β-catenin/Tcf signalling and their focus was to target this active pathway in cancer treatment [379]. These studies, like the research presented in this thesis used the similar and different properties of the cell lines to benefit their research.

Table 32 Candidate protein interactors of β-catenin common to SW480 and HT29.

*W denotes WT; N denotes non-phosphorylatable; M denotes phosphomimetic

*White denotes absence of that protein in the dataset

**Grey denotes presence of that protein in the dataset

		HT29			SW480		
Accession number	**Protein**	**W**	**N**	**M**	**W**	**N**	**M**
Q9C0C2	182 kDa tankyrase-1-binding protein						
P62277	40S ribosomal protein S13						
P62263	40S ribosomal protein S14						
P62269	40S ribosomal protein S18						
P15880	40S ribosomal protein S2						
P60866	40S ribosomal protein S20						
P23396	40S ribosomal protein S3						
P61247	40S ribosomal protein S3a						
P46781	40S ribosomal protein S9						
P08865	40S ribosomal protein SA						
P05388	60S acidic ribosomal protein P0						
P62906	60S ribosomal protein L10a						
P62913	60S ribosomal protein L11						
P62829	60S ribosomal protein L23						
P18124	60S ribosomal protein L7						
P11021	78 kDa glucose-regulated protein						
P60709	Actin, cytoplasmic 1						
P63267	Actin, gamma-enteric smooth muscle						
P61160	Actin-related protein 2						
P59998	Actin-related protein 2/3 complex subunit 4						
P11766	Alcohol dehydrogenase class-3						
P02765	Alpha-2-HS-glycoprotein						
P06733	Alpha-enolase						
P04114	Apolipoprotein B-100						
P25705	ATP synthase subunit alpha, mitochondrial						
P61221	ATP-binding cassette sub-family E member 1						
O00571	ATP-dependent RNA helicase DDX3X						
P11586	C-1-tetrahydrofolate synthase, cytoplasmic						
P27708	CAD protein						
P35221	Catenin alpha-1						
P35222	Catenin beta-1						

		HT29			SW480		
Accession number	**Protein**	**W**	**N**	**M**	**W**	**N**	**M**
O60716	Catenin delta-1						
Q00610	Clathrin heavy chain 1						
P35606	Coatomer subunit beta'						
Q9NX63	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial						
P21291	Cysteine and glycine-rich protein 1						
P00403	Cytochrome c oxidase subunit 2						
P60981	Destrin						
P33992	DNA replication licensing factor MCM5						
P33993	DNA replication licensing factor MCM7						
Q14258	E3 ubiquitin/ISG15 ligase TRIM25						
P49411	Elongation factor Tu, mitochondrial						
P14625	Endoplasmin						
O15371	Eukaryotic translation initiation factor 3 subunit D						
P15311	Ezrin						
P49327	Fatty acid synthase						
P30711	Glutathione S-transferase theta-1						
P04406	Glyceraldehyde-3-phosphate dehydrogenase						
O14556	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific						
P11216	Glycogen phosphorylase, brain form						
P63244	Guanine nucleotide-binding protein subunit beta-2-like 1						
P08107	Heat shock 70 kDa protein 1A/1B						
Q12931	Heat shock protein 75 kDa, mitochondrial						
P07900	Heat shock protein HSP 90-alpha						
P31943	Heterogeneous nuclear ribonucleoprotein H						
O60506	Heterogeneous nuclear ribonucleoprotein Q						
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1						
Q96KK5	Histone H2A type 1-H						
O60814	Histone H2B type 1-K						
Q15181	Inorganic pyrophosphatase						
Q9Y6M1	Insulin-like growth factor 2 mRNA-binding protein 2						
P14923	Junction plakoglobin						
Q9NSK0	Kinesin light chain 4						
P20700	Lamin-B1						
Q14847	LIM and SH3 domain protein 1						
Q86W92	Liprin-beta-1						

		HT29			SW480		
Accession number	**Protein**	**W**	**N**	**M**	**W**	**N**	**M**
P00338	L-lactate dehydrogenase A chain						
P10619	Lysosomal protective protein						
Q14697	Neutral alpha-glucosidase AB						
P50479	PDZ and LIM domain protein 4						
Q9NR12	PDZ and LIM domain protein 7						
P62937	Peptidyl-prolyl cis-trans isomerase A						
Q06830	Peroxiredoxin-1						
P32119	Peroxiredoxin-2						
Q15365	Poly(rC)-binding protein 1						
Q13310	Polyadenylate-binding protein 4						
P26599	Polypyrimidine tract-binding protein 1						
P02545	Prelamin-A/C						
Q92841	Probable ATP-dependent RNA helicase DDX17						
P17844	Probable ATP-dependent RNA helicase DDX5						
Q15185	Prostaglandin E synthase 3						
P21980	Protein-glutamine gamma-glutamyltransferase 2						
P14618	Pyruvate kinase isozymes M1/M2						
P61026	Ras-related protein Rab-10						
P51148	Ras-related protein Rab-5C						
Q8WUF5	RelA-associated inhibitor						
Q96PK6	RNA-binding protein 14						
Q01844	RNA-binding protein EWS						
P35637	RNA-binding protein FUS						
Q9Y265	RuvB-like 1						
Q9Y230	RuvB-like 2						
Q00796	Sorbitol dehydrogenase						
Q14247	Src substrate cortactin						
P38646	Stress-70 protein, mitochondrial						
O14907	Tax1-binding protein 3						
P40227	T-complex protein 1 subunit zeta						
Q13263	Transcription intermediary factor 1-beta						
P29401	Transketolase						
Q14134	Tripartite motif-containing protein 29						
P68363	Tubulin alpha-1B chain						
P68366	Tubulin alpha-4A chain						
P07437	Tubulin beta chain						
Q13885	Tubulin beta-2A chain						
P68371	Tubulin beta-4B chain						

		HT29			SW480		
Accession number	**Protein**	**W**	**N**	**M**	**W**	**N**	**M**
Q9BUF5	Tubulin beta-6 chain						
O00159	Unconventional myosin-Ic						
O95292	Vesicle-associated membrane protein-associated protein B/C						
P21796	Voltage-dependent anion-selective channel protein 1						
P45880	Voltage-dependent anion-selective channel protein 2						
P12956	X-ray repair cross-complementing protein 6						

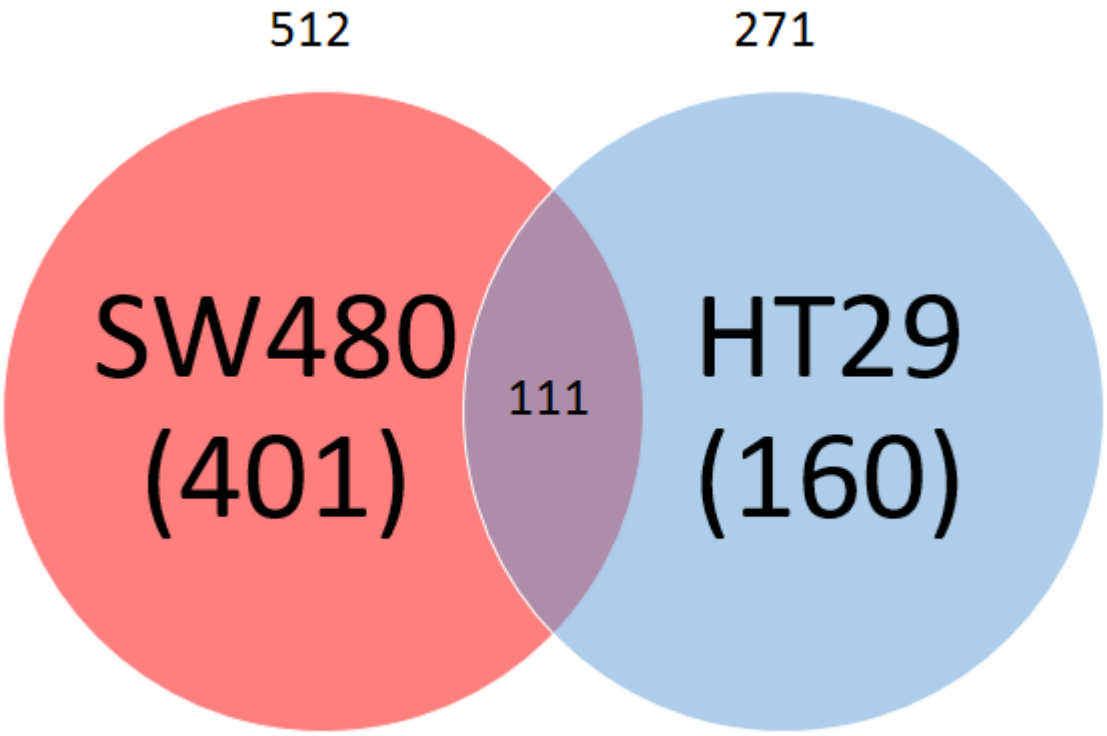


Figure 33 Venn diagram portraying protein binders of β-catenin found across both SW480 and HT29 datasets

4.4 Conclusion

In conclusion, novel candidate interactors of β-catenin were pulled-down in HT29 by use of β-catenin-MBP constructs and a SILAC MS strategy. Some of the proteins detected in this chapter which were also found in SW480 are followed-up in Chapter 5 (validations chapter), due to them being found to interact with β-catenin in two colon cancer cell lines, supporting the notion their interaction with β-catenin is associated with cancer. These proteins include COPB, mtHSP70, KCL4 and Tab182.

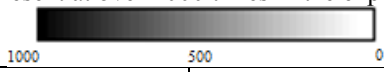
5 Validation of candidate protein binding partners of β-catenin

5.1 Introduction

Putative β-catenin protein binding partners were identified by MS in Chapters 3 and 4 and were selected for further study (Table 33). These proteins were selected based on their function and subcellular location. That is, protein binders with a function of interest (eg. Nuclear proteins that may aid β-catenin in shuttling) or protein binders implicating β-catenin to be at a novel organelle (eg. Mitochondrial or golgi proteins) were selected. In this chapter, immunoprecipitation (IP) and immunofluorescence (IF) techniques were explored in efforts to confirm the validity of these proteins as novel β-catenin protein binders. Identification of such β-catenin protein interactors would likely provide new insights into the functional activities of β-catenin and its regulatory roles in cancer.

For IPs, β-catenin antibody (Table 7) was used for protein isolation in SW480 cell lysate followed by western blot using a specific antibody against the novel candidate protein of interest. For IF, antibodies against β-catenin and the novel candidate protein were used in SW480, HT29 or HEK293T (non-cancer control) cell lines in order to shed light on their potential interaction or co-localisation. The following putative β-catenin binding proteins were investigated in this chapter by methods of IP, pull-down and IF imaging – COPB, mtHSP70, Tab182, KCL4, hnRNPK and Opa1. From these studies, the most promising new β-catenin binding proteins, COPB and mtHSP70, are discussed in-depth within the context of β-catenin biology because they showed positive results across all investigative methods – MS, IP, pull-down and IF. Table 34 lists the proteins above in the context of the methods used to validate them.

Table 33 Putative novel protein interactors of β-catenin.
*_WT_ = WT dataset; _pD_ = non-phosphorylatable dataset, _pM_ = phosphomimetic dataset
**Numbers in _WT, Y654F, Y654E_ columns are fold-change (β-catenin:MBP)
***Bar denotes fold-change where '0' means absent and '1000' means present at over 1000 times in the experimental vs control



			SW480			HT29			
Protein name	**Subcellular location**	**Function**	*WT*	*Y654F*	*Y654E*	*WT*	*Y654F*	*Y654E*	**Justification for pursuit**
182 kDa tankyrase-1-binding protein (Tab182) (Q9C0C2)	Cytoplasm	Ankyrin-binding protein associated with cortical actin in the cytoplasm but also resides in the nucleus [380]	796			786		345	Bound β-catenin in both cell lines with high fold-change compared to control. Tab182 binds tankyrase 1 which downregulates axin and thereby helps stabilise β-catenin [381, 382], implicating Tab182 in regulating the stability of β-catenin. Tab182 also binds ankyrin and ankyrin regulates Wnt signaling by altering β-catenin localisation [383].
Coatomer subunit beta' (COPB') (P35606)	Golgi	Part of Golgi coatomer 7-protein complex that coats non-clathrin-coated vesicles, involved in protein transport from ER, required for Golgi budding/vesicular trafficking [384]	1000					478	Novel putative role and localisation of β-catenin at the Golgi. 4 out of 7 subunits of the whole protein complex were identified by proteomics. Bound β-catenin in both cell lines. COPB selected as collaborators were working on it and had convincing results and antibody was available
COP9 signalosome complex subunit 5 (COPS5) (Q92905)	Cytoplasm	Part of the COP9 signalosome complex, roles in cellular and developmental processes, ubiquitin conjugation pathway [385]	325						Bound β-catenin in SW480 cells. COP9 signalosome may have a role in β-catenin degradation
Dynamin-like 120 kDa protein, mitochondrial (Opa1)	Mitochondria	Regulates mitochondrial fusion and cristae structure in the inner mitochondrial membrane [386-388]		268					Novel putative role and localisation of β-catenin at the mitochondria

			SW480			HT29			
Protein name	**Subcellular location**	**Function**	*WT*	*Y654 F*	*Y654 E*	*WT*	*Y654 F*	*Y654 E*	**Justification for pursuit**
(O60313)									
Heat shock 70 kDa protein 1A/1B, mitochondrial (mtHSP70) (P08107)	Mitochondria	Molecular chaperone stabilising proteins against aggregation and misfolding, stabilising or degrading mutant proteins [389]		3	3	6	5	9	Novel putative role and localisation of β-catenin at the mitochondria. Bound β-catenin in both cell lines at similar fold-changes
Heat shock protein HSP 90-alpha (HSP90) (P07900)	Cytoplasm	Molecular chaperone binding and folding proteins into their functional structure [390, 391]			3	4			Another heat shock protein and molecular chaperone (aside from mtHSP70) that may have a role at the mitochondria [392]
Heterogeneous nuclear ribonucleoprotein K (hnRNPK) (P61978)	Nucleus	Binds RNA, roles in pre-mRNA processing and mRNA metabolism/transport, transcription activation and repression [393]		8					Numerous hnRNPs bound β-catenin across both cell lines. hnRNP interaction with β-catenin could be involved with the β-catenin nuclear shuttling.
Kinesin light chain 4 (KCL4) (Q9NSK0)	Cytoplasm	Microtubule-associated protein, roles in organelle transport, light chain involved in coupling cargo to heavy chain [394]	920	649				572	Bound β-catenin in both cell lines with high fold-change compared to control
Ras-related protein Rab-5A (Rab5a) (P20339)	Cytoplasm	Regulate intracellular membrane trafficking, form transport vesicles [395-398]	798						Numerous Rabs bound β-catenin in both cell lines including Rab1b, 4a, 5b, 5c, 8a, 10, 14

Table 34 Methods used to validate novel candidate protein interactors of β-catenin.

Protein	Mass spectrometry	Pull-down	Immunoprecipitation (IP)	Immunofluorescence (IF)
COPB	Positive	Positive	Positive	Positive
mtHSP70	Positive	Positive	Positive	Positive
Tab182	Positive	Positive	Positive (length of Tab182 makes results unclear)	Positive
KCL4	Positive	Positive but very faint signal	High background. Reverse-IP positive	Positive
hnRNPK	Positive	N/A	High background	Need more data
Opa1	Positive	Positive	High background	Positive

5.2 Results

The immunoprecipitation assay (referred to here as IP) was one method used to validate protein interactions. An IP control experiment was performed, where APC, a well-characterised interactor of β-catenin was detected in an IP with α-β-catenin antibody, but not with the control IgG antibody (Figure 34). This provides insight into the quality of the IP protocol where minimal false signal is detected in the control lane for a known, high-affinity binder.

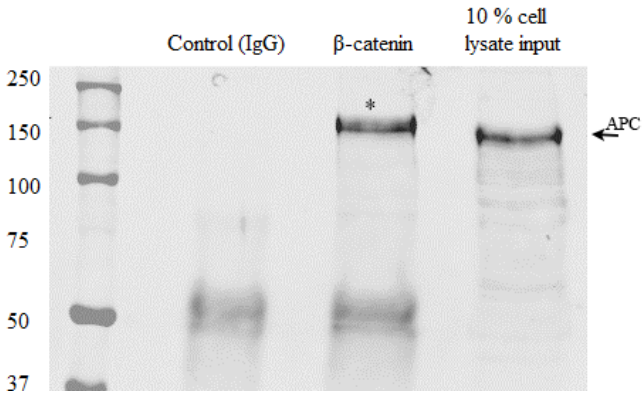


Figure 34 Validation of IP protocol by IP using α-β-catenin antibody and probing for known interactor, APC.

Mutant endogenous form of APC (~160 kDa) is immunoprecipitated by α-β-catenin antibody (*) but not the IgG control, in extract from SW480 cells, supporting the IP protocol utilised within. Note that the mutant form of APC detected here is known to retain β-catenin binding sites.

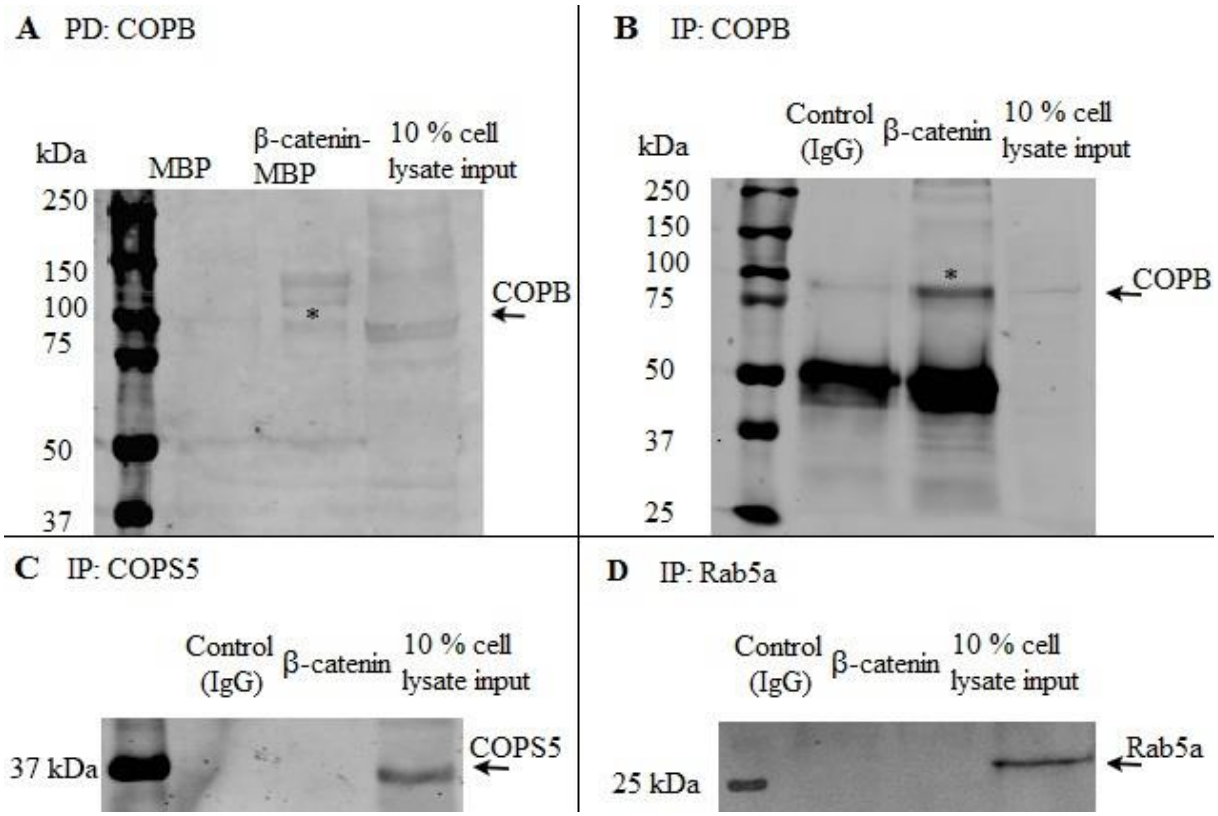
5.2.1 Coatomer subunit beta (COPB)

i. Validation by immunoprecipitation

Coatomer subunit beta (COPB), a known golgi protein [399, 400] was identified by MS to bind to WT full-length β-catenin-MBP in SW480 and the phosphomimetic in HT29 cells. A protein pull-down strategy with β-catenin-MBP and western blot for COPB demonstrated the potential for interaction in SW480 cells (Figure 35A). Despite identifying COPB in the phosphomimetic condition in HT29 this could not be readily validated by IP as no reliable phosphospecific antibody was available for β-catenin Y654.

To further investigate this potential novel interaction in SW480, a series of IPs were performed using α-β-catenin antibody and subsequent western blot using α-COPB antibody (Figure 35B). Taken together with the pull-down data this provides evidence for a novel interaction of β-catenin with COPB.

Two other vesicle-related proteins of interest (COPS5 and Rab5a) were also detected to interact with β-catenin by mass spectrometry and assessed in IP experiments, but neither provided evidence for β-catenin interaction and so investigation of these proteins was not continued. Thus, the confirmation of interaction between COPB and β-catenin is rather specific.



<u>Figure 35</u> Detection of novel β-catenin–COPB protein interactions by immunoprecipitation.

SW480 total cell extract was subjected to pull-down or IP.

(A) In a pull-down, SW480 extract was mixed with beads coated with β-catenin-MBP, then the complexes washed, separated by SDS-PAGE and blotted for detection of COPB (110 kDa). As shown, a COPB specific antibody detected COPB in the input sample and modestly in the β-catenin-MBP sample lane (*), but not with MBP control.

(B) When SW480 cell extract was subjected to IP using α-β-catenin antibody, COPB (*) was clearly detected, whereas very little COPB was captured by the IgG control.

(C) COPS5 (37 kDa) and; (D) Rab5a (25 kDa) were not immunoprecipitated by α-β-catenin antibody.

ii. Validation by immunofluorescence microscopy

Immunofluorescent imaging in SW480 cells was performed as an orthogonal approach to confirm the interaction of β-catenin and COPB. First, an experiment was performed to establish that COPB is at the golgi using golgi-marker TGN46.

SW480 and HT29 cells were incubated fixed with methanol-acetone and blocked with 3 % BSA/PBS. The cells were incubated with α-COPB and α-TGN46 antibody followed by incubation with secondary antibody (includes Hoechst stain for nuclei). Slides were then mounted for microscopy using Vectashield and cells imaged on the DeltaVision (GE Lifesciences) at x100 magnification. Images were analysed by SoftWoRx Explorer 1.3 (Figure 36). For image analysis, a co-localisation was deemed positive if the protein staining patterns showed at least partial overlap. Utilisation of red (AlexFluor®594) and green (AlexFluor®488) fluorophores meant that co-localisation was identified by the production of yellow colouration in the merged images. Figure 36 shows spots of co-localisation of COPB (green) and TGN46 (red), depicted by yellow colour or partial overlap when images were compared. There are some spots of co-localisation although there does not seem to be a strong overlap between the two proteins. It is suggested that COPB can be at the golgi although it cannot be considered as a golgi-marker.

After confirming some co-localisation of COPB to the golgi cells were grown on slides, fixed with methanol-acetone and blocked with 3 % BSA/PBS before being incubated with α-β-catenin antibody and α-COPB antibody. The cells were then incubated with secondary antibody (includes Hoechst stain for nuclei) and the slides mounted for microscopy using Vectashield. The cells were imaged on the DeltaVision (GE Lifesciences) at x100 magnification and images analysed by SoftWoRx Explorer 1.3 (Figure 37). A zoom of an inset of cells is shown in Figure 38. Figure 39 shows a secondary antibody control.

Prior to fixation, a mild cytoplasmic extraction (CSK Triton X-100 detergent wash, 1 min) was performed in the SW480 cells, causing mild permeabilisation of the plasma membrane and progressive loss of soluble proteins from the cell, leading to a reduced background. Only immobile and anchored proteins are resilient to the extraction process. In this instance, the CSK extraction caused loss of some cytoplasmic β-catenin staining and allowed a clearer detection of potential co-localisation with COPB.

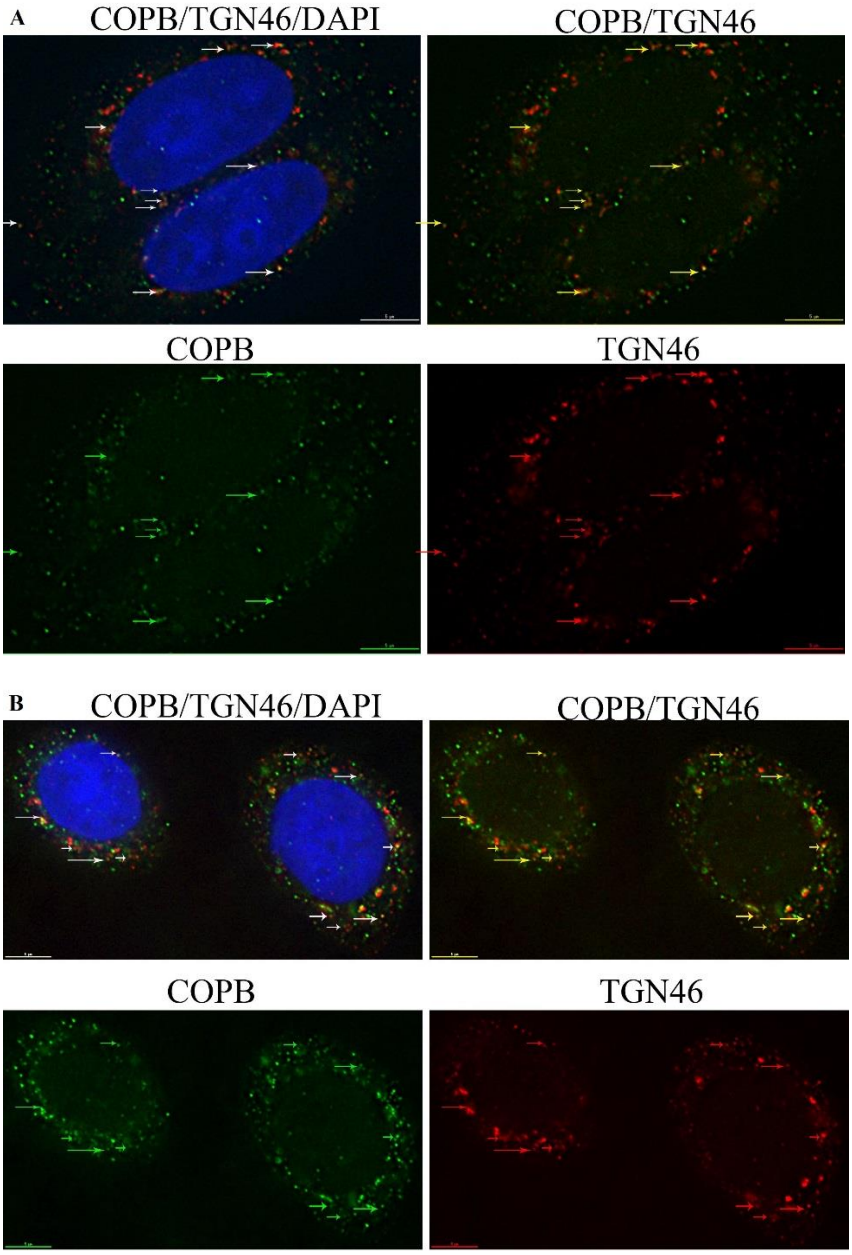


Figure 36 Co-localisation of COPB and golgi-marker (TGN46) in A) SW480 and B) HT29 cells (scalebar = 5 µm).

Cells were fixed with methanol-acetone, blocked with 3 % BSA/PBS and incubated with α-COPB and α-TGN46 antibody. The cells were then incubated with secondary antibody and slides mounted for microscopy on the DeltaVision (magnification x100). Although not strong, there is some co-localisation of COPB (green) with the TGN46 (red) indicating that COPB can sometimes be found at the golgi although it cannot be considered as a golgi-marker.

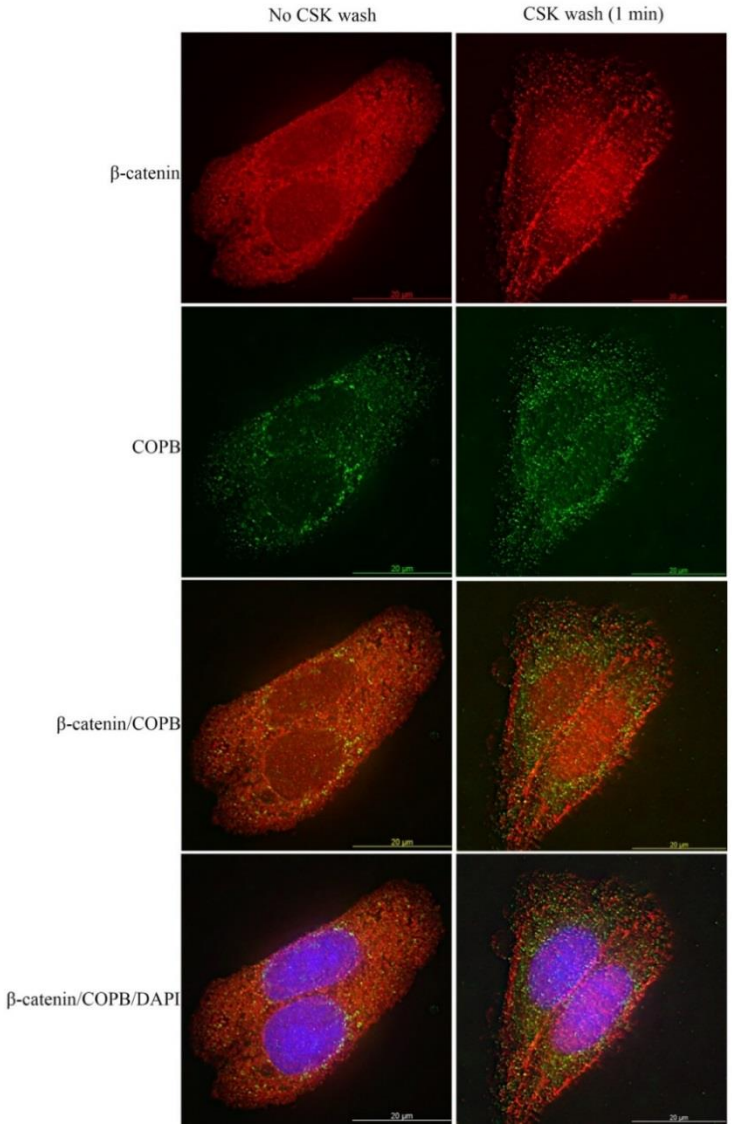


Figure 37 Co-localisation of β-catenin and COPB in SW480 cells before and after CSK detergent extraction of soluble proteins (scalebar = 20 µm).

SW480 cells underwent a mild CSK-wash followed by fixation with methanol-acetone. The cells were blocked with 3 % BSA/PBS and incubated with primary antibody (β-catenin and COPB). The cells were then incubated with secondary antibody and slides mounted for microscopy on the DeltaVision at magnification x100. The non-CSK washed SW480 cells (left) have a highly saturated signal of cytoplasmic β-catenin (red). This image demonstrates the need for a CSK wash to release non-anchored cytoplasmic β-catenin in order allow for detection of spots of co-localisation with COPB (green).

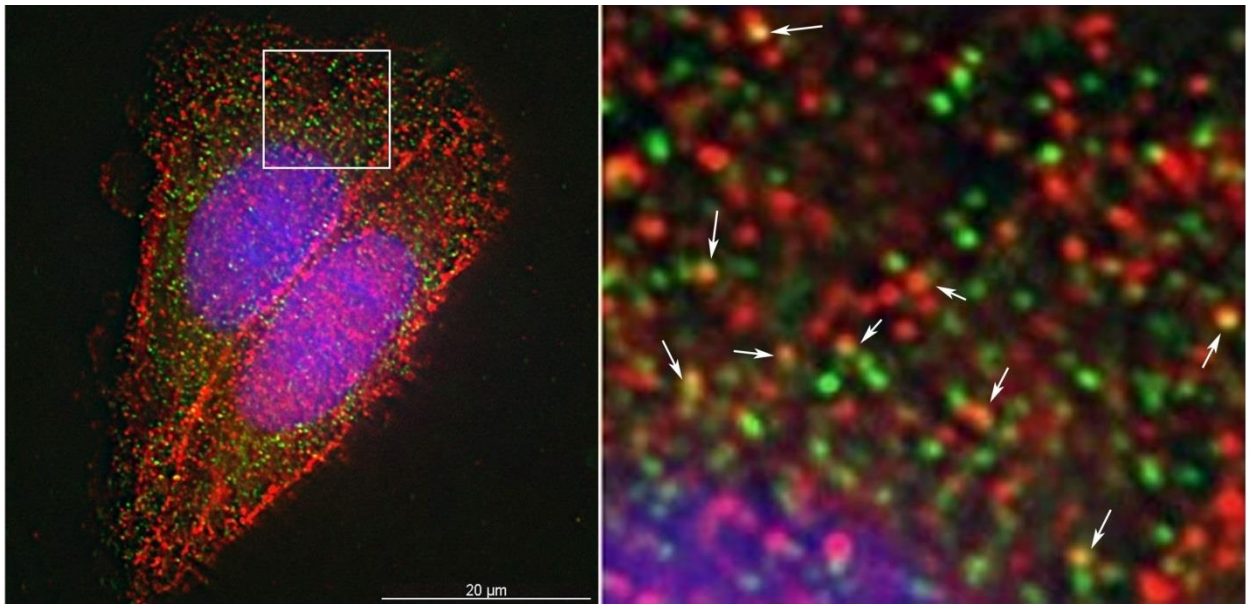


Figure 38 Co-localisation of β-catenin and COPB in SW480 (inset zoom of Figure 37). Image on left was taken at x100 magnification.

An inset zoom of a CSK washed SW480 IF experiment of β-catenin (red) and COPB (green). Arrows on the right point to sites of co-localisation.

Figure 39 A secondary antibody control demonstrates the specificity of the secondary antibodies utilised.

SW480 cells were fixed with methanol-acetone and blocked with 3 % BSA/PBS. Cells were not incubated with primary antibody but instead incubated with secondary antibody α-rabbit AlexaFluor®594 (red) and α-mouse AlexaFluor®488 (green) and slides mounted for microscopy on the DeltaVision at magnification x100. There is very low background, that is, there is very low non-specific binding of the secondary antibodies.

5.2.2 Mitochondrial heat shock protein 70 (mtHSP70)

i. Validation by immunoprecipitation

Mitochondrial heat shock protein 70 (mtHSP70), a known mitochondrial protein [401], was found to bind all constructs (WT, phosphomimetic and non- phosphorylatable β-catenin) across both cell lines (SW480 and HT29) by MS, meaning that this interaction is not dependant on phosphorylation at Y654. Since β-catenin has been not been previously detected at mitochondria, it was decided to further investigate this potential interaction. Thus, IPs were performed in SW480 cell extract using α-β-catenin antibody and subsequent western blot using α-mtHSP70 antibody.

A pull-down of mtHSP70 by β-catenin-MBP is shown in Figure 40A and an IP of mtHSP70 by α-β-catenin antibody is displayed in Figure 40B. Two bands (~ 75 kDa and ~ 100 kDa) were detected by the mtHSP70 antibody in Figure 40B. The band of interest is the lower band due to the mass of mtHSP70 (70 kDa) and the fact that the migration pattern of mtHSP70 has been detected at this molecular mass before using the same antibody (refer to Figure 40A).

The IP of another chaperone, HSP90 by α-β-catenin antibody is shown in Figure 40C, followed by a pull-down of HSP90 by β-catenin-MBP (Figure 40D). This is a very interesting result because researchers have documented that HSP90 and mtHSP70 are associated with tumorigenesis, possibly by regulation of cell cycle components including p53 [402, 403] (discussed in 5.3.2). Furthermore, mitochondrial HSP90 is also involved in transport of proteins into the mitochondria. mtHSP70 was further explored on its own in order to begin initial investigations for β-catenin at the mitochondria.

Another chaperone, nuclear-related hnRNPK was immunoprecipitated by α-β-catenin antibody (Figure 40E) however, this was not specific as it also bound to the IgG control. Furthermore, a reverse-IP by α-hnRNPK antibody immunoprecipitated β-catenin, however, this was not specific as it also bound to the IgG control (Figure 40F). Repeat experiments need to be performed in order to either confirm or deny an interaction of hnRNPK with β-catenin.

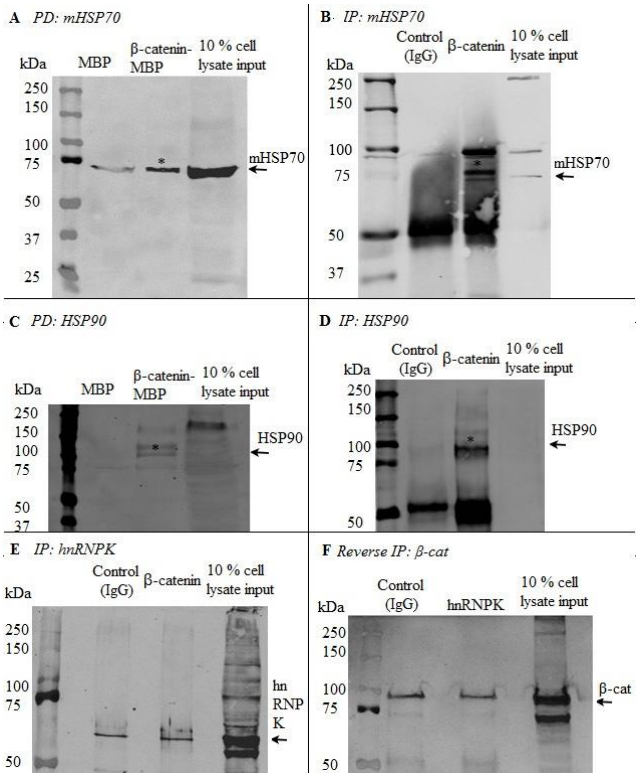


Figure 40 Evidence for interaction between β-catenin and mtHSP70.

(A) In a pull-down, SW480 extract was mixed with beads and coated with β-catenin-MBP, then complexes washed, separated by SDS-PAGE and blotted for detection of mtHSP70 (70 kDa). As shown, a mtHSP70 specific antibody detected mtHSP70 in the input sample and in the β-catenin-MBP lane (*) with low signal for the MBP control.

(B) When SW480 cell extract was subjected to IP using α-β-catenin antibody, mtHSP70 was clearly detected (*), whereas no mtHSP70 was captured by the IgG control.

(C) A pull-down performed in SW480 extract using β-catenin-MBP was blotted for detection of HSP90. As shown, a HSP90 specific antibody detected HSP90 modestly in the input lane and clearly in the β-catenin-MBP lane (*) with no signal for the MBP control.

(D) When SW480 extract was subjected to IP using α-β-catenin antibody, HSP90 was clearly detected (*) whereas no HSP90 was captured by the IgG control.

(E) When SW480 cell extract was subjected to IP using α-β-catenin antibody, hnRNPK was clearly detected in the β-catenin sample but was also captured by the IgG control.

(F) A reverse-IP was performed in SW480 extract using α-hnRNPK antibody and β-catenin was detected in the hnRNPK sample but also the IgG control.

ii. Validation by immunofluorescence

Immunofluorescence was performed in order to investigate and further study the potential interaction of β-catenin and mtHSP70 in whole cells. First, an experiment was performed to establish that mtHSP70 is at the mitochondria, and confirm it is a mitochondrial marker as the literature states [404].

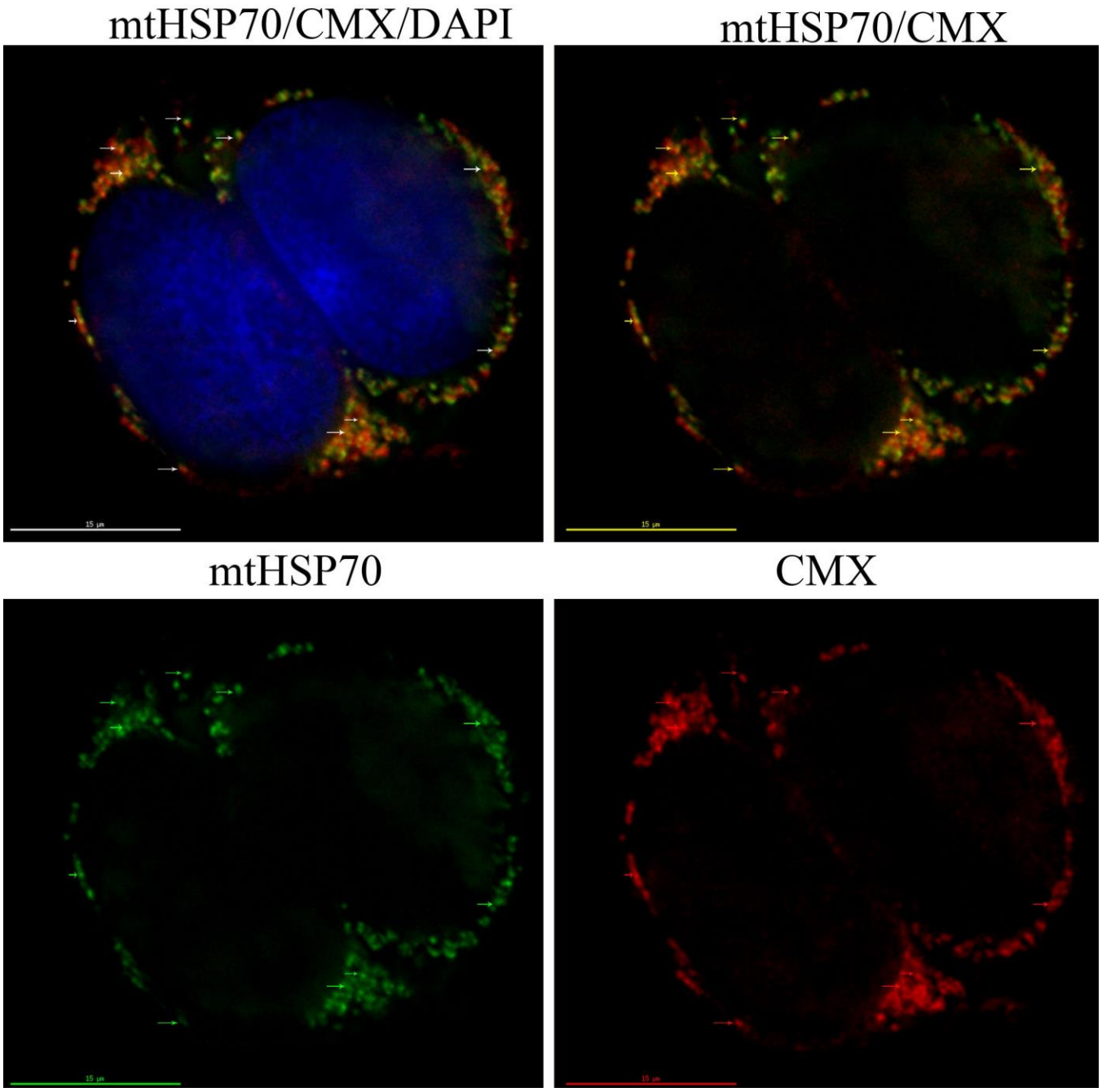
SW480 and HT29 cells were incubated with mitochondrial marker CMX-ros (MitoTracker®, LifeTech) to stain mitochondria and cells were then fixed with methanol-acetone and blocked with 3 % BSA/PBS. The cells were incubated with α-mtHSP70 antibody followed by incubation with secondary antibody (includes Hoechst stain for nuclei) and then they were mounted for microscopy using Vectashield. The cells were imaged on the DeltaVision (GE Lifesciences) at x100 magnification and images analysed by SoftWoRx Explorer 1.3 (Figure 41). The image displays obvious and almost total co-localisation of mtHSP70 and the mitochondria.

After establishing mtHSP70 as a mitochondrial marker, another IF experiment was performed. SW480 and HEK293T (non-cancer) cells underwent a mild CSK-wash (1 min), were fixed with methanol-acetone and blocked with 3 % BSA/PBS before being incubated with α-β-catenin antibody and α-mtHSP70 antibody. The cells were then incubated with secondary antibody and mounted for microscopy. The cells were imaged on the DeltaVision (GE Lifesciences) at x100 magnification and images analysed by SoftWoRx Explorer 1.3 (Figure 43). A zoom of an inset of SW480 cells is shown in Figure 44.

For image analysis, a co-localisation was deemed positive if the protein staining patterns showed at least partial overlap. Utilisation of red (AlexFluor®594) and green (AlexFluor®488) fluorophores meant that co-localisation was identified by the production of yellow colouration in the merged images. Figure 44 shows spots of co-localisation of β-catenin (red) with mtHSP70 (green), depicted by yellow colour or partial overlap patterning when images were compared.

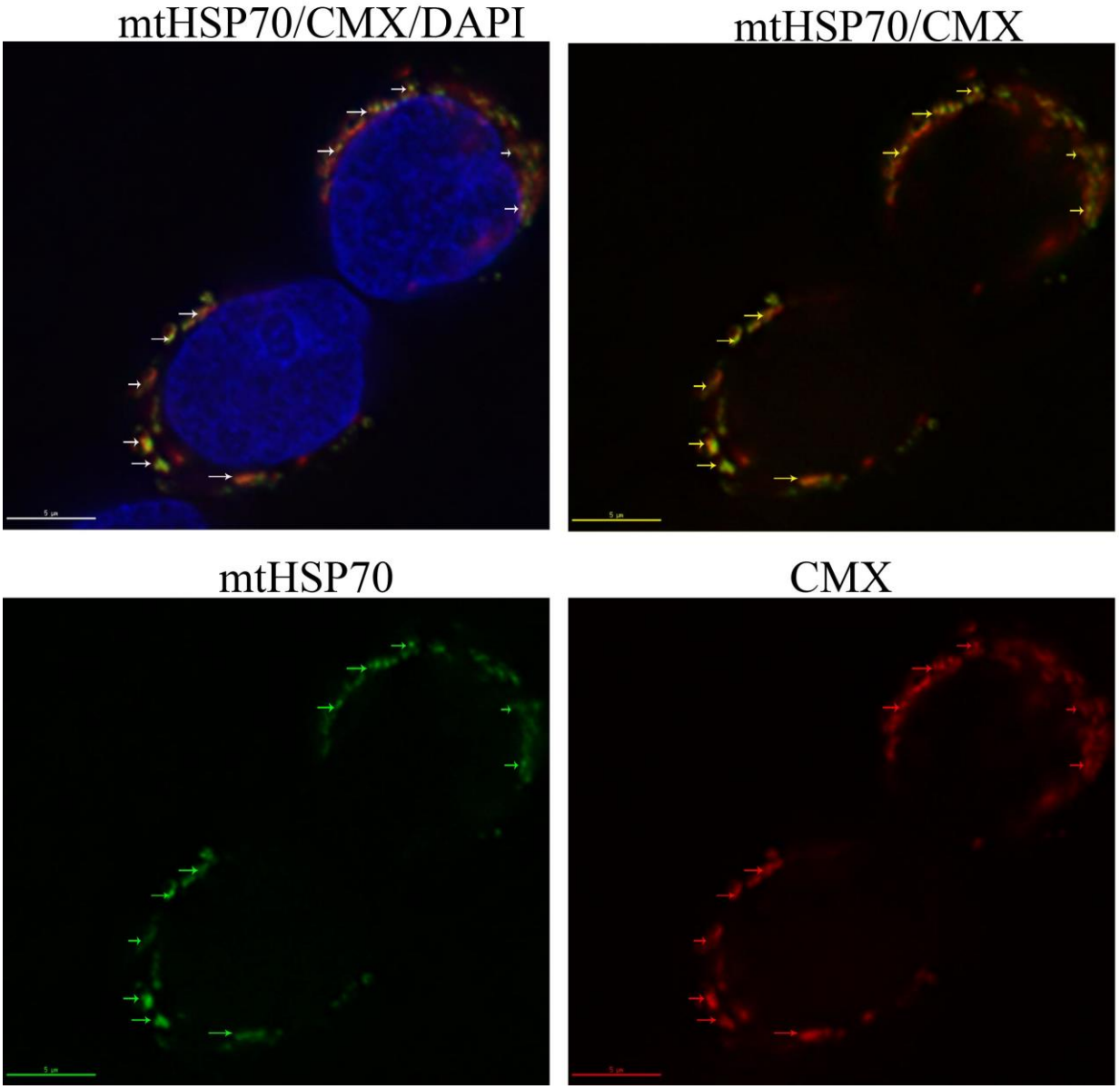
While it is often difficult to detect β-catenin at the mitochondria, under conditions where β-catenin background was decreased by CSK-extraction there was visible co-localisation with mtHSP70, which at least partly supports the MS, IP and pull-down data for complex formation and co-localisation.

If further IF experiments do not demonstrate β-catenin and mtHSP70 to interact (contradicting MS, IP and pull-down data), a reasoning behind this may be that the proteomics data identifying mtHSP70 and other mitochondrial proteins as potential β-catenin partners, was analysed from a pull-down performed in RIPA lysed cells. This type of cell lysis generates total cell lysate comprising a soup of proteins which allows for the opportunity of non-specific interactions to occur [344]. In fact, other researchers have used RIPA buffer (Triton X-100) to rupture the cell membrane and mitochondrial membranes specifically [345-348]. Therefore, although this binding in whole cell lysate is detectable, it may not be possible *in vivo* where partitioning of cell membranes would block such interactions.



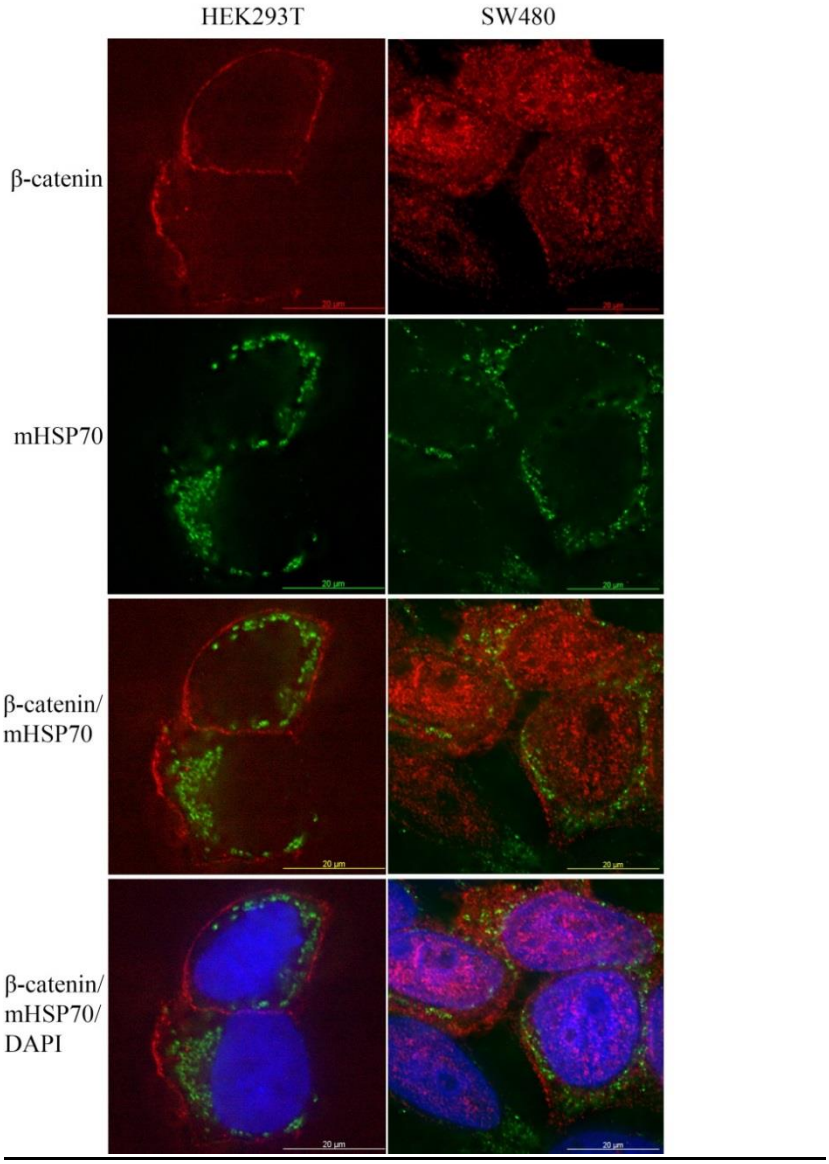
<u>Figure 41</u> Co-localisation of mtHSP70 and mitochondria in SW480 (scalebar = 15 µm)

SW480 cells were stained with CMX-ros, a mitochondrial dye. Cells were then fixed with methanol-acetone, blocked with 3 % BSA/PBS and incubated with α-mtHSP70 antibody. The cells were then incubated with secondary antibody (includes Hoechst stain for nuclei) and slides mounted for microscopy on the DeltaVision at magnification x100. The SW480 (left) and HT29 (right) cells have mtHSP70 (green) localising to mitochondria (red) almost completely. Arrows point out some points of co-localisation.



<u>Figure 42</u> Co-localisation of mtHSP70 and mitochondria in HT29 cells (scalebar = 5 µm).

HT29 cells were stained with CMX-ros, a mitochondrial dye. Cells were then fixed with methanol-acetone, blocked with 3 % BSA/PBS and incubated with α-mtHSP70 antibody. The cells were then incubated with secondary antibody (includes Hoechst stain for nuclei) and slides mounted for microscopy on the DeltaVision at magnification x100. The SW480 (left) and HT29 (right) cells have mtHSP70 (green) localising to mitochondria (red) almost completely. Arrows point out some points of co-localisation.



<u>Figure 43</u> Co-localisation of β-catenin and mtHSP70 in HEK293T (non-cancer control) and SW480 cells (scalebar = 20 µm).

SW480 cells underwent a mild CSK-wash followed by being fixed by methanol-acetone. The cells were then blocked with 3 % BSA/PBS and incubated with primary antibody (β-catenin or mtHSP70). The cells were then incubated with secondary antibody (includes Hoechst stain for nuclei) and slides mounted for microscopy on the DeltaVision at magnification x100. The HEK293T cells (left) have β-catenin (red) localising to the cell membrane while the SW480 cells (right) have β-catenin (red) localising to the cytoplasm and nucleus. The localisation for β-catenin in the SW480 cells allows for a higher chance of interaction with mtHSP70 (green).

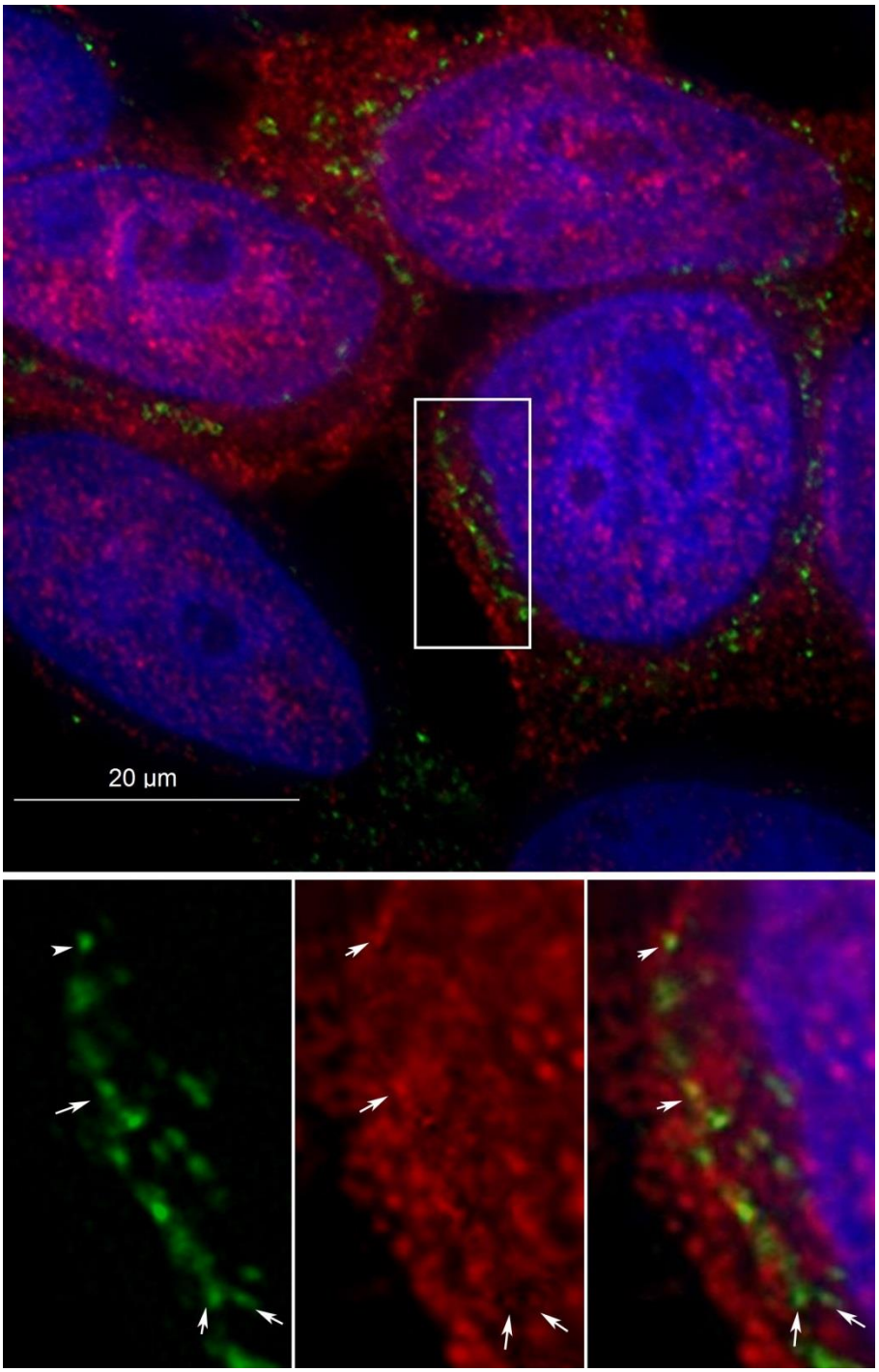


Figure 44 Co-localisation of β-catenin and mtHSP70 in SW480 (inset zoom).

An inset zoom of a CSK washed SW480 IF experiment of β-catenin (red) and mtHSP70 (green). Arrows on the bottom panel point to sites of co-localisation. It should be noted that co-localising spots are not always yellow, but rather the pattern of overlap should be considered.

5.2.3 Other novel protein interactor candidates

Our study pulled-down hundreds of potential novel protein interactors of β-catenin. Only a small subset of these could be further tested and this subset was chosen carefully and justified (Table 33). The following proteins were subjected to further testing but were met with unclear results.

i. 182 kDa tankyrase-1-binding protein (Tab182)

Tab182, a nuclear protein potentially possessing two nuclear localisation sequences [380], was identified by MS to bind WT β-catenin in both colon cancer cell lines (SW480 and HT29) and the phosphomimetic in HT29. It was further investigated as a potential protein interactor of β-catenin.

Upon pull-down using β-catenin-MBP and western blot with Tab182 antibody, no specific signal at the expected molecular weight for Tab182 (182 kDa) was observed in SW480 cell lysates and so an antibody characterisation was performed (Figure 45A). Results showed that for SW480, Tab182 was detected at ~ 50 kDa and ~ 85 kDa.

A pull-down performed in SW480 using β-catenin-MBP showed that for Tab182 a band (~ 85 kDa) was present in the β-catenin-MBP lane but not the control (Figure 45B). An IP was performed in SW480 cells and a signal was detected at ~ 85 kDa for β-catenin with much lower signal present for the control (Figure 45C). However, low signal in the input lanes makes these results unclear because detection in the input lanes was inconsistent.

The absence of signal at the expected molecular mass (182 kDa) makes it impossible to determine if an interaction occurs using these approaches. However, we recently discovered that a truncated version exists at 83 kDa (source: Uniprot). Tab182 western blots presented in this thesis present a band at this expected weight, potentially meaning that β-catenin is interacting with this mutated, truncated form.

IF experiments were also performed in CSK-washed SW480 cells using β-catenin and Tab182 antibody (Figure 46). Results suggest that binding of β-catenin and Tab182 occurs in the nucleus – evidence pointing towards an interaction of β-catenin being implicated in nuclear shuttling as they are seen in the nucleus together.

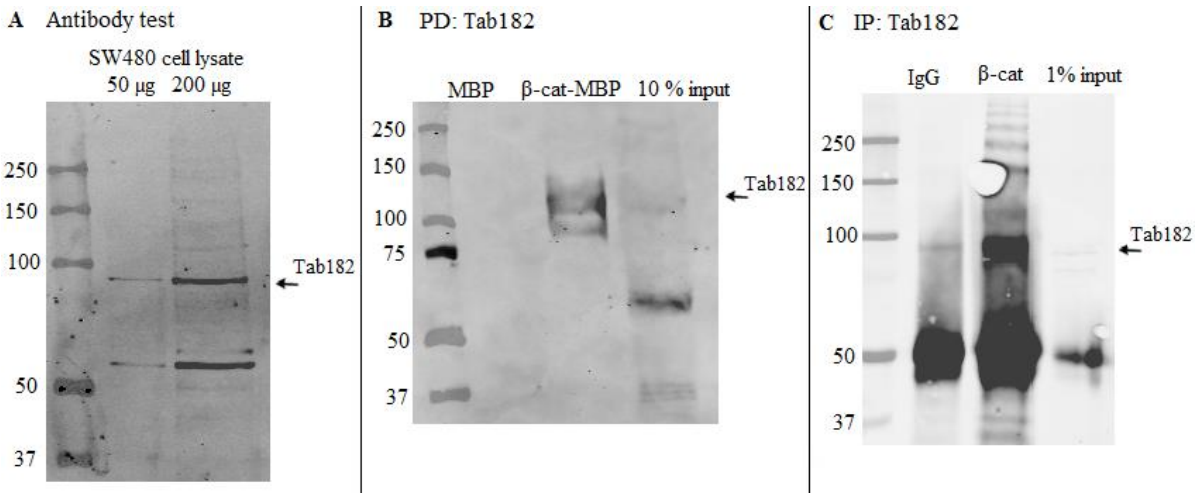


Figure 45 Evidence for interaction of β-catenin and Tab182 in SW480.

A) Antibody characterisation for Tab182 showing that in SW480, two bands are clear, at ~ 50 kDa and ~ 85 kDa.

B) A pull-down was performed in SW480 using β-catenin-MBP demonstrating that Tab182 (~ 85 kDa) bound to the β-catenin-MBP but not the MBP control.

C) IP performed in SW480 using β-catenin antibody showed that here is a clear band for Tab182 (~ 85 kDa) and lower signal detected for the IgG control. However, lack of a band in the input makes the result unclear.

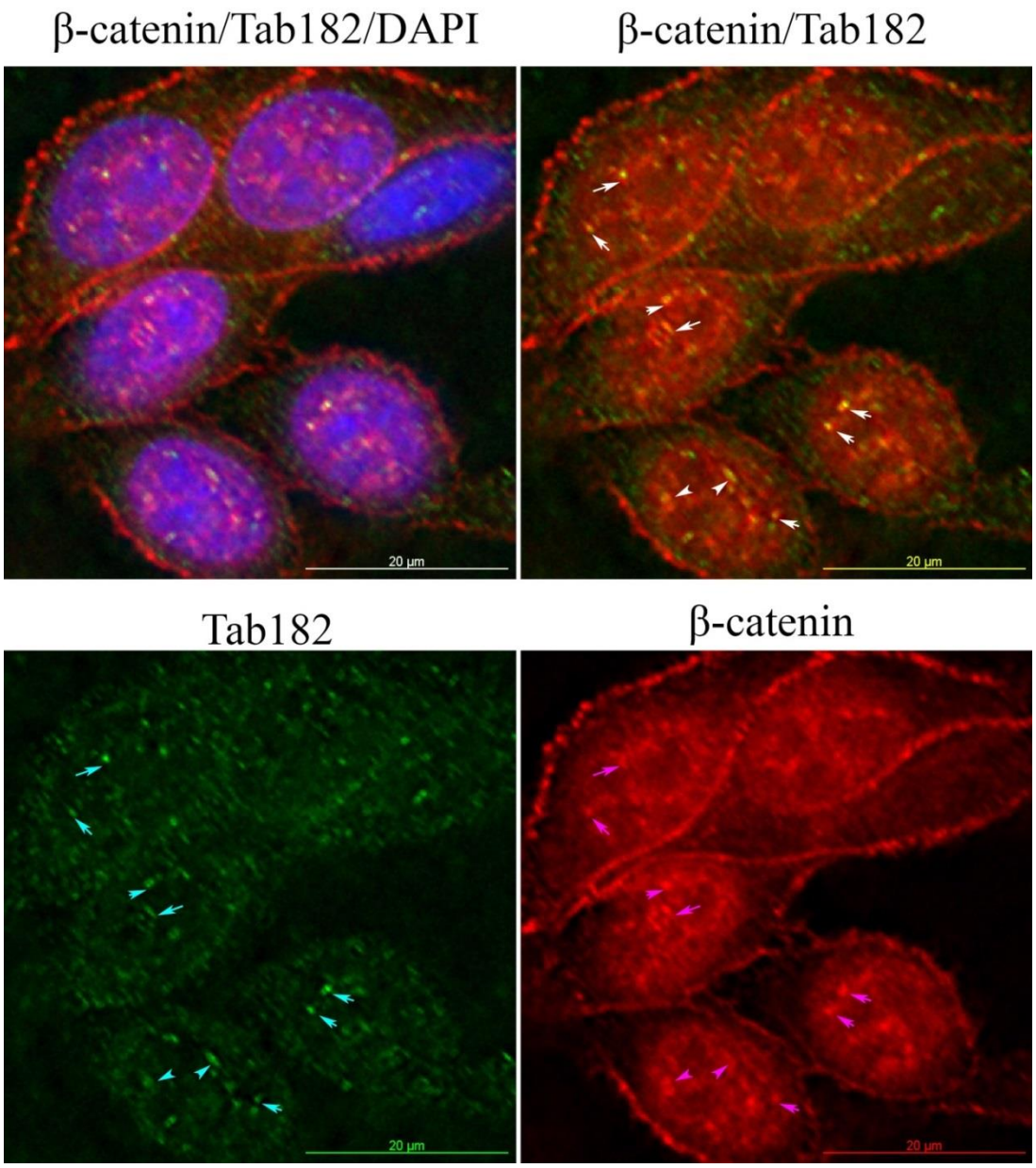


Figure 46 Co-localisation of β-catenin and Tab182 in SW480 (scalebar = 20 µm).

SW480 cells underwent a mild CSK-wash followed by being fixed by methanol-acetone and blocked with 3 % BSA/PBS and incubated with primary antibody (β-catenin and Tab182). The cells were then incubated with secondary antibody and slides mounted for microscopy on the DeltaVision at magnification x60. Results suggest that β-catenin (red) and Tab182 (green) interact in the nucleus. Arrows point to potential sites of interaction depicted by either yellow colour or overlap patterning.

ii. Kinesin light chain 4 (KCL4)

KCL4 was identified by an MS screen to bind to WT and non-phosphorylatable-Y654-β-catenin in SW480 while binding to phosphomimetic-Y654E-β-catenin in HT29. This interaction was investigated further and a reverse-IP was performed using α-KCL4-antibody to IP β-catenin (90 kDa) in SW480 cells which was positive (Figure 47A).

Furthermore, a pull-down performed using β-catenin-MBP in SW480 cells had a very slight signal for KCL4 (Figure 47B) while an IP using β-catenin antibody and probing for KCL4 (69 kDa) appeared negative (Figure 47C).

The most convincing evidence for an interaction of β-catenin and KCL4 comes from an IF experiment where antibodies against β-catenin and KCL4 were utilised in SW480 cells (Figure 48).

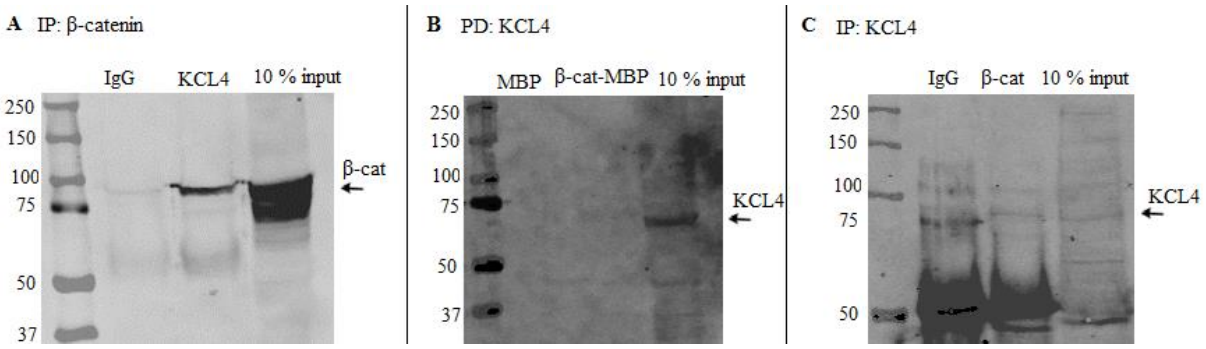
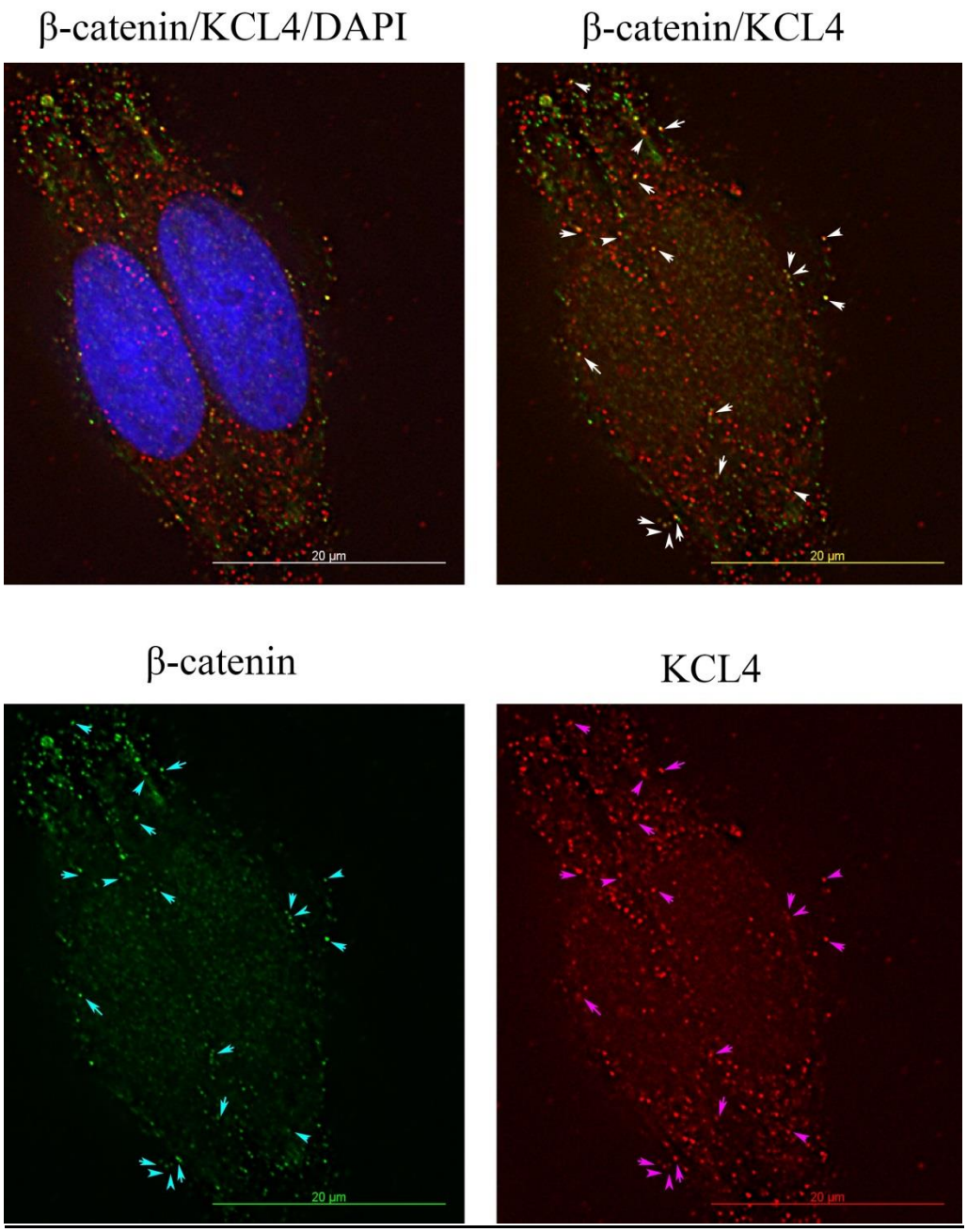


Figure 47 Evidence for interaction of β-catenin and KCL4 in SW480.

A) A reverse-IP performed in SW480 using KCL4 antibody to IP β-catenin was positive where signal for β-catenin was detected for the KCL4 lane but not the control. β-catenin was also detected in the input.

B) After a pull-down performed in SW480 using β-catenin-MBP, a very faint band can be seen for KCL4.

C) An IP performed in SW480 using β-catenin antibody has KCL4 signal in both the IgG and β-catenin experiments.



<u>Figure 48</u> Co-localisation of β-catenin and KCL4 in SW480 (scalebar = 20 µm).

SW480 cells underwent a mild CSK-wash followed by being fixed by methanol-acetone and blocked with 3 % BSA/PBS and incubated with primary antibody (β-catenin and KCL4). The cells were then incubated with secondary antibody and slides mounted for microscopy on the DeltaVision at magnification x100. Results suggest that β-catenin (green) and Tab182 (red) as indicated by arrows pointing to potential sites of interaction depicted by either yellow colour or overlap patterning.

iii. Heterogeneous nuclear ribonucleoprotein K (hnRNPK)

The weight of hnRNPK (60 kDa) made it a difficult protein to observe after western blot of an IP in SW480 as the β-catenin antibody heavy chain at 50 kDa tended to overshadow the 60 kDa molecular weight band of hnRNPK (Figure 49A). After multiple attempts at gel separation, it was possible to observe the 60 kDa weight band after IP with β-catenin in SW480 (Figure 49B). There is signal for both the β-catenin lane and the IgG control lane. However, this was performed once and needs to be further confirmed.

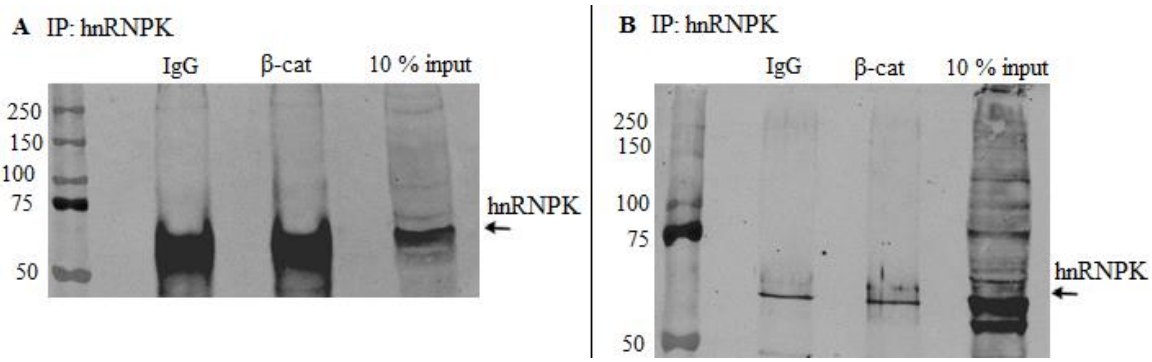


Figure 49 Evidence of an interaction between β-catenin and hnRNPK.

A) IPs were performed using β-catenin antibody in SW480 cell lysate and upon western blot with hnRNPK antibody, a band for hnRNPK (60 kDa) could not be seen due to an overshadowing 50 kDa heavy chain.

B) Gel separation allowed hnRNPK to be observed at 60 kDa after an IP performed using β-catenin antibody in SW480 however background (IgG) was high. It should be noted that the heavy chain ran just below the 50 kDa weight marker and ran off the gel as it was being run.

iv. Dynamin-like 120 kDa protein, mitochondrial (Opa1)

Opa1 had conflicting results where multiple pull-downs (followed by MS and western blot) demonstrated a binding of β-catenin to Opa1, however, IPs showed that Opa1 was also binding to the control (Figure 50). Furthermore, MS data found that Opa1 bound the non-phosphorylatable construct (Table 33) while pull-downs followed by western blot found that it bound all constructs albeit with different strengths (Figure 50A). The signal for Opa1 binding the various β-catenin-MBP constructs was 2-6 times stronger than the MBP control when signal intensities were compared. Figure 50B shows that Opa1 binds β-catenin antibody but also the control in the IP. However, it should be noted that based on the MS data (strongest binding of Opa1 to non-phosphorylatable β-catenin) in order to successfully IP Opa1 the phosphorylation status of β-catenin may need to be controlled. Use of WT β-catenin antibody during the IP meant that phosphorylation was not controlled as kinases would still be active within the lysate. Here, validations could not confirm initial MS data.

IF experiments were also conducted to investigate the interaction of β-catenin and Opa1. First, it was tested that Opa1 does mainly localise to the mitochondria because the intention of validating Opa1 was not just to focus on the interaction of it with β-catenin but to also find some evidence for β-catenin being at the mitochondria (to show that β-catenin interacts with Opa1 at the mitochondria). This was achieved by IF experiments in SW480 using Opa1 antibody and CMX-Ros dye which stains mitochondria [405-407] (Figure 51).

After confirming that Opa1 did mainly localise to the mitochondria, an IF experiment was performed in CSK-washed SW480 cells, HT29 cells and HEK293T cells using β-catenin and Opa1 antibody (Figure 52). Results showed that Opa1 may have the opportunity to interact with β-catenin in SW480 as β-catenin is not sequestered at the cell membrane. However, β-catenin localisation at the cell membrane in HT29 and HEK293T makes it less likely that β-catenin interacts with Opa1 in these cells lines.

Figure 53 demonstrates that there is some co-localisation of Opa1 and β-catenin in SW480 based on their staining patterns.

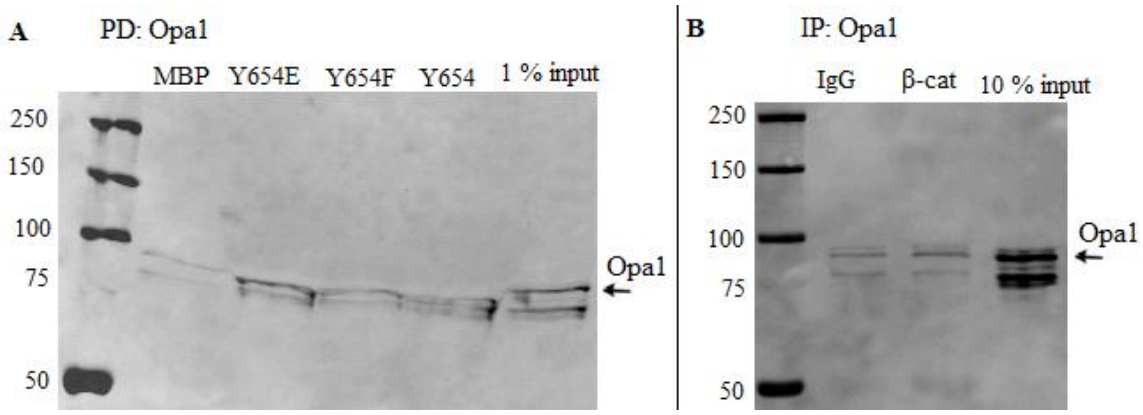
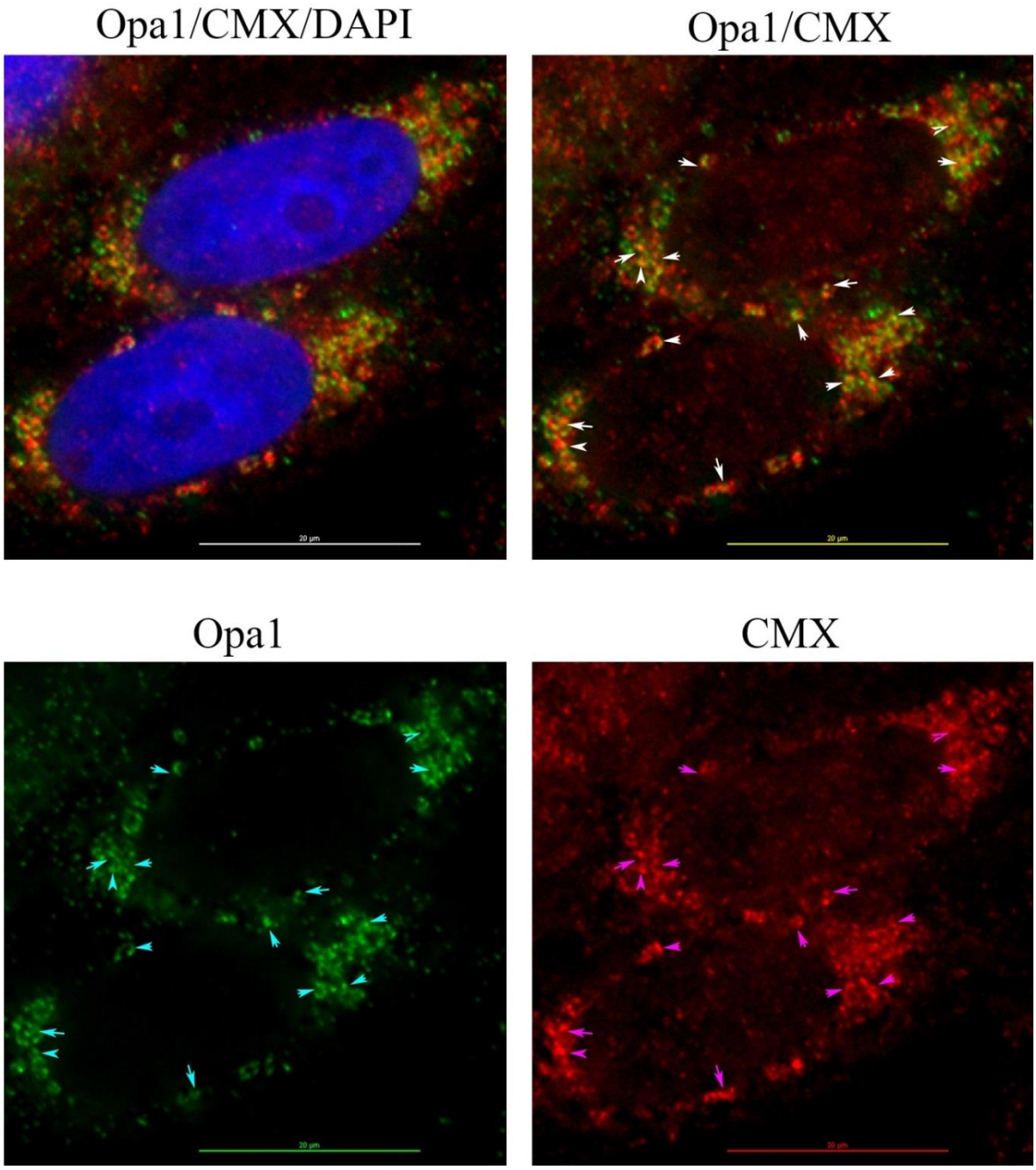


Figure 50 Evidence for the interaction of β-catenin and Opa1

A) Upon a pull-down in SW480 cell lysate using β-catenin-MBP constructs (phosphomimetic, non-phosphorylatable and WT), it was found that all constructs bound Opa1 (80/100 kDa) but not MBP control. The constructs bound Opa1 with different strengths, as indicated by their signal intensities (I.I). (MBP = 1.29 I.I, Y654E = 5.02 I.I, Y654F = 2.24 I.I, Y654 = 5.11 I.I, input = 10.21 I.I).

B) IP of Opa1 by β-catenin antibody in SW480 was successful, however the background (IgG control) signal is high . (IgG = 1.96 I.I, β-catenin = 2.99 I.I, input = 25.54 I.I).



<u>Figure 51</u> Co-localisation of Opa1 and the mitochondria (scalebar = 20 µm).

SW480 cells were stained with Hoechst (nuclear stain), Opa1 and CMX-Ros (mitochondrial dye). Cells were imaged using the DeltaVision at magnification x100. As indicated by arrows and comparison of Opa1 and CMX-Ros staining patterns, Opa1 does co-localise to the mitochondria.

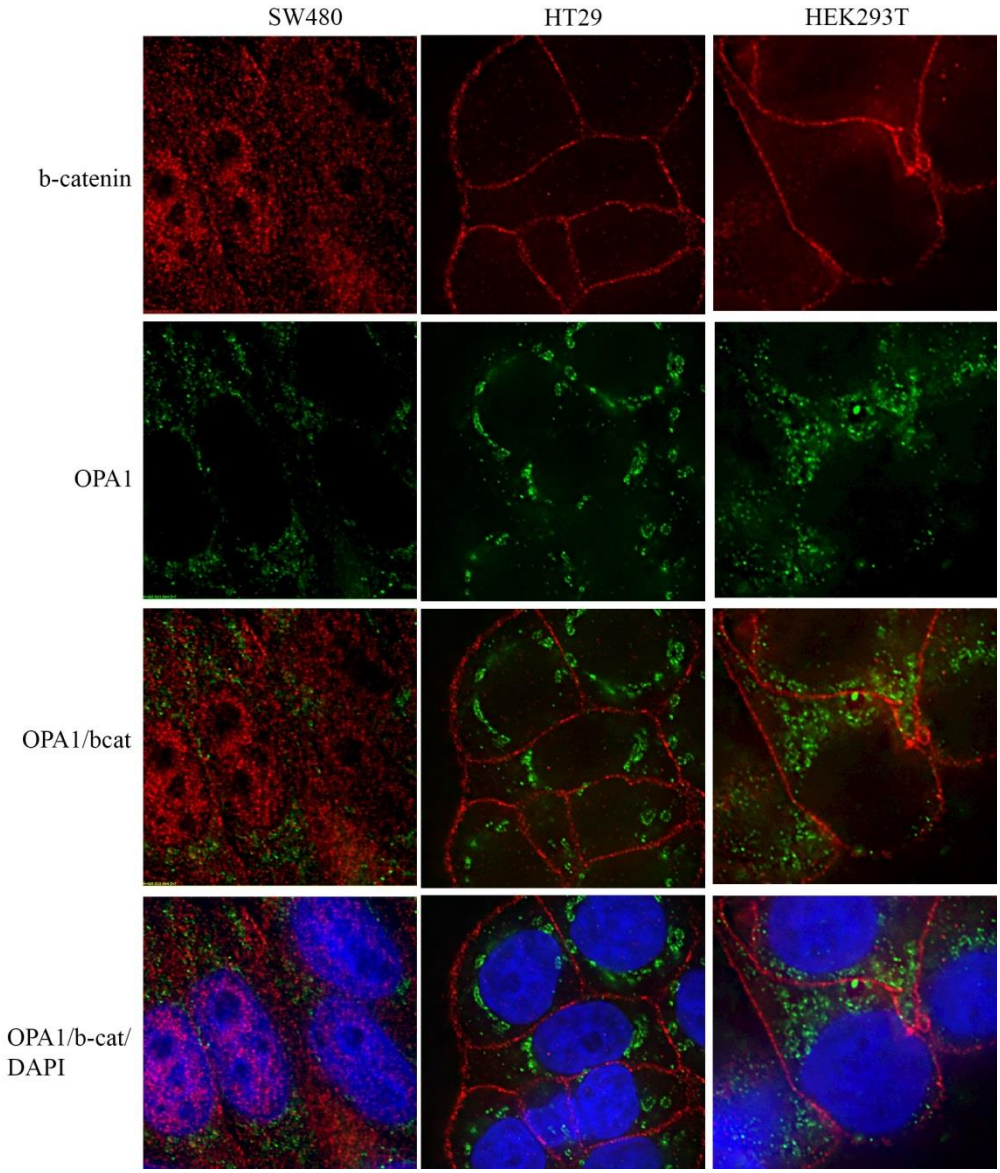
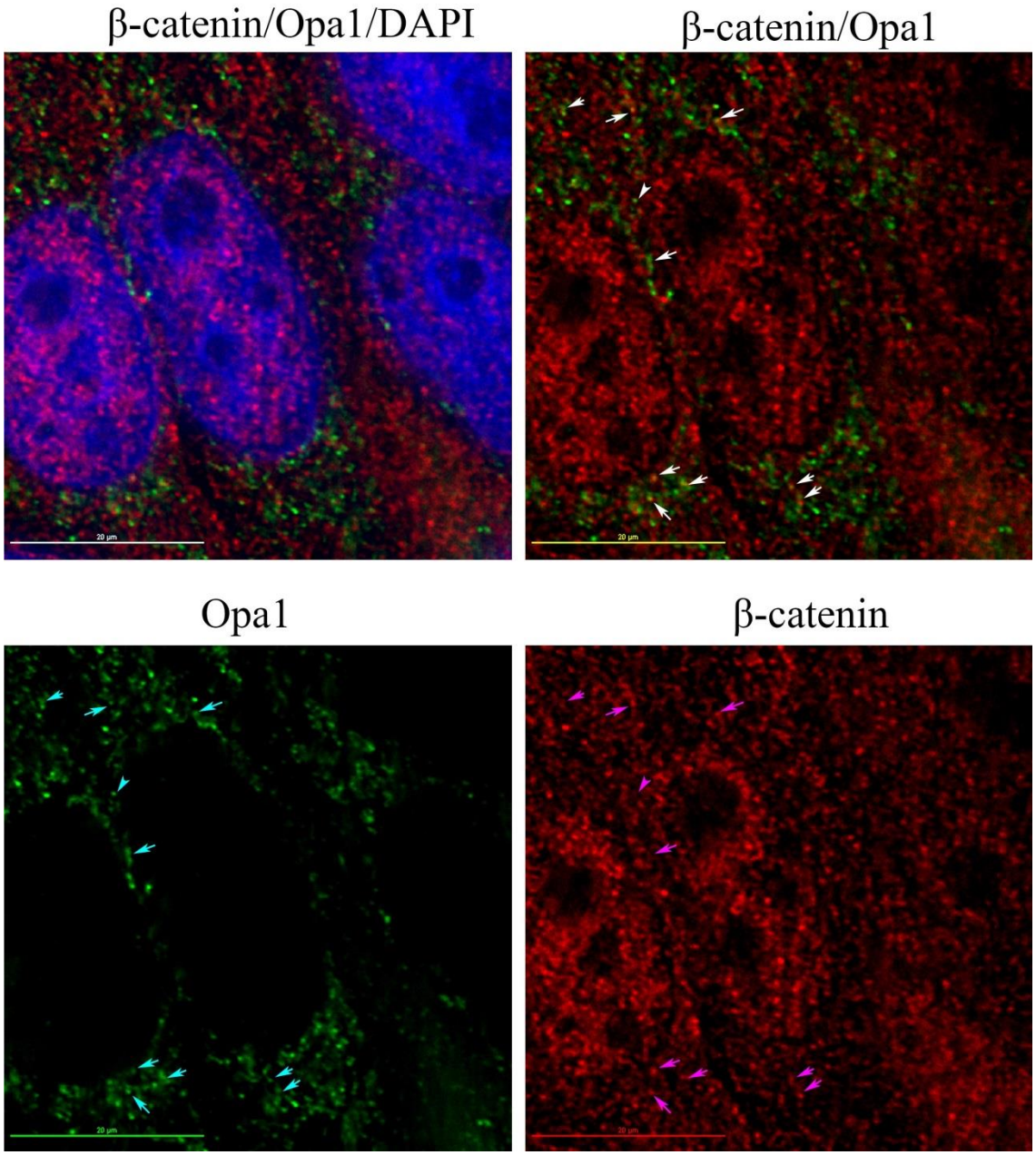


Figure 52 Co-localisation of β-catenin and Opa1 in SW480, HT29 and HEK293T (non-cancer control) (scalebar = 20 µm).

SW480 (CSK-washed), HT29 and HEK293T cells were fixed by methanol-acetone and blocked with 3 % BSA/PBS followed by incubation with primary antibody (β-catenin and Opa1). The cells were then incubated with secondary antibody (includes Hoechst stain for nuclei) and slides mounted for microscopy on the DeltaVision at magnification x100. The HT29 and HEK293T cells have β-catenin (red) localising to the cell membrane while the SW480 cells have β-catenin (red) localising to the cytoplasm and nucleus. The localisation for β-catenin in the SW480 cells allows for a higher chance of interaction with Opa1 (green) at the mitochondria.



<u>Figure 53</u> Co-localisation of β-catenin and Opa1 in SW480 CSK-washed cells.

SW480 cells underwent a mild CSK-wash followed by being fixed by methanol-acetone and blocked with 3 % BSA/PBS and incubated with primary antibody (β-catenin and Opa1). The cells were then incubated with secondary antibody and slides mounted for microscopy on the DeltaVision at magnification x100. Results suggest that β-catenin (red) and Opa1 (green) may interact or co-localise at the mitochondria (as indicated by arrows).

5.3 Discussion

5.3.1 β-catenin at the golgi

As stated earlier, currently there is not much scientific evidence on β-catenin localising to the golgi. Research conducted in this thesis resulted in the pull-down a number of golgi-related proteins (8 % of the candidate protein interactor) including a number of coatomer subunits including as COPB'. Another subunit, COPB was found to interact with β-catenin in MCF-7 breast cancer cells by our collaborators (discussed in the following section) and so with the availability of a specific COPB' antibody, COPB' was chosen to use in the investigations of β-catenin at the golgi in this thesis.

As shown in 5.2.1, a pull-down by β-catenin-MBP and subsequent western blot using COPB antibody was positive (Figure 35A) (correlates with MS data) while an IP was also positive where COPB was found to bind β-catenin by IP in SW480 whole cell lysate (Figure 35B). In SW480 CSK-washed whole cells, IF was performed using β-catenin and COPB antibody and results indicated an interaction of the two proteins (Figure 38).

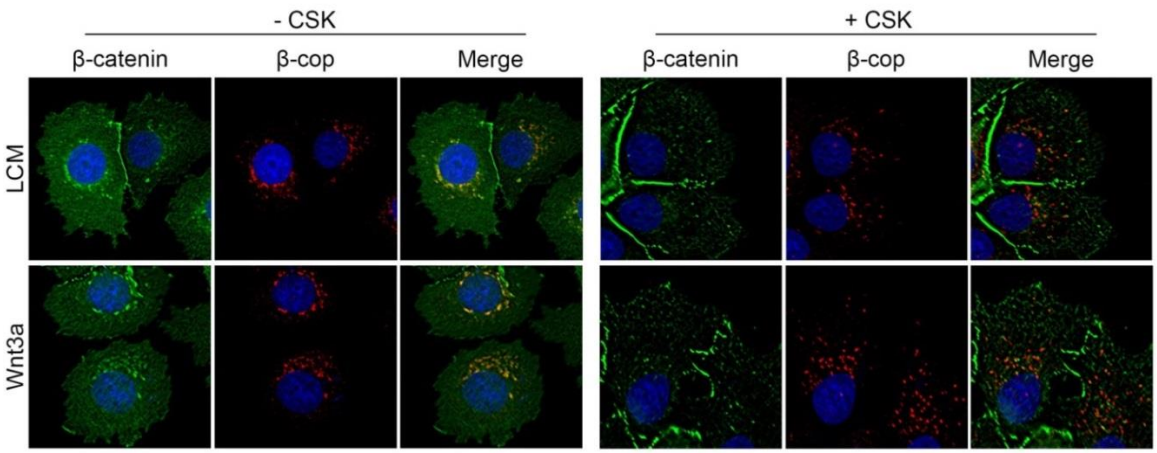
i. Evidence of β-catenin and COPB interaction in MCF-7

Our collaborators at Westmead Millennium Institute (Henderson lab) have found evidence for the binding of COPB to β-catenin in breast cancer cell line MCF-7 (Figure 54) [364].

Generally, it is mutations in components of the β-catenin destruction complex that lead to its stabilisation and nuclear accumulation and ~ 90 % of cases in colorectal cancer harbour these mutations (discussed in 1.3.1) [72, 107, 119, 142]. However, in breast cancer these mutations are rare [408] and so our collaborators investigated the subcellular location of β-catenin in breast cancer.

Excessive Wnt activation occurs in breast cancer due to overexpression of Wnt ligands and receptors [409-412] and upon Wnt treatment of MCF-7 breast cancer cells, cytoplasmic and membrane-bound β-catenin atypically did not relocate to the nucleus due to unusually low expression of LEF/TCF [364]. Upon reconstitution of LEF-1 or TCF4 expression, β-catenin was able to localise to the nucleus after Wnt treatment. In the cytoplasm, they found that β-catenin accumulated in recycling endosomes, golgi and at COPB positive coatomer complexes. The peripheral association with endosomes declined after Wnt treatment, releasing β-catenin into the cytoplasm for nuclear entry.

It was proposed that β-catenin may have a role in cytoplasmic functions such as golgi-ER transport in addition to its normal roles in the nucleus [364].



<u>Figure 54</u> β-catenin co-localisation with COPB at the golgi in breast cancer MCF-7. Figure from Jamieson *et al.* (2016) [364].

Co-localisation of β-catenin with COPB at transport vesicles (often overlap the golgi) with Wnt3a (stimulates the Wnt pathway) or control L-cell conditioned media (LCM). In the left panel (- CSK) and (+/- exposure to Wnt3a) revealed co-localisation in the perinuclear region. In the right panel (+CSK) the relative intensity of β-catenin diminished in the cytoplasm and in particular at COPB vesicles.

ii. β-catenin at the golgi in the current literature

Brunner *et al.* (2006) analysed the expression and subcellular location of β-catenin/E-cadherin in human meningioma by reason that a hallmark of meningioma tumorigenesis is loss of a tumour suppressor that controls cell-cell adhesion (neurofibromatosis type 2 gene, NF2) [290]. Loss of β-catenin at the cell membrane was found in meningioma, and it was then mostly localised to the golgi [290]. There was no correlation between loss of NF2 and loss of β-catenin at the cell membrane, showing that altered localisation is independent of NF2. Thus, interactors of β-catenin at the golgi (such as COPB) are brought into the spotlight when considering tumorigenesis. Current studies such as this can localise β-catenin to the golgi, but have not investigated binding partners, making this research and results unique and novel to the current literature.

Shtutman *et al.* (2011) identified the COPZ gene as essential for various tumours but not normal cells [413]. The study found isoform COPZI in particular as a potential cancer-specific target because its depletion induced cell death in dividing and non-dividing tumour cells, but not normal cells. Furthermore, normal cells expressed two isoforms of COPZ (COPZ1 and COPZ2) while tumour cells expressed only COPZ1 due to silencing of the COPZ2 gene. This is evidence for the importance of COPI subunits in cancer.

Some research indicates that the epidermal growth factor receptor (EGFR) family localises in the nucleus [414]. A retrograde route from golgi-ER is thought to be involved in the EGFR trafficking to the nucleus, yet the mechanism remains unknown [414]. Wang *et al.* (2010) found that membrane-embedded vesicular trafficking was involved in the nuclear transport of EGFR and that blocking retrograde trafficking (by disassembly of the COPI coat to the golgi) inhibited EGFR transport to the nucleus. It was concluded that nuclear transport of EGFR is regulated by COPI-mediated vesicular trafficking. Because β-catenin shuttles in and out of the nucleus freely, it may be implicated in the localisation of EGFR to the nucleus by its interaction with COPI.

Though there is some literature reporting localisation of β-catenin at the golgi, it is yet to be determined which golgi proteins associate with β-catenin at the golgi, and/or mediate its localisation to that organelle. In lieu of this, the proteomics screen in this thesis resulted in the pull-down of 4/7 subunits of COPI. This interaction of β-catenin and COPI may function in the formation of the COPI vesicular coat or transport of the vesicles between the ER and golgi [415], implicating the golgi as a site of vesicle transport for β-catenin.

5.3.2 β-catenin at the mitochondria

As previously stated, the localisation of β-catenin at the mitochondria has been difficult for studies to confirm by cell staining and imaging. This means that any mitochondrial proteins pulled-down by our mass spectrometric screen do not only provide a novel candidate protein interactor, but also a novel localisation for β-catenin at the mitochondria. 10 % of the novel candidate protein interactor list is comprised of proteins with reported roles at mitochondria such as mtHSP70. mtHSP70, a well-known mitochondrial marker [404], was found to bind both the phosphomimetic and non-phosphorylatable constructs of β-catenin-MBP in both cell lines.

As shown in 5.2.2, pull-down using β-catenin-MBP in SW480 followed by western blot using mtHSP70 antibody was positive (Figure 40A), along with an IP performed using β-catenin antibody which immunoprecipitated mtHSP70 (Figure 40B).

IF experiments were also performed in SW480 cells and non-cancer control cell line HEK293T. For the HEK293T cells, it was clear that mtHSP70, which is normally mitochondria-specific in its localisation pattern, did not bind β-catenin because the β-catenin was mainly localised to the cell membrane (Figure 43). For SW480, there were spots of co-localisation, suggesting an interaction of β-catenin and mtHSP70 or at least a co-localisation of β-catenin and the mitochondria (Figure 44).

i. β-catenin and the mitochondria in the current literature

Little is known about the roles of β-catenin in mitochondrial activity and cellular respiration [288]. One study was able to show that β-catenin-deficient hepatocytes demonstrated mitochondrial dysfunctions, including impairments in oxidative phosphorylation and decreased ATP production [288]. The study concluded that β-catenin was important in maintenance of mitochondrial homeostasis and that conditions altering β-catenin signalling may serve as a novel therapeutic treatment for liver disease.

Another study showed that altered respiratory activity and mitochondrial DNA transcription are important features of cancer cells. They found that a proinflammatory mediator implicated in chronic inflammation and cancer causes the translocation of β-catenin to the mitochondria and to the nucleus [287]. At the mitochondria they showed β-catenin to associate with Bcl-2 (identifying a role of β-catenin in cell survival) while fulfilling its normal roles of transcription in the nucleus [287].

Yoon *et al*. (2010) identified that Wnt proteins, including β-catenin, were associated with mitochondrial abundance, regulation and function. They reported that increased Wnt signalling was a potent activator of mitochondrial biogenesis and the generation of reactive oxygen species [416].

ii. β-catenin and mtHSP70

The heat shock proteins (HSP) increase in expression during stressful conditions such as heat shock, oxidative stress or inflammation [417]. In cell survival, their increased expression results in protection and disaggregation of stress-labile proteins and the breakdown of damaged proteins [418, 419]. Family member mtHSP70 (and Hsp90) is thought to affect tumorigenesis by regulating proteins involved in cell cycle control such as p53 [402, 403]. mtHSP70 has been previously described in 3.3.1.

Although not previously reported, β-catenin cross-talk with mitochondrial protein mtHSP70 in particular could be due to their interaction with p53. mtHSP70 has been shown to inactivate tumour suppressor p53 [350] while β-catenin has been reported to be down-regulated by p53 [420]. An illustration of this cross-talk is presented in Figure 55.

The accumulation of β-catenin, characteristic of several types of cancers and tumours as described earlier can also be associated with mutational inactivation of p53 [420]. Sadot *et* al. (2001) showed that overexpression of p53 down-regulated β-catenin but that this was not the case for a common tumour-associated inactive mutant p53 [420]. It has also been found that the down-regulation of β-catenin by p53 involves changes in the rate of β-catenin phosphorylation [421].

Human tumour cells (including colon cancer cells and breast cancer cells) have high levels of cytoplasmic WT p53, and for colon carcinomas, in particular, this accumulation correlates with poor prognosis [422]. It is thought that HSP70 family members are involved in this cytoplasmic sequestration of p53 [417]. It has been proposed that p53 has three potential nuclear localisation sequences that allow it to enter into the nucleus but that mutant importin-α (identified in breast cancer cells) is deficient in its ability to import WT p53 into the nucleus, resulting in p53 cytoplasmic accumulation [423, 424]. Members of the HSP70 family that perform functions in the mitochondria have been shown to interact with accumulated cytoplasmic WT p53 [425] and so it has been proposed that molecular chaperones such as mtHSP70 are involved in the localisation of p53 [417].

Although the increased expression of HSP70 (and other heat shock proteins) is part of an adaptive mechanism for stress tolerance, this same adaptive mechanism can also result in negative effects on regulation of cell growth and apoptosis [417]. These unknown effects of HSP70 can present problems for new therapeutic applications which involve regulation of HSP70 expression to induce apoptosis in cancer cells [426] and the use of HSP70 for new types of adjuvants for vaccines that specifically target tumour cells [427-429].

As stated earlier in 3.3.1, mtHSP70 mediates the folding and unfolding of proteins in response to stressful conditions, controlling their stability and transport in the cell. Of particular interest is p53, which mtHSP70 assists into the outer mitochondrial membrane [350]. Given the results for the interaction of β-catenin and mtHSP70, perhaps mtHSP70 is playing a similar role for β-catenin that it does for p53, aiding transport of β-catenin into the mitochondria. Furthermore, given the fact that mtHSP70 inactivates the protein that inactivates β-catenin (p53), it is possible that by inactivating the p53 tumour suppressor, mtHSP70 inhibits the down-regulation of β-catenin, allowing for the overexpression of β-catenin, and therefore the progression of cancer.

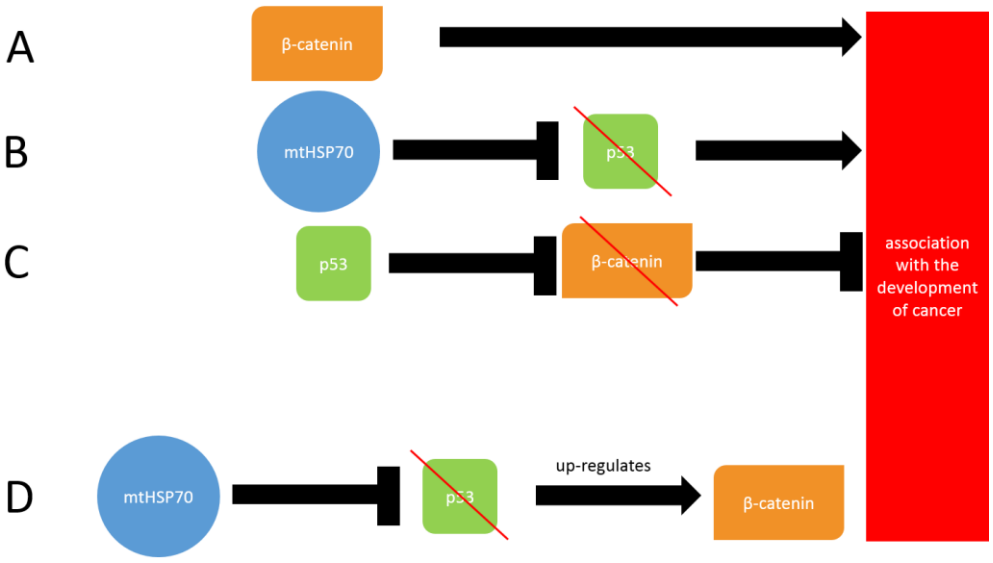


Figure 55 Illustration of potential cross-talk between β-catenin, mtHSP70 and p53.

A) β-catenin is a protein associated with the progression of several human cancers.

B) mtHSP70 is known to inactivate p53, and inactivation of p53 is associated with several cancers [350].

C) β-catenin is known to down-regulated by p53 [418].

D) Merging the information from the literature, as presented in A-C, there is potential for cross-talk between β-catenin, mtHSP70 and p53. mtHSP70 inactivation of p53 may result in up-regulation of β-catenin and therefore the development or progression of cancer.

5.4 Conclusion

In summary, this chapter validated some data founded by MS in chapters 3 and 4. In particular, COPB and mtHSP70 have some convincing evidence behind them in the form of pull-down and MS, IP and IF that they bind β-catenin in SW480. Functional analysis needs to be performed in future studies in order to clarify the purpose and importance of these interactions. However, the interaction of these two proteins with β-catenin also presents a novel localisation of β-catenin at the golgi (COPB) and mitochondria (mtHSP70). This presents β-catenin as a protein with roles at the golgi and involvement in health and regulation of the mitochondria.

The effect of phosphorylation on the interaction of β-catenin and COPB in particular also could be taken further. A phospho-Y654-β-catenin antibody should be characterised followed by other experiments such as kinase inhibitions (on C-Src kinase which phosphorylates Y654) that would serve to observe the effect of phosphorylation on Y654 and the protein interactors of β-catenin. Phosphomutants were not examined further in this thesis due to lack of specific antibody availability for non-phosphorylatable β-catenin and β-catenin phosphorylated at Y654. Section 6.5.3 discusses alternative methods of examining the phosphomutant datasets in the context of cell signalling and pathways.

The other suite of proteins investigated has had positive results, however, because they have been unsuccessful in at least one method of validation conducted in this thesis, they are not discussed further. It should be noted that the ambiguity of these results is not negative but more experimental work needs to be undertaken before these proteins are discarded as potential protein interactors of β-catenin. Tab182 and KCL4 in particular look to be promising if additional research is conducted on them or repeated.

6 General discussion

6.1 Overview and key novel findings

β-catenin, a signal transducer of the Wnt signalling pathway, is involved in the progression of several human cancers, including colon cancer. Research has shown that the involvement of β-catenin in the progression of cancer may be linked to its nuclear translocation and accumulation. However, β-catenin is a multi-functional protein and interacts with other proteins to regulate its subcellular localisation and function. It has been speculated that protein phosphorylation may influence these protein interactions. Thus, the objective of this thesis was to explore β-catenin protein interactions in the context of colon cancer and focus on detecting putative new interactors by proteomics and validating these by affinity enrichments and imaging.

Using SILAC quantitation and high-resolution mass spectrometry hundreds of candidate β-catenin protein interactors were detected in SW480 and HT29 colon cancer cells. A novel aspect of this thesis was the investigation of β-catenin Y654 phosphorylation in the Arm 10-12 domain and how this affects protein interactions. Three recombinant β-catenin-MBP constructs were purified – wildtype, phosphomimetic-Y654E and non-phosphorylatable-Y654F. These purified proteins were utilised in pull-down experiments performed in triplicate in two SILAC labelled colon cancer cell lines SW480 (Chapter 3) and HT29 (Chapter 4). The two cell lines have the same endogenous β-catenin mutational status but β-catenin localises to different subcellular locations (SW480 cytoplasmic/nuclear; HT29 membrane).

High-resolution mass spectrometry using an Orbitrap mass analyser was used and peptide identification and SILAC quantitation performed using Proteome Discoverer 1.3. A series of parameters for data acceptance was developed which included protein presence in all three replicates, statistical analysis involving p-value < 0.05 and fold-change between experimental:control of 2.

For validation studies, β-catenin candidate interactors were selected based on current literature, function of the candidate proteins in the context of cancer and subcellular localisation. IP and IF were used in efforts to validate the novel interaction with β-catenin that was originally proposed from the MS data (Chapter 5).

Several novel candidate protein interactors of β-catenin were confirmed, with COPB and mtHSP70 showing the strongest results evidenced by pull-down, MS, IP and IF. Both these proteins not only present a novel interaction of β-catenin but also a novel co-localisation of β-catenin at the golgi and mitochondria respectively. This is novel because the literature details and characterises β-catenin in the nucleus and cytoplasm but no other organelles.

6.2 Protein interactions of β-catenin in colon cancer

Utilising SILAC-labelled SW480 cells, pull-downs using wild-type β-catenin-MBP and mass spectrometry, over 350 putative protein interactors of β-catenin were detected (Chapter 3).

Of these protein interaction candidates, 4 % were proteins involved in nuclear import and export, 10 % were mitochondrial proteins and 8 % were classified as golgi proteins. The remaining proteins were grouped into 'other main processes' which includes proteins involved in other functions such as cytoskeletal proteins or particular pathways such as glycogenolysis.

These groupings were assigned based on GO annotation and manual grouping of each protein. Of particular interest were the nuclear import and export proteins, mitochondrial proteins and golgi-related proteins due to the role of nuclear accumulation of β-catenin in cancer and the fact that β-catenin had not been definitively reported at the mitochondria or golgi previously. It should be noted that the interactions with the β-catenin constructs were detected in whole lysed cells and specific subcellular structures were not isolated by centrifugation prior to complex purification. Although there was insufficient time to carry out those experiments in this thesis it is a recommendation for future work that the experiments be repeated using enriched fractions of mitochondria and golgi vesicles.

Protein interactors of β-catenin were investigated further in another colon cancer cell line, HT29 (Chapter 4). Mutational status of β-catenin is the same in HT29 as it is in SW480 in that it is wildtype β-catenin, although differences exist in APC truncations. Upon data analysis of the pull-downs performed in triplicate (across all three β-catenin-MBP constructs), a smaller list of putative β-catenin interactors was observed in the HT29 cells, perhaps due to the sequestering of endogenous β-catenin at the plasma membrane in complexes with E-cadherin. The method used for cell lysate preparation does not accommodate for cell membrane proteins as the high spin at 10,000 rpm would be insufficient at enriching these proteins and thus they would be absent from analysis.

6.2.1 Significant of β-catenin binding to COPB and mtHSP70

Importantly, particular proteins found in the HT29 dataset reinforced empirical evidence obtained from the SW480 dataset and was an added reason why specific proteins were further validated. These proteins include COPB, mtHSP70, KCL4 and Tab182. Only COPB and mtHSP70 are discussed at depth in this thesis because they had positive, unambiguous results across all methods of validation.

i. Impact of a β-catenin/COPB interaction

During protein synthesis, proteins are targeted to the ER (for synthesis, folding and modification) and then transported to the golgi for further processing in vesicles encased by COP complexes [362]. COPI coated vesicles (a type of protein vesicular carrier system) are implicated in anterograde (ER-golgi) and retrograde (golgi-ER) transport and are composed of several subunits including COPB [363]. These vesicles also mediate protein and lipid transport in the early secretory pathway [430].

Our collaborators discovered that a large pool of cytoplasmic β-catenin accumulated in recycling endosomes, golgi and COPB positive COPI complexes in breast cancer MCF-7 cells [364]. These findings linked β-catenin to functions in transport between the ER and golgi and an implication of this interaction is that β-catenin may regulate assembly of the COPI vesicular coat or transport of the COPI vesicles which is required for transport between golgi and ER.

Another effect of β-catenin/COPB can be inferred by examining bryostatin 1, a protein kinase D (PKD) modulator with antineoplastic qualities. In recent years, it has been used in clinical trials to treat human cancers with minimal success and so researchers have looked into the mechanism behind it in order to improve treatment [431]. PKD1 regulates β-catenin/E-cadherin activity and upon activation of PKD1 by bryostatin 1, the cytoplasmic pool of β-catenin co-localises with PKD1, trans-golgi markers, and proteins associated with vesicular trafficking [432]. Downregulation of PKD1 also decreased the expression and transcriptional activity of β-catenin [432].

Perhaps, golgi proteins such as COPB sequester β-catenin to the golgi once β-catenin has re-localised upon PKD1 activation, decreasing its transcriptional activity and its role in cancer progression. Complementary to this, it was found that the inhibitory effect of PKD1 on cell proliferation was rescued by β-catenin, suggesting PKD1 decreases cell proliferation, motility, and invasion while the loss of it increases expression of β-catenin and cell proliferation [433]. Modulation of β-catenin signalling by bryostatin 1 through PKD1 identifies a novel mechanism to improve the efficacy of cancer treatment using bryostatin 1, and COPB may be a protein of interest allowing β-catenin to remain at the golgi as opposed to performing its nuclear roles that aid in cancer progression.

Conversely, another study correlated subcellular re-localisation of β-catenin with cancer when it was found that β-catenin at cell membrane re-localised to the golgi in human meningioma [290]. This is another example where COPB may sequester β-catenin to the golgi, however in this case golgi localisation is involved in tumorigenesis.

Nuclear transport of epidermal growth factor receptor (EGFR) is thought to be controlled by COPI-mediated vesicular trafficking [414]. Although this provides a mechanism for EGFR movement, the mechanism of EGFR transport from these vesicles to the nucleus is yet to be elucidated. Results presented in this thesis and that in the literature suggest that EGFR and COPI interact, and upon binding of β-catenin, EGFR may be able to translocate into the nucleus. Nuclear EGFR ultimately results in cell proliferation and metastasis and so research has attempted to inhibit its activity [434]. Here, targeting COPB/β-catenin may also be useful.

ii. Impact of a β-catenin/mtHSP70 interaction

The notion of mtHSP70/mitochondria/β-catenin complexing and co-localising has been discussed previously in 3.3.1 and 5.3.2. In summary, mtHSP70 is involved in the folding of proteins and degrading of misfolded proteins and assisting movement of p53 into the outer mitochondrial membrane [350]. Its roles in mitochondrial activity and biogenesis, and the fact that it is upregulated in human cancer cell lines and tumours [350, 353, 355-357] makes it stand out as a protein of interest that may aid β-catenin in its role in cancer progression. The impact of β-catenin/mtHSP70, β-catenin at the mitochondria, or targeting such interactions in cancer therapy can be inferred from the literature.

Mitochondrial protein and marker mtHSP70 is thought to affect tumorigenesis by regulation of cell cycle proteins such as p53. mtHSP70 inactivates tumour suppressor p53 [350] while it has also been reported that β-catenin is down-regulated by p53 [420]. Here, the link between β-catenin and mtHSP70 could be the common binder p53. This is possible due to the ability of mtHSP70 to suppress the role of p53 in tumour suppression and aiding the development of cancer while the downregulation of β-catenin by p53 should not aid the progression of cancer because accumulation of β-catenin is associated with the progression of cancer (described in sections 1.4.3 and 1.4.4). Here perhaps, while mtHSP70 disrupts the role of p53 in tumour suppression, it could also be disrupting the downregulation of β-catenin by p53. Both of these traits have the opposite effect of keeping cancer at bay. It should also be noted that accumulation of β-catenin has in fact been previously found to be associated with inactivation of p53 [420], which means this theory is feasible.

If mtHSP70 is responsible for inactivation of tumour suppressor p53 and consequential disruption of downregulation of β-catenin, one may assume that mtHSP70 be a potential target for cancer therapy. However, other functions of mtHSP70 would also be affected. These roles include those of mtHSP70 in adapting to stress [417] and transport of proteins into the outer mitochondrial membrane [350].

Collecting data on specificity, affinity and kinetics of the potential mtHSP70/p53/β-catenin interaction will be important components of validation. Biacore (uses surface plasmon resonance biosensors) is a method capable of validating this interaction, elucidating its function and defining the stability of the complex. Another strategy to examine the link between mtHSP0/p53/β-catenin includes knocking-down one of the proteins in the complex using siRNA and following the effects it has on the other two proteins of the complex (eg. Follow their expression and see if they are upregulated or downregulated).

6.2.2 Conclusion

In conclusion, the data in this thesis provides insight into protein interactors of β-catenin, demonstrating the β-catenin does not just hold the traditional roles at the plasma membrane or in the nucleus. Rather, it is a dynamic protein that can interact with a number of proteins that are involved in a myriad of functions and localises at a number of organelles – demonstrated here by pull-down of more than just plasma membrane and nuclear-related proteins.

6.3 The effect of phosphorylation

Upon utilising constructs with point mutations at Y654 to mimic a phosphorylatable or non-phosphorylatable amino acid, the same pull-downs were performed in triplicate in SW480. For the phosphomimetic, the candidate list consisted of 92 proteins while the non-phosphorylatable data set contained 129. For the phosphomimetic list, 5 % were grouped into nuclear import and export related proteins, 7 % mitochondrial related and 5 % golgi related. For the non-phosphorylatable list, 2 % were grouped into nuclear import and export related proteins, 9 % mitochondrial related and 5 % golgi related.

When comparing the phosphorylation mutant datasets there were distinctions between the lists, for example, kinesin light chain 4 (KCL4) bound to β-catenin only in the absence of phosphorylation at Y654. That is, KCL4 bound to the non-phosphorylatable construct and the WT. Due to the nature of the experiments, the contrast in binding-partners of β-catenin across phosphomimetic and non-phosphorylatable datasets can be attributed to the phosphorylation at Y654.

The literature has reported that phosphorylation at this site can either inhibit protein interactions or mediate them. For example, the negative charge that a phosphorylation imposes can block an interaction (eg. E-cadherin [257, 285], potentially KCL4) while in other cases (eg. TATA-binding protein [257]) phosphorylation at this amino acid opens up the Arm domain from the C-terminus, abolishing C-terminal steric hindrance and allowing for proteins to bind the Arm domain. In summary, it has been shown in the literature and in this thesis that phosphorylation at Y654 can effect binding partners (block or enhance them), by changing β-catenin tertiary structure and charge.

Future directions validating the effect of phosphorylation beyond the initial proteomic screen are discussed in 6.5.3.

6.4 Evaluation of methodology

6.4.1 Use of an MBP tag

The research in this thesis was conducted using purified recombinant protein with a maltose-binding-protein (MBP) tag. Although other affinity tags could have been used (eg. β-catenin-GST), there were very well established protocols for MBP constructs (expression, purification) available in the lab, in particular for β-catenin-MBP. Use of the MBP tag comes with other advantages.

Some tags, including MBP, are known to protect the target protein from degradation during expression by mediating translocation of the protein to a cellular location where fewer proteases exist [435, 436]. For MBP, the target protein is passed from the cytosol of *E. coli* to the cell membrane [437]. An important aspect to consider when choosing a fusion tag is the purpose for which it is needed, for example, purposes of solubility improvement or affinity purification. The research in this thesis requires both these factors and MBP is suitable for both [438, 439]. In fact, MBP was ranked as the best affinity tag for making soluble fusions [440, 441]. Among 8 other tags, it was also ranked as the most cost-effective [442]. Furthermore, MBP allows for gentle protein purification conditions [443] and has strong translational initiation signals, resulting in high expression [444].

Furthermore, MBP is also not only known to attract chaperones, driving its partner protein into a chaperone-mediated pathway but also has intrinsic chaperone-like activity in itself [436]. In some cases when fusion proteins are produced, they aggregate as insoluble folding intermediates known as inclusion bodies. Using large affinity tags such as MBP leads to the avoidance of inclusion bodies [445]. Hydrophobic patches of MBP interact with partially folded passenger proteins, encouraging proper folding and preventing self-aggregation [438].

A disadvantage of the MBP tag is its large size (43 kDa) which can potentially influence the structure or biological activity of the partner [446]. However, the fact that known interactors of β-catenin were isolated from pull-downs suggests this did not negatively impact the experiment.

Antibodies can also be utilised to investigate interactors of particular proteins (as opposed to recombinant proteins) however the use of β-catenin antibody was not appropriate for our study because there was no phosphomimetic or non-phophorylatable β-catenin-Y654 antibody available at the conception of the project.

An advantage of using antibodies is the feature that endogenous β-catenin is immunoprecipitated, consequentially pulling out protein interactors already bound to β-catenin. Our method of adding exogenous β-catenin-MBP suggests the potential to miss true protein binders which are bound to endogenous β-catenin. However, the introduction of excess β-catenin-MBP must result in competition for binders between it and the endogenous β-catenin because several known interactors were pulled-down using our method.

6.4.2 Pull-down experiments

Pull-down experiments are useful for *in vitro* determination of protein interactions. The pull-downs conducted in this thesis involve the addition of β-catenin-MBP purified protein, or 'bait', to cell lysate.

i. Pull-down (using purified protein) in preference of IP (using antibody)

An advantage of pull-downs over immunoprecipitations (use antibody as opposed to purified protein) is that when there is no appropriate or specific antibody available (which was the case in this thesis where Y654 phospho-mutants were required), the exact protein desired can be designed and produced (eg. mutants, certain domains).

However, both methods may allow for isolation of non-specific proteins, for example proteins that bind the amylose beads. To combat non-specific binding using the pull-down method, the lysate is 'pre-cleared'. That is, the lysate is incubated with the amylose beads, allowing for non-specific binders to bind the beads. The beads are then discarded and the lysate incubated with fresh beads that have been bound to the purified protein.

Furthermore, pull-down of known interactors validated that the method utilised did mimic *in vivo* conditions.

ii. Cell lysis method

The lysis method of cells (RIPA buffer with 1 % Triton X-100) which creates whole cell lysate used for pull-downs could be altered. If looking specifically for proteins involved in nuclear transport, perhaps a nuclear fractionation could be performed and pull-downs performed in that fraction. This, however, may provide an artificial preference for interaction. That is, if β-catenin-MBP is only allowed the option of interacting with nuclear proteins, there may be artificial interactions occurring by chance. This is potentially something that occurred when β-catenin was allowed to interact with mitochondrial or golgi proteins using our lysis method where organelle partitioning was eliminated. Disruption of cell membranes interrupts cell compartmentalisation as discussed in 5.2.2.ii. This may lead to protein binders that are not relevant, but rather are artefacts. These potential artefacts are one of the many reasons protein binders need to be validated by other methodologies, including methodologies that immunoprecipitate endogenous β-catenin, or investigate interactions in whole cells (IF).

However, when β-catenin-MBP is permitted to interact with proteins in whole cell lysate, this may be a more accurate reflection of *in vivo* interactions because a myriad of proteins would be competing for the interaction. Another explanation is that these proteins may reside in other accessible locations as a result of a mutation in the protein (eg. protein truncation). The proteomics approach used cannot decipher this and high-resolution imaging is needed to confirm the interaction *in vivo*.

6.5 Future directions

The research in this thesis has established a foundation upon which future investigations on β-catenin's involvement in cancer can be carried out. These future studies will advance knowledge of the biological relevance and function of the protein interactions found in this thesis and may even target these proteins or interactions in cancer therapy. In order to validate the candidate interactors of β-catenin explored in this thesis, the following ideas can be explored.

6.5.1 Functional relevance of protein interactors

Future directions should include functional assays that lead to elucidation of the relevance of the novel interactions of β-catenin found in this thesis.

Early experiments should consist of knock-down/overexpression of β-catenin or its interactor and looking at the effect by observing expression or function of the other. Experiments involving FRAP assays (fluorescence recovery after photobleaching) could be used to assess the effect of these interactions on the rate of nuclear import or export, or even import or export in other organelles such as the mitochondria. Other examples of assays that demonstrate the functionality of β-catenin interactions includes cell proliferation, migration or invasion assays that can assess the effect on the progression of cancer.

6.5.2 Other cell lines

A limited number of cell lines were used in this thesis - two colon cancer cell lines. Non-cancer cell lines should be used as a control or baseline. A non-cancer cell line such as HEK293T could be used as it has a low endogenous level of β-catenin, unlike SW480.

Colon cancer cell lines with mutated β-catenin could be utilised (HCT116) to contrast the interactors identified in β-catenin WT cell lines (SW480 and HT29) to shed light on the effect of mutated β-catenin on interactors.

6.5.3 Effect of phosphorylation at Y654

Regarding the phosphorylation component of this thesis, there is much that can be taken on by future studies. The research presented here focused on protein interactors of phosphorylated and non-phosphorylatable β-catenin at Y654 using purified protein in pull-down experiments. The importance of phosphorylation could be further investigated utilising a specific antibody against phospho-Y654-β-catenin. At the inception of this project such an antibody was unavailable, and so in order to investigate the effect of phosphorylation, the constructs created in this thesis had to be used. Should a reliable phospho-specific β-catenin Y654 antibody become available, one could perform IPs using the antibody or co-localisation experiments using the antibody for IF.

Kinase inhibitions or knock-downs could be performed on C-Src kinase (CSK), the kinase that phosphorylates Y654, and then protein interactions thought to be affected by phosphorylation based on data presented in this thesis could be monitored. Finally, β-catenin may be stabilised by activation of the Wnt pathway using Wnt3a or LiCl (inhibits GSK3β which would normally result in the phosphorylation and destruction of β-catenin) and effect on interactors or subcellular location observed.

6.5.4 Conclusion

This thesis has investigated protein interactors of β-catenin in colon cancer cell lines and proposed many new putative interactors and validated a few by affinity enrichment and immunofluorescent imaging. The effect of phosphorylation at Y654 on binding partners of β-catenin has been explored.

The most significant findings are the immune-validation of β-catenin interacting with golgi protein COPB and mitochondrial protein mtHSP70. Future research will elucidate the functional relevance of such interactions, giving new knowledge to the molecular cell biology regulated through these interactions. With such knowledge, the potential for therapeutic disruption of specific β-catenin interactions can be investigated.

7 References

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8 Appendices

8.1 Candidate novel interacting proteins of full-length WT β-catenin in SW480.

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
Q9C0C2	182 kDa tankyrase-1-binding protein OS=Homo sapiens GN=TNKS1BP1 PE=1 SV=4 - [TB182_HUMAN]	4	5	lost	0.003	0.001	629.31	0.0005	lost	3	3
P19174	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-1 OS=Homo sapiens GN=PLCG1 PE=1 SV=1 - [PLCG1_HUMAN]	1	2	lost	n/a	n/a	0	0	lost	1	1
P62333	26S protease regulatory subunit 10B OS=Homo sapiens GN=PSMC6 PE=1 SV=1 - [PRS10_HUMAN]	6	6	0.017	0.002	0.001	93.02	5.8E-05	6	3	1
P17980	26S protease regulatory subunit 6A OS=Homo sapiens GN=PSMC3 PE=1 SV=3 - [PRS6A_HUMAN]	8	12	0.220	0.137	0.185	13.27	0.003	8	8	6
P43686	26S protease regulatory subunit 6B OS=Homo sapiens GN=PSMC4 PE=1 SV=2 - [PRS6B_HUMAN]	6	7	0.008	0.043	0.001	37.19	0.0004	4	6	5
P62195	26S protease regulatory subunit 8 OS=Homo sapiens GN=PSMC5 PE=1 SV=1 - [PRS8_HUMAN]	8	9	0.344	0.023	0.015	3.43	0.04	8	6	4
Q99460	26S proteasome non-ATPase regulatory subunit 1 OS=Homo sapiens GN=PSMD1 PE=1 SV=2 - [PSMD1_HUMAN]	5	6	n/a	0.002	0.015	80.95	0.004	2	3	2
O00487	26S proteasome non-ATPase regulatory subunit 14 OS=Homo sapiens GN=PSMD14 PE=1 SV=1 - [PSDE_HUMAN]	3	3	0.008	0.233	0.284	3.83	0.03	2	3	2
Q13200	26S proteasome non-ATPase regulatory subunit 2 OS=Homo sapiens GN=PSMD2 PE=1 SV=3 - [PSMD2_HUMAN]	13	14	0.265	0.259	0.223	18.85	0.001	9	8	6

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
O43242	26S proteasome non-ATPase regulatory subunit 3 OS=Homo sapiens GN=PSMD3 PE=1 SV=2 - [PSMD3_HUMAN]	13	14	0.275	0.232	0.001	3.88	0.03	7	7	7
Q02218	2-oxoglutarate dehydrogenase, mitochondrial OS=Homo sapiens GN=OGDH PE=1 SV=3 - [ODO1_HUMAN]	3	4	n/a	n/a	0.001			1	2	3
Q9BZE1	39S ribosomal protein L37, mitochondrial OS=Homo sapiens GN=MRPL37 PE=1 SV=2 - [RM37_HUMAN]	6	7	0.039	0.015	0.001	43.61	0.0003	5	2	3
Q99714	3-hydroxyacyl-CoA dehydrogenase type-2 OS=Homo sapiens GN=HSD17B10 PE=1 SV=3 - [HCD2_HUMAN]	6	6	0.008	0.266	0.194	4.48	0.02	1	6	5
P62269	40S ribosomal protein S18 OS=Homo sapiens GN=RPS18 PE=1 SV=3 - [RS18_HUMAN]	12	13	0.283	0.388	0.176	3.56	0.04	9	10	11
P23396	40S ribosomal protein S3 OS=Homo sapiens GN=RPS3 PE=1 SV=2 - [RS3_HUMAN]	19	19	0.224	0.423	0.213	3.13	0.04	18	19	17
P49189	4-trimethylaminobutyraldehyde dehydrogenase OS=Homo sapiens GN=ALDH9A1 PE=1 SV=3 - [AL9A1_HUMAN]	3	4	0.001	0.002	n/a	3982.02	8.0E-05	3	2	1
P62906	60S ribosomal protein L10a OS=Homo sapiens GN=RPL10A PE=1 SV=2 - [RL10A_HUMAN]	10	10	0.360	0.440	0.273	2.94	0.05	5	7	9
Q01813	6-phosphofructokinase type C OS=Homo sapiens GN=PFKP PE=1 SV=2 - [K6PP_HUMAN]	7	12	0.061	0.248	0.001	5.34	0.02	6	9	3
P17858	6-phosphofructokinase, liver type OS=Homo sapiens GN=PFKL PE=1 SV=6 - [K6PL_HUMAN]	2	6	n/a	0.002	n/a			3	4	1
P08237	6-phosphofructokinase, muscle type OS=Homo sapiens GN=PFKM PE=1 SV=2 - [K6PF_HUMAN]	4	6	0.002	0.160	0.011	8.64	0.007	3	4	3
Q9UBM7	7-dehydrocholesterol reductase OS=Homo sapiens GN=DHCR7 PE=1 SV=1 - [DHCR7_HUMAN]	5	5	0.272	0.033	0.017	4.75	0.02	5	3	2

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
Q9NR19	Acetyl-coenzyme A synthetase, cytoplasmic OS=Homo sapiens GN=ACSS2 PE=1 SV=1 - [ACSA_HUMAN]	4	5	n/a	0.001	0.001	0	0	3	3	3
Q99798	Aconitate hydratase, mitochondrial OS=Homo sapiens GN=ACO2 PE=1 SV=2 - [ACON_HUMAN]	3	5	0.097	0.004	n/a	9.59	0.03	2	2	1
P68032	Actin, alpha cardiac muscle 1 OS=Homo sapiens GN=ACTC1 PE=1 SV=1 - [ACTC_HUMAN]	1	12	0.332	0.406	0.257	3.91	0.03	11	12	10
P61160	Actin-related protein 2 OS=Homo sapiens GN=ACTR2 PE=1 SV=1 - [ARP2_HUMAN]	7	8	0.265	0.002	0.001	4.68	0.02	4	5	3
O15145	Actin-related protein 2/3 complex subunit 3 OS=Homo sapiens GN=ARPC3 PE=1 SV=3 - [ARPC3_HUMAN]	6	6	0.002	0.262	0.321	3.11	0.04	1	5	5
P59998	Actin-related protein 2/3 complex subunit 4 OS=Homo sapiens GN=ARPC4 PE=1 SV=3 - [ARPC4_HUMAN]	6	6	0.380	0.352	0.205	3.46	0.04	4	5	4
O00767	Acyl-CoA desaturase OS=Homo sapiens GN=SCD PE=1 SV=2 - [ACOD_HUMAN]	2	2	0.004	0.282	0.013	4.39	0.02	1	2	2
Q86TX2	Acyl-coenzyme A thioesterase 1 OS=Homo sapiens GN=ACOT1 PE=1 SV=1 - [ACOT1_HUMAN]	2	2	0.021	0.359	0.001	3.21	0.04	2	1	1
P25054	Adenomatous polyposis coli protein OS=Homo sapiens GN=APC PE=1 SV=2 - [APC_HUMAN]	5	7	lost	0.002	0.001	985.72	0.0003	lost	5	5
P12235	ADP/ATP translocase 1 OS=Homo sapiens GN=SLC25A4 PE=1 SV=4 - [ADT1_HUMAN]	2	9	n/a	0.425	n/a			4	8	6
P05141	ADP/ATP translocase 2 OS=Homo sapiens GN=SLC25A5 PE=1 SV=7 - [ADT2_HUMAN]	5	14	0.321	0.355	0.286	8.96	0.006	10	11	9
P84085	ADP-ribosylation factor 5 OS=Homo sapiens GN=ARF5 PE=1 SV=2 - [ARF5_HUMAN]	1	4	n/a	n/a	0.001			4	4	4

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
P62330	ADP-ribosylation factor 6 OS=Homo sapiens GN=ARF6 PE=1 SV=2 - [ARF6_HUMAN]	2	4	n/a	0.409	n/a			1	4	1
O00170	AH receptor-interacting protein OS=Homo sapiens GN=AIP PE=1 SV=2 - [AIP_HUMAN]	6	8	0.001	0.373	0.001	3.02	0.05	2	4	6
P11766	Alcohol dehydrogenase class-3 OS=Homo sapiens GN=ADH5 PE=1 SV=4 - [ADHX_HUMAN]	9	9	0.386	0.331	0.275	5.28	0.02	2	7	7
P01009	Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3 - [A1AT_HUMAN]	24	24	0.001	0.001	0.001	6445.46	1.2E-08	8	24	15
P02765	Alpha-2-HS-glycoprotein OS=Homo sapiens GN=AHSG PE=1 SV=1 - [FETUA_HUMAN]	4	5	0.001	0.001	0.001	12489.61	3.2E-09	5	5	4
P01023	Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=3 - [A2MG_HUMAN]	5	6	0.001	0.001	0.001	3518.04	4.0E-08	4	6	5
O43707	Alpha-actinin-4 OS=Homo sapiens GN=ACTN4 PE=1 SV=2 - [ACTN4_HUMAN]	33	53	0.411	0.358	0.267	3.68	0.03	45	47	43
P02771	Alpha-fetoprotein OS=Homo sapiens GN=AFP PE=1 SV=1 - [FETA_HUMAN]	3	4	n/a	0.001	0.001	0	0	2	2	3
Q8IY63	Angiomotin-like protein 1 OS=Homo sapiens GN=AMOTL1 PE=1 SV=1 - [AMOL1_HUMAN]	1	2	lost	n/a	0.352			lost	2	1
P50995	Annexin A11 OS=Homo sapiens GN=ANXA11 PE=1 SV=1 - [ANX11_HUMAN]	5	6	0.059	0.001	n/a	16.19	0.02	4	2	2
P01008	Antithrombin-III OS=Homo sapiens GN=SERPINC1 PE=1 SV=1 - [ANT3_HUMAN]	5	5	0.001	0.002	n/a	5266.17	6.0E-05	4	2	1
P63010	AP-2 complex subunit beta OS=Homo sapiens GN=AP2B1 PE=1 SV=1 - [AP2B1_HUMAN]	6	7	n/a	0.020	0.001	52.86	0.006	1	6	2

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
P02647	Apolipoprotein A-I OS=Homo sapiens GN=APOA1 PE=1 SV=1 - [APOA1_HUMAN]	6	7	n/a	0.001	0.001	0	0	1	6	1
P04114	Apolipoprotein B-100 OS=Homo sapiens GN=APOB PE=1 SV=2 - [APOB_HUMAN]	52	53	n/a	0.001	0.001	0	0	1	52	29
P78540	Arginase-2, mitochondrial OS=Homo sapiens GN=ARG2 PE=1 SV=1 - [ARGI2_HUMAN]	6	6	0.161	0.377	0.155	3.68	0.03	3	4	4
Q9UBB4	Ataxin-10 OS=Homo sapiens GN=ATXN10 PE=1 SV=1 - [ATX10_HUMAN]	5	6	0.004	0.001	n/a	373.21	0.0009	6	4	3
P25705	ATP synthase subunit alpha, mitochondrial OS=Homo sapiens GN=ATP5A1 PE=1 SV=1 - [ATPA_HUMAN]	14	14	0.291	0.311	0.114	4.17	0.03	10	10	7
P48047	ATP synthase subunit O, mitochondrial OS=Homo sapiens GN=ATP5O PE=1 SV=1 - [ATPO_HUMAN]	9	9	0.008	0.158	0.084	9.61	0.005	6	7	6
Q5T9A4	ATPase family AAA domain-containing protein 3B OS=Homo sapiens GN=ATAD3B PE=1 SV=1 - [ATD3B_HUMAN]	6	7	0.026	0.001	0.001	59.25	0.0001	3	5	5
Q99758	ATP-binding cassette sub-family A member 3 OS=Homo sapiens GN=ABCA3 PE=1 SV=2 - [ABCA3_HUMAN]	1	1	lost	0.001	0.001	0	0	lost	1	1
P61221	ATP-binding cassette sub-family E member 1 OS=Homo sapiens GN=ABCE1 PE=1 SV=1 - [ABCE1_HUMAN]	10	10	0.203	0.002	0.194	5.58	0.02	6	7	5
P46063	ATP-dependent DNA helicase Q1 OS=Homo sapiens GN=RECQL PE=1 SV=3 - [RECQ1_HUMAN]	5	5	n/a	0.362	n/a			1	5	2
O00148	ATP-dependent RNA helicase DDX39A OS=Homo sapiens GN=DDX39A PE=1 SV=2 - [DX39A_HUMAN]	2	11	0.289	0.015	0.282	3.38	0.04	7	9	5
Q9Y6E2	Basic leucine zipper and W2 domain-containing protein 2 OS=Homo sapiens GN=BZW2 PE=1 SV=1 -	3	6	0.002	0.001	0.001	1544.42	2.1E-07	4	4	4

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
	[BZW2_HUMAN]										
P16278	Beta-galactosidase OS=Homo sapiens GN=GLB1 PE=1 SV=2 - [BGAL_HUMAN]	4	5	0.001	0.001	0.001	3117 7.21	5.1E-10	3	2	1
Q9HBI1	Beta-parvin OS=Homo sapiens GN=PARVB PE=1 SV=1 - [PARVB_HUMAN]	1	2	n/a	n/a	0.001			1	1	2
P53004	Biliverdin reductase A OS=Homo sapiens GN=BLVRA PE=1 SV=2 - [BIEA_HUMAN]	5	6	0.259	0.015	0.001	4.87	0.02	3	3	2
Q9NWV8	BRISC and BRCA1-A complex member 1 OS=Homo sapiens GN=BABAM1 PE=1 SV=1 - [BABA1_HUMAN]	1	1	n/a	n/a	0.987			1	1	1
P11586	C-1-tetrahydrofolate synthase, cytoplasmic OS=Homo sapiens GN=MTHFD1 PE=1 SV=3 - [C1TC_HUMAN]	25	25	0.308	0.276	0.001	3.13	0.04	21	22	15
Q9Y376	Calcium-binding protein 39 OS=Homo sapiens GN=CAB39 PE=1 SV=1 - [CAB39_HUMAN]	2	3	0.337	n/a	n/a			2	3	1
P07384	Calpain-1 catalytic subunit OS=Homo sapiens GN=CAPN1 PE=1 SV=1 - [CAN1_HUMAN]	6	6	0.280	0.166	0.001	4.33	0.02	3	6	3
Q99439	Calponin-2 OS=Homo sapiens GN=CNN2 PE=1 SV=4 - [CNN2_HUMAN]	6	6	0.001	0.391	0.001	2.84	0.05	2	3	4
O75828	Carbonyl reductase [NADPH] 3 OS=Homo sapiens GN=CBR3 PE=1 SV=3 - [CBR3_HUMAN]	1	3	n/a	n/a	0.073			1	2	3
Q9BXW7	Cat eye syndrome critical region protein 5 OS=Homo sapiens GN=CECR5 PE=1 SV=1 - [CECR5_HUMAN]	3	3	0.002	n/a	n/a			1	2	2
P35221	Catenin alpha-1 OS=Homo sapiens GN=CTNNA1 PE=1 SV=1 - [CTNA1_HUMAN]	33	35	0.003	0.003	0.001	646.09	1.2E-06	30	28	23

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
P35222	Catenin beta-1 OS=Homo sapiens GN=CTNNB1 PE=1 SV=1 - [CTNB1_HUMAN]	32	39	0.001	0.001	0.001	4188.69	2.8E-08	36	37	39
O60716	Catenin delta-1 OS=Homo sapiens GN=CTNND1 PE=1 SV=1 - [CTND1_HUMAN]	4	4	0.002	0.002	0.001	1615.70	1.9E-07	1	3	2
P11717	Cation-independent mannose-6-phosphate receptor OS=Homo sapiens GN=IGF2R PE=1 SV=3 - [MPRI_HUMAN]	11	11	lost	0.003	0.001	615.51	0.0005	lost	2	11
A5YKK6	CCR4-NOT transcription complex subunit 1 OS=Homo sapiens GN=CNOT1 PE=1 SV=2 - [CNOT1_HUMAN]	6	6	lost	0.001	0.001	23714.67	1.3E-05	lost	2	6
Q7LBR1	Charged multivesicular body protein 1b OS=Homo sapiens GN=CHMP1B PE=1 SV=1 - [CHM1B_HUMAN]	4	4	n/a	n/a	n/a	0	0	1	3	1
Q13185	Chromobox protein homolog 3 OS=Homo sapiens GN=CBX3 PE=1 SV=4 - [CBX3_HUMAN]	5	5	0.001	0.016	0.028	62.41	0.0001	1	3	4
Q14839	Chromodomain-helicase-DNA-binding protein 4 OS=Homo sapiens GN=CHD4 PE=1 SV=2 - [CHD4_HUMAN]	4	7	n/a	n/a	0.001			1	3	6
P09496	Clathrin light chain A OS=Homo sapiens GN=CLTA PE=1 SV=1 - [CLCA_HUMAN]	4	5	0.291	0.016	0.197	4.12	0.03	3	3	5
Q16630	Cleavage and polyadenylation specificity factor subunit 6 OS=Homo sapiens GN=CPSF6 PE=1 SV=2 - [CPSF6_HUMAN]	2	2	0.096	0.002	n/a	9.57	0.03	1	2	1
P53621	Coatomer subunit alpha OS=Homo sapiens GN=COPA PE=1 SV=2 - [COPA_HUMAN]	19	20	0.002	0.297	0.001	4.06	0.03	6	15	15
P35606	Coatomer subunit beta' OS=Homo sapiens GN=COPB2 PE=1 SV=2 - [COPB2_HUMAN]	7	8	n/a	n/a	0.001			4	3	3
O14579	Coatomer subunit epsilon OS=Homo sapiens GN=COPE PE=1	3	5	n/a	0.444	n/a			2	4	5

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
	SV=3 - [COPE_HUMAN]										
P61923	Coatomer subunit zeta-1 OS=Homo sapiens GN=COPZ1 PE=1 SV=1 - [COPZ1_HUMAN]	3	3	0.460	0.462	0.423	4.06	0.03	2	2	1
Q9Y281	Cofilin-2 OS=Homo sapiens GN=CFL2 PE=1 SV=1 - [COF2_HUMAN]	1	5	0.464	n/a	n/a			3	4	4
Q9NVE4	Coiled-coil domain-containing protein 87 OS=Homo sapiens GN=CCDC87 PE=2 SV=2 - [CCD87_HUMAN]	1	2	0.001	0.001	0.001	0	0	2	1	1
Q9NX63	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial OS=Homo sapiens GN=CHCHD3 PE=1 SV=1 - [CHCH3_HUMAN]	4	4	0.092	n/a	0.135	17.92	0.02	3	2	2
P00746	Complement factor D OS=Homo sapiens GN=CFD PE=1 SV=5 - [CFAD_HUMAN]	1	1	0.001	0.001	0.001	0	0	1	1	1
Q15021	Condensin complex subunit 1 OS=Homo sapiens GN=NCAPD2 PE=1 SV=3 - [CND1_HUMAN]	9	10	lost	0.001	0.001	0	0	lost	7	7
Q9NZB2	Constitutive coactivator of PPAR-gamma-like protein 1 OS=Homo sapiens GN=FAM120A PE=1 SV=2 - [F120A_HUMAN]	5	5	0.418	n/a	n/a			2	3	3
Q92905	COP9 signalosome complex subunit 5 OS=Homo sapiens GN=COPS5 PE=1 SV=4 - [CSN5_HUMAN]	4	4	0.010	0.001	0.224	5.77	0.01	1	2	1
P12277	Creatine kinase B-type OS=Homo sapiens GN=CKB PE=1 SV=1 - [KCRB_HUMAN]	5	5	0.381	0.018	0.167	2.95	0.05	3	3	2
Q86VP6	Cullin-associated NEDD8-dissociated protein 1 OS=Homo sapiens GN=CAND1 PE=1 SV=2 - [CAND1_HUMAN]	12	14	0.020	0.292	0.001	4.21	0.03	5	9	5
P06493	Cyclin-dependent kinase 1 OS=Homo sapiens GN=CDK1 PE=1	11	13	0.075	0.227	0.131	8.02	0.008	7	10	12

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
	SV=3 - [CDK1_HUMAN]										
P24941	Cyclin-dependent kinase 2 OS=Homo sapiens GN=CDK2 PE=1 SV=2 - [CDK2_HUMAN]	3	6	0.007	0.001	0.001	230.87	9.4E-06	5	4	3
P21291	Cysteine and glycine-rich protein 1 OS=Homo sapiens GN=CSRP1 PE=1 SV=3 - [CSRP1_HUMAN]	5	5	0.061	0.201	0.117	9.16	0.006	3	3	4
P00403	Cytochrome c oxidase subunit 2 OS=Homo sapiens GN=MT-CO2 PE=1 SV=1 - [COX2_HUMAN]	4	4	0.355	0.001	0.014	3.26	0.04	3	4	2
Q8NCM8	Cytoplasmic dynein 2 heavy chain 1 OS=Homo sapiens GN=DYNC2H1 PE=1 SV=4 - [DYHC2_HUMAN]	1	4	n/a	0.004	n/a			3	2	1
Q7L576	Cytoplasmic FMR1-interacting protein 1 OS=Homo sapiens GN=CYFIP1 PE=1 SV=1 - [CYFP1_HUMAN]	5	6	0.011	0.272	0.001	4.57	0.02	4	3	4
Q14008	Cytoskeleton-associated protein 5 OS=Homo sapiens GN=CKAP5 PE=1 SV=3 - [CKAP5_HUMAN]	4	4	lost	0.024	0.001	42.61	0.007	lost	2	4
Q9BZ29	Dedicator of cytokinesis protein 9 OS=Homo sapiens GN=DOCK9 PE=1 SV=2 - [DOCK9_HUMAN]	15	17	lost	0.002	0.001	1106.67	0.0003	lost	9	17
P54886	Delta-1-pyrroline-5-carboxylate synthase OS=Homo sapiens GN=ALDH18A1 PE=1 SV=2 - [P5CS_HUMAN]	4	5	0.003	0.001	0.001	680.08	1.1E-06	4	2	1
P36957	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial OS=Homo sapiens GN=DLST PE=1 SV=4 - [ODO2_HUMAN]	4	5	0.056	0.003	0.001	26.61	0.0007	2	3	2
Q9NY33	Dipeptidyl peptidase 3 OS=Homo sapiens GN=DPP3 PE=1 SV=2 - [DPP3_HUMAN]	3	4	0.465	n/a	n/a			1	3	1
P26358	DNA (cytosine-5)-methyltransferase 1 OS=Homo sapiens GN=DNMT1 PE=1 SV=2 - [DNMT1_HUMAN]	2	2	lost	0.002	0.001	1888.73	0.0002	lost	1	2

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
P28340	DNA polymerase delta catalytic subunit OS=Homo sapiens GN=POLD1 PE=1 SV=2 - [DPOD1_HUMAN]	2	3	n/a	n/a	n/a	0	0	1	2	2
P49736	DNA replication licensing factor MCM2 OS=Homo sapiens GN=MCM2 PE=1 SV=4 - [MCM2_HUMAN]	12	14	0.022	0.305	0.001	3.98	0.03	12	10	6
P25205	DNA replication licensing factor MCM3 OS=Homo sapiens GN=MCM3 PE=1 SV=3 - [MCM3_HUMAN]	17	18	0.255	0.345	0.001	2.91	0.05	14	12	9
P33991	DNA replication licensing factor MCM4 OS=Homo sapiens GN=MCM4 PE=1 SV=5 - [MCM4_HUMAN]	12	13	0.249	0.128	0.001	5.24	0.02	7	11	7
P33992	DNA replication licensing factor MCM5 OS=Homo sapiens GN=MCM5 PE=1 SV=5 - [MCM5_HUMAN]	22	25	0.104	0.001	0.001	13.56	0.003	11	18	15
P33993	DNA replication licensing factor MCM7 OS=Homo sapiens GN=MCM7 PE=1 SV=4 - [MCM7_HUMAN]	20	20	0.143	0.222	0.001	5.84	0.01	14	11	9
P27695	DNA-(apurinic or apyrimidinic site) lyase OS=Homo sapiens GN=APEX1 PE=1 SV=2 - [APEX1_HUMAN]	6	8	0.148	0.308	0.164	5.77	0.01	4	7	6
P78527	DNA-dependent protein kinase catalytic subunit OS=Homo sapiens GN=PRKDC PE=1 SV=3 - [PRKDC_HUMAN]	44	52	lost	0.196	0.136	11.10	0.03	lost	34	45
P04843	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 OS=Homo sapiens GN=RPN1 PE=1 SV=1 - [RPN1_HUMAN]	13	15	0.282	0.001	0.020	4.40	0.02	12	8	5
Q5VZB9	Doublesex- and mab-3-related transcription factor A1 OS=Homo sapiens GN=DMRTA1 PE=2 SV=1 - [DMRTA_HUMAN]	1	3	n/a	n/a	n/a	0	0	2	3	2
Q02750	Dual specificity mitogen-activated protein kinase kinase 1 OS=Homo sapiens GN=MAP2K1 PE=1 SV=2 -	1	3	n/a	n/a	n/a	0	0	1	2	3

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
	[MP2K1_HUMAN]										
P36507	Dual specificity mitogen-activated protein kinase kinase 2 OS=Homo sapiens GN=MAP2K2 PE=1 SV=1 - [MP2K2_HUMAN]	1	2	n/a	n/a	n/a	0	0	2	2	1
Q13561	Dynactin subunit 2 OS=Homo sapiens GN=DCTN2 PE=1 SV=4 - [DCTN2_HUMAN]	3	3	0.030	0.001	0.016	58.84	0.0001	3	2	2
Q7Z6Z7	E3 ubiquitin-protein ligase HUWE1 OS=Homo sapiens GN=HUWE1 PE=1 SV=3 - [HUWE1_HUMAN]	12	14	lost	0.002	0.001	1211.67	0.0003	lost	7	10
P62877	E3 ubiquitin-protein ligase RBX1 OS=Homo sapiens GN=RBX1 PE=1 SV=1 - [RBX1_HUMAN]	2	2	n/a	0.214	n/a			1	2	1
Q13049	E3 ubiquitin-protein ligase TRIM32 OS=Homo sapiens GN=TRIM32 PE=1 SV=2 - [TRI32_HUMAN]	3	3	0.001	0.004	0.001	567.98	1.5E-06	2	2	1
Q5T4S7	E3 ubiquitin-protein ligase UBR4 OS=Homo sapiens GN=UBR4 PE=1 SV=1 - [UBR4_HUMAN]	4	6	lost	0.002	0.001	1198.51	0.0003	lost	4	4
Q96C19	EF-hand domain-containing protein D2 OS=Homo sapiens GN=EFHD2 PE=1 SV=1 - [EFHD2_HUMAN]	9	11	0.348	0.437	0.254	2.91	0.05	6	9	9
P38117	Electron transfer flavoprotein subunit beta OS=Homo sapiens GN=ETFB PE=1 SV=3 - [ETFB_HUMAN]	9	9	0.006	0.313	0.196	3.68	0.03	3	7	6
P43897	Elongation factor Ts, mitochondrial OS=Homo sapiens GN=TSFM PE=1 SV=2 - [EFTS_HUMAN]	4	5	0.177	0.335	0.012	3.50	0.04	2	3	3
P49411	Elongation factor Tu, mitochondrial OS=Homo sapiens GN=TUFM PE=1 SV=2 - [EFTU_HUMAN]	15	16	0.456	0.420	0.340	2.78	0.05	13	11	8
Q14240	Eukaryotic initiation factor 4A-II OS=Homo sapiens GN=EIF4A2 PE=1 SV=2 - [IF4A2_HUMAN]	4	15	0.221	0.017	0.011	6.05	0.01	12	10	11

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
Q99613	Eukaryotic translation initiation factor 3 subunit C OS=Homo sapiens GN=EIF3C PE=1 SV=1 - [EIF3C_HUMAN]	5	5	0.026	0.001	0.001	57.98	0.0001	4	4	2
O15372	Eukaryotic translation initiation factor 3 subunit H OS=Homo sapiens GN=EIF3H PE=1 SV=1 - [EIF3H_HUMAN]	3	4	0.004	0.001	0.001	475.29	2.2E-06	3	3	3
P23588	Eukaryotic translation initiation factor 4B OS=Homo sapiens GN=EIF4B PE=1 SV=2 - [IF4B_HUMAN]	8	8	0.381	0.141	0.016	2.99	0.05	6	4	2
Q9NQT5	Exosome complex component RRP40 OS=Homo sapiens GN=EXOSC3 PE=1 SV=3 - [EXOS3_HUMAN]	3	3	0.073	0.045	0.001	22.03	0.001	1	3	2
Q9HAV4	Exportin-5 OS=Homo sapiens GN=XPO5 PE=1 SV=1 - [XPO5_HUMAN]	2	5	lost	n/a	n/a	0	0	lost	3	2
Q9UIA9	Exportin-7 OS=Homo sapiens GN=XPO7 PE=1 SV=3 - [XPO7_HUMAN]	4	4	0.003	0.001	n/a	643.77	0.0005	2	2	1
Q9BSJ8	Extended synaptotagmin-1 OS=Homo sapiens GN=ESYT1 PE=1 SV=1 - [ESYT1_HUMAN]	7	8	0.371	0.003	0.001	3.04	0.05	3	4	3
P52907	F-actin-capping protein subunit alpha-1 OS=Homo sapiens GN=CAPZA1 PE=1 SV=3 - [CAZA1_HUMAN]	3	6	0.347	0.299	n/a	7.31	0.04	4	3	4
Q92945	Far upstream element-binding protein 2 OS=Homo sapiens GN=KHSRP PE=1 SV=4 - [FUBP2_HUMAN]	4	6	0.312	0.391	0.330	6.51	0.01	5	2	3
P49327	Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3 - [FAS_HUMAN]	65	69	0.087	0.371	0.235	3.29	0.04	4	59	61
Q2WGJ9	Fer-1-like protein 6 OS=Homo sapiens GN=FER1L6 PE=2 SV=2 - [FR1L6_HUMAN]	1	2	lost	n/a	n/a	0	0	lost	1	2
Q14192	Four and a half LIM domains protein 2 OS=Homo sapiens GN=FHL2 PE=1 SV=3 - [FHL2_HUMAN]	13	13	0.001	0.001	0.001	1964 9.29	1.3E-09	6	11	11

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
P07954	Fumarate hydratase, mitochondrial OS=Homo sapiens GN=FH PE=1 SV=3 - [FUMH_HUMAN]	5	6	0.003	0.001	0.009	194.62	1.3E-05	6	2	2
P16930	Fumarylacetoacetase OS=Homo sapiens GN=FAH PE=1 SV=2 - [FAAA_HUMAN]	1	1	0.004	0.324	0.001	3.64	0.03	1	1	1
P51570	Galactokinase OS=Homo sapiens GN=GALK1 PE=1 SV=1 - [GALK1_HUMAN]	3	3	0.001	n/a	0.001	2589.56	0.0001	3	1	1
Q8TEQ6	Gem-associated protein 5 OS=Homo sapiens GN=GEMIN5 PE=1 SV=3 - [GEMI5_HUMAN]	7	8	lost	0.025	0.019	156.68	0.002	lost	5	5
P78347	General transcription factor II-I OS=Homo sapiens GN=GTF2I PE=1 SV=2 - [GTF2I_HUMAN]	7	9	0.003	0.005	0.001	425.74	2.8E-06	3	5	3
P11413	Glucose-6-phosphate 1-dehydrogenase OS=Homo sapiens GN=G6PD PE=1 SV=4 - [G6PD_HUMAN]	9	9	0.062	0.001	0.013	25.35	0.0008	5	4	3
P14314	Glucosidase 2 subunit beta OS=Homo sapiens GN=PRKCSH PE=1 SV=2 - [GLU2B_HUMAN]	6	6	0.051	0.003	0.180	7.97	0.008	2	4	4
P78417	Glutathione S-transferase omega-1 OS=Homo sapiens GN=GSTO1 PE=1 SV=2 - [GSTO1_HUMAN]	11	12	0.267	0.349	0.148	4.20	0.03	6	10	10
P30711	Glutathione S-transferase theta-1 OS=Homo sapiens GN=GSTT1 PE=1 SV=4 - [GSTT1_HUMAN]	1	1	0.001	0.001	0.001	0	0	1	1	1
P04406	Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3 - [G3P_HUMAN]	12	13	0.427	0.373	0.253	2.90	0.05	10	10	10
P11216	Glycogen phosphorylase, brain form OS=Homo sapiens GN=PYGB PE=1 SV=5 - [PYGB_HUMAN]	7	11	0.249	0.001	0.112	5.30	0.02	2	10	7
P06737	Glycogen phosphorylase, liver form OS=Homo sapiens GN=PYGL PE=1 SV=4 - [PYGL_HUMAN]	9	15	0.001	0.001	0.001	4849.75	2.1E-08	2	7	11

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
Q92538	Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1 OS=Homo sapiens GN=GBF1 PE=1 SV=2 - [GBF1_HUMAN]	1	3	n/a	n/a	0.001			2	2	2
Q9UIJ7	GTP:AMP phosphotransferase, mitochondrial OS=Homo sapiens GN=AK3 PE=1 SV=4 - [KAD3_HUMAN]	3	4	0.055	0.300	0.179	4.54	0.02	1	3	2
Q14C86	GTPase-activating protein and VPS9 domain-containing protein 1 OS=Homo sapiens GN=GAPVD1 PE=1 SV=2 - [GAPD1_HUMAN]	2	3	lost	0.003	n/a			lost	3	1
Q15382	GTP-binding protein Rheb OS=Homo sapiens GN=RHEB PE=1 SV=1 - [RHEB_HUMAN]	2	2	0.307	0.001	0.001	3.89	0.03	1	1	1
Q9Y6B6	GTP-binding protein SAR1b OS=Homo sapiens GN=SAR1B PE=1 SV=1 - [SAR1B_HUMAN]	1	3	n/a	0.227	n/a			2	2	2
Q9UBI6	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-12 OS=Homo sapiens GN=GNG12 PE=1 SV=3 - [GBG12_HUMAN]	4	4	0.002	0.032	0.010	55.34	0.0002	3	4	2
P63244	Guanine nucleotide-binding protein subunit beta-2-like 1 OS=Homo sapiens GN=GNB2L1 PE=1 SV=3 - [GBLP_HUMAN]	17	18	0.375	0.405	0.217	2.87	0.05	13	14	15
P14866	Heterogeneous nuclear ribonucleoprotein L OS=Homo sapiens GN=HNRNPL PE=1 SV=2 - [HNRPL_HUMAN]	4	4	0.022	0.002	n/a	47.85	0.007	1	4	2
Q1KMD3	Heterogeneous nuclear ribonucleoprotein U-like protein 2 OS=Homo sapiens GN=HNRNPUL2 PE=1 SV=1 - [HNRL2_HUMAN]	2	3	0.001	n/a	0.001	0	0	2	2	1
P52789	Hexokinase-2 OS=Homo sapiens GN=HK2 PE=1 SV=2 -	1	3	n/a	n/a	n/a	0	0	1	3	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
	[HXK2_HUMAN]										
Q92522	Histone H1x OS=Homo sapiens GN=H1FX PE=1 SV=1 - [H1X_HUMAN]	2	2	0.085	0.278	0.149	5.82	0.01	1	1	1
Q96KK5	Histone H2A type 1-H OS=Homo sapiens GN=HIST1H2AH PE=1 SV=3 - [H2A1H_HUMAN]	1	4	0.237	0.366	0.015	2.86	0.05	4	3	3
Q8IUE6	Histone H2A type 2-B OS=Homo sapiens GN=HIST2H2AB PE=1 SV=3 - [H2A2B_HUMAN]	1	3	0.480	n/a	n/a			3	1	1
Q09028	Histone-binding protein RBBP4 OS=Homo sapiens GN=RBBP4 PE=1 SV=3 - [RBBP4_HUMAN]	2	8	0.002	n/a	0.001	728.99	0.0004	6	6	6
Q16576	Histone-binding protein RBBP7 OS=Homo sapiens GN=RBBP7 PE=1 SV=1 - [RBBP7_HUMAN]	2	7	n/a	0.001	0.001	7056.58	4.5E-05	5	6	5
Q86YZ3	Hornerin OS=Homo sapiens GN=HRNR PE=1 SV=2 - [HORN_HUMAN]	4	4	lost	0.007	0.001	174.76	0.002	lost	3	3
Q16543	Hsp90 co-chaperone Cdc37 OS=Homo sapiens GN=CDC37 PE=1 SV=1 - [CDC37_HUMAN]	4	5	0.009	0.020	n/a	85.07	0.004	4	2	2
Q9Y4L1	Hypoxia up-regulated protein 1 OS=Homo sapiens GN=HYOU1 PE=1 SV=1 - [HYOU1_HUMAN]	14	14	lost	0.005	0.001	262.86	0.001	lost	11	7
P01857	Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1 - [IGHG1_HUMAN]	2	6	0.001	0.001	0.001	0	0	2	5	3
P52292	Importin subunit alpha-2 OS=Homo sapiens GN=KPNA2 PE=1 SV=1 - [IMA2_HUMAN]	5	5	0.057	0.003	0.001	26.00	0.0007	4	2	2
Q8NI35	InaD-like protein OS=Homo sapiens GN=INADL PE=1 SV=3 - [INADL_HUMAN]	1	1	0.016	0.001	0.001	100.07	5.0E-05	1	1	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
Q9Y2U8	Inner nuclear membrane protein Man1 OS=Homo sapiens GN=LEMD3 PE=1 SV=2 - [MAN1_HUMAN]	1	1	0.381	n/a	n/a			1	1	1
Q9H2U2	Inorganic pyrophosphatase 2, mitochondrial OS=Homo sapiens GN=PPA2 PE=1 SV=2 - [IPYR2_HUMAN]	3	4	0.306	0.001	0.001	3.91	0.03	2	3	2
Q15181	Inorganic pyrophosphatase OS=Homo sapiens GN=PPA1 PE=1 SV=2 - [IPYR_HUMAN]	8	10	0.327	0.368	0.295	8.12	0.007	6	8	7
P29218	Inositol monophosphatase 1 OS=Homo sapiens GN=IMPA1 PE=1 SV=1 - [IMPA1_HUMAN]	3	3	0.002	0.001	0.020	79.27	8.0E-05	2	3	2
Q9NZI8	Insulin-like growth factor 2 mRNA-binding protein 1 OS=Homo sapiens GN=IGF2BP1 PE=1 SV=2 - [IF2B1_HUMAN]	11	12	0.256	0.020	0.296	3.60	0.03	8	6	8
O00425	Insulin-like growth factor 2 mRNA-binding protein 3 OS=Homo sapiens GN=IGF2BP3 PE=1 SV=2 - [IF2B3_HUMAN]	3	5	n/a	n/a	n/a	0	0	2	4	2
Q13418	Integrin-linked protein kinase OS=Homo sapiens GN=ILK PE=1 SV=2 - [ILK_HUMAN]	7	7	0.039	0.001	0.001	38.31	0.0003	6	3	2
P19823	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Homo sapiens GN=ITIH2 PE=1 SV=2 - [ITIH2_HUMAN]	3	3	0.001	0.001	0.001	9290.78	5.8E-09	2	2	3
O60306	Intron-binding protein aquarius OS=Homo sapiens GN=AQR PE=1 SV=4 - [AQR_HUMAN]	1	1	lost	0.451	n/a			lost	1	1
Q96CN7	Isochorismatase domain-containing protein 1 OS=Homo sapiens GN=ISOC1 PE=1 SV=3 - [ISOC1_HUMAN]	3	4	0.003	0.001	n/a	423.96	0.0008	1	2	2
O43837	Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial OS=Homo sapiens GN=IDH3B PE=1 SV=2 -	4	4	0.264	0.001	0.001	4.69	0.02	1	2	3

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
	[IDH3B_HUMAN]										
P48735	Isocitrate dehydrogenase [NADP], mitochondrial OS=Homo sapiens GN=IDH2 PE=1 SV=2 - [IDHP_HUMAN]	4	6	0.028	0.001	n/a	36.05	0.009	4	3	1
P41252	Isoleucine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=IARS PE=1 SV=2 - [SYIC_HUMAN]	15	18	0.004	0.025	0.001	64.78	0.0001	4	14	13
Q13907	Isopentenyl-diphosphate Delta-isomerase 1 OS=Homo sapiens GN=IDI1 PE=1 SV=2 - [IDI1_HUMAN]	6	7	0.277	0.013	0.010	4.52	0.02	1	6	4
P14923	Junction plakoglobin OS=Homo sapiens GN=JUP PE=1 SV=3 - [PLAK_HUMAN]	2	11	n/a	n/a	n/a	0	0	6	8	10
Q9NSK0	Kinesin light chain 4 OS=Homo sapiens GN=KLC4 PE=1 SV=3 - [KLC4_HUMAN]	7	10	0.001	0.001	0.001	4774.71	2.2E-08	3	4	6
Q14678	KN motif and ankyrin repeat domain-containing protein 1 OS=Homo sapiens GN=KANK1 PE=1 SV=3 - [KANK1_HUMAN]	1	2	lost	n/a	n/a	0	0	lost	1	2
P20700	Lamin-B1 OS=Homo sapiens GN=LMNB1 PE=1 SV=2 - [LMNB1_HUMAN]	5	6	0.040	0.001	0.001	37.27	0.0004	5	2	2
Q01650	Large neutral amino acids transporter small subunit 1 OS=Homo sapiens GN=SLC7A5 PE=1 SV=2 - [LAT1_HUMAN]	5	5	0.262	0.187	0.127	7.91	0.008	2	4	4
Q9NQ48	Leucine zipper transcription factor-like protein 1 OS=Homo sapiens GN=LZTFL1 PE=1 SV=1 - [LZTL1_HUMAN]	5	6	0.004	0.321	0.022	3.74	0.03	2	5	3
A6NHZ5	Leucine-rich repeat-containing protein 14B OS=Homo sapiens GN=LRRC14B PE=3 SV=3 - [LR14B_HUMAN]	1	1	0.001	0.003	0.001	655.85	1.2E-06	1	1	1
P09960	Leukotriene A-4 hydrolase OS=Homo sapiens GN=LTA4H	11	13	0.272	0.002	0.001	4.53	0.02	8	8	5

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
	PE=1 SV=2 - [LKHA4_HUMAN]										
P48059	LIM and senescent cell antigen-like-containing domain protein 1 OS=Homo sapiens GN=LIMS1 PE=1 SV=4 - [LIMS1_HUMAN]	4	6	n/a	0.001	0.014	79.58	0.004	3	3	3
P0CW20	LIM and senescent cell antigen-like-containing domain protein 3-like OS=Homo sapiens GN=LIMS3L PE=2 SV=1 - [LIM3L_HUMAN]	4	4	n/a	0.019	0.001	54.62	0.006	2	2	1
Q14847	LIM and SH3 domain protein 1 OS=Homo sapiens GN=LASP1 PE=1 SV=2 - [LASP1_HUMAN]	12	13	0.355	0.431	0.259	3.04	0.05	8	12	12
P50851	Lipopolysaccharide-responsive and beige-like anchor protein OS=Homo sapiens GN=LRBA PE=1 SV=4 - [LRBA_HUMAN]	5	5	lost	0.036	0.020	57.66	0.006	lost	4	3
Q86W92	Liprin-beta-1 OS=Homo sapiens GN=PPFIBP1 PE=1 SV=2 - [LIPB1_HUMAN]	2	3	n/a	n/a	n/a	0	0	1	3	1
Q9BYZ2	L-lactate dehydrogenase A-like 6B OS=Homo sapiens GN=LDHAL6B PE=1 SV=3 - [LDH6B_HUMAN]	1	3	n/a	0.001	n/a			1	3	2
P36776	Lon protease homolog, mitochondrial OS=Homo sapiens GN=LONP1 PE=1 SV=2 - [LONM_HUMAN]	2	2	lost	n/a	0.001			lost	1	1
O95573	Long-chain-fatty-acid--CoA ligase 3 OS=Homo sapiens GN=ACSL3 PE=1 SV=3 - [ACSL3_HUMAN]	2	3	n/a	n/a	n/a	0	0	2	2	1
O60488	Long-chain-fatty-acid--CoA ligase 4 OS=Homo sapiens GN=ACSL4 PE=1 SV=2 - [ACSL4_HUMAN]	3	4	0.001	0.001	n/a	4864.28	6.5E-05	3	2	1
P10619	Lysosomal protective protein OS=Homo sapiens GN=CTSA PE=1 SV=2 - [PPGB_HUMAN]	8	8	0.001	0.032	0.001	47.83	0.0002	3	5	7

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
Q14165	Malectin OS=Homo sapiens GN=MLEC PE=1 SV=1 - [MLEC_HUMAN]	4	4	0.293	0.398	0.396	3.97	0.03	1	4	4
Q96IJ6	Mannose-1-phosphate guanyltransferase alpha OS=Homo sapiens GN=GMPPA PE=1 SV=1 - [GMPPA_HUMAN]	5	5	0.002	n/a	0.001	972.69	0.0003	5	1	1
O75352	Mannose-P-dolichol utilization defect 1 protein OS=Homo sapiens GN=MPDU1 PE=1 SV=2 - [MPU1_HUMAN]	1	1	0.205	0.076	0.001	6.82	0.01	1	1	1
Q96QZ7	Membrane-associated guanylate kinase, WW and PDZ domain-containing protein 1 OS=Homo sapiens GN=MAGI1 PE=1 SV=3 - [MAGI1_HUMAN]	4	6	lost	n/a	0.001			lost	2	4
O00264	Membrane-associated progesterone receptor component 1 OS=Homo sapiens GN=PGRMC1 PE=1 SV=3 - [PGRC1_HUMAN]	2	5	n/a	0.219	0.200	30.88	0.01	1	3	4
Q9UPN3	Microtubule-actin cross-linking factor 1, isoforms 1/2/3/5 OS=Homo sapiens GN=MACF1 PE=1 SV=4 - [MACF1_HUMAN]	33	37	lost	0.002	0.001	1408.87	0.0002	lost	23	26
Q8N4C8	Misshapen-like kinase 1 OS=Homo sapiens GN=MINK1 PE=1 SV=2 - [MINK1_HUMAN]	1	2	n/a	n/a	n/a	0	0	1	2	1
Q9Y3D6	Mitochondrial fission 1 protein OS=Homo sapiens GN=FIS1 PE=1 SV=2 - [FIS1_HUMAN]	3	3	0.365	0.361	0.135	2.80	0.05	2	3	2
Q3ZCQ8	Mitochondrial import inner membrane translocase subunit TIM50 OS=Homo sapiens GN=TIMM50 PE=1 SV=2 - [TIM50_HUMAN]	6	7	0.259	0.021	0.001	4.91	0.02	4	4	4
O75439	Mitochondrial-processing peptidase subunit beta OS=Homo sapiens GN=PMPCB PE=1 SV=2 - [MPPB_HUMAN]	2	2	n/a	n/a	n/a	0	0	2	1	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
O43684	Mitotic checkpoint protein BUB3 OS=Homo sapiens GN=BUB3 PE=1 SV=1 - [BUB3_HUMAN]	5	5	0.033	0.164	0.139	9.66	0.005	4	5	4
P78406	mRNA export factor OS=Homo sapiens GN=RAE1 PE=1 SV=1 - [RAE1L_HUMAN]	4	5	0.015	0.002	0.001	109.47	4.2E-05	3	4	4
Q9NR56	Muscleblind-like protein 1 OS=Homo sapiens GN=MBNL1 PE=1 SV=2 - [MBNL1_HUMAN]	4	4	0.215	0.300	0.010	3.78	0.03	2	4	3
Q9NZM1	Myoferlin OS=Homo sapiens GN=MYOF PE=1 SV=1 - [MYOF_HUMAN]	4	6	lost	n/a	0.020			lost	1	5
O14745	Na(+)/H(+) exchange regulatory cofactor NHE-RF1 OS=Homo sapiens GN=SLC9A3R1 PE=1 SV=4 - [NHRF1_HUMAN]	5	6	0.025	0.001	0.001	62.38	0.0001	5	3	4
Q15599	Na(+)/H(+) exchange regulatory cofactor NHE-RF2 OS=Homo sapiens GN=SLC9A3R2 PE=1 SV=2 - [NHRF2_HUMAN]	5	5	n/a	0.002	0.001	1867.29	0.0002	1	2	5
O75489	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial OS=Homo sapiens GN=NDUFS3 PE=1 SV=1 - [NDUS3_HUMAN]	2	3	0.407	n/a	n/a			1	1	3
Q9GZZ1	N-alpha-acetyltransferase 50 OS=Homo sapiens GN=NAA50 PE=1 SV=1 - [NAA50_HUMAN]	4	5	0.115	0.284	0.013	4.59	0.02	2	4	2
O43847	Nardilysin OS=Homo sapiens GN=NRD1 PE=1 SV=2 - [NRDC_HUMAN]	2	2	lost	n/a	n/a	0	0	lost	1	1
Q6X4W1	Nasal embryonic luteinizing hormone-releasing hormone factor OS=Homo sapiens GN=NELF PE=1 SV=1 - [NELF_HUMAN]	2	3	0.002	0.002	0.001	1289.60	3.0E-07	1	2	3
O76041	Nebulette OS=Homo sapiens GN=NEBL PE=1 SV=1 - [NEBL_HUMAN]	2	3	lost	0.263	n/a			lost	1	3
P12036	Neurofilament heavy polypeptide OS=Homo sapiens	1	4	n/a	n/a	n/a	0	0	2	3	3

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
	GN=NEFH PE=1 SV=4 - [NFH_HUMAN]										
Q14697	Neutral alpha-glucosidase AB OS=Homo sapiens GN=GANAB PE=1 SV=3 - [GANAB_HUMAN]	15	15	0.379	0.023	0.001	2.98	0.05	9	10	8
Q9Y314	Nitric oxide synthase-interacting protein OS=Homo sapiens GN=NOSIP PE=1 SV=1 - [NOSIP_HUMAN]	5	5	0.002	0.002	0.191	6.92	0.01	2	3	3
Q15233	Non-POU domain-containing octamer-binding protein OS=Homo sapiens GN=NONO PE=1 SV=4 - [NONO_HUMAN]	5	8	0.090	n/a	0.016	12.12	0.03	4	4	5
Q9NVX2	Notchless protein homolog 1 OS=Homo sapiens GN=NLE1 PE=1 SV=4 - [NLE1_HUMAN]	1	1	0.010	n/a	n/a			1	1	1
P49321	Nuclear autoantigenic sperm protein OS=Homo sapiens GN=NASP PE=1 SV=2 - [NASP_HUMAN]	4	4	0.395	0.331	0.229	3.77	0.03	2	2	3
Q15596	Nuclear receptor coactivator 2 OS=Homo sapiens GN=NCOA2 PE=1 SV=2 - [NCOA2_HUMAN]	1	1	lost	0.001	0.001	0	0	lost	1	1
P67809	Nuclease-sensitive element-binding protein 1 OS=Homo sapiens GN=YBX1 PE=1 SV=3 - [YBOX1_HUMAN]	2	5	0.029	0.045	0.247	5.62	0.02	4	4	3
P12270	Nucleoprotein TPR OS=Homo sapiens GN=TPR PE=1 SV=3 - [TPR_HUMAN]	8	10	lost	n/a	0.012			lost	4	8
P55209	Nucleosome assembly protein 1-like 1 OS=Homo sapiens GN=NAP1L1 PE=1 SV=1 - [NP1L1_HUMAN]	3	4	0.001	0.014	0.010	129.28	3.0E-05	2	3	4
Q9Y239	Nucleotide-binding oligomerization domain-containing protein 1 OS=Homo sapiens GN=NOD1 PE=1 SV=1 - [NOD1_HUMAN]	1	2	lost	0.001	0.001	0	0	lost	2	1
Q96RS6	NudC domain-containing protein 1 OS=Homo sapiens	2	2	n/a	0.001	n/a			1	1	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
	GN=NUDCD1 PE=1 SV=2 - [NUDC1_HUMAN]										
P50479	PDZ and LIM domain protein 4 OS=Homo sapiens GN=PDLIM4 PE=1 SV=2 - [PDLI4_HUMAN]	5	6	0.002	0.001	0.001	1925.81	1.3E-07	4	5	4
Q9NR12	PDZ and LIM domain protein 7 OS=Homo sapiens GN=PDLIM7 PE=1 SV=1 - [PDLI7_HUMAN]	10	10	0.001	0.001	0.001	0	0	9	7	7
Q08752	Peptidyl-prolyl cis-trans isomerase D OS=Homo sapiens GN=PPID PE=1 SV=3 - [PPID_HUMAN]	6	8	0.049	0.001	n/a	19.94	0.02	6	3	2
O43447	Peptidyl-prolyl cis-trans isomerase H OS=Homo sapiens GN=PPIH PE=1 SV=1 - [PPIH_HUMAN]	3	4	n/a	0.017	n/a			1	2	3
P30044	Peroxiredoxin-5, mitochondrial OS=Homo sapiens GN=PRDX5 PE=1 SV=4 - [PRDX5_HUMAN]	6	6	0.036	0.354	0.001	3.29	0.04	3	6	5
Q08426	Peroxisomal bifunctional enzyme OS=Homo sapiens GN=EHHADH PE=1 SV=3 - [ECHP_HUMAN]	3	3	0.006	n/a	n/a			2	1	2
O75420	PERQ amino acid-rich with GYF domain-containing protein 1 OS=Homo sapiens GN=GIGYF1 PE=1 SV=2 - [PERQ1_HUMAN]	7	8	0.015	0.002	0.001	114.20	3.8E-05	2	3	5
Q8WW12	PEST proteolytic signal-containing nuclear protein OS=Homo sapiens GN=PCNP PE=1 SV=2 - [PCNP_HUMAN]	2	2	n/a	0.112	n/a			1	1	2
O00443	Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit alpha OS=Homo sapiens GN=PIK3C2A PE=1 SV=2 - [P3C2A_HUMAN]	5	6	lost	n/a	0.030			lost	4	4
Q9Y617	Phosphoserine aminotransferase OS=Homo sapiens GN=PSAT1 PE=1 SV=2 - [SERC_HUMAN]	9	10	0.130	0.254	0.218	8.13	0.007	8	6	5
Q99959	Plakophilin-2 OS=Homo sapiens GN=PKP2 PE=1 SV=2 -	7	9	0.004	0.002	0.001	497.0	2.0E-	5	5	5

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
	[PKP2_HUMAN]						8	06			
Q15365	Poly(rC)-binding protein 1 OS=Homo sapiens GN=PCBP1 PE=1 SV=2 - [PCBP1_HUMAN]	4	6	0.413	0.396	0.251	2.86	0.05	6	6	5
P26599	Polypyrimidine tract-binding protein 1 OS=Homo sapiens GN=PTBP1 PE=1 SV=1 - [PTBP1_HUMAN]	6	6	0.020	n/a	0.017	313.7 8	0.001	3	5	3
P20742	Pregnancy zone protein OS=Homo sapiens GN=PZP PE=1 SV=4 - [PZP_HUMAN]	1	2	0.001	0.001	0.001	0	0	2	2	2
Q13206	Probable ATP-dependent RNA helicase DDX10 OS=Homo sapiens GN=DDX10 PE=1 SV=2 - [DDX10_HUMAN]	1	2	lost	n/a	n/a	0	0	lost	1	1
P25788	Proteasome subunit alpha type-3 OS=Homo sapiens GN=PSMA3 PE=1 SV=2 - [PSA3_HUMAN]	7	8	0.417	0.416	0.347	4.62	0.02	6	3	5
P25789	Proteasome subunit alpha type-4 OS=Homo sapiens GN=PSMA4 PE=1 SV=1 - [PSA4_HUMAN]	6	6	0.440	0.377	0.308	3.28	0.04	5	6	4
P28066	Proteasome subunit alpha type-5 OS=Homo sapiens GN=PSMA5 PE=1 SV=3 - [PSA5_HUMAN]	5	6	0.001	0.282	0.307	3.10	0.05	3	5	6
P60900	Proteasome subunit alpha type-6 OS=Homo sapiens GN=PSMA6 PE=1 SV=1 - [PSA6_HUMAN]	10	10	0.434	0.411	0.379	5.78	0.01	5	7	9
Q99873	Protein arginine N-methyltransferase 1 OS=Homo sapiens GN=PRMT1 PE=1 SV=2 - [ANM1_HUMAN]	11	11	0.320	0.344	0.217	5.27	0.02	8	8	5
O14744	Protein arginine N-methyltransferase 5 OS=Homo sapiens GN=PRMT5 PE=1 SV=4 - [ANM5_HUMAN]	4	5	0.007	0.001	n/a	156.1 7	0.002	3	3	1
Q9Y2B0	Protein canopy homolog 2 OS=Homo sapiens GN=CNPY2 PE=1 SV=1 - [CNPY2_HUMAN]	3	3	n/a	0.221	n/a			1	3	2

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
Q9NUQ9	Protein FAM49B OS=Homo sapiens GN=FAM49B PE=1 SV=1 - [FA49B_HUMAN]	2	2	n/a	0.001	0.001	0	0	1	2	1
Q13045	Protein flightless-1 homolog OS=Homo sapiens GN=FLII PE=1 SV=2 - [FLII_HUMAN]	8	9	lost	0.003	0.001	573.46	0.0006	lost	5	4
P61326	Protein mago nashi homolog OS=Homo sapiens GN=MAGOH PE=1 SV=1 - [MGN_HUMAN]	3	3	n/a	0.342	n/a			1	1	1
Q92597	Protein NDRG1 OS=Homo sapiens GN=NDRG1 PE=1 SV=1 - [NDRG1_HUMAN]	2	3	0.077	0.022	0.001	20.72	0.001	2	3	2
Q9BPW8	Protein NipSnap homolog 1 OS=Homo sapiens GN=NIPSNAP1 PE=1 SV=1 - [NIPS1_HUMAN]	6	7	n/a	0.001	0.130	6.73	0.05	3	6	4
O75323	Protein NipSnap homolog 2 OS=Homo sapiens GN=GBAS PE=1 SV=1 - [NIPS2_HUMAN]	4	5	n/a	0.107	0.001	8.39	0.04	1	4	3
Q9H7J1	Protein phosphatase 1 regulatory subunit 3E OS=Homo sapiens GN=PPP1R3E PE=2 SV=2 - [PPR3E_HUMAN]	2	3	n/a	n/a	0.209			2	1	1
O15355	Protein phosphatase 1G OS=Homo sapiens GN=PPM1G PE=1 SV=1 - [PPM1G_HUMAN]	5	5	0.002	0.001	0.021	74.15	9.1E-05	2	3	2
P41236	Protein phosphatase inhibitor 2 OS=Homo sapiens GN=PPP1R2 PE=1 SV=2 - [IPP2_HUMAN]	3	3	0.004	0.261	0.147	4.88	0.02	1	3	2
Q9Y570	Protein phosphatase methylesterase 1 OS=Homo sapiens GN=PPME1 PE=1 SV=3 - [PPME1_HUMAN]	5	5	0.002	0.328	0.001	3.58	0.04	4	3	4
P48634	Protein PRRC2A OS=Homo sapiens GN=PRRC2A PE=1 SV=3 - [PRC2A_HUMAN]	3	3	lost	0.033	0.001	30.47	0.01	lost	2	2
Q9P258	Protein RCC2 OS=Homo sapiens GN=RCC2 PE=1 SV=2 - [RCC2_HUMAN]	4	4	0.002	0.001	0.001	1640.56	1.9E-07	2	2	3

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
Q01105	Protein SET OS=Homo sapiens GN=SET PE=1 SV=3 - [SET_HUMAN]	4	4	0.026	0.250	0.001	5.15	0.02	3	3	4
Q969Z0	Protein TBRG4 OS=Homo sapiens GN=TBRG4 PE=1 SV=1 - [TBRG4_HUMAN]	2	2	0.002	n/a	n/a			1	1	2
P60468	Protein transport protein Sec61 subunit beta OS=Homo sapiens GN=SEC61B PE=1 SV=2 - [SC61B_HUMAN]	3	3	0.001	0.131	0.026	11.28	0.004	1	3	3
Q7L2E3	Putative ATP-dependent RNA helicase DHX30 OS=Homo sapiens GN=DHX30 PE=1 SV=1 - [DHX30_HUMAN]	6	7	lost	0.056	n/a			lost	4	4
Q9BZK3	Putative nascent polypeptide-associated complex subunit alpha-like protein OS=Homo sapiens GN=NACAP1 PE=5 SV=1 - [NACP1_HUMAN]	1	2	0.379	n/a	n/a			1	1	1
Q9NQ29	Putative RNA-binding protein Luc7-like 1 OS=Homo sapiens GN=LUC7L PE=1 SV=1 - [LUC7L_HUMAN]	1	3	n/a	0.001	0.001	0	0	3	1	2
Q9Y383	Putative RNA-binding protein Luc7-like 2 OS=Homo sapiens GN=LUC7L2 PE=1 SV=2 - [LC7L2_HUMAN]	4	6	0.065	0.048	0.018	33.30	0.0005	4	3	3
O00764	Pyridoxal kinase OS=Homo sapiens GN=PDXK PE=1 SV=1 - [PDXK_HUMAN]	5	6	n/a	0.032	0.141	7.55	0.04	1	5	5
P32322	Pyrroline-5-carboxylate reductase 1, mitochondrial OS=Homo sapiens GN=PYCR1 PE=1 SV=2 - [P5CR1_HUMAN]	5	6	0.188	0.115	0.001	7.34	0.009	5	2	4
Q96C36	Pyrroline-5-carboxylate reductase 2 OS=Homo sapiens GN=PYCR2 PE=1 SV=1 - [P5CR2_HUMAN]	7	8	0.003	0.048	0.001	31.27	0.0005	4	5	6
P61026	Ras-related protein Rab-10 OS=Homo sapiens GN=RAB10 PE=1 SV=1 - [RAB10_HUMAN]	4	8	0.399	0.337	0.352	7.36	0.009	4	6	7
P62820	Ras-related protein Rab-1A OS=Homo sapiens GN=RAB1A	4	13	0.368	0.422	0.253	3.05	0.05	12	9	8

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
	PE=1 SV=3 - [RAB1A_HUMAN]										
Q9H0U4	Ras-related protein Rab-1B OS=Homo sapiens GN=RAB1B PE=1 SV=1 - [RAB1B_HUMAN]	2	10	0.272	0.235	0.243	22.34	0.001	9	9	8
Q9UL25	Ras-related protein Rab-21 OS=Homo sapiens GN=RAB21 PE=1 SV=3 - [RAB21_HUMAN]	2	2	0.267	0.300	0.001	3.29	0.04	1	1	2
P20338	Ras-related protein Rab-4A OS=Homo sapiens GN=RAB4A PE=1 SV=3 - [RAB4A_HUMAN]	1	2	n/a	n/a	0.001			1	1	2
P20339	Ras-related protein Rab-5A OS=Homo sapiens GN=RAB5A PE=1 SV=2 - [RAB5A_HUMAN]	1	4	n/a	0.001	n/a			3	3	2
P61020	Ras-related protein Rab-5B OS=Homo sapiens GN=RAB5B PE=1 SV=1 - [RAB5B_HUMAN]	2	4	n/a	0.388	n/a			2	4	3
P51148	Ras-related protein Rab-5C OS=Homo sapiens GN=RAB5C PE=1 SV=2 - [RAB5C_HUMAN]	3	5	0.284	0.036	0.134	4.82	0.02	5	5	5
P61006	Ras-related protein Rab-8A OS=Homo sapiens GN=RAB8A PE=1 SV=1 - [RAB8A_HUMAN]	3	8	n/a	0.015	n/a			4	6	8
Q9NQG5	Regulation of nuclear pre-mRNA domain-containing protein 1B OS=Homo sapiens GN=RPRD1B PE=1 SV=1 - [RPR1B_HUMAN]	2	2	n/a	n/a	0.001			1	1	1
Q92900	Regulator of nonsense transcripts 1 OS=Homo sapiens GN=UPF1 PE=1 SV=2 - [RENT1_HUMAN]	12	12	0.001	0.075	0.001	19.31	0.001	3	5	10
Q8WUF5	RelA-associated inhibitor OS=Homo sapiens GN=PPP1R13L PE=1 SV=4 - [IASPP_HUMAN]	12	13	0.002	0.001	0.001	1373.16	2.7E-07	5	3	9
P40938	Replication factor C subunit 3 OS=Homo sapiens GN=RFC3 PE=1 SV=2 - [RFC3_HUMAN]	2	2	0.004	0.002	0.318	3.74	0.03	1	2	1

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Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
Q15293	Reticulocalbin-1 OS=Homo sapiens GN=RCN1 PE=1 SV=1 - [RCN1_HUMAN]	7	7	0.339	0.004	0.001	3.43	0.04	6	5	5
Q9NQC3	Reticulon-4 OS=Homo sapiens GN=RTN4 PE=1 SV=2 - [RTN4_HUMAN]	3	4	0.326	0.017	0.001	3.64	0.03	4	2	3
Q8TC12	Retinol dehydrogenase 11 OS=Homo sapiens GN=RDH11 PE=1 SV=2 - [RDH11_HUMAN]	2	2	n/a	0.005	n/a			1	1	1
P78346	Ribonuclease P protein subunit p30 OS=Homo sapiens GN=RPP30 PE=1 SV=1 - [RPP30_HUMAN]	5	6	n/a	0.001	0.001	0	0	2	4	3
P11908	Ribose-phosphate pyrophosphokinase 2 OS=Homo sapiens GN=PRPS2 PE=1 SV=2 - [PRPS2_HUMAN]	1	4	0.012	n/a	0.001	91.63	0.003	3	2	2
P38159	RNA-binding motif protein, X chromosome OS=Homo sapiens GN=RBMX PE=1 SV=3 - [RBMX_HUMAN]	5	6	0.287	0.010	0.261	3.56	0.04	5	4	2
P35637	RNA-binding protein FUS OS=Homo sapiens GN=FUS PE=1 SV=1 - [FUS_HUMAN]	2	2	0.126	0.237	0.013	5.79	0.01	2	2	2
Q9Y265	RuvB-like 1 OS=Homo sapiens GN=RUVBL1 PE=1 SV=1 - [RUVB1_HUMAN]	19	19	0.004	0.002	0.001	562.41	1.6E-06	16	14	16
Q9Y230	RuvB-like 2 OS=Homo sapiens GN=RUVBL2 PE=1 SV=3 - [RUVB2_HUMAN]	25	25	0.017	0.001	0.001	91.06	6.0E-05	20	20	23
P31153	S-adenosylmethionine synthase isoform type-2 OS=Homo sapiens GN=MAT2A PE=1 SV=1 - [METK2_HUMAN]	6	6	0.032	0.338	0.014	3.54	0.04	5	4	4
Q9NVA2	Septin-11 OS=Homo sapiens GN=SEPT11 PE=1 SV=3 - [SEP11_HUMAN]	5	7	0.428	0.329	0.344	4.29	0.03	5	5	5
Q15019	Septin-2 OS=Homo sapiens GN=SEPT2 PE=1 SV=1 - [SEPT2_HUMAN]	8	8	0.048	0.280	0.317	3.38	0.04	7	5	4

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
Q01130	Serine/arginine-rich splicing factor 2 OS=Homo sapiens GN=SRSF2 PE=1 SV=4 - [SRSF2_HUMAN]	5	6	0.342	0.279	0.253	7.85	0.008	3	4	4
Q13043	Serine/threonine-protein kinase 4 OS=Homo sapiens GN=STK4 PE=1 SV=2 - [STK4_HUMAN]	1	1	0.019	0.002	n/a	56.66	0.006	1	1	1
P63151	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform OS=Homo sapiens GN=PPP2R2A PE=1 SV=1 - [2ABA_HUMAN]	4	5	n/a	0.014	0.001	78.29	0.004	1	3	4
P36873	Serine/threonine-protein phosphatase PP1-gamma catalytic subunit OS=Homo sapiens GN=PPP1CC PE=1 SV=1 - [PP1G_HUMAN]	2	8	0.003	n/a	n/a			2	8	8
P49591	Serine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=SARS PE=1 SV=3 - [SYSC_HUMAN]	9	9	0.004	0.010	0.001	180.42	1.5E-05	2	5	6
P50454	Serpin H1 OS=Homo sapiens GN=SERPINH1 PE=1 SV=2 - [SERPH_HUMAN]	12	13	0.284	0.401	0.123	2.87	0.05	9	6	8
Q9NR45	Sialic acid synthase OS=Homo sapiens GN=NANS PE=1 SV=2 - [SIAS_HUMAN]	3	3	0.002	0.017	0.001	97.44	5.3E-05	2	3	2
Q15005	Signal peptidase complex subunit 2 OS=Homo sapiens GN=SPCS2 PE=1 SV=3 - [SPCS2_HUMAN]	1	2	n/a	0.391	n/a			1	2	1
Q9Y5M8	Signal recognition particle receptor subunit beta OS=Homo sapiens GN=SRPRB PE=1 SV=3 - [SRPRB_HUMAN]	7	10	0.202	0.019	0.079	7.42	0.009	4	7	3
P42224	Signal transducer and activator of transcription 1-alpha/beta OS=Homo sapiens GN=STAT1 PE=1 SV=2 - [STAT1_HUMAN]	7	8	0.395	0.001	0.001	2.80	0.05	2	6	4
P62304	Small nuclear ribonucleoprotein E OS=Homo sapiens	2	2	0.230	0.256	0.116	7.00	0.01	2	2	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
	GN=SNRPE PE=1 SV=1 - [RUXE_HUMAN]										
P62318	Small nuclear ribonucleoprotein Sm D3 OS=Homo sapiens GN=SNRPD3 PE=1 SV=1 - [SMD3_HUMAN]	2	2	0.257	0.433	0.189	2.85	0.05	2	1	1
P05023	Sodium/potassium-transporting ATPase subunit alpha-1 OS=Homo sapiens GN=ATP1A1 PE=1 SV=1 - [AT1A1_HUMAN]	15	16	0.272	0.034	0.001	4.67	0.02	7	12	8
Q9BX66	Sorbin and SH3 domain-containing protein 1 OS=Homo sapiens GN=SORBS1 PE=1 SV=3 - [SRBS1_HUMAN]	4	5	0.037	n/a	0.001	32.85	0.0005	4	1	1
Q00796	Sorbitol dehydrogenase OS=Homo sapiens GN=SORD PE=1 SV=4 - [DHSO_HUMAN]	5	6	0.031	0.168	0.001	8.45	0.007	5	4	3
Q9Y5X3	Sorting nexin-5 OS=Homo sapiens GN=SNX5 PE=1 SV=1 - [SNX5_HUMAN]	4	5	0.005	n/a	0.001	233.17	0.001	3	1	1
P63208	S-phase kinase-associated protein 1 OS=Homo sapiens GN=SKP1 PE=1 SV=2 - [SKP1_HUMAN]	3	4	n/a	0.020	0.015	214.85	0.001	2	3	2
Q13838	Spliceosome RNA helicase DDX39B OS=Homo sapiens GN=DDX39B PE=1 SV=1 - [DX39B_HUMAN]	2	11	0.001	0.024	0.265	4.79	0.02	7	9	5
Q12874	Splicing factor 3A subunit 3 OS=Homo sapiens GN=SF3A3 PE=1 SV=1 - [SF3A3_HUMAN]	2	3	0.001	n/a	n/a			1	2	1
O75533	Splicing factor 3B subunit 1 OS=Homo sapiens GN=SF3B1 PE=1 SV=3 - [SF3B1_HUMAN]	15	16	0.008	0.004	0.001	262.38	7.3E-06	5	10	12
Q15393	Splicing factor 3B subunit 3 OS=Homo sapiens GN=SF3B3 PE=1 SV=4 - [SF3B3_HUMAN]	3	4	lost	n/a	0.001			lost	3	3
P23246	Splicing factor, proline- and glutamine-rich OS=Homo sapiens GN=SFPQ PE=1 SV=2 - [SFPQ_HUMAN]	4	5	n/a	0.002	0.001	678.98	0.0005	2	4	5

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
Q9UJZ1	Stomatin-like protein 2 OS=Homo sapiens GN=STOML2 PE=1 SV=1 - [STML2_HUMAN]	6	7	0.244	0.058	0.024	5.71	0.01	6	3	3
A6NHR9	Structural maintenance of chromosomes flexible hinge domain-containing protein 1 OS=Homo sapiens GN=SMCHD1 PE=1 SV=2 - [SMHD1_HUMAN]	1	2	lost	n/a	0.266			lost	1	1
Q9UQE7	Structural maintenance of chromosomes protein 3 OS=Homo sapiens GN=SMC3 PE=1 SV=2 - [SMC3_HUMAN]	9	12	lost	0.009	0.001	121.33	0.003	lost	7	7
P53597	Succinyl-CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial OS=Homo sapiens GN=SUCLG1 PE=1 SV=4 - [SUCA_HUMAN]	5	5	0.325	0.362	n/a	8.39	0.04	2	5	4
Q9UBE0	SUMO-activating enzyme subunit 1 OS=Homo sapiens GN=SAE1 PE=1 SV=1 - [SAE1_HUMAN]	6	7	0.259	0.393	0.311	4.62	0.02	5	3	6
Q9UBT2	SUMO-activating enzyme subunit 2 OS=Homo sapiens GN=UBA2 PE=1 SV=2 - [SAE2_HUMAN]	10	12	0.356	0.001	0.001	3.22	0.04	8	7	6
Q9Y4G6	Talin-2 OS=Homo sapiens GN=TLN2 PE=1 SV=4 - [TLN2_HUMAN]	1	4	lost	n/a	n/a	0	0	lost	2	3
O60343	TBC1 domain family member 4 OS=Homo sapiens GN=TBC1D4 PE=1 SV=2 - [TBCD4_HUMAN]	9	10	lost	0.002	0.001	1809.01	0.0002	lost	8	6
P78371	T-complex protein 1 subunit beta OS=Homo sapiens GN=CCT2 PE=1 SV=4 - [TCPB_HUMAN]	15	16	0.399	0.017	0.001	2.78	0.05	11	11	7
Q9UGI8	Testin OS=Homo sapiens GN=TES PE=1 SV=1 - [TES_HUMAN]	10	10	0.319	0.269	0.003	3.09	0.05	6	7	4
P04183	Thymidine kinase, cytosolic OS=Homo sapiens GN=TK1 PE=1 SV=2 - [KITH_HUMAN]	2	2	0.004	n/a	n/a			1	1	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
Q9Y2W1	Thyroid hormone receptor-associated protein 3 OS=Homo sapiens GN=THRAP3 PE=1 SV=2 - [TR150_HUMAN]	2	2	lost	0.001	0.001	3553.00	9.0E-05	lost	2	2
Q9UDY2	Tight junction protein ZO-2 OS=Homo sapiens GN=TJP2 PE=1 SV=2 - [ZO2_HUMAN]	6	7	lost	0.241	0.176	8.94	0.04	lost	6	2
Q8WZ42	Titin OS=Homo sapiens GN=TTN PE=1 SV=4 - [TITIN_HUMAN]	2	22	n/a	n/a	n/a	0	0	12	8	8
O00267	Transcription elongation factor SPT5 OS=Homo sapiens GN=SUPT5H PE=1 SV=1 - [SPT5H_HUMAN]	4	4	0.005	0.003	0.014	155.17	2.1E-05	1	1	4
Q13263	Transcription intermediary factor 1-beta OS=Homo sapiens GN=TRIM28 PE=1 SV=5 - [TIF1B_HUMAN]	8	8	0.033	0.286	0.001	4.37	0.02	6	7	6
Q92616	Translational activator GCN1 OS=Homo sapiens GN=GCN1L1 PE=1 SV=6 - [GCN1L_HUMAN]	28	31	lost	0.002	0.001	798.75	0.0004	lost	21	24
Q15631	Translin OS=Homo sapiens GN=TSN PE=1 SV=1 - [TSN_HUMAN]	4	6	0.083	0.388	0.089	3.12	0.04	2	3	5
P02766	Transthyretin OS=Homo sapiens GN=TTR PE=1 SV=1 - [TTHY_HUMAN]	5	5	0.001	0.002	0.001	2628.75	7.2E-08	2	5	3
P40939	Trifunctional enzyme subunit alpha, mitochondrial OS=Homo sapiens GN=HADHA PE=1 SV=2 - [ECHA_HUMAN]	11	11	0.351	0.019	0.001	3.30	0.04	9	4	5
Q14134	Tripartite motif-containing protein 29 OS=Homo sapiens GN=TRIM29 PE=1 SV=2 - [TRI29_HUMAN]	6	6	0.001	0.002	0.001	2171.14	1.1E-07	3	3	5
P29144	Tripeptidyl-peptidase 2 OS=Homo sapiens GN=TPP2 PE=1 SV=4 - [TPP2_HUMAN]	9	9	lost	0.005	0.001	234.66	0.001	lost	3	7
P67936	Tropomyosin alpha-4 chain OS=Homo sapiens GN=TPM4 PE=1 SV=3 - [TPM4_HUMAN]	5	7	0.033	0.023	n/a	96.49	0.003	4	6	4

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
Q13885	Tubulin beta-2A chain OS=Homo sapiens GN=TUBB2A PE=1 SV=1 - [TBB2A_HUMAN]	1	17	0.001	n/a	n/a			17	17	17
Q9BUF5	Tubulin beta-6 chain OS=Homo sapiens GN=TUBB6 PE=1 SV=1 - [TBB6_HUMAN]	7	14	0.007	0.398	0.001	2.78	0.05	12	12	13
Q9BTW9	Tubulin-specific chaperone D OS=Homo sapiens GN=TBCD PE=1 SV=2 - [TBCD_HUMAN]	2	3	n/a	n/a	n/a	0	0	1	1	1
P18031	Tyrosine-protein phosphatase non-receptor type 1 OS=Homo sapiens GN=PTPN1 PE=1 SV=1 - [PTN1_HUMAN]	6	7	0.379	0.001	0.001	2.96	0.05	4	4	4
Q9H3S7	Tyrosine-protein phosphatase non-receptor type 23 OS=Homo sapiens GN=PTPN23 PE=1 SV=1 - [PTN23_HUMAN]	2	2	lost	n/a	n/a	0	0	lost	1	1
P45974	Ubiquitin carboxyl-terminal hydrolase 5 OS=Homo sapiens GN=USP5 PE=1 SV=2 - [UBP5_HUMAN]	7	9	0.068	0.001	0.001	21.30	0.001	5	5	2
Q13404	Ubiquitin-conjugating enzyme E2 variant 1 OS=Homo sapiens GN=UBE2V1 PE=1 SV=2 - [UB2V1_HUMAN]	1	5	0.013	0.241	0.001	5.31	0.02	4	5	3
Q15819	Ubiquitin-conjugating enzyme E2 variant 2 OS=Homo sapiens GN=UBE2V2 PE=1 SV=4 - [UB2V2_HUMAN]	2	4	0.003	0.084	n/a	11.30	0.03	3	4	2
P22314	Ubiquitin-like modifier-activating enzyme 1 OS=Homo sapiens GN=UBA1 PE=1 SV=3 - [UBA1_HUMAN]	15	16	0.388	0.017	0.001	2.88	0.05	15	12	10
Q04323	UBX domain-containing protein 1 OS=Homo sapiens GN=UBXN1 PE=1 SV=2 - [UBXN1_HUMAN]	3	4	n/a	n/a	0.001			1	1	2
Q86XI8	Uncharacterized protein C19orf68 OS=Homo sapiens GN=C19orf68 PE=1 SV=2 - [CS068_HUMAN]	1	2	n/a	n/a	n/a	0	0	2	2	1
O00159	Unconventional myosin-Ic OS=Homo sapiens GN=MYO1C PE=1 SV=4 - [MYO1C_HUMAN]	15	18	0.181	0.008	0.116	7.90	0.008	11	7	7

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
O94832	Unconventional myosin-Id OS=Homo sapiens GN=MYO1D PE=1 SV=2 - [MYO1D_HUMAN]	4	4	0.014	n/a	0.001	78.38	0.004	1	1	2
Q9HB07	UPF0160 protein MYG1, mitochondrial OS=Homo sapiens GN=C12orf10 PE=1 SV=2 - [MYG1_HUMAN]	5	5	0.044	n/a	n/a			3	2	1
Q9Y224	UPF0568 protein C14orf166 OS=Homo sapiens GN=C14orf166 PE=1 SV=1 - [CN166_HUMAN]	6	6	0.006	0.001	0.001	296.62	5.7E-06	1	3	5
Q9UBQ0	Vacuolar protein sorting-associated protein 29 OS=Homo sapiens GN=VPS29 PE=1 SV=1 - [VPS29_HUMAN]	5	5	0.007	0.327	0.017	3.65	0.03	2	4	1
Q96QK1	Vacuolar protein sorting-associated protein 35 OS=Homo sapiens GN=VPS35 PE=1 SV=2 - [VPS35_HUMAN]	8	8	0.256	0.001	0.001	4.86	0.02	3	6	4
P26640	Valine--tRNA ligase OS=Homo sapiens GN=VARS PE=1 SV=4 - [SYVC_HUMAN]	15	17	0.021	0.188	0.010	7.42	0.009	8	8	16
Q15836	Vesicle-associated membrane protein 3 OS=Homo sapiens GN=VAMP3 PE=1 SV=3 - [VAMP3_HUMAN]	3	3	0.001	0.019	n/a	54.67	0.006	2	3	1
O95292	Vesicle-associated membrane protein-associated protein B/C OS=Homo sapiens GN=VAPB PE=1 SV=3 - [VAPB_HUMAN]	6	9	n/a	0.002	0.022	48.84	0.007	3	7	7
Q12907	Vesicular integral-membrane protein VIP36 OS=Homo sapiens GN=LMAN2 PE=1 SV=1 - [LMAN2_HUMAN]	3	4	n/a	0.002	n/a			2	2	2
Q00341	Vigilin OS=Homo sapiens GN=HDLBP PE=1 SV=2 - [VIGLN_HUMAN]	5	7	lost	0.001	n/a			lost	5	2
P18206	Vinculin OS=Homo sapiens GN=VCL PE=1 SV=4 - [VINC_HUMAN]	18	18	0.371	0.006	0.001	3.05	0.05	12	13	11
P21796	Voltage-dependent anion-selective channel protein 1 OS=Homo	6	7	0.368	0.425	0.280	3.38	0.04	7	6	6

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]	5	6	0.001	n/a	n/a			4	3	2
	sapiens GN=VDAC1 PE=1 SV=2 - [VDAC1_HUMAN]										
P45880	Voltage-dependent anion-selective channel protein 2 OS=Homo sapiens GN=VDAC2 PE=1 SV=2 - [VDAC2_HUMAN]	6	6	0.308	0.442	0.251	2.93	0.05	3	5	4
P21281	V-type proton ATPase subunit B, brain isoform OS=Homo sapiens GN=ATP6V1B2 PE=1 SV=3 - [VATB2_HUMAN]	3	3	0.001	0.002	0.001	2858.15	6.1E-08	2	1	1
Q9Y5K8	V-type proton ATPase subunit D OS=Homo sapiens GN=ATP6V1D PE=1 SV=1 - [VATD_HUMAN]	6	6	0.002	0.001	0.005	379.94	3.5E-06	2	4	5
P12956	X-ray repair cross-complementing protein 6 OS=Homo sapiens GN=XRCC6 PE=1 SV=2 - [XRCC6_HUMAN]	20	21	0.161	0.013	0.193	6.81	0.01	11	13	10
Q9Y5A9	YTH domain family protein 2 OS=Homo sapiens GN=YTHDF2 PE=1 SV=2 - [YTHD2_HUMAN]	1	4	n/a	n/a	n/a	0	0	2	2	2

8.2 Candidate novel interacting proteins of the phosphomimetic mutant of β-catenin at tyrosine-654 in SW480.

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
P62277	40S ribosomal protein S13 OS=Homo sapiens GN=RPS13 PE=1 SV=2 - [RS13_HUMAN]	4	4	0.432		0.546			4	4	1
P62263	40S ribosomal protein S14 OS=Homo sapiens GN=RPS14 PE=1 SV=3 - [RS14_HUMAN]	2	2	0.396	0.295	0.385	4.4	0.02	2	2	1
P15880	40S ribosomal protein S2 OS=Homo sapiens GN=RPS2 PE=1 SV=2 - [RS2_HUMAN]	8	8	0.427	0.443				7	3	1
P61247	40S ribosomal protein S3a OS=Homo sapiens GN=RPS3A PE=1 SV=2 - [RS3A_HUMAN]	7	7	0.507	0.508	0.520	2.9	0.05	4	5	1
P46781	40S ribosomal protein S9 OS=Homo sapiens GN=RPS9 PE=1 SV=3 - [RS9_HUMAN]	7	7	0.465	0.529				7	1	1
P10809	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2 - [CH60_HUMAN]	10	10	0.225	0.189	0.159	16.2	0.002	7	3	8
P05388	60S acidic ribosomal protein P0 OS=Homo sapiens GN=RPLP0 PE=1 SV=1 - [RLA0_HUMAN]	3	3	0.480	0.450	0.441	3.7	0.03	3	2	1
P62913	60S ribosomal protein L11 OS=Homo sapiens GN=RPL11 PE=1 SV=2 - [RL11_HUMAN]	5	5	0.280	0.297	0.334	12.4	0.003	5	3	3
P62829	60S ribosomal protein L23 OS=Homo sapiens GN=RPL23 PE=1 SV=1 - [RL23_HUMAN]	4	4	0.505	0.506	0.506	13.0	0.003	4	4	1
P18124	60S ribosomal protein L7 OS=Homo sapiens GN=RPL7 PE=1 SV=1 - [RL7_HUMAN]	5	5	0.381	0.479				5	3	1
P11021	78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2 - [GRP78_HUMAN]	18	20	0.242	0.174	0.234	13.2	0.003	15	10	13
P68032	Actin, alpha cardiac muscle 1 OS=Homo sapiens GN=ACTC1	1	10		0.414				8	10	5

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
	PE=1 SV=1 - [ACTC_HUMAN]										
P60709	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 - [ACTB_HUMAN]	1	14			0.477			12	12	8
P02765	Alpha-2-HS-glycoprotein OS=Homo sapiens GN=AHSG PE=1 SV=1 - [FETUA_HUMAN]	3	3	0.001	0.001	0.001	5401 3.2	1.71E -10	3	2	2
P12814	Alpha-actinin-1 OS=Homo sapiens GN=ACTN1 PE=1 SV=2 - [ACTN1_HUMAN]	1	9		0.275	0.336			6	8	8
O43707	Alpha-actinin-4 OS=Homo sapiens GN=ACTN4 PE=1 SV=2 - [ACTN4_HUMAN]	7	15	0.030	0.226	0.319	3.6	0.03	10	10	11
P04745	Alpha-amylase 1 OS=Homo sapiens GN=AMY1A PE=1 SV=2 - [AMY1_HUMAN]	1	6	0.001					5	4	4
P06733	Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2 - [ENOA_HUMAN]	6	6	0.387	0.300	0.436	3.1	0.04	2	6	4
P61221	ATP-binding cassette sub-family E member 1 OS=Homo sapiens GN=ABCE1 PE=1 SV=1 - [ABCE1_HUMAN]	6	6	0.035	0.002				4	2	1
Q08211	ATP-dependent RNA helicase A OS=Homo sapiens GN=DHX9 PE=1 SV=4 - [DHX9_HUMAN]	1	1	0.012	lost				1		1
P16278	Beta-galactosidase OS=Homo sapiens GN=GLB1 PE=1 SV=2 - [BGAL_HUMAN]	4	4	0.001	0.001	0.001	1305 6.0	2.93E -09	4	3	4
P31939	Bifunctional purine biosynthesis protein PURH OS=Homo sapiens GN=ATIC PE=1 SV=3 - [PUR9_HUMAN]	6	6	0.282	0.266	0.345	8.4	0.007	3	1	4
P27708	CAD protein OS=Homo sapiens GN=CAD PE=1 SV=3 - [PYR1_HUMAN]	18	18	0.370	lost	0.373	85.5	0.004	15		7
P17655	Calpain-2 catalytic subunit OS=Homo sapiens GN=CAPN2 PE=1 SV=6 - [CAN2_HUMAN]	4	4	0.304	0.137	0.240	5.6	0.02	2	1	3
P35221	Catenin alpha-1 OS=Homo sapiens GN=CTNNA1 PE=1 SV=1 - [CTNA1_HUMAN]	12	12	0.002	0.001	0.001	3296. 9	4.60E -08	2	7	9

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
P35222	Catenin beta-1 OS=Homo sapiens GN=CTNNB1 PE=1 SV=1 - [CTNB1_HUMAN]	32	37	0.001	0.001	0.001	0	0	36	30	36
Q00610	Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5 - [CLH1_HUMAN]	20	20	0.340	lost	0.360	15.4	0.02	17		15
P06732	Creatine kinase M-type OS=Homo sapiens GN=CKM PE=1 SV=2 - [KCRM_HUMAN]	1	1	0.002	0.003	0.001	1120.3	3.98E-07	1	1	1
Q14204	Cytoplasmic dynein 1 heavy chain 1 OS=Homo sapiens GN=DYNC1H1 PE=1 SV=5 - [DYHC1_HUMAN]	3	3	0.005	lost				2		1
P81605	Dermcidin OS=Homo sapiens GN=DCD PE=1 SV=2 - [DCD_HUMAN]	2	2	0.001	0.001	0.002	2002.6	1.25E-07	2	2	2
P15924	Desmoplakin OS=Homo sapiens GN=DSP PE=1 SV=3 - [DESP_HUMAN]	8	8	0.006	0.005	0.009	412.9	2.93E-06	3	3	3
P60981	Destrin OS=Homo sapiens GN=DSTN PE=1 SV=3 - [DEST_HUMAN]	4	5	0.335	0.286	0.310	13.6	0.003	5	1	2
P16989	DNA-binding protein A OS=Homo sapiens GN=CSDA PE=1 SV=4 - [DBPA_HUMAN]	1	3				0	0	1	3	1
Q14258	E3 ubiquitin/ISG15 ligase TRIM25 OS=Homo sapiens GN=TRIM25 PE=1 SV=2 - [TRI25_HUMAN]	6	6	0.441	0.432	0.401	6.2	0.01	6	2	3
P14625	Endoplasmin OS=Homo sapiens GN=HSP90B1 PE=1 SV=1 - [ENPL_HUMAN]	1	2				0	0	1	1	2
O15371	Eukaryotic translation initiation factor 3 subunit D OS=Homo sapiens GN=EIF3D PE=1 SV=1 - [EIF3D_HUMAN]	1	1	0.002		0.321			1	1	1
P49327	Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3 - [FAS_HUMAN]	39	39	0.414	lost	0.425	14.8	0.02	34		23
P21333	Filamin-A OS=Homo sapiens GN=FLNA PE=1 SV=4 - [FLNA_HUMAN]	7	7	0.280	lost	0.291	37.8	0.008	5		3
O75369	Filamin-B OS=Homo sapiens GN=FLNB PE=1 SV=2 -	5	5	0.061	lost	0.036	36.5	0.009	4		2

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
	[FLNB_HUMAN]										
P04075	Fructose-bisphosphate aldolase A OS=Homo sapiens GN=ALDOA PE=1 SV=2 - [ALDOA_HUMAN]	3	3			0.278			1	1	1
P06744	Glucose-6-phosphate isomerase OS=Homo sapiens GN=GPI PE=1 SV=4 - [G6PI_HUMAN]	2	2	0.391	0.365	0.367	14.8	0.002	1	2	2
P30711	Glutathione S-transferase theta-1 OS=Homo sapiens GN=GSTT1 PE=1 SV=4 - [GSTT1_HUMAN]	1	1	0.001	0.002	0.001	2366.7	8.93E-08	1	1	1
O14556	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific OS=Homo sapiens GN=GAPDHS PE=1 SV=2 - [G3PT_HUMAN]	1	2			0.001			1	1	2
P41250	Glycine--tRNA ligase OS=Homo sapiens GN=GARS PE=1 SV=3 - [SYG_HUMAN]	4	4	0.322		0.032			2	1	3
P49915	GMP synthase [glutamine-hydrolyzing] OS=Homo sapiens GN=GMPS PE=1 SV=1 - [GUAA_HUMAN]	5	5	0.334	0.241	0.199	6.1	0.01	3	2	3
P08107	Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5 - [HSP71_HUMAN]	5	8	0.268	0.353	0.367	5.6	0.02	6	3	6
Q12931	Heat shock protein 75 kDa, mitochondrial OS=Homo sapiens GN=TRAP1 PE=1 SV=3 - [TRAP1_HUMAN]	2	3				0	0	1	1	3
P07900	Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1 PE=1 SV=5 - [HS90A_HUMAN]	7	15	0.354	0.242	0.292	6.3	0.01	11	12	13
O60814	Histone H2B type 1-K OS=Homo sapiens GN=HIST1H2BK PE=1 SV=3 - [H2B1K_HUMAN]	3	3	0.318	0.344	0.380	8.5	0.007	2	1	1
Q86YZ3	Hornerin OS=Homo sapiens GN=HRNR PE=1 SV=2 - [HORN_HUMAN]	5	5	0.024	0.021	0.004	75.7	8.72E-05	3	3	1
Q9NZI8	Insulin-like growth factor 2 mRNA-binding protein 1 OS=Homo sapiens GN=IGF2BP1 PE=1 SV=2 - [IF2B1_HUMAN]	6	8	0.299	0.330				6	4	2

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q9Y6M1	Insulin-like growth factor 2 mRNA-binding protein 2 OS=Homo sapiens GN=IGF2BP2 PE=1 SV=2 - [IF2B2_HUMAN]	7	9	0.279	0.336	0.192	5.5	0.02	8	6	5
O00425	Insulin-like growth factor 2 mRNA-binding protein 3 OS=Homo sapiens GN=IGF2BP3 PE=1 SV=2 - [IF2B3_HUMAN]	1	4			0.007			3	1	1
P14923	Junction plakoglobin OS=Homo sapiens GN=JUP PE=1 SV=3 - [PLAK_HUMAN]	3	8		0.008				5	8	5
P00338	L-lactate dehydrogenase A chain OS=Homo sapiens GN=LDHA PE=1 SV=2 - [LDHA_HUMAN]	11	15	0.416	0.401	0.473	3.2	0.04	14	7	9
Q9BYZ2	L-lactate dehydrogenase A-like 6B OS=Homo sapiens GN=LDHAL6B PE=1 SV=3 - [LDH6B_HUMAN]	1	3	0.001	0.001				3	2	1
P10619	Lysosomal protective protein OS=Homo sapiens GN=CTSA PE=1 SV=2 - [PPGB_HUMAN]	2	2	0.010		0.002			2	2	2
P35580	Myosin-10 OS=Homo sapiens GN=MYH10 PE=1 SV=3 - [MYH10_HUMAN]	2	10	0.015	lost				10		4
P35579	Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4 - [MYH9_HUMAN]	28	35	0.349	lost	0.379	9.2	0.03	33		18
P04746	Pancreatic alpha-amylase OS=Homo sapiens GN=AMY2A PE=1 SV=2 - [AMYP_HUMAN]	1	6	0.001	0.001				5	5	4
P62937	Peptidyl-prolyl cis-trans isomerase A OS=Homo sapiens GN=PPIA PE=1 SV=2 - [PPIA_HUMAN]	8	8	0.362		0.432			7	3	4
Q06830	Peroxiredoxin-1 OS=Homo sapiens GN=PRDX1 PE=1 SV=1 - [PRDX1_HUMAN]	6	7	0.418	0.338	0.365	5.4	0.02	7	2	2
P32119	Peroxiredoxin-2 OS=Homo sapiens GN=PRDX2 PE=1 SV=5 - [PRDX2_HUMAN]	2	3	0.340	0.213				2	1	1
Q15365	Poly(rC)-binding protein 1 OS=Homo sapiens GN=PCBP1	4	5	0.413	0.432	0.448	6.9	0.01	3	3	3

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
	PE=1 SV=2 - [PCBP1_HUMAN]										
Q13310	Polyadenylate-binding protein 4 OS=Homo sapiens GN=PABPC4 PE=1 SV=1 - [PABP4_HUMAN]	1	4			0.448			4	3	3
P02545	Prelamin-A/C OS=Homo sapiens GN=LMNA PE=1 SV=1 - [LMNA_HUMAN]	10	10	0.336		0.426			9	3	6
Q92841	Probable ATP-dependent RNA helicase DDX17 OS=Homo sapiens GN=DDX17 PE=1 SV=2 - [DDX17_HUMAN]	2	4	0.004					3	1	2
P17844	Probable ATP-dependent RNA helicase DDX5 OS=Homo sapiens GN=DDX5 PE=1 SV=1 - [DDX5_HUMAN]	4	6			0.534			3	1	5
P07737	Profilin-1 OS=Homo sapiens GN=PFN1 PE=1 SV=2 - [PROF1_HUMAN]	3	3	0.028		0.329			3	1	2
O14744	Protein arginine N-methyltransferase 5 OS=Homo sapiens GN=PRMT5 PE=1 SV=4 - [ANM5_HUMAN]	3	3	0.029	0.003	0.313	3.9	0.03	3	1	1
P21980	Protein-glutamine gamma-glutamyltransferase 2 OS=Homo sapiens GN=TGM2 PE=1 SV=2 - [TGM2_HUMAN]	9	9	0.241	0.003	0.193	4.9	0.02	8	4	9
Q9Y5H6	Protocadherin alpha-8 OS=Homo sapiens GN=PCDHA8 PE=2 SV=1 - [PCDA8_HUMAN]	1	1	0.001	0.001	0.001	0	0	1	1	1
P14618	Pyruvate kinase isozymes M1/M2 OS=Homo sapiens GN=PKM PE=1 SV=4 - [KPYM_HUMAN]	12	12	0.444	0.375	0.448	3.3	0.04	11	9	11
O00472	RNA polymerase II elongation factor ELL2 OS=Homo sapiens GN=ELL2 PE=1 SV=2 - [ELL2_HUMAN]	1	1	0.004	0.002	0.002	901.0	6.16E -07	1	1	1
Q9Y230	RuvB-like 2 OS=Homo sapiens GN=RUVBL2 PE=1 SV=3 - [RUVB2_HUMAN]	13	13	0.004	0.001	0.001	3756 4.4	3.54E -10	5	11	5
Q9Y5X1	Sorting nexin-9 OS=Homo sapiens GN=SNX9 PE=1 SV=1 - [SNX9_HUMAN]	3	3	0.059		0.007			2	1	1
Q13813	Spectrin alpha chain, non-erythrocytic 1 OS=Homo sapiens GN=SPTAN1 PE=1 SV=3 - [SPTN1_HUMAN]	22	22	0.208	lost	0.218	56.2	0.006	20		7

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
P38646	Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2 - [GRP75_HUMAN]	8	8	0.015	0.001	0.071	22.2	0.001	5	4	6
O14907	Tax1-binding protein 3 OS=Homo sapiens GN=TAX1BP3 PE=1 SV=2 - [TX1B3_HUMAN]	1	1	0.001		0.002			1	1	1
Q99832	T-complex protein 1 subunit eta OS=Homo sapiens GN=CCT7 PE=1 SV=2 - [TCPH_HUMAN]	2	2	0.437	0.424	0.361	3.9	0.03	1	1	2
P55072	Transitional endoplasmic reticulum ATPase OS=Homo sapiens GN=VCP PE=1 SV=4 - [TERA_HUMAN]	1	1	0.405	0.389	0.383	15.9	0.002	1	1	1
P29401	Transketolase OS=Homo sapiens GN=TKT PE=1 SV=3 - [TKT_HUMAN]	8	8	0.307	0.027	0.329	2.9	0.05	6	2	4
P60174	Triosephosphate isomerase OS=Homo sapiens GN=TPI1 PE=1 SV=3 - [TPIS_HUMAN]	5	5	0.330	0.312	0.318	34.4	0.0004	5	4	2
Q14134	Tripartite motif-containing protein 29 OS=Homo sapiens GN=TRIM29 PE=1 SV=2 - [TRI29_HUMAN]	3	3	0.005	0.002				1	1	1
Q71U36	Tubulin alpha-1A chain OS=Homo sapiens GN=TUBA1A PE=1 SV=1 - [TBA1A_HUMAN]	4	9	0.373	0.346	0.429	4.8	0.02	4	9	5
P68366	Tubulin alpha-4A chain OS=Homo sapiens GN=TUBA4A PE=1 SV=1 - [TBA4A_HUMAN]	1	6	0.002	0.001				4	6	2
P07437	Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2 - [TBB5_HUMAN]	1	12	0.369	0.418	0.434	4.7	0.02	10	9	5
Q13885	Tubulin beta-2A chain OS=Homo sapiens GN=TUBB2A PE=1 SV=1 - [TBB2A_HUMAN]	2	11		0.031	0.045			8	8	6
P04350	Tubulin beta-4A chain OS=Homo sapiens GN=TUBB4A PE=1 SV=2 - [TBB4A_HUMAN]	2	11				0	0	8	8	4
P62979	Ubiquitin-40S ribosomal protein S27a OS=Homo sapiens GN=RPS27A PE=1 SV=2 - [RS27A_HUMAN]	1	3				0	0	2	1	2
P62987	Ubiquitin-60S ribosomal protein L40 OS=Homo sapiens	1	3	0.476					2	1	2

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
	GN=UBA52 PE=1 SV=2 - [RL40_HUMAN]										
P26640	Valine--tRNA ligase OS=Homo sapiens GN=VARS PE=1 SV=4 - [SYVC_HUMAN]	2	2	0.008	lost	0.018	102.7	0.003	2		1

8.3 Candidate novel interacting proteins of the non-phosphorylatable mutant of β-catenin at tyrosine-654 in SW480.

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
P35998	26S protease regulatory subunit 7 OS=Homo sapiens GN=PSMC2 PE=1 SV=3 - [PRS7_HUMAN]	1	1	0.230	0.144	0.004	5.66	0.01	1	1	1
P60866	40S ribosomal protein S20 OS=Homo sapiens GN=RPS20 PE=1 SV=1 - [RS20_HUMAN]	3	3	0.383	0.391	0.234	3.21	0.04	2	2	3
P08865	40S ribosomal protein SA OS=Homo sapiens GN=RPSA PE=1 SV=4 - [RSSA_HUMAN]	3	3	0.276	0.309	0.218	8.75	0.006	3	2	3
P62906	60S ribosomal protein L10a OS=Homo sapiens GN=RPL10A PE=1 SV=2 - [RL10A_HUMAN]	2	2	0.386					2	1	1
P62913	60S ribosomal protein L11 OS=Homo sapiens GN=RPL11 PE=1 SV=2 - [RL11_HUMAN]	4	4	0.153	0.223	0.147	13.33	0.003	3	4	3
Q01813	6-phosphofructokinase type C OS=Homo sapiens GN=PFKP PE=1 SV=2 - [K6PP_HUMAN]	3	5			0.001			2	2	4
P17858	6-phosphofructokinase, liver type OS=Homo sapiens GN=PFKL PE=1 SV=6 - [K6PL_HUMAN]	2	4	0.170					1	1	3
P11021	78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2 - [GRP78_HUMAN]	7	9	0.357	0.252	0.266	6.30	0.01	5	4	7
Q7L2J0	7SK snRNA methylphosphate capping enzyme OS=Homo sapiens GN=MEPCE PE=1 SV=1 - [MEPCE_HUMAN]	1	1			0.134			1	1	1
P60709	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 - [ACTB_HUMAN]	1	13			0.425			11	8	11
P63267	Actin, gamma-enteric smooth muscle OS=Homo sapiens GN=ACTG2 PE=1 SV=1 - [ACTH_HUMAN]	1	9			0.357			7	5	8

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q01518	Adenylyl cyclase-associated protein 1 OS=Homo sapiens GN=CAP1 PE=1 SV=5 - [CAP1_HUMAN]	2	2		0.526				1	1	1
Q8N6T3	ADP-ribosylation factor GTPase-activating protein 1 OS=Homo sapiens GN=ARFGAP1 PE=1 SV=2 - [ARFG1_HUMAN]	4	4	0.005	0.004	0.001	432.94	2.7E-06	1	2	2
P02765	Alpha-2-HS-glycoprotein OS=Homo sapiens GN=AHSG PE=1 SV=1 - [FETUA_HUMAN]	3	3	0.004	0.008	0.001	245.62	8.3E-06	2	3	3
P12814	Alpha-actinin-1 OS=Homo sapiens GN=ACTN1 PE=1 SV=2 - [ACTN1_HUMAN]	4	16				0	0	10	11	9
P04745	Alpha-amylase 1 OS=Homo sapiens GN=AMY1A PE=1 SV=2 - [AMY1_HUMAN]	1	1		0.001	0.001	3920.84	8.1E-05	1	1	1
P08243	Asparagine synthetase [glutamine-hydrolyzing] OS=Homo sapiens GN=ASNS PE=1 SV=4 - [ASNS_HUMAN]	2	2	0.080	0.279	0.150	5.66	0.01	1	2	1
P53396	ATP-citrate synthase OS=Homo sapiens GN=ACLY PE=1 SV=3 - [ACLY_HUMAN]	2	2	0.418	0.406		15.18	0.02	2	1	1
O00571	ATP-dependent RNA helicase DDX3X OS=Homo sapiens GN=DDX3X PE=1 SV=3 - [DDX3X_HUMAN]	14	14	0.001	0.067	0.001	21.86	0.001	12	9	5
Q9NXV2	BTB/POZ domain-containing protein KCTD5 OS=Homo sapiens GN=KCTD5 PE=1 SV=1 - [KCTD5_HUMAN]	3	3	0.001	0.002	0.001	1215.00	3.4E-07	3	2	2
P11586	C-1-tetrahydrofolate synthase, cytoplasmic OS=Homo sapiens GN=MTHFD1 PE=1 SV=3 - [C1TC_HUMAN]	7	7	0.004	0.056	0.001	26.66	0.00070222	4	4	4
Q99439	Calponin-2 OS=Homo sapiens GN=CNN2 PE=1 SV=4 - [CNN2_HUMAN]	3	3	0.086	0.010	0.061	19.98	0.001	3	3	3
P35221	Catenin alpha-1 OS=Homo sapiens GN=CTNNA1 PE=1 SV=1 - [CTNA1_HUMAN]	8	8	0.002	0.001	0.001	2106.15	1.1E-07	6	4	6

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
P35222	Catenin beta-1 OS=Homo sapiens GN=CTNNB1 PE=1 SV=1 - [CTNB1_HUMAN]	31	35	0.001	0.001	0.001	18669.24	1.4E-09	31	31	30
O60716	Catenin delta-1 OS=Homo sapiens GN=CTNND1 PE=1 SV=1 - [CTND1_HUMAN]	4	4		0.005				1	2	2
P62633	Cellular nucleic acid-binding protein OS=Homo sapiens GN=CNBP PE=1 SV=1 - [CNBP_HUMAN]	4	4	0.168	0.184	0.002	6.56	0.01	3	4	2
Q9Y696	Chloride intracellular channel protein 4 OS=Homo sapiens GN=CLIC4 PE=1 SV=4 - [CLIC4_HUMAN]	2	2	0.332	0.333	0.270	9.06	0.006	1	2	1
P48444	Coatomer subunit delta OS=Homo sapiens GN=ARCN1 PE=1 SV=1 - [COPD_HUMAN]	3	3			0.002			1	1	2
Q567U6	Coiled-coil domain-containing protein 93 OS=Homo sapiens GN=CCDC93 PE=1 SV=2 - [CCD93_HUMAN]	2	2	0.004		0.001	341.30	0.00093264 5	1	1	2
Q16527	Cysteine and glycine-rich protein 2 OS=Homo sapiens GN=CSRP2 PE=1 SV=3 - [CSRP2_HUMAN]	2	2	0.004	0.040		26.32	0.01	1	2	1
Q13409	Cytoplasmic dynein 1 intermediate chain 2 OS=Homo sapiens GN=DYNC1I2 PE=1 SV=3 - [DC1I2_HUMAN]	2	2	0.415	0.332	0.399	4.62	0.02	2	1	1
P33316	Deoxyuridine 5'-triphosphate nucleotidohydrolase, mitochondrial OS=Homo sapiens GN=DUT PE=1 SV=4 - [DUT_HUMAN]	1	1	0.278	0.300		19.60	0.02	1	1	1
P33992	DNA replication licensing factor MCM5 OS=Homo sapiens GN=MCM5 PE=1 SV=5 - [MCM5_HUMAN]	3	3			0.164			2	1	1
P16989	DNA-binding protein A OS=Homo sapiens GN=CSDA PE=1 SV=4 - [DBPA_HUMAN]	2	4			0.001			2	1	2
P31689	DnaJ homolog subfamily A member 1 OS=Homo sapiens GN=DNAJA1 PE=1 SV=2 - [DNJA1_HUMAN]	1	1		0.370				1	1	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q8TEA8	D-tyrosyl-tRNA(Tyr) deacylase 1 OS=Homo sapiens GN=DTD1 PE=1 SV=2 - [DTD1_HUMAN]	1	1	0.409	0.289	0.157	2.94	0.05	1	1	1
O60313	Dynamin-like 120 kDa protein, mitochondrial OS=Homo sapiens GN=OPA1 PE=1 SV=3 - [OPA1_HUMAN]	4	4	0.003	0.013	0.002	147.60	2.3E-05	2	2	3
P62877	E3 ubiquitin-protein ligase RBX1 OS=Homo sapiens GN=RBX1 PE=1 SV=1 - [RBX1_HUMAN]	2	2	0.006	0.241	0.002	5.28	0.02	1	2	1
P38117	Electron transfer flavoprotein subunit beta OS=Homo sapiens GN=ETFB PE=1 SV=3 - [ETFB_HUMAN]	2	2	0.215	0.174	0.039	6.72	0.01	1	2	1
P49411	Elongation factor Tu, mitochondrial OS=Homo sapiens GN=TUFM PE=1 SV=2 - [EFTU_HUMAN]	4	4	0.011	0.219	0.075	6.46	0.01	3	2	2
Q9BY44	Eukaryotic translation initiation factor 2A OS=Homo sapiens GN=EIF2A PE=1 SV=3 - [EIF2A_HUMAN]	2	2			0.004			1	1	2
P23588	Eukaryotic translation initiation factor 4B OS=Homo sapiens GN=EIF4B PE=1 SV=2 - [IF4B_HUMAN]	5	5	0.022	0.206	0.153	6.82	0.01	3	3	4
O60573	Eukaryotic translation initiation factor 4E type 2 OS=Homo sapiens GN=EIF4E2 PE=1 SV=1 - [IF4E2_HUMAN]	3	3	0.001	0.026	0.001	59.64	0.000140511	2	1	1
P15311	Ezrin OS=Homo sapiens GN=EZR PE=1 SV=4 - [EZRI_HUMAN]	5	6	0.039	0.120		10.34	0.03	3	3	1
P49327	Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3 - [FAS_HUMAN]	27	27	0.331	0.309	0.200	5.41	0.02	22	17	3
Q14192	Four and a half LIM domains protein 2 OS=Homo sapiens GN=FHL2 PE=1 SV=3 - [FHL2_HUMAN]	4	4	0.003	0.014	0.004	132.39	2.9E-05	3	4	2
P04406	Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3 - [G3P_HUMAN]	8	8	0.435	0.429	0.336	3.13	0.04	8	5	4
P06737	Glycogen phosphorylase, liver form OS=Homo sapiens	5	6	0.314	0.34	0.266	7.99	0.008	3	4	3

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
	GN=PYGL PE=1 SV=4 - [PYGL_HUMAN]				8						
P08107	Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5 - [HSP71_HUMAN]	6	8	0.287	0.336	0.306	13.27	0.003	3	4	6
P52597	Heterogeneous nuclear ribonucleoprotein F OS=Homo sapiens GN=HNRNPF PE=1 SV=3 - [HNRPF_HUMAN]	1	3	0.093	0.118	0.061	24.94	0.0008018 55	3	3	2
P31943	Heterogeneous nuclear ribonucleoprotein H OS=Homo sapiens GN=HNRNPH1 PE=1 SV=4 - [HNRH1_HUMAN]	4	6	0.001	0.068	0.001	21.49	0.001	5	4	3
P61978	Heterogeneous nuclear ribonucleoprotein K OS=Homo sapiens GN=HNRNPK PE=1 SV=1 - [HNRPK_HUMAN]	10	10	0.172	0.177	0.075	10.80	0.004	7	6	9
O60506	Heterogeneous nuclear ribonucleoprotein Q OS=Homo sapiens GN=SYNCRIP PE=1 SV=2 - [HNRPQ_HUMAN]	3	3	0.218	0.230	0.126	9.44	0.006	1	3	3
Q00839	Heterogeneous nuclear ribonucleoprotein U OS=Homo sapiens GN=HNRNPU PE=1 SV=6 - [HNRPU_HUMAN]	1	1	0.004	0.013	0.001	135.15	2.7E-05	1	1	1
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1 OS=Homo sapiens GN=HNRNPA2B1 PE=1 SV=2 - [ROA2_HUMAN]	4	4	0.282	0.051	0.229	4.49	0.02	2	2	2
Q16576	Histone-binding protein RBBP7 OS=Homo sapiens GN=RBBP7 PE=1 SV=1 - [RBBP7_HUMAN]	1	1		0.050				1	1	1
P52294	Importin subunit alpha-5 OS=Homo sapiens GN=KPNA1 PE=1 SV=3 - [IMA5_HUMAN]	2	2	0.002	0.006	0.001	332.63	4.5E-06	1	1	1
Q9Y6M1	Insulin-like growth factor 2 mRNA-binding protein 2 OS=Homo sapiens GN=IGF2BP2 PE=1 SV=2 - [IF2B2_HUMAN]	6	6	0.032	0.052	0.010	39.47	0.0003206 8	3	3	3
P48735	Isocitrate dehydrogenase [NADP], mitochondrial OS=Homo sapiens GN=IDH2 PE=1 SV=2 - [IDHP_HUMAN]	1	1			0.002			1	1	1
P14923	Junction plakoglobin OS=Homo sapiens GN=JUP PE=1	1	5				0	0	4	5	4

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
	SV=3 - [PLAK_HUMAN]										
Q9NSK0	Kinesin light chain 4 OS=Homo sapiens GN=KLC4 PE=1 SV=3 - [KLC4_HUMAN]	4	4	0.001	0.004	0.001	535.79	1.7E-06	3	1	1
A6NHZ5	Leucine-rich repeat-containing protein 14B OS=Homo sapiens GN=LRRC14B PE=3 SV=3 - [LR14B_HUMAN]	1	1	0.002	0.001	0.001	2777.81	6.5E-08	1	1	1
Q8N1G4	Leucine-rich repeat-containing protein 47 OS=Homo sapiens GN=LRRC47 PE=1 SV=1 - [LRC47_HUMAN]	2	2			0.015			1	2	2
P09960	Leukotriene A-4 hydrolase OS=Homo sapiens GN=LTA4H PE=1 SV=2 - [LKHA4_HUMAN]	1	1		0.016	0.002	67.65	0.005	1	1	1
P48059	LIM and senescent cell antigen-like-containing domain protein 1 OS=Homo sapiens GN=LIMS1 PE=1 SV=4 - [LIMS1_HUMAN]	2	2	0.004	0.006		524.28	0.00060713 9	1	1	1
P0CW20	LIM and senescent cell antigen-like-containing domain protein 3-like OS=Homo sapiens GN=LIMS3L PE=2 SV=1 - [LIM3L_HUMAN]	1	1	0.278	0.073	0.002	4.61	0.02	1	1	1
Q14847	LIM and SH3 domain protein 1 OS=Homo sapiens GN=LASP1 PE=1 SV=2 - [LASP1_HUMAN]	11	11	0.130	0.165	0.053	11.61	0.004	8	4	8
Q8WWI1	LIM domain only protein 7 OS=Homo sapiens GN=LMO7 PE=1 SV=3 - [LMO7_HUMAN]	1	1	0.005	0.047	0.001	32.82	0.00046367 4	1	1	1
P00338	L-lactate dehydrogenase A chain OS=Homo sapiens GN=LDHA PE=1 SV=2 - [LDHA_HUMAN]	6	8	0.009	0.150	0.389	2.86	0.05	6	6	6
P05455	Lupus La protein OS=Homo sapiens GN=SSB PE=1 SV=2 - [LA_HUMAN]	3	3	0.273	0.052	0.015	4.80	0.02	2	2	2
Q7Z4W1	L-xylulose reductase OS=Homo sapiens GN=DCXR PE=1 SV=2 - [DCXR_HUMAN]	2	2	0.404					1	2	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
P10619	Lysosomal protective protein OS=Homo sapiens GN=CTSA PE=1 SV=2 - [PPGB_HUMAN]	2	2	0.011	0.008	0.007	475.75	2.2E-06	2	1	1
O43684	Mitotic checkpoint protein BUB3 OS=Homo sapiens GN=BUB3 PE=1 SV=1 - [BUB3_HUMAN]	3	3	0.001		0.001	2080.75	0.00015297 8	3	1	2
O76041	Nebulette OS=Homo sapiens GN=NEBL PE=1 SV=1 - [NEBL_HUMAN]	2	2	0.001	0.014	0.004	130.14	3.0E-05	2	2	2
Q14697	Neutral alpha-glucosidase AB OS=Homo sapiens GN=GANAB PE=1 SV=3 - [GANAB_HUMAN]	3	3		0.025	0.022	341.62	0.00093176 3	2	3	1
P19338	Nucleolin OS=Homo sapiens GN=NCL PE=1 SV=3 - [NUCL_HUMAN]	4	4	0.327	0.337		33.58	0.01	2	2	1
Q9BRP8	Partner of Y14 and mago OS=Homo sapiens GN=WIBG PE=1 SV=1 - [WIBG_HUMAN]	1	1	0.157	0.307	0.002	3.92	0.03	1	1	1
P49023	Paxillin OS=Homo sapiens GN=PXN PE=1 SV=3 - [PAXI_HUMAN]	3	3	0.003	0.008	0.001	247.03	8.2E-06	3	1	2
P50479	PDZ and LIM domain protein 4 OS=Homo sapiens GN=PDLIM4 PE=1 SV=2 - [PDLI4_HUMAN]	7	7	0.001	0.004	0.001	522.40	1.8E-06	6	2	5
Q9UBV8	Peflin OS=Homo sapiens GN=PEF1 PE=1 SV=1 - [PEF1_HUMAN]	1	1	0.011	0.017	0.002	110.65	4.1E-05	1	1	1
P32119	Peroxiredoxin-2 OS=Homo sapiens GN=PRDX2 PE=1 SV=5 - [PRDX2_HUMAN]	1	2		0.427				1	2	1
O75420	PERQ amino acid-rich with GYF domain-containing protein 1 OS=Homo sapiens GN=GIGYF1 PE=1 SV=2 - [PERQ1_HUMAN]	7	7	0.001	0.003	0.001	836.54	7.1E-07	7	3	3
Q6Y7W6	PERQ amino acid-rich with GYF domain-containing protein 2	10	10	0.012	0.01	0.003	174.6	1.6E-	9	5	2

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
	OS=Homo sapiens GN=GIGYF2 PE=1 SV=1 - [PERQ2_HUMAN]				0		6	05			
Q8WW12	PEST proteolytic signal-containing nuclear protein OS=Homo sapiens GN=PCNP PE=1 SV=2 - [PCNP_HUMAN]	2	2		0.269	0.233	14.03	0.02	1	2	1
Q86UU1	Pleckstrin homology-like domain family B member 1 OS=Homo sapiens GN=PHLDB1 PE=1 SV=1 - [PHLB1_HUMAN]	7	7	0.002	0.022	0.004	77.95	8.2E-05	6	2	1
Q15365	Poly(rC)-binding protein 1 OS=Homo sapiens GN=PCBP1 PE=1 SV=2 - [PCBP1_HUMAN]	3	4	0.345	0.294	0.237	6.68	0.01	2	3	3
Q92841	Probable ATP-dependent RNA helicase DDX17 OS=Homo sapiens GN=DDX17 PE=1 SV=2 - [DDX17_HUMAN]	2	3	0.001	0.003	0.001	675.69	1.1E-06	2	2	1
Q15185	Prostaglandin E synthase 3 OS=Homo sapiens GN=PTGES3 PE=1 SV=1 - [TEBP_HUMAN]	1	1		0.452				1	1	1
P07237	Protein disulfide-isomerase OS=Homo sapiens GN=P4HB PE=1 SV=3 - [PDIA1_HUMAN]	4	4		0.061	0.029	28.21	0.01	1	3	2
O15355	Protein phosphatase 1G OS=Homo sapiens GN=PPM1G PE=1 SV=1 - [PPM1G_HUMAN]	3	3	0.002		0.036	28.39	0.01	2	1	2
P60468	Protein transport protein Sec61 subunit beta OS=Homo sapiens GN=SEC61B PE=1 SV=2 - [SC61B_HUMAN]	1	1	0.003	0.005	0.001	425.94	2.8E-06	1	1	1
P21980	Protein-glutamine gamma-glutamyltransferase 2 OS=Homo sapiens GN=TGM2 PE=1 SV=2 - [TGM2_HUMAN]	6	6	0.187	0.224	0.235	19.51	0.001	4	5	2
Q9UN86	Ras GTPase-activating protein-binding protein 2 OS=Homo sapiens GN=G3BP2 PE=1 SV=2 - [G3BP2_HUMAN]	1	1	0.064	0.036	0.239	6.09	0.01	1	1	1
Q8WUF5	RelA-associated inhibitor OS=Homo sapiens GN=PPP1R13L PE=1 SV=4 - [IASPP_HUMAN]	12	12	0.001	0.003	0.001	938.55	5.7E-07	9	3	5
P60891	Ribose-phosphate pyrophosphokinase 1 OS=Homo sapiens	1	1				0	0	1	1	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
	GN=PRPS1 PE=1 SV=2 - [PRPS1_HUMAN]										
Q96PK6	RNA-binding protein 14 OS=Homo sapiens GN=RBM14 PE=1 SV=2 - [RBM14_HUMAN]	3	3	0.004	0.015	0.018	112.72	3.9E-05	2	1	2
Q01844	RNA-binding protein EWS OS=Homo sapiens GN=EWSR1 PE=1 SV=1 - [EWS_HUMAN]	1	1		0.003				1	1	1
Q9Y265	RuvB-like 1 OS=Homo sapiens GN=RUVBL1 PE=1 SV=1 - [RUVB1_HUMAN]	8	8	0.003	0.004	0.001	551.79	1.6E-06	5	4	4
Q9Y230	RuvB-like 2 OS=Homo sapiens GN=RUVBL2 PE=1 SV=3 - [RUVB2_HUMAN]	17	17	0.001	0.003	0.001	990.90	5.1E-07	12	11	13
P62140	Serine/threonine-protein phosphatase PP1-beta catalytic subunit OS=Homo sapiens GN=PPP1CB PE=1 SV=3 - [PP1B_HUMAN]	1	1	0.001	0.004	0.028	57.86	0.00014929696	1	1	1
O76094	Signal recognition particle 72 kDa protein OS=Homo sapiens GN=SRP72 PE=1 SV=3 - [SRP72_HUMAN]	1	1			0.266			1	1	1
Q9Y5M8	Signal recognition particle receptor subunit beta OS=Homo sapiens GN=SRPRB PE=1 SV=3 - [SRPRB_HUMAN]	1	1	0.010	0.017	0.003	117.24	3.6E-05	1	1	1
Q04837	Single-stranded DNA-binding protein, mitochondrial OS=Homo sapiens GN=SSBP1 PE=1 SV=1 - [SSBP_HUMAN]	1	1	0.126	0.163	0.041	10.81	0.004	1	1	1
P62306	Small nuclear ribonucleoprotein F OS=Homo sapiens GN=SNRPF PE=1 SV=1 - [RUXF_HUMAN]	1	1		0.347				1	1	1
Q07889	Son of sevenless homolog 1 OS=Homo sapiens GN=SOS1 PE=1 SV=1 - [SOS1_HUMAN]	1	1	0.001	0.001	0.001	0	0	1	1	1
Q9Y5X1	Sorting nexin-9 OS=Homo sapiens GN=SNX9 PE=1 SV=1 - [SNX9_HUMAN]	3	3	0.004	0.029	0.001	55.81	0.00016047575	2	1	3

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
Q14247	Src substrate cortactin OS=Homo sapiens GN=CTTN PE=1 SV=2 - [SRC8_HUMAN]	4	4		0.010				2	4	1
P38646	Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2 - [GRP75_HUMAN]	4	4	0.002	0.212	0.155	6.01	0.01	2	2	3
P53597	Succinyl-CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial OS=Homo sapiens GN=SUCLG1 PE=1 SV=4 - [SUCA_HUMAN]	2	2		0.006	0.001	218.32	0.001	1	1	1
O14907	Tax1-binding protein 3 OS=Homo sapiens GN=TAX1BP3 PE=1 SV=2 - [TX1B3_HUMAN]	3	3	0.002	0.001	0.001	1271.35	3.1E-07	2	2	2
P40227	T-complex protein 1 subunit zeta OS=Homo sapiens GN=CCT6A PE=1 SV=3 - [TCPZ_HUMAN]	2	2	0.529	0.520	0.524	9.12	0.006	1	1	1
Q9UGI8	Testin OS=Homo sapiens GN=TES PE=1 SV=1 - [TES_HUMAN]	5	5	0.007	0.037	0.010	50.90	0.0001928 7	4	5	2
P04183	Thymidine kinase, cytosolic OS=Homo sapiens GN=TK1 PE=1 SV=2 - [KITH_HUMAN]	1	1	0.004	0.016	0.002	112.66	3.9E-05	1	1	1
Q15654	Thyroid receptor-interacting protein 6 OS=Homo sapiens GN=TRIP6 PE=1 SV=3 - [TRIP6_HUMAN]	1	1	0.007	0.003	0.001	292.53	5.8E-06	1	1	1
Q15369	Transcription elongation factor B polypeptide 1 OS=Homo sapiens GN=TCEB1 PE=1 SV=1 - [ELOC_HUMAN]	2	2	0.058	0.118	0.142	15.68	0.002	1	2	1
P29401	Transketolase OS=Homo sapiens GN=TKT PE=1 SV=3 - [TKT_HUMAN]	6	6	0.486		0.481	6.00	0.05	3	1	3
P40939	Trifunctional enzyme subunit alpha, mitochondrial OS=Homo sapiens GN=HADHA PE=1 SV=2 - [ECHA_HUMAN]	2	2	0.002		0.008	150.45	0.002	1	1	2
Q14134	Tripartite motif-containing protein 29 OS=Homo sapiens GN=TRIM29 PE=1 SV=2 - [TRI29_HUMAN]	4	4	0.005		0.003	430.64	0.0007391	3	2	4

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 Peptides	R2 Peptides	R3 Peptides
								62			
P06753	Tropomyosin alpha-3 chain OS=Homo sapiens GN=TPM3 PE=1 SV=2 - [TPM3_HUMAN]	1	2		0.013				1	2	1
P68363	Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1 - [TBA1B_HUMAN]	8	8	0.442	0.394	0.330	3.42	0.04	5	4	5
P07437	Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2 - [TBB5_HUMAN]	1	12	0.228	0.270		12.18	0.03	8	12	8
Q9H4B7	Tubulin beta-1 chain OS=Homo sapiens GN=TUBB1 PE=1 SV=1 - [TBB1_HUMAN]	0	4				0	0	1	4	2
Q13885	Tubulin beta-2A chain OS=Homo sapiens GN=TUBB2A PE=1 SV=1 - [TBB2A_HUMAN]	1	11		0.003				7	11	8
Q9BVA1	Tubulin beta-2B chain OS=Homo sapiens GN=TUBB2B PE=1 SV=1 - [TBB2B_HUMAN]	1	11		0.348				7	11	8
Q13509	Tubulin beta-3 chain OS=Homo sapiens GN=TUBB3 PE=1 SV=2 - [TBB3_HUMAN]	1	10	0.001		0.001	3557.07	8.9E-05	6	9	6
P04350	Tubulin beta-4A chain OS=Homo sapiens GN=TUBB4A PE=1 SV=2 - [TBB4A_HUMAN]	1	10		0.300				6	9	6
P68371	Tubulin beta-4B chain OS=Homo sapiens GN=TUBB4B PE=1 SV=1 - [TBB4B_HUMAN]	1	12	0.001	0.001	0.015	103.95	4.6E-05	8	11	9
P62979	Ubiquitin-40S ribosomal protein S27a OS=Homo sapiens GN=RPS27A PE=1 SV=2 - [RS27A_HUMAN]	4	4	0.445	0.337	0.262	2.86	0.05	2	4	1
Q6UXN9	WD repeat-containing protein 82 OS=Homo sapiens GN=WDR82 PE=1 SV=1 - [WDR82_HUMAN]	1	1				0	0	1	1	1

8.4 Candidate novel interacting proteins of β-catenin that may be affected by phosphorylation.

*PM denotes phosphomimetic and NP denotes non-phosphorylatable

**White denotes absence of that protein in the dataset

***Grey denotes presence of that protein in the dataset

Acc #	Protein	PM	NP	Wildtype
P62277	40S ribosomal protein S13 OS=Homo sapiens GN=RPS13 PE=1 SV=2 - [RS13_HUMAN]			
P62263	40S ribosomal protein S14 OS=Homo sapiens GN=RPS14 PE=1 SV=3 - [RS14_HUMAN]			
P15880	40S ribosomal protein S2 OS=Homo sapiens GN=RPS2 PE=1 SV=2 - [RS2_HUMAN]			
P61247	40S ribosomal protein S3a OS=Homo sapiens GN=RPS3A PE=1 SV=2 - [RS3A_HUMAN]			
P46781	40S ribosomal protein S9 OS=Homo sapiens GN=RPS9 PE=1 SV=3 - [RS9_HUMAN]			
P10809	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2 - [CH60_HUMAN]			
P05388	60S acidic ribosomal protein P0 OS=Homo sapiens GN=RPLP0 PE=1 SV=1 - [RLA0_HUMAN]			
P62829	60S ribosomal protein L23 OS=Homo sapiens GN=RPL23 PE=1 SV=1 - [RL23_HUMAN]			
P18124	60S ribosomal protein L7 OS=Homo sapiens GN=RPL7 PE=1 SV=1 - [RL7_HUMAN]			
P06733	Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2 - [ENOA_HUMAN]			
Q08211	ATP-dependent RNA helicase A OS=Homo sapiens GN=DHX9 PE=1 SV=4 - [DHX9_HUMAN]			
P31939	Bifunctional purine biosynthesis protein PURH OS=Homo sapiens GN=ATIC PE=1 SV=3 - [PUR9_HUMAN]			
P27708	CAD protein OS=Homo sapiens GN=CAD PE=1 SV=3 - [PYR1_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
P17655	Calpain-2 catalytic subunit OS=Homo sapiens GN=CAPN2 PE=1 SV=6 - [CAN2_HUMAN]			
Q00610	Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5 - [CLH1_HUMAN]			
P06732	Creatine kinase M-type OS=Homo sapiens GN=CKM PE=1 SV=2 - [KCRM_HUMAN]			
Q14204	Cytoplasmic dynein 1 heavy chain 1 OS=Homo sapiens GN=DYNC1H1 PE=1 SV=5 - [DYHC1_HUMAN]			
P81605	Dermcidin OS=Homo sapiens GN=DCD PE=1 SV=2 - [DCD_HUMAN]			
P15924	Desmoplakin OS=Homo sapiens GN=DSP PE=1 SV=3 - [DESP_HUMAN]			
P60981	Destrin OS=Homo sapiens GN=DSTN PE=1 SV=3 - [DEST_HUMAN]			
Q14258	E3 ubiquitin/ISG15 ligase TRIM25 OS=Homo sapiens GN=TRIM25 PE=1 SV=2 - [TRI25_HUMAN]			
P14625	Endoplasmin OS=Homo sapiens GN=HSP90B1 PE=1 SV=1 - [ENPL_HUMAN]			
O15371	Eukaryotic translation initiation factor 3 subunit D OS=Homo sapiens GN=EIF3D PE=1 SV=1 - [EIF3D_HUMAN]			
P21333	Filamin-A OS=Homo sapiens GN=FLNA PE=1 SV=4 - [FLNA_HUMAN]			
O75369	Filamin-B OS=Homo sapiens GN=FLNB PE=1 SV=2 - [FLNB_HUMAN]			
P04075	Fructose-bisphosphate aldolase A OS=Homo sapiens GN=ALDOA PE=1 SV=2 - [ALDOA_HUMAN]			
P06744	Glucose-6-phosphate isomerase OS=Homo sapiens GN=GPI PE=1 SV=4 - [G6PI_HUMAN]			
O14556	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific OS=Homo sapiens GN=GAPDHS PE=1 SV=2 - [G3PT_HUMAN]			
P41250	Glycine--tRNA ligase OS=Homo sapiens GN=GARS PE=1 SV=3 - [SYG_HUMAN]			
P49915	GMP synthase [glutamine-hydrolyzing] OS=Homo sapiens GN=GMPS PE=1 SV=1 - [GUAA_HUMAN]			
Q12931	Heat shock protein 75 kDa, mitochondrial OS=Homo sapiens GN=TRAP1 PE=1 SV=3 - [TRAP1_HUMAN]			
P07900	Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1 PE=1 SV=5 - [HS90A_HUMAN]			
O60814	Histone H2B type 1-K OS=Homo sapiens GN=HIST1H2BK PE=1 SV=3 - [H2B1K_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
P35580	Myosin-10 OS=Homo sapiens GN=MYH10 PE=1 SV=3 - [MYH10_HUMAN]			
P35579	Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4 - [MYH9_HUMAN]			
P04746	Pancreatic alpha-amylase OS=Homo sapiens GN=AMY2A PE=1 SV=2 - [AMYP_HUMAN]			
P62937	Peptidyl-prolyl cis-trans isomerase A OS=Homo sapiens GN=PPIA PE=1 SV=2 - [PPIA_HUMAN]			
Q06830	Peroxiredoxin-1 OS=Homo sapiens GN=PRDX1 PE=1 SV=1 - [PRDX1_HUMAN]			
Q13310	Polyadenylate-binding protein 4 OS=Homo sapiens GN=PABPC4 PE=1 SV=1 - [PABP4_HUMAN]			
P02545	Prelamin-A/C OS=Homo sapiens GN=LMNA PE=1 SV=1 - [LMNA_HUMAN]			
P17844	Probable ATP-dependent RNA helicase DDX5 OS=Homo sapiens GN=DDX5 PE=1 SV=1 - [DDX5_HUMAN]			
P07737	Profilin-1 OS=Homo sapiens GN=PFN1 PE=1 SV=2 - [PROF1_HUMAN]			
Q9Y5H6	Protocadherin alpha-8 OS=Homo sapiens GN=PCDHA8 PE=2 SV=1 - [PCDA8_HUMAN]			
P14618	Pyruvate kinase isozymes M1/M2 OS=Homo sapiens GN=PKM PE=1 SV=4 - [KPYM_HUMAN]			
O00472	RNA polymerase II elongation factor ELL2 OS=Homo sapiens GN=ELL2 PE=1 SV=2 - [ELL2_HUMAN]			
Q13813	Spectrin alpha chain, non-erythrocytic 1 OS=Homo sapiens GN=SPTAN1 PE=1 SV=3 - [SPTN1_HUMAN]			
Q99832	T-complex protein 1 subunit eta OS=Homo sapiens GN=CCT7 PE=1 SV=2 - [TCPH_HUMAN]			
P55072	Transitional endoplasmic reticulum ATPase OS=Homo sapiens GN=VCP PE=1 SV=4 - [TERA_HUMAN]			
P60174	Triosephosphate isomerase OS=Homo sapiens GN=TPI1 PE=1 SV=3 - [TPIS_HUMAN]			
Q71U36	Tubulin alpha-1A chain OS=Homo sapiens GN=TUBA1A PE=1 SV=1 - [TBA1A_HUMAN]			
P68366	Tubulin alpha-4A chain OS=Homo sapiens GN=TUBA4A PE=1 SV=1 - [TBA4A_HUMAN]			
P62987	Ubiquitin-60S ribosomal protein L40 OS=Homo sapiens GN=UBA52 PE=1 SV=2 - [RL40_HUMAN]			
P35998	26S protease regulatory subunit 7 OS=Homo sapiens GN=PSMC2 PE=1 SV=3 - [PRS7_HUMAN]			
P60866	40S ribosomal protein S20 OS=Homo sapiens GN=RPS20 PE=1 SV=1 - [RS20_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
P08865	40S ribosomal protein SA OS=Homo sapiens GN=RPSA PE=1 SV=4 - [RSSA_HUMAN]			
Q7L2J0	7SK snRNA methylphosphate capping enzyme OS=Homo sapiens GN=MEPCE PE=1 SV=1 - [MEPCE_HUMAN]			
P63267	Actin, gamma-enteric smooth muscle OS=Homo sapiens GN=ACTG2 PE=1 SV=1 - [ACTH_HUMAN]			
Q01518	Adenylyl cyclase-associated protein 1 OS=Homo sapiens GN=CAP1 PE=1 SV=5 - [CAP1_HUMAN]			
Q8N6T3	ADP-ribosylation factor GTPase-activating protein 1 OS=Homo sapiens GN=ARFGAP1 PE=1 SV=2 - [ARFG1_HUMAN]			
P08243	Asparagine synthetase [glutamine-hydrolyzing] OS=Homo sapiens GN=ASNS PE=1 SV=4 - [ASNS_HUMAN]			
P53396	ATP-citrate synthase OS=Homo sapiens GN=ACLY PE=1 SV=3 - [ACLY_HUMAN]			
O00571	ATP-dependent RNA helicase DDX3X OS=Homo sapiens GN=DDX3X PE=1 SV=3 - [DDX3X_HUMAN]			
Q9NXV2	BTB/POZ domain-containing protein KCTD5 OS=Homo sapiens GN=KCTD5 PE=1 SV=1 - [KCTD5_HUMAN]			
P62633	Cellular nucleic acid-binding protein OS=Homo sapiens GN=CNBP PE=1 SV=1 - [CNBP_HUMAN]			
Q9Y696	Chloride intracellular channel protein 4 OS=Homo sapiens GN=CLIC4 PE=1 SV=4 - [CLIC4_HUMAN]			
P48444	Coatomer subunit delta OS=Homo sapiens GN=ARCN1 PE=1 SV=1 - [COPD_HUMAN]			
Q567U6	Coiled-coil domain-containing protein 93 OS=Homo sapiens GN=CCDC93 PE=1 SV=2 - [CCD93_HUMAN]			
Q16527	Cysteine and glycine-rich protein 2 OS=Homo sapiens GN=CSRP2 PE=1 SV=3 - [CSRP2_HUMAN]			
Q13409	Cytoplasmic dynein 1 intermediate chain 2 OS=Homo sapiens GN=DYNC1I2 PE=1 SV=3 - [DC1I2_HUMAN]			
P33316	Deoxyuridine 5'-triphosphate nucleotidohydrolase, mitochondrial OS=Homo sapiens GN=DUT PE=1 SV=4 - [DUT_HUMAN]			
P31689	DnaJ homolog subfamily A member 1 OS=Homo sapiens GN=DNAJA1 PE=1 SV=2 - [DNJA1_HUMAN]			
Q8TEA8	D-tyrosyl-tRNA(Tyr) deacylase 1 OS=Homo sapiens GN=DTD1 PE=1 SV=2 - [DTD1_HUMAN]			
O60313	Dynamin-like 120 kDa protein, mitochondrial OS=Homo sapiens GN=OPA1 PE=1 SV=3 - [OPA1_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
Q9BY44	Eukaryotic translation initiation factor 2A OS=Homo sapiens GN=EIF2A PE=1 SV=3 - [EIF2A_HUMAN]			
O60573	Eukaryotic translation initiation factor 4E type 2 OS=Homo sapiens GN=EIF4E2 PE=1 SV=1 - [IF4E2_HUMAN]			
P15311	Ezrin OS=Homo sapiens GN=EZR PE=1 SV=4 - [EZRI_HUMAN]			
P52597	Heterogeneous nuclear ribonucleoprotein F OS=Homo sapiens GN=HNRNPF PE=1 SV=3 - [HNRPF_HUMAN]			
P31943	Heterogeneous nuclear ribonucleoprotein H OS=Homo sapiens GN=HNRNPH1 PE=1 SV=4 - [HNRH1_HUMAN]			
P61978	Heterogeneous nuclear ribonucleoprotein K OS=Homo sapiens GN=HNRNPK PE=1 SV=1 - [HNRPK_HUMAN]			
O60506	Heterogeneous nuclear ribonucleoprotein Q OS=Homo sapiens GN=SYNCRIP PE=1 SV=2 - [HNRPQ_HUMAN]			
Q00839	Heterogeneous nuclear ribonucleoprotein U OS=Homo sapiens GN=HNRNPU PE=1 SV=6 - [HNRPU_HUMAN]			
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1 OS=Homo sapiens GN=HNRNPA2B1 PE=1 SV=2 - [ROA2_HUMAN]			
P52294	Importin subunit alpha-5 OS=Homo sapiens GN=KPNA1 PE=1 SV=3 - [IMA5_HUMAN]			
Q8N1G4	Leucine-rich repeat-containing protein 47 OS=Homo sapiens GN=LRRC47 PE=1 SV=1 - [LRC47_HUMAN]			
Q8WWI1	LIM domain only protein 7 OS=Homo sapiens GN=LMO7 PE=1 SV=3 - [LMO7_HUMAN]			
P05455	Lupus La protein OS=Homo sapiens GN=SSB PE=1 SV=2 - [LA_HUMAN]			
Q7Z4W1	L-xylulose reductase OS=Homo sapiens GN=DCXR PE=1 SV=2 - [DCXR_HUMAN]			
P19338	Nucleolin OS=Homo sapiens GN=NCL PE=1 SV=3 - [NUCL_HUMAN]			
Q9BRP8	Partner of Y14 and mago OS=Homo sapiens GN=WIBG PE=1 SV=1 - [WIBG_HUMAN]			
P49023	Paxillin OS=Homo sapiens GN=PXN PE=1 SV=3 - [PAXI_HUMAN]			
Q9UBV8	Peflin OS=Homo sapiens GN=PEF1 PE=1 SV=1 - [PEF1_HUMAN]			
Q6Y7W6	PERQ amino acid-rich with GYF domain-containing protein 2 OS=Homo sapiens GN=GIGYF2 PE=1 SV=1 - [PERQ2_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
Q86UU1	Pleckstrin homology-like domain family B member 1 OS=Homo sapiens GN=PHLDB1 PE=1 SV=1 - [PHLB1_HUMAN]			
Q15185	Prostaglandin E synthase 3 OS=Homo sapiens GN=PTGES3 PE=1 SV=1 - [TEBP_HUMAN]			
P07237	Protein disulfide-isomerase OS=Homo sapiens GN=P4HB PE=1 SV=3 - [PDIA1_HUMAN]			
Q9UN86	Ras GTPase-activating protein-binding protein 2 OS=Homo sapiens GN=G3BP2 PE=1 SV=2 - [G3BP2_HUMAN]			
P60891	Ribose-phosphate pyrophosphokinase 1 OS=Homo sapiens GN=PRPS1 PE=1 SV=2 - [PRPS1_HUMAN]			
Q96PK6	RNA-binding protein 14 OS=Homo sapiens GN=RBM14 PE=1 SV=2 - [RBM14_HUMAN]			
Q01844	RNA-binding protein EWS OS=Homo sapiens GN=EWSR1 PE=1 SV=1 - [EWS_HUMAN]			
P62140	Serine/threonine-protein phosphatase PP1-beta catalytic subunit OS=Homo sapiens GN=PPP1CB PE=1 SV=3 - [PP1B_HUMAN]			
O76094	Signal recognition particle 72 kDa protein OS=Homo sapiens GN=SRP72 PE=1 SV=3 - [SRP72_HUMAN]			
Q04837	Single-stranded DNA-binding protein, mitochondrial OS=Homo sapiens GN=SSBP1 PE=1 SV=1 - [SSBP_HUMAN]			
P62306	Small nuclear ribonucleoprotein F OS=Homo sapiens GN=SNRPF PE=1 SV=1 - [RUXF_HUMAN]			
Q07889	Son of sevenless homolog 1 OS=Homo sapiens GN=SOS1 PE=1 SV=1 - [SOS1_HUMAN]			
Q14247	Src substrate cortactin OS=Homo sapiens GN=CTTN PE=1 SV=2 - [SRC8_HUMAN]			
P40227	T-complex protein 1 subunit zeta OS=Homo sapiens GN=CCT6A PE=1 SV=3 - [TCPZ_HUMAN]			
Q15654	Thyroid receptor-interacting protein 6 OS=Homo sapiens GN=TRIP6 PE=1 SV=3 - [TRIP6_HUMAN]			
Q15369	Transcription elongation factor B polypeptide 1 OS=Homo sapiens GN=TCEB1 PE=1 SV=1 - [ELOC_HUMAN]			
P06753	Tropomyosin alpha-3 chain OS=Homo sapiens GN=TPM3 PE=1 SV=2 - [TPM3_HUMAN]			
P68363	Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1 - [TBA1B_HUMAN]			
Q9H4B7	Tubulin beta-1 chain OS=Homo sapiens GN=TUBB1 PE=1 SV=1 - [TBB1_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
Q9BVA1	Tubulin beta-2B chain OS=Homo sapiens GN=TUBB2B PE=1 SV=1 - [TBB2B_HUMAN]			
Q13509	Tubulin beta-3 chain OS=Homo sapiens GN=TUBB3 PE=1 SV=2 - [TBB3_HUMAN]			
P68371	Tubulin beta-4B chain OS=Homo sapiens GN=TUBB4B PE=1 SV=1 - [TBB4B_HUMAN]			
Q6UXN9	WD repeat-containing protein 82 OS=Homo sapiens GN=WDR82 PE=1 SV=1 - [WDR82_HUMAN]			
P62913	60S ribosomal protein L11 OS=Homo sapiens GN=RPL11 PE=1 SV=2 - [RL11_HUMAN]			
P11021	78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2 - [GRP78_HUMAN]			
P60709	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 - [ACTB_HUMAN]			
P12814	Alpha-actinin-1 OS=Homo sapiens GN=ACTN1 PE=1 SV=2 - [ACTN1_HUMAN]			
P04745	Alpha-amylase 1 OS=Homo sapiens GN=AMY1A PE=1 SV=2 - [AMY1_HUMAN]			
P16989	DNA-binding protein A OS=Homo sapiens GN=CSDA PE=1 SV=4 - [DBPA_HUMAN]			
P08107	Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5 - [HSP71_HUMAN]			
Q9Y6M1	Insulin-like growth factor 2 mRNA-binding protein 2 OS=Homo sapiens GN=IGF2BP2 PE=1 SV=2 - [IF2B2_HUMAN]			
P00338	L-lactate dehydrogenase A chain OS=Homo sapiens GN=LDHA PE=1 SV=2 - [LDHA_HUMAN]			
P32119	Peroxiredoxin-2 OS=Homo sapiens GN=PRDX2 PE=1 SV=5 - [PRDX2_HUMAN]			
Q92841	Probable ATP-dependent RNA helicase DDX17 OS=Homo sapiens GN=DDX17 PE=1 SV=2 - [DDX17_HUMAN]			
P21980	Protein-glutamine gamma-glutamyltransferase 2 OS=Homo sapiens GN=TGM2 PE=1 SV=2 - [TGM2_HUMAN]			
Q9Y5X1	Sorting nexin-9 OS=Homo sapiens GN=SNX9 PE=1 SV=1 - [SNX9_HUMAN]			
P38646	Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2 - [GRP75_HUMAN]			
O14907	Tax1-binding protein 3 OS=Homo sapiens GN=TAX1BP3 PE=1 SV=2 - [TX1B3_HUMAN]			
P29401	Transketolase OS=Homo sapiens GN=TKT PE=1 SV=3 - [TKT_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
P07437	Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2 - [TBB5_HUMAN]			
P04350	Tubulin beta-4A chain OS=Homo sapiens GN=TUBB4A PE=1 SV=2 - [TBB4A_HUMAN]			
P62979	Ubiquitin-40S ribosomal protein S27a OS=Homo sapiens GN=RPS27A PE=1 SV=2 - [RS27A_HUMAN]			
Q15029	116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=1 SV=1 - [U5S1_HUMAN]			
Q9C0C2	182 kDa tankyrase-1-binding protein OS=Homo sapiens GN=TNKS1BP1 PE=1 SV=4 - [TB182_HUMAN]			
P19174	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-1 OS=Homo sapiens GN=PLCG1 PE=1 SV=1 - [PLCG1_HUMAN]			
P62333	26S protease regulatory subunit 10B OS=Homo sapiens GN=PSMC6 PE=1 SV=1 - [PRS10_HUMAN]			
P17980	26S protease regulatory subunit 6A OS=Homo sapiens GN=PSMC3 PE=1 SV=3 - [PRS6A_HUMAN]			
P43686	26S protease regulatory subunit 6B OS=Homo sapiens GN=PSMC4 PE=1 SV=2 - [PRS6B_HUMAN]			
P62195	26S protease regulatory subunit 8 OS=Homo sapiens GN=PSMC5 PE=1 SV=1 - [PRS8_HUMAN]			
Q99460	26S proteasome non-ATPase regulatory subunit 1 OS=Homo sapiens GN=PSMD1 PE=1 SV=2 - [PSMD1_HUMAN]			
O00487	26S proteasome non-ATPase regulatory subunit 14 OS=Homo sapiens GN=PSMD14 PE=1 SV=1 - [PSDE_HUMAN]			
Q13200	26S proteasome non-ATPase regulatory subunit 2 OS=Homo sapiens GN=PSMD2 PE=1 SV=3 - [PSMD2_HUMAN]			
O43242	26S proteasome non-ATPase regulatory subunit 3 OS=Homo sapiens GN=PSMD3 PE=1 SV=2 - [PSMD3_HUMAN]			
Q02218	2-oxoglutarate dehydrogenase, mitochondrial OS=Homo sapiens GN=OGDH PE=1 SV=3 - [ODO1_HUMAN]			
Q9BZE1	39S ribosomal protein L37, mitochondrial OS=Homo sapiens GN=MRPL37 PE=1 SV=2 - [RM37_HUMAN]			
Q99714	3-hydroxyacyl-CoA dehydrogenase type-2 OS=Homo sapiens GN=HSD17B10 PE=1 SV=3 - [HCD2_HUMAN]			
P62269	40S ribosomal protein S18 OS=Homo sapiens GN=RPS18 PE=1 SV=3 - [RS18_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
P23396	40S ribosomal protein S3 OS=Homo sapiens GN=RPS3 PE=1 SV=2 - [RS3_HUMAN]			
P49189	4-trimethylaminobutyraldehyde dehydrogenase OS=Homo sapiens GN=ALDH9A1 PE=1 SV=3 - [AL9A1_HUMAN]			
P08237	6-phosphofructokinase, muscle type OS=Homo sapiens GN=PFKM PE=1 SV=2 - [K6PF_HUMAN]			
Q9UBM7	7-dehydrocholesterol reductase OS=Homo sapiens GN=DHCR7 PE=1 SV=1 - [DHCR7_HUMAN]			
Q9NR19	Acetyl-coenzyme A synthetase, cytoplasmic OS=Homo sapiens GN=ACSS2 PE=1 SV=1 - [ACSA_HUMAN]			
Q99798	Aconitate hydratase, mitochondrial OS=Homo sapiens GN=ACO2 PE=1 SV=2 - [ACON_HUMAN]			
P61160	Actin-related protein 2 OS=Homo sapiens GN=ACTR2 PE=1 SV=1 - [ARP2_HUMAN]			
O15145	Actin-related protein 2/3 complex subunit 3 OS=Homo sapiens GN=ARPC3 PE=1 SV=3 - [ARPC3_HUMAN]			
P59998	Actin-related protein 2/3 complex subunit 4 OS=Homo sapiens GN=ARPC4 PE=1 SV=3 - [ARPC4_HUMAN]			
O00767	Acyl-CoA desaturase OS=Homo sapiens GN=SCD PE=1 SV=2 - [ACOD_HUMAN]			
Q86TX2	Acyl-coenzyme A thioesterase 1 OS=Homo sapiens GN=ACOT1 PE=1 SV=1 - [ACOT1_HUMAN]			
P25054	Adenomatous polyposis coli protein OS=Homo sapiens GN=APC PE=1 SV=2 - [APC_HUMAN]			
P12235	ADP/ATP translocase 1 OS=Homo sapiens GN=SLC25A4 PE=1 SV=4 - [ADT1_HUMAN]			
P05141	ADP/ATP translocase 2 OS=Homo sapiens GN=SLC25A5 PE=1 SV=7 - [ADT2_HUMAN]			
P84085	ADP-ribosylation factor 5 OS=Homo sapiens GN=ARF5 PE=1 SV=2 - [ARF5_HUMAN]			
P62330	ADP-ribosylation factor 6 OS=Homo sapiens GN=ARF6 PE=1 SV=2 - [ARF6_HUMAN]			
O00170	AH receptor-interacting protein OS=Homo sapiens GN=AIP PE=1 SV=2 - [AIP_HUMAN]			
P11766	Alcohol dehydrogenase class-3 OS=Homo sapiens GN=ADH5 PE=1 SV=4 - [ADHX_HUMAN]			
P01009	Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3 - [A1AT_HUMAN]			
P01023	Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=3 - [A2MG_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
P02771	Alpha-fetoprotein OS=Homo sapiens GN=AFP PE=1 SV=1 - [FETA_HUMAN]			
Q8IY63	Angiomotin-like protein 1 OS=Homo sapiens GN=AMOTL1 PE=1 SV=1 - [AMOL1_HUMAN]			
P50995	Annexin A11 OS=Homo sapiens GN=ANXA11 PE=1 SV=1 - [ANX11_HUMAN]			
P01008	Antithrombin-III OS=Homo sapiens GN=SERPINC1 PE=1 SV=1 - [ANT3_HUMAN]			
P63010	AP-2 complex subunit beta OS=Homo sapiens GN=AP2B1 PE=1 SV=1 - [AP2B1_HUMAN]			
P02647	Apolipoprotein A-I OS=Homo sapiens GN=APOA1 PE=1 SV=1 - [APOA1_HUMAN]			
P04114	Apolipoprotein B-100 OS=Homo sapiens GN=APOB PE=1 SV=2 - [APOB_HUMAN]			
P78540	Arginase-2, mitochondrial OS=Homo sapiens GN=ARG2 PE=1 SV=1 - [ARGI2_HUMAN]			
Q9UBB4	Ataxin-10 OS=Homo sapiens GN=ATXN10 PE=1 SV=1 - [ATX10_HUMAN]			
P25705	ATP synthase subunit alpha, mitochondrial OS=Homo sapiens GN=ATP5A1 PE=1 SV=1 - [ATPA_HUMAN]			
P48047	ATP synthase subunit O, mitochondrial OS=Homo sapiens GN=ATP5O PE=1 SV=1 - [ATPO_HUMAN]			
Q5T9A4	ATPase family AAA domain-containing protein 3B OS=Homo sapiens GN=ATAD3B PE=1 SV=1 - [ATD3B_HUMAN]			
Q99758	ATP-binding cassette sub-family A member 3 OS=Homo sapiens GN=ABCA3 PE=1 SV=2 - [ABCA3_HUMAN]			
P46063	ATP-dependent DNA helicase Q1 OS=Homo sapiens GN=RECQL PE=1 SV=3 - [RECQ1_HUMAN]			
O00148	ATP-dependent RNA helicase DDX39A OS=Homo sapiens GN=DDX39A PE=1 SV=2 - [DX39A_HUMAN]			
Q9Y6E2	Basic leucine zipper and W2 domain-containing protein 2 OS=Homo sapiens GN=BZW2 PE=1 SV=1 - [BZW2_HUMAN]			
Q9HBI1	Beta-parvin OS=Homo sapiens GN=PARVB PE=1 SV=1 - [PARVB_HUMAN]			
P53004	Biliverdin reductase A OS=Homo sapiens GN=BLVRA PE=1 SV=2 - [BIEA_HUMAN]			
Q9NWV8	BRISC and BRCA1-A complex member 1 OS=Homo sapiens GN=BABAM1 PE=1 SV=1 - [BABA1_HUMAN]			
Q9Y376	Calcium-binding protein 39 OS=Homo sapiens GN=CAB39 PE=1 SV=1 - [CAB39_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
P07384	Calpain-1 catalytic subunit OS=Homo sapiens GN=CAPN1 PE=1 SV=1 - [CAN1_HUMAN]			
O75828	Carbonyl reductase [NADPH] 3 OS=Homo sapiens GN=CBR3 PE=1 SV=3 - [CBR3_HUMAN]			
Q9BXW7	Cat eye syndrome critical region protein 5 OS=Homo sapiens GN=CECR5 PE=1 SV=1 - [CECR5_HUMAN]			
P11717	Cation-independent mannose-6-phosphate receptor OS=Homo sapiens GN=IGF2R PE=1 SV=3 - [MPRI_HUMAN]			
A5YKK6	CCR4-NOT transcription complex subunit 1 OS=Homo sapiens GN=CNOT1 PE=1 SV=2 - [CNOT1_HUMAN]			
Q7LBR1	Charged multivesicular body protein 1b OS=Homo sapiens GN=CHMP1B PE=1 SV=1 - [CHM1B_HUMAN]			
Q13185	Chromobox protein homolog 3 OS=Homo sapiens GN=CBX3 PE=1 SV=4 - [CBX3_HUMAN]			
Q14839	Chromodomain-helicase-DNA-binding protein 4 OS=Homo sapiens GN=CHD4 PE=1 SV=2 - [CHD4_HUMAN]			
P24941	chuk			
P09496	Clathrin light chain A OS=Homo sapiens GN=CLTA PE=1 SV=1 - [CLCA_HUMAN]			
Q16630	Cleavage and polyadenylation specificity factor subunit 6 OS=Homo sapiens GN=CPSF6 PE=1 SV=2 - [CPSF6_HUMAN]			
P53621	Coatomer subunit alpha OS=Homo sapiens GN=COPA PE=1 SV=2 - [COPA_HUMAN]			
P35606	Coatomer subunit beta' OS=Homo sapiens GN=COPB2 PE=1 SV=2 - [COPB2_HUMAN]			
O14579	Coatomer subunit epsilon OS=Homo sapiens GN=COPE PE=1 SV=3 - [COPE_HUMAN]			
P61923	Coatomer subunit zeta-1 OS=Homo sapiens GN=COPZ1 PE=1 SV=1 - [COPZ1_HUMAN]			
Q9Y281	Cofilin-2 OS=Homo sapiens GN=CFL2 PE=1 SV=1 - [COF2_HUMAN]			
Q9NVE4	Coiled-coil domain-containing protein 87 OS=Homo sapiens GN=CCDC87 PE=2 SV=2 - [CCD87_HUMAN]			
Q9NX63	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial OS=Homo sapiens GN=CHCHD3 PE=1 SV=1 - [CHCH3_HUMAN]			
P00746	Complement factor D OS=Homo sapiens GN=CFD PE=1 SV=5 - [CFAD_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
Q15021	Condensin complex subunit 1 OS=Homo sapiens GN=NCAPD2 PE=1 SV=3 - [CND1_HUMAN]			
Q9NZB2	Constitutive coactivator of PPAR-gamma-like protein 1 OS=Homo sapiens GN=FAM120A PE=1 SV=2 - [F120A_HUMAN]			
Q92905	COP9 signalosome complex subunit 5 OS=Homo sapiens GN=COPS5 PE=1 SV=4 - [CSN5_HUMAN]			
P12277	Creatine kinase B-type OS=Homo sapiens GN=CKB PE=1 SV=1 - [KCRB_HUMAN]			
Q86VP6	Cullin-associated NEDD8-dissociated protein 1 OS=Homo sapiens GN=CAND1 PE=1 SV=2 - [CAND1_HUMAN]			
P06493	Cyclin-dependent kinase 1 OS=Homo sapiens GN=CDK1 PE=1 SV=3 - [CDK1_HUMAN]			
P21291	Cysteine and glycine-rich protein 1 OS=Homo sapiens GN=CSRP1 PE=1 SV=3 - [CSRP1_HUMAN]			
P00403	Cytochrome c oxidase subunit 2 OS=Homo sapiens GN=MT-CO2 PE=1 SV=1 - [COX2_HUMAN]			
Q8NCM8	Cytoplasmic dynein 2 heavy chain 1 OS=Homo sapiens GN=DYNC2H1 PE=1 SV=4 - [DYHC2_HUMAN]			
Q7L576	Cytoplasmic FMR1-interacting protein 1 OS=Homo sapiens GN=CYFIP1 PE=1 SV=1 - [CYFP1_HUMAN]			
Q14008	Cytoskeleton-associated protein 5 OS=Homo sapiens GN=CKAP5 PE=1 SV=3 - [CKAP5_HUMAN]			
Q9BZ29	Dedicator of cytokinesis protein 9 OS=Homo sapiens GN=DOCK9 PE=1 SV=2 - [DOCK9_HUMAN]			
P54886	Delta-1-pyrroline-5-carboxylate synthase OS=Homo sapiens GN=ALDH18A1 PE=1 SV=2 - [P5CS_HUMAN]			
P36957	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial OS=Homo sapiens GN=DLST PE=1 SV=4 - [ODO2_HUMAN]			
Q9NY33	Dipeptidyl peptidase 3 OS=Homo sapiens GN=DPP3 PE=1 SV=2 - [DPP3_HUMAN]			
P26358	DNA (cytosine-5)-methyltransferase 1 OS=Homo sapiens GN=DNMT1 PE=1 SV=2 - [DNMT1_HUMAN]			
P28340	DNA polymerase delta catalytic subunit OS=Homo sapiens GN=POLD1 PE=1 SV=2 - [DPOD1_HUMAN]			
P49736	DNA replication licensing factor MCM2 OS=Homo sapiens GN=MCM2 PE=1 SV=4 - [MCM2_HUMAN]			
P25205	DNA replication licensing factor MCM3 OS=Homo sapiens GN=MCM3 PE=1 SV=3 - [MCM3_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
P33991	DNA replication licensing factor MCM4 OS=Homo sapiens GN=MCM4 PE=1 SV=5 - [MCM4_HUMAN]			
P33993	DNA replication licensing factor MCM7 OS=Homo sapiens GN=MCM7 PE=1 SV=4 - [MCM7_HUMAN]			
P27695	DNA-(apurinic or apyrimidinic site) lyase OS=Homo sapiens GN=APEX1 PE=1 SV=2 - [APEX1_HUMAN]			
P78527	DNA-dependent protein kinase catalytic subunit OS=Homo sapiens GN=PRKDC PE=1 SV=3 - [PRKDC_HUMAN]			
P04843	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 OS=Homo sapiens GN=RPN1 PE=1 SV=1 - [RPN1_HUMAN]			
Q5VZB9	Doublesex- and mab-3-related transcription factor A1 OS=Homo sapiens GN=DMRTA1 PE=2 SV=1 - [DMRTA_HUMAN]			
Q02750	Dual specificity mitogen-activated protein kinase kinase 1 OS=Homo sapiens GN=MAP2K1 PE=1 SV=2 - [MP2K1_HUMAN]			
P36507	Dual specificity mitogen-activated protein kinase kinase 2 OS=Homo sapiens GN=MAP2K2 PE=1 SV=1 - [MP2K2_HUMAN]			
Q13561	Dynactin subunit 2 OS=Homo sapiens GN=DCTN2 PE=1 SV=4 - [DCTN2_HUMAN]			
Q7Z6Z7	E3 ubiquitin-protein ligase HUWE1 OS=Homo sapiens GN=HUWE1 PE=1 SV=3 - [HUWE1_HUMAN]			
Q13049	E3 ubiquitin-protein ligase TRIM32 OS=Homo sapiens GN=TRIM32 PE=1 SV=2 - [TRI32_HUMAN]			
Q5T4S7	E3 ubiquitin-protein ligase UBR4 OS=Homo sapiens GN=UBR4 PE=1 SV=1 - [UBR4_HUMAN]			
Q96C19	EF-hand domain-containing protein D2 OS=Homo sapiens GN=EFHD2 PE=1 SV=1 - [EFHD2_HUMAN]			
P43897	Elongation factor Ts, mitochondrial OS=Homo sapiens GN=TSFM PE=1 SV=2 - [EFTS_HUMAN]			
Q14240	Eukaryotic initiation factor 4A-II OS=Homo sapiens GN=EIF4A2 PE=1 SV=2 - [IF4A2_HUMAN]			
Q99613	Eukaryotic translation initiation factor 3 subunit C OS=Homo sapiens GN=EIF3C PE=1 SV=1 - [EIF3C_HUMAN]			
O15372	Eukaryotic translation initiation factor 3 subunit H OS=Homo sapiens GN=EIF3H PE=1 SV=1 - [EIF3H_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
Q9NQT5	Exosome complex component RRP40 OS=Homo sapiens GN=EXOSC3 PE=1 SV=3 - [EXOS3_HUMAN]			
Q9HAV4	Exportin-5 OS=Homo sapiens GN=XPO5 PE=1 SV=1 - [XPO5_HUMAN]			
Q9UIA9	Exportin-7 OS=Homo sapiens GN=XPO7 PE=1 SV=3 - [XPO7_HUMAN]			
Q9BSJ8	Extended synaptotagmin-1 OS=Homo sapiens GN=ESYT1 PE=1 SV=1 - [ESYT1_HUMAN]			
P52907	F-actin-capping protein subunit alpha-1 OS=Homo sapiens GN=CAPZA1 PE=1 SV=3 - [CAZA1_HUMAN]			
Q92945	Far upstream element-binding protein 2 OS=Homo sapiens GN=KHSRP PE=1 SV=4 - [FUBP2_HUMAN]			
Q2WGJ9	Fer-1-like protein 6 OS=Homo sapiens GN=FER1L6 PE=2 SV=2 - [FR1L6_HUMAN]			
P07954	Fumarate hydratase, mitochondrial OS=Homo sapiens GN=FH PE=1 SV=3 - [FUMH_HUMAN]			
P16930	Fumarylacetoacetase OS=Homo sapiens GN=FAH PE=1 SV=2 - [FAAA_HUMAN]			
P51570	Galactokinase OS=Homo sapiens GN=GALK1 PE=1 SV=1 - [GALK1_HUMAN]			
Q8TEQ6	Gem-associated protein 5 OS=Homo sapiens GN=GEMIN5 PE=1 SV=3 - [GEMI5_HUMAN]			
P78347	General transcription factor II-I OS=Homo sapiens GN=GTF2I PE=1 SV=2 - [GTF2I_HUMAN]			
P11413	Glucose-6-phosphate 1-dehydrogenase OS=Homo sapiens GN=G6PD PE=1 SV=4 - [G6PD_HUMAN]			
P14314	Glucosidase 2 subunit beta OS=Homo sapiens GN=PRKCSH PE=1 SV=2 - [GLU2B_HUMAN]			
P78417	Glutathione S-transferase omega-1 OS=Homo sapiens GN=GSTO1 PE=1 SV=2 - [GSTO1_HUMAN]			
P11216	Glycogen phosphorylase, brain form OS=Homo sapiens GN=PYGB PE=1 SV=5 - [PYGB_HUMAN]			
Q92538	Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1 OS=Homo sapiens GN=GBF1 PE=1 SV=2 - [GBF1_HUMAN]			
Q9UIJ7	GTP:AMP phosphotransferase, mitochondrial OS=Homo sapiens GN=AK3 PE=1 SV=4 - [KAD3_HUMAN]			
Q14C86	GTPase-activating protein and VPS9 domain-containing protein 1 OS=Homo sapiens GN=GAPVD1 PE=1 SV=2 - [GAPD1_HUMAN]			
Q15382	GTP-binding protein Rheb OS=Homo sapiens GN=RHEB PE=1 SV=1 - [RHEB_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
Q9Y6B6	GTP-binding protein SAR1b OS=Homo sapiens GN=SAR1B PE=1 SV=1 - [SAR1B_HUMAN]			
Q9UBI6	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-12 OS=Homo sapiens GN=GNG12 PE=1 SV=3 - [GBG12_HUMAN]			
P63244	Guanine nucleotide-binding protein subunit beta-2-like 1 OS=Homo sapiens GN=GNB2L1 PE=1 SV=3 - [GBLP_HUMAN]			
P14866	Heterogeneous nuclear ribonucleoprotein L OS=Homo sapiens GN=HNRNPL PE=1 SV=2 - [HNRPL_HUMAN]			
Q1KMD3	Heterogeneous nuclear ribonucleoprotein U-like protein 2 OS=Homo sapiens GN=HNRNPUL2 PE=1 SV=1 - [HNRL2_HUMAN]			
P52789	Hexokinase-2 OS=Homo sapiens GN=HK2 PE=1 SV=2 - [HXK2_HUMAN]			
Q92522	Histone H1x OS=Homo sapiens GN=H1FX PE=1 SV=1 - [H1X_HUMAN]			
Q96KK5	Histone H2A type 1-H OS=Homo sapiens GN=HIST1H2AH PE=1 SV=3 - [H2A1H_HUMAN]			
Q8IUE6	Histone H2A type 2-B OS=Homo sapiens GN=HIST2H2AB PE=1 SV=3 - [H2A2B_HUMAN]			
Q09028	Histone-binding protein RBBP4 OS=Homo sapiens GN=RBBP4 PE=1 SV=3 - [RBBP4_HUMAN]			
Q16543	Hsp90 co-chaperone Cdc37 OS=Homo sapiens GN=CDC37 PE=1 SV=1 - [CDC37_HUMAN]			
Q9Y4L1	Hypoxia up-regulated protein 1 OS=Homo sapiens GN=HYOU1 PE=1 SV=1 - [HYOU1_HUMAN]			
P01857	Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1 - [IGHG1_HUMAN]			
P52292	Importin subunit alpha-2 OS=Homo sapiens GN=KPNA2 PE=1 SV=1 - [IMA2_HUMAN]			
Q8NI35	InaD-like protein OS=Homo sapiens GN=INADL PE=1 SV=3 - [INADL_HUMAN]			
Q9Y2U8	Inner nuclear membrane protein Man1 OS=Homo sapiens GN=LEMD3 PE=1 SV=2 - [MAN1_HUMAN]			
Q9H2U2	Inorganic pyrophosphatase 2, mitochondrial OS=Homo sapiens GN=PPA2 PE=1 SV=2 - [IPYR2_HUMAN]			
Q15181	Inorganic pyrophosphatase OS=Homo sapiens GN=PPA1 PE=1 SV=2 - [IPYR_HUMAN]			
P29218	Inositol monophosphatase 1 OS=Homo sapiens GN=IMPA1 PE=1 SV=1 - [IMPA1_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
Q13418	Integrin-linked protein kinase OS=Homo sapiens GN=ILK PE=1 SV=2 - [ILK_HUMAN]			
P19823	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Homo sapiens GN=ITIH2 PE=1 SV=2 - [ITIH2_HUMAN]			
O60306	Intron-binding protein aquarius OS=Homo sapiens GN=AQR PE=1 SV=4 - [AQR_HUMAN]			
Q96CN7	Isochorismatase domain-containing protein 1 OS=Homo sapiens GN=ISOC1 PE=1 SV=3 - [ISOC1_HUMAN]			
O43837	Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial OS=Homo sapiens GN=IDH3B PE=1 SV=2 - [IDH3B_HUMAN]			
P41252	Isoleucine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=IARS PE=1 SV=2 - [SYIC_HUMAN]			
Q13907	Isopentenyl-diphosphate Delta-isomerase 1 OS=Homo sapiens GN=IDI1 PE=1 SV=2 - [IDI1_HUMAN]			
Q14678	KN motif and ankyrin repeat domain-containing protein 1 OS=Homo sapiens GN=KANK1 PE=1 SV=3 - [KANK1_HUMAN]			
P20700	Lamin-B1 OS=Homo sapiens GN=LMNB1 PE=1 SV=2 - [LMNB1_HUMAN]			
Q01650	Large neutral amino acids transporter small subunit 1 OS=Homo sapiens GN=SLC7A5 PE=1 SV=2 - [LAT1_HUMAN]			
Q9NQ48	Leucine zipper transcription factor-like protein 1 OS=Homo sapiens GN=LZTFL1 PE=1 SV=1 - [LZTL1_HUMAN]			
P50851	Lipopolysaccharide-responsive and beige-like anchor protein OS=Homo sapiens GN=LRBA PE=1 SV=4 - [LRBA_HUMAN]			
Q86W92	Liprin-beta-1 OS=Homo sapiens GN=PPFIBP1 PE=1 SV=2 - [LIPB1_HUMAN]			
P36776	Lon protease homolog, mitochondrial OS=Homo sapiens GN=LONP1 PE=1 SV=2 - [LONM_HUMAN]			
O95573	Long-chain-fatty-acid--CoA ligase 3 OS=Homo sapiens GN=ACSL3 PE=1 SV=3 - [ACSL3_HUMAN]			
O60488	Long-chain-fatty-acid--CoA ligase 4 OS=Homo sapiens GN=ACSL4 PE=1 SV=2 - [ACSL4_HUMAN]			
Q14165	Malectin OS=Homo sapiens GN=MLEC PE=1 SV=1 - [MLEC_HUMAN]			
Q96IJ6	Mannose-1-phosphate guanyltransferase alpha OS=Homo sapiens GN=GMPPA PE=1 SV=1 - [GMPPA_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
O75352	Mannose-P-dolichol utilization defect 1 protein OS=Homo sapiens GN=MPDU1 PE=1 SV=2 - [MPU1_HUMAN]			
Q96QZ7	Membrane-associated guanylate kinase, WW and PDZ domain-containing protein 1 OS=Homo sapiens GN=MAGI1 PE=1 SV=3 - [MAGI1_HUMAN]			
O00264	Membrane-associated progesterone receptor component 1 OS=Homo sapiens GN=PGRMC1 PE=1 SV=3 - [PGRC1_HUMAN]			
Q9UPN3	Microtubule-actin cross-linking factor 1, isoforms 1/2/3/5 OS=Homo sapiens GN=MACF1 PE=1 SV=4 - [MACF1_HUMAN]			
Q8N4C8	Misshapen-like kinase 1 OS=Homo sapiens GN=MINK1 PE=1 SV=2 - [MINK1_HUMAN]			
Q9Y3D6	Mitochondrial fission 1 protein OS=Homo sapiens GN=FIS1 PE=1 SV=2 - [FIS1_HUMAN]			
Q3ZCQ8	Mitochondrial import inner membrane translocase subunit TIM50 OS=Homo sapiens GN=TIMM50 PE=1 SV=2 - [TIM50_HUMAN]			
O75439	Mitochondrial-processing peptidase subunit beta OS=Homo sapiens GN=PMPCB PE=1 SV=2 - [MPPB_HUMAN]			
P78406	mRNA export factor OS=Homo sapiens GN=RAE1 PE=1 SV=1 - [RAE1L_HUMAN]			
Q9NR56	Muscleblind-like protein 1 OS=Homo sapiens GN=MBNL1 PE=1 SV=2 - [MBNL1_HUMAN]			
Q9NZM1	Myoferlin OS=Homo sapiens GN=MYOF PE=1 SV=1 - [MYOF_HUMAN]			
O14745	Na(+)/H(+) exchange regulatory cofactor NHE-RF1 OS=Homo sapiens GN=SLC9A3R1 PE=1 SV=4 - [NHRF1_HUMAN]			
Q15599	Na(+)/H(+) exchange regulatory cofactor NHE-RF2 OS=Homo sapiens GN=SLC9A3R2 PE=1 SV=2 - [NHRF2_HUMAN]			
O75489	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial OS=Homo sapiens GN=NDUFS3 PE=1 SV=1 - [NDUS3_HUMAN]			
Q9GZZ1	N-alpha-acetyltransferase 50 OS=Homo sapiens GN=NAA50 PE=1 SV=1 - [NAA50_HUMAN]			
O43847	Nardilysin OS=Homo sapiens GN=NRD1 PE=1 SV=2 - [NRDC_HUMAN]			
Q6X4W1	Nasal embryonic luteinizing hormone-releasing hormone factor OS=Homo sapiens GN=NELF PE=1 SV=1 -			

Acc #	Protein	PM	NP	Wildtype
	[NELF_HUMAN]			
P12036	Neurofilament heavy polypeptide OS=Homo sapiens GN=NEFH PE=1 SV=4 - [NFH_HUMAN]			
Q9Y314	Nitric oxide synthase-interacting protein OS=Homo sapiens GN=NOSIP PE=1 SV=1 - [NOSIP_HUMAN]			
Q15233	Non-POU domain-containing octamer-binding protein OS=Homo sapiens GN=NONO PE=1 SV=4 - [NONO_HUMAN]			
Q9NVX2	Notchless protein homolog 1 OS=Homo sapiens GN=NLE1 PE=1 SV=4 - [NLE1_HUMAN]			
P49321	Nuclear autoantigenic sperm protein OS=Homo sapiens GN=NASP PE=1 SV=2 - [NASP_HUMAN]			
Q15596	Nuclear receptor coactivator 2 OS=Homo sapiens GN=NCOA2 PE=1 SV=2 - [NCOA2_HUMAN]			
P67809	Nuclease-sensitive element-binding protein 1 OS=Homo sapiens GN=YBX1 PE=1 SV=3 - [YBOX1_HUMAN]			
P12270	Nucleoprotein TPR OS=Homo sapiens GN=TPR PE=1 SV=3 - [TPR_HUMAN]			
P55209	Nucleosome assembly protein 1-like 1 OS=Homo sapiens GN=NAP1L1 PE=1 SV=1 - [NP1L1_HUMAN]			
Q9Y239	Nucleotide-binding oligomerization domain-containing protein 1 OS=Homo sapiens GN=NOD1 PE=1 SV=1 - [NOD1_HUMAN]			
Q96RS6	NudC domain-containing protein 1 OS=Homo sapiens GN=NUDCD1 PE=1 SV=2 - [NUDC1_HUMAN]			
Q9NR12	PDZ and LIM domain protein 7 OS=Homo sapiens GN=PDLIM7 PE=1 SV=1 - [PDLI7_HUMAN]			
Q08752	Peptidyl-prolyl cis-trans isomerase D OS=Homo sapiens GN=PPID PE=1 SV=3 - [PPID_HUMAN]			
O43447	Peptidyl-prolyl cis-trans isomerase H OS=Homo sapiens GN=PPIH PE=1 SV=1 - [PPIH_HUMAN]			
P30044	Peroxiredoxin-5, mitochondrial OS=Homo sapiens GN=PRDX5 PE=1 SV=4 - [PRDX5_HUMAN]			
Q08426	Peroxisomal bifunctional enzyme OS=Homo sapiens GN=EHHADH PE=1 SV=3 - [ECHP_HUMAN]			
O00443	Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit alpha OS=Homo sapiens GN=PIK3C2A PE=1 SV=2 - [P3C2A_HUMAN]			
Q9Y617	Phosphoserine aminotransferase OS=Homo sapiens GN=PSAT1 PE=1 SV=2 - [SERC_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
Q99959	Plakophilin-2 OS=Homo sapiens GN=PKP2 PE=1 SV=2 - [PKP2_HUMAN]			
P26599	Polypyrimidine tract-binding protein 1 OS=Homo sapiens GN=PTBP1 PE=1 SV=1 - [PTBP1_HUMAN]			
P20742	Pregnancy zone protein OS=Homo sapiens GN=PZP PE=1 SV=4 - [PZP_HUMAN]			
Q13206	Probable ATP-dependent RNA helicase DDX10 OS=Homo sapiens GN=DDX10 PE=1 SV=2 - [DDX10_HUMAN]			
P25788	Proteasome subunit alpha type-3 OS=Homo sapiens GN=PSMA3 PE=1 SV=2 - [PSA3_HUMAN]			
P25789	Proteasome subunit alpha type-4 OS=Homo sapiens GN=PSMA4 PE=1 SV=1 - [PSA4_HUMAN]			
P28066	Proteasome subunit alpha type-5 OS=Homo sapiens GN=PSMA5 PE=1 SV=3 - [PSA5_HUMAN]			
P60900	Proteasome subunit alpha type-6 OS=Homo sapiens GN=PSMA6 PE=1 SV=1 - [PSA6_HUMAN]			
Q99873	Protein arginine N-methyltransferase 1 OS=Homo sapiens GN=PRMT1 PE=1 SV=2 - [ANM1_HUMAN]			
Q9Y2B0	Protein canopy homolog 2 OS=Homo sapiens GN=CNPY2 PE=1 SV=1 - [CNPY2_HUMAN]			
Q9NUQ9	Protein FAM49B OS=Homo sapiens GN=FAM49B PE=1 SV=1 - [FA49B_HUMAN]			
Q13045	Protein flightless-1 homolog OS=Homo sapiens GN=FLII PE=1 SV=2 - [FLII_HUMAN]			
P61326	Protein mago nashi homolog OS=Homo sapiens GN=MAGOH PE=1 SV=1 - [MGN_HUMAN]			
Q92597	Protein NDRG1 OS=Homo sapiens GN=NDRG1 PE=1 SV=1 - [NDRG1_HUMAN]			
Q9BPW8	Protein NipSnap homolog 1 OS=Homo sapiens GN=NIPSNAP1 PE=1 SV=1 - [NIPS1_HUMAN]			
O75323	Protein NipSnap homolog 2 OS=Homo sapiens GN=GBAS PE=1 SV=1 - [NIPS2_HUMAN]			
Q9H7J1	Protein phosphatase 1 regulatory subunit 3E OS=Homo sapiens GN=PPP1R3E PE=2 SV=2 - [PPR3E_HUMAN]			
P41236	Protein phosphatase inhibitor 2 OS=Homo sapiens GN=PPP1R2 PE=1 SV=2 - [IPP2_HUMAN]			
Q9Y570	Protein phosphatase methylesterase 1 OS=Homo sapiens GN=PPME1 PE=1 SV=3 - [PPME1_HUMAN]			
P48634	Protein PRRC2A OS=Homo sapiens GN=PRRC2A PE=1 SV=3 - [PRC2A_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
Q9P258	Protein RCC2 OS=Homo sapiens GN=RCC2 PE=1 SV=2 - [RCC2_HUMAN]			
Q01105	Protein SET OS=Homo sapiens GN=SET PE=1 SV=3 - [SET_HUMAN]			
Q969Z0	Protein TBRG4 OS=Homo sapiens GN=TBRG4 PE=1 SV=1 - [TBRG4_HUMAN]			
Q7L2E3	Putative ATP-dependent RNA helicase DHX30 OS=Homo sapiens GN=DHX30 PE=1 SV=1 - [DHX30_HUMAN]			
Q9BZK3	Putative nascent polypeptide-associated complex subunit alpha-like protein OS=Homo sapiens GN=NACAP1 PE=5 SV=1 - [NACP1_HUMAN]			
Q9NQ29	Putative RNA-binding protein Luc7-like 1 OS=Homo sapiens GN=LUC7L PE=1 SV=1 - [LUC7L_HUMAN]			
Q9Y383	Putative RNA-binding protein Luc7-like 2 OS=Homo sapiens GN=LUC7L2 PE=1 SV=2 - [LC7L2_HUMAN]			
O00764	Pyridoxal kinase OS=Homo sapiens GN=PDXK PE=1 SV=1 - [PDXK_HUMAN]			
P32322	Pyrroline-5-carboxylate reductase 1, mitochondrial OS=Homo sapiens GN=PYCR1 PE=1 SV=2 - [P5CR1_HUMAN]			
Q96C36	Pyrroline-5-carboxylate reductase 2 OS=Homo sapiens GN=PYCR2 PE=1 SV=1 - [P5CR2_HUMAN]			
P61026	Ras-related protein Rab-10 OS=Homo sapiens GN=RAB10 PE=1 SV=1 - [RAB10_HUMAN]			
P62820	Ras-related protein Rab-1A OS=Homo sapiens GN=RAB1A PE=1 SV=3 - [RAB1A_HUMAN]			
Q9H0U4	Ras-related protein Rab-1B OS=Homo sapiens GN=RAB1B PE=1 SV=1 - [RAB1B_HUMAN]			
Q9UL25	Ras-related protein Rab-21 OS=Homo sapiens GN=RAB21 PE=1 SV=3 - [RAB21_HUMAN]			
P20338	Ras-related protein Rab-4A OS=Homo sapiens GN=RAB4A PE=1 SV=3 - [RAB4A_HUMAN]			
P20339	Ras-related protein Rab-5A OS=Homo sapiens GN=RAB5A PE=1 SV=2 - [RAB5A_HUMAN]			
P61020	Ras-related protein Rab-5B OS=Homo sapiens GN=RAB5B PE=1 SV=1 - [RAB5B_HUMAN]			
P51148	Ras-related protein Rab-5C OS=Homo sapiens GN=RAB5C PE=1 SV=2 - [RAB5C_HUMAN]			
P61006	Ras-related protein Rab-8A OS=Homo sapiens GN=RAB8A PE=1 SV=1 - [RAB8A_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
Q9NQG5	Regulation of nuclear pre-mRNA domain-containing protein 1B OS=Homo sapiens GN=RPRD1B PE=1 SV=1 - [RPR1B_HUMAN]			
Q92900	Regulator of nonsense transcripts 1 OS=Homo sapiens GN=UPF1 PE=1 SV=2 - [RENT1_HUMAN]			
P40938	Replication factor C subunit 3 OS=Homo sapiens GN=RFC3 PE=1 SV=2 - [RFC3_HUMAN]			
Q15293	Reticulocalbin-1 OS=Homo sapiens GN=RCN1 PE=1 SV=1 - [RCN1_HUMAN]			
Q9NQC3	Reticulon-4 OS=Homo sapiens GN=RTN4 PE=1 SV=2 - [RTN4_HUMAN]			
Q8TC12	Retinol dehydrogenase 11 OS=Homo sapiens GN=RDH11 PE=1 SV=2 - [RDH11_HUMAN]			
P78346	Ribonuclease P protein subunit p30 OS=Homo sapiens GN=RPP30 PE=1 SV=1 - [RPP30_HUMAN]			
P11908	Ribose-phosphate pyrophosphokinase 2 OS=Homo sapiens GN=PRPS2 PE=1 SV=2 - [PRPS2_HUMAN]			
P38159	RNA-binding motif protein, X chromosome OS=Homo sapiens GN=RBMX PE=1 SV=3 - [RBMX_HUMAN]			
P35637	RNA-binding protein FUS OS=Homo sapiens GN=FUS PE=1 SV=1 - [FUS_HUMAN]			
P31153	S-adenosylmethionine synthase isoform type-2 OS=Homo sapiens GN=MAT2A PE=1 SV=1 - [METK2_HUMAN]			
Q9NVA2	Septin-11 OS=Homo sapiens GN=SEPT11 PE=1 SV=3 - [SEP11_HUMAN]			
Q15019	Septin-2 OS=Homo sapiens GN=SEPT2 PE=1 SV=1 - [SEPT2_HUMAN]			
Q01130	Serine/arginine-rich splicing factor 2 OS=Homo sapiens GN=SRSF2 PE=1 SV=4 - [SRSF2_HUMAN]			
Q13043	Serine/threonine-protein kinase 4 OS=Homo sapiens GN=STK4 PE=1 SV=2 - [STK4_HUMAN]			
P63151	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform OS=Homo sapiens GN=PPP2R2A PE=1 SV=1 - [2ABA_HUMAN]			
P36873	Serine/threonine-protein phosphatase PP1-gamma catalytic subunit OS=Homo sapiens GN=PPP1CC PE=1 SV=1 - [PP1G_HUMAN]			
P49591	Serine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=SARS PE=1 SV=3 - [SYSC_HUMAN]			
P50454	Serpin H1 OS=Homo sapiens GN=SERPINH1 PE=1 SV=2 - [SERPH_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
Q9NR45	Sialic acid synthase OS=Homo sapiens GN=NANS PE=1 SV=2 - [SIAS_HUMAN]			
Q15005	Signal peptidase complex subunit 2 OS=Homo sapiens GN=SPCS2 PE=1 SV=3 - [SPCS2_HUMAN]			
P42224	Signal transducer and activator of transcription 1-alpha/beta OS=Homo sapiens GN=STAT1 PE=1 SV=2 - [STAT1_HUMAN]			
P62304	Small nuclear ribonucleoprotein E OS=Homo sapiens GN=SNRPE PE=1 SV=1 - [RUXE_HUMAN]			
P62318	Small nuclear ribonucleoprotein Sm D3 OS=Homo sapiens GN=SNRPD3 PE=1 SV=1 - [SMD3_HUMAN]			
P05023	Sodium/potassium-transporting ATPase subunit alpha-1 OS=Homo sapiens GN=ATP1A1 PE=1 SV=1 - [AT1A1_HUMAN]			
Q9BX66	Sorbin and SH3 domain-containing protein 1 OS=Homo sapiens GN=SORBS1 PE=1 SV=3 - [SRBS1_HUMAN]			
Q00796	Sorbitol dehydrogenase OS=Homo sapiens GN=SORD PE=1 SV=4 - [DHSO_HUMAN]			
Q9Y5X3	Sorting nexin-5 OS=Homo sapiens GN=SNX5 PE=1 SV=1 - [SNX5_HUMAN]			
P63208	S-phase kinase-associated protein 1 OS=Homo sapiens GN=SKP1 PE=1 SV=2 - [SKP1_HUMAN]			
Q13838	Spliceosome RNA helicase DDX39B OS=Homo sapiens GN=DDX39B PE=1 SV=1 - [DX39B_HUMAN]			
Q12874	Splicing factor 3A subunit 3 OS=Homo sapiens GN=SF3A3 PE=1 SV=1 - [SF3A3_HUMAN]			
O75533	Splicing factor 3B subunit 1 OS=Homo sapiens GN=SF3B1 PE=1 SV=3 - [SF3B1_HUMAN]			
Q15393	Splicing factor 3B subunit 3 OS=Homo sapiens GN=SF3B3 PE=1 SV=4 - [SF3B3_HUMAN]			
P23246	Splicing factor, proline- and glutamine-rich OS=Homo sapiens GN=SFPQ PE=1 SV=2 - [SFPQ_HUMAN]			
Q9UJZ1	Stomatin-like protein 2 OS=Homo sapiens GN=STOML2 PE=1 SV=1 - [STML2_HUMAN]			
A6NHR9	Structural maintenance of chromosomes flexible hinge domain-containing protein 1 OS=Homo sapiens GN=SMCHD1 PE=1 SV=2 - [SMHD1_HUMAN]			
Q9UQE7	Structural maintenance of chromosomes protein 3 OS=Homo sapiens GN=SMC3 PE=1 SV=2 - [SMC3_HUMAN]			
Q9UBE0	SUMO-activating enzyme subunit 1 OS=Homo sapiens GN=SAE1 PE=1 SV=1 - [SAE1_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
Q9UBT2	SUMO-activating enzyme subunit 2 OS=Homo sapiens GN=UBA2 PE=1 SV=2 - [SAE2_HUMAN]			
Q9Y4G6	Talin-2 OS=Homo sapiens GN=TLN2 PE=1 SV=4 - [TLN2_HUMAN]			
O60343	TBC1 domain family member 4 OS=Homo sapiens GN=TBC1D4 PE=1 SV=2 - [TBCD4_HUMAN]			
P78371	T-complex protein 1 subunit beta OS=Homo sapiens GN=CCT2 PE=1 SV=4 - [TCPB_HUMAN]			
Q9Y2W1	Thyroid hormone receptor-associated protein 3 OS=Homo sapiens GN=THRAP3 PE=1 SV=2 - [TR150_HUMAN]			
Q9UDY2	Tight junction protein ZO-2 OS=Homo sapiens GN=TJP2 PE=1 SV=2 - [ZO2_HUMAN]			
Q8WZ42	Titin OS=Homo sapiens GN=TTN PE=1 SV=4 - [TITIN_HUMAN]			
O00267	Transcription elongation factor SPT5 OS=Homo sapiens GN=SUPT5H PE=1 SV=1 - [SPT5H_HUMAN]			
Q13263	Transcription intermediary factor 1-beta OS=Homo sapiens GN=TRIM28 PE=1 SV=5 - [TIF1B_HUMAN]			
Q92616	Translational activator GCN1 OS=Homo sapiens GN=GCN1L1 PE=1 SV=6 - [GCN1L_HUMAN]			
Q15631	Translin OS=Homo sapiens GN=TSN PE=1 SV=1 - [TSN_HUMAN]			
P02766	Transthyretin OS=Homo sapiens GN=TTR PE=1 SV=1 - [TTHY_HUMAN]			
P29144	Tripeptidyl-peptidase 2 OS=Homo sapiens GN=TPP2 PE=1 SV=4 - [TPP2_HUMAN]			
P67936	Tropomyosin alpha-4 chain OS=Homo sapiens GN=TPM4 PE=1 SV=3 - [TPM4_HUMAN]			
Q9BUF5	Tubulin beta-6 chain OS=Homo sapiens GN=TUBB6 PE=1 SV=1 - [TBB6_HUMAN]			
Q9BTW9	Tubulin-specific chaperone D OS=Homo sapiens GN=TBCD PE=1 SV=2 - [TBCD_HUMAN]			
P18031	Tyrosine-protein phosphatase non-receptor type 1 OS=Homo sapiens GN=PTPN1 PE=1 SV=1 - [PTN1_HUMAN]			
Q9H3S7	Tyrosine-protein phosphatase non-receptor type 23 OS=Homo sapiens GN=PTPN23 PE=1 SV=1 - [PTN23_HUMAN]			
P45974	Ubiquitin carboxyl-terminal hydrolase 5 OS=Homo sapiens GN=USP5 PE=1 SV=2 - [UBP5_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
Q13404	Ubiquitin-conjugating enzyme E2 variant 1 OS=Homo sapiens GN=UBE2V1 PE=1 SV=2 - [UB2V1_HUMAN]			
Q15819	Ubiquitin-conjugating enzyme E2 variant 2 OS=Homo sapiens GN=UBE2V2 PE=1 SV=4 - [UB2V2_HUMAN]			
P22314	Ubiquitin-like modifier-activating enzyme 1 OS=Homo sapiens GN=UBA1 PE=1 SV=3 - [UBA1_HUMAN]			
Q04323	UBX domain-containing protein 1 OS=Homo sapiens GN=UBXN1 PE=1 SV=2 - [UBXN1_HUMAN]			
Q86XI8	Uncharacterized protein C19orf68 OS=Homo sapiens GN=C19orf68 PE=1 SV=2 - [CS068_HUMAN]			
O00159	Unconventional myosin-Ic OS=Homo sapiens GN=MYO1C PE=1 SV=4 - [MYO1C_HUMAN]			
O94832	Unconventional myosin-Id OS=Homo sapiens GN=MYO1D PE=1 SV=2 - [MYO1D_HUMAN]			
Q9HB07	UPF0160 protein MYG1, mitochondrial OS=Homo sapiens GN=C12orf10 PE=1 SV=2 - [MYG1_HUMAN]			
Q9Y224	UPF0568 protein C14orf166 OS=Homo sapiens GN=C14orf166 PE=1 SV=1 - [CN166_HUMAN]			
Q9UBQ0	Vacuolar protein sorting-associated protein 29 OS=Homo sapiens GN=VPS29 PE=1 SV=1 - [VPS29_HUMAN]			
Q96QK1	Vacuolar protein sorting-associated protein 35 OS=Homo sapiens GN=VPS35 PE=1 SV=2 - [VPS35_HUMAN]			
Q15836	Vesicle-associated membrane protein 3 OS=Homo sapiens GN=VAMP3 PE=1 SV=3 - [VAMP3_HUMAN]			
O95292	Vesicle-associated membrane protein-associated protein B/C OS=Homo sapiens GN=VAPB PE=1 SV=3 - [VAPB_HUMAN]			
Q12907	Vesicular integral-membrane protein VIP36 OS=Homo sapiens GN=LMAN2 PE=1 SV=1 - [LMAN2_HUMAN]			
Q00341	Vigilin OS=Homo sapiens GN=HDLBP PE=1 SV=2 - [VIGLN_HUMAN]			
P18206	Vinculin OS=Homo sapiens GN=VCL PE=1 SV=4 - [VINC_HUMAN]			
P21796	Voltage-dependent anion-selective channel protein 1 OS=Homo sapiens GN=VDAC1 PE=1 SV=2 - [VDAC1_HUMAN]			
P45880	Voltage-dependent anion-selective channel protein 2 OS=Homo sapiens GN=VDAC2 PE=1 SV=2 - [VDAC2_HUMAN]			
P21281	V-type proton ATPase subunit B, brain isoform OS=Homo sapiens GN=ATP6V1B2 PE=1 SV=3 - [VATB2_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
Q9Y5K8	V-type proton ATPase subunit D OS=Homo sapiens GN=ATP6V1D PE=1 SV=1 - [VATD_HUMAN]			
P12956	X-ray repair cross-complementing protein 6 OS=Homo sapiens GN=XRCC6 PE=1 SV=2 - [XRCC6_HUMAN]			
Q9Y5A9	YTH domain family protein 2 OS=Homo sapiens GN=YTHDF2 PE=1 SV=2 - [YTHD2_HUMAN]			
P68032	Actin, alpha cardiac muscle 1 OS=Homo sapiens GN=ACTC1 PE=1 SV=1 - [ACTC_HUMAN]			
O43707	Alpha-actinin-4 OS=Homo sapiens GN=ACTN4 PE=1 SV=2 - [ACTN4_HUMAN]			
P61221	ATP-binding cassette sub-family E member 1 OS=Homo sapiens GN=ABCE1 PE=1 SV=1 - [ABCE1_HUMAN]			
P16278	Beta-galactosidase OS=Homo sapiens GN=GLB1 PE=1 SV=2 - [BGAL_HUMAN]			
P30711	Glutathione S-transferase theta-1 OS=Homo sapiens GN=GSTT1 PE=1 SV=4 - [GSTT1_HUMAN]			
Q86YZ3	Hornerin OS=Homo sapiens GN=HRNR PE=1 SV=2 - [HORN_HUMAN]			
Q9NZI8	Insulin-like growth factor 2 mRNA-binding protein 1 OS=Homo sapiens GN=IGF2BP1 PE=1 SV=2 - [IF2B1_HUMAN]			
O00425	Insulin-like growth factor 2 mRNA-binding protein 3 OS=Homo sapiens GN=IGF2BP3 PE=1 SV=2 - [IF2B3_HUMAN]			
Q9BYZ2	L-lactate dehydrogenase A-like 6B OS=Homo sapiens GN=LDHAL6B PE=1 SV=3 - [LDH6B_HUMAN]			
O14744	Protein arginine N-methyltransferase 5 OS=Homo sapiens GN=PRMT5 PE=1 SV=4 - [ANM5_HUMAN]			
P26640	Valine--tRNA ligase OS=Homo sapiens GN=VARS PE=1 SV=4 - [SYVC_HUMAN]			
P62906	60S ribosomal protein L10a OS=Homo sapiens GN=RPL10A PE=1 SV=2 - [RL10A_HUMAN]			
Q01813	6-phosphofructokinase type C OS=Homo sapiens GN=PFKP PE=1 SV=2 - [K6PP_HUMAN]			
P17858	6-phosphofructokinase, liver type OS=Homo sapiens GN=PFKL PE=1 SV=6 - [K6PL_HUMAN]			
P11586	C-1-tetrahydrofolate synthase, cytoplasmic OS=Homo sapiens GN=MTHFD1 PE=1 SV=3 - [C1TC_HUMAN]			
Q99439	Calponin-2 OS=Homo sapiens GN=CNN2 PE=1 SV=4 - [CNN2_HUMAN]			
O60716	Catenin delta-1 OS=Homo sapiens GN=CTNND1 PE=1 SV=1 - [CTND1_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
P33992	DNA replication licensing factor MCM5 OS=Homo sapiens GN=MCM5 PE=1 SV=5 - [MCM5_HUMAN]			
P62877	E3 ubiquitin-protein ligase RBX1 OS=Homo sapiens GN=RBX1 PE=1 SV=1 - [RBX1_HUMAN]			
P38117	Electron transfer flavoprotein subunit beta OS=Homo sapiens GN=ETFB PE=1 SV=3 - [ETFB_HUMAN]			
P49411	Elongation factor Tu, mitochondrial OS=Homo sapiens GN=TUFM PE=1 SV=2 - [EFTU_HUMAN]			
P23588	Eukaryotic translation initiation factor 4B OS=Homo sapiens GN=EIF4B PE=1 SV=2 - [IF4B_HUMAN]			
Q14192	Four and a half LIM domains protein 2 OS=Homo sapiens GN=FHL2 PE=1 SV=3 - [FHL2_HUMAN]			
P04406	Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3 - [G3P_HUMAN]			
P06737	Glycogen phosphorylase, liver form OS=Homo sapiens GN=PYGL PE=1 SV=4 - [PYGL_HUMAN]			
Q16576	Histone-binding protein RBBP7 OS=Homo sapiens GN=RBBP7 PE=1 SV=1 - [RBBP7_HUMAN]			
P48735	Isocitrate dehydrogenase [NADP], mitochondrial OS=Homo sapiens GN=IDH2 PE=1 SV=2 - [IDHP_HUMAN]			
Q9NSK0	Kinesin light chain 4 OS=Homo sapiens GN=KLC4 PE=1 SV=3 - [KLC4_HUMAN]			
A6NHZ5	Leucine-rich repeat-containing protein 14B OS=Homo sapiens GN=LRRC14B PE=3 SV=3 - [LR14B_HUMAN]			
P09960	Leukotriene A-4 hydrolase OS=Homo sapiens GN=LTA4H PE=1 SV=2 - [LKHA4_HUMAN]			
P48059	LIM and senescent cell antigen-like-containing domain protein 1 OS=Homo sapiens GN=LIMS1 PE=1 SV=4 - [LIMS1_HUMAN]			
P0CW20	LIM and senescent cell antigen-like-containing domain protein 3-like OS=Homo sapiens GN=LIMS3L PE=2 SV=1 - [LIM3L_HUMAN]			
Q14847	LIM and SH3 domain protein 1 OS=Homo sapiens GN=LASP1 PE=1 SV=2 - [LASP1_HUMAN]			
O43684	Mitotic checkpoint protein BUB3 OS=Homo sapiens GN=BUB3 PE=1 SV=1 - [BUB3_HUMAN]			
O76041	Nebulette OS=Homo sapiens GN=NEBL PE=1 SV=1 - [NEBL_HUMAN]			
Q14697	Neutral alpha-glucosidase AB OS=Homo sapiens GN=GANAB PE=1 SV=3 - [GANAB_HUMAN]			
P50479	PDZ and LIM domain protein 4 OS=Homo sapiens GN=PDLIM4 PE=1 SV=2 - [PDLI4_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
O75420	PERQ amino acid-rich with GYF domain-containing protein 1 OS=Homo sapiens GN=GIGYF1 PE=1 SV=2 - [PERQ1_HUMAN]			
Q8WW12	PEST proteolytic signal-containing nuclear protein OS=Homo sapiens GN=PCNP PE=1 SV=2 - [PCNP_HUMAN]			
O15355	Protein phosphatase 1G OS=Homo sapiens GN=PPM1G PE=1 SV=1 - [PPM1G_HUMAN]			
P60468	Protein transport protein Sec61 subunit beta OS=Homo sapiens GN=SEC61B PE=1 SV=2 - [SC61B_HUMAN]			
Q8WUF5	RelA-associated inhibitor OS=Homo sapiens GN=PPP1R13L PE=1 SV=4 - [IASPP_HUMAN]			
Q9Y265	RuvB-like 1 OS=Homo sapiens GN=RUVBL1 PE=1 SV=1 - [RUVB1_HUMAN]			
Q9Y5M8	Signal recognition particle receptor subunit beta OS=Homo sapiens GN=SRPRB PE=1 SV=3 - [SRPRB_HUMAN]			
P53597	Succinyl-CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial OS=Homo sapiens GN=SUCLG1 PE=1 SV=4 - [SUCA_HUMAN]			
Q9UGI8	Testin OS=Homo sapiens GN=TES PE=1 SV=1 - [TES_HUMAN]			
P04183	Thymidine kinase, cytosolic OS=Homo sapiens GN=TK1 PE=1 SV=2 - [KITH_HUMAN]			
P40939	Trifunctional enzyme subunit alpha, mitochondrial OS=Homo sapiens GN=HADHA PE=1 SV=2 - [ECHA_HUMAN]			
P02765	Alpha-2-HS-glycoprotein OS=Homo sapiens GN=AHSG PE=1 SV=1 - [FETUA_HUMAN]			
P35221	Catenin alpha-1 OS=Homo sapiens GN=CTNNA1 PE=1 SV=1 - [CTNA1_HUMAN]			
P35222	Catenin beta-1 OS=Homo sapiens GN=CTNNB1 PE=1 SV=1 - [CTNB1_HUMAN]			
P49327	Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3 - [FAS_HUMAN]			
P14923	Junction plakoglobin OS=Homo sapiens GN=JUP PE=1 SV=3 - [PLAK_HUMAN]			
P10619	Lysosomal protective protein OS=Homo sapiens GN=CTSA PE=1 SV=2 - [PPGB_HUMAN]			
Q15365	Poly(rC)-binding protein 1 OS=Homo sapiens GN=PCBP1 PE=1 SV=2 - [PCBP1_HUMAN]			

Acc #	Protein	PM	NP	Wildtype
Q9Y230	RuvB-like 2 OS=Homo sapiens GN=RUVBL2 PE=1 SV=3 - [RUVB2_HUMAN]			
Q14134	Tripartite motif-containing protein 29 OS=Homo sapiens GN=TRIM29 PE=1 SV=2 - [TRI29_HUMAN]			
Q13885	Tubulin beta-2A chain OS=Homo sapiens GN=TUBB2A PE=1 SV=1 - [TBB2A_HUMAN]			

8.5 Candidate novel interacting proteins of full-length WT β-catenin in HT29.

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
P62258	14-3-3 protein epsilon OS=Homo sapiens GN=YWHAE PE=1 SV=1 - [1433E_HUMAN]	3	6	0.322					4	3	2
P61981	14-3-3 protein gamma OS=Homo sapiens GN=YWHAG PE=1 SV=2 - [1433G_HUMAN]	2	6	0.282	0.282	0.368	6.6	0.01	4	4	4
P27348	14-3-3 protein theta OS=Homo sapiens GN=YWHAQ PE=1 SV=1 - [1433T_HUMAN]	1	5	0.345					3	2	2
P63104	14-3-3 protein zeta/delta OS=Homo sapiens GN=YWHAZ PE=1 SV=1 - [1433Z_HUMAN]	4	7	0.170	0.096		9.899	0.03205	4	4	3
Q9C0C2	182 kDa tankyrase-1-binding protein OS=Homo sapiens GN=TNKS1BP1 PE=1 SV=4 - [TB182_HUMAN]	11	11	0.001	0.003	0.001	837.0	7.14E-07	3	8	1
P62280	40S ribosomal protein S11 OS=Homo sapiens GN=RPS11 PE=1 SV=3 - [RS11_HUMAN]	4	4	0.168	0.221	0.250	12.0	0.003	1	4	2
P62277	40S ribosomal protein S13 OS=Homo sapiens GN=RPS13 PE=1 SV=2 - [RS13_HUMAN]	3	3	0.194	0.278	0.211	10.7	0.004	3	3	1
P62263	40S ribosomal protein S14 OS=Homo sapiens GN=RPS14 PE=1 SV=3 - [RS14_HUMAN]	2	2		0.125	0.111	54.17	0.00588	1	2	2
P62244	40S ribosomal protein S15a OS=Homo sapiens GN=RPS15A PE=1 SV=2 - [RS15A_HUMAN]	3	3	0.188	0.310	0.001	3.7	0.03	2	3	2
P62249	40S ribosomal protein S16 OS=Homo sapiens GN=RPS16 PE=1 SV=2 - [RS16_HUMAN]	5	5	0.180	0.287	0.178	8.0	0.008	3	4	2
P62269	40S ribosomal protein S18 OS=Homo sapiens GN=RPS18 PE=1 SV=3 - [RS18_HUMAN]	6	6	0.233	0.299	0.205	9.1	0.006	5	6	4

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
P39019	40S ribosomal protein S19 OS=Homo sapiens GN=RPS19 PE=1 SV=2 - [RS19_HUMAN]	5	5	0.163	0.219		11.06	0.0287	2	2	3
P15880	40S ribosomal protein S2 OS=Homo sapiens GN=RPS2 PE=1 SV=2 - [RS2_HUMAN]	6	6	0.135	0.180	0.152	26.0	0.0007	4	4	3
P62851	40S ribosomal protein S25 OS=Homo sapiens GN=RPS25 PE=1 SV=1 - [RS25_HUMAN]	3	3	0.241	0.323	0.280	9.2	0.006	3	3	1
P42677	40S ribosomal protein S27 OS=Homo sapiens GN=RPS27 PE=1 SV=3 - [RS27_HUMAN]	3	3	0.067	0.190	0.176	9.1	0.006	1	3	2
P23396	40S ribosomal protein S3 OS=Homo sapiens GN=RPS3 PE=1 SV=2 - [RS3_HUMAN]	13	13	0.304	0.295	0.151	5.1	0.02	10	11	8
P61247	40S ribosomal protein S3a OS=Homo sapiens GN=RPS3A PE=1 SV=2 - [RS3A_HUMAN]	7	7	0.160	0.232	0.012	5.7	0.01	6	4	4
P62701	40S ribosomal protein S4, X isoform OS=Homo sapiens GN=RPS4X PE=1 SV=2 - [RS4X_HUMAN]	7	7	0.191	0.246	0.092	7.2	0.009	3	6	4
P62753	40S ribosomal protein S6 OS=Homo sapiens GN=RPS6 PE=1 SV=1 - [RS6_HUMAN]	5	5	0.178	0.236	0.179	15.9	0.002	5	5	4
P62241	40S ribosomal protein S8 OS=Homo sapiens GN=RPS8 PE=1 SV=2 - [RS8_HUMAN]	7	7	0.190	0.294	0.189	7.9	0.008	4	5	5
P46781	40S ribosomal protein S9 OS=Homo sapiens GN=RPS9 PE=1 SV=3 - [RS9_HUMAN]	7	7	0.155	0.236	0.001	5.3	0.02	5	5	4
P05388	60S acidic ribosomal protein P0 OS=Homo sapiens GN=RPLP0 PE=1 SV=1 - [RLA0_HUMAN]	5	5	0.238	0.235	0.001	4.4	0.02	4	5	1
P62913	60S ribosomal protein L11 OS=Homo sapiens GN=RPL11 PE=1 SV=2 - [RL11_HUMAN]	4	4	0.133	0.153	0.012	9.1	0.006	3	4	3
P26373	60S ribosomal protein L13 OS=Homo sapiens	4	4	0.130	0.220	0.001	6.0	0.01	2	3	3

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	GN=RPL13 PE=1 SV=4 - [RL13_HUMAN]										
P40429	60S ribosomal protein L13a OS=Homo sapiens GN=RPL13A PE=1 SV=2 - [RL13A_HUMAN]	4	4	0.226	0.017	0.176	5.7	0.01	3	4	2
P50914	60S ribosomal protein L14 OS=Homo sapiens GN=RPL14 PE=1 SV=4 - [RL14_HUMAN]	3	3	0.143	0.002	0.167	7.7	0.008	2	3	1
P61313	60S ribosomal protein L15 OS=Homo sapiens GN=RPL15 PE=1 SV=2 - [RL15_HUMAN]	4	4	0.128	0.225		6.649	0.04752	3	3	1
Q07020	60S ribosomal protein L18 OS=Homo sapiens GN=RPL18 PE=1 SV=2 - [RL18_HUMAN]	4	4	0.161	0.238	0.223	12.3	0.003	4	4	2
P84098	60S ribosomal protein L19 OS=Homo sapiens GN=RPL19 PE=1 SV=1 - [RL19_HUMAN]	2	2		0.246				1	2	1
P35268	60S ribosomal protein L22 OS=Homo sapiens GN=RPL22 PE=1 SV=2 - [RL22_HUMAN]	2	2	0.121	0.028	0.156	10.4	0.005	2	1	2
P62750	60S ribosomal protein L23a OS=Homo sapiens GN=RPL23A PE=1 SV=1 - [RL23A_HUMAN]	3	3	0.193	0.207	0.191	59.7	0.0001	2	3	3
P83731	60S ribosomal protein L24 OS=Homo sapiens GN=RPL24 PE=1 SV=1 - [RL24_HUMAN]	5	5	0.172	0.296	0.013	4.1	0.03	4	4	2
P61353	60S ribosomal protein L27 OS=Homo sapiens GN=RPL27 PE=1 SV=2 - [RL27_HUMAN]	1	1		0.283				1	1	1
P46776	60S ribosomal protein L27a OS=Homo sapiens GN=RPL27A PE=1 SV=2 - [RL27A_HUMAN]	3	3	0.179	0.258		7.078	0.04468	2	3	2
P46779	60S ribosomal protein L28 OS=Homo sapiens GN=RPL28 PE=1 SV=3 - [RL28_HUMAN]	4	4	0.183	0.261		7.184	0.04403	1	2	3
P62888	60S ribosomal protein L30 OS=Homo sapiens GN=RPL30 PE=1 SV=2 - [RL30_HUMAN]	2	3	0.134		0.001	6.496	0.04862	3	2	1
P62899	60S ribosomal protein L31 OS=Homo sapiens GN=RPL31 PE=1 SV=1 - [RL31_HUMAN]	2	2	0.117	0.222	0.001	6.0	0.01	1	2	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
P18077	60S ribosomal protein L35a OS=Homo sapiens GN=RPL35A PE=1 SV=2 - [RL35A_HUMAN]	3	3	0.128	0.307	0.231	5.4	0.02	2	2	3
Q9Y3U8	60S ribosomal protein L36 OS=Homo sapiens GN=RPL36 PE=1 SV=3 - [RL36_HUMAN]	3	3	0.135	0.149	0.171	33.0	0.0005	3	3	2
P46777	60S ribosomal protein L5 OS=Homo sapiens GN=RPL5 PE=1 SV=3 - [RL5_HUMAN]	5	5	0.151	0.208		11.24	0.02825	2	4	2
Q02878	60S ribosomal protein L6 OS=Homo sapiens GN=RPL6 PE=1 SV=3 - [RL6_HUMAN]	2	2	0.136	0.241	0.059	6.7	0.01	2	2	1
P18124	60S ribosomal protein L7 OS=Homo sapiens GN=RPL7 PE=1 SV=1 - [RL7_HUMAN]	5	5	0.263	0.324	0.001	3.1	0.05	2	3	3
P63261	Actin, cytoplasmic 2 OS=Homo sapiens GN=ACTG1 PE=1 SV=1 - [ACTG_HUMAN]	1	9				0.0	0	6	9	8
P53999	Activated RNA polymerase II transcriptional coactivator p15 OS=Homo sapiens GN=SUB1 PE=1 SV=3 - [TCP4_HUMAN]	2	2	0.145	0.164		36.86	0.00863	1	2	1
P23526	Adenosylhomocysteinase OS=Homo sapiens GN=AHCY PE=1 SV=4 - [SAHH_HUMAN]	4	4		0.002	0.001	754	0.00042	1	2	3
P02765	Alpha-2-HS-glycoprotein OS=Homo sapiens GN=AHSG PE=1 SV=1 - [FETUA_HUMAN]	1	1	0.001	0.001	0.001	4095.4	2.98E-08	1	1	1
P06733	Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2 - [ENOA_HUMAN]	8	8	0.244	0.304	0.282	12.9	0.003	1	5	5
P07355	Annexin A2 OS=Homo sapiens GN=ANXA2 PE=1 SV=2 - [ANXA2_HUMAN]	12	12	0.146	0.163	0.016	8.5	0.007	5	12	7
P09525	Annexin A4 OS=Homo sapiens GN=ANXA4 PE=1 SV=4 - [ANXA4_HUMAN]	3	3	0.001	0.001	0.001	0.0	0	2	3	1
P08758	Annexin A5 OS=Homo sapiens GN=ANXA5 PE=1 SV=2 - [ANXA5_HUMAN]	3	3	0.139					2	2	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
P25705	ATP synthase subunit alpha, mitochondrial OS=Homo sapiens GN=ATP5A1 PE=1 SV=1 - [ATPA_HUMAN]	3	3			0.001			1	2	1
P27708	CAD protein OS=Homo sapiens GN=CAD PE=1 SV=3 - [PYR1_HUMAN]	31	31	0.001	0.121	0.001	11.5	0.004	19	26	1
Q9HB71	Calcyclin-binding protein OS=Homo sapiens GN=CACYBP PE=1 SV=2 - [CYBP_HUMAN]	2	2		0.067				1	1	2
P35222	Catenin beta-1 OS=Homo sapiens GN=CTNNB1 PE=1 SV=1 - [CTNB1_HUMAN]	28	34	0.001	0.001	0.001	0.0	0	33	34	31
Q00610	Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5 - [CLH1_HUMAN]	20	20	0.002	0.007	0.001	256.3	7.61E-06	8	16	1
P60981	Destrin OS=Homo sapiens GN=DSTN PE=1 SV=3 - [DEST_HUMAN]	1	2				0.0	0	1	1	2
Q14919	Dr1-associated corepressor OS=Homo sapiens GN=DRAP1 PE=1 SV=3 - [NC2A_HUMAN]	1	1	0.070	0.108		21.87	0.01454	1	1	1
P68104	Elongation factor 1-alpha 1 OS=Homo sapiens GN=EEF1A1 PE=1 SV=1 - [EF1A1_HUMAN]	5	5	0.166	0.213	0.207	20.7	0.001	4	5	4
P60842	Eukaryotic initiation factor 4A-I OS=Homo sapiens GN=EIF4A1 PE=1 SV=1 - [IF4A1_HUMAN]	4	4		0.239	0.191	11.98	0.02651	1	4	3
P41091	Eukaryotic translation initiation factor 2 subunit 3 OS=Homo sapiens GN=EIF2S3 PE=1 SV=3 - [IF2G_HUMAN]	3	3	0.185	0.268	0.210	11.3	0.004	1	3	1
P47755	F-actin-capping protein subunit alpha-2 OS=Homo sapiens GN=CAPZA2 PE=1 SV=3 - [CAZA2_HUMAN]	2	2	0.001	0.125		7.061	0.04478	2	2	1
P47756	F-actin-capping protein subunit beta OS=Homo	3	3		0.001				1	2	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	sapiens GN=CAPZB PE=1 SV=4 - [CAPZB_HUMAN]										
P49327	Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3 - [FAS_HUMAN]	46	46	0.001	0.001	0.001	0.0	0	26	44	2
P56470	Galectin-4 OS=Homo sapiens GN=LGALS4 PE=1 SV=1 - [LEG4_HUMAN]	2	2	0.001	0.001	0.001	0.0	0	1	2	1
P04406	Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3 - [G3P_HUMAN]	10	11	0.133	0.147	0.116	40.4	0.0003	4	8	8
O14556	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific OS=Homo sapiens GN=GAPDHS PE=1 SV=2 - [G3PT_HUMAN]	1	2		0.001	0.001	0	0	1	2	2
P62826	GTP-binding nuclear protein Ran OS=Homo sapiens GN=RAN PE=1 SV=3 - [RAN_HUMAN]	4	4	0.328	0.332	0.438	3.7	0.03	3	4	4
P62879	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2 OS=Homo sapiens GN=GNB2 PE=1 SV=3 - [GBB2_HUMAN]	3	3	0.139	0.094		17.17	0.01851	2	1	1
P63244	Guanine nucleotide-binding protein subunit beta-2-like 1 OS=Homo sapiens GN=GNB2L1 PE=1 SV=3 - [GBLP_HUMAN]	9	9	0.146	0.161	0.001	7.8	0.008	6	8	4
P08107	Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5 - [HSP71_HUMAN]	6	9	0.158					6	5	1
P04792	Heat shock protein beta-1 OS=Homo sapiens GN=HSPB1 PE=1 SV=2 - [HSPB1_HUMAN]	3	3	0.351	0.368		16.42	0.01936	3	2	2

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
P07900	Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1 PE=1 SV=5 - [HS90A_HUMAN]	6	14	0.217	0.267		10.32	0.03074	14	7	3
P08238	Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4 - [HS90B_HUMAN]	6	16	0.107	0.026		10.71	0.02965	15	8	3
Q14103	Heterogeneous nuclear ribonucleoprotein D0 OS=Homo sapiens GN=HNRNPD PE=1 SV=1 - [HNRPD_HUMAN]	2	2		0.240				1	2	1
P52272	Heterogeneous nuclear ribonucleoprotein M OS=Homo sapiens GN=HNRNPM PE=1 SV=3 - [HNRPM_HUMAN]	10	10	0.008	0.018		97.26	0.00327	7	2	1
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1 OS=Homo sapiens GN=HNRNPA2B1 PE=1 SV=2 - [ROA2_HUMAN]	4	4	0.010	0.021		87.01	0.00366	2	4	1
O15347	High mobility group protein B3 OS=Homo sapiens GN=HMGB3 PE=1 SV=4 - [HMGB3_HUMAN]	2	2	0.125	0.013	0.001	11.5	0.004	1	2	1
P16403	Histone H1.2 OS=Homo sapiens GN=HIST1H1C PE=1 SV=2 - [H12_HUMAN]	3	3	0.121	0.109	0.009	11.8	0.004	2	3	1
P16401	Histone H1.5 OS=Homo sapiens GN=HIST1H1B PE=1 SV=3 - [H15_HUMAN]	2	2	0.121	0.167	0.001	8.2	0.007	2	2	1
Q96KK5	Histone H2A type 1-H OS=Homo sapiens GN=HIST1H2AH PE=1 SV=3 - [H2A1H_HUMAN]	1	2		0.374				1	2	1
P0C0S5	Histone H2A.Z OS=Homo sapiens GN=H2AFZ PE=1 SV=2 - [H2AZ_HUMAN]	1	2				0.0	0	2	1	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
O60814	Histone H2B type 1-K OS=Homo sapiens GN=HIST1H2BK PE=1 SV=3 - [H2B1K_HUMAN]	2	2	0.290	0.405	0.280	4.4	0.02	1	2	2
P12268	Inosine-5'-monophosphate dehydrogenase 2 OS=Homo sapiens GN=IMPDH2 PE=1 SV=2 - [IMDH2_HUMAN]	4	4	0.001	0.002	0.001	2884.4	6.01E-08	1	3	2
P14923	Junction plakoglobin OS=Homo sapiens GN=JUP PE=1 SV=3 - [PLAK_HUMAN]	4	10	0.009		0.001	129.4	0.00246	9	6	7
Q14847	LIM and SH3 domain protein 1 OS=Homo sapiens GN=LASP1 PE=1 SV=2 - [LASP1_HUMAN]	5	5	0.085	0.106	0.001	13.6	0.003	1	3	5
P00338	L-lactate dehydrogenase A chain OS=Homo sapiens GN=LDHA PE=1 SV=2 - [LDHA_HUMAN]	9	10	0.201	0.198	0.224	35.7	0.0004	4	9	6
P07195	L-lactate dehydrogenase B chain OS=Homo sapiens GN=LDHB PE=1 SV=2 - [LDHB_HUMAN]	6	7	0.146	0.113	0.010	10.0	0.005	4	5	4
P10619	Lysosomal protective protein OS=Homo sapiens GN=CTSA PE=1 SV=2 - [PPGB_HUMAN]	3	3	0.001	0.001		0	0	2	2	1
P60660	Myosin light polypeptide 6 OS=Homo sapiens GN=MYL6 PE=1 SV=2 - [MYL6_HUMAN]	3	3	0.167	0.219		11.69	0.02716	3	1	1
P19105	Myosin regulatory light chain 12A OS=Homo sapiens GN=MYL12A PE=1 SV=2 - [ML12A_HUMAN]	4	4	0.197	0.016	0.001	6.8	0.01	3	4	2
Q86U44	N6-adenosine-methyltransferase 70 kDa subunit OS=Homo sapiens GN=METTL3 PE=1 SV=2 - [MTA70_HUMAN]	1	1			0.001			1	1	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
P49116	Nuclear receptor subfamily 2 group C member 2 OS=Homo sapiens GN=NR2C2 PE=1 SV=1 - [NR2C2_HUMAN]	1	1	0.001	0.001	0.001	0.0	0	1	1	1
Q9NR12	PDZ and LIM domain protein 7 OS=Homo sapiens GN=PDLIM7 PE=1 SV=1 - [PDLI7_HUMAN]	5	5		0.001				1	4	2
P62937	Peptidyl-prolyl cis-trans isomerase A OS=Homo sapiens GN=PPIA PE=1 SV=2 - [PPIA_HUMAN]	8	8	0.135	0.138	0.219	12.2	0.003	4	8	4
P23284	Peptidyl-prolyl cis-trans isomerase B OS=Homo sapiens GN=PPIB PE=1 SV=2 - [PPIB_HUMAN]	3	3	0.127	0.001	0.001	10.9	0.004	2	1	2
Q06830	Peroxiredoxin-1 OS=Homo sapiens GN=PRDX1 PE=1 SV=1 - [PRDX1_HUMAN]	7	7	0.252	0.255	0.001	3.9	0.03	5	5	3
Q15149	Plectin OS=Homo sapiens GN=PLEC PE=1 SV=3 - [PLEC_HUMAN]	164	167	0.207	0.303	0.219	8.5	0.007	122	148	10
P09874	Poly [ADP-ribose] polymerase 1 OS=Homo sapiens GN=PARP1 PE=1 SV=4 - [PARP1_HUMAN]	5	5	0.001	0.001	0.140	9.8	0.005	3	3	3
P11940	Polyadenylate-binding protein 1 OS=Homo sapiens GN=PABPC1 PE=1 SV=2 - [PABP1_HUMAN]	7	9	0.084	0.089		181.5	0.00175	8	3	1
Q13310	Polyadenylate-binding protein 4 OS=Homo sapiens GN=PABPC4 PE=1 SV=1 - [PABP4_HUMAN]	2	4	0.001					4	1	1
P02545	Prelamin-A/C OS=Homo sapiens GN=LMNA PE=1 SV=1 - [LMNA_HUMAN]	23	23	0.165	0.004	0.014	8.4	0.007	21	6	3

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
P17844	Probable ATP-dependent RNA helicase DDX5 OS=Homo sapiens GN=DDX5 PE=1 SV=1 - [DDX5_HUMAN]	4	8	0.001	0.001		0	0	7	5	1
P35232	Prohibitin OS=Homo sapiens GN=PHB PE=1 SV=1 - [PHB_HUMAN]	4	4	0.275	0.273	0.001	3.5	0.04	2	4	2
P30101	Protein disulfide-isomerase A3 OS=Homo sapiens GN=PDIA3 PE=1 SV=4 - [PDIA3_HUMAN]	3	3		0.001	0.001	2880	0.00011	1	2	3
Q6ZRV2	Protein FAM83H OS=Homo sapiens GN=FAM83H PE=1 SV=3 - [FA83H_HUMAN]	3	3	0.001	0.001	0.001	0.0	0	1	3	1
Q92928	Putative Ras-related protein Rab-1C OS=Homo sapiens GN=RAB1C PE=5 SV=2 - [RAB1C_HUMAN]	2	3		0.001				2	3	1
P14618	Pyruvate kinase isozymes M1/M2 OS=Homo sapiens GN=PKM PE=1 SV=4 - [KPYM_HUMAN]	11	11	0.153	0.126	0.177	23.4	0.001	9	8	7
P61106	Ras-related protein Rab-14 OS=Homo sapiens GN=RAB14 PE=1 SV=4 - [RAB14_HUMAN]	1	2				0.0	0	1	1	2
Q9Y265	RuvB-like 1 OS=Homo sapiens GN=RUVBL1 PE=1 SV=1 - [RUVB1_HUMAN]	6	6		0.001	0.001	0	0	1	4	4
Q9Y230	RuvB-like 2 OS=Homo sapiens GN=RUVBL2 PE=1 SV=3 - [RUVB2_HUMAN]	16	16	0.001	0.001	0.001	0.0	0	5	15	12
P62136	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit OS=Homo sapiens GN=PPP1CA PE=1 SV=1 - [PP1A_HUMAN]	2	2		0.001	0.001	0	0	1	1	1
O14907	Tax1-binding protein 3 OS=Homo sapiens GN=TAX1BP3 PE=1 SV=2 -	1	1	0.001	0.001	0.001	0.0	0	1	1	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	[TX1B3_HUMAN]										
P50991	T-complex protein 1 subunit delta OS=Homo sapiens GN=CCT4 PE=1 SV=4 - [TCPD_HUMAN]	1	1			0.204			1	1	1
P49368	T-complex protein 1 subunit gamma OS=Homo sapiens GN=CCT3 PE=1 SV=4 - [TCPG_HUMAN]	4	4	0.001					2	2	1
P40227	T-complex protein 1 subunit zeta OS=Homo sapiens GN=CCT6A PE=1 SV=3 - [TCPZ_HUMAN]	2	2	0.146	0.001	0.140	8.5	0.007	1	2	1
P68363	Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1 - [TBA1B_HUMAN]	3	10		0.246	0.272	18.59	0.0171	5	7	6
P68366	Tubulin alpha-4A chain OS=Homo sapiens GN=TUBA4A PE=1 SV=1 - [TBA4A_HUMAN]	2	9		0.240	0.220	26.72	0.01191	3	8	5
P07437	Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2 - [TBB5_HUMAN]	2	12		0.226	0.196	19.17	0.01659	5	11	9
Q13885	Tubulin beta-2A chain OS=Homo sapiens GN=TUBB2A PE=1 SV=1 - [TBB2A_HUMAN]	1	11	0.135					5	8	8
P68371	Tubulin beta-4B chain OS=Homo sapiens GN=TUBB4B PE=1 SV=1 - [TBB4B_HUMAN]	3	13		0.127	0.013	7.559	0.04187	4	10	9
P61088	Ubiquitin-conjugating enzyme E2 N OS=Homo sapiens GN=UBE2N PE=1 SV=1 - [UBE2N_HUMAN]	1	1				0.0	0	1	1	1
P08670	Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4 - [VIME_HUMAN]	17	20	0.267	0.338	0.291	9.7	0.005	8	16	12
P21796	Voltage-dependent anion-selective channel	4	5	0.421	0.404	0.330	4.1	0.03	3	4	3

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	protein 1 OS=Homo sapiens GN=VDAC1 PE=1 SV=2 - [VDAC1_HUMAN]										

8.6 Candidate novel interacting proteins of the phosphomimetic mutant of β-catenin at tyrosine-654 in HT29.

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
P31946	14-3-3 protein beta/alpha OS=Homo sapiens GN=YWHAB PE=1 SV=3 - [1433B_HUMAN]	2	7	0.220	0.239	0.285	13.2	0.003	4	5	6
Q04917	14-3-3 protein eta OS=Homo sapiens GN=YWHAH PE=1 SV=4 - [1433F_HUMAN]	1	5		0.308				3	4	3
Q9C0C2	182 kDa tankyrase-1-binding protein OS=Homo sapiens GN=TNKS1BP1 PE=1 SV=4 - [TB182_HUMAN]	4	4	0.003	0.004	0.002	1317.1	2.88E-07	3	1	2
P62280	40S ribosomal protein S11 OS=Homo sapiens GN=RPS11 PE=1 SV=3 - [RS11_HUMAN]	7	7	0.001	0.219	0.179	5.5	0.02	3	4	7
P62277	40S ribosomal protein S13 OS=Homo sapiens GN=RPS13 PE=1 SV=2 - [RS13_HUMAN]	4	4	0.139	0.211	0.205	13.7	0.003	3	3	3
P62263	40S ribosomal protein S14 OS=Homo sapiens GN=RPS14 PE=1 SV=3 - [RS14_HUMAN]	3	3	0.079	0.131	0.158	16.4	0.002	1	3	3
P62244	40S ribosomal protein S15a OS=Homo sapiens GN=RPS15A PE=1 SV=2 - [RS15A_HUMAN]	5	5	0.151	0.141	0.260	8.3	0.007	3	4	5
P62249	40S ribosomal protein S16 OS=Homo sapiens GN=RPS16 PE=1 SV=2 - [RS16_HUMAN]	4	4	0.098	0.173	0.201	11.2	0.004	3	4	2
P62269	40S ribosomal protein S18 OS=Homo sapiens GN=RPS18 PE=1 SV=3 - [RS18_HUMAN]	5	5	0.170	0.234	0.293	7.6	0.009	5	5	5
P39019	40S ribosomal protein S19 OS=Homo sapiens GN=RPS19 PE=1 SV=2 - [RS19_HUMAN]	8	8	0.163	0.265	0.266	7.9	0.008	4	6	7
P15880	40S ribosomal protein S2 OS=Homo sapiens GN=RPS2 PE=1 SV=2 - [RS2_HUMAN]	8	8	0.082	0.151	0.145	16.9	0.002	7	6	6

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
P60866	40S ribosomal protein S20 OS=Homo sapiens GN=RPS20 PE=1 SV=1 - [RS20_HUMAN]	2	2	0.159	0.269	0.281	6.8	0.01	1	2	1
P62266	40S ribosomal protein S23 OS=Homo sapiens GN=RPS23 PE=1 SV=3 - [RS23_HUMAN]	1	1	0.114	0.213	0.212	9.8	0.005	1	1	1
P62847	40S ribosomal protein S24 OS=Homo sapiens GN=RPS24 PE=1 SV=1 - [RS24_HUMAN]	1	1	0.112		0.175	11.3	0.03	1	1	1
P62851	40S ribosomal protein S25 OS=Homo sapiens GN=RPS25 PE=1 SV=1 - [RS25_HUMAN]	5	5	0.178	0.248	0.242	12.5	0.003	3	4	4
P42677	40S ribosomal protein S27 OS=Homo sapiens GN=RPS27 PE=1 SV=3 - [RS27_HUMAN]	1	3				0.0	0	2	2	3
Q71UM5	40S ribosomal protein S27-like OS=Homo sapiens GN=RPS27L PE=1 SV=3 - [RS27L_HUMAN]	1	3		0.093	0.180	8.3	0.04	2	3	3
P23396	40S ribosomal protein S3 OS=Homo sapiens GN=RPS3 PE=1 SV=2 - [RS3_HUMAN]	14	14	0.197	0.298	0.315	6.2	0.01	13	11	12
P62861	40S ribosomal protein S30 OS=Homo sapiens GN=FAU PE=1 SV=1 - [RS30_HUMAN]	1	1	0.142	0.177	0.160	34.0	0.0004	1	1	1
P61247	40S ribosomal protein S3a OS=Homo sapiens GN=RPS3A PE=1 SV=2 - [RS3A_HUMAN]	8	8	0.124	0.217	0.178	12.2	0.003	5	5	7
P62701	40S ribosomal protein S4, X isoform OS=Homo sapiens GN=RPS4X PE=1 SV=2 - [RS4X_HUMAN]	7	7	0.121	0.211	0.197	11.6	0.004	6	7	4
P46782	40S ribosomal protein S5 OS=Homo sapiens GN=RPS5 PE=1 SV=4 - [RS5_HUMAN]	4	4	0.225	0.253	0.306	10.0	0.005	3	3	2
P62753	40S ribosomal protein S6 OS=Homo sapiens GN=RPS6 PE=1 SV=1 - [RS6_HUMAN]	5	5	0.112	0.157	0.160	23.1	0.0009	4	4	5
P62081	40S ribosomal protein S7 OS=Homo sapiens	2	2	0.144	0.207	0.186	17.2	0.002	1	2	2

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	GN=RPS7 PE=1 SV=1 - [RS7_HUMAN]										
P62241	40S ribosomal protein S8 OS=Homo sapiens GN=RPS8 PE=1 SV=2 - [RS8_HUMAN]	5	5	0.104	0.225	0.186	9.2	0.006	3	3	4
P46781	40S ribosomal protein S9 OS=Homo sapiens GN=RPS9 PE=1 SV=3 - [RS9_HUMAN]	7	7	0.107	0.199	0.172	12.4	0.003	6	6	6
P08865	40S ribosomal protein SA OS=Homo sapiens GN=RPSA PE=1 SV=4 - [RSSA_HUMAN]	4	4	0.011	0.252	0.154	5.2	0.02	4	2	2
P05388	60S acidic ribosomal protein P0 OS=Homo sapiens GN=RPLP0 PE=1 SV=1 - [RLA0_HUMAN]	5	5	0.166	0.287	0.227	7.9	0.008	4	5	2
P27635	60S ribosomal protein L10 OS=Homo sapiens GN=RPL10 PE=1 SV=4 - [RL10_HUMAN]	4	4	0.012	0.159	0.174	7.4	0.009	4	4	3
P62906	60S ribosomal protein L10a OS=Homo sapiens GN=RPL10A PE=1 SV=2 - [RL10A_HUMAN]	3	3		0.240	0.218	24.2	0.01	2	2	2
P62913	60S ribosomal protein L11 OS=Homo sapiens GN=RPL11 PE=1 SV=2 - [RL11_HUMAN]	4	4	0.115	0.220	0.204	9.7	0.005	4	3	3
P30050	60S ribosomal protein L12 OS=Homo sapiens GN=RPL12 PE=1 SV=1 - [RL12_HUMAN]	4	4		0.261	0.205	9.5	0.03	1	3	4
P26373	60S ribosomal protein L13 OS=Homo sapiens GN=RPL13 PE=1 SV=4 - [RL13_HUMAN]	6	6	0.094	0.124	0.142	27.3	0.0007	5	4	4
P40429	60S ribosomal protein L13a OS=Homo sapiens GN=RPL13A PE=1 SV=2 - [RL13A_HUMAN]	4	4	0.137	0.224	0.209	11.5	0.004	1	3	3
P50914	60S ribosomal protein L14 OS=Homo sapiens GN=RPL14 PE=1 SV=4 - [RL14_HUMAN]	6	6	0.002	0.138	0.179	7.3	0.009	3	4	6
P61313	60S ribosomal protein L15 OS=Homo sapiens GN=RPL15 PE=1 SV=2 - [RL15_HUMAN]	4	4	0.009	0.159	0.163	7.7	0.008	4	4	4

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
Q07020	60S ribosomal protein L18 OS=Homo sapiens GN=RPL18 PE=1 SV=2 - [RL18_HUMAN]	5	5	0.113	0.014	0.165	9.1	0.006	4	3	5
Q02543	60S ribosomal protein L18a OS=Homo sapiens GN=RPL18A PE=1 SV=2 - [RL18A_HUMAN]	2	2	0.001	0.162	0.204	6.1	0.01	1	2	2
P84098	60S ribosomal protein L19 OS=Homo sapiens GN=RPL19 PE=1 SV=1 - [RL19_HUMAN]	3	3	0.115	0.184	0.136	17.5	0.002	1	2	2
P35268	60S ribosomal protein L22 OS=Homo sapiens GN=RPL22 PE=1 SV=2 - [RL22_HUMAN]	3	3		0.233	0.204	19.3	0.02	2	2	3
P62829	60S ribosomal protein L23 OS=Homo sapiens GN=RPL23 PE=1 SV=1 - [RL23_HUMAN]	4	4	0.089	0.197	0.121	11.4	0.004	4	2	3
P62750	60S ribosomal protein L23a OS=Homo sapiens GN=RPL23A PE=1 SV=1 - [RL23A_HUMAN]	4	4	0.122	0.195	0.196	13.5	0.003	3	3	4
P83731	60S ribosomal protein L24 OS=Homo sapiens GN=RPL24 PE=1 SV=1 - [RL24_HUMAN]	4	4	0.103	0.174	0.181	13.9	0.003	4	3	4
P61254	60S ribosomal protein L26 OS=Homo sapiens GN=RPL26 PE=1 SV=1 - [RL26_HUMAN]	4	4	0.108	0.202	0.191	11.2	0.004	4	2	4
P61353	60S ribosomal protein L27 OS=Homo sapiens GN=RPL27 PE=1 SV=2 - [RL27_HUMAN]	1	1	0.106	0.255	0.001	5.1	0.02	1	1	1
P46776	60S ribosomal protein L27a OS=Homo sapiens GN=RPL27A PE=1 SV=2 - [RL27A_HUMAN]	3	3	0.107	0.155	0.178	16.9	0.002	3	2	3
P46779	60S ribosomal protein L28 OS=Homo sapiens GN=RPL28 PE=1 SV=3 - [RL28_HUMAN]	2	2	0.107	0.179	0.216	10.4	0.005	1	2	2
P62888	60S ribosomal protein L30 OS=Homo sapiens GN=RPL30 PE=1 SV=2 - [RL30_HUMAN]	4	5		0.192	0.191	1623.3	0.0002	2	3	4
P49207	60S ribosomal protein L34 OS=Homo sapiens GN=RPL34 PE=1 SV=3 - [RL34_HUMAN]	2	2	0.095	0.123	0.181	14.5	0.002	1	2	1
P18077	60S ribosomal protein L35a OS=Homo sapiens	4	4	0.103	0.193	0.186	11.8	0.004	3	3	3

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	GN=RPL35A PE=1 SV=2 - [RL35A_HUMAN]										
Q9Y3U8	60S ribosomal protein L36 OS=Homo sapiens GN=RPL36 PE=1 SV=3 - [RL36_HUMAN]	3	3	0.111	0.011	0.179	8.2	0.007	3	3	3
P83881	60S ribosomal protein L36a OS=Homo sapiens GN=RPL36A PE=1 SV=2 - [RL36A_HUMAN]	1	1	0.059	0.154	0.001	9.6	0.005	1	1	1
P61513	60S ribosomal protein L37a OS=Homo sapiens GN=RPL37A PE=1 SV=2 - [RL37A_HUMAN]	1	1			0.205			1	1	1
P36578	60S ribosomal protein L4 OS=Homo sapiens GN=RPL4 PE=1 SV=5 - [RL4_HUMAN]	7	7	0.066	0.097	0.124	24.2	0.0009	5	2	4
P46777	60S ribosomal protein L5 OS=Homo sapiens GN=RPL5 PE=1 SV=3 - [RL5_HUMAN]	3	3	0.113	0.180	0.128	17.5	0.002	1	2	2
Q02878	60S ribosomal protein L6 OS=Homo sapiens GN=RPL6 PE=1 SV=3 - [RL6_HUMAN]	5	5	0.010	0.129	0.150	9.2	0.006	3	2	4
P18124	60S ribosomal protein L7 OS=Homo sapiens GN=RPL7 PE=1 SV=1 - [RL7_HUMAN]	7	7	0.167	0.234	0.253	10.9	0.004	5	5	6
P62424	60S ribosomal protein L7a OS=Homo sapiens GN=RPL7A PE=1 SV=2 - [RL7A_HUMAN]	6	6	0.145	0.206	0.192	17.4	0.002	2	4	5
P62917	60S ribosomal protein L8 OS=Homo sapiens GN=RPL8 PE=1 SV=2 - [RL8_HUMAN]	2	2				0.0	0	2	2	2
P11021	78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2 - [GRP78_HUMAN]	7	9	0.137	0.285	0.224	6.6	0.01	7	7	4
P60709	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 - [ACTB_HUMAN]	10	10	0.184	0.327	0.241	6.0	0.01	7	8	10
P61160	Actin-related protein 2 OS=Homo sapiens GN=ACTR2 PE=1 SV=1 - [ARP2_HUMAN]	2	2	0.091	0.205	0.001	6.8	0.01	1	2	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
P59998	Actin-related protein 2/3 complex subunit 4 OS=Homo sapiens GN=ARPC4 PE=1 SV=3 - [ARPC4_HUMAN]	2	2	0.001	0.015	0.238	5.4	0.02	2	2	2
P53999	Activated RNA polymerase II transcriptional coactivator p15 OS=Homo sapiens GN=SUB1 PE=1 SV=3 - [TCP4_HUMAN]	2	2	0.131	0.306	0.201	5.7	0.01	1	2	1
P18085	ADP-ribosylation factor 4 OS=Homo sapiens GN=ARF4 PE=1 SV=3 - [ARF4_HUMAN]	3	4	0.015	0.355	0.158	3.3	0.04	3	3	4
P11766	Alcohol dehydrogenase class-3 OS=Homo sapiens GN=ADH5 PE=1 SV=4 - [ADHX_HUMAN]	1	1			0.160			1	1	1
P02765	Alpha-2-HS-glycoprotein OS=Homo sapiens GN=AHSG PE=1 SV=1 - [FETUA_HUMAN]	2	2	0.001	0.001	0.002	2208.1	1.03E-07	1	2	2
P07355	Annexin A2 OS=Homo sapiens GN=ANXA2 PE=1 SV=2 - [ANXA2_HUMAN]	16	16	0.188	0.330	0.276	5.7	0.01	9	14	7
P09525	Annexin A4 OS=Homo sapiens GN=ANXA4 PE=1 SV=4 - [ANXA4_HUMAN]	5	5	0.001	0.001	0.004	466.3	2.30E-06	3	2	2
P00505	Aspartate aminotransferase, mitochondrial OS=Homo sapiens GN=GOT2 PE=1 SV=3 - [AATM_HUMAN]	1	1		0.001	0.001	0.0	0	1	1	1
Q9NVI7	ATPase family AAA domain-containing protein 3A OS=Homo sapiens GN=ATAD3A PE=1 SV=2 - [ATD3A_HUMAN]	2	2	0.001		0.002	1419.3	0.0002	2	1	1
P61221	ATP-binding cassette sub-family E member 1 OS=Homo sapiens GN=ABCE1 PE=1 SV=1 - [ABCE1_HUMAN]	4	4	0.001	0.212	0.199	5.3	0.02	4	2	1
Q9UG6	ATP-binding cassette sub-family F member 2	2	2	0.127		0.115	66.3	0.005	1	1	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
3	OS=Homo sapiens GN=ABCF2 PE=1 SV=2 - [ABCF2_HUMAN]										
O00571	ATP-dependent RNA helicase DDX3X OS=Homo sapiens GN=DDX3X PE=1 SV=3 - [DDX3X_HUMAN]	7	8	0.007	0.001	0.139	10.0	0.005	6	5	3
O95816	BAG family molecular chaperone regulator 2 OS=Homo sapiens GN=BAG2 PE=1 SV=1 - [BAG2_HUMAN]	1	1	0.001		0.001	0.0	0	1	1	1
O75531	Barrier-to-autointegration factor OS=Homo sapiens GN=BANF1 PE=1 SV=1 - [BAF_HUMAN]	1	1	0.189	0.244		10.1	0.03	1	1	1
P11586	C-1-tetrahydrofolate synthase, cytoplasmic OS=Homo sapiens GN=MTHFD1 PE=1 SV=3 - [C1TC_HUMAN]	7	7	0.001	0.202	0.002	6.4	0.01	7	4	5
P27708	CAD protein OS=Homo sapiens GN=CAD PE=1 SV=3 - [PYR1_HUMAN]	15	15	0.001	0.002	0.006	338.4	4.37E-06	13	5	1
Q9HB71	Calcyclin-binding protein OS=Homo sapiens GN=CACYBP PE=1 SV=2 - [CYBP_HUMAN]	1	1	0.198	0.396	0.373	2.8	0.05	1	1	1
P35221	Catenin alpha-1 OS=Homo sapiens GN=CTNNA1 PE=1 SV=1 - [CTNA1_HUMAN]	16	16	0.001	0.001	0.002	2197.2	1.04E-07	12	9	4
P35222	Catenin beta-1 OS=Homo sapiens GN=CTNNB1 PE=1 SV=1 - [CTNB1_HUMAN]	28	34	0.001	0.001	0.001	0.0	0	31	33	31
O60716	Catenin delta-1 OS=Homo sapiens GN=CTNND1 PE=1 SV=1 - [CTND1_HUMAN]	1	1	0.001	0.001	0.003	951.7	5.52E-07	1	1	1
P07339	Cathepsin D OS=Homo sapiens GN=CTSD	2	3			0.194			2	1	2

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	PE=1 SV=1 - [CATD_HUMAN]										
P60953	Cell division control protein 42 homolog OS=Homo sapiens GN=CDC42 PE=1 SV=2 - [CDC42_HUMAN]	2	3		0.001				1	1	2
Q05048	Cleavage stimulation factor subunit 1 OS=Homo sapiens GN=CSTF1 PE=1 SV=1 - [CSTF1_HUMAN]	2	2	0.001	0.001	0.001	0.0	0	1	1	2
P35606	Coatomer subunit beta' OS=Homo sapiens GN=COPB2 PE=1 SV=2 - [COPB2_HUMAN]	1	2	0.001	0.399	0.002	2.8	0.05	1	2	1
Q9NX63	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial OS=Homo sapiens GN=CHCHD3 PE=1 SV=1 - [CHCH3_HUMAN]	4	4				0.0	0	1	2	3
P21291	Cysteine and glycine-rich protein 1 OS=Homo sapiens GN=CSRP1 PE=1 SV=3 - [CSRP1_HUMAN]	3	3			0.120			2	2	3
P00403	Cytochrome c oxidase subunit 2 OS=Homo sapiens GN=MT-CO2 PE=1 SV=1 - [COX2_HUMAN]	1	1		0.190	0.184	106.7	0.003	1	1	1
P61962	DDB1- and CUL4-associated factor 7 OS=Homo sapiens GN=DCAF7 PE=1 SV=1 - [DCAF7_HUMAN]	1	1				0.0	0	1	1	1
P33992	DNA replication licensing factor MCM5 OS=Homo sapiens GN=MCM5 PE=1 SV=5 - [MCM5_HUMAN]	3	3		0.001	0.080	11.6	0.03	1	1	2
P33993	DNA replication licensing factor MCM7 OS=Homo sapiens GN=MCM7 PE=1 SV=4 -	7	7	0.001	0.001	0.001	10177.1	4.83E-09	3	3	3

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	[MCM7_HUMAN]										
O95793	Double-stranded RNA-binding protein Staufen homolog 1 OS=Homo sapiens GN=STAU1 PE=1 SV=2 - [STAU1_HUMAN]	4	4	0.001	0.001	0.005	402.2	3.09E-06	3	2	2
Q14258	E3 ubiquitin/ISG15 ligase TRIM25 OS=Homo sapiens GN=TRIM25 PE=1 SV=2 - [TRI25_HUMAN]	4	4	0.001	0.001	0.119	11.6	0.004	3	2	1
Q15717	ELAV-like protein 1 OS=Homo sapiens GN=ELAVL1 PE=1 SV=2 - [ELAV1_HUMAN]	2	2		0.255				1	1	1
P68104	Elongation factor 1-alpha 1 OS=Homo sapiens GN=EEF1A1 PE=1 SV=1 - [EF1A1_HUMAN]	7	7	0.141	0.233	0.206	11.3	0.004	4	4	6
P13639	Elongation factor 2 OS=Homo sapiens GN=EEF2 PE=1 SV=4 - [EF2_HUMAN]	12	12	0.176	0.344	0.212	5.0	0.02	9	9	5
P49411	Elongation factor Tu, mitochondrial OS=Homo sapiens GN=TUFM PE=1 SV=2 - [EFTU_HUMAN]	4	4	0.008	0.158	0.136	8.5	0.007	3	2	2
P50402	Emerin OS=Homo sapiens GN=EMD PE=1 SV=1 - [EMD_HUMAN]	2	2	0.001		0.001	10011.8	3.18E-05	1	1	1
P14625	Endoplasmin OS=Homo sapiens GN=HSP90B1 PE=1 SV=1 - [ENPL_HUMAN]	5	7	0.185	0.345	0.208	5.1	0.02	6	3	3
P60842	Eukaryotic initiation factor 4A-I OS=Homo sapiens GN=EIF4A1 PE=1 SV=1 - [IF4A1_HUMAN]	4	4	0.116	0.232	0.211	8.8	0.006	4	3	2
P05198	Eukaryotic translation initiation factor 2 subunit 1 OS=Homo sapiens GN=EIF2S1 PE=1 SV=3 - [IF2A_HUMAN]	5	5	0.127	0.384	0.312	2.9	0.05	3	3	1
P20042	Eukaryotic translation initiation factor 2 subunit	6	6	0.263	0.404	0.389	3.3	0.04	5	3	5

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	2 OS=Homo sapiens GN=EIF2S2 PE=1 SV=2 - [IF2B_HUMAN]										
O15371	Eukaryotic translation initiation factor 3 subunit D OS=Homo sapiens GN=EIF3D PE=1 SV=1 - [EIF3D_HUMAN]	1	1	0.001	0.001	0.002	2568.6	7.58E-08	1	1	1
P15311	Ezrin OS=Homo sapiens GN=EZR PE=1 SV=4 - [EZRI_HUMAN]	3	6	0.011					6	1	2
P47756	F-actin-capping protein subunit beta OS=Homo sapiens GN=CAPZB PE=1 SV=4 - [CAPZB_HUMAN]	4	4	0.082	0.014	0.143	11.2	0.004	1	4	1
P49327	Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3 - [FAS_HUMAN]	21	22	0.001	0.001	0.002	2012.6	1.23E-07	20	5	2
P56470	Galectin-4 OS=Homo sapiens GN=LGALS4 PE=1 SV=1 - [LEG4_HUMAN]	2	2		0.001	0.001	4261.2	7.47E-05	1	2	1
P30711	Glutathione S-transferase theta-1 OS=Homo sapiens GN=GSTT1 PE=1 SV=4 - [GSTT1_HUMAN]	1	1	0.001	0.001	0.001	0.0	0	1	1	1
P04406	Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3 - [G3P_HUMAN]	9	10	0.108	0.170	0.142	20.1	0.001	9	9	7
O14556	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific OS=Homo sapiens GN=GAPDHS PE=1 SV=2 - [G3PT_HUMAN]	2	3	0.001					2	3	1
P11216	Glycogen phosphorylase, brain form OS=Homo sapiens GN=PYGB PE=1 SV=5 - [PYGB_HUMAN]	11	11	0.001	0.006	0.002	309.1	5.23E-06	7	9	6
P62826	GTP-binding nuclear protein Ran OS=Homo	5	5	0.227	0.433	0.326	2.9	0.05	3	5	4

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	sapiens GN=RAN PE=1 SV=3 - [RAN_HUMAN]										
P62879	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2 OS=Homo sapiens GN=GNB2 PE=1 SV=3 - [GBB2_HUMAN]	4	4	0.044	0.023	0.009	46.3	0.0002	2	3	2
P63244	Guanine nucleotide-binding protein subunit beta-2-like 1 OS=Homo sapiens GN=GNB2L1 PE=1 SV=3 - [GBLP_HUMAN]	12	12	0.096	0.165	0.148	17.6	0.002	10	10	7
P08107	Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5 - [HSP71_HUMAN]	7	9	0.084	0.157	0.128	17.9	0.002	8	6	4
O95757	Heat shock 70 kDa protein 4L OS=Homo sapiens GN=HSPA4L PE=1 SV=3 - [HS74L_HUMAN]	1	3				0.0	0	2	2	1
P11142	Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1 - [HSP7C_HUMAN]	11	14	0.189	0.348	0.265	5.1	0.02	11	12	5
Q12931	Heat shock protein 75 kDa, mitochondrial OS=Homo sapiens GN=TRAP1 PE=1 SV=3 - [TRAP1_HUMAN]	1	2		0.001				2	2	1
P04792	Heat shock protein beta-1 OS=Homo sapiens GN=HSPB1 PE=1 SV=2 - [HSPB1_HUMAN]	4	4	0.234	0.399	0.353	3.5	0.04	3	4	3
P08238	Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4 - [HS90B_HUMAN]	7	17	0.010	0.244	0.014	5.3	0.02	14	15	7
P09651	Heterogeneous nuclear ribonucleoprotein A1 OS=Homo sapiens GN=HNRNPA1 PE=1 SV=5	3	3	0.001	0.294	0.002	4.1	0.03	2	1	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	- [ROA1_HUMAN]										
P31943	Heterogeneous nuclear ribonucleoprotein H OS=Homo sapiens GN=HNRNPH1 PE=1 SV=4 - [HNRH1_HUMAN]	4	5	0.001	0.001	0.001	3072.5	5.30E-08	2	3	3
P52272	Heterogeneous nuclear ribonucleoprotein M OS=Homo sapiens GN=HNRNPM PE=1 SV=3 - [HNRPM_HUMAN]	10	10	0.001		0.062	15.3	0.02	7	4	4
O60506	Heterogeneous nuclear ribonucleoprotein Q OS=Homo sapiens GN=SYNCRIP PE=1 SV=2 - [HNRPQ_HUMAN]	8	8	0.148	0.327	0.005	3.6	0.03	6	3	2
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1 OS=Homo sapiens GN=HNRNPA2B1 PE=1 SV=2 - [ROA2_HUMAN]	6	6	0.001	0.001	0.019	82.8	7.30E-05	2	3	4
O15347	High mobility group protein B3 OS=Homo sapiens GN=HMGB3 PE=1 SV=4 - [HMGB3_HUMAN]	3	3	0.009	0.195	0.163	6.6	0.01	2	1	2
P17096	High mobility group protein HMG-I/HMG-Y OS=Homo sapiens GN=HMGA1 PE=1 SV=3 - [HMGA1_HUMAN]	2	2	0.001	0.046	0.002	32.1	0.0005	1	1	2
P16403	Histone H1.2 OS=Homo sapiens GN=HIST1H1C PE=1 SV=2 - [H12_HUMAN]	4	4		0.001	0.102	8.9	0.04	1	2	4
P16401	Histone H1.5 OS=Homo sapiens GN=HIST1H1B PE=1 SV=3 - [H15_HUMAN]	2	2	0.069	0.104	0.074	38.0	0.0003	2	2	2
Q96KK5	Histone H2A type 1-H OS=Homo sapiens GN=HIST1H2AH PE=1 SV=3 - [H2A1H_HUMAN]	2	3		0.253				2	2	1
O60814	Histone H2B type 1-K OS=Homo sapiens	5	5	0.198	0.277	0.370	4.4	0.02	4	3	3

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	GN=HIST1H2BK PE=1 SV=3 - [H2B1K_HUMAN]										
P68431	Histone H3.1 OS=Homo sapiens GN=HIST1H3A PE=1 SV=2 - [H31_HUMAN]	2	2	0.185	0.278	0.341	5.1	0.02	2	2	2
P62805	Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2 - [H4_HUMAN]	5	5	0.210	0.259	0.357	5.2	0.02	4	4	4
Q14974	Importin subunit beta-1 OS=Homo sapiens GN=KPNB1 PE=1 SV=2 - [IMB1_HUMAN]	2	2	0.238					1	2	1
Q15181	Inorganic pyrophosphatase OS=Homo sapiens GN=PPA1 PE=1 SV=2 - [IPYR_HUMAN]	2	2	0.001					1	1	1
P12268	Inosine-5'-monophosphate dehydrogenase 2 OS=Homo sapiens GN=IMPDH2 PE=1 SV=2 - [IMDH2_HUMAN]	5	5			0.001			2	1	4
Q9Y6M1	Insulin-like growth factor 2 mRNA-binding protein 2 OS=Homo sapiens GN=IGF2BP2 PE=1 SV=2 - [IF2B2_HUMAN]	1	2	0.329	0.355	0.346	20.8	0.001	1	2	1
P19525	Interferon-induced, double-stranded RNA-activated protein kinase OS=Homo sapiens GN=EIF2AK2 PE=1 SV=2 - [E2AK2_HUMAN]	1	1	0.001		0.001	14815 9.1	2.15E-06	1	1	1
P14923	Junction plakoglobin OS=Homo sapiens GN=JUP PE=1 SV=3 - [PLAK_HUMAN]	2	8	0.001	0.001	0.003	897.4	6.21E-07	8	7	8
Q9NSK0	Kinesin light chain 4 OS=Homo sapiens GN=KLC4 PE=1 SV=3 - [KLC4_HUMAN]	4	4	0.002	0.001	0.004	614.9	1.32E-06	2	2	1
P20700	Lamin-B1 OS=Homo sapiens GN=LMNB1 PE=1 SV=2 - [LMNB1_HUMAN]	4	5	0.094	0.015	0.296	4.4	0.02	3	4	2
P30740	Leukocyte elastase inhibitor OS=Homo sapiens GN=SERPINB1 PE=1 SV=1 - [ILEU_HUMAN]	2	2	0.001		0.001	0.0	0	2	1	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
Q14847	LIM and SH3 domain protein 1 OS=Homo sapiens GN=LASP1 PE=1 SV=2 - [LASP1_HUMAN]	6	6	0.001	0.152	0.092	9.5	0.005	4	4	5
Q86W92	Liprin-beta-1 OS=Homo sapiens GN=PPFIBP1 PE=1 SV=2 - [LIPB1_HUMAN]	4	4	0.001	0.001	0.155	8.7	0.006	1	2	4
P00338	L-lactate dehydrogenase A chain OS=Homo sapiens GN=LDHA PE=1 SV=2 - [LDHA_HUMAN]	8	10	0.165	0.270	0.189	9.2	0.006	7	8	6
P07195	L-lactate dehydrogenase B chain OS=Homo sapiens GN=LDHB PE=1 SV=2 - [LDHB_HUMAN]	7	9	0.160	0.263	0.154	8.7	0.006	6	7	8
P10619	Lysosomal protective protein OS=Homo sapiens GN=CTSA PE=1 SV=2 - [PPGB_HUMAN]	3	3	0.006	0.012	0.016	176.0	1.61E-05	2	2	2
Q16891	Mitochondrial inner membrane protein OS=Homo sapiens GN=IMMT PE=1 SV=1 - [IMMT_HUMAN]	1	1	0.001		0.004	330.7	0.001	1	1	1
P60660	Myosin light polypeptide 6 OS=Homo sapiens GN=MYL6 PE=1 SV=2 - [MYL6_HUMAN]	4	4	0.001	0.118	0.166	8.3	0.007	1	4	3
P19105	Myosin regulatory light chain 12A OS=Homo sapiens GN=MYL12A PE=1 SV=2 - [ML12A_HUMAN]	5	5	0.009	0.135	0.169	8.1	0.007	4	5	4
Q13765	Nascent polypeptide-associated complex subunit alpha OS=Homo sapiens GN=NACA PE=1 SV=1 - [NACA_HUMAN]	2	2	0.154	0.350	0.211	4.5	0.02	2	1	2
Q14697	Neutral alpha-glucosidase AB OS=Homo sapiens GN=GANAB PE=1 SV=3 - [GANAB_HUMAN]	7	7	0.001	0.001	0.002	2054.0	1.19E-07	4	5	3

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
P49116	Nuclear receptor subfamily 2 group C member 2 OS=Homo sapiens GN=NR2C2 PE=1 SV=1 - [NR2C2_HUMAN]	1	1				0.0	0	1	1	1
Q9NZP5	Olfactory receptor 5AC2 OS=Homo sapiens GN=OR5AC2 PE=2 SV=2 - [O5AC2_HUMAN]	1	1				0.0	0	1	1	1
P50479	PDZ and LIM domain protein 4 OS=Homo sapiens GN=PDLIM4 PE=1 SV=2 - [PDLI4_HUMAN]	2	2	0.001	0.001	0.001	10015.5	4.98E-09	2	2	1
Q9NR12	PDZ and LIM domain protein 7 OS=Homo sapiens GN=PDLIM7 PE=1 SV=1 - [PDLI7_HUMAN]	4	4	0.162	0.203	0.015	6.5	0.01	2	1	4
P62937	Peptidyl-prolyl cis-trans isomerase A OS=Homo sapiens GN=PPIA PE=1 SV=2 - [PPIA_HUMAN]	8	8	0.134	0.239	0.162	10.3	0.005	6	5	5
P23284	Peptidyl-prolyl cis-trans isomerase B OS=Homo sapiens GN=PPIB PE=1 SV=2 - [PPIB_HUMAN]	6	6	0.162	0.001	0.281	4.3	0.02	2	6	3
Q06830	Peroxiredoxin-1 OS=Homo sapiens GN=PRDX1 PE=1 SV=1 - [PRDX1_HUMAN]	7	8	0.265	0.376	0.411	3.4	0.04	7	8	6
P32119	Peroxiredoxin-2 OS=Homo sapiens GN=PRDX2 PE=1 SV=5 - [PRDX2_HUMAN]	3	4		0.525				2	3	2
Q9Y446	Plakophilin-3 OS=Homo sapiens GN=PKP3 PE=1 SV=1 - [PKP3_HUMAN]	5	5	0.008	0.014	0.015	207.1	1.17E-05	4	2	4
Q15149	Plectin OS=Homo sapiens GN=PLEC PE=1 SV=3 - [PLEC_HUMAN]	91	94	0.112	0.121		85.5	0.004	88	42	5
P09874	Poly [ADP-ribose] polymerase 1 OS=Homo sapiens GN=PARP1 PE=1 SV=4 -	8	8	0.099	0.199	0.182	11.0	0.004	3	5	7

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	[PARP1_HUMAN]										
Q15365	Poly(rC)-binding protein 1 OS=Homo sapiens GN=PCBP1 PE=1 SV=2 - [PCBP1_HUMAN]	5	6	0.013	0.241	0.093	5.8	0.01	3	4	5
P11940	Polyadenylate-binding protein 1 OS=Homo sapiens GN=PABPC1 PE=1 SV=2 - [PABP1_HUMAN]	7	10	0.095	0.171	0.113	16.3	0.002	8	8	5
Q13310	Polyadenylate-binding protein 4 OS=Homo sapiens GN=PABPC4 PE=1 SV=1 - [PABP4_HUMAN]	1	4	0.001		0.002	1746.7	0.0002	4	3	2
P26599	Polypyrimidine tract-binding protein 1 OS=Homo sapiens GN=PTBP1 PE=1 SV=1 - [PTBP1_HUMAN]	3	3		0.001	0.002	1179.8	0.0003	1	3	2
P02545	Prelamin-A/C OS=Homo sapiens GN=LMNA PE=1 SV=1 - [LMNA_HUMAN]	19	20	0.095	0.174	0.177	13.1	0.003	17	14	10
Q92841	Probable ATP-dependent RNA helicase DDX17 OS=Homo sapiens GN=DDX17 PE=1 SV=2 - [DDX17_HUMAN]	4	8	0.001					6	5	4
P17844	Probable ATP-dependent RNA helicase DDX5 OS=Homo sapiens GN=DDX5 PE=1 SV=1 - [DDX5_HUMAN]	6	10	0.001	0.008	0.002	211.5	1.12E-05	7	8	7
Q8WUM4	Programmed cell death 6-interacting protein OS=Homo sapiens GN=PDCD6IP PE=1 SV=1 - [PDC6I_HUMAN]	4	4	0.001	0.001	0.002	1086.3	4.24E-07	3	1	3
P35232	Prohibitin OS=Homo sapiens GN=PHB PE=1 SV=1 - [PHB_HUMAN]	8	8	0.122	0.272	0.244	6.2	0.01	5	3	5
Q99623	Prohibitin-2 OS=Homo sapiens GN=PHB2 PE=1 SV=2 - [PHB2_HUMAN]	3	3	0.163	0.283	0.305	5.6	0.01	3	2	2

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
Q15084	Protein disulfide-isomerase A6 OS=Homo sapiens GN=PDIA6 PE=1 SV=1 - [PDIA6_HUMAN]	5	5	0.209	0.376	0.101	3.4	0.04	3	3	2
Q8WVV4	Protein POF1B OS=Homo sapiens GN=POF1B PE=1 SV=3 - [POF1B_HUMAN]	3	3	0.001	0.001	0.001	8218.5	7.40E-09	1	3	2
P06703	Protein S100-A6 OS=Homo sapiens GN=S100A6 PE=1 SV=1 - [S10A6_HUMAN]	1	1	0.001		0.006	216.8	0.001	1	1	1
Q92734	Protein TFG OS=Homo sapiens GN=TFG PE=1 SV=2 - [TFG_HUMAN]	3	3	0.038	0.001	0.015	44.0	0.0003	2	2	1
Q15436	Protein transport protein Sec23A OS=Homo sapiens GN=SEC23A PE=1 SV=2 - [SC23A_HUMAN]	1	2	0.002					1	1	1
Q15437	Protein transport protein Sec23B OS=Homo sapiens GN=SEC23B PE=1 SV=2 - [SC23B_HUMAN]	1	2	0.001	0.001	0.005	438.8	2.60E-06	1	2	2
P21980	Protein-glutamine gamma-glutamyltransferase 2 OS=Homo sapiens GN=TGM2 PE=1 SV=2 - [TGM2_HUMAN]	5	5		0.179				2	4	1
P14618	Pyruvate kinase isozymes M1/M2 OS=Homo sapiens GN=PKM PE=1 SV=4 - [KPYM_HUMAN]	14	14	0.174	0.263	0.204	10.9	0.004	12	11	6
P63000	Ras-related C3 botulinum toxin substrate 1 OS=Homo sapiens GN=RAC1 PE=1 SV=1 - [RAC1_HUMAN]	3	4	0.292	0.424	0.327	3.9	0.03	2	3	3
P61026	Ras-related protein Rab-10 OS=Homo sapiens GN=RAB10 PE=1 SV=1 - [RAB10_HUMAN]	1	2				0.0	0	2	2	2
P61106	Ras-related protein Rab-14 OS=Homo sapiens	1	2				0.0	0	2	1	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	GN=RAB14 PE=1 SV=4 - [RAB14_HUMAN]										
P51148	Ras-related protein Rab-5C OS=Homo sapiens GN=RAB5C PE=1 SV=2 - [RAB5C_HUMAN]	2	2			0.316			1	1	1
P61224	Ras-related protein Rap-1b OS=Homo sapiens GN=RAP1B PE=1 SV=1 - [RAP1B_HUMAN]	2	2	0.326	0.333	0.377	9.8	0.005	1	2	2
P52565	Rho GDP-dissociation inhibitor 1 OS=Homo sapiens GN=ARHGDIA PE=1 SV=3 - [GDIR1_HUMAN]	1	1	0.182	0.321	0.295	5.5	0.02	1	1	1
P13489	Ribonuclease inhibitor OS=Homo sapiens GN=RNH1 PE=1 SV=2 - [RINI_HUMAN]	6	6	0.095	0.135	0.004	10.9	0.004	4	3	5
Q96PK6	RNA-binding protein 14 OS=Homo sapiens GN=RBM14 PE=1 SV=2 - [RBM14_HUMAN]	2	2	0.001		0.006	194.4	0.002	1	1	1
Q01844	RNA-binding protein EWS OS=Homo sapiens GN=EWSR1 PE=1 SV=1 - [EWS_HUMAN]	1	1	0.001	0.001		0.0	0	1	1	1
P35637	RNA-binding protein FUS OS=Homo sapiens GN=FUS PE=1 SV=1 - [FUS_HUMAN]	3	3		0.139	0.001	6.3	0.05	3	2	2
Q9Y265	RuvB-like 1 OS=Homo sapiens GN=RUVBL1 PE=1 SV=1 - [RUVB1_HUMAN]	8	8	0.044	0.001	0.001	33.5	0.0004	4	5	5
Q9Y230	RuvB-like 2 OS=Homo sapiens GN=RUVBL2 PE=1 SV=3 - [RUVB2_HUMAN]	12	12	0.001	0.001	0.006	306.5	5.32E-06	9	8	9
Q07955	Serine/arginine-rich splicing factor 1 OS=Homo sapiens GN=SRSF1 PE=1 SV=2 - [SRSF1_HUMAN]	2	2				0.0	0	1	1	2
Q9UHD2	Serine/threonine-protein kinase TBK1 OS=Homo sapiens GN=TBK1 PE=1 SV=1 - [TBK1_HUMAN]	6	6	0.003	0.001	0.026	62.1	0.0001	5	1	2
P62136	Serine/threonine-protein phosphatase PP1-alpha	3	3	0.001	0.001	0.001	0.0	0	1	1	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	catalytic subunit OS=Homo sapiens GN=PPP1CA PE=1 SV=1 - [PP1A_HUMAN]										
Q00796	Sorbitol dehydrogenase OS=Homo sapiens GN=SORD PE=1 SV=4 - [DHSO_HUMAN]	3	3				0.0	0	1	1	1
Q14247	Src substrate cortactin OS=Homo sapiens GN=CTTN PE=1 SV=2 - [SRC8_HUMAN]	6	6	0.001	0.001	0.002	1597.6	1.96E-07	5	2	2
P38646	Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2 - [GRP75_HUMAN]	4	4	0.164	0.278	0.322	5.2	0.02	4	3	2
O14907	Tax1-binding protein 3 OS=Homo sapiens GN=TAX1BP3 PE=1 SV=2 - [TX1B3_HUMAN]	1	1	0.021	0.033	0.021	117.1	3.65E-05	1	1	1
P49368	T-complex protein 1 subunit gamma OS=Homo sapiens GN=CCT3 PE=1 SV=4 - [TCPG_HUMAN]	5	5	0.142	0.017	0.022	10.7	0.004	3	3	2
P26639	Threonine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=TARS PE=1 SV=3 - [SYTC_HUMAN]	4	4	0.001	0.001	0.002	2265.8	9.74E-08	2	2	2
Q9Y4K3	TNF receptor-associated factor 6 OS=Homo sapiens GN=TRAF6 PE=1 SV=1 - [TRAF6_HUMAN]	1	1	0.001		0.002	1780.1	0.0002	1	1	1
Q13263	Transcription intermediary factor 1-beta OS=Homo sapiens GN=TRIM28 PE=1 SV=5 - [TIF1B_HUMAN]	4	4	0.001	0.001	0.007	268.6	6.93E-06	2	2	1
P37802	Transgelin-2 OS=Homo sapiens GN=TAGLN2 PE=1 SV=3 - [TAGL2_HUMAN]	2	2		0.288				1	2	1
P29401	Transketolase OS=Homo sapiens GN=TKT	3	3	0.001	0.117	0.003	12.0	0.003	2	3	2

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	PE=1 SV=3 - [TKT_HUMAN]										
Q9UNL2	Translocon-associated protein subunit gamma OS=Homo sapiens GN=SSR3 PE=1 SV=1 - [SSRG_HUMAN]	1	1	0.001	0.001	0.211	6.1	0.01	1	1	1
Q14134	Tripartite motif-containing protein 29 OS=Homo sapiens GN=TRIM29 PE=1 SV=2 - [TRI29_HUMAN]	7	7	0.001	0.001	0.160	8.4	0.007	5	4	2
Q9Y3I0	tRNA-splicing ligase RtcB homolog OS=Homo sapiens GN=C22orf28 PE=1 SV=1 - [RTCB_HUMAN]	2	2	0.001		0.001	10854.1	2.93E-05	2	1	1
Q9BQE3	Tubulin alpha-1C chain OS=Homo sapiens GN=TUBA1C PE=1 SV=1 - [TBA1C_HUMAN]	4	11	0.193	0.017	0.176	6.6	0.01	8	5	7
P07437	Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2 - [TBB5_HUMAN]	3	14	0.109	0.238		6.2	0.01	9	11	10
P68371	Tubulin beta-4B chain OS=Homo sapiens GN=TUBB4B PE=1 SV=1 - [TBB4B_HUMAN]	4	15		0.172	0.135	19.1	0.02	6	12	11
Q9BUF5	Tubulin beta-6 chain OS=Homo sapiens GN=TUBB6 PE=1 SV=1 - [TBB6_HUMAN]	1	7		0.245	0.157	6.8	0.05	4	5	5
P61088	Ubiquitin-conjugating enzyme E2 N OS=Homo sapiens GN=UBE2N PE=1 SV=1 - [UBE2N_HUMAN]	1	1	0.120	0.209	0.268	7.0	0.01	1	1	1
O00159	Unconventional myosin-Ic OS=Homo sapiens GN=MYO1C PE=1 SV=4 - [MYO1C_HUMAN]	6	6	0.001	0.001	0.005	333.4	4.50E-06	5	3	2
O95292	Vesicle-associated membrane protein-associated protein B/C OS=Homo sapiens GN=VAPB PE=1 SV=3 - [VAPB_HUMAN]	2	2	0.094	0.001		9.7	0.03	1	1	1
P08670	Vimentin OS=Homo sapiens GN=VIM PE=1	14	17	0.138	0.260	0.252	7.2	0.009	14	10	12

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	SV=4 - [VIME_HUMAN]										
P21796	Voltage-dependent anion-selective channel protein 1 OS=Homo sapiens GN=VDAC1 PE=1 SV=2 - [VDAC1_HUMAN]	4	5	0.148	0.293	0.273	5.8	0.01	4	3	4
P45880	Voltage-dependent anion-selective channel protein 2 OS=Homo sapiens GN=VDAC2 PE=1 SV=2 - [VDAC2_HUMAN]	5	5	0.015	0.178	0.002	7.7	0.008	3	2	2
Q9Y277	Voltage-dependent anion-selective channel protein 3 OS=Homo sapiens GN=VDAC3 PE=1 SV=1 - [VDAC3_HUMAN]	3	4		0.254	0.197	9.8	0.03	2	3	4
P13010	X-ray repair cross-complementing protein 5 OS=Homo sapiens GN=XRCC5 PE=1 SV=3 - [XRCC5_HUMAN]	5	5	0.109	0.001	0.167	8.4	0.007	3	4	3
P12956	X-ray repair cross-complementing protein 6 OS=Homo sapiens GN=XRCC6 PE=1 SV=2 - [XRCC6_HUMAN]	6	6	0.143	0.305	0.237	5.8	0.01	6	3	4
Q7Z2W4	Zinc finger CCCH-type antiviral protein 1 OS=Homo sapiens GN=ZC3HAV1 PE=1 SV=3 - [ZCCHV_HUMAN]	3	3	0.001	0.001	0.001	11418.0	3.84E-09	3	1	1

8.7 Candidate novel interacting proteins of the non-phosphorylatable mutant of β-catenin at tyrosine-654 in HT29.

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
P61981	14-3-3 protein gamma OS=Homo sapiens GN=YWHAG PE=1 SV=2 - [1433G_HUMAN]	2	4		0.513				2	4	3
P62857	40S ribosomal protein S28 OS=Homo sapiens GN=RPS28 PE=1 SV=1 - [RS28_HUMAN]	1	1				0.0	0	1	1	1
P63261	Actin, cytoplasmic 2 OS=Homo sapiens GN=ACTG1 PE=1 SV=1 - [ACTG_HUMAN]	1	10	0.356					8	8	9
P63267	Actin, gamma-enteric smooth muscle OS=Homo sapiens GN=ACTG2 PE=1 SV=1 - [ACTH_HUMAN]	1	7				0.0	0	6	5	7
P61204	ADP-ribosylation factor 3 OS=Homo sapiens GN=ARF3 PE=1 SV=2 - [ARF3_HUMAN]	1	2		0.440	0.433	19.51	0.0163007	1	2	2
P18085	ADP-ribosylation factor 4 OS=Homo sapiens GN=ARF4 PE=1 SV=3 - [ARF4_HUMAN]	1	2				0.0	0	1	2	1
P04114	Apolipoprotein B-100 OS=Homo sapiens GN=APOB PE=1 SV=2 - [APOB_HUMAN]	3	3	0.001	0.001	0.006	281.0	6.331E-06	2	1	1
O95816	BAG family molecular chaperone regulator 2 OS=Homo sapiens GN=BAG2 PE=1 SV=1 - [BAG2_HUMAN]	1	1	0.001	0.001		0	0	1	1	1
P35221	Catenin alpha-1 OS=Homo sapiens GN=CTNNA1 PE=1 SV=1 - [CTNA1_HUMAN]	13	13	0.019	0.002	0.077	20.6	0.0011731	11	4	9
P35222	Catenin beta-1 OS=Homo sapiens GN=CTNNB1 PE=1 SV=1 - [CTNB1_HUMAN]	25	31	0.001	0.001	0.001	0.0	0	29	26	28

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
Q14919	Dr1-associated corepressor OS=Homo sapiens GN=DRAP1 PE=1 SV=3 - [NC2A_HUMAN]	1	1	0.259	0.256		190.94	0.0016671	1	1	1
P13804	Electron transfer flavoprotein subunit alpha, mitochondrial OS=Homo sapiens GN=ETFA PE=1 SV=1 - [ETFA_HUMAN]	1	1	0.245	0.275	0.359	6.1	0.0130481	1	1	1
P58107	Epiplakin OS=Homo sapiens GN=EPPK1 PE=1 SV=2 - [EPIPL_HUMAN]	38	41	0.001	0.001	0.007	261.0	7.339E-06	35	34	1
P55060	Exportin-2 OS=Homo sapiens GN=CSE1L PE=1 SV=3 - [XPO2_HUMAN]	1	2	0.429					2	1	1
P49327	Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3 - [FAS_HUMAN]	49	50	0.355	0.367	0.289	6.7	0.0106536	44	43	4
P56470	Galectin-4 OS=Homo sapiens GN=LGALS4 PE=1 SV=1 - [LEG4_HUMAN]	3	3	0.002	0.002	0.002	6675.2	1.122E-08	1	2	2
P30711	Glutathione S-transferase theta-1 OS=Homo sapiens GN=GSTT1 PE=1 SV=4 - [GSTT1_HUMAN]	1	1	0.001	0.001	0.001	0.0	0	1	1	1
O14556	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific OS=Homo sapiens GN=GAPDHS PE=1 SV=2 - [G3PT_HUMAN]	1	2		0.001				1	2	1
P11216	Glycogen phosphorylase, brain form OS=Homo sapiens GN=PYGB PE=1 SV=5 - [PYGB_HUMAN]	13	13	0.063	0.003	0.166	8.9	0.0062229	11	4	7
P08107	Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5 - [HSP71_HUMAN]	5	7	0.192	0.213	0.277	10.7	0.0043067	7	7	6
P08238	Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4 -	6	16	0.345	0.335	0.467	2.8	0.0549589	16	9	14

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	[HS90B_HUMAN]										
P02042	Hemoglobin subunit delta OS=Homo sapiens GN=HBD PE=1 SV=2 - [HBD_HUMAN]	1	1	0.001	0.001	0.001	0.0	0	1	1	1
Q14103	Heterogeneous nuclear ribonucleoprotein D0 OS=Homo sapiens GN=HNRNPD PE=1 SV=1 - [HNRPD_HUMAN]	2	2		0.483				1	2	1
Q02241	Kinesin-like protein KIF23 OS=Homo sapiens GN=KIF23 PE=1 SV=3 - [KIF23_HUMAN]	6	6	0.237	0.345	0.285	6.7	0.0106946	4	3	4
P10619	Lysosomal protective protein OS=Homo sapiens GN=CTSA PE=1 SV=2 - [PPGB_HUMAN]	3	3	0.001	0.001		0	0	2	2	1
P40926	Malate dehydrogenase, mitochondrial OS=Homo sapiens GN=MDH2 PE=1 SV=3 - [MDHM_HUMAN]	3	3			0.006			2	1	2
Q7Z406	Myosin-14 OS=Homo sapiens GN=MYH14 PE=1 SV=2 - [MYH14_HUMAN]	31	39	0.002	0.002		10911	2.917E-05	30	30	1
Q13765	Nascent polypeptide-associated complex subunit alpha OS=Homo sapiens GN=NACA PE=1 SV=1 - [NACA_HUMAN]	3	3	0.091	0.284	0.311	3.9	0.0297548	1	3	2
Q14697	Neutral alpha-glucosidase AB OS=Homo sapiens GN=GANAB PE=1 SV=3 - [GANAB_HUMAN]	6	6	0.335	0.405	0.388	5.9	0.013844	6	4	4
P23284	Peptidyl-prolyl cis-trans isomerase B OS=Homo sapiens GN=PPIB PE=1 SV=2 - [PPIB_HUMAN]	5	5	0.157	0.174		39.611	0.0080341	2	3	2
Q9Y446	Plakophilin-3 OS=Homo sapiens GN=PKP3 PE=1 SV=1 - [PKP3_HUMAN]	11	11	0.323	0.287	0.458	2.8	0.0547806	11	2	7
Q15149	Plectin OS=Homo sapiens GN=PLEC PE=1	157	160	0.341	0.371	0.462	3.0	0.04789	144	139	18

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
	SV=3 - [PLEC_HUMAN]							26			
Q15185	Prostaglandin E synthase 3 OS=Homo sapiens GN=PTGES3 PE=1 SV=1 - [TEBP_HUMAN]	1	1				0.0	0	1	1	1
Q92530	Proteasome inhibitor PI31 subunit OS=Homo sapiens GN=PSMF1 PE=1 SV=2 - [PSMF1_HUMAN]	1	1				0.0	0	1	1	1
Q01658	Protein Dr1 OS=Homo sapiens GN=DR1 PE=1 SV=1 - [NC2B_HUMAN]	1	1	0.227	0.274	0.315	9.0	0.0060951	1	1	1
Q8WVV4	Protein POF1B OS=Homo sapiens GN=POF1B PE=1 SV=3 - [POF1B_HUMAN]	5	5	0.001	0.003	0.005	510.8	1.916E-06	5	2	2
Q8WUF5	RelA-associated inhibitor OS=Homo sapiens GN=PPP1R13L PE=1 SV=4 - [IASPP_HUMAN]	3	3	0.021		0.020	554.62	0.0005739	3	1	2
P13489	Ribonuclease inhibitor OS=Homo sapiens GN=RNH1 PE=1 SV=2 - [RINI_HUMAN]	3	3		0.423				2	2	1
Q01844	RNA-binding protein EWS OS=Homo sapiens GN=EWSR1 PE=1 SV=1 - [EWS_HUMAN]	1	1			0.543			1	1	1
Q9Y230	RuvB-like 2 OS=Homo sapiens GN=RUVBL2 PE=1 SV=3 - [RUVB2_HUMAN]	14	14	0.001	0.002	0.003	813.3	7.558E-07	10	12	13
Q9Y3F4	Serine-threonine kinase receptor-associated protein OS=Homo sapiens GN=STRAP PE=1 SV=1 - [STRAP_HUMAN]	1	1			0.430			1	1	1
Q00796	Sorbitol dehydrogenase OS=Homo sapiens GN=SORD PE=1 SV=4 - [DHSO_HUMAN]	2	2		0.020				2	2	2
Q8WU68	Splicing factor U2AF 26 kDa subunit OS=Homo sapiens GN=U2AF1L4 PE=1 SV=2 - [U2AF4_HUMAN]	1	1	0.006	0.001		217.17	0.0014657	1	1	1

Acc #	Protein	Unique peptides	Peptides	R1 H/L	R2 H/L	R3 H/L	tstat	pval	R1 peptides	R2 peptides	R3 peptides
Q14247	Src substrate cortactin OS=Homo sapiens GN=CTTN PE=1 SV=2 - [SRC8_HUMAN]	8	9	0.174	0.225	0.354	4.7	0.0215845	7	2	6
Q8WXE9	Stonin-2 OS=Homo sapiens GN=STON2 PE=1 SV=1 - [STON2_HUMAN]	1	1		0.001	0.001	0	0	1	1	1
P38646	Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2 - [GRP75_HUMAN]	8	8	0.244	0.384	0.398	3.2	0.0425633	5	3	5
P37837	Transaldolase OS=Homo sapiens GN=TALDO1 PE=1 SV=2 - [TALDO_HUMAN]	1	1			0.260			1	1	1
P29401	Transketolase OS=Homo sapiens GN=TKT PE=1 SV=3 - [TKT_HUMAN]	4	4	0.267	0.302	0.345	8.6	0.0066406	3	2	2

8.8 Candidate novel interacting proteins of β-catenin that may be effect by phosphorylation.

*PM denotes phosphomimetic and NP denotes non-phosphorylatable

**White denotes absence of that protein in the dataset

***Grey denotes presence of that protein in the dataset

Acc #	Protein	NP	Wildtype	PM
P62857	40S ribosomal protein S28 OS=Homo sapiens GN=RPS28 PE=1 SV=1 - [RS28_HUMAN]			
P63267	Actin, gamma-enteric smooth muscle OS=Homo sapiens GN=ACTG2 PE=1 SV=1 - [ACTH_HUMAN]			
P61204	ADP-ribosylation factor 3 OS=Homo sapiens GN=ARF3 PE=1 SV=2 - [ARF3_HUMAN]			
P04114	Apolipoprotein B-100 OS=Homo sapiens GN=APOB PE=1 SV=2 - [APOB_HUMAN]			
P13804	Electron transfer flavoprotein subunit alpha, mitochondrial OS=Homo sapiens GN=ETFA PE=1 SV=1 - [ETFA_HUMAN]			
P58107	Epiplakin OS=Homo sapiens GN=EPPK1 PE=1 SV=2 - [EPIPL_HUMAN]			
P55060	Exportin-2 OS=Homo sapiens GN=CSE1L PE=1 SV=3 - [XPO2_HUMAN]			
P02042	Hemoglobin subunit delta OS=Homo sapiens GN=HBD PE=1 SV=2 - [HBD_HUMAN]			
Q02241	Kinesin-like protein KIF23 OS=Homo sapiens GN=KIF23 PE=1 SV=3 - [KIF23_HUMAN]			
P40926	Malate dehydrogenase, mitochondrial OS=Homo sapiens GN=MDH2 PE=1 SV=3 - [MDHM_HUMAN]			
Q7Z406	Myosin-14 OS=Homo sapiens GN=MYH14 PE=1 SV=2 - [MYH14_HUMAN]			
Q15185	Prostaglandin E synthase 3 OS=Homo sapiens GN=PTGES3 PE=1 SV=1 - [TEBP_HUMAN]			
Q92530	Proteasome inhibitor PI31 subunit OS=Homo sapiens GN=PSMF1 PE=1 SV=2 - [PSMF1_HUMAN]			

Acc #	Protein	NP	Wildtype	PM
Q01658	Protein Dr1 OS=Homo sapiens GN=DR1 PE=1 SV=1 - [NC2B_HUMAN]			
Q8WUF5	RelA-associated inhibitor OS=Homo sapiens GN=PPP1R13L PE=1 SV=4 - [IASPP_HUMAN]			
Q9Y3F4	Serine-threonine kinase receptor-associated protein OS=Homo sapiens GN=STRAP PE=1 SV=1 - [STRAP_HUMAN]			
Q8WU68	Splicing factor U2AF 26 kDa subunit OS=Homo sapiens GN=U2AF1L4 PE=1 SV=2 - [U2AF4_HUMAN]			
Q8WXE9	Stonin-2 OS=Homo sapiens GN=STON2 PE=1 SV=1 - [STON2_HUMAN]			
P37837	Transaldolase OS=Homo sapiens GN=TALDO1 PE=1 SV=2 - [TALDO_HUMAN]			
P62258	14-3-3 protein epsilon OS=Homo sapiens GN=YWHAE PE=1 SV=1 - [1433E_HUMAN]			
P27348	14-3-3 protein theta OS=Homo sapiens GN=YWHAQ PE=1 SV=1 - [1433T_HUMAN]			
P63104	14-3-3 protein zeta/delta OS=Homo sapiens GN=YWHAZ PE=1 SV=1 - [1433Z_HUMAN]			
P62899	60S ribosomal protein L31 OS=Homo sapiens GN=RPL31 PE=1 SV=1 - [RL31_HUMAN]			
P23526	Adenosylhomocysteinase OS=Homo sapiens GN=AHCY PE=1 SV=4 - [SAHH_HUMAN]			
P06733	Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2 - [ENOA_HUMAN]			
P08758	Annexin A5 OS=Homo sapiens GN=ANXA5 PE=1 SV=2 - [ANXA5_HUMAN]			
P25705	ATP synthase subunit alpha, mitochondrial OS=Homo sapiens GN=ATP5A1 PE=1 SV=1 - [ATPA_HUMAN]			
Q00610	Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5 - [CLH1_HUMAN]			
P60981	Destrin OS=Homo sapiens GN=DSTN PE=1 SV=3 - [DEST_HUMAN]			
P41091	Eukaryotic translation initiation factor 2 subunit 3 OS=Homo sapiens GN=EIF2S3 PE=1 SV=3 - [IF2G_HUMAN]			
P47755	F-actin-capping protein subunit alpha-2 OS=Homo sapiens GN=CAPZA2 PE=1 SV=3 - [CAZA2_HUMAN]			

Acc #	Protein	NP	Wildtype	PM
P07900	Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1 PE=1 SV=5 - [HS90A_HUMAN]			
P0C0S5	Histone H2A.Z OS=Homo sapiens GN=H2AFZ PE=1 SV=2 - [H2AZ_HUMAN]			
Q86U44	N6-adenosine-methyltransferase 70 kDa subunit OS=Homo sapiens GN=METTL3 PE=1 SV=2 - [MTA70_HUMAN]			
P30101	Protein disulfide-isomerase A3 OS=Homo sapiens GN=PDIA3 PE=1 SV=4 - [PDIA3_HUMAN]			
Q6ZRV2	Protein FAM83H OS=Homo sapiens GN=FAM83H PE=1 SV=3 - [FA83H_HUMAN]			
Q92928	Putative Ras-related protein Rab-1C OS=Homo sapiens GN=RAB1C PE=5 SV=2 - [RAB1C_HUMAN]			
P50991	T-complex protein 1 subunit delta OS=Homo sapiens GN=CCT4 PE=1 SV=4 - [TCPD_HUMAN]			
P40227	T-complex protein 1 subunit zeta OS=Homo sapiens GN=CCT6A PE=1 SV=3 - [TCPZ_HUMAN]			
P68363	Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1 - [TBA1B_HUMAN]			
P68366	Tubulin alpha-4A chain OS=Homo sapiens GN=TUBA4A PE=1 SV=1 - [TBA4A_HUMAN]			
Q13885	Tubulin beta-2A chain OS=Homo sapiens GN=TUBB2A PE=1 SV=1 - [TBB2A_HUMAN]			
P61981	14-3-3 protein gamma OS=Homo sapiens GN=YWHAG PE=1 SV=2 - [1433G_HUMAN]			
P63261	Actin, cytoplasmic 2 OS=Homo sapiens GN=ACTG1 PE=1 SV=1 - [ACTG_HUMAN]			
Q14919	Dr1-associated corepressor OS=Homo sapiens GN=DRAP1 PE=1 SV=3 - [NC2A_HUMAN]			
Q14103	Heterogeneous nuclear ribonucleoprotein D0 OS=Homo sapiens GN=HNRNPD PE=1 SV=1 - [HNRPD_HUMAN]			
P31946	14-3-3 protein beta/alpha OS=Homo sapiens GN=YWHAB PE=1 SV=3 - [1433B_HUMAN]			
Q04917	14-3-3 protein eta OS=Homo sapiens GN=YWHAH PE=1 SV=4 - [1433F_HUMAN]			
P60866	40S ribosomal protein S20 OS=Homo sapiens GN=RPS20 PE=1 SV=1 - [RS20_HUMAN]			
P62266	40S ribosomal protein S23 OS=Homo sapiens GN=RPS23 PE=1 SV=3 - [RS23_HUMAN]			
P62847	40S ribosomal protein S24 OS=Homo sapiens GN=RPS24 PE=1 SV=1 - [RS24_HUMAN]			

Acc #	Protein	NP	Wildtype	PM
Q71UM5	40S ribosomal protein S27-like OS=Homo sapiens GN=RPS27L PE=1 SV=3 - [RS27L_HUMAN]			
P62861	40S ribosomal protein S30 OS=Homo sapiens GN=FAU PE=1 SV=1 - [RS30_HUMAN]			
P46782	40S ribosomal protein S5 OS=Homo sapiens GN=RPS5 PE=1 SV=4 - [RS5_HUMAN]			
P62081	40S ribosomal protein S7 OS=Homo sapiens GN=RPS7 PE=1 SV=1 - [RS7_HUMAN]			
P08865	40S ribosomal protein SA OS=Homo sapiens GN=RPSA PE=1 SV=4 - [RSSA_HUMAN]			
P27635	60S ribosomal protein L10 OS=Homo sapiens GN=RPL10 PE=1 SV=4 - [RL10_HUMAN]			
P62906	60S ribosomal protein L10a OS=Homo sapiens GN=RPL10A PE=1 SV=2 - [RL10A_HUMAN]			
P30050	60S ribosomal protein L12 OS=Homo sapiens GN=RPL12 PE=1 SV=1 - [RL12_HUMAN]			
P61513	60S ribosomal protein L15 OS=Homo sapiens GN=RPL15 PE=1 SV=2 - [RL15_HUMAN]			
Q02543	60S ribosomal protein L18a OS=Homo sapiens GN=RPL18A PE=1 SV=2 - [RL18A_HUMAN]			
P62829	60S ribosomal protein L23 OS=Homo sapiens GN=RPL23 PE=1 SV=1 - [RL23_HUMAN]			
P61254	60S ribosomal protein L26 OS=Homo sapiens GN=RPL26 PE=1 SV=1 - [RL26_HUMAN]			
P49207	60S ribosomal protein L34 OS=Homo sapiens GN=RPL34 PE=1 SV=3 - [RL34_HUMAN]			
P83881	60S ribosomal protein L36a OS=Homo sapiens GN=RPL36A PE=1 SV=2 - [RL36A_HUMAN]			
P36578	60S ribosomal protein L4 OS=Homo sapiens GN=RPL4 PE=1 SV=5 - [RL4_HUMAN]			
P62424	60S ribosomal protein L7a OS=Homo sapiens GN=RPL7A PE=1 SV=2 - [RL7A_HUMAN]			
P62917	60S ribosomal protein L8 OS=Homo sapiens GN=RPL8 PE=1 SV=2 - [RL8_HUMAN]			
P11021	78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2 - [GRP78_HUMAN]			
P60709	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 - [ACTB_HUMAN]			
P61160	Actin-related protein 2 OS=Homo sapiens GN=ACTR2 PE=1 SV=1 - [ARP2_HUMAN]			
P59998	Actin-related protein 2/3 complex subunit 4 OS=Homo sapiens GN=ARPC4 PE=1 SV=3 -			

Acc #	Protein	NP	Wildtype	PM
	[ARPC4_HUMAN]			
P11766	Alcohol dehydrogenase class-3 OS=Homo sapiens GN=ADH5 PE=1 SV=4 - [ADHX_HUMAN]			
P00505	Aspartate aminotransferase, mitochondrial OS=Homo sapiens GN=GOT2 PE=1 SV=3 - [AATM_HUMAN]			
Q9NVI7	ATPase family AAA domain-containing protein 3A OS=Homo sapiens GN=ATAD3A PE=1 SV=2 - [ATD3A_HUMAN]			
P61221	ATP-binding cassette sub-family E member 1 OS=Homo sapiens GN=ABCE1 PE=1 SV=1 - [ABCE1_HUMAN]			
Q9UG63	ATP-binding cassette sub-family F member 2 OS=Homo sapiens GN=ABCF2 PE=1 SV=2 - [ABCF2_HUMAN]			
O00571	ATP-dependent RNA helicase DDX3X OS=Homo sapiens GN=DDX3X PE=1 SV=3 - [DDX3X_HUMAN]			
O75531	Barrier-to-autointegration factor OS=Homo sapiens GN=BANF1 PE=1 SV=1 - [BAF_HUMAN]			
P11586	C-1-tetrahydrofolate synthase, cytoplasmic OS=Homo sapiens GN=MTHFD1 PE=1 SV=3 - [C1TC_HUMAN]			
O60716	Catenin delta-1 OS=Homo sapiens GN=CTNND1 PE=1 SV=1 - [CTND1_HUMAN]			
P07339	Cathepsin D OS=Homo sapiens GN=CTSD PE=1 SV=1 - [CATD_HUMAN]			
P60953	Cell division control protein 42 homolog OS=Homo sapiens GN=CDC42 PE=1 SV=2 - [CDC42_HUMAN]			
Q05048	Cleavage stimulation factor subunit 1 OS=Homo sapiens GN=CSTF1 PE=1 SV=1 - [CSTF1_HUMAN]			
P35606	Coatomer subunit beta' OS=Homo sapiens GN=COPB2 PE=1 SV=2 - [COPB2_HUMAN]			
Q9NX63	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial OS=Homo sapiens GN=CHCHD3 PE=1 SV=1 - [CHCH3_HUMAN]			
P21291	Cysteine and glycine-rich protein 1 OS=Homo sapiens GN=CSRP1 PE=1 SV=3 - [CSRP1_HUMAN]			
P00403	Cytochrome c oxidase subunit 2 OS=Homo sapiens GN=MT-CO2 PE=1 SV=1 - [COX2_HUMAN]			

Acc #	Protein	NP	Wildtype	PM
P61962	DDB1- and CUL4-associated factor 7 OS=Homo sapiens GN=DCAF7 PE=1 SV=1 - [DCAF7_HUMAN]			
P33992	DNA replication licensing factor MCM5 OS=Homo sapiens GN=MCM5 PE=1 SV=5 - [MCM5_HUMAN]			
P33993	DNA replication licensing factor MCM7 OS=Homo sapiens GN=MCM7 PE=1 SV=4 - [MCM7_HUMAN]			
O95793	Double-stranded RNA-binding protein Staufen homolog 1 OS=Homo sapiens GN=STAU1 PE=1 SV=2 - [STAU1_HUMAN]			
Q14258	E3 ubiquitin/ISG15 ligase TRIM25 OS=Homo sapiens GN=TRIM25 PE=1 SV=2 - [TRI25_HUMAN]			
Q15717	ELAV-like protein 1 OS=Homo sapiens GN=ELAVL1 PE=1 SV=2 - [ELAV1_HUMAN]			
P13639	Elongation factor 2 OS=Homo sapiens GN=EEF2 PE=1 SV=4 - [EF2_HUMAN]			
P49411	Elongation factor Tu, mitochondrial OS=Homo sapiens GN=TUFM PE=1 SV=2 - [EFTU_HUMAN]			
P50402	Emerin OS=Homo sapiens GN=EMD PE=1 SV=1 - [EMD_HUMAN]			
P14625	Endoplasmin OS=Homo sapiens GN=HSP90B1 PE=1 SV=1 - [ENPL_HUMAN]			
P05198	Eukaryotic translation initiation factor 2 subunit 1 OS=Homo sapiens GN=EIF2S1 PE=1 SV=3 - [IF2A_HUMAN]			
P20042	Eukaryotic translation initiation factor 2 subunit 2 OS=Homo sapiens GN=EIF2S2 PE=1 SV=2 - [IF2B_HUMAN]			
O15371	Eukaryotic translation initiation factor 3 subunit D OS=Homo sapiens GN=EIF3D PE=1 SV=1 - [EIF3D_HUMAN]			
P15311	Ezrin OS=Homo sapiens GN=EZR PE=1 SV=4 - [EZRI_HUMAN]			
O95757	Heat shock 70 kDa protein 4L OS=Homo sapiens GN=HSPA4L PE=1 SV=3 - [HS74L_HUMAN]			
P11142	Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1 - [HSP7C_HUMAN]			
Q12931	Heat shock protein 75 kDa, mitochondrial OS=Homo sapiens GN=TRAP1 PE=1 SV=3 - [TRAP1_HUMAN]			

Acc #	Protein	NP	Wildtype	PM
P09651	Heterogeneous nuclear ribonucleoprotein A1 OS=Homo sapiens GN=HNRNPA1 PE=1 SV=5 - [ROA1_HUMAN]			
P31943	Heterogeneous nuclear ribonucleoprotein H OS=Homo sapiens GN=HNRNPH1 PE=1 SV=4 - [HNRH1_HUMAN]			
O60506	Heterogeneous nuclear ribonucleoprotein Q OS=Homo sapiens GN=SYNCRIP PE=1 SV=2 - [HNRPQ_HUMAN]			
P17096	High mobility group protein HMG-I/HMG-Y OS=Homo sapiens GN=HMGA1 PE=1 SV=3 - [HMGA1_HUMAN]			
P68431	Histone H3.1 OS=Homo sapiens GN=HIST1H3A PE=1 SV=2 - [H31_HUMAN]			
P62805	Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2 - [H4_HUMAN]			
Q14974	Importin subunit beta-1 OS=Homo sapiens GN=KPNB1 PE=1 SV=2 - [IMB1_HUMAN]			
Q15181	Inorganic pyrophosphatase OS=Homo sapiens GN=PPA1 PE=1 SV=2 - [IPYR_HUMAN]			
Q9Y6M1	Insulin-like growth factor 2 mRNA-binding protein 2 OS=Homo sapiens GN=IGF2BP2 PE=1 SV=2 - [IF2B2_HUMAN]			
P19525	Interferon-induced, double-stranded RNA-activated protein kinase OS=Homo sapiens GN=EIF2AK2 PE=1 SV=2 - [E2AK2_HUMAN]			
Q9NSK0	Kinesin light chain 4 OS=Homo sapiens GN=KLC4 PE=1 SV=3 - [KLC4_HUMAN]			
P20700	Lamin-B1 OS=Homo sapiens GN=LMNB1 PE=1 SV=2 - [LMNB1_HUMAN]			
P30740	Leukocyte elastase inhibitor OS=Homo sapiens GN=SERPINB1 PE=1 SV=1 - [ILEU_HUMAN]			
Q86W92	Liprin-beta-1 OS=Homo sapiens GN=PPFIBP1 PE=1 SV=2 - [LIPB1_HUMAN]			
Q16891	Mitochondrial inner membrane protein OS=Homo sapiens GN=IMMT PE=1 SV=1 - [IMMT_HUMAN]			
Q9NZP5	Olfactory receptor 5AC2 OS=Homo sapiens GN=OR5AC2 PE=2 SV=2 - [O5AC2_HUMAN]			
P50479	PDZ and LIM domain protein 4 OS=Homo sapiens GN=PDLIM4 PE=1 SV=2 - [PDLI4_HUMAN]			
P32119	Peroxiredoxin-2 OS=Homo sapiens GN=PRDX2 PE=1 SV=5 - [PRDX2_HUMAN]			

Acc #	Protein	NP	Wildtype	PM
Q15365	Poly(rC)-binding protein 1 OS=Homo sapiens GN=PCBP1 PE=1 SV=2 - [PCBP1_HUMAN]			
P26599	Polypyrimidine tract-binding protein 1 OS=Homo sapiens GN=PTBP1 PE=1 SV=1 - [PTBP1_HUMAN]			
Q92841	Probable ATP-dependent RNA helicase DDX17 OS=Homo sapiens GN=DDX17 PE=1 SV=2 - [DDX17_HUMAN]			
Q8WUM4	Programmed cell death 6-interacting protein OS=Homo sapiens GN=PDCD6IP PE=1 SV=1 - [PDC6I_HUMAN]			
Q99623	Prohibitin-2 OS=Homo sapiens GN=PHB2 PE=1 SV=2 - [PHB2_HUMAN]			
Q15084	Protein disulfide-isomerase A6 OS=Homo sapiens GN=PDIA6 PE=1 SV=1 - [PDIA6_HUMAN]			
P06703	Protein S100-A6 OS=Homo sapiens GN=S100A6 PE=1 SV=1 - [S10A6_HUMAN]			
Q92734	Protein TFG OS=Homo sapiens GN=TFG PE=1 SV=2 - [TFG_HUMAN]			
Q15436	Protein transport protein Sec23A OS=Homo sapiens GN=SEC23A PE=1 SV=2 - [SC23A_HUMAN]			
Q15437	Protein transport protein Sec23B OS=Homo sapiens GN=SEC23B PE=1 SV=2 - [SC23B_HUMAN]			
P21980	Protein-glutamine gamma-glutamyltransferase 2 OS=Homo sapiens GN=TGM2 PE=1 SV=2 - [TGM2_HUMAN]			
P63000	Ras-related C3 botulinum toxin substrate 1 OS=Homo sapiens GN=RAC1 PE=1 SV=1 - [RAC1_HUMAN]			
P61026	Ras-related protein Rab-10 OS=Homo sapiens GN=RAB10 PE=1 SV=1 - [RAB10_HUMAN]			
P51148	Ras-related protein Rab-5C OS=Homo sapiens GN=RAB5C PE=1 SV=2 - [RAB5C_HUMAN]			
P61224	Ras-related protein Rap-1b OS=Homo sapiens GN=RAP1B PE=1 SV=1 - [RAP1B_HUMAN]			
P52565	Rho GDP-dissociation inhibitor 1 OS=Homo sapiens GN=ARHGDIA PE=1 SV=3 - [GDIR1_HUMAN]			
Q96PK6	RNA-binding protein 14 OS=Homo sapiens GN=RBM14 PE=1 SV=2 - [RBM14_HUMAN]			
P35637	RNA-binding protein FUS OS=Homo sapiens GN=FUS PE=1 SV=1 - [FUS_HUMAN]			
Q07955	Serine/arginine-rich splicing factor 1 OS=Homo sapiens GN=SRSF1 PE=1 SV=2 - [SRSF1_HUMAN]			

Acc #	Protein	NP	Wildtype	PM
Q9UHD2	Serine/threonine-protein kinase TBK1 OS=Homo sapiens GN=TBK1 PE=1 SV=1 - [TBK1_HUMAN]			
P26639	Threonine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=TARS PE=1 SV=3 - [SYTC_HUMAN]			
Q9Y4K3	TNF receptor-associated factor 6 OS=Homo sapiens GN=TRAF6 PE=1 SV=1 - [TRAF6_HUMAN]			
Q13263	Transcription intermediary factor 1-beta OS=Homo sapiens GN=TRIM28 PE=1 SV=5 - [TIF1B_HUMAN]			
P37802	Transgelin-2 OS=Homo sapiens GN=TAGLN2 PE=1 SV=3 - [TAGL2_HUMAN]			
Q9UNL2	Translocon-associated protein subunit gamma OS=Homo sapiens GN=SSR3 PE=1 SV=1 - [SSRG_HUMAN]			
Q14134	Tripartite motif-containing protein 29 OS=Homo sapiens GN=TRIM29 PE=1 SV=2 - [TRI29_HUMAN]			
Q9Y3I0	tRNA-splicing ligase RtcB homolog OS=Homo sapiens GN=C22orf28 PE=1 SV=1 - [RTCB_HUMAN]			
Q9BQE3	Tubulin alpha-1C chain OS=Homo sapiens GN=TUBA1C PE=1 SV=1 - [TBA1C_HUMAN]			
Q9BUF5	Tubulin beta-6 chain OS=Homo sapiens GN=TUBB6 PE=1 SV=1 - [TBB6_HUMAN]			
O00159	Unconventional myosin-Ic OS=Homo sapiens GN=MYO1C PE=1 SV=4 - [MYO1C_HUMAN]			
O95292	Vesicle-associated membrane protein-associated protein B/C OS=Homo sapiens GN=VAPB PE=1 SV=3 - [VAPB_HUMAN]			
P45880	Voltage-dependent anion-selective channel protein 2 OS=Homo sapiens GN=VDAC2 PE=1 SV=2 - [VDAC2_HUMAN]			
Q9Y277	Voltage-dependent anion-selective channel protein 3 OS=Homo sapiens GN=VDAC3 PE=1 SV=1 - [VDAC3_HUMAN]			
P13010	X-ray repair cross-complementing protein 5 OS=Homo sapiens GN=XRCC5 PE=1 SV=3 - [XRCC5_HUMAN]			
P12956	X-ray repair cross-complementing protein 6 OS=Homo sapiens GN=XRCC6 PE=1 SV=2 - [XRCC6_HUMAN]			
Q7Z2W4	Zinc finger CCCH-type antiviral protein 1 OS=Homo sapiens GN=ZC3HAV1 PE=1 SV=3 - [ZCCHV_HUMAN]			

Acc #	Protein	NP	Wildtype	PM
P18085	ADP-ribosylation factor 4 OS=Homo sapiens GN=ARF4 PE=1 SV=3 - [ARF4_HUMAN]			
O95816	BAG family molecular chaperone regulator 2 OS=Homo sapiens GN=BAG2 PE=1 SV=1 - [BAG2_HUMAN]			
P35221	Catenin alpha-1 OS=Homo sapiens GN=CTNNA1 PE=1 SV=1 - [CTNA1_HUMAN]			
P30711	Glutathione S-transferase theta-1 OS=Homo sapiens GN=GSTT1 PE=1 SV=4 - [GSTT1_HUMAN]			
P11216	Glycogen phosphorylase, brain form OS=Homo sapiens GN=PYGB PE=1 SV=5 - [PYGB_HUMAN]			
Q13765	Nascent polypeptide-associated complex subunit alpha OS=Homo sapiens GN=NACA PE=1 SV=1 - [NACA_HUMAN]			
Q14697	Neutral alpha-glucosidase AB OS=Homo sapiens GN=GANAB PE=1 SV=3 - [GANAB_HUMAN]			
Q9Y446	Plakophilin-3 OS=Homo sapiens GN=PKP3 PE=1 SV=1 - [PKP3_HUMAN]			
Q8WVV4	Protein POF1B OS=Homo sapiens GN=POF1B PE=1 SV=3 - [POF1B_HUMAN]			
P13489	Ribonuclease inhibitor OS=Homo sapiens GN=RNH1 PE=1 SV=2 - [RINI_HUMAN]			
Q01844	RNA-binding protein EWS OS=Homo sapiens GN=EWSR1 PE=1 SV=1 - [EWS_HUMAN]			
Q00796	Sorbitol dehydrogenase OS=Homo sapiens GN=SORD PE=1 SV=4 - [DHSO_HUMAN]			
Q14247	Src substrate cortactin OS=Homo sapiens GN=CTTN PE=1 SV=2 - [SRC8_HUMAN]			
P38646	Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2 - [GRP75_HUMAN]			
P29401	Transketolase OS=Homo sapiens GN=TKT PE=1 SV=3 - [TKT_HUMAN]			
Q9C0C2	182 kDa tankyrase-1-binding protein OS=Homo sapiens GN=TNKS1BP1 PE=1 SV=4 - [TB182_HUMAN]			
P62280	40S ribosomal protein S11 OS=Homo sapiens GN=RPS11 PE=1 SV=3 - [RS11_HUMAN]			
P62277	40S ribosomal protein S13 OS=Homo sapiens GN=RPS13 PE=1 SV=2 - [RS13_HUMAN]			
P62263	40S ribosomal protein S14 OS=Homo sapiens GN=RPS14 PE=1 SV=3 - [RS14_HUMAN]			

Acc #	Protein	NP	Wildtype	PM
P62244	40S ribosomal protein S15a OS=Homo sapiens GN=RPS15A PE=1 SV=2 - [RS15A_HUMAN]			
P62249	40S ribosomal protein S16 OS=Homo sapiens GN=RPS16 PE=1 SV=2 - [RS16_HUMAN]			
P62269	40S ribosomal protein S18 OS=Homo sapiens GN=RPS18 PE=1 SV=3 - [RS18_HUMAN]			
P39019	40S ribosomal protein S19 OS=Homo sapiens GN=RPS19 PE=1 SV=2 - [RS19_HUMAN]			
P15880	40S ribosomal protein S2 OS=Homo sapiens GN=RPS2 PE=1 SV=2 - [RS2_HUMAN]			
P62851	40S ribosomal protein S25 OS=Homo sapiens GN=RPS25 PE=1 SV=1 - [RS25_HUMAN]			
P42677	40S ribosomal protein S27 OS=Homo sapiens GN=RPS27 PE=1 SV=3 - [RS27_HUMAN]			
P23396	40S ribosomal protein S3 OS=Homo sapiens GN=RPS3 PE=1 SV=2 - [RS3_HUMAN]			
P61247	40S ribosomal protein S3a OS=Homo sapiens GN=RPS3A PE=1 SV=2 - [RS3A_HUMAN]			
P62701	40S ribosomal protein S4, X isoform OS=Homo sapiens GN=RPS4X PE=1 SV=2 - [RS4X_HUMAN]			
P62753	40S ribosomal protein S6 OS=Homo sapiens GN=RPS6 PE=1 SV=1 - [RS6_HUMAN]			
P62241	40S ribosomal protein S8 OS=Homo sapiens GN=RPS8 PE=1 SV=2 - [RS8_HUMAN]			
P46781	40S ribosomal protein S9 OS=Homo sapiens GN=RPS9 PE=1 SV=3 - [RS9_HUMAN]			
P05388	60S acidic ribosomal protein P0 OS=Homo sapiens GN=RPLP0 PE=1 SV=1 - [RLA0_HUMAN]			
P62913	60S ribosomal protein L11 OS=Homo sapiens GN=RPL11 PE=1 SV=2 - [RL11_HUMAN]			
P26373	60S ribosomal protein L13 OS=Homo sapiens GN=RPL13 PE=1 SV=4 - [RL13_HUMAN]			
P40429	60S ribosomal protein L13a OS=Homo sapiens GN=RPL13A PE=1 SV=2 - [RL13A_HUMAN]			
P50914	60S ribosomal protein L14 OS=Homo sapiens GN=RPL14 PE=1 SV=4 - [RL14_HUMAN]			
P61313	60S ribosomal protein L15 OS=Homo sapiens GN=RPL15 PE=1 SV=2 - [RL15_HUMAN]			
Q07020	60S ribosomal protein L18 OS=Homo sapiens GN=RPL18 PE=1 SV=2 - [RL18_HUMAN]			
P84098	60S ribosomal protein L19 OS=Homo sapiens GN=RPL19 PE=1 SV=1 - [RL19_HUMAN]			

Acc #	Protein	NP	Wildtype	PM
P35268	60S ribosomal protein L22 OS=Homo sapiens GN=RPL22 PE=1 SV=2 - [RL22_HUMAN]			
P62750	60S ribosomal protein L23a OS=Homo sapiens GN=RPL23A PE=1 SV=1 - [RL23A_HUMAN]			
P83731	60S ribosomal protein L24 OS=Homo sapiens GN=RPL24 PE=1 SV=1 - [RL24_HUMAN]			
P61353	60S ribosomal protein L27 OS=Homo sapiens GN=RPL27 PE=1 SV=2 - [RL27_HUMAN]			
P46776	60S ribosomal protein L27a OS=Homo sapiens GN=RPL27A PE=1 SV=2 - [RL27A_HUMAN]			
P46779	60S ribosomal protein L28 OS=Homo sapiens GN=RPL28 PE=1 SV=3 - [RL28_HUMAN]			
P62888	60S ribosomal protein L30 OS=Homo sapiens GN=RPL30 PE=1 SV=2 - [RL30_HUMAN]			
P18077	60S ribosomal protein L35a OS=Homo sapiens GN=RPL35A PE=1 SV=2 - [RL35A_HUMAN]			
Q9Y3U8	60S ribosomal protein L36 OS=Homo sapiens GN=RPL36 PE=1 SV=3 - [RL36_HUMAN]			
P46777	60S ribosomal protein L5 OS=Homo sapiens GN=RPL5 PE=1 SV=3 - [RL5_HUMAN]			
Q02878	60S ribosomal protein L6 OS=Homo sapiens GN=RPL6 PE=1 SV=3 - [RL6_HUMAN]			
P18124	60S ribosomal protein L7 OS=Homo sapiens GN=RPL7 PE=1 SV=1 - [RL7_HUMAN]			
P53999	Activated RNA polymerase II transcriptional coactivator p15 OS=Homo sapiens GN=SUB1 PE=1 SV=3 - [TCP4_HUMAN]			
P02765	Alpha-2-HS-glycoprotein OS=Homo sapiens GN=AHSG PE=1 SV=1 - [FETUA_HUMAN]			
P07355	Annexin A2 OS=Homo sapiens GN=ANXA2 PE=1 SV=2 - [ANXA2_HUMAN]			
P09525	Annexin A4 OS=Homo sapiens GN=ANXA4 PE=1 SV=4 - [ANXA4_HUMAN]			
P27708	CAD protein OS=Homo sapiens GN=CAD PE=1 SV=3 - [PYR1_HUMAN]			
Q9HB71	Calcyclin-binding protein OS=Homo sapiens GN=CACYBP PE=1 SV=2 - [CYBP_HUMAN]			
P68104	Elongation factor 1-alpha 1 OS=Homo sapiens GN=EEF1A1 PE=1 SV=1 - [EF1A1_HUMAN]			
P60842	Eukaryotic initiation factor 4A-I OS=Homo sapiens GN=EIF4A1 PE=1 SV=1 - [IF4A1_HUMAN]			

Acc #	Protein	NP	Wildtype	PM
P47756	F-actin-capping protein subunit beta OS=Homo sapiens GN=CAPZB PE=1 SV=4 - [CAPZB_HUMAN]			
P04406	Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3 - [G3P_HUMAN]			
P62826	GTP-binding nuclear protein Ran OS=Homo sapiens GN=RAN PE=1 SV=3 - [RAN_HUMAN]			
P62879	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2 OS=Homo sapiens GN=GNB2 PE=1 SV=3 - [GBB2_HUMAN]			
P63244	Guanine nucleotide-binding protein subunit beta-2-like 1 OS=Homo sapiens GN=GNB2L1 PE=1 SV=3 - [GBLP_HUMAN]			
P04792	Heat shock protein beta-1 OS=Homo sapiens GN=HSPB1 PE=1 SV=2 - [HSPB1_HUMAN]			
P52272	Heterogeneous nuclear ribonucleoprotein M OS=Homo sapiens GN=HNRNPM PE=1 SV=3 - [HNRPM_HUMAN]			
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1 OS=Homo sapiens GN=HNRNPA2B1 PE=1 SV=2 - [ROA2_HUMAN]			
O15347	High mobility group protein B3 OS=Homo sapiens GN=HMGB3 PE=1 SV=4 - [HMGB3_HUMAN]			
P16403	Histone H1.2 OS=Homo sapiens GN=HIST1H1C PE=1 SV=2 - [H12_HUMAN]			
P16401	Histone H1.5 OS=Homo sapiens GN=HIST1H1B PE=1 SV=3 - [H15_HUMAN]			
Q96KK5	Histone H2A type 1-H OS=Homo sapiens GN=HIST1H2AH PE=1 SV=3 - [H2A1H_HUMAN]			
O60814	Histone H2B type 1-K OS=Homo sapiens GN=HIST1H2BK PE=1 SV=3 - [H2B1K_HUMAN]			
P12268	Inosine-5'-monophosphate dehydrogenase 2 OS=Homo sapiens GN=IMPDH2 PE=1 SV=2 - [IMDH2_HUMAN]			
P14923	Junction plakoglobin OS=Homo sapiens GN=JUP PE=1 SV=3 - [PLAK_HUMAN]			
Q14847	LIM and SH3 domain protein 1 OS=Homo sapiens GN=LASP1 PE=1 SV=2 - [LASP1_HUMAN]			
P00338	L-lactate dehydrogenase A chain OS=Homo sapiens GN=LDHA PE=1 SV=2 - [LDHA_HUMAN]			
P07195	L-lactate dehydrogenase B chain OS=Homo sapiens GN=LDHB PE=1 SV=2 - [LDHB_HUMAN]			

Acc #	Protein	NP	Wildtype	PM
P60660	Myosin light polypeptide 6 OS=Homo sapiens GN=MYL6 PE=1 SV=2 - [MYL6_HUMAN]			
P19105	Myosin regulatory light chain 12A OS=Homo sapiens GN=MYL12A PE=1 SV=2 - [ML12A_HUMAN]			
P49116	Nuclear receptor subfamily 2 group C member 2 OS=Homo sapiens GN=NR2C2 PE=1 SV=1 - [NR2C2_HUMAN]			
Q9NR12	PDZ and LIM domain protein 7 OS=Homo sapiens GN=PDLIM7 PE=1 SV=1 - [PDLI7_HUMAN]			
P62937	Peptidyl-prolyl cis-trans isomerase A OS=Homo sapiens GN=PPIA PE=1 SV=2 - [PPIA_HUMAN]			
Q06830	Peroxiredoxin-1 OS=Homo sapiens GN=PRDX1 PE=1 SV=1 - [PRDX1_HUMAN]			
P09874	Poly [ADP-ribose] polymerase 1 OS=Homo sapiens GN=PARP1 PE=1 SV=4 - [PARP1_HUMAN]			
P11940	Polyadenylate-binding protein 1 OS=Homo sapiens GN=PABPC1 PE=1 SV=2 - [PABP1_HUMAN]			
Q13310	Polyadenylate-binding protein 4 OS=Homo sapiens GN=PABPC4 PE=1 SV=1 - [PABP4_HUMAN]			
P02545	Prelamin-A/C OS=Homo sapiens GN=LMNA PE=1 SV=1 - [LMNA_HUMAN]			
P17844	Probable ATP-dependent RNA helicase DDX5 OS=Homo sapiens GN=DDX5 PE=1 SV=1 - [DDX5_HUMAN]			
P35232	Prohibitin OS=Homo sapiens GN=PHB PE=1 SV=1 - [PHB_HUMAN]			
P14618	Pyruvate kinase isozymes M1/M2 OS=Homo sapiens GN=PKM PE=1 SV=4 - [KPYM_HUMAN]			
P61106	Ras-related protein Rab-14 OS=Homo sapiens GN=RAB14 PE=1 SV=4 - [RAB14_HUMAN]			
Q9Y265	RuvB-like 1 OS=Homo sapiens GN=RUVBL1 PE=1 SV=1 - [RUVB1_HUMAN]			
P62136	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit OS=Homo sapiens GN=PPP1CA PE=1 SV=1 - [PP1A_HUMAN]			
O14907	Tax1-binding protein 3 OS=Homo sapiens GN=TAX1BP3 PE=1 SV=2 - [TX1B3_HUMAN]			
P49368	T-complex protein 1 subunit gamma OS=Homo sapiens GN=CCT3 PE=1 SV=4 - [TCPG_HUMAN]			
P07437	Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2 - [TBB5_HUMAN]			

Acc #	Protein	NP	Wildtype	PM
P68371	Tubulin beta-4B chain OS=Homo sapiens GN=TUBB4B PE=1 SV=1 - [TBB4B_HUMAN]			
P61088	Ubiquitin-conjugating enzyme E2 N OS=Homo sapiens GN=UBE2N PE=1 SV=1 - [UBE2N_HUMAN]			
P08670	Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4 - [VIME_HUMAN]			
P21796	Voltage-dependent anion-selective channel protein 1 OS=Homo sapiens GN=VDAC1 PE=1 SV=2 - [VDAC1_HUMAN]			
P35222	Catenin beta-1 OS=Homo sapiens GN=CTNNB1 PE=1 SV=1 - [CTNB1_HUMAN]			
P49327	Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3 - [FAS_HUMAN]			
P56470	Galectin-4 OS=Homo sapiens GN=LGALS4 PE=1 SV=1 - [LEG4_HUMAN]			
O14556	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific OS=Homo sapiens GN=GAPDHS PE=1 SV=2 - [G3PT_HUMAN]			
P08107	Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5 - [HSP71_HUMAN]			
P08238	Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4 - [HS90B_HUMAN]			
P10619	Lysosomal protective protein OS=Homo sapiens GN=CTSA PE=1 SV=2 - [PPGB_HUMAN]			
P23284	Peptidyl-prolyl cis-trans isomerase B OS=Homo sapiens GN=PPIB PE=1 SV=2 - [PPIB_HUMAN]			
Q15149	Plectin OS=Homo sapiens GN=PLEC PE=1 SV=3 - [PLEC_HUMAN]			
Q9Y230	RuvB-like 2 OS=Homo sapiens GN=RUVBL2 PE=1 SV=3 - [RUVB2_HUMAN]			