Table S1. Known palmitoylated platelet proteins that were not considered significantly enriched

Several highly abundant platelet proteins that have been previously identified as palmitoylated were identified in the list of 1,300 purified proteins but failed to demonstrate a HA+/HA- ratio ≥ 3. Thus, they did not meet the stringent cutoff values used to define the significant dataset. Shown is the HA+/HA- ratio and corresponding p-value.

Table S2. Comparison of known proteins of the platelet palmitoylome with palmitoyl proteins in prostate cancer cells: Localization to lipid rafts

The raft localization for 78 of the known palmitoylated proteins identified in the platelet palmitoylome were assigned based on the localization of these proteins identified in a proteomic study of palmitoylated proteins present in human prostate cancer cells. Of the 78 proteins, 6 were present only in lipid rafts, 33 were present only in non-raft fractions, and 39 were present in both raft and non-raft fractions.

Figure S1. Manual verification of platelet palmitoyl protein candidates identified based on one unique peptide

For each verified identification, the IPI accession number, protein name, peptide sequence, and annotated tandem mass spectrum were provided.

Figure S2. A fraction of platelet TLT-1 associates with lipid rafts

Platelets were either exposed to 100 µM SFLLRN for 20 mins (*Activated*) or left unstimulated (*Resting*). Samples were then lysed with cold 1% Triton X-100 and subjected to sucrose gradient centrifugation (Sardjono *et al*, 2006). Equal volume aliquots of each fraction were analyzed for TLT-1 by Western blot analysis. Dot blot analysis of each fraction indicated the location of lipid rafts in the gradient at fractions two and three as demonstrated by Flotillin-1 content.

Table S1. Previously established palmitoylated platelet proteins that did not meet the cutoff values

Protein Name	Gene Name	Ratio	P-value
P-selectin	SELP	0.65	0.726517
Tubulin	TUBB1	1.19	0.376411
Platelet glycoprotein Ib	GP1BA	1.92	0.150071
Platelet endothelial cell adhesion molecule	PECAM1	2.11	0.119245
G(z)	GNAZ	2.29	0.096928
Platelet glycoprotein IX	GP9	2.65	0.064166
CD9 antigen	CD9	2.81	0.053393

Table S2. Comparison of known proteins of the platelet palmitoylome with palmitoyl proteins in prostate cancer cells: localization to lipid rafts

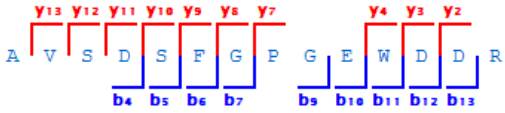
Protein Name	Gene Name	Present in lipid raft fraction?
55 kDa erythrocyte membrane protein	MPP1	Non-raft
Abhydrolase domain-containing protein FAM108B1	FAM108B1	Both
Acid ceramidase	ASAH1	Non-raft
ADP-ribosylation factor-like protein 15	ARL15	Non-raft
ATP-binding cassette sub-family B member 6	ABCB6	Non-raft
Calnexin	CANX	Both
Casein kinase I gamma 1 isoform	CSNK1G1	Raft
Casein kinase I isoform gamma-3	CSNK1G3	Raft
Cation-dependent mannose-6-phosphate receptor	M6PR	Both
CD151 antigen	CD151	Non-raft
CD63 antigen	CD63	Non-raft
CDC42 small effector protein 2	CDC42SE2	Non-raft
Cell division control protein 42 homolog	CDC42	Non-raft
Choline transporter-like protein 1	CTL1	Non-raft
Choline transporter-like protein 2	CTL2	Non-raft
Claudin-3	CLDN3	Both
CTD small phosphatase-like protein	CTDSPL	Non-raft
Cytochrome b5 type B	CYB5B	Non-raft
Desmoplakin	DSP	Both
Disheveled-associated activator of morphogenesis 1	DAAM1	Non-raft
DnaJ homolog subfamily C member 5	DNAJC5	Non-raft
Endothelin-converting enzyme 1	ECE1	Non-raft
Erbb2-interacting protein	ERBB2IP	Both
F-box/LRR-repeat protein 20	FBXL20	Non-raft
Flotillin-1	FLOT1	Both
Flotillin-2	FLOT2	Both
G(i) alpha-1	GNAI1	Both
G(i) alpha-2	GNAI2	Both
G(i) alpha-3	GNAI3	Both
G(q) alpha	GNAQ	Both
G(s) alpha	GNAS	Both
Galpha-11	GNA11	Both
Galpha-13	GNA13	Both
Glutaminase kidney isoform	GLS	Non-raft
Golgin subfamily A member 7	GOLGA7	Both
GTPase Hras	HRAS	Non-raft
GTPase Nras	NRAS	Both
HLA class I histocompatibility antigen, B-27 alpha chain	HLA-B	Both
Linker for activation of T-cells family member 1	LAT	Raft
Linker for activation of T-cells family member 2	LAT2	Raft
Multidrug resistance-associated protein 4	ABCC4	Non-raft

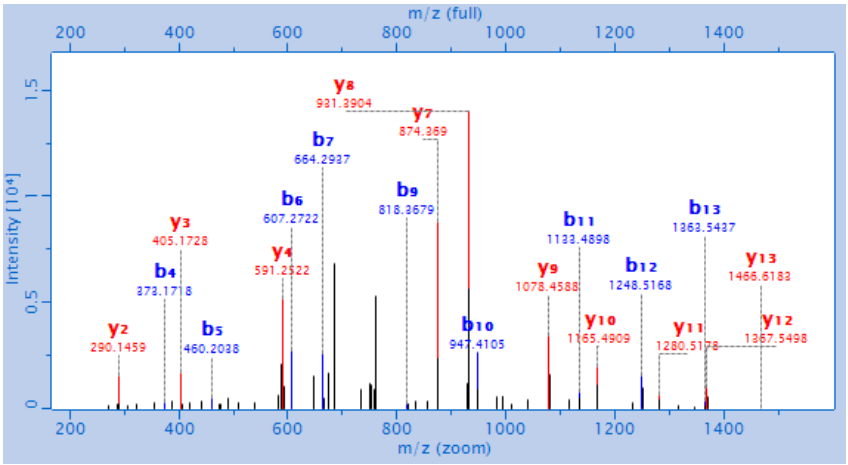
Protein Name	Gene Name	Present in lipid raft fraction?
Phosphatidylinositol 4-kinase type 2-alpha	PI4K2A	Both
Phosphatidylinositol 4-kinase type 2-beta	PI4K2B	Non-raft
Phospholipid scramblase 1	PLSCR1	Both
Phospholipid scramblase 3	PLSCR3	Non-raft
Pituitary tumor-transforming gene 1 protein-interacting protein	PTTG1IP	Both
PRA1 family protein 2	PRAF2	Non-raft
Probable phospholipid-transporting ATPase IF	ATP11B	Non-raft
Protein FAM49B	FAM49B	Both
Protein LYRICgene-1 protein	LYRIC	Both
Protein tweety homolog 3	TTYH3	Non-raft
Proto-oncogene tyrosine-protein kinase Yes	YES1	Both
Ras-related protein Rap-2a	RAP2A	Non-raft
Ras-related protein Rap-2b	RAP2B	Both
Ras-related protein Rap-2c	RAP2C	Non-raft
Ras-related protein R-Ras	RRAS	Both
Regulator of G-protein signaling 19	RGS19	Raft
Secretory carrier-associated membrane protein 1	SCAMP1	Both
Secretory carrier-associated membrane protein 2	SCAMP2	Non-raft
Secretory carrier-associated membrane protein 3	SCAMP3	Both
Secretory carrier-associated membrane protein 4	SCAMP4	Non-raft
Sn1-specific diacylglycerol lipase beta	DAGLB	Non-raft
SNAP-23	SNAP23	Both
Sodium/potassium-transporting ATPase subunit alpha-1	ATP1A1	Both
Stomatin	STOM	Both
Syntaxin-8	STX8	Both
Tetraspanin-9	TSPAN9	Non-raft
Trafficking protein particle complex subunit 3	TRAPPC3	Both
Transferrin receptor protein 1	TFRC	Both
Transmembrane protein 55B	TMEM55B	Both
Transmembrane protein 63A	TMEM63A	Non-raft
Transmembrane protein 63B	TMEM63B	Both
Type I inositol-1,4,5-trisphosphate 5-phosphatase	INPP5A	Raft
Tyrosine-protein kinase Lyn	LYN	Both
UPF0404 protein C11orf59	C11orf59	Both
Vesicle-associated membrane protein 3	VAMP3	Both
Vesicle-associated membrane protein 4	VAMP4	Non-raft
Vesicle-associated membrane protein 7	VAMP7	Non-raft

Known palmitoylated proteins identified in this study were assigned as raft, non-raft or present in both fractions based on a comparison with a previous study detailing the localization of palmitoylated proteins found in human prostate cancer cells (Yang *et al*, 2010).

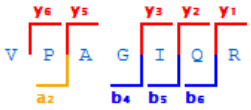
Figure S1. Manual verification of platelet palmitoyl protein candidates identified based on one unique peptide

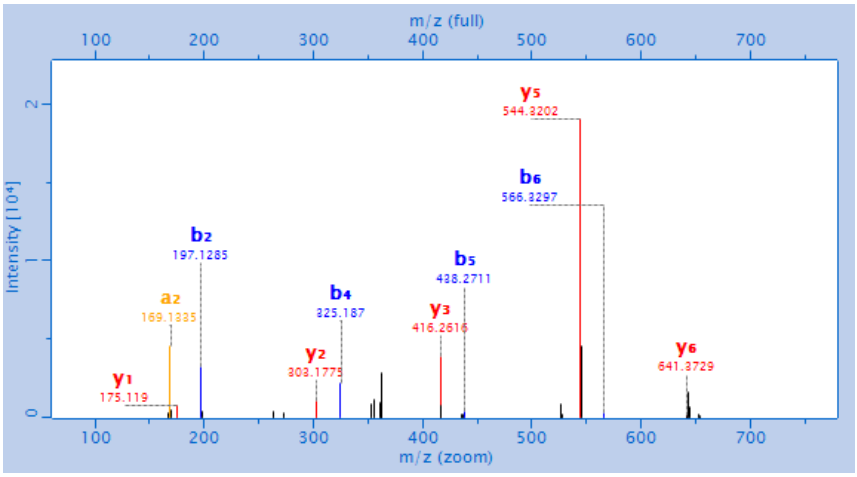
IPI00290452 Transmembrane BAX inhibitor motif-containing protein 1



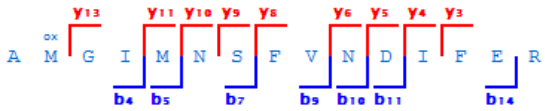


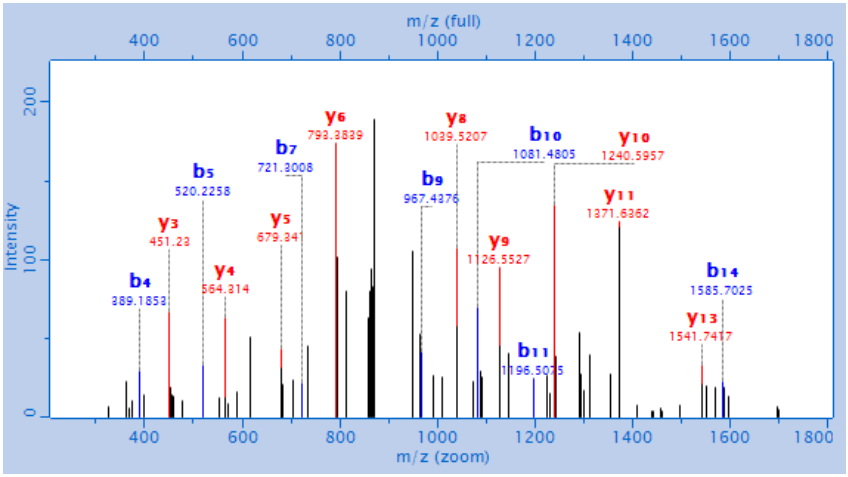
IPI00002127 Dynein heavy chain 1, axonemal



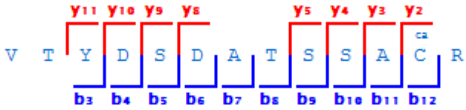


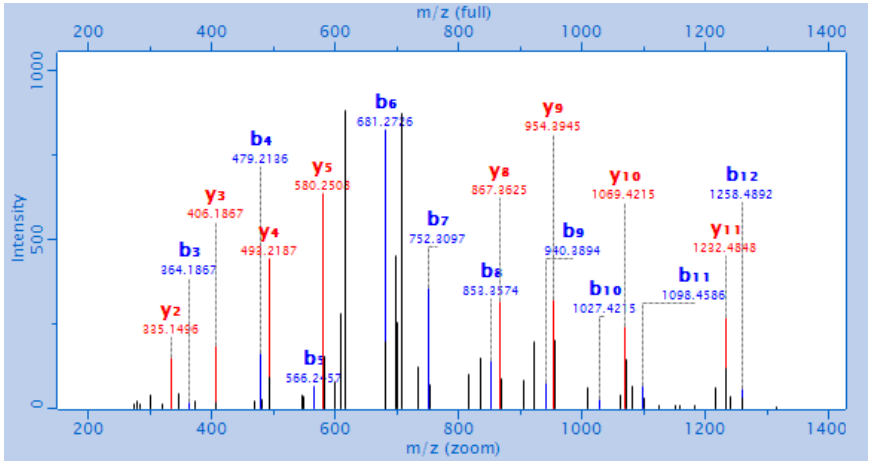
IPI00794461 Histone H2B type 1-N



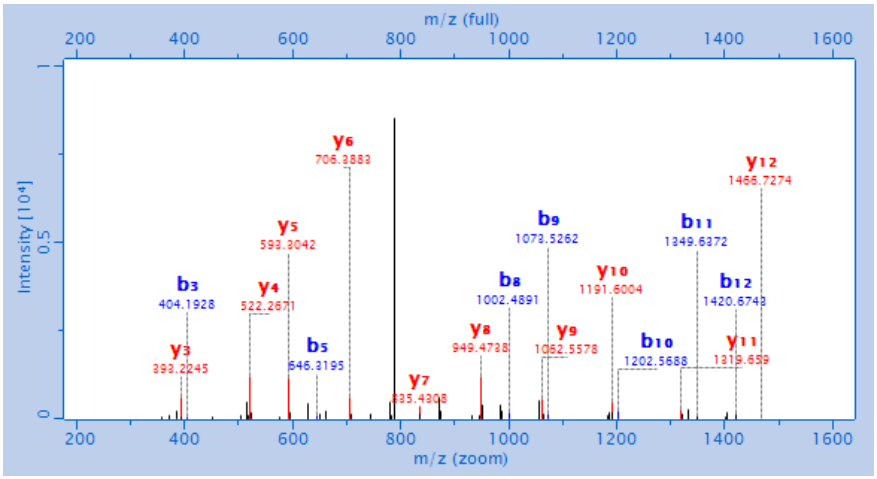


IPI00872769 Leptin receptor gene-related protein

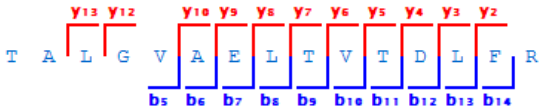


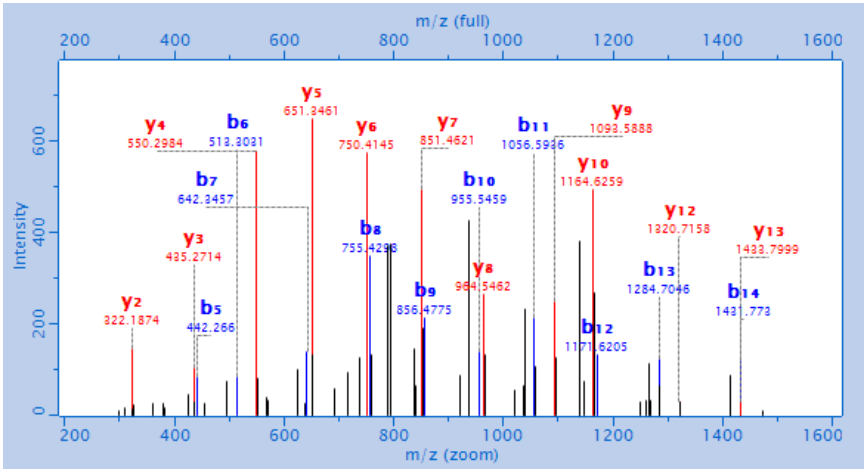


IPI00303699 Calcium release-activated calcium channel protein 1

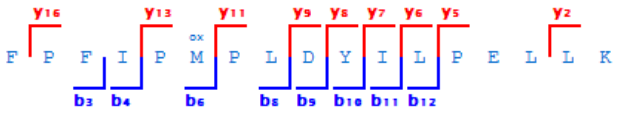


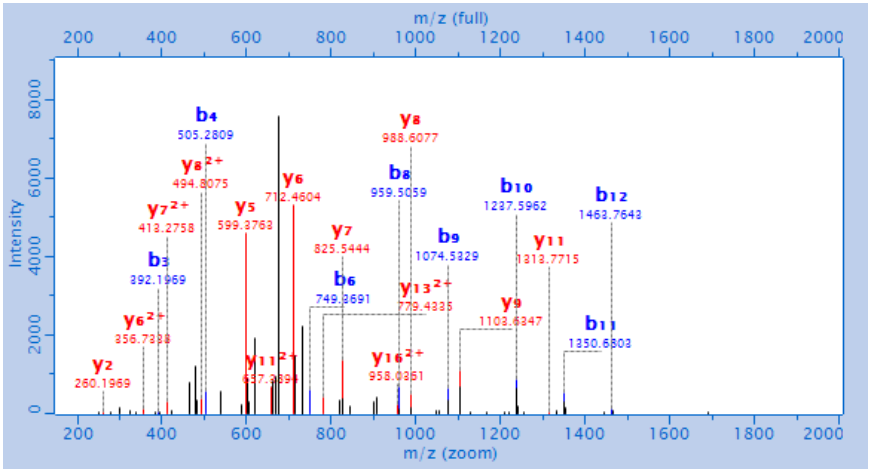
IPI00171626 Lysophosphatidylcholine acyltransferase 1



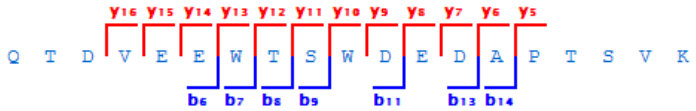


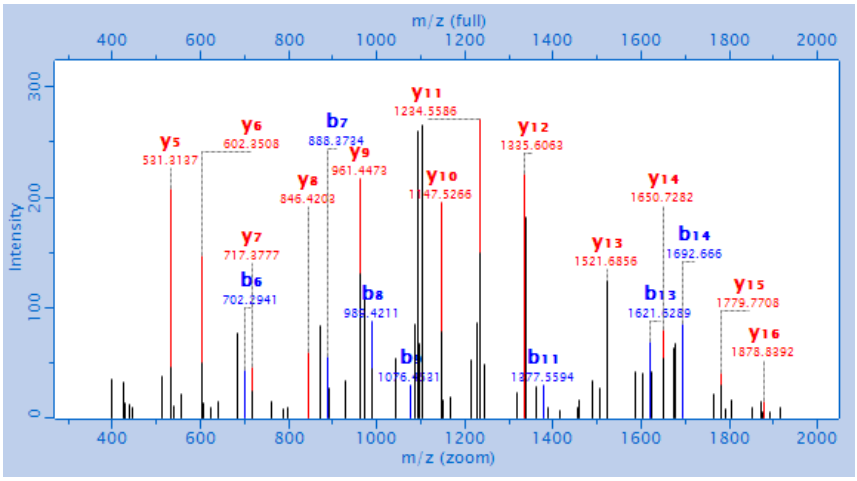
IPI00298612 3-methyl-2-oxobutanoate dehydrogenase (lipoamide) kinase, mitochondrial



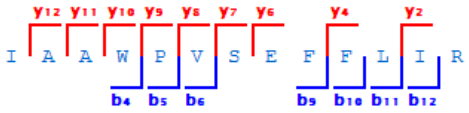


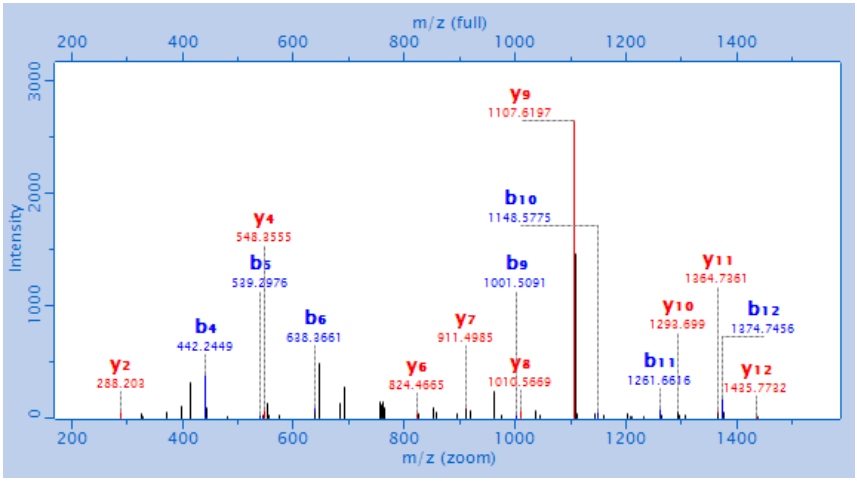
IPI00477518 Placenta derived apoptotic factor



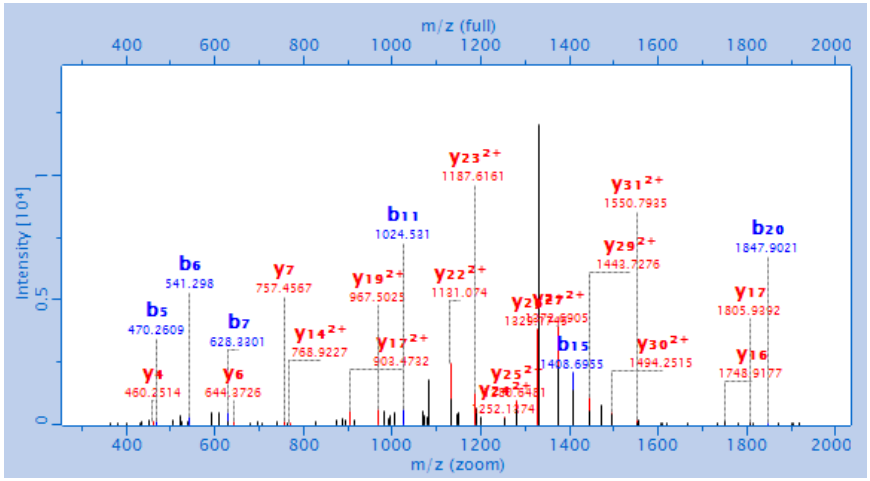


IPI00329410 Dolichyldiphosphatase 1

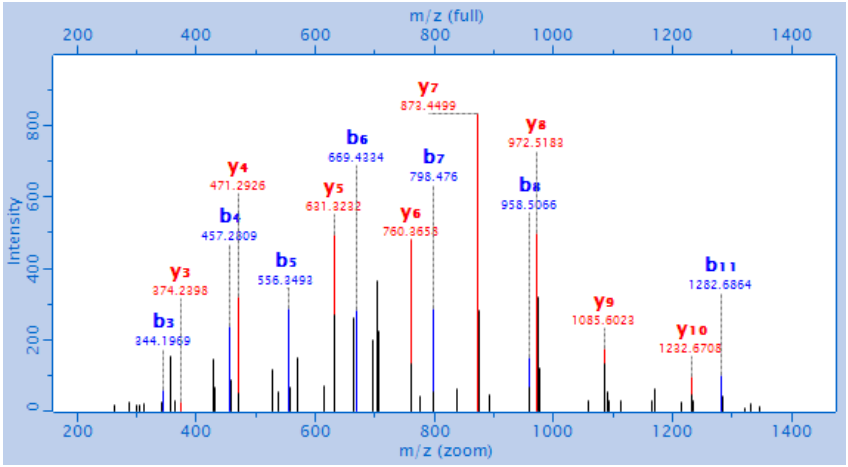




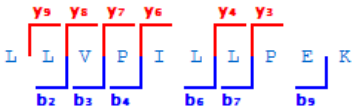
IPI00022471 Minor histocompatibility protein HA-1

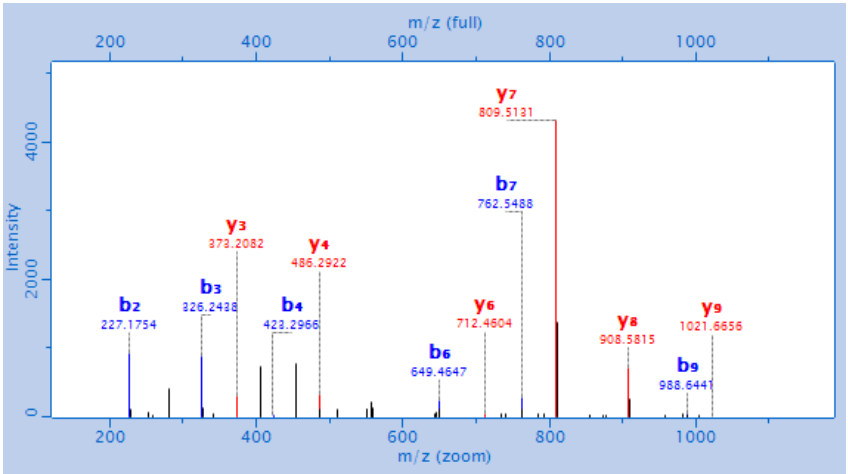


IPI00183603 Oligosaccharyltransferase complex subunit OSTC

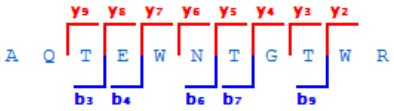


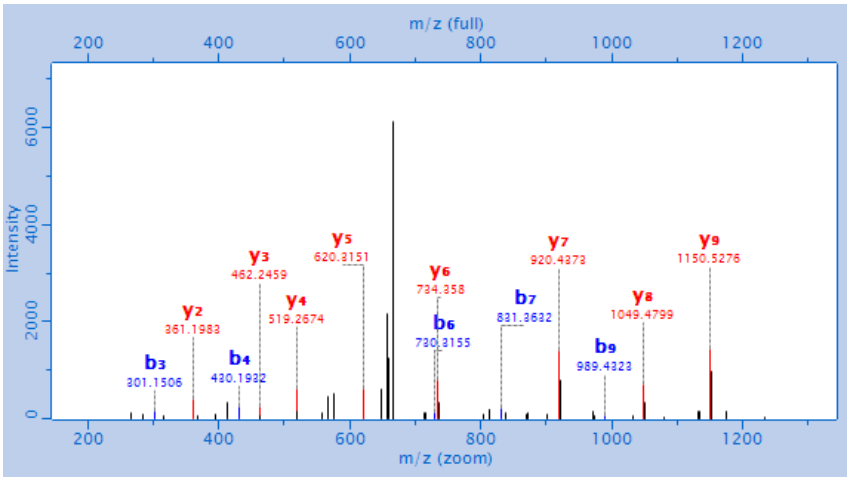
IPI00759799 HBeAg-binding protein 2 binding protein A



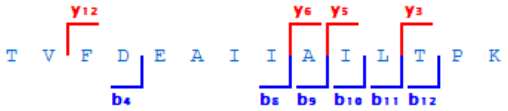


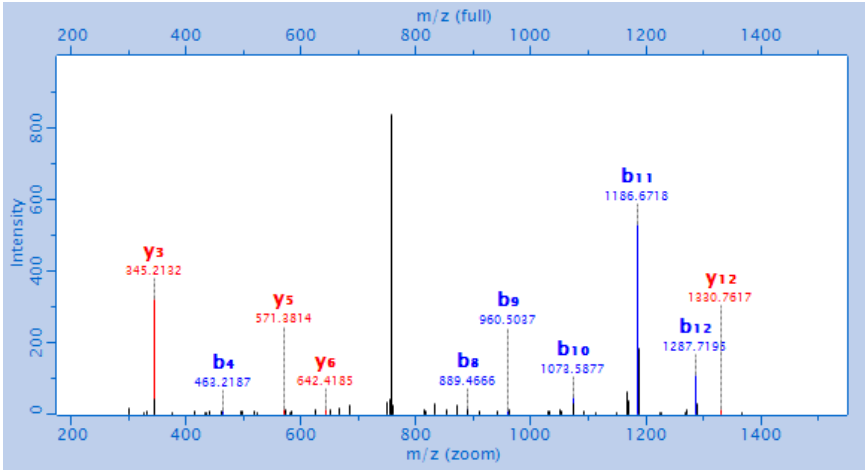
IPI00056310 Secretory carrier-associated membrane protein 4



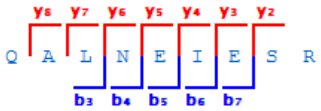


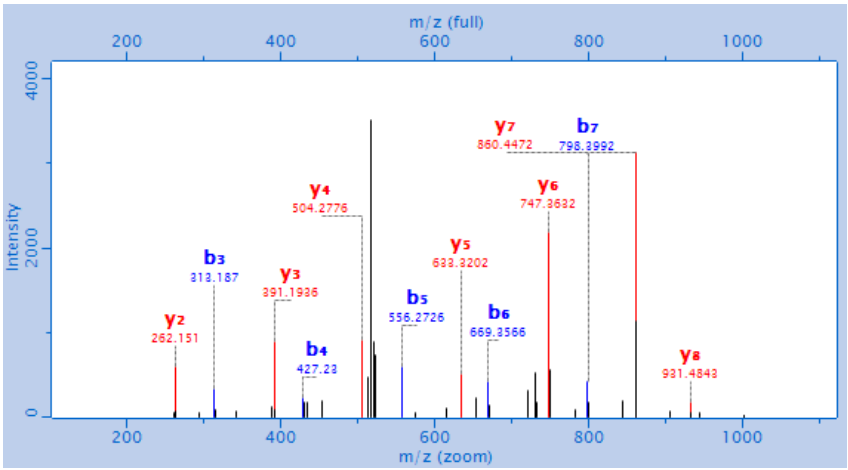
IPI00012511 Rho-related GTP-binding protein RhoQ



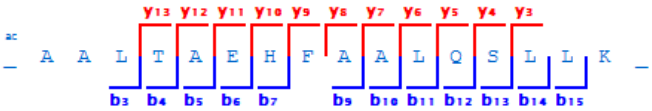


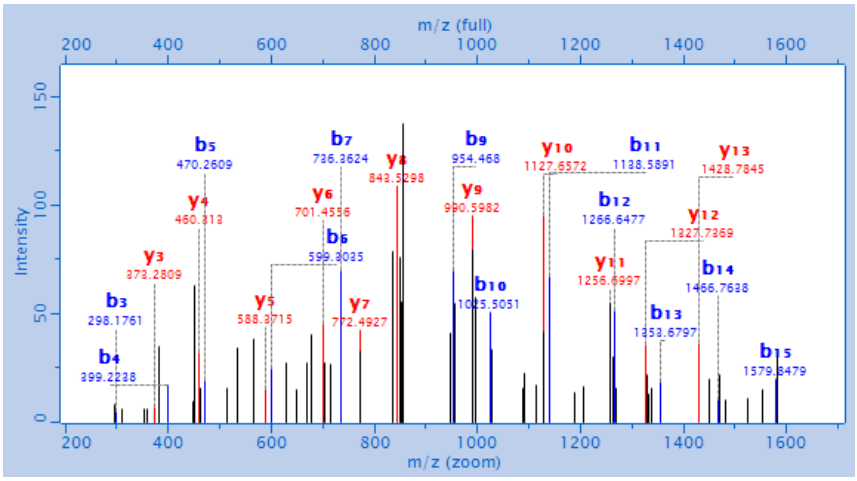
IPI00336034 Syntaxin-2



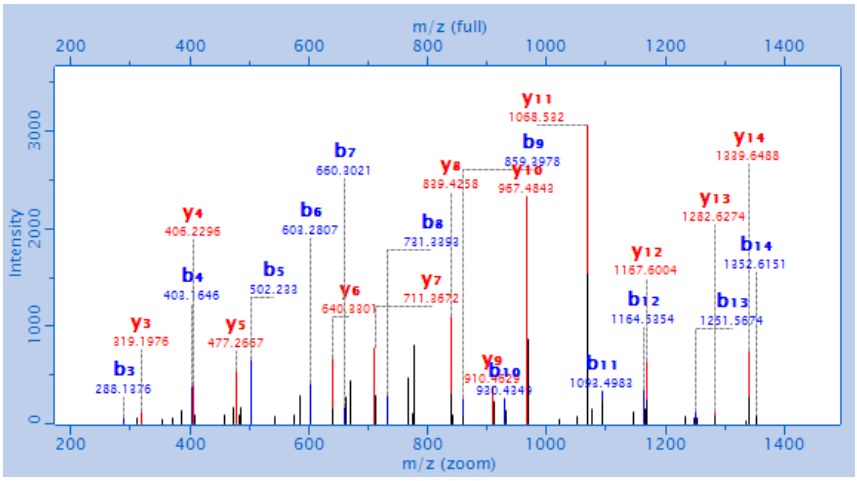


IPI00305212 COMM domain-containing protein 9

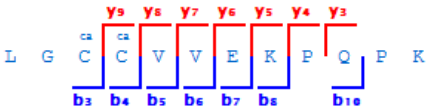


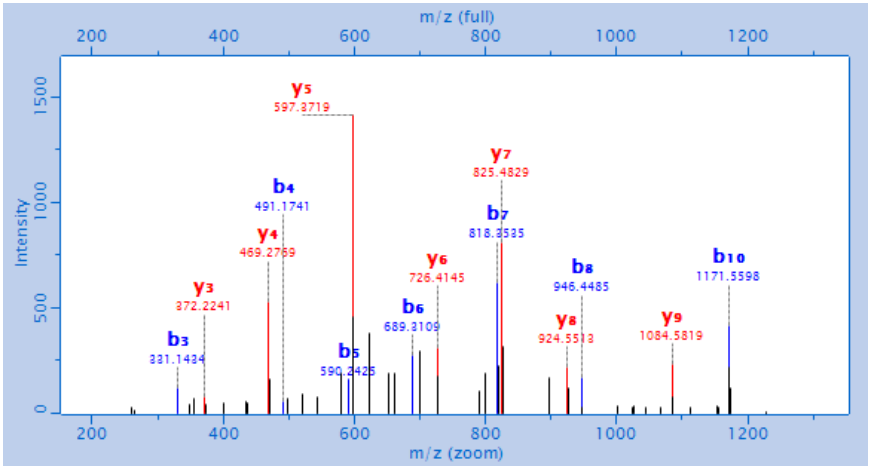


IPI00303726 Interferon-induced transmembrane protein 3

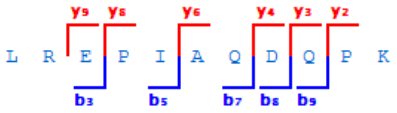


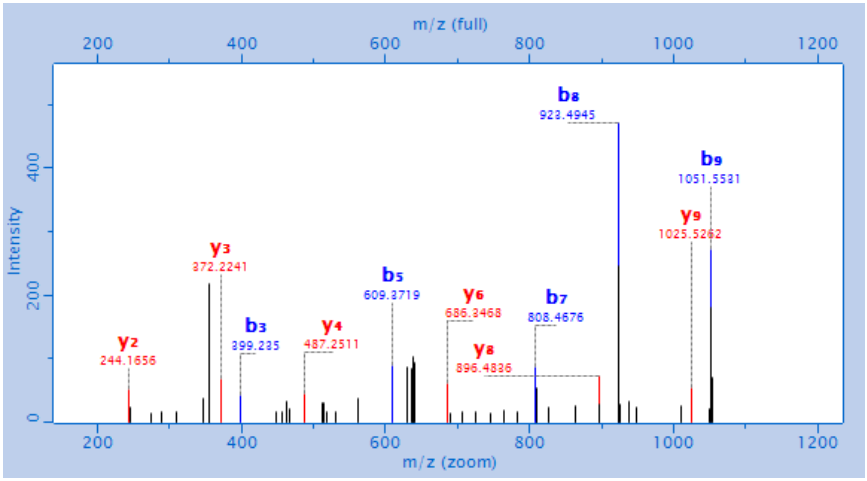
IPI00024972 CDC42 small effector protein 1



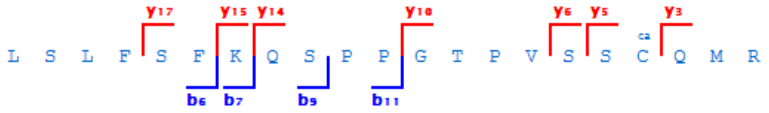


IPI00384349 Protein FAM78A





IPI00018033 Nuclear receptor subfamily 4 group A member 2



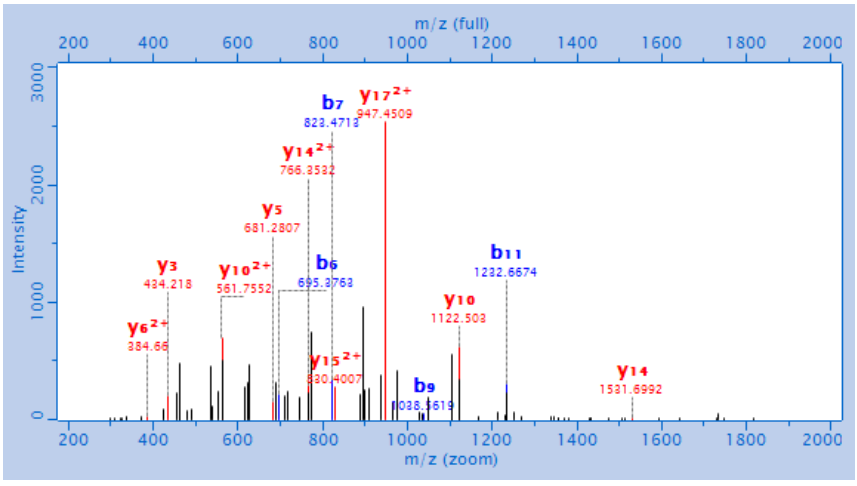


Figure S2. Western blot analysis of TLT-1 in sucrose density gradient fractions in resting and SFLLRN-activated platelets