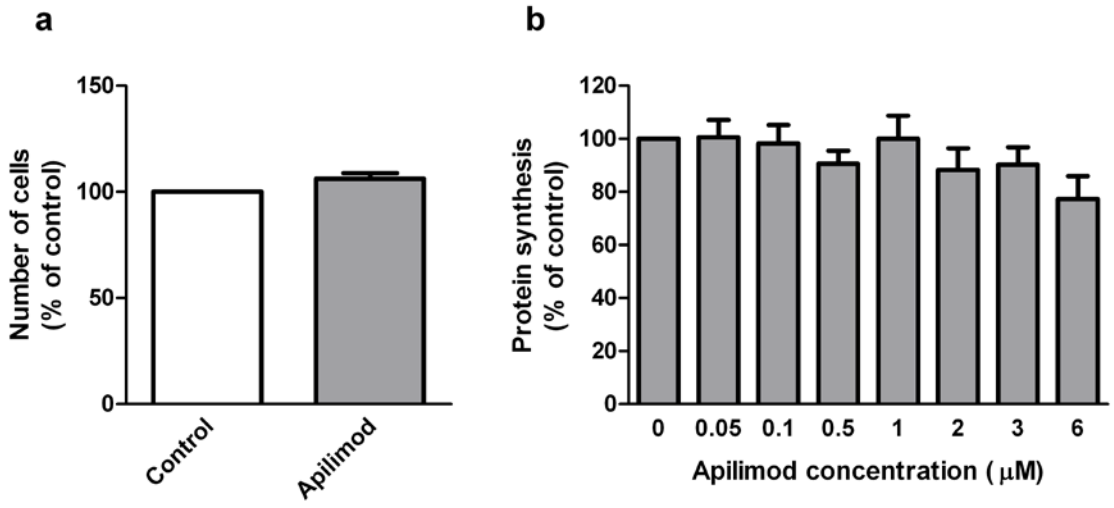
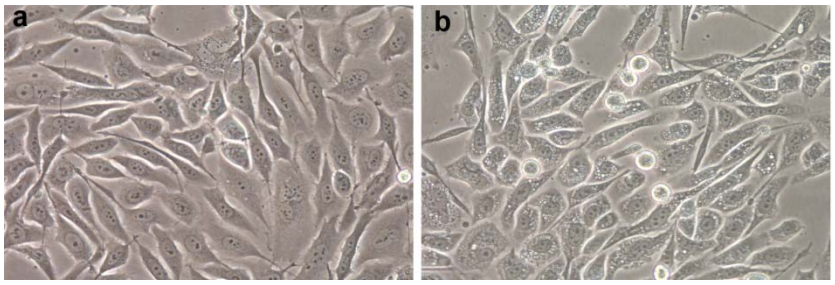
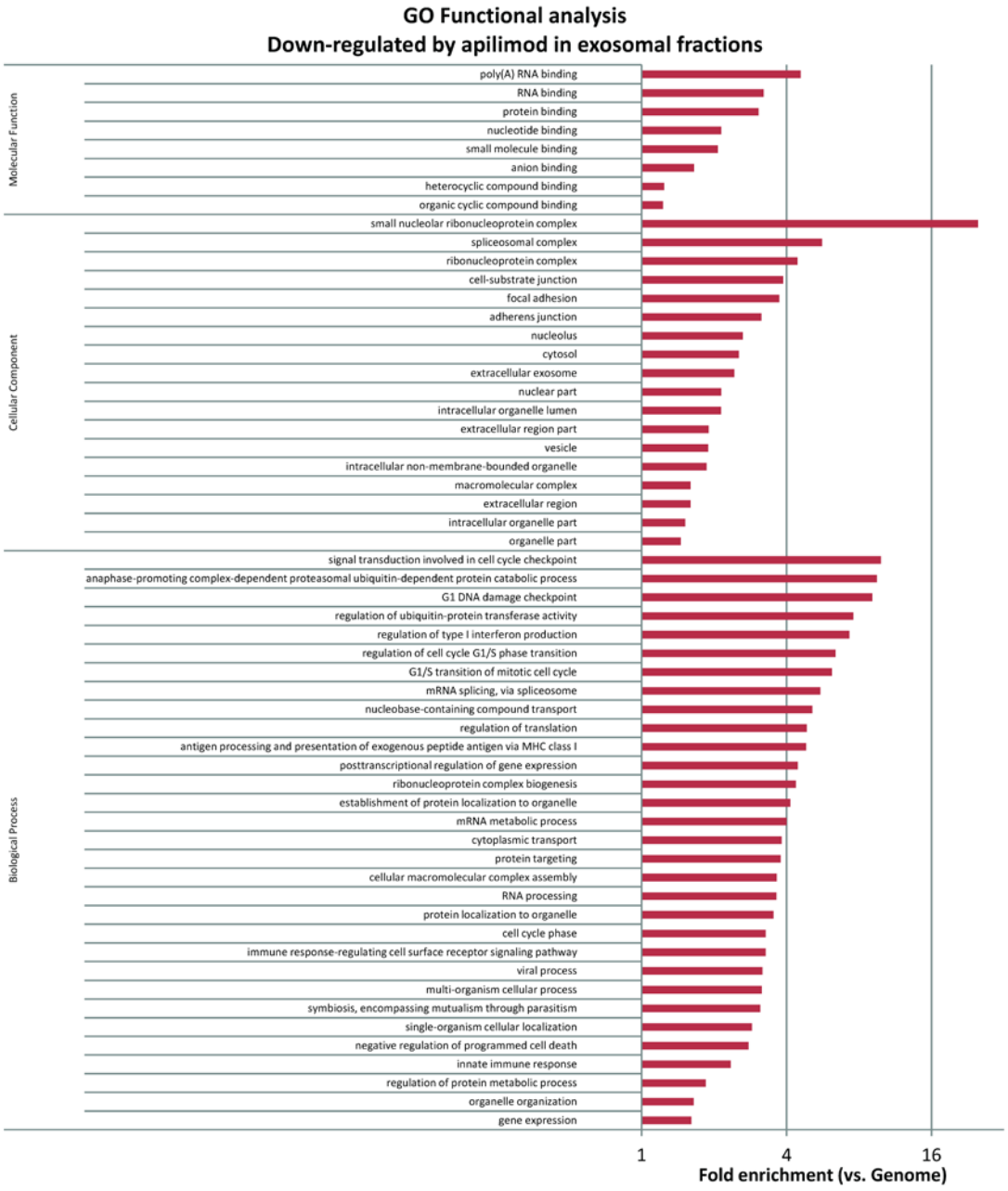
Supplementary Information



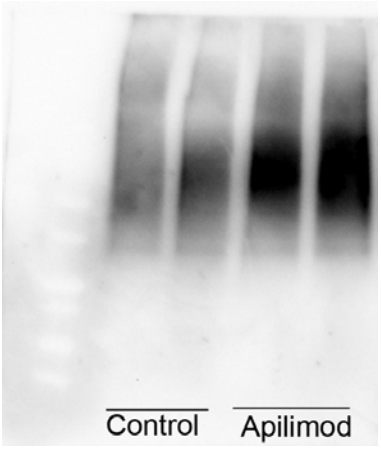
Supplementary Fig. 1 Apilimod treatment is well tolerated by PC-3 cells. **a** PC-3 cells were treated for 21 h with apilimod (0.5 μM) or control (0.1% DMSO) before the number of cells was counted. **b** PC-3 cells were treated for 21 h with different concentrations of apilimod before incorporation of [^{3}H]leucine into newly synthesized proteins was measured. The results are expressed as percentage of control and plotted as mean values + standard error of the mean, n=3



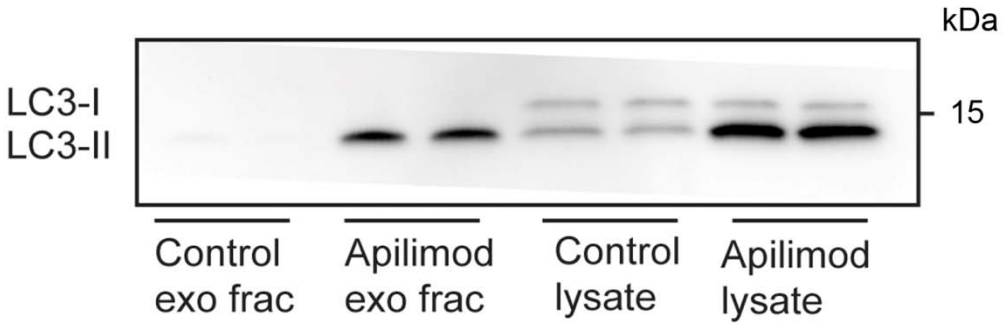
Supplementary Fig. 2 Induction of vacuoles, a phenotypic hallmark of PIKfyve inhibition was observed by light microscopy in PC-3 cells after PIKfyve inhibition. **a** Control cells and **b** cells treated with apilimod (0.5 μM).



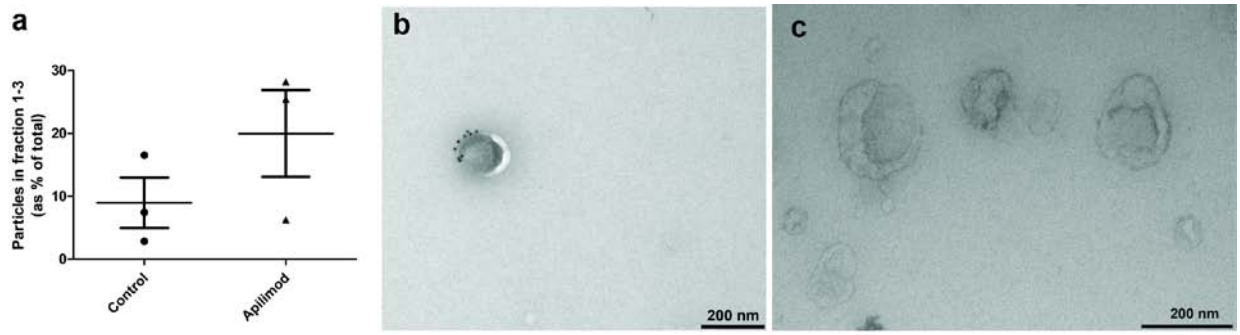
Supplementary Fig. 3 Pathways down-regulated in exosomal fractions after apilimod treatment. Functional analysis was performed on proteins in exosomal fractions that were significantly changed by apilimod. Analysis performed with DAVID-algorithm (http://david.abcc.ncifcrf.gov/) and fold-enrichment of GO-entries compared to genome



Supplementary Fig. 4 Ubiquitin is increased in exosomal fractions after apilimod treatment. Representative immunoblot showing ubiquitin in exosome preparations after apilimod and control treatment



Supplementary Fig. 5 Only the lipidated form of LC3 (LC3-II) is found in exosomal fractions. Representative immunoblot showing LC3 in exosomal fractions (exo frac) and lysates after apilimod and control treatment



Supplementary Fig. 6 Analysis of density gradient fractions by NTA and EM.

Exosomes were separated from p62-positive structures by OptiPrep density gradient (5-30%) centrifugation at 100,000 g for 18 h. **a** The fractions positive for p62, LC3-II and NBR1 (fraction 1-3) were combined and the fractions positive for caveolin-1, Tsg101 and Alix (fraction 4-9) were combined, before they were analyzed by NTA. **b** Immuno-EM on fraction 1-3 after apilimod treatment, labeled with p62. **c** Immuno-EM on fraction 4-9 after apilimod treatment, labeled with p62.

	Gene name	Uniprot Accession Number	T-test; api exo frac vs ctr exo frac	Ratio api exo frac vs ctr exo frac
Thymidine phosphorylase	TYMP	P19971	0.00	inf
Ferrochelatase, mitochondrial	FECH	P22830	0.00	inf
Cyclin-dependent kinase 2	CDK2	P24941	0.01	inf
Seizure 6-like protein 2	SEZ6L2	Q6UXD5	0.01	inf
E3 ubiquitin-protein ligase RNF13	RNF13	O43567	0.02	inf
WD repeat domain phosphoinositide-interacting protein 2	WIPI2	Q9Y4P8	0.05	inf
Sphingosine 1-phosphate receptor 2	S1PR2	O95136	0.02	68.91
Neurolysin, mitochondrial	NLN	Q9BYT8	0.01	62.30
Optineurin	OPTN	Q96CV9	0.19	43.58
Integrin beta-1-binding protein 1	ITGB1BP1	O14713	0.04	9.15
Endothelial cell-selective adhesion molecule	ESAM	Q96AP7	0.02	8.21
Histamine H1 receptor	HRH1	P35367	0.02	7.20
Probable G-protein coupled receptor 37	GPR37	O15354	0.03	4.81
CD82 antigen	CD82	P27701	0.03	4.52
Microtubule-associated proteins 1A/1B light chain 3B	MAP1LC3B	Q9GZQ8	0.01	4.31
Sequestosome-1	SQSTM1	Q13501	0.13	3.62
Ferritin light chain	FTL	P02792	0.04	3.58
Next to BRCA1 gene 1 protein	NBR1	Q14596	0.20	3.49
Tetraspanin-8	TSPAN8	P19075	0.00	3.39
Calcium-binding and coiled-coil domain-containing protein 2	CALCOCO2	Q13137	0.16	3.19
Brain protein I3	BRI3	O95415	0.02	3.04
Protein spinster homolog 1	SPNS1	Q9H2V7	0.01	2.99
Dystrobrevin alpha	DTNA	Q9Y4J8	0.03	2.84
Solute carrier family 52, riboflavin transporter, member 2	SLC52A2	Q9HAB3	0.02	2.74
Secretory carrier-associated membrane protein 2	SCAMP2	O15127	0.02	2.68
Gamma-aminobutyric acid receptor-associated protein-like 2	GABARAPL2	P60520	0.09	2.62
Large neutral amino acids transporter small subunit 3	SLC43A1	O75387	0.02	2.54
Ragulator complex protein LAMTOR1	LAMTOR1	Q6IAA8	0.01	2.49
Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	GNB1	P62873	0.02	2.48
Disintegrin and metalloproteinase domain-containing protein 10	ADAM10	O14672	0.04	2.41
Guanine nucleotide-binding protein subunit beta-4	GNB4	Q9HAV0	0.04	2.41
Phosphatase and actin regulator 4	PHACTR4	Q8IZ21	0.05	2.26
Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	GNB2	P62879	0.02	2.21
Actin, alpha cardiac muscle 1	ACTC1	P68032	0.03	2.19
Myeloid-associated differentiation marker	MYADM	Q96S97	0.04	2.10

1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-1	PLCG1	P19174	0.01	2.08
Somatostatin receptor type 5	SSTR5	P35346	0.04	2.04
L-lactate dehydrogenase A chain	LDHA	P00338	0.03	2.02
Solute carrier family 2, facilitated glucose transporter member 1	SLC2A1	P11166	0.03	2.01
GTPase KRas	KRAS	P01116	0.02	1.99
Vesicle-associated membrane protein 3	VAMP3	Q15836	0.05	1.94
MOB kinase activator 1B	MOB1B	Q7L9L4	0.03	1.93
Tyrosine-protein kinase JAK1	JAK1	P23458	0.02	1.86
Antithrombin-III	SERPINC1	P01008	0.04	1.81
Roundabout homolog 1	ROBO1	Q9Y6N7	0.03	1.80
Tyrosine-protein phosphatase non-receptor type substrate 1	SIRPA	P78324	0.03	1.72
E3 ubiquitin-protein ligase RNF167	RNF167	Q9H6Y7	0.00	1.66
Acid sphingomyelinase-like phosphodiesterase 3b	SMPDL3B	Q92485	0.01	1.64
Toll-interacting protein	TOLLIP	Q9H0E2	0.01	1.63
Integrin alpha-1	ITGA1	P56199	0.02	1.63
Serine incorporator 3	SERINC3	Q13530	0.03	1.60
Peripheral plasma membrane protein CASK	CASK	O14936	0.03	1.59
GTPase HRas	HRAS	P01112	0.01	1.57
Retinoic acid-induced protein 3	GPRC5A	Q8NFJ5	0.01	1.54
Neuroplastin	NPTN	Q9Y639	0.01	1.53
DnaJ homolog subfamily A member 1	DNAJA1	P31689	0.01	1.52
Ferritin heavy chain	FTH1	P02794	0.04	1.49
Syntaxin-binding protein 3	STXBP3	O00186	0.02	1.48
Band 4.1-like protein 3	EPB41L3	Q9Y2J2	0.05	1.47
Solute carrier organic anion transporter family member 4A1	SLCO4A1	Q96BD0	0.02	1.46
Anion exchange protein 2	SLC4A2	P04920	0.02	1.45
Equilibrative nucleoside transporter 1	SLC29A1	Q99808	0.03	1.43
Pannexin-1	PANX1	Q96RD7	0.01	1.42
Protein numb homolog	NUMB	P49757	0.02	1.41
Lysosomal-associated transmembrane protein 4A	LAPTM4A	Q15012	0.05	1.41
Cytochrome c	CYCS	P99999	0.03	1.40
Casein kinase I isoform gamma-3	CSNK1G3	Q9Y6M4	0.05	1.39
Lysosome membrane protein 2	SCARB2	Q14108	0.02	1.27
Proteasome subunit alpha type-6	PSMA6	P60900	0.01	1.23
Guanine nucleotide-binding protein G(i) subunit alpha-2	GNAI2	P04899	0.05	1.23
Na(+)/H(+) exchange regulatory cofactor NHE-RF1	SLC9A3R1	O14745	0.01	1.22
Vacuolar protein sorting-associated protein 28 homolog	VPS28	Q9UK41	0.00	1.16

Supplementary Table 1 Significantly up-regulated proteins in exosomal fractions after apilimod treatment. Ratio api exo frac vs ctr exo frac; calculated as average TOP3TIC for exosomal fraction (exo frac) after apilimod (api) divided by average TOP3TIC for exosomal fraction after

control (ctr) treatment. inf; infinite ratio (not found in control exosomal fractions). All protein reference data are from www.uniprot.org

	Gene Name	Uniprot Accession Number	T-test; api exo frac vs ctr exo frac	Ratio api exo frac vs ctr exo frac
28S ribosomal protein S34, mitochondrial	MRPS34	P82930	**0.000**	0.00
SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5	SMARCA5	O60264	**0.000**	0.00
Serine/arginine-rich splicing factor 5	SRSF5	Q13243	**0.000**	0.00
Protein FAM195A	FAM195A	Q9BUT9	**0.001**	0.00
Ribose-5-phosphate isomerase	RPIA	P49247	**0.001**	0.00
Epsin-2	EPN2	O95208	**0.002**	0.00
Nuclear pore complex protein Nup98-Nup96	NUP98	P52948	**0.003**	0.00
WD repeat-containing protein 92	WDR92	Q96MX6	**0.003**	0.00
ATP synthase subunit gamma, mitochondrial	ATP5C1	P36542	**0.003**	0.00
Vacuolar protein-sorting-associated protein 36	VPS36	Q86VN1	**0.003**	0.00
Survival motor neuron protein	SMN1	Q16637	**0.005**	0.00
Golgin subfamily A member 3	GOLGA3	Q08378	**0.005**	0.00
E3 ubiquitin-protein ligase synoviolin	SYVN1	Q86TM6	**0.006**	0.00
Ankyrin repeat domain-containing protein 40	ANKRD40	Q6AI12	**0.009**	0.00
Beta-arrestin-2	ARRB2	P32121	**0.009**	0.00
Mitochondrial import inner membrane translocase subunit Tim8 B	TIMM8B	Q9Y5J9	**0.010**	0.00
Heat shock protein 75 kDa, mitochondrial	TRAP1	Q12931	**0.011**	0.00
Condensin complex subunit 1	NCAPD2	Q15021	**0.011**	0.00
Caspase-2	CASP2	P42575	**0.012**	0.00
Cytoplasmic dynein 1 light intermediate chain 2	DYNC1LI2	O43237	**0.013**	0.00
Mitochondrial import inner membrane translocase subunit Tim10	TIMM10	P62072	**0.015**	0.00
PHD finger-like domain-containing protein 5A	PHF5A	Q7RTV0	**0.016**	0.00
TBC1 domain family member 23	TBC1D23	Q9NUY8	**0.018**	0.00
Nexilin	NEXN	Q0ZGT2	**0.018**	0.00
Chromatin target of PRMT1 protein	CHTOP	Q9Y3Y2	**0.018**	0.00
NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial	NDUFV1	P49821	**0.018**	0.00
UPF0488 protein C8orf33	C8orf33	Q9H7E9	**0.019**	0.00
NCK-interacting protein with SH3 domain	NCKIPSD	Q9NZQ3	**0.024**	0.00
Transcriptional repressor CTCF	CTCF	P49711	**0.029**	0.00
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 7	NDUFA7	O95182	**0.030**	0.00
28S ribosomal protein S2, mitochondrial	MRPS2	Q9Y399	**0.030**	0.00
ATP synthase subunit g, mitochondrial	ATP5L	O75964	**0.034**	0.00

SURP and G-patch domain-containing protein 1	SUGP1	Q8IWZ8	**0.035**	0.00
Proteasome activator complex subunit 3	PSME3	P61289	**0.035**	0.00
Peptidyl-prolyl cis-trans isomerase G	PPIG	Q13427	**0.036**	0.00
N-acylneuraminate cytidylyltransferase	CMAS	Q8NFW8	**0.039**	0.00
Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	ACADVL	P49748	**0.040**	0.00
ATP synthase subunit d, mitochondrial	ATP5H	O75947	**0.047**	0.00
PERQ amino acid-rich with GYF domain-containing protein 2	GIGYF2	Q6Y7W6	**0.028**	0.020
Nuclear pore complex protein Nup205	NUP205	Q92621	**0.004**	0.020
Pre-mRNA-splicing regulator WTAP	WTAP	Q15007	**0.042**	0.038
Nuclear pore complex protein Nup153	NUP153	P49790	**0.020**	0.039
Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial	CHCHD3	Q9NX63	**0.024**	0.041
Structural maintenance of chromosomes flexible hinge domain-containing protein 1	SMCHD1	A6NHR9	**0.033**	0.051
SAP domain-containing ribonucleoprotein	SARNP	P82979	**0.021**	0.053
DNA fragmentation factor subunit alpha	DFFA	O00273	**0.027**	0.053
Hepatoma-derived growth factor-related protein 2	HDGFRP2	Q7Z4V5	**0.009**	0.060
Pre-mRNA-splicing factor SPF27	BCAS2	O75934	**0.022**	0.061
Gephyrin	GPHN	Q9NQX3	**0.002**	0.064
DNA replication licensing factor MCM2	MCM2	P49736	**0.022**	0.067
E3 ubiquitin-protein ligase KCMF1	KCMF1	Q9P0J7	**0.005**	0.074
UDP-glucose 4-epimerase	GALE	Q14376	**0.025**	0.077
15-hydroxyprostaglandin dehydrogenase [NAD(+)]	HPGD	P15428	**0.019**	0.079
Serine/arginine repetitive matrix protein 2	SRRM2	Q9UQ35	**0.027**	0.081
Calcium/calmodulin-dependent protein kinase type II subunit delta	CAMK2D	Q13557	**0.027**	0.082
Trinucleotide repeat-containing gene 6B protein	TNRC6B	Q9UPQ9	**0.005**	0.084
Ubiquitin-conjugating enzyme E2 A	UBE2A	P49459	**0.050**	0.084
PHD finger protein 6	PHF6	Q8IWS0	**0.036**	0.086
Activity-dependent neuroprotector homeobox protein	ADNP	Q9H2P0	**0.014**	0.090
Nucleoredoxin	NXN	Q6DKJ4	**0.001**	0.099
HIG1 domain family member 1A, mitochondrial	HIGD1A	Q9Y241	**0.018**	0.099
5'-3' exoribonuclease 2	XRN2	Q9H0D6	**0.046**	0.102
Nuclear receptor coactivator 5	NCOA5	Q9HCD5	**0.041**	0.105
Mycophenolic acid acyl-glucuronide esterase, mitochondrial	ABHD10	Q9NUJ1	**0.038**	0.105
3-ketoacyl-CoA thiolase, mitochondrial	ACAA2	P42765	**0.002**	0.106
Isocitrate dehydrogenase [NADP], mitochondrial	IDH2	P48735	**0.006**	0.107
Centrosomal protein of 170 kDa	CEP170	Q5SW79	**0.019**	0.111
MMS19 nucleotide excision repair protein homolog	MMS19	Q96T76	**0.019**	0.111
Mitochondrial import inner membrane translocase subunit Tim8 A	TIMM8A	O60220	**0.003**	0.114
Histone deacetylase 2	HDAC2	Q92769	**0.018**	0.121
DBIRD complex subunit ZNF326	ZNF326	Q5BKZ1	**0.032**	0.122
Leucine zipper protein 1	LUZP1	Q86V48	**0.005**	0.124

Laminin subunit beta-2	LAMB2	P55268	**0.041**	0.125
Protein PRRC2A	PRRC2A	P48634	**0.004**	0.128
Protein max	MAX	P61244	**0.036**	0.129
Citrate synthase, mitochondrial	CS	O75390	**0.029**	0.134
Telomere length regulation protein TEL2 homolog	TELO2	Q9Y4R8	**0.004**	0.140
Exportin-5	XPO5	Q9HAV4	**0.050**	0.142
Zinc finger protein 281	ZNF281	Q9Y2X9	**0.012**	0.147
PC4 and SFRS1-interacting protein	PSIP1	O75475	**0.008**	0.151
U6 snRNA-associated Sm-like protein LSm4	LSM4	Q9Y4Z0	**0.038**	0.154
Epididymal secretory protein E1	NPC2	P61916	**0.000**	0.166
PCI domain-containing protein 2	PCID2	Q5JVF3	**0.024**	0.168
Calponin-3	CNN3	Q15417	**0.003**	0.169
Nuclear factor 1 X-type	NFIX	Q14938	**0.035**	0.173
Lamina-associated polypeptide 2, isoforms beta/gamma	TMPO	P42167	**0.034**	0.175
H/ACA ribonucleoprotein complex subunit 2	NHP2	Q9NX24	**0.033**	0.176
Sphingolipid delta(4)-desaturase DES1	DEGS1	O15121	**0.023**	0.178
Cleavage and polyadenylation specificity factor subunit 5	NUDT21	O43809	**0.003**	0.179
General transcription factor IIF subunit 1	GTF2F1	P35269	**0.044**	0.183
Cytochrome b-c1 complex subunit 6, mitochondrial	UQCRH	P07919	**0.019**	0.193
Dual specificity mitogen-activated protein kinase kinase 3	MAP2K3	P46734	**0.039**	0.194
AN1-type zinc finger protein 1	ZFAND1	Q8TCF1	**0.013**	0.196
Squamous cell carcinoma antigen recognized by T-cells 3	SART3	Q15020	**0.001**	0.196
Splicing factor, arginine/serine-rich 15	SCAF4	O95104	**0.016**	0.198
Hexokinase-2	HK2	P52789	**0.044**	0.201
Protein CYR61	CYR61	O00622	**0.029**	0.201
Golgin subfamily A member 4	GOLGA4	Q13439	**0.050**	0.209
Chromobox protein homolog 8	CBX8	Q9HC52	**0.019**	0.211
Zinc finger protein 787	ZNF787	Q6DD87	**0.014**	0.212
LIM domain and actin-binding protein 1	LIMA1	Q9UHB6	**0.044**	0.215
Inosine triphosphate pyrophosphatase	ITPA	Q9BY32	**0.002**	0.216
Macrophage migration inhibitory factor	MIF	P14174	**0.003**	0.216
Reticulocalbin-1	RCN1	Q15293	**0.043**	0.218
Cell differentiation protein RCD1 homolog	RQCD1	Q92600	**0.047**	0.219
Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	SDHB	P21912	**0.050**	0.220
Malectin	MLEC	Q14165	**0.023**	0.222
Acylpyruvase FAHD1, mitochondrial	FAHD1	Q6P587	**0.004**	0.222
Zinc finger CCCH domain-containing protein 15	ZC3H15	Q8WU90	**0.017**	0.226
Early endosome antigen 1	EEA1	Q15075	**0.021**	0.230
Eukaryotic translation initiation factor 3 subunit E	EIF3E	P60228	**0.009**	0.235
Opioid growth factor receptor	OGFR	Q9NZT2	**0.019**	0.235
Succinyl-CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial	SUCLG1	P53597	**0.004**	0.235
Transcription elongation factor SPT5	SUPT5H	O00267	**0.009**	0.240

Signal recognition particle 9 kDa protein	SRP9	P49458	**0.020**	0.246
C-Myc-binding protein	MYCBP	Q99417	**0.000**	0.254
Transcription factor p65	RELA	Q04206	**0.045**	0.258
LIM domain-containing protein 1	LIMD1	Q9UGP4	**0.049**	0.260
Double-stranded RNA-specific adenosine deaminase	ADAR	P55265	**0.005**	0.261
SUMO-activating enzyme subunit 2	UBA2	Q9UBT2	**0.004**	0.261
Cytoplasmic dynein 1 light intermediate chain 1	DYNC1LI1	Q9Y6G9	**0.002**	0.267
60S ribosomal export protein NMD3	NMD3	Q96D46	**0.015**	0.268
YTH domain family protein 2	YTHDF2	Q9Y5A9	**0.014**	0.269
Zinc finger RNA-binding protein	ZFR	Q96KR1	**0.040**	0.270
ADP/ATP translocase 3	SLC25A6	P12236	**0.029**	0.270
Replication protein A 14 kDa subunit	RPA3	P35244	**0.002**	0.272
Glutathione S-transferase kappa 1	GSTK1	Q9Y2Q3	**0.036**	0.272
Apolipoprotein O	APOO	Q9BUR5	**0.041**	0.276
Eukaryotic translation initiation factor 3 subunit M	EIF3M	Q7L2H7	**0.024**	0.276
ATP-dependent RNA helicase DDX50	DDX50	Q9BQ39	**0.024**	0.277
FACT complex subunit SSRP1	SSRP1	Q08945	**0.009**	0.278
TAR DNA-binding protein 43	TARDBP	Q13148	**0.019**	0.282
Transmembrane protein 205	TMEM205	Q6UW68	**0.028**	0.284
Thyroid receptor-interacting protein 6	TRIP6	Q15654	**0.003**	0.285
Cleavage stimulation factor subunit 2 tau variant	CSTF2T	Q9H0L4	**0.019**	0.288
Ubiquitin-protein ligase E3C	UBE3C	Q15386	**0.029**	0.288
PRKC apoptosis WT1 regulator protein	PAWR	Q96IZ0	**0.027**	0.289
Thioredoxin domain-containing protein 12	TXNDC12	O95881	**0.007**	0.301
Single-stranded DNA-binding protein, mitochondrial	SSBP1	Q04837	**0.032**	0.307
Electron transfer flavoprotein subunit alpha, mitochondrial	ETFA	P13804	**0.024**	0.309
Angio-associated migratory cell protein	AAMP	Q13685	**0.026**	0.311
BAG family molecular chaperone regulator 2	BAG2	O95816	**0.014**	0.313
Pantothenate kinase 4	PANK4	Q9NVE7	**0.048**	0.319
Nucleoprotein TPR	TPR	P12270	**0.005**	0.321
Serine hydroxymethyltransferase, mitochondrial	SHMT2	P34897	**0.046**	0.327
Splicing factor 1	SF1	Q15637	**0.006**	0.327
Vesicle-associated membrane protein-associated protein B/C	VAPB	O95292	**0.035**	0.327
Microtubule-associated protein 1B	MAP1B	P46821	**0.003**	0.328
Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	PDHA1	P08559	**0.040**	0.332
Ribonucleases P/MRP protein subunit POP1	POP1	Q99575	**0.041**	0.333
Protein FAM203A	HGH1	Q9BTY7	**0.031**	0.333
26S proteasome non-ATPase regulatory subunit 13	PSMD13	Q9UNM6	**0.035**	0.336
Ubiquitin conjugation factor E4 A	UBE4A	Q14139	**0.032**	0.337
ES1 protein homolog, mitochondrial	C21orf33	P30042	**0.022**	0.340
Lysophospholipid acyltransferase 7	MBOAT7	Q96N66	**0.020**	0.346
Serine/threonine-protein phosphatase 4 regulatory subunit 2	PPP4R2	Q9NY27	**0.020**	0.347

Protein mago nashi homolog 2	MAGOHB	Q96A72	**0.039**	0.347
Density-regulated protein	DENR	O43583	**0.034**	0.352
Anterior gradient protein 2 homolog	AGR2	O95994	**0.024**	0.353
DnaJ homolog subfamily C member 8	DNAJC8	O75937	**0.041**	0.356
Amyloid-like protein 2	APLP2	Q06481	**0.013**	0.357
Tubulin-folding cofactor B	TBCB	Q99426	**0.014**	0.362
THO complex subunit 4	ALYREF	Q86V81	**0.019**	0.362
Exportin-7	XPO7	Q9UIA9	**0.025**	0.365
Small nuclear ribonucleoprotein E	SNRPE	P62304	**0.011**	0.366
Dynamin-like 120 kDa protein, mitochondrial	OPA1	O60313	**0.029**	0.366
Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	RPN1	P04843	**0.005**	0.368
Splicing factor U2AF 35 kDa subunit	U2AF1	Q01081	**0.030**	0.370
Histidine triad nucleotide-binding protein 1	HINT1	P49773	**0.032**	0.370
26S proteasome non-ATPase regulatory subunit 14	PSMD14	O00487	**0.013**	0.376
Sorting nexin-1	SNX1	Q13596	**0.047**	0.383
Acyl-protein thioesterase 1	LYPLA1	O75608	**0.024**	0.385
Thioredoxin-related transmembrane protein 2	TMX2	Q9Y320	**0.035**	0.388
Leucine-rich PPR motif-containing protein, mitochondrial	LRPPRC	P42704	**0.038**	0.388
Heterogeneous nuclear ribonucleoprotein A0	HNRNPA0	Q13151	**0.017**	0.389
Alpha/beta hydrolase domain-containing protein 14B	ABHD14B	Q96IU4	**0.038**	0.391
Probable ATP-dependent RNA helicase DHX36	DHX36	Q9H2U1	**0.036**	0.395
Tubulin gamma-1 chain	TUBG1	P23258	**0.005**	0.397
Eukaryotic translation initiation factor 2 subunit 2	EIF2S2	P20042	**0.014**	0.398
Phenylalanine--tRNA ligase beta subunit	FARSB	Q9NSD9	**0.007**	0.398
Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	HADH	Q16836	**0.011**	0.402
Translational activator GCN1	GCN1L1	Q92616	**0.050**	0.403
Inositol monophosphatase 1	IMPA1	P29218	**0.003**	0.403
Hypoxia up-regulated protein 1	HYOU1	Q9Y4L1	**0.044**	0.405
Putative RNA-binding protein Luc7-like 2	LUC7L2	Q9Y383	**0.021**	0.410
Atlastin-3	ATL3	Q6DD88	**0.009**	0.413
N-alpha-acetyltransferase 15, NatA auxiliary subunit	NAA15	Q9BXJ9	**0.027**	0.418
Phosphate carrier protein, mitochondrial	SLC25A3	Q00325	**0.014**	0.418
N-alpha-acetyltransferase 50	NAA50	Q9GZZ1	**0.007**	0.422
BRISC and BRCA1-A complex member 1	BABAM1	Q9NWV8	**0.028**	0.422
CUGBP Elav-like family member 1	CELF1	Q92879	**0.008**	0.427
Uncharacterized protein C19orf43	C19orf43	Q9BQ61	**0.006**	0.429
Protein SET	SET	Q01105	**0.011**	0.429
Protein canopy homolog 2	CNPY2	Q9Y2B0	**0.013**	0.433
Serine/arginine-rich splicing factor 3	SRSF3	P84103	**0.032**	0.437
60S ribosomal protein L23	RPL23	P62829	**0.039**	0.439
PEST proteolytic signal-containing nuclear protein	PCNP	Q8WW12	**0.027**	0.441
Nucleolin	NCL	P19338	**0.040**	0.441

Non-POU domain-containing octamer-binding protein	NONO	Q15233	**0.019**	0.442
Septin-10	10-sep	Q9P0V9	**0.012**	0.444
Interferon-induced, double-stranded RNA-activated protein kinase	EIF2AK2	P19525	**0.041**	0.444
Transcription elongation factor A protein-like 3	TCEAL3	Q969E4	**0.000**	0.452
Tumor suppressor p53-binding protein 1	TP53BP1	Q12888	**0.021**	0.457
Minor histocompatibility antigen H13	HM13	Q8TCT9	**0.001**	0.457
Signal recognition particle 54 kDa protein	SRP54	P61011	**0.035**	0.463
DAZ-associated protein 1	DAZAP1	Q96EP5	**0.009**	0.463
DNA replication licensing factor MCM3	MCM3	P25205	**0.002**	0.467
DNA-dependent protein kinase catalytic subunit	PRKDC	P78527	**0.012**	0.467
Dihydropyrimidinase-related protein 2	DPYSL2	Q16555	**0.004**	0.469
Histone H1.3	HIST1H1D	P16402	**0.010**	0.474
DNA replication licensing factor MCM7	MCM7	P33993	**0.027**	0.476
NADH-cytochrome b5 reductase 3	CYB5R3	P00387	**0.037**	0.478
Heterogeneous nuclear ribonucleoprotein A/B	HNRNPAB	Q99729	**0.002**	0.483
YLP motif-containing protein 1	YLPM1	P49750	**0.011**	0.485
Rho-related GTP-binding protein RhoB	RHOB	P62745	**0.030**	0.485
High mobility group protein B3	HMGB3	O15347	**0.036**	0.488
Splicing factor 3B subunit 3	SF3B3	Q15393	**0.026**	0.493
UBX domain-containing protein 1	UBXN1	Q04323	**0.043**	0.493
Heterogeneous nuclear ribonucleoprotein A3	HNRNPA3	P51991	**0.031**	0.498
Serine--tRNA ligase, cytoplasmic	SARS	P49591	**0.004**	0.500
tRNA (cytosine(34)-C(5))-methyltransferase	NSUN2	Q08J23	**0.045**	0.500
Secernin-1	SCRN1	Q12765	**0.035**	0.505
Ras GTPase-activating protein-binding protein 1	G3BP1	Q13283	**0.014**	0.508
Heterogeneous nuclear ribonucleoprotein Q	SYNCRIP	O60506	**0.032**	0.513
Nucleolar protein 56	NOP56	O00567	**0.018**	0.518
Torsin-1A-interacting protein 2	TOR1AIP2	Q8NFQ8	**0.012**	0.521
Histone H1.4	HIST1H1E	P10412	**0.039**	0.524
26S proteasome non-ATPase regulatory subunit 7	PSMD7	P51665	**0.038**	0.524
Protein RCC2	RCC2	Q9P258	**0.013**	0.526
60 kDa heat shock protein, mitochondrial	HSPD1	P10809	**0.014**	0.529
Heterogeneous nuclear ribonucleoprotein D0	HNRNPD	Q14103	**0.007**	0.532
X-ray repair cross-complementing protein 5	XRCC5	P13010	**0.044**	0.535
Arf-GAP domain and FG repeat-containing protein 1	AGFG1	P52594	**0.046**	0.535
Apoptosis-inducing factor 1, mitochondrial	AIFM1	O95831	**0.000**	0.535
Pleiotropic regulator 1	PLRG1	O43660	**0.005**	0.535
3-hydroxyacyl-CoA dehydrogenase type-2	HSD17B10	Q99714	**0.036**	0.538
Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	ATP2A2	P16615	**0.034**	0.541
Heterogeneous nuclear ribonucleoprotein A1	HNRNPA1	P09651	**0.027**	0.549
GTP-binding protein 1	GTPBP1	O00178	**0.003**	0.549
40S ribosomal protein SA	RPSA	P08865	**0.035**	0.565

Far upstream element-binding protein 2	KHSRP	Q92945	**0.033**	0.565
60S ribosomal protein L5	RPL5	P46777	**0.030**	0.565
Translation initiation factor eIF-2B subunit gamma	EIF2B3	Q9NR50	**0.032**	0.568
Polyadenylate-binding protein 4	PABPC4	Q13310	**0.039**	0.568
Vimentin	VIM	P08670	**0.050**	0.571
Ribosomal RNA-processing protein 8	RRP8	O43159	**0.019**	0.575
Probable ATP-dependent RNA helicase DDX17	DDX17	Q92841	**0.040**	0.581
Arginine--tRNA ligase, cytoplasmic	RARS	P54136	**0.025**	0.585
Dihydrolipoyl dehydrogenase, mitochondrial	DLD	P09622	**0.039**	0.588
Heterogeneous nuclear ribonucleoprotein D-like	HNRNPDL	O14979	**0.016**	0.588
Ribosomal protein S6 kinase alpha-3	RPS6KA3	P51812	**0.035**	0.592
Cytoskeleton-associated protein 5	CKAP5	Q14008	**0.039**	0.592
Eukaryotic translation initiation factor 2 subunit 1	EIF2S1	P05198	**0.008**	0.592
60S ribosomal protein L10a	RPL10A	P62906	**0.025**	0.595
Phosphoribosylformylglycinamidine synthase	PFAS	O15067	**0.026**	0.595
Transformer-2 protein homolog beta	TRA2B	P62995	**0.026**	0.599
Stress-70 protein, mitochondrial	HSPA9	P38646	**0.025**	0.610
60S ribosomal protein L8	RPL8	P62917	**0.030**	0.613
Poly [ADP-ribose] polymerase 1	PARP1	P09874	**0.008**	0.613
Aspartate--tRNA ligase, cytoplasmic	DARS	P14868	**0.035**	0.625
Testin	TES	Q9UGI8	**0.020**	0.625
Isoleucine--tRNA ligase, cytoplasmic	IARS	P41252	**0.010**	0.629
V-type proton ATPase subunit B, brain isoform	ATP6V1B2	P21281	**0.046**	0.629
Mitogen-activated protein kinase 1	MAPK1	P28482	**0.043**	0.633
E3 ubiquitin-protein ligase TRIP12	TRIP12	Q14669	**0.038**	0.637
Low-density lipoprotein receptor	LDLR	P01130	**0.035**	0.641
GrpE protein homolog 1, mitochondrial	GRPEL1	Q9HAV7	**0.037**	0.641
Rho-associated protein kinase 2	ROCK2	O75116	**0.019**	0.645
60S ribosomal protein L7a	RPL7A	P62424	**0.026**	0.645
Hexokinase-1	HK1	P19367	**0.043**	0.649
Cytosol aminopeptidase	LAP3	P28838	**0.050**	0.649
Histone H1.2	HIST1H1C	P16403	**0.011**	0.654
tRNA (adenine(58)-N(1))-methyltransferase non-catalytic subunit TRM6	TRMT6	Q9UJA5	**0.047**	0.662
26S protease regulatory subunit 7	PSMC2	P35998	**0.012**	0.662
Nucleosome assembly protein 1-like 4	NAP1L4	Q99733	**0.044**	0.662
Ankyrin repeat and FYVE domain-containing protein 1	ANKFY1	Q9P2R3	**0.029**	0.671
Eukaryotic translation initiation factor 2A	EIF2A	Q9BY44	**0.009**	0.671
Heterogeneous nuclear ribonucleoproteins C1/C2	HNRNPC	P07910	**0.014**	0.671
Coatomer subunit beta'	COPB2	P35606	**0.048**	0.676
60S ribosomal protein L27a	RPL27A	P46776	**0.019**	0.685
GDP-L-fucose synthase	TSTA3	Q13630	**0.042**	0.690
Cytoskeleton-associated protein 4	CKAP4	Q07065	**0.034**	0.694

Acylamino-acid-releasing enzyme	APEH	P13798	**0.038**	0.704
Mannose-1-phosphate guanyltransferase beta	GMPPB	Q9Y5P6	**0.045**	0.704
Spectrin beta chain, non-erythrocytic 1	SPTBN1	Q01082	**0.012**	0.709
Nucleoside diphosphate kinase A	NME1	P15531	**0.015**	0.709
Cytoplasmic aconitate hydratase	ACO1	P21399	**0.036**	0.714
Exportin-2	CSE1L	P55060	**0.045**	0.714
78 kDa glucose-regulated protein	HSPA5	P11021	**0.035**	0.714
Lupus La protein	SSB	P05455	**0.003**	0.730
60S acidic ribosomal protein P2	RPLP2	P05387	**0.045**	0.730
Pigment epithelium-derived factor	SERPINF1	P36955	**0.034**	0.735
Actin-related protein 2	ACTR2	P61160	**0.047**	0.741
Vigilin	HDLBP	Q00341	**0.041**	0.752
Actin-related protein 2/3 complex subunit 2	ARPC2	O15144	**0.045**	0.763
Polyadenylate-binding protein 1	PABPC1	P11940	**0.034**	0.763
Valine--tRNA ligase	VARS	P26640	**0.014**	0.775
DNA dC->dU-editing enzyme APOBEC-3C	APOBEC3C	Q9NRW3	**0.035**	0.781
ADP-ribosylation factor GTPase-activating protein 1	ARFGAP1	Q8N6T3	**0.041**	0.787
Rho GDP-dissociation inhibitor 1	ARHGDIA	P52565	**0.025**	0.800
Thioredoxin-like protein 1	TXNL1	O43396	**0.047**	0.806

Supplementary Table 2 Significantly down-regulated proteins in exosomal fractions after apilimod treatment. Ratio api exo frac vs ctr exo frac; calculated as average TOP3TIC for exosomal fraction (exo frac) after apilimod (api) divided by average TOP3TIC for exosomal fraction after control (ctr) treatment. inf; infinite ratio (not found in control exosomal fractions). All protein reference data are from www.uniprot.org