

SUPPLEMENTARY INFORMATION

Supplementary table legends

Table S1. List of proteins included in the siRNA screen.

Table S2. Sequences for all siRNAs included in the siRNA screen.

Table S3. Proteins that were affected ($p \leq 0.01$; fold change ≥ 1.4) in sEVs released from cells depleted of SNAP29 compared to control cells. Blue color indicates downregulated proteins, pink color indicates upregulated proteins; $FDR \leq 0.1$. Four independent analysis were performed.

Supplementary figure legends

Fig. S1. Size distribution of sEVs shown as number of particles normalized by total number of particles for each condition. PC-3 cells were depleted for STX2 (A), STX3 (B), STX18 (C), VAMP8 (D), and (E) SNAP29, before sEVs were isolated by sequential centrifugation and their size was measured by NTA. The data shows mean + SEM from 3-4 independent experiments.

Fig. S2. Confocal microscopy analysis of SNAP29 in MCF7, MDA-MB-231 and Caco2 cell. SNAP29 labelling (green) in (A) MCF7, (B) MDA-MB-231 and (C) Caco2 cells. In all cases, cells were fixed and permeabilized before incubation with SNAP29 antibody and the corresponding secondary antibodies. Cells were then washed and mounted with ProLong Gold antifade mounting medium containing DAPI to stain the nuclei (blue) and imaged using a Nikon ECLIPSE Ti2-E confocal spinning disk microscope. Images were captured with a x100 objective. Scale bars are indicated (10 μm).

Fig. S3. Confocal microscopy analysis of SNAP29 in control PC-3 cells. (A-C) Cells showing SNAP29 labelling (green), EEA1 (early endosome) labelling (red), and both proteins together. (D-F) Cells showing SNAP29 labelling (green), GM130 (Golgi apparatus) labelling (red), and both proteins together. (G-H) Control cells showing SNAP29 labelling (green), MitoTracker (mitochondria) staining (red), and a combination of both. In all cases, cells were washed and mounted with ProLong Gold antifade mounting medium containing DAPI to stain the nuclei (blue) and imaged using a Nikon ECLIPSE Ti2-E confocal spinning disk microscope. Images were captured with a x100 objective. Scale bars are indicated (10 μm).

Table S1. List of proteins included in the siRNA screen.

Category	Gene Symbol	UniprotKB name
SNARE and SNARE-associated proteins	YKT6	Synaptobrevin homolog YKT6
	SNAP23	Synaptosomal-associated protein 23
	SNAP25	Synaptosomal-associated protein 25
	SNAP29	Synaptosomal-associated protein 29
	BNIP1	Vesicle transport protein SEC20
	SEC22A	Vesicle-trafficking protein SEC22a
	SEC22B	Vesicle-trafficking protein SEC22b
	SEC22C	Vesicle-trafficking protein SEC22c
	VAMP7	Vesicle-associated membrane protein 7
	VAMP8	Vesicle-associated membrane protein 8
	STX2	Syntaxin-2
	STX3	Syntaxin-3
	STX4	Syntaxin-4
	STX7	Syntaxin-7
	STX16	Syntaxin-16
	STX18	Syntaxin-18
	STXBP3	Syntaxin-binding protein 3
	USE1	Vesicle transport protein USE1
	VTI1B	Vesicle transport through interaction with t-SNAREs homolog 1B
	GOSR1	Golgi SNAP receptor complex member 1
	GOSR2	Golgi SNAP receptor complex member 2
	BET1	BET1 homolog
	BET1L	BET1-like protein
SYT1	Synaptotagmin-1	
SYTL2	Synaptotagmin-like protein 2	
Ras superfamily of small GTPases	RAB27A	Ras-related protein Rab-27A
	RAB27B	Ras-related protein Rab-27B
	CDC42	Cell division control protein 42 homolog
	RHOD	Rho-related GTP-binding protein RhoD
	RHOF	Rho-related GTP-binding protein RhoF
	RND3	Rho-related GTP-binding protein RhoE
	ARL13B	ADP-ribosylation factor-like protein 13B
	ARL15	ADP-ribosylation factor-like protein 15
Phospholipases	PLCB4	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-4
	PLCD1	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase delta-1
	PLCD3	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase delta-3
	PLD1	Phospholipase D1
	PLD2	Phospholipase D2
Tight junctions	CLDN1	Claudin-1
	CLDN3	Claudin-2
	CLDN4	Claudin-3
Phospholipid scramblases	PLSCR1	Phospholipid scramblase 1
	PLSCR3	Phospholipid scramblase 3
Sortin nexins	SNX11	Sorting nexin-11
	SNX17	Sorting nexin-17
Others	GRASP	GRP1-associated scaffold protein
	GORASP1	Golgi reassembly-stacking protein 1
	GORASP2	Golgi reassembly-stacking protein 2
	SDCBP	Syntenin-1

Table S2. Sequences for all siRNAs included in the siRNA screen.

Duplex Catalog Number	Gene Symbol	GENE ID	Gene Accession	GI Number	Sequence
J-004667-05	RAB27A	5873	NM_183236	34485710	CCAUAGCACUCGCAGAGAA
J-004667-06	RAB27A	5873	NM_183236	34485710	CAGGAGAGGUUUCGUAGCU
J-004667-07	RAB27A	5873	NM_183236	34485710	UCACAACAGUGGGCAUUGA
J-004667-08	RAB27A	5873	NM_183236	34485710	CUACGGAUCAGUUAAUGUGA
J-004228-05	RAB27B	5874	NM_004163	21314628	GGAACUGGCUGACAAAUAU
J-004228-06	RAB27B	5874	NM_004163	21314628	GCAAAUGCUUAUUGUGAAA
J-004228-07	RAB27B	5874	NM_004163	21314628	GGAAGUCAAUGAACGGCAA
J-004228-08	RAB27B	5874	NM_004163	21314628	UGAAACAAGUGCAGCAACU
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J-019237-10	YKT6	10652	NM_006555	34304384	CUAUAAAACUGCCCGGAAA
J-019237-11	YKT6	10652	NM_006555	34304384	AUACCAGAACCCACGAGAA
J-019237-12	YKT6	10652	NM_006555	34304384	CUAAAAGUGCAGGCCGAACU
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J-011959-07	GOSR1	9527	NM_001007025	55770857	GAAGUCAAUUCACAGCAA
J-011959-08	GOSR1	9527	NM_001007025	55770857	GGGAGAAUCUUAUGGGAUC
J-011959-09	GOSR1	9527	NM_001007025	55770857	GAGACAUUAUUGCAGGAUUA
J-010980-06	GOSR2	9570	NM_054022	60499002	ACGAAUCACUGCAGUUUAA
J-010980-07	GOSR2	9570	NM_054022	60499002	CGAAAUCCAAGCAAGCAUA
J-010980-08	GOSR2	9570	NM_054022	60499002	GAUCCAGUCUUGCAUGGGA
J-010980-09	GOSR2	9570	NM_054022	60499002	UUJAGAUGGGCAAAUUAUU
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J-017545-06	SNAP23	8773	NM_130798	18765730	CAACUAAAACCGCAUAGAAG
J-017545-07	SNAP23	8773	NM_130798	18765730	GAAACUCAUUGACAGCUAA
J-017545-08	SNAP23	8773	NM_130798	18765730	ACAGAGAUUCGUUAUUGAUU
J-011394-09	SNAP25	6616	NM_130811	18765734	CUGGAAAGCACCCGUCGUA
J-011394-10	SNAP25	6616	NM_130811	18765734	CAGAAUCGCCAGAUCGACA
J-011394-11	SNAP25	6616	NM_130811	18765734	GUGUAGUGGACGAACGGGA
J-011394-12	SNAP25	6616	NM_130811	18765734	ACAAAUGAUGCCCGAGAAA
J-011935-05	SNAP29	9342	NM_004782	18765736	GAAGCUAUAAGUACAAGUA
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J-021379-08	STX2	2054	NM_001980	37577286	GUUAAAAGGCUAUUGAACAA

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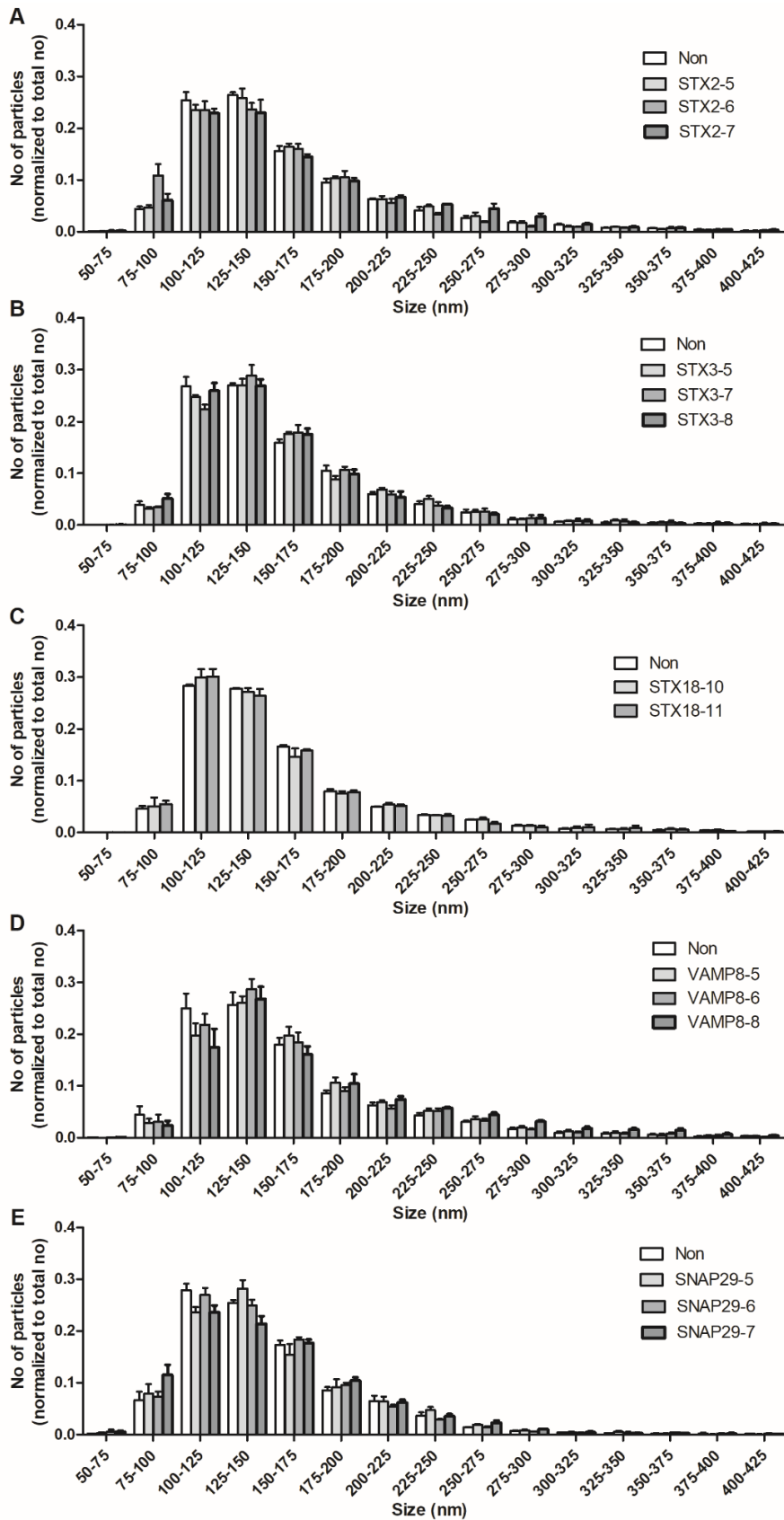
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J-003729-09	PLSCR1	5359	NM_021105	10863876	CCAAAUCAGCCAGUGUAUA
J-003729-10	PLSCR1	5359	NM_021105	10863876	GUAGUGGAUUAGUGAAAGU
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J-010255-06	PLSCR3	57048	NM_020360	31543416	GCAGAUGACUUUGGCCUAC
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J-020044-06	SYT1	6857	NM_005639	5032138	GGGCACAUCUGAUCCUUAC
J-020044-07	SYT1	6857	NM_005639	5032138	GAUCGUUUCUCUAAGCAUG
J-020044-08	SYT1	6857	NM_005639	5032138	GUAAGAGGCUGAAGAAGAA
J-013555-05	SYTL2	54843	NM_032379	46255060	GAAACUAGCUCUCCAGUAU
J-013555-06	SYTL2	54843	NM_032379	46255060	CAUUUAAGCGCAAUAGUUU
J-013555-07	SYTL2	54843	NM_032379	46255060	UGUAAAGGCCUAUUUGCUA
J-013555-08	SYTL2	54843	NM_032379	46255060	GGACUGGGAUAACAAACAG
J-019626-09	STXBP3	6814	NM_007269	6005885	AGAGUGACAUGAUUCGUAA
J-019626-10	STXBP3	6814	NM_007269	6005885	UCAACAAGGCAAACCGUUA
J-019626-11	STXBP3	6814	NM_007269	6005885	GUAAAUCGGAGAACAAGUA
J-019626-12	STXBP3	6814	NM_007269	6005885	GAAGAUGACCUCUGGGUUA
J-008270-05	SDCBP	6386	NM_001007068	55749503	GGAGAGAAGAUUACCAUGA
J-008270-06	SDCBP	6386	NM_001007068	55749503	GACCAAGUACUUCAGAUCA
J-008270-07	SDCBP	6386	NM_001007068	55749503	GGAUGGUCUUAGAAUUUU
J-008270-08	SDCBP	6386	NM_001007068	55749503	GCAUUUGACUCUUAAGAUU

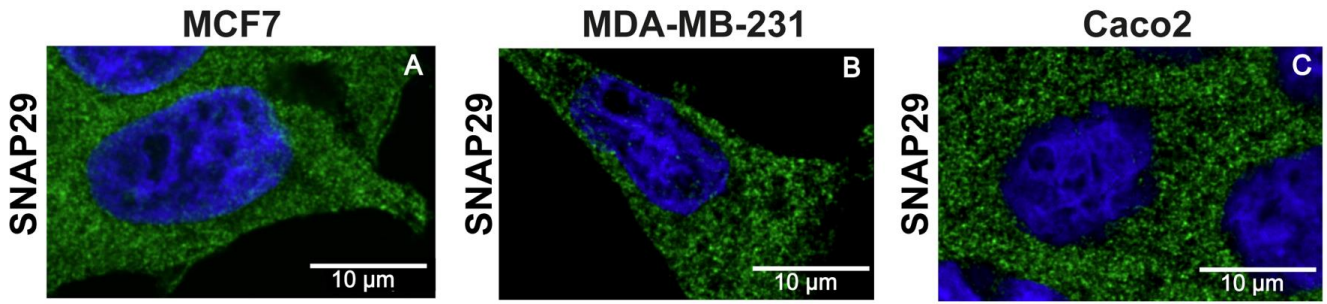
Table S3. Proteins affected ($p \leq 0.01$; fold change ≥ 1.4) in small EVs released from SNAP29 depleted compared to control cells.

Protein names	Gene names	p-value	Fold change SNAP29/control
Dedicator of cytokinesis protein 10	DOCK10	0,00015	-1,69
Anion exchange protein	SLC4A7	0,00033	1,5
Choline transporter-like protein 2	SLC44A2	0,00138	-1,71
Podocalyxin	PODXL	0,00144	-1,78
1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-4	PLCB4	0,00186	1,43
Anoctamin-1	ANO1	0,00203	-1,7
Thioredoxin	TXN	0,00227	1,46
Adenylyl cyclase-associated protein 1	CAP1	0,00344	-1,4
Guanine nucleotide-binding protein subunit alpha-11	GNA11	0,00362	1,43
Annexin A1	ANXA1	0,00409	1,49
G-protein coupled receptor family C group 5 member C	GPRC5C	0,00478	-1,89
Na(+)/H(+) exchange regulatory cofactor NHE-RF1	SLC9A3R1	0,00539	-1,44
Ras-related protein R-Ras2	RRAS2	0,00684	-1,5
Alkaline phosphatase	ALPP	0,00690	-1,5
F-actin-capping protein subunit beta	CAPZB	0,00795	-1,64
Hyaluronan synthase 3	HAS3	0,00866	1,78
Tyrosine-protein kinase transmembrane receptor ROR1	ROR1	0,00881	-1,76

Supplementary figure 1



Supplementary figure 2



Supplementary figure 3

