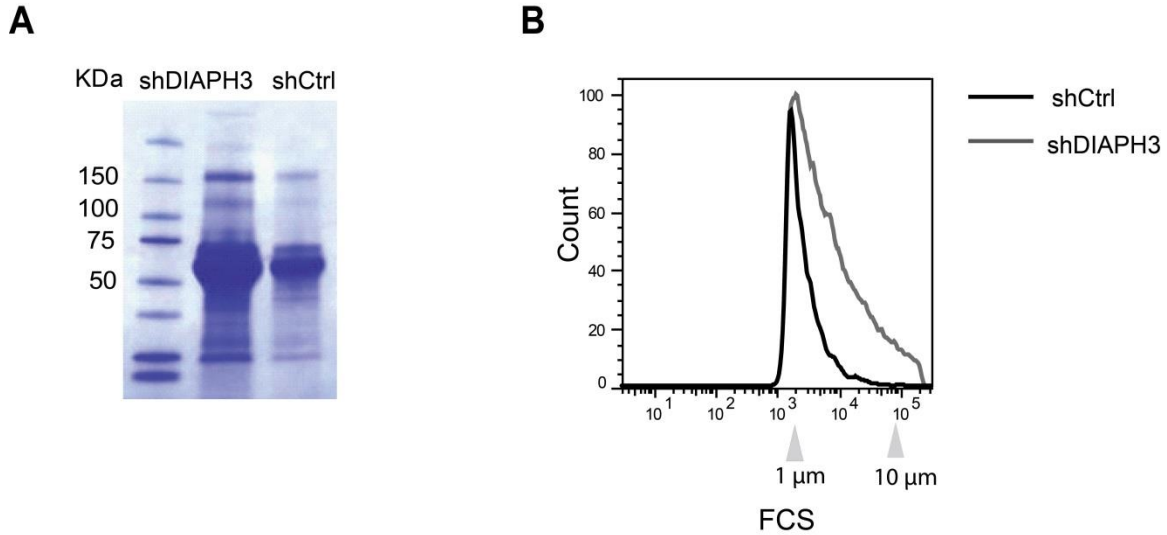


## Large oncosomes contain distinct protein cargo and represent a separate functional class of tumor-derived extracellular vesicles

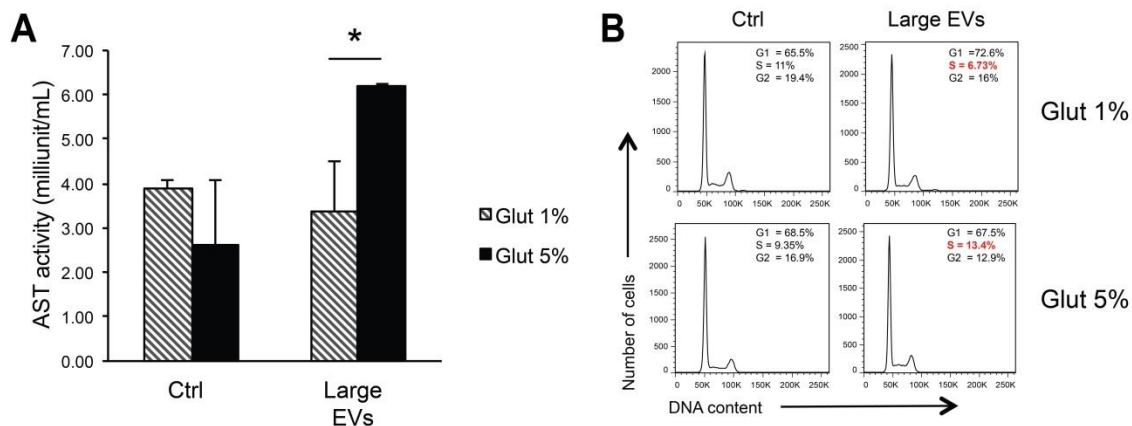
### Supplementary Material



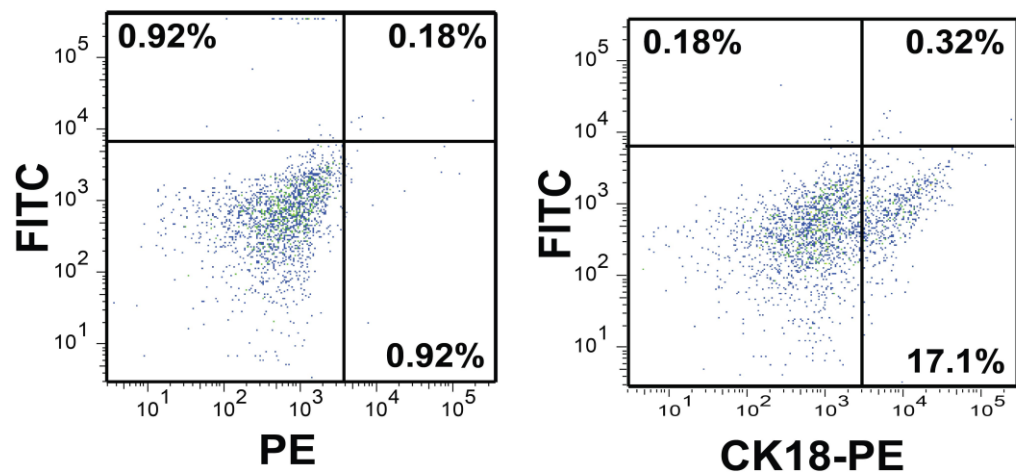
**Figure S1:** (A) Protein lysates from large EVs, isolated from the growth medium of DIAPH3-silenced or unsilenced control DU145 cells, were resolved by SDS-PAGE and stained with Coomassie Blue. Notice protein abundance is higher in EVs from DIAPH3-silenced cells. (B) Histogram of large EVs derived from DU145 cells stably transfected with either control or DIAPH3 shRNAs, showing increased EV shedding, compatible with large oncosomes, from the latter.

A. List of proteins enriched in large EVs.				B. List of proteins enriched in nano-sized EVs.			
Protein ID	Symbol	Large EVs/nano-sized EVs	FDR	Protein ID	Symbol	Large EVs/nano-sized EVs	FDR
P12236	SLC25A6	87.575	< 0.001	G8JLH6	CD9	10.133	0.01
J3KPX7	PHB2	73.812	< 0.001	A6NMH8	CD81	9.484	0.003
P06576	ATP5B	61.432	< 0.001	P11047	LAMC1	8.821	0.01
Q00325	SLC25A3	43.373	< 0.001	O94985	CLSTN1	8.374	0.003
P21796	VDAC1	41.622	< 0.001	P10253	P10253	7.527	0.013
P00505	GOT2	29.161	< 0.001	Q14118	DAG1	6.995	0.016
Q9UJZ1	STOML2	27.436	< 0.001	P01137	TGFB1	6.973	0.016
P40926	MDH2	27.318	< 0.001	P11166	SLC2A1	6.867	0.016
P38646	HSPA9	20.08	< 0.001	Q15758	SLC1A5	5.523	0.014
P45880	VDAC2	16.884	< 0.001	O75787	ATP6AP2	5.523	0.009
P05141	SLC25A5	15.667	0.001	P10909-2	CLU	5.377	0.009
P02751-15	FN1	10.702	0.003	P08572	COL4A2	5.353	0.024
P34897	SHMT2	9.967	0.003	P62879	GNB2	5.346	0.011
P16104	H2AFX	9.772	0.011	P26006	ITGA3	5.326	0.012
P10809	HSPD1	8.491	0.003	J3KN08	MATN2	5.215	0.024
Q13011	ECH1	8.138	0.008	P19021-5	PAM	5.107	0.025
P30048	PRDX3	7.905	0.008	P27105	STOM	5.047	0.025
Q02978	SLC25A11	7.786	0.008	O00468	AGRN	4.904	0.05
P52272	HNRNPM	6.915	0.01	P06756	ITGAV	4.748	0.026
B4DR52	HIST2H2BF	6.262	0.004	Q08431	MFGE8	4.693	0.029
Q9H3N1	TMX1	5.466	0.01	P18065	IGFBP2	4.692	0.012
F8VTL3	MYH10	5.25	0.018	P35052	GPC1	4.626	0.029
F5H7K4	NCEH1	5.208	0.01	Q13641	TPBG	4.588	0.03
P30084	ECHS1	4.924	0.01	P31431	SDC4	4.55	0.032
Q969H8	C19orf10	4.899	0.021	P16070	CD44	4.465	0.046
P51571	SSR4	4.836	0.021	P05362	ICAM1	4.449	0.034
P23284	PPIB	4.683	0.021	Q9Y4K0	LOXL2	4.354	0.004
P06744-2	GPI	4.677	0.018	P62873	GNB1	4.26	0.038
Q15149	PLEC	4.613	0.024	I3NI00	BSG	4.249	0.001
P14314	PRKCSH	4.604	0.005	O00159	MYO1C	4.205	0.04
P20618	PSMB1	4.446	0.024	B7Z5Z2	RRAS2	4.198	0.041
Q96AG4	LRRC59	4.409	0.025	J3KNY4	CTSA	4.151	0.003
P17174	GOT1	4.171	0.032	P07996	THBS1	4.123	0.018
Q00839	HNRNPU	4.012	0.04	Q9NS15	LTBP3	4.068	0.03
P04843	RPN1	4.007	0.033	Q92820	GGH	4.043	0.044
P05783	CK18	3.977	0.003	P00749	PLAU	3.955	0.047
P84103	SRSF3	3.94	0.013	O43854	EDIL3	3.886	0.002
P27797	CALR	3.896	0.031	P21589	NT5E	3.87	0.048
P30101	PDIA3	3.89	0.038	P05067	APP	3.853	0.002
P62805	HIST1H4A	3.865	0.014	P16035	TIMP2	3.508	0.023
P06748	P06748	3.839	0.038	Q9UBV8	PEF1	3.354	0.01
P28074	PSMB5	3.718	0.03	P07339	CTSD	3.12	0.037
P07237	P4HB	3.708	0.049	F5H3A1	ATP1A1	3.106	0.026
P13667	PDIA4	3.444	0.046	G8JLA8	TGFBI	3.008	0.045
P49720	PSMB3	3.43	0.041				
P42704	LRPPRC	3.414	0.048				
P11021	HSPA5	3.359	0.014				
P30040	ERP29	3.259	0.034				
P35579	MYH9	3.256	0.01				
F5H098	MDH1	3.217	0.006				
E7EPA7	TKT	2.963	0.033				
P07195	LDHB	2.851	0.019				
P04406	GAPDH	2.777	0.017				
B5MDF5	RAN	2.776	0.023				
Q15084-2	PDIA6	2.528	0.041				
P46782	RPS5	2.362	0.023				
P07910	HNRNPC	2.006	0.027				

**Figure S2:** Ratio of significant differentially expressed protein (DEPs) in large and nano-sized EVs (A) and vice-versa (B). FDR <0.01.



**Figure S3:** (A) The results from 2 experiments in DU145 cells treated with large oncosomes or vehicle are displayed as AST enzymatic activity (milliunit/mL), in cells cultured in 5% or 1% glutamine to highlight the enzymatic activity dynamically ( $p=0.023$ ). (B) Cell-cycle analysis of DU145 cells treated with large oncosomes or vehicle in presence of 1% or 5% glutamine for 24 hours. After treatment, the cells were incubated with propidium iodide for DNA content-based evaluation of cell-cycle phase distribution. The percentage of cells in S-phase was increased by treatment with large oncosomes in 5% (13.4%) in comparison with 1% glutamine (6.73%). See also Fig 4.



**Figure S4:** DIAPH3-silenced DU145 cell-derived large EVs unstained (left panel), and stained with CK18 (right panel) were analyzed by FACS.