Supplementary Information

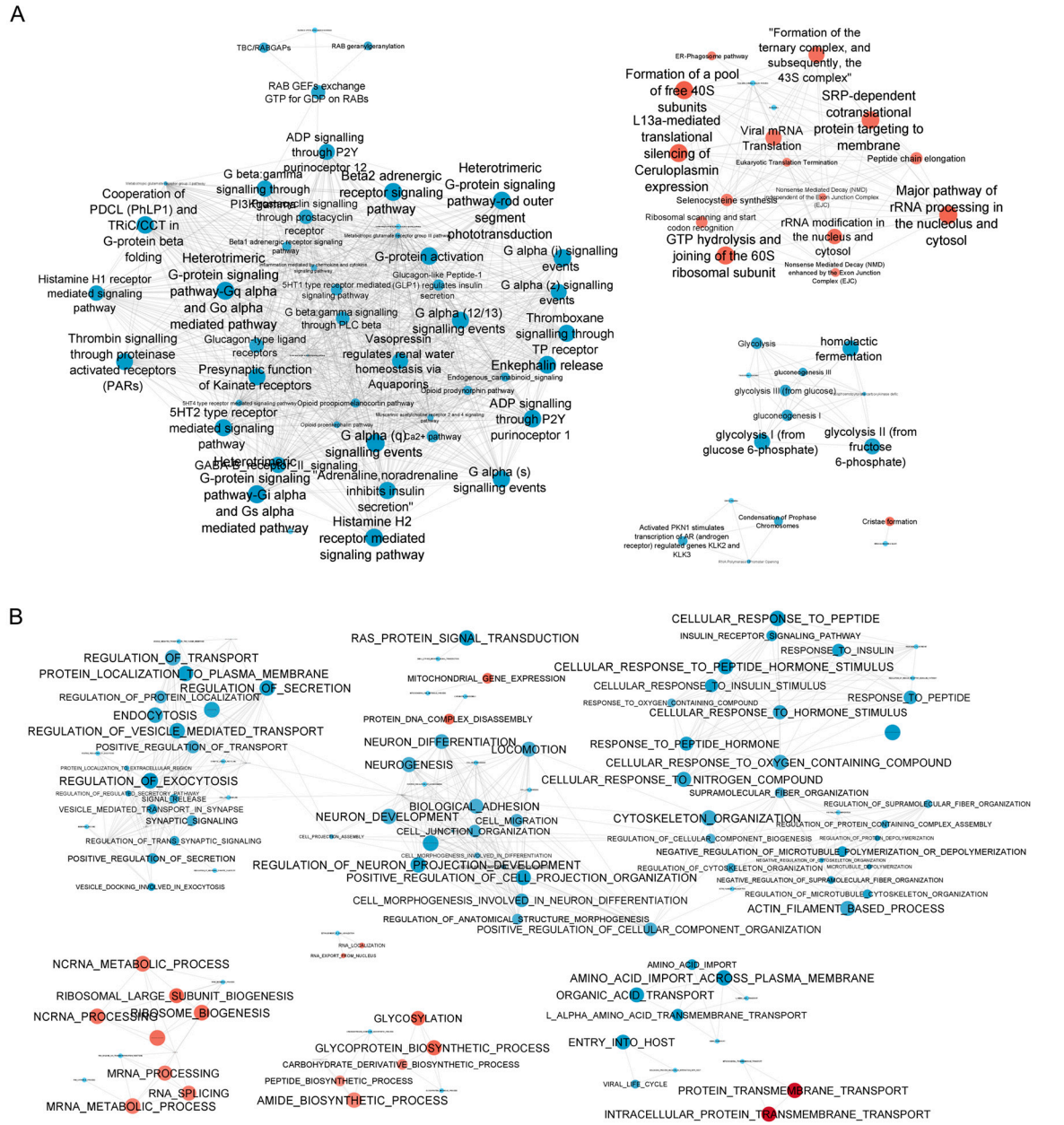


Figure S1: Kelly and KellyCis83 sEV have differentially expressed proteomes. Gene Set Enrichment Analysis using the “Pathway Commons” (A) and “Gene Ontology Biological Processes” (B) databases. A threshold of 20% overlapping proteins was used for network construction of significantly enriched pathways (adjusted p-val < 0.05). Pathways

upregulated in KellyCis83 sEV were coloured red and downregulated pathways were coloured blue. The size of the nodes was proportionally set to -log10(adjusted p-val) (C). Heatmap of normalised LFQ intensities of proteins related to mRNA processing, glycolysis, extracellular vesicle, G-protein signalling in Kelly and KellyCis83 sEV. A darker orange colour indicates a higher protein abundance.

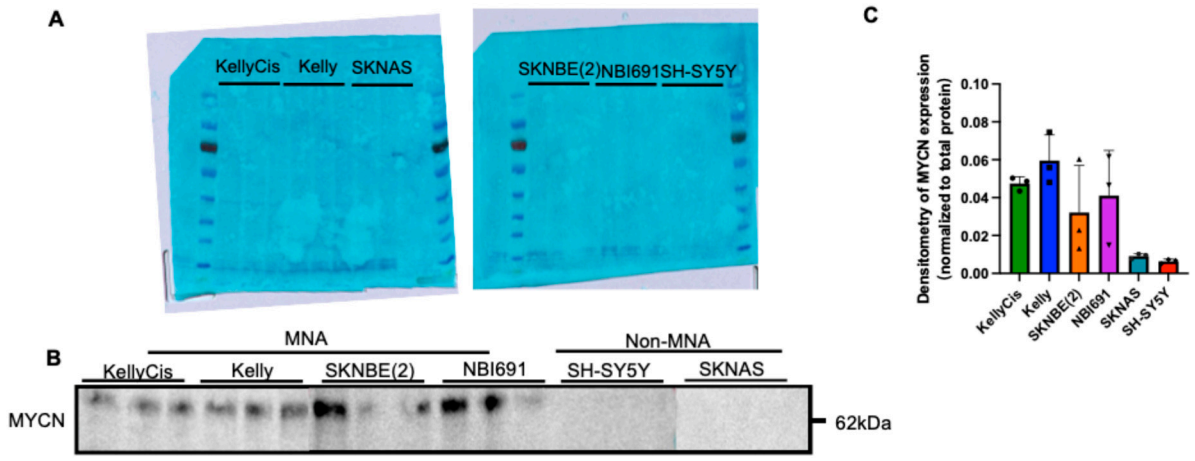
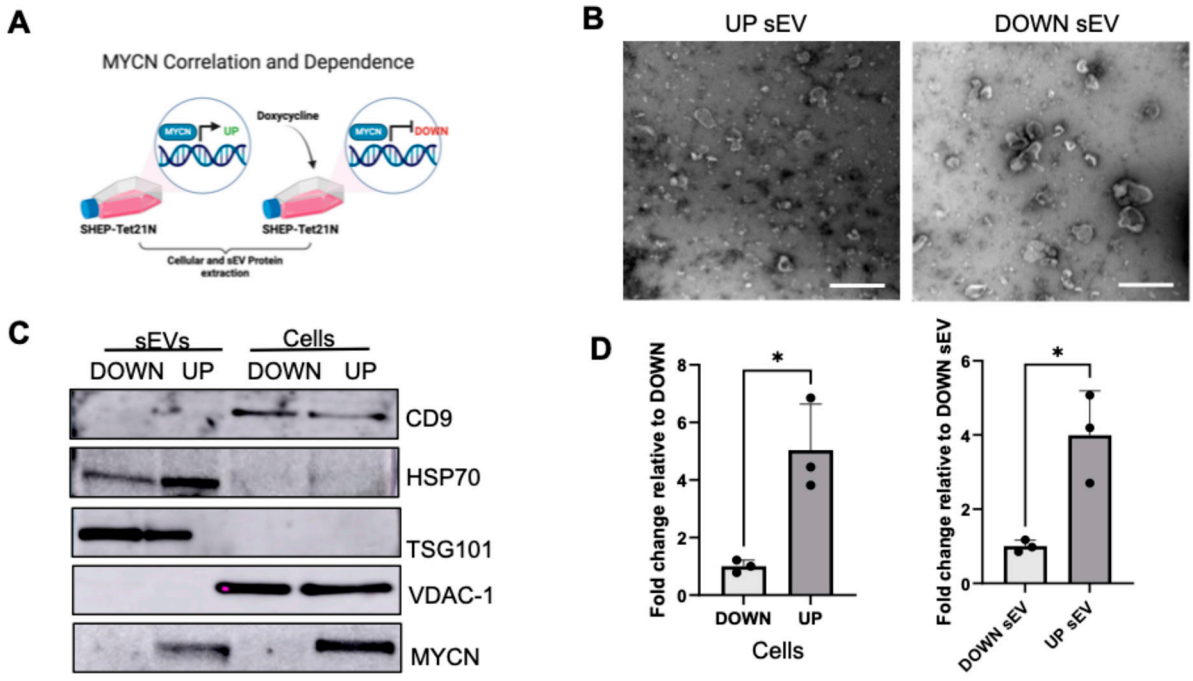


Figure S2: NB cell line-derived sEVs examined with anti-MYCN antibody. MYCN-amplified NB derived sEVs: KellyCis83, Kelly, SK-N-BE(2), and NB-1691. Non-MYCN-amplified NB derived sEVs: SH-SY5Y and SK-N-AS. (A) Total sEV protein load stained with Reversible Protein Stain Kit for Nitrocellulose (ThermoFisher, Cat no. 24580), and imaged using Amersham Imager 600 (GE Healthcare Lifescience) for protein normalization. Samples were loaded in 3 biological replicates. (B) Western blot analysis of MYCN enrichment in sEVs. (C) Densitometry analysis of MYCN detection in sEVs.



Supplementary Information

Figure S3: Isolation of sEVs from the inducible SHEP-Tet-21N cell system. (A) SHEP-Tet-21N cells were grown in the presence (MYCN-DOWN) and absence (MYCN-UP) of doxycycline, and cellular and sEV proteins were extracted for a western blot to assess whether the expression of selected glycolytic proteins is MYCN dependent. (B) TEM images of MYCN-DOWN and MYCN-UP sEV (scale bar = 500 nm); (C) Representative blot of three independent experiments, showing expression of CD9, HSP70, TSG101, and VDAC-1 in MYCN-DOWN and MYCN-UP cells and sEVs. Images for VDAC-1 and TSG101 are derived from the same blot, cut into two, prior to primary antibody incubation. The same is for CD9 and HSP70 images; (D) Densitometry analysis of MYCN in cellular and sEVs in response to doxycycline treatment . Data represented as mean ± standard deviation. Statistical significance was calculated by an unpaired t-test (* $p \leq 0.05$ and ** $p \leq 0.01$, only statistically significant values graphed). Image created with Biorender.com.

Table S1: Primary antibodies used for immunodetection

Antibody	Species	Mw (kDa)	Manufacturer	Cat no.
EGFR	Rb	175	Cell Signalling Technology	2963S
GAPDH	Ms	36	Bio-Rad, Cat no	MCA4740
ANXA5	Rb	30	Cell Signalling Technology	8555S
CD9	Rb	22, 24, 35	Cell Signalling Technology	13174S
HSP70	Rb	70	Cell Signalling Technology	4876S
FLOT1	Rb	49	Cell Signalling Technology	18634S
TSG101	Ms	44	Santa Cruz Biotechnology	sc-7964
VDAC-1	Rb	32	Cell Signalling Technology	4661S
TUBA1A	Rt	50	Bio-Rad	MCA78G
SMAD4	Rb	70	Cell Signalling Technology	38454
LIN28B	Rb	32, 21	Cell Signalling Technology	4196S
LDHA	Rb	37	Cell Signalling Technology	3582
PKM2	Rb	60	Cell Signalling Technology	4053
PKM1/2	Rb	60	Cell Signalling Technology	3990

Abbreviations: Mw = molecular weight, Rb = rabbit, Ms = mouse, Rt = rat, EGFR = epidermal growth factor receptor, GAPDH = glyceraldehyde-3-phosphate dehydrogenase, ANXA5 = annexin A5, HSP70 = heat shock protein 70, FLOT1 = flotillin, VDAC-1 = voltage-dependent anion-selective channel protein 1, TUBA1A = tubulin alpha-1A chain, SMAD4 = mothers against decapentaplegic homolog 4, LIN28B = protein lin-28 homolog B, LDHA = Lactate Dehydrogenase A, PKM1/2= Pyruvate Kinase M1/M2, PKM2= Pyruvate Kinase M2.

Supplementary Information

Table S2: List of proteins with their fold change.

Q12972_PPP1R8	1.41135372	0.09383154	GOBP_MRNA_PROCESSING
A9Z1X7_SRRM1	-1.5827254	0.16193087	GOBP_MRNA_PROCESSING
O15116_LSM1	-1.2154771	0.16720434	GOBP_MRNA_PROCESSING
Q15427_SF3B4	3.18273407	0.19337478	GOBP_MRNA_PROCESSING
Q15427_SF3B4	3.18273407	0.19337478	GOBP_MRNA_PROCESSING
M0R0G9_SNRPA	-0.9624677	0.23032851	GOBP_MRNA_PROCESSING
Q9H307_PNN	1.08054492	0.24146631	GOBP_MRNA_PROCESSING
P62306_SNRPF	-0.9908117	0.25757046	GOBP_MRNA_PROCESSING
Q6NZY4_ZCCHC8	2.08119875	0.2821599	GOBP_MRNA_PROCESSING
Q9H0S4_DDX47	-1.6054489	0.28324677	GOBP_MRNA_PROCESSING
O14908_GIPC1	-1.5462703	0.09354307	GOBP_REGULATION_OF_EXOCYTOSIS
P61026_RAB10	-1.6860884	0.16193087	GOBP_REGULATION_OF_EXOCYTOSIS
P20336_RAB3A	-1.1481692	0.17966354	GOBP_REGULATION_OF_EXOCYTOSIS
E3W994_CLASP2	-1.5254781	0.18220687	GOBP_REGULATION_OF_EXOCYTOSIS
Q9BT88_SYT11	-1.9633812	0.22810019	GOBP_REGULATION_OF_EXOCYTOSIS
P15153_RAC2	-2.1926907	0.23032851	GOBP_REGULATION_OF_EXOCYTOSIS
P61266_STX1B	-2.4821263	0.23999056	GOBP_REGULATION_OF_EXOCYTOSIS
O43581_SYT7	-2.4539054	0.26956446	GOBP_REGULATION_OF_EXOCYTOSIS
P61006_RAB8A	-1.1416837	0.27894719	GOBP_REGULATION_OF_EXOCYTOSIS
P62760_VSNL1	-2.0763637	0.31383915	GOBP_REGULATION_OF_EXOCYTOSIS
H3BTN5_PKM	-1.7824983	0.09354307	P00024::Glycolysis
P15259_PGAM2	-1.6568107	0.17966354	P00024::Glycolysis
P17858_PFKL	-1.5345469	0.23032851	P00024::Glycolysis
P09104_ENO2	-1.0807737	0.23032851	P00024::Glycolysis
P09972_ALDOC	-1.7563193	0.23032851	P00024::Glycolysis
P06744_GPI	-1.1924603	0.30110379	P00024::Glycolysis
A0A2R8Y891_PFKM	-0.7550127	0.32832224	P00024::Glycolysis
P06733_ENO1	-0.5723848	0.4165649	P00024::Glycolysis
P60174_TPI1	-0.5777126	0.42029655	P00024::Glycolysis
P04075_ALDOA	-1.0048515	0.43368964	P00024::Glycolysis
A0A0A0MSK4_GPSM1	-3.0966506	0.08914164	P00027::Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway
P50150_GNG4	-2.2020755	0.16193087	P00027::Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway
P09497_CLTB	-1.2261096	0.17966354	P00027::Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway
P63215_GNG3	-2.282809	0.17966354	P00027::Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway

P62873_GNB1	-1.5366645	0.19027923	P00027::Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway
Q9HAV0_GNB4	-1.5097493	0.19039491	P00027::Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway
P81274_GPSM2	-2.1536448	0.19337478	P00027::Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway
P63218_GNG5	-1.6819556	0.20675868	P00027::Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway
P49798_RGS4	-1.7940742	0.20675868	P00027::Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway
P59768_GNG2	-1.8081367	0.23032851	P00027::Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway

Table S3: Neuroblastoma cell lines and MYCN amplification status

Cell Line	Origin	MYCN Status
Kelly	1 year old female NB patient	Amplified
KellyCis83	Kelly-derived cisplatin-resistant cell line (Piskareva Lab)	Amplified
NB-1691	1 – 3 year old unspecified NB patient	Amplified
SH-SY5Y	4 year old female NB patient	Non-amplified
SK-N-AS	6 year old female NB patient	Non-amplified
SK-N-BE(2)	2 year, 2 month old male NB patient	Amplified