

A549 EVs

Nx Hx

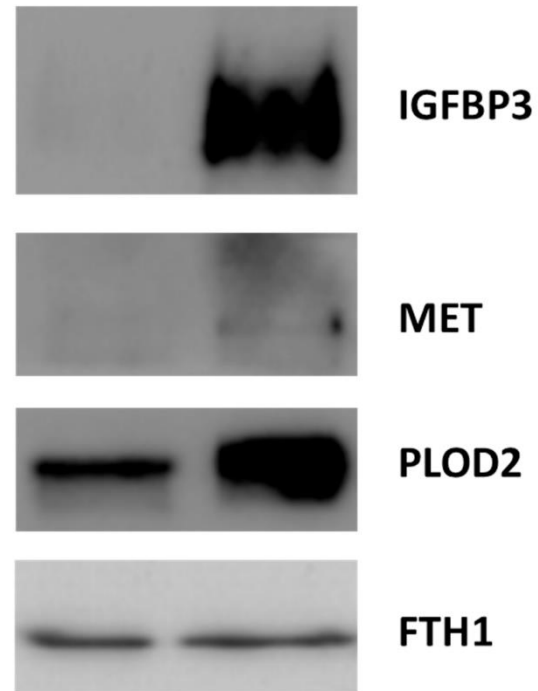


Figure S1. Validation of newly synthesized proteins using Western blots. Several EVs proteins that were classified as newly synthesised in Table 1 were selected and validated using non-SILAC labelled EVs isolated from A549 lung cancer cells cultured under normal culture conditions or hypoxia. Experiment was repeated with 3 biological replicate.

Protein Name (Gene Symbol)	GO:0005604~basement membrane	GO:0030198~extracellular matrix organization	GO:0031012~extracellular matrix	H/L	emPAI
matrilin 2(MATN2)				100.00	0.05
Fraser extracellular matrix complex subunit 1(FRAS1)				6.77	0.29
TIMP metalloproteinase inhibitor 1(TIMPI)				10.85	3.33
cystatin C(CST3)				8.57	0.59
amyloid beta precursor protein(APP)				20.85	0.90
cadherin 1(CDH1)				2.67	0.19
thrombospondin 1(THBS1)				3.32	4.62
serpin family E member 1(SERPINE1)				2.88	1.02
secreted phosphoprotein 1(SPP1)				100.00	0.70
EGF containing fibulin like extracellular matrix protein 1(EFEMP1)				35.79	0.30
hyaluronan and proteoglycan link protein 3(HAPLN3)				7.02	0.62
collagen type XII alpha 1 chain(COL12A1)				100.00	0.03
galectin 3 binding protein(LGALS3BP)				6.32	173.75
bone morphogenetic protein 1(BMP1)				13.78	0.17
cartilage associated protein(CRTAP)				7.44	0.98
SPARC/osteonectin, cwcv and kazal like domains proteoglycan 1(SPOCK1)				79.73	0.59
matrix metalloproteinase 7(MMP7)				100.00	0.19
matrix metalloproteinase 2(MMP2)				100.00	0.19
cochlin(COCH)				65.09	0.16
dystroglycan 1(DAG1)				100.00	0.10
lysyl oxidase like 2(LOXL2)				9.92	1.11
fibrillin 2(FBN2)				8.87	0.18
collagen type V alpha 2 chain(COL5A2)				11.70	0.14
collagen type VI alpha 1 chain(COL6A1)				5.98	1.13
fibronectin 1(FN1)				7.73	6.78
collagen type IV alpha 2 chain(COL4A2)				4.32	0.15
agrin(AGRN)				4.39	8.58
heparan sulfate proteoglycan 2(HSPG2)				5.31	5.16
fibrillin 1(FBN1)				52.19	0.06
secreted protein acidic and cysteine rich(SPARC)				5.63	0.97
fibulin 1(FBLN1)				8.99	0.73
transforming growth factor beta induced(TGFB1)				7.86	7.96
collagen type XVIII alpha 1 chain(COL18A1)				4.35	0.61
collagen type V alpha 1 chain(COL5A1)				4.34	0.82
collagen type VII alpha 1 chain(COL7A1)				3.02	1.19
coiled-coil domain containing 80(CCDC80)				68.76	0.22
laminin subunit gamma 1(LAMC1)				2.55	15.28
nidogen 2(NID2)				37.98	0.12
laminin subunit alpha 5(LAMA5)				4.18	6.93
laminin subunit alpha 3(LAMA3)				67.13	0.11
von Willebrand factor A domain containing 1(VWA1)				8.14	1.15

Figure S2. Annotation clusters of extracellular matrix related protein found in EVs derived from A549 cells cultured under normal culture conditions. EV proteins associated with the corresponding gene terms are shown in green. Proteins not reported to be associated with the gene terms are shown in black. H/L ratios and relative abundance (emPAI) of each proteins were extracted from pSILAC data.

Protein Name (Gene Symbol)	GO:0043202~lysosomal lumen	GO:0005764~lysosome	Hydrolase	H/L	emPAI
heparan sulfate proteoglycan 2(HSPG2)				3.41	3.06
tubulointerstitial nephritis antigen like 1(TINAGL1)				100.00	0.21
cystatin C(CST3)				6.53	1.51
carboxypeptidase, vitellogenic like(CPVL)				56.67	0.19
eukaryotic translation initiation factor 4A3(EIF4A3)				2.38	0.32
amyloid beta precursor protein(APP)				14.24	2.25
pappalysin 1(PAPPA)				2.42	11.23
protein tyrosine phosphatase, receptor type D(PTPRD)				3.36	0.13
fucosidase, alpha-L- 2, plasma(FUCA2)				3.15	3.26
complement C1r(C1R)				11.47	16.30
bone morphogenetic protein 1(BMP1)				6.49	0.37
protein tyrosine phosphatase, receptor type J(PTPRJ)				11.23	0.27
complement C1s(C1S)				16.01	8.35
protein tyrosine phosphatase, receptor type S(PTPRS)				5.42	0.40
complement factor D(CFD)				100.00	0.39
matrix metalloproteinase 7(MMP7)				100.00	0.43
matrix metalloproteinase 2(MMP2)				53.76	0.12
plasminogen activator, urokinase(PLAU)				100.00	0.09
protease, serine 23(PRSS23)				3.44	0.23
low density lipoprotein receptor(LDLR)				4.31	0.98
transmembrane protein 59(TMEM59)				100.00	0.13
mannosidase alpha class 2B member 2(MAN2B2)				66.07	0.36
NPC intracellular cholesterol transporter 2(NPC2)				2.32	0.23
fucosidase, alpha-L- 1, tissue(FUCA1)				3.13	0.20
proprotein convertase subtilisin/kexin type 9(PCSK9)				94.84	0.69
gamma-glutamyl hydrolase(GGH)				3.89	21.39
prosaposin(PSAP)				11.83	18.68
hexosaminidase subunit alpha(HEXA)				2.37	1.68
hexosaminidase subunit beta(HEXB)				2.34	2.28
galactosidase beta 1(GLB1)				100.00	0.23
galactosamine (N-acetyl)				4.85	0.49
mannosidase alpha class 2B member 1(MAN2B1)				3.15	0.49
mannosidase beta(MANBA)				61.97	0.44
deoxyribonuclease 2, lysosomal(DNASE2)				100.00	0.31
aspartylglucosaminidase(AGA)				2.88	0.59
cathepsin B(CTSB)				8.41	145.78
cathepsin C(CTSC)				4.97	1.87
legumain(LGMN)				8.38	0.62
palmitoyl-protein thioesterase 1(PPT1)				69.78	0.17
glucosamine (N-acetyl)				3.98	2.05
cathepsin A(CTSA)				21.02	0.74
neuraminidase 1(NEU1)				32.96	0.82
galactosidase alpha(GLA)				3.36	0.64
cathepsin D(CTSD)				6.79	271.13
tripeptidyl peptidase 1(TPP1)				45.03	0.98
N-acylsphingosine amidohydrolase 1(ASAH1)				2.37	0.61
glucosidase alpha, acid(GAA)				7.25	0.47

Figure S3. Annotation clusters of extracellular matrix related protein found in EVs derived from A549 cells cultured under hypoxia (Hx). EV proteins associated with the corresponding gene terms are shown in green. Proteins not reported to be associated with the gene terms are shown in black. H/L ratios and relative abundance (emPAI) of each proteins were extracted from pSILAC data.

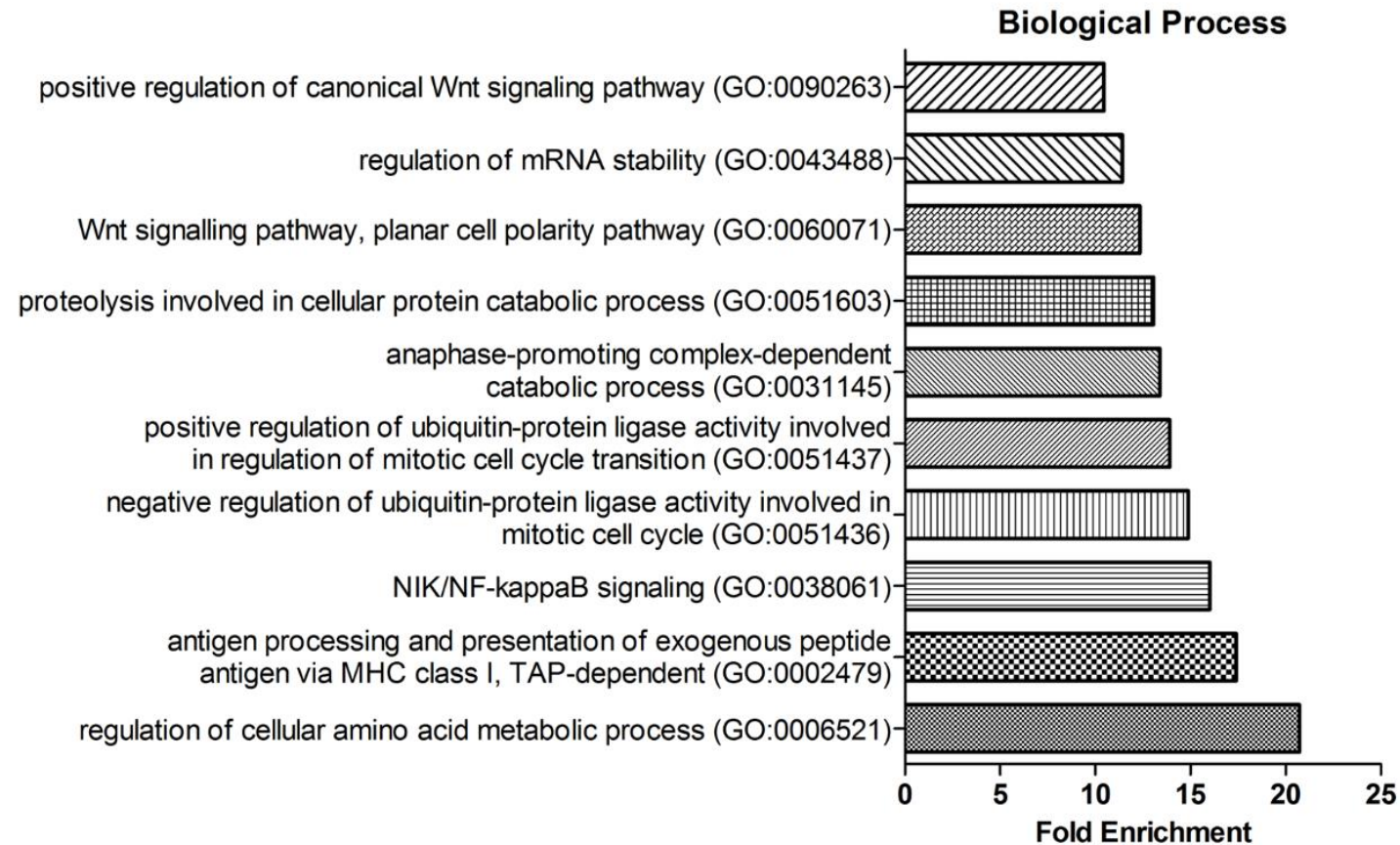
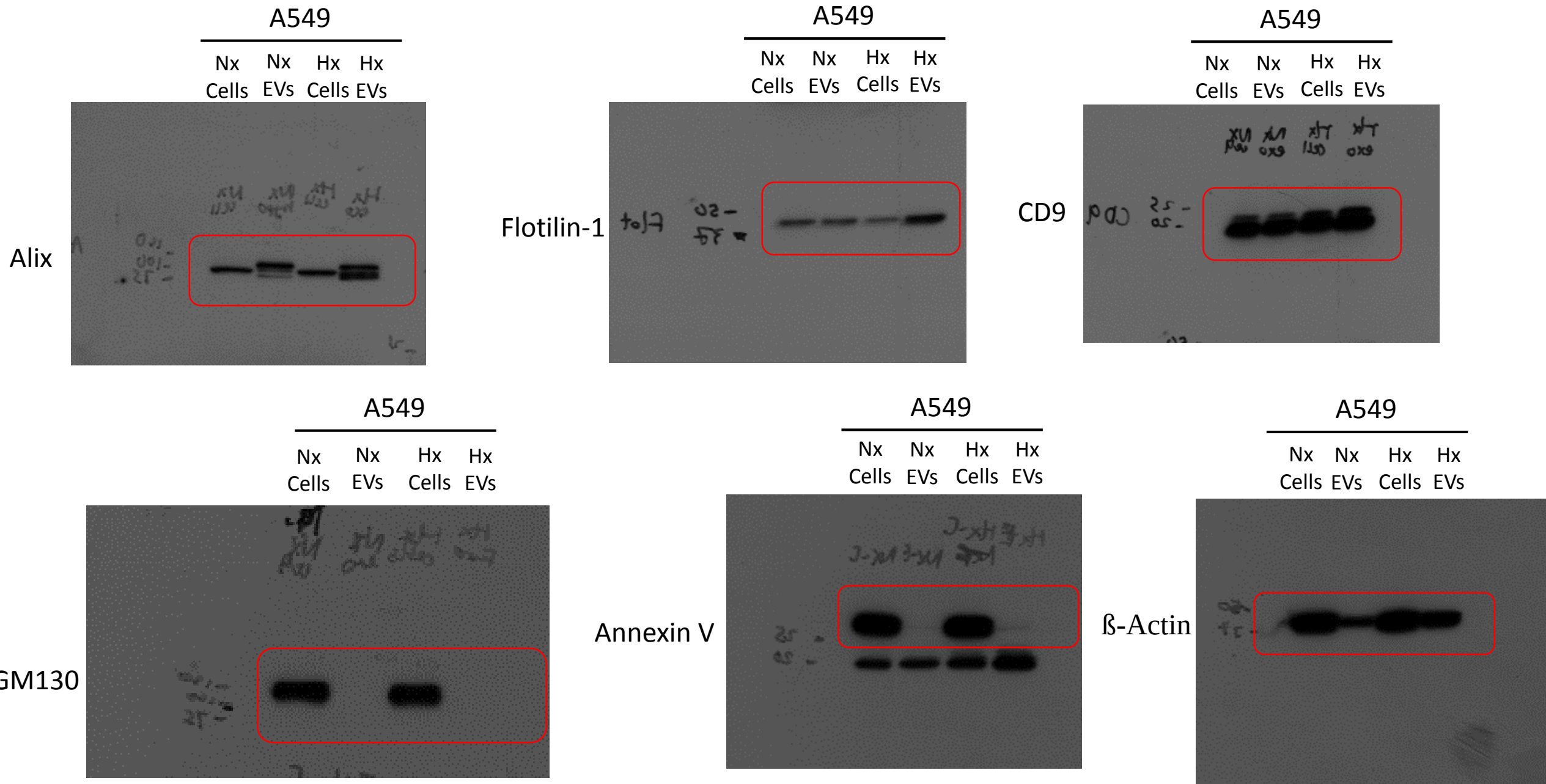
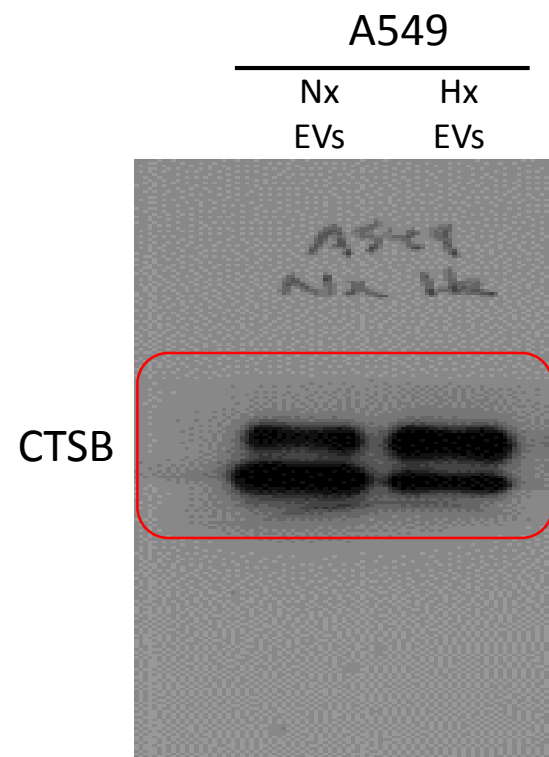
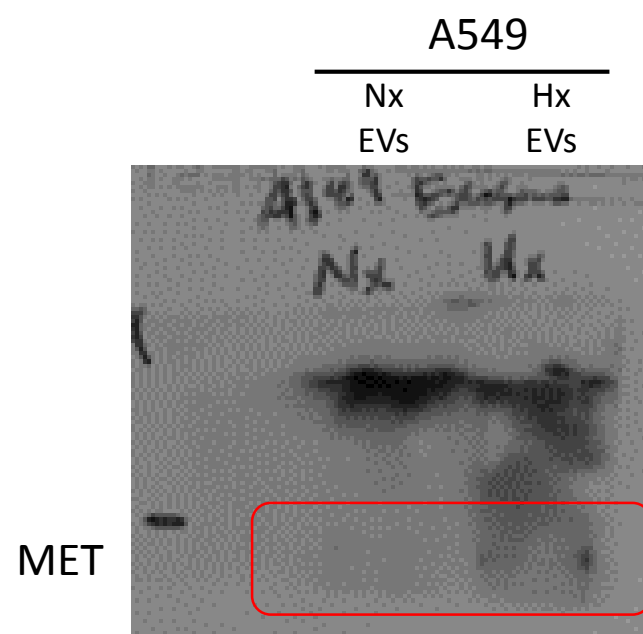
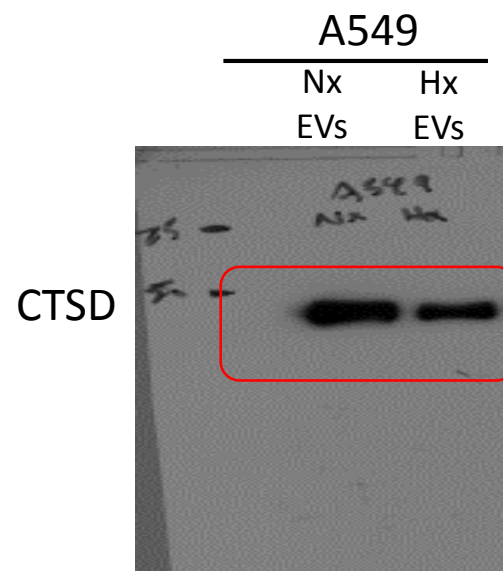
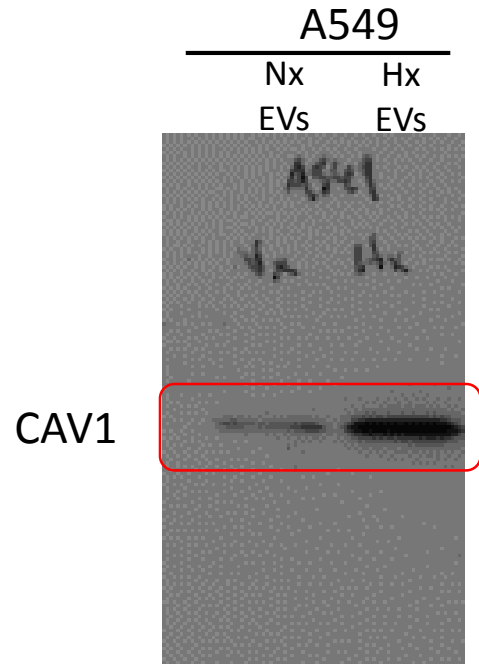


Figure S4. Gene ontology analysis revealed biological process associated with proteins (n=443) found in both condition (Nx and Hx) that had similar H/L ratios.

Supplementary Figure 5: Uncropped western blot images

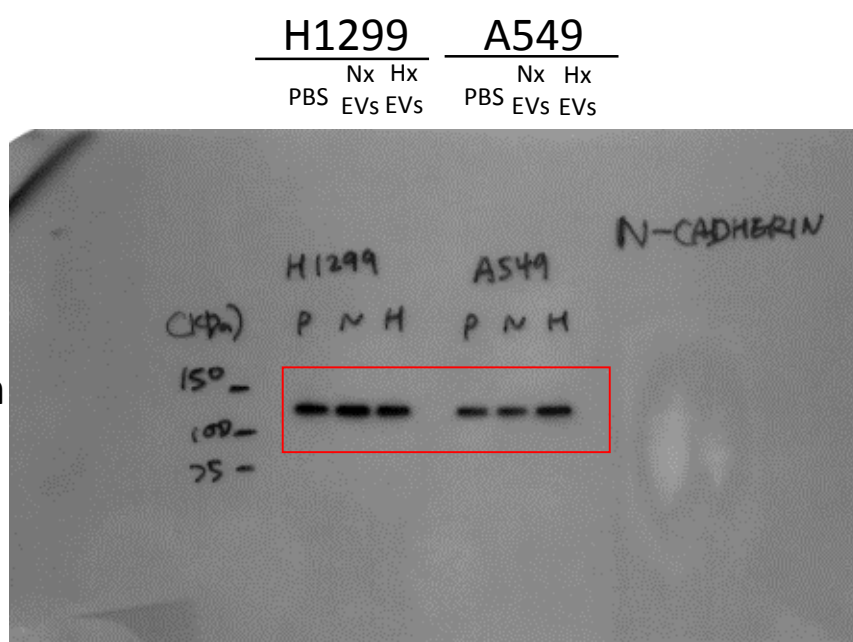


Uncropped western blot images for Figure 1D

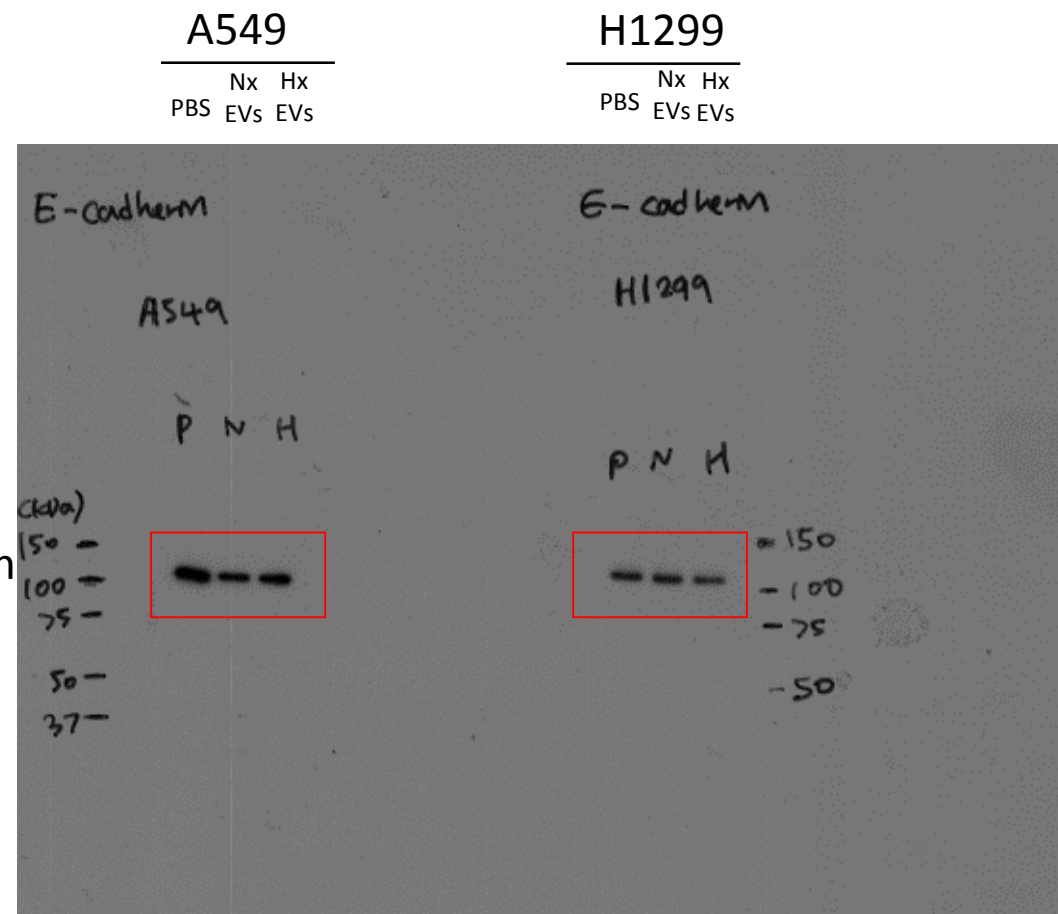


Uncropped western blot images for Figure 3B

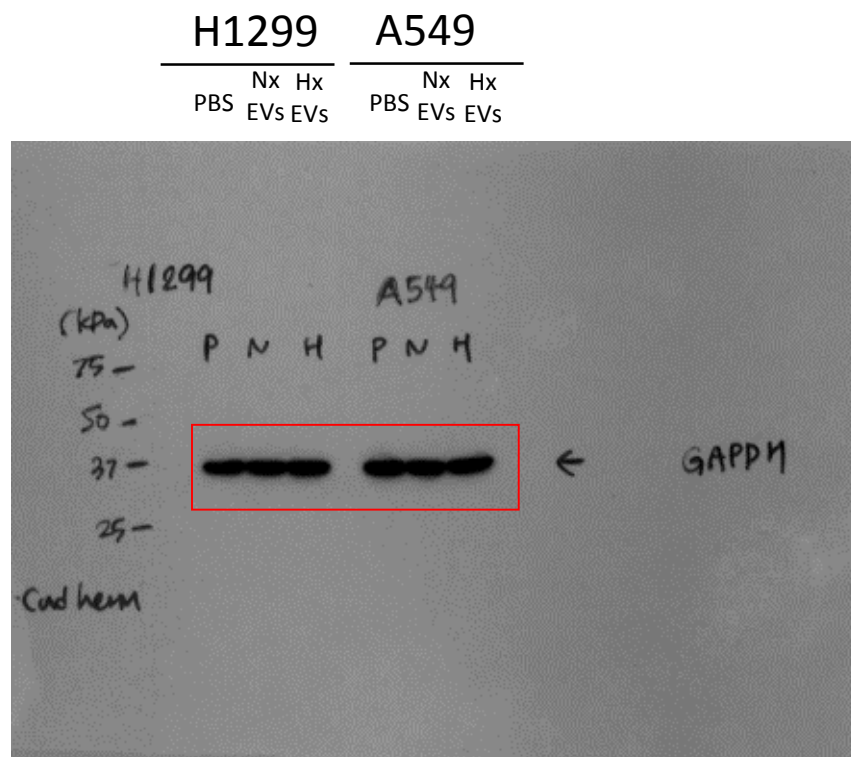
N-cadherin



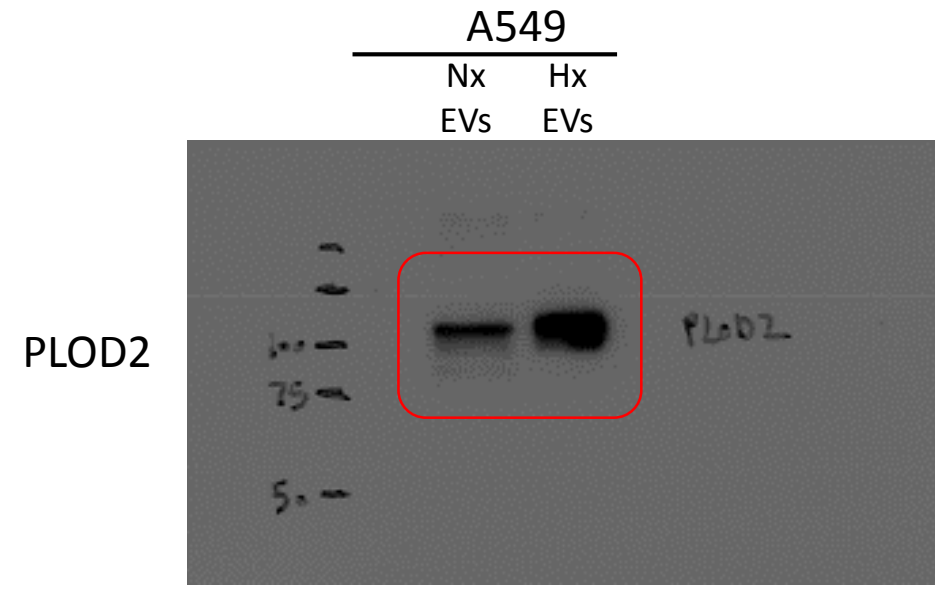
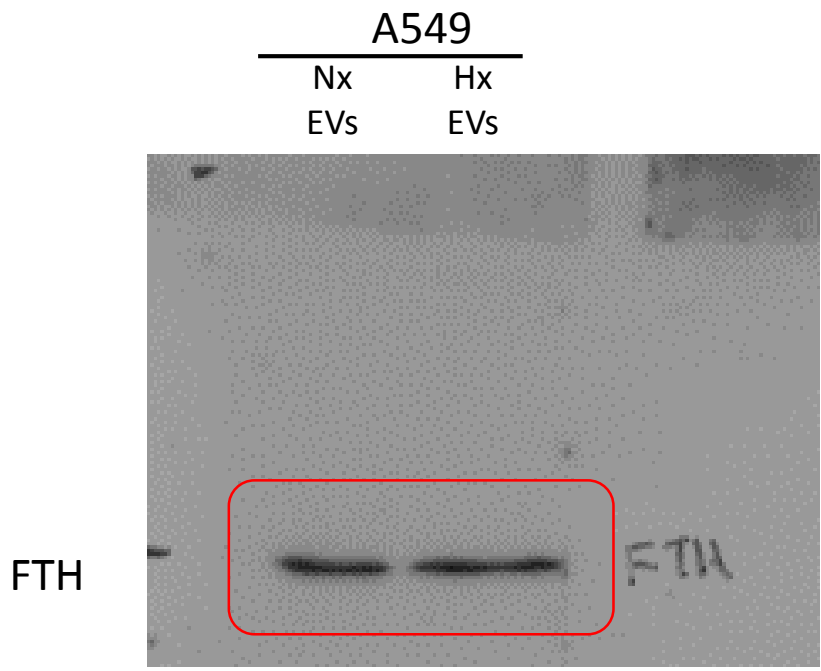
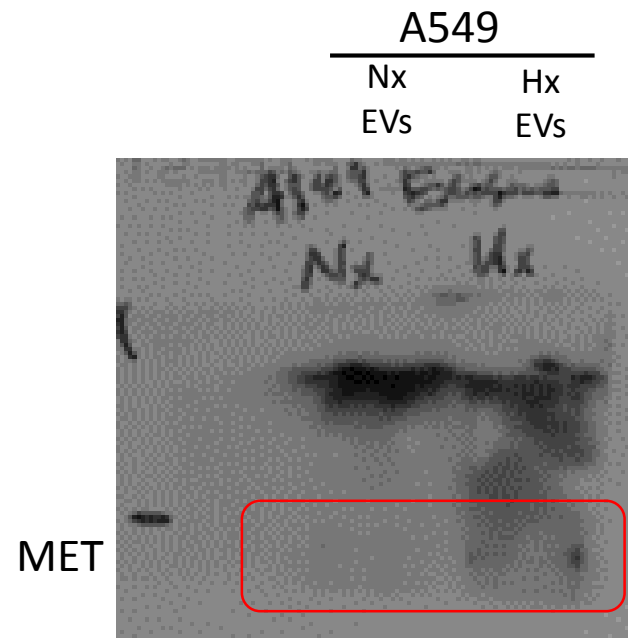
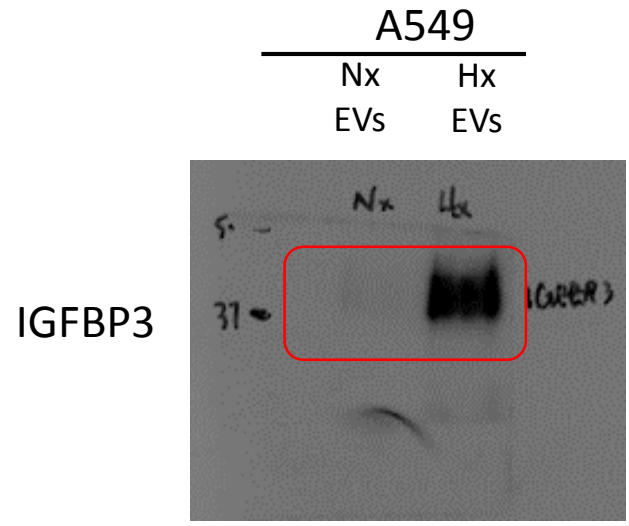
E-cadherin



GAPDH



Uncropped western blot image of Figure 4C



Uncropped western blot images for Figure S1