

Table S1. Top 100 proteins identified in the isolated EVs compared to Vesiclepedia database*Top 100 EV proteins listed in the Vesiclepedia database were downloaded on 02/14/2021.*

Vesiclepedia information		Expression in	
No.	Gene Symbol	Naïve D3-EV	Naïve RAW-EV
1	PDCD6IP	Not detected	Q80Y09_MOUSE Pcd6ip protein OS=Mus musculus OX=10090 GN=Pcd6ip PE=2 SV=1
2	GAPDH	G3P_HUMAN Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens OX=9606 GN=GAPDH PE=1 SV=3	D2KHZ9_MOUSE Glyceraldehyde-3-phosphate dehydrogenase OS=Mus musculus OX=10090 GN=GAPDH PE=2 SV=1
3	HSPA8	Not detected	Q3UBA6_MOUSE Uncharacterized protein OS=Mus musculus OX=10090 GN=Hspa8 PE=2 SV=1
4	ACTB	Not detected	B2RRX1_MOUSE Actin, beta OS=Mus musculus OX=10090 GN=Actb PE=2 SV=1
5	ANXA2	ANXA2_HUMAN Annexin A2 OS=Homo sapiens OX=9606 GN=ANXA2 PE=1 SV=2	Not detected
6	CD9	Not detected	CD9_MOUSE CD9 antigen OS=Mus musculus OX=10090 GN=Cd9 PE=1 SV=2
7	PKM	KPYM_HUMAN Pyruvate kinase PKM OS=Homo sapiens OX=9606 GN=PKM PE=1 SV=4	KPYM_MOUSE Pyruvate kinase PKM OS=Mus musculus OX=10090 GN=Pkm PE=1 SV=4
8	HSP90AA1	HS90A_HUMAN Isoform 2 of Heat shock protein HSP 90-alpha OS=Homo sapiens OX=9606 GN=HSP90AA1	Not detected
9	ENO1	ENOA_HUMAN Alpha-enolase OS=Homo sapiens OX=9606 GN=ENO1 PE=1 SV=2	ENOA_MOUSE Alpha-enolase OS=Mus musculus OX=10090 GN=Eno1 PE=1 SV=3
10	ANXA5	Not detected	ANXA5_MOUSE Annexin A5 OS=Mus musculus OX=10090 GN=Anxa5 PE=1 SV=1

Vesiclepedia information		Expression in	
No.	Gene Symbol	Naïve D3-EV	Naïve RAW-EV
11	HSP90AB1	Not detected	1) ENPL_MOUSE Endoplasmin OS=Mus musculus OX=10090 GN=Hsp90b1 PE=1 SV=2 2) HS90B_MOUSE Heat shock protein HSP 90-beta OS=Mus musculus OX=10090 GN=Hsp90ab1 PE=1 SV=3
12	CD63	Not detected	Not detected
13	YWHAZ	1433Z_HUMAN 14-3-3 protein zeta/delta OS=Homo sapiens OX=9606 GN=YWHAZ PE=1 SV=1	Not detected
14	YWHAE	1433E_HUMAN 14-3-3 protein epsilon OS=Homo sapiens OX=9606 GN=YWHAE PE=1 SV=1	Not detected
15	EEF1A1	Not detected	EF1A1_MOUSE Elongation factor 1-alpha 1 OS=Mus musculus OX=10090 GN=Eef1a1 PE=1 SV=3
16	PGK1	Not detected	PGK1_MOUSE Phosphoglycerate kinase 1 OS=Mus musculus OX=10090 GN=Pgk1 PE=1 SV=4
17	CLTC	CLH1_HUMAN Clathrin heavy chain 1 OS=Homo sapiens OX=9606 GN=CLTC PE=1 SV=5	Q5SXR6_MOUSE Clathrin heavy chain OS=Mus musculus OX=10090 GN=Cltc PE=1 SV=1
18	PPIA	Not detected	PPIA_MOUSE Peptidyl-prolyl cis-trans isomerase A OS=Mus musculus OX=10090 GN=Ppia PE=1 SV=2
19	SDCBP	Not detected	Not detected
20	ALDOA	ALDOA_HUMAN Fructose-bisphosphate aldolase A OS=Homo sapiens OX=9606 GN=ALDOA PE=1 SV=2	A6ZI44_MOUSE Fructose-bisphosphate aldolase OS=Mus musculus OX=10090 GN=Aldoa PE=1 SV=1

Vesiclepedia information		Expression in	
No.	Gene Symbol	Naïve D3-EV	Naïve RAW-EV
21	EEF2	Not detected	EF2_MOUSE Elongation factor 2 OS=Mus musculus OX=10090 GN=Eef2 PE=1 SV=2
22	ALB	ALBU_HUMAN Serum albumin OS=Homo sapiens OX=9606 GN=ALB PE=1 SV=2	ALBU_MOUSE Serum albumin OS=Mus musculus OX=10090 GN=Alb PE=1 SV=3
23	TPI1	TPIS_HUMAN Isoform 2 of Triosephosphate isomerase OS=Homo sapiens OX=9606 GN=TPI1	TPIS_MOUSE Triosephosphate isomerase OS=Mus musculus OX=10090 GN=Tpi1 PE=1 SV=4
24	VCP	Not detected	TERA_MOUSE Transitional endoplasmic reticulum ATPase OS=Mus musculus OX=10090 GN=Vcp PE=1 SV=4
25	CFL1	Not detected	COF1_MOUSE Cofilin-1 OS=Mus musculus OX=10090 GN=Cfl1 PE=1 SV=3
26	MSN	Not detected	MOES_MOUSE Moesin OS=Mus musculus OX=10090 GN=Msn PE=1 SV=3
27	ATP1A1	Not detected	AT1A1_MOUSE Sodium/potassium-transporting ATPase subunit alpha-1 OS=Mus musculus OX=10090 GN=Atp1a1 PE=1 SV=1
28	PRDX1	Not detected	Not detected
29	MYH9	MYH9_HUMAN Myosin-9 OS=Homo sapiens OX=9606 GN=MYH9 PE=1 SV=4	MYH9_MOUSE Myosin-9 OS=Mus musculus OX=10090 GN=Myh9 PE=1 SV=4
30	EZR	Not detected	Not detected
31	CD81	Not detected	Not detected
32	ANXA6	Not detected	Not detected
33	FLOT1	Not detected	Not detected
34	YWHAB	Not detected	Not detected
35	LDHB	Not detected	Not detected
36	SLC3A2	4F2_HUMAN 4F2 cell-surface antigen heavy chain OS=Homo sapiens OX=9606 GN=SLC3A2 PE=1 SV=3	4F2_MOUSE 4F2 cell-surface antigen heavy chain OS=Mus musculus OX=10090 GN=Slc3a2 PE=1 SV=1

Vesiclepedia information		Expression in	
No.	Gene Symbol	Naïve D3-EV	Naïve RAW-EV
37	GNB1	Not detected	
38	PFN1	Not detected	PROF1_MOUSE Profilin-1 OS=Mus musculus OX=10090 GN=Pfn1 PE=1 SV=2
39	TSG101	Not detected	Not detected
40	YWHAQ	1433T_HUMAN 14-3-3 protein theta OS=Homo sapiens OX=9606 GN=YWHAQ PE=1 SV=1	Not detected
41	GNAI2	Not detected	Not detected
42	CLIC1	Not detected	Q542F1_MOUSE Chloride intracellular channel protein OS=Mus musculus OX=10090 GN=Clic1 PE=1 SV=1
43	ANXA1	ANXA1_HUMAN Annexin A1 OS=Homo sapiens OX=9606 GN=ANXA1 PE=1 SV=2	Not detected
44	ITGB1	Not detected	1) ITB2_MOUSE Integrin beta- 2 OS=Mus musculus OX=10090 GN=Itgb2 PE=1 SV=2 2) ITB1_MOUSE Integrin beta-1 OS=Mus musculus OX=10090 GN=Itgb1 PE=1 SV=1
45	LDHA	LDHA_HUMAN Isoform 3 of L-lactate dehydrogenase A chain OS=Homo sapiens OX=9606 GN=LDHA	A0A1B0GSR9_MOUSE L- lactate dehydrogenase OS=Mus musculus OX=10090 GN=Ldha PE=1 SV=1
46	FASN	FAS_HUMAN Fatty acid synthase OS=Homo sapiens OX=9606 GN=FASN PE=1 SV=3	FAS_MOUSE Fatty acid synthase OS=Mus musculus OX=10090 GN=Fasn PE=1 SV=2
47	CDC42	Not detected	Not detected
48	RAP1B	Not detected	Not detected
49	CCT2	TCPB_HUMAN T- complex protein 1 subunit beta OS=Homo sapiens OX=9606 GN=CCT2 PE=1 SV=4	TCPB_MOUSE T-complex protein 1 subunit beta OS=Mus musculus OX=10090 GN=Cct2 PE=1 SV=4

Vesiclepedia information		Expression in	
No.	Gene Symbol	Naïve D3-EV	Naïve RAW-EV
50	YWHAG	1433G_HUMAN 14-3-3 protein gamma OS=Homo sapiens OX=9606 GN=YWHAG PE=1 SV=2	Not detected
51	GNB2	Not detected	Not detected
52	ACTN4	ACTN4_HUMAN Alpha-actinin-4 OS=Homo sapiens OX=9606 GN=ACTN4 PE=1 SV=2	Not detected
53	RAB5C	Not detected	RAB5C_MOUSE Ras-related protein Rab-5C OS=Mus musculus OX=10090 GN=Rab5c PE=1 SV=2
54	C3	Not detected	Not detected
55	RAB10	Not detected	Not detected
56	HIST1H4A	H4_HUMAN Histone H4 OS=Homo sapiens OX=9606 GN=HIST1H4A PE=1 SV=2	Not detected
57	KRT1	1) K2C1_HUMAN Keratin, type II cytoskeletal 1 OS=Homo sapiens OX=9606 GN=KRT1 PE=1 SV=6 2) K1C9_HUMAN Keratin, type I cytoskeletal 9 OS=Homo sapiens OX=9606 GN=KRT9 PE=1 SV=3 3) K1C10_HUMAN Keratin, type I cytoskeletal 10 OS=Homo sapiens OX=9606 GN=KRT10 PE=1	E9Q1Z0_MOUSE Keratin 90 OS=Mus musculus OX=10090 GN=Krt90 PE=1 SV=1
58	FN1	FINC_HUMAN Fibronectin OS=Homo sapiens OX=9606 GN=FN1 PE=1 SV=4	Not detected
59	AHCY	SAHH_HUMAN Adenosylhomocysteinase OS=Homo sapiens OX=9606 GN=AHCY PE=1 SV=4	Not detected
60	A2M	Not detected	Not detected
61	BSG	Not detected	Not detected

Vesiclepedia information		Expression in	
No.	Gene Symbol	Naïve D3-EV	Naïve RAW-EV
62	ACTN1	ACTN1_HUMAN Isoform 3 of Alpha-actinin-1 OS=Homo sapiens OX=9606 GN=ACTN1	Not detected
63	ANXA7	Not detected	Not detected
64	ACLY	ACLY_HUMAN ATP-citrate synthase OS=Homo sapiens OX=9606 GN=ACLY PE=1 SV=3	Not detected
65	HIST1H4B	Not detected	Not detected
66	GDI2	Not detected	Q3TIY6_MOUSE Rab GDP dissociation inhibitor OS=Mus musculus OX=10090 GN=Gdi2 PE=2 SV=1
67	FLNA	FLNA_HUMAN Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4	Not detected
68	UBA1	Not detected	A0A1S6GWH5_MOUSE Uncharacterized protein OS=Mus musculus OX=10090 GN=Uba1 PE=2 SV=1
69	GNAS	Not detected	Not detected
70	GSN	Not detected	Not detected
71	CCT4	TCPD_HUMAN T-complex protein 1 subunit delta OS=Homo sapiens OX=9606 GN=CCT4 PE=1 SV=4	TCPD_MOUSE T-complex protein 1 subunit delta OS=Mus musculus OX=10090 GN=Cct4 PE=1 SV=3
72	RAN	Not detected	Not detected
73	PRDX2	Not detected	Not detected
74	RHOA	Not detected	Not detected
75	CCT3	Not detected	TCPG_MOUSE T-complex protein 1 subunit gamma OS=Mus musculus OX=10090 GN=Cct3 PE=1 SV=1
76	RAC1	Not detected	Not detected
77	LGALS3BP	LG3BP_HUMAN Galectin-3-binding protein OS=Homo sapiens OX=9606 GN=LGALS3BP PE=1 SV=1	LG3BP_MOUSE Galectin-3-binding protein OS=Mus musculus OX=10090 GN=Lgals3bp PE=1 SV=1

Vesiclepedia information		Expression in	
No.	Gene Symbol	Naïve D3-EV	Naïve RAW-EV
78	TCP1	Not detected	TCPA_MOUSE T-complex protein 1 subunit alpha OS=Mus musculus OX=10090 GN=Tcp1 PE=1 SV=3
79	KRT10	Not detected	
80	CAP1	Not detected	CAP1_MOUSE Adenylyl cyclase-associated protein 1 OS=Mus musculus OX=10090 GN=Cap1 PE=1 SV=4
81	RAB7A	Not detected	Not detected
82	TUBB4B	1) TBB5_HUMAN Tubulin beta chain OS=Homo sapiens OX=9606 GN=TUBB PE=1 SV=2 2) TBB4B_HUMAN Tubulin beta-4B chain OS=Homo sapiens OX=9606 GN=TUBB4B PE=1 SV=1	Not detected
83	HSPA5	Not detected	BIP_MOUSE Endoplasmic reticulum chaperone BiP OS=Mus musculus OX=10090 GN=Hspa5 PE=1 SV=3
84	IQGAP1	Not detected	Not detected
85	GPI	Not detected	Not detected
86	RALA	Not detected	Not detected
87	KPNB1	IMB1_HUMAN Importin subunit beta-1 OS=Homo sapiens OX=9606 GN=KPNB1 PE=1 SV=2	Not detected
88	HIST1H4I	Not detected	Not detected
89	TFRC	Not detected	Not detected
90	EIF4A1	1) EIF3L_HUMAN Eukaryotic translation initiation factor 3 subunit L OS=Homo sapiens OX=9606 GN=EIF3L PE=1 SV=1 2) DDX17_HUMAN Probable ATP-dependent RNA helicase DDX17	1) B7ZWF1_MOUSE Ddx3x protein OS=Mus musculus OX=10090 GN=Ddx3x PE=2 SV=1 2) IF4A3_MOUSE Eukaryotic initiation factor 4A-III OS=Mus musculus OX=10090 GN=Eif4a3 PE=1 SV=3

Vesiclepedia information		Expression in	
No.	Gene Symbol	Naïve D3-EV	Naïve RAW-EV
		OS=Homo sapiens OX=9606 GN=DDX17 PE=1 SV=2	3) Q8VH52_MOUSE Translation initiation factor-3 subunit 5 (Fragment) OS=Mus musculus OX=10090 GN=Eif3f PE=2 SV=1
91	HIST4H4	Not detected	Not detected
92	CCT8	TCPQ_HUMAN T-complex protein 1 subunit theta OS=Homo sapiens OX=9606 GN=CCT8 PE=1 SV=4	Q8BVY8_MOUSE Uncharacterized protein OS=Mus musculus OX=10090 GN=Cct8 PE=2 SV=1
93	TLN1	Not detected	Q80TM2_MOUSE MKIAA1027 protein (Fragment) OS=Mus musculus OX=10090 GN=Tln1 PE=2 SV=4
94	HIST1H4K	Not detected	Not detected
95	HIST1H4H	Not detected	Not detected
96	CCT6A	TCPZ_HUMAN T-complex protein 1 subunit zeta OS=Homo sapiens OX=9606 GN=CCT6A PE=1 SV=3	Not detected
97	ANXA11	Not detected	Not detected
98	HIST1H4J	Not detected	Not detected
99	HIST1H4F	Not detected	Not detected
100	HIST1H4D	Not detected	Not detected

Table S2. Gene IDs for D3-EVs versus RAW-EVs

Gene ID	
Naïve D3-EV	Naïve RAW-EV
GAPDH	PDCD6IP
ANXA2	GAPDH
PKM	HSPA8
HSP90AA1	ACTB
ENO1	CD9
YWHAZ	PKM
YWHAE	ENO1
CLTC	ANXA5
ALDOA	HSP90AB1
ALB	EEF1A1
TPI1	PGK1
MYH9	CLTC
SLC3A2	PPIA
YWHAQ	ALDOA
ANXA1	EEF2
LDHA	ALB
FASN	TPI1
CCT2	VCP
YWHAG	CFL1
ACTN4	MSN
HIST1H4A	ATP1A1
KRT1	MYH9
FN1	SLC3A2
AHCY	PFN1
ACTN1	CLIC1
ACLY	ITGB1
FLNA	LDHA
CCT4	FASN
LGALS3BP	CCT2
TUBB4B	RAB5C
KPNB1	KRT1
EIF4A1	GDI2
CCT8	UBA1
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	TLN1
	CCT3
	LGALS3BP
	TCP1
	CAP1
	HSPA5

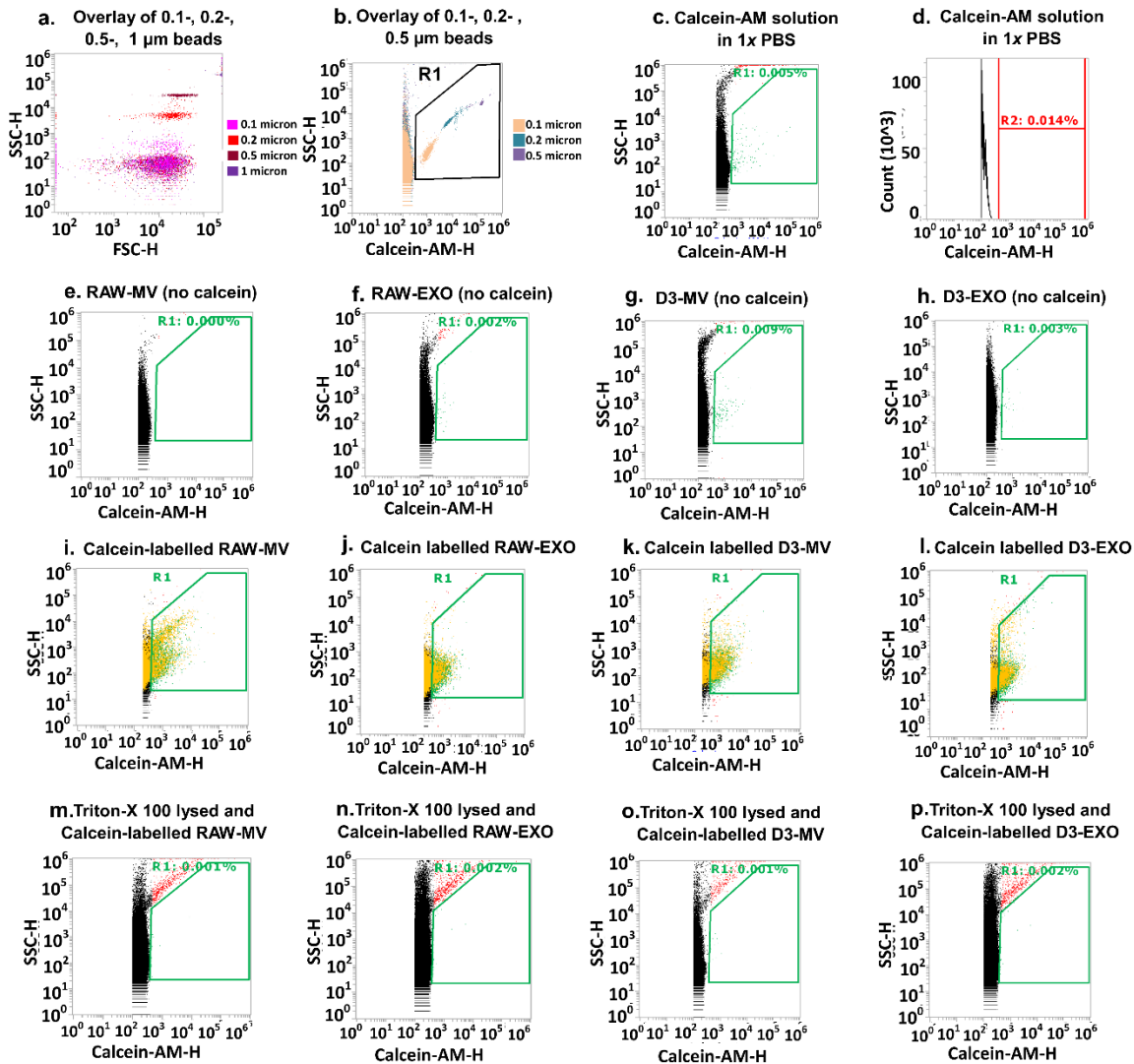
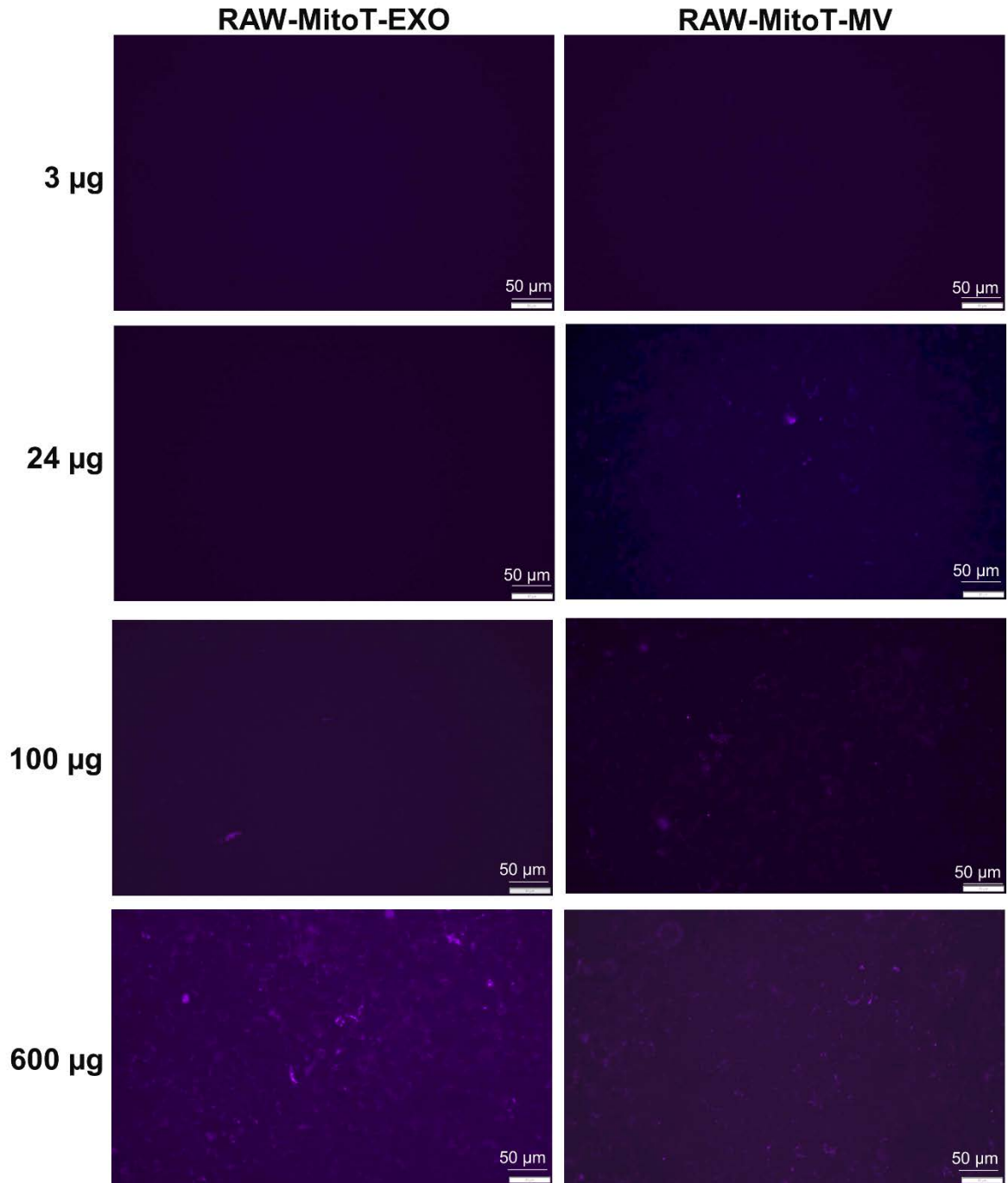
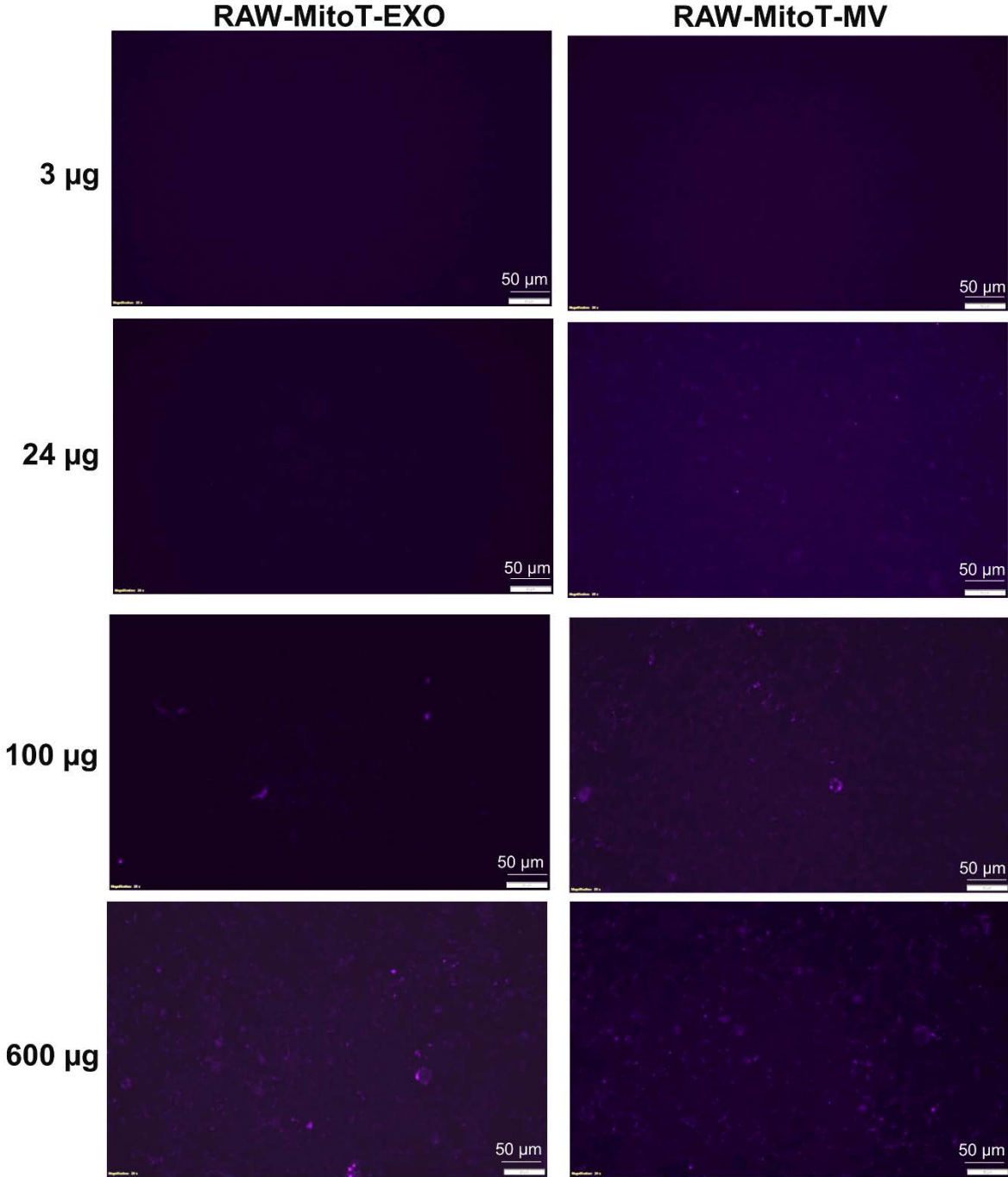


Figure S1. Flow cytometry analysis of EVs. (a) Side scatter (SSC)/Forward scatter (FSC) dot plots of 0.1, 0.2, and 0.5 μm microbeads obtained from an Attune NxT Acoustic Focusing Cytometer. The EV gate was defined below 0.5 μm , (b) SSC/Calcein-AM (BL1) fluorescent profiles of 0.1, 0.2, and 0.5 μm microbeads used to set the EV gate and establish the measurement settings, (c) SSC/Calcein-AM scatter plots and (d) histogram of fluorescent signals demonstrating separation of the instrument noise and background signals for free calcein-AM solution in 1x PBS. EVs were isolated from hCMEC/D3 and RAW 264.7 cell lines and resuspended in 1x PBS. Non-stained RAW-MV (e), RAW-EXO (f), D3-MV (g) and D3-EXO (h) were used as negative controls to verify the absence of fluorescent signals in the gated area. Scatter plots of calcein-stained RAW-MV (i), RAW-EXO (j), D3-MV (k) and D3-EXO (l). Scatter plots of calcein-stained samples treated with Triton X (1 % v/v): (m) RAW-MV (n) RAW-EXO (o) D3-MV and (p) D3-EXO. The data shown are representative plots of n=3 samples.

a. 24 h incubation



b. 48 h incubation



c. 72 h incubation

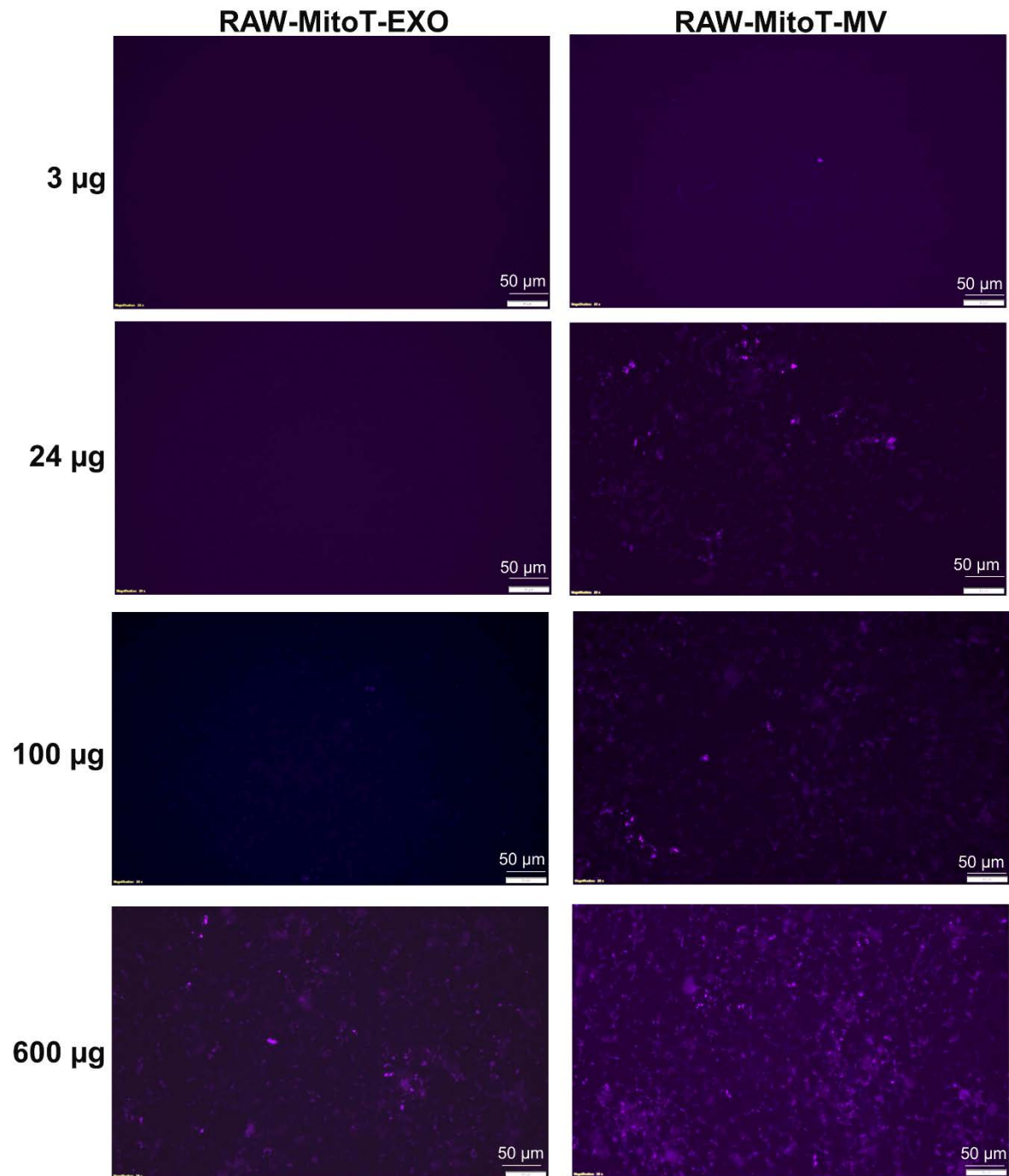


Figure S2. Transfer of mitochondria from RAW-MitoT-EXO and RAW-MitoT-MV to the recipient hCMEC/D3 endothelial cells. The donor/source RAW 264.7 macrophages were stained with MitoTracker Deep-Red (MitoT) (250 nM for 30 min) to specifically label polarized mitochondria following which the MitoT-EVs were isolated from conditioned media. The

recipient hCMEC/D3 endothelial cells were treated with RAW-MitoT-EXO and RAW-MitoT-MV at the indicated protein doses and observed under an Olympus IX 73 epifluorescent inverted microscope (Olympus, Pittsburgh, PA) under the Cy5 channel settings at 24 h, 48 h and 72 h post-treatment. The presented data are representative images from three independent experiments (n=3 per experiment). Scale bar = 50 μ m.

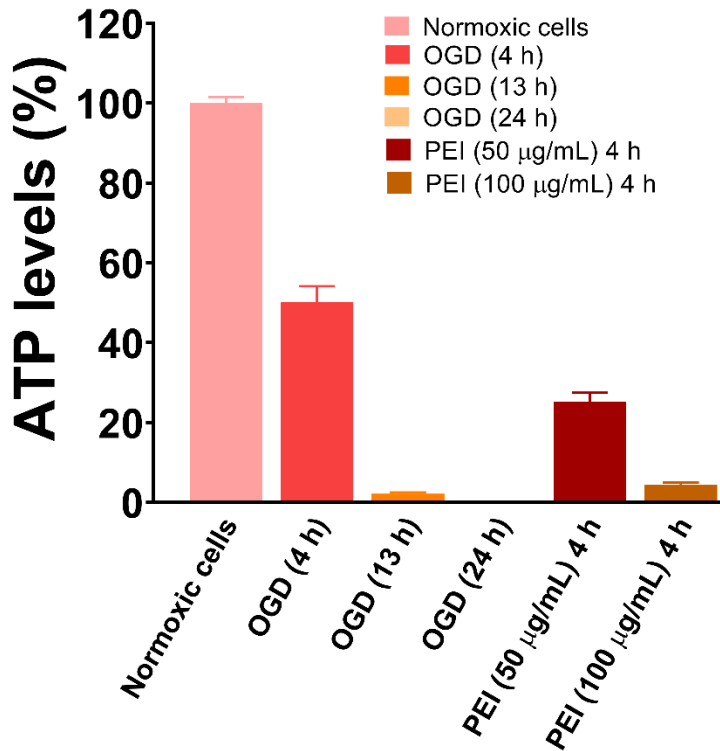


Figure S3. Modelling oxygen-glucose deprivation (OGD)-induced cell death in hCMEC/D3 endothelial cells. Confluent hCMEC/D3 endothelial cells seeded in a 96-well plate were subjected to OGD conditions for the indicated times. OGD was induced by exposing the cells in a sealed hypoxic (90% N₂, 5% H₂, 5% CO₂) chamber and glucose-free media at 37 °C in a humidified incubator. An ATP assay was performed to measure the cell viability post-OGD exposure. The resulting ATP levels were compared to normoxic cells cultured in complete media. Normoxic cells treated with polyethyleneimine (PEI) for 4 h were used as a positive control. Data are represented as mean \pm SD (n = 6).