Table S1. Nomenclature of Extracellular Vesicles and Nanoparticles

Name	Category	EV class[a]	Size	Typical sedimentation	Protein markers	Biogenesis	Comments	Principal References
Classical exosome	Exosome	Small EV	40 - 150 nm	100,000 - 200,000 × *g*[c]	CD63, (CD81, CD9)	MVE	CD63 generally most accepted, CD81 and CD9 likely less specific but present. CD63 strongly labels MVEs[h]	Kowal et al 2016, van Niel et al 2018, this work
Non-classical exosome	Exosome	Small EV	40 - 150 nm	100,000 - 200,000 × *g*	? (CD63-, CD81- and CD9-negative)	MVE	Hypothetical CD63/CD81/CD9-negative exosome. Subpopulations of ILVs and MVEs may exist[i]	van Niel et al 2011, Edgar et al 2014, this work
Classical microvesicle	Microvesicle	Large EV	~ 150 - 1000 nm[b]	10,000 - 20,000 × *g*	Annexin A1, (ARF6, Annexin A2?)	Plasma membrane	This work identifies Annexin A1 as a marker of classical microvesicles	Muralidharan-Charivan et al 2009, this work
Large oncosome	Microvesicle	Large EV	1 - 10 µm	10,000 × *g*	Annexin A1, (ARF6, Annexin A2?)	Plasma membrane	This work identifies Annexin A1 as a likely marker of large oncosomes	Minciacci et al 2015, Minciacci et al 2017, this work
ARMM	Microvesicle	Small EV	~ 40 - 100 nm	100,000 - 200,000 × *g*	ARRDC1, (TSG101)[f]	Plasma membrane	Small EV-sized budding microvesicles that depend on ARRDC1 and TSG101 for biogenesis	Nabhan et al 2012, Wang and Lu 2017, this work
Apoptotic body	Apoptotic EV	Large EV	1 - 5 µm	1,000 - 10,000 × *g*	Annexin V (PS exposure)	Apoptosis	Generally accepted size range for apoptotic bodies	Hristov et al 2004, Crescitelli et al 2013,
Apoptotic vesicle	Apoptotic EV	Small to large EV	~ 100 - 1000 nm	10,000 - 200,000 × *g*[d]	Annexin V (PS exposure)	Apoptosis	Apoptosis-related vesicle, this work demonstrates classical exosomes do not express Annexin V[j]	Hristov et al 2004, Schiller et al 2008, this work
Autophagic EV	Autophagic EV	Small to large EV	40 - 1000 nm?	10,000 - 200,000 × *g*[e]	LC3B-PE, p62	Autophagy	Does not express CD63, CD81 and CD9, extracellular release is induced by inhibition of autophagic flux	Hessvik et al 2016, this work
Exomere	Nanoparticle	Non-EV	~ 35 - 50 nm	100,000 - 200,000 × *g*	HSP90, HSPA13[g]	Unknown	Perhaps represents composite/aggregation of secreted proteins and extracellular debris	Zhang et al 2018, Zhang et al 2019, this work
NV fractions	Nanoparticle	Non-EV	? (vaults: ~ 70 nm)	100,000 - 200,000 × *g*	Fibronectin, Histones, MVP, HSPA13[g]	Unknown	Perhaps represents composite/aggregation of secreted proteins and extracellular debris including vaults	This work

EV, extracellular vesicle; sEV, small extracellular vesicle; MVE, multivesicular endosome; SIM, Structured Illumination Microscopy; ARMM, arrestin-domain-containing protein 1 (ARRDC1)-mediated microvesicle; PS, phosphatidylserine; NV, non-vesicular.

[a]The terms Small EV (<200 nm) and Large EV (>200 nm) for lipid bilayer-delimited extracellular vesicles (EVs) are somewhat arbitrary but widely adopted. In this work, samples were passed through a 220 nm pore filter before isolation of small EV samples.

[b]In this work, we observe Anenxin A1-positive vesicles down to about 150 nm in size using SIM, which is at the resolution limit of reliable identification of a vesicle budding from the plasma membrane. Better resolution may allow <150 nm classical microvesicles to be observed.

[c]Significant sedimentation of exosomes/sEVs occurs already at 33,000 × *g* (Jeppesen et al 2014a). Differential ultracentrifugation will produce enrichment of specific EVs but is incapable of generating samples of purely one type of EV.

[d]Presumed, based on the size-range of vesicles bearing Annexin V.

[e]Presumed, based on the size-range of vesicles bearing LC3B-PE.

[f]In this work, we demonstrate that at least some ARRDC1-positive sEVs also express CD9 and CD81. In particular, CD9-positive sEVs from the Gli36 cell line strongly expressed ARRDC1. The expression of TSG101 was very low in CD63- and CD81-positive sEVs but robust in CD9-positive sEVs.

[g]HSP90 was associated with exomeres (Zhang et al 2018) and is absent from classical exosomes (this work). HSPA13 was associated with exomeres (Zhang et al 2018, Zhang et al 2019), and NV fractions (this work). Other heat shock proteins may be associated with exosomes or sEVs, including HSC70 (Kowal et al 2016, this work).

[h]In this work, we demonstrate that some CD63-positive organelles also stain for LC3B, indicative of amphisomes - the fusion products of MVEs and autophagosomes. However, CD63-positive exosomes do not express LC3B-PE.

[i]There is evidence for CD63-dependent and -independent ILVs (van Niel et al 2011), and for CD63-dependent and Hrs-dependent subpopulations of ILVs in the same MVEs (Edgar et al 2014). sEVs that are distinct from classical exosomes must be significant carriers of extracellular proteins previously associated with exosomes (this work), but these sEVs could be non-classical, MVE-derived, exosome.

[j]At least under conditions where apoptosis is not deliberately induced. In this work, we demonstrate that classical exosomes do express Annexin V, but Annexin V is present in gradient purified sEV fractions.

Table S1. Nomenclature of Extracellular Vesicles and Nanoparticles, Related to Figures 1-7.

Nomenclature of terms used to describe extracellular vesicles and nanoparticles in the context of this study. Note that the term ‘extracellular vesicles’ refers exclusively to lipid bilayer-membrane enclosed vesicles and thus distinct from non-membrane enclosed nanoparticles such as exomeres and the NV fractions, and from lipoprotein complexes such as HDL.

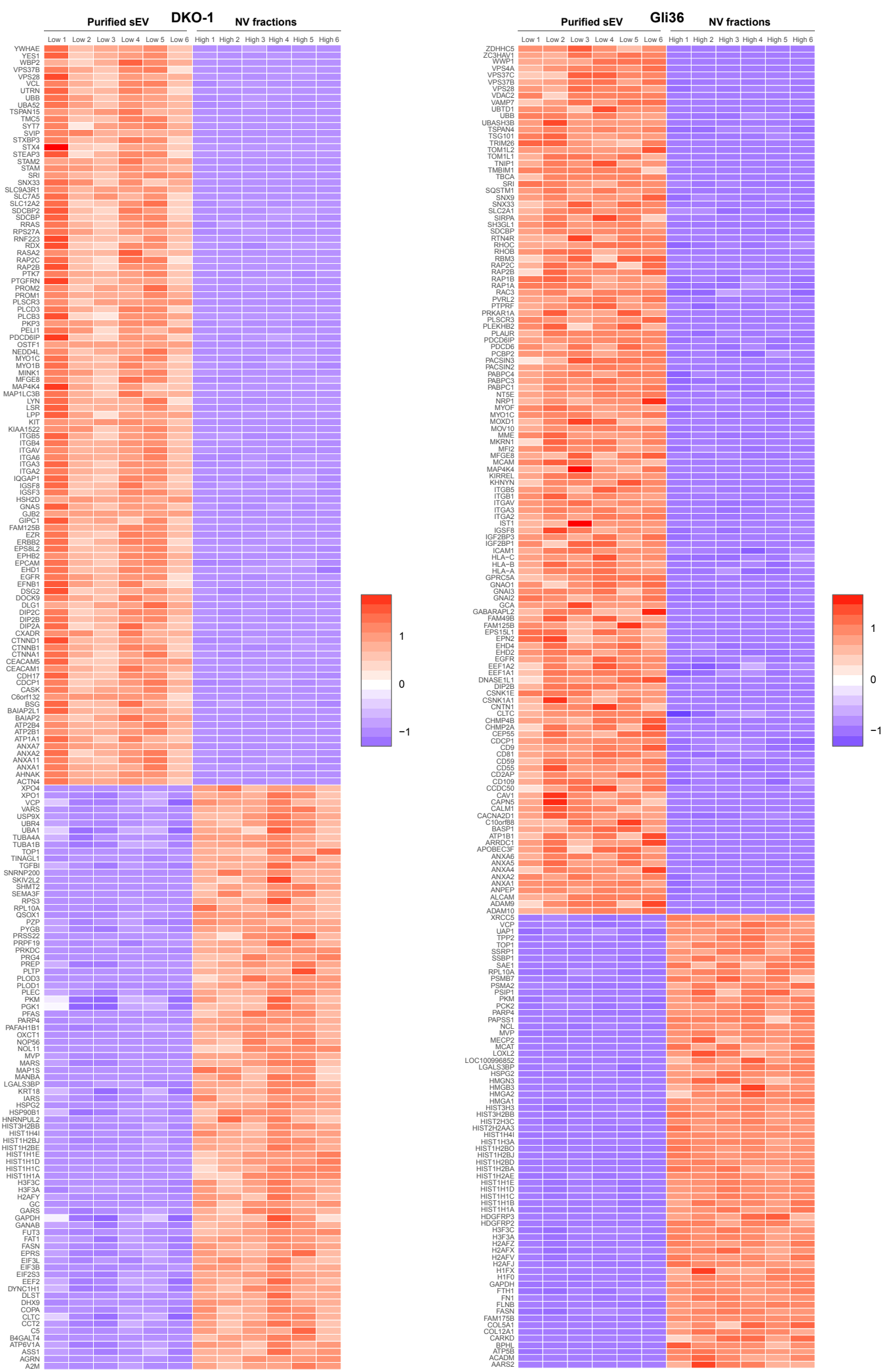

Table S2. Top 200 proteins most significantly differentially expressed between purified sEV and non-vesicular (NV) fractions, Related to Figure 2. Small EVs (low density) and non-vesicular (high density) fractions were separated by high-resolution density gradient centrifugation followed by proteomic analysis. Heat map scale indicates intensity, defined as Δ(spectral counts – mean spectral counts)/standard deviation.

DKO-1

Cell	Large EV	Small EV	Non-vesicular
GAPDH	AHNAK	PDCD6IP	PKM
EEF2	GAPDH	PDCD6IP	CLTC
HSP90AA1	PKM	AHNAK	PKM
HSP90AB1	ACTG1	SDCBP	PKM
KRT18	PKM	SDCBP	DYNC1H1
KRT8	PKM	SDCBP	GAPDH
EEF1A1	ANXA2	NT5E	MVP
FLNA	HSPG2	NT5E	FAT1
PKM	FLNA	ITGB4	FASN
AHNAK	FLNB	ITGB4	PGK1
ACTG1	IQGAP1	DIP2B	HSPG2
ENO1	DYNC1H1	PKM	HSP90AA1
PLEC	ENO1	GAPDH	EEF2
PLEC	CLTC	PKM	FLNB
TUBB	EZR	CLTC	MYH9
PKM	AGRN	ITGA6	PRKDC
PKM	MYH9	PKM	HSP90AB1
HSPD1	MYO1C	ITGA6	UBR4
TUBB4B	PGK1	ACTG1	AGRN
FASN	ALDOA	PTGFRN	VCP
HSPA8	SPTAN1	CTNND1	PLEC
TUBB4A	HSP90AA1	EZR	PLEC
TUBB2B	ACTN4	MYO1C	UBA1
CLTC	ITGB4	HSPA8	TUBB4B
MYH9	SPTBN1	IQGAP1	ENO1
TUBA1B	ITGB4	DYNC1H1	TUBB
SPTAN1	ACTA1	MYOF	HIST1H4I
FLNB	CTNND1	CD81	TUBA1B
SPTBN1	SPTBN1	ENO1	EPRS
DYNC1H1	HSP90AB1	SLC12A2	TGFBI
TUBA1C	MYO1B	ANXA2	KIAA1199
SPTBN1	MYO1B	PLXNB2	PARP4
NCL	ACTG2	PGK1	TUBA1C
PRKDC	PLEC	ITGB1	ACTG1
TUBB2A	PLEC	UBB	TUBB4A
TUBA4A	MYOF	ATP1A1	CLTCL1
EEF1A2	NT5E	RPS27A	TUBB2A
HIST1H4I	HSPA8	UBA52	TUBB2B
ALDOA	MYO1D	FLNB	PDCD6IP
HSP90B1	ITGA6	ITGAV	LGALS3BP
VCP	ITGA6	CTNNB1	PDCD6IP
XRCC5	PLXNB2	MYO1D	GANAB
HNRNPU	YWHAZ	HSP90AA1	TUBA4A
LRPPRC	NT5E	ITGA3	HSPA8
HSPA5	POTEE	HSPA1A	GANAB
HNRNPU	FASN	MFGE8	CCT2
UBA1	LDHA	ADAM10	HSP90B1
CCT2	EEF1A1	CYFIP1	IARS
ANXA2	EEF2	IGSF8	HIST1H2BE
ACTN4	ITGB1	CASK	QSOX1
EPRS	ATP1A1	EPCAM	FLNA
ACTA1	PDCD6IP	CD9	LDHA
GANAB	PDCD6IP	CTNNA1	XPO1
XRCC6	CTNNA1	ALDOA	HIST1H2BJ
GANAB	TUBB	SLC2A1	TUBB3
LDHB	EPCAM	MYO1B	WDR1
HNRNPA2B1	RDX	FAT1	KRT18
TUBA8	HSPA5	MYO1B	PYGB
KRT19	TUBB4B	PDCD6IP	ALDOA
GCN1L1	VCP	HSP90AB1	GFPT1
HNRNPA1	SLC2A1	JUP	PYGL
LDHA	HIST1H4I	EPS8L2	CAND1
LMNA	TPI1	GNAI2	COPA
P4HB	CTNNB1	ITGA2	HIST3H2BB
CCT4	KRT8	TLN1	KPNB1
TUBB6	UBA1	ACTA1	DPYSL2
RPL3	UTRN	FLNA	ASS1
NPM1	TLN1	LDHA	HSPA5
RPL4	ACTG2	SLC3A2	TLN1
HNRNPK	FAM129B	ATP2B1	KRT8
ACTG2	GPI	RDX	PGM1
LMNA	ACTBL2	NCKAP1	IPO7
TUBA3E	KRT18	GNAI3	RNPEP
HIST1H2BE	JUP	UTRN	PSMD2
PDIA3	ACTN1	YWHAZ	CCT8
HSPA9	EPS8L2	FASN	ACLY
YWHAZ	MYH14	EEF1A1	NAMPT
EZR	CFL1	PKP3	MYH14
EIF3A	GNAI3	CDCP1	SNRNP200
TPI1	ITGAV	MYH9	HSPA1A
TUBB3	GNAI2	RAP1B	TUBA8
POTEE	TUBA1B	EGFR	CRYZ
SNRNP200	TUBB2B	POTEE	LDHA
TLN1	GFPT1	NEDD4L	EIF3L
PGK1	TUBB4A	CD55	VARS
DSP	POTEJ	ACTN4	CCT4
SYNCRIP	VCL	MINK1	TUBA3E
NPM1	ANXA1	ACTG2	PGM1
CFL1	SFN	TPI1	PRSS23
CCT6A	LDHA	VCP	GARS
HSPA4	HSPA1A	AP2B1	ERO1L
IPO5	TUBB2A	PTK7	CSE1L
HIST1H2AM	HSP90B1	LLGL2	PLOD1
HSPH1	TUBA1C	IGSF3	XRCC6
HIST1H3A	WDR1	UBA1	ACTA1
IPO7	CAP1	PTK7	USP9X
HIST2H2AC	CAND1	EHD1	CCT5
HMGB1	POTEF	RAP1A	HUWE1
VCL	ANXA5	FAM129B	EEF1A1
CALR	MYO6	SDCBP2	SDCBP

Gli36

Small EV	Non-vesicular
CLTC	GAPDH
PDCD6IP	HIST1H4I
PDCD6IP	MVP
GAPDH	PGK1
NT5E	CLTC
ANPEP	FTH1
SDCBP	PKM
SDCBP	HSP90AB1
HSPA8	PKM
EEF1A1	HIST1H3A
UBB	FASN
HSPA1A	VCP
ENO1	FLNB
PGK1	DYNC1H1
HSP90AB1	FN1
PABPC1	HIST2H3C
EEF1A2	TUBB
HLA-A	FLNA
HSPA5	PCK2
HSP90AA1	HSP90AA1
PKM	ATP5B
DYNC1H1	PARP4
ACTG1	H3F3A
MYO1C	TUBB4B
EHD2	HIST2H2AA3
PABPC4	ACTG1
FTH1	HSP90B1
MOV10	EEF2
MME	HIST1H2BD
PKM	UBA1
HLA-B	HSPA8
CLTCL1	H2AFZ
ANXA6	H2AFJ
ANXA2	PYGL
TLN1	TUBB2A
TUBB	SERPINE1
SQSTM1	TUBB4A
MYOF	HIST1H2BO
TUBA1B	VIM
MFI2	HSPA5
FLNA	NAMPT
EGFR	H2AFY
SH3GL1	HIST1H2AE
CACNA2D1	TOP1
SLC3A2	H2AFY
ITGB1	TUBA1B
TUBA1C	MYH9
HSP90B1	HIST3H3
PPIA	ENO1
ANXA5	H2AFV
LDHA	HSPA1A
TUBB4B	HSPG2
EEF2	HIST1H2BJ
IGSF8	TUBA1C
DIP2B	LGALS3BP
KRT1	GARS
ITGAV	H2AFX
ICAM1	H3F3C
SNX33	TUBB6
TUBA4A	XRCC6
HSPA1L	HIST1H1D
PTPRF	HIST1H1C
EHD1	EPRS
TUBB2A	HIST1H1E
PABPC3	TLN1
FLNB	TUBA4A
UBA1	TUBB3
TUBB4A	XRCC5
TPI1	H1F0
CDCP1	ACTC1
VPS37B	FLNC
HSPA2	GANAB
FASN	OAT
ACTC1	FSCN1
KRT10	ATIC
TSG101	HIST2H2AB
FSCN1	ACTG2
EHD4	CS
VIM	ALDH1A3
HLA-C	EEF1A1
MSN	MIPEP
CD2AP	NDRG1
HIST1H4I	HYOU1
MYH9	BANF1
CD81	HSPA9
HLA-C	HIST1H1B
SNX9	PDCD6IP
ADAM10	AARS
CFL1	UAP1
ANXA11	FTL
VCP	PDCD6IP
VPS28	HIST3H2BB
ALDOA	PXDN
CD55	TUBA3E
PTGFRN	IARS2
PCK2	PLEC
KRT2	PYGB
CD59	CLTCL1
MFGE8	HSPA4
PYGL	SSBP1

Table S5. Top 100 most abundant proteins identified in cells, large EV and high-resolution density gradient purified sEV and non-vesicular fractions, Related to Figure 2.

Proteomic analysis of DKO-1 cells, large EVs, small EVs and non-vesicluar fractions (left), and Gli36 small EVs and non-vesicular fractions (right). Small EVs and non-vesicular fractions were separated by high-resolution density gradient fractionation. Proteins are ranked from top to bottom based on the number of spectral counts.

Table S6. Differential expression of extracellular miRNA between sEV and non-vesicular (NV) fractions, Related to Figure 2.

DKO-1

Enriched in sEV

miRNA	Fold enrichment	p-value (FDR)
miR-1249	Infinite	0.005121695
miR-23a-5p	Infinite	0.002511446
miR-6087	18.512	0.00013301
miR-4516	18.190	0.020737292
miR-1260b	12.783	0.001518903
let-7d-5p	12.229	8.24324E-14
let-7e-5p	10.010	1.87266E-12
miR-5100	8.929	0.00132335
let-7b-5p	8.496	2.01003E-13
miR-3178	8.395	0.013110886
miR-92a-1-5p	7.330	5.38855E-06
miR-25-5p	7.090	3.34438E-05
miR-30b-3p	6.686	0.002309714
miR-4508	5.818	0.003926944
miR-3182	5.522	9.77007E-05
miR-492	5.251	0.016848952
let-7c	4.999	3.99027E-08
miR-98-5p	4.883	9.07274E-09
miR-197-3p	4.862	1.91292E-07
let-7a-5p	4.816	7.87557E-07
miR-1301	4.788	8.35249E-05
miR-139-3p	4.621	0.004441666
miR-1306-5p	4.584	0.014890867
miR-1323	4.194	0.031742701
miR-374b-5p	3.874	0.000281787
miR-374c-3p	3.874	0.000281787
miR-641	3.654	0.001083684
miR-185-5p	3.490	6.79962E-06
miR-516b-5p	3.342	0.005355404
miR-576-5p	3.300	0.005355404
miR-29b-1-5p	3.290	0.012357964
miR-424-3p	3.154	0.001816149
miR-331-3p	3.023	0.016116464
miR-1247-3p	2.928	0.000171117
miR-196a-5p	2.862	0.000944707
miR-26a-5p	2.590	0.000326817
miR-1180	2.522	0.00132335
miR-125a-3p	2.507	0.018563296
miR-196b-5p	2.353	0.00211413
miR-652-3p	2.206	0.031366541
miR-132-3p	2.091	0.006074342
miR-320a	1.975	0.007662384
let-7i-5p	1.970	0.039864818
miR-27a-5p	1.819	0.039609269

Enriched in NV

miRNA	Fold enrichment	p-value (FDR)
miR-1291	14.504	1.91292E-07
miR-4479	10.377	0.011221734
miR-1307-5p	10.150	8.34514E-20
miR-22-3p	10.095	1.11159E-38
miR-3074-5p	9.794	1.50141E-36
miR-24-3p	9.233	6.37581E-35
miR-193a-5p	8.566	1.00825E-09
miR-9-3p	8.494	7.4573E-10
miR-629-5p	6.588	7.97809E-25
miR-129-5p	6.384	3.71236E-05
miR-10b-3p	6.174	3.12024E-07
let-7i-3p	5.932	0.002671421
miR-3191-5p	5.372	0.005121695
miR-101-3p	5.162	6.61305E-18
miR-3190-3p	5.130	0.002671421
miR-335-5p	4.859	6.73765E-16
miR-1246	4.649	0.008675648
miR-10a-3p	4.447	8.09872E-16
miR-378c	4.288	2.48378E-09
miR-3605-3p	3.790	0.000219997
miR-629-3p	3.389	0.000826114
miR-320e	3.330	0.003394286
miR-130b-3p	3.328	0.001518903
miR-320d	3.315	0.001029466
miR-95	3.180	7.35126E-09
miR-378d	3.161	0.000606018
miR-107	3.059	1.45028E-06
miR-29c-3p	2.878	0.001816149
miR-193b-5p	2.758	0.004859571
miR-215	2.574	1.14086E-05
miR-140-3p	2.568	1.77963E-06
miR-181a-5p	2.438	4.95428E-05
miR-328	2.437	6.64054E-05
miR-92b-3p	2.356	0.011375891
miR-181b-5p	2.315	7.90088E-05
miR-29a-3p	2.280	0.00015546
miR-3184-5p	2.280	1.4716E-05
miR-152	2.269	3.71236E-05
miR-1287	2.261	0.0443721
miR-192-5p	2.201	0.000432721
miR-3615	2.182	0.000321644
miR-423-3p	2.149	6.64054E-05
miR-203b-3p	2.139	0.00115975
miR-375	2.106	8.68019E-05
miR-500a-3p	2.024	0.020431581
miR-320c	1.934	0.01540494
miR-3928	1.930	0.033329499
miR-378a-3p	1.928	0.002243453
miR-1304-3p	1.909	0.033329499
miR-651	1.890	0.02257969
miR-103a-3p	1.878	0.001152357
miR-103b	1.878	0.001152357
miR-484	1.862	0.00763591
miR-20a-5p	1.829	0.004196148
miR-584-5p	1.811	0.011512276
miR-92a-3p	1.811	0.005121695
miR-210	1.771	0.019523623
miR-146a-5p	1.762	0.015843339
miR-221-3p	1.720	0.0443721
miR-28-3p	1.715	0.011190766

Gli36

Enriched in sEV

miRNA	Fold enrichment	p-value (FDR)
miR-1236-5p	Infinite	2.38577E-12
miR-1225-5p	Infinite	1.25306E-05
miR-3676-5p	Infinite	0.001888493
miR-3620-3p	Infinite	0.003500437
miR-3940-3p	Infinite	0.006605787
miR-4700-3p	Infinite	0.006756923
miR-574-5p	Infinite	0.007004365
miR-6511b-5p	Infinite	0.023992038
miR-4443	190.216	8.83084E-11
miR-1275	106.311	1.61415E-29
miR-1229-5p	70.218	0.002860958
miR-324-5p	49.582	0.000375731
miR-23a-5p	42.661	3.59764E-39
miR-365a-5p	32.555	1.95513E-57
miR-378a-5p	31.077	2.51746E-14
miR-197-5p	29.640	3.07266E-29
miR-877-3p	24.591	0.001126995
miR-671-5p	22.256	2.72141E-19
miR-34a-5p	20.585	0.002213908
miR-149-3p	18.990	2.71965E-20
miR-365b-5p	17.913	2.93753E-38
miR-30b-3p	17.480	1.42828E-22
miR-769-3p	16.750	0.013099709
miR-30c-1-3p	16.153	0.010617826
miR-1296	15.663	0.022946487
miR-5100	14.119	2.5596E-14
miR-92b-5p	13.471	1.85341E-23
miR-664b-5p	13.311	0.038311784
miR-1226-5p	13.283	0.019381796
miR-2277-5p	12.573	2.48366E-10
miR-331-3p	11.439	0.000272393
miR-29b-1-5p	11.037	3.22655E-12
miR-1908	10.123	9.64036E-18
miR-1236-3p	9.068	5.19374E-13
miR-3182	8.536	0.000599701
miR-503-3p	8.476	9.53712E-05
miR-652-3p	8.244	6.82654E-06
miR-532-3p	7.980	0.00336107
miR-193b-5p	7.439	4.76071E-20
let-7b-5p	7.327	1.21871E-26
miR-140-5p	7.275	2.44209E-22
let-7e-5p	6.944	1.56845E-20
miR-195-5p	6.768	3.37288E-05
miR-935	6.483	0.0025355
miR-342-5p	6.407	0.037863457
miR-93-3p	6.305	0.005811746
miR-193b-3p	6.203	2.8574E-09
miR-25-5p	5.731	5.63155E-06
miR-1260b	5.501	0.03745708
miR-151a-5p	5.246	5.25486E-06
miR-744-5p	5.149	1.05909E-14
miR-138-5p	5.099	0.000692993
miR-339-5p	4.965	0.000272393
miR-374b-5p	4.764	9.76307E-10
miR-374c-3p	4.764	9.76307E-10
miR-874	4.553	0.001167393
miR-454-3p	4.436	0.000802099
miR-23b-5p	4.390	0.004608123
miR-16-5p	4.241	1.63666E-11
miR-99b-3p	4.053	1.65409E-07
miR-4521	3.938	0.043802516
miR-92a-1-5p	3.916	2.55567E-07
miR-1180	3.890	7.69145E-08
miR-125a-3p	3.884	0.002764717
miR-126-5p	3.870	0.009899547
miR-769-5p	3.809	1.99282E-07
miR-365b-3p	3.724	0.048480747
miR-365a-3p	3.724	0.048480747
miR-374a-5p	3.455	4.26559E-08
let-7d-5p	3.245	1.31339E-07
miR-425-3p	3.223	0.006307783
miR-455-3p	3.104	0.001975098
miR-210	3.078	3.43344E-08
miR-15b-5p	2.942	0.001630291
miR-425-5p	2.861	9.33411E-06
miR-1307-3p	2.693	3.57584E-05
miR-19b-3p	2.450	0.020382871
miR-30b-5p	2.408	0.000192772
miR-671-3p	2.387	0.000802463
miR-31-5p	2.201	7.32209E-05
miR-30c-2-3p	1.973	0.042146601
miR-361-3p	1.966	0.009899547
miR-423-3p	1.835	0.006605787
miR-3184-5p	1.757	0.016278778
miR-28-5p	1.756	0.034548805

Enriched in NV

miRNA	Fold enrichment	p-value (FDR)
miR-4661-5p	Infinite	0.027843937
miR-215	59.698	1.87176E-06
miR-452-5p	53.656	0.000115088
miR-335-5p	35.639	0.000886052
miR-335-3p	35.545	4.04217E-05
miR-148a-3p	35.514	1.10994E-36
miR-200a-5p	24.988	6.4363E-05
miR-200b-3p	23.785	2.06953E-50
miR-200c-3p	19.975	4.58537E-52
miR-375	19.483	1.59982E-50
miR-1291	17.893	0.001707206
miR-22-3p	17.802	6.88043E-79
miR-10b-5p	16.450	1.19132E-49
miR-3074-5p	15.061	6.15373E-71
miR-224-5p	15.030	1.4267E-17
miR-184	14.606	1.40258E-11
miR-200b-5p	14.231	5.82675E-05
miR-193a-5p	13.711	5.6144E-66
miR-320d	12.881	0.000199904
miR-24-3p	11.854	1.84227E-62
miR-3909	11.754	0.000684607
miR-1304-3p	10.863	0.000248829
miR-200a-3p	10.715	3.19293E-40
miR-1307-5p	9.487	7.44722E-19
miR-629-5p	9.358	9.49043E-17
miR-378i	9.172	1.3915E-12
miR-625-5p	9.001	0.004073497
miR-429	8.703	1.36806E-06
miR-141-3p	7.625	1.55299E-07
miR-3605-3p	7.587	1.25876E-11
miR-92b-3p	7.541	5.23634E-30
miR-378c	7.331	1.81494E-12
miR-192-5p	7.007	1.86856E-09
miR-625-3p	6.177	0.000203018
miR-320c	6.119	5.63863E-06
miR-27b-5p	5.868	0.000101367
miR-378a-3p	5.857	5.85365E-13
miR-140-3p	5.526	2.88794E-26
miR-339-3p	5.513	1.67232E-23
miR-3158-5p	5.352	0.008418044
miR-152	5.276	4.44765E-14
miR-10a-5p	5.275	1.14243E-29
miR-10a-3p	5.239	2.03899E-10
miR-320b	5.091	4.3029E-09
miR-363-3p	4.756	6.57425E-06
let-7d-3p	4.679	1.82846E-10
miR-378d	4.111	0.001877832
let-7f-5p	3.881	1.06414E-19
miR-128	3.770	2.91475E-13
miR-9-3p	3.691	8.12733E-09
miR-3615	3.625	2.93304E-06
miR-708-3p	3.503	0.023523389
miR-125a-5p	3.470	2.44266E-17
miR-181b-5p	3.431	9.09106E-09
miR-130b-3p	3.405	0.000225281
miR-3158-3p	3.232	0.039211927
miR-1468	3.221	0.009693567
miR-130b-5p	3.090	4.8246E-07
miR-30e-3p	3.070	5.18956E-11
miR-30a-3p	3.041	3.98371E-12
miR-22-5p	2.887	1.75393E-06
miR-27b-3p	2.843	9.09835E-12
let-7b-3p	2.751	0.016885707
miR-584-5p	2.609	0.009899547
miR-107	2.508	0.00011606
miR-501-3p	2.500	0.022367484
miR-345-5p	2.479	0.003479991
miR-130a-3p	2.427	3.86817E-05
miR-95	2.412	0.000120622
miR-103a-3p	2.410	4.37759E-09
miR-103b	2.410	4.37759E-09
miR-2682-5p	2.407	5.82675E-05
miR-203a	2.349	2.1339E-05
miR-203b-5p	2.337	2.70771E-05
miR-450b-5p	2.312	0.042146601
miR-99a-5p	2.260	9.83661E-06
miR-194-5p	2.008	0.01364046
miR-99b-5p	1.987	1.47442E-05
miR-25-3p	1.944	9.88793E-05
miR-330-3p	1.930	0.008066346
miR-28-3p	1.910	0.000493414
miR-27a-3p	1.829	0.000410262
miR-92a-3p	1.814	0.000272456
miR-182-5p	1.766	0.001946666
miR-183-5p	1.702	0.006605787
miR-146b-5p	1.652	0.043436673
let-7g-5p	1.619	0.011136507
miR-101-3p	1.612	0.015431956
miR-532-5p	1.590	0.029444596
miR-320a	1.572	0.019766344
miR-29a-3p	1.561	0.014872613
miR-26a-5p	1.518	0.042146601

Table S6. Differential expression of extracellular miRNA between sEV and non-vesicular (NV) fractions, Related to Figure 2. Significant differential expression of miRNAs between density gradient purified sEV (low density) and NV (high density) fractions. Included are miRNA that are significantly (FDR-adjusted p-value < 0.05) different between samples.

Table S7. Differential expression of miRNA between sEVs and parental cells, Related to Figure 2.

DKO-1

Enriched in sEV		
miRNA	Fold enrichment	p-value (FDR)
miR-3655	Infinite	4.70978E-13
miR-105-5p	Infinite	2.04769E-08
miR-4680-3p	Infinite	1.31609E-06
miR-4492	Infinite	8.60079E-06
miR-708-3p	Infinite	0.000157405
miR-767-5p	Infinite	0.000161031
miR-223-5p	Infinite	0.019209336
miR-4488	1804.609	1.49977E-10
miR-3178	422.181	5.09375E-35
miR-552	283.755	1.25171E-95
miR-6087	262.881	8.51081E-05
miR-4516	255.845	8.66747E-21
miR-492	206.371	3.782E-53
miR-3656	145.064	1.21256E-11
miR-1244	111.079	1.26063E-08
miR-4508	91.519	3.27675E-54
miR-4680-5p	74.011	7.12838E-06
miR-4709-3p	72.361	1.85865E-05
miR-622	44.538	1.61814E-10
miR-205-5p	41.887	0.000278353
miR-4700-5p	39.351	4.05853E-08
miR-320e	35.159	3.37923E-81
miR-320d	33.232	0
miR-1246	32.538	2.0532E-153
miR-139-3p	31.587	4.67079E-48
miR-139-5p	31.327	0
miR-4497	28.123	3.00828E-17
miR-125a-5p	27.806	0
miR-95	24.348	2.67207E-67
miR-4647	24.301	0.000355041
miR-543	23.631	0.02386302
miR-3929	23.380	0.00020019
miR-4709-5p	21.275	0.043838734
miR-3617-5p	20.627	9.37596E-08
miR-135b-5p	15.809	3.2324E-164
miR-125a-3p	15.024	1.1762E-84
miR-127-3p	14.979	0.042644537
miR-509-3p	13.908	0.03328933
miR-153	13.583	0.000149454
miR-4724-5p	12.996	0.000101085
miR-4510	11.846	5.60716E-05
miR-3605-3p	11.724	3.13392E-37
miR-320c	10.690	1.6548E-26
miR-1468	10.514	2.63989E-21
miR-3614-5p	9.232	0.003394943
miR-664a-5p	9.177	9.17537E-46
miR-99b-5p	8.674	0
miR-181a-5p	8.266	2.2839E-200
let-7e-5p	8.159	9.0465E-187
miR-516a-5p	8.126	3.37996E-05
miR-141-3p	7.820	0
miR-3182	7.612	3.10853E-24
miR-3605-5p	7.433	0.000232411
miR-224-5p	7.397	2.9691E-174
miR-338-5p	7.351	3.97394E-16
miR-1277-3p	7.244	0.013084336
miR-10b-5p	7.239	1.9747E-151
miR-375	7.233	0
let-7c	6.781	7.2929E-194
miR-934	6.757	3.37263E-07
miR-99a-5p	6.613	2.77785E-17
miR-146b-5p	6.520	1.3862E-131
let-7b-5p	6.493	1.1294E-191
miR-523-3p	6.422	0.033308577
let-7a-5p	6.363	2.0037E-161
miR-27a-3p	6.363	5.6018E-189
miR-184	6.146	0.001677301
miR-23a-3p	5.955	7.832E-168
miR-939-5p	5.921	0.029833036
miR-616-3p	5.701	0.019209336
miR-320b	5.689	1.49E-139
miR-451a	5.629	1.92422E-06
miR-203a	5.608	8.9862E-162
miR-203b-5p	5.596	2.4618E-161
miR-142-5p	5.534	6.4154E-15
miR-4708-5p	5.501	0.020320854
miR-129-5p	5.276	0.00193896
miR-642a-3p	5.197	2.57271E-08
miR-642b-5p	5.197	2.57271E-08
miR-664a-3p	4.966	5.81721E-13
miR-483-5p	4.924	0.003890983
miR-514a-3p	4.651	6.64653E-05
miR-3074-3p	4.570	7.72471E-22
miR-6716-3p	4.565	0.00016912
miR-100-5p	4.487	9.55594E-73
miR-328	4.432	2.09627E-16
miR-3065-3p	4.401	8.51598E-12
miR-222-3p	4.365	1.86175E-76
miR-143-3p	4.305	1.02415E-08
miR-210	4.056	3.47579E-46

Enriched in Cell		
miRNA	Fold enrichment	p-value (FDR)
miR-1275	Infinite	9.81446E-09
miR-3662	Infinite	1.24759E-08
miR-573	Infinite	0.00065315
miR-652-5p	Infinite	0.003185544
miR-4791	Infinite	0.00352673
miR-3170	Infinite	0.005831891
miR-4517	Infinite	0.009013818
miR-1257	Infinite	0.013635142
miR-4521	27.560	3.08294E-09
miR-1305	26.512	0.002824
miR-3145-3p	25.235	0.001106078
miR-556-3p	24.370	2.02458E-05
miR-3116	22.543	0.007677828
miR-19b-1-5p	22.436	0.009511301
miR-2277-5p	21.970	4.12297E-28
miR-5701	18.417	0.000203264
miR-3944-3p	16.542	0.042765388
miR-3619-5p	14.771	1.21377E-07
miR-188-5p	12.827	2.78424E-06
miR-4802-3p	11.573	7.58056E-12
miR-4687-3p	11.379	0.011941098
miR-181b-3p	11.272	1.5175E-24
miR-671-5p	11.257	1.01949E-41
miR-18a-5p	10.597	1.62546E-43
miR-4470	9.766	0.012175725
miR-1296	9.129	4.75862E-19
miR-582-5p	8.571	8.67023E-06
miR-7-5p	8.195	1.96983E-62
miR-3529-3p	8.184	7.71032E-62
miR-1914-5p	8.135	0.005130632
miR-16-1-3p	7.533	2.57177E-06
miR-548u	6.735	0.013705458
miR-449c-5p	6.662	9.81144E-06
miR-185-3p	6.283	3.55976E-15
miR-491-5p	6.150	2.69102E-09
miR-15a-5p	6.146	1.76282E-13
miR-500a-5p	6.080	0.011770198
miR-33b-3p	5.952	0.000199165
miR-4659a-3p	5.763	0.003599157
miR-4446-3p	5.688	0.02358975
miR-5001-3p	5.633	4.96868E-07
miR-301a-3p	5.423	0.000822508
miR-17-5p	5.074	2.88082E-32
miR-3200-3p	4.926	1.12411E-10
miR-362-5p	4.774	1.34627E-12
miR-326	4.736	0.00095297
miR-3187-3p	4.647	1.4951E-05
miR-107	4.489	4.52082E-27
miR-15b-5p	4.470	6.92977E-33
miR-542-3p	4.359	8.53609E-16
miR-877-3p	4.323	9.89344E-08
miR-548n	4.322	0.040189372
miR-500a-3p	4.290	1.53307E-21
miR-373-3p	4.206	1.21078E-08
miR-4474-3p	4.181	0.006004349
miR-106b-5p	4.045	2.15194E-32
miR-4664-3p	4.034	7.78091E-11
miR-301a-5p	4.017	4.13582E-18

Gli36

Enriched in sEV		
miRNA	Fold enrichment	p-value (FDR)
miR-934	Infinite	0.007209507
miR-1225-3p	Infinite	0.00697912
miR-451a	Infinite	0.000128247
miR-1244	Infinite	1.60859E-05
miR-4700-3p	Infinite	1.16049E-08
miR-516b-5p	Infinite	4.41676E-10
miR-4658	Infinite	3.62694E-12
miR-1225-5p	Infinite	1.70566E-15
miR-1236-5p	606.177	9.08161E-26
miR-4443	335.398	8.15279E-40
miR-1236-3p	203.409	4.61871E-41
miR-5100	192.482	6.02661E-72
miR-365a-5p	190.159	4.83915E-24
miR-197-5p	126.403	3.75988E-52
miR-193b-5p	107.762	3.53243E-52
miR-3656	91.708	0.021041802
miR-149-3p	87.745	7.57006E-35
miR-1229-5p	82.042	3.1972E-07
miR-224-5p	74.012	7.69927E-09
miR-6087	63.541	1.15049E-07
miR-3676-5p	55.660	2.11248E-06
miR-503-3p	51.613	5.89674E-18
miR-365b-5p	46.484	2.72704E-32
miR-5088	46.212	0.018914824
miR-141-3p	34.608	2.02316E-07
miR-23a-5p	27.072	1.20113E-21
miR-6721-5p	25.843	7.30788E-05
miR-4497	24.846	5.05748E-06
miR-6511b-5p	23.142	0.001597103
miR-3913-5p	22.609	0.007209507
miR-3913-3p	22.609	0.007209507
miR-375	21.857	2.3239E-08
miR-1226-5p	21.192	2.7597E-09
miR-509-3-5p	20.382	0.002079343
miR-1275	19.215	1.1459E-13
miR-146b-3p	18.258	3.86336E-06
miR-664b-5p	16.341	0.00023185
miR-92b-5p	15.861	7.7856E-16
miR-3929	15.664	0.000167102
miR-216a-5p	15.621	0.027277456
miR-1268b	15.526	0.000121051
miR-216a-3p	13.283	0.031330801
miR-146a-5p	13.043	1.22906E-17
miR-181c-5p	12.510	0.00023185
miR-200a-3p	12.046	0.000277134
miR-210	11.899	1.48126E-18
miR-146a-3p	10.962	0.002239386
miR-184	10.915	0.002920092
miR-135b-5p	10.815	0.042449535
miR-92a-1-5p	10.689	2.57097E-08
miR-574-5p	9.811	0.013012527
miR-95	9.533	5.24033E-08
miR-3605-3p	9.404	0.011151548
miR-483-5p	9.287	0.047348198
miR-1	9.244	0.001615857
miR-708-5p	8.928	0.008218985
miR-30b-3p	8.361	1.76847E-08
miR-671-5p	8.170	4.41676E-10
miR-2277-5p	7.653	2.44688E-07
miR-193b-3p	7.648	8.15671E-07
miR-3687	7.104	0.0401428
miR-3182	7.078	0.002157102
miR-1908	6.619	3.90877E-07
miR-139-3p	6.210	9.5823E-05
miR-195-5p	6.072	0.000844722
miR-181a-5p	5.971	6.56181E-13
miR-671-3p	5.840	9.62043E-07
miR-194-3p	5.769	0.007590316
miR-615-5p	5.443	0.008373052
miR-148a-3p	5.116	0.002450874
miR-203b-5p	5.036	2.3518E-07
miR-203a	5.016	2.31697E-07
miR-324-5p	4.960	0.004022675
miR-200b-3p	4.785	0.00522479
miR-140-5p	4.556	4.17038E-09
miR-146b-5p	4.553	8.02533E-07
miR-423-3p	4.236	3.91414E-10
miR-935	4.135	0.046277211
miR-3184-5p	4.040	1.18266E-09

Enriched in Cell		
miRNA	Fold enrichment	p-value (FDR)
miR-494	Infinite	0.015048719
miR-4661-5p	Infinite	0.014350076
miR-654-3p	Infinite	0.001097456
miR-543	Infinite	6.42481E-07
miR-381-3p	Infinite	1.74435E-12
miR-379-5p	Infinite	2.36629E-15
miR-409-3p	Infinite	1.75648E-16
miR-382-5p	38.140	0.002239386
miR-411-5p	37.370	0.000316201
miR-7-5p	24.722	2.60627E-42
miR-3529-3p	24.483	8.2222E-47
miR-584-5p	20.511	6.85083E-42
miR-1304-3p	15.093	1.97592E-06
miR-760	12.322	2.51759E-05
miR-2276	11.924	0.00682725
miR-937-3p	11.618	0.00499653
miR-27b-5p	11.146	2.81223E-09
miR-3934-5p	10.292	0.001308124
miR-1301	9.430	7.05544E-09
miR-378a-3p	9.298	1.28122E-23
miR-3909	9.124	0.012784519
miR-15b-3p	8.073	4.37141E-05
miR-942	6.564	0.011501358
miR-378i	6.493	1.24626E-06
miR-185-5p	6.228	3.06863E-26
miR-378c	6.024	2.80384E-08
miR-105-5p	5.852	3.12374E-08
miR-98-5p	5.570	9.08161E-26
miR-128	5.219	1.05998E-25
miR-378d	5.089	0.000604114
miR-18a-5p	4.981	0.003447325
let-7f-5p	4.899	2.40093E-28
miR-25-3p	4.829	1.50062E-26
miR-3615	4.522	1.25035E-07
miR-501-3p	4.512	5.62657E-06
miR-93-5p	4.455	4.92139E-23
miR-500a-3p	4.452	0.000253891
miR-421	4.321	1.52476E-06
miR-767-5p	4.287	3.79845E-05

Table S7. Differential expression of miRNA between sEVs and parental cells, Related to Figure 2.

Significant differential expression of miRNAs between density gradient purified sEVs and their parental cell type. Included are miRNAs with at least a four fold-change enrichment and an FDR-adjusted p-value < 0.05.