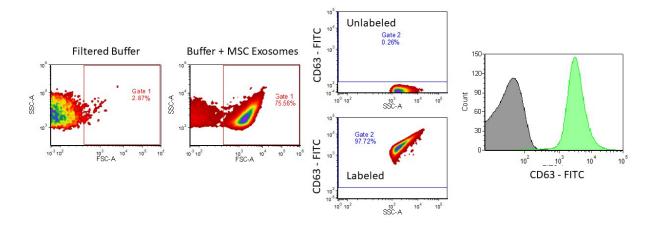
## The microRNA regulatory landscape of MSC-derived exosomes: a systems view

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## **Supplementary Information**



**Supplementary Figure 1:** Analysis of exosomes for CD63 expression by flow cytometry. 50µg MSC exosomes in 100µl of filtered PBS were incubated with 5µl of anti-CD63-FITC antibody (Miltenyi) at 4°C for 1 hour. Samples were analyzed on the MacsQuant flow cytometer using manufacturer suggested settings for exosomes.

miR-23a-3p

miR-424-5p miR-144-3p miR-130a-3p

miR-145-5p miR-29b-3p miR-29a-3p miR-25-3p

miR-221-5p miR-21-5p

miR-125b-5p miR-22-3p

miR-199a-3p

Let-7a-5p

Let-7i-5p

Let-7b-5p

miR-630

miR-191-5p

miR-1246

miR-451a

miR-100-5p

416

174

168

166

74

72

61

43

33

Number of genes targeted	Circulatory System Development	Growth	
1069	104	176	
956	94	195	
780	85	136	
742	99	180	
660	66	117	
635	65	129	
629	65	127	
604	75	120	
456	55	107	
439	61	104	
436	43	93	
422	44	95	
422	44	95	

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**Biological Processes** 

Circulatory System Development	<ul> <li>Circulatory System Development</li> <li>Heart Development</li> <li>Cardiovascular System Development</li> <li>Blood Vessel Development</li> <li>Blood Vessel Morphogenesis</li> <li>Tube Development</li> <li>Tube Morphogenesis</li> <li>Fissue Morphogenesis</li> <li>Morphogenesis of an Epithelium</li> </ul>
Growth	<ul> <li>Developmental Growth         <ul> <li>Regulation of Developmental Growth</li> <li>Cell-Cell Signaling by Wnt</li> <li>Wnt-Signaling Pathway</li> <li>Urogenital System Development</li> <li>in utero Embryonic Development</li> </ul> </li> </ul>

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	Number of	GnRH	Angio	Integrin	CCKR	Wnt	TGF-beta	PDGF	EGF	PI3K	P53
	Genes	receptor	genesis	signaling							
	Targeted	•	Ŭ	0 0	map	0 0	0 0	0 0	0 0	0 0	
miR-23a-3p	1069	21	10	12	18	14	4	9	10	5	5
miR-424-5p	956	28	13	13	15	16	8	12	9	6	3
miR-144-3p	780	19	6	10	9	22	5	10	9	5	4
miR-130a-3p	742	20	14	8	14	18	10	12	9	3	5
miR-145-5p	660	16	9	12	11	10	9	10	4	1	0
miR-29b-3p	635	11	10	25	11	15	4	5	9	6	6
miR-29a-3p	629	11	8	25	11	15	4	6	8	5	6
miR-25-3p	604	10	3	11	10	10	5	3	4	2	2
miR-221-5p	456	11	8	5	6	16	6	4	4	1	2
miR-21-5p	439	10	4	6	6	5	10	5	6	1	0
miR-125b-5p	436	10	4	3	9	5	2	6	7	1	1
miR-22-3p	422	12	6	2	8	5	7	4	6	4	6
miR-199a-3p	416	13	7	9	10	9	4	3	8	2	0
Let-7a-5p	174	3	2	5	0	3	4	2	1	1	1
Let-7i-5p	168	3	1	6	0	3	4	3	1	1	1
Let-7b-5p	166	3	1	5	0	3	4	3	1	1	1
miR-630	74	13	6	2	7	7	4	3	4	1	1
miR-191-5p	72	4	3	2	1	1	2	0	0	0	1
miR-1246	61	2	1	0	1	3	1	0	1	0	0
miR-451a	43	1	0	0	1	1	1	0	1	0	1
miR-100-5p	33	1	0	2	0	2	0	0	0	0	0

**Supplementary Figure 2.** (**A**) Number of genes targeted by the top 23 miRNAs found in MSC exosomes categorized into total number of genes targeted, genes related to circulatory system development, and genes related to growth. (**B**) Biological processes that are categorized into circulatory system development and growth, (**C**) Number of genes targeted categorized into total number of genes targeted and enriched pathways.

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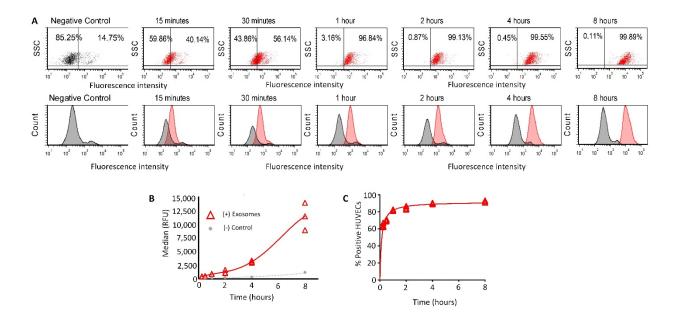
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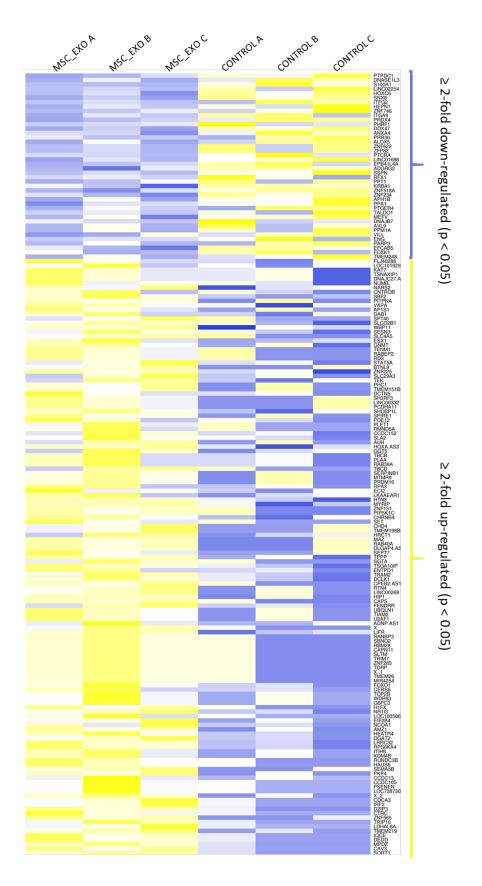
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**Supplementary Figure 3.** Cellular binding and uptake of exosomes into HUVECs as a function of time (0.5h, 1h, 2h, 4h, 6h, and 8h).

To assess uptake kinetics of exosomes into endothelial cells, HUVECs were incubated with Bodipy-TR-labeled exosomes for up to eight hours. Exosome uptake increased with increasing incubation time. A 12-fold increase in exosomal binding or uptake was observed at 8 h of incubation compared to 1h and 2h of incubation. At 30 minutes of incubation, only 56% of cells showed exosome binding and or uptake, which increased to ~95% after 1 h and further to 99.1% and 99.9% after 2 to 8 hours of incubation. Further, the percentage of exosome-positive HUVECs increased over time (~1,000 MFI at 1 h vs. ~12,500 MFI at 8 h).



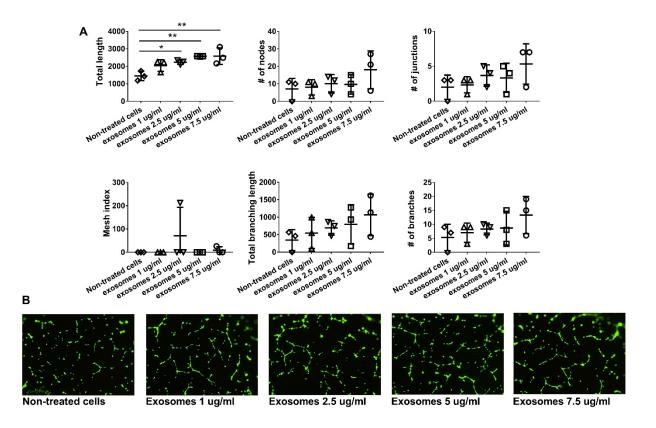
**Supplementary Figure 4.** Heatmap illustrating all genes in HUVECs that were up- or down-regulated ( $\geq$  2-fold) as a result of exposure to MSC exosomes achieving statistical significance at p < 0.05. RNA reads were aligned using STAR version 2.5.3a. The resultant SAM files were converted to BAM in Galaxy and then analyzed using htseq-count, followed by DEseq2 which provides fold changes as well as statistical significance.

Unique Gene Targets of miR-199a-3p	Other Non-Unique Gene Targets of miR-199a-3p			
CASP9	CRIM1	ERBB4	NF1	
CABLES1	NET1	ATRX	DNMT3A	
LKB1	GRHL3	PAK1	CNOT7	
NEUROD1	PRKACB	MECP2	CDC42	
RB1	GREM1	CDC14A	ITGA6	
DEPDC1B	NR4A3	ARHGEF12	VANGL1	
KIAA0141	MAP3K1	DCBLD2	PIK3R1	
ITGA3	ANGPT1	UFM1	PTPN3	
MAP3K5	PRKCE	YWHAE	PTPRJ	
CHMP5	CITED2	SEMA3A	YAP1	
ARHGEF2	GPR37	MEIS2	AREL1	
SLC46A2	DDIT4	SH3GLB1	PTPRU	
PRPF40A	NTRK2	MAPRE1	SGK3	
SERPINE2	PLCB1	RARB	TAOK1	
DLX5	FOS	RUNX1	GOLGA4	
ARG2	CXCL12	FN1	TRIM36	
ATP6V1C2	CPEB4	GDF9	CYP1B1	
SRA1	MAP3K4	CARD6	BCL2L14	
PAK4	FBXW11	CHL1		
CELSR2	UBQLN1	PDE8A		
USP8	SLC39A10	PAWR		
HGF	HNF1B	ARHGEF3		

**Supplementary Table 3**: Gene targets of the miRNAs were compared to the GO Terms: Regulation of Cell Death (GO:0010941), Growth (GO:0040007), Cell Cycle (GO:0051726) and Wnt-signaling Pathway (GO:0016055). Twenty two genes curated in regulation of cell death, growth, cell cycle, or Wnt-signaling pathway were unique to miR-199a-3p.

	hsa-miR-23a-3p		hsa-miR-130a	-3p
KLF5 ZEB1 APOLD1 HRG ANKRD17 AQP2 SIRT1 MEIS1 MESP1 AMBRA1 HAS2 TGFBR3 SOX11 RBPMS2 NOX4 GREM1 PRDM1 CALCRL PRDM1 CALCRL PRDM1 CALCRL PRDM1 CALCRL OL15A1 PTK2B MEF2C SIX4 COL4A3BP MAP3K3 FZD5 TSPAN12 HEXIM1 HOXA1 FOXA1 TCAP SGCD RORA ESM1 LGR4 SRPK2 FBN1 CCM2 SLC9A1 JAM3 PTPRB	HNRNPU NKX2-1 EDNRB PKNOX1 CTNNBIP1 C5orf42 LGR5 ERBB4 PDPK1 E2F8 TNFAIP3 PRCP JAG1 DDAH1 CHD7 PDGFA TENM4 TMEM2 PKP2 TIPARP RYR2 KCNAB1 NCOA6 BCL2 AGGF1 EPAS1 INTU GGNBP2 SPRY2 MAPK14 HOXD13 FGF2 RUNX1 RAPGEF2 TBX5 RDH10 ARG1 FOXP2 MED12 FZD7 LRP5 ADAM19 ALDH1A2 PDLIM5	CSF1 ITGB8 PTEN ROBO2 TGFBR2 FZD4 MET STK4 NUS1 GJA1 QKI ROBO1 NFIB HOXA3 SMAD5	EFNB2 LRP6 EGR3 CDKN1A STAT3 SOX4 DNM2 HOXB3 SIK1 SGCB UBE4B SASH1 FOXP1 JARID2 HEG1 SDC4 EPHA4 PTPRM TEK RASA1 TSC1 HHEX FOSL1 CELSR1 MIB1 NRP2 SLC8A1 EPB41L5 DLL1 LRP2 EPHB4 KIT MYH11 E2F7 FZD6 BCL2L11 NOV WNT2B THSD7A KLHL3 NUP133 BMPR2 PPARG PDGFRA	EREG AGO1 EDA HECA ARHGAP24 EDN1 HOXA5 F3 LIPA STARD13 SPHK2 FOXF2 MAP2K1 PARVA AHI1 IGF1 MAML1 ITPK1 ZEB2 WNT1 BTG1 ZFPM2 S1PR1 MEOX2 S1PR1 MEOX2 S1PR1 MEOX2 S1PR1 NPNT PHF14 SMAD4 ERBB3 ELK3 ESR1 RB1CC1 CREB1 SULF1 TFDP2 VAV2 CBY1 PDGFD ACVR1

**Supplementary Figure 5.** The Venn diagram depicts the 104 and 99 genes related to "Circulatory System Development" (Supplementary Figure 2A) for hsa-miR-23a-3p and hsa-miR-130a-3p.



**Supplementary Figure 6.** (A) Effect of unmodified MSC exosomes on angiogenesis in HUVECs as analyzed using the ImageJ angiogenesis analyzer. (B) Calcein stained HUVECs treated with increasing concentrations of exosomes. Data are mean  $\pm$  sd, n=3, by one-way ANOVA with Tukey post-test (\*p<0.05, \*\*p<0.01).