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Supplemental Information

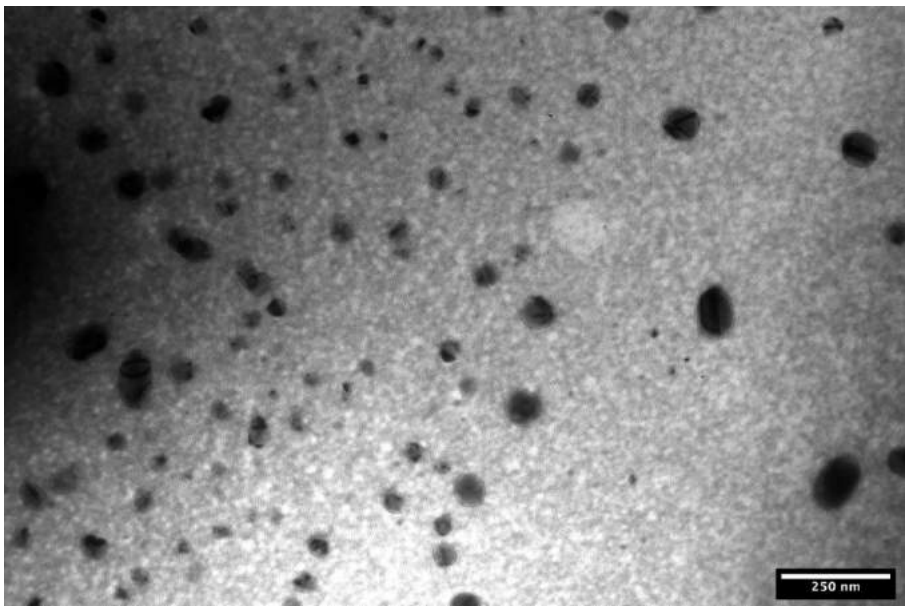
Bioinspired artificial exosomes based on lipid nanoparticles carrying let-7b-5p promote angiogenesis *in vitro* and *in vivo*

Sezin Aday, Inbal Hazan-Halevy, Aranzazu Chamorro-Jorganes, Maryam Anwar, Meir Goldsmith, Nicholas Beazley-Long, Susmita Sahoo, Navneet Dogra, Walid Sweaad, Francesco Catapano, Sho Ozaki-Tan, Gianni D. Angelini, Paolo Madeddu, Andrew V. Benest, Dan Peer, and Costanza Emanueli

Supplemental Figures

Figure S1

A



B

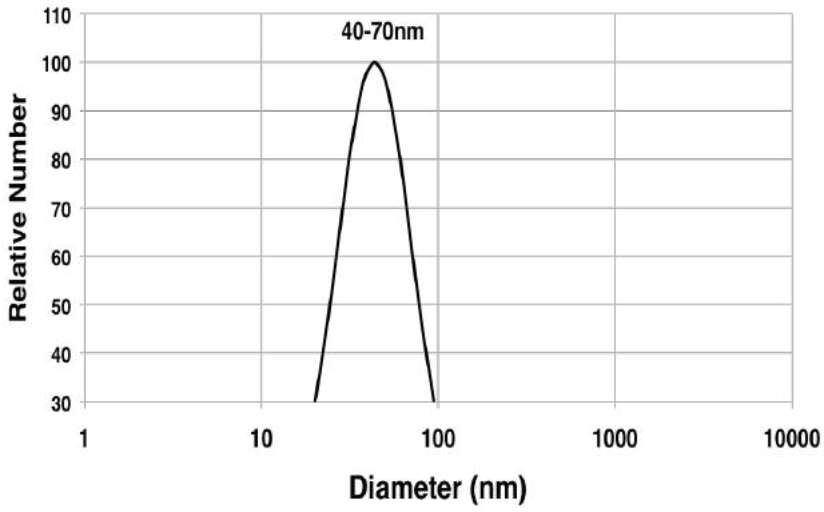


Figure S1. Further Characterization of Pericyte (hSVP) EVs by Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS). (A) TEM image shows the characteristic size distribution of hSVP EVs. Scale bar, 250 nm. (B) DLS size distribution measurement of isolated hSVP EVs demonstrates a single peak (in the range of 40–110 nm diameter) indicating they are free of contamination.

Figure S2

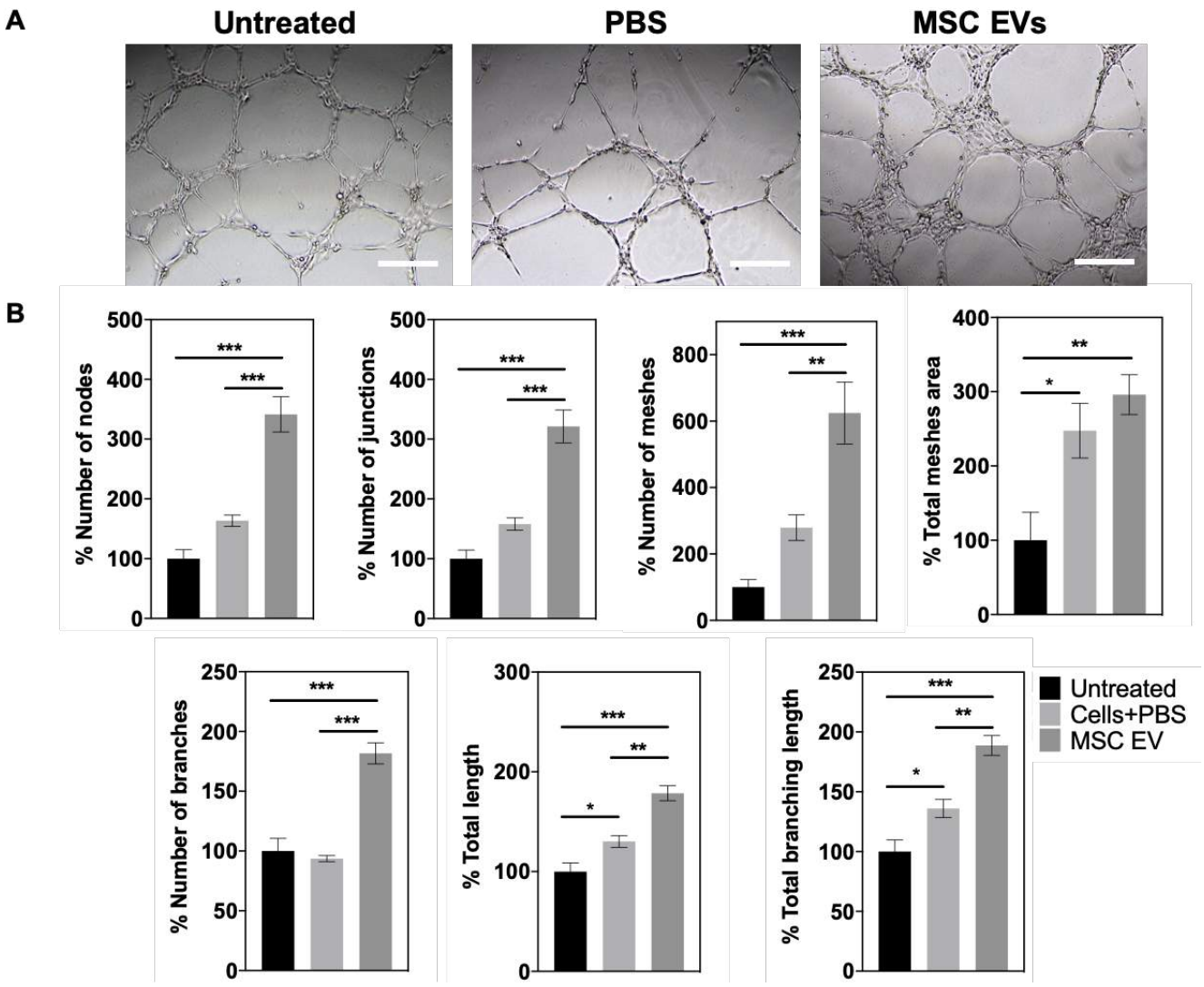


Figure S2. MSC EVs increase sprouting of HUVECs on Matrigel. (A) Matrigel figures for untreated, PBS-treated and MSC EV-treated HUVECs. Scale bars, 200μm. (B) Evaluation of different parameters in Matrigel assay using ImageJ Angiogenesis Analyzer tool. In all graphs, values are given as average ± SEM (n=6-8). **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001, and *****P* ≤ 0.0001.

Figure S3

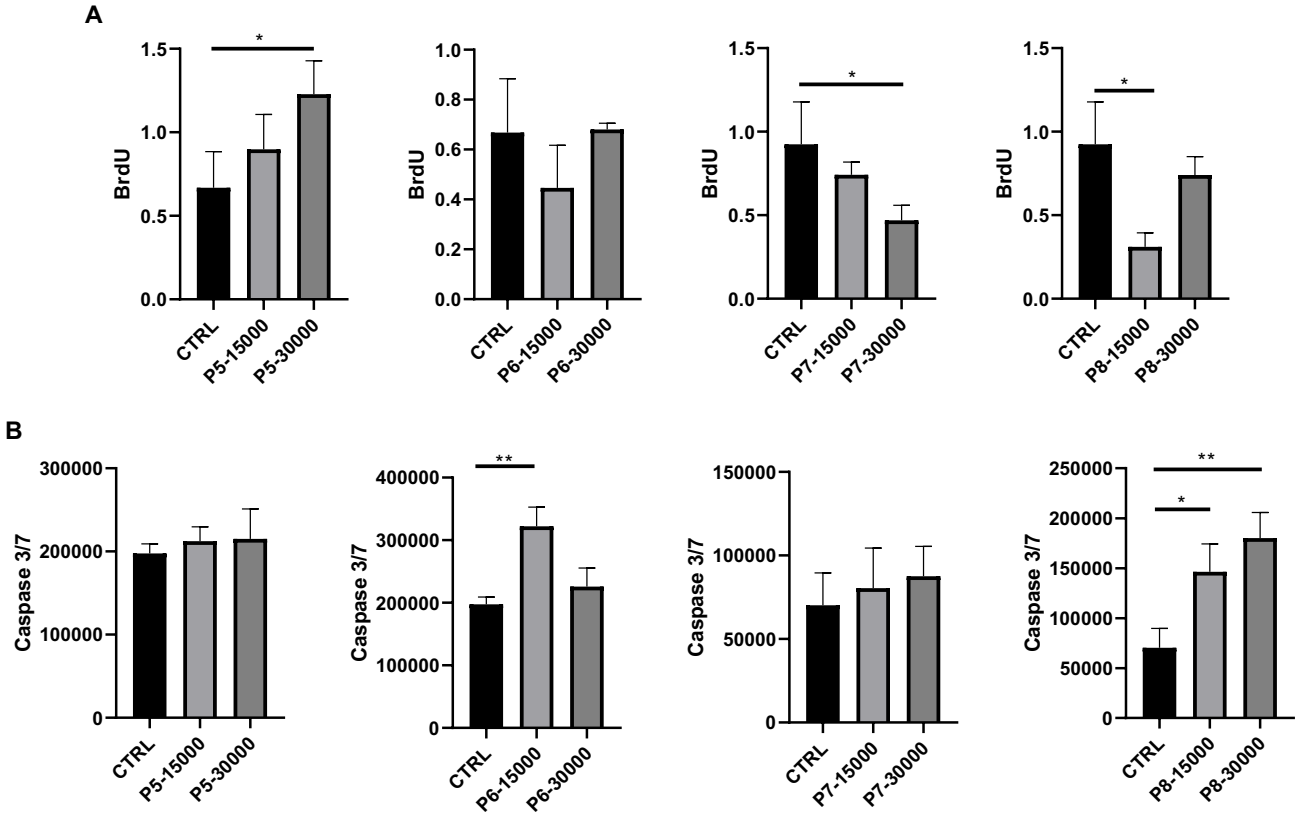


Figure S3. Function of MSC-sEVs depends on the passage number. (A) HUVECs proliferation and (B) HUVECs apoptosis after 48 h incubation with MSC-sEVs at P5-8 at two different concentrations ($1.5x10^4$ and $3x10^4$ nanoparticles). Proliferation was measured by BrdU incorporation and apoptosis by Caspase-3 activity assay. In all graphs, values are given as average ± SEM (n=4). *$p \leq 0.05$ and **$p \leq 0.001$.

Figure S4

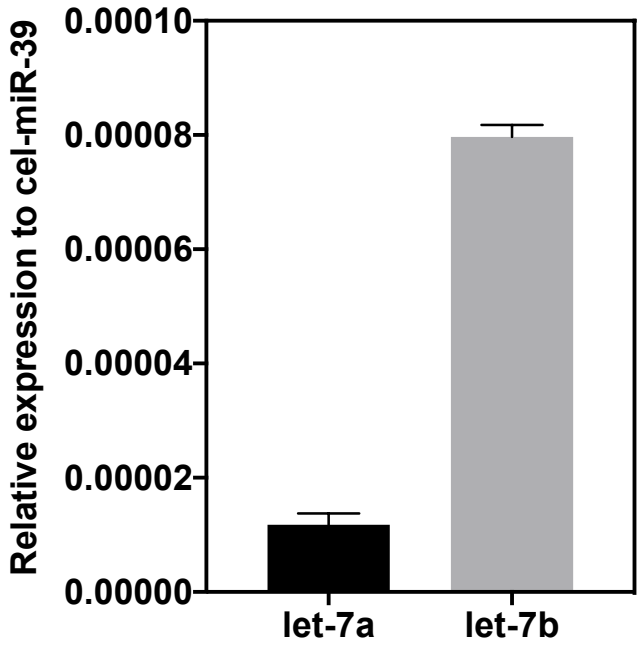


Figure S4. Relative (to spike-in Cel-miR-39) expression of let-7a and let-7b in MSC sEVs. Values are given as average ± SEM (n=3).

Figure S5

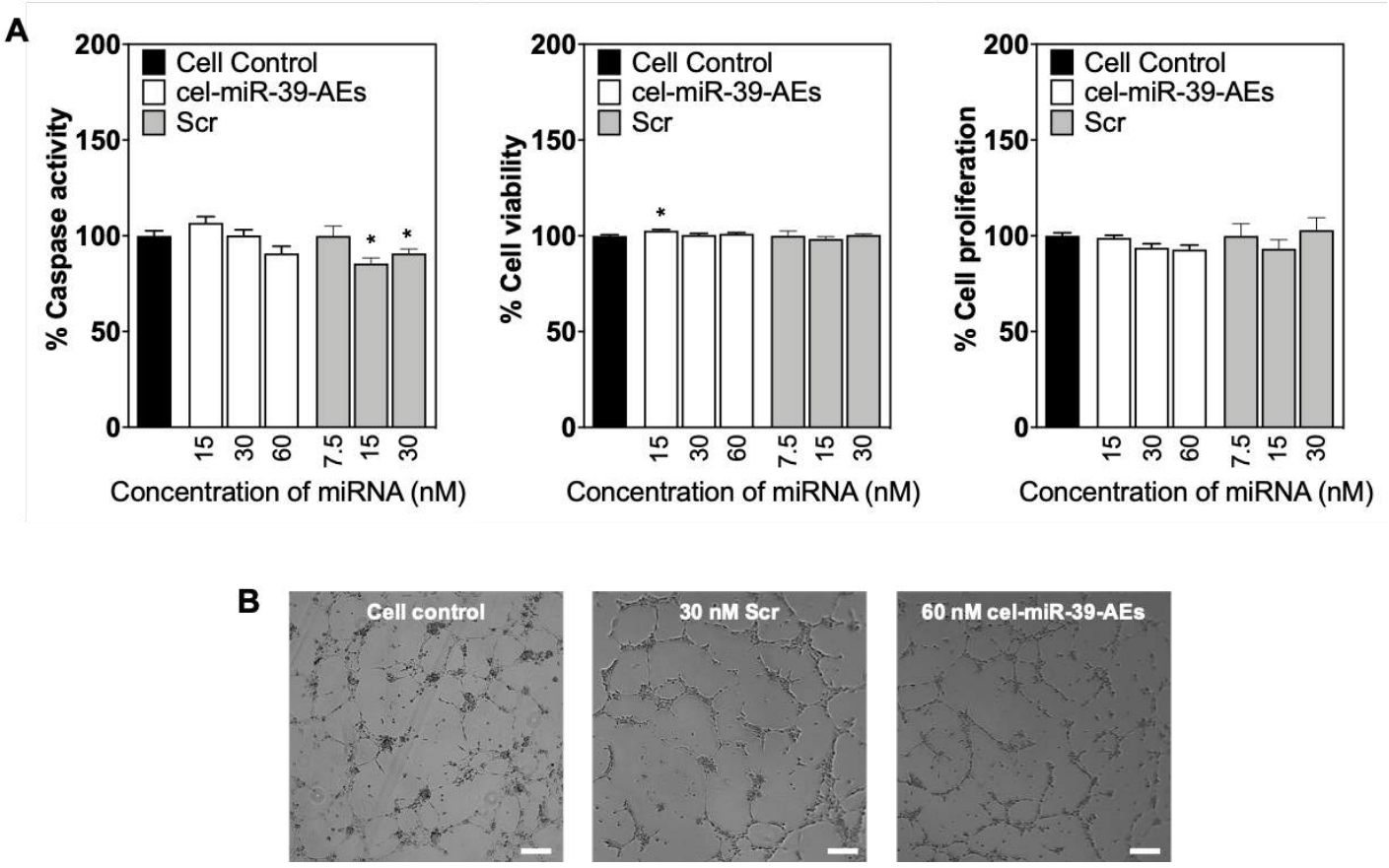


Figure S5. Cell control data for functional assays. Scr and cel-miR-39-AE controls used in the experiments do not negatively affect cell survival and proliferation (A) or tubule formation on Matrigel (B) under hypoxic conditions (1% O_2). Images are given for 30 nM Scr control and 60 nM cel-miR-39-AEs. In experiments with let-7a and let-7b single/double transfection, the highest concentration of miRNA was 30 nM while in the experiments with AEs, it was 60 nM. Even at the highest concentrations, corresponding controls (30 nM Scr control and 60 nM cel-miR-39-AEs) do not negatively affect cell behavior. In all graphs, values are given as average ± SEM (n=5-6). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, and ****$P \leq 0.0001$. Scale bars, 200μm.

Figure S6

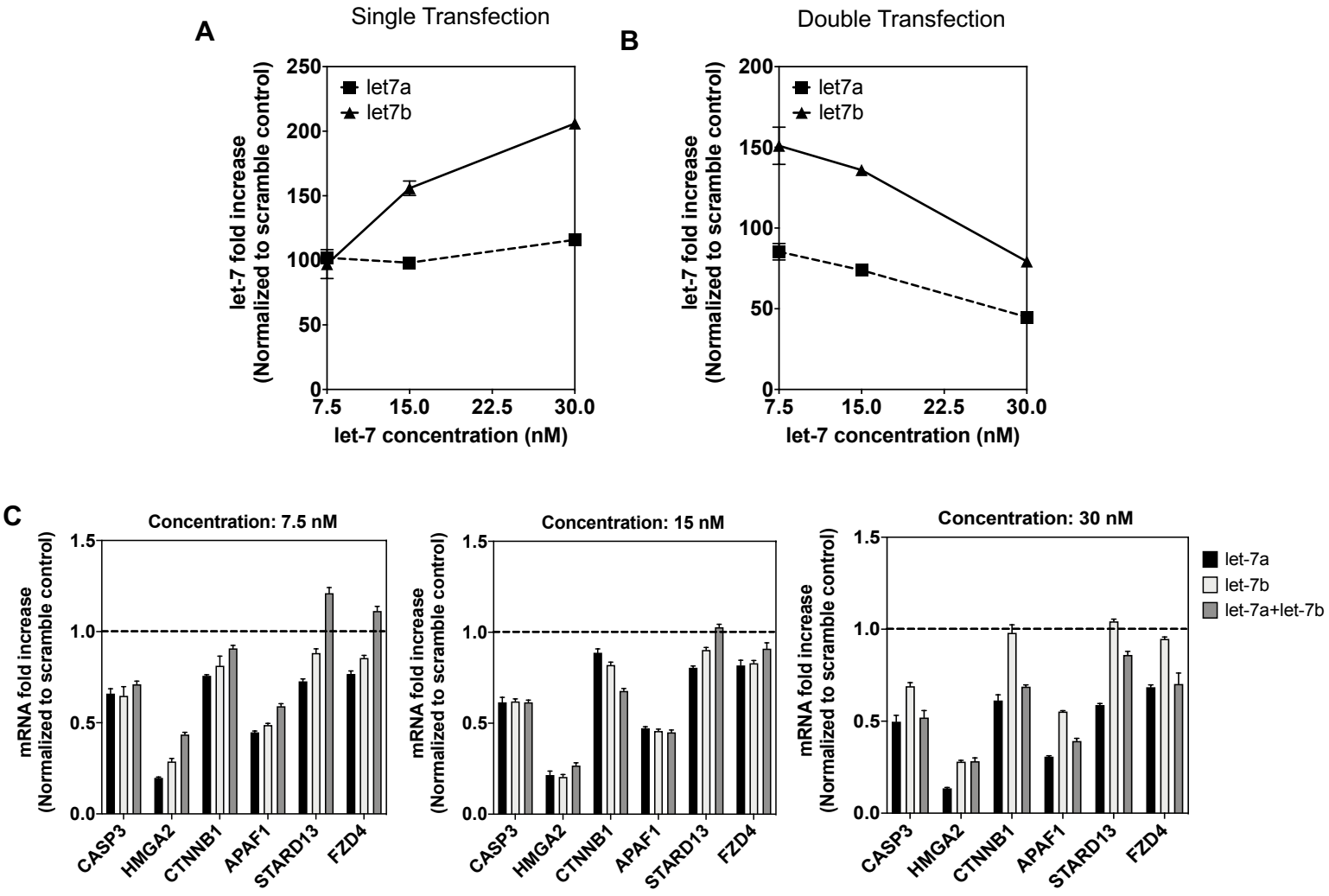


Figure S6. Kinetics of expression for let-7a and let-7b and their mutual gene targets. (A) let-7a or let-7b expression when HUVECs are treated with different concentrations of single (i.e. let-7a or let-7b) miRNAs. (B) Co-transfection of let-7a and let-7b show different kinetics compared with single transfections. (C) let-7 transfection effectively downregulates mutual gene targets after 24 h. In all graphs, values are given as average ± SEM (n=4). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, and ****$P \leq 0.0001$.

Figure S7

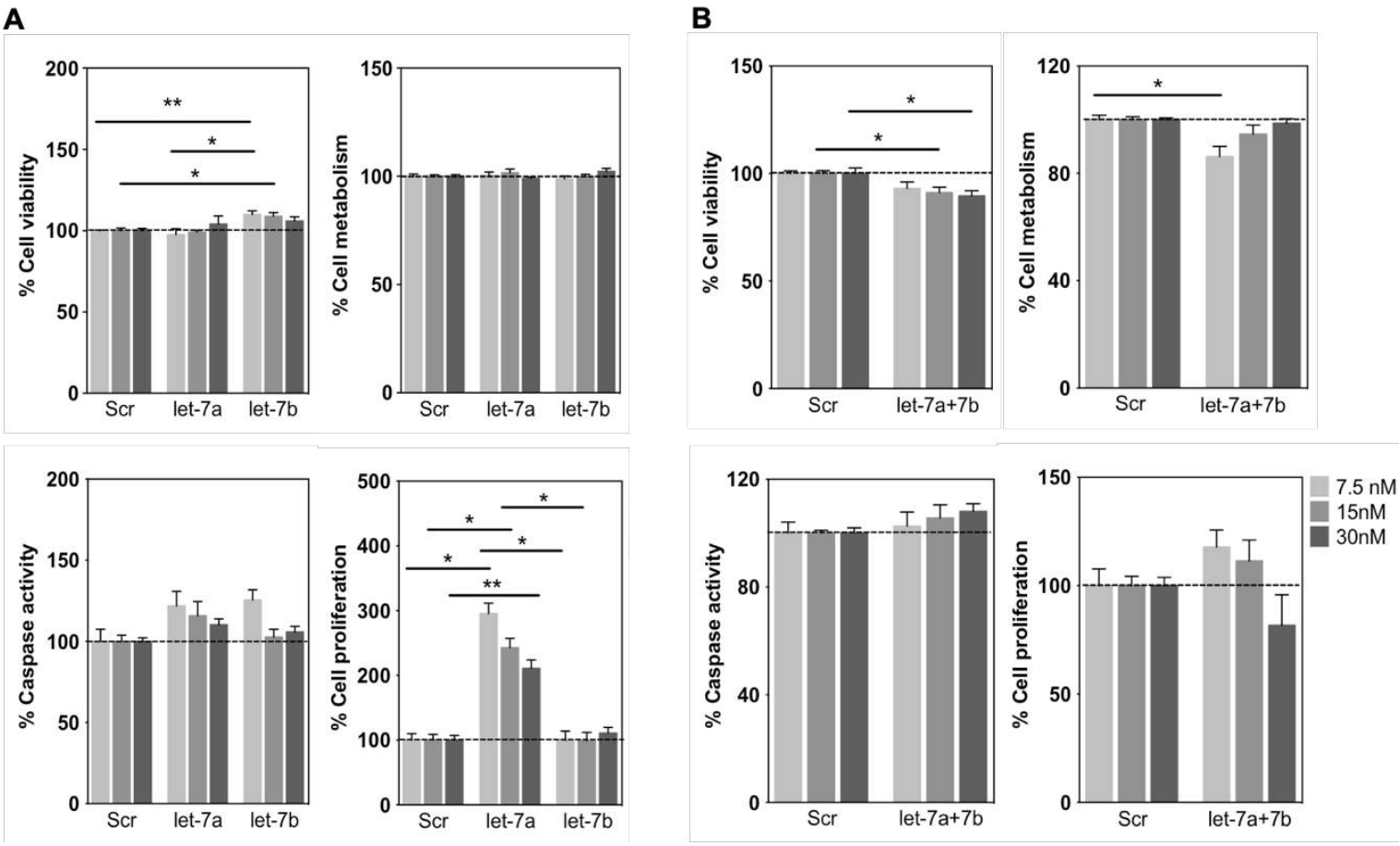


Figure S7. The effect of let-7a, let-7b and dual transfection of them on HUVEC activity under hypoxia (1% O_2). (A) Neither let-7a nor let-7b negatively affect HUVEC activity. (B) Dual transfection of let-7a and let-7b decreases cell viability and metabolism. In all graphs, values are given as average ± SEM (n=5-10). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, and ****$P \leq 0.0001$.

Figure S8

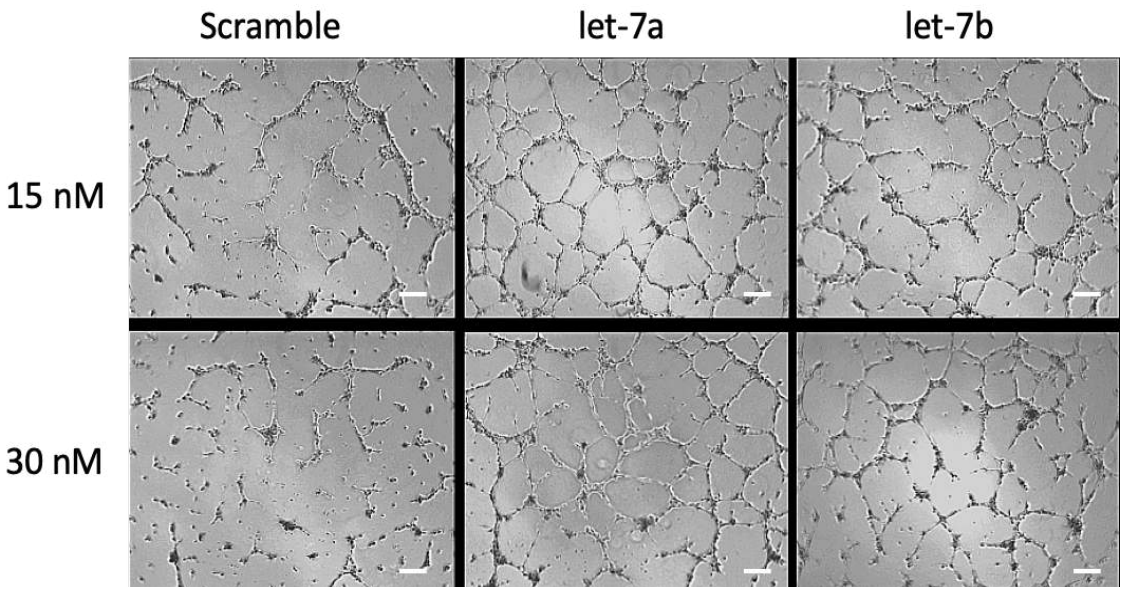


Figure S8. Matrigel figures for let-7a- or let-7b-treated HUVECs under hypoxia (1% O_2). Scale bars, 200μm.

Figure S9

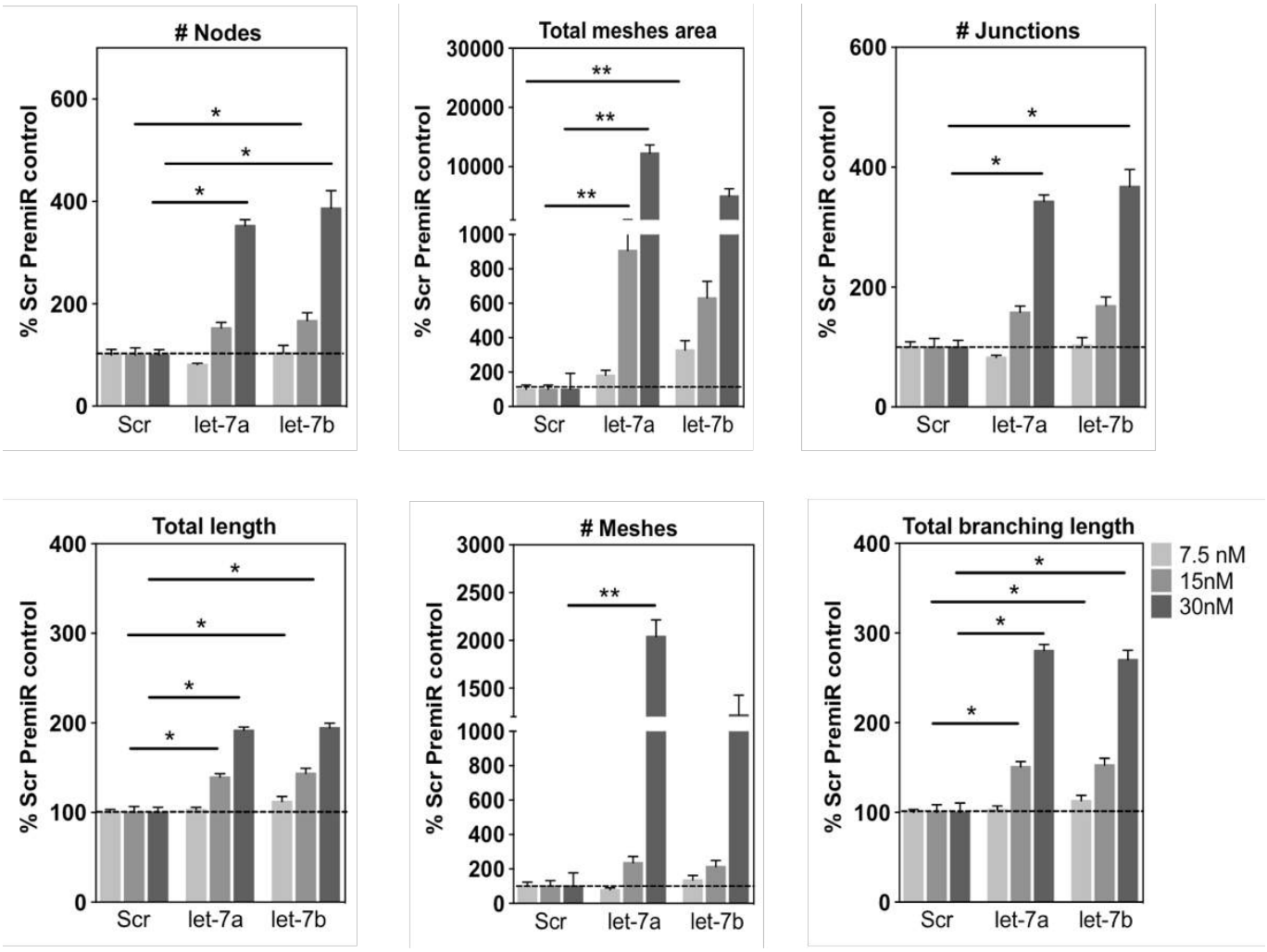


Figure S9. Analysis of different angiogenesis parameters for HUVECs treated with let-7a or let-7b in hypoxia (1% O_2) using ImageJ Angiogenesis Analyzer tool. In all graphs, values are given as average ± SEM (n=5-10). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, and ****$P \leq 0.0001$.

Figure S10

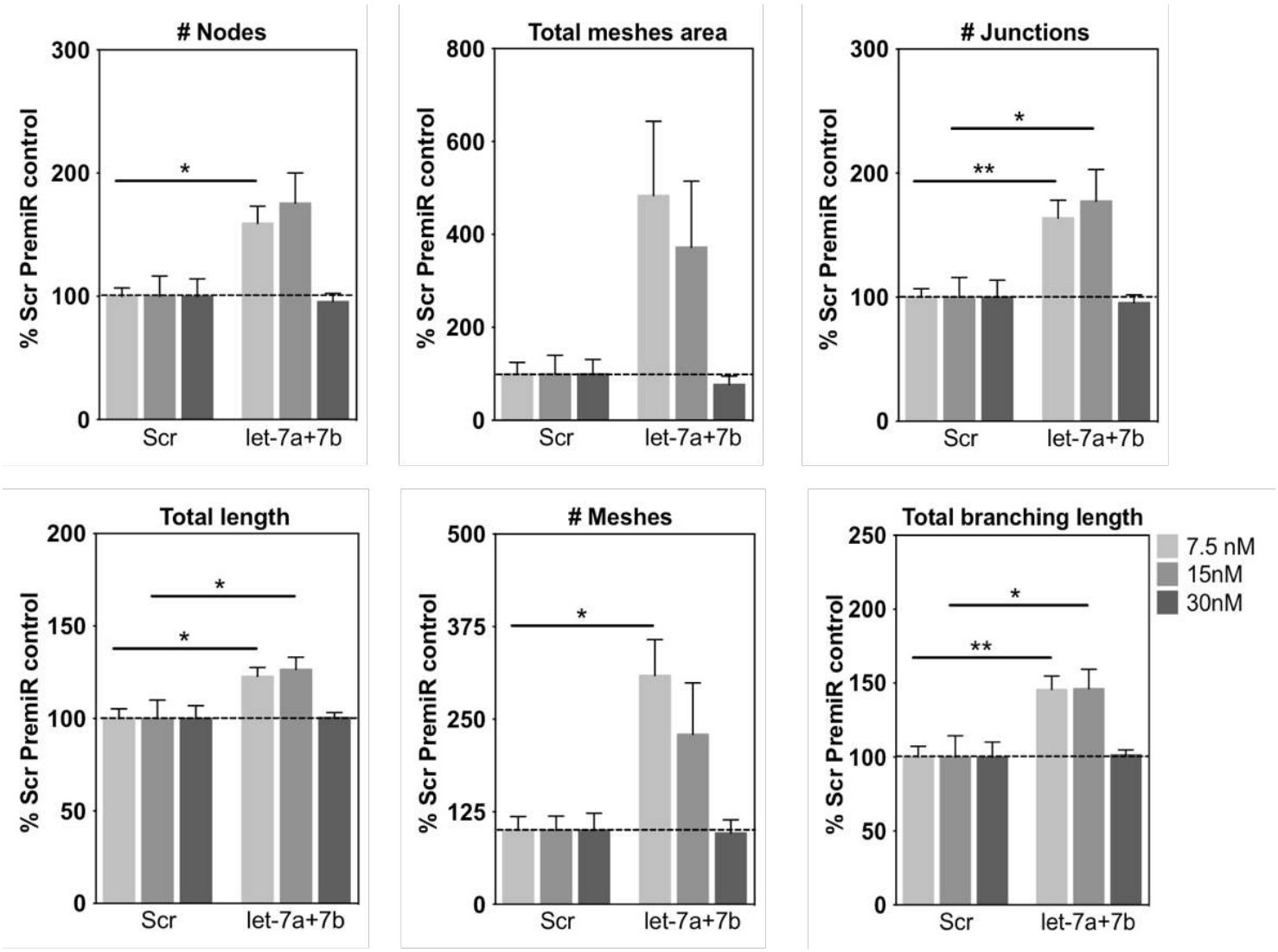


Figure S10. Analysis of different angiogenesis parameters for HUVECs co-transfected with let-7a and let-7b in hypoxia (1% O_2) using ImageJ Angiogenesis Analyzer tool. In all graphs, values are given as average ± SEM (n=5-10). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, and ****$P \leq 0.0001$.

Figure S11

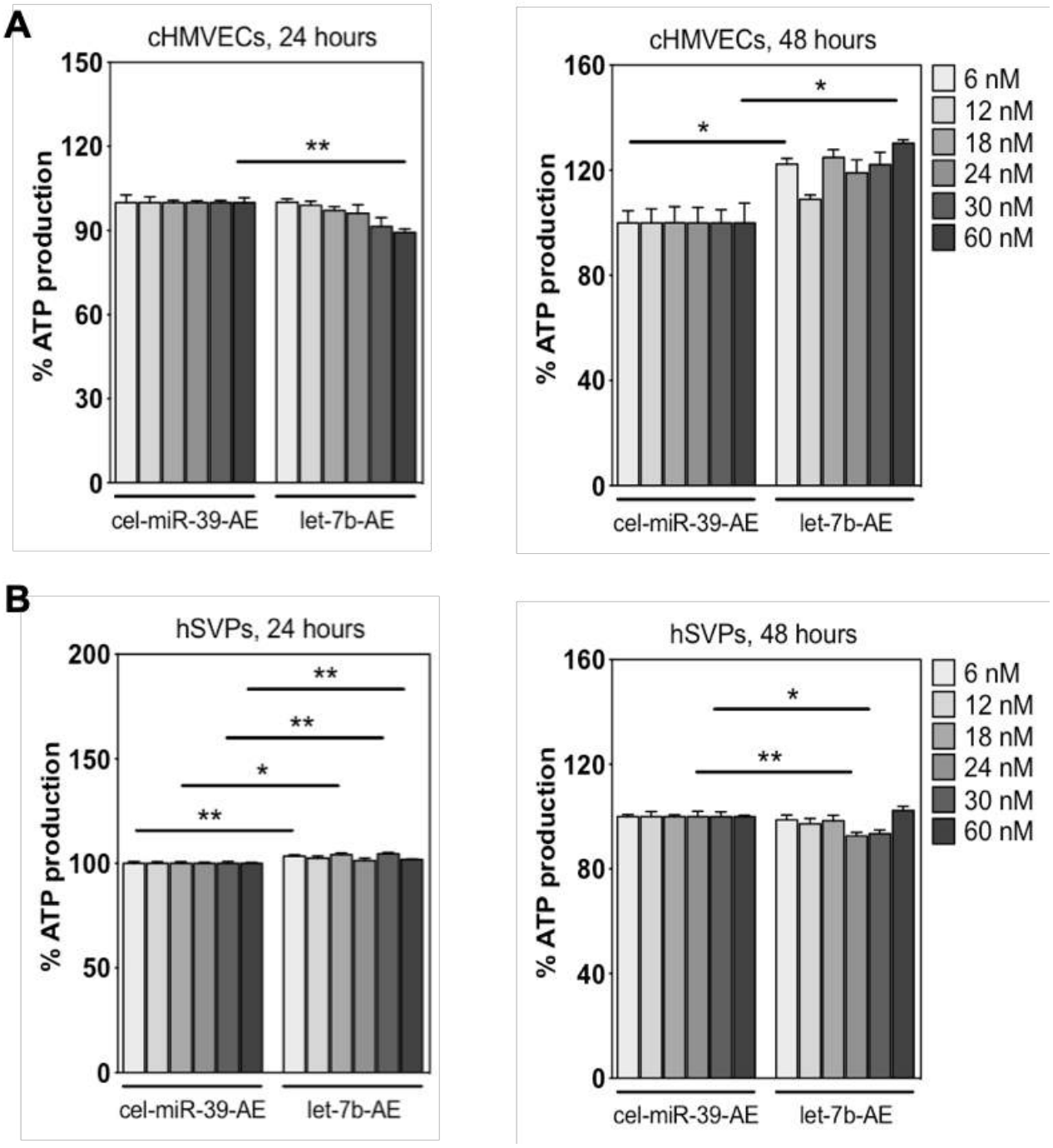


Figure S11. Analysis of AEs cytotoxic effects on cHMEVs and hSVPs. AEs do not cause any significant toxicity on (A) cHMEVs or (B) hSVPs up to 48-hour transfection. Cells were cultured under normoxic conditions (20% O_2). In all graphs, values are given as average ± SEM (n=5-10). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, and ****$P \leq 0.0001$.

Figure S12

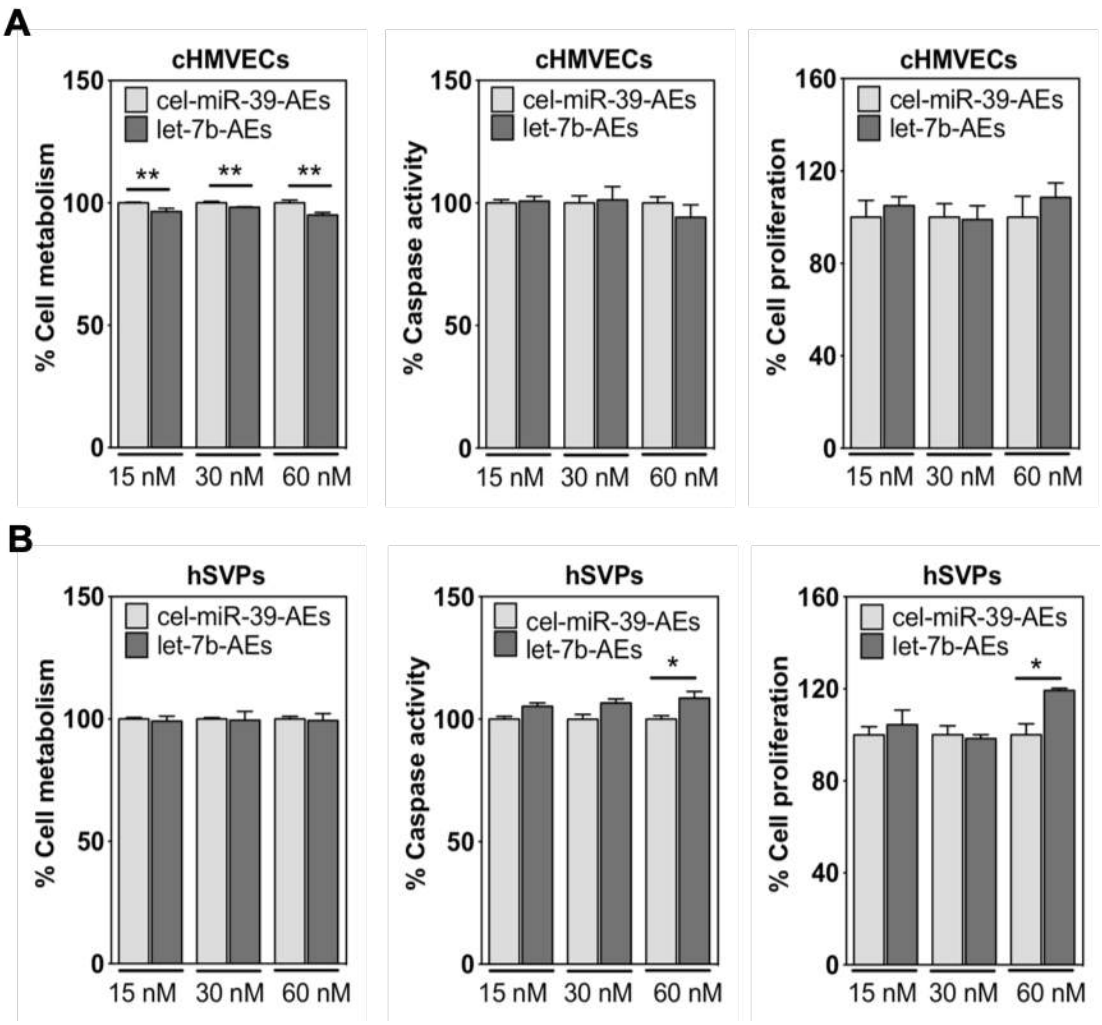


Figure S12. The effect of AEs on cHMEV and hSVP activities. AEs have no negative effect on (A) cHMEVs or (B) hSVPs' activity under hypoxic conditions (1% O_2). In all graphs, values are given as average ± SEM (n=4-5). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, and ****$P \leq 0.0001$.

Figure S13

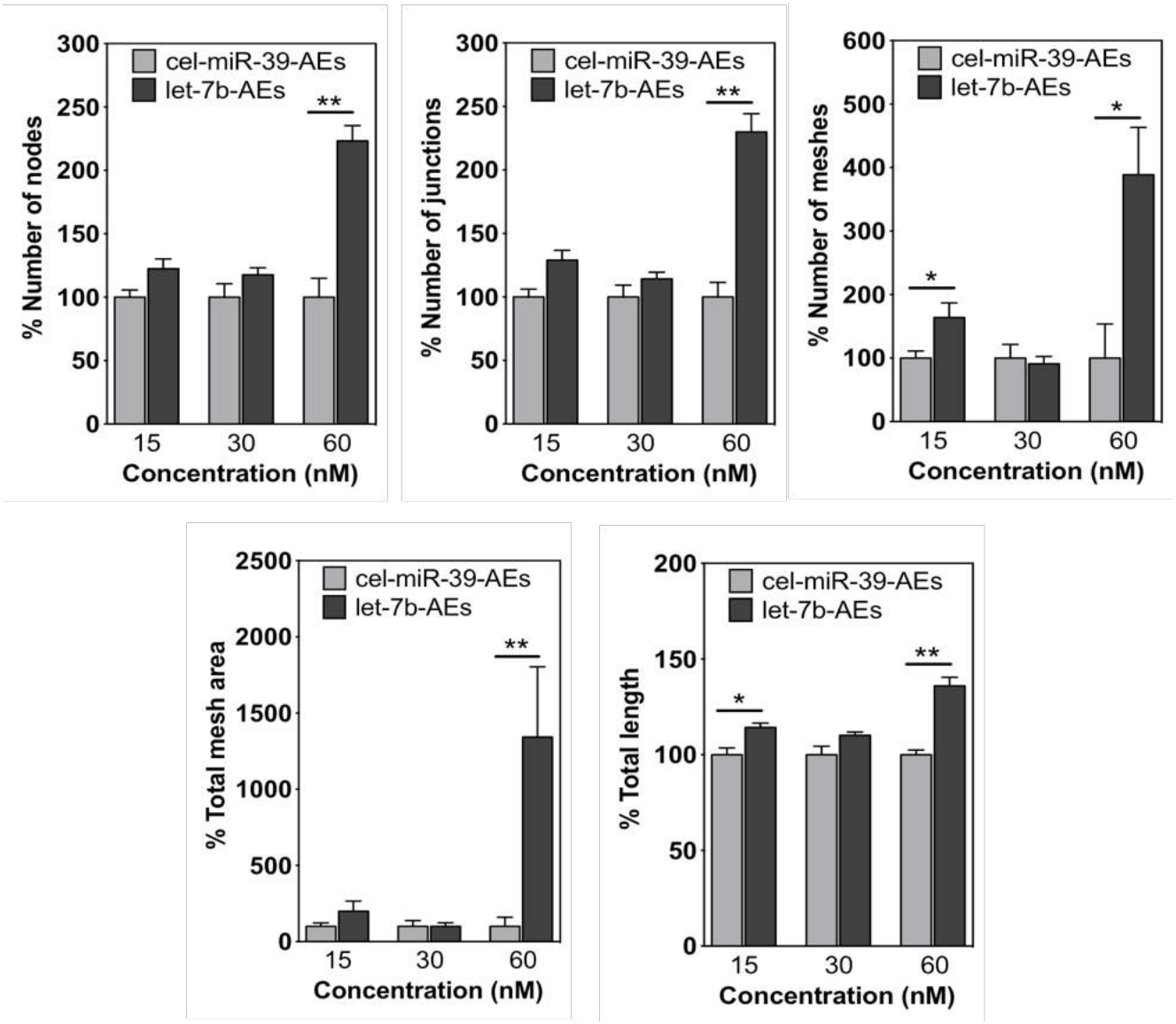


Figure S13. Additional parameters of Matrigel for HUVECs treated with cel-miR-39-AEs or let-7b-AEs under hypoxic conditions (1% O_2). In all graphs, values are given as average ± SEM (n=5). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, and ****$P \leq 0.0001$.

Figure S14

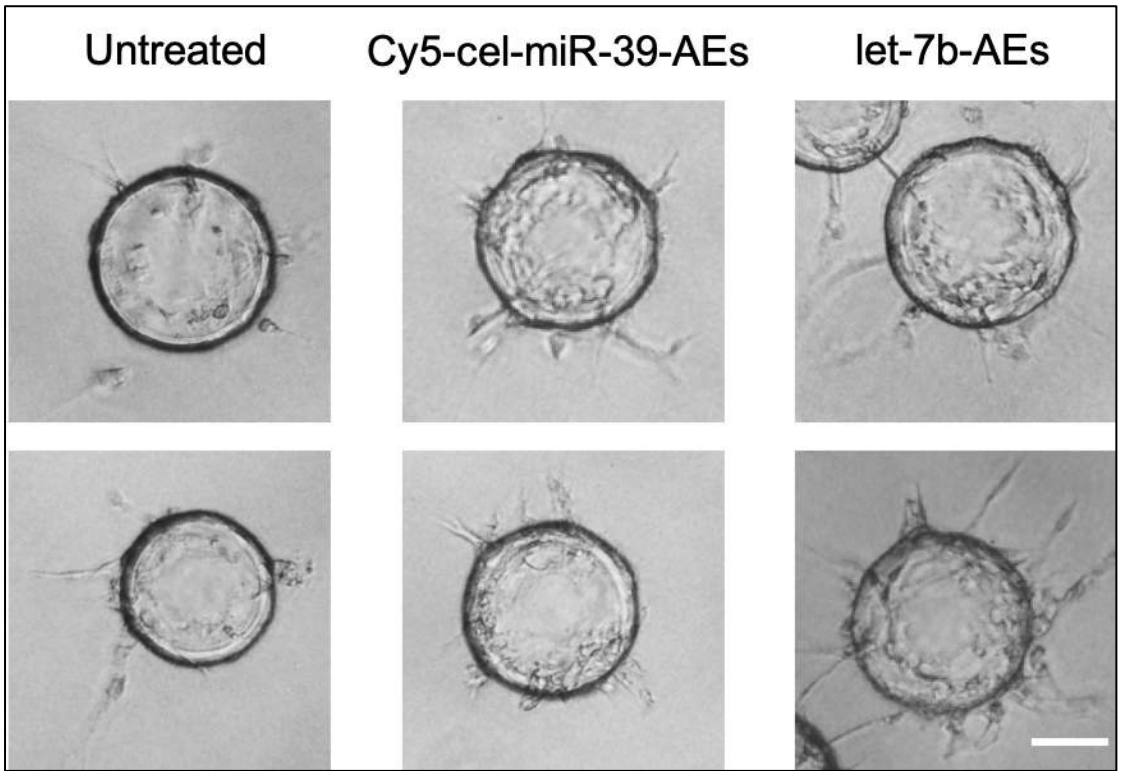


Figure S14. Higher magnification images for fibrin gel bead assay. Images of individual beads from different experimental conditions show the sprouts in fibrin gel. Scale bar, 75 μm.

Figure S15

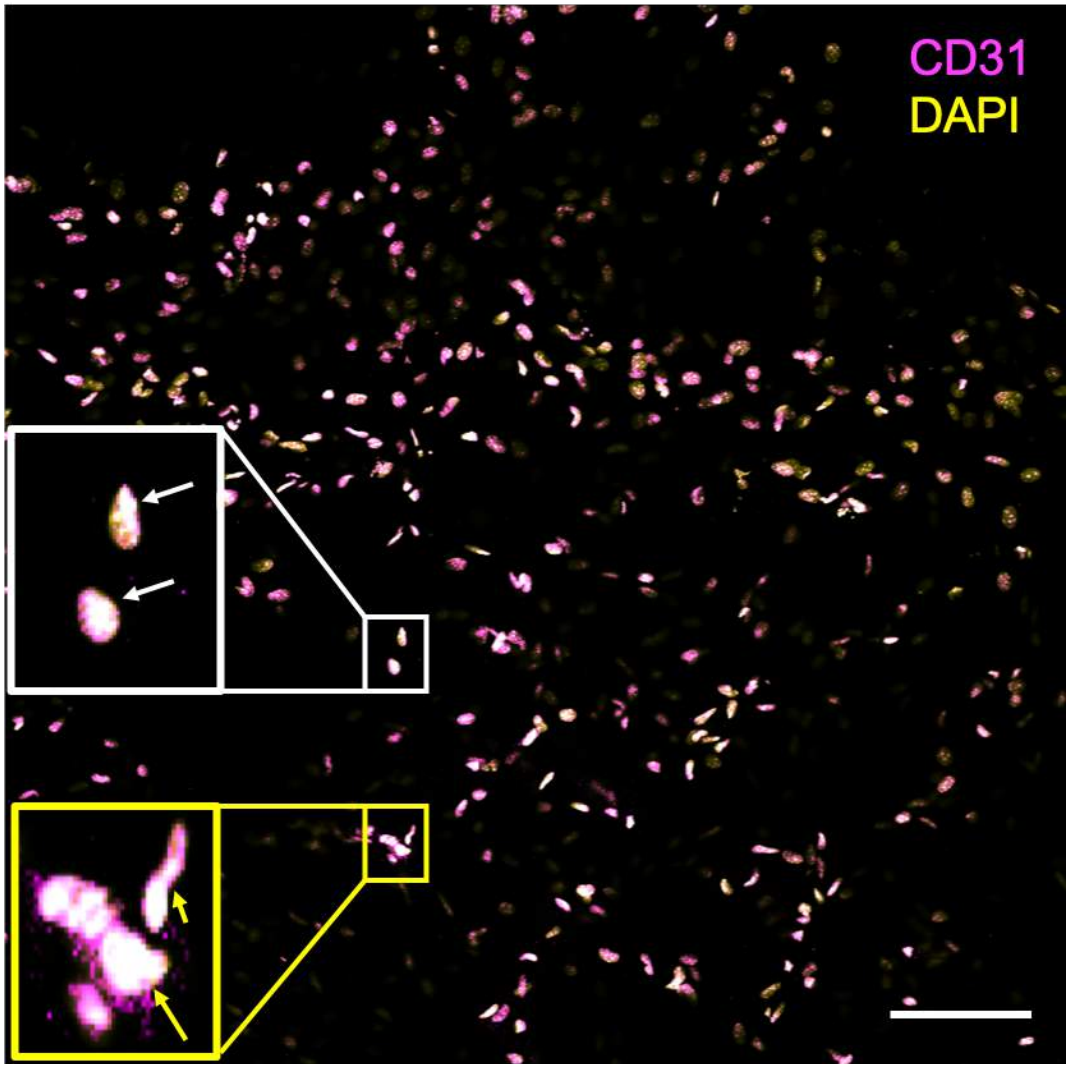


Figure S15. Identification of vessel like structures in xenograft Matrigel plugs. Individual ECs ($CD31^+DAPI^+$) can be seen (white box, white arrows) but also 'vessel-like structures' comprising elongated fragments of multiple $CD31^+DAPI^+$ (yellow box, yellow arrows). In image, pseudocolors were used (red channel shown in magenta and blue channel in yellow) to make cells easier to see. Scale bar, 50 μm.

Supplemental Tables.

Table S1. miRNA Panel for pericardial fluid extracellular vesicles. AVR: Aortic valve replacement, CABG: coronary artery bypass graft. When the Ct≥40, the miRNA was "undetermined". If a miRNA was undetermined in any of the replicates from AVR patients, it was not considered for the analysis. The full data for miRNA panels can be found at GEO with accession number: GSE118103. cel-miR-39-3p, UniSp6 and UniSp3 IPC show spike-in controls. Table is uploaded separately as an Excel file (Table S1).

Table S2. miRNA Panel for human saphenous vein extracellular vesicles. When the Ct≥40, the miRNA was "undetermined". If a miRNA was undetermined in any of the replicates, it was not considered for the analysis. The full data for miRNA panels can be found at GEO with accession number: GSE118855. cel-miR-39-3p, UniSp6 and UniSp3 IPC show spike-in controls.

miRNA Name	*Replicate-1*	*Replicate-2*	*Replicate-3*
cel-miR-39-3p	16.229	15.757	16.364
UniSp6	17.197	17.208	17.348
UniSp3 IPC	18.238	18.221	17.965
UniSp3 IPC	18.317	18.228	18.141
UniSp3 IPC	18.216	18.361	18.150
UniSp3 IPC	18.208	18.457	18.134
UniSp3 IPC	18.408	18.191	18.240
UniSp3 IPC	18.575	18.435	18.224
hsa-miR-27b-5p	32.947	13.979	14.243
hsa-miR-100-5p	32.480	32.174	30.391
hsa-miR-21-5p	33.930	31.704	29.722
hsa-miR-125b-5p	32.787	32.456	30.240
hsa-miR-1260a	32.874	32.516	31.227
hsa-miR-451a	32.856	33.519	30.276
hsa-miR-23a-3p	34.909	31.704	30.740
hsa-miR-221-3p	34.033	33.419	30.070
hsa-miR-24-3p	35.239	32.023	31.057
hsa-miR-191-5p	26.626	38.674	33.528
hsa-let-7a-5p	32.510	34.447	31.921
hsa-miR-34a-3p	34.341	32.978	33.128
hsa-let-7b-5p	34.627	33.975	31.859
hsa-miR-19b-3p	33.632	34.969	32.124
hsa-miR-222-3p	37.534	33.988	31.461
hsa-miR-92a-3p	35.631	34.117	33.308
hsa-miR-31-5p	36.593	33.619	32.911
hsa-miR-29a-3p	36.174	35.221	32.281
hsa-miR-23b-3p	38.060	34.391	31.798
hsa-miR-16-5p	34.627	39.478	30.423
hsa-miR-127-3p	34.238	37.329	33.367

hsa-miR-548c-5p	34.714	35.109	35.602
hsa-miR-188-3p	34.988	34.782	35.664
hsa-miR-663b	36.968	35.945	33.525
hsa-miR-142-3p	35.333	37.926	34.219
hsa-miR-29c-3p	37.355	36.513	33.788
hsa-miR-27a-5p	34.805	36.540	36.402
hsa-miR-144-3p	36.876	35.638	35.267
hsa-miR-940	35.028	38.869	34.493
hsa-miR-376c-3p	36.825	39.130	32.699
hsa-miR-1972	38.216	36.918	34.256
hsa-miR-329-3p	36.989	37.812	36.004
hsa-miR-1471	38.905	38.457	38.050
hsa-miR-23a-5p	39.374	38.768	39.076

Table S3. Info for the patients whose small extracellular vesicles were used in PF miRNA panels. NYHA: New York Heart Association Classification.

	Age	Gender	Diabetes	CABG Vessel #	Hypertension	NYHA
CABG Replicate-1	**62**	**M**	**No**	**2**	**Yes**	**II**
CABG Replicate-2	**56**	**M**	**No**	**2**	**Yes**	**II**
CABG Replicate-3	**68**	**M**	**No**	**3**	**Yes**	**II**
CABG Replicate-4	**71**	**M**	**No**	**3**	**Yes**	**II**
AVR Replicate-1	**83**	**M**	**No**	**N/A**	**Yes**	**II**
AVR Replicate-2	**66**	**M**	**No**	**N/A**	**Yes**	**II**
AVR Replicate-3	**76**	**M**	**No**	**N/A**	**Yes**	**III**
AVR Replicate-4	**76**	**M**	**No**	**N/A**	**Yes**	**II**

Table S4. Major Resources Table

Animals (*in vivo* studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Mouse	Charles River	Crl:CD1-*Foxn1*^{nu}	M	https://www.criver.com/products-services/find-model/cd-1-nude-mouse?region=3611

Antibodies/Other Reagents

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot #	Persistent ID / URL
Human CD31	Abcam	ab28364	1:50	GR3247742-11	https://www.abcam.com/cd31-antibody-ab28364.html
Rabbit IgG	Jackson Immunoresearch	711-605-152	1:200	132485	https://www.jacksonimmuno.com/catalog/products/711-605-152
Matrigel	Corning	354230	Not diluted (*in vitro*) 1:2 (*in vivo*)	6032001 (9.3 mg/mL)	https://ecatalog.corning.com/life-sciences/b2c/US/en/Surfaces/Extracellular-Matrices-ECMs/Corning%C2%AE-Matrigel%C2%AE-Matrix/p/354230
Matrigel	Corning	354230	Diluted 1:2 (*in vivo*)	9028255 (8.6 mg/mL)	https://ecatalog.corning.com/life-sciences/b2c/US/en/Surfaces/Extracellular-Matrices-ECMs/Corning%C2%AE-Matrigel%C2%AE-Matrix/p/354230

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
Human umbilical vein endothelial cells (HUVEC)	LONZA (Cat#C2519A)	M	Lot# 0000437550
Human cardiac microvascular endothelial cells (cHMVEC)	LONZA (Cat#CC-7030)	M	Lot# 0000399195
Human bone marrow-derived mesenchymal stem cells (BM-MSCs)	Rooster Bio (RoosterVial-hBM-10M [MSC-001])	M	https://www.roosterbio.com/products/roostervial-hbm-10m-msc-001/

Data & Code Availability

Description	Source / Repository	Persistent ID / URL
miRNA Panel Data for Human Pericardial Fluid Extracellular Vesicles	GEO	GSE118103
miRNA Panel Data for Human Saphenous Vein Extracellular Vesicles	GEO	GSE118855
miRNA Array Data for Mesenchymal Stem Cell Extracellular Vesicles	GEO	GSE71241

Table S5. ID numbers for Taqman® primers used in qRT-PCR experiments.

Oligo Name	miRbase Accession Number	Assay ID
cel-miR-39-3p	MIMAT0000010	000200
let-7a-5p	MIMAT0000062	000377
let-7b-5p	MIMAT0000063	002619
U6 small nuclear RNA	N/A	001973

Table S6. Sequences for the SYBR® Green primers used in qRT-PCR experiments.

Oligo Name	Forward Sequence	Reverse Sequence
APAF1	AAGCTCTCCAAATTGAAAGG	CCTTCTAAAGGGAATGATCTC
CASP3	AAAGCACTGGAATGACATC	CGCATCAATTCCACAATTTC
CTNNB1	CAACTAAACAGGAAGGGATG	CACAGGTGACCACATTTATATC
FZD4	GCAGTTCTTCCTTTGTTCTG	AGGCAAATCCAAATTCCTTC
GAPDH	CTGGGCTACACTGAGCACC	AAGTGGTCGTTGAGGGCAATG
HMGA2	AGCTCAAAAGAAAGCAGAAG	CCCTTCAAAAGATCCAACTG
STARD13	AGGATTCACAATTTCCCATC	AAAGAGGTTCTACAAGGTCC

Table S7. Gene targets of synergistic miRNA regulation for let-7a and let-7b were determined using TriplexRNA database.

GENE ID	REFSEQ ID	MIRNA1 ID	MIRNA2 ID	SEED DISTANCE (NT)	TRIPLEX ID	FREE ENERGY (KCAL/MOL)	ENERGY GAIN (KCAL/MOL)	SEED BINDING	PATTERN
GGA3	NM_138619	hsa-let-7a	hsa-let-7b	30	39186	-37.86	-12.08	Yes	Target self-complementarity
KCTD14	NM_023930	hsa-let-7a	hsa-let-7b	28	163780	-31.86	-8.18	Yes	Canonical triplex
KCTD14	NM_023930	hsa-let-7a	hsa-let-7b	28	163786	-31.76	-8.18	Yes	Canonical triplex
CTPS2	NM_019857	hsa-let-7a	hsa-let-7b	24	165638	-42.26	-19.08	Yes	Canonical triplex
NQO1	NM_000903	hsa-let-7a	hsa-let-7b	18	184240	-31.76	-11.98	Yes	Canonical triplex
CISH	NM_145071	hsa-let-7a	hsa-let-7b	16	225705	-31.16	-12.78	Yes	Canonical triplex
HOXA1	NM_153620	hsa-let-7a	hsa-let-7b	18	238991	-35.76	-8.48	Yes	Target self-complementarity
STARD13	NM_178006	hsa-let-7a	hsa-let-7b	17	258303	-26.56	-8.48	Yes	Canonical triplex
C18ORF21	NM_031446	hsa-let-7a	hsa-let-7b	32	310565	-35.16	-7.18	Yes	Canonical triplex
IL10	NM_000572	hsa-let-7a	hsa-let-7b	23	413608	-29.46	-11.78	Yes	Canonical triplex
IL10	NM_000572	hsa-let-7a	hsa-let-7b	32	413619	-30.76	-11.68	Yes	Canonical triplex
FASLG	AF288573	hsa-let-7a	hsa-let-7b	22	419431	-33.86	-5.98	No	Target self-complementarity
HMGA2	NM_003483	hsa-let-7a	hsa-let-7b	19	504515	-33.86	-11.58	Yes	Canonical triplex
DLC1	NM_182643	hsa-let-7a	hsa-let-7b	26	651299	-27.06	-8.88	Yes	Canonical triplex
EPB41L4A	NM_022140	hsa-let-7a	hsa-let-7b	30	655303	-28.46	-12.08	No	Canonical triplex

Table S8. miRNA sequences used in the preparation of AEs.

Oligo Name	Sequence (5'-3')
cel-miR-39-3p-sense	[AmC6F]CAAGCUGAUUUACACCCGGUUGA[dT][dT][Cyanine5]
cel-miR-39-3p-antisense	UCACCGGGUGUAAAUCAGCUUG
let-7b-5p-sense	[AmC6F]AACCACACAACCUACUACCUUCA[dT][dT]
let-7b-5p-antisense	UGAGGUAGUAGGUUGUGUGGUU