A. DETAILED METHODS

HUVEC Transfection

Human umbilical vein endothelial cells (HUVECs), passages 2-6, were cultured in Medium 199 (Mediatech) containing 20% fetal calf serum (Hyclone), endothelial Migogen (Biomedical Technologies Inc.), penicillin-streptomycin, glutamine, and Heparin. HUVECs were split 1:4 and transfected 24 hours later using siPORT NeoFX and Opti-MEM (Invitrogen) according to the protocol (Applied Biosystems). Cells were transfected with either 100 nM pre-miR-21 precursor and pre-miR miRNA precursor negative control #1 (referred to as control), or anti-miR-21 (100 nM) and anti-miR inhibitor negative control #1 (also referred to as control), all oligonucleotides were purchased from Applied Biosystems. Twenty-four hours after transfection Opti-MEM (reduced serum) was replaced by regular 20% medium. Twenty-four hours later, cells were harvested or subjected to treatments (i.e. USS, LPS, LY294002) for an additional 24 hours. LPS (Lipopolysaccharide, Sigma) was diluted in water and used in a 10 ng/μl concentration in medium containing 0.5% fetal calf serum, added 48 hours after transfection. LY294002 (2-(4-Morpholinyl)-8-phenyl-4H-1-benzopyran-4-one, Biosource) was dissolved in DMSO (Sigma); final concentration was 10μM and it was added 24 hours after transfection.

microRNA q-RT-PCR

microRNA reverse transcription was performed using the TaqMan microRNA Reverse transcription Kit (Applied Biosystems) at 16°C for 30 min, 42°C for 30 min, and denaturation of the enzyme at 85°C for 5 min. In the samples where cells were treated with the miR-21 inhibitor, miRNA levels were quantified using the Qiagen miScript Reverse Transcription Kit according to the protocol. The RT reaction was performed at 37°C for 1 hour followed by 5 min at 95°C. TaqMan microRNA assays (Applied Biosystems) were performed using the 7500 Fast Real-Time PCR System at the 9600 emulation run mode. Ct values were converted into copy numbers (copy# = $2^{(-Ct)}$) and normalized to RNU48. Real Time PCR for detection of miRNA reverse transcribed with Qiagen miScript was performed using QuantiTect SYBR Green PCR Master Mix and the miScript Primer Assays for miR-21 and RNU6B. The 7500 Fast Real Time PCR System from Applied Biosystems was used in the 7500 standard run mode, but cycle times and temperatures were changed to: 95°C-15 min; 94°C-15 s; 55°C-30 s; 70°C -30 s; 40 cycles. Ct values were converted into copy numbers (copy# = $2^{(-Ct)}$) and normalized to RNU6B.

Caspase 3 activity

Caspase-3 activity was measured with the ApoAlert Caspase 3 Colorimetric Assay Kit (Clontech) according to the manufacturer's instructions. Briefly, cells were harvested and washed in PBS, the cell pellet was resuspended in Cell Lysis Buffer and incubated on ice for 10 min. After centrifugation for 10 min, supernatants were incubated with Reaction Buffer/DTT mix and 50 μM Caspase-3 Substrate (DEVD-pNA, p-nitroanilin linked to a synthetic tetrapeptide) for 1 hour at 37°C. A standard curve with p-nitroanilin was created and the sample signals were determined at 405 nm.

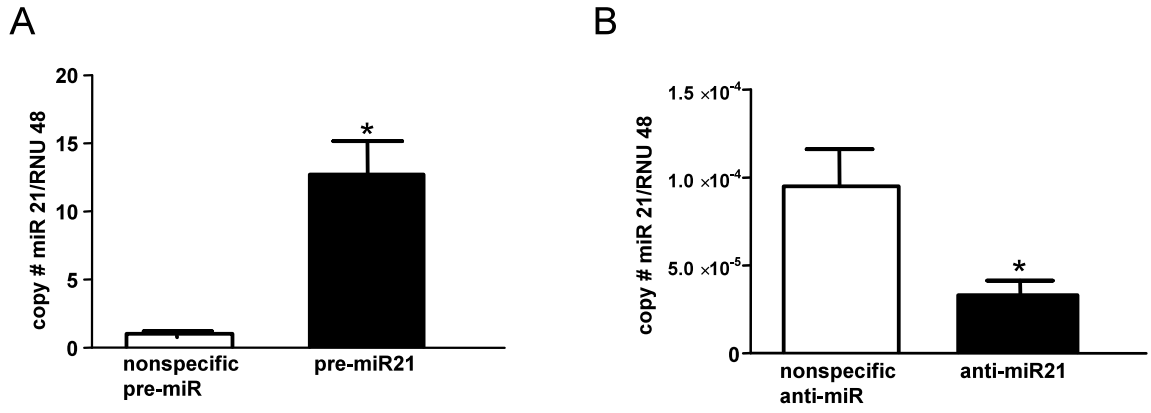
NO measurements

Measurement of NO was assessed by electron spin resonance (ESR). Briefly, HUVECs were rinsed with modified Kreb's/HEPES buffer prior to incubation with $Fe^{2+}(DETC)_2$ (0.5mmol, Sigma) in Kreb's/HEPES buffer containing the agonist (5 μM 4-Bromo-calcium-ionophore A23187, Sigma). After incubation at 37ºC for 1 hour, the cells were scraped with a cell scraper and collected as a suspension in 1ml syringes. These syringes were snap-frozen with liquid N_2. The frozen sample column was loaded into a finger Dewar and analyzed at low temperature with a Bruker EMX ESR spectrometer (Bruker Instruments, Billerica) at microwave power of 10mW and modulation amplitude of 3G.

Statistical Analysis

Data were analyzed using GraphPad Prism, paired or unpaired t-test with two-tailed p values.

B. SUPPLEMENTAL FIGURES



Supplemental Figure 1: miR-21 expression levels (assessed by qRT-PCR) in HUVECs transfected with pre- or anti-miR-21. **A:** miR-21 levels in cells transfected with pre-miR-21, but not subjected to unidirectional shear stress (USS), were 16.6 fold higher compared to control (*p=0.008, n=3-4). **B:** miR-21 levels in cells tranfected with anti-miR-21 and subjected to USS (15 dynes/cm^2 for 24 hours) were decreased 65% compared to control (*p=0.026, n=5)

C. SUPPLEMENTAL TABLE

qRT-PCR (TaqMan-based) screen of microRNA expression in HUVECs subjected to USS (15 dynes/cm^2 for 24 hours). Reported are all miRNAs whose Ct values were < 35. Fold change is compared to static cells.

miRNA	fold change	p value
hsa-let-7c	2.165239	0.0209
hsa-miR-19a	3.957717	0.0104
hsa-miR-21	5.195693	0.002
hsa-miR-23b	3.314732	0.0065
hsa-miR-24	2.139921	0.0225
hsa-miR-27b	1.652452	0.0404
hsa-miR-30a-5p	2.519366	0.022
hsa-miR-30d	1.907097	0.0313
hsa-miR-34a	2.417133	0.0057
hsa-miR-126	2.389289	0.0443
hsa-miR-155	3.298047	0.0053

hsa-miR-195	1.919694	0.0359
hsa-miR-376a	1.941718	0.0108
hsa-let-7a	1.456283	>0.05
hsa-let-7b	1.652452	>0.05
hsa-let-7d	1.094418	>0.05
hsa-let-7e	1.527945	>0.05
hsa-let-7f	1.749982	>0.05
hsa-let-7g	1.569928	>0.05
hsa-miR-7	1.886231	>0.05
hsa-miR-10a	1.334634	>0.05
hsa-miR-10b	1.418706	>0.05
hsa-miR-15a	0.835914	>0.05
hsa-miR-15b	1.116556	>0.05
hsa-miR-16	1.398043	>0.05
hsa-miR-17-3p	0.849569	>0.05
hsa-miR-17-5p	1.265627	>0.05
hsa-miR-18a	0.967873	>0.05
hsa-miR-19b	0.885657	>0.05
hsa-miR-20a	0.80673	>0.05
hsa-miR-20b	1.232948	>0.05
hsa-miR-22	1.654083	>0.05
hsa-miR-23a	1.550029	>0.05
hsa-miR-25	1.305279	>0.05
hsa-miR-26a	1.595546	>0.05
hsa-miR-26b	2.117428	>0.05
hsa-miR-27a	1.531088	>0.05
hsa-miR-28	1.404643	>0.05
hsa-miR-29a	1.385113	>0.05
hsa-miR-29c	2.967963	>0.05

hsa-miR-30a-3p	1.891644	>0.05
hsa-miR-30b	1.779293	>0.05
hsa-miR-30c	1.4097	>0.05
hsa-miR-30e-3p	1.671238	>0.05
hsa-miR-30e-5p	5.953496	>0.05
hsa-miR-31	1.281475	>0.05
hsa-miR-92	1.216592	>0.05
hsa-miR-93	1.354219	>0.05
hsa-miR-98	3.461685	>0.05
hsa-miR-99a	0.841991	>0.05
hsa-miR-99b	1.266058	>0.05
hsa-miR-100	1.272092	>0.05
hsa-miR-101	1.795415	>0.05
hsa-miR-103	1.517289	>0.05
hsa-miR-106b	1.501333	>0.05
hsa-miR-107	0.225514	>0.05
hsa-miR-125a	1.561171	>0.05
hsa-miR-125b	1.13252	>0.05
hsa-miR-127	1.653399	>0.05
hsa-miR-130a	0.917949	>0.05
hsa-miR-130b	1.220525	>0.05
hsa-miR-132	1.471617	>0.05
hsa-miR-133b	1.359988	>0.05
hsa-miR-134	1.816696	>0.05
hsa-miR-137	1.101529	>0.05
hsa-miR-139	1.864663	>0.05
hsa-miR-140	1.564705	>0.05
hsa-miR-146a	0.63044	>0.05
hsa-miR-146b	1.581084	>0.05

hsa-miR-148b	1.576618	>0.05
hsa-miR-149	1.439483	>0.05
hsa-miR-151	1.084858	>0.05
hsa-miR-152	1.68394	>0.05
hsa-miR-181b	1.105462	>0.05
hsa-miR-181c	4.187537	>0.05
hsa-miR-181d	1.037452	>0.05
hsa-miR-186	1.156313	>0.05
hsa-miR-191	0.984388	>0.05
hsa-miR-192	1.69774	>0.05
hsa-miR-193a	2.068048	>0.05
hsa-miR-194	1.01392	>0.05
hsa-miR-196a	1.766842	>0.05
hsa-miR-196b	1.810122	>0.05
hsa-miR-197	1.322092	>0.05
hsa-miR-199a	1.208104	>0.05
hsa-miR-204	0.631978	>0.05
hsa-miR-210	0.767099	>0.05
hsa-miR-213	1.162646	>0.05
hsa-miR-214	1.23155	>0.05
hsa-miR-216	1.356563	>0.05
hsa-miR-218	1.291514	>0.05
hsa-miR-219	1.783671	>0.05
hsa-miR-221	0.729211	>0.05
hsa-miR-222	0.800003	>0.05
hsa-miR-223	0.030096	>0.05
hsa-miR-224	1.493814	>0.05
hsa-miR-296	2.21395	>0.05
hsa-miR-299-5p	1.264429	>0.05
hsa-miR-301	1.408488	>0.05

hsa-miR-320	1.250014	>0.05
hsa-miR-323	1.438143	>0.05
hsa-miR-324-3p	0.898928	>0.05
hsa-miR-324-5p	1.216787	>0.05
hsa-miR-326	0.773437	>0.05
hsa-miR-328	1.273096	>0.05
hsa-miR-330	1.347405	>0.05
hsa-miR-331	1.308656	>0.05
hsa-miR-335	1.611039	>0.05
hsa-miR-339	0.725258	>0.05
hsa-miR-340	1.901842	>0.05
hsa-miR-342	1.111678	>0.05
hsa-miR-345	1.239641	>0.05
hsa-miR-361	1.130184	>0.05
hsa-miR-362	1.241492	>0.05
hsa-miR-365	1.190561	>0.05
hsa-miR-374	2.139966	>0.05
hsa-miR-379	1.523383	>0.05
hsa-miR-382	1.346672	>0.05
hsa-miR-383	0.616925	>0.05
hsa-miR-410	1.443092	>0.05
hsa-miR-411	1.147077	>0.05
hsa-miR-423	1.168924	>0.05
hsa-miR-425	1.341101	>0.05
hsa-miR-425-5p	1.309365	>0.05
hsa-miR-432	1.263486	>0.05
hsa-miR-433	0.769696	>0.05
hsa-miR-449b	0.737638	>0.05
hsa-miR-452	1.94065	>0.05
hsa-miR-484	1.146406	>0.05

hsa-miR-485-3p	1.542929	>0.05
hsa-miR-485-5p	1.088145	>0.05
hsa-miR-486	6.026851	>0.05
hsa-miR-487b	0.818626	>0.05
hsa-miR-491	1.334769	>0.05
hsa-miR-497	1.159712	>0.05
hsa-miR-501	1.443936	>0.05
hsa-miR-502	0.928923	>0.05
hsa-miR-515-3p	0.550345	>0.05
hsa-miR-517c	0.56083	>0.05
hsa-miR-518e	0.549041	>0.05
hsa-miR-518f	0.516897	>0.05
hsa-miR-519c	0.981187	>0.05
hsa-miR-519e	0.639661	>0.05
hsa-miR-520a	0.870298	>0.05
hsa-miR-520g	0.687553	>0.05
hsa-miR-521	0.543887	>0.05
hsa-miR-532	1.501457	>0.05
hsa-miR-550	0.881381	>0.05
hsa-miR-564	1.178718	>0.05
hsa-miR-565	1.001632	>0.05
hsa-miR-572	2.249813	>0.05
hsa-miR-589	1.486408	>0.05
hsa-miR-594	1.109588	>0.05
hsa-miR-629	0.90299	>0.05
hsa-miR-650	0.785581	>0.05
hsa-miR-659	1.276303	>0.05
hsa-miR-660	1.238177	>0.05