

ONLINE SUPPLEMENT

Endogenous microRNAs in Human Microvascular Endothelial Cells Regulate mRNAs Encoded by Hypertension-Related Genes

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Running title: Endothelial miRNAs relevant to hypertension

Supplementary methods, 3 tables and 2 figures

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Note: Supplemental Tables were converted to PDF per journal requirements. Datasets in analyzable formats such as Excel are available upon request.

Supplemental Methods-

Cell culture- Human dermal microvascular endothelial cells (HMVEC-D) were cultured in EGM – MV medium (Clonetics).¹ Cells were subcultured at 80% confluency for propagation.

RNA extraction- RNA for small RNA deep sequencing was extracted using a chloroform/trizol method, as previously described,² from cells cultured in 35 mm dishes and collected at >90% confluency. Total RNA was quantified by Nanodrop. The RNA from the transfected cells was extracted from 96 well plates using the Aurum Total RNA 96 Kit (BioRad) and resuspended in RNase-free water. Samples were stored at -80°C until the RNA was analyzed.

Small RNA deep sequencing- Equal amounts of RNA from 6 plates of HMVEC-D cells were pooled, to produce 2 pools (3 plates/pool). Small RNA libraries were created using the TruSeq Small RNA Sample Preparation Kit (Illumina) and cluster generation and sequencing performed using TruSeq PE Cluster Kit v3-cBot-HS, TruSeq SBS kit v3-HS, and a HiSeq 2000 sequencer (Illumina), as previously described.³

Analysis of small RNA deep sequencing data- Small RNA deep sequencing data was analyzed as previously described,³ except that sequencing reads were mapped against miRBase v18. miRNA expression levels were normalized using the quartile method.³

Identification of genes involved with blood pressure regulation and hypertension- The literature was reviewed to identify and compile a list of genes involved in blood pressure regulation and/or hypertension and likely expressed in endothelial cells. Hypertension risk genes identified by human GWAS were obtained from the literature.^{4,5}

Inhibition of endogenous miRNA using LNA anti-miR- HMVEC-D cells were subcultured onto 96 well plates and transfected at >90% confluency with locked nucleic acid (LNA)-modified anti-miRs from Exiqon.^{6,7} Transfections of LNA anti-miR at a final concentration of 10 nM were performed using Lipofectamine 2000 (following manufacturer instructions).

Real-time PCR- mRNA expression levels of the blood pressure/hypertension related genes predicted to be targeted by miRNAs inhibited by anti-miR transfection were measured by qPCR using SYBR green chemistry, as previously described.² These genes included *ADM1*, *ADRA1B*, *ADRA1D*, *ADRA2A*, *ADRA2B*, *ADRB1*, *ATP2B1*, *CAT*, *EDN1*, *EDNRA*, *EDNRB*, *FGF5*, *FURIN-FES*, *GOSR2*, *NOX2*, *NOX4*, *PLECKHA7*, *SH2B3*, *TBX3*, and *TBX5*. Primer sequences are listed in **Supplemental Table S3**.

Chronic Blood Pressure Measurement in anti-miR treated mice- Male C57BL/6J mice (Jackson Lab) were maintained on AIN-76A diet (Dyets) containing 0.4% NaCl after receipt. Telemeters were implanted into the carotid artery at 10 weeks of age, and animals were allowed to recover for 5 days prior to the start of 24 hour blood pressure monitoring. Mice received intraperitoneal injection of LNA-anti-miR-21 or scrambled LNA-anti-miR (Exiqon) (10 mg/kg body weight, in saline) or switched to AIN-76A diet containing 4% NaCl as explained in Results.

Measurement of hydrogen peroxide (H₂O₂) release- HMVEC-D were cultured and transfected with LNA-anti-miR as described above. At 48 hours after transfection an Amplex Red Assay (Life Technologies) was performed to measure the H₂O₂ released from the cells over a 5.5 hour period. H₂O₂ signal was normalized by total protein in each well to account for cell number.

References:

1. Liang M, Pietrusz JL. Thiol-related genes in diabetic complications: a novel protective role for endogenous thioredoxin 2. *Arteriosclerosis, thrombosis, and vascular biology*. 2007;27:77-83.
2. Kriegel AJ, Fang Y, Liu Y, et al. MicroRNA-target pairs in human renal epithelial cells treated with transforming growth factor beta 1: a novel role of miR-382. *Nucleic acids research*. 2010;38:8338-8347.
3. Kriegel AJ, Liu Y, Liu P, Baker MA, Hodges MR, Hua X, Liang M. Characteristics of microRNAs enriched in specific cell types and primary tissue types in solid organs. *Physiol Genomics*. 2013;45:1144-1156.
4. Ehret GB. Genome-wide association studies: contribution of genomics to understanding blood pressure and essential hypertension. *Curr Hypertens Rep*. 2010;12:17-25.
5. International Consortium for Blood Pressure Genome-Wide Association Studies, Ehret GB, Munroe PB, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*. 2011;478:103-109.
6. Mladinov D, Liu Y, Mattson DL, Liang M. MicroRNAs contribute to the maintenance of cell-type-specific physiological characteristics: miR-192 targets Na⁺/K⁺-ATPase beta1. *Nucleic acids research*. 2013;41:1273-1283.
7. Xu X, Kriegel AJ, Liu Y, Usa K, Mladinov D, Liu H, Fang Y, Ding X, Liang M. Delayed ischemic preconditioning contributes to renal protection by upregulation of miR-21. *Kidney international*. 2012;82:1167-1175.

Supplemental Table S1. Small RNA deep sequencing result.

miRbase v18 ID	miRNA category	HMVEC-D pool 1	HMVEC-D pool 2	Pool Average
hsa-miR-10b-5p	mature_unique	2,034,760	2,658,905	2,346,832
hsa-miR-22-3p	mature_unique	1,132,311	1,132,635	1,132,473
hsa-miR-10a-5p	mature_unique	901,041	1,145,679	1,023,360
hsa-miR-126-5p	mature_unique	881,777	897,159	889,468
hsa-let-7a-5p	mature_unique	759,738	991,392	875,565
hsa-miR-21-5p	mature_unique	663,316	743,919	703,617
hsa-miR-222-3p	mature_unique	647,314	547,468	597,391
hsa-miR-127-3p	mature_unique	494,543	535,541	515,042
hsa-miR-191-5p	mature_unique	420,577	455,747	438,162
hsa-miR-100-5p	mature_unique	255,959	323,501	289,730
hsa-miR-181a-5p	mature_unique	274,708	268,503	271,605
hsa-miR-30a-5p	mature_unique	272,270	207,750	240,010
hsa-let-7f-5p	mature_unique	170,010	191,778	180,894
hsa-miR-26a-5p	mature_unique	164,653	188,534	176,594
hsa-miR-92a-3p	mature_unique	161,253	170,748	166,001
hsa-miR-151a-3p	mature_unique	174,508	156,345	165,426
hsa-miR-221-3p	mature_unique	128,975	179,397	154,186
hsa-miR-27b-3p	mature_unique	129,628	131,514	130,571
hsa-miR-125a-5p	mature_unique	115,068	107,589	111,329
hsa-miR-151a-5p	mature_unique	94,328	100,719	97,523
hsa-miR-28-3p	mature_unique	106,964	88,583	97,773
hsa-miR-99b-5p	mature_unique	78,635	84,502	81,569
hsa-let-7i-5p	mature_unique	86,155	71,738	78,947
hsa-miR-126-3p	mature_unique	51,748	66,542	59,145
hsa-let-7e-5p	mature_unique	51,988	60,613	56,301
hsa-miR-92b-3p	mature_unique	53,637	56,532	55,085
hsa-miR-146a-5p	mature_unique	46,838	48,999	47,919
hsa-miR-186-5p	mature_unique	49,310	44,152	46,731
hsa-let-7b-5p	mature_unique	42,580	48,581	45,580
hsa-miR-98	mature_unique	39,180	48,651	43,915
hsa-miR-423-3p	mature_unique	49,756	35,433	42,595
hsa-miR-30d-5p	mature_unique	38,768	38,363	38,565
hsa-miR-27a-3p	mature_unique	40,485	36,096	38,290
hsa-miR-21-3p	mature_unique	44,983	26,993	35,988
hsa-miR-181b-5p	mature_unique	40,176	30,062	35,119
hsa-miR-125b-5p	mature_unique	33,137	31,422	32,279
hsa-miR-16-5p	mature_unique	26,818	32,643	29,731
hsa-miR-409-3p	mature_unique	26,956	27,133	27,044
hsa-let-7g-5p	mature_unique	21,084	32,434	26,759
hsa-miR-654-3p	mature_unique	22,698	29,609	26,153
hsa-miR-423-5p	mature_unique	31,557	20,297	25,927
hsa-miR-31-5p	mature_unique	23,144	23,680	23,412
hsa-miR-3182	mature_unique	24,552	22,320	23,436
hsa-miR-411-5p	mature_unique	21,668	22,320	21,994

hsa-miR-103a-3p	mature_unique	18,680	23,541	21,110
hsa-miR-103b	mature_unique	17,993	21,378	19,686
hsa-miR-3184-3p	mature_unique	23,247	14,473	18,860
hsa-miR-30e-5p	mature_unique	20,637	16,217	18,427
hsa-miR-25-3p	mature_unique	17,135	18,484	17,809
hsa-let-7c	mature_unique	15,006	15,868	15,437
hsa-miR-148b-3p	mature_unique	14,010	16,356	15,183
hsa-miR-381	mature_unique	16,105	13,253	14,679
hsa-miR-29a-3p	mature_unique	13,495	13,915	13,705
hsa-miR-30c-5p	mature_unique	12,740	13,148	12,944
hsa-miR-23a-3p	mature_unique	10,576	13,601	12,089
hsa-miR-24-3p	mature_unique	11,984	11,021	11,502
hsa-miR-221-5p	mature_unique	8,070	16,287	12,178
hsa-miR-30a-3p	mature_unique	9,546	11,823	10,684
hsa-miR-26b-5p	mature_unique	8,276	12,695	10,485
hsa-miR-941	mature_unique	9,134	8,789	8,961
hsa-miR-345-5p	mature_unique	9,649	8,091	8,870
hsa-miR-181a-3p	mature_unique	7,211	7,777	7,494
hsa-miR-3074-5p	mature_unique	8,276	6,591	7,433
hsa-miR-769-5p	mature_unique	6,181	8,649	7,415
hsa-let-7d-5p	mature_unique	6,833	7,463	7,148
hsa-miR-410	mature_unique	6,250	7,359	6,804
hsa-miR-125b-1-3p	mature_unique	7,314	5,057	6,185
hsa-miR-136-3p	mature_unique	6,353	5,266	5,809
hsa-miR-28-5p	mature_unique	4,910	5,615	5,263
hsa-miR-320a	mature_unique	5,563	4,499	5,031
hsa-miR-484	mature_unique	4,670	4,708	4,689
hsa-miR-486-5p	mature_unique	4,567	4,464	4,516
hsa-miR-500a-3p	mature_unique	4,395	4,290	4,342
hsa-miR-1260b	mature_unique	3,915	3,697	3,806
hsa-miR-589-5p	mature_unique	4,086	3,522	3,804
hsa-miR-874	mature_unique	3,606	3,836	3,721
hsa-miR-34a-5p	mature_unique	3,331	3,836	3,584
hsa-miR-378a-3p	mature_unique	3,846	3,104	3,475
hsa-miR-125b-2-3p	mature_unique	3,983	2,964	3,474
hsa-miR-93-5p	mature_unique	3,228	3,627	3,427
hsa-miR-130b-3p	mature_unique	2,987	3,522	3,255
hsa-miR-574-3p	mature_unique	3,159	3,174	3,166
hsa-miR-155-5p	mature_unique	3,262	2,999	3,131
hsa-miR-23b-3p	mature_unique	2,335	4,011	3,173
hsa-miR-134	mature_unique	2,953	3,069	3,011
hsa-miR-130a-3p	mature_unique	2,884	2,825	2,855
hsa-miR-224-5p	mature_unique	2,816	2,860	2,838
hsa-miR-192-5p	mature_unique	2,987	2,511	2,749
hsa-miR-140-3p	mature_unique	2,541	2,651	2,596
hsa-miR-889	mature_unique	2,301	2,755	2,528
hsa-miR-199a-3p#hsa-rr	mature_unique	2,507	2,197	2,352

hsa-miR-671-3p	mature_unique	2,747	1,883	2,315
hsa-miR-30e-3p	mature_unique	1,751	2,825	2,288
hsa-miR-361-5p	mature_unique	1,717	2,790	2,253
hsa-miR-101-3p	mature_unique	2,472	1,814	2,143
hsa-miR-100-3p	mature_unique	2,404	1,535	1,969
hsa-miR-20a-5p	mature_unique	1,580	2,197	1,888
hsa-miR-340-5p	mature_unique	1,957	1,709	1,833
hsa-miR-197-3p	mature_unique	1,545	2,162	1,854
hsa-miR-421	mature_unique	1,820	1,709	1,764
hsa-miR-584-5p	mature_unique	2,095	1,395	1,745
hsa-miR-532-5p	mature_unique	1,751	1,604	1,678
hsa-miR-744-5p	mature_unique	2,129	1,290	1,710
hsa-miR-193a-5p	mature_unique	1,374	1,988	1,681
hsa-miR-181a-2-3p	mature_unique	1,889	1,360	1,624
hsa-let-7b-3p	mature_unique	1,477	1,674	1,575
hsa-miR-15b-5p	mature_unique	1,133	2,058	1,595
hsa-miR-99a-5p	mature_unique	1,133	1,988	1,561
hsa-miR-450b-5p	mature_unique	1,580	1,395	1,487
hsa-miR-130b-5p	mature_unique	1,580	1,221	1,400
hsa-miR-181c-5p	mature_unique	1,168	1,569	1,368
hsa-miR-19b-3p	mature_unique	1,236	1,430	1,333
hsa-miR-146b-5p	mature_unique	1,442	1,186	1,314
hsa-miR-424-5p	mature_unique	1,305	1,290	1,298
hsa-miR-432-5p	mature_unique	1,442	1,151	1,297
hsa-miR-143-3p	mature_unique	1,717	907	1,312
hsa-miR-27a-5p	mature_unique	1,236	1,116	1,176
hsa-let-7d-3p	mature_unique	1,236	1,011	1,124
hsa-miR-152	mature_unique	790	1,500	1,145
hsa-miR-29c-3p	mature_unique	1,408	837	1,122
hsa-miR-128	mature_unique	927	1,221	1,074
hsa-miR-301a-3p	mature_unique	893	1,151	1,022
hsa-miR-17-5p	mature_unique	961	1,046	1,004
hsa-miR-370	mature_unique	961	1,046	1,004
hsa-miR-181c-3p	mature_unique	893	1,011	952
hsa-miR-431-5p	mature_unique	961	907	934
hsa-miR-1307-3p	mature_unique	1,064	802	933
hsa-miR-758	mature_unique	824	977	900
hsa-miR-1304-3p	mature_unique	927	837	882
hsa-miR-501-3p	mature_unique	961	802	882
hsa-miR-424-3p	mature_unique	790	977	883
hsa-miR-374a-3p	mature_unique	755	942	849
hsa-miR-4455	mature_unique	824	802	813
hsa-miR-339-3p	mature_unique	893	663	778
hsa-miR-181d	mature_unique	618	942	780
hsa-miR-342-3p	mature_unique	515	1,116	816
hsa-miR-15a-5p	mature_unique	652	872	762
hsa-miR-195-5p	mature_unique	549	872	711

hsa-miR-374c-3p	mature_unique	927	488	708
hsa-miR-30b-5p	mature_unique	549	802	676
hsa-miR-365a-3p#hsa-rr	mature_unique	652	663	658
hsa-miR-369-5p	mature_unique	721	558	640
hsa-miR-23a-5p	mature_unique	515	767	641
hsa-miR-1910	mature_unique	652	593	623
hsa-miR-654-5p	mature_unique	584	663	623
hsa-miR-455-3p	mature_unique	481	732	607
hsa-miR-425-5p	mature_unique	309	1,116	713
hsa-miR-301b	mature_unique	618	558	588
hsa-miR-582-3p	mature_unique	515	663	589
hsa-miR-183-5p	mature_unique	2,369	140	1,254
hsa-miR-455-5p	mature_unique	652	488	570
hsa-miR-139-5p	mature_unique	412	767	590
hsa-miR-450a-5p	mature_unique	687	453	570
hsa-miR-3184-5p	mature_unique	481	628	554
hsa-miR-193b-3p	mature_unique	549	523	536
hsa-miR-379-5p	mature_unique	584	453	519
hsa-miR-30c-2-3p	mature_unique	515	488	502
hsa-miR-497-5p	mature_unique	515	488	502
hsa-miR-665	mature_unique	515	488	502
hsa-miR-574-5p	mature_unique	412	593	502
hsa-miR-106b-3p	mature_unique	515	453	484
hsa-miR-548k	mature_unique	549	419	484
hsa-miR-299-3p	mature_unique	481	419	450
hsa-miR-1307-5p	mature_unique	824	244	534
hsa-miR-660-5p	mature_unique	275	732	504
hsa-miR-374a-5p	mature_unique	412	488	450
hsa-miR-3168	mature_unique	618	314	466
hsa-miR-29b-3p	mature_unique	721	244	483
hsa-miR-485-5p	mature_unique	721	244	483
hsa-miR-323b-3p	mature_unique	446	349	398
hsa-miR-330-5p	mature_unique	378	384	381
hsa-miR-720	mature_unique	378	384	381
hsa-miR-4532	mature_unique	824	174	499
hsa-miR-4677-3p	mature_unique	343	419	381
hsa-miR-487b	mature_unique	412	349	380
hsa-miR-411-3p	mature_unique	343	384	364
hsa-miR-887	mature_unique	618	209	414
hsa-miR-1296	mature_unique	275	453	364
hsa-miR-323a-3p	mature_unique	309	384	346
hsa-miR-149-5p	mature_unique	172	558	365
hsa-miR-542-3p	mature_unique	275	349	312
hsa-miR-222-5p	mature_unique	275	314	294
hsa-miR-376c	mature_unique	309	244	277
hsa-miR-452-5p	mature_unique	309	244	277
hsa-miR-935	mature_unique	309	244	277

hsa-miR-2682-5p	mature_unique	515	140	327
hsa-miR-4301	mature_unique	343	209	276
hsa-miR-361-3p	mature_unique	240	279	260
hsa-miR-10a-3p	mature_unique	172	349	260
hsa-miR-379-3p	mature_unique	172	314	243
hsa-miR-125a-3p	mature_unique	309	174	242
hsa-miR-19a-3p	mature_unique	309	174	242
hsa-miR-26b-3p	mature_unique	309	174	242
hsa-miR-3605-3p	mature_unique	309	174	242
hsa-miR-3909	mature_unique	206	244	225
hsa-miR-339-5p	mature_unique	137	314	226
hsa-miR-27b-5p	mature_unique	240	174	207
hsa-miR-132-3p	mature_unique	275	140	207
hsa-miR-377-5p	mature_unique	275	140	207
hsa-miR-4448	mature_unique	275	140	207
hsa-miR-138-5p	mature_unique	172	209	190
hsa-miR-337-3p	mature_unique	172	209	190
hsa-miR-107	mature_unique	137	244	191
hsa-miR-30c-1-3p	mature_unique	137	244	191
hsa-miR-22-5p	mature_unique	172	174	173
hsa-miR-335-3p	mature_unique	172	174	173
hsa-miR-127-5p	mature_unique	206	140	173
hsa-miR-382-5p	mature_unique	172	140	156
hsa-miR-503	mature_unique	172	140	156
hsa-miR-708-3p	mature_unique	172	140	156
hsa-miR-369-3p	mature_unique	137	140	138
hsa-miR-433	mature_unique	137	140	138
hsa-miR-328	mature_unique	584	0	292
hsa-miR-454-3p	mature_unique	0	419	209
hsa-miR-409-5p	mature_unique	0	349	174
hsa-miR-548o-3p	mature_unique	0	314	157
hsa-miR-1185-1-3p	mature_unique	309	0	155
hsa-miR-196b-5p	mature_unique	275	0	137
hsa-miR-664-3p	mature_unique	275	0	137
hsa-miR-548g-5p#hsa-rr	mature_unique	0	244	122
hsa-miR-138-1-3p	mature_unique	240	0	120
hsa-let-7a-3p	mature_unique	0	209	105
hsa-miR-24-2-5p	mature_unique	0	209	105
hsa-miR-3152-5p	mature_unique	0	209	105
hsa-miR-362-5p	mature_unique	0	209	105
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hsa-miR-136-5p	mature_unique	206	0	103
hsa-miR-376a-3p	mature_unique	206	0	103
hsa-miR-485-3p	mature_unique	206	0	103
hsa-let-7f-2-3p	mature_unique	0	174	87
hsa-miR-1180	mature_unique	0	174	87
hsa-miR-190a	mature_unique	0	174	87

hsa-miR-26a-2-3p	mature_unique	0	174	87
hsa-miR-340-3p	mature_unique	0	174	87
hsa-miR-3529-3p	mature_unique	0	174	87
hsa-miR-377-3p	mature_unique	0	174	87
hsa-miR-1271-5p	mature_unique	172	0	86
hsa-miR-214-3p	mature_unique	172	0	86
hsa-miR-34a-3p	mature_unique	172	0	86
hsa-miR-561-5p	mature_unique	172	0	86
hsa-miR-629-5p	mature_unique	172	0	86
hsa-miR-656	mature_unique	172	0	86
hsa-let-7f-1-3p	mature_unique	0	140	70
hsa-miR-182-5p	mature_unique	0	140	70
hsa-miR-29c-5p	mature_unique	0	140	70
hsa-miR-454-5p	mature_unique	0	140	70
hsa-miR-652-3p	mature_unique	0	140	70
hsa-miR-668	mature_unique	0	140	70
hsa-miR-93-3p	mature_unique	0	140	70
hsa-miR-106b-5p	mature_unique	137	0	69
hsa-miR-10b-3p	mature_unique	137	0	69
hsa-miR-1246	mature_unique	137	0	69
hsa-miR-1908	mature_unique	137	0	69
hsa-miR-195-3p	mature_unique	137	0	69
hsa-miR-2467-5p	mature_unique	137	0	69
hsa-miR-25-5p	mature_unique	137	0	69
hsa-miR-34c-5p	mature_unique	137	0	69
hsa-miR-3615	mature_unique	137	0	69
hsa-miR-4435	mature_unique	137	0	69
hsa-miR-505-3p	mature_unique	137	0	69
hsa-miR-671-5p	mature_unique	137	0	69
hsa-miR-7-5p	mature_unique	137	0	69
hsa-miR-99b-3p	mature_unique	137	0	69
hsa-let-7a-2-3p	mature_unique	0	0	0
hsa-let-7e-3p	mature_unique	0	0	0
hsa-let-7g-3p	mature_unique	0	0	0
hsa-let-7i-3p	mature_unique	0	0	0
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hsa-miR-1185-2-3p	mature_unique	0	0	0
hsa-miR-1185-5p	mature_unique	0	0	0
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hsa-miR-1197	mature_unique	0	0	0
hsa-miR-1226-3p	mature_unique	0	0	0
hsa-miR-1228-3p	mature_unique	0	0	0
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hsa-miR-1249	mature_unique	0	0	0
hsa-miR-1254	mature_unique	0	0	0

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hsa-miR-1273g-3p	mature_unique	0	0	0
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hsa-miR-1276	mature_unique	0	0	0
hsa-miR-1277-5p	mature_unique	0	0	0
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hsa-miR-1285-5p	mature_unique	0	0	0
hsa-miR-1287	mature_unique	0	0	0
hsa-miR-1290	mature_unique	0	0	0
hsa-miR-1293	mature_unique	0	0	0
hsa-miR-1294	mature_unique	0	0	0
hsa-miR-1301	mature_unique	0	0	0
hsa-miR-1303	mature_unique	0	0	0
hsa-miR-1306-5p	mature_unique	0	0	0
hsa-miR-1323	mature_unique	0	0	0
hsa-miR-132-5p	mature_unique	0	0	0
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hsa-miR-1343	mature_unique	0	0	0
hsa-miR-135b-3p	mature_unique	0	0	0
hsa-miR-135b-5p	mature_unique	0	0	0
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hsa-miR-140-5p	mature_unique	0	0	0
hsa-miR-141-3p	mature_unique	0	0	0
hsa-miR-141-5p	mature_unique	0	0	0
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hsa-miR-145-5p	mature_unique	0	0	0
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hsa-miR-146b-3p	mature_unique	0	0	0
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hsa-miR-204-5p	mature_unique	0	0	0
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hsa-miR-2116-3p	mature_unique	0	0	0
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hsa-miR-218-5p	mature_unique	0	0	0
hsa-miR-219-1-3p	mature_unique	0	0	0
hsa-miR-224-3p	mature_unique	0	0	0
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hsa-miR-29b-1-5p	mature_unique	0	0	0
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hsa-miR-4662a-5p	mature_unique	0	0	0
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hsa-miR-548ah-3p	mature_unique	0	0	0
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hsa-miR-548f	mature_unique	0	0	0
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hsa-miR-550a-5p	mature_unique	0	0	0
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hsa-miR-589-3p	mature_unique	0	0	0
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hsa-miR-625-5p	mature_unique	0	0	0
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hsa-miR-628-3p	mature_unique	0	0	0

hsa-miR-628-5p	mature_unique	0	0	0
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hsa-miR-644b-3p	mature_unique	0	0	0
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hsa-miR-655	mature_unique	0	0	0
hsa-miR-659-5p	mature_unique	0	0	0
hsa-miR-664-5p	mature_unique	0	0	0
hsa-miR-708-5p	mature_unique	0	0	0
hsa-miR-7-1-3p	mature_unique	0	0	0
hsa-miR-744-3p	mature_unique	0	0	0
hsa-miR-760	mature_unique	0	0	0
hsa-miR-766-3p	mature_unique	0	0	0
hsa-miR-769-3p	mature_unique	0	0	0
hsa-miR-770-5p	mature_unique	0	0	0
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hsa-miR-891a	mature_unique	0	0	0
hsa-miR-92a-1-5p	mature_unique	0	0	0
hsa-miR-92b-5p	mature_unique	0	0	0
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hsa-miR-940	mature_unique	0	0	0
hsa-miR-942	mature_unique	0	0	0
hsa-miR-9-5p	mature_unique	0	0	0
hsa-miR-96-5p	mature_unique	0	0	0
hsa-miR-99a-3p	mature_unique	0	0	0
aca-miR-10c	maturehomolog_u	0	0	
aly-miR161.1	maturehomolog_u	0	0	
ath-miR5658	maturehomolog_u	0	0	
bfl-let-7b#rno-miR-3596	maturehomolog_u	59,543	70,622	
bfl-miR-125b#rno-miR-3	maturehomolog_u	26,578	26,017	
bfl-miR-29a*	maturehomolog_u	0	0	
bmo-miR-2779	maturehomolog_u	378	174	
bmo-miR-2780a*	maturehomolog_u	0	0	
bmo-miR-2842	maturehomolog_u	0	0	
bta-miR-1434*	maturehomolog_u	0	0	
bta-miR-1940	maturehomolog_u	0	0	
bta-miR-2340	maturehomolog_u	54,358	30,202	
bta-miR-2354	maturehomolog_u	0	0	
bta-miR-2404	maturehomolog_u	0	0	
bta-miR-2478	maturehomolog_u	2,953	1,779	
bta-miR-2484	maturehomolog_u	0	0	
bta-miR-2487	maturehomolog_u	275	209	
bta-miR-26c	maturehomolog_u	177,530	200,775	
bta-miR-2887	maturehomolog_u	0	0	
bta-miR-2889	maturehomolog_u	1,889	1,674	

bta-miR-2898	maturehomolog_ui	2,198	1,325
bta-miR-2904	maturehomolog_ui	0	0
bta-miR-2957	maturehomolog_ui	0	0
bta-miR-3578	maturehomolog_ui	0	0
bta-miR-3596	maturehomolog_ui	40,107	41,745
bta-miR-3600	maturehomolog_ui	1,129,014	1,125,660
bta-miR-3601	maturehomolog_ui	0	0
bta-miR-3604	maturehomolog_ui	2,919	2,755
bta-miR-669	maturehomolog_ui	2,472	2,720
cel-miR-39-3p	maturehomolog_ui	0	0
cfa-miR-1839#mmu-miF	maturehomolog_ui	0	209
cfa-miR-1843	maturehomolog_ui	0	0
cin-miR-3575-5p	maturehomolog_ui	0	0
cin-miR-4185-3p	maturehomolog_ui	0	0
dme-miR-10-3p#bmo-m	maturehomolog_ui	32,931	43,071
dme-miR-10-5p#aae-mi	maturehomolog_ui	378	349
dme-miR-4949-5p	maturehomolog_ui	0	0
dme-miR-4951-5p	maturehomolog_ui	0	0
dme-miR-979-5p	maturehomolog_ui	0	0
dre-miR-125a#fru-miR-1	maturehomolog_ui	0	0
dre-miR-27e#fru-miR-27	maturehomolog_ui	0	0
ebv-miR-BART10*	maturehomolog_ui	0	0
eca-miR-199a-3p	maturehomolog_ui	0	0
gga-miR-1565	maturehomolog_ui	0	0
gga-miR-1622	maturehomolog_ui	0	0
gga-miR-1692	maturehomolog_ui	172	0
gga-miR-1816	maturehomolog_ui	206	279
gga-miR-3535	maturehomolog_ui	0	140
gma-miR1520h	maturehomolog_ui	0	0
hcmv-miR-US25-1*	maturehomolog_ui	0	0
hsv1-miR-H17	maturehomolog_ui	137	0
mdo-miR-151	maturehomolog_ui	0	0
mmu-miR-1195	maturehomolog_ui	0	0
mmu-miR-1839-3p	maturehomolog_ui	0	0
mmu-miR-1843-3p	maturehomolog_ui	0	0
mmu-miR-1843-5p	maturehomolog_ui	0	0
mmu-miR-1957	maturehomolog_ui	0	0
mmu-miR-1983	maturehomolog_ui	0	244
mmu-miR-2137	maturehomolog_ui	1,408	1,151
mmu-miR-300-3p#rno-r	maturehomolog_ui	0	0
mmu-miR-3071-3p	maturehomolog_ui	0	0
mmu-miR-3096-5p	maturehomolog_ui	5,391	5,824
mmu-miR-3096b-5p	maturehomolog_ui	5,219	5,162
mmu-miR-3963	maturehomolog_ui	0	0
mmu-miR-3968	maturehomolog_ui	0	0
mmu-miR-5097	maturehomolog_ui	1,614	1,709
mmu-miR-5105	maturehomolog_ui	0	0

mmu-miR-5106	maturehomolog_ui	0	0
mmu-miR-5109	maturehomolog_ui	0	0
mmu-miR-5115	maturehomolog_ui	19,710	17,368
mmu-miR-5119	maturehomolog_ui	0	0
mmu-miR-5124	maturehomolog_ui	0	0
mne-miR-140	maturehomolog_ui	0	0
oan-miR-1357	maturehomolog_ui	0	0
oan-miR-1386	maturehomolog_ui	1,030	488
oar-miR-3955-5p	maturehomolog_ui	0	0
oar-miR-654-3p	maturehomolog_ui	0	0
pma-let-7b	maturehomolog_ui	0	0
rno-miR-218b	maturehomolog_ui	0	0
rno-miR-3548	maturehomolog_ui	0	0
rno-miR-3549	maturehomolog_ui	412	593
rno-miR-3553	maturehomolog_ui	2,335	279
rno-miR-3554	maturehomolog_ui	0	0
rno-miR-3556a	maturehomolog_ui	0	0
rno-miR-3556b	maturehomolog_ui	1,202	977
rno-miR-3557-3p	maturehomolog_ui	0	0
rno-miR-3557-5p	maturehomolog_ui	3,400	3,069
rno-miR-3560	maturehomolog_ui	0	0
rno-miR-3570	maturehomolog_ui	7,726	6,975
rno-miR-3574	maturehomolog_ui	0	0
rno-miR-3576	maturehomolog_ui	0	0
rno-miR-3578	maturehomolog_ui	996	558
rno-miR-3581	maturehomolog_ui	35,575	36,584
rno-miR-3586-3p	maturehomolog_ui	39,077	42,548
rno-miR-3587	maturehomolog_ui	721	209
rno-miR-3589	maturehomolog_ui	0	0
rno-miR-3590-5p	maturehomolog_ui	927	1,604
rno-miR-3592	maturehomolog_ui	0	0
rno-miR-3596b	maturehomolog_ui	8,413	10,637
rno-miR-3596c	maturehomolog_ui	4,086	3,522
rno-miR-3596d	maturehomolog_ui	961	907
sha-miR-716b	maturehomolog_ui	0	0
spu-miR-10	maturehomolog_ui	0	0
spu-miR-183	maturehomolog_ui	446	0
ssc-miR-1285	maturehomolog_ui	0	0
ssc-miR-2476	maturehomolog_ui	15,212	9,242
ssc-miR-4332	maturehomolog_ui	0	0
tca-miR-100-5p	maturehomolog_ui	5,185	7,847
xbo-miR-92b	maturehomolog_ui	0	0
xtr-miR-143#mdo-miR-1	maturehomolog_ui	0	0
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chrX_49768083_497680	newMicroRNA_gen	0	0
chrX_49773036_497731	newMicroRNA_gen	4,086	3,871
chrX_49773536_497735	newMicroRNA_gen	0	0
chrX_49774319_497744	newMicroRNA_gen	1,339	0
chrX_49777829_497777	newMicroRNA_gen	481	767
chrX_49779187_497791	newMicroRNA_gen	0	0
chrX_8094981_809511	newMicroRNA_gen	0	0
chrX_85158629_851587	newMicroRNA_gen	1,889	3,348
chrY_10035593_100355	newMicroRNA_gen	0	0
chr10_53059307_530593	newMicroRNA_hor	0	0
chr1_1102463_110259	newMicroRNA_hor	0	0
chr1_1103213_110335	newMicroRNA_hor	0	0
chr11_121970442_121970	newMicroRNA_hor	0	0
chr11_568081_568204	newMicroRNA_hor	0	0
chr1_172113664_172113	newMicroRNA_hor	2,919	2,930
chr1_198828160_198828	newMicroRNA_hor	243,837	234,674
chr1_207975173_207975	newMicroRNA_hor	0	732
chr12_127650667_127650	newMicroRNA_hor	0	0
chr12_7073246_707336	newMicroRNA_hor	0	0
chr14_101340813_101340	newMicroRNA_hor	0	349
chr14_101351030_101351	newMicroRNA_hor	6,902	5,615
chr14_101490105_101490	newMicroRNA_hor	858	732
chr14_101491317_101491	newMicroRNA_hor	0	0
chr14_101505993_101505	newMicroRNA_hor	412	209
chr14_101506543_101506	newMicroRNA_hor	824	802

chr14_101507105_1015newMicroRNA_hor	0	0
chr14_101531620_1015newMicroRNA_hor	36,605	37,770
chr1_41220012_412201newMicroRNA_hor	0	0
chr1_65524089_655242newMicroRNA_hor	858	942
chr16_73091853_73091newMicroRNA_hor	0	0
chr17_1617166_161731newMicroRNA_hor	1,131,452	1,129,183
chr17_29887014_29887newMicroRNA_hor	1,820	2,337
chr17_46114512_46114newMicroRNA_hor	790	1,779
chr18_19405632_19405newMicroRNA_hor	0	0
chr19_10928064_10928newMicroRNA_hor	2,919	2,930
chr19_46000191_46000newMicroRNA_hor	0	0
chr19_52196009_52196newMicroRNA_hor	56,212	64,693
chr20_61162109_61162newMicroRNA_hor	0	0
chr21_17911405_17911newMicroRNA_hor	0	0
chr21_17962543_17962newMicroRNA_hor	28,776	29,365
chr2_133011947_13301newMicroRNA_hor	0	0
chr2_133038048_13303newMicroRNA_hor	0	0
chr2_177015047_17701newMicroRNA_hor	0	0
chr22_46508592_46508newMicroRNA_hor	3,400	3,592
chr22_46509540_46509newMicroRNA_hor	47,799	48,825
chr3_188406540_18840newMicroRNA_hor	549	279
chr3_38010883_38010newMicroRNA_hor	181,719	205,100
chr3_49058030_490581newMicroRNA_hor	376,933	410,200
chr4_38869631_388697newMicroRNA_hor	2,472	2,720
chr5_122990785_12299newMicroRNA_hor	0	0
chr5_149112349_14911newMicroRNA_hor	3,400	3,069
chr5_150747981_15074newMicroRNA_hor	652	244
chr5_71146764_711468newMicroRNA_hor	0	0
chr7_129410216_12941newMicroRNA_hor	0	0
chr7_129414722_12941newMicroRNA_hor	2,163	279
chr7_25989535_259896newMicroRNA_hor	0	0
chr8_141060651_14106newMicroRNA_hor	0	0
chr8_141742644_14174newMicroRNA_hor	155,588	135,629
chr9_127454723_12745newMicroRNA_hor	243,700	234,674
chr9_131006998_13100newMicroRNA_hor	0	2,930
chr9_139565038_13956newMicroRNA_hor	941,252	957,981
chr9_21512092_215122newMicroRNA_hor	26,647	27,551
chr9_28888846_288889newMicroRNA_hor	0	0
chr9_96941082_969412newMicroRNA_hor	8,756	10,846
chrX_133675369_13367newMicroRNA_hor	0	488
chrX_139006302_13900newMicroRNA_hor	0	0

Supplemental Table S2. Selected hypertension-related genes

<u>Category</u>	<u>Gene</u>	<u>Gene symbol</u>
GWAS of hypertension	Mov10 RISC complex RNA helicase	MOV10
	solute carrier family 4, sodium bicarbonate cotransporter, member 7	SLC4A7
	MDS1 and EVI1 complex locus	MECOM
	solute carrier family 39 (metal ion transporter), member 8	SLC39A8
	calcium channel, voltage-dependent, beta 2 subunit	CACNB2
	guanylate cyclase 1, soluble, alpha 3	GUCY1A3
	guanylate cyclase 1, soluble, beta 3	GUCY1B3
	early B cell factor 1	EBF1
	hemochromatosis	HFE
	branched-chain amino acid transaminase/abhydrolase domain containing 16A	BAT2-BAT5
	phospholipase C, epsilon 1	PLCE
	adrenomedullin	ADM
	furin (paired basic amino acid cleaving enzyme)-FES proto-oncogene, tyrosine kinase	FURIN-FES
	golgi SNAP receptor complex member 2	GOSR2
	jagged 1	JAG1
	GNAS complex locus	GNAS
	methylenetetrahydrofolate reductase (NAD(P)H)	MTHFR
	unc-51 like kinase 4	ULK4
	fibroblast growth factor 5	FGF5
	cytochrome P450 17A1	CYP17a1
	5'-nucleotidase, cytosolic II	NT5C2
	pleckstrin homology domain containing, family A member 7	PLEKHA7
	ATPase, Ca ⁺⁺ transporting, plasma membrane 1	ATP2B1
	SH2B adaptor protein 3	SH2B3
	T-box 5	TBX5
	T-box 3	TBX3
	cytochrome P450, family 1, subfamily A, polypeptide 1	CYP1A1
	unc-51 like kinase 3	ULK3
	zinc finger protein 652	ZNF652

<u>Category</u>	<u>Gene</u>	<u>Gene symbol</u>
Cortisol Processing	11 β -hydroxysteroid dehydrogenase type 1	HSD11B1
	11 β -hydroxysteroid dehydrogenase type 2	HSD11B2
Sympathetic signaling	alpha 1A receptor	ADRA1A
	alpha 1B receptor	ADRA1B
	alpha 1D receptor	ADRA1D
	alpha 2A receptor	ADRA2A
	alpha 2B receptor	ADRA2B
	alpha 2C receptor	ADRA2C

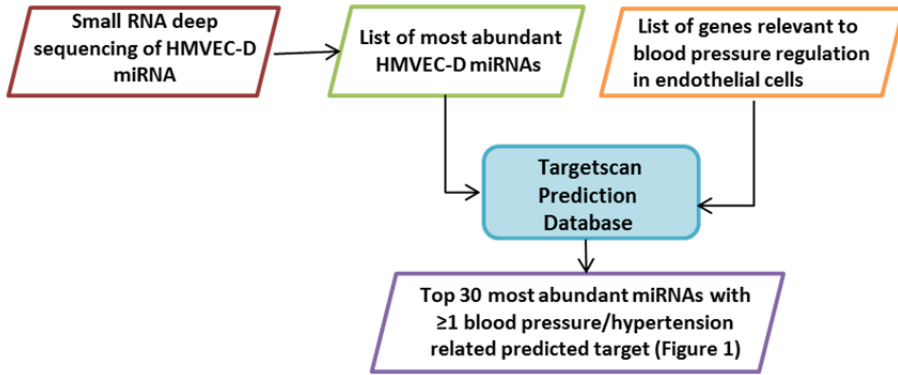
	beta 1 receptor	ADRB1
	beta 2 receptor	ADRB2
	beta 3 receptor	ADRB3
Vasoconstriction	calcium channel, voltage-dependent, L type, alpha 1C subunit	CACNA1C
	calcium channel, voltage-dependent, L type, alpha 1D subunit	CACNA1D
	calcium channel, voltage-dependent, beta 2 subunit	CACNB2
	nitric oxide synthase 3 (endothelial)	NOS3
Endothelin	Endothelin-1 (EDN1)	EDN1
	Endothelin (EDN3)*	EDN3
	Endothelin Receptor A	EDNRA
	Endothelin Receptor B	EDNRB
Oxidative Stress	superoxide dismutase 1	SOD1
	superoxide dismutase 2	SOD-2
	superoxide dismutase 3	SOD-3
	catalase	CAT
	NADPH oxidase 1	NOX-1
	NADPH oxidase 2	NOX-2
	NADPH oxidase 3	NOX-3
	NADPH oxidase 4	NOX-4
	NADPH oxidase 5	NOX-5

Supplemental Table S3. Primer sequences.

Primer name	Sequence (5'--> 3')
hu ADM fw	CTGATGTACCTGGGTTTCGCTCGCCTTCCTA
hu ADM rv	GACATCCGCAGTTCCTCTTCC
hu ADRA1B fw	AACCCTTCTATGCCCTCTTCTCC
hu ADRA1B rv	CCTCTAGGTTCTTGGTGGTTCTCTT
hu ADRA1D fw	CCTACGAATTGGCCGACTACAG
hu ADRA1D rv	TGGGGTCCTTAAATATCGGTCTC
hu ADRA2A fw	CCTCCGTGCTTTTCTGGTTG
hu ADRA2A rv	CTGCCCTCCTCCCCTGACT
hu ADRA2B fw	GGGGGAGGAAGAAACGAAGAC
hu ADRA2B rv	GAACCACAGATCCGAAAACAGG
hu ADRB1 fw	GAAGCCCACAATCCTCGTCTG
hu ADRB1 rv	CTCCCATCCCTTCCCAAACCT
hu ADRB2 fw	CGCTACTTTGCCATTACTTCACC
hu ADRB2 rv	CACCCGGGCCTTATTCTTG
hu ADRB3 fw	TGAGAGCAGCAGGGTAGAAAAGAC
hu ADRB3 rv	GAGCAGTAATAAGGGAACCAGAGATA
hu ATP2B1 fw	GACTGATGCCGAAGATGATG
hu ATP2B1 rv	GTTGGGAGAGGGTGGAGGAC
hu CACNA1C fw	CATCGGGTTTGGTTTTCTTGATTA
hu CACNA1C rv	CCTGCAGCGGCTTCTATTGA
hu CACNA1D fw	GAGCCTGAGGAAACAAAACGAG
hu CACNA1D rv	AGGGGACGGTGGGTGGTA
hu CACNB2 fw	CTCCCCACCACCGACTCC
hu CACNB2 rv	CATTTTGCCCTGATTTCTTCTGA
hu catalase fw	GCAGGACAATCAGGGTGGTG
hu catalase rv	GAGTAACGTTATCATCATTGGCAGTG
hu EDN1 fw	CTGGGGATGACAATGGACCTC
hu EDN1 rv	ACCCGAATTCGAATGTGACTGT
hu EDNRA fw	ATTTTGAAGTGGCCAGATGAGTTTAT
hu EDNRA rv	AGATGTTGGGGTGGGGAGTTAG
hu EDNRB fw	TTGCATTTTTCGGACACTGGA
hu EDNRB rv	AATGCTGAGGTTTGGGCTTCTA
hu ENaC γ fw	AGCCCGAGAGCGAGCAGA
hu ENaC γ rv	GGCGAGGATGGGACTTTGAG
hu FGF5 fw	CCGCACAGGGGCTACAGAG
hu FGF5 rv	AGAAGAGGAGGAGGAGGAAGGAC
hu Furin fw	ACCGTGGCCTCATCTCCTACA
hu Furin rv	GGGCGCTCTGGTCTTTGATAA
hu GOSR2 fw	CACTGTAAGAAAAGCCAAACTCCA
hu GOSR2 rv	GGCAAAGGGTGTACAGAACTCC
hu JAG1 fw	CGCGTGACCTGTGATGACTACT
hu JAG1 rv	TGGGGCGGCAGAACTTATT
hu MTHFR fw	GAACCAGGGAGGAGAGGAAATG
hu MTHFR rv	CTAGCAGGCACAGCACAGACC

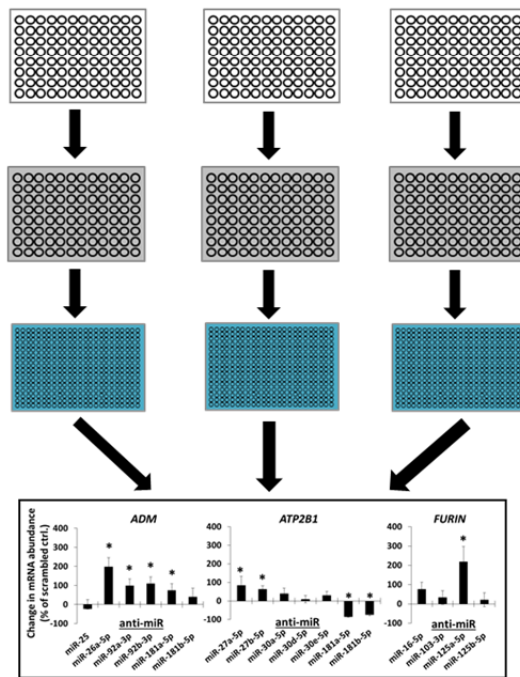
hu NHE2 fw	GGTTGGACTTCTACTAGGTGGGATTAT
hu NHE2 rv	TTCATTGCAGGGGGAGACTTC
hu NOX2 fw	TGTCAAGTGCCCAAAGGTGTC
hu NOX2 rv	AGGGGCGGATGTCAGTGTA
hu NOX4 fw	AGATGTTGGGGCTAGGATTGTGT
hu NOX4 rv	AAGGATAAGGCTGCAGTTGAGGT
hu PLEKHA7 fw	TCCAGCAGTCGTCCACCATT
hu PLEKHA7 rv	GGCGCTCCAAGGCACTCT
hu RAC1 fw	ATGGGATACAGCTGGACAAGAAG
hu RAC1 rv	AAGGGAAAAGCAAATTAAGAACACA
hu SH2B3 fw	TGGCCACTGTCCCTTCTAAATG
hu SH2B3 rv	AACTGTTCTTGCCACCCTCCTAT
hu Slc4a7 fw	TAAAGCCAATGATTCCCCTCTG
hu Slc4a7 rv	AACTCCTGATTATTTACGCCTACTTAT
hu TBX3 fw	GCGCGACTATGTTTGCTGATT
hu TBX3 rv	AAAGGGAGGAGGGGAAGTGTC
hu TBX5 fw	CACTCAAGGTCGCTGGATGC
hu TBX5 rv	GTGGCTGGTTGATGGGTGTG

A. Strategy for identifying relevant miRNA-mRNA target pairs in HMVEC-D

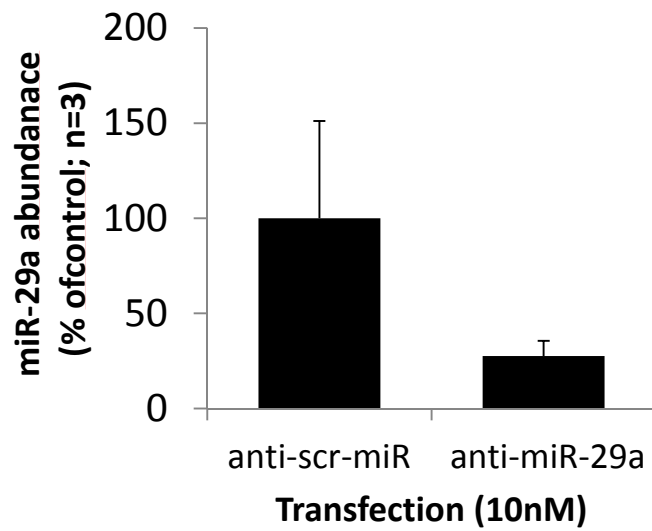


B. Strategy for evaluating miRNA regulation of gene expression

- 1.) HMVEC-D culture (3x 96 well plates)
- 2.) Transfection with 30 LNA-anti-miR's (n=3/plate; control n=6)
- 3.) RNA Extraction (BioRad Aurum Kit)
- 4.) qRT-PCR analysis (384 well format, numerous plates; 3 technical reps/sample)
- 5.) mRNA Abundance analysis (reported as % of avg. control from each plate)



Supplemental Figure S1. Study strategy. A) We performed small RNA deep sequencing on HMVEC-D, identifying the most abundant miRNAs. We compiled a list of protein-coding genes that have been associated with hypertension in human genome-wide association studies or are known to be functionally relevant to vascular endothelial cells and the regulation of blood pressure or development of hypertension. Target prediction databases were searched to identify the 30 most abundant miRNAs that had predicted targets on our list. We then performed our cell culture and qPCR analysis (B). LNA-anti-miRs targeting the identified miRNAs of interest were transfected in HMVEC-D in triplicate in 3 separate plates, for a total of 9 replicates per LNA anti-miR. The Aurum kit (Biorad) kit was used to isolate RNA for qRT-PCR analysis.



Supplemental Figure S2. Verification of the efficiency of anti-miR transfection at 10 nM. HMVEC-D were transfected with 10 nM anti-scr-miR or anti-miR-29a for 48 hours and the abundance of miR-29a was measured.