

MicroRNA-223-3p inhibits vascular calcification and the osteogenic switch of vascular smooth muscle cells

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Running title: MiR-223-3p inhibits vascular calcification in mice

Keywords: vascular smooth muscle cell; interleukin-6 (IL-6); signal transducer and activator of transcription 3 (STAT3); osteogenic phenotype; vascular calcification; miR-223-3p; epigenetics.

Supporting Information

Figure S1. Representative Alizarin Red S and HE staining of the aortic roots and BCAs of WT and aged ApoE^{-/-} mice.

Figure S2. Representative Oil Red O staining (**A**) and quantification (**B**) in the aortic roots of aged ApoE^{-/-} and DKO mice. Data are expressed as the mean \pm SD. ** $P < 0.01$ by unpaired two-tailed Student's *t*-test

Figure S3. MiR-223-3p level in VSMCs(A), MOVAS(B) and 3T3 cells(C) detected by RT-PCR 24 h after transfection with Ctrl mimics or miR-223-3p (100nM). Data are expressed as the mean \pm SD. *** $P < 0.001$ by unpaired two-tailed Student's *t*-test.

Figure S4. A. Representative western blotting of STAT3 in VSMCs 48h after transfection with 50nM Ctrl siRNA (si-Ctrl) or STAT3 siRNA (si-Stat3) cultured with mIL-6/sIL-6R complex (50ng/mL). B. Total calcium content in VSMCs transfected with si-Ctrl or si-STAT3 cultured in OIM with PBS or mIL-6/sIL-6R complex (50ng/mL) for 14 d ($n=3$). * $P < 0.05$, ** $P < 0.01$ compared with PBS group, # $P < 0.05$ compared with by mIL-6/sIL-6R + si-Ctrl group unpaired two-tailed Student's *t*-test.

Figure S1

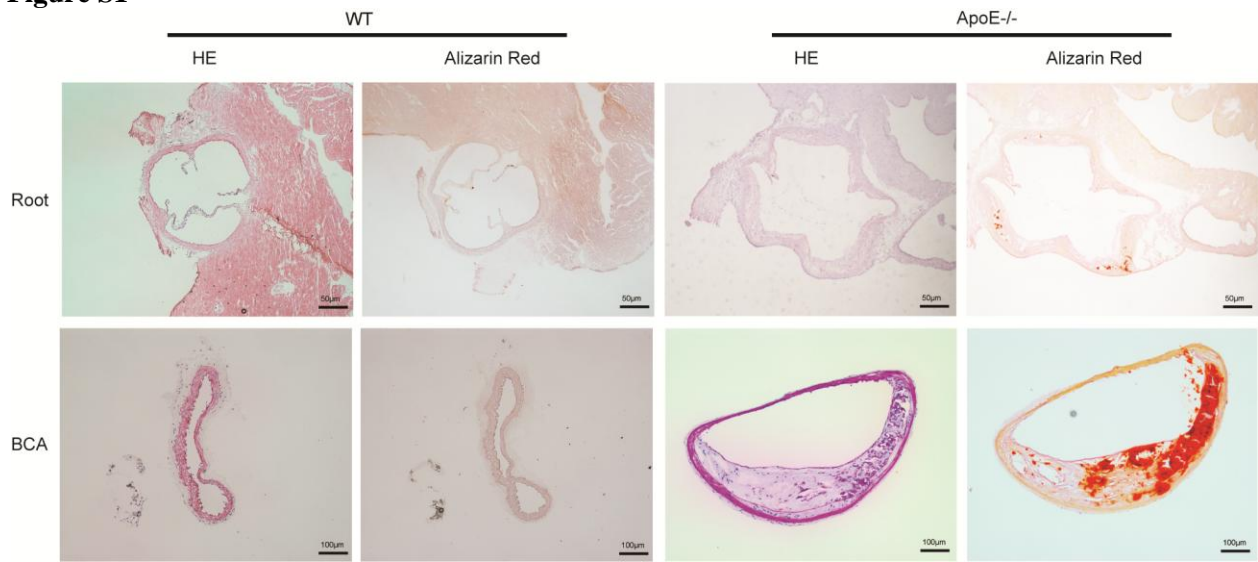
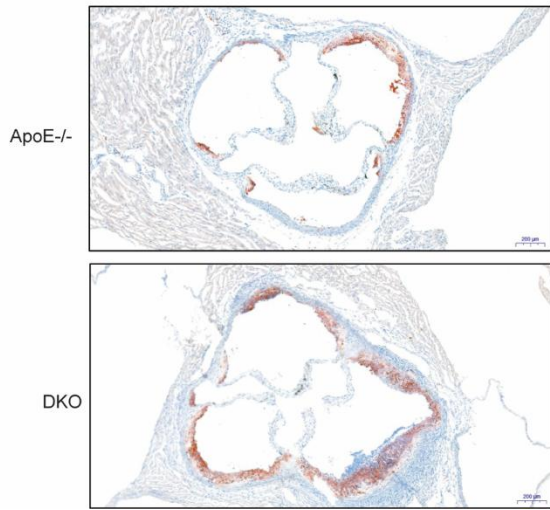


Figure S2

A



B

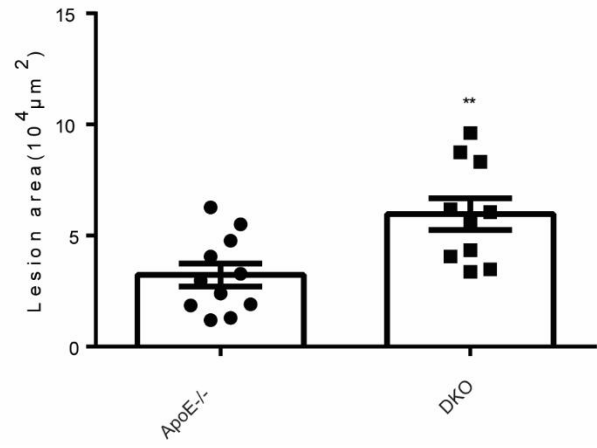


Figure S3

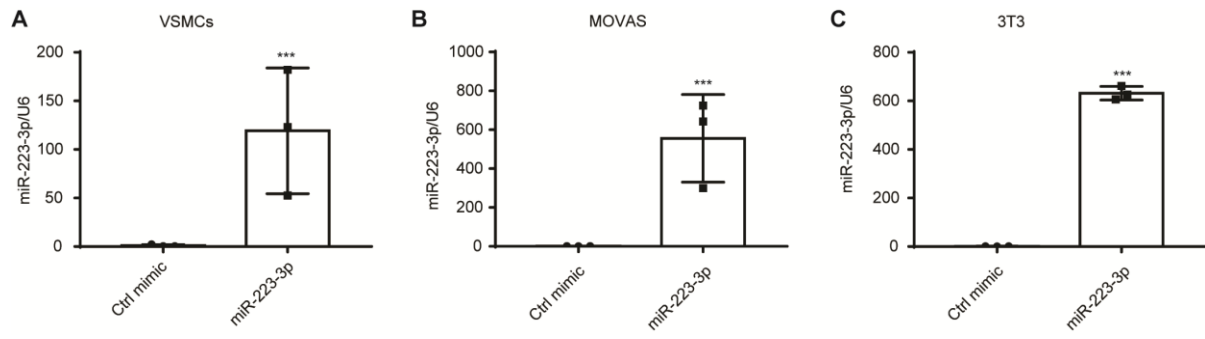
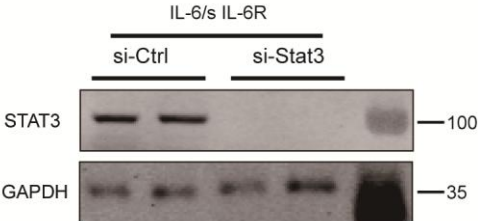
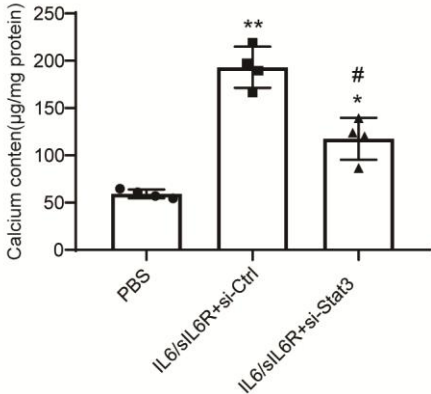


Figure S4

A



B



Supplemental Table 1

Table S1 Up and down regulated miRNA(FC>1.5 or FC<0.67, P-value<0.01)

Symbol	TPM (Transcripts Per Million)				FC	Log2FC	P-value	up/ down
	CKD1	CKD2	Ctrl1	Ctrl2				
mmu-miR-486-5p	7809	54409	1347	3571	4.931929	2.302152	0.005089	up
mmu-miR-708-5p	1130	548	91	165	2.308931	1.207225	0.004568	up
mmu-miR-139-5p	948	669	93	182	2.108748	1.076387	0.006727	up
mmu-miR-29b-3p	8292	6041	1023	1469	2.014048	1.010098	0.000165	up
mmu-miR-224-5p	19670	19734	3174	4365	1.843375	0.88235	0.000123	up
mmu-miR-223-3p	2571	3243	449	695	1.823771	0.866925	0.00735	up
mmu-miR-221-3p	23812	23046	3906	5485	1.760691	0.816142	0.000306	up
mmu-miR-29a-3p	364038	266464	56603	72345	1.696042	0.762172	3.50E-05	up
mmu-miR-214-3p	22521	15807	3464	4641	1.644455	0.71761	0.001686	up
mmu-miR-21a-5p	245048	301972	50375	68933	1.627499	0.702656	0.000224	up
mmu-miR-145a-5p	1191694	1261500	585527	797366	0.626268	-0.67515	0.001792	down
mmu-miR-101a-3p	35100	30096	16411	20009	0.621783	-0.68552	0.004563	down
mmu-let-7i-5p	102833	111059	53827	70128	0.60736	-0.71938	0.001173	down
mmu-miR-148a-3p	61781	53222	28620	44385	0.558224	-0.84108	0.000114	down
mmu-miR-28a-3p	1430	1365	883	1110	0.490119	-1.0288	0.001511	down
mmu-miR-423-3p	1568	1410	914	1292	0.475438	-1.07267	0.00066	down
mmu-miR-299a-3p	299	267	182	250	0.460233	-1.11957	0.006718	down
mmu-miR-199b-5p	6122	6116	4352	6007	0.416725	-1.26283	3.48E-06	down
mmu-miR-140-3p	637443	508006	440095	519111	0.412631	-1.27708	1.43E-06	down
mmu-miR-30c-2-3p	574	488	415	683	0.344254	-1.53846	9.02E-06	down
mmu-miR-381-3p	531	490	490	582	0.330915	-1.59547	1.08E-05	down
mmu-miR-196b-5p	192	196	208	204	0.323147	-1.62974	0.000283	down
mmu-let-7i-3p	318	301	354	390	0.287515	-1.79829	4.78E-06	down
mmu-miR-143-3p	7212255	6793865	13984725	12239910	0.181072	-2.46537	4.83E-07	down
mmu-miR-5099	300	279	596	662	0.159066	-2.6523	1.22E-12	down
mmu-miR-196a-5p	43	38	99	86	0.148033	-2.75601	2.92E-06	down

Supplemental Table 2

Gene		Primer
<i>Il6</i>	F	GGGAAATCGTGGAATGAGA
	R	TTCTGCAAGTGCATCATCGT
<i>Il6r</i>	F	CCTGAGACTCAAGCAGAAATGG
	R	AGAAGGAAGGTCGGCTTCAGT
<i>Stat3</i>	F	CAATACCATTGACCTGCCGAT
	R	GAGCGACTCAAAC TGCCCT
<i>Alp</i>	F	CCA ACTCTTTTGTGCCAGAGA
	R	GGCTACATTGGTGTTGAGCTTTT
<i>Ocn</i>	F	AGCAGAGTGAGCAGAAAGATG
	R	GAACAGACAAGTCCCACACAG
<i>Runx2</i>	F	CCGTCCACTGTCACTTTAATAGC
	R	GTAGCCAGGTTCAACGATCTG
<i>Gapdh</i>	F	AATGCATCCTGCACCACC
	R	ATGCCAGTGAGCTTCCCG