

Supplemental Information

MicroRNA-216a is essential for cardiac angiogenesis

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Figure S1

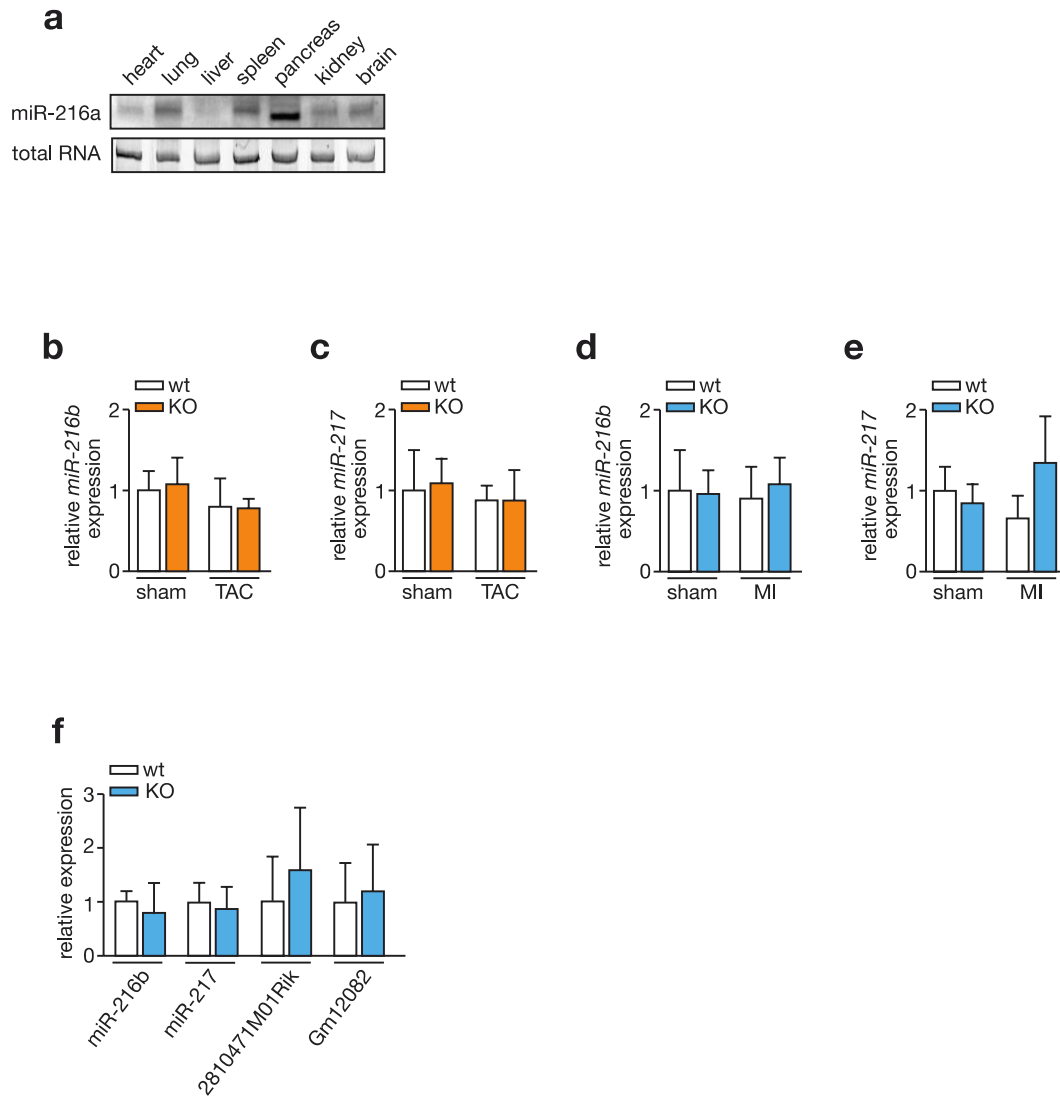


Figure S1 | (a) Northern blotting analysis of *miR-216a-5p* expression in different organs from wild-type mice. *Rnu6-2* was used as a loading control; (b-e) QPCR data showing that other cluster members are not affected under conditions of cardiac stress; n refers to number of hearts: n=3 per group. (f) QPCR data showing that our *miR-216a* target strategy does not affect other cluster members nor the neighboring lncRNAs. n refers to number of hearts: n=3 per group.

Figure S2

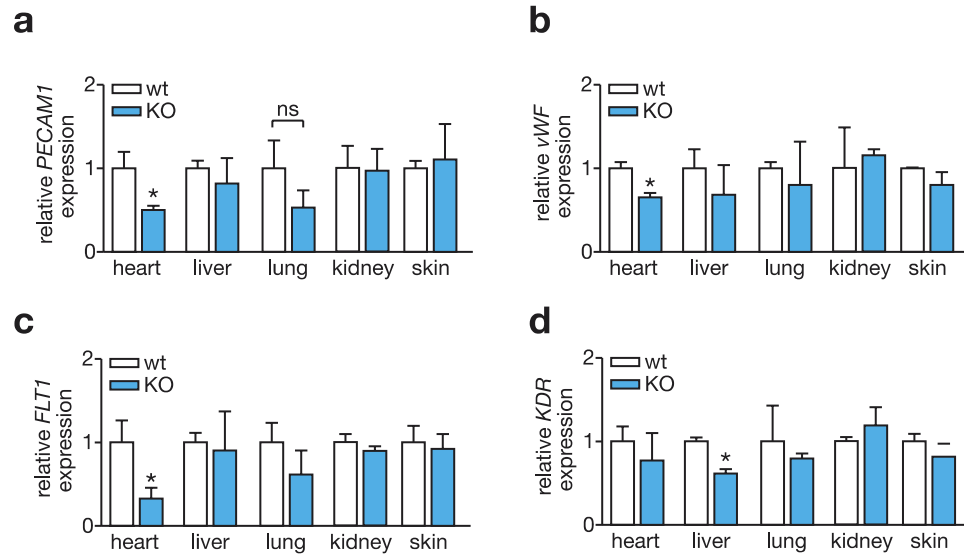


Figure S2 |(a-d) Real-time PCR analysis of transcript abundance for several endothelial cell markers: *Pecam1*, *vwf*, *Flt1* and *Kdr*, in different tissues derived from wt and KO mice. n refers to number of hearts: n=4 per group.

Figure S3

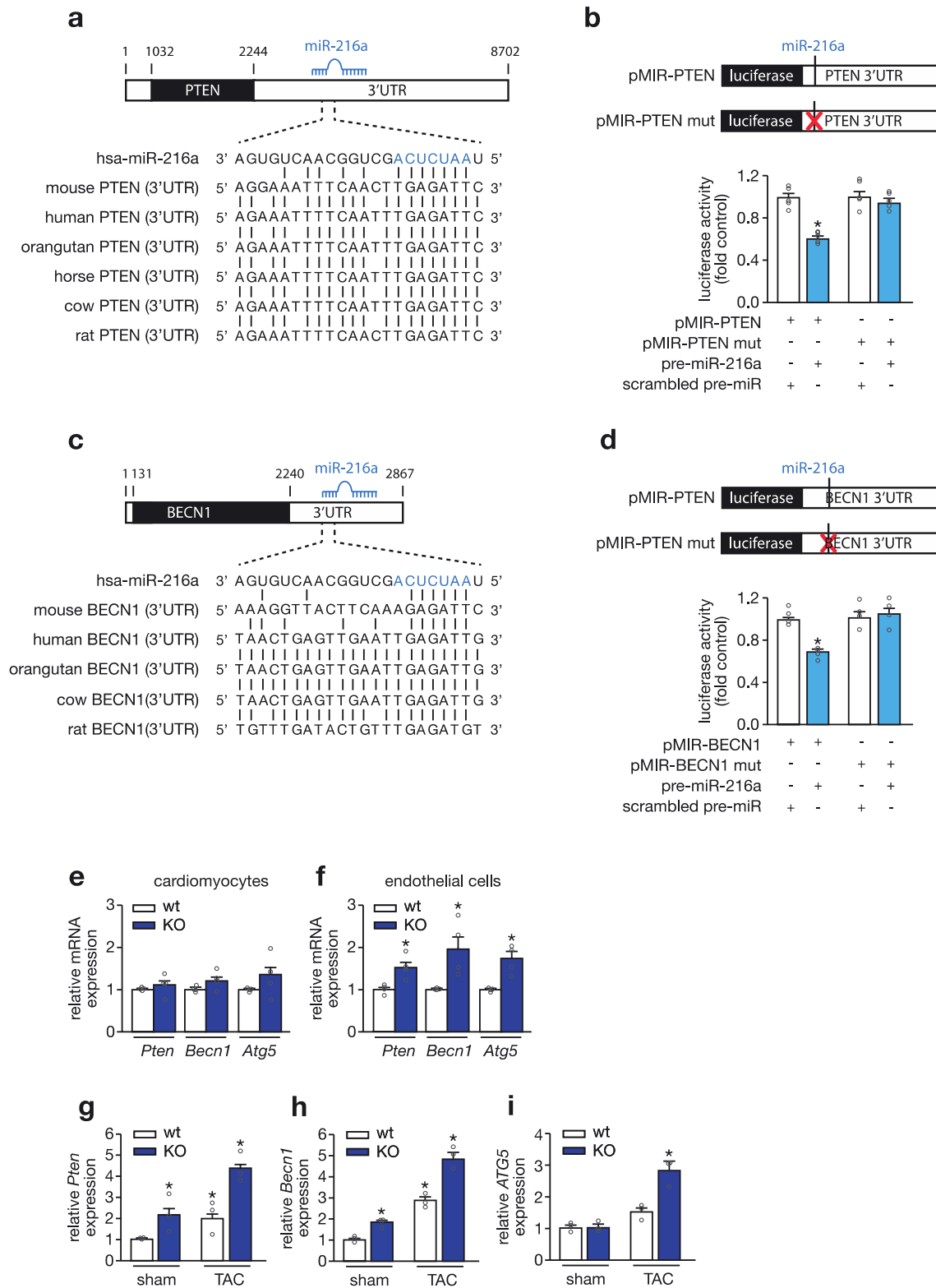


Figure S3 | (a) Location and evolutionary conservation of *hsa-miR-216a-5p* seed region on *PTEN* 3'UTR. (b) Schematic representation of luciferase reporter constructs harboring either the intact or the mutated *miR-216a* binding site on *PTEN* 3'UTR (top panel) and quantification of luciferase activity after co-transfection of HUVECs with *pre-miR-216a* (bottom panel); a scrambled precursor molecule was used as control; n refers to independent experiments (n=5). (c) Location and evolutionary conservation of *hsa-miR-216a-5p* seed region on *BECN1* 3'UTR. (d) Schematic representation of luciferase reporter constructs harboring either the intact or the mutated *miR-216a* binding site on *BECN1* 3'UTR (top panel) and quantification of luciferase activity after co-transfection of HUVECs with *pre-miR-216a* (bottom panel); a scrambled precursor molecule was used as control; n refers to independent experiments (n=4). (e,f) Real-time PCR analysis of the expression of *Pten*, *Becn1* and *Atg5* in (e) cardiomyocytes and (f) endothelial cells isolated from myocardial tissue of wt and KO mice, n = 4 hearts per group. (g-i) Real-time PCR analysis of the expression of *Pten* (g), *Becn1* (h) and *Atg5* (i) in myocardial tissue of wt and KO mice subjected to sham or TAC surgery, n refers to number of hearts (n=3 for each condition). In all panels numerical data are presented as mean (error bars show s.e.m.); statistical significance was calculated using two-tailed unpaired t-test when comparing two experimental groups or two-way ANOVA followed by Tukey's multiple comparison test, when comparing more than two experimental groups. *P<0.05 versus corresponding control group.

Table S1 | microRNA screen results

(excel file)

Table S2: RNA sequencing results showing most up and downregulated genes upon miR-216a overexpression in endothelial cells.

ENSEMBL gene ID	baseMean	log2FoldChange	pvalue	padj	hgnc_symbol
ENSG00000163513	673.708.710.753.919	-0.611990155141959	2,27E-122	2,32E-119	TGFB2
ENSG00000171033	131.200.903.152.731	100.048.797.073.057	1,18E-45	6,08E-41	PKIA
ENSG00000142867	123.773.927.304.601	-0.593092906548889	4,37E-23	1,49E-18	BCL10
ENSG00000087842	602.556.621.044.263	-0.725944134842392	6,26E-08	9,18E-05	PIR
ENSG00000071967	288.313.975.937.435	0.736262568051788	5,22E-02	4,12E+01	CYBRD1
ENSG00000124145	500.080.636.288.422	-0.674079717031292	5,16E-01	3,41E+02	SDC4
ENSG00000171720	387.666.140.250.179	-0.68117650612579	3,79E+00	1,94E+03	HDAC3
ENSG00000059804	392.803.858.033.049	-0.637160843353113	7,41E+03	2,24E+06	SLC2A3
ENSG00000106688	256.609.367.262.772	-0.687150753693311	1,66E+04	4,27E+05	SLC1A1
ENSG00000283239	163.331.423.147.394	-349.223.466.653.225	3,04E+05	6,12E+07	KBTBD11-OT1
ENSG00000260342	486.038.280.826.969	250.745.449.392.809	3,22E+05	6,25E+07	
ENSG00000090339	949.230.615.292.808	-0.920524710166	9,78E+05	1,79E+08	ICAM1
ENSG00000130821	22.619.076.325.122	-0.606701516918977	2,27E+06	3,76E+08	SLC6A8
ENSG00000164938	177.529.154.934.185	-0.64614706301234	1,29E+07	1,87E+09	TP53INP1
ENSG00000164494	19.185.752.461.795	-0.631177914354996	2,61E+07	3,39E+09	PDSS2
ENSG00000141562	259.029.209.859.613	0.605109599524017	1,50E+08	0.000158364679480824	NARF
ENSG00000259623	210.143.723.351.988	0.598990700238616	3,06E+08	0.000293852365673068	
ENSG00000157111	873.007.108.596.805	-0.730204166795773	9,19E+08	0.0007727535459837	TMEM171
ENSG00000139645	157.249.711.531.211	-121.755.981.137.284	2,20E+09	0.00163728696194362	ANKRD52
ENSG00000136859	23.439.383.347.952	0.585882775413566	3,05E+09	0.0021126788589627	ANGPTL2
ENSG00000110080	108.863.523.856.776	-0.636357046235934	4,46E+09	0.0028814499234559	ST3GAL4
ENSG00000099804	490.903.947.075.811	-0.934650519410166	6,53E+09	0.00373991888720579	CDC34
ENSG00000137575	348.968.353.315.411	-0.669418883549087	9,28E+09	0.00490961409946267	SDCBP
ENSG00000173875	249.713.312.360.511	0.837284494600489	0.000129678992552782	0.0066869710610653	ZNF791

Table S3. Primer sequences used for quantitative real time PCR.

Gene name	Gene identification	sequence
<i>Nppa</i>	(NM_008725)	5'-TCTTCCTCGTCTTGGCCTTT3'
		5'-CCAGGTGGTCTAGCAGGTTTC-3'
<i>Nppb</i>	(NM_008726)	5'-TGGGAGGTCACCTCTATCCT-3'
		5'-GGCCATTTCTCCGACTTT-3'
<i>Acta1</i>	(NM_009606)	5'-CCGGGAGAAGATGACTCAA-3'
		5'-GTAGTACGGCC GGAAGCATA-3'
<i>Myh7</i>	(NM_080728)	5'-CGGACCTTGAAGACCAGAT-3'
		5'-GACAGC TCCCATTCTCTGT-3'
<i>Rcan1.4</i>	(NM_019466)	5'-GCTTGACTGAGAGCGGAGTC-3'
		5'-CCACACAAGCAATCAGGGAGC-3'
<i>L7</i>	(NM_011291)	5'-GAAGCTCATCTATGAGAAGGC-3'
		5'-AAGACGAAGGAGCTGCAGAAC-3'
<i>FLT1</i>	(NM_001159920)	5'-CCGGCTCTCTATGAAAGTGAAG-3'
		5'-CGAGTAGCCACGAGTCAAATAG-3'
<i>KDR</i>	(NM_002253)	5'-AGCAGGATGGCAAAGACTAC-3'
		5'-TACTTCCTCCTCCTCCATACAG-3'
<i>CDH5</i>	(NM_001795)	5'-GTTACCTTCTGCGAGGATATG-3'
		5'-GATGGTGAGGATGCAGAGTAAG-3'
<i>PECAM1</i>	(NM_000442)	5'-GGTGGATGAGTCCAGATTTTC-3'
		5'-GATGGGACCAGATCCTTCATTC-3'
<i>ANGTL2</i>	(NM_0012098)	5'-CACCATCCATCACCCACTTATC
		5'-GAGTACAGCAGAGCGGAAG-3'
<i>CLDN12</i>	(NM_001185072)	5'-GCATACTTACTCACAGCCCTATT-3'
		5'-GGGAATGTGAGGCTACAAGAA-3'
<i>TGFBR2</i>	(NM_001024847)	5'-TTAGAGAGGGACTGGTAGTGAG-3'
		5'-AGACCTTCCACCATCCAACAGAGC-3'
<i>HDAC3</i>	(NM_003883)	5'-GACTGAGATTGCCTCTGACTTC-3'
		5'-CATCTGGGATTCAGGTGTTAGG-3'
<i>BCL10</i>	(NM_003921)	5'-GCCACCATGCCTCACTAAT-3'
		5'-GGTGGCTCGTCTGTAATC-3'
<i>ICAM1</i>	(NM_000201)	5'-GTAGCAGCCGAGTCATAAT-3'
		5'-GGGCCTGTTGAGTCTGTATT-3'