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Supplemental information

Circular RNA circSmoc1-2 regulates vascular calcification by acting as a miR-874-3p sponge in vascular smooth muscle cells

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Supplementary Table 1.

Expression of circSmoc1-2 in various cell types

circBase was searched to find the expression of circSmoc1-2 in other cell types.

Organism	Position	circRNA ID	Samples	Best transcript	Gene symbol	circRNA study
Human	chr14: 70442431-70461197	hsa_circ_0032358	Hepg2, A549, diencephalon, cerebellum, occipital_lobe, frontal_cortex, parietal_lobe, temporal_lobe	NM_001034852	SMOC1	Rybak2015, Salzman2013
Mouse	chr12: 82236762-82253740	mmu_circ_0004229	forebrain, hindbrain, PN_D07, PN_D21	ENSMUST00000110347	Smoc1	Rybak2015

Supplementary Table 2.

Protein coding potential of circSmoc1-2

circRNADb was utilized to assess the protein-coding potential of circSmoc1-2. Abesence of open reading frame or R score<1.6 denotes poor protein-coding potential.

circRNADb			
Circ ID	hsa_circ_21776 (Transcipt ID: NM_001034582)		
Location (hg19)	chr14 : 70442431-70461197		
Gene Symbol	Smoc1		
IRES Elements	Parameter Index		
	Position (start--end)	R Score	With Pseudoknot (Y/N)
	34--170	1.608008	Y
	21--172	1.364798	Y
Open Reading Frame	None (Protein length less than 100aa)		
Protein Features	The possibility of encoding protein is relatively low		

Supplementary Table 3.

PCR primer The PCR primer sequences for circRNA and mRNA.

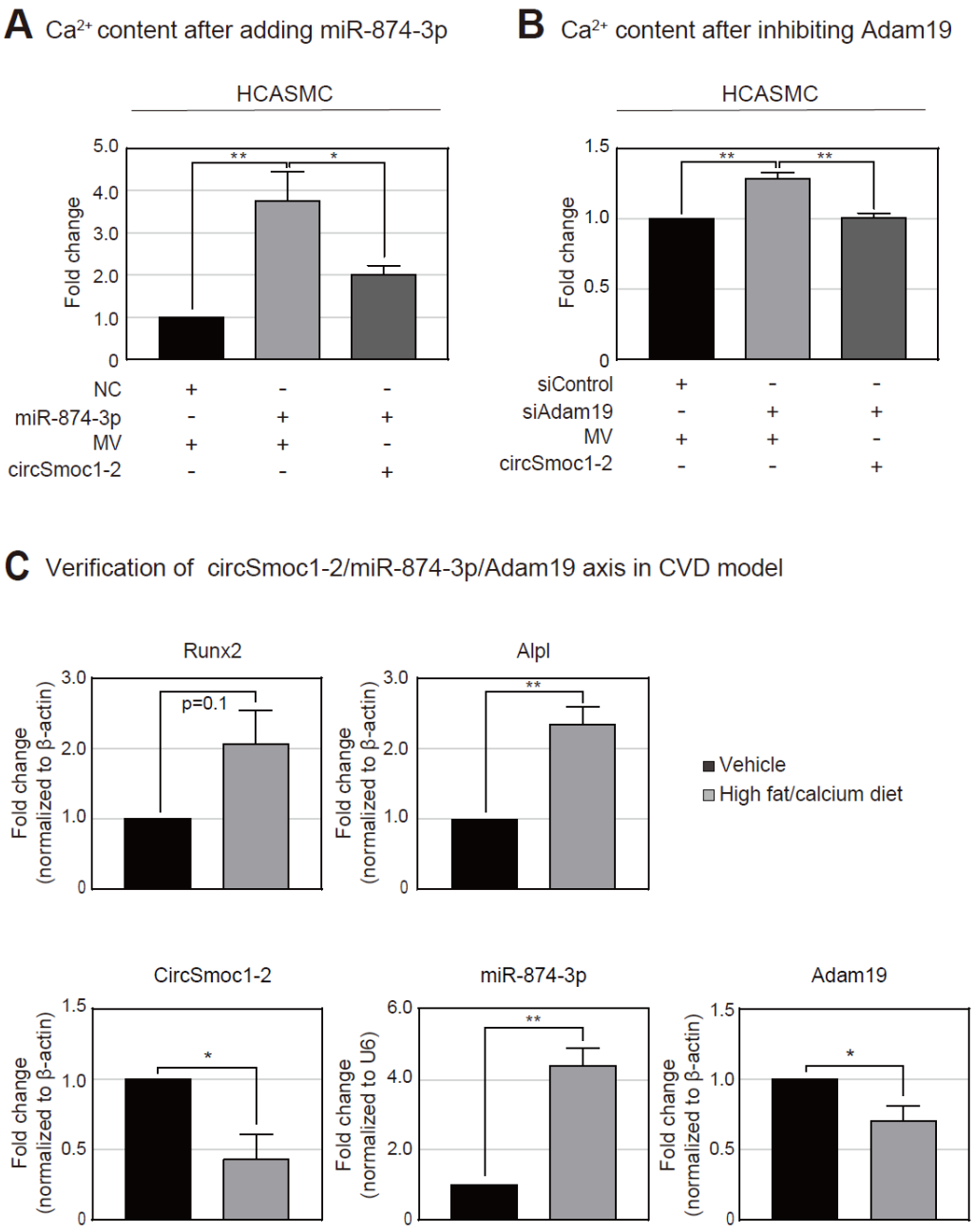
Name of Primer	Sequence (5′→3′)
circSmoc1-2_rat_Fwd	CTG CAC GGA CGA GCC ACT GAT G
circSmoc1-2_rat_Rev	CAC AGC CCC TAC CTT ATG GAT TAA GC
circSmoc1-2_mouse_Fwd	CTG CAC GGA AGA ACC ACT GAT G
circSmoc1-2_mouse_Rev	CAC AGC CCC CAC CTT ATG GAT TA
circSmoc1-2_human_Fwd	CTG CAC AGA AGA GCC ACT GAT G
circSmoc1-2_human_Rev	CAC AGC CCC AAC TCT ATG GAT TAA AC
Smoc1_mRNA_rat_Fwd	GTG CAG TGT CAT ACG TAC AC
Smoc1_mRNA_rat_Rev	CTG AAT TTC TTA CAT TGG TGT TAT TC
Adam19_rat_Fwd	GGC AAA GGC TTG AAT GGC AA
Adam19_rat_Rev	ATA GCA CCA GGA CTG CCA AC
Adam19_mouse_Fwd	AGG ACT GGG CCC TTC AGT TT
Adam19_mouse_Rev	AGC CAC CAG GTA AAG CTC CA
Adam19_human_Fwd	ATG GGC CAC AAC TTT GGC AT
Adam19_human_Rev	ATG ATG CAC CCA CCA TCA GC
Runx2_mouse_Fwd	TGG GAC TGT GGT TAC CGT CA
Runx2_mouse_Rev	CTC CGG CCC ACA AAT CTC AG
Alpl_mouse_Fwd	CAA CCT GAC TGA CCC TTC GC
Alpl_mouse_Rev	CAG AGC CTG CTT GGC CTT AC
Pre-Gapdh_ rat_Fwd	CCA TGG TGC AGC GAT GCT TT
Pre-Gapdh_ rat_Rev	ACG GCC AAA TCT GAG GCA AG
Gapdh_ rat_Fwd	GTA TCG GAC GCC TGG TTA C
Gapdh_ rat_Rev	CTT GCC GTG GGT AGA GTC AT
ActinB_ mouse_Fwd	CTG TAT TCC CCT CCA TCG TG
ActinB_ mouse_Rev	AGC TCA TTG TAG AAG GTG TGG

Cloning primer The PCR primer sequences for cloning to construct circSmoc1-2 overexpression vector. Uppercase letters denote nucleotide sequence of circSmoc1-2, while lowercase letters indicate vector sequence and enzyme sites. Restriction enzyme sites are underlined.

Name of Primer	Sequence (5′→3′)
Smoc1-2_rat_Fwd	tttatgcagaaattaattaa GTGCAGTGTCATACGTAC
Smoc1-2_rat_Rev	gaatacttacgctccgcgg CTGAATTTCTTACATTGG
Smoc1-2_human_Fwd	tttatgcagaaattaattaa GTGCAGTGCCATACTTACAC
Smoc1-2_human_Rev	gaatacttacgctccgcgg CTGAATTTCTTATGTTGGTGTTGTTC

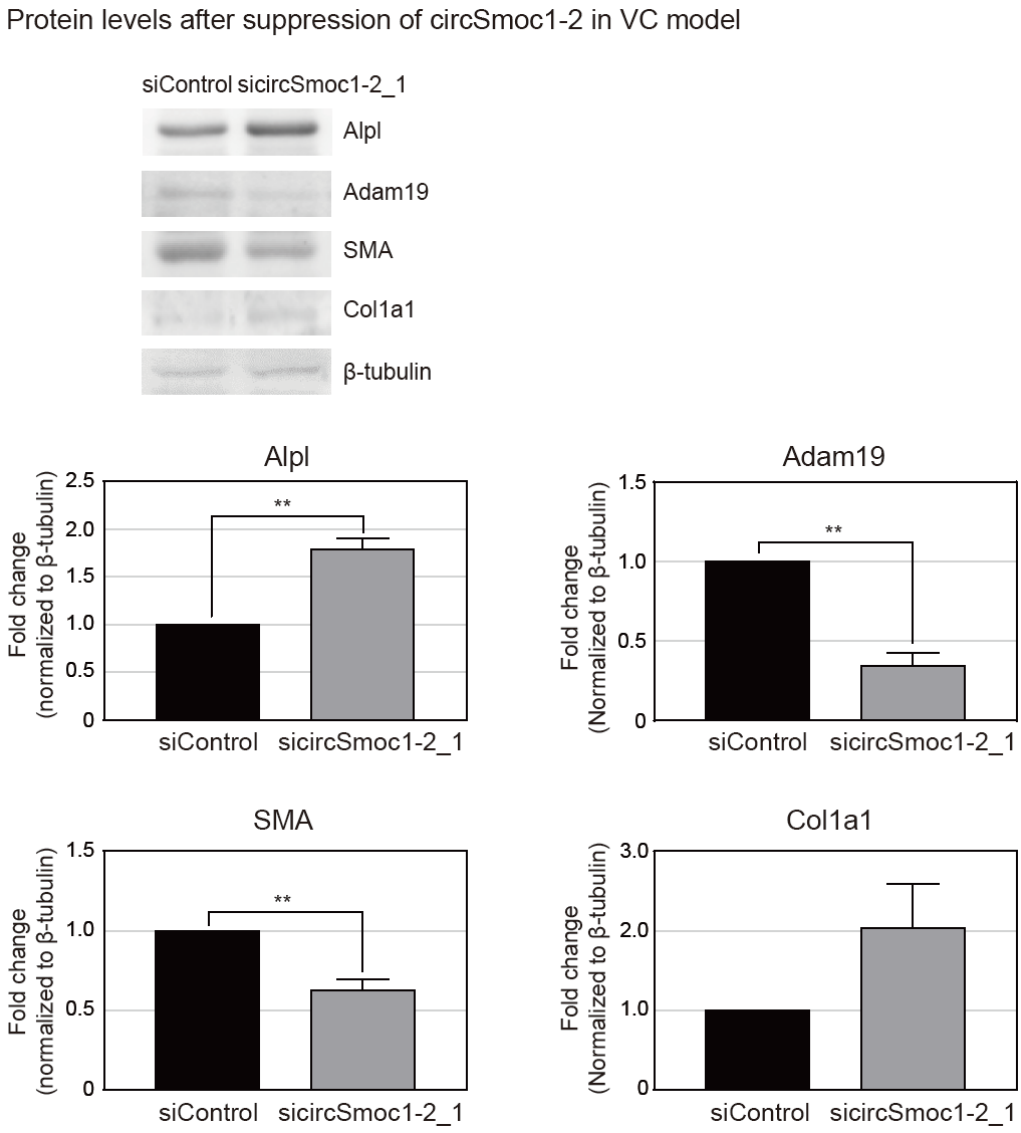
siRNA sequence The siRNA sequences to inhibit circSmoc1-2 and Adam19, respectively.

Name of siRNA	Sequence (5′→3′)
sicircSmoc1-2_1_rat_sense	AAAUUCAGGUGCAGUGUCUUU
sicircSmoc1-2_1_rat_antisense	UGACACUGCACCUGAAUUUUU
sicircSmoc1-2_2_rat_sense	AGAAAUUCAGGUGCAGUUUUU
sicircSmoc1-2_2_rat_antisense	ACACUGCACCUGAAUUUCUUU
sicircSmoc1-2_1_human_sense	AAAUUCAGGUGCAGUGCCUUU
sicircSmoc1-2_1_human_antisense	UGGCACUGCACCUGAAUUUUU
siAdam19_ 1_human_sense	GGAGGGAGCUGGACAGGUAUU
siAdam19_ 1_human_antisense	UACCUGUCCAGCUCCCUCCUU

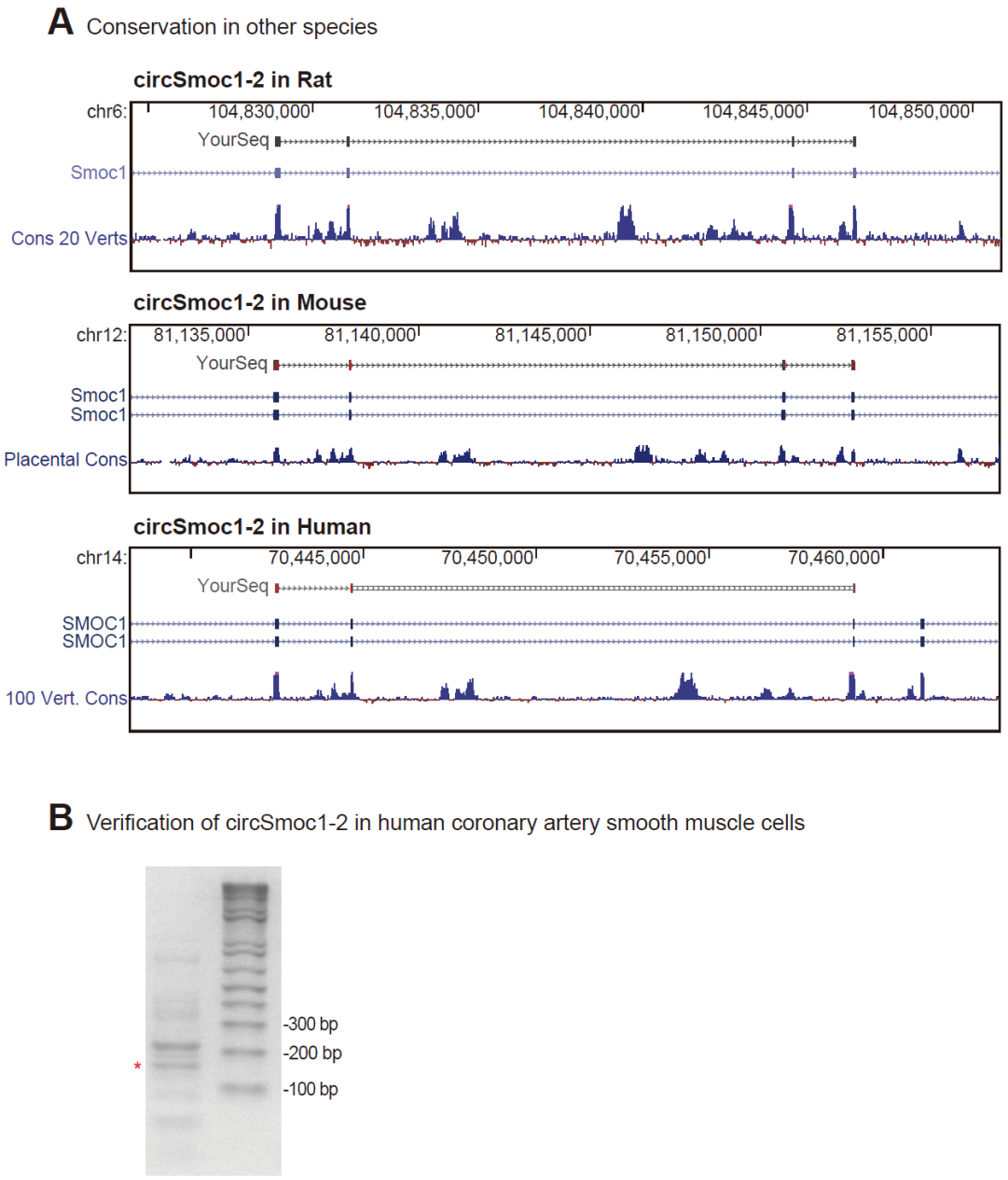


Supplementary Figure 1. Verification of circSmoc1-2/miR-874-3p/Adam19 regulatory axis in human coronary artery vascular smooth muscle cells (HCASMCs) and vascular calcification (VC) *in vivo* model. (A) Calcium concentrations (mg Ca/mg protein) after addition of miR-874-3p in HCASMCs (n=3) (B) Calcium concentration (mg Ca/mg protein) after Adam19 inhibition in HCASMCs (n=3). Cells were treated with 2 mM Pi and then cultured for an additional three days post transfection. NC indicates negative control siRNA, while MV means mock Laccase2 vector without insert. (C) Expression levels of calcification

markers and circSmoc1-2/miR-874-3p/Adam19 axis in an VC *in vivo* model (n=3). High fat/calcium diet was fed to *ApoE* KO mice for 8 weeks to induce VC. Then total RNA was isolated from mouse aorta and was used for quantitative real time PCR. Calcification markers, Runx2 and Alpl, were upregulated in *ApoE* KO mice with high fat/calcium diet. CircSmoc1-2 and Adam19 was downregulated, while miR-874-3p was upregulated in the *in vivo* VC model. Expression of calcification markers, circSmoc1-2, and Adam19 were normalized against β-actin, while expression of miR-874-3p was normalized against U6. Data represent the mean ± SEM. Statistical significance was determined using the Student's t-test. *: $p \leq 0.05$, **: $p \leq 0.01$.



Supplementary Figure 2. Confirmation of protein levels of Adam19, smooth muscle actin, and collagen marker after inhibition of circSmoc1-2. A10 cells were transfected with negative control siRNA or sicircSmoc1-2_1. Then cells were treated with 2 mM Pi and cultured for an additional three days post transfection. Alpl, a calcification marker, was evaluated to assess VC induction. Col1a1 was used as a collagen marker. Protein expression levels were normalized against β-tubulin (n=3). Data represent the mean ± SEM. Statistical significance was determined using the Student's t-test. **: $p \leq 0.01$.



Supplementary Figure 3. Conservation of circSmoc1-2 in humans and mice (A) The circSmoc1-2 locus in the human, mouse, and rat genomes is depicted in a screenshot modified from the UCSC genome browser (http://genome.ucsc.edu/) (B) Confirmation of circSmoc1-2 expression using RT-PCR in human coronary artery smooth muscle cells. The red asterisk (*) indicates the expected size for circSmoc1-2, which was verified by Sanger sequencing.