Supplementary information

Long noncoding RNAs in vascular smooth muscle cells regulate vascular calcification

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Supplementary Table S1. A list of lncRNAs identified in this study, along with their expression change after induction of vascular calcification in VSMCs. Those lncRNAs with an average FPKM value greater than 10 and not 0 in any sample are shown. The lncRNAs presented in Figure 2A are highlighted.

Supplementary Table S2. Full-length sequences and the exon-intron boundary of lncRNAs. The exonexon junction is indicated with asterisk (*).

(A) Linc00116

TGGACTGGAGGAAGCGGAGGCTGCAGGACAAGCTGGCGACGACTCAGAAAAAGCTGGA CCTGGCCTGAGCACGTTGGGTTCTCTCCAAGCCTCGTGCAGCCCGTATCCGGGGAGCTGC GCGACTACACGCCCTGAGTCCCGGCTCGTCCTTGCACGGCTTGCAGGAACGTGGCTTGCT TCCAGACCTCAGAAAGAAAATAGTTTTGTCTTCGCTAACAGCCTGTGCTCAG*CTTGTCG AAGATGGATATAAAGCACGGGAAAGAAAACATTTCTTGCACTTTATGAATCTATTTTAA AATAAAAAATTTTAAACATC

(B) Lrrc75a-as1

CTCTGCGGCCATCATGGGATCATGTTCCTCATCTTCCTGTAGCTGGTACTTTCGCCATGGCC TGCACTCGGCGGGCCGCAGCCATGCTGAGCCCGGGCCCGGTCTCCCAGCACCGCCCTGGG CGCTGCGCAGATCTAGGGGAGCCTG*ACAGGACGTTGACATCGTGGAATAGGAGCACTAT CCCACCATGAG*GTTGGGAAGTTAGGGTGGAAGATTGCCAGCAGCCATGGCCTCCCATGA G*GGTGTCCCTTTCAAGTTTGAGAACGCACTGGGGGGACTGCAGATGGTGTTGACAGCAGC TGAG*GCTACATTCCCAGGAGTCTCCAGCCATCACCCCAAAAGCAAGGTGTGGGGTCAGCC AACTTTTGTTCTGGACAGGCACGGTTGGTGGTGCTCATCTCCCACAGGATCTTCTGTACCAT TTAATAAAGTTTTGTTTATAAGACTGAT

(C) Snhg1

TCATTTTCCTTTGCTCTGG*GTTTAAGGTACTCTTTTACATGCGAAAGAATGGGAGCACGC TCTCATTTCAAA*ATATGGCTAATGTCAAAGCCGTTTGCACTTTGGAGCCAGGCCTGTTCA ATTTTACAAATAGCAAGTTTTTCTTACAATCAG*GGTACGGTGGCAACGTGACTTGAAAC CCCCAGAATCATACCCGGGATGAATGCAG*GTTTTCTTGGTGGTGATTATCTTAAAG*GA GGAGGTTGTTCCTGCACGCCATCCATCTCCAGCGTTCGAAG*GTTCTGTTAGCAGTGCTGT TACTTGGTGCTTCAGAG*ATATGGTGACTGGCCGAGCGCAAAGGAGCCATGCCAG*GAAA ACTAAGAGCACATCTTCATAAAGTCCAGGCTAAAGTAAG*GTCTGAGGAGAGGGATGAG GTGTGTGTTAGAG*GAACTGAAATCAAAGATCCGATTACCTAGGCCAGTGGCCACGGCAA ACTTCAGAACCCATGGAATACAGCCTGTTGATTCCTTGTGGATTTTTAGCATCTGAGCAG TATCAGTGGCACTCCAAGTGAAATGGTTGATCTCTTCAGTACCGCTGGAGCAGATTAATA TTTATTTCACTGCCAGAGGCTATATTTCTCTGCCCCCCATACCCCCTTCCTCTTCTCCTTA GACTACTTAAGATTGAAATATTTGCAACTCCAAATTTATCTTCCTTGCTTACCACTTTTT TTCGGAGCTGGGGACCAAACCCAGGGCCTTGCGCTTCCTAGGTAAGCGCTCTACCACTGA GCTAAATCCCCAGCCCCGCTTACCACATTGTTAATCTGATCCTTCCGCAGTTATTTGTGCT GTTCTTTTGTTCCTGGCAATAAATGCTTTTTATGCTA

(D) Snhg16

CTTTCCGAGGTCCTCTGCCGCGTGCACTAAAGTTATGGCCGGTCCTGTCCGTGGTTCACTC TCTGAAAGGCCTAGGGTTTGGGTGATG*GCACGCCATTGCACATGGGACGCAAGCCGCA GGTTCCATTTCAGAAGTAATGGCTGGATCCCTGGAAGCAGATGGACTCGGTGTGGTTAGA GGGCAGAGGGTAGATGATGCTGCGTATTGAGCCCTCCTCAGCCCACTTTG*ATTGAGGTGT TGCGCGGTGAGGAAGGGCCTTCGTGACCAGTCTGCATG*GGGGAGGCTGAACCATAGTTT CTGGGCCTTCAAGGGGTGTTGCTGCAGCCACGGGGGCAGCCCTCCTGCGCCTGGACTTCT GCCTGCGAGCAGAGGGGCTTCCACACACACGCTCGTCAGAGCATCCGTGCTTCTGCGCTAA GTGGCAG*ATTCCAGGACGGCTCCAGGGGGCATCATCGAAAGGCCGTGGCACATCAGGGC CTTCAGGATGGCTGCGTCCTG*GTTATTGCCCTGTGGTGCCAGAGAGAACCACACAGCCT GCTGCTGCTGCCTTTAATTTCCCAAAGAGCCATCAAAAGACCTTGGACTTTGTTCTGC AAGAGGAGCTAGCAAAATGGAGTGACTGTGGTCACCCTGGAAGCCTTTGACAGCTGGGG AGGCCCTGCTGAGCTTAGAAAACCATCTGATAGATTTCCTGAGTAAGGATTGGGCTTGG ATTTTGAAGCACAAATAAACTTGATAAAATTAAA Supplementary Table S3. The sequences of PCR primers to measure lncRNA and mRNA expression.

Linc00116 forward primer	5'-TTGCTTCCAGACCTCAGAAAG-3'
Linc00116 reverse primer	5'-TCCCGTGCTTTATATCCATCTTC-3'
Lrrc75a-as1 forward primer	5'-TTTCGCCATGGCCTGCACTC-3'
Lrrc75a-as1 reverse primer	5'-GCCATGGCTGCTGGCAATCT-3'
Snhg1 forward primer	5'-CAAAGATCCGATTACCTAGGCC-3'
Snhg1 reverse primer	5'-CACAAGGAATCAACAGGCTG-3'
Snhg16 forward primer	5'-TCGAAAGGCGTGGCACATCA-3'
Snhg16 reverse primer	5'-CTCTGGCACCACAGGGCAAT-3'
Malat1 forward primer	5'-AGTTCTGTTGGCACACTGGGT-3'
Malat1 reverse primer	5'-CCAAAGCTGCACTGTGCTGTA-3'
Gas5 forward primer	5'-ACATGTGGGTCCCTTTAACTG-3'
Gas5 reverse primer	5'-GGCAAATCTTCTGTTCAAGCATC-3'
Runx2 forward primer	5'-CGATGGGACCGTGGTTACCG-3'
Runx2 reverse primer	5'-GTCCGCTCCGGCCTACAAAT-3'
Msx2 forward primer	5'-GGCCTCTCGTCAAGCCCTTC-3'
Msx2 reverse primer	5'-AGTATCTGCCGGGCTCCTGT-3'
Bmp2 forward primer	5'-CGACGTCCTCAGCGAGTTTG-3'
Bmp2 reverse primer	5'-GGGTGGGTCTCTGCTTCAGG-3'
Pre-Gapdh forward primer	5'-CCATGGTGCAGCGATGCTTT-3'
Pre-Gapdh reverse primer	5'-ACGGCCAAATCTGAGGCAAG-3'
Gapdh forward primer	5'-ATGACTCTACCCACGGCAAG-3'
Gapdh reverse primer	5'-CTGGAAGATGGTGATGGGTT-3'
Actb forward primer	5'-CACTTTCTACAATGAGCTGCG-3'
Actb reverse primer	5'-CTGGATGGCTACGTACATGG-3'

Linc00116 5' GSP	5'-GAGCACAGGCTGTTAGCGAAGAC-3'
Linc00116 Nested 5' GSP	5'-AAGAGCAAGCCACGTTCCTGCAAG-3'
Lrrc75a-as1 5' GSP	5'-CTATTCCACGATGTCAACGTCCTG -3'
Lrrc75a-as1 Nested 5' GSP	5'-AGGCTCCCCTAGATCTGCGCAG-3'
Snhg1 5' GSP	5'-TTCAAGTCACGTTGCCACCGTACC-3'
Snhg1 Nested 5' GSP	5'-GTGCAAACGGCTTTGACATTAGCC-3'
Snhg16 5' GSP	5'-CCAGGGATCCAGCCATTACTTCTG-3'
Snhg16 Nested 5' GSP	5'-GCTTGCGTCCCATGTGCAATGG-3'
Linc00116 3' GSP	5'-GGCTTGCTTCCAGACCTCAGAAAG-3'
Linc00116 Nested 3' GSP	5'-TCTTCGCTAACAGCCTGTGCTCAG-3'
Lrrc75a-as1 3' GSP	5'-ATTCCCAGGAGTCTCCAGCCATC-3'
Lrrc75a-as1 Nested 3' GSP	5'-AAAGCAAGGTGTGGGGTCAGCCAAC-3'
Snhg1 3' GSP	5'-TCTGAGGAGAGGGATGAGGTGTG-3'
Snhg1 Nested 3' GSP	5'-CAAAGATCCGATTACCTAGGCCAG-3'
Snhg16 3' GSP	5'-TGGAGTGACTGTGGTCACCCTGG-3'
Snhg16 Nested 3' GSP	5'-ACCCTGGAAGCCTTTGACAGCTG-3'

Supplementary Table S4. The sequences of gene-specific primers (GSPs) used in RACE experiments.

Supplementary Table S5. The sequences of PCR primers for cloning of lncRNAs.

Linc00116 forward primer	5'-TGGACTGGAGGAAGCGGAGGCTGC-3'
Linc00116 reverse primer	5'-GATGTTTAAAATTTTTTTTTATTTTA-3'
Lrrc75a-as1 forward primer	5'-CTCTGCGGCCATCATGGGATCAT-3'
Lrrc75a-as1 reverse primer	5'-ATCAGTCTTATAAACAAAACTTT-3'
Snhg1 forward primer	5'-TCATTTTCCTTTGCTCTGGGTTT-3'
Snhg1 reverse primer	5'-TAGCATAAAAAGCATTTATTGCC-3'
Snhg16 forward primer	5'-CTTTCCGAGGTCCTCTGCCGC-3'
Snhg16 reverse primer	5'-TTTAATTTTATCAAGTTTATTTG-3'



Enriched KEGG pathway (6day/control)

Supplementary Figure S1. Pathway analysis of RNA-seq data. The decreased mRNAs at the day 6 sample compared to the control sample with fold change (6 day/control) below 0.5 were selected. Pathway analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) gene set analysis (reference) at MSigDB database ^{22,23}.



Measurement of IncRNA after siRNA treatment

Supplementary Figure S2. Measurement of lncRNA level after the treatment with siRNA for each gene.

chr2:	110,150,000	110,20	0,000l	110,3	250,000	110,300,000
	*******			RP11-1223D1	19.1 }>>>>>>>>>	I MIR4436B
	······kk	****				
······ MALL			LINC00116	←	_	
			LINC00116			
Gene locus of	1500011K16Rik	(LINC00116) o	on mouse (mm	n10)		
chr2: 127,750,0	00 127,760,000 127	,770,000 127,780,0	00 127,790,000	127,800,000	127,810,000 127,820,0	000 127,830,000
Nphp1 (++++++++++++++++++++++++++++++++++++	·····	···· 4···· •••••••••••••••••••••••••••••		Bub1	ente ditta de calendaria en la construcción de dise	
Como loculo of		man (ha29)				
Gene locus of	SNHG16 on hur	nan (ng38)				
hr17: 76,560,000	76,565,000	76,570,	000 76,5	575,000	76,580,000	76,585,000
SNHG16 SNORD1C						
SNC	RD1B SNORD1A ST6GALNA	02			·····	
				-	•	
	40400000000	(O-h-10)				
Gene locus of	1810032008Rik	(Snhg16) on n	nouse (mm10))		
hr11: 🕞	116,675,000	116,680,000	116,68	5,000	116,690,000	116,695,000
1810032008Rik	al					
Shord I	Snord1b Snord1a					
	St6galnac2		********	************	······	
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Gene locus of	LRRC75A-AS1	on human (hg3	38)			
Gene locus of	LRRC75A-AS1	on human (hg3	38) 16,450,000 16,4	460,000 16	6,470,000 16,480,0	16,490,000
Gene locus of 16,420,0 TRPV2	LRRC75A-AS1	on human (hg3 16,440,000 ⊷ →+→→+	38) 16,450,000 16,4	460,000 16	5,470,000 16,480,0	000 16,490,000
Gene locus of 16,420,0 TRPV2	LRRC75A-AS1	on human (hg3	38) 16,450,000 16,4 D49A	460,000 16	5,470,000 16,480,0	16,490,000
Gene locus of 16,420,0 TRPV2	LRRC75A-AS1	on human (hg3 16,440,000) IRC75A-AS1 SNORD49B11 SNOR ISNO	38) 16,450,000 16,4 D49A RD65	460,000 10	5,470,000 16,480,0	16,490,000
Gene locus of 16,420,0 TRPV2 I	LRRC75A-AS1	on human (hg3 16,440,000) RC75A-AS1 SNORD49B11 SNOR LRRC75A	38) 16,450,000 16,4 D49A RD65	460,000 16	5,470,000 16,480,0	16,490,000
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Gene locus of	LRRC75A-AS1	on human (hg3 16,440,000) IRC75A-AS1 SNORD49B11 SNOR LRRC75A (Lrrc75a-as1)	38) ^{16,450,000} 16,4 D49A RD65 	460,000 16 	5,470,000 16,480,0	
Gene locus of TRPV2 I +++ Gene locus of hr11: 62,580,000]	LRRC75A-AS1	on human (hg3 16,440,000) IRC75A-AS1 SNORD49B11 SNOR ISNO LRRC75A-as1) (Lrrc75a-as1) 000] 62,610,000]	38) 16,450,000 16,4 D49A RD65 b con mouse (mn 62,620,000 62,6	460,000) 16 	5,470,000) 16,480,0	62,660,000]
Gene locus of TRPV2 ++++ Gene locus of hr11: 62,580,000 Trpv2 ++++++++++++++++++++++++++++++++++++	LRRC75A-AS1	on human (hg3 16,440,000) IRC75A-AS1 SNORD49B11 SNOR ISNO LRRC75A-as1) (Lrrc75a-as1) 1000 62,610,000]	38) 16,450,000 16,4 D49A RD65 	460,000 16 n10) 530,000 62,6	5,470,000) 16,480,0	62,660,000]
Gene locus of TRPV2	LRRC75A-AS1	on human (hg3 16,440,000) IRC75A-AS1 SNORD49B II SNOR ISNO LRRC75A (Lrrc75a-as1) (Lrrc75a-as1) (Lrrc75a-as1) HIGRik ISNOR IS	38) 16,450,000 16,4 D49A RD65 	460,000 16 	5,470,000) 16,480,0	62,660,000]
Gene locus of TRPV2	LRRC75A-AS1	on human (hg3 16,440,000) IRC75A-AS1 SNORD49B II SNOR ISNO LRRC75A (Lrrc75a-as1) (Lrrc75a-as1) (Lrrc75a-as1) (H16Rik ISnord49a ISnord49a ISnord49a	38) 16,450,000 16,4 D49A RD65 	460,000 16 	5,470,000 16,480,0	62,660,000]
Gene locus of TRPV2	LRRC75A-AS1	on human (hg3 16,440,000) IRC75A-AS1 SNORD49B II SNOR ISNO LRC75A-as1) (Lrrc75a-as1) (Lrrc75a-as1) (Lrrc75a-as1) ISNORD49B II SNOR (Lrrc75a-as1) ISNORD49B II SNOR Lrrc75a-as1) ISNORD49B II SNOR ISNORD49B II SNOR ISNO ISNORD49B II SNOR ISNO ISNORD49B II SNOR ISNO ISNORD49B II SNOR ISNO ISNORD49B II SNOR ISNORD49B II SNORD49B II SNOR ISNORD49B II SNORD49B	38) 16,450,000 16,4 D49A RD65 	460,000 16 n10) 530,000 62,6	5,470,000 16,480,0	62,660,000
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Gene locus of TRPV2 ++++ Gene locus of hr11: 62,580,000 Trpv2 ++++++++++++++++++++++++++++++++++++	LRRC75A-AS1	on human (hg3 16,440,000) IRC75A-AS1 SNORD49B II SNOR ISNO LRRC75A = (Lrrc75a-as1) 000] 62,610,000] H16Rik ISNOrd49b II SNord49a ISNOrd49b II SNord49a ISNOrd49b II SNord49a ISNOrd65 Lrrc75a = Lrrc75a = Lr	38) 16,450,000 16,4 16,450,000 16,4 16,450,000 62,6 00 mouse (mn 62,620,000 62,6 	460,000 16 n10) 530,000 62,6	5,470,000 16,480,0 40,000 62,650,000 	000 16,490,000
Gene locus of TRPV2	LRRC75A-AS1	on human (hg3 16,440,000) IRC75A-AS1 SNORD49B II SNOR ISNO LRRC75A	38) 16,450,000 16,4 16,450,000 16,4 16,450,000 62,6 00 mouse (mn 62,620,000 62,6 	460,000 16	5,470,000 16,480,0 40,000 62,650,000 	000 16,490,000
Gene locus of TRPV2 ++++ Gene locus of hr11: 62,580,000 Trpv2 ++++++++++++++++++++++++++++++++++++	LRRC75A-AS1	on human (hg3 16,440,000) IRC75A-AS1 SNORD49B II SNOR ISNO LRRC75A	38) 16,450,000 16,4 16,450,000 16,4 16,450,000 62,6 00 mouse (mn 62,620,000 62,6 62,870,000 6	460,000 10 	5,470,000 16,480,0 40,000 62,650,000 	000 16,490,000
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Gene locus of TRPV2 +++ Gene locus of hr11: 62,580,000] Trpv2 ++++ Gene locus of hr11: 62,855,000] SNORD30] SNORD30] IS SNORD30] IS SNORD3	LRRC75A-AS1	on human (hg3 16,440,000) IRC75A-AS1 SNORD49B11 SNOR ISNO LRRC75A	38) 16,450,000 16,4 D49A RD65 con mouse (mn 62,620,000 62,6 62,870,000 6 62,870,000 6	460,000 16 	5,470,000 16,480,0 40,000 62,650,000 	000] 16,490,000 62,660,000] ,,885,000]
Gene locus of TRPV2 +++ Gene locus of hr11: 62,580,000 Trpv2 ++++ Gene locus of hr11: 62,855,000 SNORD22 IS SNORD22 IS SNORD22 IS SNORD21 IS SNORD22 IS SNORD20 SLC3.	LRRC75A-AS1	on human (hg3 16,440,000) 16,440,000 16,440,000 ISNORD49BIJ SNOR ISNO LRRC75A-AS1 (Lrrc75a-as1) (100] 62,610,000 H16Rik ISnord49a ISNO ISNO ISNO ISNO ISNO ISNO ISNO ISNO	38) 16,450,000 16,4 D49A RD65 0n mouse (mn 62,620,000 62,6 62,870,000 6 62,870,000 6	460,000 16 	5,470,000 16,480,0 40,000 62,650,000 	16,490,000 62,660,000 ,885,000
Gene locus of TRPV2 +++ Gene locus of hr11: 62,580,000 Trpv2 ++++ Gene locus of hr11: 62,855,000 SNORD22 IS SNORD22 IS SNORD22 IS SNORD21 IS SNORD21 IS SNORD20 SLC3.	LRRC75A-AS1	on human (hg3 16,440,000) 16,440,000 16,440,000 16,240,000 LRRC75A-AS1 SNORD49B II SNOR ISNO (Lrrc75a-as1) (100] 62,610,000 H16Rik ISnord49a	38) 16,450,000 16,4 D49A RD65 0n mouse (mn 62,620,000 62,6 62,870,000 6 62,870,000 6	460,000 16 	5,470,000 16,480,0 40,000 62,650,000 	16,490,000
Gene locus of TRPV2 +++ Gene locus of hr11: 62,580,000 Trpv2 ++++ Gene locus of hr11: 62,855,000 SNORD22 IS SNORD22 IS SNORD22 IS SNORD21 IS SNORD21 IS SNORD29 SLC3. Gene locus of hr19:	LRRC75A-AS1	on human (hg3 16,440,000) 16,440,000 16,440,000 16,240,000 LRRC75A-AS1 SNORD49B II SNOR ISNO LRRC75A-aS1) (100 62,610,000 H16Rik ISnord49a ISnord49 ISnord49a ISno	38) 16,450,000 16,4 D49A RD65 0n mouse (mn 62,620,000 62,6 62,870,000 6 62,870,000 6 1,11111111111111111111111111111111111	460,000 16 	5,470,000) 16,480,0 40,000] 62,650,000] 	000 16,490,000 62,660,000 ,885,000 ,885,000
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Supplementary Figure S3. The human and mouse genomic regions near lncRNAs analyzed in this study.



A Measurement of calcium deposition after Snhg16 knockdown





Supplementary Figure S4. (a) Measurement of calcium deposition after knockdown of Snhg16. Six independent experiments were performed, and the P value was calculated by a two-sided paired *t*-test.
(b) Expression change of the osteoblast-related factors after overexpression of Snhg16. Error bars indicate standard error between three independent experiments.



Supplementary Figure S5. The expression level of Trpv2, the neighboring gene of Lrrc75a-as1. FPKM values from RNA-seq data were averaged. Error bars indicate the deviation from two samples.