



Supplementary Material

Human lncRNA *SUGCT-AS1* regulates the proinflammatory response of macrophage

Yeong-Hwan Lim, Gwangho Yoon, Yeongseo Ryu, Dahee Jeong, Juhyun Song, Yong Sook Kim, Youngkeun Ahn, Hyun Kook, Young-Kook Kim*

* Corresponding Author: Young-Kook Kim; Email: ykk@jnu.ac.kr

1. Supplementary Tables

Table S1. The sequences of PCR primer, siRNA, and GapmeR.

Human PCR primers.

Gene	Forward (5' -> 3')	Reverse (5' -> 3')	Amplicon size (bp)
ACTB	ACTCTTCCAGCCTTCCTTCC	CAATGCCAGGGTACATGGTG	149
GAPDH	CCCTGGCCAAGGTCATCCAT	GGGCCATCCACAGTCTTCTG	97
RPPH1	GCTTGGAACAGACTCACGG	TCTGAATTGGGTTATGAGGTC C	84
pre-GAPDH	CCGTCTTCCGAAACCAGAT	CCAGCTACAGAAAGGTCAGC	76
MALAT1	AGACAGCAGCAGACAGGATT	AGCTTCCTTCACCAAATCGC	132
TNF	GAACCCCGAGTGACAAGC	TGGGAGTAGATGAGGTACAG G	166
IL1B	TCTTCGACACATGGGATAACG	ACAAAGGACATGGAGAACAC C	178
IL6	GTACATCCTCGACGGCATC	ACCTCAAACCTCCAAAAGACC AG	198
PTGS2	CCTTCTGCCTGACACCTTTC	TCTGGTCAATGGAAGCCTGT	197
CXCL10	TCTAAGTGGCATTCAAGGAGT AC	TGGCCTTCGATTCTGGATTC	200
CXCL8	GTGTAAACATGACTTCCAAGC TG	GTCCACTCTCAATCACTCTCA G	183
CCL2	CTGAGACTAACCCAGAAACA TCC	TGCACTGAGATCTTCCTATTG G	196
CCR7	GGGAAACCAATGAAAAGCGT G	TGGAGCACAAAGACTCGAAC	139
TLR4	TCTACAAAATCCCCGACAAC C	GCTCTGATATGCCCCATCTTC	167
IL10	GAGAACCAAGACCCAGACAT C	TCACTCATGGCTTTGTAGATG C	185
CD200R1	CATCGTGGGATTCATTTGGTT G	CAGATGCCTTCACCTTGTTTG	167

MRC1	CATTTGCCAGCGACATAACA G	GAGACCAGATTCCCTCCAAA G	198
CD163	GAGTCCCTTCACCATTACTGT G	GACTTTCACCTCCACTCTCCC	133
TGFB1	AACAATTCCTGGCGATACCTC	GTAGTGAACCCGTTGATGTCC	197
LGALS3	GTGCCTTATAACCTGCCTTTG	AGCGTGGGTAAAGTGGAAG	142
CD68	TCATTCTTTCACCAGCTGTCC	AGAAGGATCAGGCCGATGA	148
CD14	CAGAGGTTTCGGAAGACTTATC G	TTCGGAGAAGTTGCAGACG	145
CD3E	GCCTCTGCCTCTTATCAGTTG	TTTATCATCCTCATCACCGCC	188
CD19	TTCTGCCTGTGTTCCCTTG	CGTCCCGTACTGGTCTG	148
TAGLN	GTCCGAACCCAGACACAAGT	CTCATGCCATAGGAAGGACC	98
CNN1	TCAAGGCCATCACCAAGTAT G	CTTCTCTGCGTACTTCACTCC	167
ACTA2	GGTGCTGTCTCTCTATGCCT	AAGGAATAGCCACGCTCAGT	186
vWF	TCAAAACAAGGAGCAGGACC	CACGTAAGGAACAGAGACCA G	172
RBM15B	CCTGGTCTCCTACTTGAAACA G	AATGTCCTTAGTGCTGACTGG	150
HNRNPU	AGAGTTTTGCTTTTCTTTGGGCC	CAGCCAATACGAACTTCATG	149
MALT1	CACCTTTGAATTCAGCCAGTG	AGAGACGCCATCAACACTTC	82
MALT1 pre-mRNA	TTTTCTGAAACAAGGAAGAA CAGA	GTAGGCTAAAAGCACTCCAC	126
Alternative splicing of MALT1 (exon 7 skip)	CGAGACAGTCAAGATAGCAA GAA	ACCTTGTCCTTCGCCAAAG	146 (113)
SUGCT-AS1 (AC004988.1)	TCTCAGGAAATCAACAGGAC TG	GCAAAACTCTTCACTGGCAC	171
SUGCT-AS1 (Cloning)	GAATTATGCTTGAAGAAGGTT AATCT	TGTGCTTTAGGTAGTCCAGTT	1771
SUGCT	TGGTCAAAAGGAAGCAAAAC G	CTATTGTGTACCCGAAGGTGG	198
RP11-1008C21.1	AACTCAGAAGCACTTGGTAC C	GGGTTGCATTCAATAACTCAG G	183
LL21NC02-1C16.2	TGGTTTCAAGTGGGCAGAG	TGTCAAAGTGGCAGAGTGAG	123
LINC01176	TGGTTTAGCATTATCCCCTTG G	AGAATGAGTCTTCCAGCCTTG	118
RP4-591C20.9	TGTGCAAGTGTATCTGTAGCC	CACCTGGCTATCATTCTGTAGG G	122
DLGAP1-AS1	TCTGTACGTTGCAATCTGTGG	AGCATTTATCACACTAGTACC GTG	150
RP11-422J8.1	CTCGGACCTAAGAACTTGAC AG	AAGAGCTTGAGATCAGCCTG	119
RP11-6N17.4	AGCTTTTACCTGCTCTTCGG	GCCTCAATCCTTCTACCACAG	79
RP11-206L10.11	TCACAATGGCATGATCTCGG	ACCCTGTGGCACTGAATATTC	148
CD63-AS1	CTATACCTCCGTGCCAACTC	AGCACAGACCTTGTCAATCC	197
RP11-452L6.5	TTTGTCACTGCCCCCTTCC	ATGTTCAAGTCCCCAATGTCC	131
RP11-63P12.6	TTGCTTGTCTGCTAAGACC	CAGCTCTGGATTTACTTCTAG GG	104
RP11-680A11.5	CTGGTGATGGTCATATCGGTG	CGGTGACAAGAGCAGGATT	137
ZBED5-AS1	TTCTCAAACCTTCACTAGCCCC	TGCAAATCCAGTGGTCCTAC	180

RP11-4B16.4	TGGATCGTGTCTGCCTAAAAG	GACATGGATGCCTCAGATGA G	141
AC079767.4	CATGAAAGCAAACACTGGGC	GCGGAAACTGTAGATGCAA G	146
RP11-452F19.3	GCAGACACCATCTTATTTCTG TTG	TGCACTTGTGTAGTCATGGG	142
XXbac-BPG246D15.8	CCAGGTGCAGCTTCAGAG	TGTGTTACCTCCTTTCCAAGC	95
AC093627.10	GGCAGAGGAAGCTGGAAG	CCAGTCCCCATAAATGTGAG AG	87
CH17-340M24.3	GGCCAATCCCTGTTTTAATCC	TGTCATTCCAATCTCTGCCTTC	150
CD27-AS1	TTGCCTGGAACCTTACCACTG	AATTTGTCCCTCTCCTTGTCC	145
RP11-344B5.2	TGACTTTGAAGCCCACTGAC	GAAGTTCTTGACCCCTGG	92

Human siRNAs

Name	Sense (overhang) 5' -> 3'	Antisense (overhang) 5' -> 3'
RBM15B siRNA #1	GGGAAGAAGGCAAGAGACA(UU)	UGUCUCUUGCCUUCUUCCC(UU)
RBM15B siRNA #2	GGGCAAGCUAGAAGAAGAA(UU)	UUCUUCUUCUAGCUUGCCC(UU)
hnRNPU siRNA #1	ACAGAAAGGCGGAGAUAAA(UU)	UUUAUCUCCGCCUUCUGU(UU)
hnRNPU siRNA #2	GAAGAAAGAUUGUGAAGUU(UU)	AACUUCACAAUCUUUCUUC(UU)

Human antisense LNA GapmeRs

Name	Sequence 5' -> 3'	Lot number
SUGCT-AS1 GapmeR #1	G*A*A*G*G*C*T*G*A*G*C*G*T*C*T*G	434942700
SUGCT-AS1 GapmeR #2	T*T*A*A*C*C*T*T*C*T*T*C*A*A*G*C	436013070
Negative control	A*A*C*A*C*G*T*C*T*A*T*A*C*G*C	10909323-1

Table S2. The sequence of *SUGCT-AS1* and predicted binding proteins**Input: *SUGCT-AS1* sequence (1,771 nucleotides)**

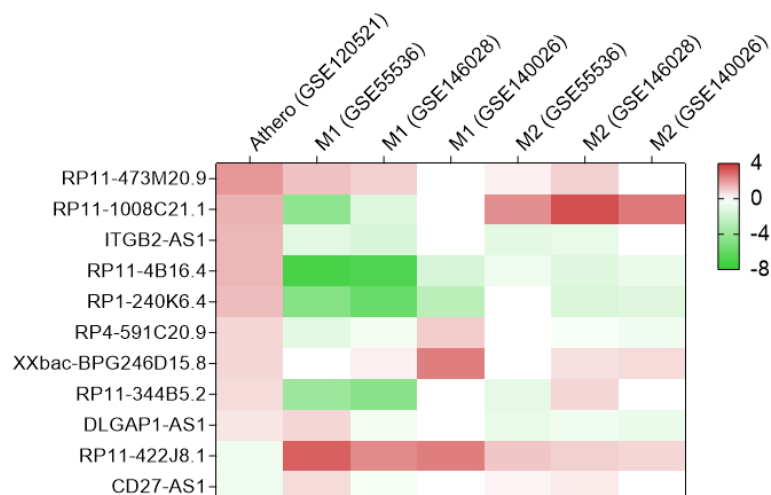
GAA TTA TGC TTG AAG AAG GTT AAT CTT ATA ATG GCA TGC AGA GCG GAG CTG GGC AAG GCC AGA GGC
 TAA ATA TGG AGC CAG AAA GAT ACT GCA GAA AGT GAG CAA AAA GGA TGA GTG GAT GGC CTG GGG TGG
 AAT CCG TGA AAA AGG GCA GAT TGT CAG TCT CAG GAA ATC AAC AGG ACT GAG TTC TAA CTT CAG CTG
 GGC TAC TAT GCC CTG GGG AAT CAG AAG ATT CTC CCT AAC ATT GGA GTC CAA AGA AGA GGA GCC ACC
 ATC TCC TTA ACT ACA AAA CAA CTG AGA GAA GAA ATG TCT CCC TTT AGT GCC AGT GAA GAG TTT TGC
 TTC TCT AGA ACA ACT GTT TAT TTT TGA TCT TAT GAA ACG CGT GAC ACT TGT AAC TGA TTT GCC TTC
 TTT TTG CTT GCT GCT AGA AAT CTC AGA ACC AAA TTC GAG GTC ATT TCT GAA TGT GCT GGA CTA CAC
 AGC ATG AAT TCT GTG TCC TCT CAC CTC ATT CAT AGA TAC ATT TTT TTA TTC CTA CCC ATC TTT TCC
 TTT CCC CAG ATT TTC CTA TTG CTC TTT TAT CAT CTT GAG TAA AGA AGG TTA AAC TAT GAA TAA AAA
 GTA CAA TCT CAA TGA CGA GTC AGG GAA AAG TAA GTT TTG GAC TTC CAT TTA GAG CCA TTT ATA GGT
 CAG CAA TCT TTT GTC TCT GTG TCT TCT TTC TGC CCT GAA CAA CCA AGG CAT CCT AGT CAG TCT CTC
 TTC ATC TGC ATA CCA CTT TAA AGA GCC CAG GGG TCT TCC AAG TAC AGT CAC TAC TAA GAA AAT GTC
 TTC AGT AAA TTT TTT TTT TTT TTT TTT GAG ATG GAG TCT CGC TCT GTC GCC CAG GCT GGA ATG TAG
 TGG CAC GAT CTC AGC TCA CTG CAA GCT CTG CCT CCC GGG TTC ACG CCA TCC TCC TGC CTC AGC CTC
 CCA AGT AGC TGA GAC TAC AGG CAC CTG CCA CCA TGC CCG GCT AAT TTT TTT TGT ATT TTT AGT AGA
 GAC GGG GTT TCA CTG TAT TAG CCA GGA TGG TCT TAA TCT CCT GAC CTC ATG ATC CGC CCG CCT CGG
 CCT CCC AAA GTG CTG GGA TTA CAG GCG TGA GCC CCC GCG CCC AGC CTT CAG TAA AAT TTT AAT GCA
 TGT TGC TTA TCA TTC TTT GTA TTG TAA GCA CCA ATC CTT AAT CTG TGC TGT GGT TTT TGT TCA GAA
 ACA TTT CTT TAA AAA AAT TTC CCC AGA CGC TCA GCC TTC ATA TTC TAA TCT TTA TTA TTC TGA ACT
 ACC TAC AAA GCT AAA TAT ACC TTG TCT ACA TGC AGT GCT TAT GTA ACA ATG GTG CTT ATT TTG TTT
 TAC ATT GCT TCA ACC TCT GAC CAG AAA CTT AGA GGA CAG AAT GCC CTG CTT TTA CAA AAA ATG TGG
 TGA CCA CAA CGG TAA CCT TAT CTA ATT GAG ACC ATT TTA GGA AGG AGG GGA GGA GAT ATT TTT ACT
 GCA AAG ACT GAG TCA TAA GCC TTC ATT TTT TAC CCT GGG TTG TGC AGA AAC TTT AAA CTT TCT ATA
 TTA TTT CAT TTC TTC CAT ACC AGG ACA TTT ATT TCC TAT TCA GTG ACA ATC TTG ATG TCT TCA TCC
 TTC AAA CTG CTC TCT AAC ATA GTT TCA AAA TCC AAG CAT TAA ACA TTA TTA ATG CTC AGG GGA TGA
 ATA TGT TCA TTA CCT TTA CTG TAG TGA TGA TTT CCT ATG GCA AAA TGT TAA ATG ATG TAA AGA TAA
 AAA TAA TTA TCA TAG TTA TTA ATC ATA GCA ATA AAA CTG GAC TAC CTA AAG CAC A

RPIseq prediction: *SUGCT-AS1*-protein interaction

	Protein	Length (amino acid)	RF classifier	SVM classifier
BARTweb and ChEA3 prediction Top 10	RELA	551	0.95	0.989
	ELK3	407	0.85	0.985
	MAX	160	0.8	0.894
	NRF1	503	0.8	0.971
	ETS1	441	0.75	0.981
	RUNX1	480	0.85	0.977
	NFATC1	943	0.8	0.996
	SP2	613	0.75	0.993
	E2F4	413	0.8	0.985
	FOXP3	431	0.8	0.995
catRAPID prediction Top 10	SND1	910	0.75	0.982
	RBM15B	890	0.75	0.99
	UPF1	1129	0.7	0.995
	ACIN1	1341	0.8	0.992
	RBM25	843	0.85	0.929
	HNRNPU	825	0.7	0.984
	RBM15	977	0.8	0.996
	KHSRP	711	0.75	0.983
	MATR3	847	0.75	0.992
	DGCR8	773	0.65	0.988

Supplementary Figures

A. Common lncRNA candidates in M1 and M2



B. Published macrophage-related lncRNAs

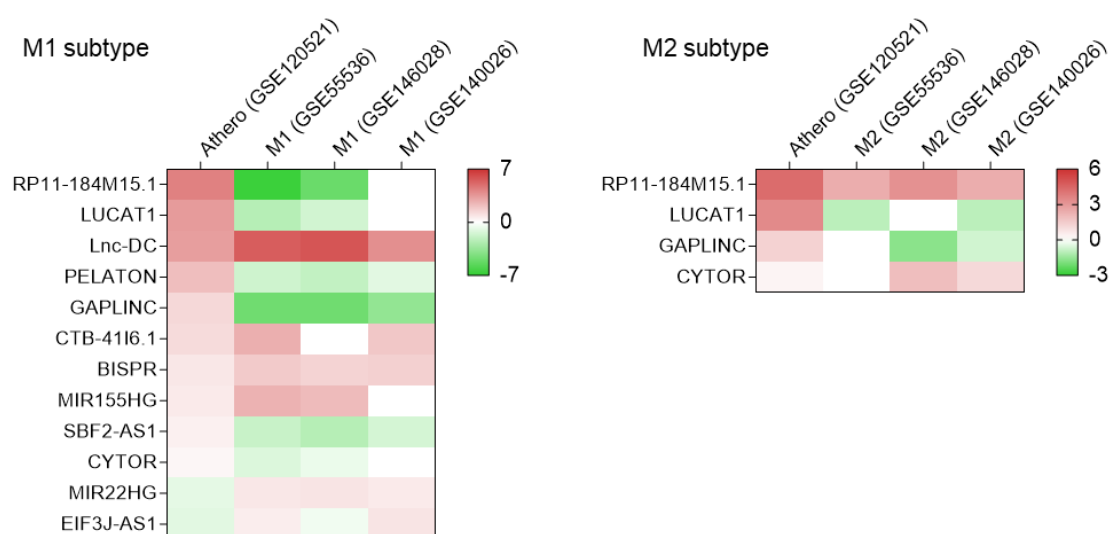


Figure S1. Comparative analysis of the lncRNA expression profiles during atherosclerosis progression and macrophage activation. (A) Atherosclerosis-related lncRNA candidates differentially expressed in both M1 and M2 macrophages. (B) Previously reported lncRNAs differentially expressed in M1 or M2 macrophages. The datasets used for this analysis were obtained from the GEO database (GSE120521, GSE55536, GSE146028, and GSE140026) [19–22].



Figure S2. Identification of the transcript structure of *SUGCT-AS1* through the analysis of RNA-seq reads. The sequencing reads were transformed into bedgraph signals and visualized in the UCSC genome browser (<https://genome.ucsc.edu/>).

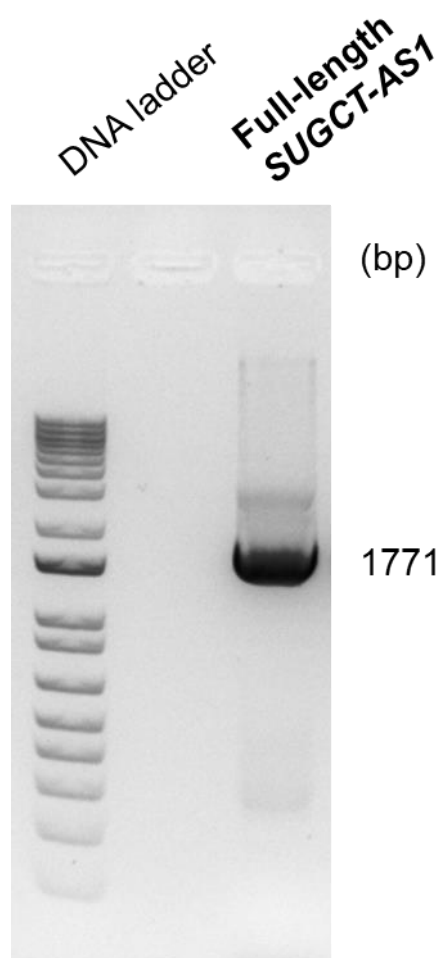


Figure S3. PCR amplification of full-length *SUGCT-AS1*. The PCR primers were designed at both ends of the RNA-seq signals near *SUGCT-AS1* locus as shown in **Figure S2**. The Sanger sequencing of the amplified PCR product confirmed that only one isoform identical to the transcript presents in the database (ENST00000415237; 1,771 base pairs) was detected. The sequences of the primer sets used are listed in **Supplementary Table S1**.

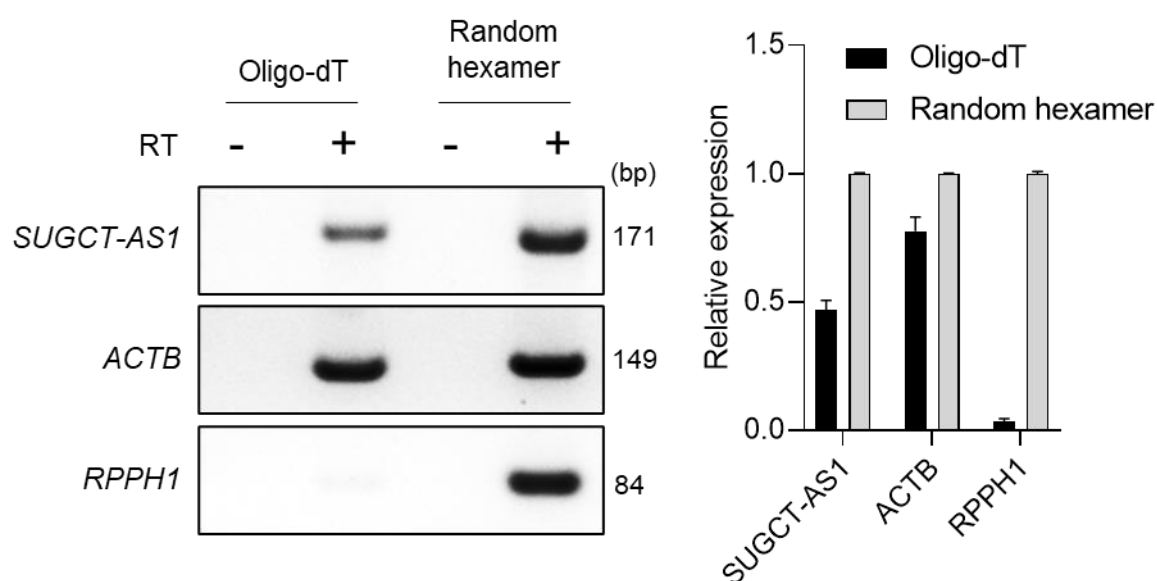


Figure S4. PCR measurement to test the presence of poly(A) tails in *SUGCT-AS1* lncRNA. Oligo-d(T) or random hexamer, respectively, was used as the primer for the reverse transcription. *ACTB* and *RPPH1* (Ribonuclease P RNA Component H1) were used as the controls for RNA with poly(A) tail and that without poly(A) tail, respectively. RT: reverse transcriptase. Data are presented as mean \pm SEM ($n = 3$).

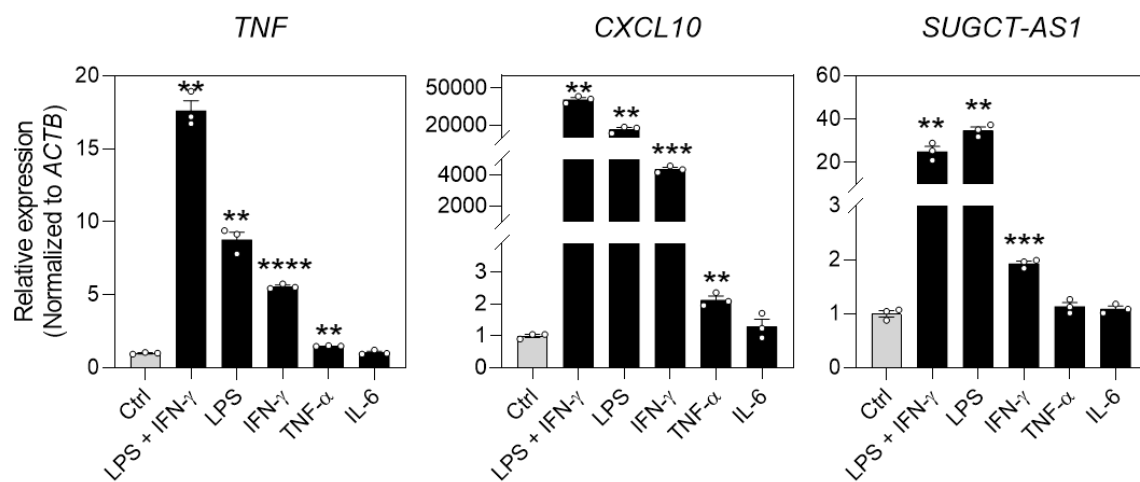
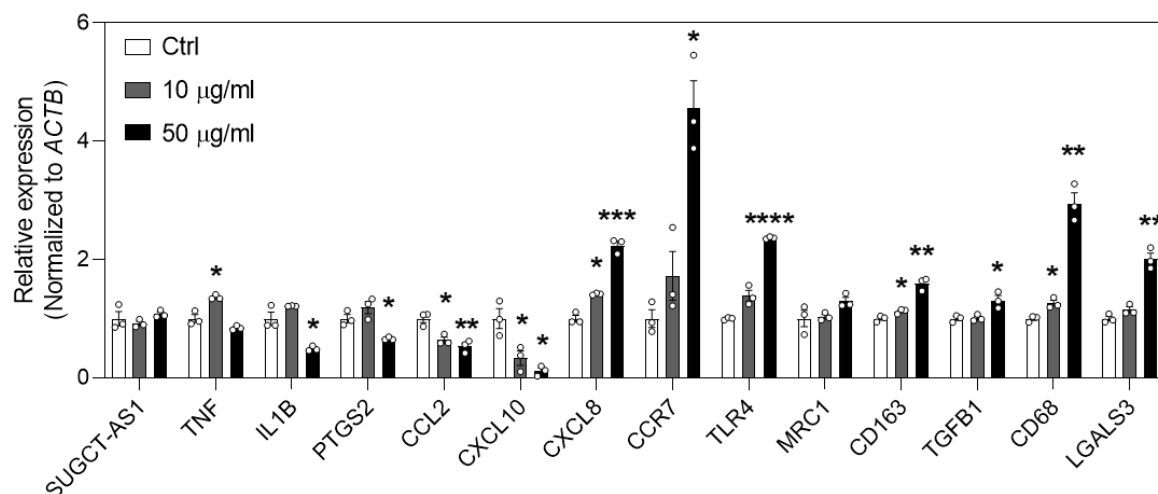
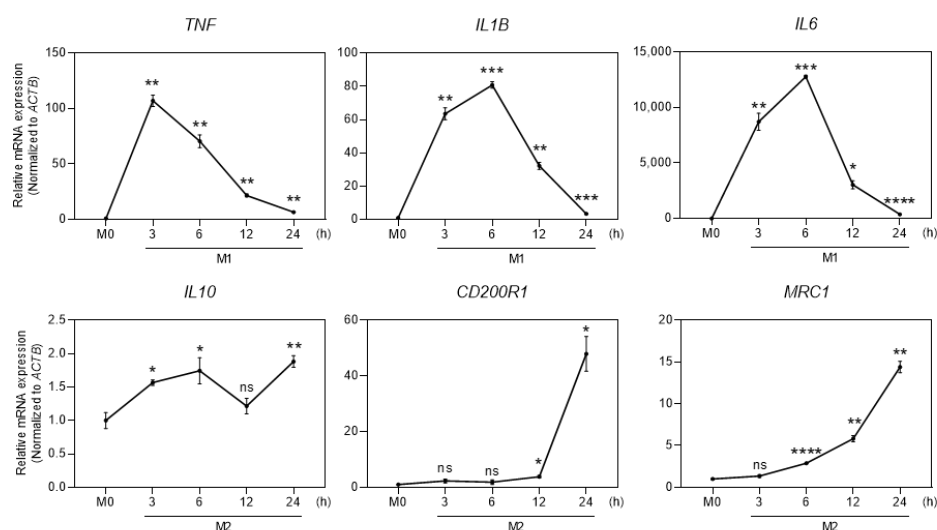
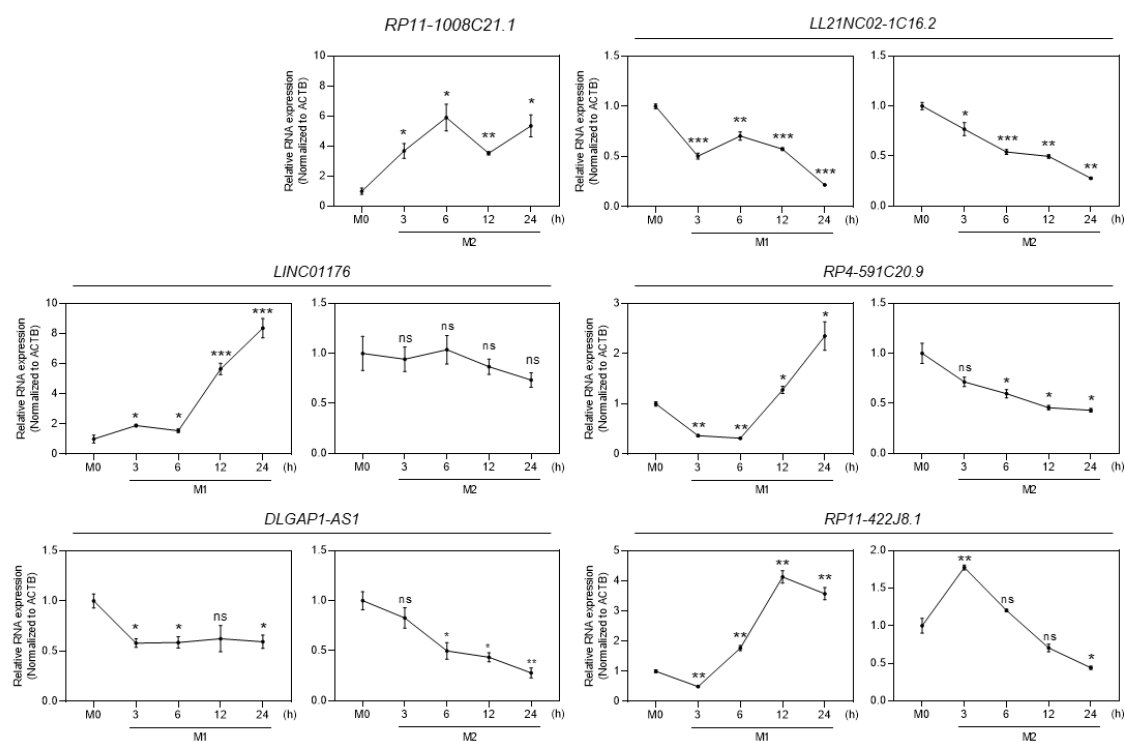
A. Response to various inflammatory stimuli (pro-inflammatory genes and *SUGCT-AS1*)**B. oxLDL-treated THP-1 macrophages**

Figure S5. Response of *SUGCT-AS1* to various inflammatory stimuli. qRT-PCR measurement of the expression of (A) inflammatory genes (*TNF* and *CXCL10*) and (B) *SUGCT-AS1* in THP-1-derived macrophages stimulated for 24 hours with various inflammatory stimuli ($n = 3$). Dosage of inflammatory stimuli: 10 ng/ml LPS, 30 ng/ml IFN- γ , 25 ng/ml TNF- α , and 20 ng/ml IL-6. qRT-PCR measurement of the expression of *SUGCT-AS1* and oxidized low-density lipoprotein (oxLDL)-related genes in THP-1-derived macrophages stimulated for 24 hours with oxLDL (10-50 μ g/ml, $n = 3$). The RNA expression was normalized to *ACTB*. Data are presented as mean \pm SEM. An unpaired two-tailed t -test with Welch's correction was used for statistical analysis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.

A. Macrophage polarization markers (M1 and M2)



B. Expression of lncRNAs (M1 and M2)



M1 stimulation (10 ng/ml LPS and 30 ng/ml IFN- γ)
M2 stimulation (20 ng/ml IL4)

Figure S6. Expression patterns of inflammatory genes and lncRNA candidates depending on the stimulation time. (A) qRT-PCR measurement of marker genes of macrophage polarization in THP-1-derived macrophages treated with stimuli (M1: 10 ng/ml LPS and 30 ng/ml IFN- γ , M2: 20 ng/ml IL-4) at different treatment times ($n = 3$). Proinflammatory (*TNF*, *IL1B*, and *IL6*) and anti-inflammatory (*IL10*, *MRC1*, and *CD200R1*) genes were used as M1 and M2 macrophage markers, respectively. THP-1-derived M0 macrophages were used as controls. (B) qRT-PCR measurement of lncRNA candidates in THP-1-derived macrophages treated with stimuli at different treatment times ($n = 3$). The RNA expression was normalized to *ACTB*. Data are presented as mean \pm SEM. An unpaired two-tailed *t*-test with Welch's correction was used for statistical analysis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$; ns: not significant

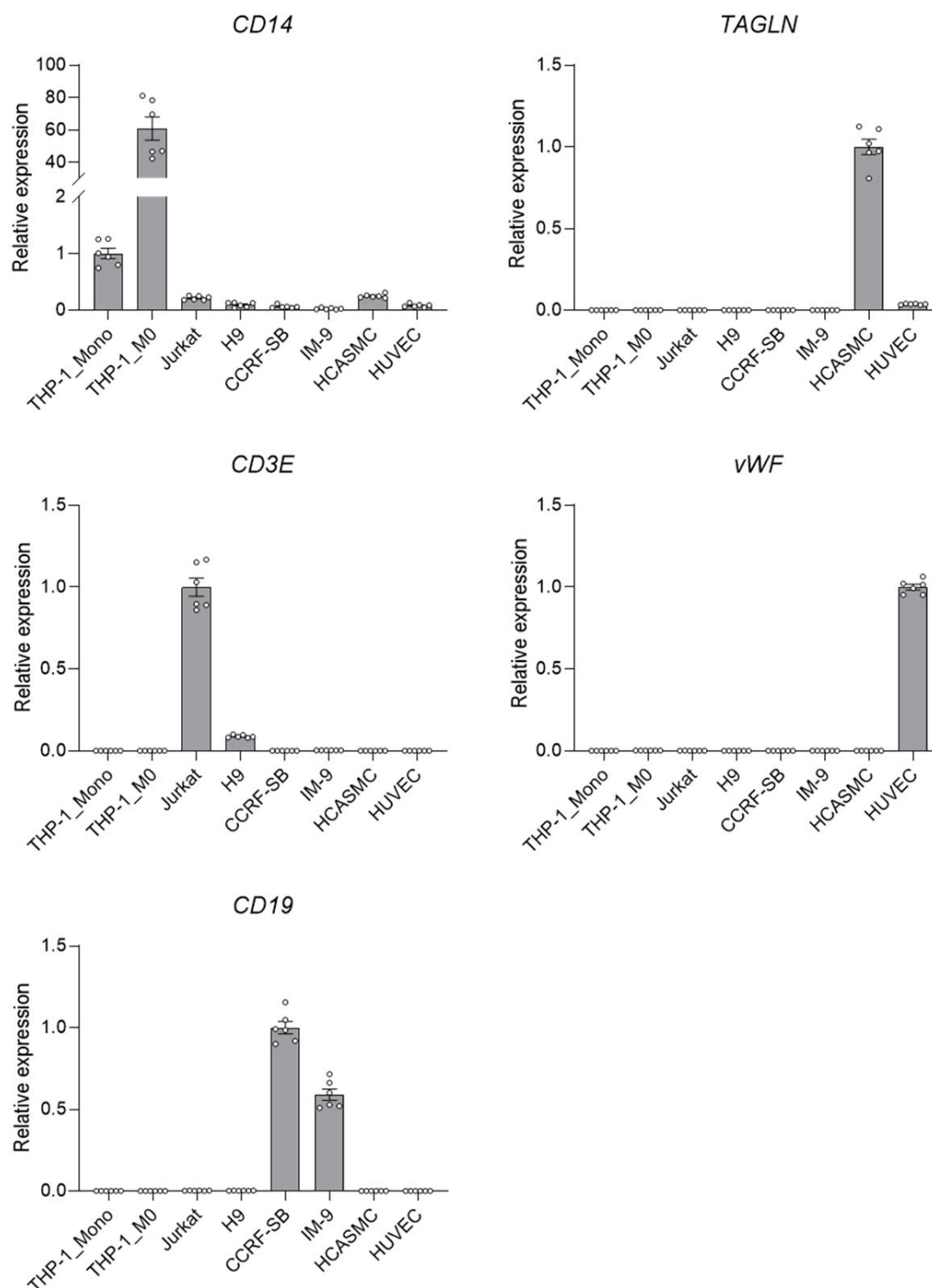
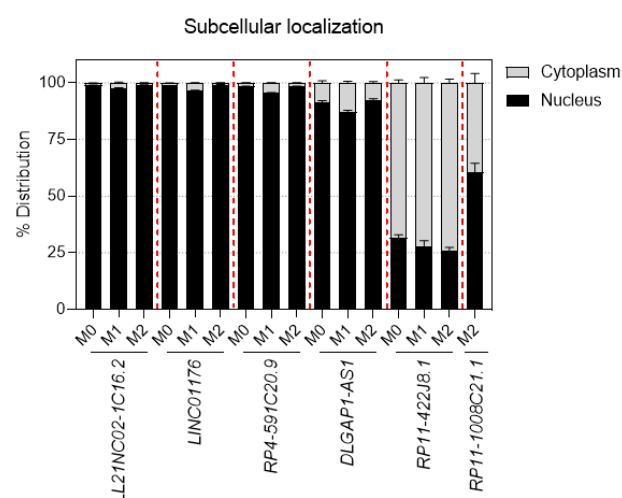
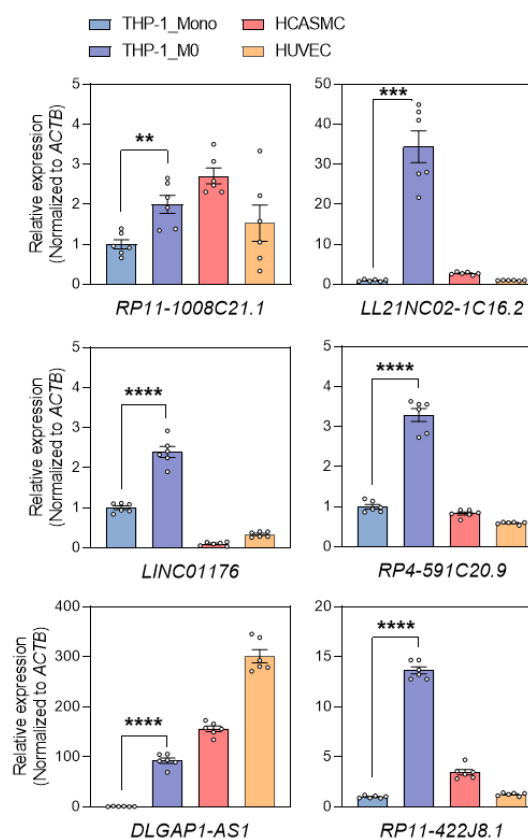


Figure S7. Vascular cell type-specific gene expression. qRT-PCR measurement of vascular cell type-specific expression of markers for each cell type ($n = 6$). THP-1_mono: monocytic THP-1 cells, THP-1_M0: THP-1-derived macrophages, Jurkat and H9: T-cell lines, IM-9 and CCRF-SB: B-cell lines, HCASMCs: human coronary artery smooth muscle cells, HUVECs: human umbilical vein endothelial cells. After normalizing the expression level of each marker gene to that of *ACTB*, their relative expression levels among the cells were compared. The marker gene information is *CD14* for monocyte (THP-1_Mono), *CD3E* for T-cell (Jurkat), *CD19* for B-cell (CCRF-SB), *TAGLN* for smooth muscle cell (HCASMC), and *vWF* for endothelial cell (HUVEC). Data are presented as mean \pm SEM.

A. Subcellular localization of lncRNAs



B. Vascular cell type-specific expression

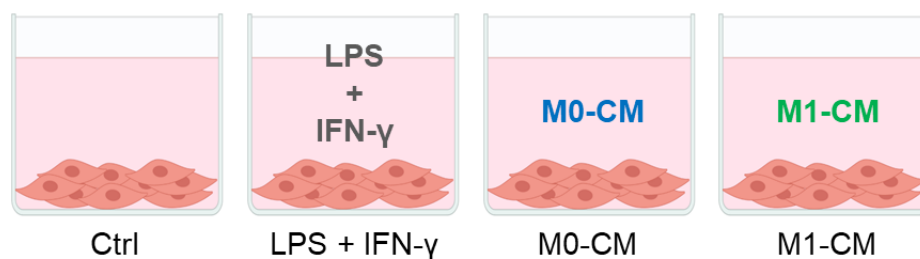


C. Protein coding potential

Gene id	Transcript id	Name	CPAT		CPC2	
			Coding Probability	Label	Coding Probability	Label
ENSG00000259225	ENST00000557883	RP11-1008C21.1	0.03805	no	0.01508	noncoding
ENSG00000272825	ENST00000609953	LL21NC02-1C16.2	0.00301	no	0.05125	noncoding
ENSG00000281404	ENST00000628121	LINC01176	0.04743	no	0.46465	noncoding
ENSG00000268858	ENST00000601296	RP4-591C20.9	0.01387	no	0.33880	noncoding
ENSG00000177337	ENST00000317114	DLGAP1-AS1	0.06284	no	0.24022	noncoding
ENSG00000233621	ENST00000424989	RP11-422J8.1	0.01945	no	0.05173	noncoding

Figure S8. Characterization of lncRNA candidates involved in the differentiation and polarization of macrophages. (A) Subcellular localization of lncRNA candidates in THP-1-derived macrophages of each subtype (M0, M1, and M2, $n = 3$). *MALAT1* and pre-*GAPDH* were used as controls for nuclear RNA, and *ACTB* and *GAPDH* were used as controls for cytoplasmic RNA (see **Figure 2C**). (B) qRT-PCR measurement of vascular cell type-specific expression of lncRNA candidates ($n = 3$). THP-1_mono: Monocytic THP-1 cells, THP-1_M0: THP-1-derived macrophages, HCASMCs: Human coronary artery smooth muscle cells, HUVECs: Human umbilical vein endothelial cells. The RNA expression was normalized to *ACTB*. (C) The protein-coding potential of lncRNA candidates. The coding probabilities of lncRNA candidates were assessed by CPAT and CPC 2.0 tools. *MALAT1* was used as a control for the non-coding RNA, and *GAPDH* was used as a control for the protein-coding RNA (see **Figure 2D**). Data are presented as mean \pm SEM. An unpaired two-tailed *t*-test with Welch's correction was used for statistical analysis. ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.

Human coronary artery smooth muscle cells (HCASMCs)



* Conditioned media (CM)

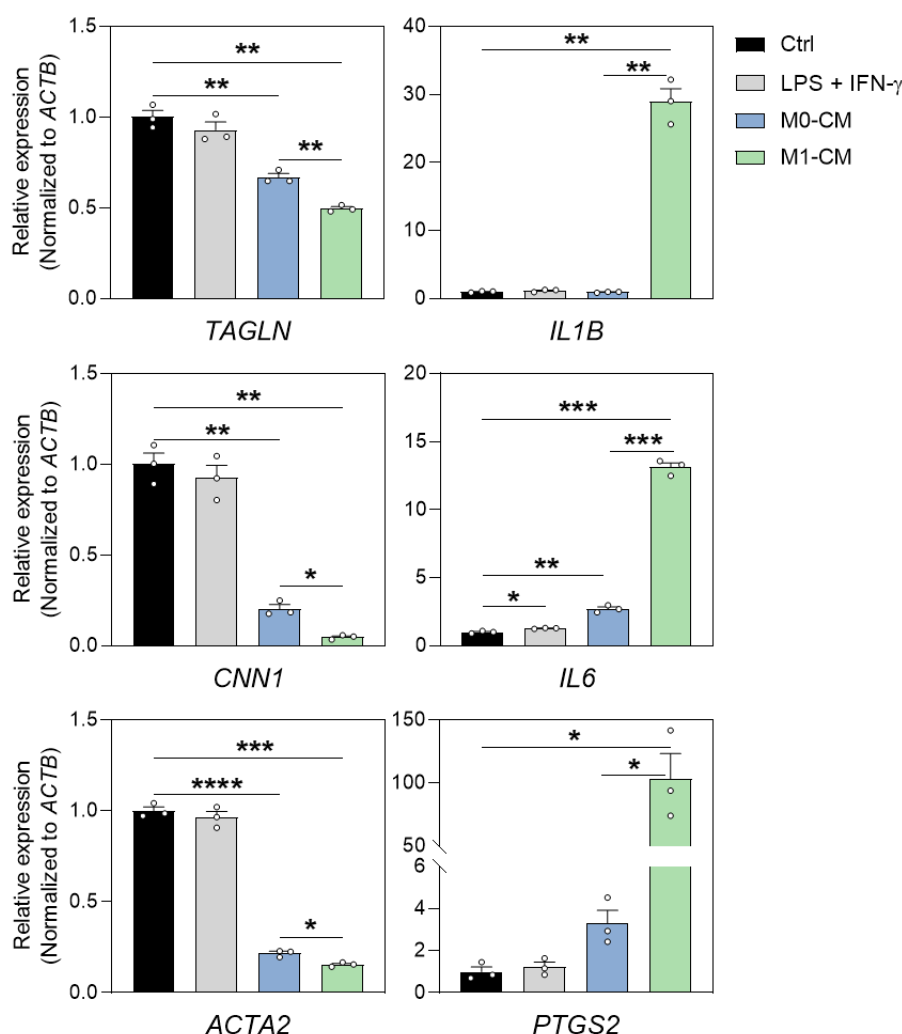


Figure S9. Establishing the treatment condition of conditioned media. Top: Each conditioned media (CM) was treated to HCASMCs for 24 hours. Ctrl (the media without treatment), LPS/IFN- γ (LPS and IFN- γ -added media incubated for one day without cells), M0-CM (the media cultured with M0 macrophages for one day), M1-CM (the media cultured with M1 macrophages for one day). Bottom: qRT-PCR measurement of contractile genes (*TAGLN*, *CNN1*, and *ACTA2*) and proinflammatory genes (*IL1B*, *IL6*, and *PTGS2*) after 24 hours of CM treatment ($n = 3$). The RNA expression was normalized to *ACTB*. Data are presented as mean \pm SEM. An unpaired two-tailed *t*-test with Welch's correction was used for statistical analysis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.

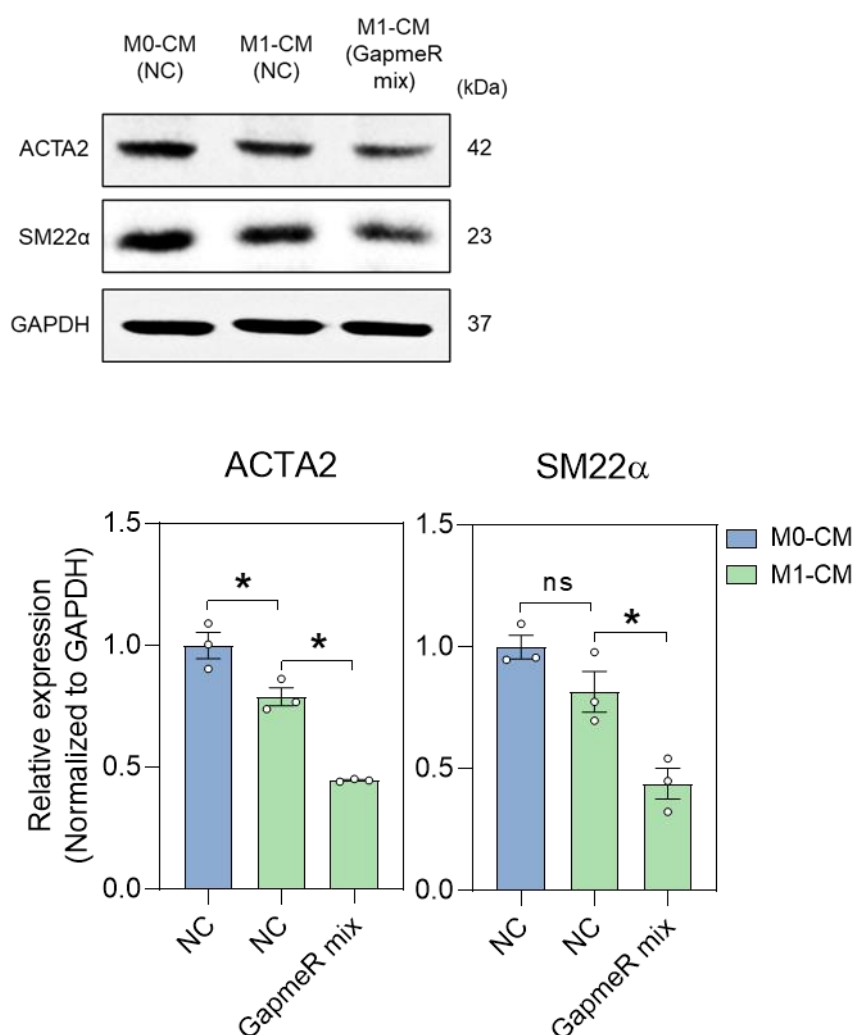


Figure S10. Effect of conditioned media of *SUGCT-AS1*-depleted THP-1 cells on the expression of contractile proteins in vascular smooth muscle cells. Western blot analysis of the expression of contractile proteins (ACTA2 and SM22 α) in HCASMCs-treated with CM of THP-1-derived macrophages (M0 and M1) transfected with GapmeRs (NC and GapmeR mix) ($n = 3$). NC indicates negative control GapmeR. GapmeR mix is a mixture of GapmeR #1 and #2. The protein expression was normalized to GAPDH. Data are presented as mean \pm SEM. An unpaired two-tailed t -test with Welch's correction was used for statistical analysis. * $p < 0.05$; ns: not significant.

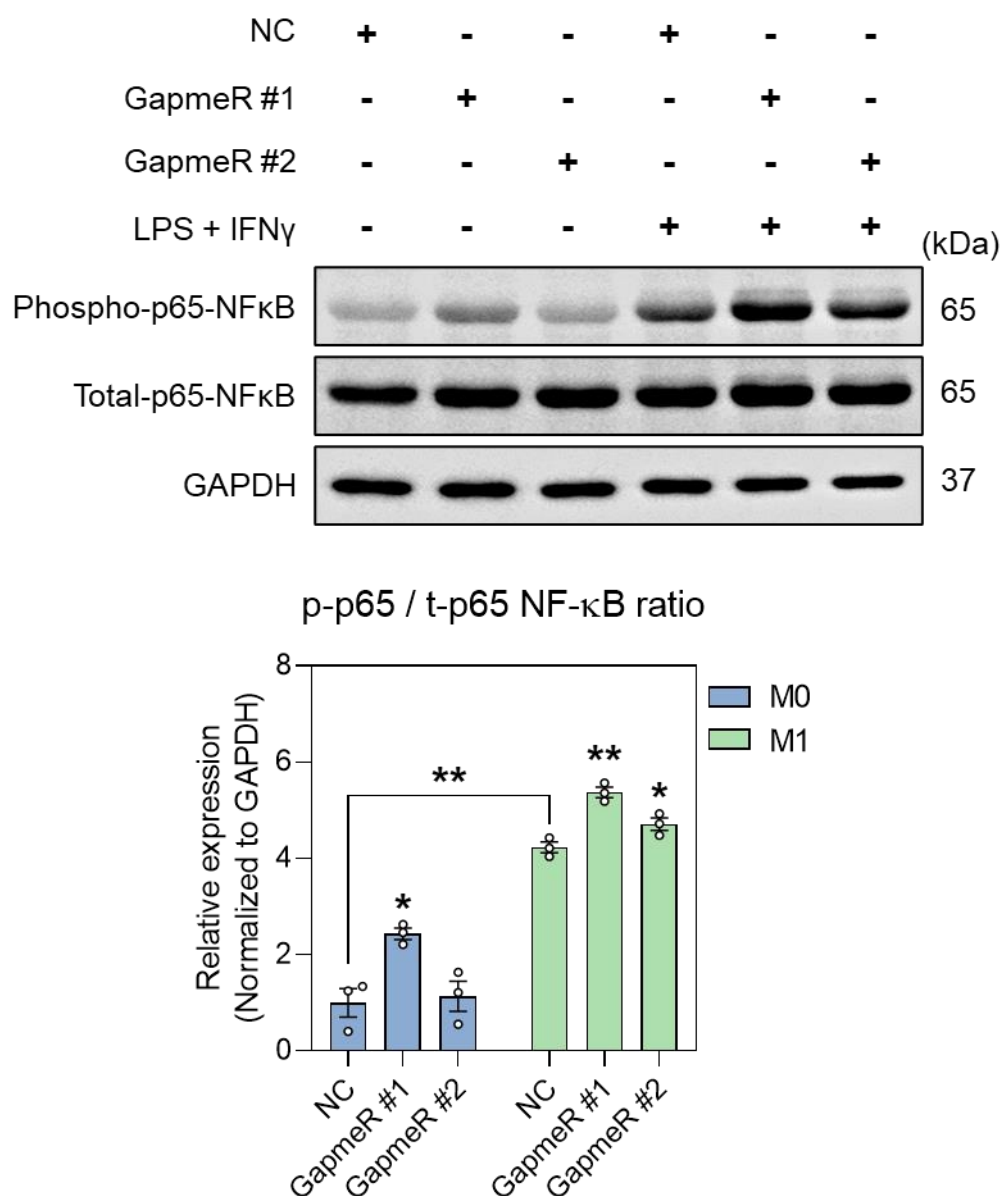


Figure S11. Effect of *SUGCT-AS1* knockdown on the activation of NF- κ B signaling pathway. Western blot analysis of NF- κ B p65 activation in THP-1-derived macrophages (M0 and M1) transfected with GapmeRs (NC, GapmeR #1, and #2) ($n = 3$). NC indicates negative control GapmeR. M1 stimulation was performed for 30 minutes. The ratio of phosphorylated p65 to total p65 was determined (phospho-p65/t-p65 ratio). The protein expression was normalized to GAPDH. Data are presented as mean \pm SEM. An unpaired two-tailed *t*-test with Welch's correction was used for statistical analysis. * $p < 0.05$, ** $p < 0.01$.

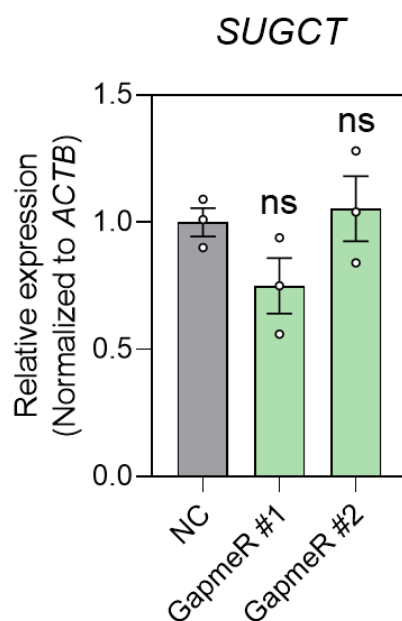


Figure S12. Effect of *SUGCT-AS1* knockdown on the expression of the neighboring gene. qRT-PCR measurement of *SUGCT* in *SUGCT-AS1*-depleted THP-1-derived M1 macrophages ($n = 3$). NC indicates negative control GapmeR. M1 stimulation was treated for 24 hours. The RNA expression was normalized to *ACTB*. Data are presented as mean \pm SEM. An unpaired two-tailed *t*-test with Welch's correction was used for statistical analysis. ns: not significant.

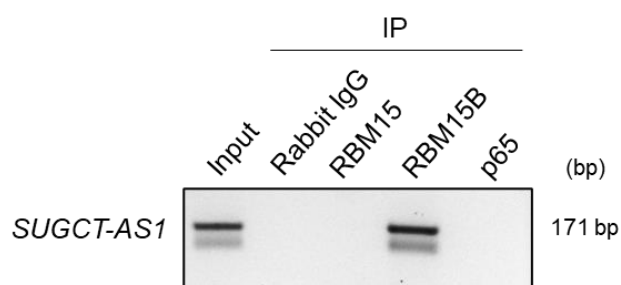
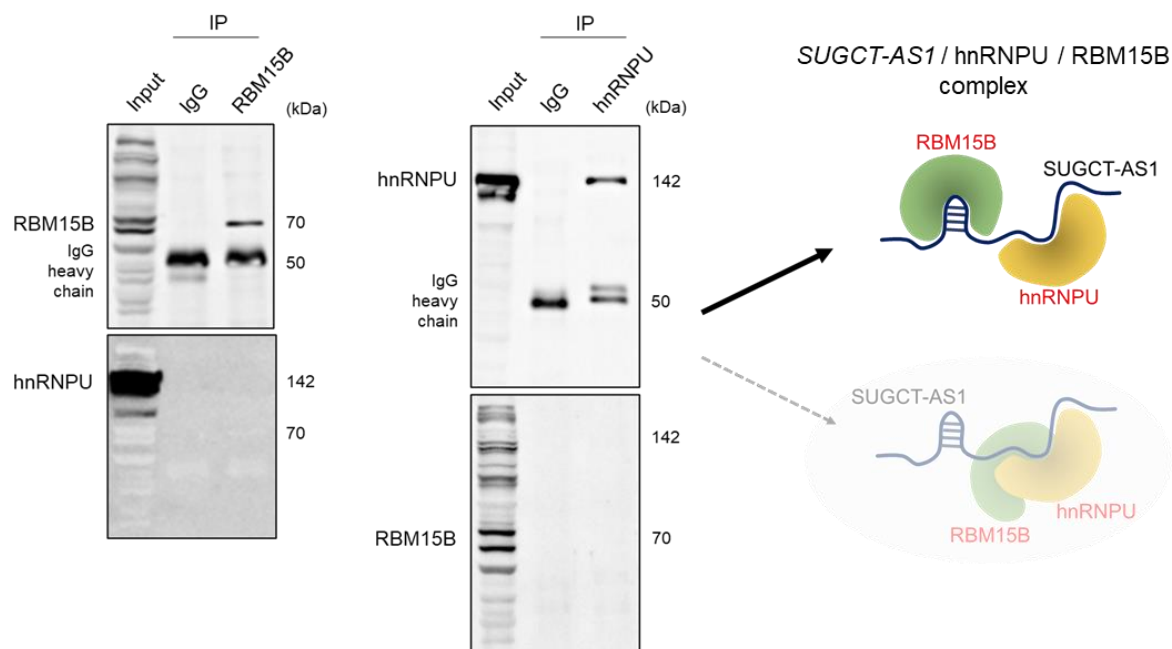
A. RNA-IP (*SUGCT-AS1*)**B. Co-IP (RBM15B and hnRNP)**

Figure S13. RNA-binding protein immunoprecipitation (RIP). (A) Confirmation of the interactions between *SUGCT-AS1* and predicted proteins (RBM15, RBM15B, and p65) in THP-1-derived M0 macrophages ($n = 3$). IP: Immunoprecipitation. (B) Co-immunoprecipitation (Co-IP) experiments to confirm whether each antibody used in this RIP efficiently binds to *SUGCT-AS1*-binding proteins (RBM15B and hnRNP) and whether there is direct binding between RBM15B and hnRNP. After blotting with each antibody (upper blot), the co-immunoprecipitation between RBM15B and hnRNP was checked by attaching different antibodies (lower blot), after stripping the previous antibody.

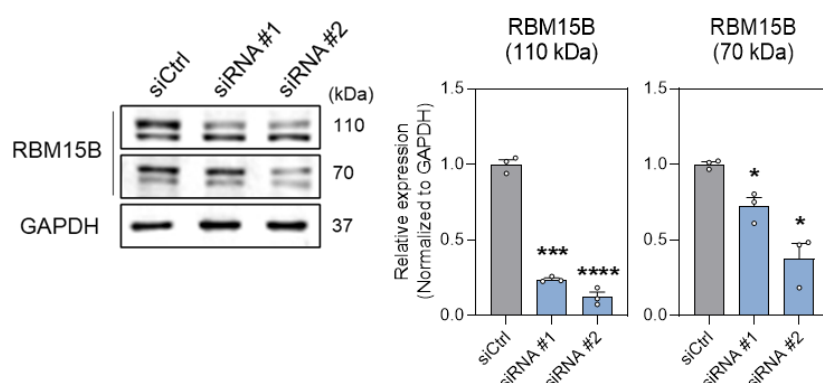
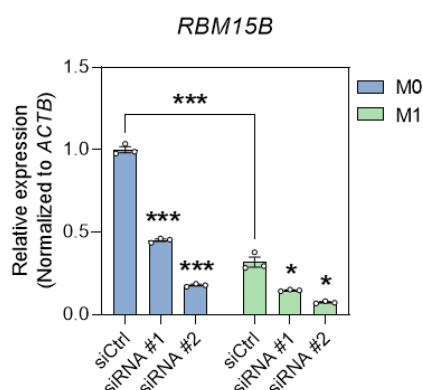
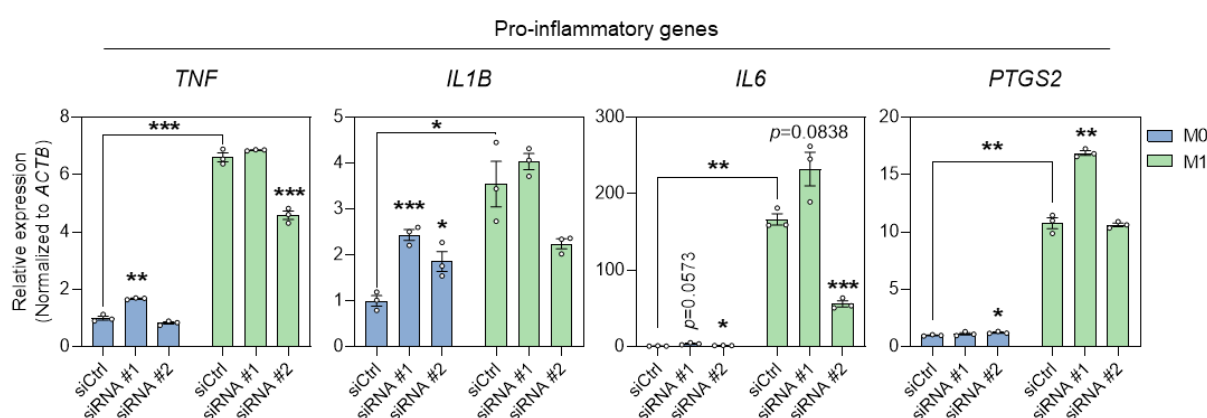
A. Western blot (RBM15B knockdown)**B. qRT-PCR (RBM15B knockdown)****C. qRT-PCR (Inflammatory genes in RBM15B knockdown)**

Figure S14. Effect of RBM15B knockdown on the expression of proinflammatory genes. (A) Verification of RBM15B knockdown by Western blot analysis in THP-1-derived M0 macrophages transfected with siRNAs (siCtrl, siRNA #1, and #2) for 24 hours ($n = 3$). The siRNAs against RBM15B effectively depleted both isoforms (110 and 70 kDa) of RBM15B. Bands marked with an asterisk are the target RBM15B. The siCtrl indicates the negative control siRNA. The protein expression was normalized to GAPDH. (B) Verification of *RBM15B* knockdown by qRT-PCR in THP-1-derived macrophages (M0 and M1) transfected with siRNAs for 24 hours. (C) qRT-PCR measurement of proinflammatory genes (*TNF*, *IL1B*, *IL6*, and *PTGS2*) in THP-1-derived macrophages (M0 and M1) transfected with siRNAs for 24 hours ($n = 3$). M1 stimulation was performed for 24 hours. The RNA expression was normalized to *ACTB*. Data are presented as mean \pm SEM. An unpaired two-tailed *t*-test with Welch's correction was used for statistical analysis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.

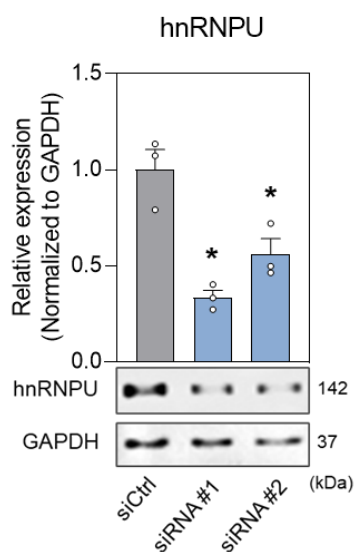
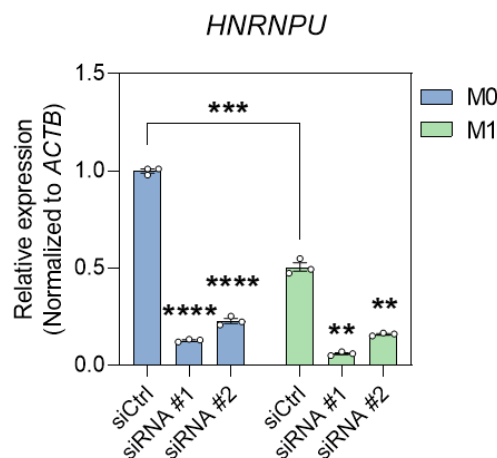
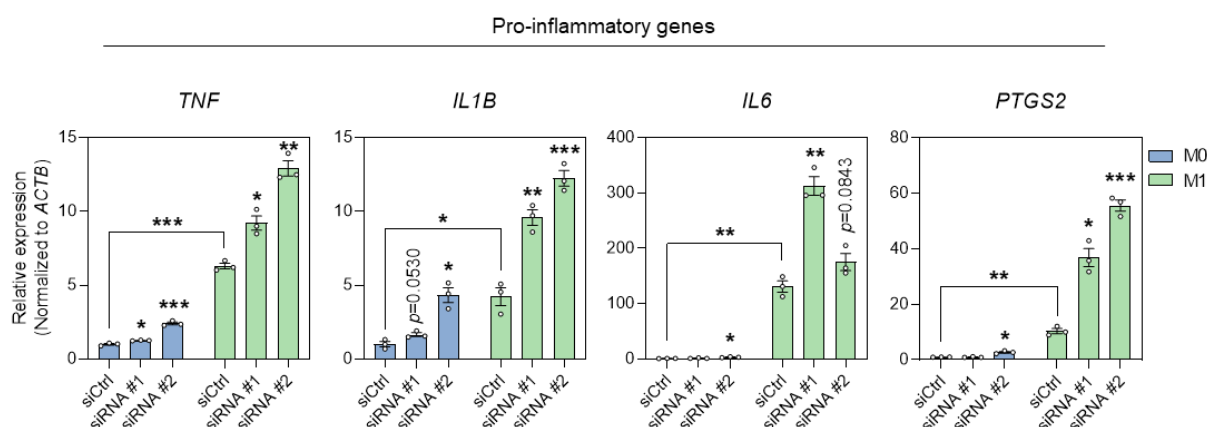
A. Western blot (hnRNPU knockdown)**B. qRT-PCR (hnRNPU knockdown)****C. qRT-PCR (Inflammatory genes in hnRNPU knockdown)**

Figure S15. Effect of hnRNPU knockdown on the expression of proinflammatory genes. (A) Verification of hnRNPU knockdown by Western blot analysis in THP-1-derived M0 macrophages transfected with siRNAs (siCtrl, siRNA #1, and #2) for 24 hours ($n = 3$). The siCtrl indicates negative control siRNA. The protein expression was normalized to GAPDH. (B) Verification of *HNRNPU* knockdown by qRT-PCR in THP-1-derived macrophages (M0 and M1) transfected with siRNAs for 24 hours. (C) qRT-PCR measurement of proinflammatory genes (*TNF*, *IL1B*, *IL6*, and *PTGS2*) in THP-1-derived macrophages (M0 and M1) transfected with siRNAs for 24 hours ($n = 3$). M1 stimulation was performed for 24 hours. The RNA expression was normalized to *ACTB*. Data are presented as mean \pm SEM. An unpaired two-tailed t -test with Welch's correction was used for statistical analysis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.

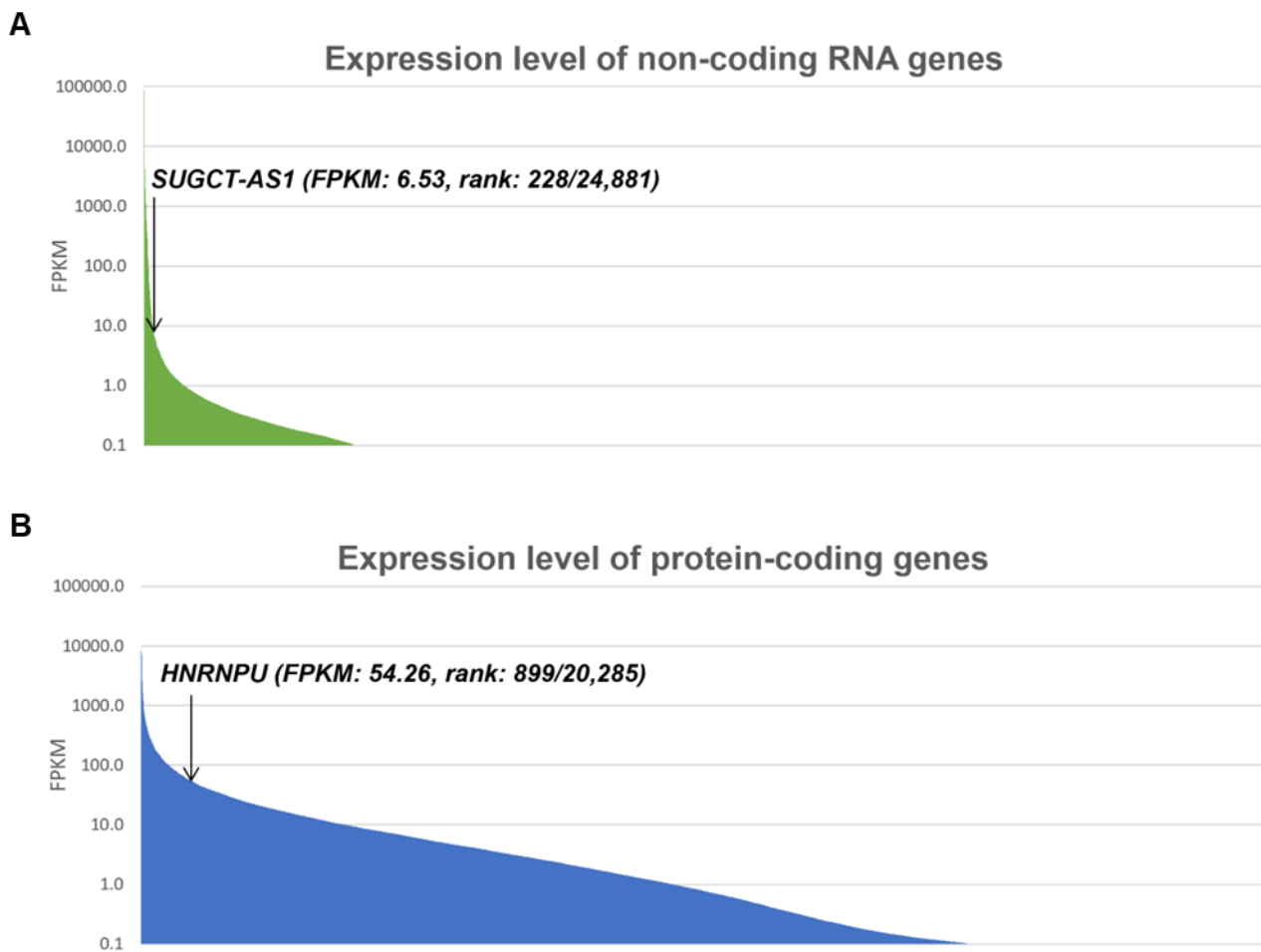


Figure S16. The relative expression level of *SUGCT-AS1* and *HNRNPU* in the THP-1-derived macrophages. The fragments per kilobase of the transcript per million mapped reads (FPKM) values of non-coding (A) and protein-coding genes (B) in THP-1-derived macrophages were depicted. According to the analysis, the expression of *SUGCT-AS1* ranked 228th out of 24,881 non-coding genes, and that of *HNRNPU* ranked 899th out of 20,285 protein-coding genes.

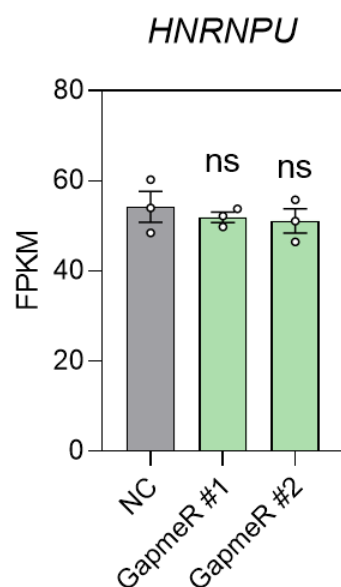
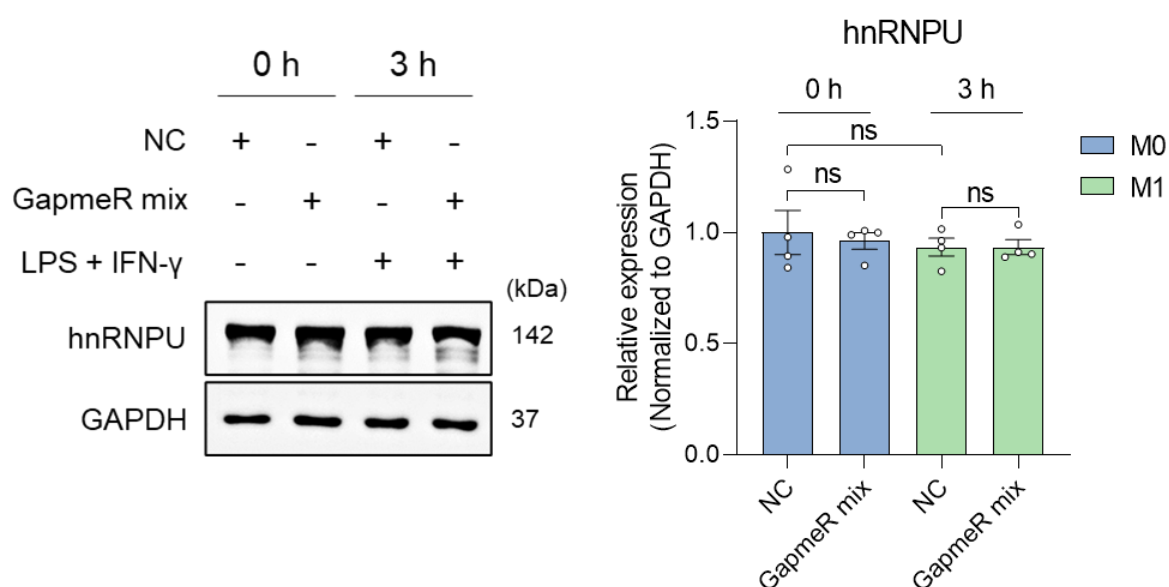
A. Expression level of *HNRNPU* (RNA-seq after *SUGCT-AS1* knockdown)**B. Western blot (hnRNPU in *SUGCT-AS1* knockdown)**

Figure S17. The expression of hnRNPU in *SUGCT-AS1*-depleted THP-1-derived macrophages. (A) FPKM values of *HNRNPU* in RNA sequencing data performed in *SUGCT-AS1*-depleted THP-1-derived M1 macrophages ($n = 3$). NC indicates negative control GapmeR. (B) Western blot analysis of the expression of hnRNPU in THP-1-derived macrophages (M0 and M1) transfected with GapmeRs (NC and GapmeR mix) ($n = 4$). M1 stimulation was performed for 3 hours. GapmeR mix is a mixture of GapmeR #1 and #2. The protein expression was normalized to GAPDH. Data are presented as mean \pm SEM. An unpaired two-tailed *t*-test with Welch's correction was used for statistical analysis; ns: not significant.

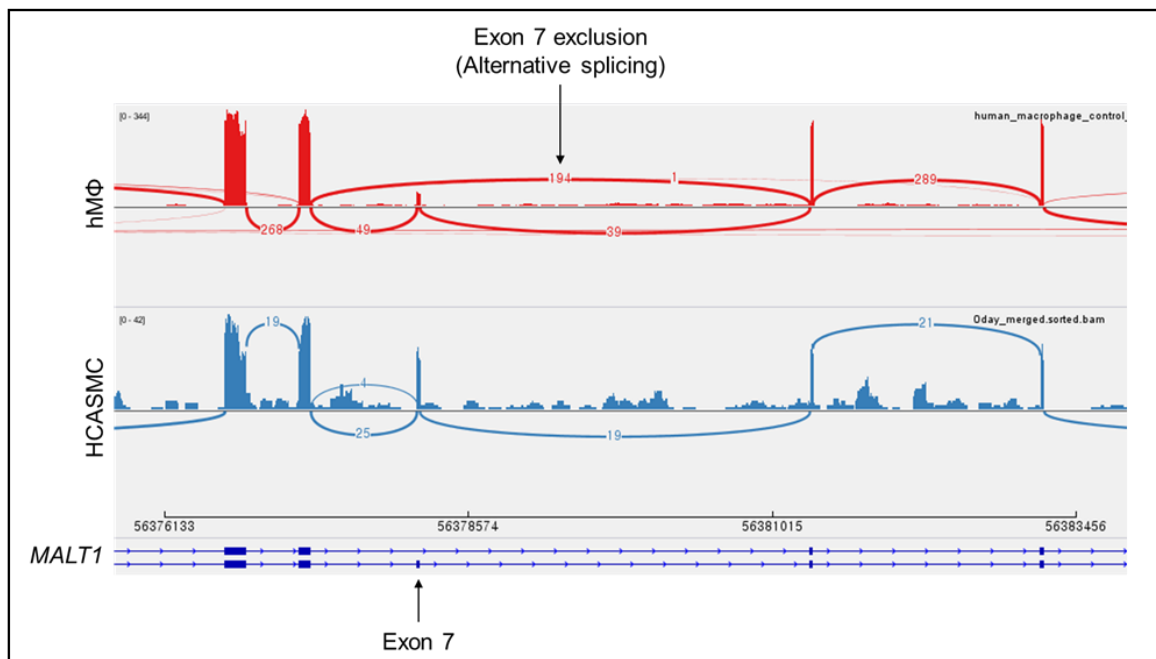
Alternative splicing of *MALT1* pre-mRNA (IGV Sashimi plot)

Figure S18. Alternative splicing of *MALT1* precursor mRNA. Alternative splicing of human *MALT1* precursor mRNA (pre-mRNA) was visualized using the Sashimi plot tool of the IGV genome browser (hg19) [43]. Our previous RNA-seq data for human coronary artery smooth muscle cells (HCASMCs) and public data for human CD14⁺ monocyte-derived macrophages (hMΦ, GSE101868) were utilized in this analysis [41, 42]. Arrows indicate the exclusion of *MALT1*'s exon 7 in human macrophages.

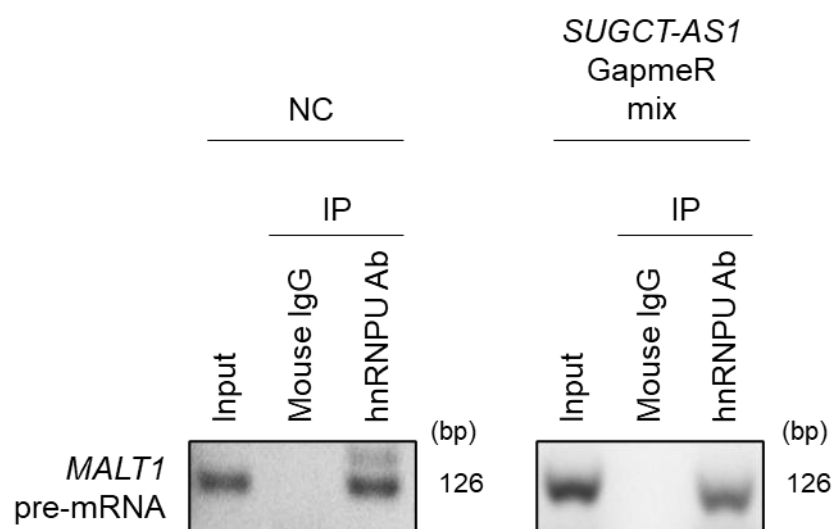


Figure S19. Depletion of *SUGCT-AS1* did not affect the binding of hnRNPU to *MALT1* precursor mRNA. RNA-binding protein immunoprecipitation (RIP) experiment of *MALT1* pre-mRNA using primary antibody against hnRNPU in THP-1-derived M0 macrophages transfected with GapmeRs (NC and GapmeR mix) ($n = 3$). NC indicates negative control GapmeR. GapmeR mix is a mixture of GapmeR #1 and #2 against *SUGCT-AS1*.

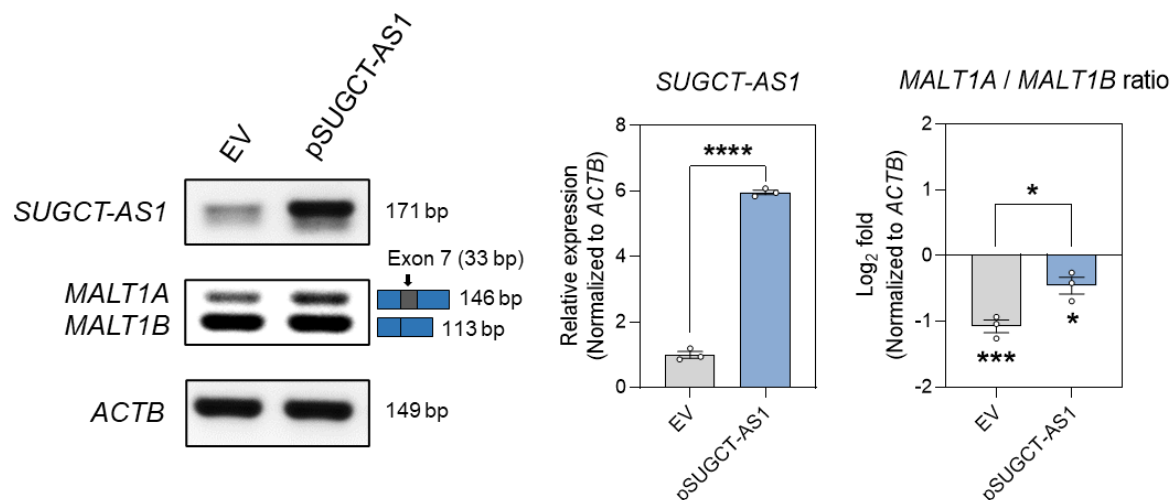
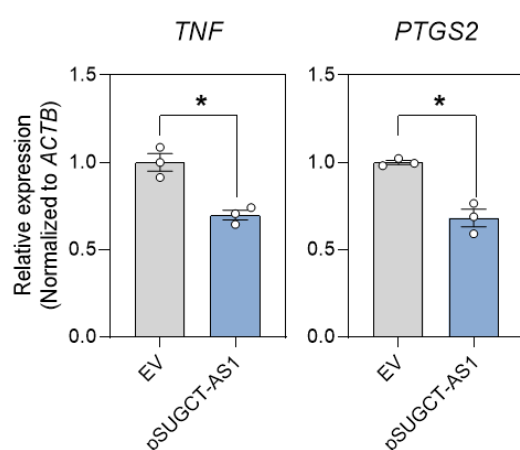
A. Semi-qPCR (*MALT1* isoforms in *SUGCT-AS1* overexpression)**B. qRT-PCR (Inflammatory genes in *SUGCT-AS1* overexpression)**

Figure S20. Effect of *SUGCT-AS1* overexpression in THP-1-derived macrophages. (A) Semi-qPCR measurement of *SUGCT-AS1* expression and the ratio of *MALT1* isoforms (*MALT1A* and *MALT1B*) in THP-1-derived M0 macrophages transfected with plasmid constructs (EV and pSUGCT-AS1) (n = 3). EV indicates an empty vector (pcDNA3). pSUGCT-AS1 is pcDNA3-*SUGCT-AS1*. *MALT1A*: Exon 7 included isoform; *MALT1B*: Exon 7 excluded isoform. (B) qRT-PCR measurement of proinflammatory genes in THP-1-derived M0 macrophages transfected with plasmid constructs (EV and pSUGCT-AS1) (n = 3). The RNA expression was normalized to *ACTB*. Data are presented as mean \pm SEM. An unpaired two-tailed *t*-test with Welch's correction was used for statistical analysis. **p* < 0.05, ****p* < 0.005, *****p* < 0.001.

Expression of *SUGCT-AS1* in atherosclerosis progression
(RNA-seq; GSE120521)

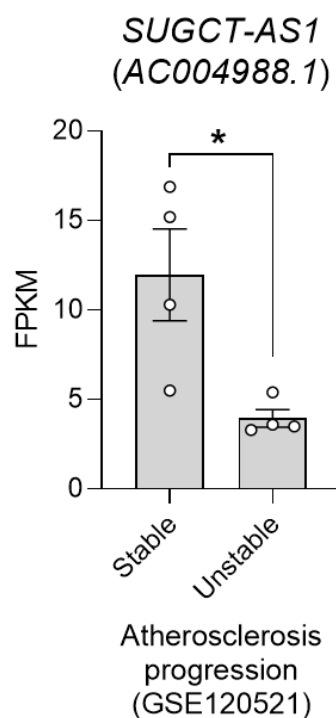


Figure S21. The expression of *SUGCT-AS1* in human atherosclerosis progression. The fragments per kilobase of the transcript per million mapped reads (FPKM) values of *SUGCT-AS1* (AC004988.1) in public RNA sequencing data for human atherosclerosis progression (GSE120521, $n = 4$) [19]. Stable plaques of atherosclerosis were used as a control and compared to unstable plaques. Data are presented as mean \pm SEM. An unpaired two-tailed t -test with Welch's correction was used for statistical analysis. $*p < 0.05$.