

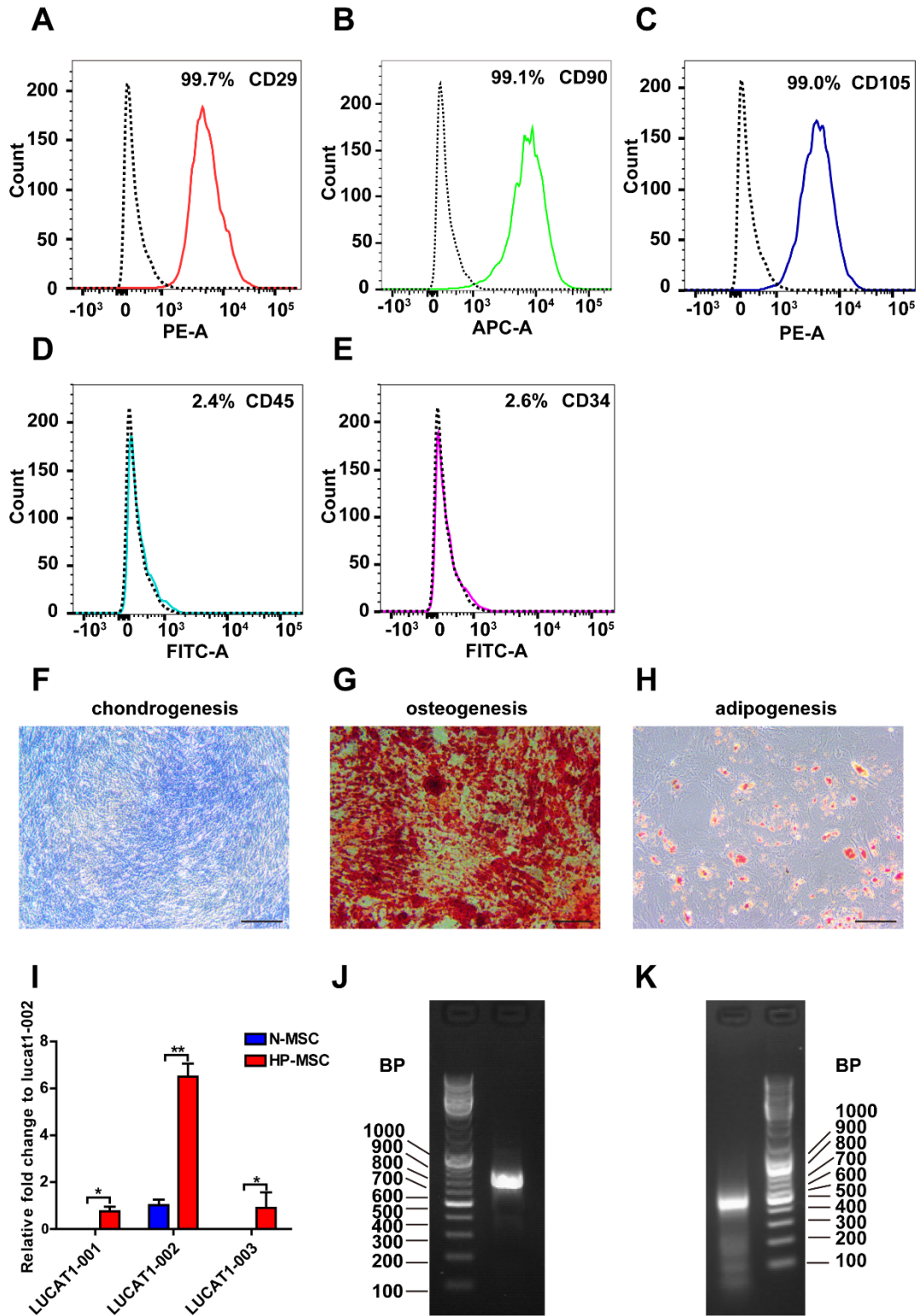
Supplemental information

**Long noncoding RNA LUCAT1 enhances the
survival and therapeutic effects of mesenchymal
stromal cells post-myocardial infarction**

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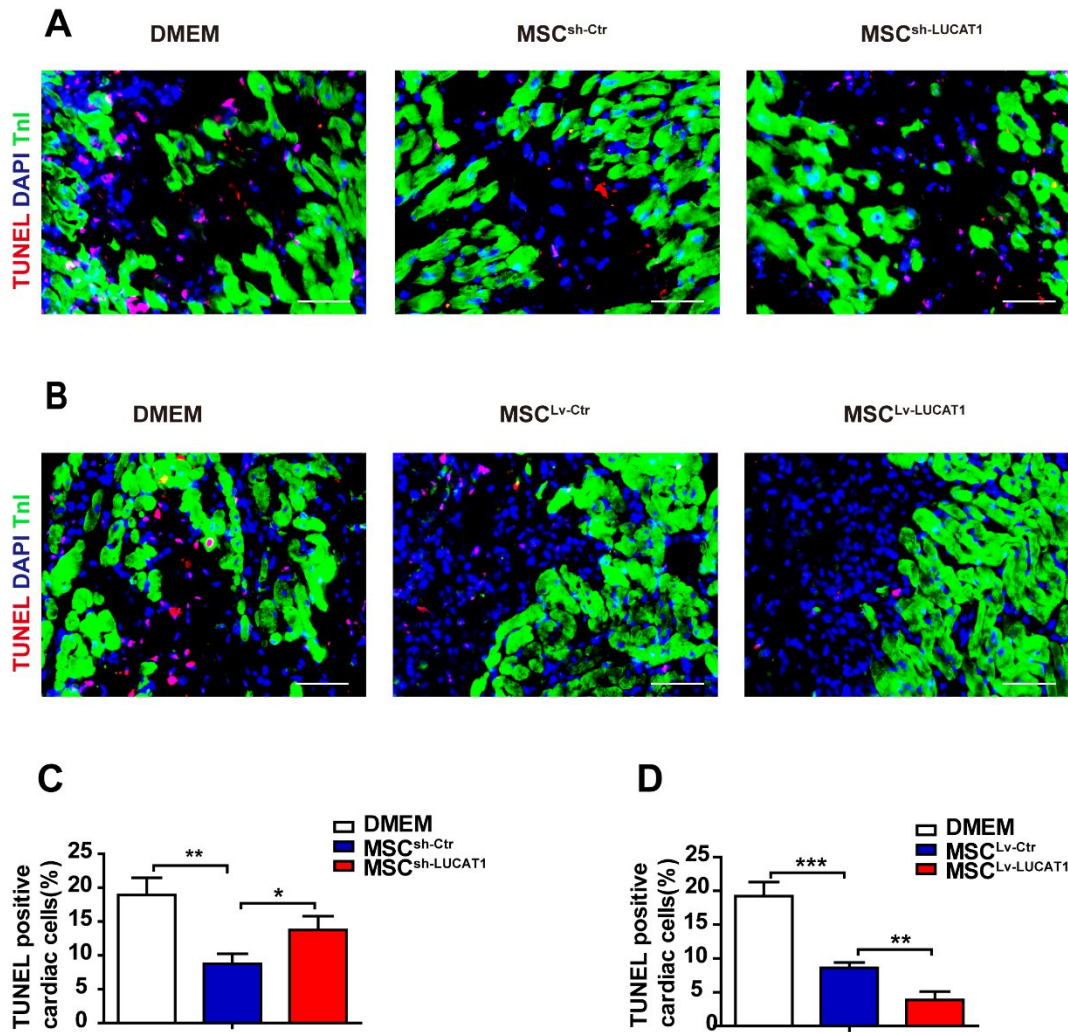
SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURES AND LEGENDS



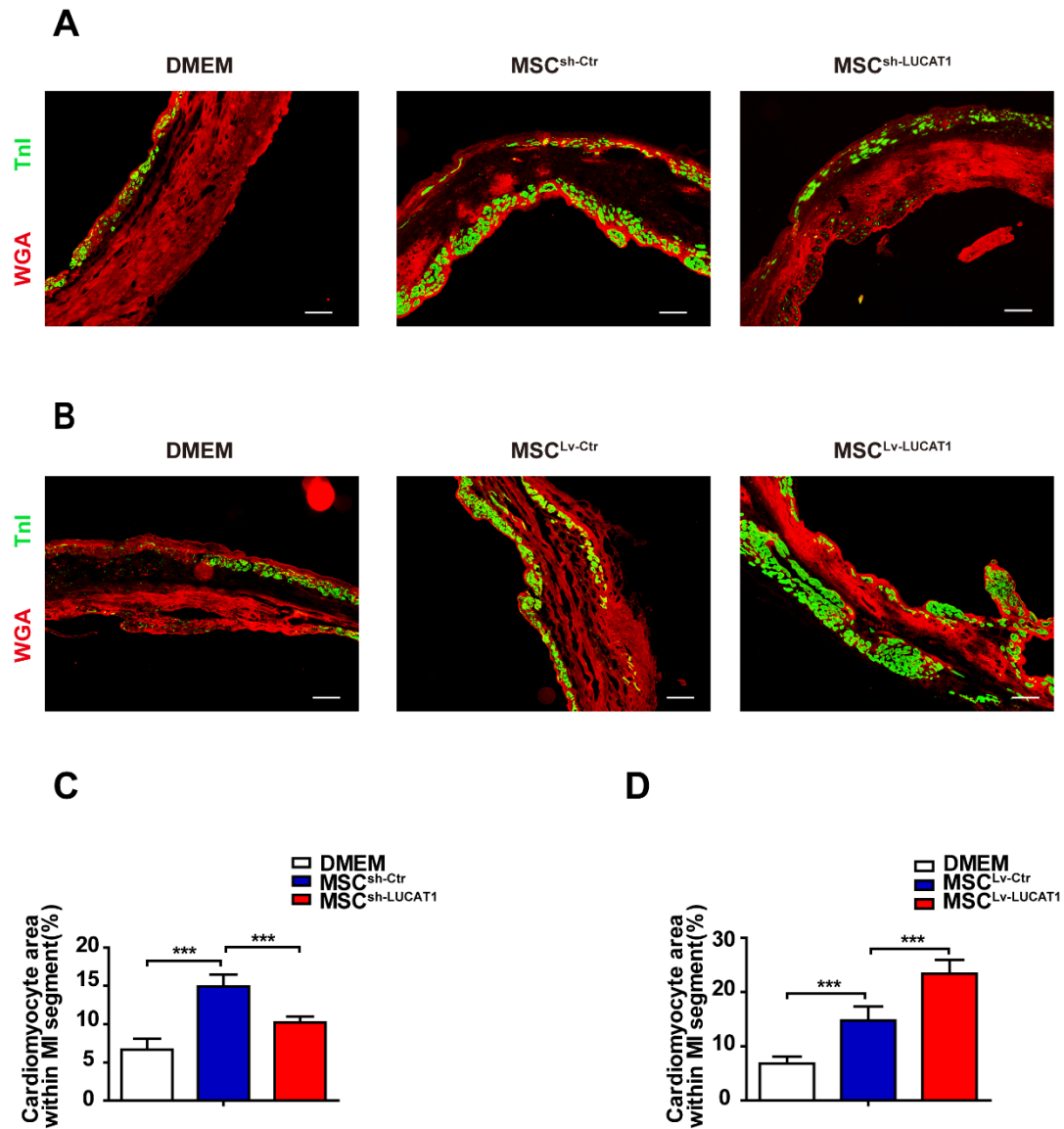
Supplementary Figure S1. MSCs identification and characterization of LUCAT1 in MSCs

Characterization of MSCs: positive for mesenchymal cell surface markers PE-CD29 (A), APC-CD90 (B), PE-CD105 (C), and negative for the hematopoietic surface marker FITC-CD45 (D) and endothelial cell surface marker FITC-CD34 (E). The chondrogenesis, osteogenesis and adipogenesis differentiation of MSCs were shown with toluidine eblue staining (F, dark blue), alizarin red staining (G, dark red) and oil red O-staining (H, red), respectively (bar = 100 μ m). I, LUCAT1-002 was the longest transcripts and expressed higher both in N-MSCs and HP-MSCs compared with other transcripts, validated by qRT-PCR with three pairs of non-overlapping primers to each transcript (n = 3). 5' (J) and 3' (K) rapid amplification of cDNA ends (RACE) assays were showed by gel electrophoresis image of PCR products. Data are presented as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



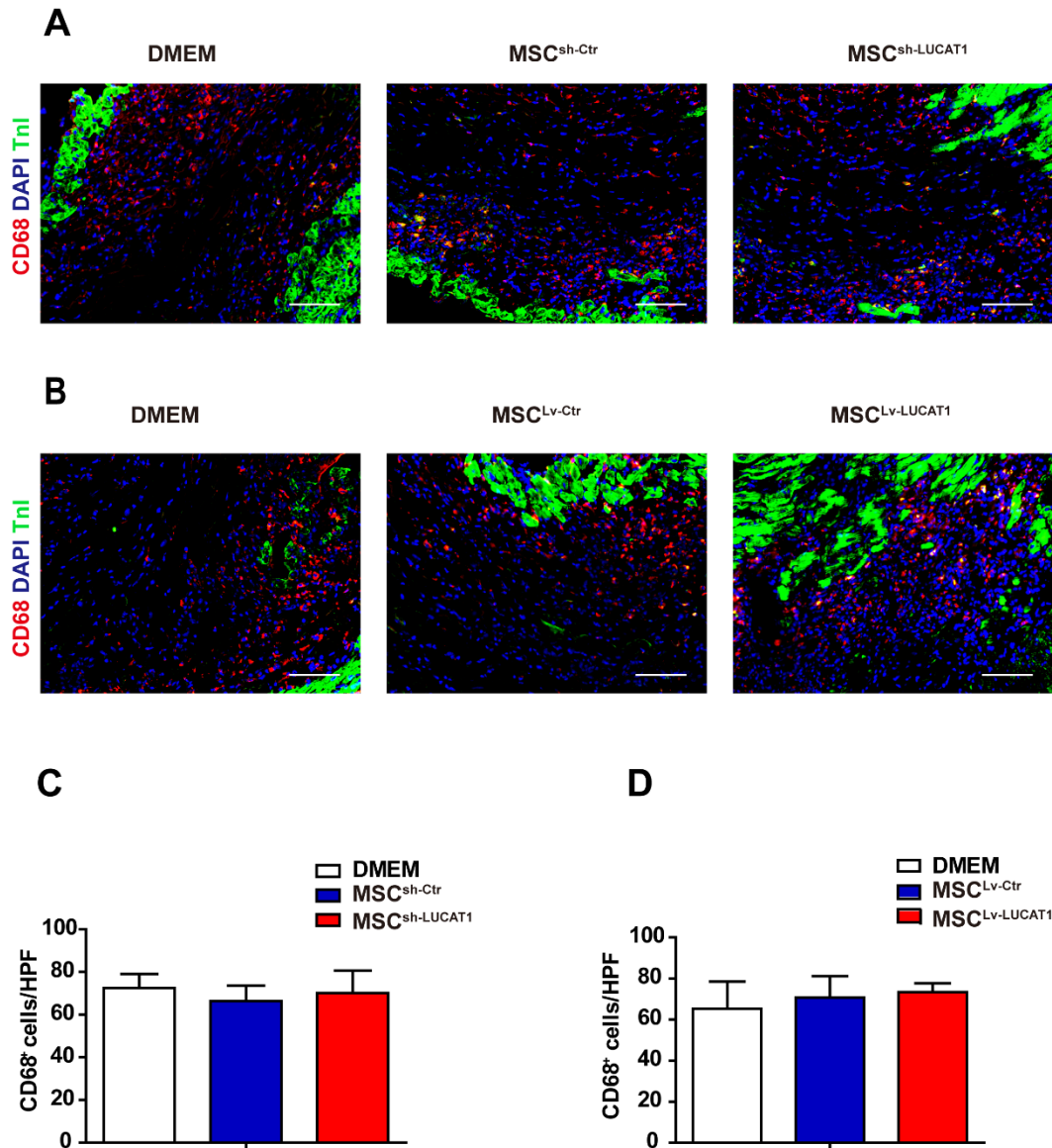
Supplementary Figure S2. The TUNEL staining of the infarct border area at 3 days post MI

A and B, TUNEL staining of apoptotic cells in border area. bar=50 μ m. C and D, Quantitative analysis of TUNEL positive cardiac cells in border zone. n=5 for each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



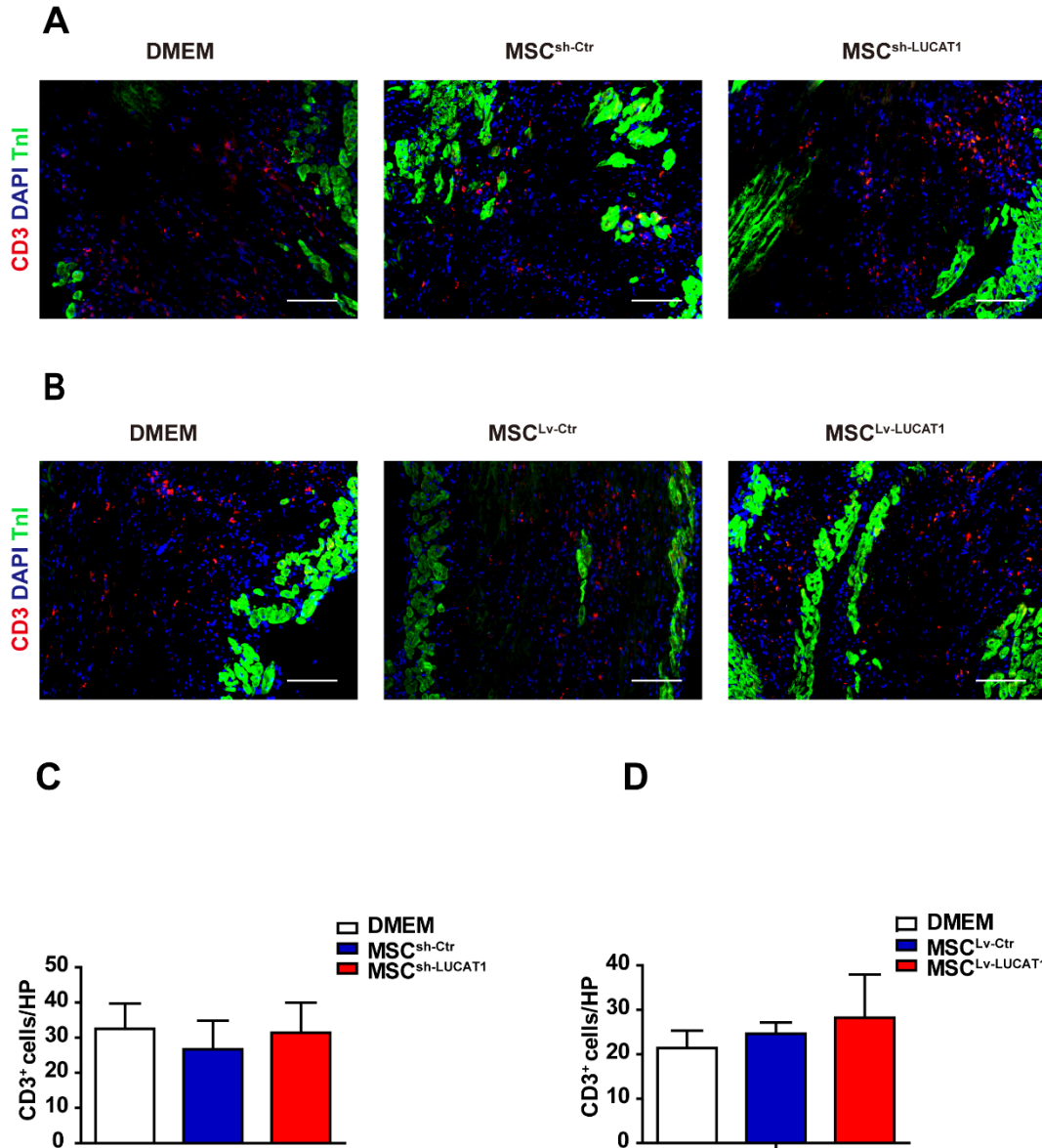
Supplementary Figure S3. The wheat-germ agglutinin (WGA) staining of MI segments at 28 days post MI treated with LUCAT1 knockdown or overexpression MSCs

A and B, The infarct area was stained with WGA (red), troponinI (TnI) (green). bar=100um. C and D, Quantitative analysis of TnI positive area within MI segment. n=5 for each group. *** $P < 0.001$.



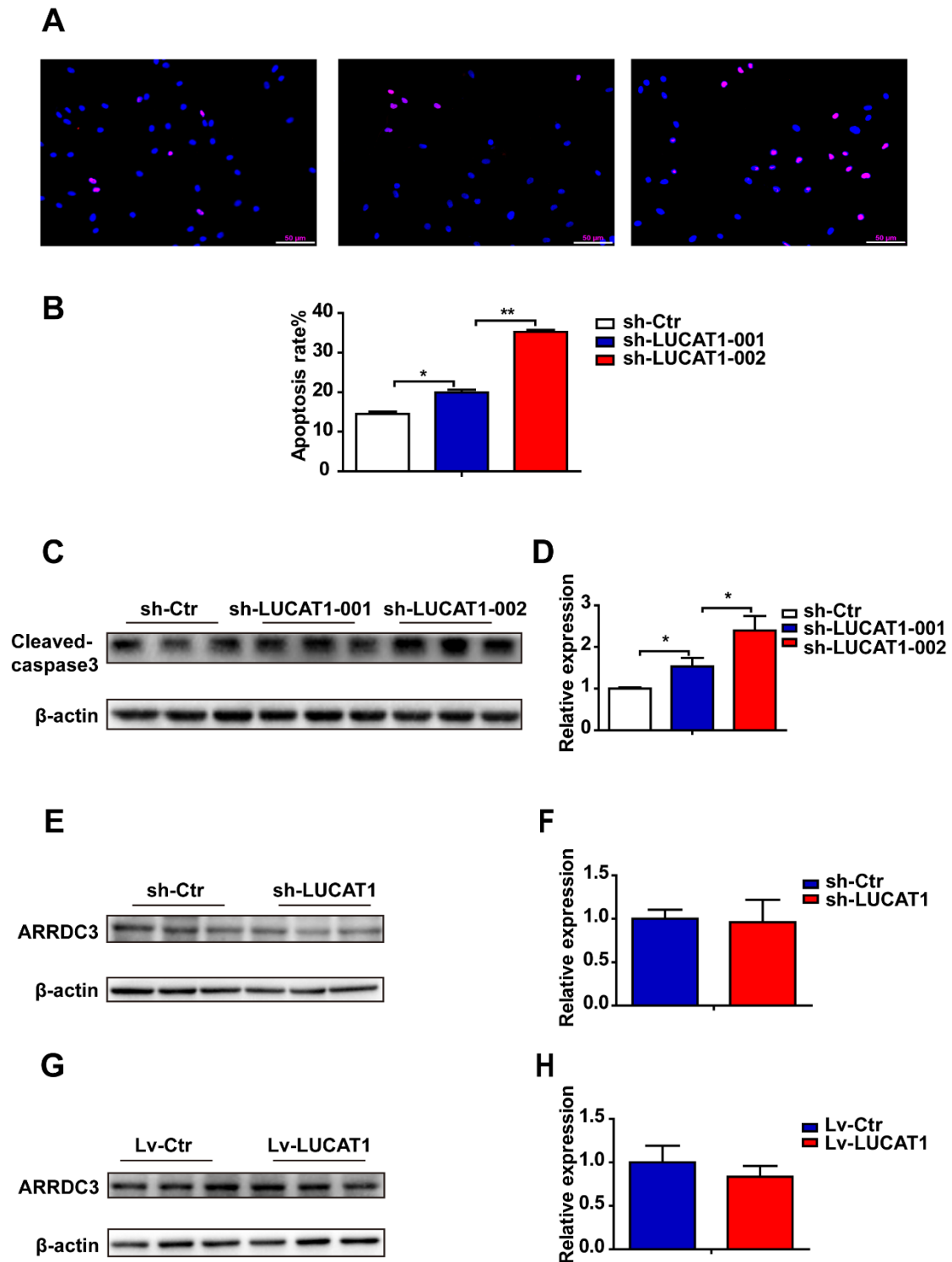
Supplementary Figure S4. Evaluation of CD68⁺ inflammation cell in the border area

A-B, Immunofluorescent staining of CD68 positive macrophages and troponin positive cardiomyocytes in the border area at 3 days post MI. bar = 100 μ m. C-D, Quantitative analysis of CD68 positive cells/HPF. n=5 for each group.



Supplementary Figure S5. Evaluation of CD3⁺ inflammation cell in the border area

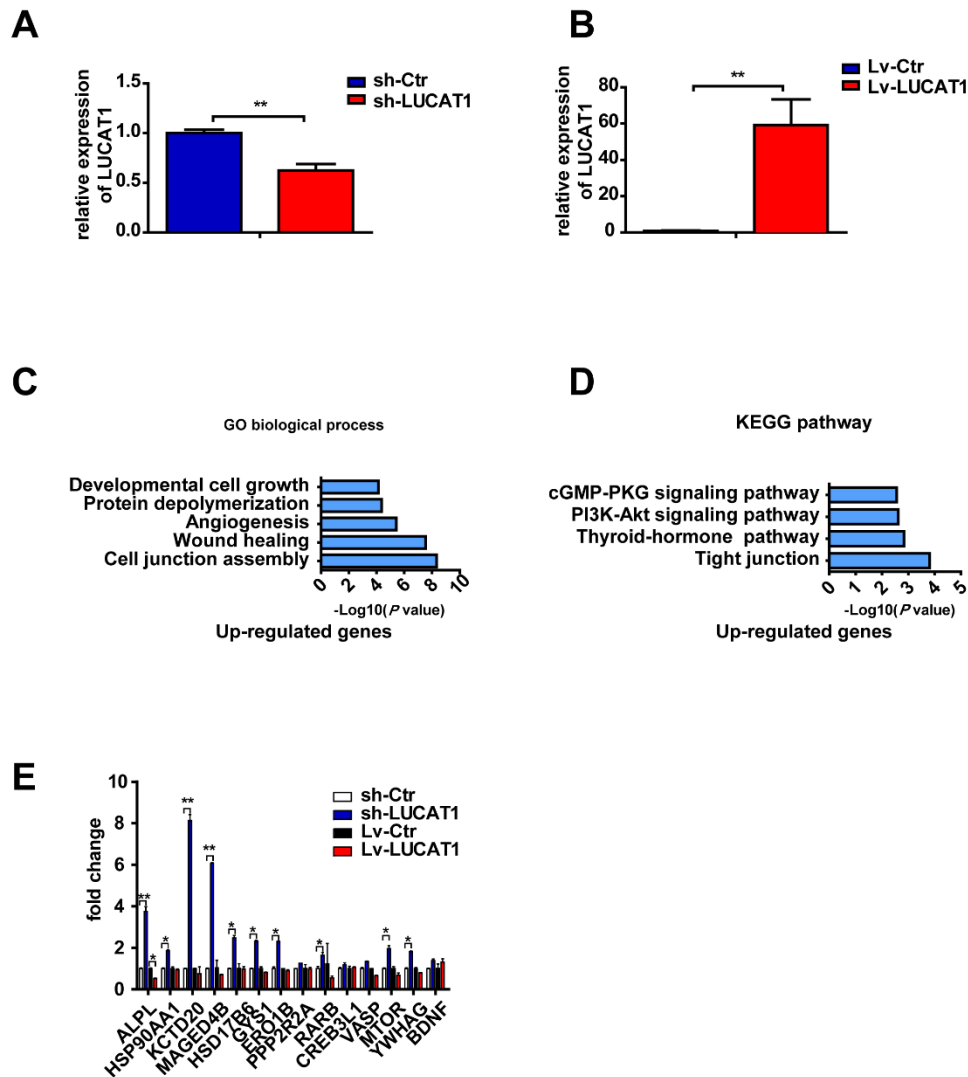
A-B, Immunofluorescent staining of CD3 positive T-lymphocytes and troponin positive cardiomyocytes in the border area at 3 days post MI. bar = 100 μ m. C-D, Quantitative analysis of CD3 positive cells/HPF. n=5 for each group.



Supplementary Figure S6. Effects of LUCAT1 on apoptosis and ARRDC3

A, TUNEL staining with nuclei as identified via DAPI staining (bar = 50 μ m) of control, sh-LUCAT1-001 and sh-LUCAT1-002 MSCs under 500 μ M H_2O_2 for 1 h. B, Quantification of TUNEL-positive nuclei (n = 3). C, Cleaved caspase 3 level was

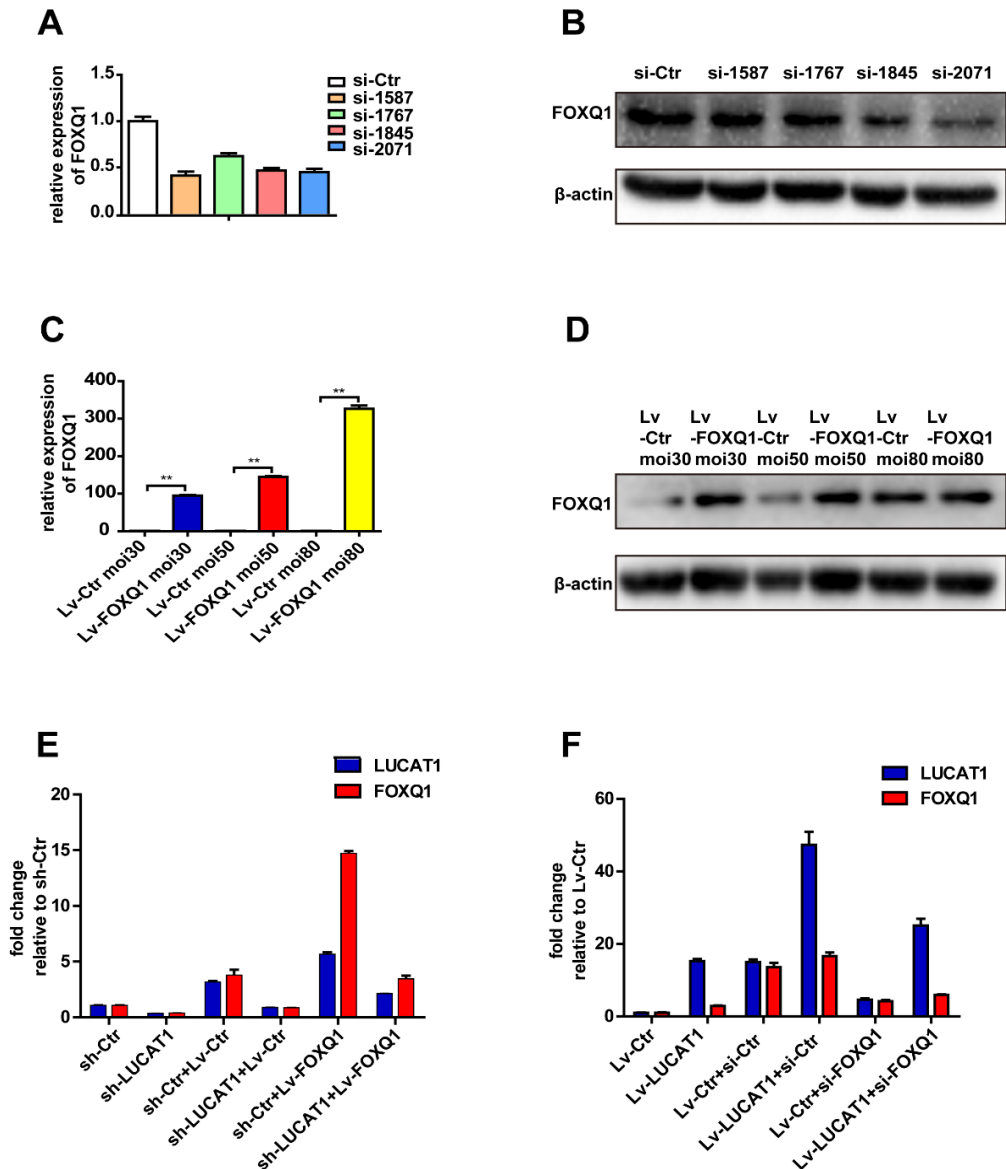
measured by western blot of control, sh-LUCAT1-001 and sh-LUCAT1-002 MSCs under conditions above. D, The quantitative analysis of Cleaved caspase 3 expression (n = 3). E, ARRDC3 level was measured by western blot of control and sh-LUCAT1 MSCs. F, The quantitative analysis of ARRDC3 expression (n = 3). G, ARRDC3 level was measured by western blot of control and Lv-LUCAT1 MSCs. H, The quantitative analysis of ARRDC3 expression (n = 3). The data are presented as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$.



Supplementary Figure S7. RNA sequencing analysis of the PCG expression in the

MSCs with LUCAT1 knockdown

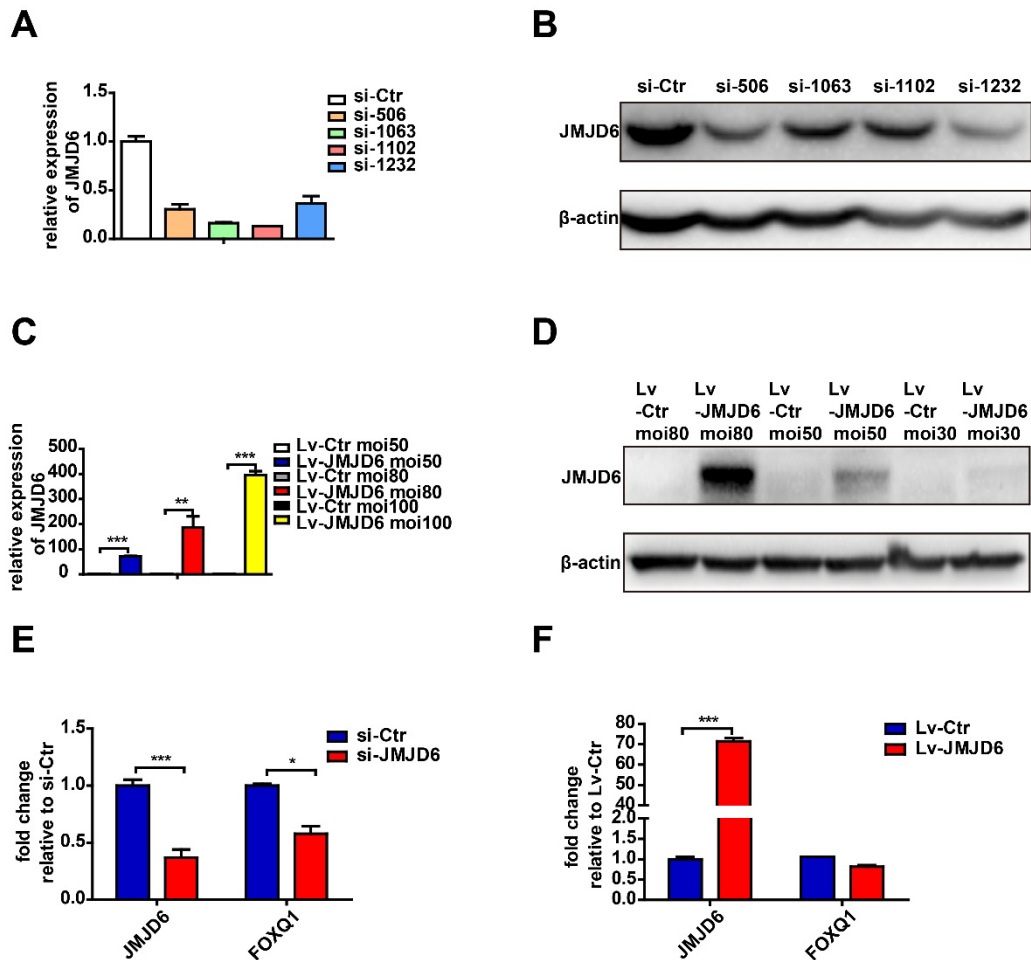
A and B, qRT-PCR showed the knockdown or overexpression efficiency of different lentivirus on LUCAT1 (n = 3). C, The GO analysis of the PCGs with downregulated expression. D, The KEGG analysis of the PCGs with downregulated expression. E, The expression of up-regulated apoptosis-related genes was analyzed through qRT-PCR when LUCAT1 was knockdown or overexpressed. Data are presented as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure S8. mRNA levels of LUCAT1 and FOXQ1 in rescue experiment

A and B, qRT-PCR and western blotting showed the knockdown efficiency of different FOXQ1 siRNA in MSCs (n = 3). C and D, qRT-PCR and western blotting revealed the efficiency of FOXQ1 overexpression lentivirus at different multiplicities of infection (MOI) (n = 3). E and F, qRT-PCR detected the expression levels of LUCAT1 and FOXQ1 under different treatments (n = 3). Data are presented as mean

± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure S9. mRNA level of FOXQ1 following JMJD6 knockdown or overexpressed treatment

A and B, qRT-PCR and western blotting showed the knockdown efficiency of JMJD6 siRNA in MSCs (n = 3). C and D, qRT-PCR and western blotting revealed the efficiency of JMJD6 overexpression lentivirus at different MOI levels (n = 3). E and F, qRT-PCR results demonstrated that the mRNA level of FOXQ1 with JMJD6 knockdown or overexpressed treatment (n = 3). Data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table S1: SiRNA sequence

siRNA	sense sequence (5'-3')	anti-sense sequence (5'-3')
FOXQ1-homo-1587	CUCCAUCAACGUGCCUUATT	UAAGGCACGUUUGAUGGAGTT
FOXQ1-homo-1767	CAGGCUUCGUCUUAUUUCUTT	AGAAAUAAGACGAAGCCUGTT
FOXQ1-homo-1845	GGGAACCUUCCACACUAUTT	AUAGUGUGGAAAGGUUCCCTT
FOXQ1-homo-2071	CAACGGGCUACAGCUUUAUTT	AUAAAGCUGUAGCCCGUUGTT
JMJD6-homo-1232	GGGAGACCAAAGUUAUCAATT	UUGAUAACUUUGGUCUCCCTT
JMJD6-homo-1063	CUGGCCACCUGAAUUCAAAATT	UUUGAAUUCAGGUGGCCAGTT
JMJD6-homo-1137	GGCAUGUUGUCCUCAUCUTT	AGAUUGAGGACAACAUGCCTT
JMJD6-homo-506	CGGUAUGAAAGACCUUACATT	UGUAAGGUCUUUCAUACCGTT

Table S2: The list of primers**lncRNA qPCR primers**

	Forward Primers	Reverse Primers
LUCAT1	CTGGCTCCTTTCTCACAAG	AGCTTGCAGTGAACCGAGAT
RP11-841O20.2	GATGGATTGAGGAGTCTGGCT	ACATTTTGCTCACTCCTGAACC
NEAT1	CTCTCCATTTCCCATCTGA	GCTGCTGCCAAACATCTACA
RP11-274H2.3	TGCCAACTCATCGGAACAGA	TTCTCAACAGCAGTGGTCCC
MIR210HG	CTATGCATTCCAGGCTCCAT	TCGGCTTGGTTATTTCTTGC

mRNA qPCR primers

	Forward Primers	Reverse Primers
ACTB	CGAAAGTTGCCTTTTATGGCTC	GCGGCGATATCATCATCCA
GAPDH	TCTCTGCTCCTCCTGTTCGAC	ACCAGAGTTAAAAGCAGCCCT
JMJD6	AAGACCTTACAAGCCCGTGG	GGGTGTTCCACCATAGCTGCT
FOXQ1	TCGGAAGACCAGGGTAGAGC	AGGGACGAACACCTCCAAC
DRP2	ACGGAATCAGAAGGTTAGAGCTG	AGGGAGCTGAAGCTTTCCAC
MAPK10	CCTTTTGTGACAGGGATTCGATAAAC	ACAAACTATGCCCTGAGCCC
RARB	A TGGAGTTGGGTGGACTTTTCT	TCAATTGATTGAGCAGTGTGCC
OLR1	GCAAATTGTTTCAGGACTTCATCCA	GGTATGTCTGGGAGACAGCG
CDH18	CACCACAGCTCCATCAAGGT	TGGAGTGCAGCTTTCCAACA
ART3	CCTGTCTCAGCTCTCACTGTC	ACACTTCAGCCTTCACCTGG
NR4A3	TCTAAAGACGGAACCGCCAC	AAAAGGTGATGAGGGCCTGG
EPHA1	TTCGAGACCTTGTGTCTGGC	CCTGACACTGGGAACACCTC
GADD45G	CAGCCAAAGTCTTGAACGTGG	TCCTCGTTGGGGTTCGAAAT
POLQ	TGACCAAACAGGATTGTCACGA	AGCTGGCGCCTATTTTCACT
ABCC2	TGCATCTAGGCAAGGTTAACGA	GTCCAGGAATGAGGAATTCCAAAAA
PCLAF	AGAAAAGTGGTGGCTGCTCG	TCCTTTTTGCCACTTGGGAGT
CCL7	CCCTCACCCCTCCAACATGAAA	TAGCTCTCCAGCCTCTGCTTA
E2F2	CAACATCCAGTGGGTAGGCA	GGCAATCACTGTCTGCTCCT
COL10A1	CAGCACGCAGAATCCATCTGA	AACTGTGTCTTGGTGTGGGT
GRM1	CAGTCCACACGGAAGGGAAT	AAGCCTCTCTCGGAGTTTGC
NR0B1	GAAGATCCTCACCACCAGGC	GGCACGTCCGGGTTAAAGA
SKA3	CGGCGCCGAGATTCAAATA	TCAAAGTCGCTTTCTCTCCG
HMMR	TTTCCAATTGGCTAACGCCG	GGAGATGGTGCACAACCAGA
CENPM	CAGGGCGGTTTGAAAGATCG	GGACTTTGCCAAGTGGACCT
MAGED4B	GGATGAGGGTAGCGACGAAG	TTTCCGGATCCAGCTGCAAT
SERF1B	GCCCGTGGAATCAACGAGA	AGACTTCTTCTCATTAGCTGCCT
ALPL	AACATCAGGGACATTGACGTG	GTATCTCGGTTTGAAGCTTTCC
KCTD20	TGACAGTGACAGGTTATTGCG	AGGCATAGTCAAGTGAGAGGTC
HSD17B6	GGACTGGTGAACAATGCAGG	ACACAGTAGCCTCCTACAAAGA
ERO1B	CCGAGGCGAAGATGATGGAG	ACAGGGTCAAAGCGGTGTTT
GJD3	GCTGTTCGTCGTCTACTCCAT	ACCGCGAAATAGAAGAGCACG
KLHDC3	CCGCATTGAGTCTTTGACAC	GTCATGGAAGTGCCGGTTC
BMF	AACCCAGCGACTCTTTTATG	GGCAATCTGTACCTCTGCTTG
LIPA	TTACAACCAGAGTTATCCTCCCA	CCAAATGAAGTCAAGATGCTCCC

Table S3: Information of antibodies

	company		Western blot	ChIP	RIP	IHC
Histone H3 [Asym-dimethyl Arg2] Antibody	Novus biologicals	NB21-1002	1:1000	1:50		
Histone H4R3me2s (symmetric) antibody (pAb)	Active motif	61187	1:1000	1:50		
Anti-JMJD6	Abcam	ab65770	1:1000	1:50	1:50	
Anti-FOXQ1	Abcam	ab51340	1:1000			
Bcl2	Cell Signaling Technology	15071	1:1000			
Bax	Cell Signaling Technology	2774	1:1000			
Cleaved Caspase3 antibody	Cell Signaling Technology	9661	1:1000			
β-Actin	Cell Signaling Technology	4970	1:3000			
Anti-GFP	Abcam	ab290				1:200
Anti-TropinI	Abcam	ab8295				1:200
Anti-CD3	Abcam	ab16669				1:200
Anti-CD68	Abcam	ab125212				1:200
Wheat germ agglutinin (WGA)	Thermofisher Scientific	W32464				100ug/ml
Donkey Anti-Mouse IgG H&L (DyLight® 488)	Abcam	ab96875				1:3000
Donkey Anti-Rabbit IgG H&L (DyLight® 550)	Abcam	ab96892				1:3000
Donkey Anti-Goat IgG H&L (DyLight® 488)	Abcam	ab96931				1:3000
Donkey polyclonal Secondary Antibody to Rabbit IgG - F	Abcam	ab150073				1:3000

ChIP:Chromatin immunoprecipitation; RIP:RNA-binding protein immunoprecipitation; IHC:immunohistochemistry

Table S4 : The list of proteins predicted to be only pulled down by sense sequence (see the attached Excel table S4 for details).

Table S5: The list of RACE and ChIP primers

RACE primers

adaptor primers:

5' adaptor	GCTGTCAACGATACGCTACGTAACGGCATGACAGTGCCCCCCCCCCCCCCCC
3' adaptor	GCTGTCAACGATACGCTACGTAACGGCATGACAGTGTTTTTTTTTTTTTTTTT
5.3' outer	TT GCTGTCAACGATACGCTACGTAAC
5.3' inner	GCTACGTAACGGCATGACAGTG
5' RACE:	
RC351-R4	TGTCTAGGACAGCTCCTAGCTCATATTTAGTGTT
RC351-R3	GAAAAATGGGATGCTAACATAAGGCCAAC
RC351-RT4	TCAGAGCTCAGGCTATACAT
RC351-RT3	CTTCCATACCAATTTGTTCA
3' RACE:	
RC351-F3	CTTTGAATGCAGCCAAACACAAATTTGTAA
RC351-F4	CATGTATTTTATGTGTGGCCCAAGACAATT

ChIP Primers

Promoter area(-)	Forward Primer(5'-3')	Reverse Primer(5'-3')
-147~-488	CCCAAAAAGCAGCCATTCCC	ACGCCGAGTTTCCTCCTTTT
-399~-766	TCCGTCGCTTTTTGTGCAAC	CACTTGTGTCCCTGCGGAAG
-757~-1156	GACACAAGTGCAGGCACAG	GCGGCGTATGTGCTTCTGTA
-1056~-1281	GCGCATACAATTTCAAGCCCA	GCAGTGACCTCTTTCGGGAG
-1261~-1643	TAAACTGCGTCCCCGAACTC	CCGGCCCAATATAACCCTGC
-1619~-2019	CCGCCGCAGGGTTATATTGG	GCTTTTTCTCTGGAGGGGCT

RACE: The 5' and 3' rapid amplification of cDNA ends

Table S6: Full length of LUCAT1 different transcripts

Full length of LUCAT1-002

TTTAAACAGAAGGCTCCAGGAGACATAACAATCAACACTCCACTCAGACAA
TGCCAGACCTCCAGAAACCATGTGTCAAGCTCGGATTGCCTTAGACAGG
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TGTTCTGACTTCTGGCTCCTTTCCTCACAAGAAGCTCACCCAGCTGGA
ACTTATGGGACCTTGGCACCAGAGACCACAAATTCCTCTTTGAAGTTTTCTA
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CAAAATTGGTTCAGCATCTACCATGGGCTACATGCTGAGCTACAGAGTTT
CGCTCTGTCGCCCAGGCTGGAGTGCAGTGGCGCGCGATCTCGGTTCACTG
CAAGCTCCACCTCCCGGGTTCACGCCATTCTCCTGCCTCAGCCTCCTGAGT
AGCTGGGACTACAGGCGCCCGCCACCACACCCAGGAATCCA
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CATGCTGTTGATGAACTGCTAAAGGGGCTG
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Full length of LUCAT1-001

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CAAATTCCTCTTTGAAGTTTTCTAACAGCAACAATGGTATTTCTGACTTGG
CTTTCTTGATTTCTCTCACGTTAACAAAATTGGTTCAGCATCTACCATGG
GCTACATGCTGAGCTACAGAGTTTCGCTCTGTGCGCCAGGCTGGAGTGCA
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