

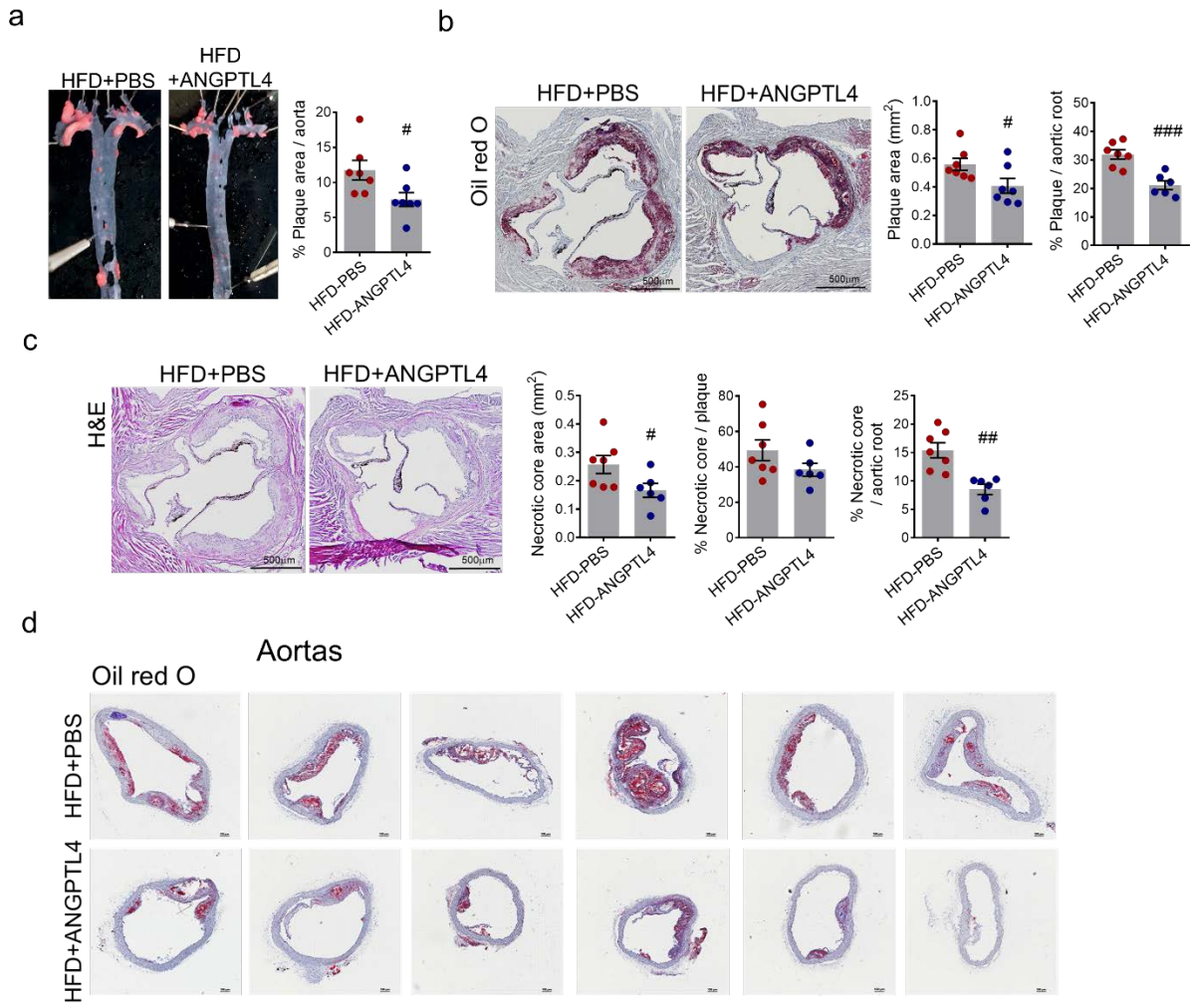
Supplementary Information

ANGPTL4 stabilizes atherosclerotic plaques and modulates phenotypic transition of vascular smooth muscle cells through KLF4 downregulation

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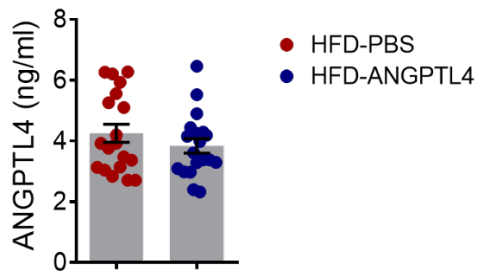
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Supplementary Fig 1. The effect of ANGPTL4 administration was examined in a high-fat diet (HFD)-fed *Ldlr*^{-/-} mouse model.

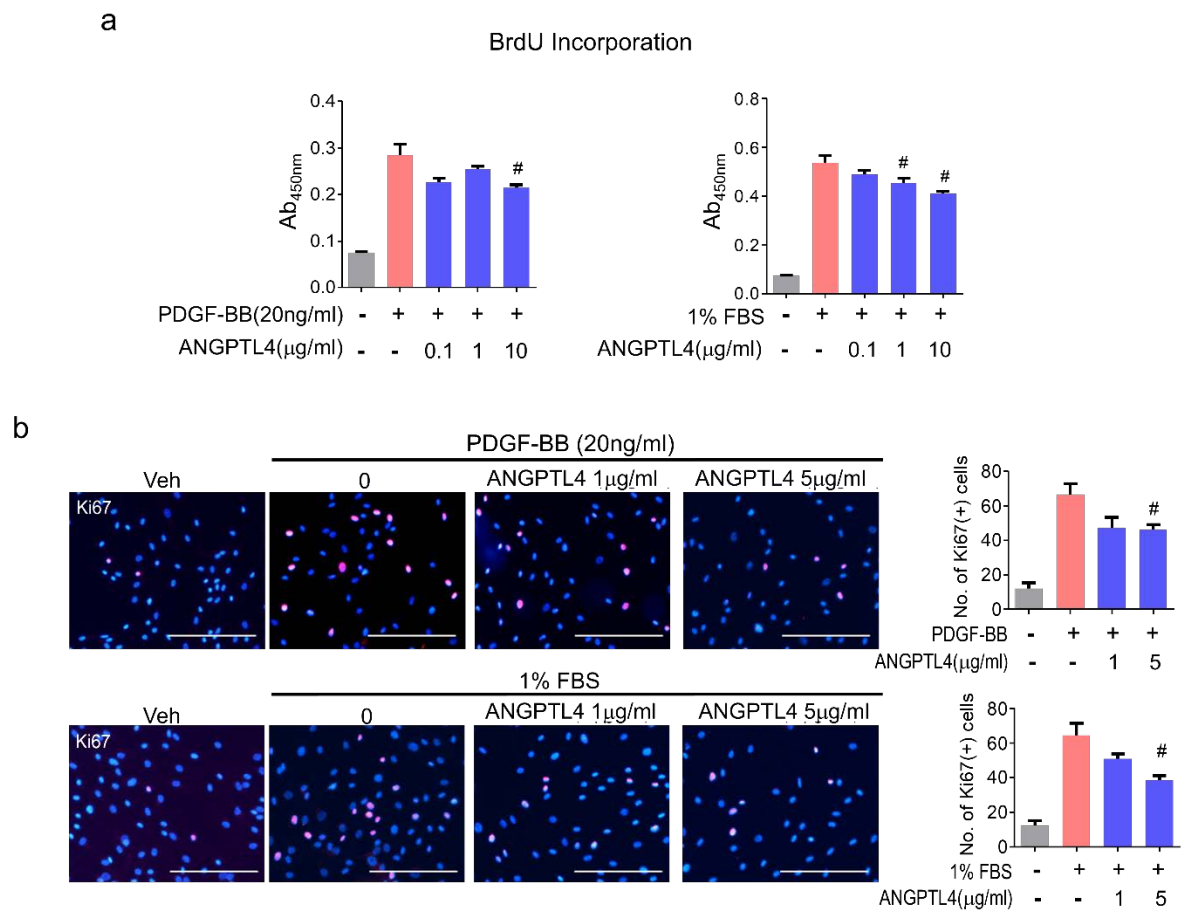
Ldlr^{-/-} mice were fed a HFD with injection of PBS or recombinant ANGPTL4 protein (2 µg per mouse, intraperitoneally twice a week) for 16 weeks, and atherosclerotic plaque area was quantified. **a**, Representative Oil-red O-stained aortas from PBS-injected and ANGPTL4-injected *Ldlr*^{-/-} mice and atherosclerotic plaque area were quantified. **b**, Lesion area measured in Oil-red O-stained cross-sections of the aortic roots from the PBS group and ANGPTL4 group. Plaque area was measured and quantified as the relative size of plaque to the aortic area. Scale bar, 500 µm. **c**, Representative images are shown for H&E-stained aortic root from the PBS and ANGPTL4 groups. Necrotic core area, qualified by anucleated area, was measured and quantified as the relative size of the necrotic core to plaque or aortic root. Scale bar, 500 µm. **d**, Representative images are shown for Oil-red O-stained aortas from the PBS and ANGPTL4 groups. Scale bar, 100 µm. Data are represented as mean ± SEM. #*p* < 0.05, ##*p* < 0.01, ###*p* < 0.001 (by Student's *t*-test).

Circulating level



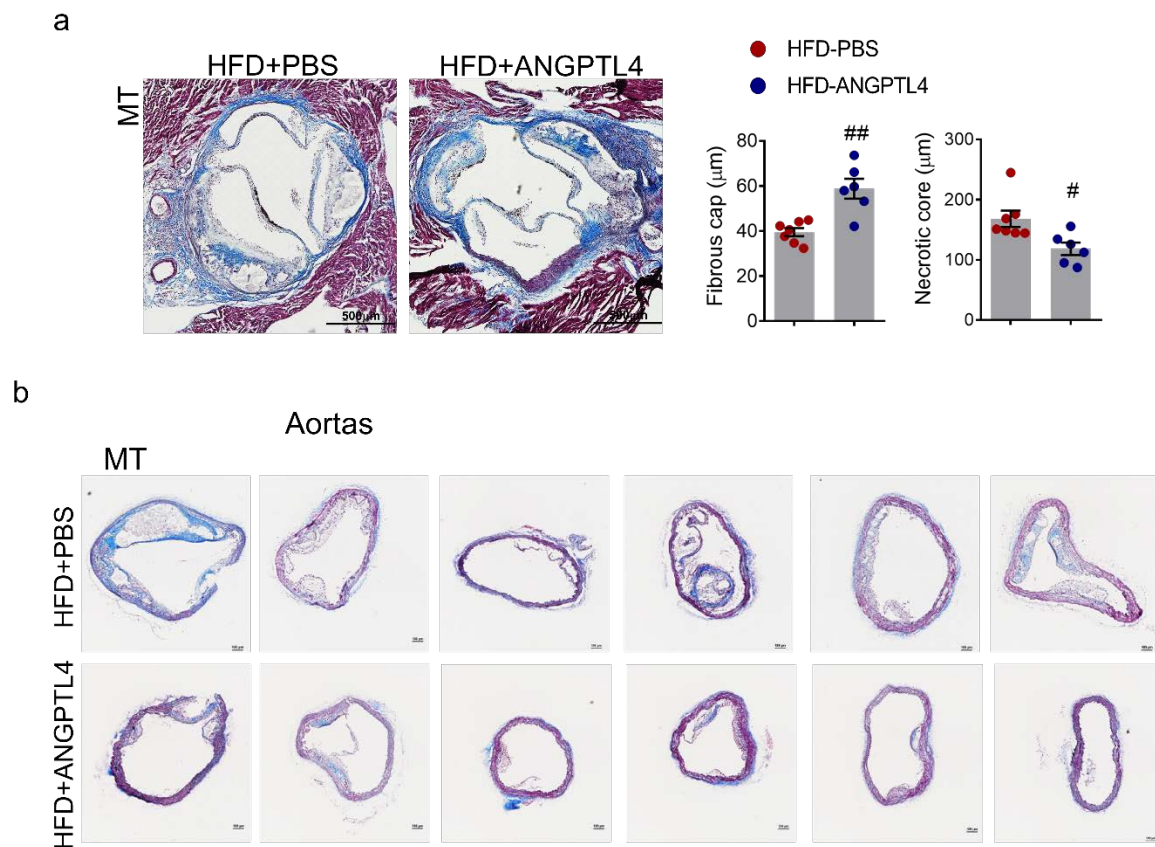
Supplementary Fig. 2. ANGPTL4 expression from plasma of *ApoE*^{-/-} mice.

Circulating ANGPTL4 was quantified in the PBS and ANGPTL4 groups.



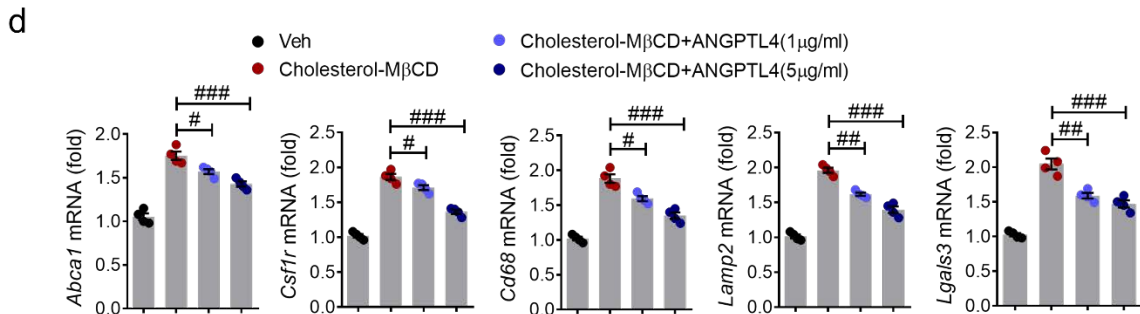
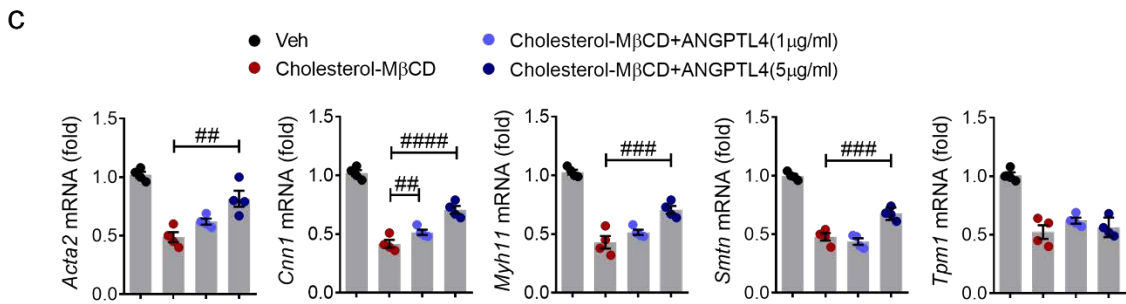
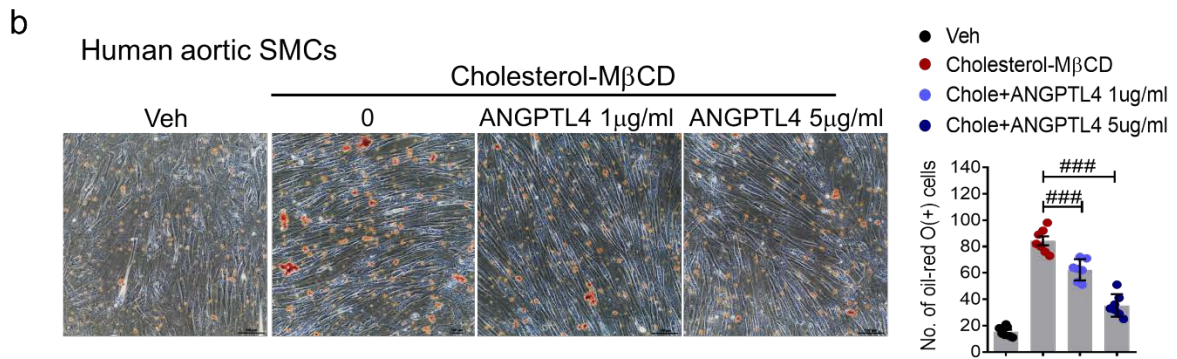
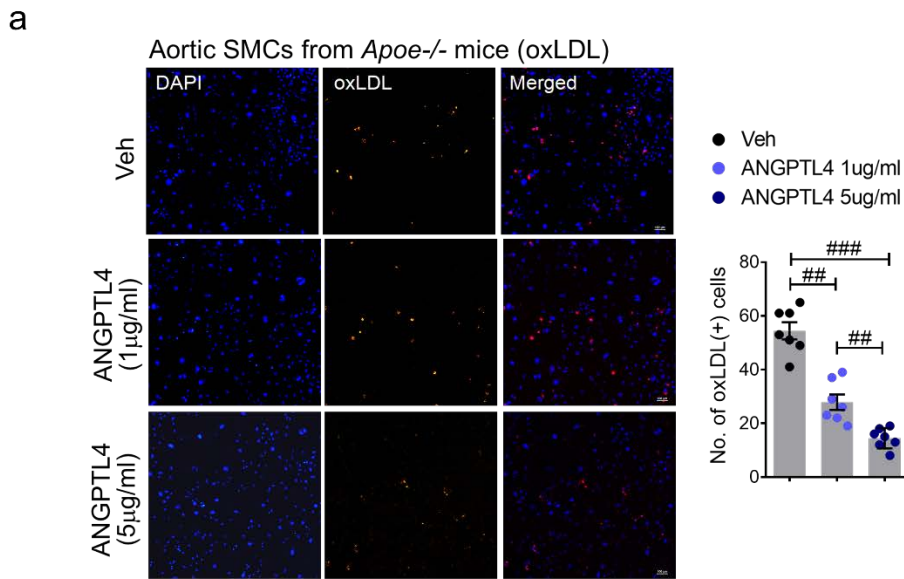
Supplementary Fig 3. The effect of ANGPTL4 on proliferation in human aortic SMCs.

a, Proliferation assay using a measurement of BrdU incorporation in human aortic SMCs. Cell proliferation was initiated with platelet-derived growth factor PDGF-BB (20 ng/mL) or 1% FBS in the presence of ANGPTL4 (0.1, 1, or 10 µg/mL). **b**, Cell proliferation marker Ki67-positive cells were counted in human aortic SMCs stimulated with PDGF-BB or 1% FBS in the presence of ANGPTL4 (1, or 5 µg/mL). Scale bar, 200 µm. Data are represented as mean ± SEM. #*p* < 0.05 (by one-way ANOVA with Bonferroni's multiple-comparisons test).



Supplementary Fig 4. Effects of ANGPTL4 on the stability of atherosclerotic plaque in the HFD-fed *Ldlr*^{-/-} mouse model.

a, The thickness of the fibrous cap and the necrotic core in the *Ldlr*^{-/-} mouse atherosclerosis model was measured from Masson trichrome staining. Scale bar, 500 μm. **b**, Representative images are shown for Masson trichrome-stained aortas from the PBS and ANGPTL4 groups. Scale bar, 100 μm. Data are represented as mean ± SEM. # $p < 0.05$, ## $p < 0.001$ (by Student's *t*-test).

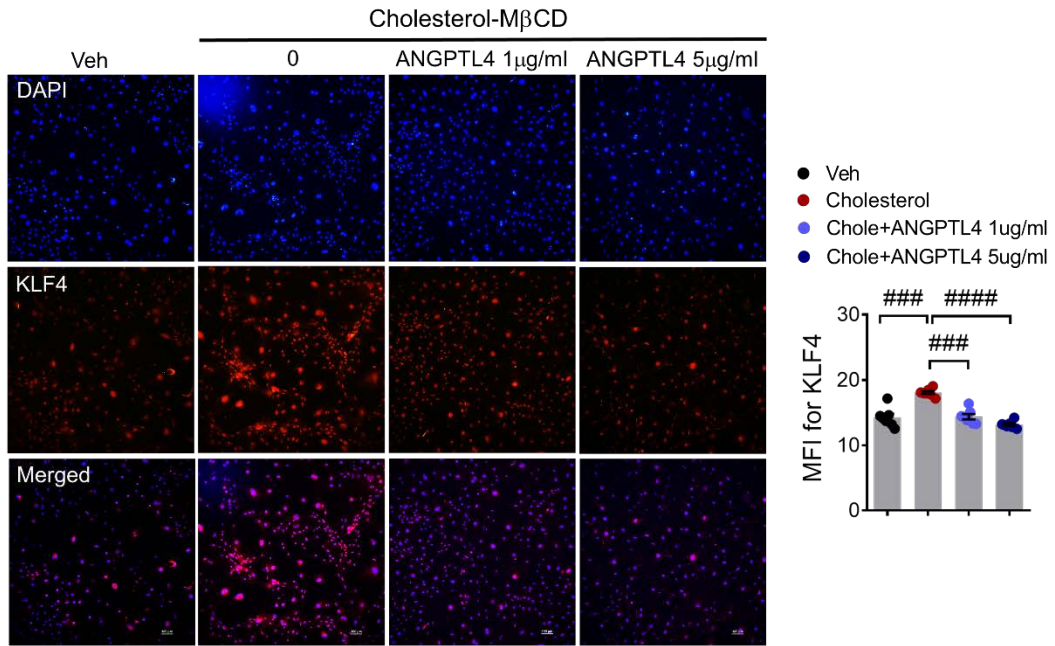


Supplementary Fig 5. The effect of ANGPTL4 treatment on lipid uptake and phenotypic change of SMCs.

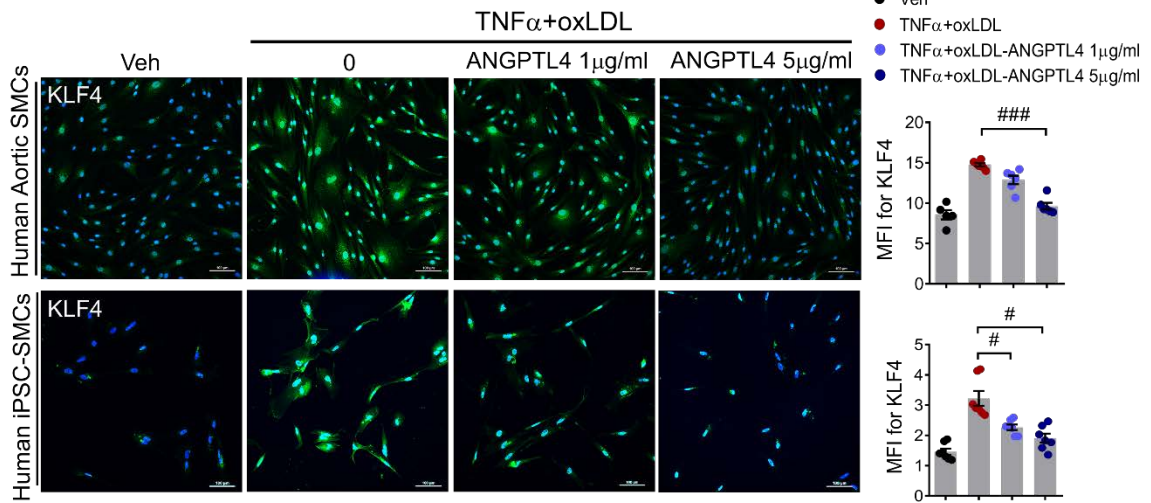
a, Aortic SMCs isolated from *Apoe*^{-/-} mice were treated with Dil-oxLDL (10 µg/ml) with or without ANGPTL4 for 24 h and then oxLDL uptake was analyzed. Scale bar, 100 µm. **b**, Human aortic SMCs with cholesterol with or without ANGPTL4 were analyzed using Oil-red O staining. Scale bar, 100 µm. Mouse aortic SMCs were stimulated with cholesterol (10 µg/ml) with or without ANGPTL4, and the relative expression of genes related to contractile (**c**) and macrophage (**d**) markers was analyzed by real-time PCR. Data are represented as mean ± SEM. #*p* < 0.05, ##*p* < 0.01, ###*p* < 0.001, ####*p* < 0.0001 (by one-way ANOVA with Bonferroni's multiple-comparisons test).

a

Aortic SMCs from *Apoe*^{-/-} mice

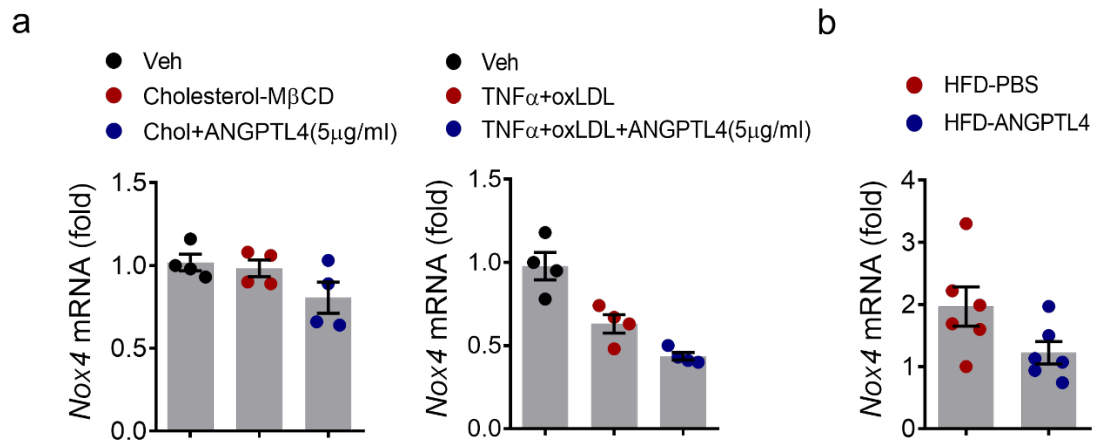


b



Supplementary Fig 6. Immunofluorescence staining of KLF4 expression in SMCs.

a, Aortic SMCs isolated from *ApoE*^{-/-} mice were pretreated with ANGPTL4 for 24 h and then stimulated with cholesterol (10 μ g/ml) for 72 h and stained with KLF4. Mean fluorescence intensity of KLF4 was quantified. Scale bar, 100 μ m. **b**, Human aortic SMCs and iPSC-SMCs were stimulated with oxLDL (10 μ g/ml) and TNF α (100 ng/ml) with or without ANGPTL4 (1 μ g/ml, 5 μ g/ml) and stained with KLF4. Scale bar, 100 μ m. Data are represented as mean \pm SEM. # p <0.05, ### p < 0.001, #### p < 0.0001 (by one-way ANOVA with Bonferroni's multiple-comparisons test).

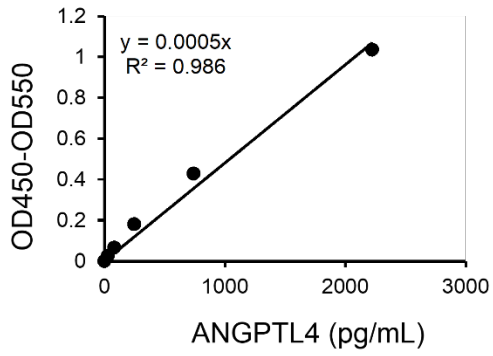


Supplementary Fig 7. Expression patterns of NOX4 in SMCs and aortas of *Apoe*^{-/-} mice.

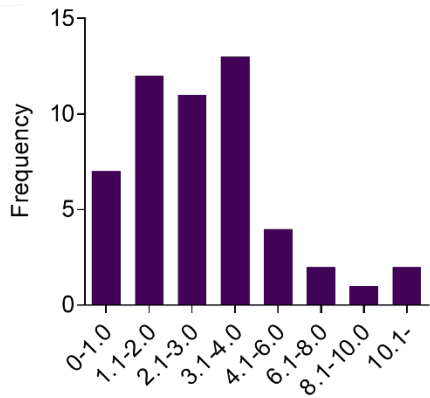
a, Aortic SMCs from atherosclerotic *Apoe*^{-/-} mice were pretreated with ANGPTL4 and cholesterol (10 μ g/ml) or oxLDL (10 μ g/ml) and TNF α (100 ng/ml) and the level of Nox4 was measured. **b**, Aortas were isolated from atherosclerotic *Apoe*^{-/-} mice treated with PBS or ANGPTL4, and the level of NOX4 was measured.

a

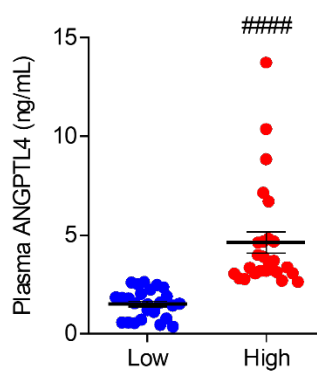
Human ELISA standard curve



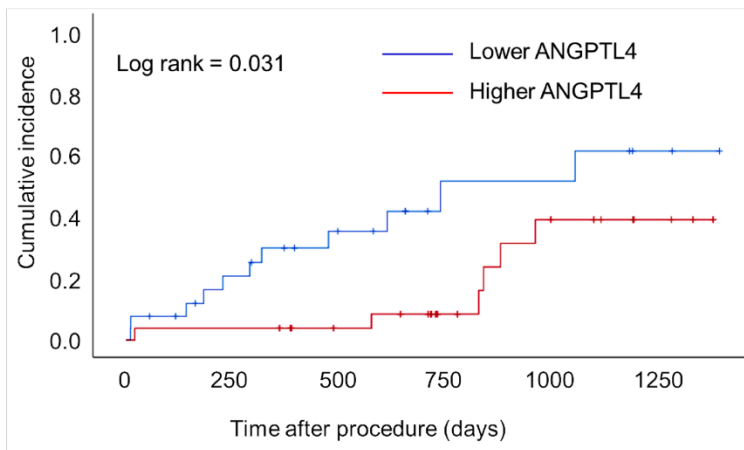
b



c



d



Supplementary Fig 8. Association of circulating ANGPTL4 levels with the incidence of heart failure. **a**, Standard curve for human ANGPTL4 ELISA. **b**, Distribution of plasma levels of ANGPTL4 measured in all patients (n=52). **c**, Plasma levels of ANGPTL4 in the Low ANGPTL4 (n=26) and high ANGPTL4 (n=26) groups. **d**, Kaplan-Meier curve illustrating vascular event incidence of patients during the follow-up period after surgery, based on plasma ANGPTL4 levels above (red) and below (blue) the median value. Data are represented as mean \pm SEM. ##### $p < 0.0001$ (by Student's *t*-test).

Supplementary Table 1. List of real-time PCR primers

Gene	Primer
Mouse <i>Angptl4</i>	Forward : 5'-GGACCTTAACTGTGCCAAGA-3' Reverse: 5'-CGTGGGATAGAGTGGAAGTATTG-3'
Mouse <i>Gapdh</i>	Forward : 5'-GGGTGTGAACACGAGAAATA-3' Reverse: 5'-GTCATGAGCCCTTCCACAAT-3'
Mouse <i>Tnfrsf11b</i>	Forward: 5'-GCCGAGAGTGTAGAGAGGATAA-3' Reverse: 5'-CTTACCATTTCCTGGTCTCTG-3'
Mouse <i>Tlr4</i>	Forward: 5'-GCTTACACCACCTCTCAAAC-3' Reverse: 5'-ACAGCCACCAGATTCTCTAAAC-3'
Mouse <i>Icam1</i>	Forward: 5'-CTGTTTGTAGCTGAGCGAGAT-3' Reverse: 5'-AACGAATACACGGTGATGGTAG-3'
Mouse <i>Vcam1</i>	Forward : 5'-CACAGCTCAGTGGACTGGAA-3' Reverse: 5'-CCACCACCACACCCATAGA-3'
Mouse <i>Il10</i>	Forward: 5'-ACAGCCGGGAAGACAATAAC-3' Reverse: 5'-CAGCTGGTCCTTTGTTTGAAAG-3'
Mouse <i>Myh11</i>	Forward: 5'-GATGAAGAGATCGCCCAGAAA-3' Reverse: 5'-CTCTGAGTCTAGGTCTCTTGT-3'
Mouse <i>Smtn</i>	Forward: 5'-GAGAAGTGGCTACACTCTCAAC-3' Reverse: 5'-GTCAACTCCTCGACATCATTCA-3'
Mouse <i>Tagln</i>	Forward: 5'-GGAACAGGTGGCTCAATTCT-3' Reverse: 5'-GCTGCCATATCCTTACCTTCAT-3'
Mouse <i>Myl9</i>	Forward: 5'-CAGAACCGAGATGGCTTCATT-3' Reverse: 5'-GTTTCATCATGCCCTCCAGATAC-3'
Mouse <i>Klf4</i>	Forward: 5'- GACCTCCTGGACCTAGACTTTA-3' Reverse: 5'- GAAGACGAGGATGAAGCTGAC-3'
Mouse <i>Arg1</i>	Forward: 5'-GATTATCGGAGCGCCTTCT-3' Reverse: 5'-AAGAATGGAAGAGTCAGTGTGG-3'
Mouse <i>Nos2</i>	Forward: 5'- GGAATCTTGGAGCGAGTTGT-3' Reverse: 5'- CCTCTTGTCTTTGACCCAGTAG -3'
Mouse <i>Ccl2</i>	Forward: 5'- GGAATCTTGGAGCGAGTTGT-3' Reverse: 5'- CCTCTTGTCTTTGACCCAGTAG -3'
Mouse <i>Acta2</i>	Forward: 5'- TCAGGGAGTAATGGTTGGAATG-3' Reverse: 5'- GGTGATGATGCCGTGTTCTA -3'
Mouse <i>Cnn1</i>	Forward: 5'- TTGAGAGAAGGCAGGAACATC-3' Reverse: 5'- GTACCCAGTTTGGGATCATAGAG -3'
Mouse <i>Cd68</i>	Forward: 5'-GACCCACAACCTGTCCTCATAA -3' Reverse: 5'-CTTCCACCCTGAATTGGGTATAG-3'
Mouse <i>Abca1</i>	Forward: 5'-GGGTGGTGTTCCTCATTAC-3' Reverse: 5'- CACATCCTCATCCTCGTCATTTC-3'
Mouse <i>Lgals3</i>	Forward: 5'- ACACGAAGCAGGACAATAACT-3' Reverse: 5'- CAGCTTCAACCAGGACTTGTA-3'

Mouse <i>Lamp2</i>	Forward: 5'- TTCAACACCCACTCCAACCTC-3' Reverse: 5'- TAGCCAGCAGACAGGTAGTAT-3'
Mouse <i>Csf1r</i>	Forward: 5'- CTGGGACAGCACGAGAATATAG-3' Reverse: 5'- CCTTCGGAGAAAGTTGAGTAGG-3'
Mouse <i>Tpm1</i>	Forward: 5'- GAGAGTGAGAGAGGCATGAAAG-3' Reverse: 5'- CATACTCCGGTCAGCATCTT-3'
Mouse <i>Nox1</i>	Forward: 5'- CGAGGCTTTAGCATGGAAGTA-3' Reverse: 5'- CAGAAGTCAGAGTGAAGGGATG-3'
Mouse <i>Nox4</i>	Forward: 5'- CCAGAATGAGGATCCCAGAAAG-3' Reverse: 5'- GGTAGAAGCTGTAACCATGAGG-3'

Supplementary Table 2. List of genotyping primers

Gene	Primer
Mouse <i>Ldlr</i> ^{-/-}	Forward: 5'-GCT CCA TAG GCT ATC TGC TC-3' Reverse: 5'-GTG TCG TAG GAC AAG TTA GG-3'
Mouse <i>ApoE</i> ^{-/-}	P1 : 5'-GCCTAGCCGAGGGAGAGCCG-3' P2 : 5'-TGTGACTTGGGAGCTCTGCAGC-3' P3 : 5'-GCCGCCCGACTGCATCT-3'

Supplementary Table 3. List of commercial antibodies

Antibodies	Supplier	Identifier	Application
CD68	Abcam	ab125212	IHC 1:100
Mac2	Cedarlane	CL8942AP	IHC 1:200
α -SMA	Sigma-Aldrich	A2547	IHC 1:100
SM-MHC	OriGene	TA323338	IHC 1:100
SM22 α	Abcam	Ab14106	IHC 1:200
Ki67	Abcam	Ab15580	ICC 1:100
KLF4	Abcam	Ab106629	IHC 1:100 WB 1:1000
CD11b(M1/70), PE	eBioscience	12-0112	FC
CD45(30-F11), Pacific Blue	eBioscience	MCD4528	FC
CD80(B7-1), PerCP Cyanine5.5	BioLegend	104721	FC
F4/80 (BM-8), APC	eBioscience	17-4801-82	FC
Human IgG Fc (HP6017)	BioLegend	409303	FC

WB, Western Blot; IHC, Immunohistochemistry; ICC, Immunocytochemistry; FC, Flow Cytometry

Supplementary Table 4. Baseline Clinical Characteristics

Factor	Low ANGPTL4 (n=26)	High ANGPTL4 (n=26)	P value
Age (years)	69.4±12.2	70.0±11.4	0.852
Men, n (%)	16 (61.5)	22 (84.6)	0.061
Systolic BP (mmHg)	123.1±40.8	119.6±19.7	0.280
Heart rate (/min)	85.9±20.6	81.7±20.9	0.466
Current or ex-smoker, n (%)	15 (57.7)	19 (73.1)	0.244
Hypertension, n (%)	19 (73.1)	11 (42.3)	0.025
Diabetes mellitus, n (%)	16 (61.5)	10 (38.5)	0.096
Dyslipidemia, n (%)	8 (30.8)	4 (15.4)	0.188
BMI (kg/m ²)	23.8±4.1	24.0±3.3	0.839
Obesity, n (%)	9 (34.6)	10 (38.5)	0.773
Familial history of CAD, n (%)	1 (3.8)	0 (0)	1.000
Previous MI, n (%)	3 (11.5)	5 (19.2)	0.703
Previous HF, n (%)	2 (7.7)	1 (3.8%)	1.000
ANGPTL4 levels (ng/mL)	1.53±0.72	4.64±2.69	<0.001
Serum creatinine (mg/dL)	1.5±1.3	1.3±1.0	0.545
Peak troponin-I (mg/dL)	60.1±79.0	68.6±62.0	0.253
Peak CK-MB (mg/dL)	57.1±91.2	93.0±100.1	0.210
Total cholesterol (mg/dL)	159.7±40.6	165.6±44.5	0.503
Triglyceride (mg/dL)	111.2±58.0	117.8±85.3	1.000
HDL-cholesterol (mg/dL)	42.9±10.3	41.9±12.6	0.515
LDL-cholesterol (mg/dL)	100.7±30.5	103.3±34.8	0.813
Serum glucose (mg/dL)	220.9±137.7	140.2±44.1	0.012
N-terminal pro BNP (pg/mL)	3,692.0±4825.3	7,169.3±11960.2	0.627
High-sensitivity CRP (mg/L)	3.6±5.9	2.3±4.0	0.493
Left ventricular EF (%)	45.8±10.9	48.5±10.7	0.374
Medications at discharge, n (%)			
Aspirin	26 (100)	26 (100)	1.000
Clopidogrel	18 (69.2)	11 (42.3)	0.051
Prasugrel or Ticagrelor	10 (38.5)	18 (69.2)	0.026
Beta-blocker	21 (80.8)	24 (92.3)	0.419
ACEi or ARB	24 (92.3)	24 (92.3)	1.000
Aldosterone antagonist	7 (26.9)	12 (46.2)	0.150
Statin	24 (92.3)	26 (100)	0.490

Values are presented as mean±SD, median [interquartile range] or number (percentage). ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin-II receptor blocker; BNP, brain-type natriuretic peptide; BP, blood pressure; CAD, coronary artery disease; CK-MB, creatine kinase-myocardial band isoenzyme; CRP, C-reactive protein; EF, ejection fraction; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Supplementary Table 5. Coronary Angiographic and Procedural Characteristics

Factor	Low ANGPTL4 (n=21)	High ANGPTL4 (n=21)	P value
Culprit LAD, n (%)	12 (46.2)	17 (65.4%)	0.163
MVD, n (%)	13 (50.0)	13 (50.0)	1.000
ACC/AHA B2/C lesion, n (%)	26 (100)	25 (96.2)	1.000
Pre-PCI TIMI flow grade 0, n (%)	14 (58.3)	10 (38.5)	0.266
Symptom-to-balloon time (min)	2482.7±4130.7	1752.7±2865.6	0.260
Post-PCI TIMI flow grade 3, n (%)	26 (100)	26 (100)	1.000

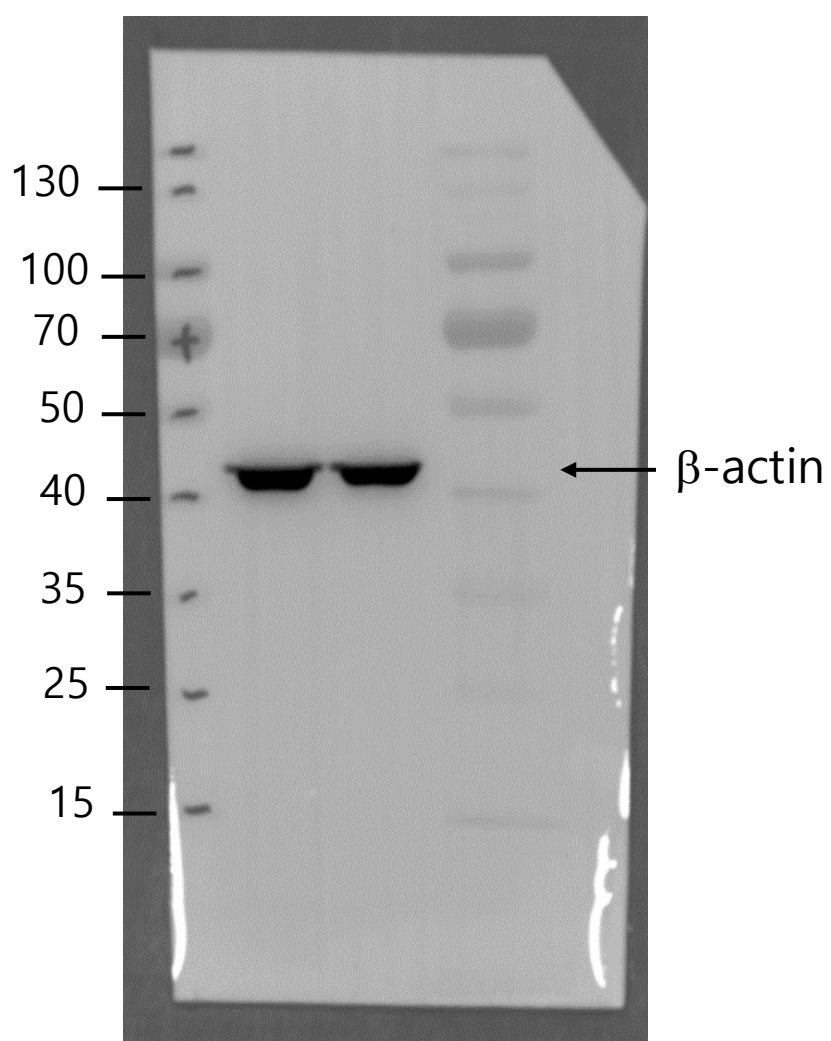
Values are presented as mean±SD or number (percentage). ACC, American College of Cardiology; AHA, American Heart Association; LAD, left anterior descending; MVD, multivessel disease; PCI, percutaneous coronary intervention; TIMI, thrombolysis in myocardial infarction.

Supplementary Table 6. Factors Associated with Recurred Heart Failure

Factor	Crude Hazard Ratio on Univariate Analysis (95% CI)	P value	Adjusted Hazard Ratio on Multivariate Analysis (95% CI)	P value
Age (per year increase)	1.078 (1.026-1.133)	0.003	1.098 (1.023-1.179)	0.010
Male	1.730 (0.603-4.962)	0.308		0.326
Killip class 3	2.762 (1.012-7.537)	0.047	2.363 (0.254-22.007)	0.450
Hypertension	0.797 (0.488-1.302)	0.365		
Diabetes mellitus	0.936 (0.580-1.511)	0.788		
Dyslipidemia	0.848 (0.479-1.501)	0.571		
Obesity	2.250 (0.730-6.939)	0.158		
Smokers	0.775 (0.286-2.101)	0.616		
Family history	4.557 (0-190080.174)	0.780		
Previous HF	4.707 (0.020-1115.518)	0.579		
Previous MI	0.832 (0.444-1.559)	0.566		
Systolic blood pressure	1.003 (0.988-1.020)	0.670		
Heart rate	1.006 (0.985-1.026)	0.599		
Pain to balloon time	1.000 (1.000-1.000)	0.115	1.001 (1.000-1.001)	0.201
MVD	1.031 (0.396-2.685)	0.951		
Culprit LAD	0.872 (0.320-2.374)	0.788		
Creatinine	1.334 (1.202-1.745)	0.035	1.277 (0.854-1.908)	0.234
ANGPTL4 levels	0.465 (0.296-0.729)	0.001	0.338 (0.166-0.686)	0.003
Peak CK-MB	0.996 (0.990-1.002)	0.231		
Peak troponin-I	0.995 (0.986-1.003)	0.186	0.998 (0.981-1.014)	0.763
hsCRP	1.131 (1.024-1.249)	0.016	1.096 (0.973-1.234)	0.131
Initial LVEF	0.990 (0.942-1.040)	0.686		
Beta-blocker at discharge	0.549 (0.200-1.511)	0.246		
ACEi or ARB at discharge	0.042 (0.000-45.106)	0.373		
Aldosterone antagonist at discharge	1.338 (0.793-2.256)	0.275		
Use of statin at discharge	1.082 (0.393-2.977)	0.879		

ACEi, angiotensin-converting enzyme inhibitor; ANGPTL4, angiotensin like 4; ARB, angiotensin-II receptor blocker; CAD, coronary artery disease; CI, confidence interval; CK-MB, creatine kinase-myocardial band isoenzyme; HF, heart failure; hsCRP, high-sensitivity C-reactive protein, LAD, left anterior descending; LVEF, left ventricular ejection fraction; MI, myocardial infarction; MVD, multivessel disease.

Full unedited gel for Figure 6C- KLF4 & β -actin



Full unedited gel for Figure 6D- KLF4 & GAPDH

