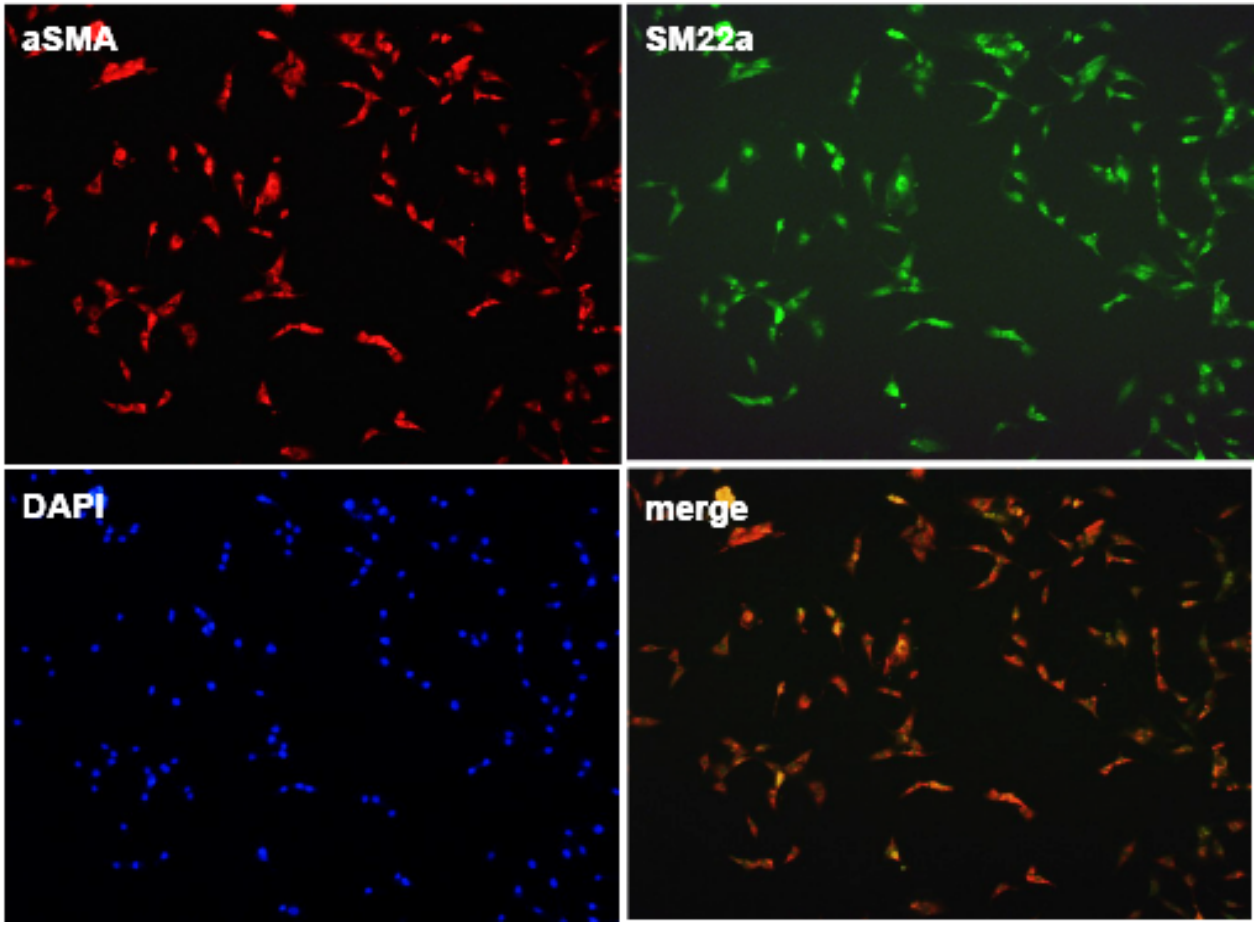
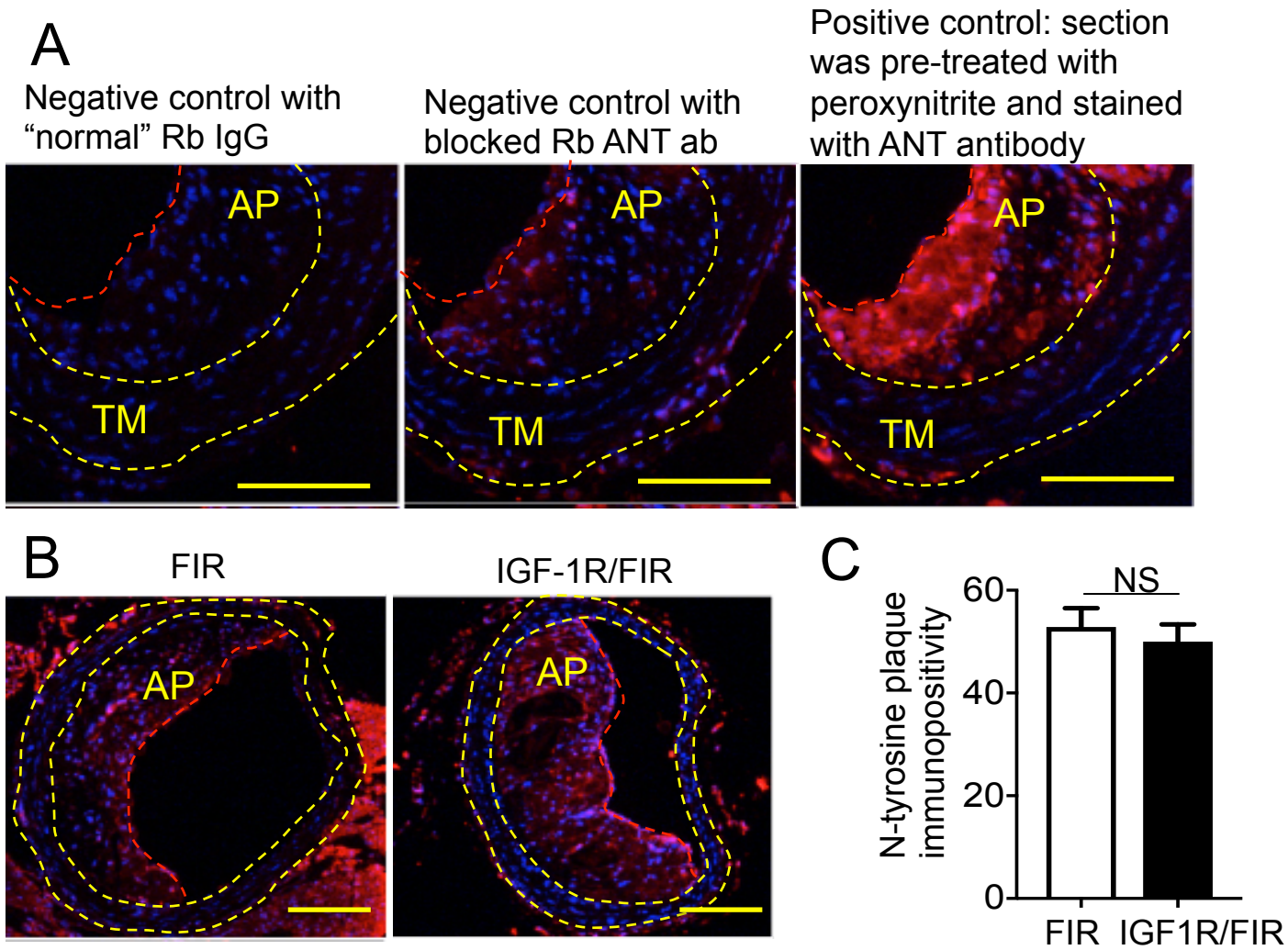
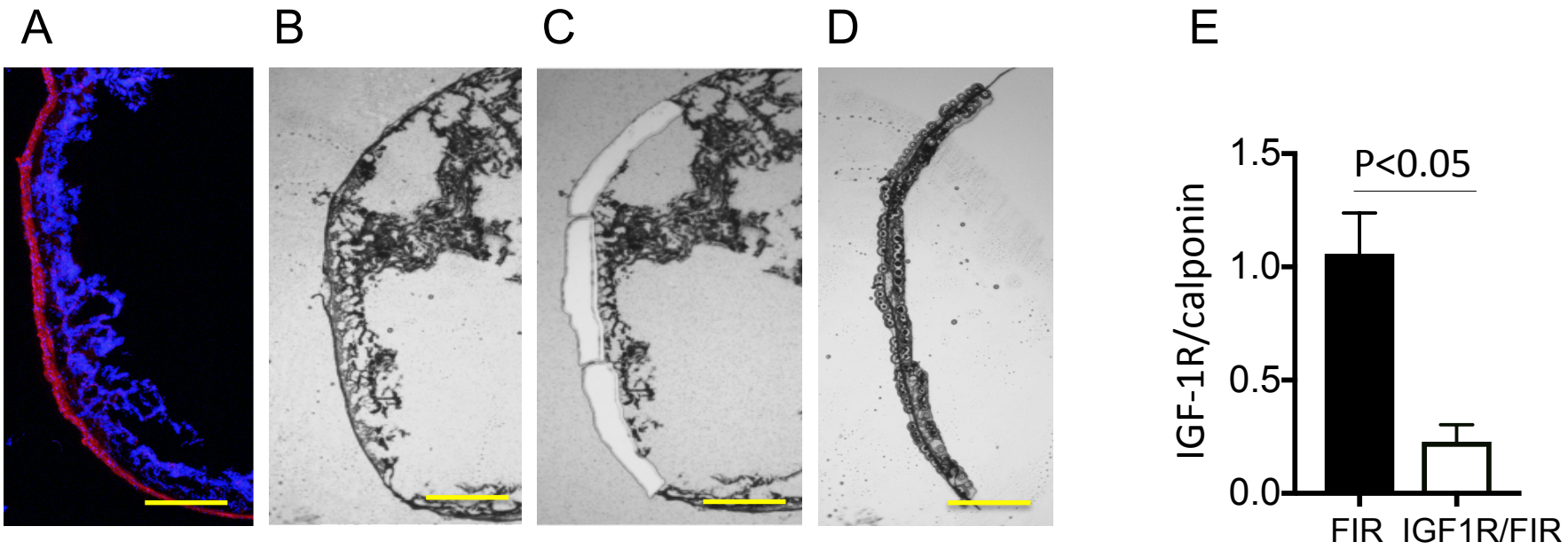
αSMA/SM22α/DAPI



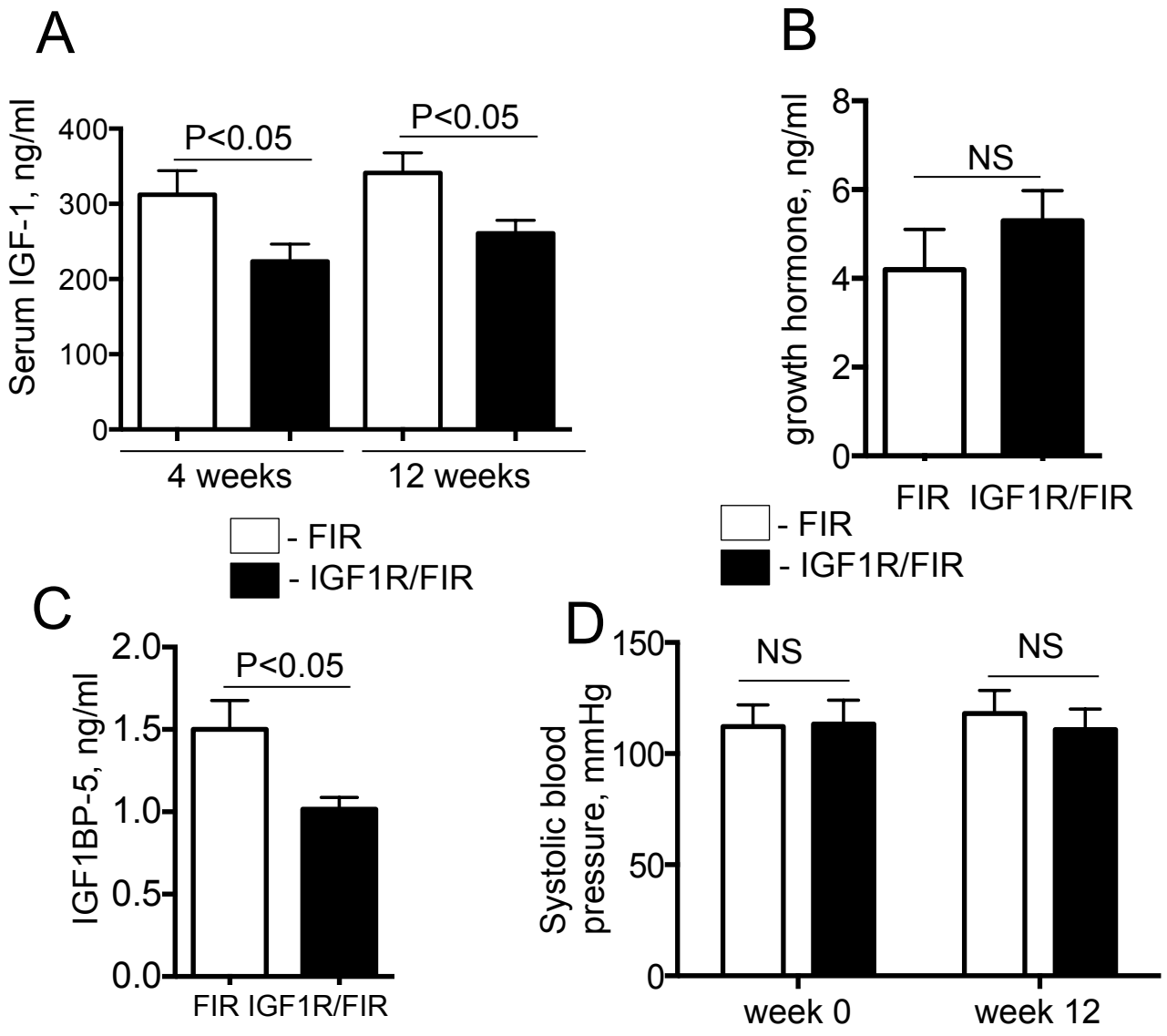
<u>Supplemental Figure I.</u> Cells isolated from mouse aortas are SMC. Aortic SMC were isolated from SM22α-CreKI/IGF1R-flox (IGF1R/FIR) and FIR mice as described in Materials and Methods. To confirm cell identity, cells were plated on chamber slide and co-stained with SMC markers: α-SMA/AlexaFluor594 and SM22α/AlexaFluor488 and with DAPI. Red and green signals were co-localized on merged images showing that these cells are SMC.



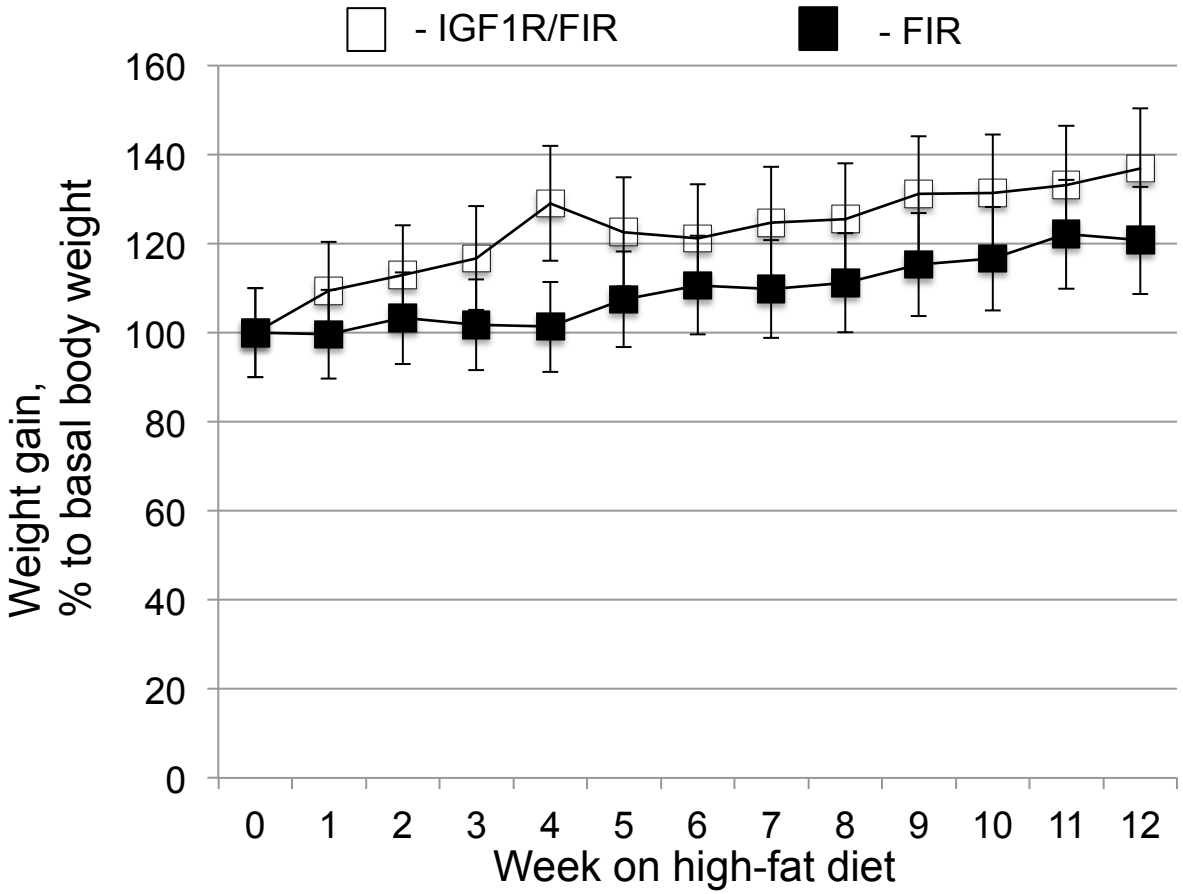
Supplemental Figure II. Atherosclerotic plaque oxidative stress. A. Controls for IHC with anti-N-tyrosine antibody (ANT). Negative controls: serial BCA cross-sections were stained with "normal" rabbit IgG or with ANT blocked by incubation with 3-Nitro-L-tyrosine. Positive control: section was pre-treated with peroxynitrite and stained with ANT. B. Representative images of BCA sections obtained from IGF-1R/FIR mice (n=7) and FIR mice (n=6) and stained with ANT. The mean "red" pixels number was normalized per plaque area and these data are shown on the graph (C). AP, atherosclerotic plaque, yellow dashed line, tunica media (TM). Scale bar, 50um.



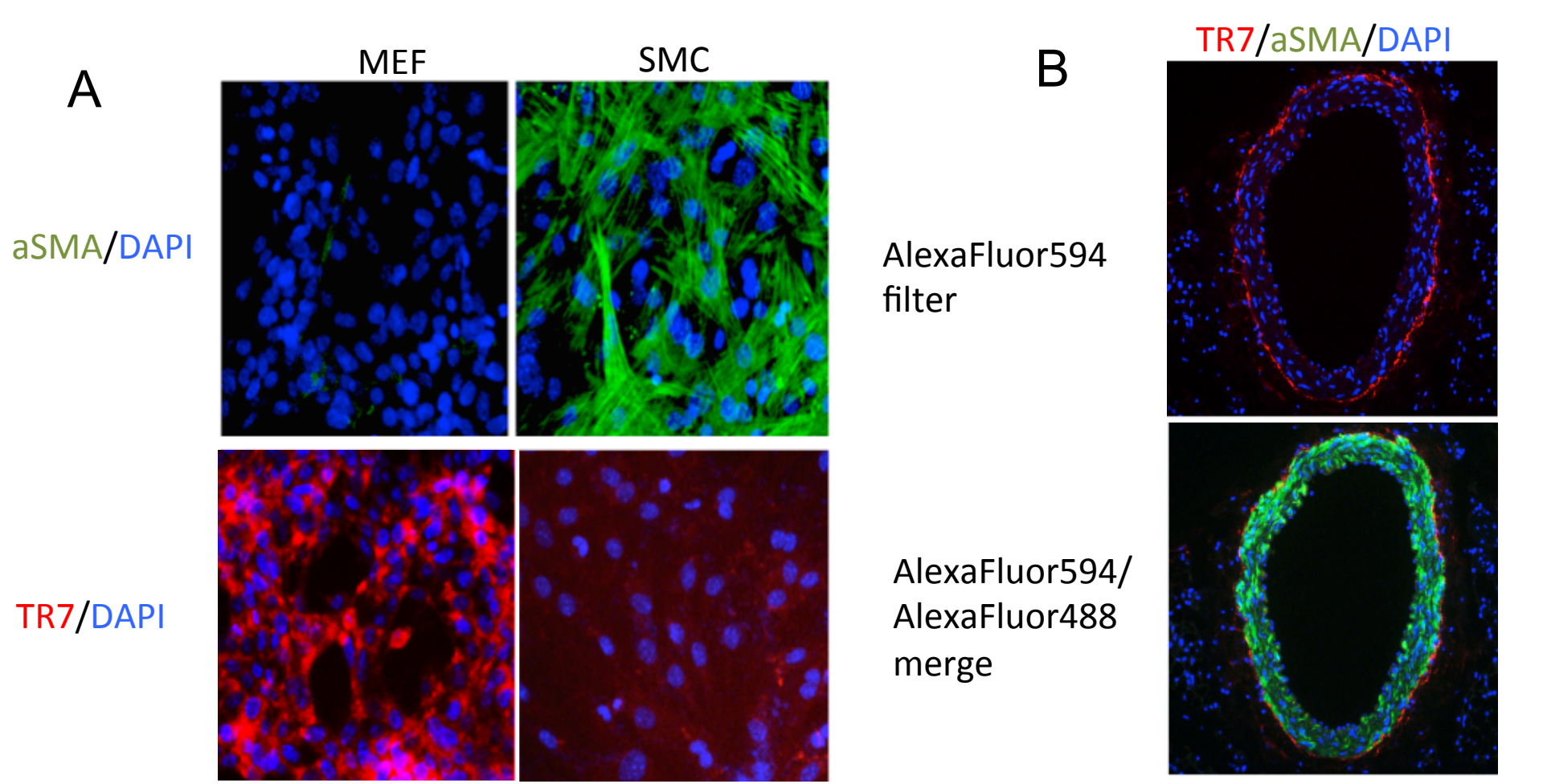
Supplemental Figure III. IGF-1R expression in non-vascular (intestinal) SMC. A-D. Laser capture microdissection (LCM) method. Terminal ileum was dissected from SM22α-CreKI/IGF1R-flox mice (IGF1R/FIR, n=4) and control mice (FIR, n=7), tissue was embedded in OCT and single cryosection was immunostained for calponin to identify SMC and serve as a reference for LCM (A). Serial sections were utilized for LCM-assisted isolation of the intestinal layer guided by reference section (B-C). Isolated tissue was transferred into LCM cap (D) and used for RNA isolation. E, RT-PCR quantitative data. Scale bar, 200 um.



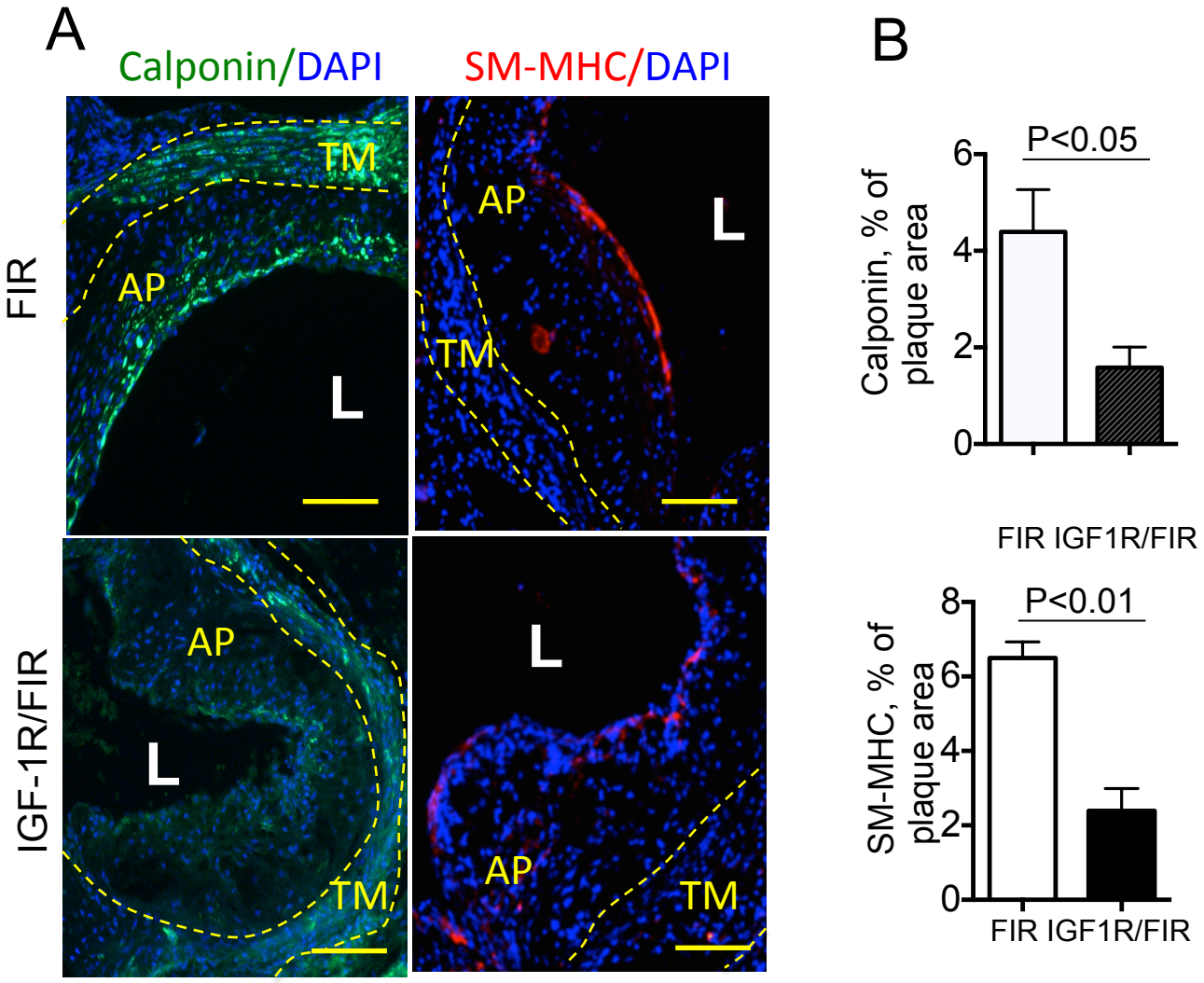
<u>Supplemental Figure IV.</u> IGF-1R deficiency reduced circulating IGF-1. Serum IGF-1 levels were quantified in SM22α-CreKI/IGF1R-flox (IGF1R/FIR) mice and FIR mice (n=6/group) after 4 and 12 weeks of mice feeding with a high-fat Western-type diet (WD) (A). Growth hormone level was quantified by ELISA after 12 weeks of feeding with WD (n=6/group) (B). IGFBP5 level was quantified by ELISA (C). Blood pressure was measured by tail cuff method (D).



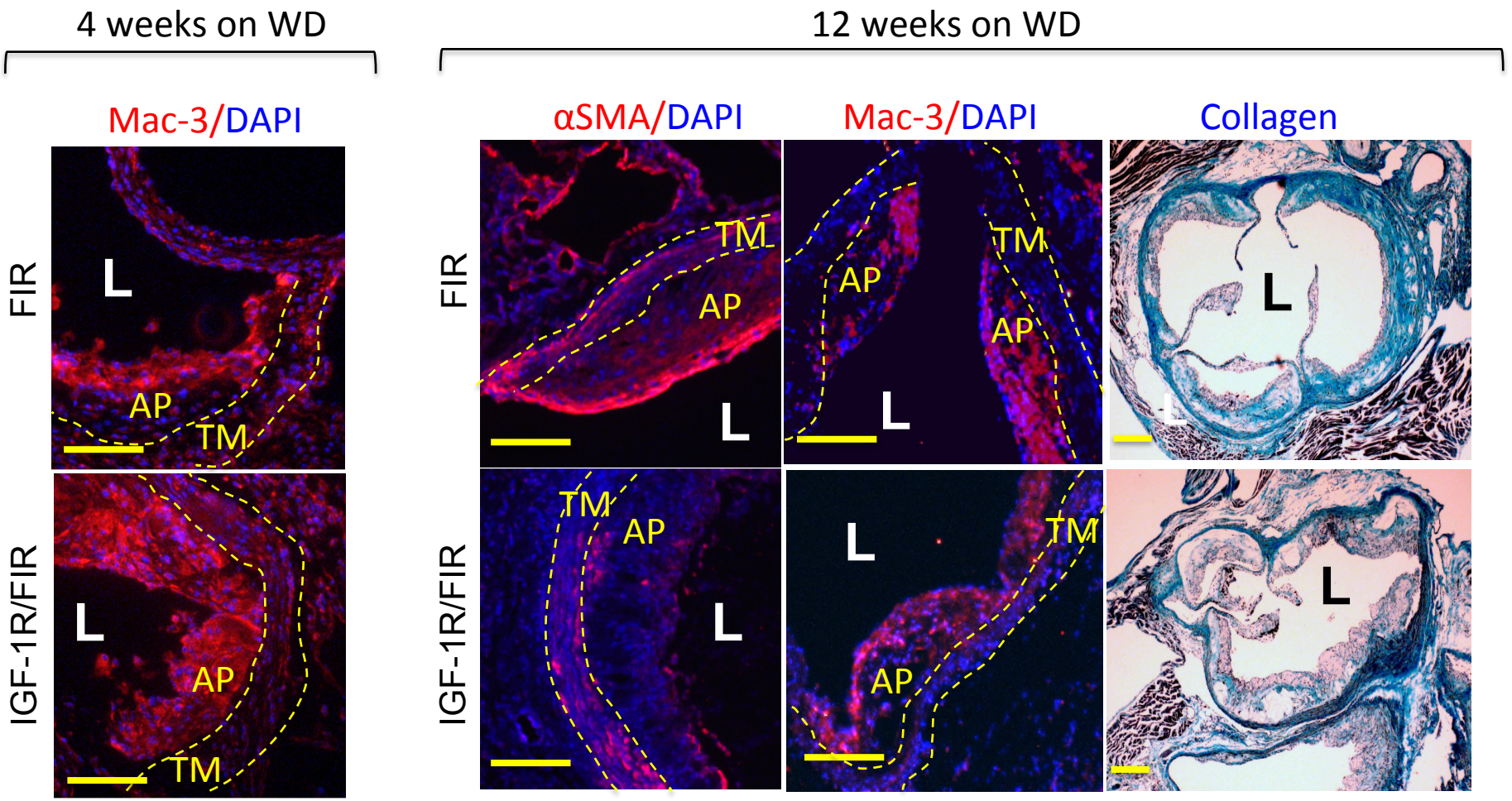
<u>Supplemental Figure V.</u> Weight gain of IGF-1R-deficient and control mice. SM22α-CreKI/IGF1R-flox mice (IGF1R/FIR, n=28) and control mice (FIR, n=55) were fed with Western type diet for 12 weeks and body weight (BW) was measured every week. Weight gain is shown as a BW percentage per time zero.



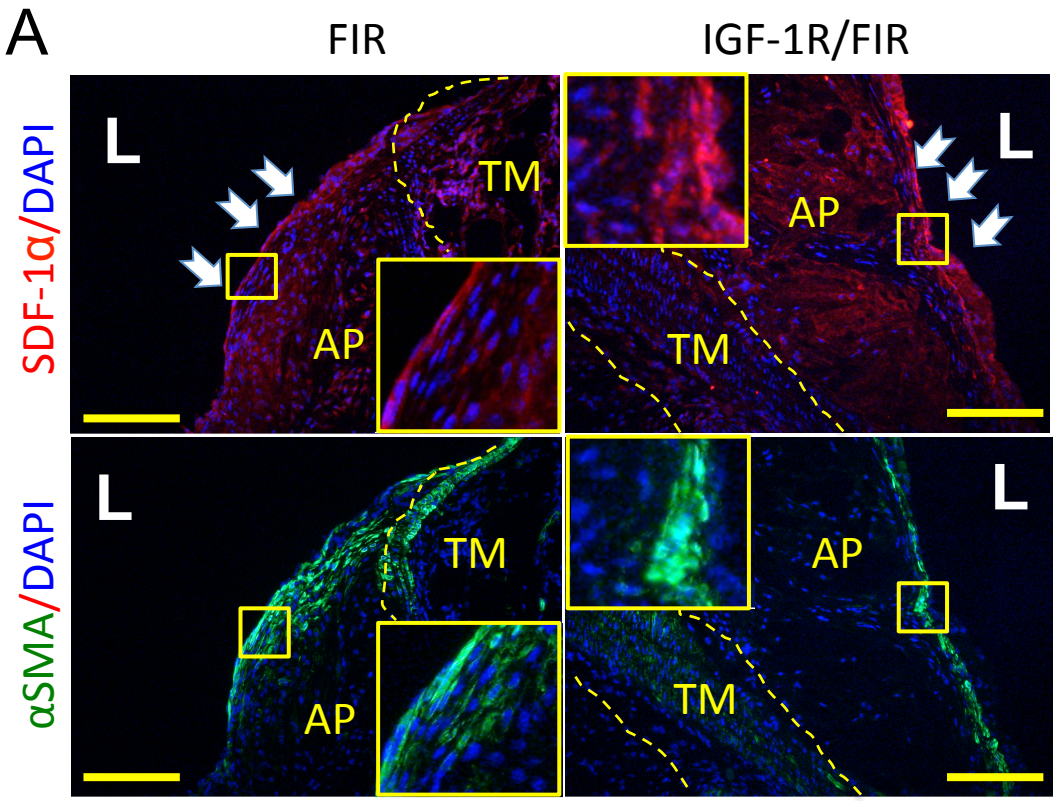
<u>Supplemental Figure VI.</u> Validation of SMC and fibroblast marker antibodies. Mouse embryonic fibroblasts (MEF**)** and SMC were stained with a mix of mouse α-SMA antibody (a SMC marker) and rat TR7 antibody (a fibroblast marker) followed by secondary antibody (a mix of anti-mouse AlexaFluor488 and anti-rat AlexaFluor594) (A). SMC marker signal was detected in SMC and no signal was found in MEF. TR7 antibody stained MEF and did not stain SMC. Sections of ascending aorta were co-stained with TR7/AlexaFluor594 and α-SMA/AlexaFluor488 (B). TR7 signal was located exclusively in the adventitial layer and the α-SMA signal was localized only in the vascular media.

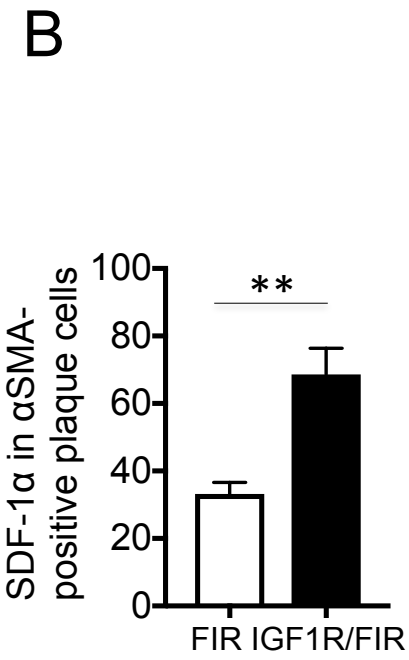


Supplemental Figure VII. IGF-1R deficiency decreased plaque SMC levels. Aortic valve cross-sections were obtained from IGF-1R-deficient (SM22α-CreKI/IGF1R-flox mice, n=14) and control mice (FIR mice, n=14/group) fed with WD for 12 weeks. SMC were identified by IHC for calponin and for smooth muscle myosin heavy chain (SM-MHC) (both are SMC makers). B, Quantitative data. Yellow dashed line, tunica media (TM), AP, atherosclerotic plaque, L, lumen. Scale bar, 50um.

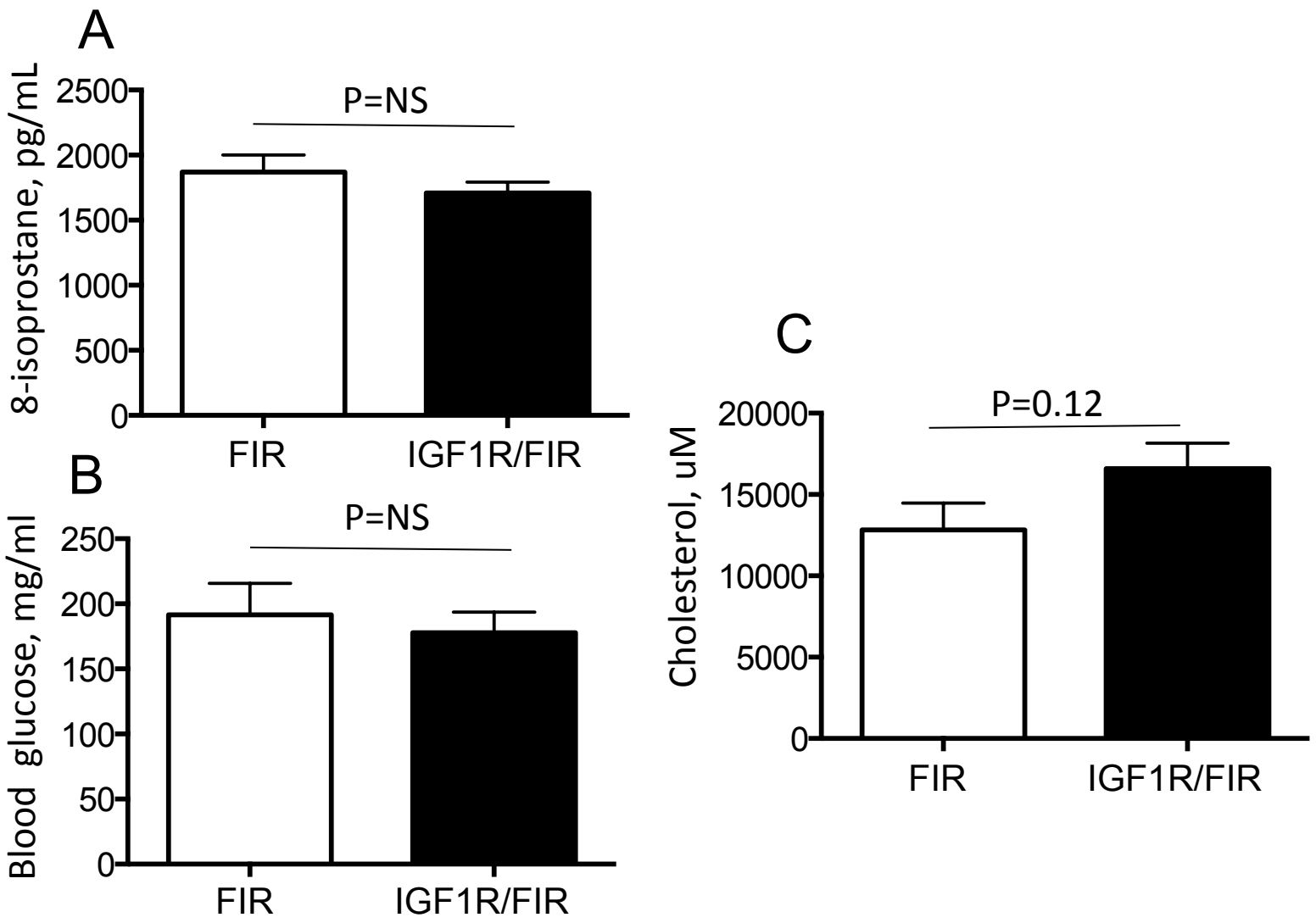


<u>Supplemental Figure VIII.</u> SMC, macrophages and collagen in the atherosclerotic plaque. SM22α-CreKI/IGF1R-flox mice (IGF-1R/FIR) and control mice (FIR mice) were fed with WD for 4 and for 12 weeks. Serial sections (6 μm) were taken throughout the entire aortic valve area and used for IHC for a-SMA (SMC marker), Mac-3 (macrophage marker) and for Trichrome staining (collagen). Representative images are shown. Yellow dashed line, tunica media (TM), AP, atherosclerotic plaque, L, lumen. Scale bar, 100 um.

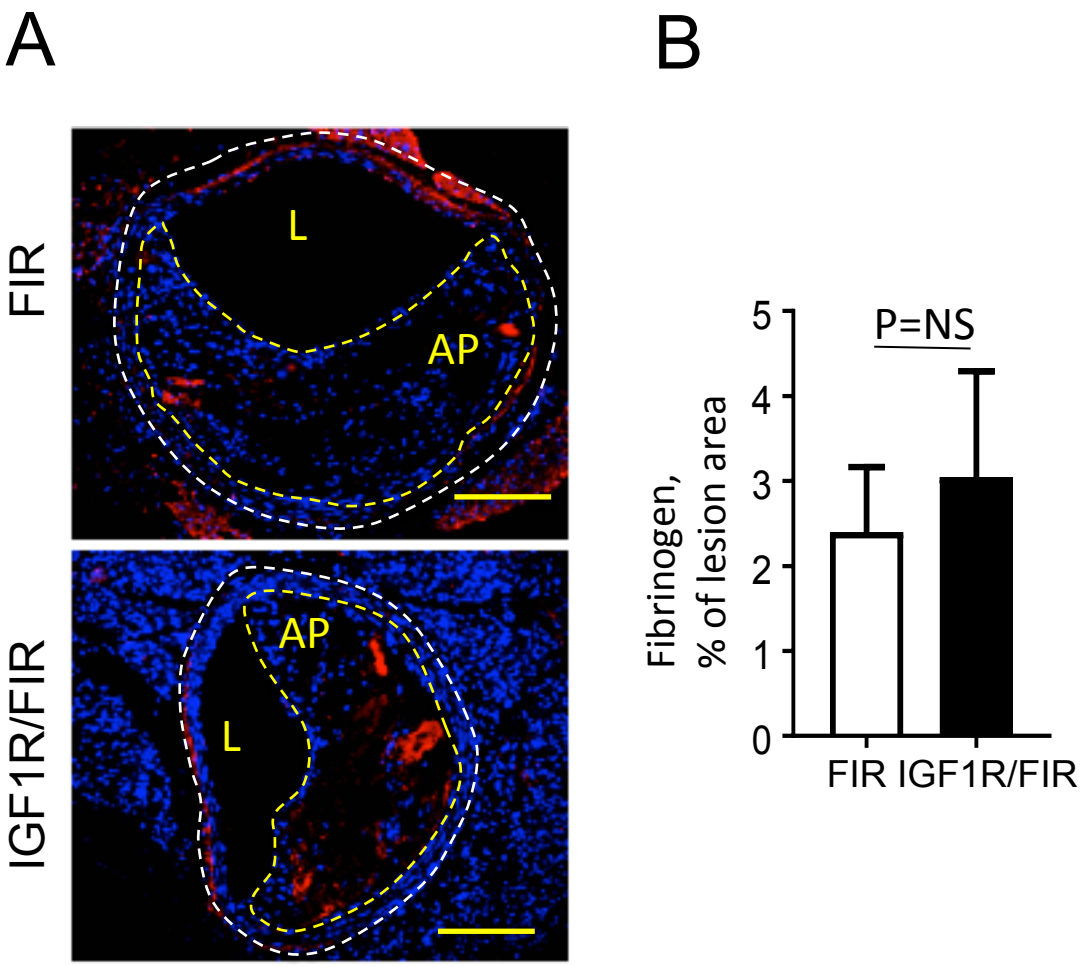




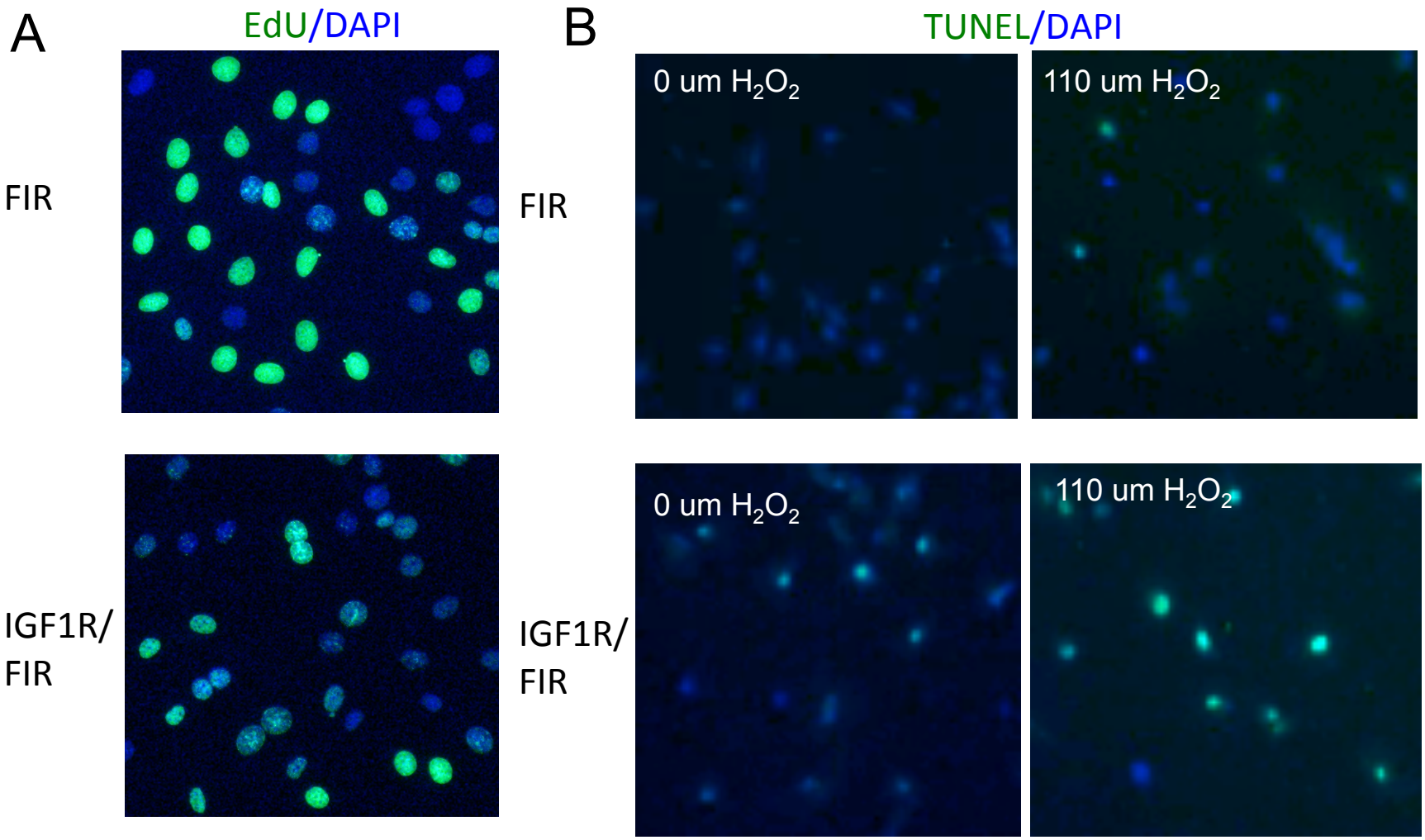
Supplemental Figure IX. IGF-1R deficiency upregulates chemokine SDF-1α (Cxcl12) in αSMA-positive plaque cells. A, SM22α-CreKI/IGF1R-flox mice (IGF-1R/FIR) and control mice (FIR mice) were fed with WD for 4 weeks and aortic valve cross-sections (n=6, IGF-1R/FIR; n=5, FIR) were co-stained with αSMA-AlexaFluor488 and with rabbit SDF-1α antibody followed by staining with DAPI and anti-rabbit-AlexaFluor594 conjugate. Representative images are shown. Square insert/white arrows, αSMA/SDF-1α-double positive plaque cells. Yellow dashed line, tunica media (TM), AP, atherosclerotic plaque, L, lumen. **P<0.01. Scale bar, 100 um. B, Quantitatve data



<u>Supplemental Figure X.</u> Eight-isoprostane (A), blood glucose (B) and cholesterol levels (C). Eight-isoprostane (a marker of systemic oxidative stress) and cholesterol levels were quantified using commercially available kits and blood glucose was measured with a glucometer (n=6/group).



Supplemental Figure XI. Fibrin deposition in the atherosclerotic plaque. Brachiocephalic arteries (BCA) were dissected from IGF-1R-deficient (n=10) and control FIR mice (n=8) and cross-sections were immunostained for fibrinogen (A, representative images). Fibrinogen-positive plaque area was normalized per lesion area. B, Quantitative data. Yellow dashed line, atherosclerotic plaque (AP), white dashed line, external elastic lamina, L, lumen. Scale bar, 50um.



<u>Supplemental Figure XII.</u> Aortic SMC isolated from IGF-1R-deficient mice had reduced proliferation and increased apoptotic rates. A, Cell proliferation rate was quantified by labeling of replicating DNA with EdU following by fluorescent-positive cell count (n=6/group). B**,** To quantify apoptosis, IGF1R/FIR- and FIR-derived aortic SMC were exposed to H_2O_2 for 16h in serum-free media followed by staining with TUNEL assay (n=4/group). Representative images are shown.

Supplemental Table 1. Aorta, aortic valve and brachiocephalic artery (BCA) morphometry

	FIR	IGF1R/FIR	P value
Aorta length*, mm	40.3±2.2	33.94±2.9	0.017
Aortic valve circumference**, $x10^3$ um	5.57±0.28	4.44±0.27	0.009
Aortic valve lumen, mm^2	1.74±0.16	1.17±0.13	0.012
Aortic valve media area, mm^2	0.37±0.01	0.21±0.02	<0.0001
Thoracic aorta circumference, $x10^3$ um	3.40±0.11	2.69±0.12	0.004
Thoracic aorta lumen, mm^2	0.61±0.05	0.44±0.05	0.099
Thoracic aorta media area, mm^2	0.21±0.02	0.12±0.01	0.002
BCA circumference, $x10^3$ um	2.26±0.12	1.88±0.13	0.092
BCA lumen, mm^2	0.26±0.03	0.17±0.04	0.049
BCA media area, mm^2	0.07±0.01	0.04±0.01	0.003

*Aorta length was quantified from the top of the left ventricle until the iliac bifurcation

**Aortic valve, thoracic aorta and brachiocephalic artery circumference were quantified at the external elastic lamina with H&E-stained cross-sections

Data were obtained for n=5/group

Supplemental Table 2. Serum cytokines levels in IGF1R/FIR and FIR mice

Cytokine	IGF1R/FIR, pg/ml	FIR, pg/ml	Fold change
IL-1α	0.34 ± 0.01	0.32 ± 0.06	1.06
IL-1β	0.44 ± 0.02	0.30 ± 0.03	1.47**
IL-2	0.29 ± 0.03	0.22 ± 0.01	1.32
IL-4	0.337 ± 0.019	0.21 ± 0.01	1.605**
IL-6	0.476 ± 0.017	0.305 ± 0.028	1.56**
IL-10	0.39 ± 0.04	0.25 ± 0.02	1.56*
IL-12	0.35 ± 0.03	0.23 ± 0.02	1.48*
IL-17α	0.358 ± 0.01	0.277 ± 0.029	1.29
IFN-γ	0.289 ± 0.039	0.245 ± 0.015	1.17
TNF-α	0.27 ± 0.03	0.19 ± 0.002	1.40
G-CSF	0.46 ± 0.12	0.43 ± 0.09	1.08
GM-CSF	0.47 ± 0.10	0.56 ± 0.082	0.84

*P<0.05, **P<0.005. N=6/group

Supplemental Table 3. Top genes overexpressed and underexpressed in aortic SMC isolated from SM22α-CreKI/IGF1R-flox mice compared to FIR mice*

Gene symbol	RefSeq number	Description	Fold change	P-value
Ackr4	**NM_145700**	**Chemokine (C-C motif) receptor-like 1**	**7.44**	**<0.001**
Cxcr3	**NM_009910**	**Chemokine (C-X-C motif) receptor 3**	**2.33**	**0.04**
Cxcl12	NM_021704	Chemokine (C-X-C motif) ligand 12	2.59	0.11
Xcl1	NM_008510	Chemokine (C motif) ligand 1	2.26	0.06
Cmtm3	NM_024217	CKLF-like MARVEL transmembrane domain containing 3	-2.56	0.12
IL6	NM_001314054	Interleukin 6	-2.31	0.08
Ccl5	NM_013653	Chemokine (C-C motif) ligand 5	-3.05	0.13
Cmklr1	NM_008153	Chemokine-like receptor 1	-3.00	0.12

*Gene expression was quantified using real-time RT² Profiler™ Mouse Chemokines & Receptors PCR Array (Qiagen) in accordance with manufacturer's instructions. N=5 for SMC isolated from SM22α-CreKI/IGF1R-flox mice and n=7 for SMC isolated from FIR mice. The P values were calculated based on a Student's t-test of the replicate 2^ (- Delta CT) values for each gene in the SM22α-CreKI/IGF1R-flox and FIR groups.

Supplemental Table 4. Relative gene expression of Ackr4, Cxcr3, Cxcl12 and Xcl1 in aortic SMC isolated from SM22α-CreKI/IGF1R-flox mice compared to FIR mice**

Gene symbol	Fold change	P-value
Ackr4	**10.77**	**<0.001**
Cxcr3	2.22	0.38
Cxcl12	**5.98**	**0.04**
Xcl1	**9.98**	**0.06**

**Relative gene expression was quantified with 40-cycle 2-step real-time PCR using sequence-specific primer pairs (Qiagen, RT² qPCR Primer Assay) in the iCycler IQ Real-Time Detection System (Bio-Rad). N=5 for SMC isolated from SM22α-CreKI/IGF1R-flox mice and n=7 for SMC isolated from FIR mice.

Major Resources Tables

Animals

Species/Strain	Vendor or Source	Background Strain	Sex
$Apoe^{-/-}$ mice	Jackson Lab	C57/Bl6	M, F
$SM22\alpha\text{-}CreKI^{+/+}$ mice	Dr. Eugene Chen	C57/Bl6	M, F
LoxP-flanked IGF-1R mice	Dr. Jens Brüning	C57/Bl6	M, F
$SM22\alpha\text{-}Cre^{+/+}/Apoe^{-/-}$ mice	Investigator generated	C57/Bl6	M, F
$LoxP^{+/+}$-IGF-1R/$Apoe^{-/-}$ mice	Investigator generated	C57/Bl6	M, F
$LoxP^{+/+}$-IGF1R/$SM22\alpha\text{-}Cre^{+/-}/Apoe^{-/-}$ mice	Investigator generated	C57/Bl6	M, F

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration, ug/ml	Lot # (preferred but not required)
IGF-1R beta (C-20)	Santa Cruz	sc-713	1.0 (WB)	
Phospho-Akt (Ser473) (D9E)	Cell Signaling	4060	5.0 (WB)	
Akt	Cell Signaling	9272	5.0 (WB)	
Phospho-p38 MAPK (Thr180/Tyr182)	Cell Signaling	4631	0.2 (WB)	
p38 MAPK	Cell Signaling	9212	0.2 (WB)	
Beta-actin	Sigma	A2228	0.05 (WB)	
Calponin (EP798Y)	Abcam	Ab46794	10.0 (IF) 5.0 (IHC)	
Alpha-actin, smooth muscle (clone ASM-1)	Millipore	CBL171	2.0 (IF) 1.0 (IHC)	
Alexa Fluor® 488 Goat anti-rabbit	Molecular Probes	A-11008	5.0 (ICC)	
Alexa Fluor® 594 Goat anti-rabbit	Molecular Probes	A-11037	1.0 (ICC)	
Mac-3 (2-4G2)	BD Biosciences	553142	10.0 (IHC)	
ER-TR7	Santa Cruz	sc-73356	2.0 (IHC)	

LARP-6 (B01P)	Abnova	H00055323 -B01P	0.25 (WB)	
N-tyrosine	Millipore Sigma	AB5411	2.0 (IHC)	
Cre recombinase (D7L7L)	Cell Signaling	15036S	0.2 (WB)	
Alpha-Tubulin (11H10)	Cell Signaling	9099	0.1 (WB)	
SM22-α	Abcam	ab14106	2.0 (IHC)	
Smooth muscle Myosin heavy chain 11	Abcam	ab53219)	2.0 (IHC)	
Fibrinogen	Abcam	ab34269	2.0 (IHC)	
SDF-1	Abcam	ab9797	2.5 (IHC)	

WB – Western blotting, IF – immunofluorescence, IHC - immunohistochemistry

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)
Aortic SMC from LoxP$^{+/+}$-IGF-1R/Apoe$^{-/-}$ mice	Investigator generated	M
Aortic SMC from LoxP$^{+/+}$-IGF1R/SM22α-Cre$^{+/-}$/Apoe$^{-/-}$ mice	Investigator generated	M
Embryonic fibroblasts from LoxP$^{+/+}$-IGF-1R/Apoe$^{-/-}$ mice	Investigator generated	M
Embryonic fibroblasts from LoxP$^{+/+}$-IGF1R/SM22α-Cre$^{+/-}$/Apoe$^{-/-}$ mice	Investigator generated	M
Skin fibroblasts from LoxP$^{+/+}$-IGF-1R/Apoe$^{-/-}$ mice	Investigator generated	M
Skin fibroblasts from LoxP$^{+/+}$-IGF1R/SM22α-Cre$^{+/-}$/Apoe$^{-/-}$ mice	Investigator generated	M
Lung fibroblasts from LoxP$^{+/+}$-IGF-1R/Apoe$^{-/-}$ mice	Investigator generated	M
Lung fibroblasts from LoxP$^{+/+}$-IGF1R/SM22α-Cre$^{+/-}$/Apoe$^{-/-}$ mice	Investigator generated	M