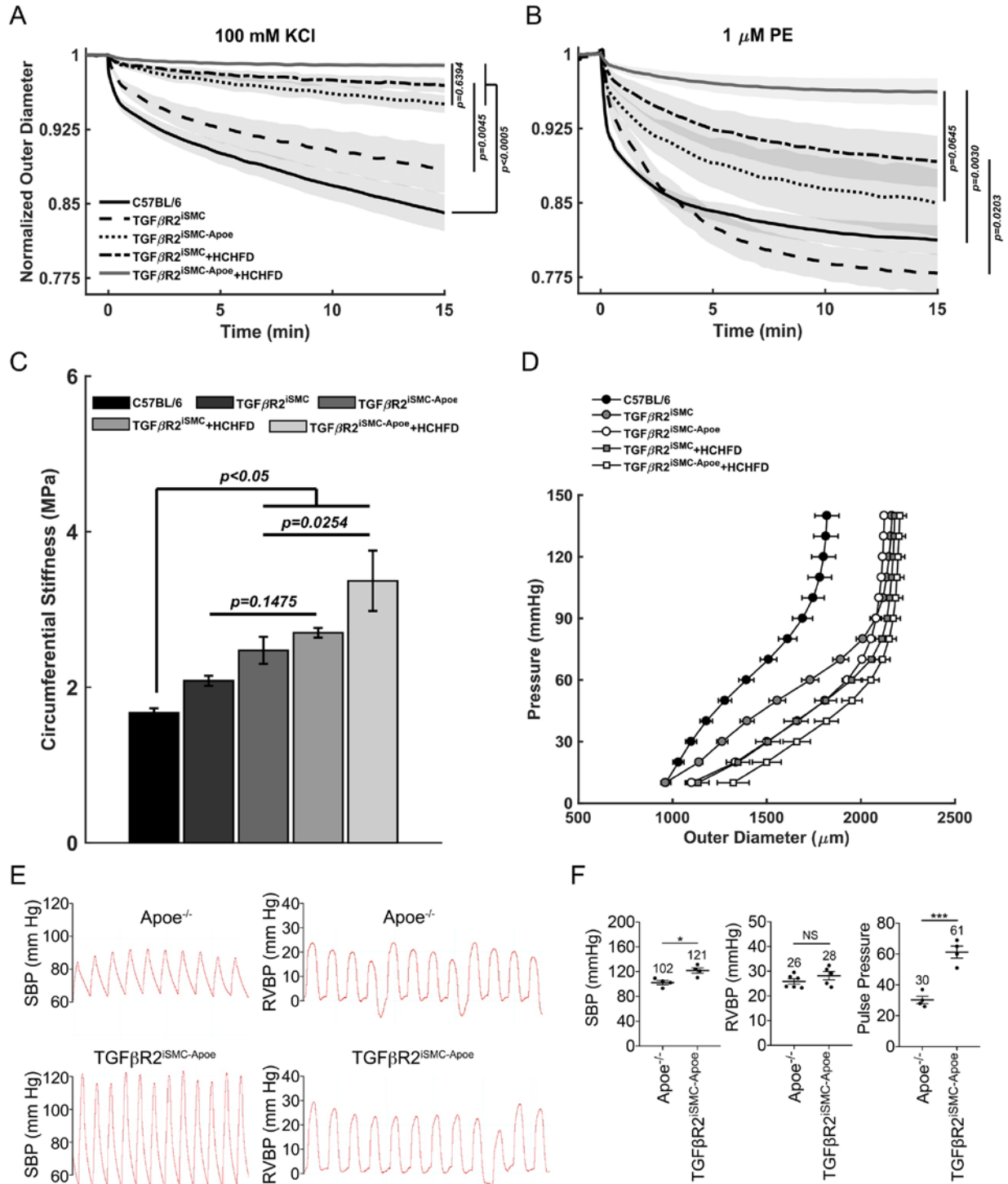


Supplementary Figure 1. Related to Figure 2. Generation and characterization of *Apoe*^{-/-} mice with smooth muscle cell-specific *Tgfbr2* ablation.

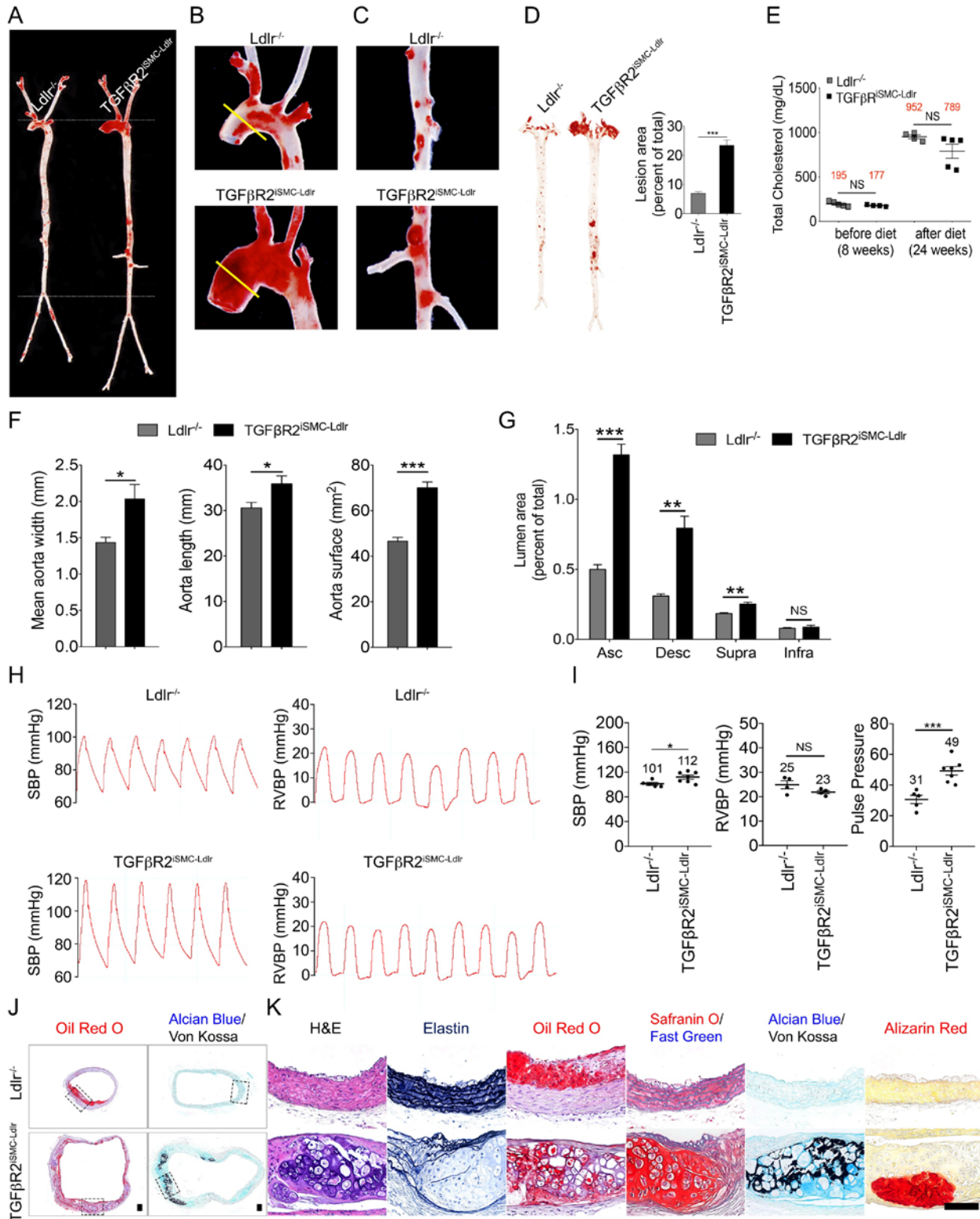
(A) Scheme of the Myh11-CreER^{T2} transgene, *Tgfbr2* floxed alleles, and R26-mTmG reporter constructs. (B) Scheme of Tamoxifen injection (1 mg/day i.p. for 5 days starting at 6 weeks old) and high cholesterol high fat diet (HCHFD) feeding. (C) PCR for *Tgfbr2* exon 2 mRNA expression using medial layers of mouse ascending aorta without adventitia. (D) Representative images of p-Smad2 immunofluorescence staining of *Apoe*^{-/-} and TGFβR2^{iSMC-Apoe} aorta. Endothelial cells are visualized by CD31 (green). L: lumen. M: Media. Nuclei were stained with DAPI (blue). N= 6 mice/group. Scale bar: 10 μm. (E) Aortic smooth muscle cells were isolated from vehicles or tamoxifen treated mice and were treated with TGFβ1 (0.5 ng/ml) for the indicated times and downstream signaling was analyzed by immunoblotting. Blots are representative of 3 independent experiments. (F) Total cholesterol and triglycerides levels from C57BL/6, TGFβR2^{iSMC}, *Apoe*^{-/-}, and TGFβR2^{iSMC-Apoe} mice after 4 months on a HCHFD (*p<0.05; **p<0.01; ***p<0.001; unpaired two-tailed Student's t test). N=3 for C57BL/6 mice; N=4 for TGFβR2^{iSMC} mice; N=10 for *Apoe*^{-/-} mice; and N=11 for TGFβR2^{iSMC-Apoe} mice. (G-I) Aorta surface area, aorta length, and width in C57BL/6, TGFβR2^{iSMC}, *Apoe*^{-/-}, and TGFβR2^{iSMC-Apoe} mice after 0, 2, 4 months on a high cholesterol high fat diet (HCHFD). All data shown as mean ± SEM (*p<0.05; ***p<0.01; ****p<0.001; unpaired two-tailed Student's t test) (for each time point N=3 for C57BL/6 mice; N=3 for TGFβR2^{iSMC} mice; N=11 for *Apoe*^{-/-} mice; and N=11 for TGFβR2^{iSMC-Apoe} mice). (J-L) Lumen, media, and vessel areas of ascending (Asc), descending (Desc), suprarenal (Supra), and infrarenal (Infra) aortas in 24-week-old mice after 4 months of HCHFD. All data shown as mean ± SEM (*p<0.05; ***p<0.01; ****p<0.001; unpaired two-tailed Student's t test) (N=3 for C57BL/6 mice; N=3 for TGFβR2^{iSMC} mice; N=11 for *Apoe*^{-/-} mice; and N=11 for TGFβR2^{iSMC-Apoe} mice). (M) Representative pictures from control and TGFβR2 knockdown HASMCs incubated with Ac-LDL (100 μg/ml) for 72 hrs and stained with Oil Red O. Nuclei were counterstained with haematoxylin. Images are representative of three independent experiments. Scale bar: 100 μm. (N) Control and TGFβR2 knockdown HASMCs were incubated with or without Ac-LDL (100 μg/ml) for 72 hrs. Quantification of cholesterol esters in control and TGFβR2 knockdown HASMCs. Data represent the mean ± SEM and are representative of three experiments in duplicate. (*p<0.05; unpaired two-tailed Student's t test). (O) Cells were treated with control and TGFβR2 shRNA for 4 days and incubated with DiI-Ac-LDL (100 μg/ml) in low LDL media (LPDS) for 0, 1, 4, 8 hrs at 37°C. At the end of labeling period, cells were rinsed with probe-free media and analyzed by FACS. Data represent the mean ± SEM and are representative of three experiments in duplicate. (***p<0.001; unpaired two-tailed Student's t test). (P) Immunohistochemical staining of Perilipin A (lipid droplet) and ApoB (major very-low-density lipoprotein component) in the ascending aortas of *Apoe*^{-/-} and TGFβR2^{iSMC-Apoe} mice after 1 month of high cholesterol high fat diet (N=3 mice/group). L: lumen. M: Media. Nuclei were counterstained with DAPI (blue). Scale bar: 16 μm. (Q) Cross-section images of ascending aorta from *Apoe*^{-/-} and TGFβR2^{iSMC-Apoe} mice 18 hrs after intravenous injection with DiI-LDL (300 μg). Immunofluorescence analysis of DiI-LDL infiltration (N=3 mice/group). I: Intima. L: lumen. M: Media. Nuclei were counterstained with DAPI (blue). Scale bar: 16 μm.



Supplementary Figure 2. Related to Figure 2. Active, passive biomechanical analysis, and blood pressure measurement of C57BL/6, TGF β R2^{ISMIC}, Apoe^{-/-}, and TGF β R2^{ISMIC}-Apoe^{-/-} mice ascending aortas.

(A,B) Time-course of changes in outer diameter of the ascending aorta following *ex vivo* exposure to (A) potassium chloride (KCl) or (B) phenylephrine (PE), with diameter normalized to the group-specific value prior to agonist exposure (time < 0 min). Transmural pressure was maintained at 90 mmHg and axial stretch at group-specific *in vivo* values λ_2^{iv} . Lines denote group means and shaded regions denote \pm SEM. Between-group *p* values are shown for C57BL/6 vs. all mutant groups as well as for mutants on normal vs. high cholesterol high fat diet (HCHF).

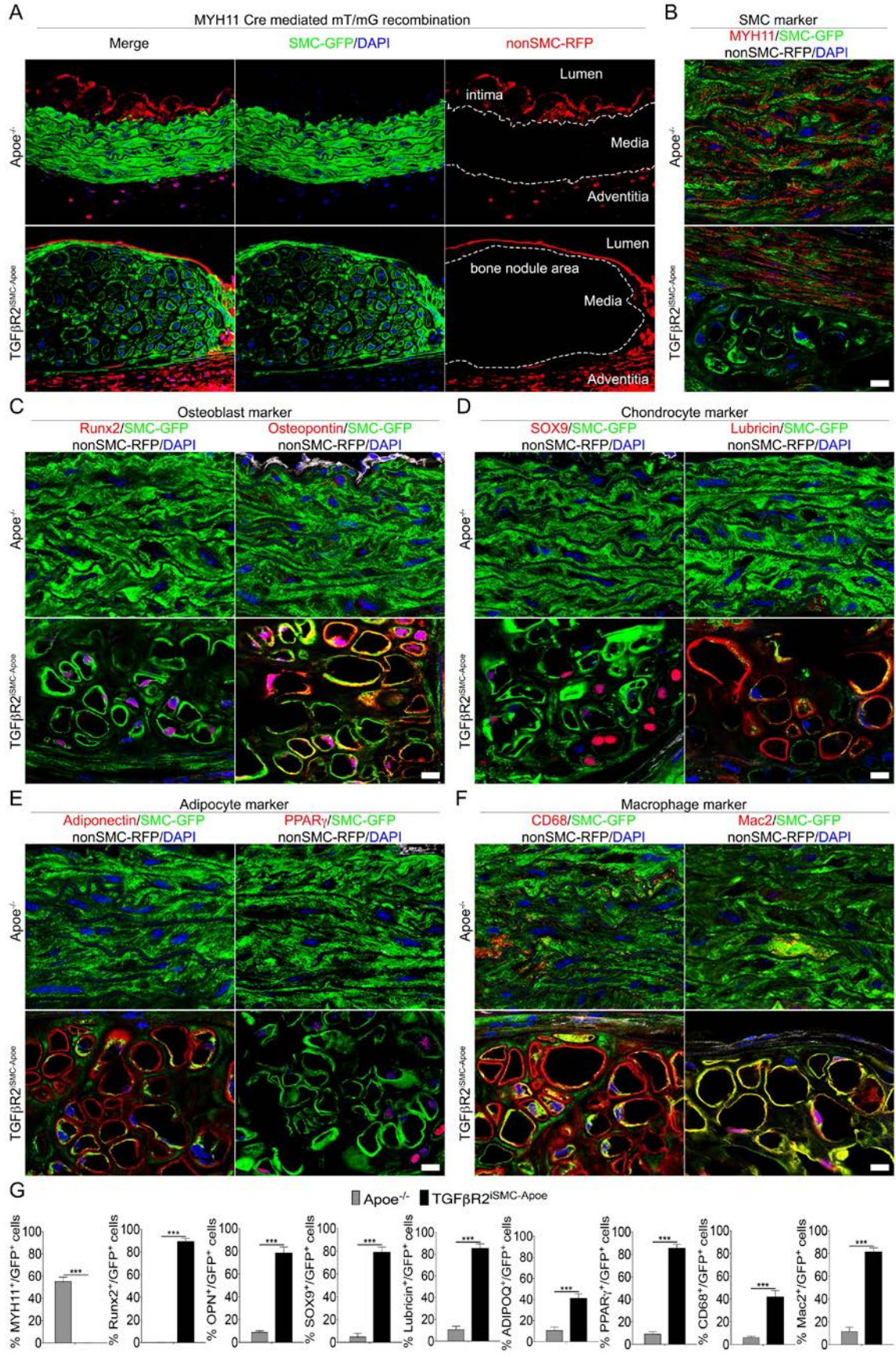
Contractile response to PE is significantly impaired in the $TGF\beta R2^{iSMC}$ group with a HCHFD compared to those on a normal diet. Though change in normalized diameter was not different between $TGF\beta R2^{iSMC-ApoE}$ on a normal diet and HCHFD, differences manifested when comparing contractile induced reductions in circumferential wall stress ($p = 0.0291$). N=5 for C57BL/6 mice; N=5 for $TGF\beta R2^{iSMC}$ mice; N=4 for $TGF\beta R2^{iSMC-ApoE}$ mice; N=5 for $TGF\beta R2^{iSMC}$ mice fed with HCHFD; and N=4 for $TGF\beta R2^{iSMC-ApoE}$ mice fed with HCHFD. (C) Circumferential material stiffness increases with lipid challenge in the $TGF\beta R2^{iSMC-ApoE}$ group alone. Data are represented as mean \pm SEM. Significance was considered when $p < 0.05$. N=5 for C57BL/6 mice; N=5 for $TGF\beta R2^{iSMC}$ mice; N=4 for $TGF\beta R2^{iSMC-ApoE}$ mice; N=5 for $TGF\beta R2^{iSMC}$ mice fed with HCHFD; and N=4 for $TGF\beta R2^{iSMC-ApoE}$ mice fed with HCHFD. (D) Pressure-diameter data from fixed-length cyclic pressurization testing at λ_z^{iv} . The pressure-diameter behavior of the $TGF\beta R2^{iSMC-ApoE} + HCHFD$ group (white squares) loses the characteristic “S”-shape, suggesting that the elastic fibers contribute less. N=5 for C57BL/6 mice; N=5 for $TGF\beta R2^{iSMC}$ mice; N=4 for $TGF\beta R2^{iSMC-ApoE}$ mice; N=5 for $TGF\beta R2^{iSMC}$ mice fed with HCHFD; and N=4 for $TGF\beta R2^{iSMC-ApoE}$ mice fed with HCHFD. (E) Representative record of SBP and RVBP in $ApoE^{-/-}$ and $TGF\beta R2^{iSMC-ApoE}$ mice after 4 months of HCHFD. N=4 mice/group. (F) Summarized data (mean \pm SEM) showing the peak value of SBP, RVBP and pulse pressure.



Supplementary Figure 3. Related to Figure 2 and Figure 3. Smooth muscle cell *Tgfr2* knockout in *Ldlr*^{-/-} background accelerates aneurysm formation and atherosclerosis plaque development.

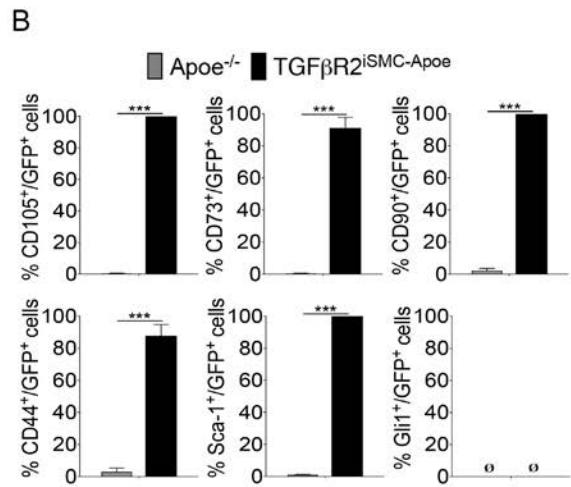
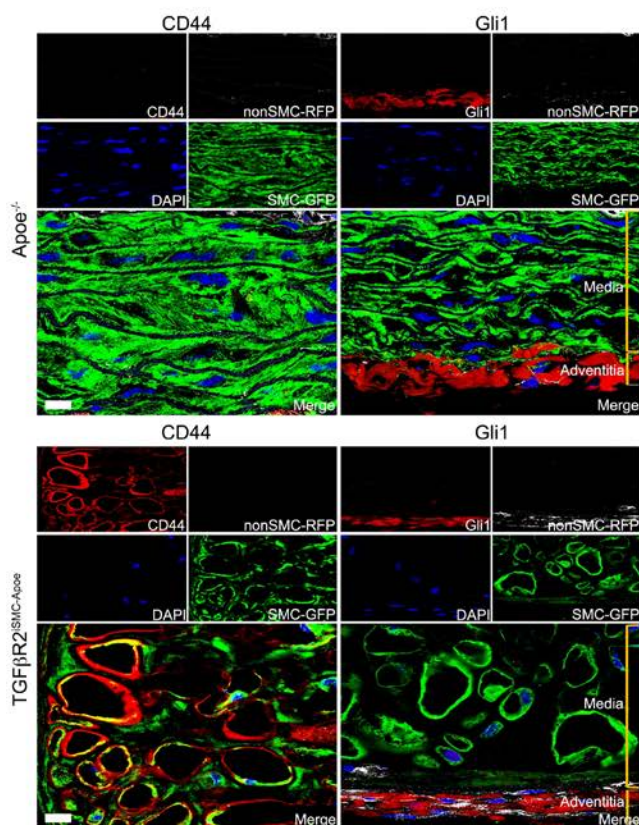
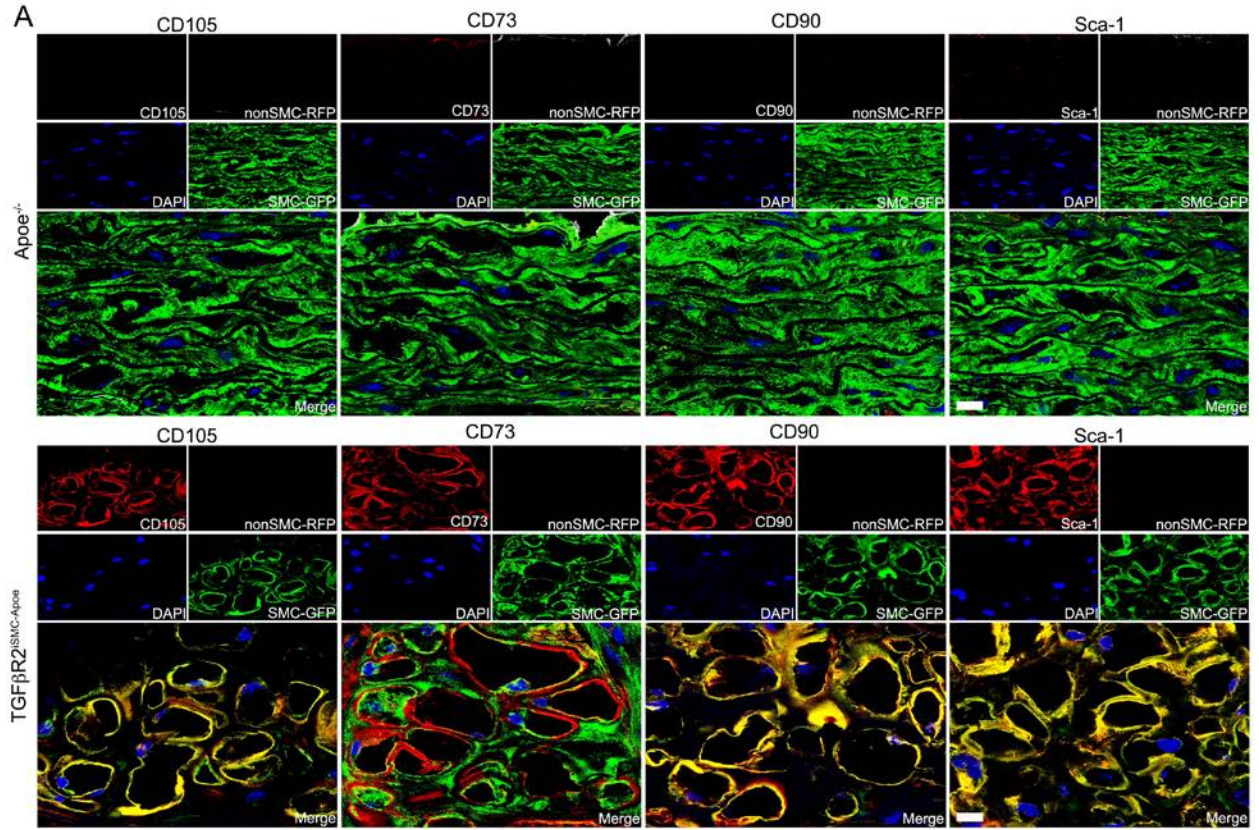
(A-C) Representative photomicrographs of Oil-Red-O stained whole aorta (A), aortic arch (B) or abdominal aorta (C) of *Ldlr*^{-/-} and *TGFβR2*^{iSMC-Ldlr} mice after 4 months of high cholesterol high fat diet (HCHFD). N=3 mice/group. (D) (left) Microphotographs of en face aortas from *Ldlr*^{-/-} and *TGFβR2*^{iSMC-Ldlr} mice after 4 months of HCHFD

stained with Oil-Red-O. (right) Lesion area quantification: % lesion area refers to Oil-Red-O stained as a % of the total aortic surface. All data shown as mean \pm SEM (\emptyset : not detected; *** $p < 0.001$; unpaired two-tailed Student's t test) (N=10 mice/group). (E) Total cholesterol levels from *Ldlr*^{-/-} and TGF β 2^{iSMC-Ldlr} mice after 4 months on a HCHFD (NS: not significant; unpaired two-tailed Student's t test). N=4 mice/group. (F) Aorta surface area, aorta length, and width in *Ldlr*^{-/-} and TGF β 2^{iSMC-Ldlr} mice after 4 months on a high cholesterol high fat diet (HCHFD). All data shown as mean \pm SEM (* $p < 0.05$; *** $p < 0.001$; unpaired two-tailed Student's t test) (N=10 mice/group). (G) Lumen areas of ascending (Asc), descending (Desc), suprarenal (Supra), and infrarenal (Infra) aortas in 24-week-old mice after 4 months of HCHFD. All data shown as mean \pm SEM (NS: not significant; ** $p < 0.01$; *** $p < 0.001$; unpaired two-tailed Student's t test) (N=10 mice/group). (H) Representative record of SBP and RVBP in *Ldlr*^{-/-} and TGF β 2^{iSMC-Ldlr} mice. (I) Summarized data (mean \pm SEM) showing the peak value of SBP, RVBP and pulse pressure. For SBP and pulse pressure measurement N=5 for *Ldlr*^{-/-} mice; and N=7 for TGF β 2^{iSMC-Ldlr} mice; for RVBP measurement N=4 mice/group. (J) Histologic analysis of mouse ascending aortas dissected from 24-week-old *Ldlr*^{-/-} and TGF β 2^{iSMC-Ldlr} mice after 4 months of high cholesterol high fat diet (HCHFD). Representative low-magnification images of Oil-Red-O and Alcian Blue (cartilage)/Von Kossa (bone) stained mouse ascending aortas. N=6 mice/group. Scale bar: 200 μ m. (K) Representative images of H&E, Elastin, Oil-Red-O, Safranin O (cartilage)/Fast Green, Alcian Blue (cartilage)/Von Kossa (bone), and Alizarin Red (calcium)-stained mouse ascending aortas from *Ldlr*^{-/-} and TGF β 2^{iSMC-Ldlr} mice (N=6 mice/group). Scale bar: 50 μ m.



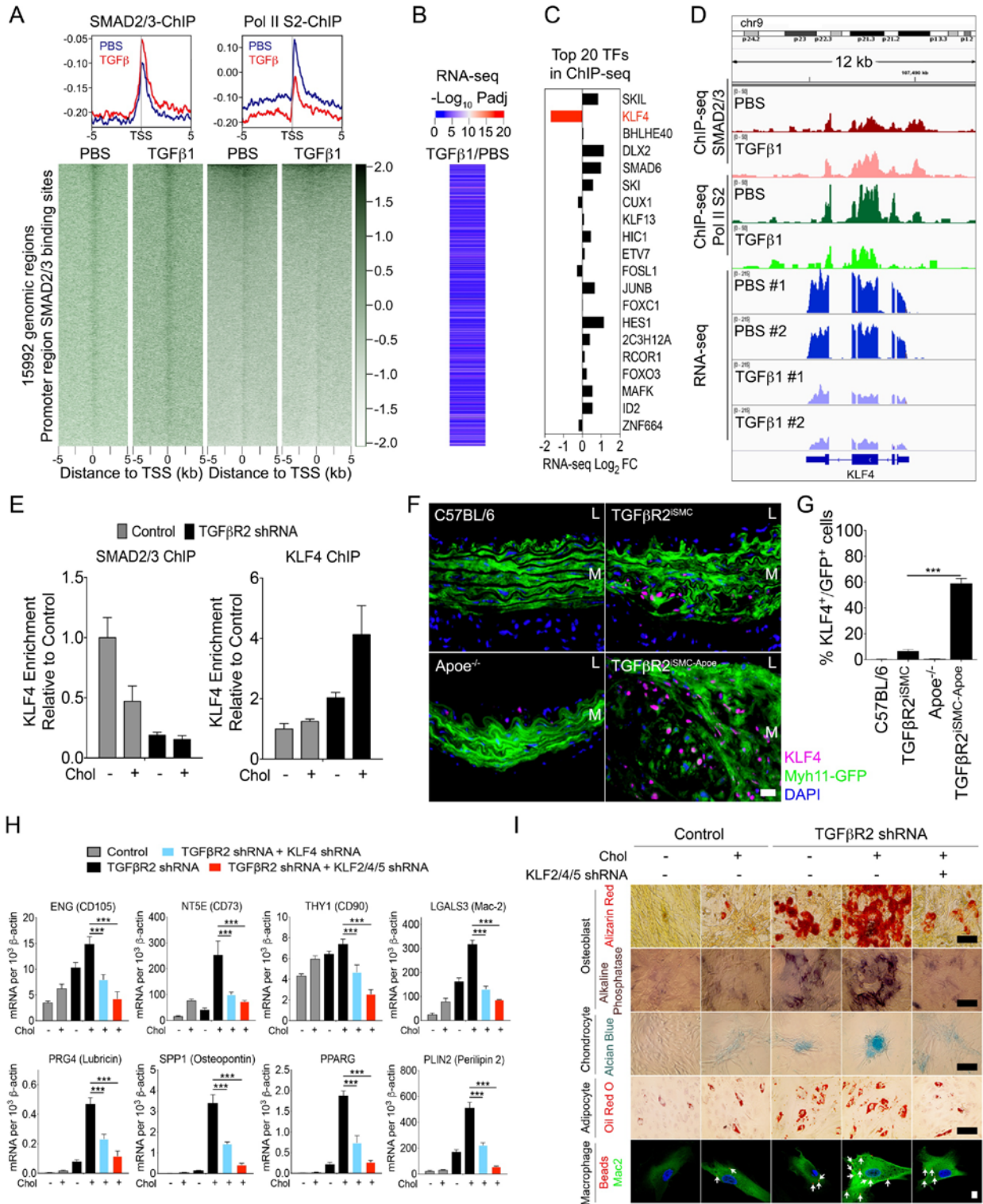
Supplementary Figure 4. Related to Figure 3 and Figure 4. SMCs express smooth muscle cell, chondrocyte, adipocyte, osteoblast, and macrophage lineage markers in $TGF\beta R2^{iSMC-ApoE}$ mice after 4 months of high cholesterol high fat diet.

(A) Z-stack confocal images of ascending aorta cross sections showing tamoxifen-dependent loss of RFP (red) and induction of GFP (green) expression by vessel wall cells selectively within the media, but not the neointima or adventitia, with DAPI-labeled nuclei (blue) in overlays (bar=10 μ m). Please note: only GFP⁺ cell but not RFP⁺ cells in the bone nodule area. N=4 mice/group. (B) Immunohistochemical staining of smooth muscle cell lineage-specific marker (MYH11) in the ascending aortas of *ApoE*^{-/-} and $TGF\beta R2^{iSMC-ApoE}$ mice after 4 months of high cholesterol high fat diet using SP8 confocal microscope. (C-F) Immunohistochemical staining of lineage-specific markers (Osteoblast, Chondrocyte, Adipocyte, and Macrophage) in the ascending aortas of *ApoE*^{-/-} and $TGF\beta R2^{iSMC-ApoE}$ mice after 4 months of high cholesterol high fat diet using SP8 confocal microscope (N=6 mice/group). Scale bar: 10 μ m. (G) Quantification of the number of ascending aortic media smooth muscle cells expressing GFP (MYH11⁺) and SMC, adipocyte, osteoblast, chondrocyte, and macrophage lineage markers (***)p<0.001; unpaired two-tailed Student's t test). N=6 mice/group.



Supplementary Figure 5. Related to Figure 4. Smooth muscle cells express stem cell markers in $TGF\beta R2^{iSMC-ApoE}$ mice after 4 months of high cholesterol high fat diet,

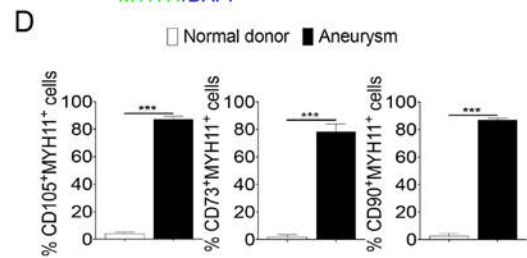
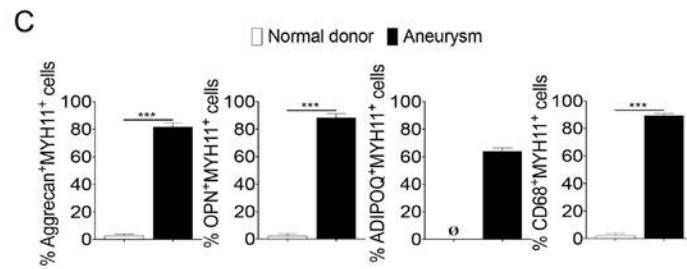
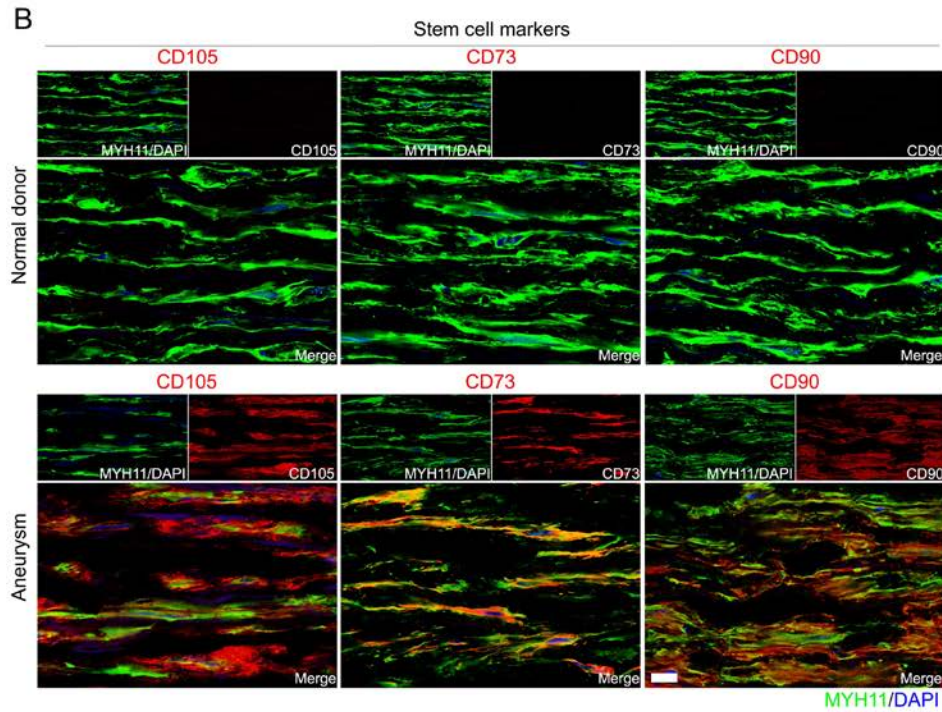
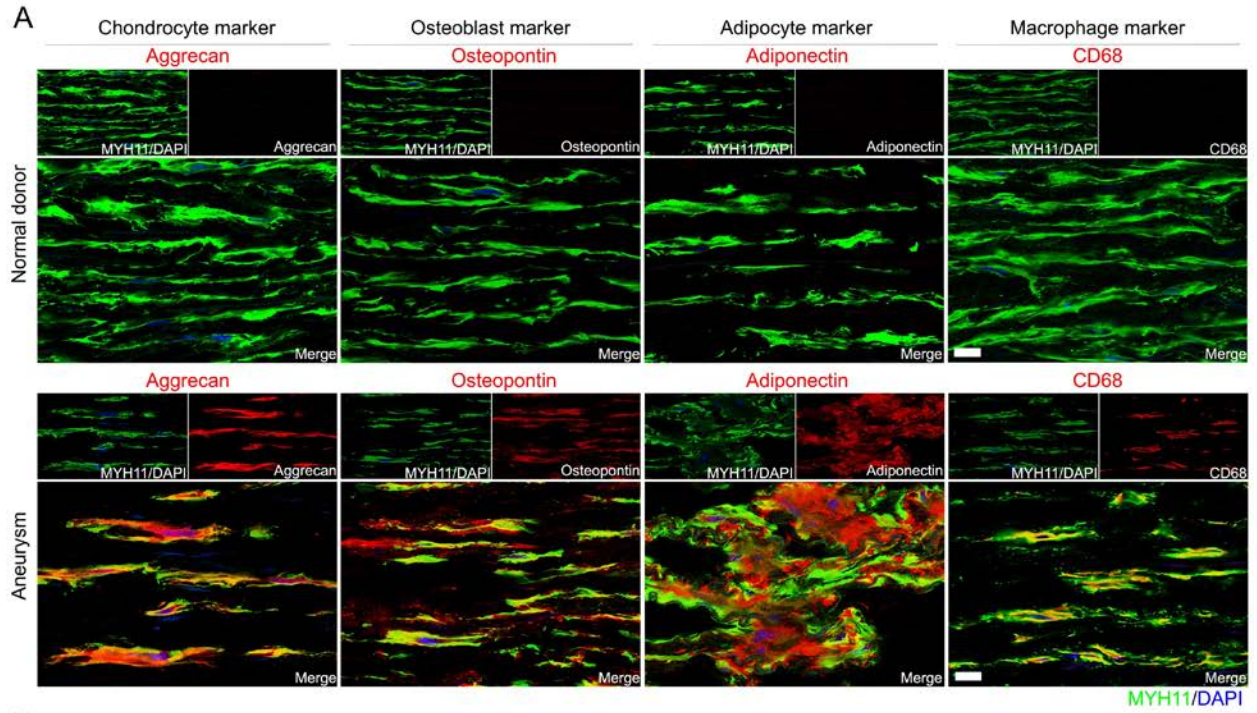
(A) Immunohistochemical staining of stem cell markers (CD105, CD73, CD90, Sca-1, CD44, Gli1) in the ascending aortas of $ApoE^{-/-}$ and $TGF\beta R2^{iSMC-ApoE}$ mice after 4 months of high cholesterol high fat diet using SP8 confocal microscope. N=6 mice/group. Scale bar: 10 μ m. (B) Quantification of the number of ascending aortic media smooth muscle cells expressing GFP (MYH11⁺) and stem cell markers (\emptyset : not detected; unpaired two-tailed Student's t test). See also Figure 4.



Supplementary Figure 6. Related to Figure 7. KLF4 is the key regulator controlling TGF β inhibition and Cholesterol responsive SMC transition.

(A) Enrichment of genomic signals on 15,992 genes identified from running differential expression analysis for bulk RNA-Seq comparison of TGF β treated vs. control groups. (Upper) Genome-wide occupancy of SMAD2/3 and Pol2-Ser2p at strong SMAD2/3 peaks in control (blue) and TGF β treated (red) SMCs. The average signal within 5 kb genomic regions flanking the center of TSS is shown. (Lower) Heatmaps of SMAD2/3 ChIP-seq and Pol2-Ser2p

read counts for targets annotated TSS regions in the strong-binding group in control and TGF β treated SMCs. Promoters are sorted in order of their SMAD2/3 intensity values. (B) Gene expression of upregulated and downregulated genes in response to TGF β treatment. (C) Top 20 up and down-regulated differentially expressed transcription factors from ChIP-seq list. (D) Genomic coverage tracks depicting binding profile of SMAD2/3 and Pol2-Ser2p in KLF4 locus in control and TGF β treated SMCs. RNA-seq analyses were also included with two biological repeats. Coverage tracks were normalized based on the number of reads. (E) Enrichment levels of SMAD2/3 and KLF4 by ChIP-qPCR at KLF4 locus in control and TGF β R2 KD SMCs in the presence or absence of CD-cholesterol. Data represent mean \pm SEM of technical triplicates. Enrichment is shown as ChIP-to-input ratio normalized to the enrichment at the non-bound regions. (F) Histological analysis of KLF4 expression in mouse ascending aortas after 4 month of high cholesterol high fat diet. Nuclei were stained with DAPI (blue). Scale bar: 16 μ m. (G) Quantification of the number of ascending aortic media smooth muscle cells expressing GFP (green) and KLF4 (magenta) (***) $p < 0.001$; unpaired two-tailed Student's t test). (N=6 for C57BL/6 mice; N=6 for TGF β R2^{iSMC} mice; N=8 for *ApoE*^{-/-} mice; and N=8 for TGF β R2^{iSMC-ApoE} mice). (H) Expression of MSC markers (ENG, NT5E, THY1) and osteoblast (SPP1), chondrocyte (PRG4), adipocyte (PPARG, PLIN2) and macrophage (LGALS3) markers in control, TGF β R2 KD, TGF β R2/KLF4 KD, and TGF β R2/KLF2/KLF4/KLF5 KD SMCs with or without CD-cholesterol. Data represent the mean \pm SEM and are representative of three experiments in duplicate. (I) Control, TGF β R2 KD, and TGF β R2/KLF2/KLF4/KLF5 KD SMCs with or without CD-cholesterol cells possess in vitro trilineage differentiation capacity toward osteoblast (Alizarin Red, Alkaline Phosphatase), chondrocyte (Alcian Blue), adipocyte (Oil Red O), and macrophage beads uptake activity. Scale bar: 100 μ m for trilineage differentiation images. Scale bar: 8 μ m for macrophage beads uptake images. Experiments were repeated three times.



Supplementary Figure 7. Related to Figure 1 and Figure 4. Smooth muscle cell express mesenchymal stem cell, bone, cartilage, and adipocyte lineage markers in older patients with aneurysm.

(A-B) Histological analysis of human ascending aortas with lineage markers (Aggrecan, Osteopontin, Adiponectin, CD68) and stem cell markers (CD105, CD73, CD90) from normal donors (N=6) and aneurysm patients (N=6).

Nuclei were stained with DAPI (blue). Scale bar: 16 μm . (C-D) Quantification of the number of ascending aortic media smooth muscle cells expressing MYH11 (green) and lineage and stem cell markers (\emptyset : not detected;

*** $p < 0.001$; unpaired two-tailed Student's t test). See also Figure 1 and Figure 4.

Table S3. List of Primers used for qRT-PCR. Related to: Quantitative Reverse Transcription PCR” in the STAR Methods.

| Gene Symbol | QIAGEN Catalog Number | RefSeq Accession | Reference Positions | Band Size (bp) |
|---------------------|--|------------------------|---------------------|-----------------------|
| ACTB | PPH00073G | NM_001101 | 730 | 174 |
| Actb | F: CCAGTTGGTAACAATGCCATGT R: GGCTGTATTCCCCTCCATCG | | | |
| ENG | PPH01140G | NM_000118 | 1381 | 116 |
| KLF2 | PPH02566A | NM_016270 | 1128 | 164 |
| KLF4 | PPH18388A | NM_004235 | 2002 | 151 |
| KLF4 (specific) | F: CTTATAACTTCCTTCGCTACAGCC R: GCCGAGTTTGTGATTTAGCTG | | | |
| KLF4 (non-specific) | F: ACAGAGTCTCCCTATATTGACCA R: AGGTAGCTCACAACATAATGCCA | | | |
| KLF5 | PPH00434A | NM_001730 | 996 | 191 |
| LGALS3 | PPH01754G | NM_002306 | 581 | 62 |
| NR1H3 | PPH01276C | NM_005693 | 1504 | 76 |
| NT5E | PPH12908E | NM_002526 | 3559 | 63 |
| PLIN2 | PPH02583A | NM_001122 | 1213 | 142 |
| PPARG | PPH02291G | NM_015869 | 1287 | 93 |
| PRG4 | PPH06096E | NM_005807 | 3453 | 131 |
| SPP1 | PPH00582E | NM_000582 | 892 | 88 |
| Tgfr2 (exon2) | F: GACCATCCATCCACTGAAACATTTTAAC R: GACTTCATGCGGCTTCTCACAG | | | |
| THY1 | PPH02406G | NM_006288 | 1759 | 134 |
| | | <u>TaqMan Assay ID</u> | | Band Size (bp) |
| Cd44 | | <u>Mm01277161_m1</u> | | 63 |
| Col2a1 | | <u>Mm01309565_m1</u> | | 66 |
| Eng | | <u>Mm00468252_m1</u> | | 77 |
| Klf2 | | <u>Mm00500486_g1</u> | | 75 |
| Klf4 | | <u>Mm00516104_m1</u> | | 77 |
| Klf5 | | <u>Mm00456521_m1</u> | | 140 |
| Lgals3 | | <u>Mm00802901_m1</u> | | 108 |
| Ly6a | | <u>Mm00726565_s1</u> | | 92 |
| Myh11 | | <u>Mm00443013_m1</u> | | 92 |
| Nt5e | | <u>Mm00501910_m1</u> | | 77 |
| Plin1 | | <u>Mm00558672_m1</u> | | 89 |
| Pparg | | <u>Mm00440940_m1</u> | | 63 |
| Prg4 | | <u>Mm01284582_m1</u> | | 63 |
| Runx2 | | <u>Mm00501584_m1</u> | | 91 |
| Sox9 | | <u>Mm00448840_m1</u> | | 101 |
| Spp1 | | <u>Mm00436767_m1</u> | | 114 |
| Thy1 | | <u>Mm00493681_m1</u> | | 68 |