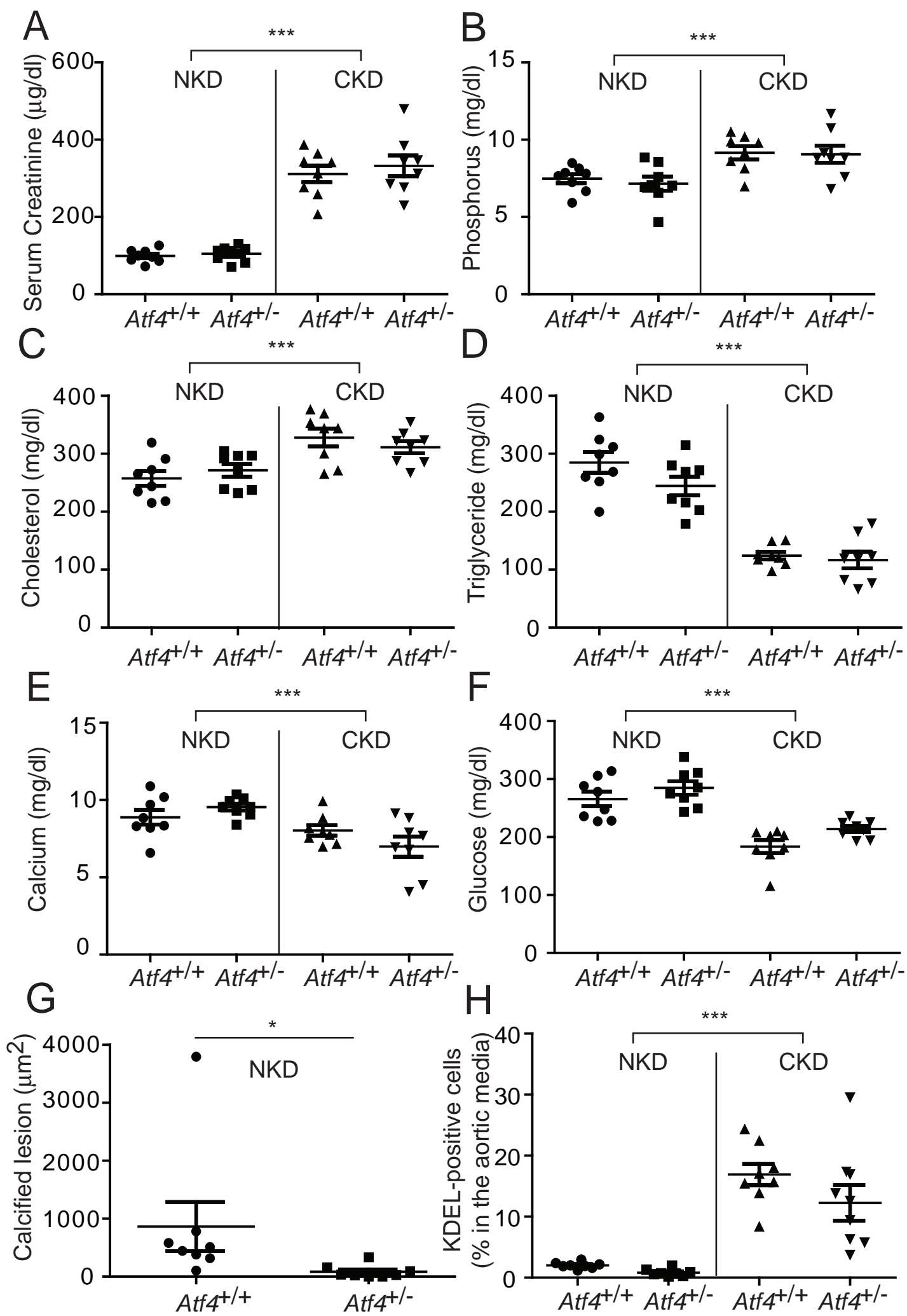


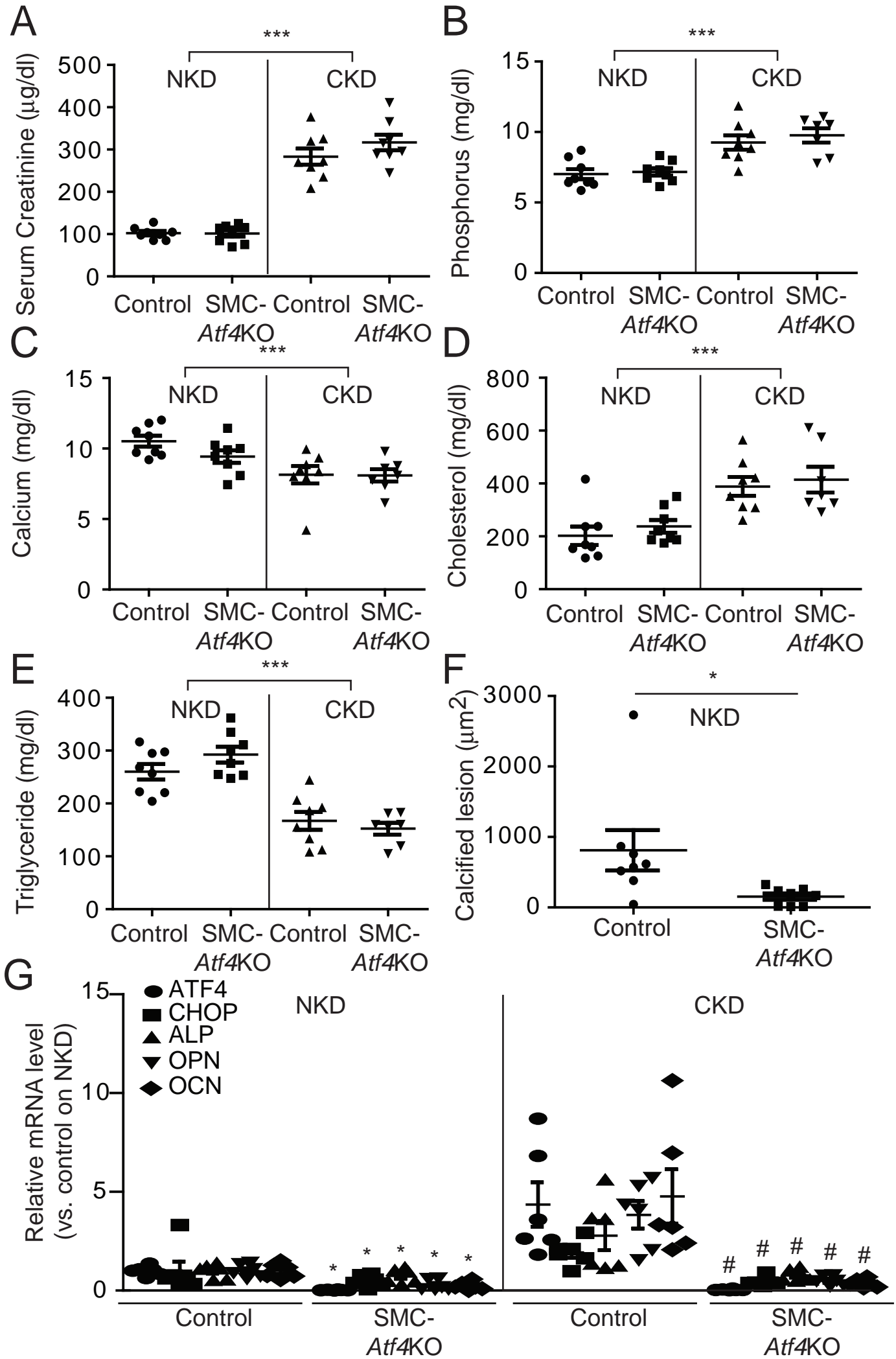
Supplemental Table I. DNA clones isolated by yeast-two hybrid screening.

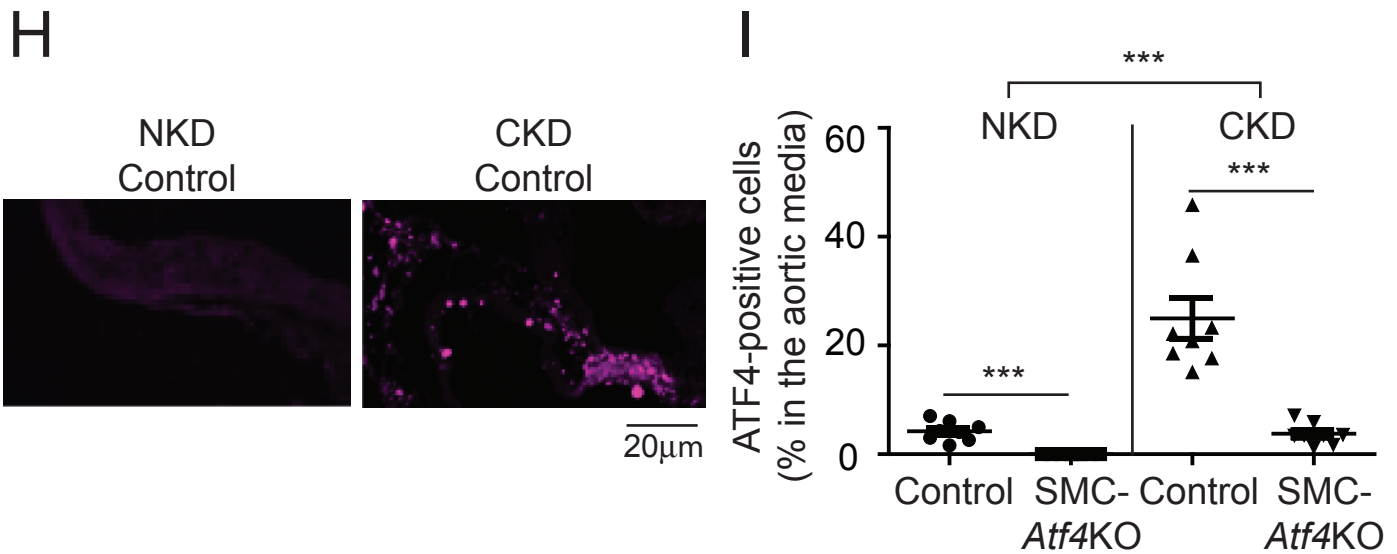
	Gene Symbol	Gene Name	# of Clone
1	Cebpb	CCAAT/enhancer binding protein (C/EBP), beta	6
2	Serp2	stress-associated endoplasmic reticulum protein family member 2	5
3	Commd2	COMM domain containing 2	4
4	Eif3a	eukaryotic translation initiation factor 3, subunit A	2
5	Cebpg	CCAAT/enhancer binding protein (C/EBP), gamma	2
6	Nrf2	Nuclear factor-like 2	2
7	Cox2	cytochrome c oxidase subunit 2	2
8	Glyr1	glyoxylate reductase 1 homolog	2
9	Vkt6	similar to <i>S. cerevisiae</i> VKT6 (YKL196C) synaptobrevin v-SNARE	2
10	Eno1	enolase 1, (alpha)	2
11	Psmb1	proteasome subunit, beta type, 1	2
12	Aup1	ancient ubiquitous protein 1	1
13	Ttc39c	tetratricopeptide repeat domain 39C	1
14	Ybx1	Y box binding protein 1	1
15	Cdc34	cell division cycle 34	1
16	Ctsd	cathepsin D	1
17	Txlng	taxilin gamma	1
18	Fam96a	family with sequence similarity 96, member a	1
19	Cmpk1	cytidine monophosphate (UMP-CMP) kinase 1	1
20	Pcna	proliferating cell nuclear antigen	1
21	Rnf167	ring finger protein 167	1
22	Lgal3	lectin, galactoside-binding, soluble, 3	1
23	Bsn	bassoon	1
24	Lgals1	lectin, galactoside-binding, soluble, 1	1
25	Neurl2	neuralized-like 2	1
26	Snap200	small nuclear ribonucleoprotein 200kDa	1
27	Ykt6	palmitoyltransferase YKT6	1
28	Pdia6	protein disulfide isomerase family A, member 6	1

The cDNA library was generated from mRNA isolated from VSMCs treated with 200  $\mu$ M stearic acid for 24 hours. ATF4-pGBKT7 was used as a bait.

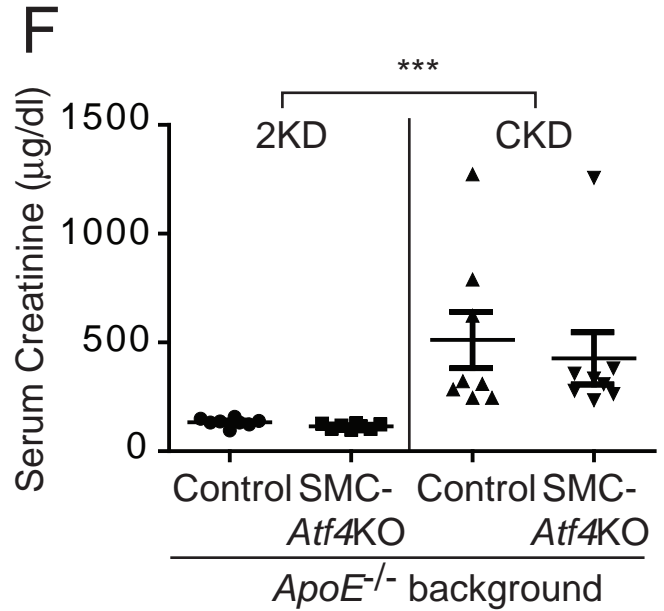
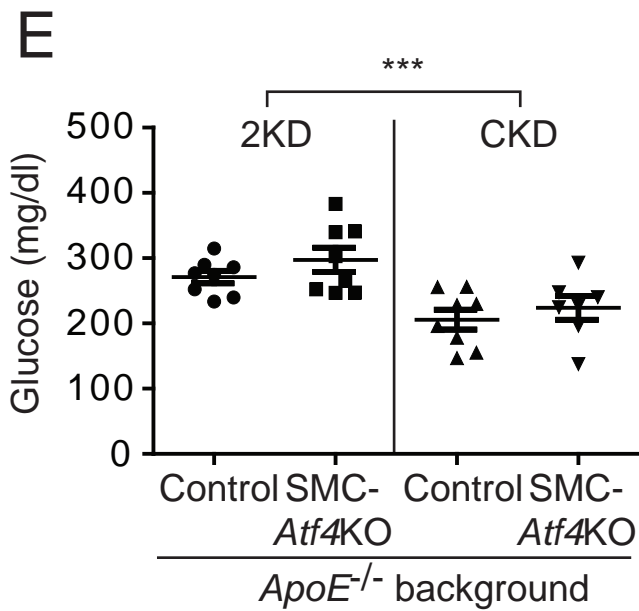
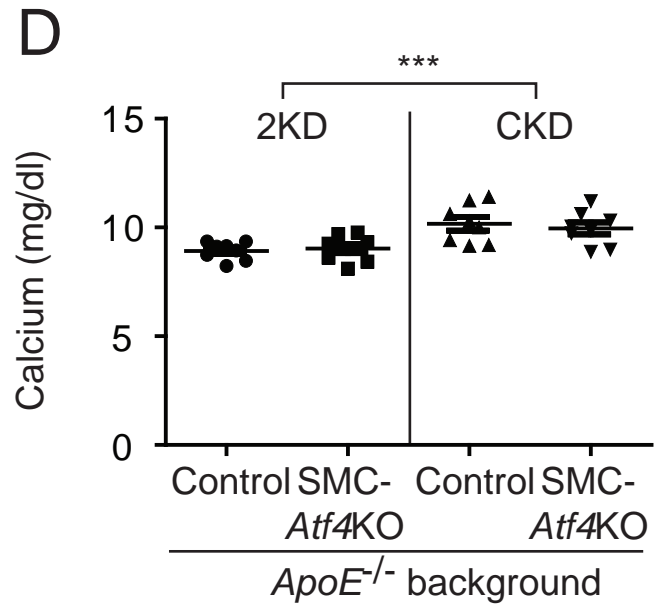
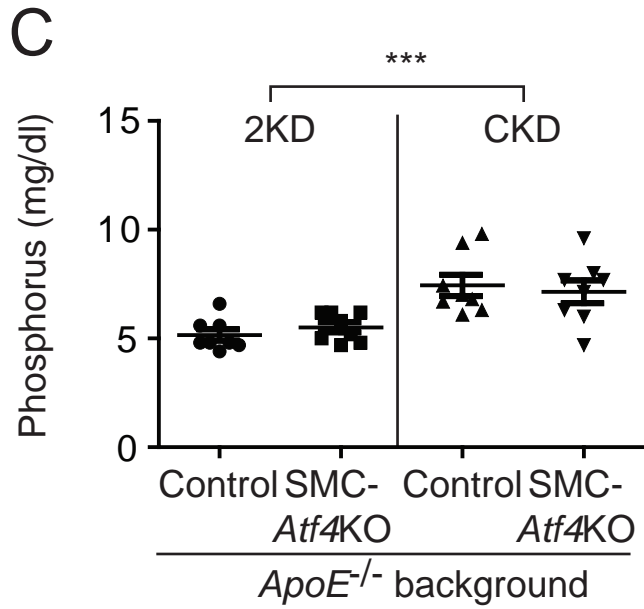
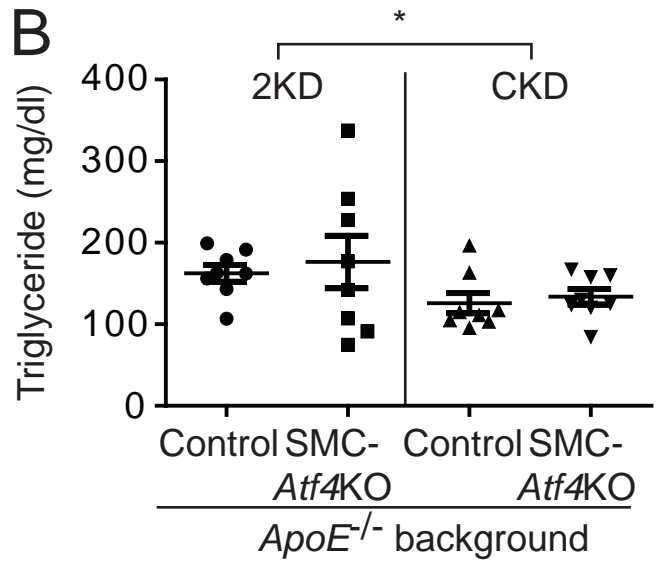
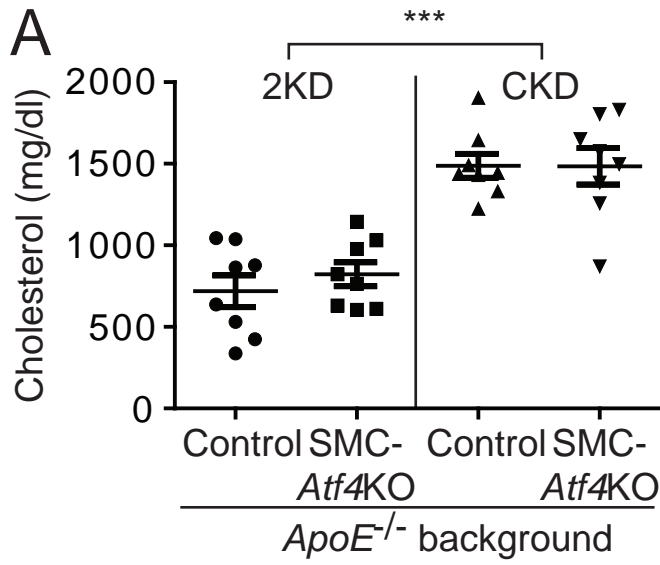


**Supplemental Figure 1. Serum and histological parameters in DBA/2J *Atf4*<sup>+/-</sup> mice under NKD and CKD.** A) Creatinine B) phosphorus, C) cholesterol, D) triglyceride, E) calcium and F) glucose in the serum of DBA/2J *Atf4*<sup>+/+</sup> and *Atf4*<sup>+/-</sup> mice (N=8) under NKD and CKD. Mice were subjected to either sham operation for NKD or 5/6 nx for CKD. The mice were sacrificed 12 weeks after the surgeries after a 4 hour fasting. Serum creatinine levels were analyzed by LC-MS/MS. Other parameters were analyzed with colorimetric assays. G) Expanded graph for calcified lesions in *Atf4*<sup>+/-</sup> mice under NKD. H) Immunofluorescence analysis of KDEL in the aortic media of *Atf4*<sup>+/-</sup> mice. Two-way ANOVA was used for comparison between NKD and CKD. Two-tailed Student's t-test was used for graph G. \*\*\*P<0.001

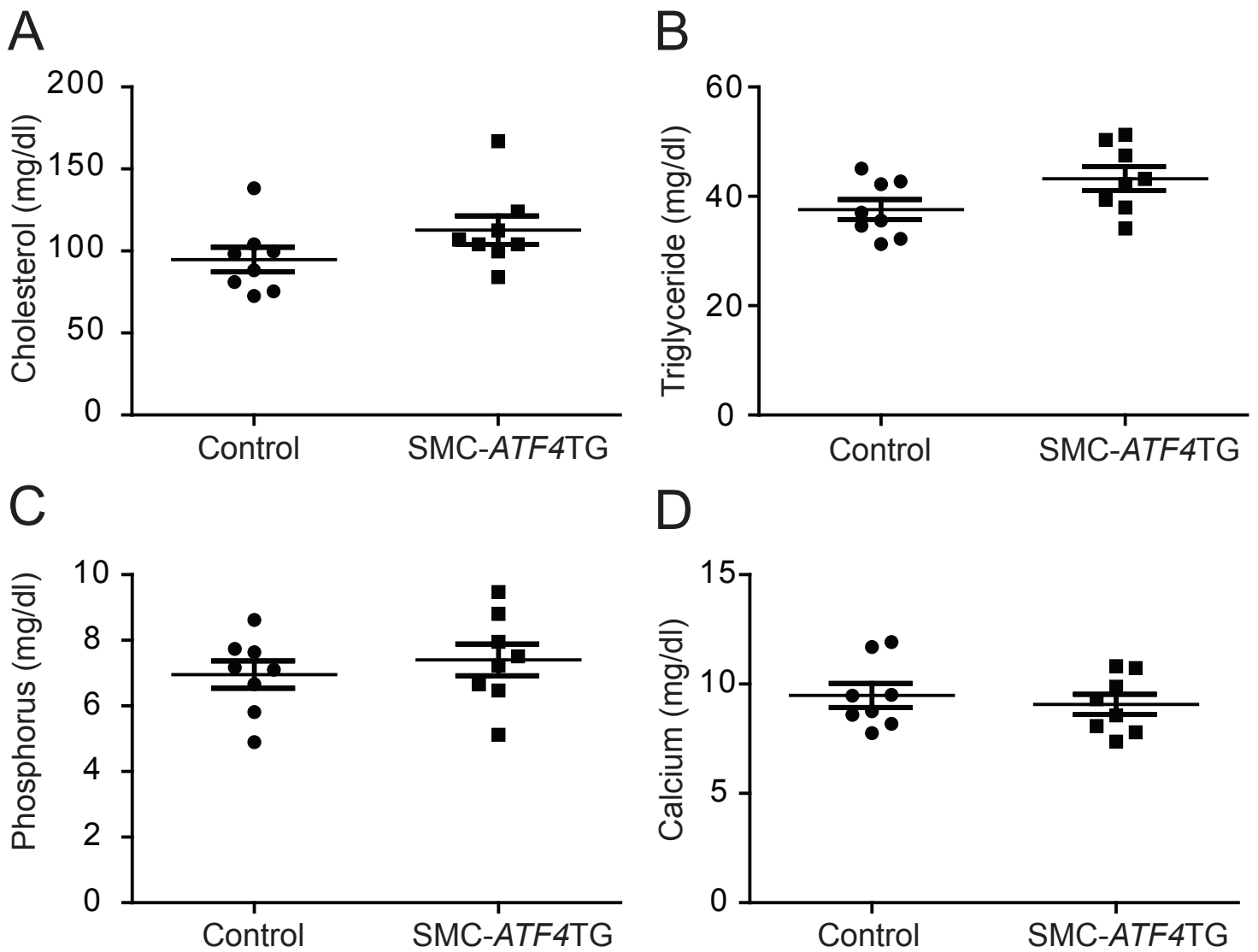




**Supplemental Figure 2. Parameters in DBA/2J SMC-*Atf4* KO mice under NKD and CKD.** A) Creatinine B) phosphorus, C) calcium, D) cholesterol and E) triglyceride in the serum of DBA/2J SMC-*Atf4* KO and control mice (N=8) under NKD and CKD. 5-week-old male mice were injected with either vehicle or 1 mg tamoxifen for 5 consecutive days. 8-week-old control and SMC-*Atf4* KO mice (N=8) were subjected to either sham operation for NKD or 5/6 nx for CKD. The mice were sacrificed 12 weeks after the surgeries after a 4-hour fasting. F) Expanded graph for calcified lesions in aortas of SMC-*Atf4* KO mice under NKD. Two-way ANOVA was used for comparison between NKD and CKD. G) Levels of ER stress and osteogenic markers in the medial layer of aortas of SMC-*Atf4* KO mice. H and I) Immunofluorescence analysis (10x) of ATF4 in the aortic media of control and SMC-*Atf4* KO mice. Two-way ANOVA was used for comparison between NKD and CKD. \*\*\*P<0.001.

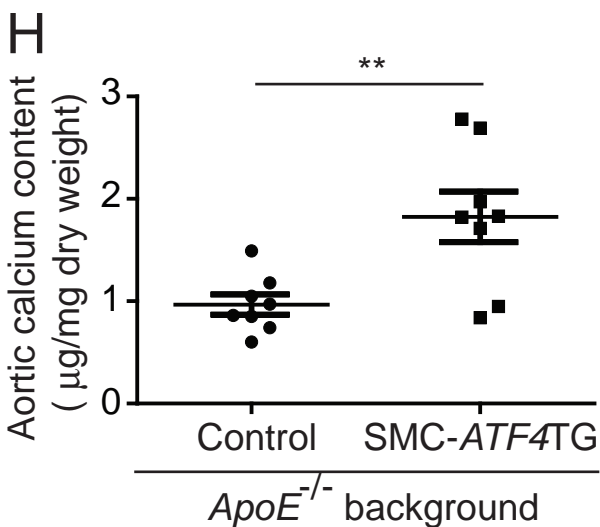
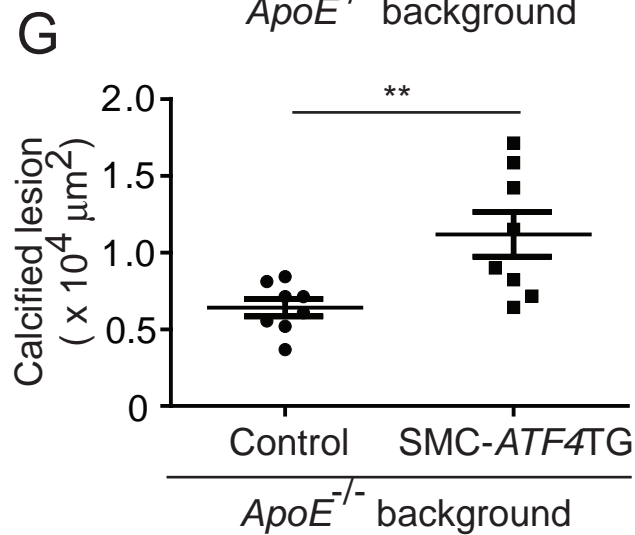
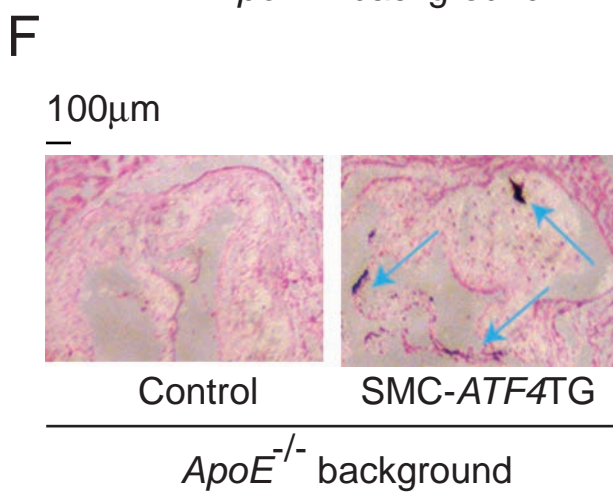
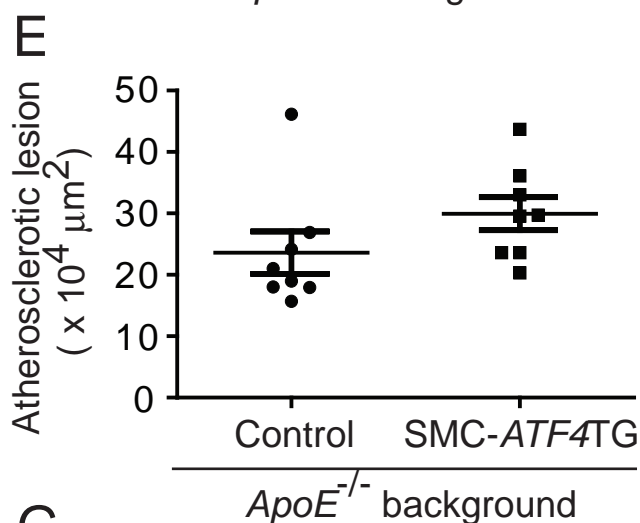
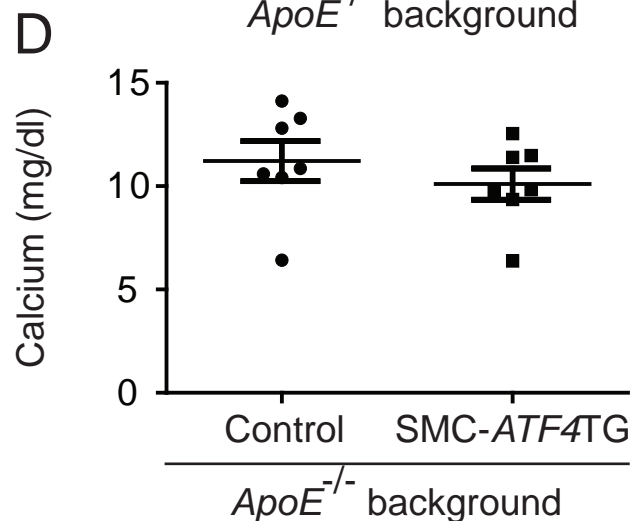
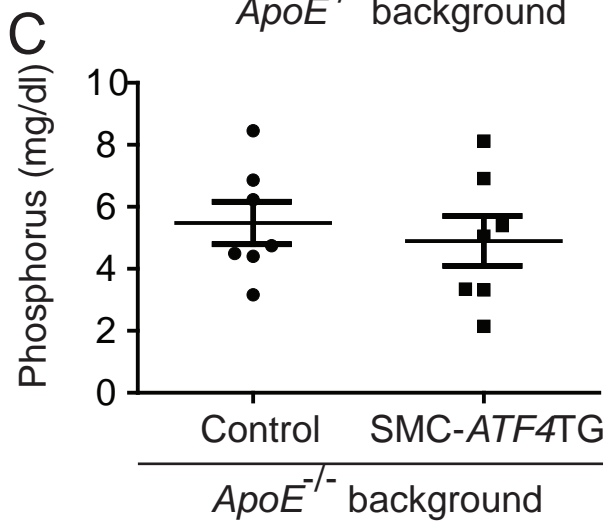
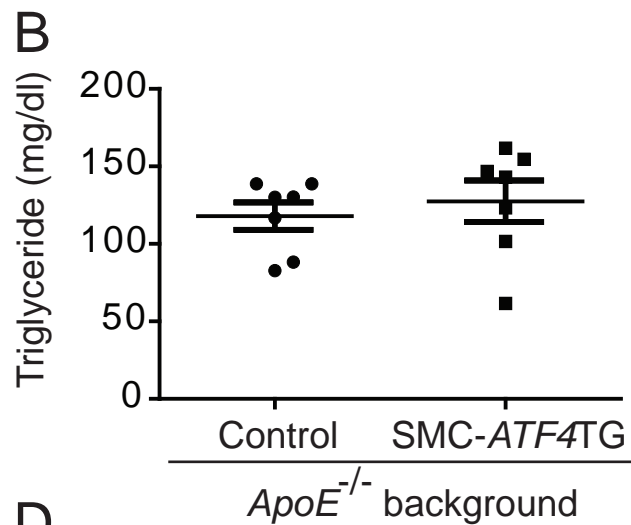
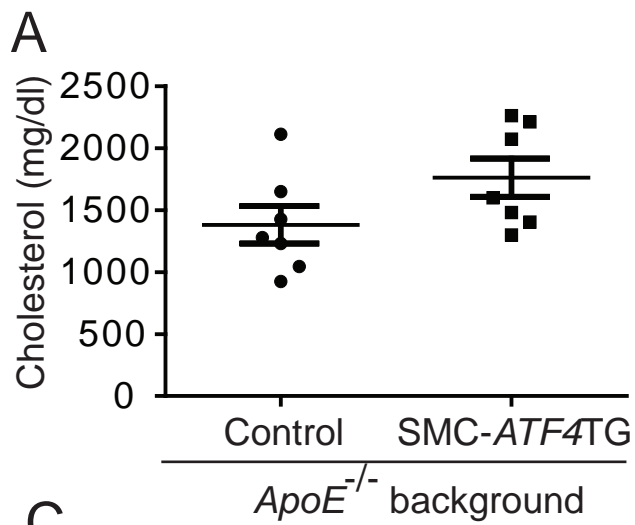


**Supplemental Figure 3. Serum parameters in SMC-Atf4 KO; ApoE<sup>-/-</sup> mice under NKD and CKD.** A) Cholesterol, B) triglyceride, C) phosphorus, D) calcium, E) glucose and F) creatinine in the serum of SMC-Atf4 KO; ApoE<sup>-/-</sup> and control ApoE<sup>-/-</sup> mice (N=8) under NKD and CKD. 5-week-old male mice were injected with either vehicle or 1 mg tamoxifen for 5 consecutive days. 8-week-old control and SMC-Atf4 KO mice (N=8) were subjected to either sham operation for NKD or 5/6 nx for CKD. The mice were sacrificed 12 weeks after the surgeries after a 4-hour fasting. Two-way ANOVA was used for comparison between NKD and CKD. \*\*\*P<0.05 and \*\*\*P<0.001.



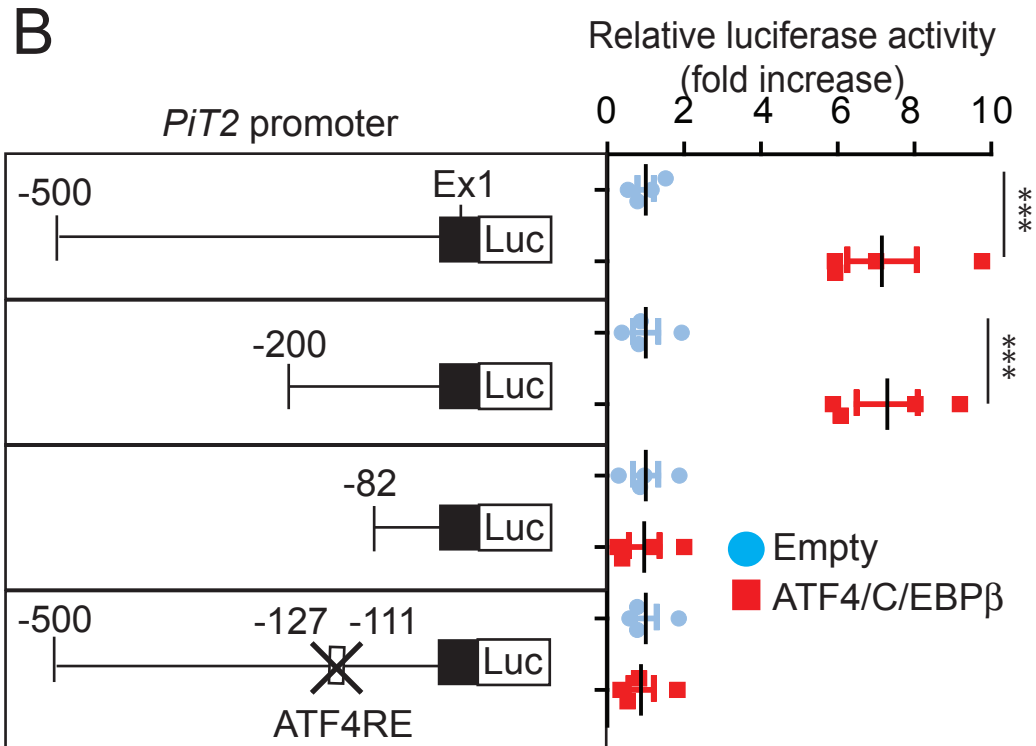
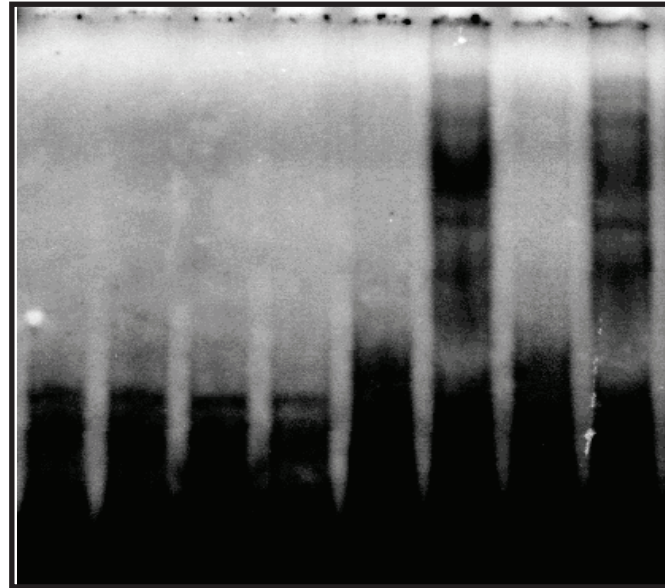
**Supplemental Figure 4. Serum parameters in DBA/2J SMC-ATF4 TG mice.** A) Cholesterol, B) triglyceride, C) phosphorus and D) calcium in the serum of SMC-ATF4 TG mice (N=8) under NKD. The mice were sacrificed at 18 weeks of age after a 4-hour fasting. E) Representative photographs (10x) of the lesions of aortic sinuses stained with von Kossa. D) Quantitative analysis of calcified lesions in the aortic sinus. Two-tailed Student's t-test was used for statistical analysis.



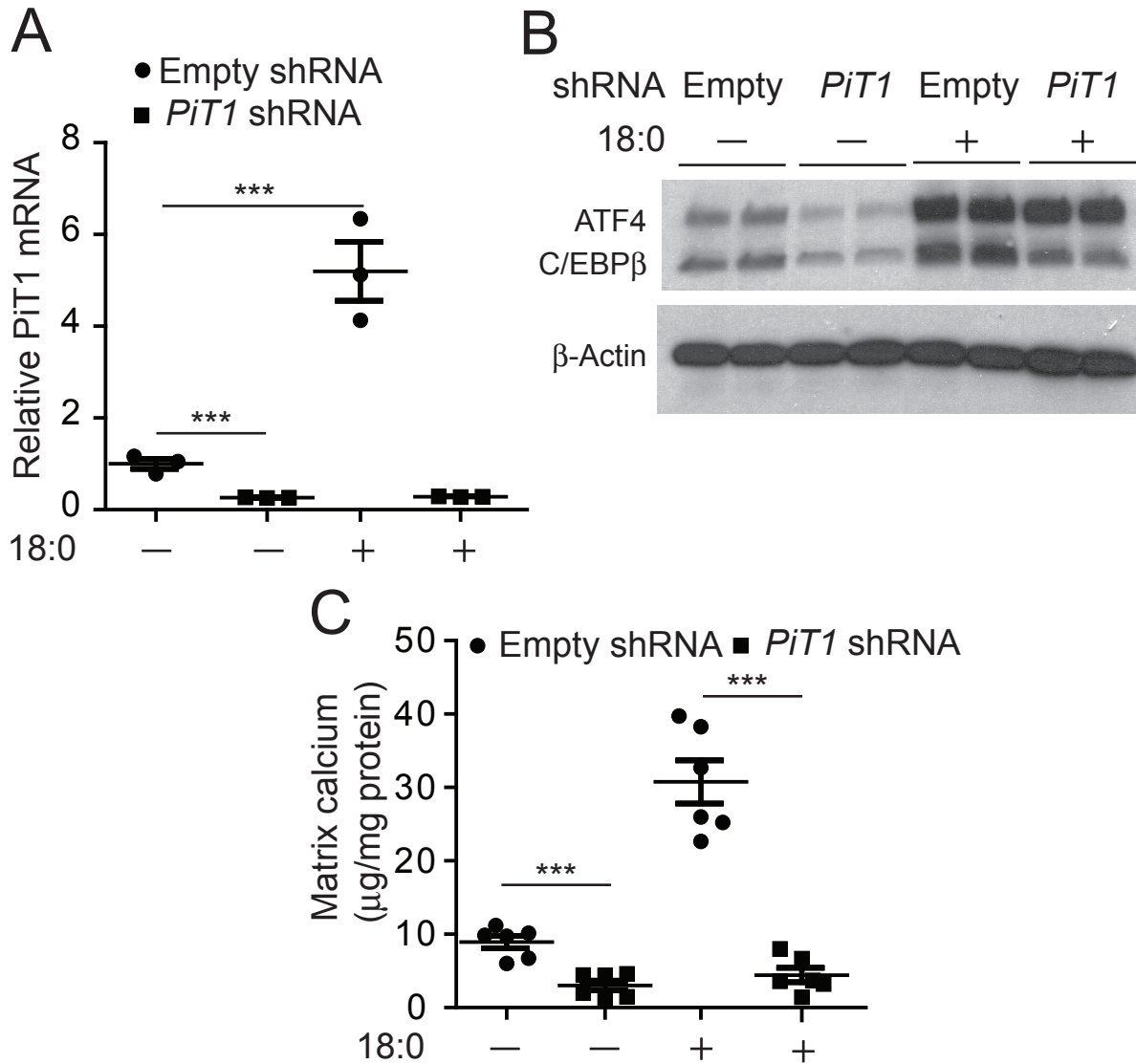


**Supplemental Figure 5. Serum and histological analysis of SMC-ATF4 TG; ApoE<sup>-/-</sup> mice.** A) Cholesterol, B) triglyceride, C) phosphorus and D) calcium in the serum of SMC-ATF4 TG; ApoE<sup>-/-</sup> mice (N=8) under NKD. The mice were sacrificed at 18 weeks of age after a 4-hour fasting. E) Quantitative analysis of atherosclerotic lesions in the aortic sinus. Aortic sinuses were stained with Oil Red O. F) Representative photographs (10x) of the lesions of aortic sinuses stained with von Kossa. The mice were sacrificed at 18 weeks of age after a 4-hour fasting. G) Quantitative analysis of calcified lesions in the aortic sinus. H) Aortic calcium content in control ApoE<sup>-/-</sup> and SMC-ATF4 TG; ApoE<sup>-/-</sup> mice. Two-tailed Student's t-test was used for statistical analysis. \*\*P<0.01.

**A** Probe            *PiT1*            *OSE1*  
 ATF4 — + + + — + + +  
 competitor — — wt mut — — wt mut



**Supplemental Figure 6. *PiT1* and *PiT2* promoter analysis.** A) EMSA was performed using  $^{32}\text{P}$ -radiolabeled double-stranded oligonucleotides corresponding to either the osteo-specific element-1 (*OSE1*) of the osteocalcin gene or the *ATF4RE* of the *PiT1* gene in the absence and presence of the wild-type ( wt) and mutant (mut) oligonucleotide competitors. Recombinant ATF4 was generated using TNT Quick Coupled Transcriptional/Translation system. B) Deletion and mutational analysis of the *PiT2* gene using a luciferase (Luc) reporter gene assay. The schematic illustrations represent the serially deleted *PiT2/Luc* reporter constructs. Results are expressed as the relative luciferase/ $\beta$ -galactosidase units of induction (n-fold) over the control value for each construct. Two-tailed Student's t-test was used for statistical analysis. \*\*\* $P < 0.001$ ..



**Supplemental Figure 7. PiT1 contributes to stearate-induced vascular calcification.** A) Stearate (18:0) treatment induces *PiT1* mRNA. VSMCs containing empty shRNA and *PiT1* shRNA were treated with 200  $\mu$ M 18:0 for 6 hours. B) ATF4 and C/EBP $\beta$  protein expression in control and *PiT1* knockdown VSMCs treated with 18:0. VSMCs containing empty shRNA and *PiT1* shRNA were treated with 200  $\mu$ M 18:0 for 6 hours. C) Calcium content in control and *PiT1* knockdown VSMCs treated with 18:0. VSMCs were treated with 200  $\mu$ M 18:0 for 7 days in the presence of 2.0mM phosphate.