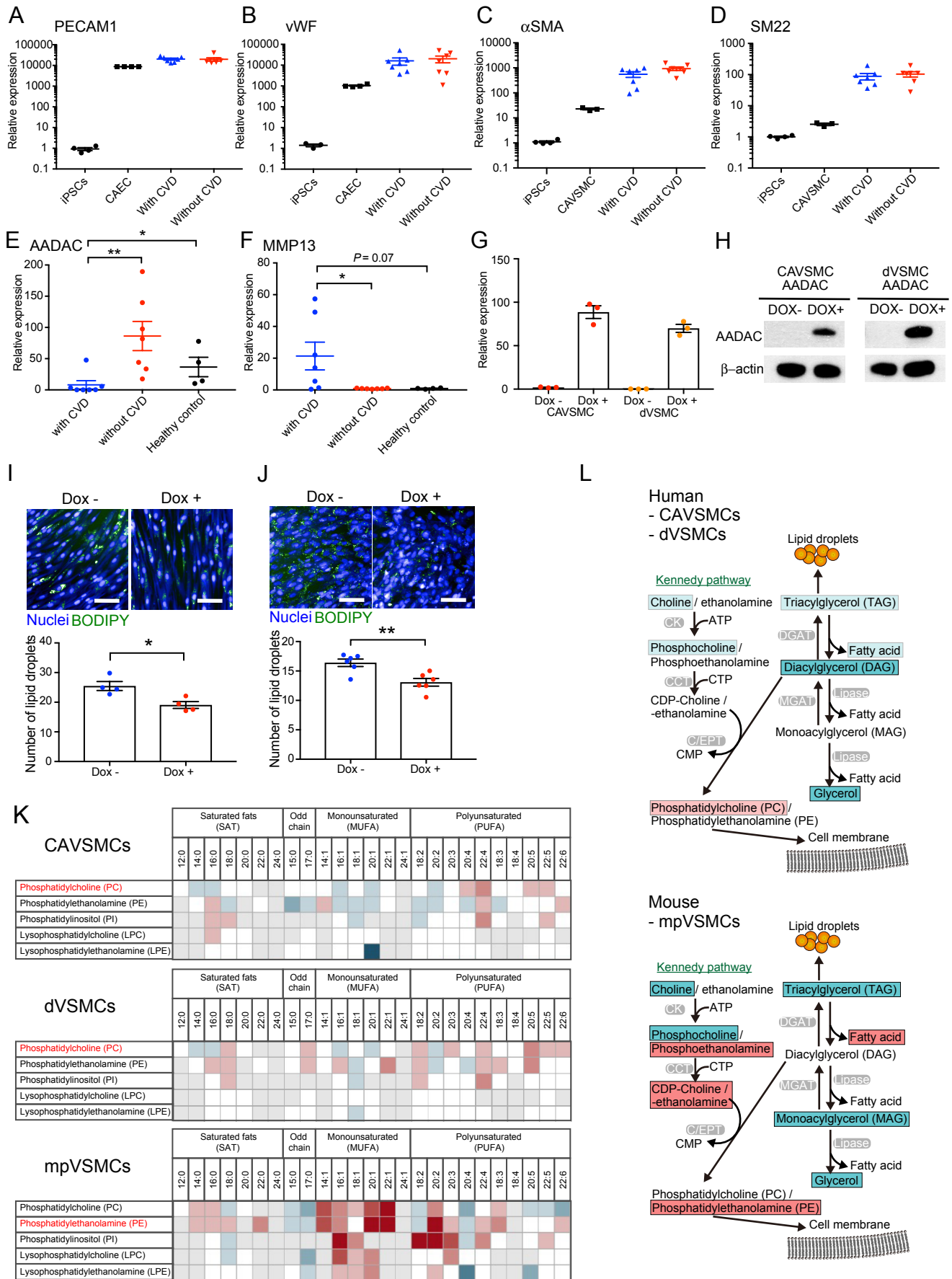


Figure S1



**Figure S1 (related to Figure 1 and 2). Characterization of the patients-derived dECs and dVSMCs, AADAC overexpression using lentivirus, and the Alterations of lipid metabolism in AADAC-overexpressing VSMCs.**

(A and B) qPCR expression analyses of platelet endothelial cell adhesion molecule1 (PECAM1) (A), vWF (B), iPSCs: n = 4, CAEC: n = 4, dECs from T2DM with CVD: n = 7, dECs from T2DM without CVD: n=7. CAEC: human primary coronary artery endothelial cells.

(C and D) qPCR expression analysis of  $\alpha$ SMA (C), SM22 (D), iPSCs: n = 4, CAVSMC: n = 3, dVSMCs from T2DM with CVD: n = 7, dVSMCs from T2DM without CVD: n=7. CAVSMC: human primary vascular smooth muscle cells.

(E and F) qPCR expression analysis of AADAC (E), and MMP13 (F) in dVSMCs, dVSMCs from T2DM with CVD: n = 7, dVSMCs from T2DM without CVD: n=7, dVSMCs from healthy control: n = 4. Mann-Whitney test for each comparison.

(G and H) mRNA (G) and protein (H) expression of AADAC in CAVSMCs and T2DM patient-derived dVSMCs infected by Dox inducible AADAC overexpression lentivirus.

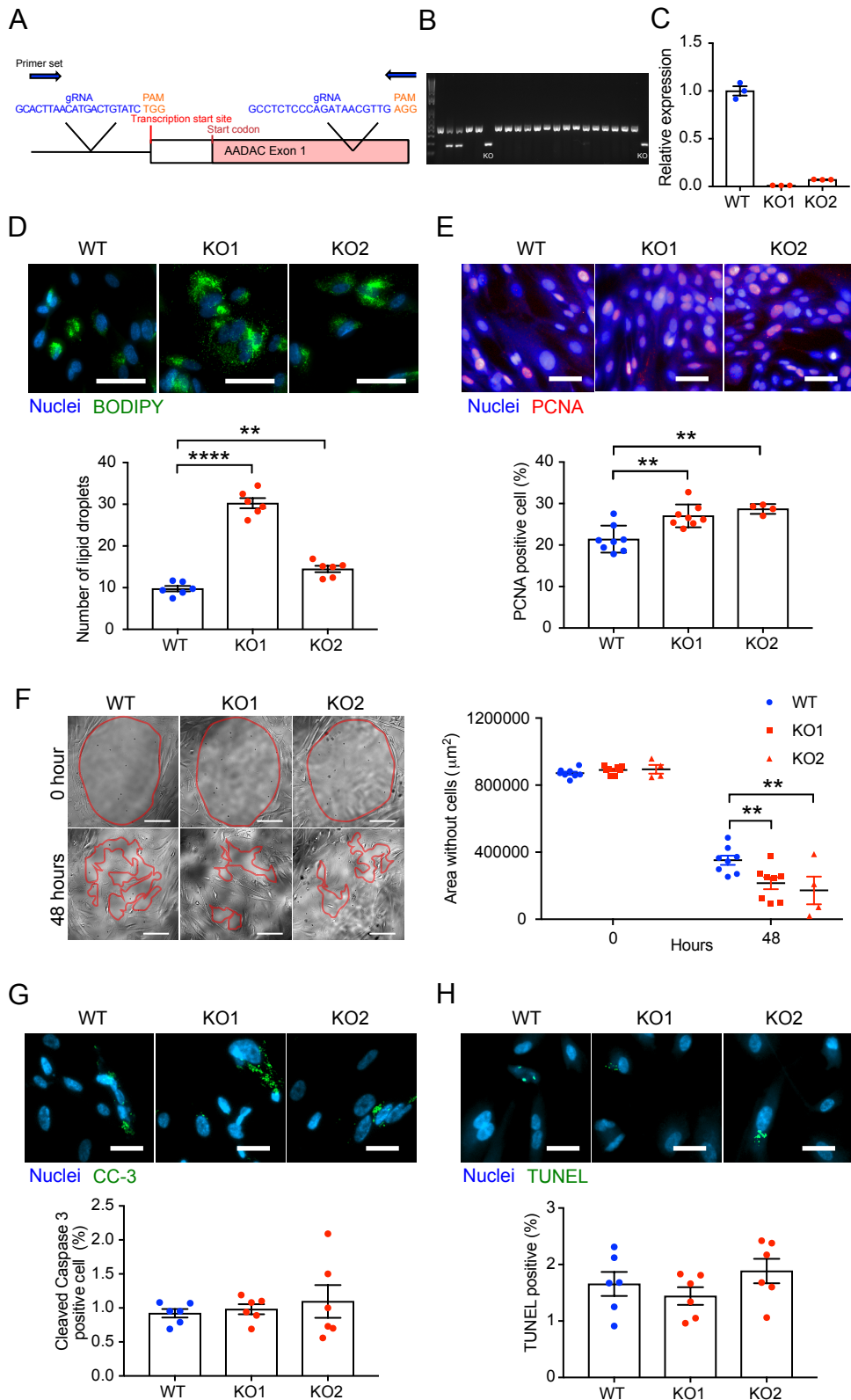
(I and J) Representative staining for neutral lipid droplets by BODIPY on culture day 7 in CAVSMCs (I) and dVSMCs (J) infected by AADAC-overexpressing lentivirus with or without Dox (Scale bar: 100  $\mu$ m.), and number of the lipid droplets per cell in CAVSMCs (n = 4) and dVSMCs (n = 6). two-tailed unpaired *t*-test.

(K) A heatmap that shows mole percentage of saturated and unsaturated phospholipids in CAVSMCs, dVSMCs, and mpVSMCs (n = 6) in relation to the total number of lipid classes detected. Illustrations show lipid classes that achieved statistically significant increase (red) and decrease (blue). Welch' s two-sample *t*-test.

(L) Potential effects of AADAC involved in storage lipid metabolism and Kennedy pathway showing significant increases (red) and decreases (blue) in CAVSMCs, dVSMCs and mpVSMCs (n = 6). As for human VSMCs, light red and light blue mean metabolites that significantly increase and decrease either in CAVSMC or dVSMC. Welch' s two-sample *t*-test.

Data represent mean  $\pm$  SEM, \**P* < 0.05, \*\**P* < 0.01.

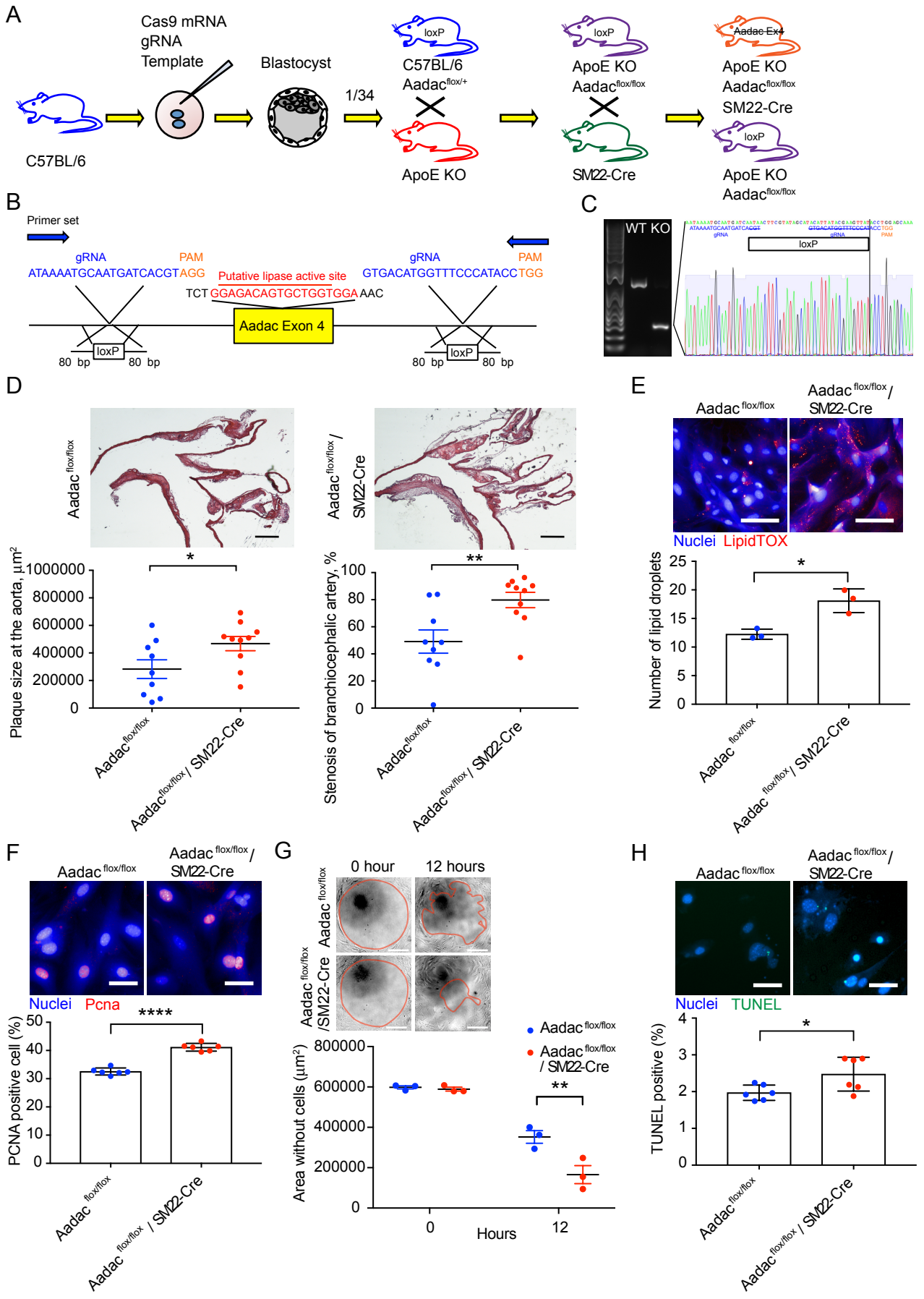
Figure S2



**Figure S2 (related to Figure 2 and 3). AADAC knockout increases the number of lipid droplets, migration, proliferation in dVSMCs derived from the T2DM patient without CVD.**

- (A) Deletion of the exon 1 of human AADAC using CRISPR/Cas9 and gRNAs in the the hiPSC line from the T2DM patient without CVD. A Primer set was designed to detect the deletion.
- (B) Confirmation of the deletion of the exon 1 by PCR. KO: homozygous knockout cell line.
- (C) qPCR expression analysis of AADAC in dVSMCs from wild type (WT) and AADAC-knockout hiPSC lines (KO1, KO2) (n = 3).
- (D) Representative staining for neutral lipid droplets by BODIPY (Scale bar: 50  $\mu\text{m}$ .) and number of the lipid droplets per cell of dVSMCs from wild type (WT) and AADAC-knockout hiPSC lines (KO1, KO2) (n = 6). one-way ANOVA with Sidak' s multiple comparisons test.
- (E) Representative immunostaining (Scale bar: 50  $\mu\text{m}$ .) and percentage of proliferating cell nuclear antigen (PCNA) positive cells in dVSMCs derived from wild type (WT; n = 8) and AADAC-knockout hiPSC lines (KO1; n = 8, KO2; n = 4). one-way ANOVA with Sidak' s multiple comparisons test.
- (F) Representative images of cell migration (Scale bar: 100  $\mu\text{m}$ .) and quantified cell migration area of dVSMCs differentiated from wild type (WT; n = 8) and AADAC-knockout hiPSC lines (KO1; n = 8, KO2; n = 4). Red lines represent the borders of cell migration. two-way ANOVA with Sidak' s multiple comparisons test.
- (G) Representative images (Scale bar: 50  $\mu\text{m}$ .) and percentage of Cleaved Caspase 3 (CC-3) positive cells in dVSMCs derived from wild type (WT) and AADAC-knockout hiPSC lines (KO1, KO2) (n = 6). one-way ANOVA with Sidak' s multiple comparisons test.
- (H) Representative images (Scale bar: 50  $\mu\text{m}$ .) and percentage of TUNEL positive cells of dVSMCs differentiated from wild type (WT) and AADAC-knockout hiPSC lines (KO1, KO2) (n = 6). one-way ANOVA with Sidak' s multiple comparisons test.
- Data represent mean  $\pm$  SEM, \*\* $P < 0.01$ , \*\*\*\* $P < 0.0001$ .

Figure S3



**Figure S3 (related to Figure 4). VSMC specific Aadac-knockout Apoe<sup>-/-</sup> mice show the aggravation of atherosclerosis.**

(A) Strategy to generate VSMC specific Aadac-knockout (KO) mice with Apoe<sup>-/-</sup> background.

(B) Integration of two loxPs before and after Aadac exon 4 including the lipase active site in C57BL/6 mouse genome. Primer set was designed to detect the integration.

(C) Confirmation of the deletion of the exon 4 by PCR and sequencing in isolated mouse VSMCs.

(D) Representative H&E staining and quantification of plaque size at the aorta (left) and percent stenosis at branchiocephalic artery (right) in Aadac<sup>fllox/fllox</sup> mice (n = 9) and Aadac<sup>fllox/fllox</sup>/ SM22-Cre mice (n = 10) (Scale bar: 500  $\mu$ m.). two-tailed unpaired *t*-test.

(E) Representative staining for neutral lipid droplets by lipidTOX (Scale bar: 100  $\mu$ m.) and number of the lipid droplets per cell in mouse VSMCs isolated from Aadac<sup>fllox/fllox</sup> and Aadac<sup>fllox/fllox</sup>/ SM22-Cre (n = 3). two-tailed unpaired *t*-test.

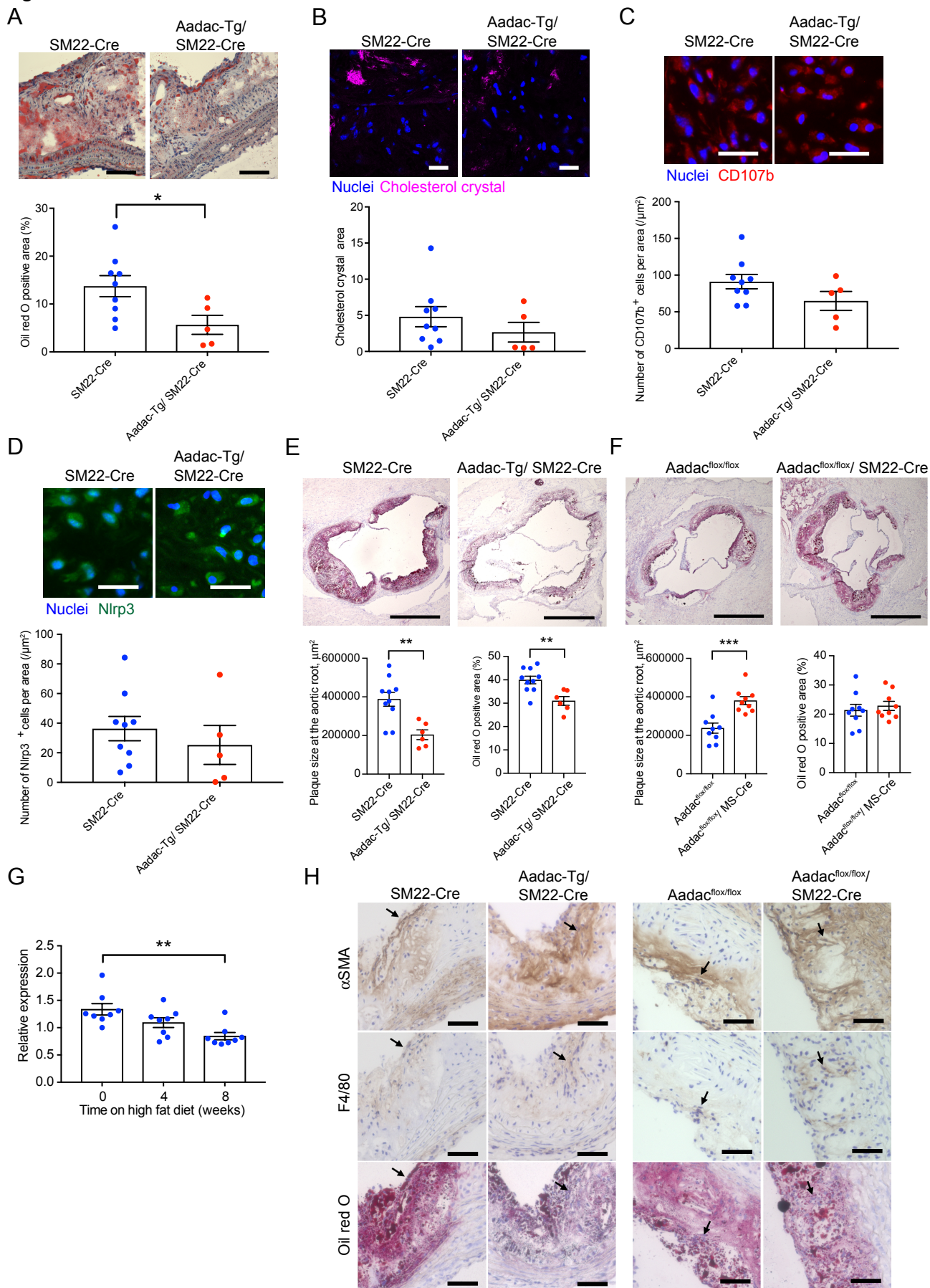
(F) Representative immunostaining (Scale bar: 50  $\mu$ m.) and percentage of PCNA positive cells in mouse VSMCs isolated from Aadac<sup>fllox/fllox</sup> and Aadac<sup>fllox/fllox</sup>/ SM22-Cre (n = 6). two-tailed unpaired *t*-test.

(G) Representative images (Scale bar: 100  $\mu$ m.) of cell migration and quantified cell migration area of mouse VSMCs isolated from Aadac<sup>fllox/fllox</sup> and Aadac<sup>fllox/fllox</sup>/ SM22-Cre (n = 3). two-way ANOVA with Sidak' s multiple comparisons test.

(H) Representative images (Scale bar: 50  $\mu$ m.) and percentage of TUNEL positive cells of mouse VSMCs isolated from Aadac<sup>fllox/fllox</sup> and Aadac<sup>fllox/fllox</sup>/ SM22-Cre (n = 6). two-tailed unpaired *t*-test.

Data represent mean  $\pm$  SEM, \**P* < 0.05, \*\**P* < 0.01, \*\*\*\**P* < 0.0001.

Figure S4



**Figure S4 (related to Figure 4). Characterization of atherosclerotic plaques in Aadac-Tg and Aadac knockout mice.**

- (A) Representative images (Scale bars: 100  $\mu\text{m}$ .) of the aorta and percentage of Oil red O positive area in atherosclerotic lesion of SM22-Cre (n = 9) and Aadac-Tg/ SM22-Cre (n = 5). two-tailed unpaired *t*-test.
- (B) Representative confocal images (Scale bars: 20  $\mu\text{m}$ .) and percentage of cholesterol crystal area in atherosclerotic lesion of SM22-Cre (n = 9) and Aadac-Tg/ SM22-Cre (n = 5). two-tailed unpaired *t*-test.
- (C and D) Representative CD107b<sup>+</sup> (C) and Nlrp3<sup>+</sup> (D) cell images (Scale bars: 20  $\mu\text{m}$ .) and the number of CD107b<sup>+</sup> (C) and Nlrp3<sup>+</sup> (D) cells per area in atherosclerotic lesion of SM22-Cre (n = 9) and Aadac-Tg/ SM22-Cre (n = 5). two-tailed unpaired *t*-test.
- (E and F) Representative Oil red O staining (Scale bar. 500  $\mu\text{m}$ .) at the aortic root, quantification of plaque size, and percentage of Oil red O positive area in atherosclerotic lesion of SM22-Cre mice (n = 10) and Aadac-Tg/ SM22-Cre mice (n = 6) fed a high fat diet for 10 weeks (E) and Aadac<sup>fllox/fllox</sup> mice (n = 9) and Aadac<sup>fllox/fllox</sup>/ SM22-Cre (n = 9) fed a high fat diet for 8 weeks (F). two-tailed unpaired *t*-test.
- (G) qPCR analysis of time-course Aadac expression in the aorta isolated from Apoe<sup>-/-</sup> mouse (n = 8) fed a high fat diet. one-way ANOVA with Sidak' s multiple comparisons test.
- (H) Representative pictures of the atherosclerotic lesions in the aortic root (Scale bar. 50  $\mu\text{m}$ .) that include Oil red O positive cells stained with both  $\alpha$ SMA (VSMC marker) and F4/80 (macrophage marker) in SM22-Cre mice, Aadac-Tg/ SM22-Cre mice, Aadac<sup>fllox/fllox</sup> mice, and Aadac<sup>fllox/fllox</sup>/ SM22-Cre. Consecutive slices from each aortic root were stained. Arrows indicate the Oil red O positive cells stained with both  $\alpha$ SMA and F4/80.

Data represent mean  $\pm$  SEM, \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.



Table S1 (related to Figure 1). Clinical data of T2D patients without CVD and T2D patients with CVD

	Age	Gender	Hypertension	Hyperlipidemia	Diabetes mellitus			Insulin therapy	Family history of Early-onset CVD	Smoking	Surgery	Ejection fraction	other vasculopathy Immune defect?
					History	Retinopahty	Nephropathy						
DM without CVD	67	M	Yes	Yes	30 years	Yes	Transplantation	Yes	No	No		75%	No
	77	F	Yes	Yes	35 years	Yes (blind)	Severe	Yes	No	Past remote		70%	No
	71	M	Yes	Yes	17 years	Unknown	Mild	Yes	No	Past remote	NAFLD liver transplantation	63%	No
	63	F	Yes	Yes	37 years	Unknown	Slight	Yes	Father	Past remote		65%	No
DM with CVD	68	M	Yes	Yes	8 years	Unknown	Slight	Yes	No. Mother died at 39 due to blood cancer	No	CABG	NA	No
	60	M	Yes	Yes	12 years	Yes	Slight	No	Father and Mother	Past remote	Stenting	65%	No
	60	M	Yes	Yes	1 year	Unknown	Slight	Yes	Father	No	CABG	73%	No
	64	M	Yes	Yes	14 years	Unknown	Slight	Yes	No	No	CABG	NA	No