Supplemental Information

Figure Legends

Supplemental Figure S1. PDK4, inhibited by canonical TGFβ/RSMADs signaling, plays crucial roles in regulating PDH phosphorylation. Related to Figure 2. A, Representative blots for PDK proteins and PDH phosphatase 1 in HUAECs treated with 7 days of TGFβ2 stimulation (10ng/ml). **B**, Representative blots for PDK proteins in HUAECs transduced with Scramble siRNA or PDK4 siRNAs for blocking PDK4. **C-D**, EndMT markers, EC markers, and PDH phosphorylation were analyzed in HUAECs transduced with siRNAs for blocking PDK1 or PDK2 separately (**C**), or for blocking PDK3 or PDK4 separately (**D**). **E-F**, The roles of canonical ALK5/RSMADs in inhibiting PDK4 expression. Constitutively active ALK5 mutant (ALK5-CA) was adenoviral delivered in HUAECs, and level of phospho-SMAD2, SM22α and PDK4 was showed (**E**). A reduction of PDK4 level induced by TGFβ2 stimulation, and the protein level of PDK4 was restored by blocking SMAD4 in HUAECs (**F**).

Supplemental Figure S2. Blocking ACSS2, but not ACLY, reverses the effects induced by TGFβ signaling in endothelial cells. Related to Figure 2. A-B, Representative blots showing EndMT markers and EC-specific gene expression in Human Aortic Endothelial cells(HAECs) treated with Scramble siRNA, Scramble siRNA + TGFβ2, or *ACSS2* siRNA + TGFβ2 (**A**) / *ACLY* siRNA + TGFβ2 (**B**) separately for 7 days. **C**, EndMT markers and EC-specific gene expression were determined in HUAECs treated with different conditions: Adv-CTL, Adv-CTL + TGFβ2, or Adv-ACSS2-HA + TGFβ2 separately for 3 days. **D**, Bulk RNA-seq analysis of metabolic-related genes expression in HUAECs with different conditions: Scramble siRNA, Scramble siRNA + TGFβ2, or *ACSS2* siRNA + TGFβ2. **E**, mRNA level of *ACSS2* and *PDK* genes in HUAECs under different conditions: Scramble siRNA, Scramble siRNA + TGFβ2, or *ACSS2* siRNA + TGFβ2. **F**, CHIP-QPCR testing of Smad-2/3 binding to SBEs on human *PDK4* promoter in HUAECs treated with different conditions: Scramble siRNA, Scramble siRNA + TGFβ2, or *ACSS2* siRNA + TGFβ2. The data in **F** were normalized to those of the non-TGFβ treated control or scramble siRNA–treated control and are presented as the mean ± SEM from three independent experiments. ****$P \leq 0.0001$. **G**, Representative blots showing the changes of TGFβ-regulated EndMT markers, PDK4 and SMAD2 phosphorylation following overexpression of PDK4 in HUAECs. **H**, Representative blots showing the changes of ALK1 and ALK5 in HUAECs treated with different conditions: Scramble siRNA, Scramble siRNA + TGFβ2, or *ACSS2* siRNA + TGFβ2.

Supplemental Figure S3. A blockade of proliferation is induced by blocking PDK4 and sodium acetate treatment. Related to Figure 2 and Figure 3. A, EdU positive cells were analyzed in HUAECs transduced with Scramble siRNA or *PDK4* siRNA for 7 days. **B**, EdU staining showing the proliferation of HUAECs treated with different concentration of sodium acetate. **C**, The pH of the EGM-2 medium containing different concentration of sodium acetate was measured using a pH meter (pHTestr 50S, OAKTON). **D-F**, Images showing the cell morphology (**D**), the staining of mesenchymal markers (**E**), or cell proliferation (**F**) of HUAECs treated with Scramble siRNA, *PDK4* siRNA, or *ACSS2* siRNA + *PDK4* siRNA. Single pair-wise comparison between two groups performed by unpaired, two-tailed Student's t-test using Prism 9. *P values < 0.05. ***P values < 0.001. ****P values < 0.0001.

Supplemental Figure S4. Acetate-generated Ac-CoA mediated by ACSS2 plays positive roles in the maintenance of endothelial TGFβ-ALK5-RSMADs signaling. Related to

Figure 4 and Figure 5. A-B, Representative blots showing phosphorylated R-SMADs in HUAECs(**A**) and HAECs(**B**) transduced with Scramble siRNA, *ACLY* siRNA, or *ACSS2* siRNA separately in the presence of TGFβ2 stimulation for 7 days. **C,** Blots of the total SMAD7 and acetylated SAMD7 in HUAECs treated with TGFβ2 stimulation for 7 days**. D-E**, Blots showing acetylated R-SMADs in HUAECs transduced with Scramble siRNA or ACSS2 siRNA separately in the presence of TGFβ2 stimulation for 7 days (**D**), or acetylated R-SMADs in HUAECs overexpressing ACSS2-HA in the presence of TGFβ2 stimulation for 3 days (**E**). **F**, Representative blots of cytosolic and nucleic RSMADs in HUAECs overexpressing ACSS2-HA for 3 days. **G-H**, mRNA(**G**) and protein(**H**) level of ALK5 in HUAECs transduced with Scramble siRNA or *PDK4* siRNA for 7 days. **I-J**, Blots showing phosphorylated R-SMADs in HUAECs with PDK4 knockdown(**I**) or pharmacological inhibition of PDKs with DCA (**J**) for 7 days. **K**, Half-life of ALK5-V5 in HUVECs transduced with Scramble siRNA or *ACSS2* siRNA separately. ALK5-V5 was adenovirally overexpressed in HUVECs followed by cycloheximide (CHX, 10 μg/ml) treatment at the indicated time points. ALK5-V5 protein level was determined by anti-V5 antibody. **L-M**, Representative blots showing the regulation of ALK5 degradation in HUVECs. HUVECs were firstly transduced with Scramble siRNA or *ACSS2* siRNA, then ALK5-V5 was adenovirally overexpressed in HUAECs followed the treatment with a proteasome inhibitor MG132 (**L**) or lysosome inhibitor chloroquine (**M**).

Supplemental Figure S5. Characterization of acetylated sites for Human ALK5. Related to Figure 5. A, Representative blots of exogenous acetylated ALK5 in 293T cells transfected with Vector or ALK5-Flag plasmids, ALK5(Flag) was immunoprecipitated followed by immunoblotting to detect Acetylated-Lysine (Ac-Lysine) and ALK5 (Flag). **B**, LC-MS/MS analysis of the digested ALK5-Flag proteins immunoprecipitated by anti-Flag beads, and Lysine 490 is the acetylated site of ALK5 by analyzing the peptides of mass spectrum. **C**, Lysine residue locates at the protein kinase domain of ALK5. **D**, 293T cells were transfected with ALK5-WT or K490R mutant bearing single lysine (K) to arginine (R) substitutions at Lysine 490 site, and ALK5-WT or K490R mutant were immunoprecipitated with anti-Flag beads. Acetylated-ALK5 protein level was determined by anti-Ac-Lysine antibody. **E**, Single K-R mutation strategy in characterizing acetylation sites for Human ALK5. There are 25 Lysine residues in Human ALK5, and 25 mutants bearing single K-R mutation were constructed. After IP-anti-FLAG / IB-anti-Ac-Lysine screening analysis, representative blots showing the major lysine residues (K223, K343, K391, K449, and K490 (by LC-MS/MS)) for acetylation.

Supplemental Figure S6. The generation of *Cdh5-CreER^{T2}*; *Acss2 $^{fl/fl}$*; *Apoe$^{-/-}$* mice and *Acss2 $^{fl/fl}$*; *Apoe$^{-/-}$* mice. Related to Figure 7. A, diagram showing the genomic information of *Acss2* gene in Acss2$^{flox/flox}$ mice. **B**, diagram showing the generation of *Acss2 $^{fl/fl}$*; *Apoe$^{-/-}$* mice and *Cdh5-CreER^{T2}*; *Acss2 $^{fl/fl}$*; *Apoe$^{-/-}$* mice. C-D, Deletion efficiency of *Acss2* gene in the lung endothelial cells of *Cdh5-CreER^{T2}*; *Acss2 $^{fl/fl}$*; *Apoe$^{-/-}$* mice. **E**, Plaques in aortas of control *ApoE$^{-/-}$* female mice (♀) and *Acss2 $^{iECKO;\ ApoE-/-}$* female mice (♀) were stained with Oil-Red-O. **F**, Oil-Red-O analysis of whole aortas, aortic arch, thoracic aorta, and abdominal aorta from control *ApoE$^{-/-}$* female mice (♀) (n=10) and *Acss2 $^{iECKO;\ ApoE-/-}$* female mice (♀) (n=14). **G-H**, total cholesterol level (**G**) and triglycerides (**H**) in plasma collected from control *ApoE$^{-/-}$* female mice (♀) and *Acss2 $^{iECKO;\ ApoE-/-}$* female mice (♀) after 3 months of high fat diet. NS, no significance. The plaque lesion area and total surface area of aortas were quantified using ImageJ software. A single pair-wise comparison between two groups performed by unpaired, two-tailed Student's t-test using Prism 9. NS, no significance, **P* values < 0.05, ***P* values < 0.01.

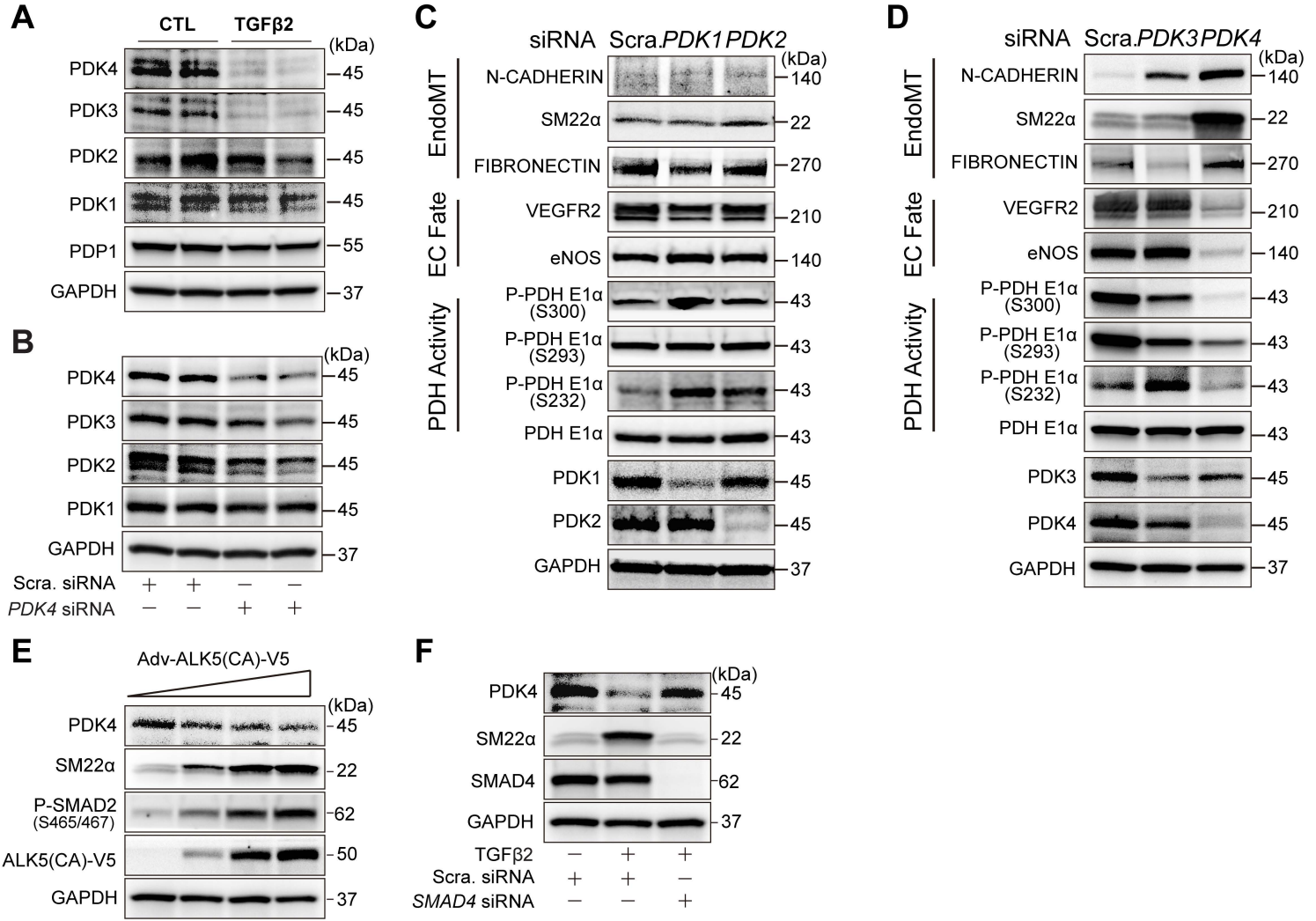
Supplemental Figure S7. Reduced development of atherosclerosis following endothelial

ACSS2 knockdown in ApoE$^{-/-}$ mice. Related to Figure 7. A, A knockdown efficiency of ACSS2 siRNA in mice artery endothelial cells (MAEC). MAECs were incubated with 20nM unmodified ACSS2 siRNAs in EGM-2/Opti-MEM medium for 10 hrs. Three days after siRNA delivery, Acss2 level in total cell lysates was analyzed by immunoblotting with anti-ACSS2 antibody. **B**, Flow diagram showing timing of atherosclerosis induction and 7C1 LNP delivery in ApoE$^{-/-}$ mice. **C-D**, Plaques in aortas of ApoE$^{-/-}$ mice were stained with Oil-Red-O. Images showing the Oil-Red-O staining of aortas dissected from 12 control ApoE$^{-/-}$ mice and 9 unmodified (siRNA 1) and 9 modified (siRNA 2) ACSS2 siRNA-treated ApoE$^{-/-}$ mice (**C**). Oil-Red-O analysis of whole aortas from control and ACSS2 siRNA-treated ApoE$^{-/-}$ mice. The plaque lesion area and total surface area of aortas were quantified using ImageJ software. A single pair-wise comparison between two groups performed by unpaired, two-tailed Student's t-test using Prism 9. ***P values < 0.001 (**D**).

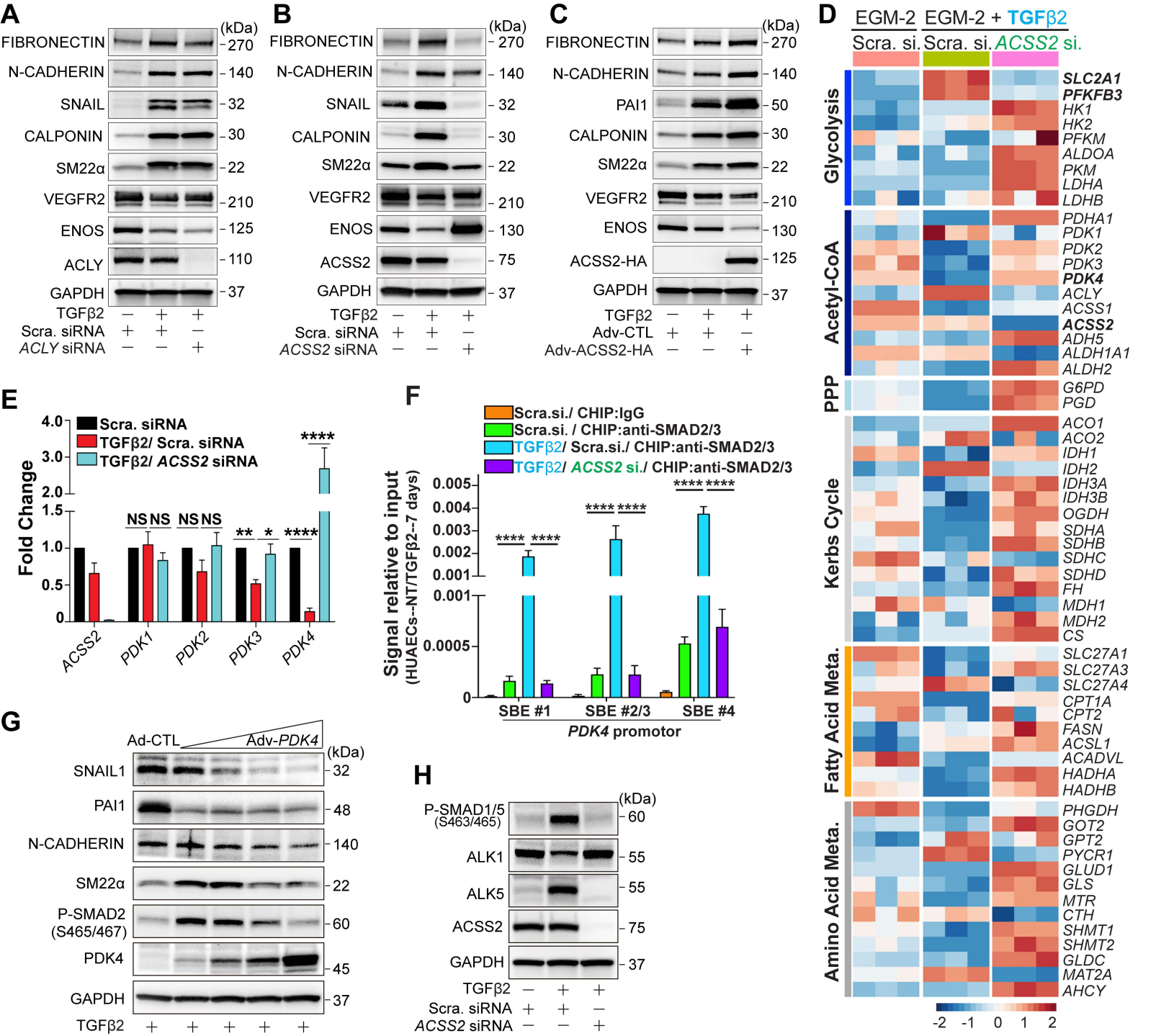
Supplemental Figure S8. ACSS2 and inhibition of PDK4 drive a positive feedback loop. A, Representative blots showing the phosphorylation of PDHE1α decreased by PDK4 knockdown can be restored by ACSS2 deficiency in HUAECs. **B-C**, Representative blots showing ACSS2 knockdown reduced EndMT markers and restored EC-specific gene expression induced by PDK4 siRNA in HUAECs(**B**) and HAECs(**C**). **D-E**, In the context of pro-EndMT TGFβ signaling, the elevated phosphorylation of PDHE1α by ACSS2 knockdown was reversed by knocking down PDK4 in HUAECs(**D**) and HAECs(**E**). **F-G**, In the presence of TGFβ signaling, the reduced EndMT markers and restored EC-specific gene expression by ACSS2 knockdown can be reversed by blocking PDK4 in HUAECs(**F**) and HAECs(**G**).

Supplemental Table 1. Information of siRNA and qPCR primers. Related to STAR Methods

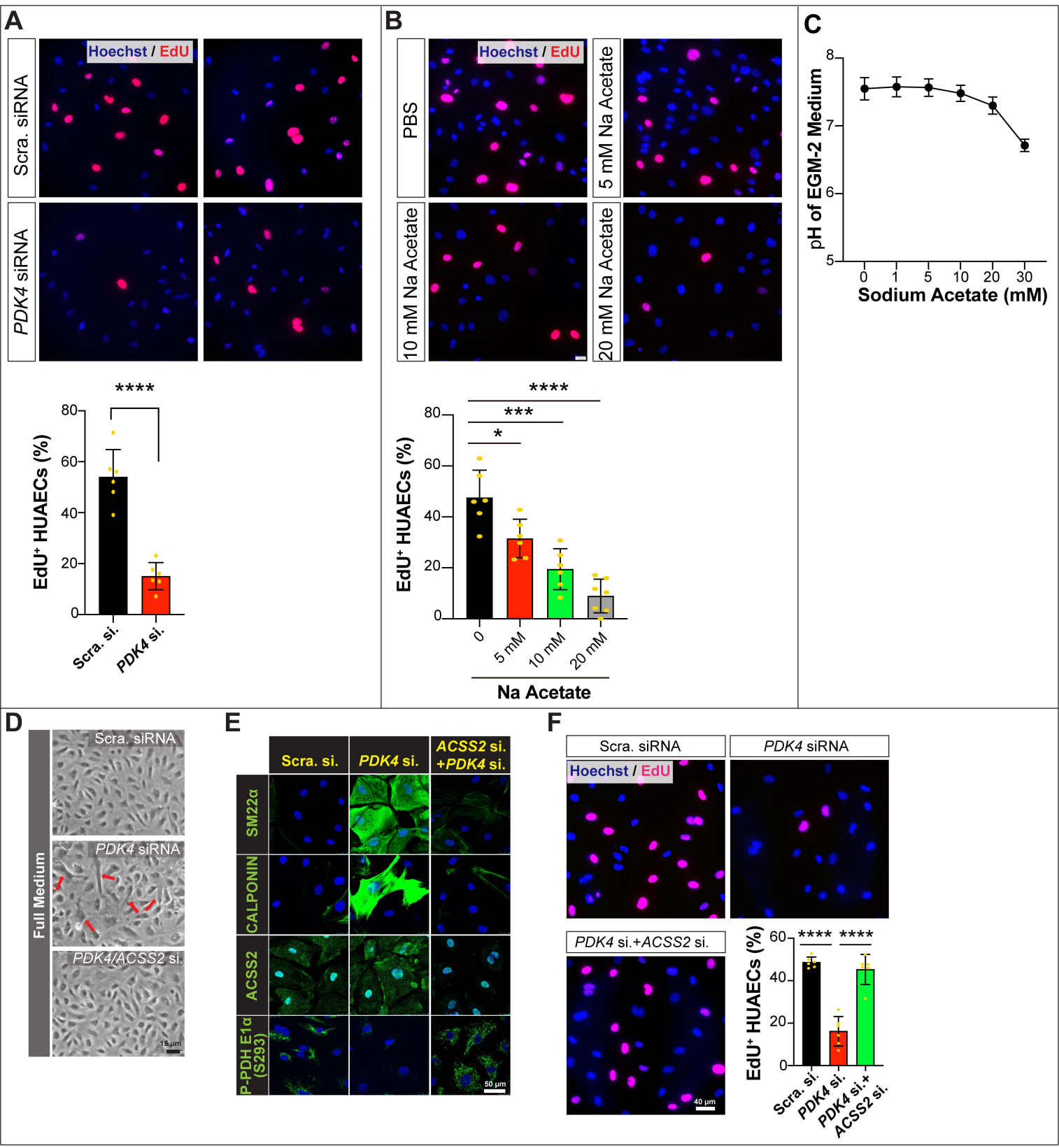
Supplemental Table 2. Information of donors. Related to Figure 6.



Supplemental Figure 1



Supplemental Figure 2



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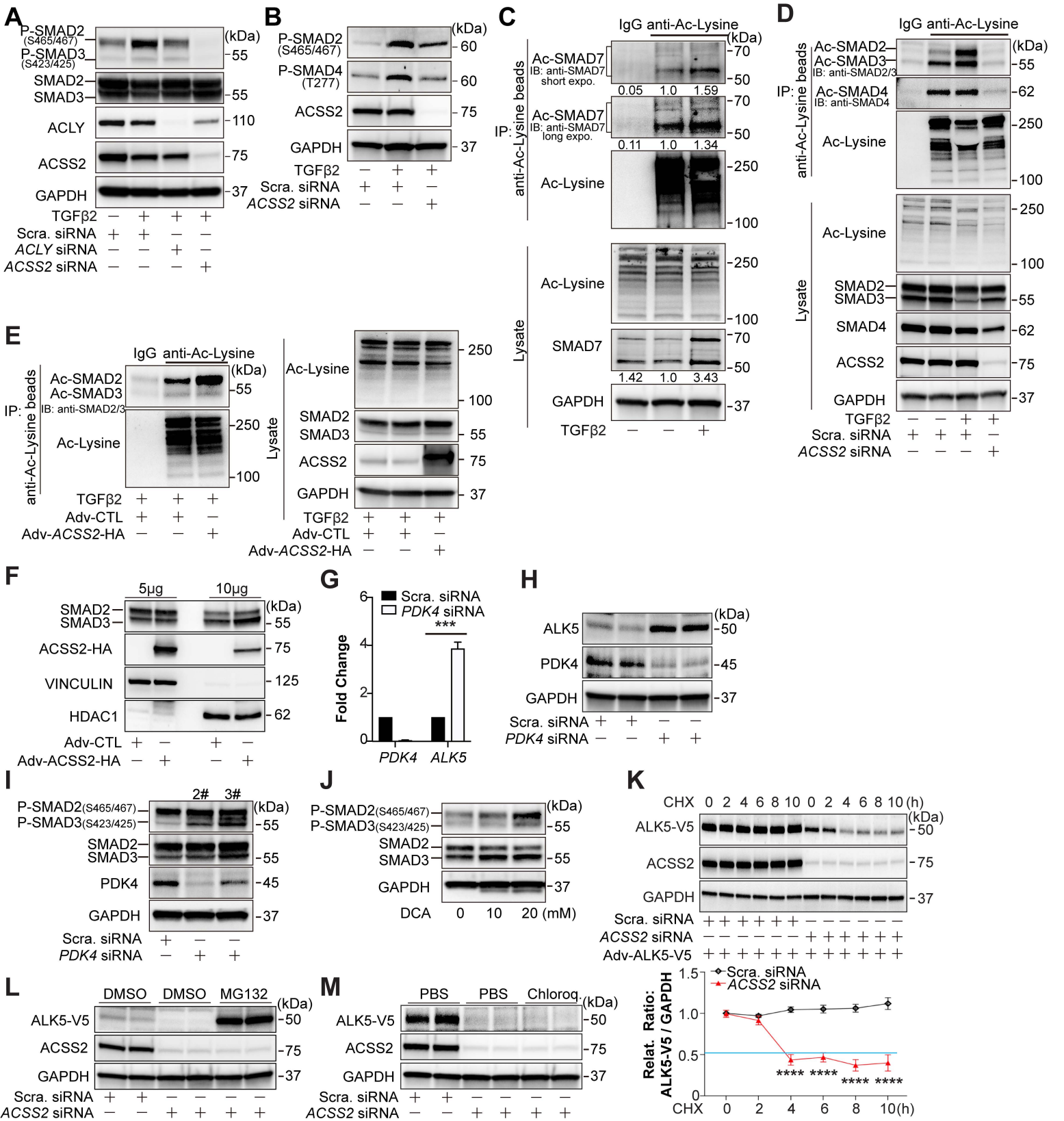

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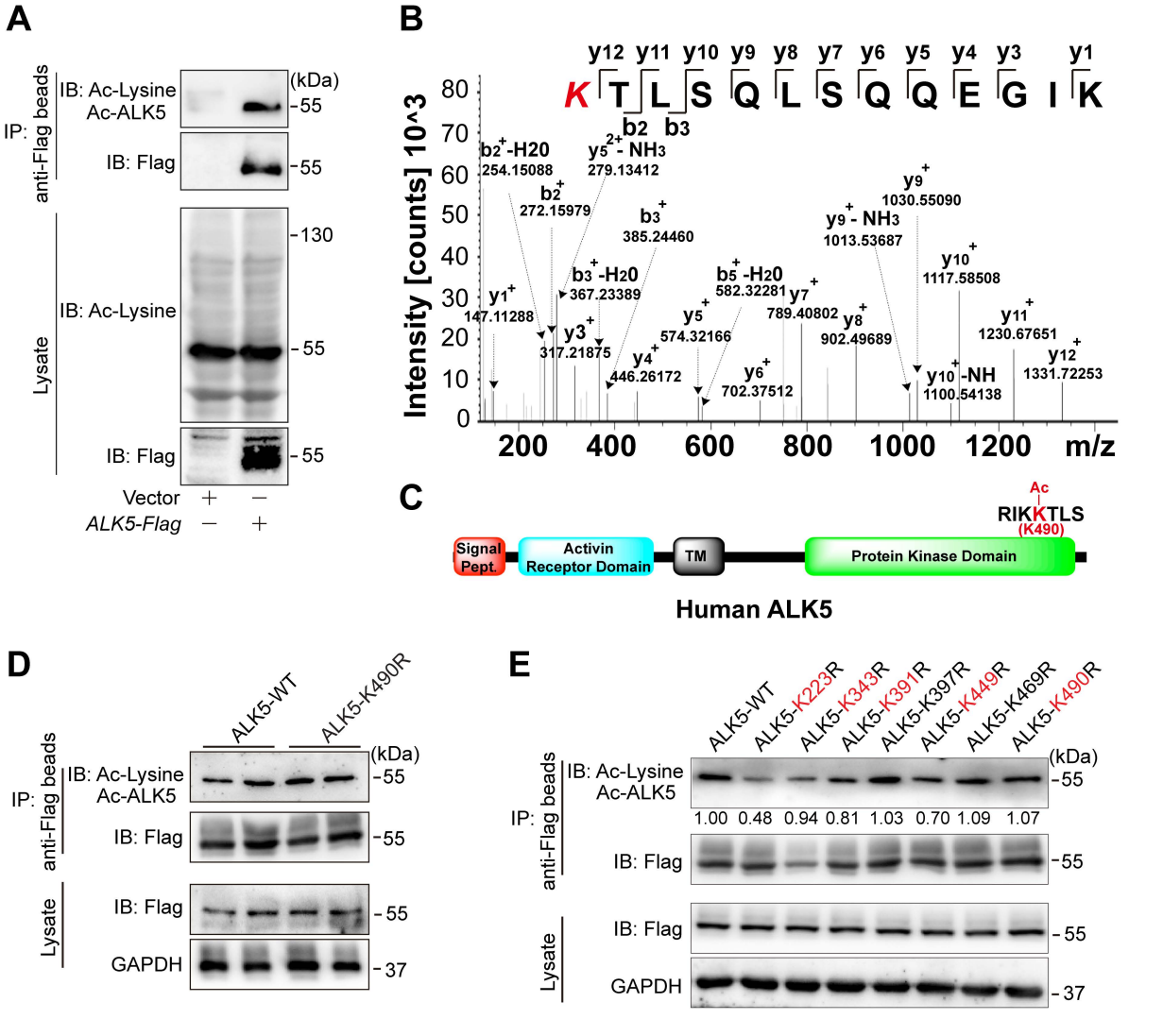

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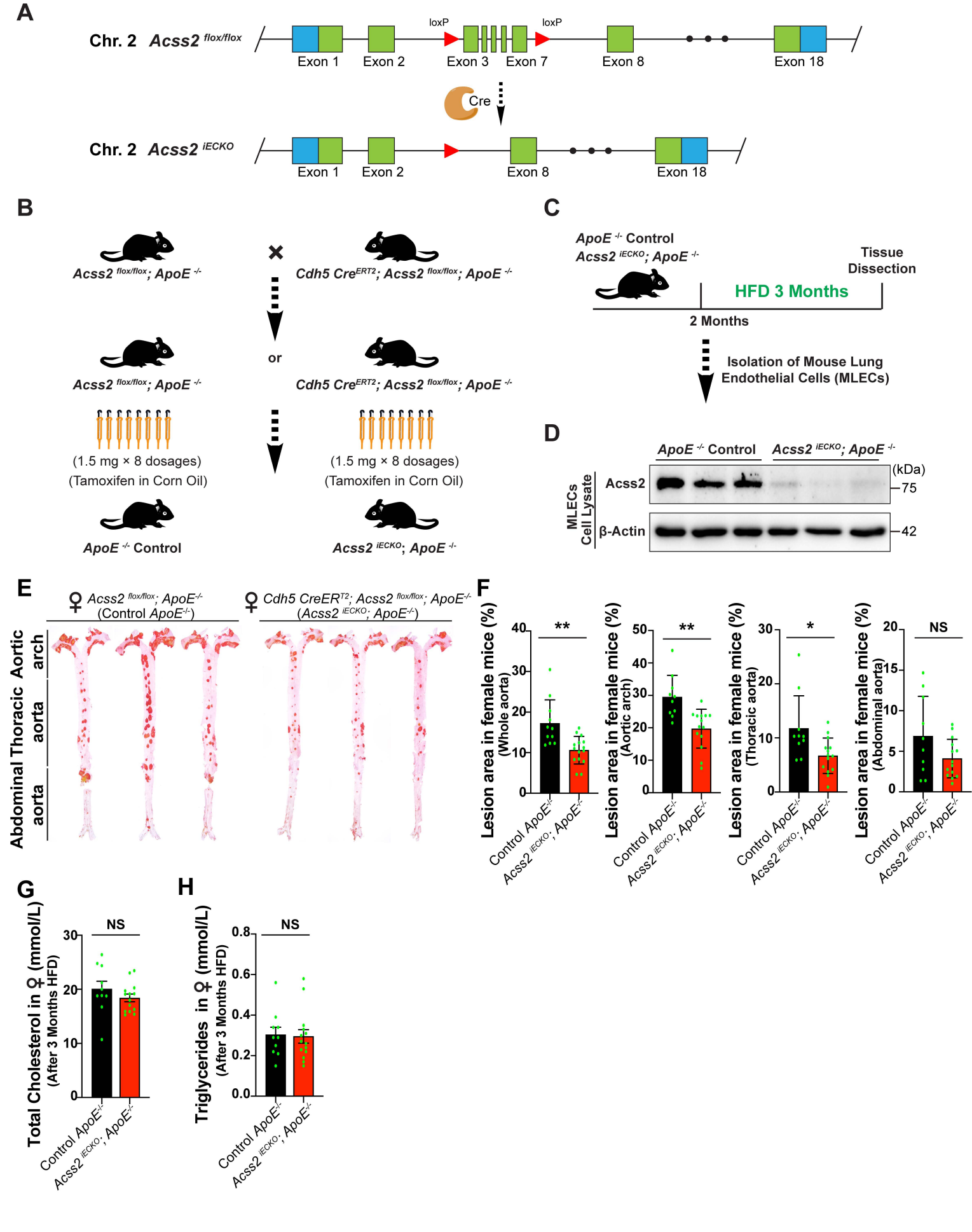

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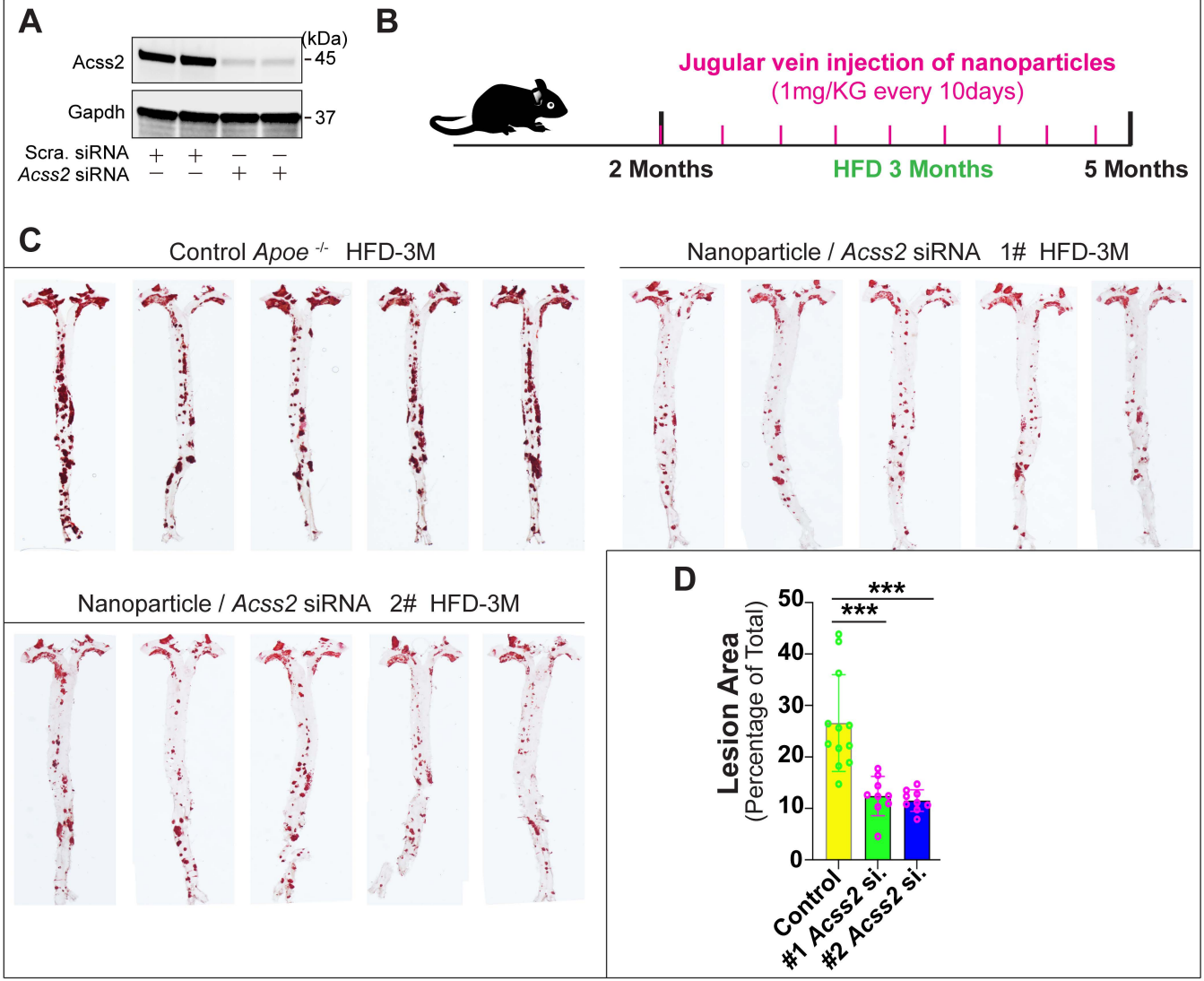
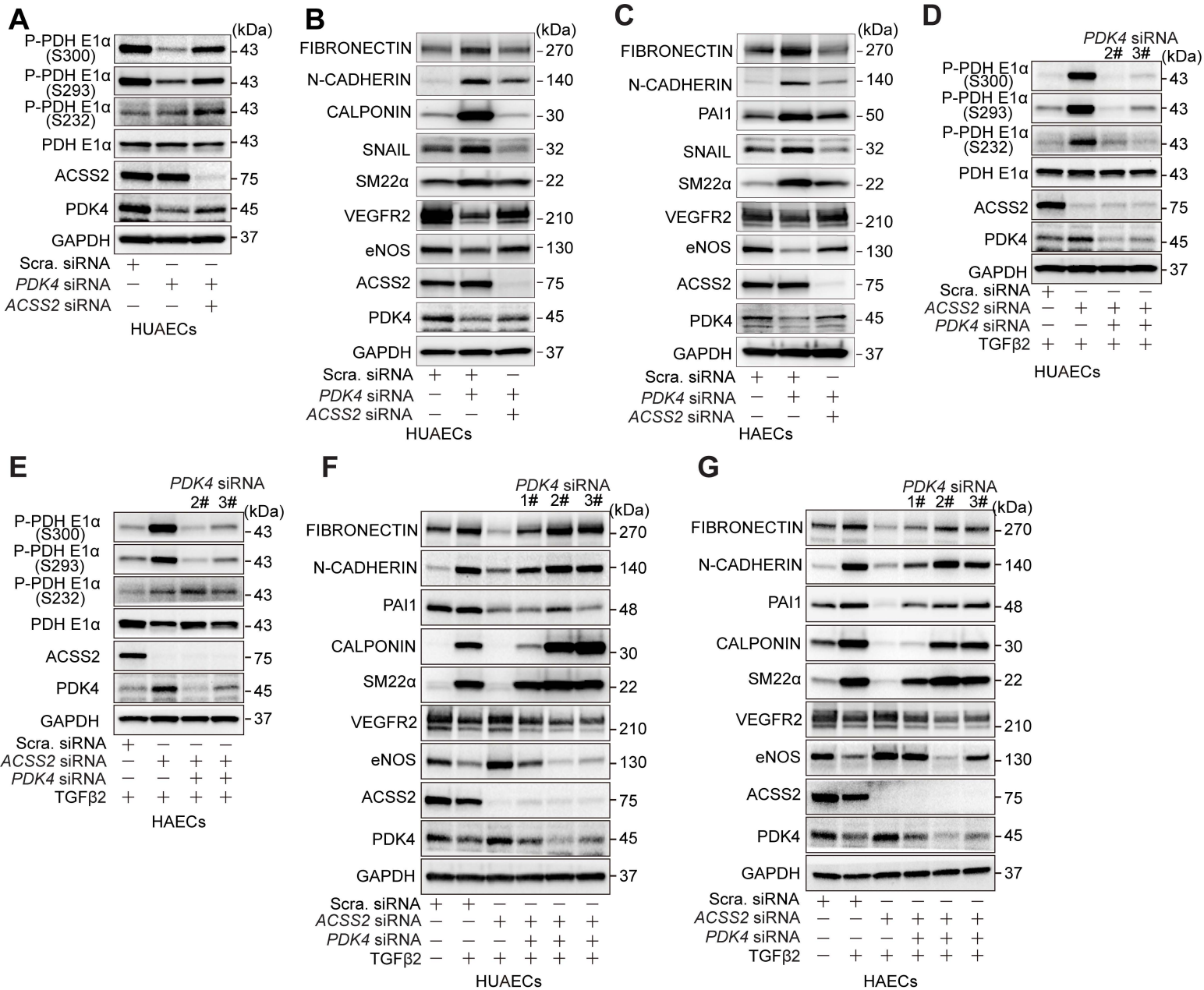


Supplemental Figure 7



Supplemental Figure 8

Supplemental Table 1

siRNA	Vendors's Name	Catalog Number / siRNA ID
ACSS2 siRNA	Horizon Discovery Biosciences Limited	E-010396-00-0005
ACLY siRNA	Thermo Fisher Scientific	s915
PDK4 siRNA 1#	Thermo Fisher Scientific	s10261
PDK4 siRNA 2#	Thermo Fisher Scientific	s10262
PDK4 siRNA 3#	Thermo Fisher Scientific	s10263
PDK1 siRNA	Thermo Fisher Scientific	s10252
PDK2 siRNA	Thermo Fisher Scientific	s10255
PDK3 siRNA	Thermo Fisher Scientific	s10258
SMAD2 siRNA	Thermo Fisher Scientific	s8397
SMAD4 siRNA	Thermo Fisher Scientific	s8403
Negative Control siRNA	Thermo Fisher Scientific	4457287

Gene (Human)	Forward primer sequence 5'- 3'	Reverse primer sequence 5'- 3'
ACLY	ATCGGTTCAAGTATGCTCGGG	GACCAAGTTTTCCACGACGTT
ACSS2	AAAGGAGCAACTACCAACATCTG	GCTGAACTGACACACTTGGAC
ALDOA	ATGCCCTACCAATATCCAGCA	GCTCCCAGTGGACTCATCTG
HK2	GAGCCACCACTCACCCTACT	CCAGGCATTCGGCAATGTG
LDHA	ATGGCAACTCTAAAGGATCAGC	CCAACCCCAACAACTGTAATCT
LDHB	CCTCAGATCGTCAAGTACAGTCC	ATCACGCGGTGTTTGGGTAAT
PDK1	CTGTGATACGGATCAGAAACCG	TCCACCAAACAATAAAGAGTGCT
PDK2	ATGAAAGAGATCAACCTGCTTCC	GGCTCTGGACATACCAGCTC
PDK3	CGCTCTCCATCAAACAATTCCT	CCACTGAAGGGCGGTTAAGTA
PDK4	GGAGCATTTCTCGCGCTACA	ACAGGCAATTCTTGTCGCAAA
PFKM	GGTGCCCGTGTCTTCTTTGT	AAGCATCATCGAAACGCTCTC
PFKFB3	ATTGCGGTTTTCGATGCCAC	GCCACAACTGTAGGGTCGT
PKM2	ATGTCGAAGCCCCATAGTGAA	TGGGTGGTGAATCAATGTCCA
SLC2A1--GLUT1	ATTGGCTCCGGTATCGTCAAC	GCTCAGATAGGACATCCAGGGTA
TGFBR1--ALK5	ACGGCGTTACAGTGTTTCTG	GCACATACAAACGGCCTATCT
β-ACTIN	ATCAAGATCATTGCTCCTCCTGAG	CTGCTTGCTGATCCACATCTG

Primers for CHIP-qPCR	Forward primer sequence 5'- 3'	Reverse primer sequence 5'- 3'
Human *PDK4* SBE-1#	AGGGTAATGTGTCTCAACCACTGTC	TTCCTTGATTACTTCTCCTCACTTC
Human *PDK4* SBE-2#/3#	AGAATAGTACCATGAAAGACCAAGG	CCTGTACGAAATGTCCTTACTCCAA
Human *PDK4* SBE-4#	GAGTGCGGGGAGACAAATAAAAC	GGCTGGGGTTTGAGGGTGC

Supplemental Table 2

Paraffin Block Label	Race/Ethnicity	Sex (M/F)	Diameter (c	Age (yı	BAV	Avg Intima Thickn	Moeckel INTIMAL THICKENING	Chin INTIMAL THICKENING	Nana INTIMAL THICKENING
Healthy Case-1	Caucasian	M	5.0	70	yes	**0.030**	minimal/Healthy	absent	absent
Healthy Case-2	ıfrican-Americaı	M	2.8	39	no	**0.028**	minimal/Healthy	absent	absent
Healthy Case-3	Hispanic	F	2.5	30	no	**0.059**	minimal/Healthy	absent	absent
Healthy Case-4	Hispanic	M	4.9	67	no	**0.048**	minimal/Healthy	absent	absent
Healthy Case-5	Caucasian	M	5.1	46	no	**0.064**	minimal/Healthy	absent	absent
Healthy Case-6	ıfrican-Americaı	F	3.4	66	no	**0.030**	minimal/Healthy	absent	absent
Healthy Case-7	Caucasian	M	5.9	65	yes	**0.037**	minimal/Healthy	absent	absent
Neointima Case-1	Caucasian	M	3.6	54	no	**0.129**	moderate	present	present
Neointima Case-2	Caucasian	M	5.2	69	yes	**0.395**	moderate	present	present
Neointima Case-3	Caucasian	M	4.5	65	no	**0.912**	mild	present	present
Neointima Case-4	ıfrican-Americaı	M	3.4	55	no	**1.475**	severe	present	present
Neointima Case-5	Caucasian	M	5.2	65	no	**0.119**	moderate	present	present
Neointima Case-6	Caucasian		6.2	74	no	**0.320**	severe	present	present