# Supplementary files

# TET1s deficiency exacerbates oscillatory shear flow-induced atherosclerosis

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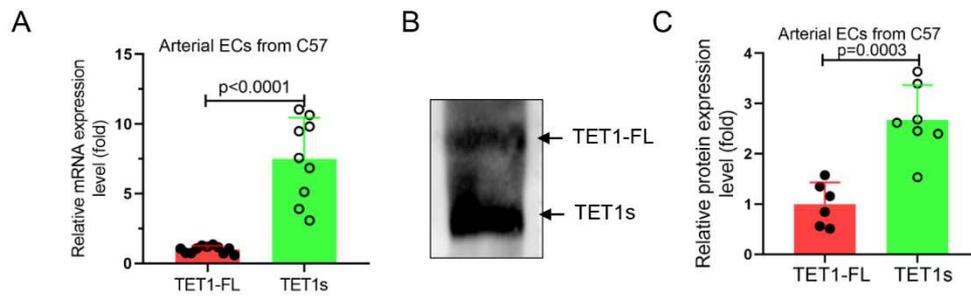
**Figure S1. TET1s is the predominant transcript compared to TET1-FL in arterial ECs from C57 mice.** (A-C) All ECs separated from the aorta of C57mice. (A) RT-qPCR was used to test the mRNA levels of TET1s and TET1-FL(n>6 per group). (B-C) The TET1s and TET1-FL protein expression level was quantified by WB (n>6 per group). All data were presented as the mean ± SD.
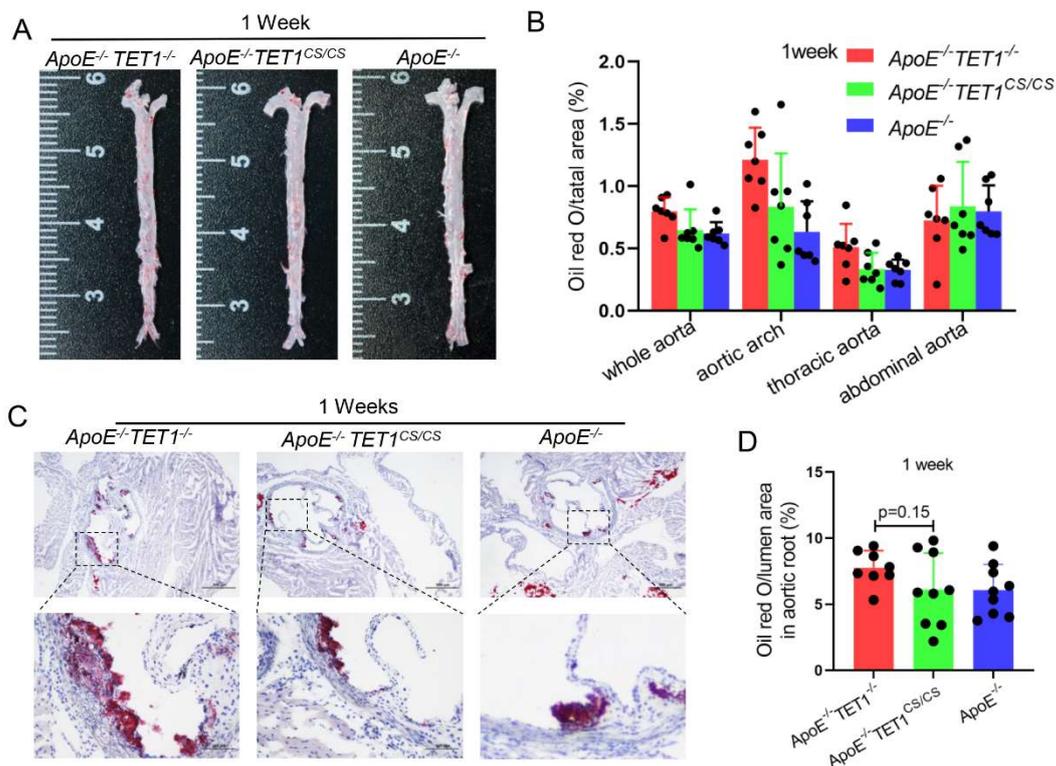


**Figure S2. The plaques in ApoE$^{-/-}$TET1cs/cs mice was no difference compared with ApoE$^{-/-}$ TET1$^{-/-}$mice fed a high-fat diet for 1 week.** (A-D) ApoE$^{-/-}$ TET1$^{-/-}$, ApoE$^{-/-}$ TET1$^{cs/cs}$ and ApoE$^{-/-}$ mice (8 weeks old) were fed a high-fat diet for 1 weeks. (A) The aortic plaques of ApoE$^{-/-}$ TET1$^{-/-}$, ApoE$^{-/-}$ TET1$^{cs/cs}$ and ApoE$^{-/-}$ mice were tested by red oil staining and en face microscopy. (B) The lesion areas in the whole aorta, aortic arch, thoracic aorta, and abdominal aorta sections were

analyzed (n>7 per group). (C-D) Representative photomicrographs of aortic root slice red oil staining and quantitative analysis of atherosclerotic plaque areas in the aortic root (n>7 per group). All data were presented as the mean ± SD.
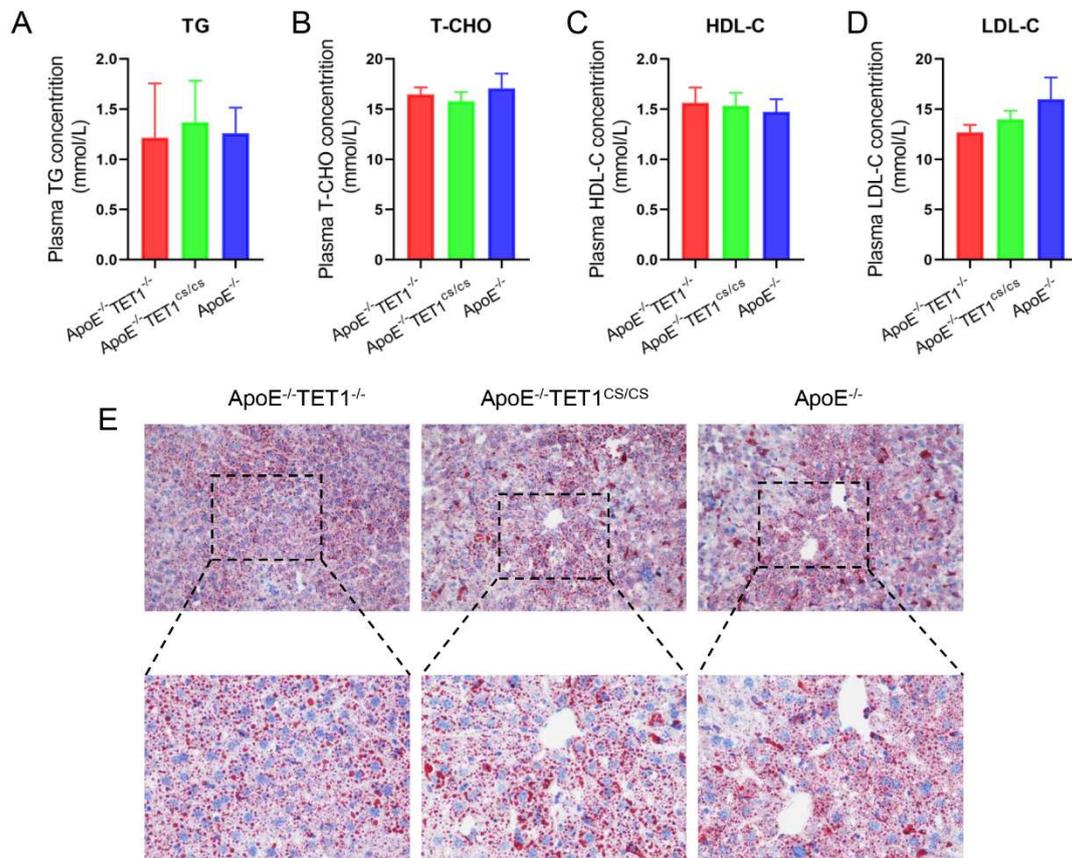


**Figure S3. Deletion of TET1s has no significant change in lipid metabolism in ApoE^{-/-} mice.** (A-E) ApoE^{-/-}TET1^{-/-}, ApoE^{-/-}TET1[CS/CS] and ApoE^{-/-} mice fed with high-fat diet for 12 weeks. (A) Quantitative analyze in triglyceride (TG) of ApoE^{-/-}TET1^{-/-}, ApoE^{-/-}TET1[CS/CS] and ApoE^{-/-} mice fed with high-fat diet for 12 weeks. (A-D) Quantitative analyze triglyceride (TG), total cholesterol (T-CHO), LDL-C and HDL-C of ApoE^{-/-}TET1^{-/-}, ApoE^{-/-}TET1[CS/CS] and ApoE^{-/-} mice (E) The representative images with hepatic slice staining with oil red O of ApoE^{-/-}TET1^{-/-}, ApoE^{-/-}TET1[CS/CS] and ApoE^{-/-} mice. All data are presented with mean value and standard deviation (SD).
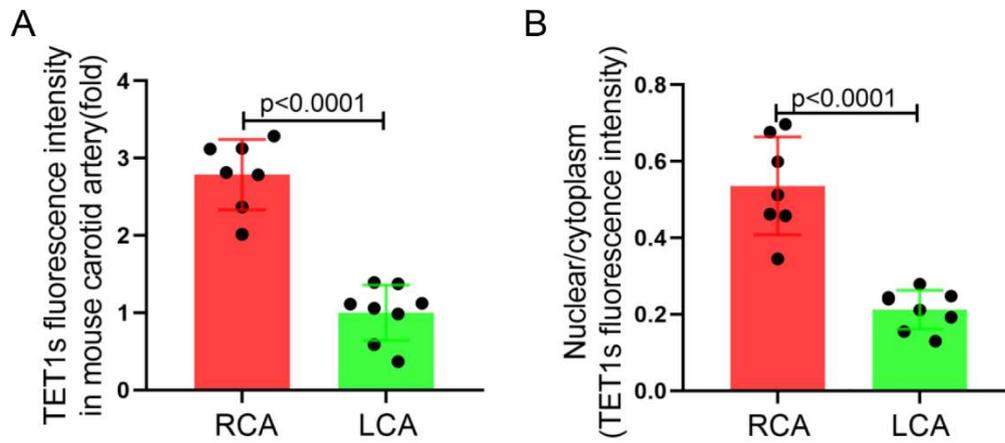
**Figure S4. OSS stimulation was decreased the expression and the nuclear/cytoplasmic ratio of TET1s in carotid ECs in TET1$^{cs/cs}$ mice.** (A-B) TET1$^{cs/cs}$ mice LCA were ligated for 2 weeks. (A) Quantitative analysis of TET1s fluorescence intensity in LCA and RCA ECs to fig.3B. (B) Quantitative analysis of the nuclear/cytoplasmic ratio of TET1s in LCA and RCA ECs to fig.2B. All data were presented as the mean ± SD.
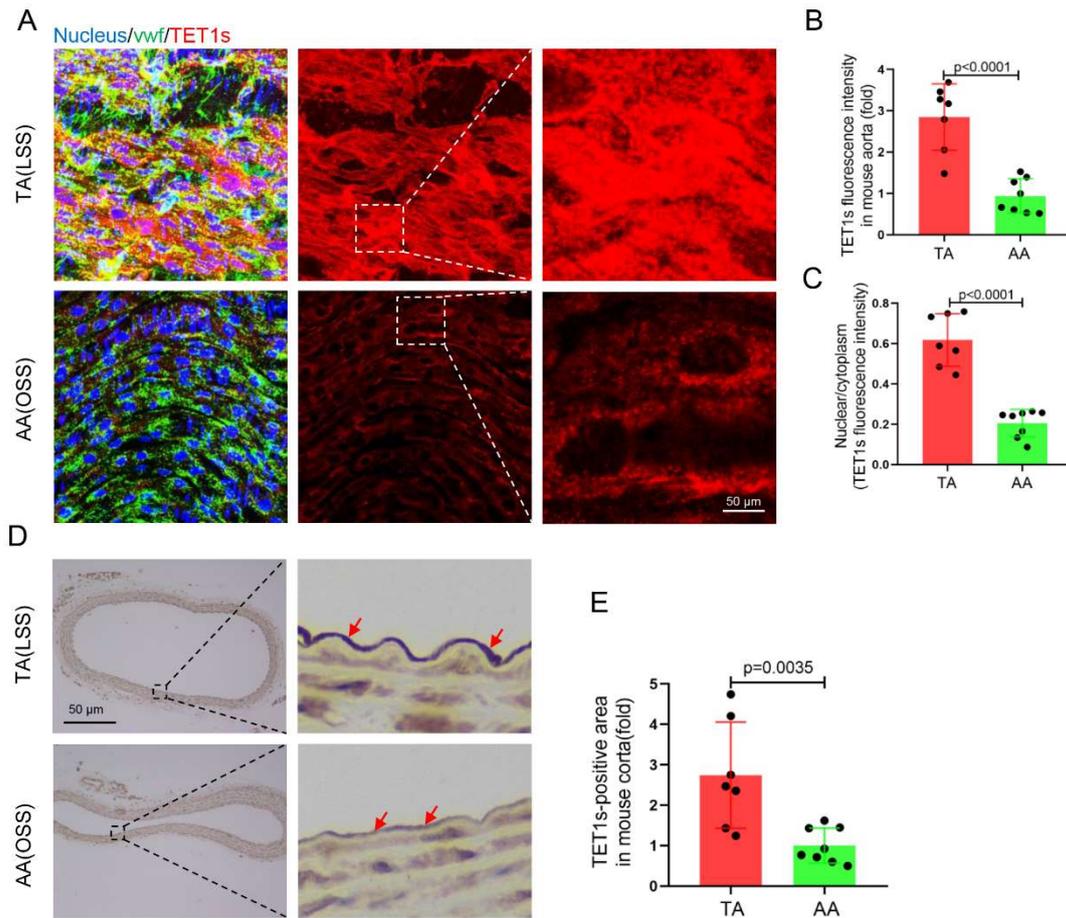
**Figure S5. OSS stimulation was decreased the expression and the nuclear/cytoplasmic ratio of TET1s in aortic ECs in TET1cs/cs mice.** (A) Immunofluorescence staining & *en face* for TET1s in AA and TA ECs (n>7 per group). (B-C) Quantitative analysis of the fluorescence intensity and the nuclear/cytoplasmic ratio of TET1s in AA and TA ECs (n>7 per group). (D-E) Immunohistochemical staining for TET1s in AA and TA slices and quantitative analysis of the TET1s-positive area; red arrows indicate the positive area in ECs (n>7 per group). All data were presented as the mean ± SD.
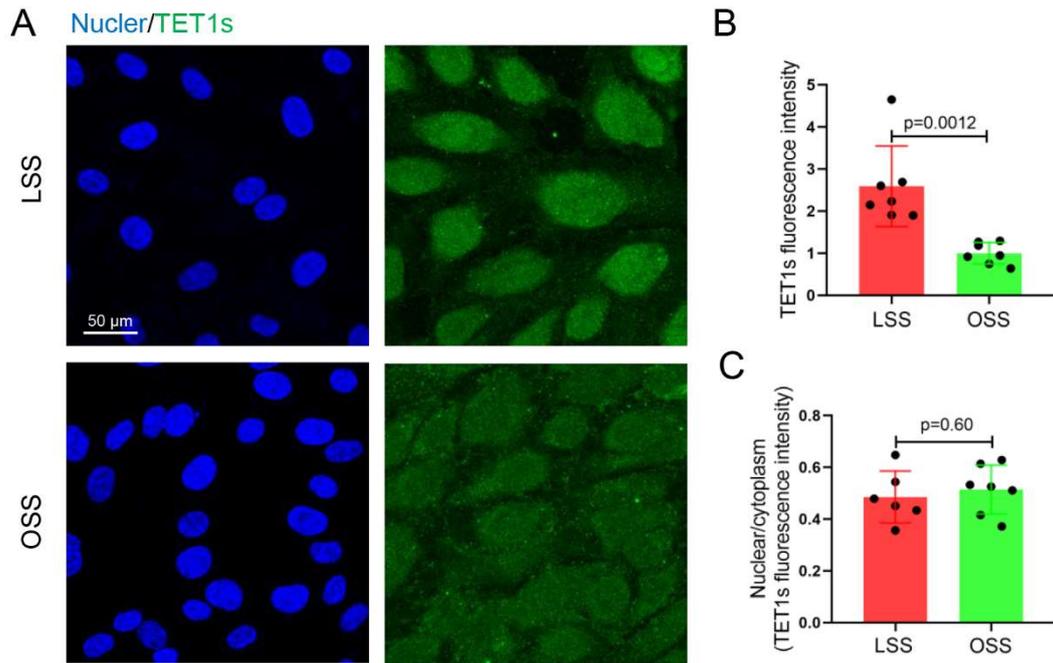
**Figure S6. OSS inhibits TET1s expression levels in primary HUVECs.** (A-C) primary HUVECs with a parallel-plate flow chamber (PPFC) for 24 h. (A) Immunofluorescence staining for TET1s in p-HUVECs. (B-C) Quantitative analysis of the fluorescence intensity and the nuclear/cytoplasmic ratio of TET1s in p-HUVECs (n=7 per group). All data were presented as the mean ± SD.
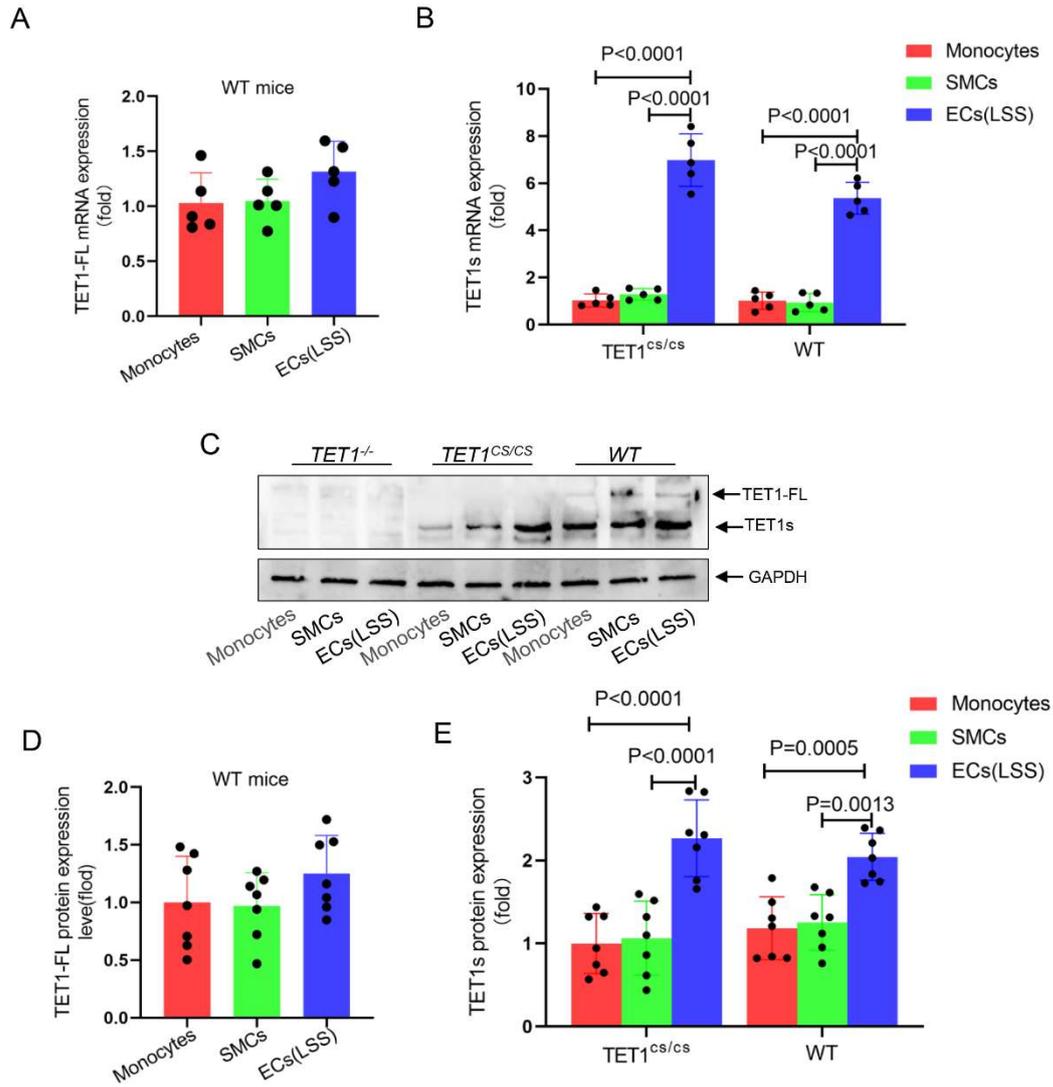
**Figure S7. Low expression levels of TET1s in vascular smooth muscle cells and macrophages.** (A-C) All ECs separated from the thoracic aorta (TA) of TET1^{-/-}, TET1[cs/cs] and WT mice. (A-B) RT-qPCR was used to test the mRNA levels of TET1s and TET1-FL (n=4 per group). (C) The TET1s and TET1-FL protein expression level was quantified by WB (n=4 per group). All data were presented as the mean ± SD.
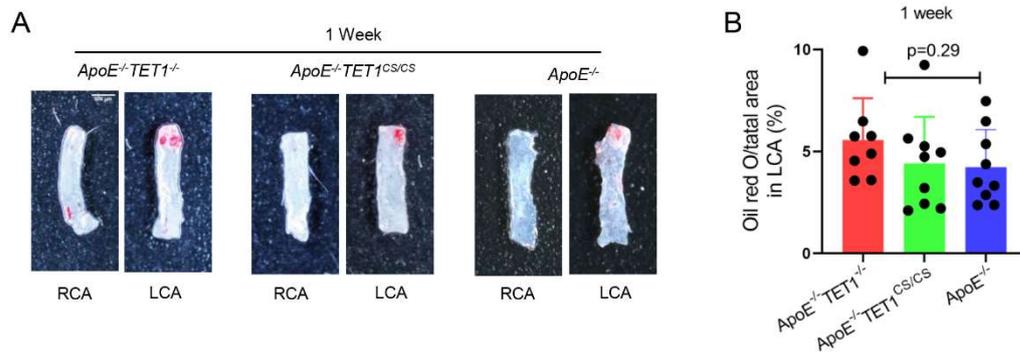
**Figure S8. The carotid plaques in ApoE$^{-/-}$TET1$^{cs/cs}$ mice with a partial carotid artery ligation is no difference compared with ApoE$^{-/-}$TET1$^{-/-}$mice fed a high-fat diet for 1 week.** (A-B) ApoE$^{-/-}$TET1cs/cs, ApoE$^{-/-}$ TET1$^{-/-}$ and ApoE$^{-/-}$ mice LCAs were ligated and fed a high-fat diet for 1 week; the lesion areas in the carotid artery were tested by red oil stain & en face and were analyzed (n=8 per group). All data were presented as the mean ± SD.
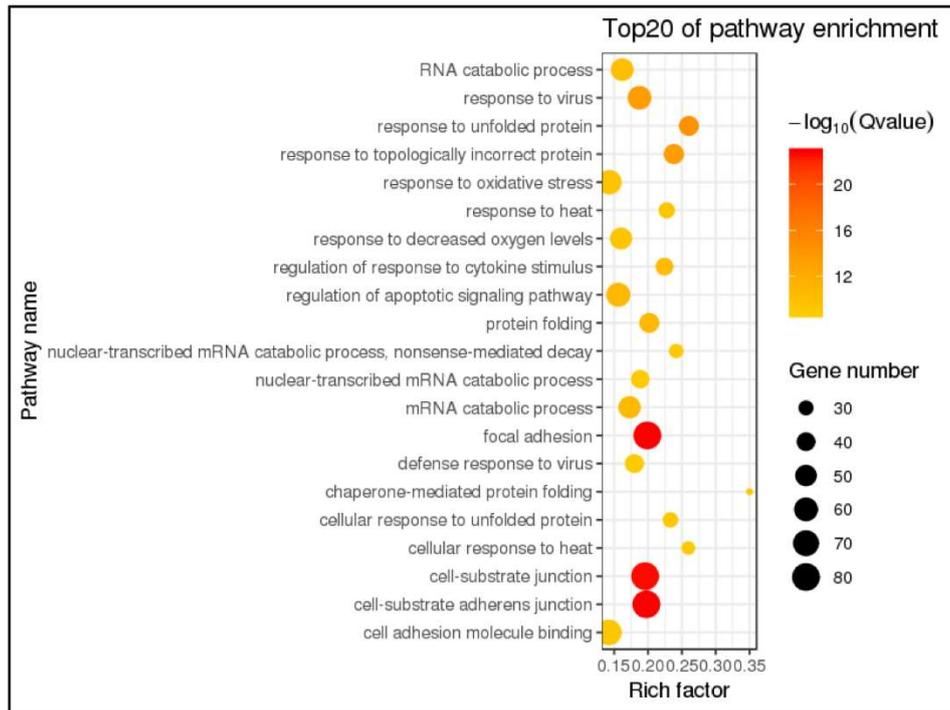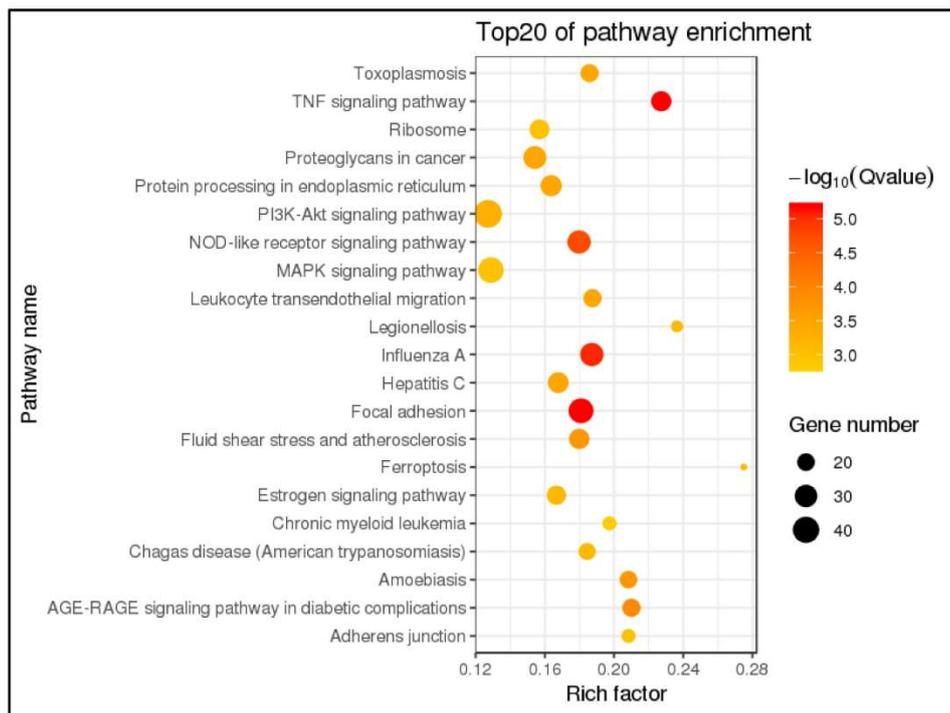
**Figure S9. Differential gene expression enrichment analysis shows that TET1s may regulate endothelial barrier function.** (A-B) RNA sequencing test the global RNA levels of TET1s-overexpressing p-HUVECs and negative control p-HUVECs; the top 20 pathways of differential gene expression GO enrichment analysis (A) and KEGG enrichment analysis (B) with RNA-

sequence data.
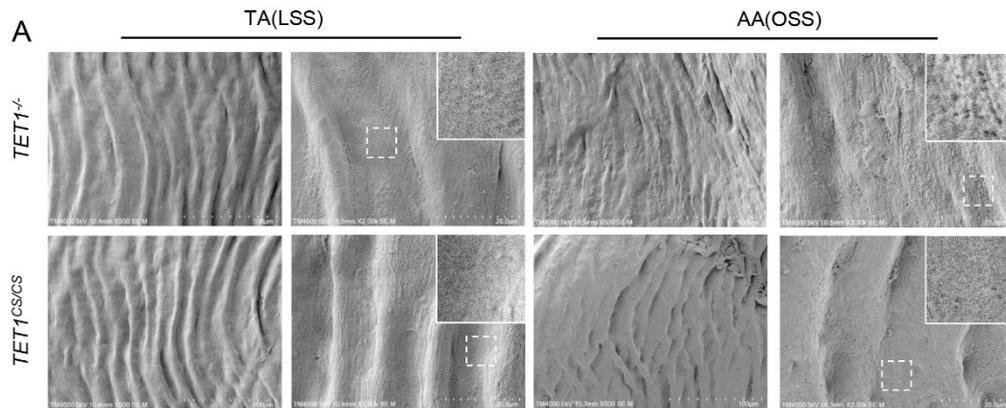


**Figure S10. The intima of the aortic arch of TET1$^{cs/cs}$ mice shows fewer holes compared with TET1$^{-/-}$mice.** (A) The morphology of ECs in AA and TA ECs by scanning electron microscopy (SEM).
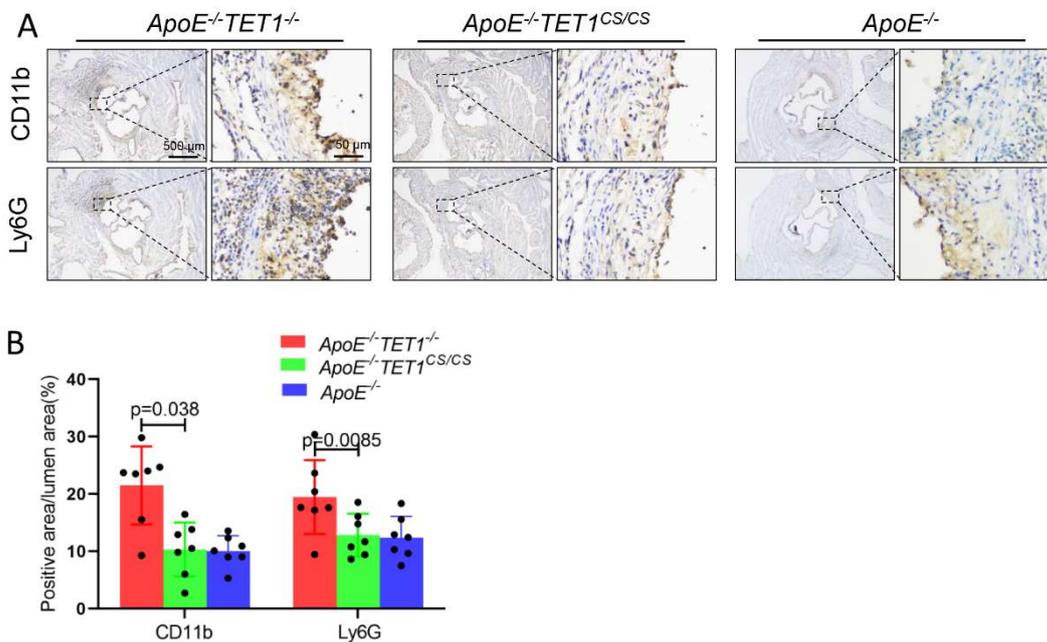


**Figure S11. Neutrophils were significantly decreased in the plaques of ApoE$^{-/-}$TET1$^{cs/cs}$ mice compared with ApoE$^{-/-}$TET1$^{-/-}$ mice.** (A-B) ApoE$^{-/-}$TET1cs/cs, ApoE$^{-/-}$ TET1$^{-/-}$ and ApoE$^{-/-}$ mice were fed a high-fat diet for 4 weeks. The aortic roots were harvested and subjected to further experiments. Representative immunohistochemical staining for Neutrophil-specific antigens CD11b and Ly6G in aortic roots and were analyzed (n=6 per group). All data are presented as the

mean ± SD.
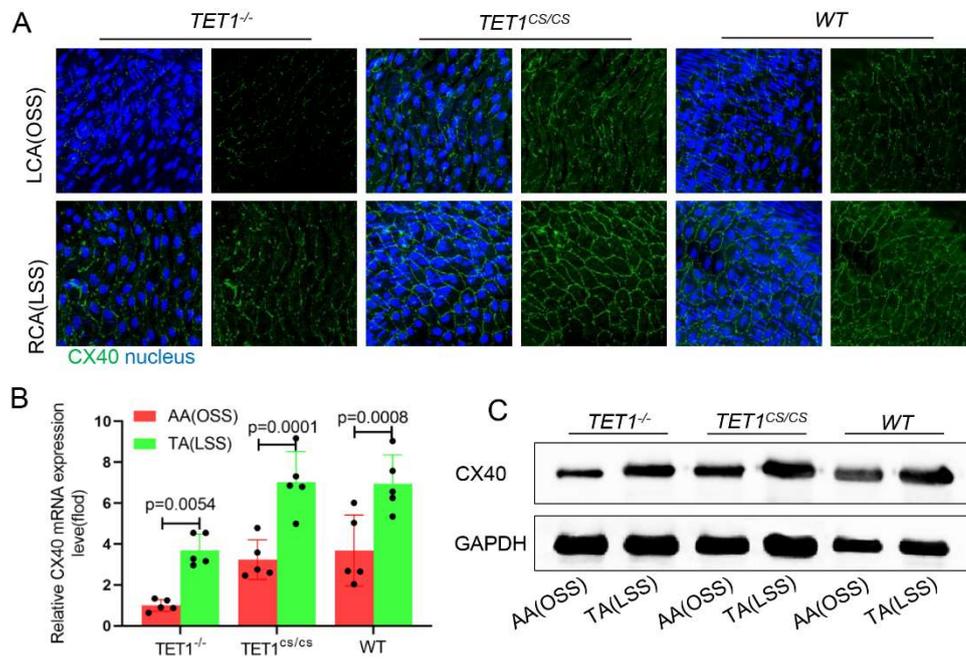


Figure S12. The endothelial CX40 expression level in TET1$^{-/-}$ and TET1$^{cs/cs}$ mice. (A) TET1$^{-/-}$, TET1$^{cs/cs}$ and WT mice LCAs were ligated for 1 week. Immunofluorescence staining for CX40 in RCA and LCA (n=3 per group). (B-C) All ECs separated from the aortic arch (AA) and thoracic aorta (TA) of TET1$^{-/-}$, TET1$^{cs/cs}$ and WT mice. (B) RT-qPCR was used to test the mRNA levels of CX40 (n=5 per group). (C-D) The CX40 protein expression level was quantified by WB (n=4 per group). All data were presented as the mean ± SD.
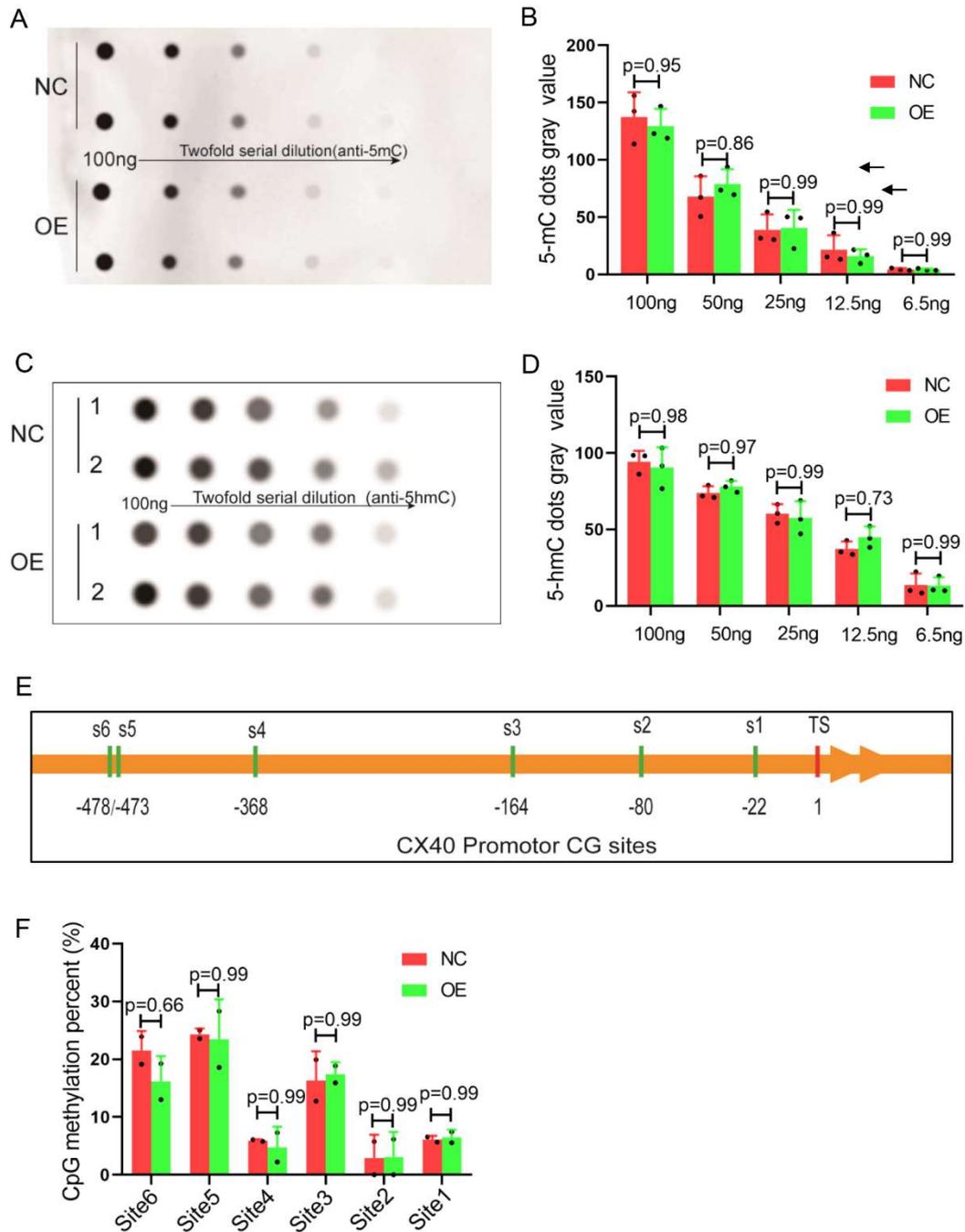
**Figure S13. TET1s overexpression did not cause a global difference in 5mC levels in p-HUVECs.** (A-F) p-HUVECs were transfected with TET1s-overexpressing adenovirus and negative control adenovirus and further tested after 48 h. (A-B) Dot blot assay was used to analyze the global 5mC levels of p-HUVECs (n=6 per group). (C-D) Dot blot assay was used to analyze the global 5hmC levels of p-HUVECs (n=6 per group). (E-F) Pyrosequencing assay analyzed the local 5hmC levels in the CX40 promoter 6 CG sites (S1-S6 indicates site 1-site 6; TS indicates transcriptional start; n=3 per group). All data were presented as the mean ± SD.

**Table S1. The primers for gene-test of knockout mice.**

| primers （name） | sequence(5'-3') |
|---|---|
| GT-apoE-OF | GCCTAGCCGAGGGAGAGCCG |
| GT-apoE-IR | TGTGACTTGGGAGCTCTGCAGC |
| GT-apoE-OR | GCCGCCCCGACTGCATCT |
| GT-TET1-F | AACTGATTCCCTTCGTGCAG |
| GT-TET1-R | TTAAAGCATGGGTGGGAGTC |
| GT-TET1CS-OF | ATCTGGGCAATGTTGTGACTC |
| GT-TET1CS-IR | CATTGTAAACCCGTTGCAAGT |
| GT-TET1CS-OR | TTCTTTCCCTTCCACTATGCA |

**Table S2. The primers for RT-qPCR.**

| primers （name） | sequence(5'-3') |
|---|---|
| H-GAPDH-F | GGAGCGAGATCCCTCCAAAAT |
| H-GAPDH-R | GGCTGTTGTCATACTTCTCATGG |
| H-TET1FL-F | GCGCGAGTTGGAAAGTTTG |
| H-TET1FL-R | GCTCAGTCACACAAGGTTTTGG |
| H-TET1s-F | CAAGCAAGATGGCTACCTCGT |
| H-TET1s-R | GGGGCCTCTTGTTTTCCTTTA |
| H-CX40-F | GCTGCCAGAATGTCTGCTAC |
| H-CX40-R | GGTACTCGTAAGAGCCAGAGC |
| H-TMEM129-F | GAGGTGACCTTCACTCTCGC |
| H-TMEM129-R | GCCCACATAGTAGCCGAGC |
| H-TGFA-F | AGGTCCGAAAACACTGTGAGT |
| H-TGFA-R | AGCAAGCGGTTCTTCCCTTC |
| H-IGF1-F | GCTCTTCAGTTCGTGTGTGGA |

| | |
|---|---|
| H-IGF1-R | GCCTCCTTAGATCACAGCTCC |
| H-APLNR-F | CCTGCATCAGCTACGTCAACA |
| H-APLNR-R | GGGATGGATTTCTCGTGCATCT |
| H-PIEZO2-F | ATGGCCTCAGAAGTGGTGTG |
| H-PIEZO2-F | ATGTCCTTGCATCGTCGTTTT |
| H-KLF2-F | CTACACCAAGAGTTCGCATCTG |
| H-KLF2-R | CCGTGTGCTTTCGGTAGTG |
| H-KLF4-F | CAGCTTCACCTATCCGATCCG |
| H-KLF4-R | GACTCCCTGCCATAGAGGAGG |
| M-TET1FL-R | TACTGCAAGAATCGAAAGAACAGCCA |
| M-TET1FL-R | CGGAAGGTGTGTGTCAGTGGGT |
| M-TET1s-F | TAAGACAGACTTTTAGGGGGAAAG |
| M-TET1s-R | GTGTGTGTCAGTGGGTAAACAGT |
| M-GAPDH-F | TGACCTCAACTACATGGTCTACA |
| M-GAPDH-R | CTTCCCATTCTCGGCCTTG |
| M-CX40-F | GGTCCACAAGCACTCCACAG |
| M-CX40-R | CTGAATGGTATCGCACCGGAA |

**Table S3. The primers for CHIP- qPCR.**

| primers（name） | sequence(5'-3') |
|---|---|
| P1-F | TCCTGTCACTGAGGAAATTCCTGTTC |
| P1-R | TGCTGTCTGAGATGGCTCTTAATGAG |
| P2-F | TCATTAAGAGCCATCTCAGACAGCAG |
| P2-R | AATCTCTGATGCTGGCCTTGC |
| P3-F | AAGGCAAGGCCAGCATCAG |
| P3-R | TCCTGTGCATGACTTTCTGGAATG |
| P4-F | CTCATTCCAGAAAGTCATGCACAGG |
| P4-R | GCCTGAAGTCAAGCTTGTCTGG |
| P5-F | CCAGACAAGCTTGACTTCAGGC |

| | |
|---|---|
| P5-R | GAGATCTTGTCCTGAGAGCATTATGCTC |

**Table S4. The primers for pyrosequencing assays.**

| primers （name） | sequence(5'-3') |
|---|---|
| 1.GJA5-1F(230bp) | TTTGGGTGAAAGTTTTATTTGGATATG |
| 1.GJA5-1R | TCCCAACTTTAATCTACTCCTATCACT |
| 1.GJA5-1S | CTCAAAAACATTTAACTTCC |
| 2.GJA5-2F(72bp) | TGGTTTTTTGGGTGAAAGTTT |
| 2.GJA5-2R | AAACCATCTCAAACAACAAATATCTATTAA |
| 2.GJA5-2S | TTTTGGGTGAAAGTTTTATT |
| 3.GJA5-3F(224bp) | AGAGGATTAGAAAAGGTAAGGTTAGTAT |
| 3.GJA5-3R | CCTCTTTAAAACCTAAAATCAAACTTATCT |
| 3.GJA5-3S | AGAGGTTTTTAAGTAAATAGTG |
| 4.GJA5-2F(221bp) | AAGGAGATTTTGTTTTGAGAGTATTATG |
| 4.GJA5-4R | AACAATACTACCCATCCTTTCAACTACCC |
| 4.GJA5-4S | ATTAAAAAGGAAGTTAGATTGT |
| 5.GJA5-5F(140bp) | TGATTTTAGGTTTTAAAGAGGAAGTTAATG |
| 5.GJA5-5R | ACTCAACCCTTCCCTAAC |
| 5.GJA5-5S | ACCCTTCCCTAACTC |