Supplementary Material

Endothelial-Specific Overexpression of Histone Deacetylase 2 Protects Mice against Endothelial Dysfunction and Atherosclerosis

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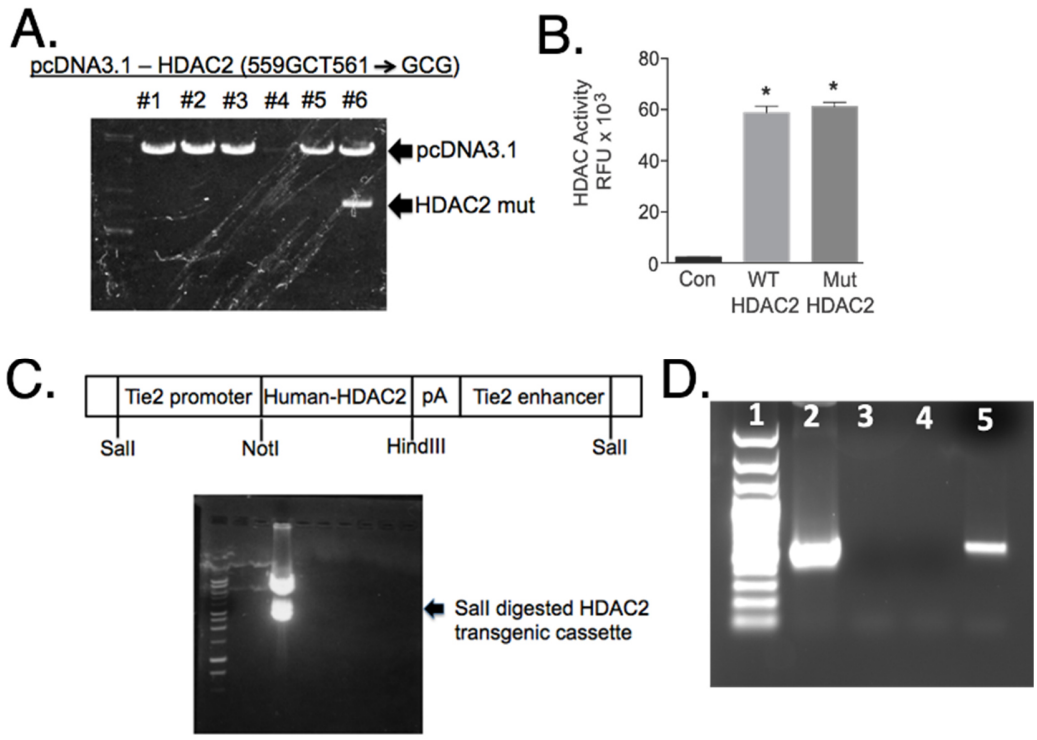
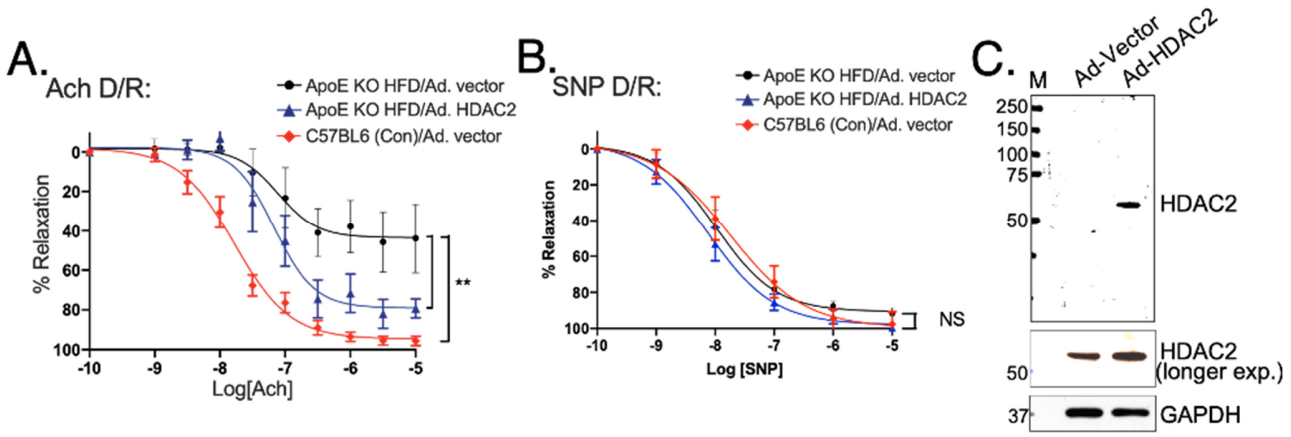
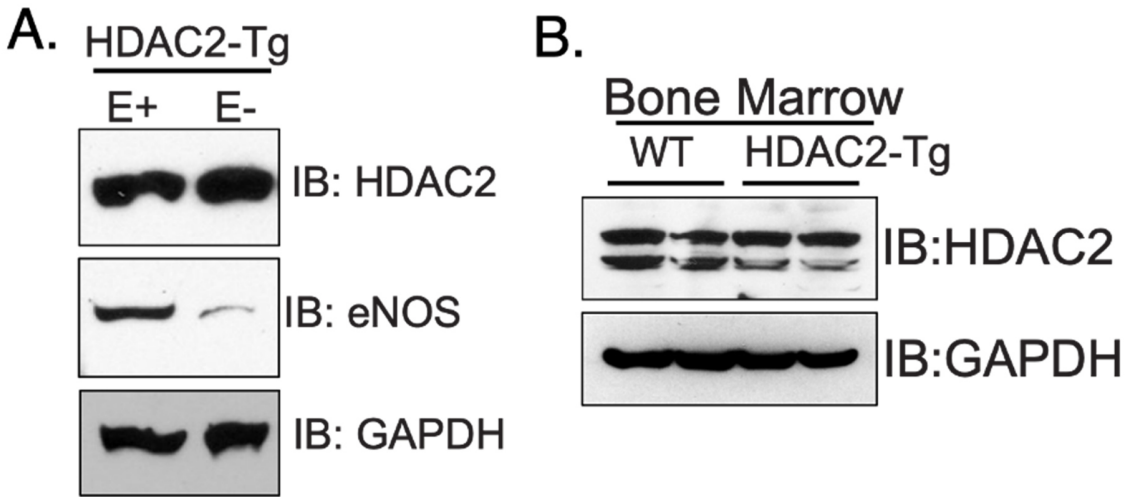


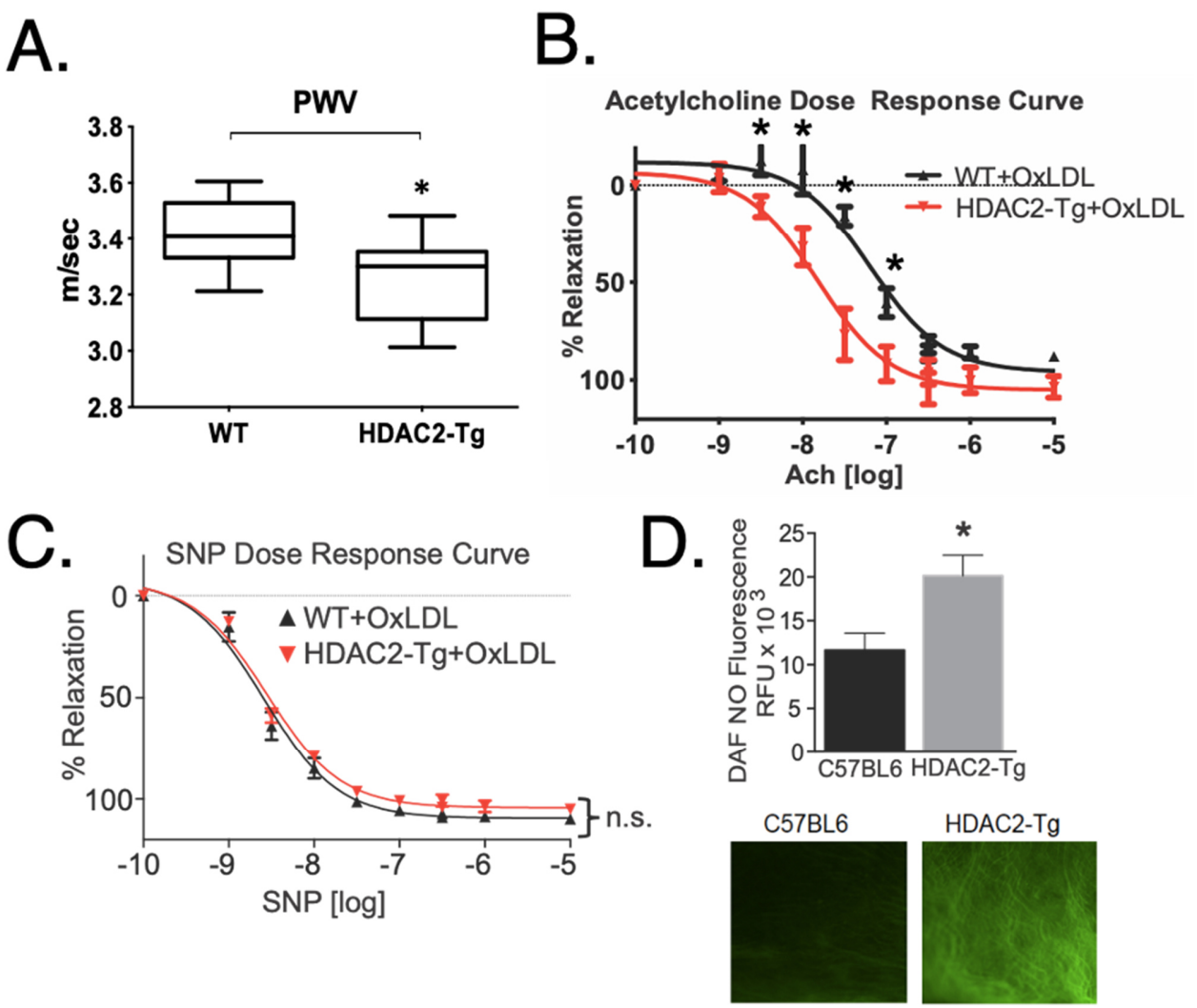
Supplementary Figure S1. Construction of an endothelial-specific human HDAC2-overexpressing (Tie2-hHDAC2) transgene. (A) HindIII restriction digestion of HDAC2 clones. The HindIII digestion site in HDAC2 was removed by mutating thymine at position 561 to guanine while preserving the amino acid coded by that codon. **(B)** HDAC activity from HEK 293 cells expressing either wild-type HDAC2 or mutant HDAC2 lacking the HindIII site was determined with the HDAC Fluorometric Activity Assay Kit (Cayman). **(C)** SalI restriction digestion of human HDAC2 cDNA that was subcloned into pSPTg.T2FpAXK (30 µg) utilizing unique HindIII and NotI restriction sites. **(D)** Primers specific to human HDAC2 were used for PCR analysis with genomic DNA isolated from the tails of the following mice as templates: for (A) C57BL/6 (lane 2), non-transgenic littermates (lane 3), and an HDAC2 transgenic founder (lane 4); a transgenic DNA construct that was injected into the murine ooytes was used as positive control template (lane 1).



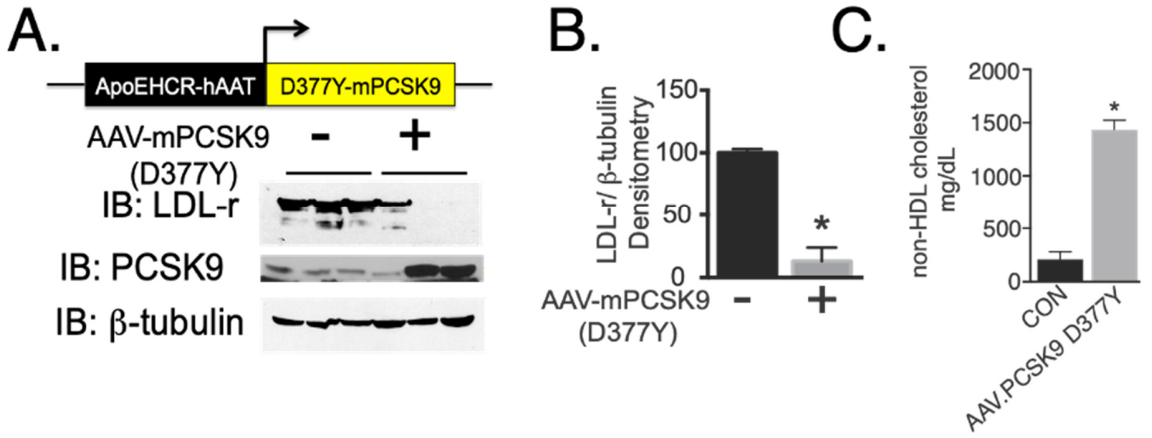
Supplementary Figure S2. HDAC2 protects endothelial-dependent vascular relaxation in atherogenic ApoE$^{-/-}$ mice. Dose-response (D/R) to **(A)** acetylcholine (Ach), **p<0.005 vs. C57BL6/Ad.vector. **(B)** sodium nitroprusside (SNP) in aortic rings isolated from ApoE$^{-/-}$ mice fed with a high-fat diet (HFD) and administered three injections of either adenoviral HDAC2 or Ad-vector (control). **(C)** HDAC2 expression in aortas isolated from HDAC2 adenovirus-injected mice fed a HFD regimen for 10 weeks.



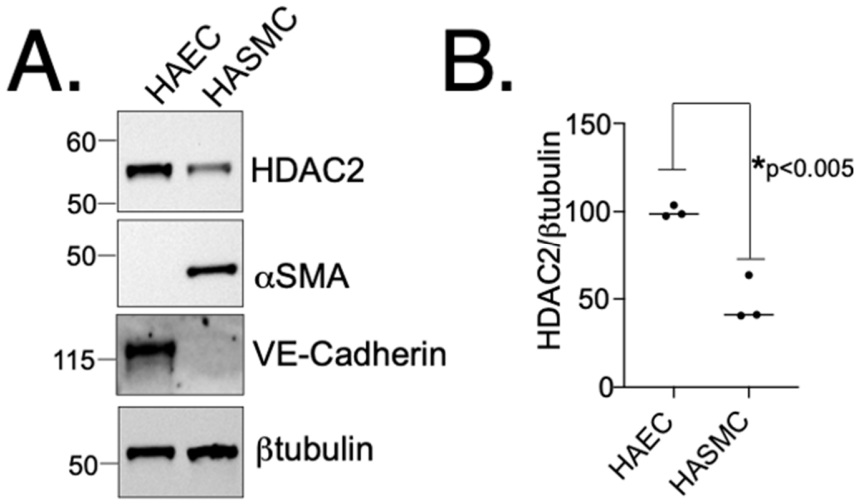
Supplementary Figure S3. Endothelial specificity of HDAC2-Tg and effect on HDAC2 expression in myeloid cells. (A) Snap-frozen, isolated endothelium-intact (E+, control) and endothelium-denuded (E-) aortas from HDAC2-Tg mice were subjected to western blotting using anti-HDAC2, anti-eNOS, and anti-GAPDH (loading control) antibodies. **(B)** Bone marrow was harvested from femurs and subjected to western blotting using anti-HDAC2 and anti-GAPDH antibodies. IB, immunoblot; WT, wild-type.



Supplementary Figure S4. Endothelial-specific HDAC2-overexpressing mice (HDAC2-Tg) have decreased vascular stiffness, enhanced endothelial nitric oxide (NO) production, and enhanced endothelial-dependent vasorelaxation at baseline. (A) Mean pulse wave velocity (PWV) was determined in HDAC2-Tg mice and age-matched wild-type (WT) controls (*p<0.05 vs. HDAC2-Tg). Isolated aortic rings from WT and HDAC2-Tg mice were incubated with or without OxLDL (50 μg/mL). After 48 hours, dose-response effects of **(B)** acetylcholine (*p<0.05 vs. WT+OxLDL) and **C)** sodium nitroprusside on vascular relaxation were determined by wire myography. **(D)** "*En face*" detection of NO production in aortic intima of WT and HDAC2-Tg mice was measured by DAF fluorescence and presented as relative fluorescence unit (RFU). *p<0.05 vs. WT+HDAC2-Tg.



Supplementary Figure S5. Mouse model of atherosclerosis with PCSK9 mutant. (A) Atherosclerosis was induced in C57BL/6 mice via one intraperitoneal injection of adeno-associated virus encoding murine PCSK9 D377Y gain-of-function cDNA controlled by a liver-specific promoter. Liver lysates were immunoblotted for PCSK9, low-density lipoprotein receptor (LDL-r), and β-actin. **(B)** Densitometric analyses of western blot shown in A (*p<0.05 vs. PBS). **(C)** Non-HDL cholesterol was determined in phosphate-buffered saline- and PCSK9 AAV-injected mice (*p<0.05 vs. PBS [Con]).



Supplementary Figure S6. Aortic endothelial cells express higher levels of HDAC2 than do smooth muscle cells. **(A)** Quiescent human aortic endothelial cell (HAEC) and human aortic smooth muscle cell (HASMC) lysates were subjected to western blotting with HDAC2, αSMA, VE-Cadherin, and β-tubulin antibodies. **(B)** Densitometric analysis of western blot shown in A. *p<0.05 vs. HAEC.