Clinical Characterization of Patients with Cardiovascular Disease

	Non-diabetes (n=5)	Diabetes (n=5)	p-value
Age (yr)	61.7 ± 7.4	61.6 ± 8.9	0.987
Gender	Males	Males	-
BMI (kg/m^2)	26.2 ± 1.9	35.9 ± 6.9	0.016
HbA1c (%)	5.6 ± 2.0	7.0 ± 1.1	0.027
SBP (mmHg)	147.0 ±11.0	134.0 ±12.0	0.149
DBP (mmHg)	81.0 ± 8.0	72.0 ± 8.0	0.095
Cholesterol (mM)	4.1 ± 0.9	4.3 ± 1.0	0.818
LDL (mM)	2.2 ± 0.7	2.0 ± 0.6	0.531
HDL (mM)	1.2 ± 0.4	1.0 ± 0.3	0.382

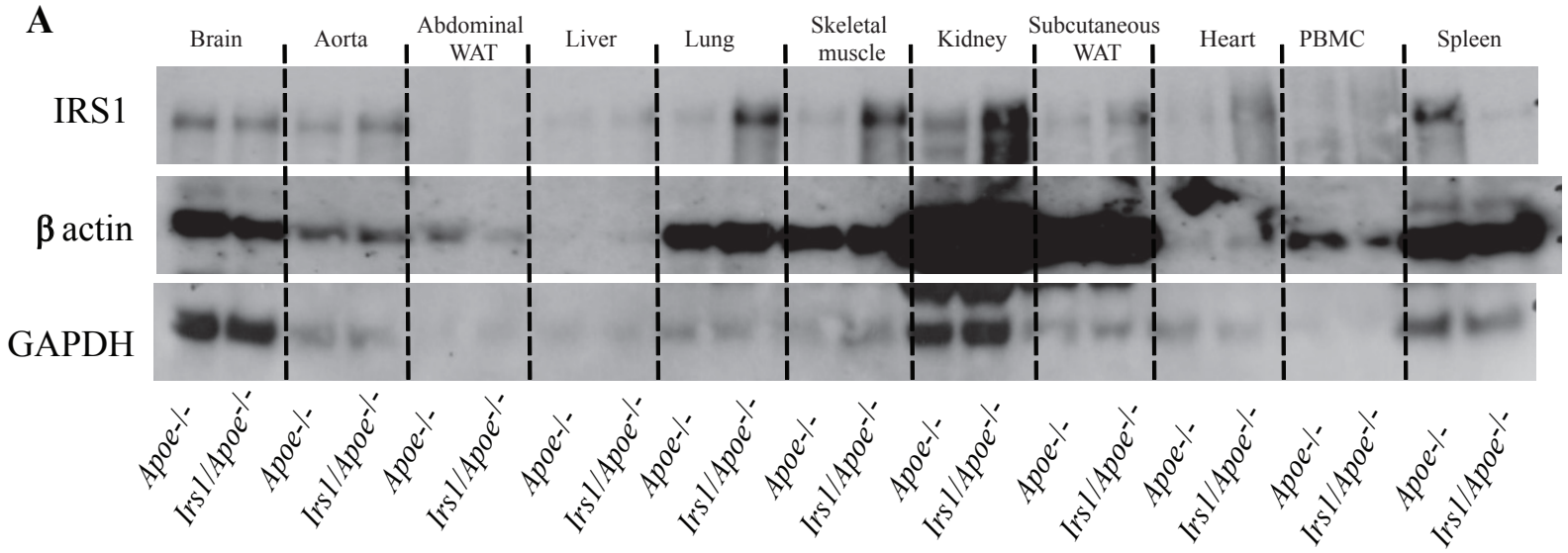
Supplemental Table 1

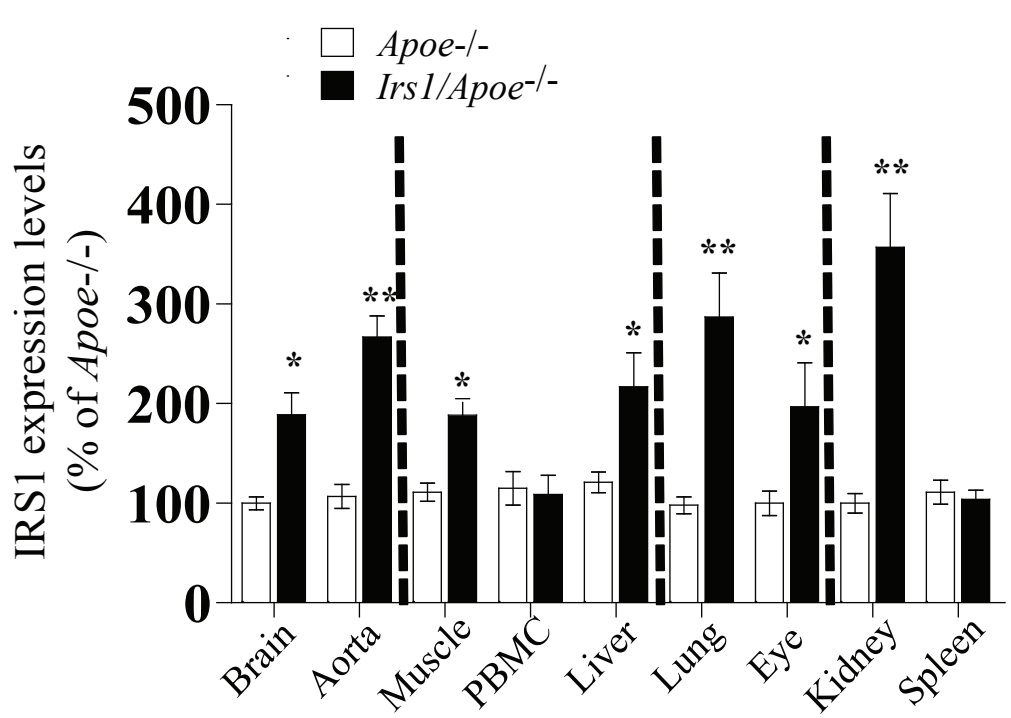
Result of ANOVA Analysis

Figure	Factor Variable	P value for main effect	Interaction	P value for interaction
Figure 1F	Gene Insulin	<0.001 <0.001	Gene#Insulin	0.01
Figure 1H	Gene Insulin	<0.001 <0.001	Gene#Insulin	0.07
Figure 2A	Gene Time Diet	0.39 <0.001 <0.001	Gene#Time Gene#Diet Time#Diet Gene#Time#Diet	0.83 0.58 <0.001 0.97
Figure 2B	Gene Time Diet	0.76 <0.001 0.01	Gene#Time Gene#Diet Time#Diet Gene#Time#Diet	0.96 0.78 <0.12 0.97
Figure 2E	Gene Diet	<0.001 <0.001	Gene#Diet	0.001
Figure 2I **p-AKT**	Gene Insulin Diet	<0.001 <0.001 <0.001	Gene#Insulin Gene#Diet Insulin#Diet Gene#Insulin#Diet	<0.01 0.14 <0.001 0.25
Figure 2I **p-eNOS**	Gene Insulin Diet	<0.001 <0.001 <0.001	Gene#Insulin Gene#Diet Insulin#Diet Gene#Insulin#Diet	<0.001 0.1 <0. 001 0.17
Figure 3B **VCAM1**	Gene Insulin Ox-LDL	0.001 0.005 <0.001	Gene#Insulin Gene#Ox-LDL Insulin#Ox-LDL Gene#Insulin#Ox-LDL	0.18 0.004 0.03 0.11
Figure 3B **p-AKT**	Gene Insulin Ox-LDL	<0.001 <0.001 <0.001	Gene#Insulin Gene#Ox-LDL Insulin#Ox-LDL Gene#Insulin#Ox-LDL	<0.001 0.19 <0.001 0.05
Figure 3C	Gene Insulin Ox-LDL	<0.001 0.04 <0.001	Gene#Insulin Gene#Ox-LDL Insulin#Ox-LDL Gene#Insulin#Ox-LDL	0.49 <0.001 0.17 0.9
Figure 3D **Insulin's effect**	Gene Insulin Ox-LDL	0.002 0.04 <0.001	Gene#Insulin Gene#Ox-LDL Insulin#Ox-LDL Gene#Insulin#Ox-LDL	0.29 0.002 0.02 0.26
Figure 3D **Antibody effect**	Gene Antibody	0.004 0.01	Gene#Antibody	<0.05
Figure 3F **p-AKT**	Gene Diet	<0.001 <0.001	Gene#Diet	0.89
Figure 3F **p-eNOS**	Gene Diet	<0.001 <0.001	Gene#Diet	0.002

Figure 4A	Gene Diet	0.4 <0.001	Gene#Diet	0.6
Figure 4B Endothelium	Gene Diet	0.02 <0.001	Gene#Diet	0.01
Figure 4B Smooth Muscle Cells	Gene Diet	<0.001 <0.001	Gene#Diet	0.004
Figure 4E	Gene Insulin	<0.001 <0.001	Gene#Insulin	<0.001
Figure 4F	Gene Time	<0.001 <0.001	Gene#Time	<0.001
Figure 5B	Insulin Inhibitor	<0.001 <0.001	Insulin#Inhibitor	0.01
Figure 5C	Gene Insulin	<0.001 <0.001	Gene#Insulin	<0.001
Figure 5F	Gene Insulin Inhibitor	<0.001 <0.001 <0.001	Gene#Insulin Gene#Inhibitor Insulin#Inhibitor Gene#Insulin#Inhibitor	<0.001 <0.001 <0.001 0.02
Figure 6D	Gene Diet Inhibitor	<0.001 <0.001 <0.001	Gene#Diet Gene#Inhibitor Diet#Inhibitor Gene#Diet#Inhibitor	<0.001 <0.001 <0.001 <0.001
Figure 7A	Gene Insulin	<0.001 <0.001	Gene#Insulin	<0.001
Figure 7C	Gene Diet	0.01 <0.001	Gene#Diet	0.02
Figure 7D	Gene	0.04	NA	NA
Figure 8B	Gene	0.003	NA	NA
Figure 8D	Gene	<0.05	NA	NA
Figure 9C	Gene Diet	0.002 <0.001	NA	NA
Figure 9E Macrophages	Gene Diet	0.003 <0.001	NA	NA
Figure 9E Collagen	Gene Diet	0.002 <0.001	NA	NA
Figure 9F	Gene Diet	0.99 <0.001	NA	NA

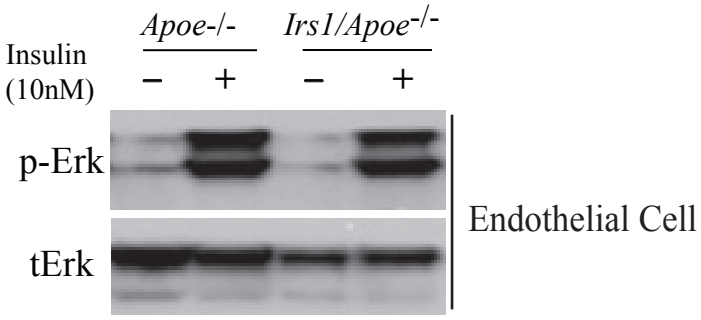
Supplemental Table 2





Supplemental Figure 1. **Distribution of IRS1 expression in various tissues.** (A and B) IB analysis (A) and quantification (B) of IRS1 expression levels in various tissues from *Apoe*$^{-/-}$ and *Irs1/Apoe*$^{-/-}$ mice. Data are represented as mean ± SEM of at least three mouse replicates. *; $p<0.05$ and **; $p<0.01$ (Two-tailed Student's t-test for pairwise comparisons).

A



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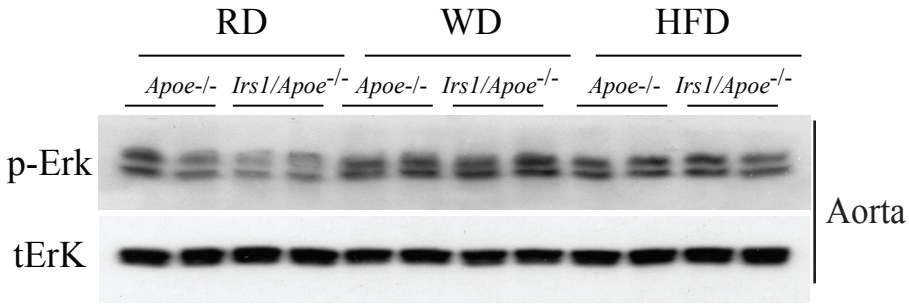
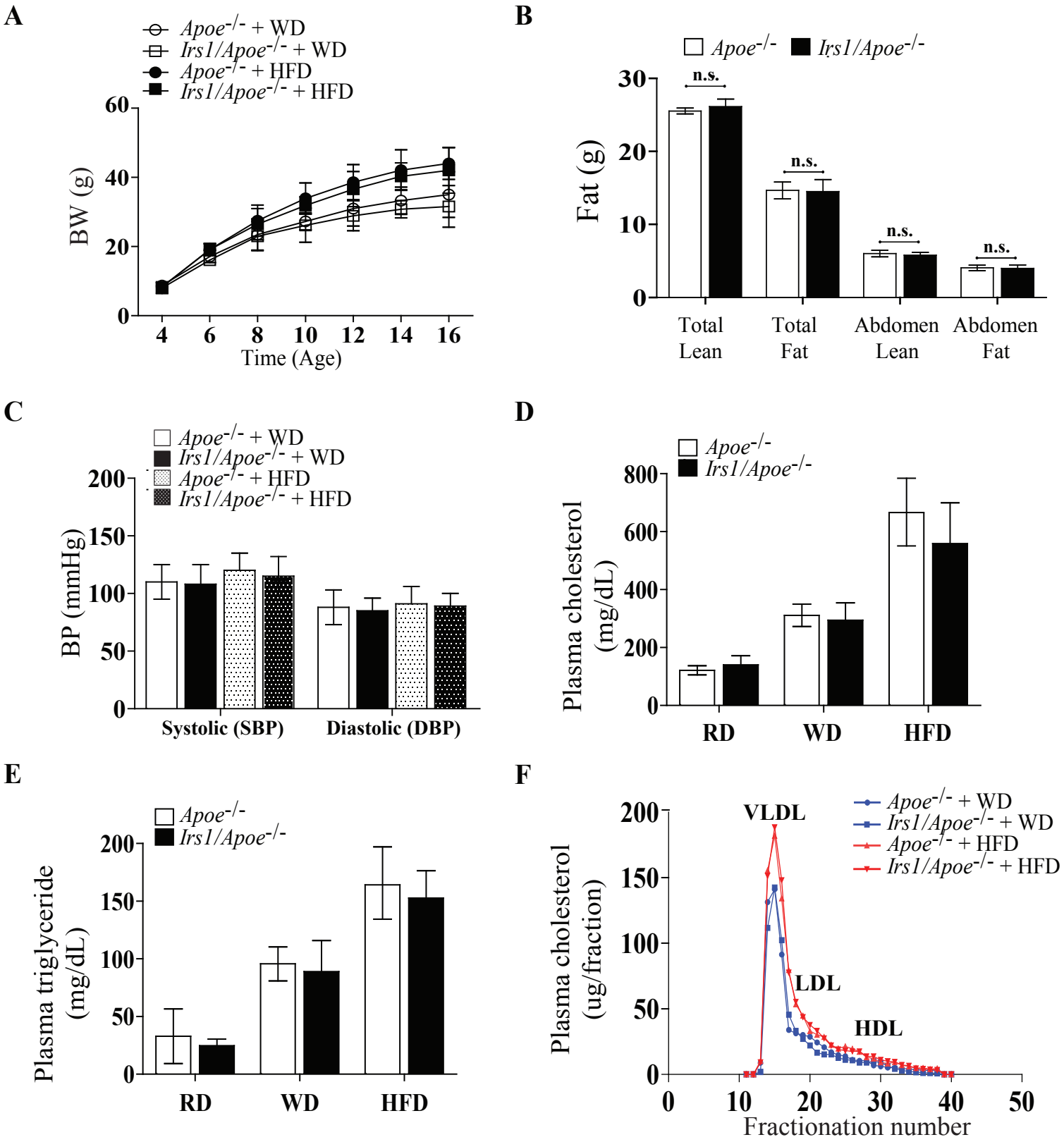
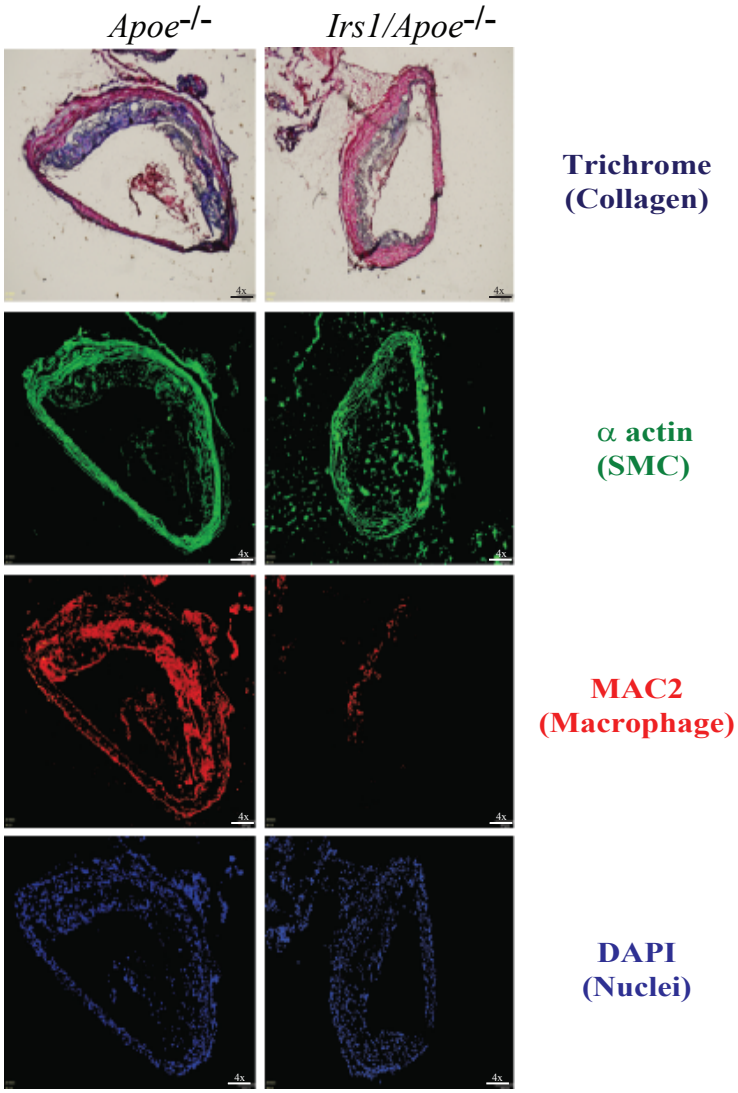


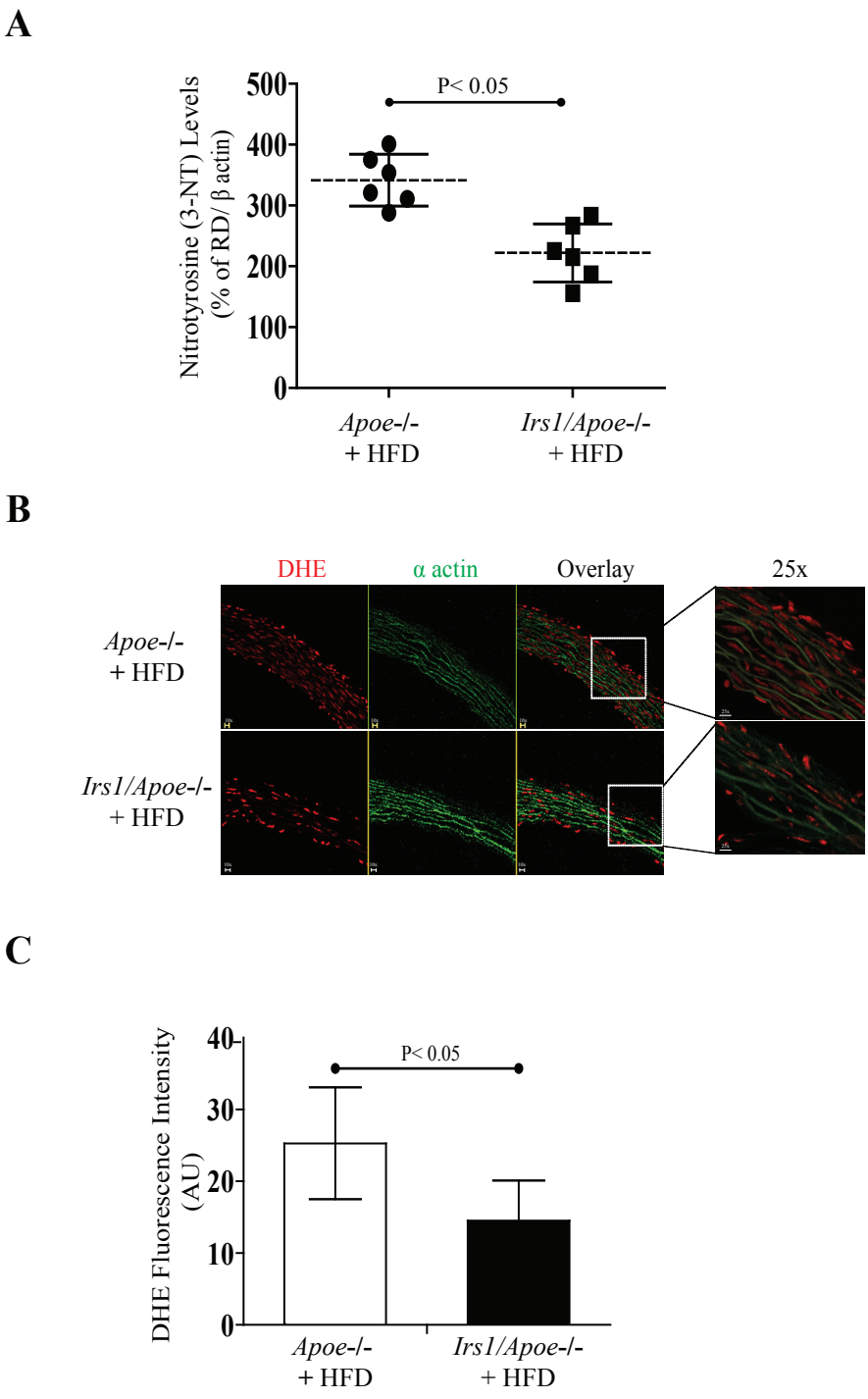
Supplemental Figure 2. **Activation of Erk in EC and aorta from *Apoe*$^{-/-}$ and *Irs1/Apoe*$^{-/-}$ mice.** (A) IB analysis of p-Erk/tErk protein levels in EC after insulin stimulation. (B) IB analysis of p-Erk/tErk levels in the aorta from *Apoe*$^{-/-}$ and *Irs1/Apoe*$^{-/-}$ mice fed on RD, WD and HFD. Data are represented as mean ± SEM of at least cellular replicates or three mouse replicates. (Two-way ANOVA for multiple comparisons involving two factorial variables and two-tailed Student's t-test for pairwise comparisons).



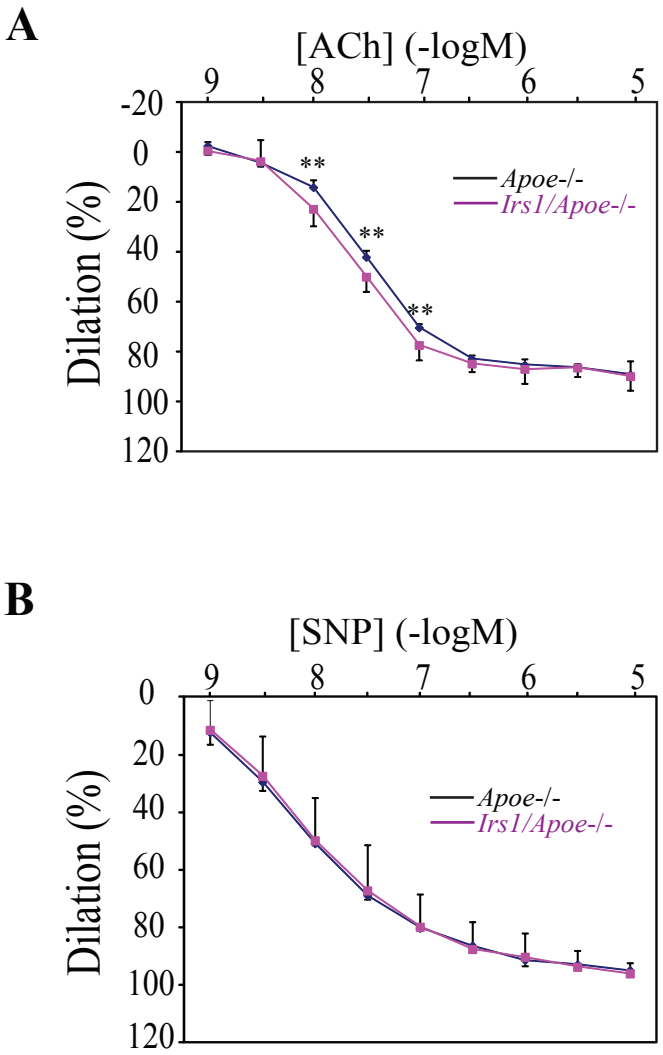
Supplemental Figure 3. **Metabolic parameters assay in *Apoe*$^{-/-}$ and *Irs1/Apoe*$^{-/-}$ mice.** (A) BW, (B) fat compositio, (C) BP, (D and E) plasma cholesterol and triglyceride, and (F) plasma lipoprotein were measured in *Apoe*$^{-/-}$ and *Irs1/Apoe*$^{-/-}$ mice. All data are represented as mean ± SEM of at least five mouse replicates. (Mixed effects model for repeated measurement and two-way ANOVA for multiple comparisons involving two/three factorial variables and two-tailed Student's t-test for pairwise comparisons).



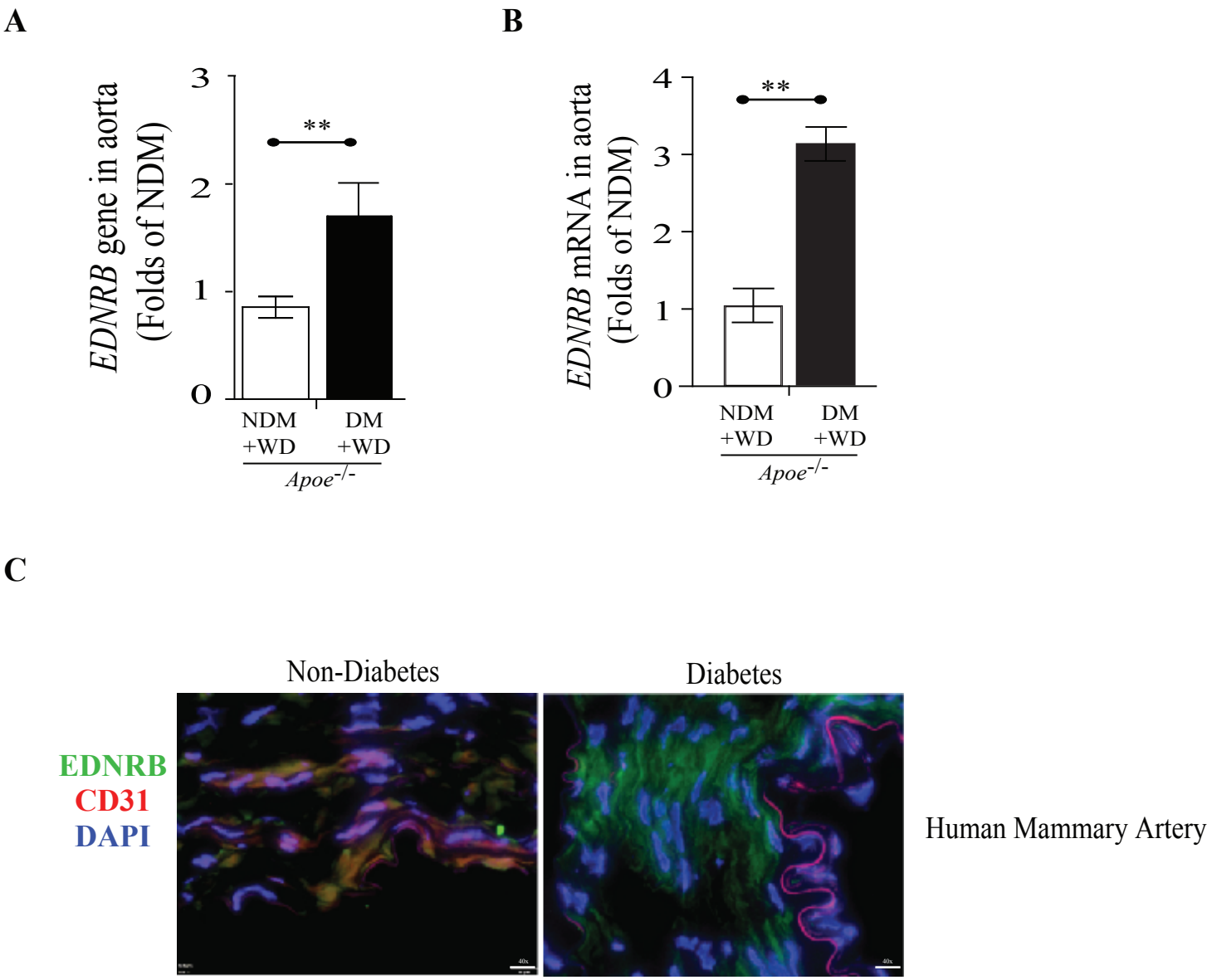
Supplemental Figure 4. **Analysis of atherogenic complexity of *Apoe*$^{-/-}$ and *Irs1/Apoe*$^{-/-}$ mice with WD feeding.** Reprsentative examples of cross-sections from the aortic sinus stained with trichrome, α actin and MAC2 after feeding on WD for 12 wks. A higher magnification (4X) and scale bar: 100um.



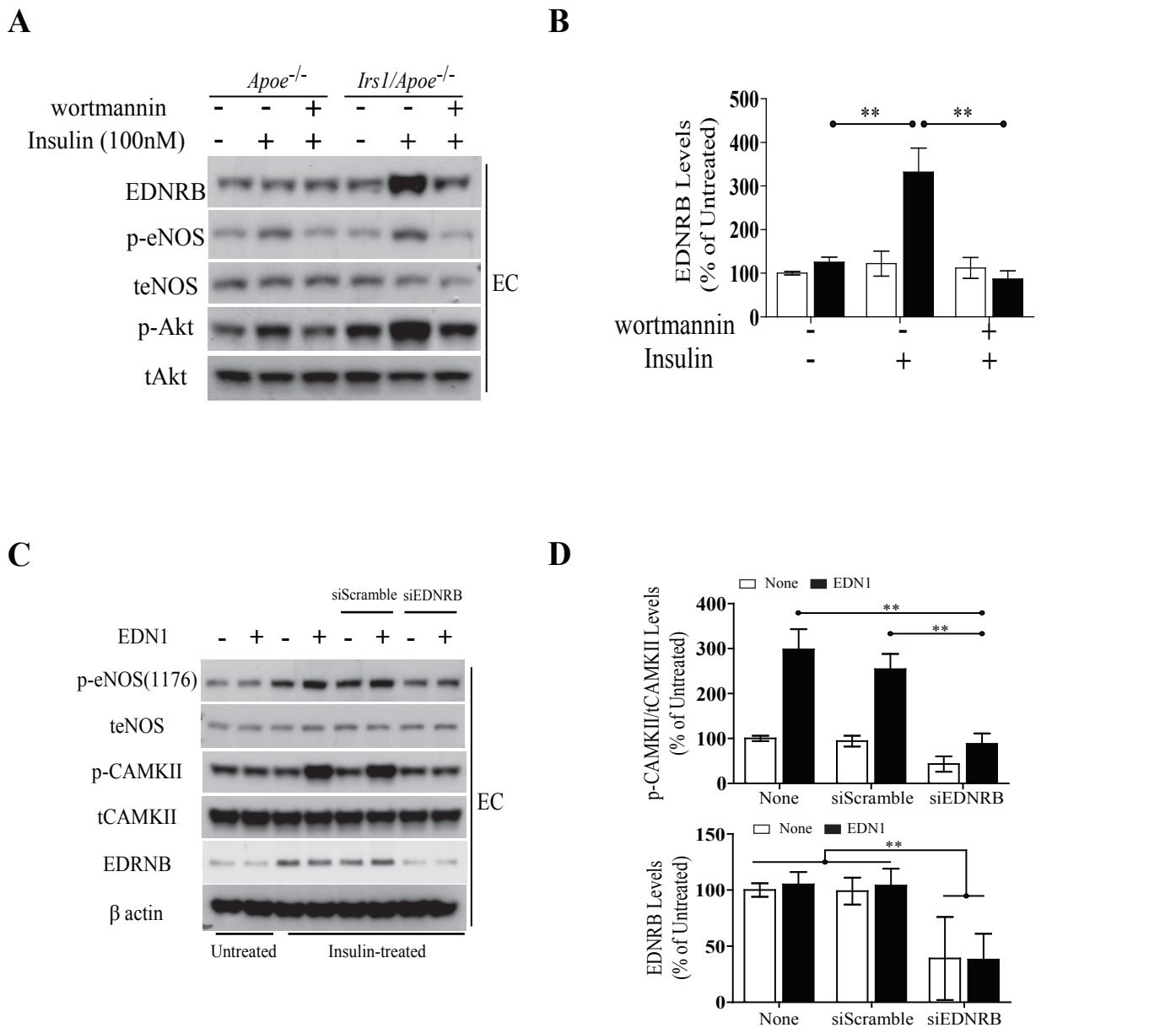
Supplemental Figure 5. Analysis of the extent of atherosclerosis and its complexity of *Apoe*$^{-/-}$ and *Irs1/Apoe*-/- mice fed on HFD. (A) NT production levels in aortas of *Apoe*$^{-/-}$ mice and *Irs1/Apoe*$^{-/-}$ mice fed on HFD for 12 wks (n = 6 per group). (B) *In situ* DHE staining of aortas from *Apoe*$^{-/-}$ mice and *Irs1/Apoe*$^{-/-}$ mice under the same condition as (A) higher magnification (10X) and scale bar: 10um. A higher magnification (25x) of the rectangle shows the VSMC (green) and DHE (red) area in the aorta. Scale bar: 10um (C) Fluorescent density analysis of DHE. Data are represented as mean ± SEM of at least six mouse replicates. (One-way ANOVA for multiple comparisons involving one factorial variable and two-tailed Student's t-test for pairwise comparisons).



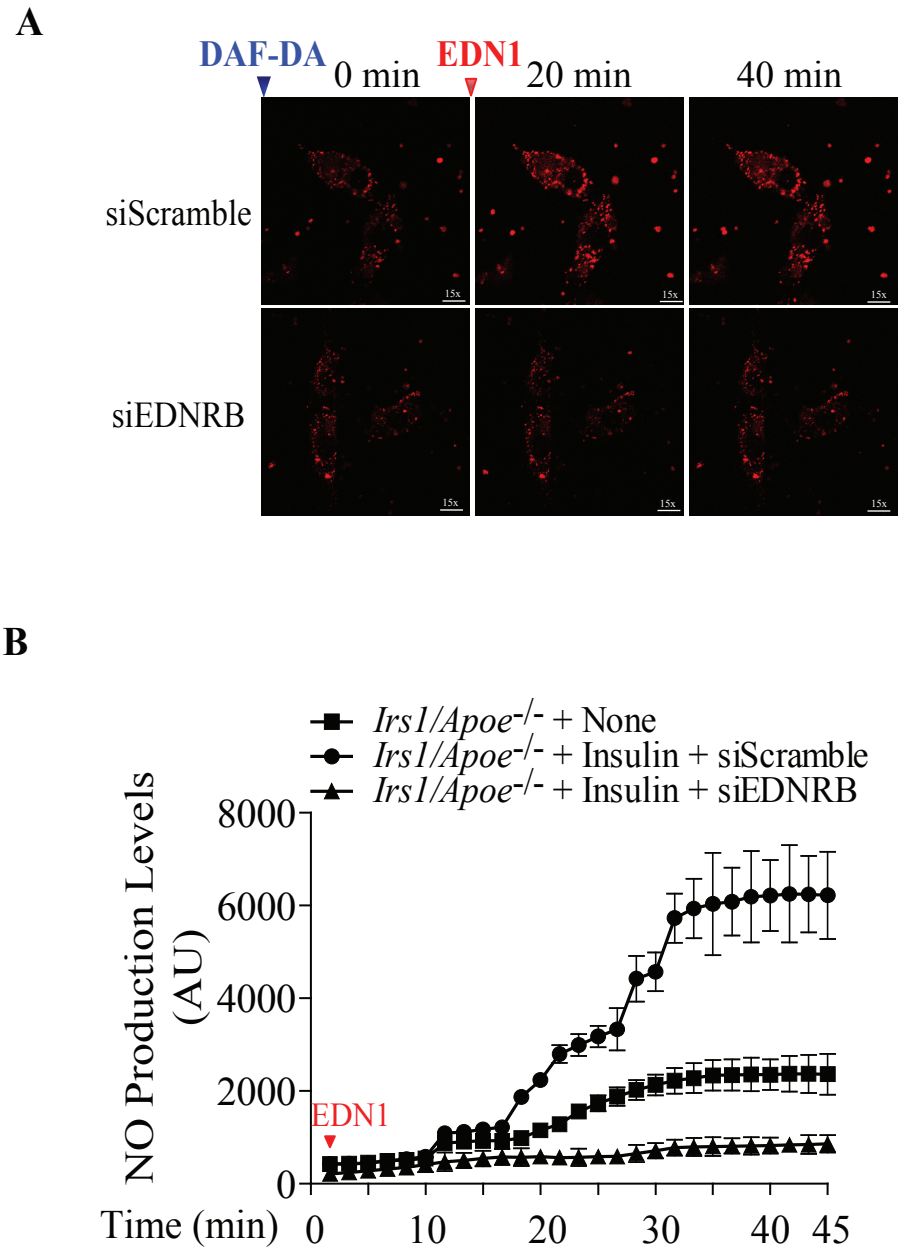
Supplemental Figure 6. **Concentration-relaxation in the femoral arteries isolated from *Apoe*$^{-/-}$ and *Irs1/Apoe*$^{-/-}$ mice.** Concentration-response curves to (A) acetylcholine and relaxation-response curves to (B) sodium nitroprusside in femoral arteries from *Apoe*$^{-/-}$ and *Irs1/Apoe*$^{-/-}$ mice. All data are represented as mean ± SEM of at least five mouse replicates. *; $p<0.05$ and **; $p<0.01$ (Mixed effects model for repeated measurement; One-way ANOVA for multiple comparisons involving one factorial variable and two-tailed Student's t-test for pairwise comparisons).



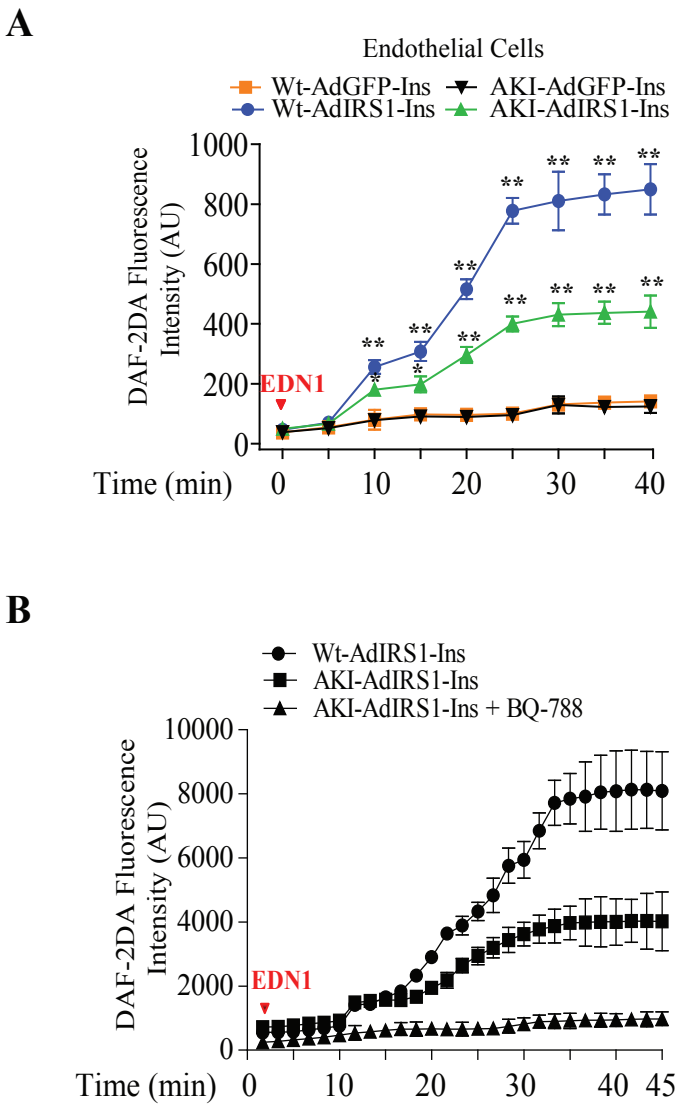
Supplemental Figure 7. **EDNRB is expressed in vascular endothelium from diabetic rodent model and diabetic patients.** *EDNRB* genes (A) and mRNA significantly altered in Aorta of non-diabetic *Apoe*$^{-/-}$ mice, compared to diabetic *Apoe*$^{-/-}$ mice fed on HFD. (C) Representative staining of EDNRB (Green), nuclei (DAPI), and CD31 (Red) in human mammary artery. A higher magnification (40X) and scale bar: 10um. Data are represented as mean ± SEM of at least three mouse replicates. **; $p<0.01$ (Two-tailed Student's t-test for pairwise comparisons).



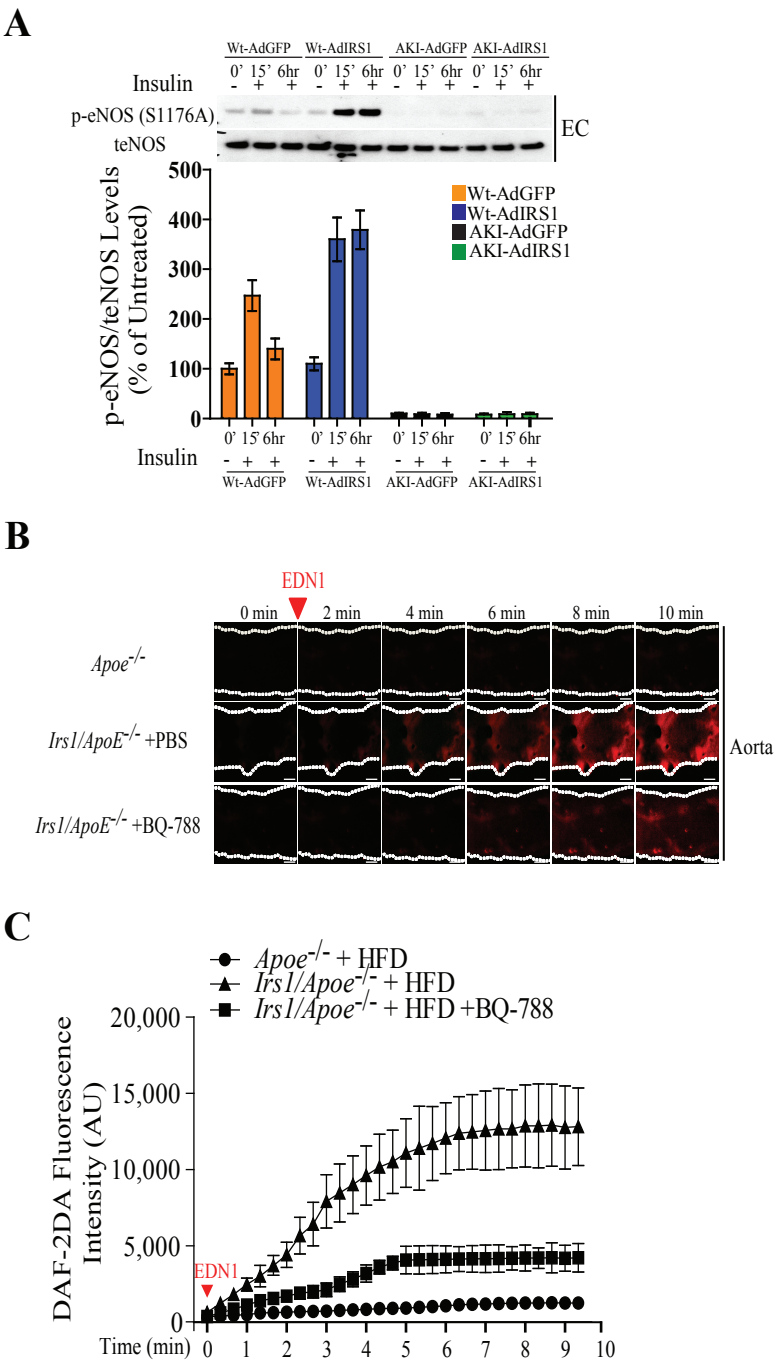
Supplemental Figure 8. Induction of NO production in EC from *Irs1/Apoe*$^{-/-}$ mice through EDN1/EDNRB/Ca^{2+}/CAMKII pathway. (A) Immunoblots of p-eNOS, p-Akt and EDNRB protein levels in EC pretreated with wortmannin. (B) Quantification of EDNRB expression level of (A). (C and D) Immunoblots and quantification of p-eNOS, p-CAMKII and EDNRB in EC from *Irs1/Apoe*$^{-/-}$mice transfected with scrambled (siScramble) or EDNRB siRNA (siEDNRB), and stimulated with insulin, and followed by EDN1 incubation. Data are represented as mean ± SEM of at least five cellular replicates for EDNRB expression experiments. **; $p<0.01$ (two-way/three-way ANOVA for multiple comparisons involving two/three factorial variables and two-tailed Student's t-test for pairwise comparisons).



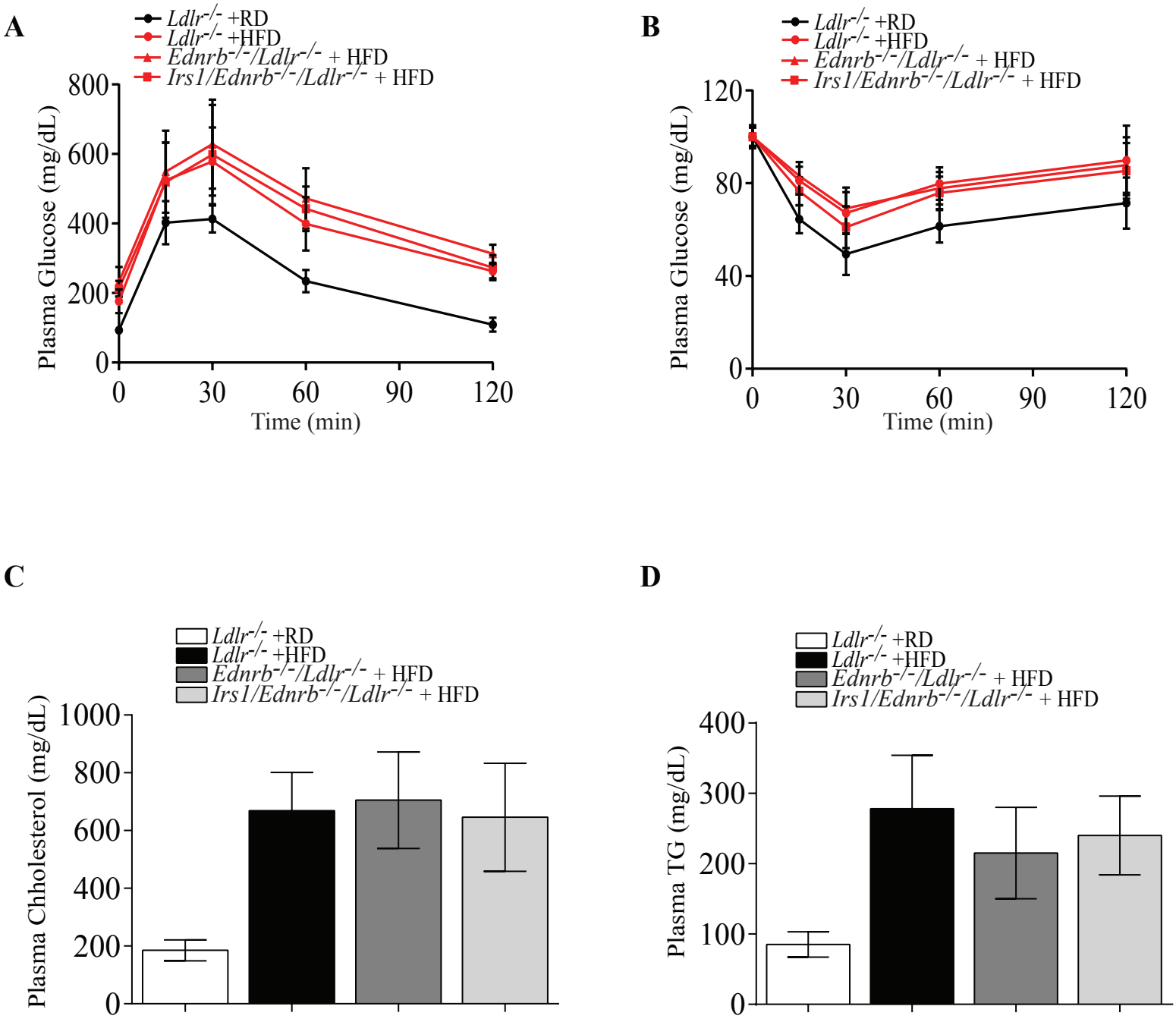
Supplemental Figure 9. Enhanced NO production through endothelial EDN1/EDNRB activation. (A) EDN1-induced NO production was reduced in the treated EC with siRNA of EDNRB, compared that of siScramble RNA. A higher magnification (15X) and scale bar: 5um. NO production was visualized by DAF-2DA fluorescence intensity (FI) (A) and quantified in (B). Data are represented as mean ± SEM of at least five cellular replicates. (Two-tailed Student's t-test for pairwise comparisons).



Supplemental Figure 10. **Effect of mutated eNOS Ser to Ala at 1176 (AKI) on NO production via EDNRB pathway.** (A) EC from *Apoe*$^{-/-}$ mice were infected with an adenovirus expressing GFP and IRS1 and followed by insulin stimulation. NO production was measured and quantified by DAF-2DA. (B) NO production was quantified in insulin-stimulated aortic EC from *Apoe*$^{-/-}$ mice treated with or without BQ-788. AU of DAF-2DA (FI). Data are represented as mean ± SEM of at least five cellular replicates, compared to Wt-AdGFP-Ins or AKI-AdGFP-Ins. *; $p<0.05$ and **; $p<0.01$ (Two-tailed Student's t-test for pairwise comparisons).



Supplemental Figure 11. **Effect of mutated eNOS Ser to Ala at 1176 (AKI) on NO production in EC or Aorta from *Apoe*$^{-/-}$ and *Irs1/Apoe*$^{-/-}$ mice**. (A) Immunoblot (Upper) and densitometry (Lower) of p-eNOS in insulin-stimulated aortic EC. (B and C) Representative example (B) of NO production visualized by DAF-2DA and quantified (C) in aorta from *Apoe*$^{-/-}$ (n=5) and *Irs1/Apoe*$^{-/-}$ (n=5) mice. AU of DAF-2DA (FI). A higher magnification (4X) and scale bar: 100um. Data are represented as mean ± SEM of at least five cellular replicates or mouse replicates, compared to *Apoe*$^{-/-}$ + HFD. (Two-tailed Student's t-test for pairwise comparisons).



Supplemental Figure 12. **Glucose tolerance, insulin resistance, and dyslipidemia in** *Ldlr*$^{-/-}$, *Ednrb*$^{-/-}$/*Ldlr*$^{-/-}$, **and** *Irs1/Ednrb*$^{-/-}$/*Ldlr*$^{-/-}$ **mice.** (A) i.p.-GTT, (B) i.p.-ITT, and (C and D) plasma cholesterol and triglyceride in *Ldlr*$^{-/-}$, *Ednrb*$^{-/-}$/*Ldlr*$^{-/-}$, and *Irs1/Ednrb*$^{-/-}$/*Ldlr*$^{-/-}$ mice after feeding HFD for 12 wks. All data are represented as mean ± SEM of at least five mouse replicates. (Mixed effects model for repeated measurement; two-way ANOVA for multiple comparisons involving two factorial variables and two-tailed Student's t-test for pairwise comparisons).