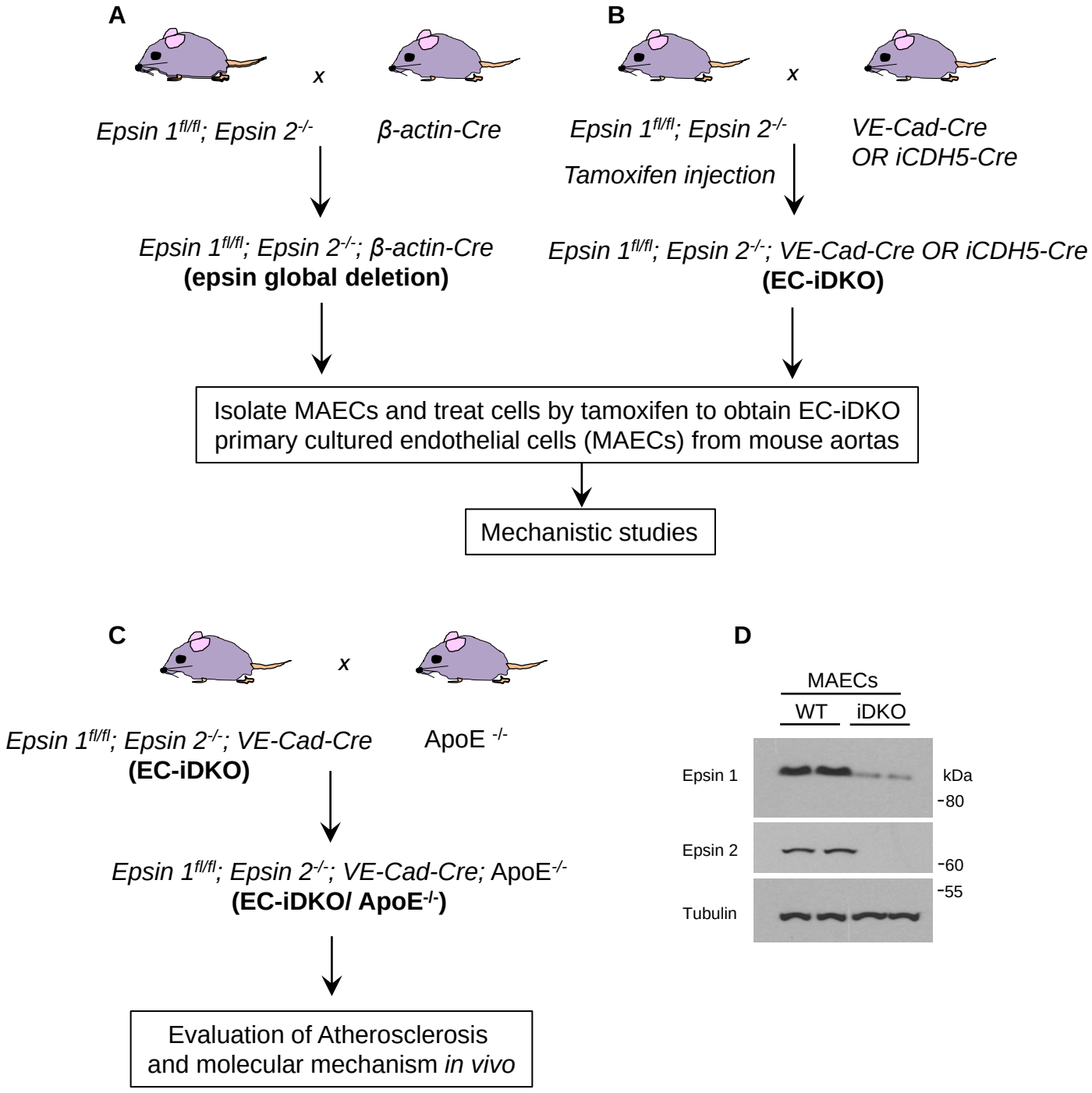

Figure S1. Procedures to generate primary cultured mouse endothelial cells (MAECs) from aortas, and epsin mutant mice in the ApoE-null murine atherosclerosis model.

(A) Global epsin double knock-out (DKO) under constitutive β-actin driven Cre. (B) Conditional DKO of epsins in endothelium was driven by the vascular endothelial (VE)-cadherin promoter. (C) Generation of epsin DKO mice in the ApoE-null mouse model. (D) Creation of epsin DKO MAECs in culture. Isolated cells were treated with 5 μM tamoxifen for 4-5 days, followed by western blot analysis.

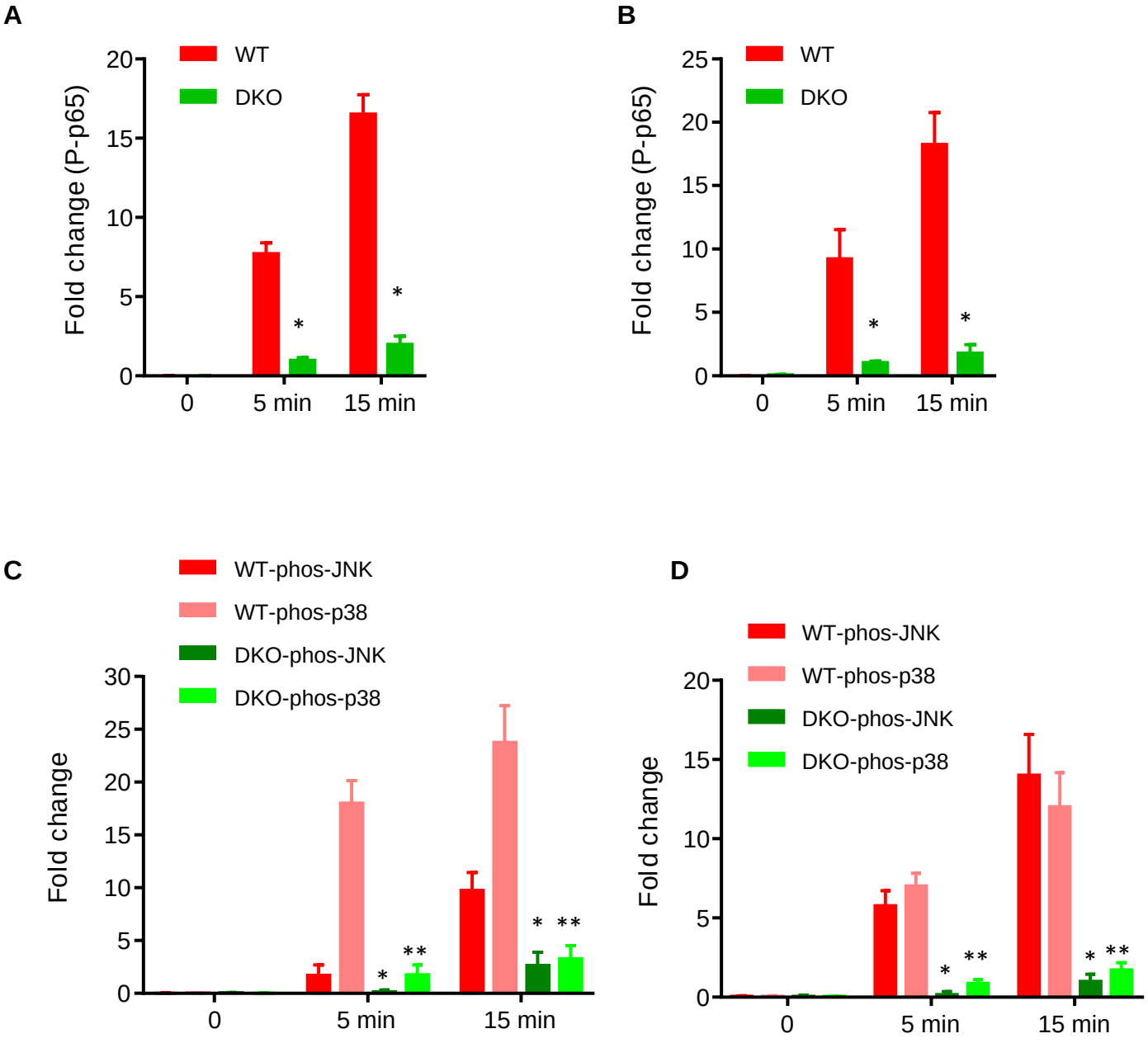

Figure S2. Quantification for Figure 1A to 1D.

(A) TNFα, phospho-p65; (B) LPS, phospho-p65; (C) TNFα, phospho-JNK and phospho-p38; (D) LPS, phospho-JNK and phospho-p38.

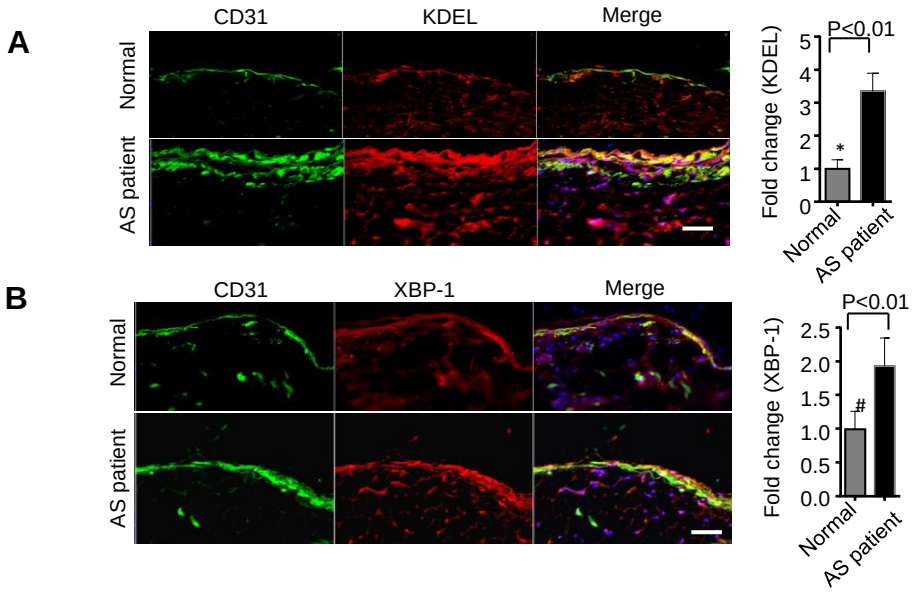

Figure S3. ER stress markers are upregulated in the aortic arches of human atherosclerosis patients, and loss of epsins in ECs attenuates ER stress.

(A, B) ER stress marker KDEL (A) and XBP-1 (B) are upregulated in atherosclerotic (AS) patients versus control patients by immunofluorescence staining (n=4-5 in each group). Scale bar: 100 μm.

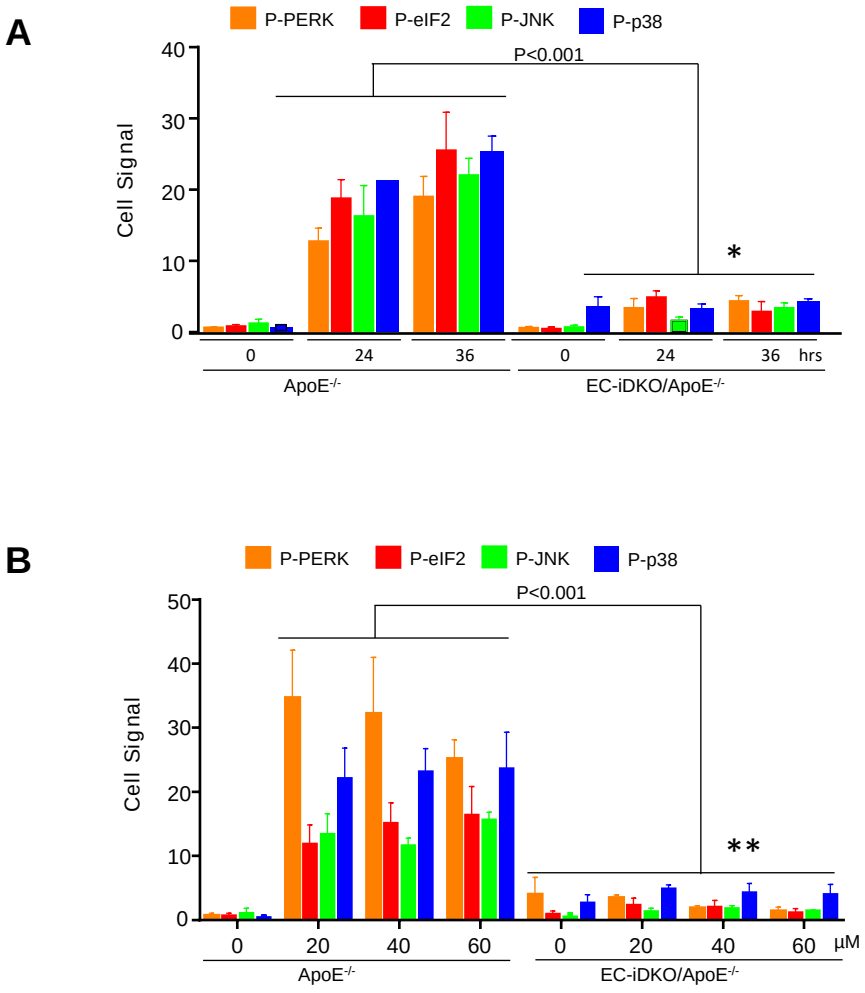

Figure S4. Quantification for Figure 2E and 2F.

MAECs were isolated from ApoE$^{-/-}$ or DKO/ApoE$^{-/-}$ mice aortas. Cells were treated with 7-KC in indicated time points (7-KC at 50 μM) or concentrations at 0, 20, 40 and 60 μM for 36 hours. Statistical analysis and quantification was shown in (A) and (B) respectively (n=4). *P* values are indicated in the histograms.

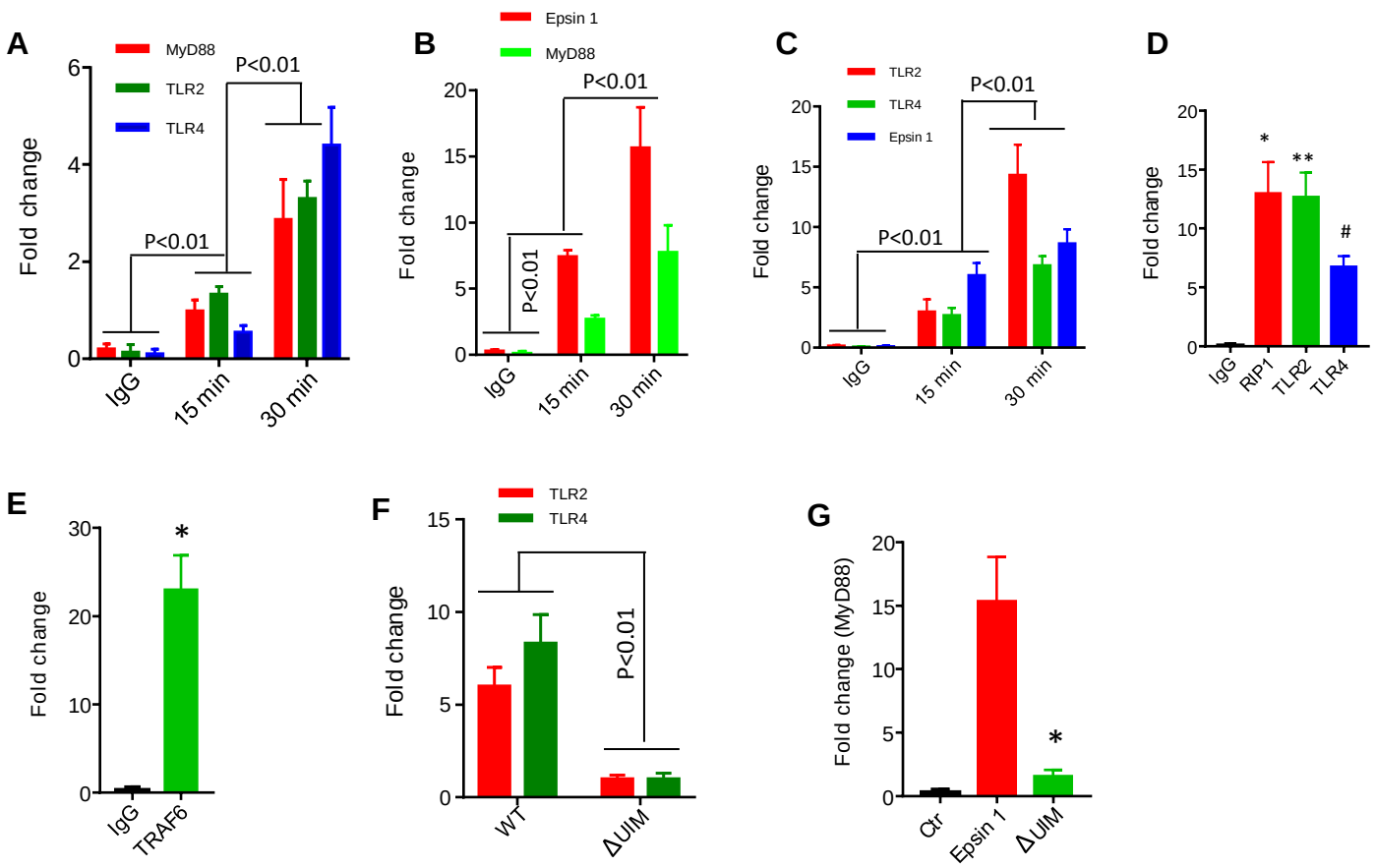

Figure S5. Quantification for Figure 6A to 6G.

(A) Wild type MAECs were treated with 100 ng/ml LPS for 15 or 30 min, cells were lysed for immunoprecipitation (IP) with epsin 1 antibody, and blotted with MyD88 or TLR2/4 antibodies. n=5. P<0.01; MyD88, TLR2 and TLR4 were quantified. **(B)** Treatment as in (**A**), while IP with TLR4 antibody, and blotted with Epsin 1, MyD88 and TLR4 antibodies. n=5. P<0.01; Epsin 1 and MyD88 were quantified. **(C)** Treatment as in (**A**), while IP with MyD88 antibody, and blotted with Epsin 1 and TLR2/4 antibodies. n=5. P<0.01; TLR2, TLR4 and Epsin 1 were quantified. **(D)** MAECs were treated with 100 ng/ml LPS for 30 min, IP with Epsin 1 antibody and blotted with RIP1 or TLR2/4 antibody. n=5. *, **, # P<0.01; RIP1, TLR2 and TLR4 were quantified. **(E)** MAECs were treated with LPS for 30 min, IP with Epsin 1 antibody and blotted with TRAF6. n=5. * P<0.001; TRAF6 was quantified. **(F)** Epsin 1^{wt} (HA tag) or epsin $1^{\Delta UIM}$ (HA tag) were transfected to wt MAECs by electroporation separately. After 30 hours, IP with HA antibody and blotted with HA or TLR2/4 antibody. n=3. WT vs. epsin $1^{\Delta UIM}$; TLR2 and TLR4 were quantified. **(G)** Epsin 1^{wt} (HA tag) or epsin $1^{\Delta UIM}$ (HA tag) were co-transfected to MAECs with MyD88 (Flag tag) by electroporation separately. After 30 hours, cells were lysed and IP with HA, followed by blotting with Flag or HA antibody.* P<0.001, Epsin 1 *vs*. epsin $1^{\Delta UIM}$.

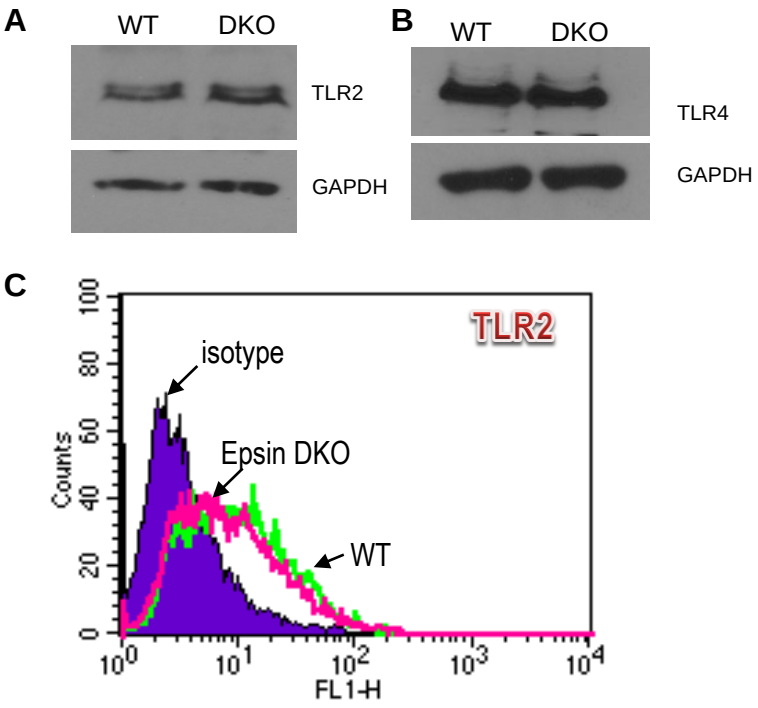

Figure S6. Loss of epsins in the endothelium did not affect the expression of TLR2 or TLR4.

(A, B) TLR2 or TLR4 expression in the MAECs of WT and DKO (n=5). (C) TLR2 is not affected by epsin loss in MAECs using flow cytometric analyses (n=5).

Table S1: qPCR primers used in this study.

Mouse Gene	Primer sequence
ICAM-1	Forward: GTG GTG AAG TCT GT CAA ACA GGA G Reverse: CCT CCT GAG CCT TCT GTA ACT TGT
VCAM-1	Forward: CTC TCC CAG GAA TAC AAC GAT CTC Reverse: GAC TCC AGA GTC TTC CAT CCT CAT
P-selectin	Forward: ATC CAG GAA GCT CTG ACG TAC TTG Reverse: CAG CGT TAG TGA AGA CTC CGT ATG
E-selectin	Forward: GTT TGA CTG TGT GGA AGG GTA CAG Reverse: GCT CAC AGG TGA AGT TAC AGG ATG
MCP-1	Forward: CCA CTC ACC TGC TGC TAC TCA Reverse: TGG TGA TCC TCT TGT AGC TCT CC
IFN-γ	Forward: AAG TGG CAT AGA TGT GGA AGA AA Reverse: TTG ACC TCA AAC TTG GCA ATA CT
TNFα	Forward: ACG GCA TGG ATC TCA AAG AC Reverse: AGA TAG CAA ATC GGC TGA CG
IL-6	Forward: CTG GAG TAC CAT AGC TAC CTG GA Reverse: CTT AGC CAC TCC TTC TGT GAC TC
IL-1β	Forward: TAC AAG GAG AAC CAA GCA ACG A Reverse: GCT TGT GAG GTG CTG ATG TAC C
IL-10	Forward: GCC ACA TGC TCC TAG AGC TG Reverse: CAG CTG GTC CTT TGT TTG AAA
GAPDH	Forward: CTC ATG ACC ACA GTC CAT GC Reverse: CAC ATT GGG GGT AGG AAC AC
β-actin	Forward: GAT CAA GAT CAT TGC TCC TCC TG Reverse: AGG GTG TAA AAC GCA GCT CA

Table S2: Body weight, blood glucose and lipid profiles of ApoE$^{-/-}$ and EC-iDKO/ApoE$^{-/-}$ mice

Items	ApoE$^{-/-}$	EC-iDKO/ApoE$^{-/-}$	P value (t test)
Body weight (g)	27.4±2.12	24.8±1.13	0.4123
Glucose (mg/dL)	127±6.90	128±14.8	0.6119
Cholesterol (mg/dL)	684.8±59.5	662±73.10	0.9419
Triglyceride (mM)	1.367±0.49	1.24±0.88	0.7463

*** n=8-10 in each group