SUPPLEMENTAL DATA

AIP1 suppresses atherosclerosis progression by limiting hyperlipidemia-induced inflammation and vascular endothelial dysfunction

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Running title: AIP1 suppresses atherosclerosis

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig.I. ApoE$^{-/-}$ and DKO adult mice were fed with Western-type diet for 10 weeks.

A-B. AIP1 deletion increases macrophage infiltration in the aorta roots. Representative histological analysis of cross-sections from the aortic roots stained with CD68 (a macrophage marker). Scale bar: 100 μm. Quantifications of CD68+ area are shown in **B**. Six sections (150 μm apart for each section) per mouse from 5 mice in each group. Data are presented are mean±SEM, *, $p<0.05$.

C. AIP1 deletion has no effects on smooth muscle cell contents. Representative histological analysis of cross-sections from the brachiocephalic arteries stained with α-smooth muscle actin (a smooth muscle cell marker). Sections were counterstained with DAPI. Scale bar: 100 μm. Six sections (150 μm apart for each section) per mouse from 5 mice in each group.

D. AIP1 deletion has no effects on circulating monocytes. Blood circulating monocytes were measured by complete blood tests via Antech Diagnostics (Irvine, CA). Data are presented are mean±SEM, n=8.

Supplemental Fig.II. AIP1 deletion has no effects on the basal EC function. ApoE$^{-/-}$ and DKO adult mice were fed with chow for 2 weeks, and aortas were harvested for vessel function assays.

A. Aortic rings were contracted with PE at a full range of doses (10^{-9}-10^{-4} M). Constriction force (mN) is shown.

B. Aortic rings were incubated with a NOS inhibitor L-NAME to remove basal NO synthesis and then contracted with PE as in **A**.

C. AIP1 deletion has no effects on vessel constriction in response to KCl. Aortic rings were contracted with 50 mM of KCl.

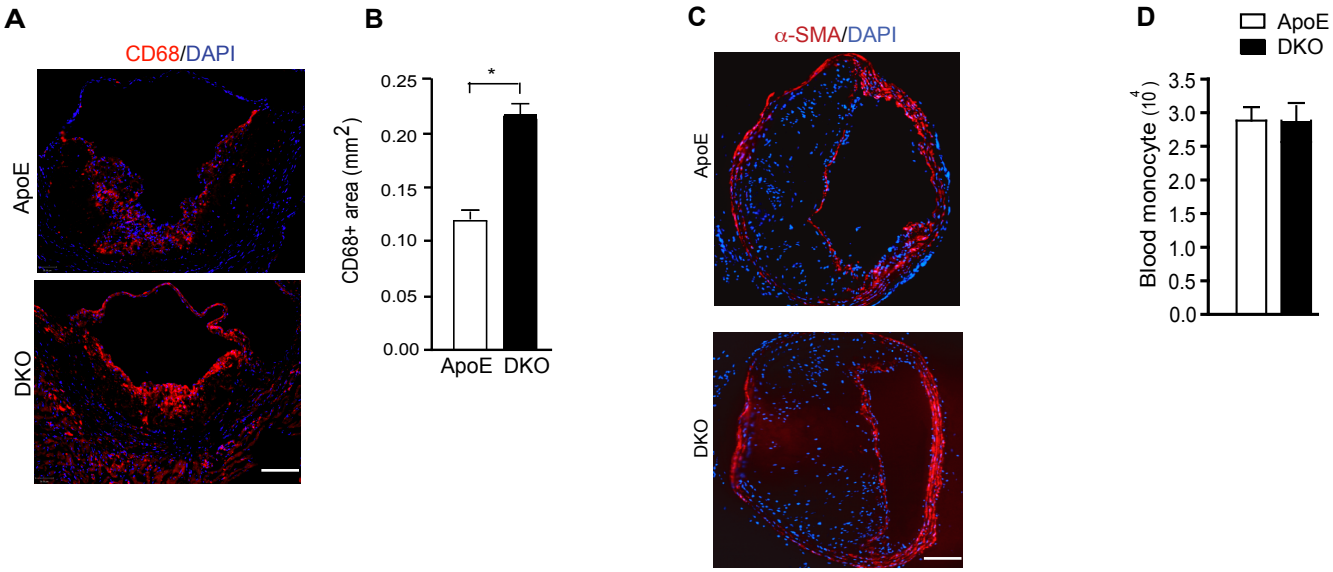
D. AIP1 deletion has no effects on vessel relaxation to the NO donor drug SNP. Aortic rings were incubated with a NOS inhibitor L-NAME to remove basal NO synthesis followed by a precontraction with PE as in **A,** and were then relaxed with SNP at a full range of doses (10^{-9}-10^{-6} M). Data in A-E are presented are mean±SEM, with n=5 animals and eight aortic rings per animal. No statistically significance was detected between DKO versus ApoE$^{-/-}$.

Supplemental Fig.III. AIP1 expression is low in macrophage and AIP1 deletion in macrophages does not significantly alter oxLDL-induced signaling. Mouse aortic EC, peritoneal macrophages and bone marrow-derived macrophage were isolated from WT and AIP1-KO mice.

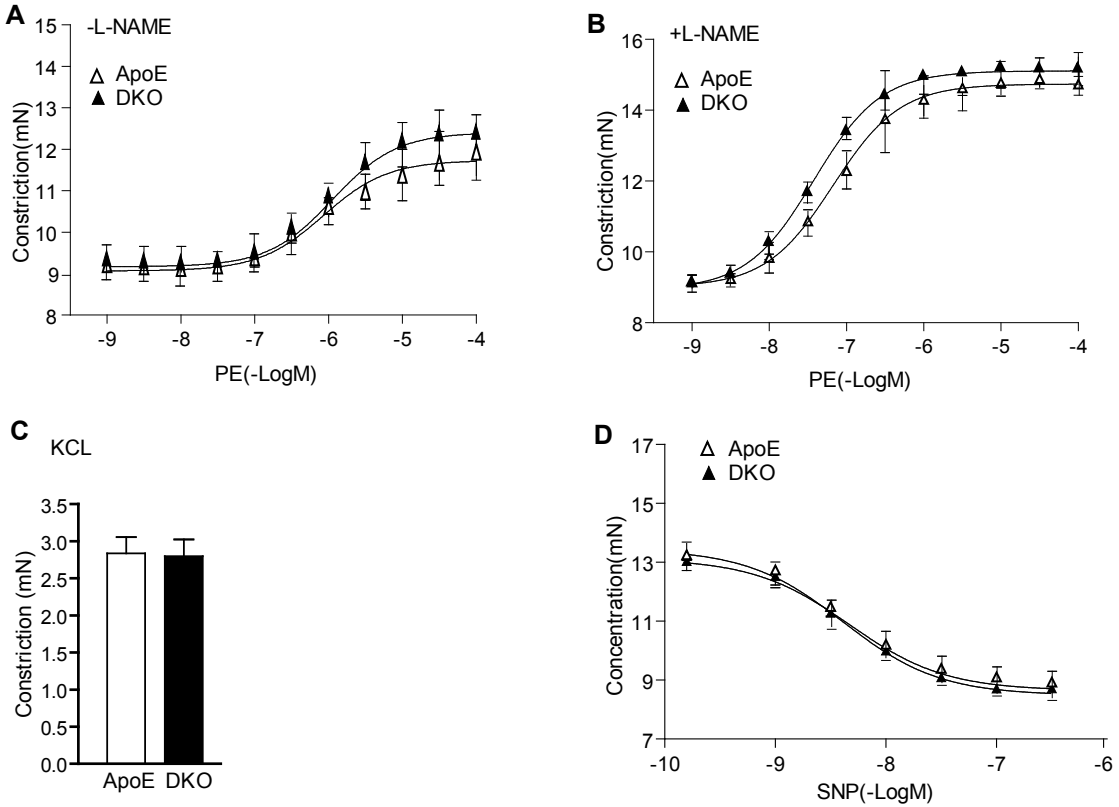
A. $1x10^6$ of peritoneal macrophages were untreated or treated with 100 μg/ml oxLDL for the indicated times. Phospho- and total p65 and p-JNK1/2 were determined by immunoblotting with the respective antibodies. Total JNK1, AIP1 and β-actin were also determined. Representative blots from three independent experiments are shown. The quantification of the ratios of p-p65/p65 and p-JNK/JNK are presented from three blots, by taking untreated WT as 1.0. *, $p<0.05$ indicate that statistically significant by comparing AIP1-KO versus WT.

B. $1x10^6$ of cells were untreated or treated with 100 μg/ml oxLDL for 12 h. AIP1 mRNA expression was determined by qRT-PCR. Data represent fold changes where aortic EC is set as 1.0. Data are mean ± SEM from 3 independent experiments. AIP1 expression was not altered by oxLDL treatment. *, $p<0.05$ comparing EC to macrophages**.**

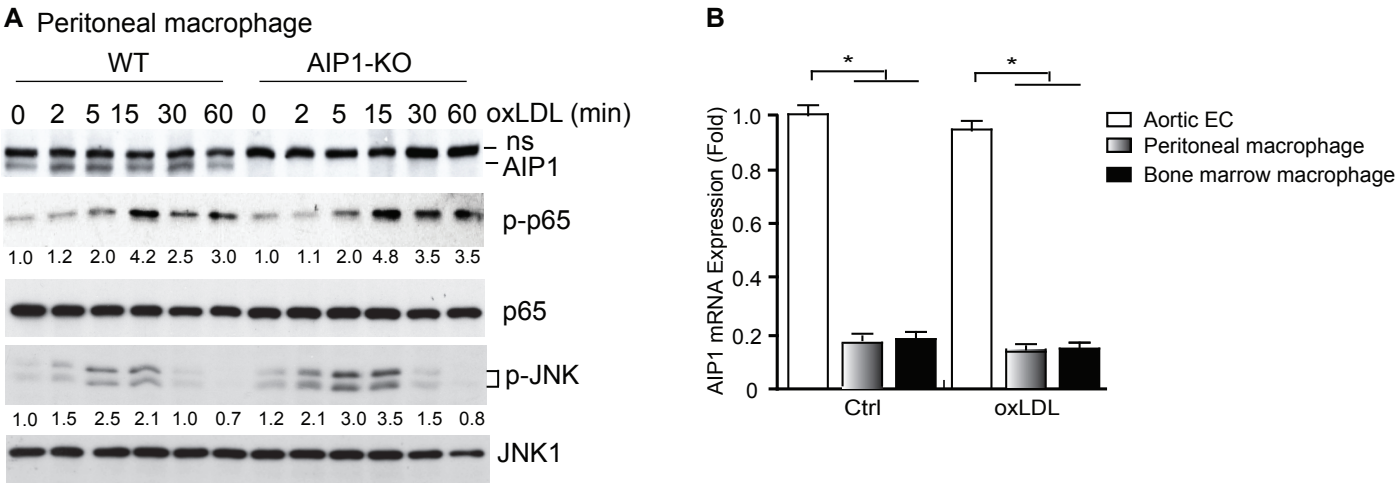
Huang, Q. et al Suppl Fig.I



Huang, Q. et al Suppl Fig.II



Huang, Q. et al Fig.III



Supplemental Table I.

	Hemoglobin (g/dL)	Hematocrit (%)	RBC (10^6/μl)	Platelet (10^6/μl)	WBC (10^3/μl)	Neutrophil (10^3/μl)	Lymphocyte (10^3/μl)	Monocyte (10^3/μl)
Basal								
ApoE$^{-/-}$	13.8	38	8.7	1.1	3.7	0.89	2.2	0.045
DKO	13.4	39	8.6	0.9	3.9	0.93	2.1	0.050
BMT								
ApoE$^{-/-}$ to ApoE$^{-/-}$	14.6	40.5	10.4	1.1	3.6	0.90	1.9	0.050
DKO to ApoE$^{-/-}$	14.9	40.8	10.6	1.2	3.7	0.99	2.2	0.053

Table I. Complete blood tests in ApoE$^{-/-}$ and DKO mice. Peripheral blood from basal ApoE$^{-/-}$, DKO, and BMT mice were collected. Complete blood tests were performed via Antech Diagnostics (Irvine, CA). RBC: red blood cell; WBC: white blood cell.