Online supplement

Fibronectin splicing variants containing extra domain A promote atherosclerosis in mice through Toll-like receptor 4

Prakash Doddapattar,[1$] Chintan Gandhi,[1$#] Prem Prakash,[1] Nirav Dhanesha,[1] Isabella M. Grumbach,[1] Michael E. Dailey,[2] Steven R. Lentz,[1] and Anil K. Chauhan[1]

[1]Department of Internal Medicine, and [2]Department of Biology,
University of Iowa, Iowa City, IA.

[#]Current address: Center for Vascular and Inflammatory Diseases, School of Medicine,
University of Maryland, Baltimore-MD

[$]These authors contributed equally to the article

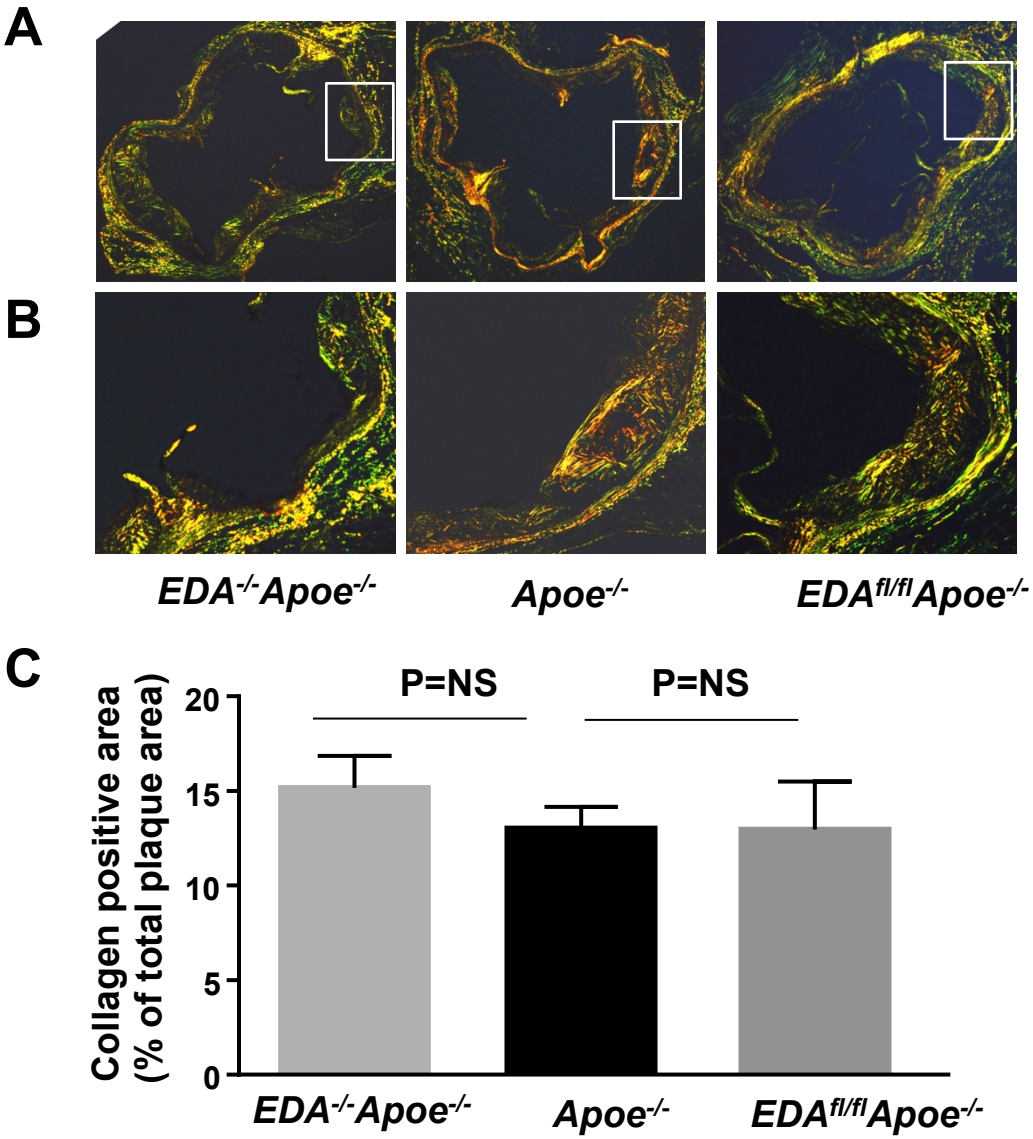
Supplementary Table I. Plasma cellular EDA^+-FN concentrations were measured from each mouse using sandwich ELISA. Value are expressed as mean ± SEM. N= 8-10 mice/group.

Mice strains	**Plasma cellular EDA^+-FN (μg/mL)**		
	6 weeks on chow diet	14 weeks on high fat Western diet	*P value*
$EDA^{-/-}Apoe^{-/-}$	0	0	
$Apoe^{-/-}$	1.5 ± 0.3*	5.8 ± 1.1*	*P<0.05 versus $EDA^{-/-}/Apoe^{-/-}$*
$EDA^{fl/fl}Apoe^{-/-}$	4.7 ± 0.6[#]	9.1 ± 0.8[#]	[#]P<0.01 versus $Apoe^{-/-}$*

Supplementary Table II. Complete Blood Counts from 8- 9 weeks old female mice. Value are expressed as mean ± SEM. N= 8-10 mice/group. *P*= Non significant versus control *$Apoe^{-/-}$* mice.

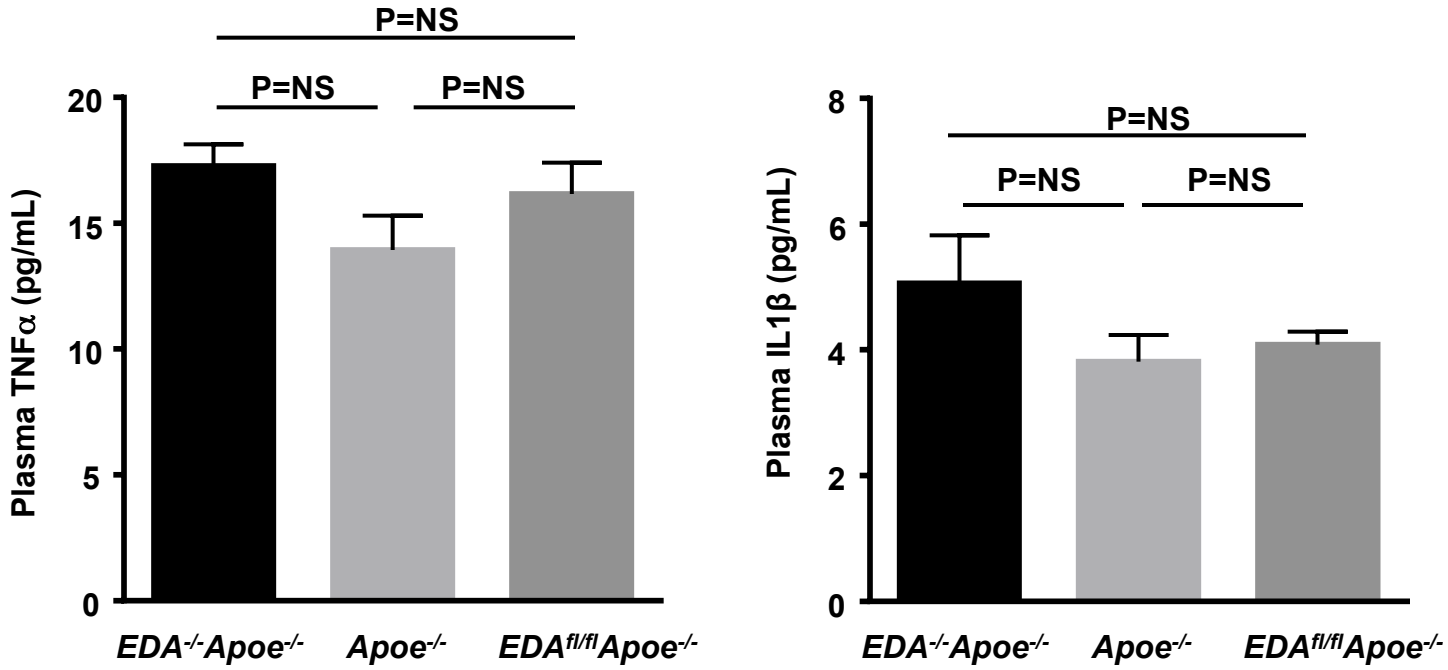
	$EDA^{-/-}Apoe^{-/-}$	***$Apoe^{-/-}$***	***$EDA^{fl/fl}Apoe^{-/-}$***
WBC (10^3/μl)	11.0 ± 0.7	9.65 ± 0.41	10.9 ± 0.9
RBC (10^6/μl)	8.8 ± 0.1	8.87 ± 0.18	9.1 ± 0.1
HGB (g/dL)	14.0 ± 0.2	14.0 ± 0.23	14.2 ± 0.1
HCT (%l)	44.5 ± 0.4	44.4 ± 0.74	44.1 ± 0.3
Platelet (10^3/μl)	1009 ± 75	1014 ± 83	936 ± 33
Neutrophil (10^3/μl)	0.8 ± 0.04	0.96 ± 0.12	0.7 ± 0.08
Lymphocytes (10^3/μl)	9.6 ± 0.64	8.0 ± 0.37	9.6 ± 0.82
Monocytes (10^3/μl)	0.3 ± 0.05	0.31 ± 0.06	0.3 ± 0.02
Eosinophils (10^3/μl)	0.2 ± 0.04	0.39 ± 0.05	0.3 ± 0.04
Basophils (10^3/μl)	0.005 ± 0.003	0.005 ± 0.003	0.008 ± 0.003

Supplementary Figure I



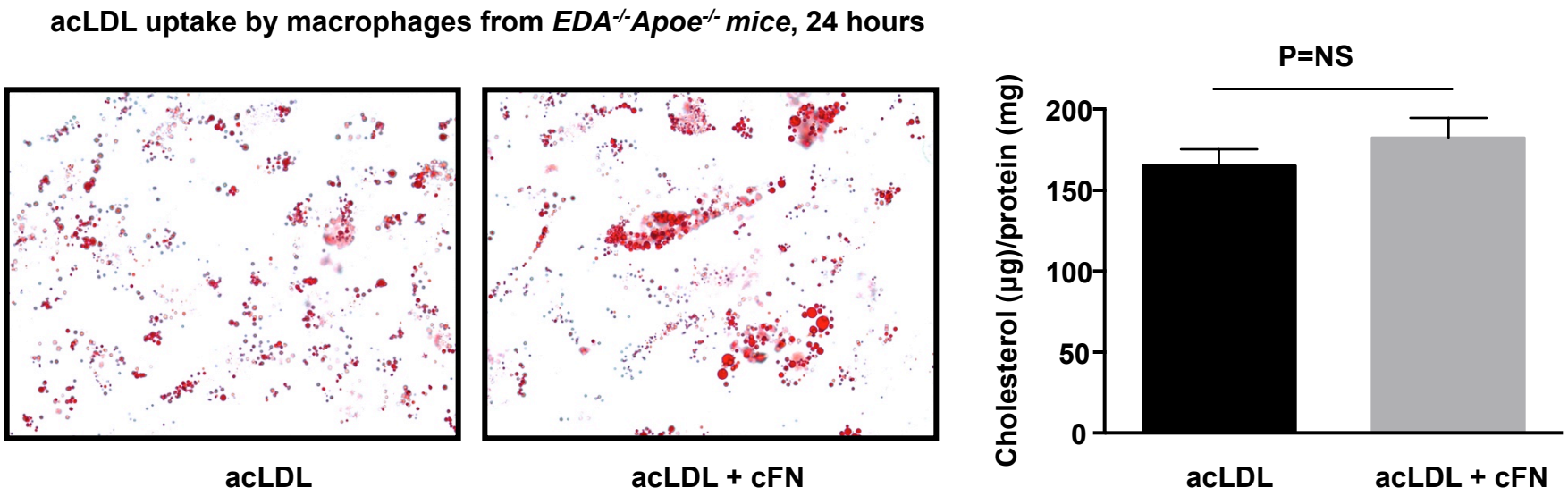
Supplementary Figure I. **Collagen staining in aortic sinus**. **A.** Representative low magnification (4X) photomicrographs stained for collagen as visualized by polarization microscope. **B.** Representative high magnification (10X) photomicrographs of the boxed region (A) stained for collagen. **C.** Quantification of collagen positive area. Data is presented as mean ± SEM. N = 5-6 mice/group.

Supplementary Figure II



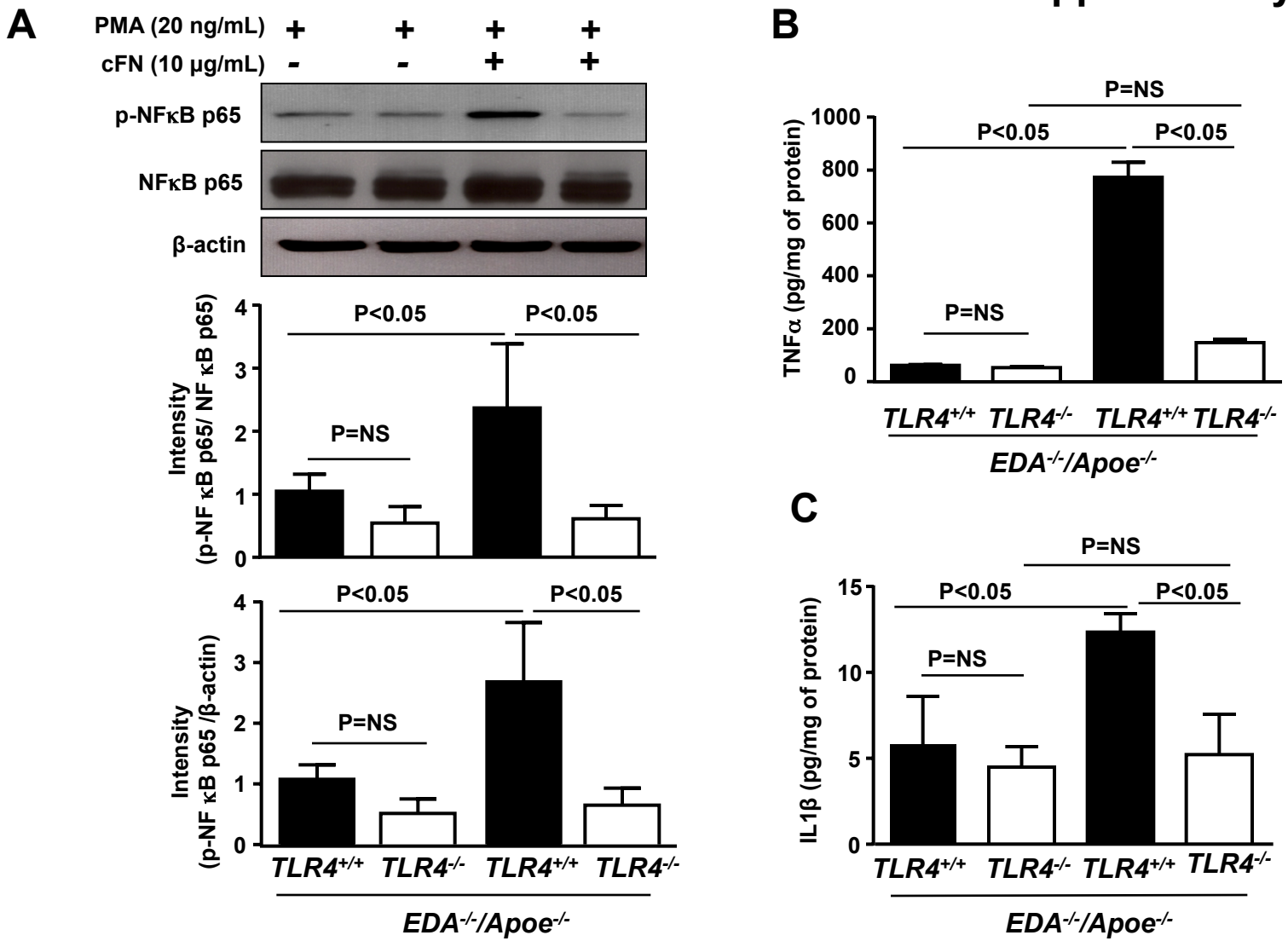
Supplementary Figure II. **Plasma TNF-α and IL-1β levels**. ELISA quantification of TNF-α and IL-1β in plasma from female *EDA*$^{-/-}$*Apoe*$^{-/-}$, *EDA*$^{fl/fl}$*Apoe*$^{-/-}$, and control *Apoe*$^{-/-}$ mice fed a high-fat Western diet for 14 weeks. Data is presented as mean ± SEM. N = 10/group.

Supplementary Figure III



Supplementary Figure III. Left panel shows staining of purified bone marrow-derived macrophages from female *EDA*$^{-/-}$*Apoe*$^{-/-}$ mice with Oil Red O 24 hour after incubating them with acLDL with or without exogenous cFN. Right panel shows quantification of total cholesterol. Values are mean ± SEM. N=6 mice/group**.** NS= non significant.

Supplementary Figure IV



Supplementary Figure IV. Cellular FN promotes inflammation in macrophages through TLR4. Bone marrow-derived macrophages from *EDA$^{-/-}$Apoe$^{-/-}$* and *EDA$^{-/-}$TLR4$^{-/-}$Apoe$^{-/-}$* were stimulated with 20 ng/mL of phorbol myristate acetate in presence or absence of cFN (10µg/mL) for 24 hours. **A.** Representative immunoblots in top panel shows expression of phosphorylated- NFκB p65, total NFκB p65 and β-actin. Bar diagram in middle and bottom panels represent quantification. β-actin was used as loading control.N = 4 mice/group. **B&C**. ELISA quantification of TNF-α and IL-1β in supernatant medium from cFN treated and untreated macrophages. Data is presented as mean ± SEM. N = 4 mice/group.