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Supplementary Materials for

Inhibition of neutrophil extracellular trap formation alleviates vascular dysfunction in type 1 diabetic mice

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This PDF file includes:

Figs. S1 to S7

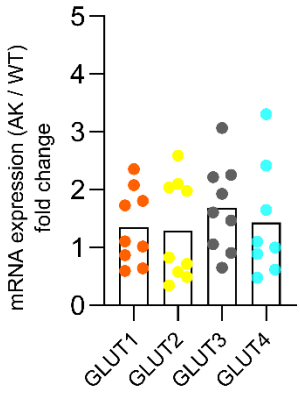

Figure S1.
Gene expression of glucose transporters in Akita and WT neutrophils. Relative mRNA coding for the indicated glucose transporters in bone-marrow neutrophils; mean for n=8-9 unique mice. Akita (AK) neutrophils were compared with WT neutrophils by unpaired Student's *t*-test and no significant difference were found.

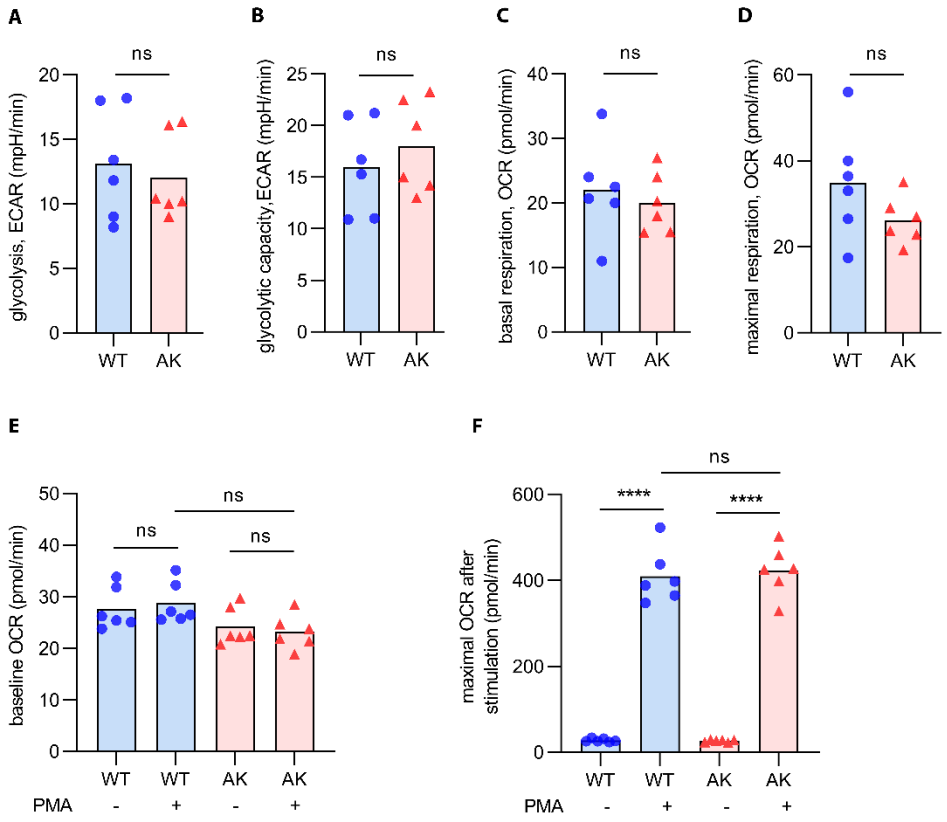

Figure S2.

Bioenergetic characterization of neutrophils in 24-week-old Akita (AK) mice with the Seahorse extracellular flux analyzer. Using the glycolysis stress test, **A,** baseline glycolysis, and **B,** glycolytic capacity was measured as an assessment of glycolysis. Using the mitochondrial stress test, **C,** basal respiration, and **D,** maximal respiration were measured as an assessment of mitochondrial respiration. For **A** to **D**, mean for n=6, ns=not significant, compared with WT mice by unpaired Student's *t*-test. In a different set of experiments, PBS or PMA was added to the neutrophils, and the oxygen consumption rate (OCR) was measured as an assessment of the oxidative burst, **E,** baseline OCR, which is the OCR before adding PBS or PMA and **F,** maximal OCR, which is the highest OCR after adding PBS or PMA. For **E** and **F**, mean for n=6, ****p<0.0001 by one-way ANOVA followed by the Holm-Šídák test.

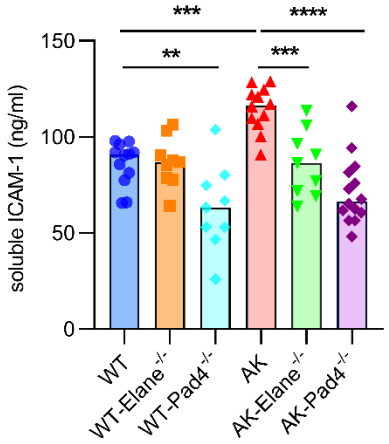

Figure S3.
Decreased soluble ICAM-1 in Akita-*Elane*$^{-/-}$ and Akita-*Pad4*$^{-/-}$ aortae as compared with Akita (AK) aortae. Values of soluble ICAM-1 by ELISA; mean for n=9-12, **p<0.01, ***p<0.001, ****p<0.0001 by one-way ANOVA followed by the Holm-Šídák test.

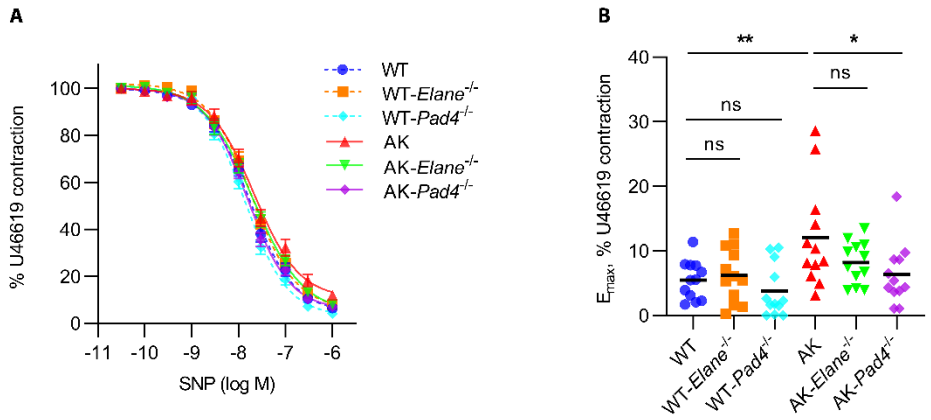

Figure S4.

Relaxation of aortic rings in response to sodium nitroprusside. A, Relaxation of aortic rings in response to sodium nitroprusside (SNP) after achieving a stable U46619 contraction plateau. Data are presented relative to the force remaining from the U46619 contraction, which was set as 100%; mean + SEM for n=12. **B,** Maximal efficacy (E_{max}) values as determined by nonlinear regression analysis; mean for n=12, *p<0.05, **p<0.01 by one-way ANOVA followed by the Holm-Šídák test.

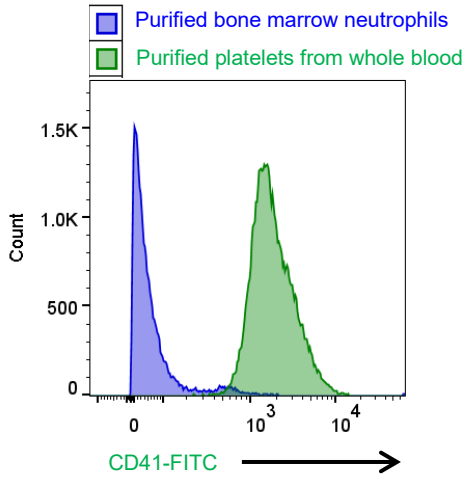

Figure S5.
Assessment for platelet contamination of bone marrow neutrophils as were used for adoptive transfer experiments. Either purified bone marrow neutrophils or platelets from whole blood were assessed by flow cytometry. Bone marrow neutrophils were consistently negative for any CD41 staining.

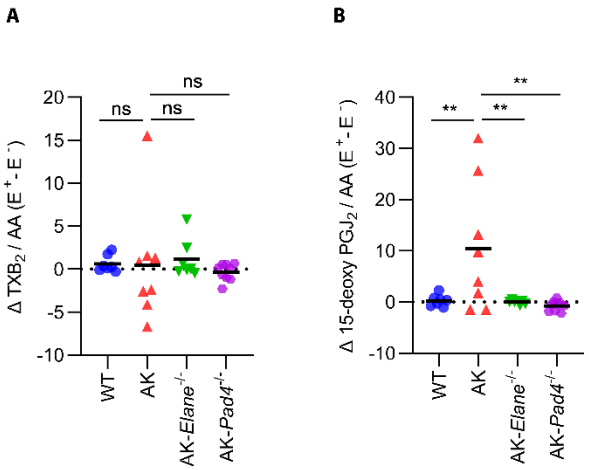

Figure S6.

Delta values of detected TXB_2 and 15-deoxy-PGJ_2. Prostanoid metabolites were measured in endothelium-intact (E^+) and endothelium-denuded (E^-) aortae of 24-week-old mice by LC/ESI-MS/MS. The values of prostanoid metabolites detected were all normalized to arachidonic acid (AA) in the aorta. **A,** Delta values of TXB_2, presented as endothelium-intact (E^+) minus endothelium-denuded (E^-). **B,** Delta values of detected 15-deoxy-PGJ_2, presented as endothelium-intact (E^+) minus endothelium-denuded (E^-); mean for n=7-9, $^{**}p<0.01$ by one-way ANOVA followed by the Holm-Šídák test, ns=non-significant.

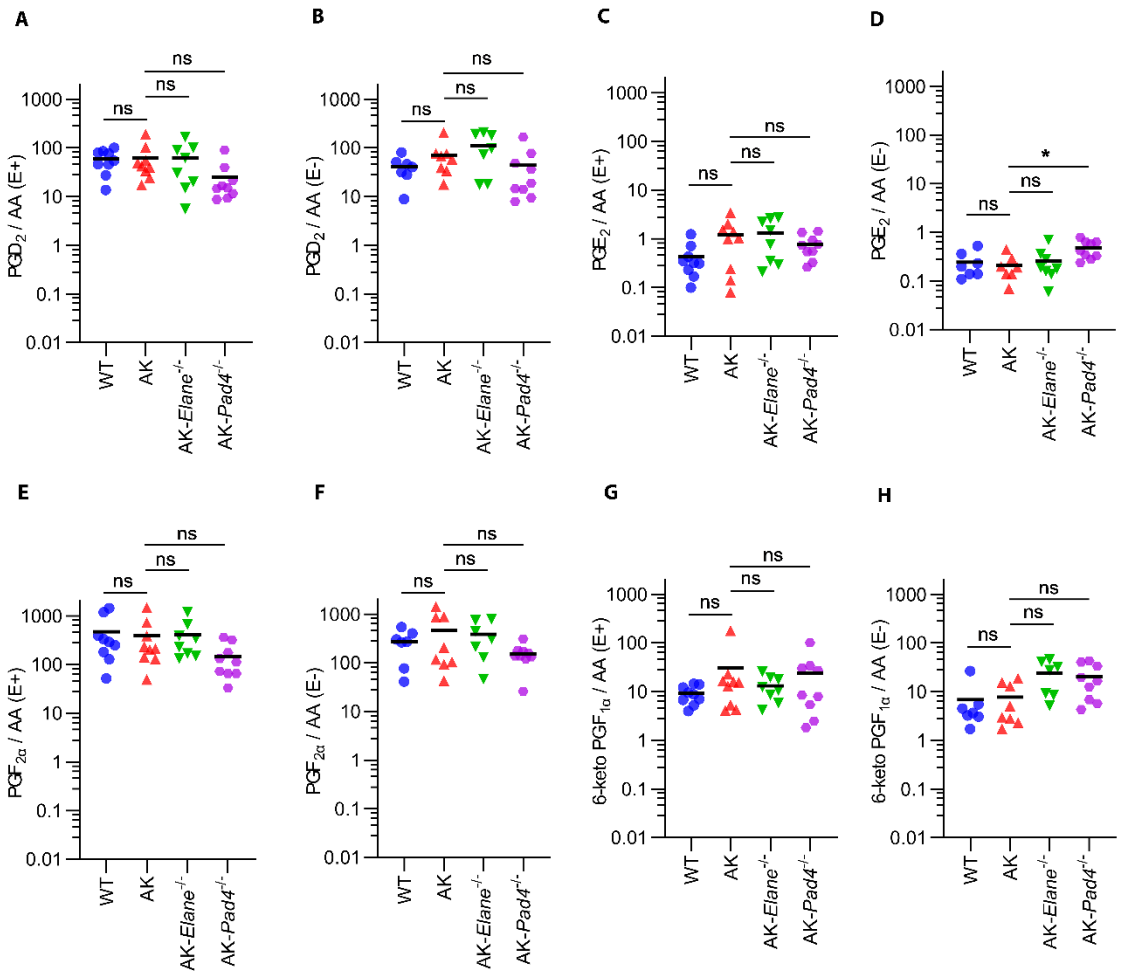

Figure S7.

Other detected prostanoid metabolites. Prostanoid metabolites were measured in endothelium-intact (E+) and endothelium-denuded (E-) aortae of 24-week-old mice by LC/ESI-MS/MS. The values of prostanoid metabolites detected were all normalized to arachidonic acid (AA) in the aorta. **A,** PGD2/AA in intact aortae. **B,** PGD2/AA in denuded aortae. **C,** PGE2/AA in intact aortae. **D,** PGE2/AA in denuded aortae. **E,** PGF2α/AA in intact aortae. **F,** PGF2α/AA in denuded aortae. **G,** 6-keto PGF1α /AA in intact aortae. **H,** 6-keto PGF1α /AA in denuded aortae. For **A-H**, mean for n=7-9, **p*<0.05 by one-way ANOVA followed by the Holm-Šídák test, ns=non-significant.