Supplemental Information

Hepatic JAK2 protects against atherosclerosis through circulating IGF-1

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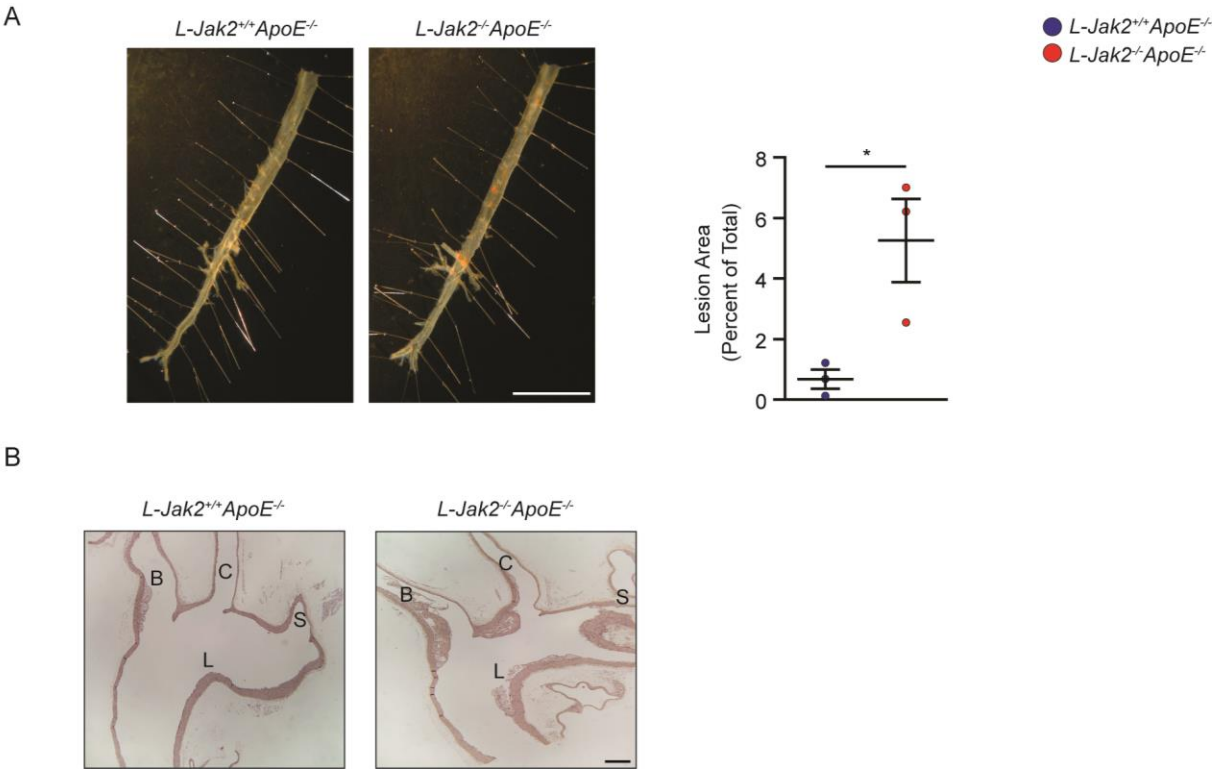
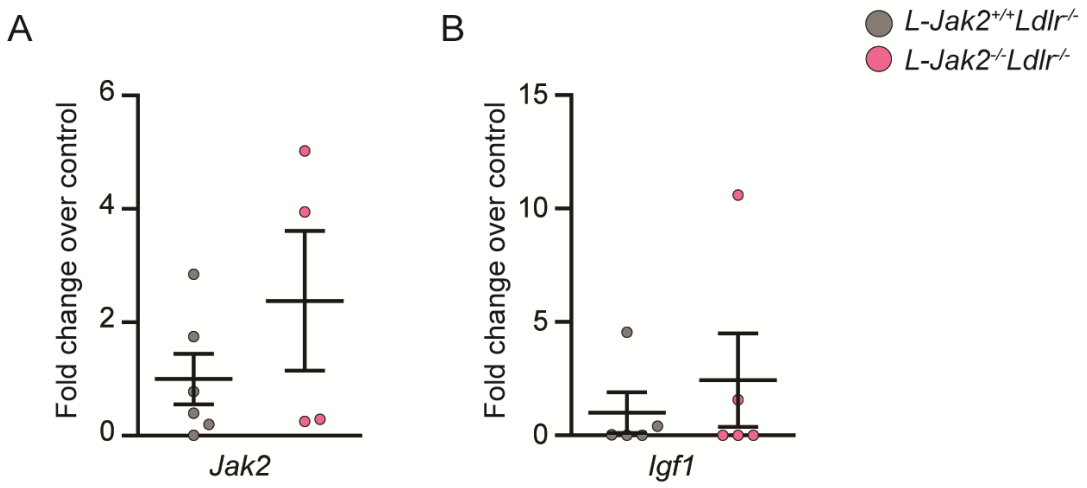
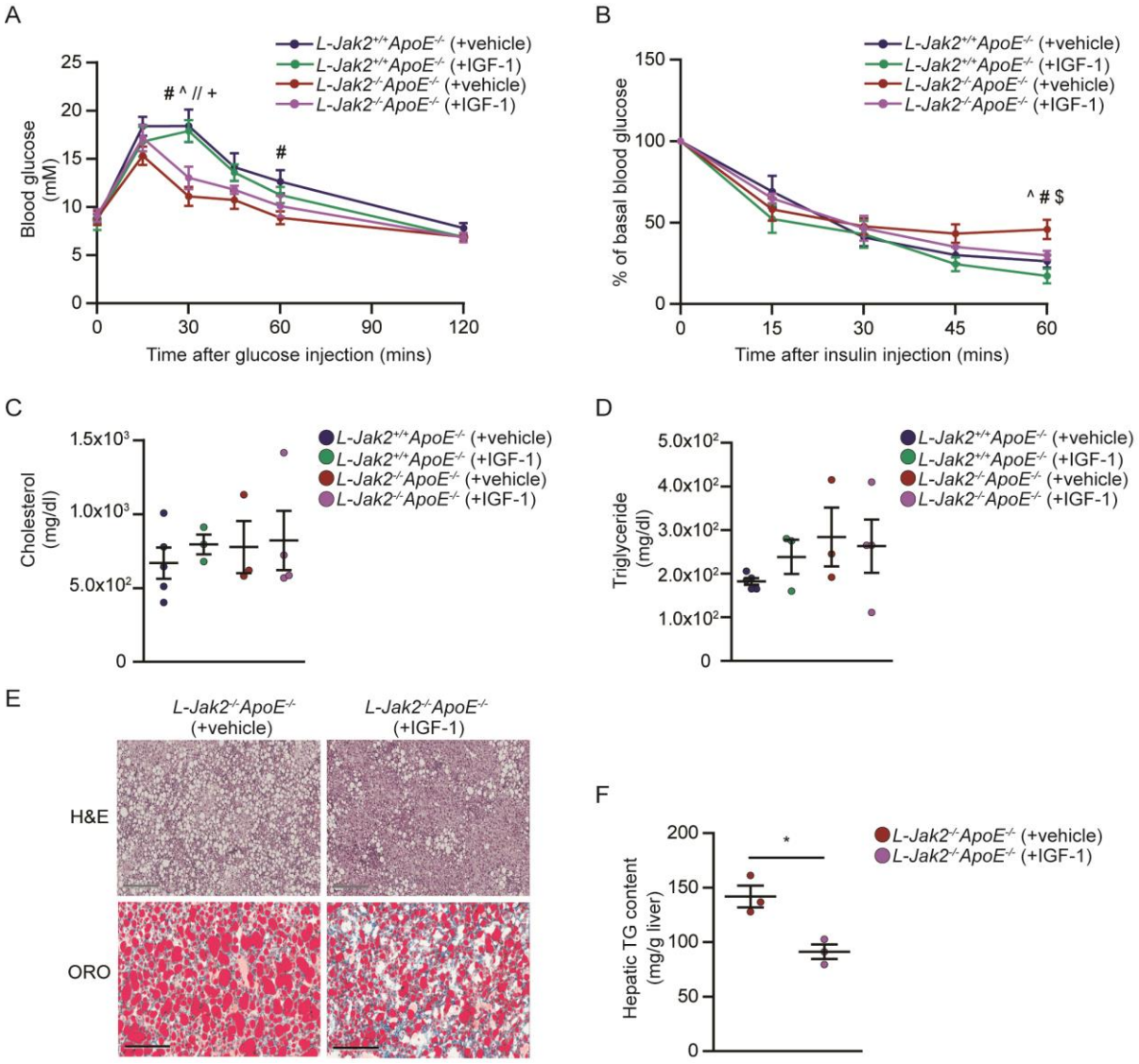


Supplemental Figure 1. *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ mice develop more atherosclerosis on chow diet. *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ and *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$ littermate controls were fed a standard rodent chow diet for 22 weeks. (**A**) Representative photographs of *en face* Oil-red-O (ORO) staining and quantification of atherosclerotic plaque area in descending aortas of 22-week-old *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ mice (n=3) and control *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$ mice (n=3). Scale bar: 1 cm. Each dot in the scatter plot indicates an individual animal. (**B**) Representative images of longitudinal sections from the aortic arch of 22-week-old *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ mice and control *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$ mice stained with H&E. B: brachiocephalic artery; C: left common carotid; S: subclavian artery; L: lesser curvature. Scale bar: 200 μm. Data represent mean ± SEM. Differences between groups were analyzed for statistical significance by Student unpaired t-test. *P < 0.05.

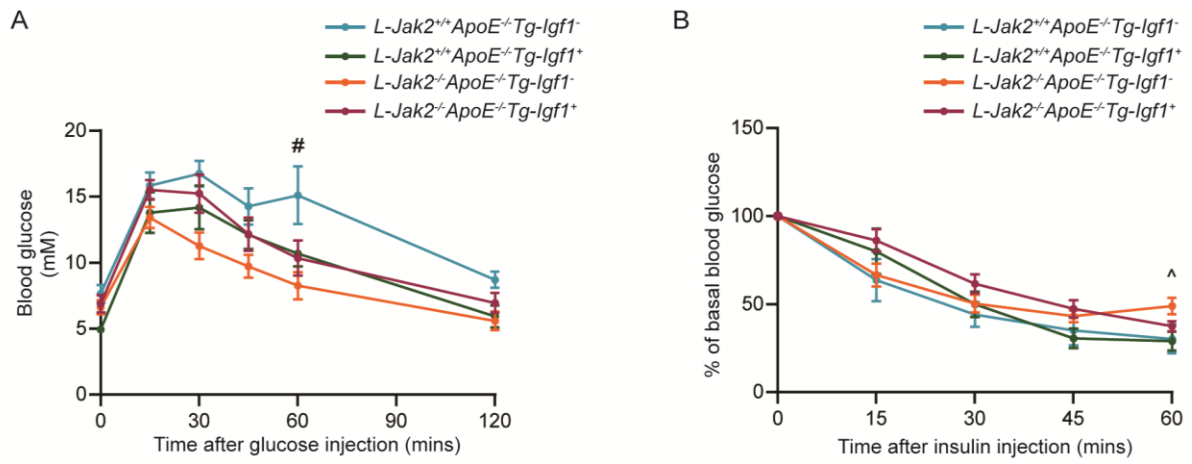


Supplemental Figure 2. Expression of *Jak2* and *Igf1* mRNA expression in the aortic arch of *L-Jak2*$^{-/-}$*Ldlr*$^{-/-}$ mice. *L-Jak2*$^{-/-}$*Ldlr*$^{-/-}$ and *L-Jak2*$^{+/+}$*Ldlr*$^{-/-}$ littermate controls were fed an atherogenic diet containing 1.25% cholesterol for 12 weeks, starting at 6 weeks of age. (**A** and **B**) Quantitative real time PCR (qRT-PCR) analysis of *Jak2* and *Igf1* mRNA expression in aortic arches from *L-Jak2*$^{-/-}$*Ldlr*$^{-/-}$ mice (n=4-5) and control *L-Jak2*$^{+/+}$*Ldlr*$^{-/-}$ mice (n=5-6). Values are normalized to 18S mRNA levels and presented as fold change over control group. Each dot in the scatter plot indicates an individual animal. Data represent mean ± SEM. Differences between groups were analyzed for statistical significance by Student unpaired t-test.



Supplemental Figure 3. IGF-1 infusion attenuates hepatic steatosis but does not affect glucose tolerance, insulin sensitivity and total serum cholesterol or triglyceride levels in *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ mice. Vehicle (saline + 10 mmol/L HCl) or human Long R3 IGF-1 (1.0 mg/kg/day), a biologically active IGF-1 analog, was administered by subcutaneous osmotic pumps into 8-week-old *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ mice and *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$ littermate controls for 12 weeks while on an atherogenic diet containing 0.2% cholesterol. (**A**) Glucose tolerance test in overnight fasted vehicle-infused *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$ (n=6), IGF-1-infused *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$ (n=4),

vehicle-infused L-Jak2$^{-/-}$ApoE$^{-/-}$ (n=8) and IGF-1-infused L-Jak2$^{-/-}$ApoE$^{-/-}$ (n=5) mice. Mice received glucose (1 g/kg) intraperitoneally and blood glucose was measured sequentially for 120 minutes. (**B**) Insulin tolerance test in 4 hour fasted vehicle-infused *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$ (n=6), IGF-1-infused *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$ (n=4), vehicle-infused *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ (n=8) and IGF-1-infused *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ (n=5) mice. Mice received insulin (0.75 units/kg) intraperitoneally and blood glucose was measured sequentially for 60 minutes. Data are expressed as a percentage of basal (fasting) glucose. (**C** and **D**) Total serum cholesterol and triglyceride from vehicle-infused *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$ (n=5), IGF-1-infused *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$ (n=3), vehicle-infused *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ (n=3) and IGF-1-infused *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ (n=4) mice. (**E**) Representative images of H&E and Oil-red-O (ORO) staining of liver sections from vehicle-infused *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ (n=7,4) and IGF-1-infused *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ (n=6,3) mice. Scale bars: 200 μm (black), 300 μm (grey). (**F**) Total hepatic triglyceride (TG) content in vehicle-infused *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ (n=3) and IGF-1-infused *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ (n=3) mice. Results are normalized to tissue weight. Each dot in the scatter plot indicates an individual animal. Data represent mean ± SEM. Differences between groups were analyzed for statistical significance by Student unpaired t-test or One-way ANOVA with Newman-Keuls post-hoc test. *$P < 0.05$; $^{\#}P < 0.05$ between *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$ + vehicle and *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ + vehicle; $^{//}P < 0.05$ between *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$ + IGF-1 and *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ + IGF-1; $^{\wedge}P < 0.05$ between *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$ + IGF-1 and *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ + vehicle; $^{+}P < 0.05$ between *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$ + vehicle and *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ + IGF-1; $^{\$}P < 0.05$ between *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ + vehicle and *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ + IGF-1.



Supplemental Figure 4. Expression of *Igf1* transgene did not affect glucose tolerance or insulin sensitivity. *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$ and *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$ controls expressing an *Igf1* transgene in the liver (*L-Jak2*$^{-/-}$*ApoE*$^{-/-}$*Tg-Igf1*$^{+}$ or *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$*Tg-Igf1*$^{+}$, respectively) and those not expressing the transgene (*L-Jak2*$^{-/-}$*ApoE*$^{-/-}$*Tg-Igf1*$^{-}$ or *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$*Tg-Igf1*$^{-}$, respectively) were fed an atherogenic diet containing 0.2% cholesterol for 13-14 weeks, starting at 8 weeks of age. (**A**) Glucose tolerance test in overnight fasted *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$*Tg-Igf1*$^{-}$ (n=4), *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$*Tg-Igf1*$^{+}$ (n=8), *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$*Tg-Igf1*$^{-}$ (n=6) and *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$*Tg-Igf1*$^{+}$ (n=10) mice. Mice received glucose (1 g/kg) intraperitoneally and blood glucose was measured sequentially for 120 minutes. (**B**) Insulin tolerance test in 4 hour fasted *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$*Tg-Igf1*$^{-}$ (n=4), *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$*Tg-Igf1*$^{+}$ (n=8), *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$*Tg-Igf1*$^{-}$ (n=6) and *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$*Tg-Igf1*$^{+}$ (n=8) mice. Mice received insulin (0.75 units/kg) intraperitoneally and blood glucose was measured sequentially for 60 minutes. Data are expressed as a percentage of basal (fasting) glucose. Data represent mean ± SEM. Differences between groups were analyzed for statistical significance by One-way ANOVA with Newman-Keuls post-hoc test. $^{\wedge}P < 0.05$ between *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$*Tg-Igf1*$^{+}$ and *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$*Tg-Igf1*$^{-}$; $^{\#}P < 0.05$ between *L-Jak2*$^{+/+}$*ApoE*$^{-/-}$*Tg-Igf1*$^{-}$ and *L-Jak2*$^{-/-}$*ApoE*$^{-/-}$*Tg-Igf1*$^{-}$.