Supplemental Information

**Hepatic JAK2 protects against atherosclerosis through circulating IGF-1**

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Supplemental Figure 1. \textit{L-Jak2}^{-/-}\textit{ApoE}^{-/-} mice develop more atherosclerosis on chow diet.

\textit{L-Jak2}^{-/-}\textit{ApoE}^{-/-} and \textit{L-Jak2}^{+/+}\textit{ApoE}^{-/-} littermate controls were fed a standard rodent chow diet for 22 weeks. (A) Representative photographs of \textit{en face} Oil-red-O (ORO) staining and quantification of atherosclerotic plaque area in descending aortas of 22-week-old \textit{L-Jak2}^{-/-}\textit{ApoE}^{-/-} mice (n=3) and control \textit{L-Jak2}^{+/+}\textit{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 \textit{L-Jak2}^{-/-}\textit{ApoE}^{-/-} mice and control \textit{L-Jak2}^{+/+}\textit{ApoE}^{-/-} mice stained with H&E. B: brachiocephalic artery; C: left common carotid; S: subclavian artery; L: lesser curvature. Scale bar: 200 \textmu m. Data represent mean \pm 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; ‡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. ^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−.