Supplementary Information

Supplementary Methods

Stromal vascular fraction (SVF) immune cell analysis and IL6 ELISA. Epididymal and inguinal white adipose tissues were removed from three control and three JAK2A mice and placed into Ham's F10 media. Following mincing, tissues were digested in 2 mg/ml Collagenase Type 1 (Worthington) with shaking (150 rpm) at 37C for 30 minutes. The SVF was pelleted at 1600 rpm for 7 minutes, resuspended in 10 ml PBS and filtered (40 um). The cells were then pelleted and resuspended in FACS buffer (PBS, 5 mM EDTA, 2.5% FBS for immunostaining flow cytometric analyses (BD FACSVerse). Antibodies were used at 1:200 and were from Biolegend and BD Biosciences: Live/dead Zombie aqua, CD45 (clone 30-F11), CD11b (clone M1/70), F4/80 (clone BM8), Ly6C (clone HK1.4), Ly6G (clone 1A8), CD206 (clone C068C2), B220 (clone RA3-6B2), CD3 (clone 145-2C11), CD49b (clone HMalpha2), Siglec-F (clone E50-2440). Samples were fixed (FACS buffer, 1.6% paraformaldehyde) and stored at 4C prior to analysis. The murine IL6 ELISA was from ALPCO (61-IL6MS-E01).

Adipose tissue histology. Mice were perfused with PBS and adipose tissues were placed directly into 10% neutral-buffered formalin. Tissues were embedded with paraffin and sectioned (5 um, UCSF Liver Center Histology Core).

Adipose tissue explant stimulated lipolysis assay. Epididymal fat pads were excised from anesthetized control and JAK2A mice into PBS and weighed. Fat pads were then minced and ~20 mg pieces placed individually into 1.5 ml microfuge tubes in 0.5 ml explant buffer (25 mM Tris, pH 7.4, 4 mM glucose, 1mM KH_2PO_4, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM KCl, 125 mM NaCl, 2% fatty acid free BSA (Sigma), 0.5 U/ml adenosine deaminase (Sigma). 10 nM isoproterenol was added with or without 100 nM insulin and tubes incubated with rocking at 37C for 24 hours. Free fatty acid levels were measured using a kit with oleic acid as standard (Wako Diagnostics).

GH treatment for adipose tissue Western blot analyses. Chow fed 4-5 month old control and JAK2A male mice were injected subcutaneously with 5mg/kg recombinant murine GH or GH buffer daily at 10:00 for 7 days. On the 7th day, mice were fasted for four hours prior to subcutaneous injection of 0.75 U/kg of insulin or PBS. After 10 minutes, mice were euthanized, and inguinal adipose tissue was removed and immediately frozen in liquid nitrogen. Inguinal adipose tissue was lysed in RIPA buffer and processed for Western blot analysis as described above. Antibodies used were anti-phospho-S473-Akt (Cell Signaling #4060), anti-total Akt (Cell Signaling #9272), anti-phospho-Y1150-IR (Cell Signaling #3918), anti-total IR (Cell Signaling Technologies #3025), anti-p85alpha (Millipore 05-212), and peroxidase-coupled anti-beta-actin (Sigma #A3854).

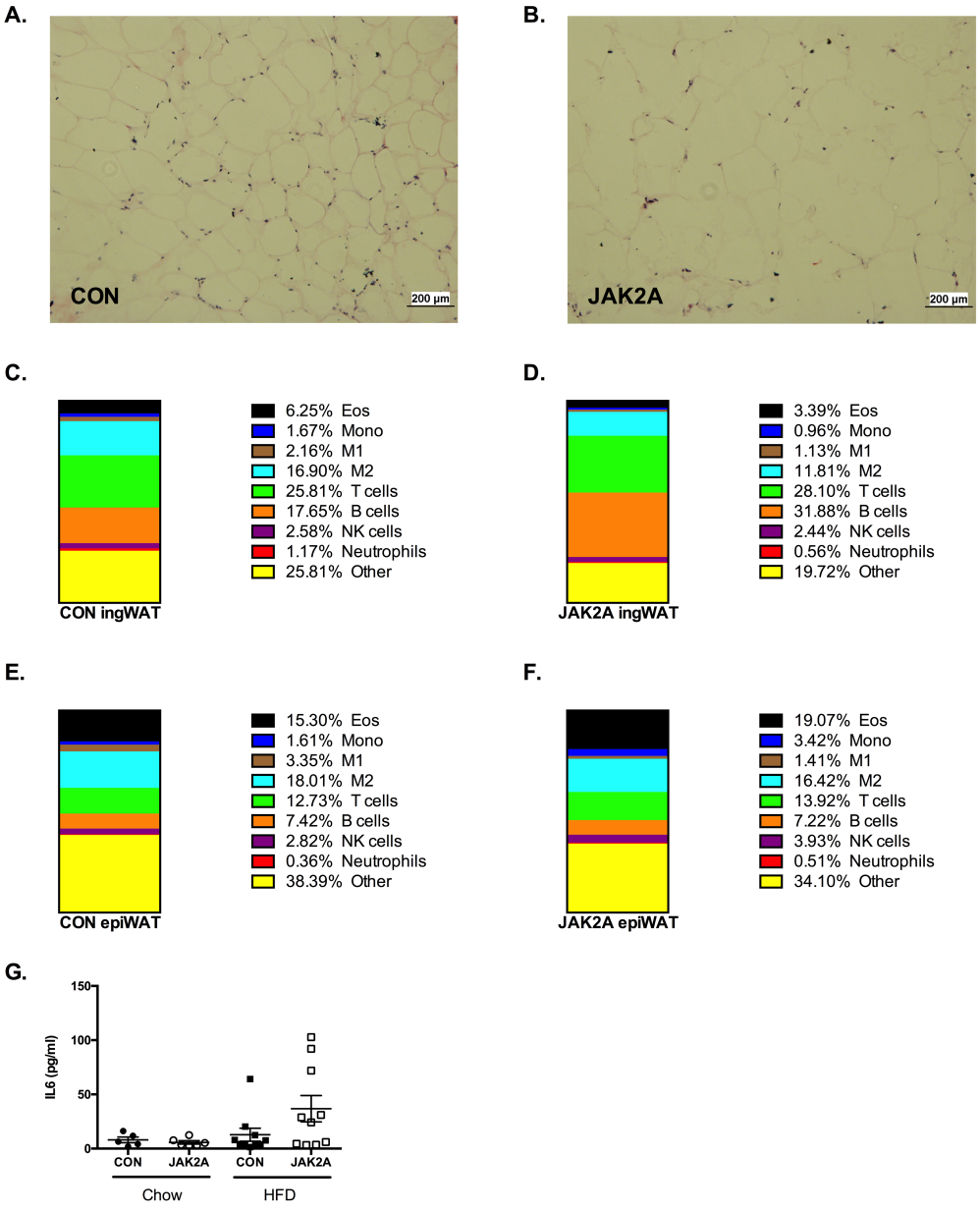
Quantitative PCR. Inguinal adipose tissue from four control and four JAK2A mice were snap frozen. Total RNA was isolated using the RNeasy Lipid Tissue Kit (QIAGEN, Valencia, California) for adipocytes. First-strand cDNA synthesis was performed using iScript Supermix for real-time quantitative PCR (Bio-Rad, Hercules, California) and oligo(dT) primers. Real-time quantitative PCR reactions were performed in a 384-well format using a Kapa Probe Fast qPCR kit (Kapa Biosystems, Woburn, Massachusetts) and total reaction volumes of 10 µl on an ABI 7900HT system (Applied Biosystems). TaqMan primer/probe sets (5′FAM/3′BHQ; Biosearch Technologies, Novato, California) were designed using Primer Express software (Applied Biosystems, Carlsbad, California). For murine *Pik3r1* the following primer/probe sets were used:

Forward: TGCACAACAGAGGCGATGAA

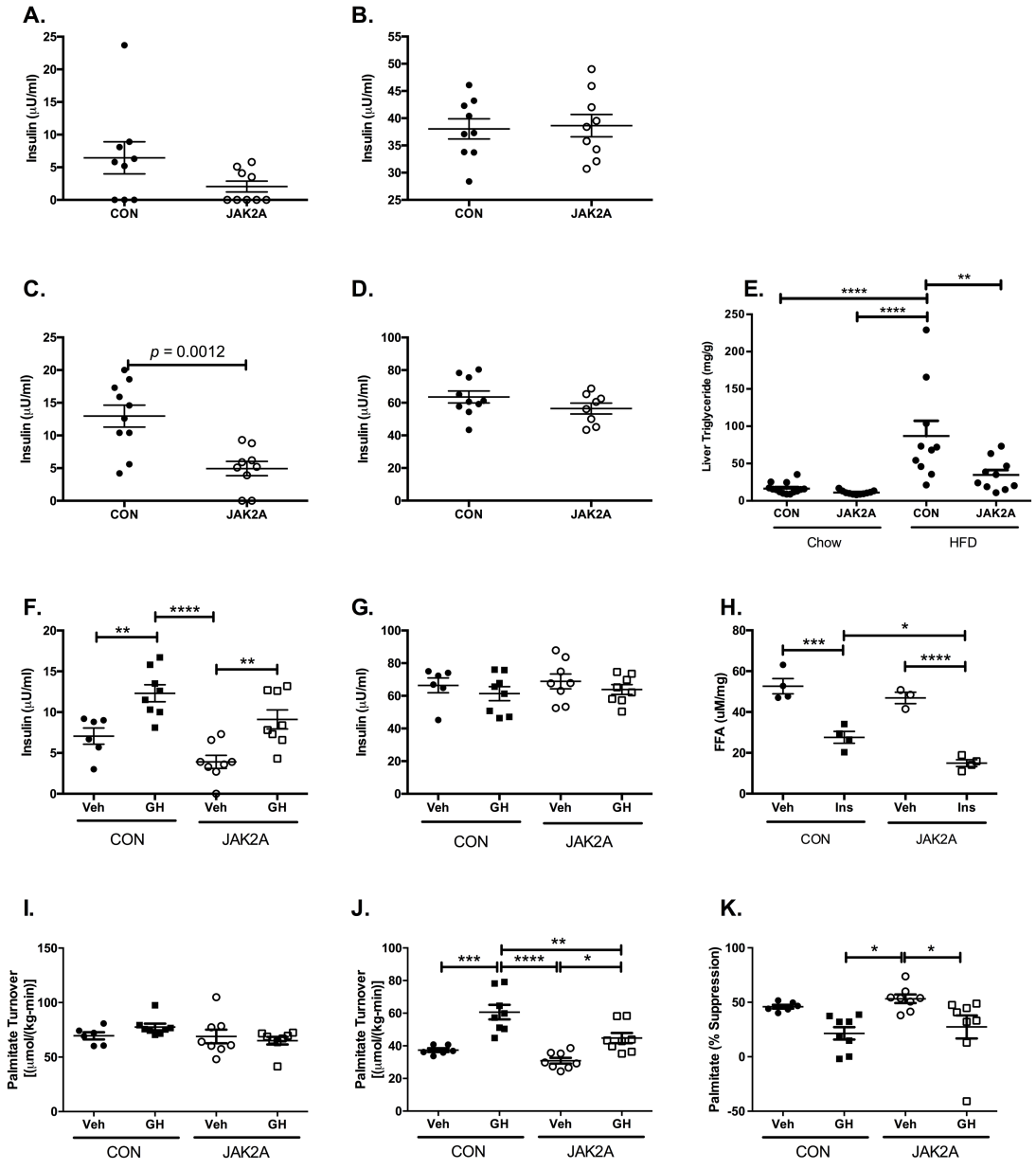
Reverse: GGAGCCCTTTGCTTCTCAGA

Probe: TGCCCTCGGATCCAGTTCCTCACC

Absolute gene expression (gene copy number) was quantified using https://genorm.cmgg.be/. The genes *Gapdh*, *β-actin*, and *Mrps9* were used for normalization.

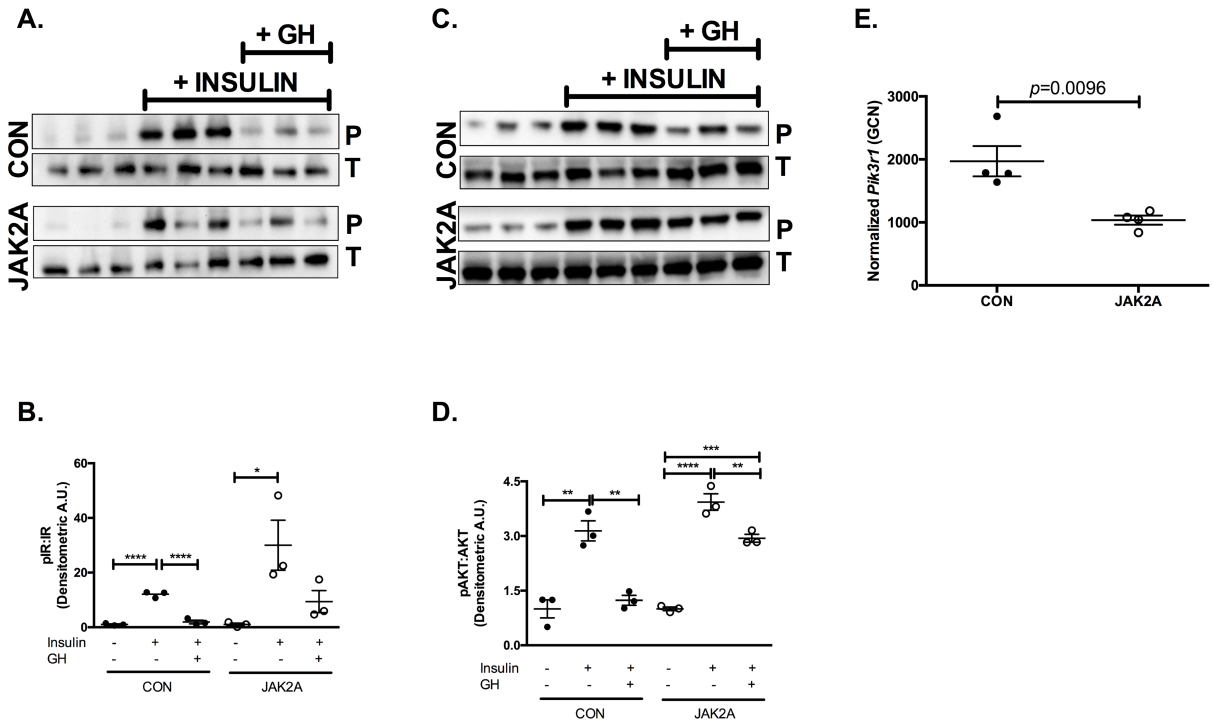


Supplemental Figure 1. Adipocyte histology and immune cell landscape. Paraffin-embedded sections of inguinal white adipose tissue from A.) control (CON) and B.) JAK2A mice. The scale bar shown is 200 um. C-F.) Representative fractions of total alive stromal vascular cells in control (CON) and JAK2A inguinal (ingWAT) and epididymal (epiWAT) white adipose tissue. Eos = eosinophils, mono = monocytes, M1 = M1 macrophages, M2 = M2 macrophages, NK cells = natural killer cells. G.) Interleukin-6 (IL6) ELISA from serum of chow and high fat diet (HFD) fed control (CON) and JAK2A mice.



Supplemental Figure 2

A.) Basal and B.) clamped plasma insulin levels in chow fed control (CON, filled circles) and JAK2A mice (open circles). C.) Basal and D.) clamped plasma insulin levels in high fat diet fed control and JAK2A mice. E.) Total liver triglyceride levels in chow and high fat diet (HFD) fed control and JAK2A mice. F.) Basal and G.) clamped plasma insulin levels in vehicle (Veh) and Growth Hormone (GH) control and JAK2A mice. H.) Isoproterenol-stimulated lipolysis as measured by release of free fatty acids (FFA) in control and JAK2A adipose tissue explants treated with vehicle or insulin (Ins). I.) Palmitate turnover in basal and J.) clamped control and JAK2A mice treated with vehicle or GH. K.) Percent suppression of palmitate turnover following insulin infusion in control and JAK2A mice treated with vehicle. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by OneWay ANOVA. N=6-11 for all cohorts. Data are +/- S.E.M.



Supplemental Figure 3. Adipose tissue insulin signaling in GH treated animals. A.) Tyrosine phosphorylated (P) and total (T) insulin receptor levels in control (CON) and JAK2A inguinal adipose tissue following intraperitoneal insulin (+INSULIN) injections. +GH = seven days daily Growth Hormone injections. B.) Densitometric quantification of phosphorylated insulin receptor (IR) to total IR ratio. C.) Serine (473) phosphorylated (P) and total (T) AKT levels in control and JAK2A inguinal adipose tissue. D.) Densitometric quantification of phosphorylated AKT to total AKT ratio. E.) Real-time qPCR of inguinal adipose tissue from control and JAK2A mice. A.U., Arbitrary Units. A-D, N=3, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by OneWay ANOVA. E, N=4, p-0.0096 by unpaired T test. Data are +/- S.E.M.