SUPPLEMENTARY DATA

General Laboratory methods

Plasma analysis: Plasma insulin (Mercodia, Sweden), leptin (duoset, R&D Systems Europe, Abingdon, United Kingdom), IL-6, TNFα and adiponectin levels (Quantikine kits, R&D Systems Europe, Abingdon, UK) were measured enzymatically. Plasma triacylglycerol (TAG) and non-esterified fatty acids (NEFA) (Bio-vision, California, USA) levels were measured as previously described(33).

Gene Expression Analysis: RNA was extracted from epididymal adipose tissue (EAT), 3T3L1 adipocytes and bone-marrow macrophages using TRI-Reagent and stored at -80°C. Single-stranded cDNA was prepared using High-Capacity cDNA Archive Kit (Applied Biosystems, Warrington, UK). Labeled primers and probes and TaqMan Universal Mastermix were obtained from Applied Biosystems. mRNA expression was quantified by real-time PCR (RT-PCR) on an ABI 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems). To control for between-sample variability, mRNA levels were normalized to GAPDH for each sample by subtracting the C_t for GAPDH from the C_t for the gene of interest producing a ΔC_t value. The ΔC_t for each treatment sample was compared to the mean ΔC_t for control samples using the relative quantification $2^{-(\Delta\Delta Ct)}$ method to determine fold-change.

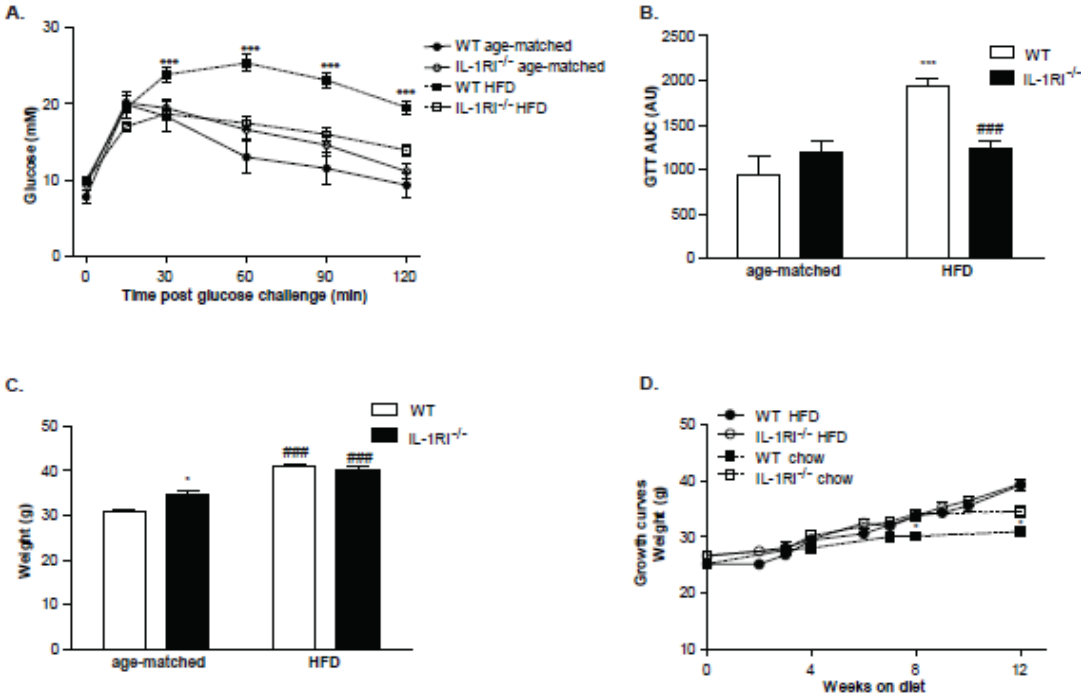
Immunoblot analysis: Protein was harvested from adipose tissue or cells by lysing in RIPA buffer containing complete protease (Roche Ltd, Dublin, Ireland) and phosphatase inhibitors. Protein concentration was quantified by Bradford assay (Bio-Rad Laboratories Inc., CA, USA). Equal concentrations of lysate (10μg) were reduced, separated by SDS-PAGE, transferred to nitrocellulose membranes, blocked and incubated overnight (at 4°C) in primary antibody. Blots were probed with antibodies to phosphorylated Akt, phosphorylated STAT3, SOCS3, serine-phosphorylated IRS-1 (Cell Signaling Technology, MA), whole cell IRS1, whole cell GLUT4, phosphorylated insulin receptor (IR) (Upstate, Millipore, MA), or β-actin (Abcam, MA). Blots were washed, incubated in secondary antibody and visualized using Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific Inc., USA). Levels of tyrosine phosphorylated IRS-1 were measured in adipose tissue protein lysates using a pathscan ELISA platform (Cell Signaling Technology, MA).

Immunohistochemistry:

Adipose tissue samples were fixed in formalin and paraffin embedded. Samples were deparaffinised and hydrated using xylene and alcohol. Antigen retrieval was performed in 0.01M citrate buffer at a PH of 6 for 30 minutes. To quench endogenous activity, sections were incubated in the dark in H_2O_2 for 30 minutes at room temperature. After washing in PBS, sections were blocked and incubated with primary F4/80 antibody overnight at 4°C. Sections were incubated with secondary antibody (ABC kit from Vectastain) for 1 hour at room temperature, before washing with PBS. Sections were detected with DAB and counterstained with H_2O_2 before being visualized using a Nikon 80i microscope.

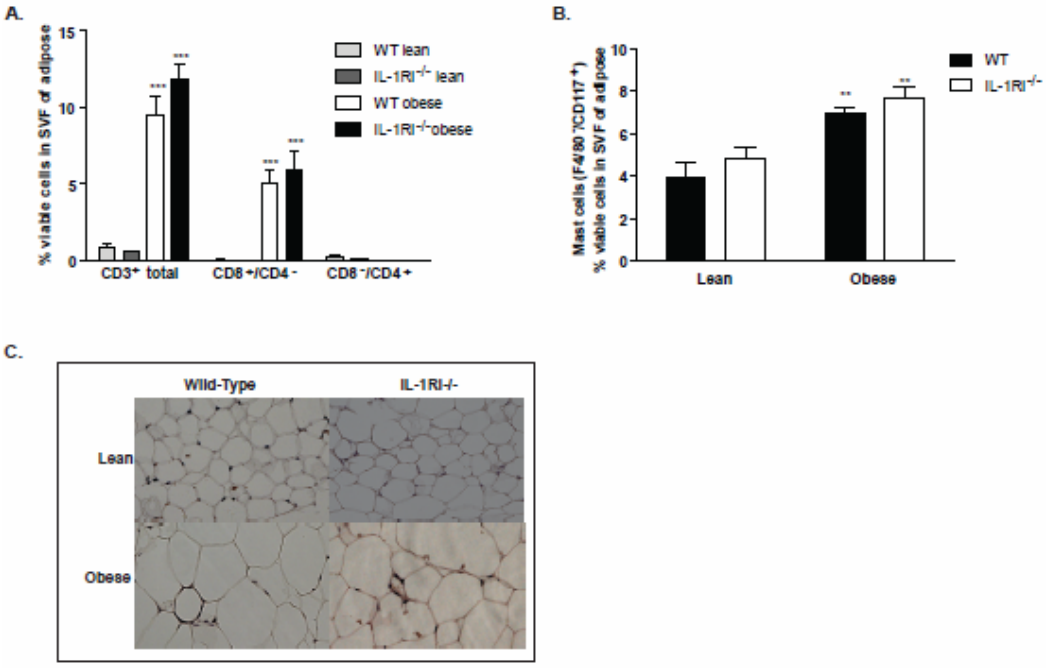
SUPPLEMENTARY DATA

Supplementary Figure 1: Comparison of glucose tolerance in high-fat fed WT and IL-1RI$^{-/-}$ mice with age-matched chow-fed controls. (A) Age-matched WT and IL-1RI$^{-/-}$ animals were placed on a chow-diet (10% kcal from fat) at 6-8wks of age for 12 weeks and GTT (1.5g/kg glucose) was performed and compared with high-fat fed animals (45% kcal from fat) (***p<0.001 w.r.t. IL-1RI$^{-/-}$ obese, n=12-31). (B) Area under the curve was calculated and expressed as arbitrary units (AU) (***p<0.001 w.r.t. age-matched counterpart; ###p<0.001 w.r.t. WT, n=8-31). (C) Weight of chow and high-fat fed animals at time of metabolic challenge (*p<0.05 w.r.t. age-matched WT, $^{###}$p<0.001 w.r.t. age-matched counterpart, n=8-31). (D) Growth curves for WT and IL-1RI$^{-/-}$ mice fed either a HFD or chow-diet for 12 weeks (*p<0.05 w.r.t. chow-fed WT mice, n=15-31).



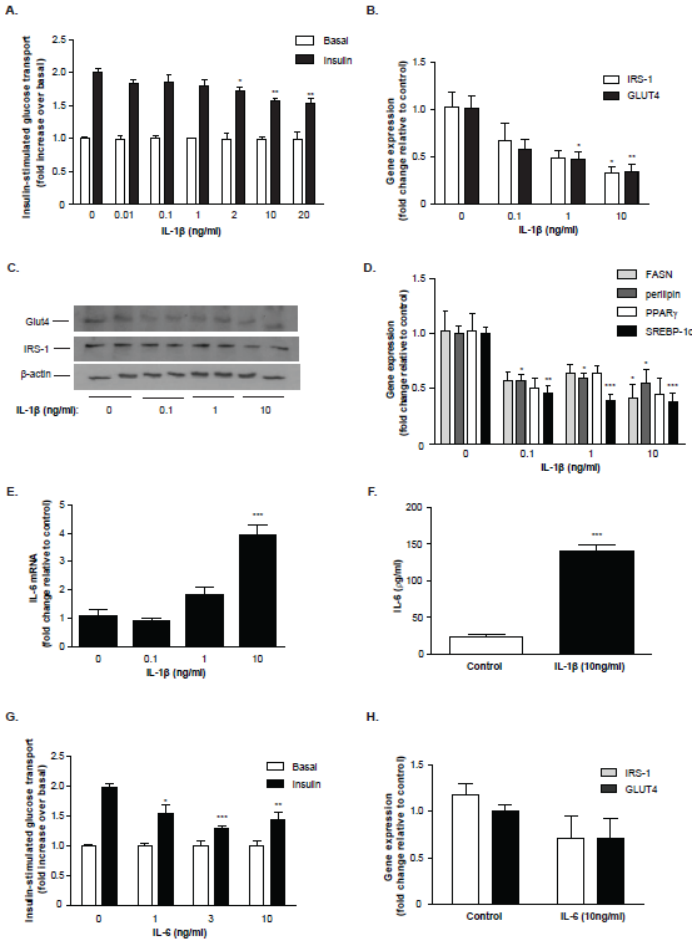
SUPPLEMENTARY DATA

Supplementary Figure 2: Immune cell numbers within adipose tissue of WT and IL-1RI$^{-/-}$ mice. (A) Recruitment of total CD3^{+} T-cells, cytotoxic T-cells (CD3^{+}/CD8^{+}/CD4^{-}) and helper T-cells (CD3^{+}/CD8^{-}/CD4^{+}) into adipose tissue of lean and obese mice (12wks HFD) was monitored by flow cytometry (***p<0.001 w.r.t. lean, n=8). (B) Recruitment of mast cells (F4/80^{-}/CD117^{+}) into adipose tissue of lean and obese WT and IL-1RI$^{-/-}$ mice was monitored by flow cytometry (**p<0.01 w.r.t. lean, n=8). (C) Adipose tissue sections from lean and obese WT and IL-1RI$^{-/-}$ were stained for the macrophage marker F4/80 to monitor presence of crown-like structures within the tissue.



SUPPLEMENTARY DATA

Supplementary Figure 3: Adverse effects of pro-inflammatory IL-1β and IL-6 on adipocyte biology. (A) The effect of 72h incubation with increasing concentrations (0.01-20ng/ml) of IL-1β on insulin (100nM)-stimulated ^{3}H-glucose transport into 3T3L1 adipocytes was evaluated. Basal and insulin-stimulated glucose transport was monitored with each dose of IL-1β and fold increase in transport over basal is presented (**p<0.05, **p<0.01 w.r.t. control cells, n=4). The effect of 72h incubation with increasing concentrations of IL-1β on insulin receptor substrate (IRS)-1 and GLUT4 (B) mRNA and (C) protein expression. Effects of 72h incubation of IL-1β on (D) PPAR□, fatty acid synthase (FASN), perilipin and SREBP-1c and (E) IL-6 mRNA expression in 3T3L1 adipocytes (*p<0.5, **p<0.01, ***p<0.001 w.r.t. control, n=4). (F) Effect of IL-1β (10ng/ml) for 24h on IL-6 secretion from 3T3L1 adipocytes (***p<0.001 w.r.t. control, n=4). (G) The effect of 72h incubation with increasing concentrations of IL-6 (1-10ng/ml) on insulin (100nM)-stimulated ^{3}H-glucose transport into 3T3L1 adipocytes. Fold increase in glucose transport over basal is presented (*p<0.05, **p<0.01, ***p<0.001 w.r.t. control, n=4). (H) The effect of 72h treatment with IL-6 (10ng/ml) on IRS-1 and GLUT4 mRNA levels (n=4).



SUPPLEMENTARY DATA

Supplementary Figure 4: Baseline inflammatory mRNA signature of WT and IL-1RI$^{-/-}$ BMM. WT and IL-1RI$^{-/-}$ bone marrow macrophages (BMM) were harvested and treated ± IL-1β (10ng/ml) for 24h. Effects of IL-1β on mRNA levels of (A) IL-6, (B) IL-10, (C) SOCS3 and (D) SOCS1 were analyzed by real-time PCR (***p<0.001 w.r.t. control; ##p<0.01, ###p<0.001 w.r.t WT, n=4).