Supplemental data

Direct control of hepatic glucose production by interleukin-13

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Supplemental Methods

Lipogenic and fat oxidation assays. For measurement of lipogenesis, hepatocytes were treated ± 10 ng/ml rIl-13 overnight followed by incubation with ^{14}C-acetate for 6 h. ^{14}C-lipids were extracted with 2:1 choloroform:methanol and normalized to protein content. Fatty acid oxidation assays were conducted using the ^{3}H palmitate tracer, following overnight rIl-13 treatment. ^{3}H$_2$O was determined and normalized to the protein concentration.

FACS and F4/80^{+} cell isolation. Livers and WAT were harvested from mice fasted for 6 h. Liver cells were released by extensive pipetting and filtered through a cell strainer (70 μm), followed by centrifugation at 50g to pellet hepatocytes. Supernatant containing immune cells was washed and collected. WAT was digested for 30 min at 37°C with 2 mg/mL collagenase, filtered through nylon mesh (250 μm) and centrifuged to pellet the stromal vascular fraction. Cells devoid of hepatocytes or adipocytes were subjected to either FACS using antibodies against F4/80 (Life Technologies) and Mgl1 (AbD Serotec) or magnetic beads conjugated with anti-F4/80 antibody (Life Technologies) for RNA isolation to determine M1/M2 gene expression in resident macrophages.
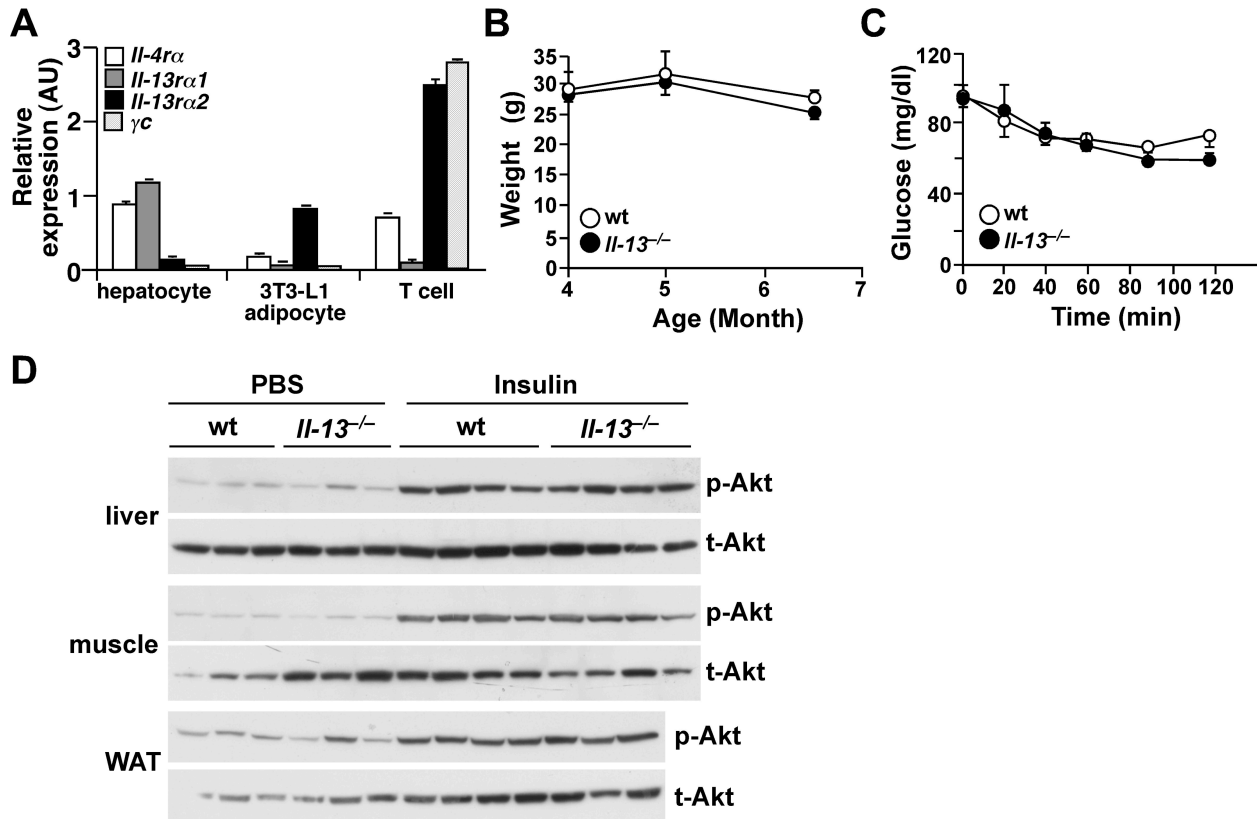
Supplemental table 1

Metabolic parameters of high fat fed BALB/c mice

BALB/c HFD	wt	*Il-13*$^{-/-}$
Weight (g)	42.78±1.82	43.66±1.31
Liver/body weight (mg/g)	33.44±1.2	34.13±1.27
WAT/body weight (mg/g)	32.7±1.5	33.32±3.12
Glucose (mg/dL)	99.20±9.40	141.20±12.16*
Insulin (ng/mL)	0.47±0.009	0.45±0.004
Triglyceride (mg/dL)	49.53±4.37	67.59±5.43*
Cholesterol (mg/dL)	111.19±5.00	125.64±5.57
Free fatty acid (mMol)	2.05±0.12	1.71±0.14
Lactate (mg/dL)	15.49±1.16	14.42±1.15

Mice were fasted 6 h (n = 8/genotype). *$p < 0.05$, wt vs. *Il-13*$^{-/-}$ mice

Supplementary figures and figure legends

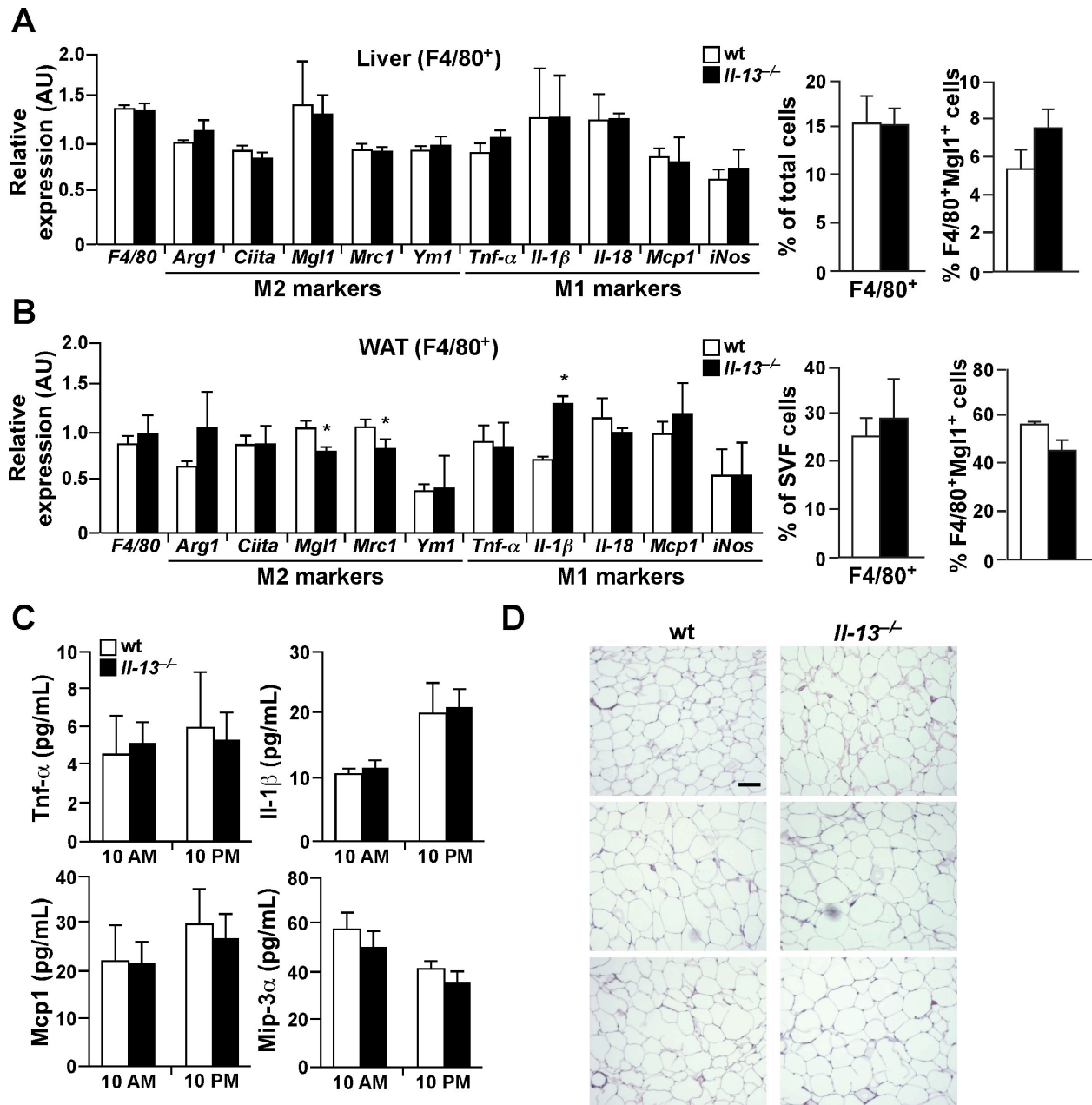**Supplemental Figure 1**
Body weight and insulin responses in chow-fed and insulin signaling in high fat fed *Il-13*^{−/−} mice in the BALB/c background. (**A**) The expression of Il-13 receptors in immune and non-immune cells determined by real-time PCR. Il-13 (and Il-4) binds to type II receptors consisting of Il-4rα/Il-13rα1 dimers. Il-13rα2 is thought to be a decoy receptor. Il-4 also binds to type I receptors consisting of Il-4rα/γc dimers, which are only expressed in immune cells, such as T lymphocytes. (**B**) Body weight and (**C**) insulin tolerance test (ITT) in wild-type (wt) or *Il-13*^{−/−} mice in the BALB/c background on a normal chow diet (9% fat). ITT was conducted in 6 month old animals (*n* = 5/genotype). (**D**) Immunoblotting of tissue insulin signaling in high fat fed wt and *Il-13*^{−/−} mice in the BALB/c background assessed by insulin stimulated Akt phosphorylation (*n* = 8/genotype). 5u/kg insulin was i.p. injected and tissues were collected 10 min later. Data are presented as mean ± SEM.
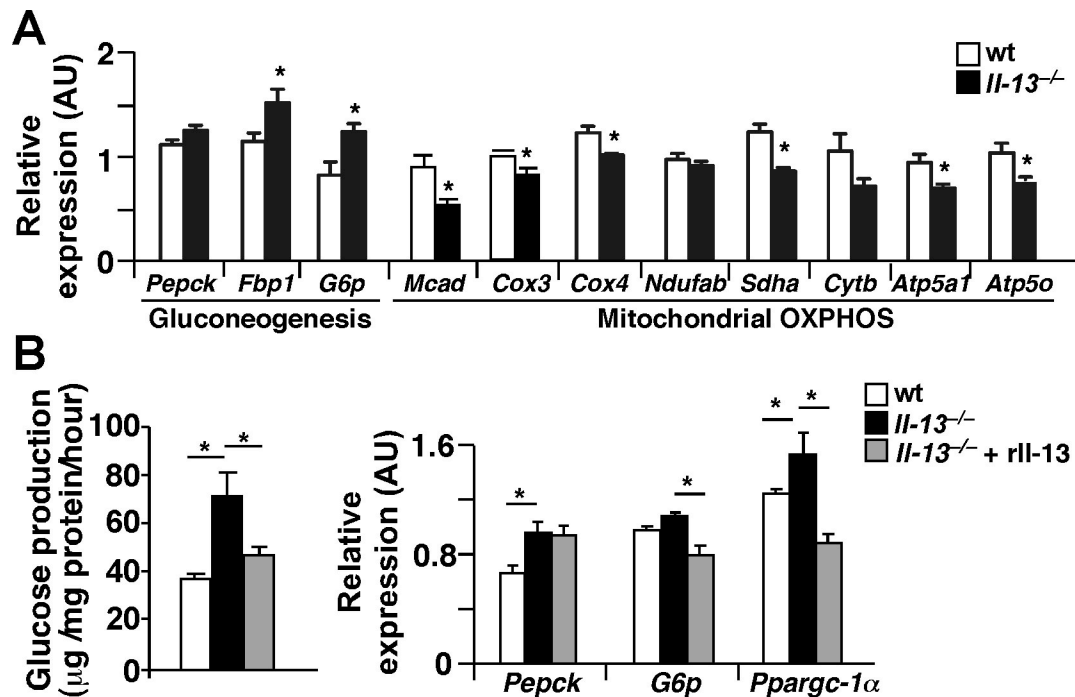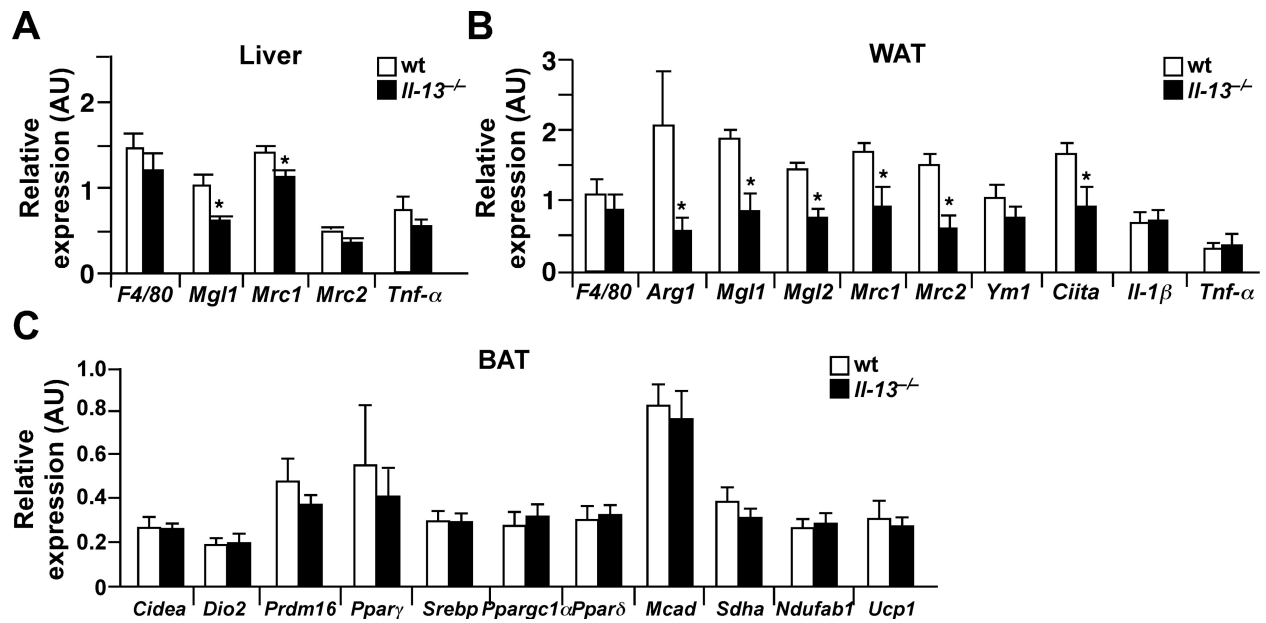
Supplemental Figure 2

Increased hepatic gluconeogenic gene expression in BALB/c $Il-13^{-/-}$ mice on high fat diet. (**A**) Metabolic gene expression in the liver. Liver samples from 6 h fasted wt and $Il-13^{-/-}$ mice in the BALB/c background (n=6, high fat diet for 6 months) were collected and gene expression was analyzed by quantitative, real-time PCR. (**B**) Glucose production and gluconeogenic gene expression are elevated in primary hepatocytes derived from $Il-13^{-/-}$ mice (BALB/c on high fat diet). rIl-13 (10 ng/ml) was given to hepatocytes for two hours followed by a 4 hour glucose production assay in the presence of rIl-13. Data are presented as mean ± SEM; *p < 0.05.
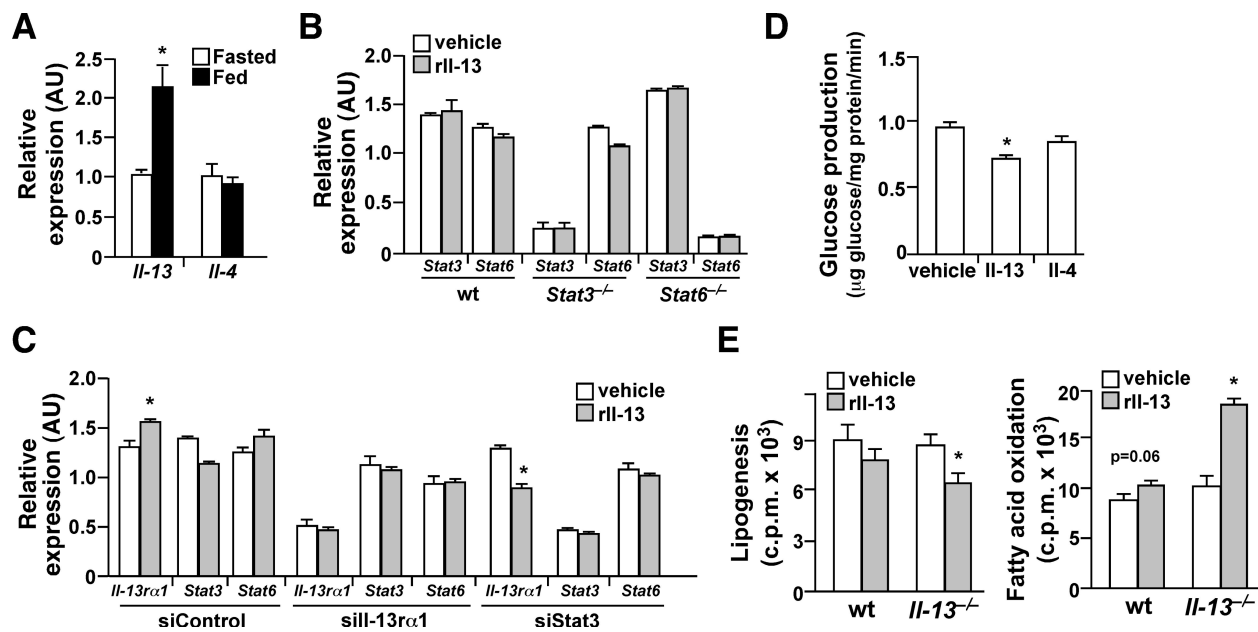
Supplemental Figure 3
Assessment of macrophage activation and tissue inflammation in C57BL/6 wt and *Il-13*^{−/−} mice on normal chow (7 month old males). (**A**) and (**B**) Gene expression analyses of inflammatory markers in F4/80^{+} cells isolated from livers and white adipose tissues (WAT) ($n = 4$). Cells were isolated using magnetic beads conjugated with anti-F4/80 antibody. Right panel: FACS analyses to examine the percentage of F4/80^{+} cells in the non-hepatocyte or non-adipocyte fraction and the expression of Mgl1 in F4/80^{+} cells. (**C**) Circulating concentrations of cytokines and chemokines determined by ELISA ($n = 7$). (**D**) WAT histology (sections from 3 individual mice). Scale bar: 100 μm. Data are presented as mean ± SEM; *p < 0.05.

Supplemental Figure 4

Inflammatory and metabolic gene expression in BALB/c wt and *Il-13*$^{-/-}$ mice on high fat diet. (**A**) and (**B**) Gene expression analyses of inflammatory markers in liver and white adipose tissue (WAT). Tissue samples from 6 h fasted wt and *Il-13*$^{-/-}$ mice in the BALB/c background (*n* = 6, high fat diet for 6 months) were collected and gene expression was analyzed by quantitative, real-time PCR. (**C**) Expression profiling of oxidative metabolism and thermogenic genes in brown adipose tissue (BAT). Data are presented as mean ± SEM; *p < 0.05.

Supplemental Figure 5

Assessment of knockout/knockdown efficiency and the role of Il-13 in fat metabolism in hepatocytes. (**A**) Hepatic expression of *Il-13* and *Il-4* at the fed or fasted state determined by quantitative real-time PCR (male C57BL/6 mice, *n* = 5). (**B**) The expression of *Stat3* and *Stat6* in wt, *Stat3*$^{-/-}$ and *Stat6*$^{-/-}$ hepatocytes ± rIl-13 (10 ng/ml) determined by quantitative real-time PCR. (**C**) The expression of *Stat3*, *Stat6* and *Il-13rα1* in control (sicontrol), Il-13rα1 siRNA (siIl-13rα1) and Stat3 siRNA (siStat3) transfected hepatocytes ± rIl-13 (10 ng/ml). (**D**) Il-4 does not suppress glucose production. Glucose production assays were conducted in primary hepatocytes ± rIl-13 or rIl-4 (10 ng/ml). (**E**) Lipogenic and fatty acid β oxidation assays in wt and *Il-13*$^{-/-}$ hepatocytes ± rIl-13. Data are presented as mean ± SEM; *p<0.05.