Supplementary Figures

Leptin signaling suppression in macrophages improves immunometabolic outcomes in obesity

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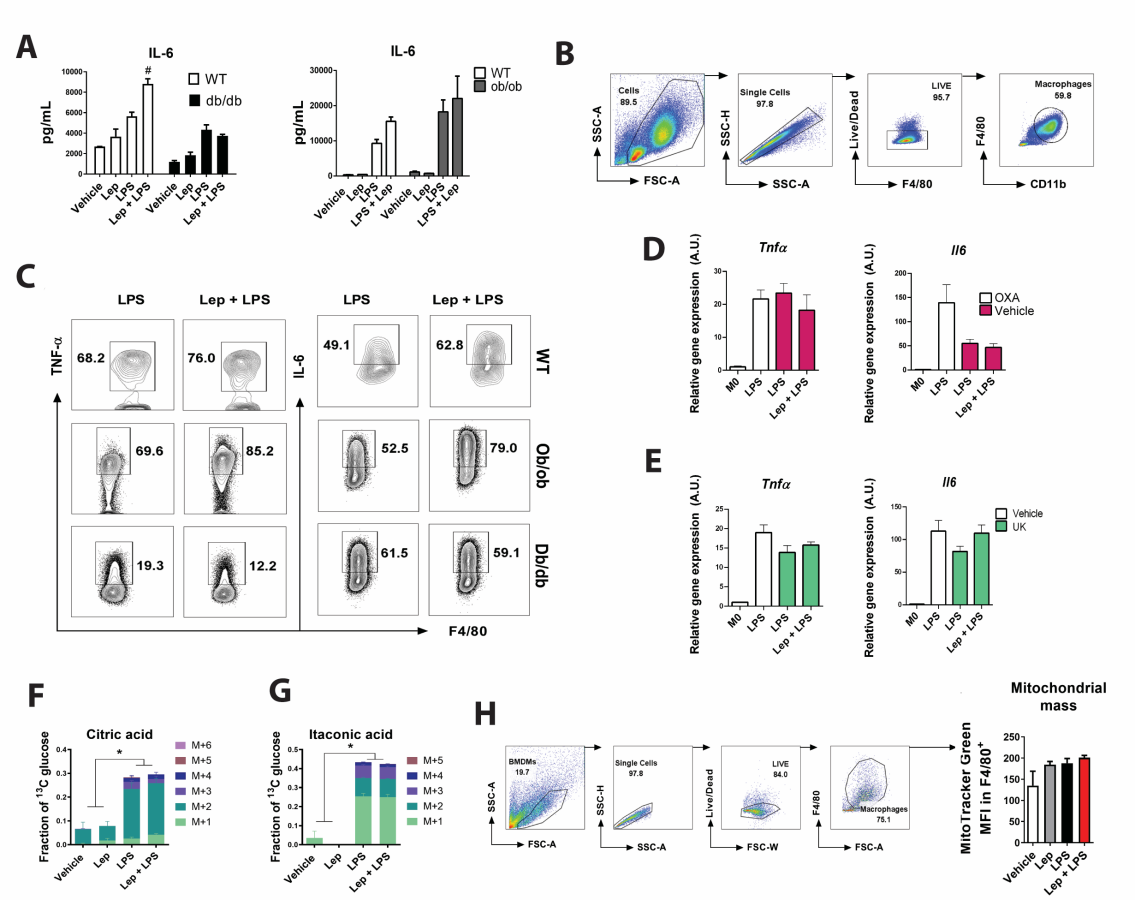


Supplementary Figure 1. Leptin directly modulates macrophage metabolism.

A) Concentration of IL-6 in the supernatant of leptin/LPS activated BMDMs from wild type (WT), db/db, or ob/ob mice by ELISA;

B) Flow cytometry gating Strategy for analysis of live peritoneal macrophages;

C) TNF-α and IL-6 positive macrophages from peritoneal lavage of WT, ob/ob and db/db mice were analyzed by flow cytometry;

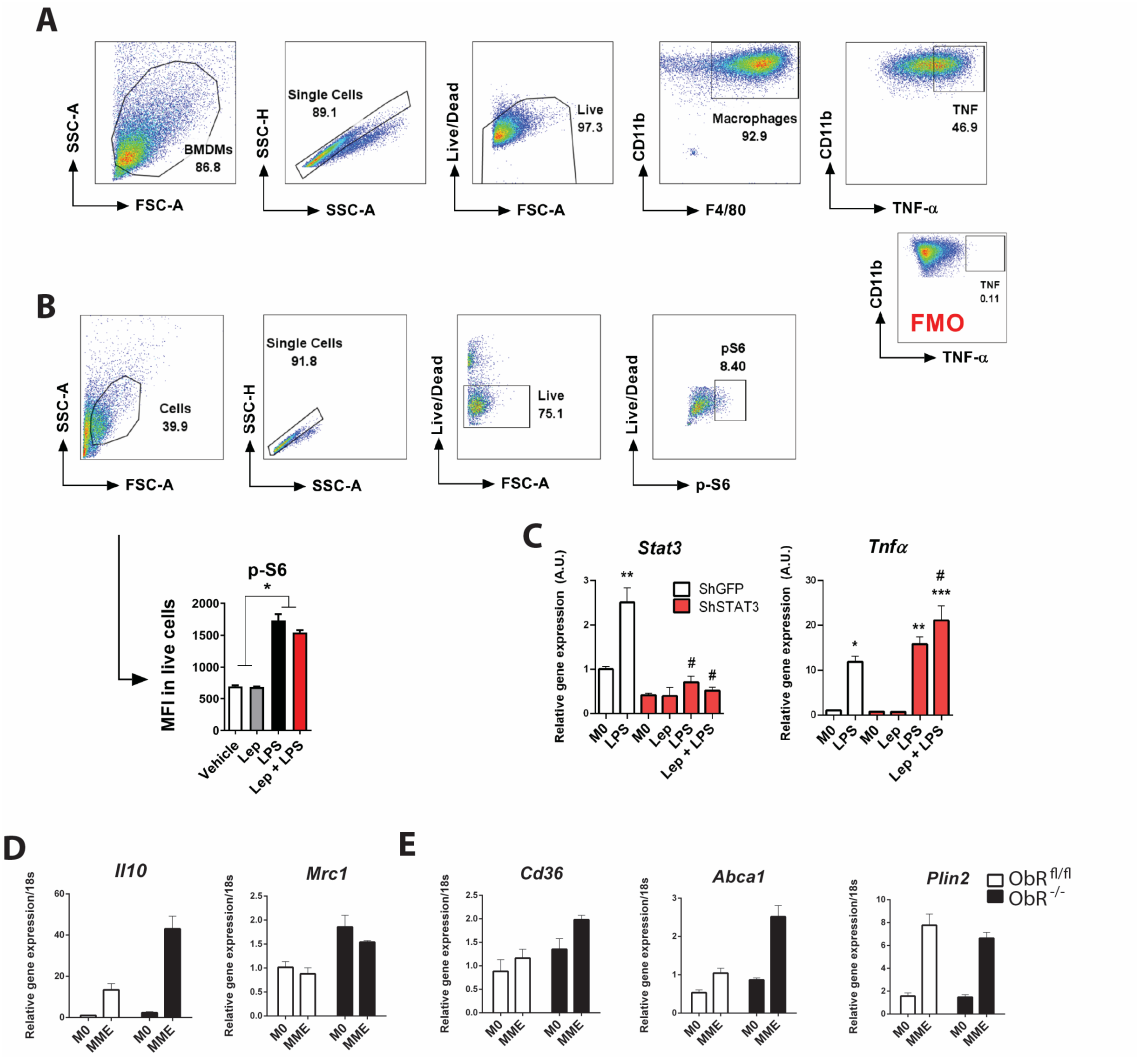
D) Relative gene expression of *Tnf*α and *Il6* from BMDMs treated with 40 mM oxamate (OXA), and/or leptin+LPS for 6 hours.

E) Relative gene expression of *Tnf*α and *Il6* from BMDMs treated with 10μM UK-5099 (UK), and/or leptin+LPS for 6 hours.

F-G) Fraction contribution of glucose carbons into citrate and G) itaconate, in macrophages stimulated with leptin/LPS for 6 hours and cultured in ^{13}C-glucose containing medium;

H) Flow cytometry gating strategy and Median of Fluorescence Intensity (MFI) of MitoTracker Green positive BMDMs after 6 hours of activation with leptin/LPS;

Data are represented as mean values ± s.e.m. n=3/5 per group. One-way ANOVA with Bonferroni's multiple comparison test (A, D-H) *p<0.05; #p<0.05 compared to all groups; Data are representative of three/two independent experiments.



Supplementary Figure 2. Strategies for studying leptin signaling in macrophages.

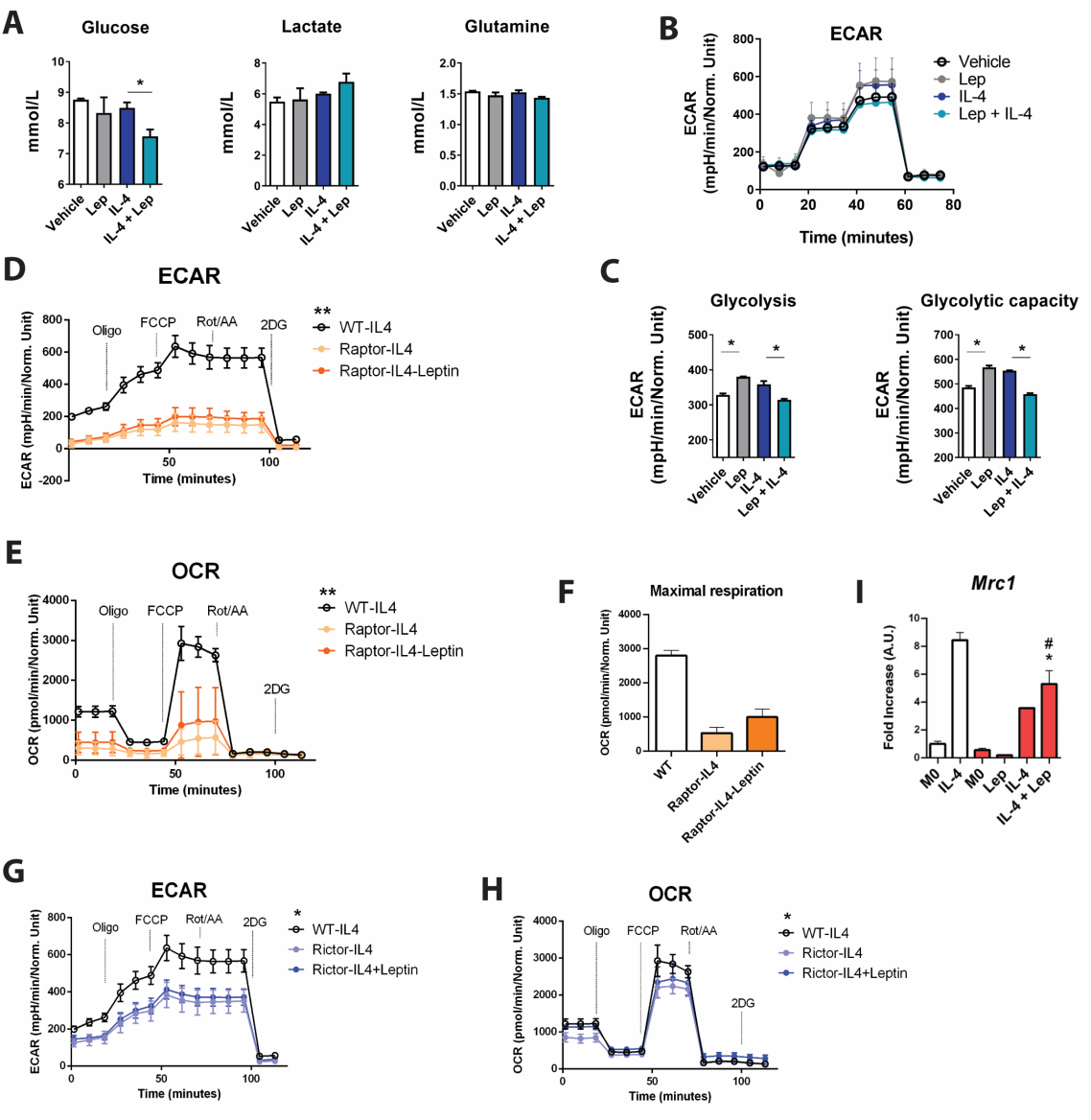
A) Representative flow cytometry gating strategy of cytokines (in this case, TNF-α) positive BMDMs after 6 hours of activation with leptin/LPS (100 ng/mL);

B) Representative flow cytometry gating strategy and MFI of phospho-S6 positive BMDMs after 30 min activation with leptin/LPS;

C) Relative gene expression of *Stat3* and *Tnfα* by shSTAT3 or shGFP (control) transduced BMDMs;
D) Relative gene expression of Il10 and Mrc1 by metabolically activated macrophages (MMe);

E) Relative gene expression of Cd36, Abca1 and Plin2 in metabolically activated macrophages (MMe);

Data are represented as mean values ± s.e.m. (C, F). n=3/5 per group. One-way ANOVA with Bonferroni's multiple comparison test (C, E). A,B representative of three independent experiments, C-F representative of two independent experiments. MFI: Median of Fluorescence Intensity.



Supplementary Figure 3. Metabolic effects of leptin on M(IL-4).

A) Concentration of Glucose, Lactate and Glutamine concentrations in the supernatant of BMDMs were measured by Bioanalyser;

B) Extracellular acidification rate (ECAR) of macrophages upon glycolytic stress (injections of glucose, oligomycin and 2-DG);

C) ECAR analysis of glycolysis (followed by glucose injection); Glycolytic capacity (followed by oligomycin injection).

D) Extracellular acidification rate (ECAR) of IL-4-treated macrophages from WT and Raptor$^{-/-}$ mice upon glycolytic stress (injections of glucose, oligomycin and 2-DG);

E) Oxygen consumption rate (OCR) of IL-4-treated macrophages from WT and Raptor$^{-/-}$ mice upon mitochondrial stress test (injections of oligomycin, FCCP, antimycin and rotenone);

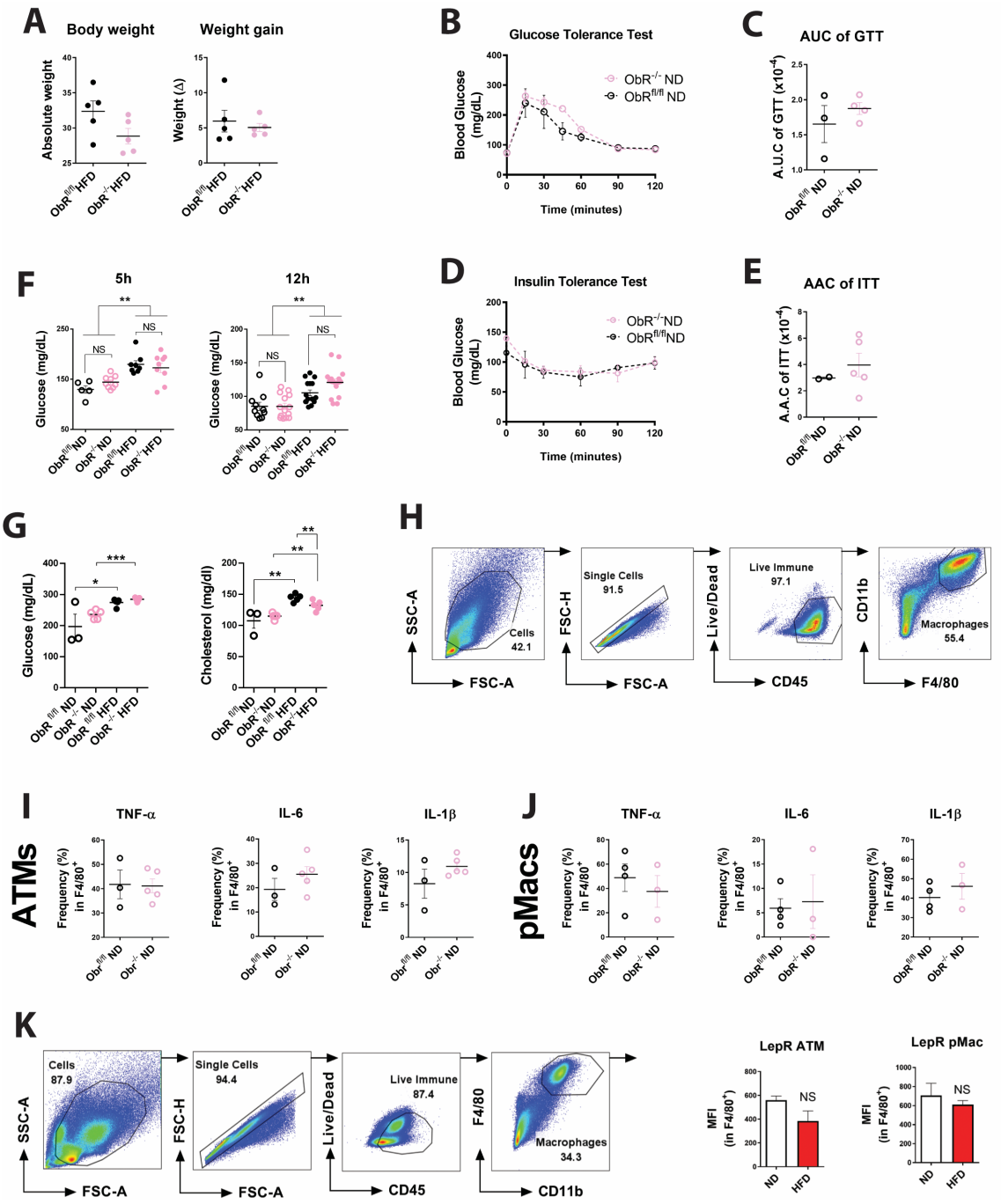
F) Maximal respiration (followed by FCCP injection) of IL-4-treated macrophages from WT and Raptor$^{-/-}$ mice;

G) Extracellular acidification rate (ECAR) of IL-4-treated macrophages from WT and Rictor$^{-/-}$ mice upon glycolytic stress (injections of glucose, oligomycin and 2-DG);

H) Oxygen consumption rate (OCR) of IL-4-treated macrophages from WT and Rictor$^{-/-}$ mice upon mitochondrial stress test (injections of oligomycin, FCCP, antimycin and rotenone);

I) Relative gene expression of Mrc1 by shSTAT3 or shGFP (control) transduced BMDMs.

Data are represented as mean values ± s.e.m. n=3/5 per group. One-way ANOVA with Bonferroni's multiple comparison test. A-H representative of two to three independent experiments.



Supplementary Figure 4. Metabolic and immune evaluation of ObR$^{fl/fl}$ and ObR$^{-/-}$ mice fed with chow and high fat diet.

A) Absolute body weight (g) and weight gain (Δ) of ObR$^{fl/fl}$ mice and ObR$^{-/-}$ mice fed a high fat diet (HFD) over the course of 16 weeks;

B) Insulin Tolerance Test (ITT) in chow-fed ObR$^{fl/fl}$ and ObR$^{-/-}$ mice*;*

C) Area under the curve (AUC) of ITT test in chow-fed ObR$^{fl/fl}$ and ObR$^{-/-}$ mice;

D) Insulin Tolerance Test (ITT) in chow-fed ObR$^{fl/fl}$ and ObR$^{-/-}$ mice*;*

E) Area above the curve (AAC) of ITT test in chow-fed ObR$^{fl/fl}$ and ObR$^{-/-}$ mice

F) Glucose levels in the blood after 5 and 12 hours of fasting;

G) Glucose and cholesterol levels in the serum of ND- and HFD-fed ObR$^{fl/fl}$ and ObR$^{-/-}$ mice;

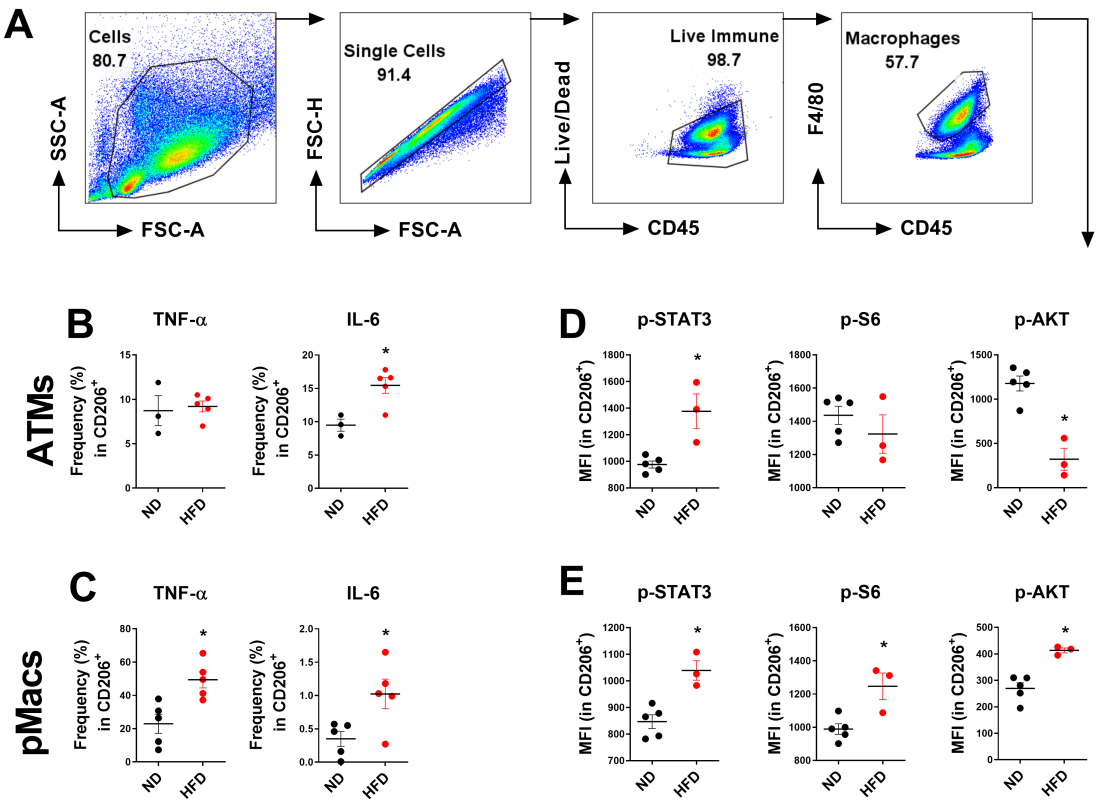
H) Flow cytometry gating strategy for evaluation of live macrophages;

I) Frequency of IL-6, IL-1β and TNF-α positive macrophages (F4/80^{+}) from the AT of chow-fed ObR$^{fl/fl}$ and ObR$^{-/-}$ mice;

J) Frequency of IL-6, IL-1β and TNF-α positive macrophages (F4/80^{+}) from the peritoneal lavage of chow-fed ObR$^{fl/fl}$ and ObR$^{-/-}$ mice;

K) Flow cytometry gating strategy for macrophage isolation and MFI analysis of ObR expression in ATMs and pMacs of chow- (normal diet - ND) and HFD-fed ObR reporter mice.

Data are represented as mean values ± s.e.m. n=3/5 per group. Two-way ANOVA Sidak's multiple comparison test Unpaired (b, d). Two-tailed Student's t test (A, C, E, I-K). One-way ANOVA with Bonferroni's multiple comparison test (F-G). A-K representative of two to three experiments. MFI: Median of Fluorescence Intensity.



Supplementary Figure 5. High fat diet impact on AT and peritoneal CD206^{+} macrophages.

A) Flow cytometry representative gating strategy to select macrophages from the AT and peritoneal lavage;

B) Frequency of TNF-α and IL-6 positive CD206^{+} macrophages from the AT of mice fed a chow normal diet (ND) or a high fat diet (HFD);

C) Frequency of TNF-α and IL-6 positive CD206^{+} macrophages from the peritoneal lavage of mice fed a chow normal diet (ND) or a high fat diet (HFD);

D) MFI of phospho-STAT3, phospho-S6 and phospho- AKT^{ser473} in CD206^{+} macrophages from the AT of mice fed a chow normal diet (ND) or a high fat diet (HFD);

E) MFI of phospho-STAT3, phospho-S6 and phospho- AKT^{ser473} in CD206^{+} macrophages from the peritoneal lavage of mice fed a chow normal diet (ND) or a high fat diet (HFD).

Data are represented as mean values ± s.e.m. n=3/5 per group. Unpaired two-tailed Student's t test (B-E), *p<0.05. Data are representative of three experiments. MFI: Median of Fluorescence Intensity.