Supporting Information

Priceman et al. 10.1073/pnas.1311557110

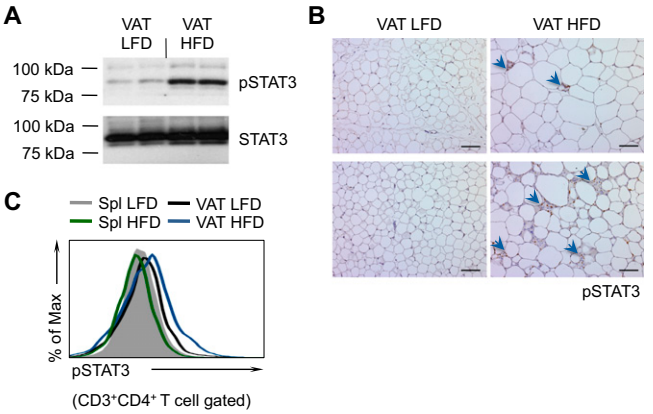

Fig. S1. Signal transducer and activator of transcription 3 (Stat3) is activated in visceral adipose tissue (VAT) and VAT-resident T cells of mice with diet-induced obesity (DIO). (*A*) Western blots of phosphoTyr^{705}-STAT3 (pSTAT3) and total STAT3 in VAT of two representative mice on a low-fat diet (LFD) or a high-fat diet (HFD) for 16 wk. (*B*) Immunohistochemistry of pSTAT3 in VAT of mice on a LFD or a HFD for 16 wk (*Upper*, females; *Lower*, males). pSTAT3 is in brown; hematoxylin is in blue. (Scale bars: 100 μm.) (*C*) Flow cytometry of pSTAT3 in CD3$^+$CD4$^+$ T cells from spleen (LFD, gray; HFD, green) and VAT (LFD, black; HFD, blue) of mice on a LFD or a HFD for 16 wk. Spl, spleen.

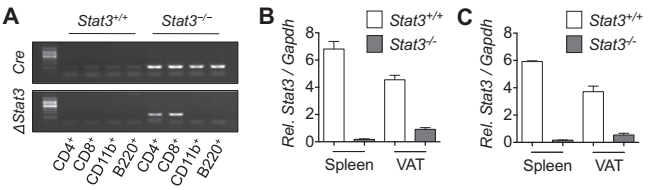

Fig. S2. Stat3 ablation in T cells. (*A*) PCR analysis of Cre and Cre-induced deletion of Stat3 (ΔStat3) of genomic DNA isolated from FACS-sorted immune subsets from *Stat3*$^{+/+}$ and *Stat3*$^{-/-}$ mice. (*B* and *C*) qRT-PCR analysis of *Stat3* mRNA expression from splenic and VAT-associated CD8$^+$ (*B*) and CD4$^+$ (*C*) T cells. Data are pooled from $n = 3$–4 per group.

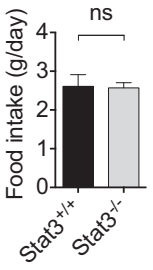

Fig. S3. Food intake of DIO mice. Food intake was monitored over the course of 1 wk in *Stat3*$^{+/+}$ and *Stat3*$^{-/-}$ mice on a HFD. $n = 2$ cages per group of $n = 3$–4 mice per cage. ns, not significant.

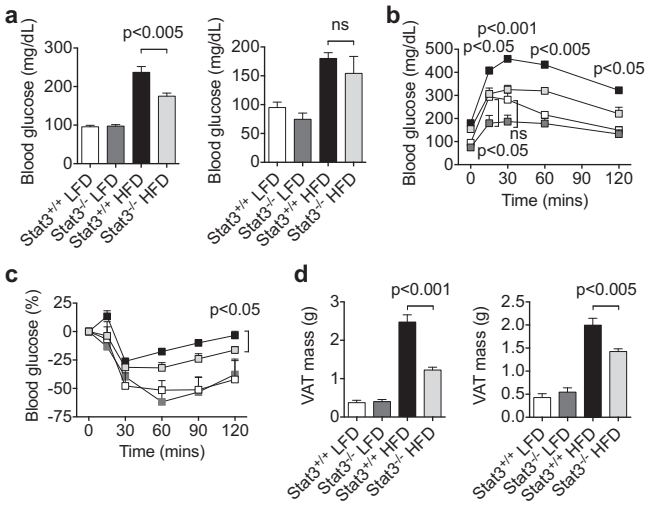

Fig. S4. Stat3 in T cells promotes insulin resistance in DIO mice. (*A*) Fasting blood glucose measurements in male mice after 6 wk (*Left*) and 12 wk (*Right*) on a LFD or HFD. $n = 4$ per group. (*B*) Glucose tolerance test (GTT) performed on male mice after 12 wk on a LFD or HFD. $n = 3$–4 per group. (*C*) Insulin tolerance test (ITT) performed on mice after 16 wk on a LFD or HFD. $n = 4$ per group. (*D*) VAT mass measured in *Stat3*$^{+/+}$ and *Stat3*$^{-/-}$ mice after 8 wk (*Left*) and 16 wk (*Right*) on a LFD or HFD. $n = 4$–8 per group. AUC, area under the curve.

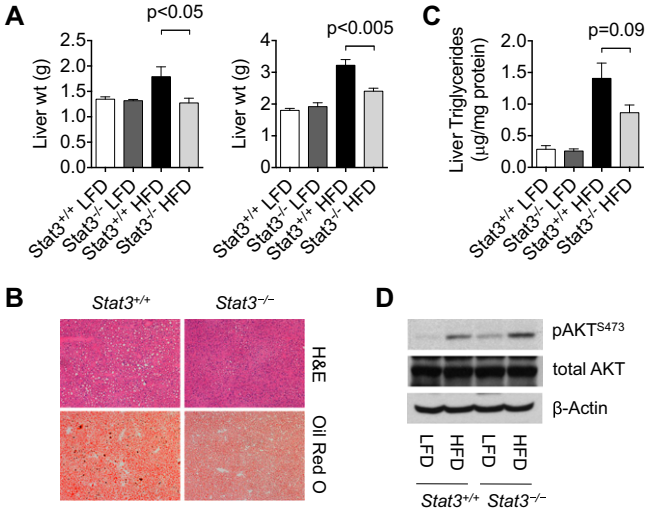

Fig. S5. Effects of Stat3 ablation in T cells on fatty liver. (*A*) Liver weights of female (*Left*; $n = 12$–14 per group) and male (*Right*; $n = 4$ per group) *Stat3*$^{+/+}$ and *Stat3*$^{-/-}$ mice on a HFD for 16 wk. (*B*) Livers from mice after 16 wk on a HFD were analyzed by H&E staining (*Upper*) and Oil Red O staining (*Lower*). (*C*) Liver triglyceride content in liver homogenates from mice after 16 wk on a HFD. $n = 4$ per group (*D*) Western blots of pAKT, total AKT, and β-actin in liver lysates from mice after 16 wk on a HFD. Representative data from $n = 4$ per group are shown.

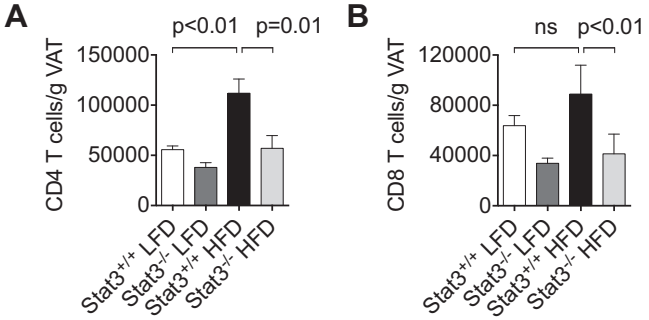

Fig. S6. Stat3 regulates VAT-associated T cells in DIO mice. CD4^{+} (*A*) and CD8^{+} (*B*) T-cell numbers in VAT of *Stat3*$^{+/+}$ and *Stat3*$^{-/-}$ mice after 16 wk on a LFD or HFD. $n = 4$ per group.

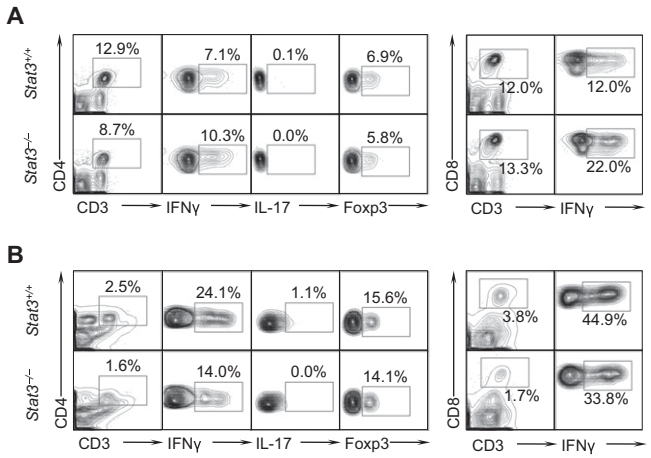

Fig. S7. Stat3 regulates VAT-associated T cells. Flow cytometry analysis of $CD4^+$ and $CD8^+$ T cells and cytokine production in spleens (*A*) and VAT (*B*) of $Stat3^{+/+}$ and $Stat3^{-/-}$ mice on a LFD for 16 wk. Representative images of $n = 4$ per group from at least three independent studies are shown.

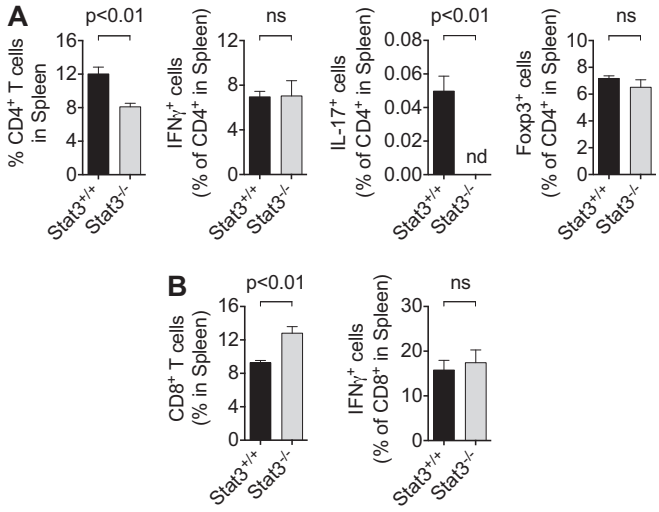

Fig. S8. Effects of Stat3 ablation on splenic T-cell subsets in DIO mice. Flow cytometry analysis of splenic $CD4^+$ (*A*) and $CD8^+$ (*B*) T-cell subsets in $Stat3^{+/+}$ and $Stat3^{-/-}$ mice after 16 wk on a HFD. nd, not detectable. $n = 4$ per group, representative of at least three independent studies.

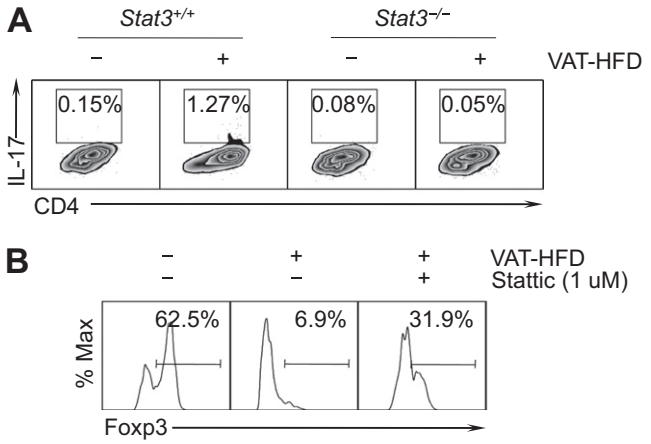

Fig. S9. Effects of Stat3 inhibition on Th subset polarization. (*A*) In vitro induced regulatory T cell polarization of naive splenic $CD4^+$ T cells from $Stat3^{+/+}$ and $Stat3^{-/-}$ mice with or without VAT- conditioned media (VAT-HFD) from HFD-fed mice for 3 d and analyzed by flow cytometry. (*B*) Under similar polarizing conditions, Stattic (1 μM) was added for 3 d, after which splenic $CD4^+$ T cells were analyzed by flow cytometry. All data are representative of at least two independent studies.

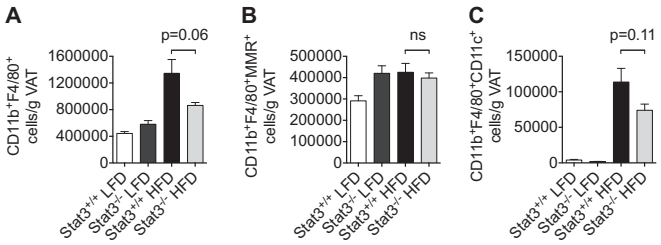

Fig. S10. Stat3 in T cells regulates adipose tissue macrophages (ATMs) in DIO mice. Total numbers of ATM (*A*), M2 (*B*), and M1 (*C*) cells in VAT of *Stat3*$^{+/+}$ and *Stat3*$^{-/-}$ mice after 16 wk on a LFD or HFD. $n = 4$ per group.