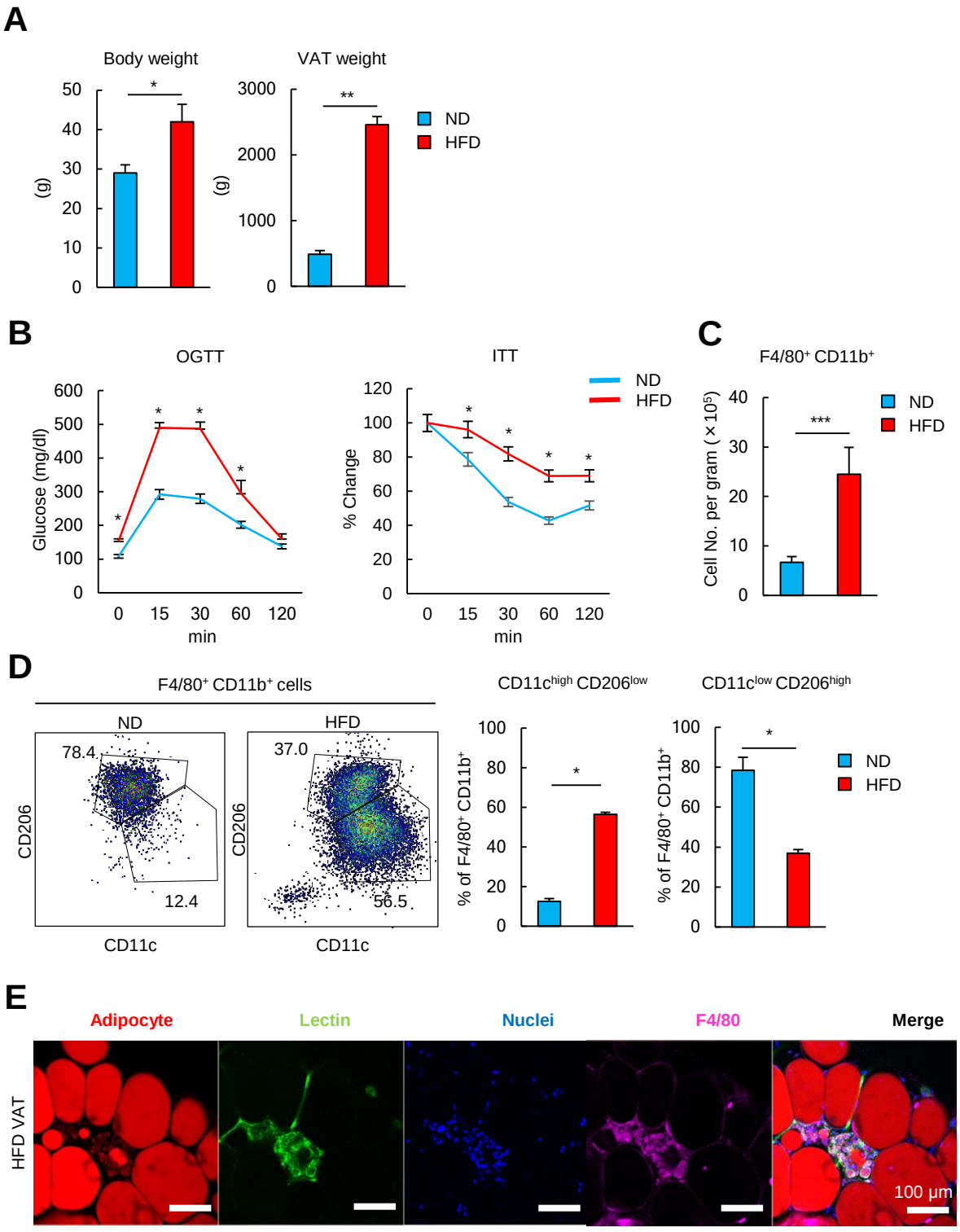
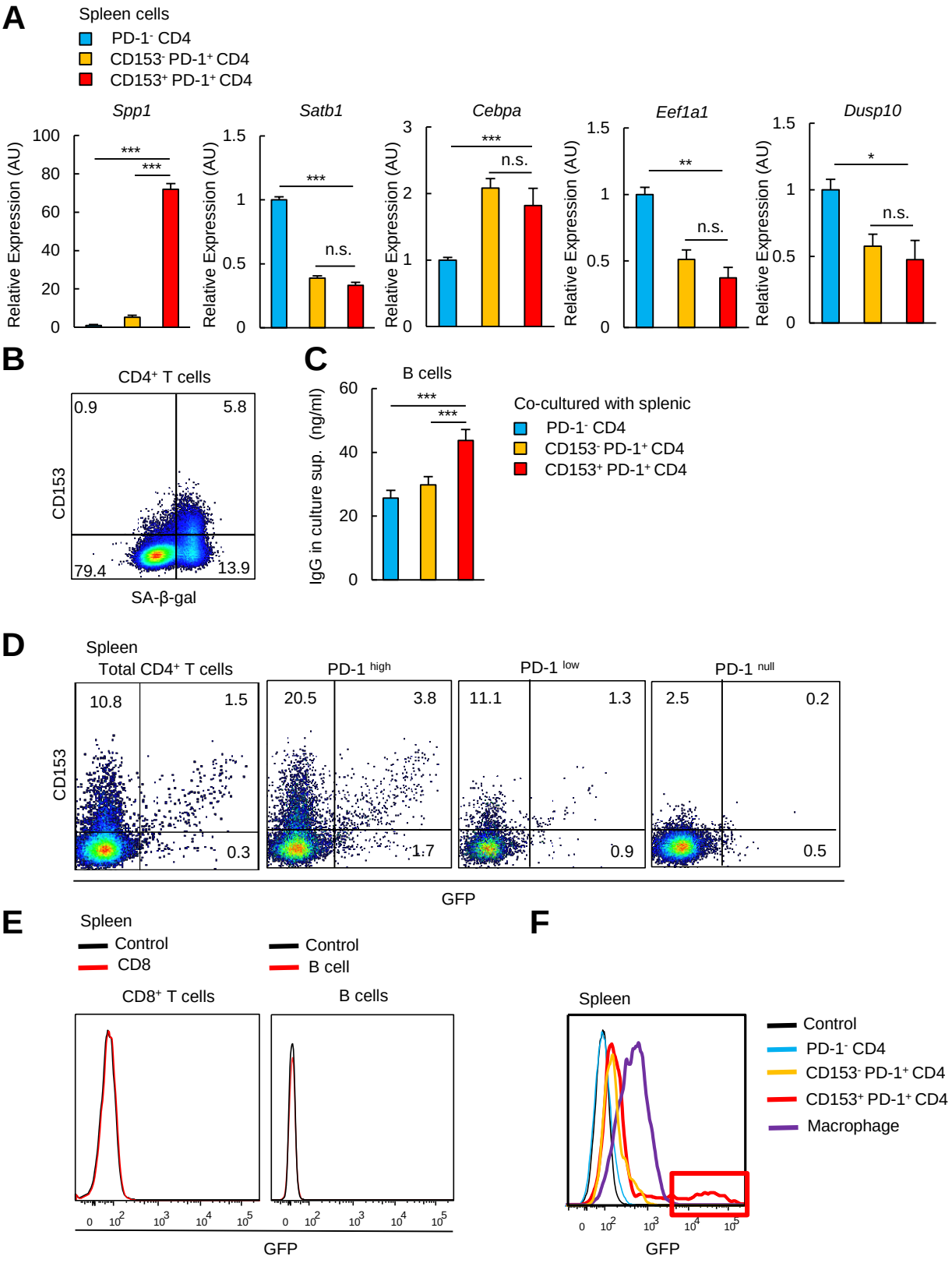
Supplementary Figure 1



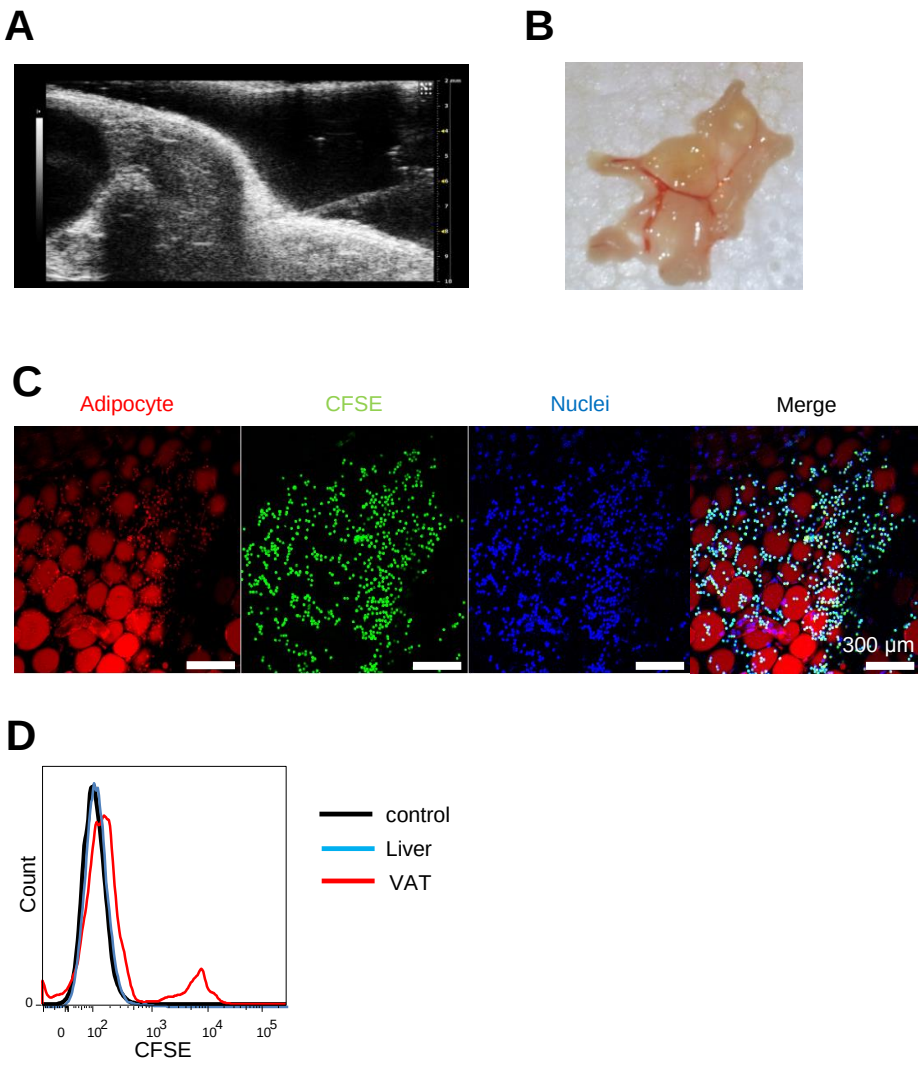
Supplementary Figure 1. High fat diet caused VAT inflammation and metabolic dysfunction.
WT mice were fed a high-fat diet (HFD) beginning at 4 weeks of age for 14 weeks. Age-matched WT mice fed a normal diet (ND) were used a control. **A.** Body and VAT weights (n = 6 mice per group). **B.** Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) (n = 6 mice per group). **C.** Quantitative analysis of macrophages (F4/80$^+$ CD11b$^+$) in VAT (n = 6 mice per group). **D.** Analysis of macrophage polarization status. CD11c^{high} CD206^{low} macrophages vs. CD11c^{low} CD206^{high} macrophages (n = 6 mice per group). **E.** Crown-like structures in VAT. Adipocytes (BODIPY, red), Lectin (yellow), Nuclei (DAPI, blue) and F4/80 (pink). Scale bars, 100 μm. Graphs show mean ± SEM; *P < 0.05; **P < 0.001; ***P < 0.0001, by two-tailed Student's *t* test.

Supplementary Figure 2



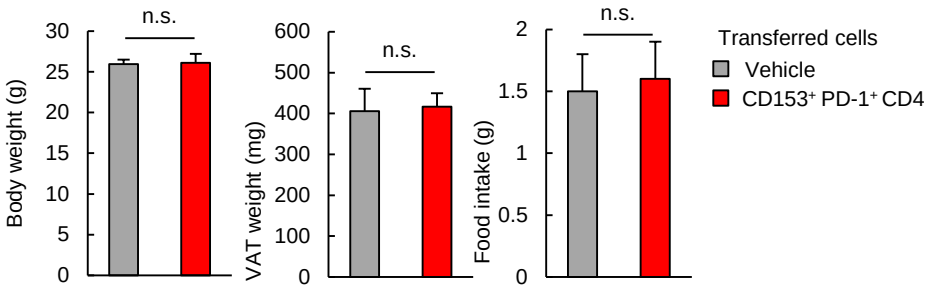
Supplementary Figure 2. Characterization of splenic CD153⁺ PD-1⁺ CD4⁺ T cells of HFD-fed obese mice.
PD-1⁻, CD153⁻ PD-1⁺, and CD153⁺ PD-1⁺ CD4⁺ T cells were separately isolated from spleen from WT mice fed a HFD. **A.** *Spp1*, *Satb1*, *Cebpa*, *Eef1a1*, and *Dusp10* expression were analyzed by real-time PCR analysis (n = 5 mice per group). **B.** A representative flow cytometric analysis demonstrated SA-β-gal activity of PD-1⁺ and PD-1⁻ CD4⁺ T cells (n = 3 mice per group). **C.** Cells were activated with anti-CD3/CD28 mAb in the presence of B cells from WT mice fed 18-week-old HFD for 7 days. IgG levels in the supernatant were determined by ELISA (n = 5 mice per group). **D.** A representative flow cytometric analysis of CD153⁺ and EGFP-*Spp1* in PD-1^{high}, PD-1^{low}, PD-1^{null} CD4⁺ T cells obtained from spleen of EGFP-*Spp1* KI reporter mice fed a HFD. **E.** A representative flow cytometric analysis of CD153 and EGFP-*Spp1* in CD8⁺ T cells and B cells obtained from VAT of EGFP-*Spp1* KI reporter mice fed a HFD. **F.** A representative flow cytometric analysis of EGFP-*Spp1* in indicated cells obtained from VAT of EGFP-Spp1 KI reporter mice fed a HFD. Red box showed high fluorescence intensity of GFP in CD153⁺ PD-1⁺ CD4⁺ T cells. Flow cytometric plots are representative of at least three independent experiments. Graphs show mean ± SEM; Not significant (n.s.); * P < 0.05; ** P < 0.001; ***P < 0.0001, by ANOVA followed by post hoc Bonferroni tests.

Supplementary Figure 3



Supplementary Figure 3. Ultrasound imaging-guided intra-adipose injections of cells.
CFSE-labeled CD4$^+$ T cells were directly injected into VAT. **A.** Ultrasound guidance allowed us to visualize the injection needle's path. **B.** VAT of recipient mice transplanted with CFSE-labeled CD4$^+$ T cells. **C.** Engraftment of injected CFSE-labeled CD4$^+$ T cells in VAT. **D.** Flow cytometric analysis of CFSE$^+$ cells within VAT and liver of recipient mice.

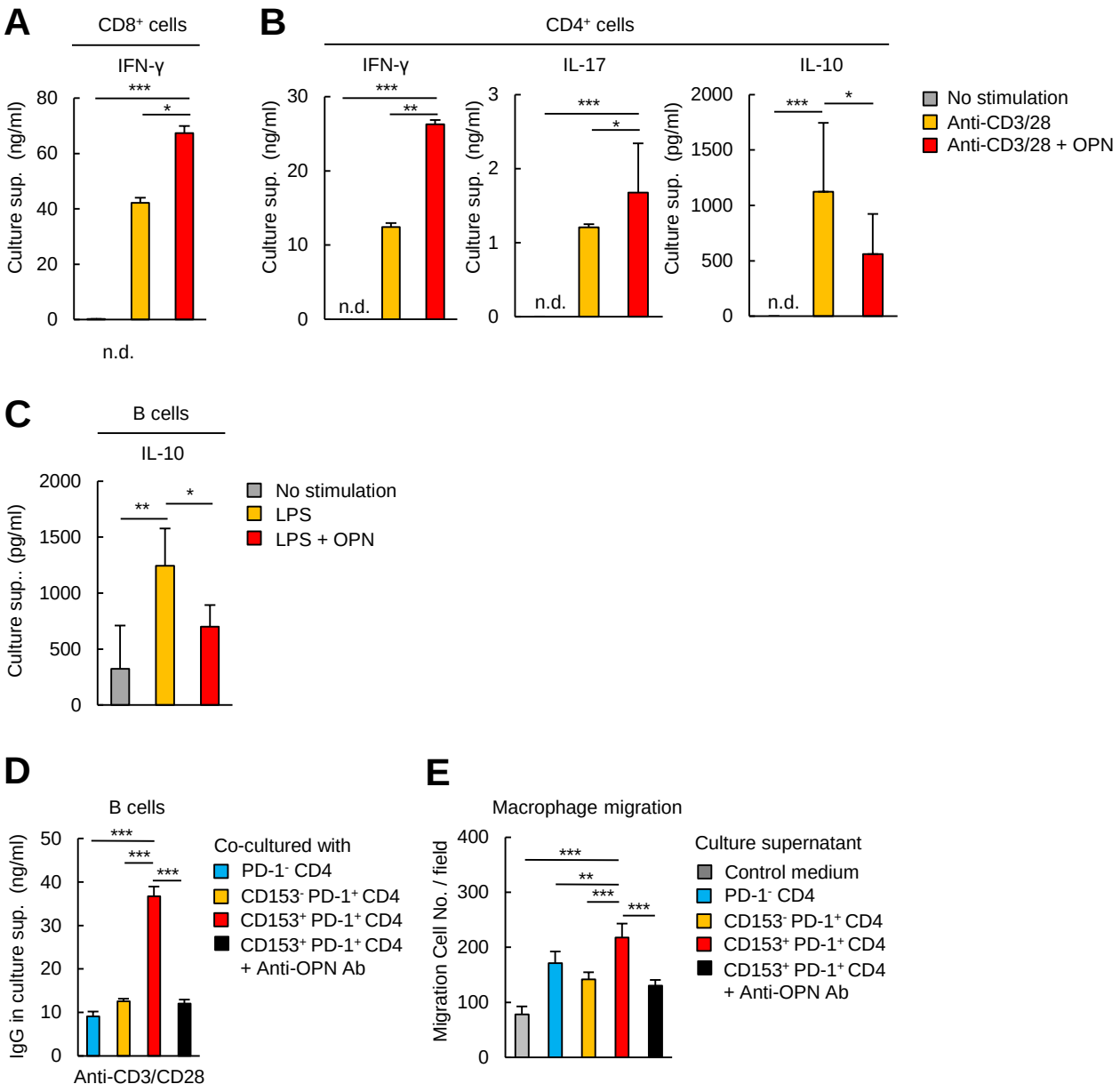
Supplementary Figure 4



Supplementary Figure 4. Adoptive transfer of CD153$^+$ PD-1$^+$ CD4$^+$ T cells induces VAT inflammation and insulin resistance in lean mice on a normal diet.

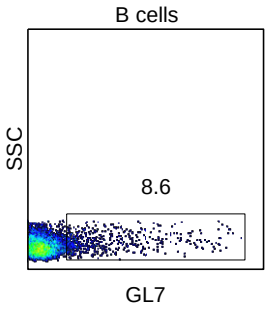
Indicated cells were separately isolated from spleen from mice fed a HFD. Next, 1×10^5 cells were transferred directly into VAT of individual recipient ND-fed lean mice 3 times per week for 2 weeks. ND-fed lean mice receiving vehicle (thermoreversible gelation polymer) were used as control group. **A.** Comparison of body weight, VAT weight, and food intake between mice transferred control regent or CD153$^+$ PD-1$^+$ CD4$^+$ T cells (n = 5 mice per group). Graphs show mean ± SEM; Not significant (n.s.), by two-tailed Student's *t* test.

Supplementary Figure 5



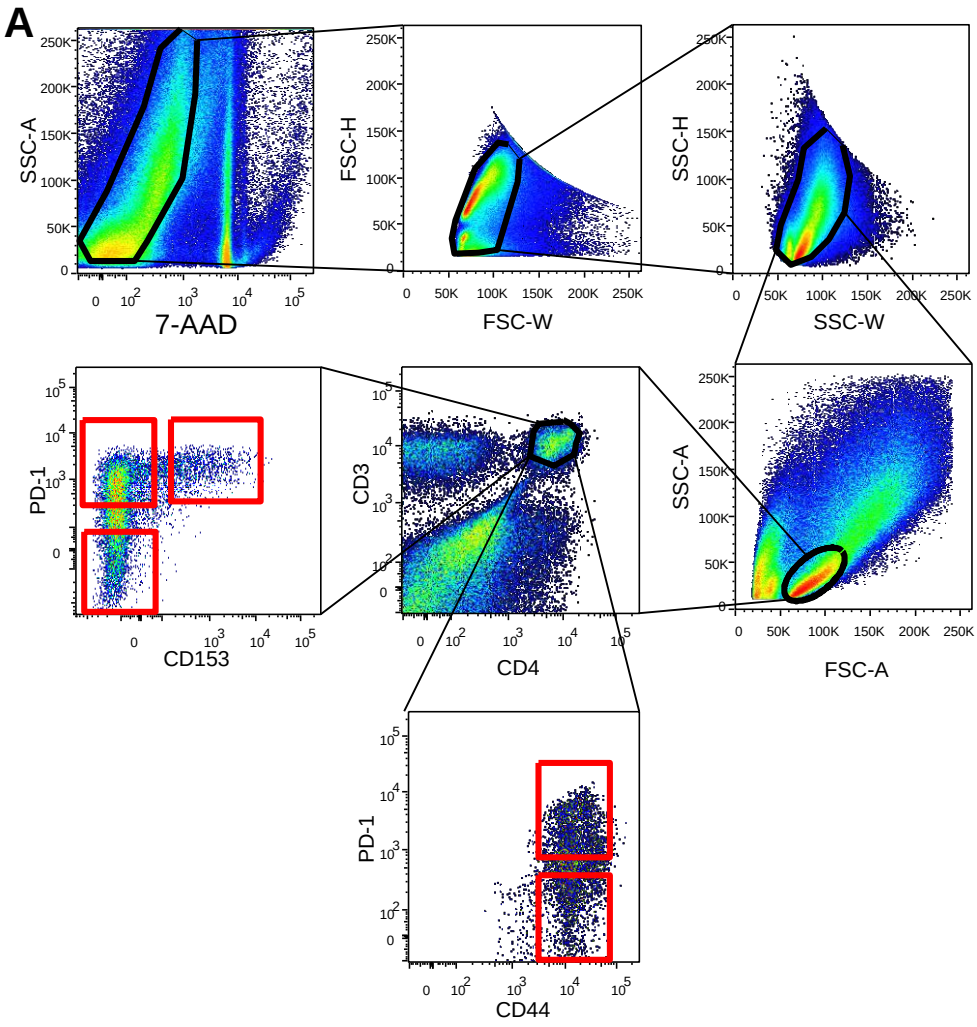
Supplementary Figure 5. The effect of OPN on immune response.
A. CD8⁺ T cells were cultured in the presence of coated anti-CD3/CD28 mAb in the presence or absence of recombinant OPN for 3 days. IFN-γ in the culture supernatants was assessed by ELISA (n = 5 mice per group). **B.** CD4⁺ T cells were cultured with coated anti-CD3/CD28 mAb in the presence or absence of recombinant OPN for 3 days. IFN-γ, IL-17, and IL-10 in the culture supernatants were assessed by ELISA (n = 5 mice per group). **C.** B cells from VAT were isolated from WT mice fed a HFD and cultured for 2 days with LPS in the presence or absence of recombinant OPN (n = 5 mice per group). IL-10 in the culture supernatants was assessed by ELISA (n = 5 mice per group). PD-1⁻, CD153⁻ PD-1⁺, and CD153⁺ PD-1⁺ CD4⁺ T cells were separately isolated from VAT of WT mice fed a HFD. **D**. Cells were activated with anti-CD3/CD28 mAb with an anti-OPN antibody or control IgG in the presence of VAT B cells from HFD-fed mice for 10 days. IgG levels in the supernatant were determined by ELISA (n = 3 mice per group). **E**. Comparative analyses for macrophage migration assay. Isolated peritoneal macrophages were plated in Boyden chambers and treated with conditioned medium of stimulated CD4⁺ T cells along with anti-OPN antibody or control IgG (n = 5 in each group). Graphs show mean ± SEM; not detected (n.d.), *P < 0.05; **P < 0.001; ***P < 0.0001, by ANOVA followed by post hoc Bonferroni tests.

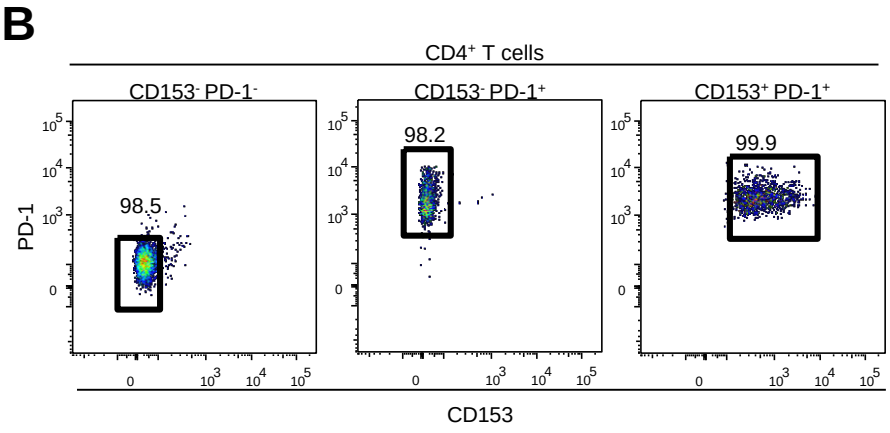
Supplementary Figure 6



Supplementary Figure 6. Obese VAT contained a significant proportion of B cells expressing GL7.
WT or μMT mice were fed either a normal diet (ND) or high-fat diet (HFD) for 14 weeks. **A.** A representative flow cytometric analysis demonstrated the expression of GL7 in adipose B cells. Flow cytometric plots are representative of at least three independent experiments.

Supplementary Figure 7





Supplementary Figure 7. Gating strategy for VAT PD-1⁺ CD4⁺ T cells and CD153⁺ PD-1⁺ CD4⁺ T cells.
A. Gating strategy for VAT PD-1⁺ CD4⁺ CD44^{high} T cells and CD153⁺ PD-1⁺ CD4⁺ T cells. **B.** Purity of sorted PD-1⁻, CD153⁻ PD-1⁺, and CD153⁺ PD-1⁺ CD4⁺ T cells.