

Supporting Information

Carrero et al. 10.1073/pnas.1713543114

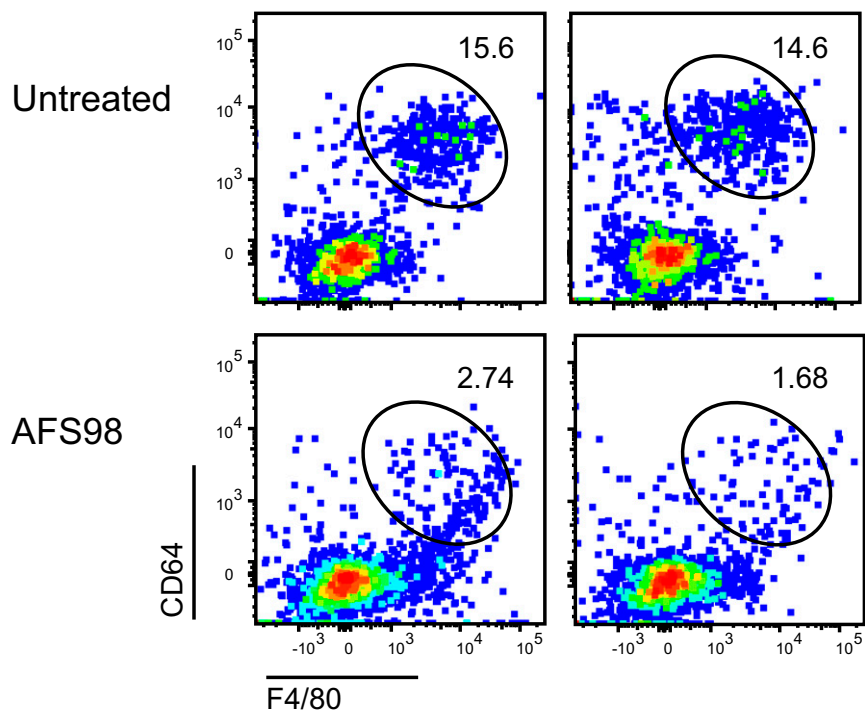


Fig. S1. Pancreatic stromal macrophages are reduced following AFS98 treatment. B6 mice were treated as in Fig. 1 and analyzed for the presence of CD45⁺F4/80⁺CD64⁺ macrophages. Numbers indicate the percentage of macrophages in the pancreatic stroma as a percent of the CD45⁺ cells. Cells were gated on FSC/SSC/CD45⁺. Data are taken from two independent mice per group and is representative of two independent experiments.

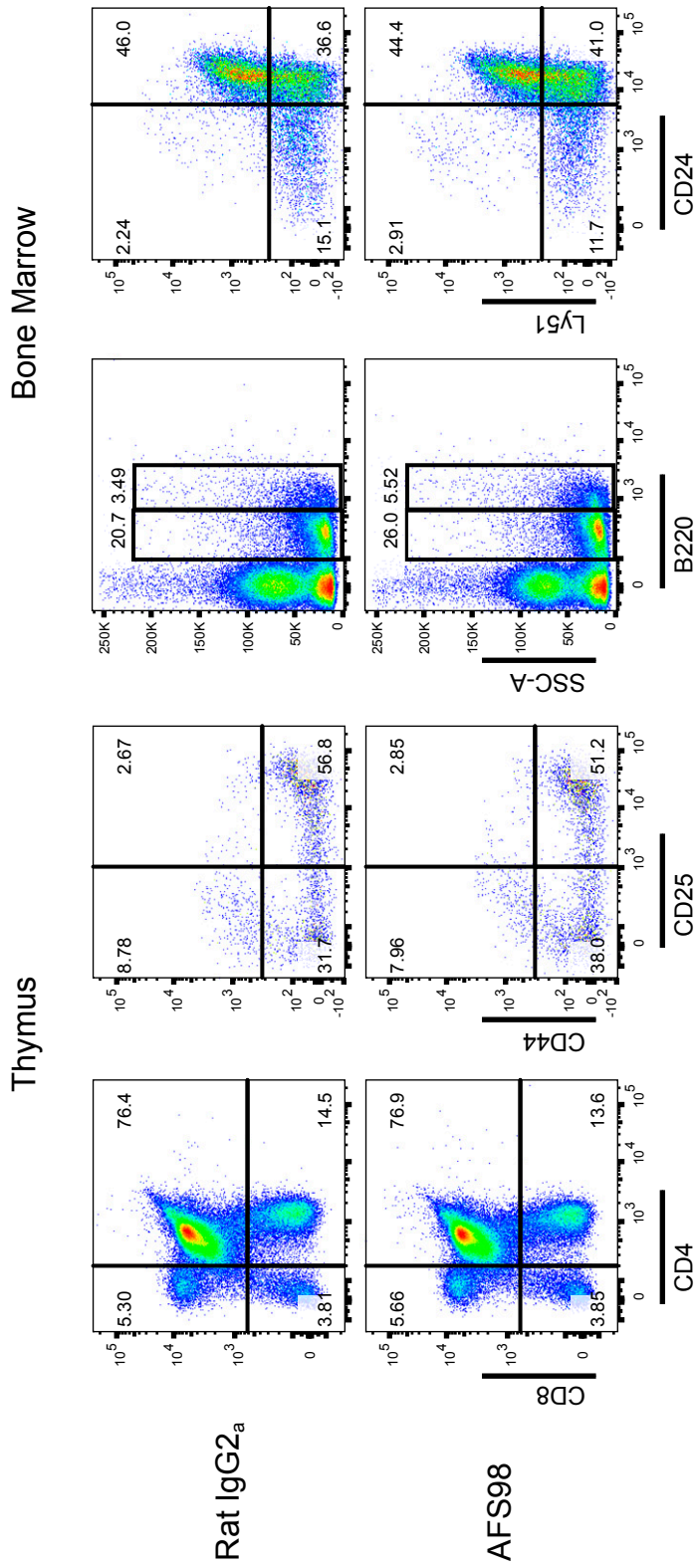


Fig. S2. Thymic T cells and bone marrow B cells are grossly normal following AFS98 treatment. Male NOD mice were injected with 2 mg of either AFS98 or Rat IgG_{2a} at 4 wk of age and then sampled at 6 wk of age. Thymus and bone marrow were harvested, and single-cell suspensions were generated. Cells were evaluated by flow cytometry. For thymus, cells were first gated on forward and side scatter, and CD4⁺ then CD4⁺CD25⁺ then CD4⁺CD25⁻ was plotted. Next, the CD4/CD8 double negative population was gated and examined for CD44 and CD25 as plotted on the *Right*. For bone marrow, cells were gated by forward and side scatter, and then plotted for B220 expression. The B220 intermediate cells were then gated and plotted for CD24 and Ly51 staining as shown on the *Right*. Numbers indicate the percentage of cells in each region as a function of the total cells in the plot. Results are representative of three individual mice per group.

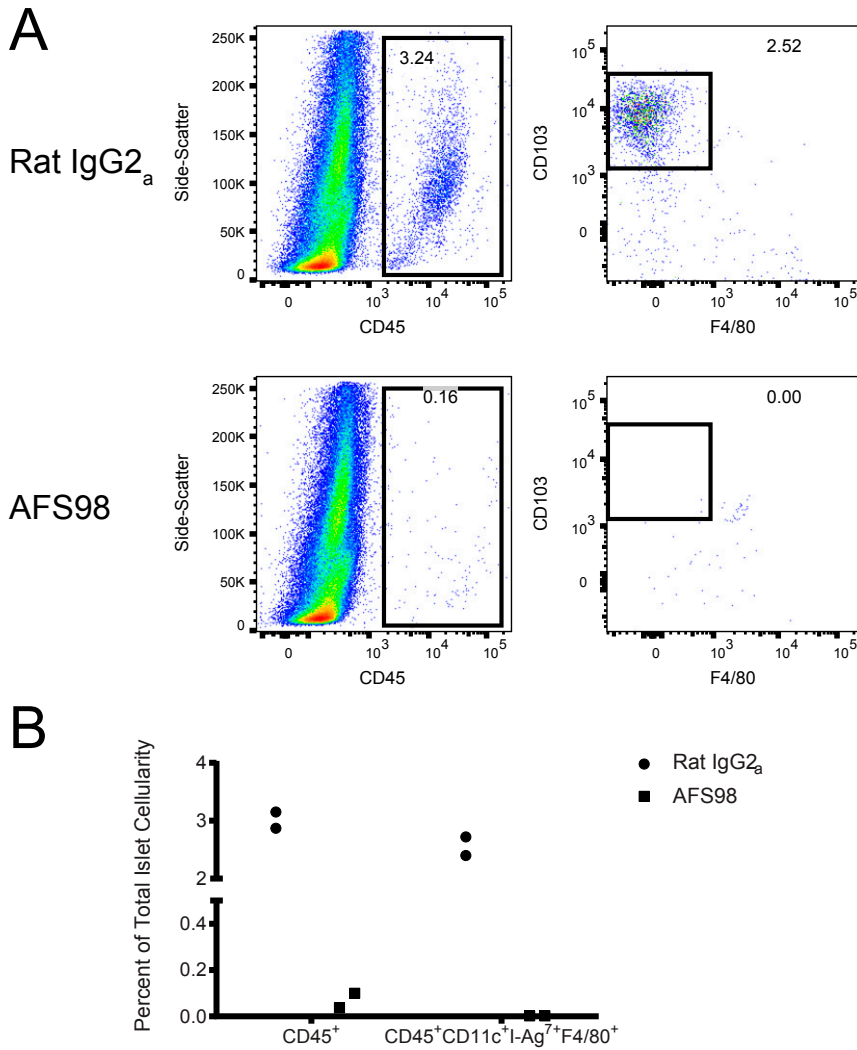


Fig. 53. AFS98 depletes islet macrophages in the NOD.*Rag1*^{-/-} mouse. Female NOD.*Rag1*^{-/-} mice were treated at 4 wk of age with 2 mg of either rat IgG_{2a} or ASF98 and then sampled at 6 wk of age. (A and B) Islet leukocytes cells were analyzed by flow cytometry. Cells were gated by forward and side scatter and then plotted for CD45 (Left). Next, cells were gated on CD45⁺CD11c⁺I-Ag⁷⁺, and the F4/80 by CD103 cells were plotted (Right). Values represent the percent of each box as a function of total islet cellularity. (B) Scatter plots of the percent of CD45⁺ or F4/80⁺CD45⁺CD11c⁺I-Ag⁷⁺ in two individual mice per group.

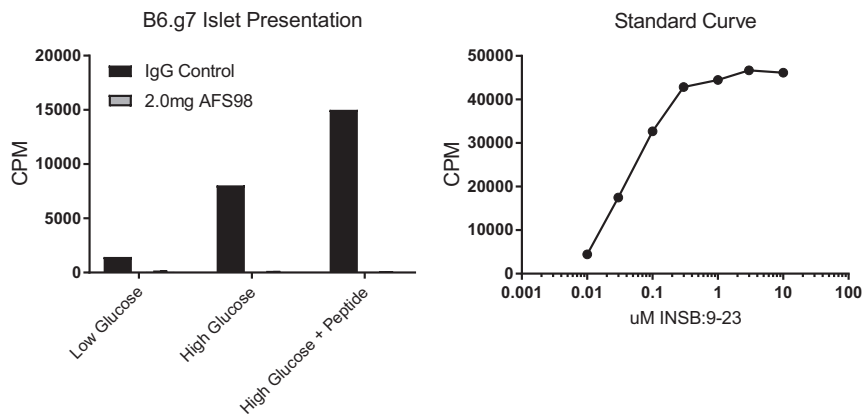


Fig. 54. Depletion of macrophages blocks MHC-II antigen presentation by whole islet cells. Antigen presentation was performed as in Fig. 3B except with 6-wk-old B6.g7 mice that were given 2 mg of either control or AFS98 antibody at 4 wk of age. Left shows the CTL2-2 assay for an anti-insulin CD4 T cell hybridoma. Right shows the peptide curve of the hybridoma using an immortalized cell line as APC.