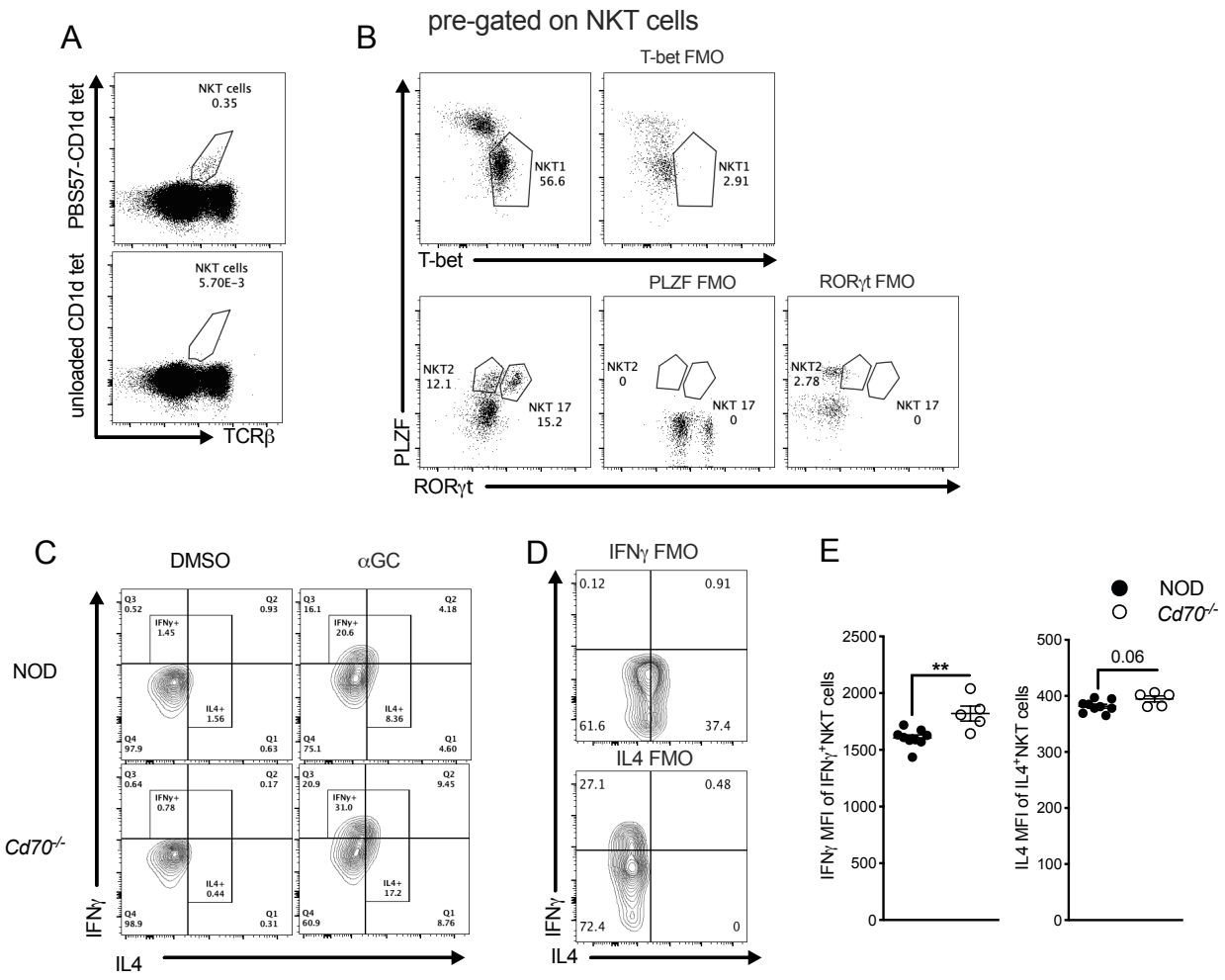
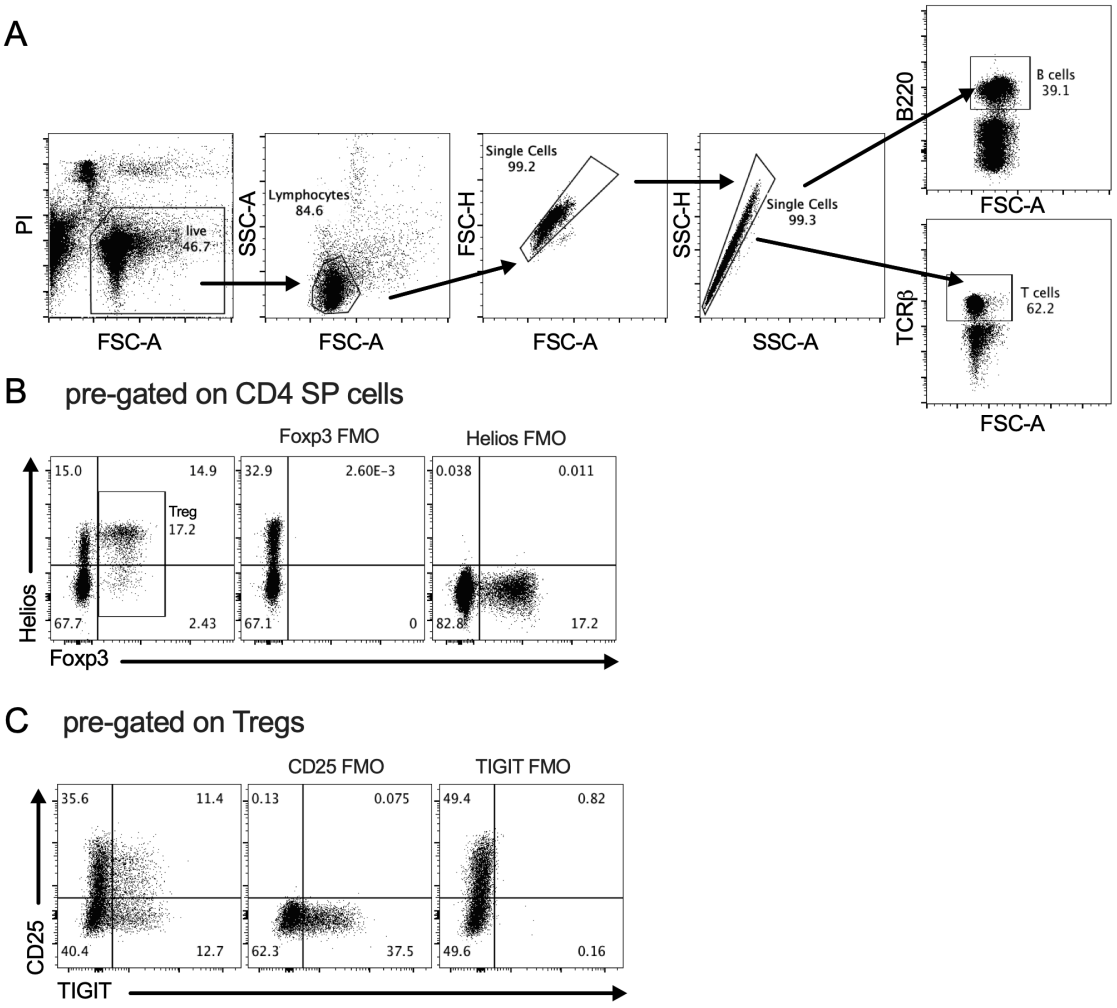
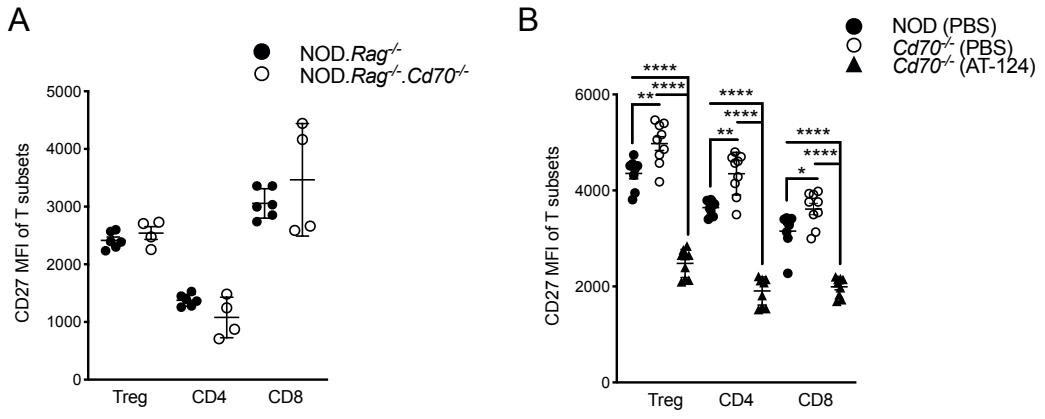


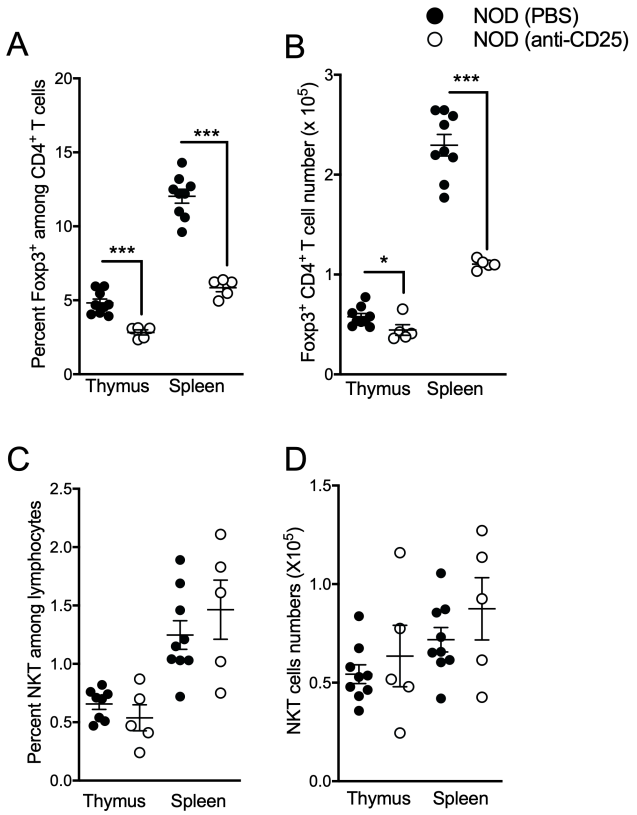
Supplemental Figure 1. Methods to identify and characterize iNKT cells. (**A**) Representative flow cytometry plots showing the gating strategy to detect iNKT cells. Splenocytes were stained with PBS57-loaded (top) and unloaded (bottom) mouse CD1d tetramer and anti-TCRβ Ab. (**B**) Fluorescence minus one (FMO) staining controls for T-bet, PLZF, and RORγt in CD1d tetramer^{+} TCRβ^{+} iNKT cells. Thymocytes were membrane labeled with CD1d tetramer and an Ab against TCRβ, fixed and permeabilized, and intracellularly stained with different combinations of T-bet, PLZF, and RORγt. (**C** & **D**) Gating strategy to measure cytokine production by α-GalCer activated iNKT cells. Splenocytes from 7-to 8-week old NOD and *Cd70$^{-/-}$* mice injected i.v. 2 h previously with 4 μg per mouse of α-GalCer or DMSO were surface stained with CD1d tetramer, anti-CD4, and anti-TCRβ then intracellularly stained for IFNγ and IL4 following fixation and permeabilization. (**C**) Representative FACS plots showing IFNγ and IL4 staining in iNKT cells. (**D)** FMO staining for IFNγ and IL4 in iNKT cells. (**E**) The mean fluorescence of intracellular IFNγ and IL4 Ab staining in NOD and *Cd70$^{-/-}$* iNKT cells. Data represent results from two independent experiments analyzed by Mann-Whitney test; error bars correspond to mean ± SEM. ***P < 0.01.* Actual P valves are provided when 0.1>P-value>0.05.



Supplemental Figure 2. Gating strategies to distinguish T cells, B cells, and Treg subsets. (**A**) Representative gating strategy to distinguish αβ T cells and B cells. αβ T cells and B cells were identified by sequentially gating on live propidium iodide (PI) negative cells, the lymphocyte singlet population, and then TCRβ$^+$ or B220$^+$ cells. FSC, forward scatter; SSC, side scatter; A, signal area; H, signal height. (**B** & **C**) Representative fluorescence minus one (FMO) staining to identify Foxp3, Helios, CD25, and TIGIT positive Tregs. Splenocytes were membrane labeled with Abs against CD4 and different combinations of TIGIT and CD25, fixed and permeabilized, and intracellularly stained with different combinations of Foxp3 and Helios. (**B**) FMO staining for Foxp3 and Helios in single positive (SP) CD4$^+$ splenocytes. (**C**) FMO staining for CD25 and TIGIT on the surface of Foxp3$^+$CD4$^+$ splenocytes.



Supplemental Figure 3. (**A**) Mean fluorescent intensity of Ab staining of CD27 on the surface of Tregs, $CD4^+$ T cells, and $CD8^+$ T cells resident within splenocytes of NOD.*Rag1*$^{-/-}$ and NOD. *Rag1*$^{-/-}$.*Cd70*$^{-/-}$ mice 7 to 10 weeks after adoptive transfer with 5 x 10^6 NOD T cells. (**B**) Mean fluorescent intensity of Ab staining of CD27 on the surface of Tregs, $CD4^+$ T cells, and $CD8^+$ T cells resident within splenocytes of NOD or *Cd70*$^{-/-}$ mice injected i.p. with 50μg of CD27 agonist Ab (AT-124) or PBS twice a week for 4 weeks. Data were analyzed by Mann-Whitney test; error bars correspond to mean ± SEM. **P < 0.05*, ***P < 0.01*, *****P < 0.0001.*



Supplemental Figure 4. Administration of anti-CD25 Ab reduces Treg frequency and number but has no effect on iNKT cells number. Three- to four-week old NOD mice were i.p. injected with 250 μg of anti-CD25 Ab (clone PC61) or PBS twice a week for 3 weeks after which their thymi and spleens were analyzed for Tregs and iNKT cells. The frequency (**A**) and number (**B**) of thymic and splenic Foxp3$^+$ CD4$^+$ T cells. The frequency (**C**) and number (**D**) of iNKT cells. Statistical differences were analyzed by the Mann-Whitney test; error bars correspond to mean ± SEM. *$P < 0.05$, ***$P < 0.001$.