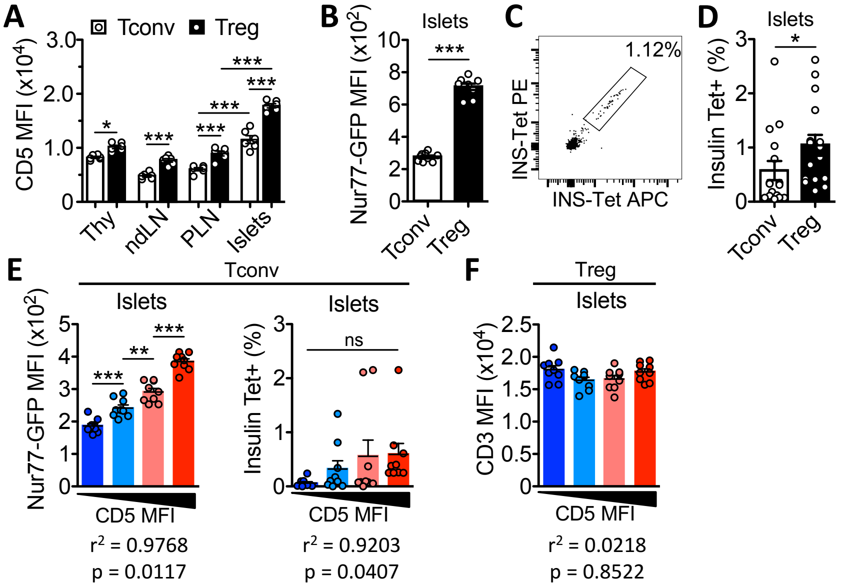
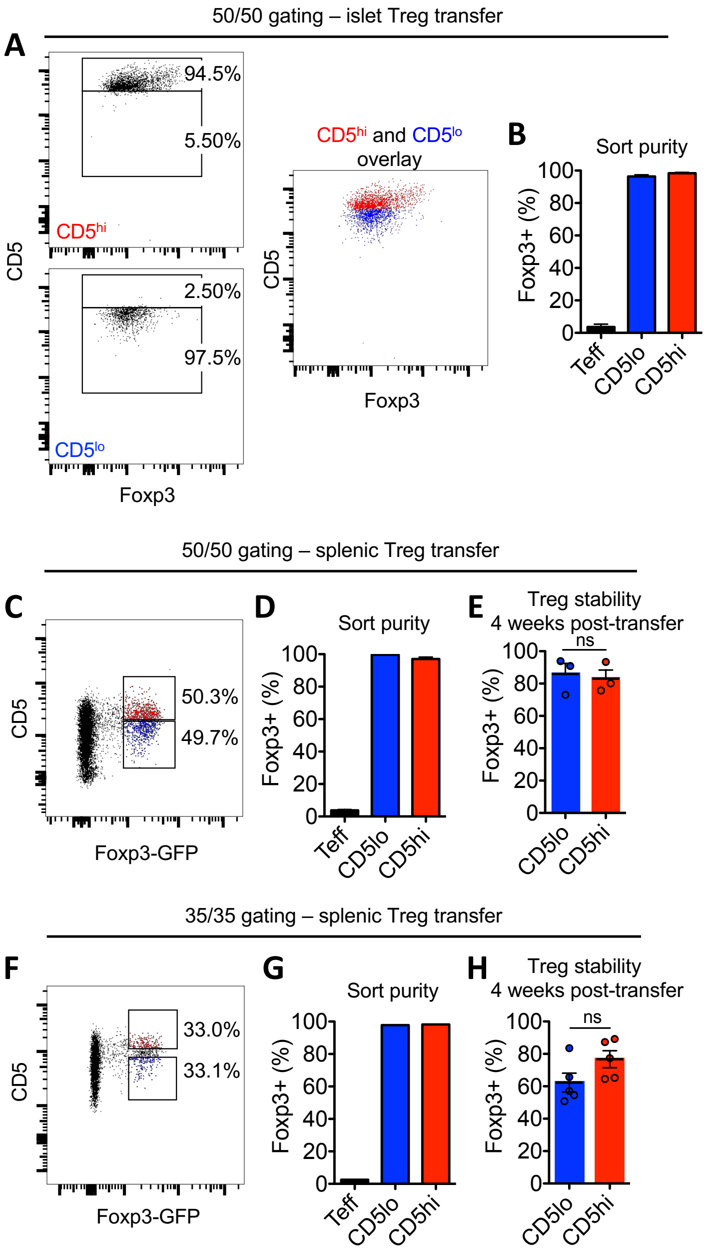
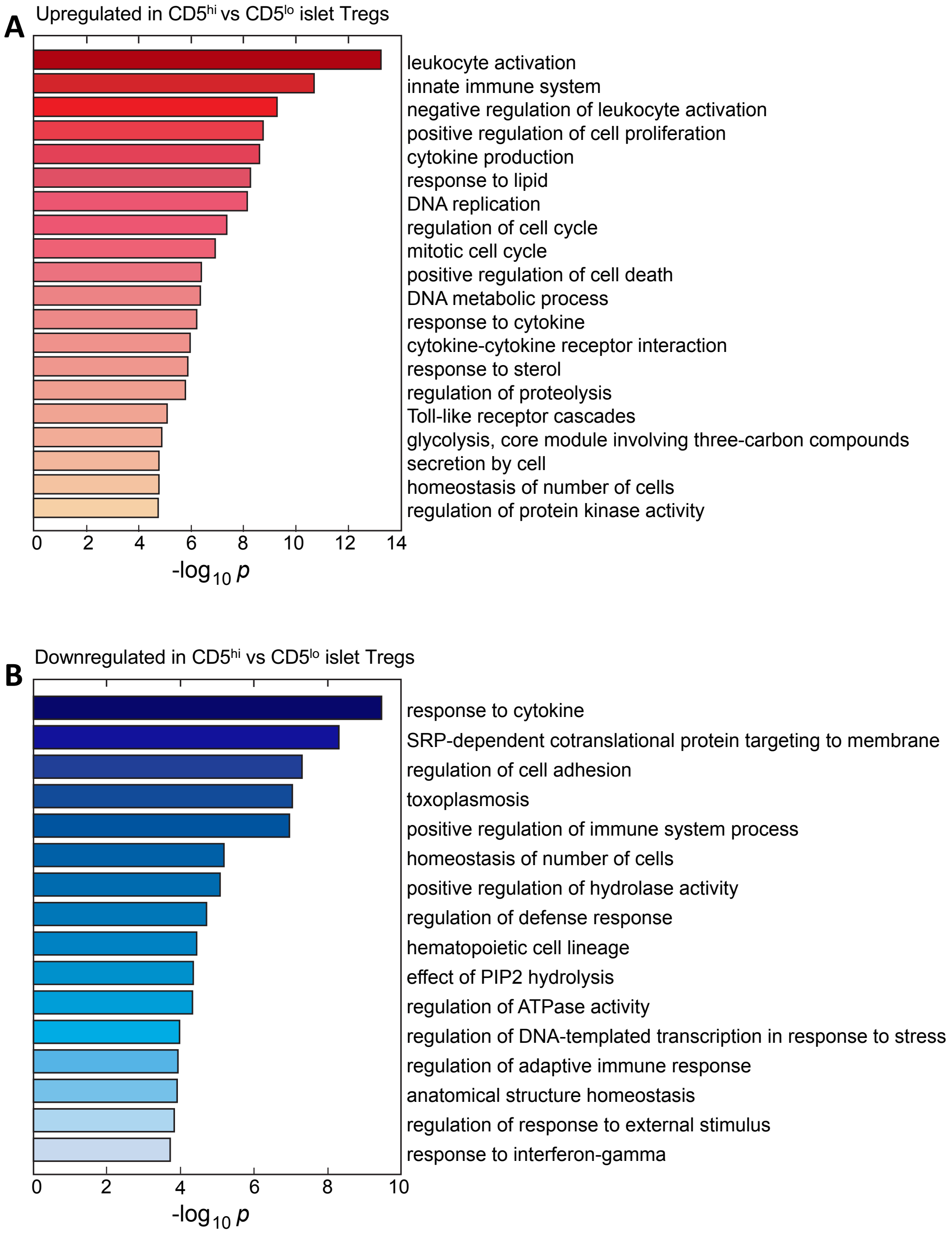


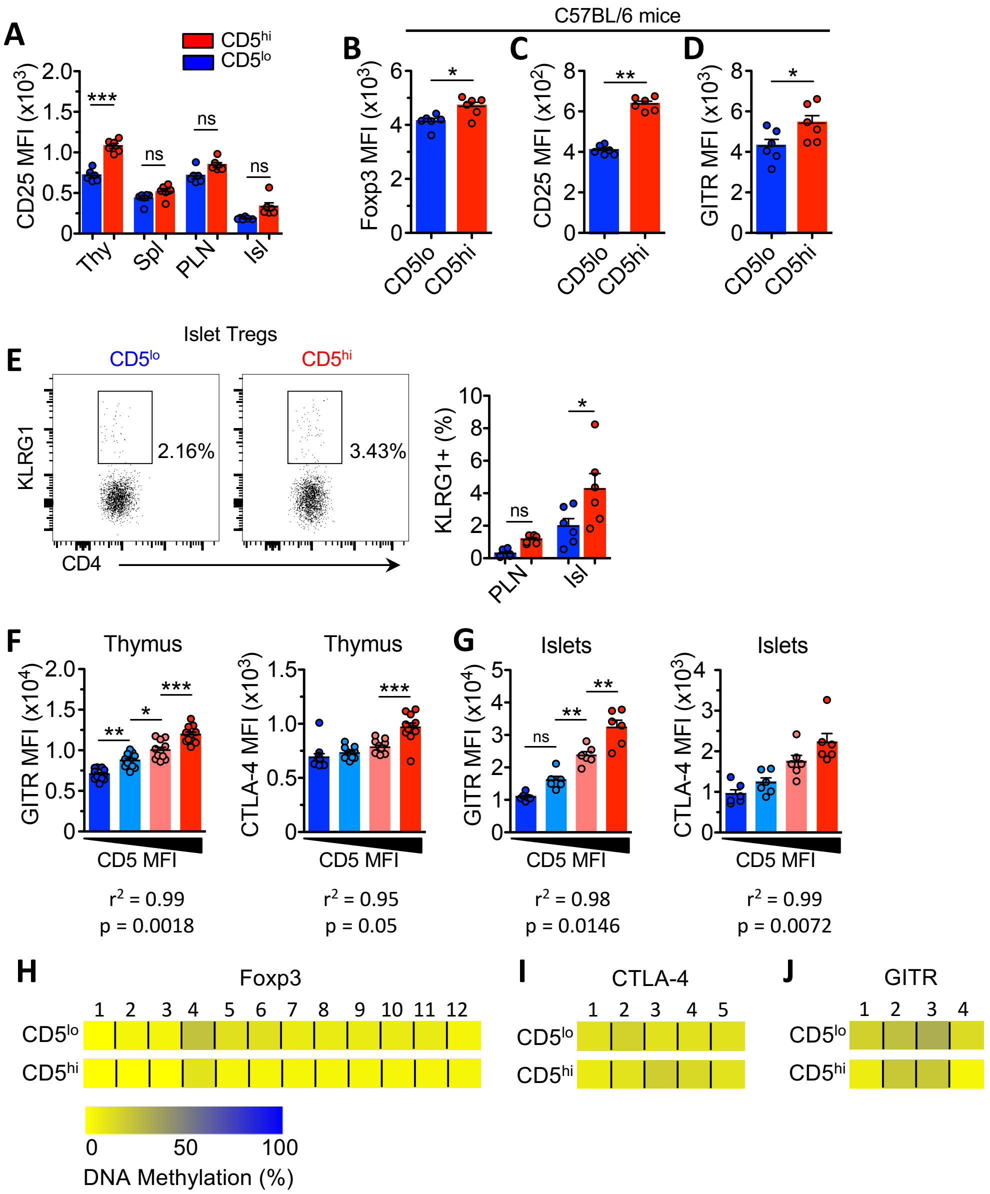
Supplemental Figure 1. CD5 correlates with self-reactivity, Related to Figure 1. (**A**) Analysis of 10-18 week old NOD female mice. CD5 expression on Tconv (CD4+CD3+Foxp3−) and Tregs (CD4+CD3+Foxp3+) in the thymus (Thy), non-draining lymph nodes (ndLN), pancreatic LN (PLN), and pancreatic islets (Islets). An average of 6 mice from two experiments is shown. (**B-F**) Analysis of 7-12 week old NOD.Nur77^{GFP} female mice. (**B**) GFP reporter of Nur77 expression in Tconv (CD4+CD3+Foxp3−) compared to Tregs (CD4+CD3+Foxp3+) in the islets. An average of 9 mice from one experiment is shown. (**C**) Representative flow plot of InsB:9-23 tetramer positive cells; analysis is gated on CD4+CD3+Foxp3+ cells. (**D**) Frequency of tetramer+ T cells in Tconv (Foxp3−) and Treg (Foxp3+) CD4+ T cells. An average of 16 mice from two experiments is shown. (**E**) Correlation between CD5 expression and GFP reporter of Nur77 expression or InsB:9-23 tetramer staining of islet-infiltrating Tconv. An average of 9 mice from one experiment is shown. (**F**) Level of CD3 expression on islet-infiltrating Tregs (CD4+CD3+Foxp3+) among the four CD5 quartiles shown in Figure 1A. Significance was determined by one-way analysis of variance (ANOVA) with Bonferroni's multiple-comparisons test (**A, E, F**), Pearson correlation (**E, F**) and Mann-Whitney *U*-test (**B, D**), The mean ± SEM is shown. ns, not significant (p>0.05), *p<0.05, **p<0.005, ***p<0.0005.



Supplemental Figure 2. Post-sort analysis of Tregs sorted from the infiltrated islets and spleen, Related to Figures 1 and 2. (**A**) Representative gating strategy of islet CD5$^{\text{hi}}$ and CD5$^{\text{lo}}$ Tregs post-sort. (**B**) Purity of CD5$^{\text{hi}}$ and CD5$^{\text{lo}}$ Tregs post-sort based on expression of Foxp3 (Gated on: CD4+CD3+Foxp3+). Data are pooled from three independent experiments. (**C**) Sort gating for 50/50 CD5$^{\text{hi}}$ and CD5$^{\text{lo}}$ splenic Treg transfers. (**D**) Purity of Tconv (Foxp3−) and Tregs (Foxp3+) post-sort. Data are pooled from two independent experiments. (**E**) Congenically marked splenic Tnaive (Foxp3$^{\text{GFP}}$−CD45RB$^{\text{hi}}$CD45.2+) and Tregs (Foxp3$^{\text{GFP}}$+CD45.1+) were transferred to NOD.*scid* recipients. Stability of Foxp3+ population in the spleen was assessed 4-weeks post transfer based on intracellular Foxp3 staining after gating on CD45.1 positive T cells. (**F**) Sort gating strategy for 35/35 CD5$^{\text{hi}}$ and CD5$^{\text{lo}}$ splenic Treg transfers. (**G**) Purity of Tconv (Foxp3−) and Tregs (Foxp3+) post-sort. Data are from one representative experiment. The mean ± SEM is shown. (**H**) Congenically marked splenic Tnaive (Foxp3$^{\text{GFP}}$−CD45RB$^{\text{hi}}$CD45.2+) and Tregs (Foxp3$^{\text{GFP}}$+CD45.1+) were transferred to NOD.*scid* recipients. Stability of Foxp3+ population in the spleen was assessed 4-weeks post transfer based on intracellular Foxp3 staining after gating on CD45.1 positive T cells. Significance was determined by Mann-Whitney *U*-test (**E, H**).



Supplemental Figure 3. Transcriptional landscape of CD5^{hi} and CD5^{lo} Tregs, Related to Figure 3. (**A**) Pathways upregulated in CD5^{hi} over CD5^{lo} islet Tregs. (**B**) Pathways down regulated in CD5^{hi} over CD5^{lo} islet Tregs.



Supplemental Figure 4. Analysis of the Treg functional phenotype, Related to Figure 4. (**A**) Quantification of CD25 expressed by CD5^{hi} or CD5^{lo} Tregs from the thymus (thy), spleen (Spl), pancreatic LN (PLN), and islets of 10-18wk old female NOD mice. Gating strategy in Figure 4A. An average of 6 mice from two experiments is shown. (**B-D**) Analysis of Tregs from spleens of C57BL/6 mice. (**B**) Foxp3, (**C**) CD25, and (**D**) GITR expression by CD4+CD3+Foxp3+ Tregs. An average of 6 mice from one experiment is shown. (**E**) Representative flow plots and combined analysis of KLRG1 expression on Tregs in PLN and islets of 10-18wk old NOD mice. An average of 6 mice from 2 experiments is shown. (**F**) Correlation between GITR, CTLA-4 and CD5 in the thymus. An average of 11 mice from three experiments is shown. (**G**) Correlation between GITR, CTLA-4 and CD5 in the islets. An average of 6 mice from two experiments is shown. (**H-J**) Bisulfite sequencing analysis of DNA isolated from CD5^{hi} and CD5^{lo} splenic Tregs sorted from five male NOD mice. (**H**) *Foxp3* intron 1 (**I**) *Ctla4* exon 2 and (**J**) *Tnfrsf18* exon 5. Significance was determined by one-way analysis of variance (ANOVA) with Bonferroni's multiple-comparisons test (**A, E, F, G**), Mann-Whitney *U*-test (**B-D**) and Pearson correlation (**F, G**). The mean ± SEM is shown. ns, not significant ($p>0.05$), *$p<0.05$, **$p<0.005$, ***$p<0.0005$.