

Supplemental Figure legends

Supplemental Figure 1. Purity of sorted populations of FcRL3⁺ T_{reg}, FcRL3⁻ T_{reg} and T_{conv}

A. Pre-FACS-sorted sample showing CD25 and CD127 expression on live CD3⁺CD4⁺ T cells (left panel), then CD25 and FcRL3 expression on the CD25^{hi}CD127^{lo} cells with either anti-FcRL3 antibody or control stain. *B.* Post-FACS-sorted FcRL3⁺ T_{reg}, FcRL3⁻ T_{reg} and T_{conv} populations showing CD25 and CD27 expression (upper panel), and CD25 and FcRL3 expression (lower panel). *C.* Unsorted samples were analyzed for FoxP3 expression, based on gates set to equivalent levels of CD25, CD127 and FcRL3 that were obtained in the sorted populations.

Supplemental Figure 2. Variability in T_{reg} FcRL3 expression is not associated with variability in FoxP3 expression

FcRL3 and FoxP3 expression were analyzed by flow cytometry as in Figure 1 in 29 healthy donors. The FcRL3 MFI, FoxP3 MFI and % FcRL3⁺ within T_{reg} were determined, together with the frequency of T_{reg} within total CD4⁺ T cells, as defined by CD25^{hi}CD127^{lo}FoxP3⁺. PBMC from the same donors were screened for the -169 C/T *FCRL3* SNP genotype by a PCR allelic discrimination assay. In (*A*) and (*B*), plots show the relationship between T_{reg} FoxP3 MFI and either T_{reg} FcRL3 MFI (*A*) or % FcRL3⁺ within T_{reg} (*B*). Statistical analysis indicates that neither parameter was significantly correlated with T_{reg} FoxP3 MFI ($P > 0.05$). r indicates the Spearman's rank correlation coefficient. In (*C*) and (*D*), donors are grouped according to genotype and shown in the category scatter plots against the T_{reg} FoxP3 MFI (*C*) or % T_{reg} in CD4⁺ T cells (*D*);

horizontal bars show the mean of each group. Differences between groups were not statistically significant as determined by unpaired Student's *t*-test ($P > 0.05$).

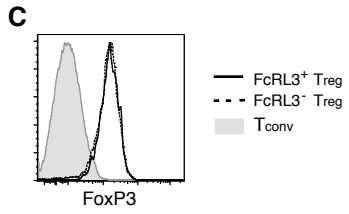
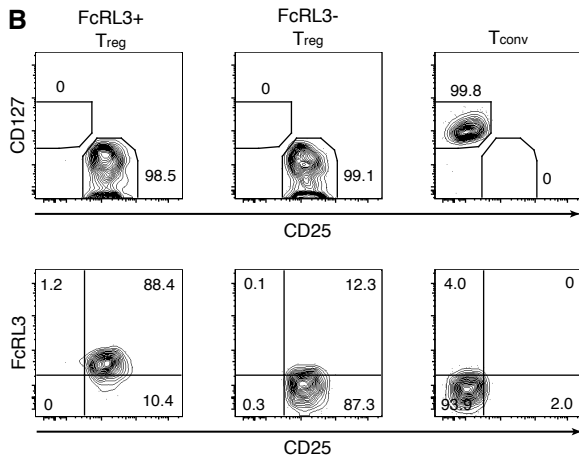
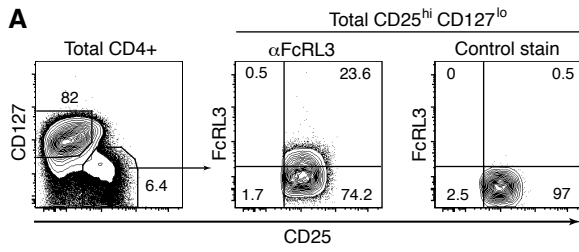
Supplemental Figure 3. FcRL3⁺ T_{reg} constitute a greater fraction of T_{reg} in the thymus than in the periphery

FcRL3 expression was analyzed by flow cytometry on fetal thymus and either mesenteric lymph node (MLN, *A*) or spleen (*B*) from paired donors. The % FcRL3⁺ within FoxP3⁺CD25^{hi} T_{reg} was determined for each tissue and donor.

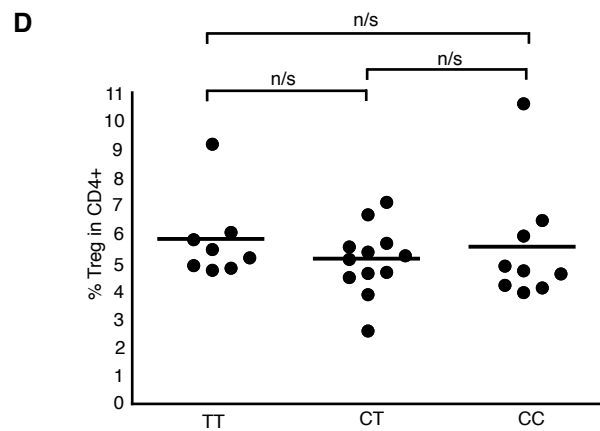
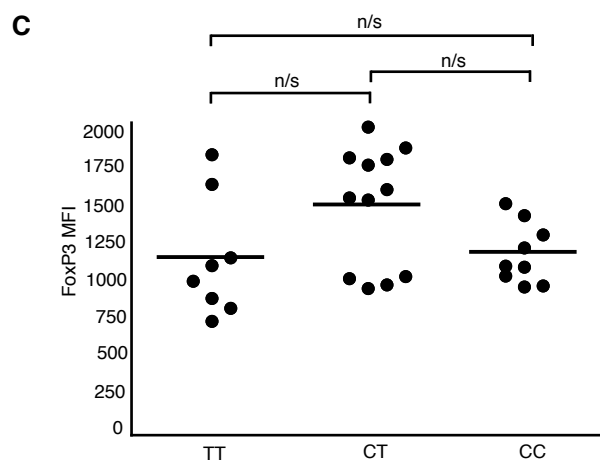
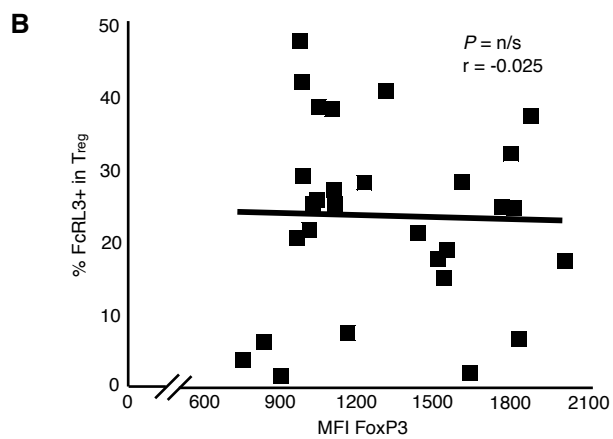
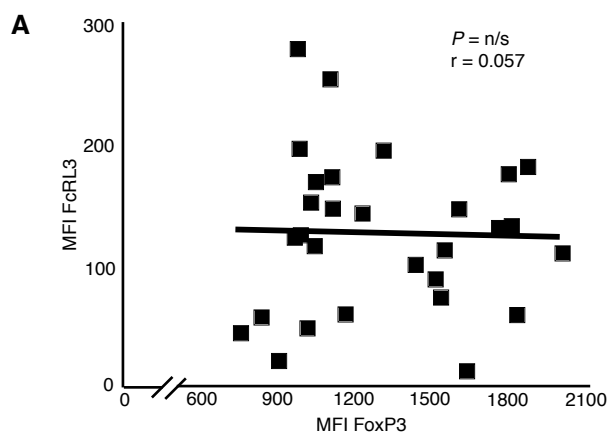
Supplemental Figure 4. FcRL3⁺ T_{reg} exhibit defective blast formation in response to αCD3/CD28 activation in the presence of IL-2

FcRL3⁺ T_{reg}, FcRL3⁻ T_{reg}, and T_{conv} were FACS sorted from adult PBMC and cultured in the presence of R10 medium alone, IL-2, αCD3/CD28 antibodies, or a combination of αCD3/CD28 antibodies and IL-2. FSC/SSC profiles of subpopulations cultured for 5 days are shown from one representative donor.

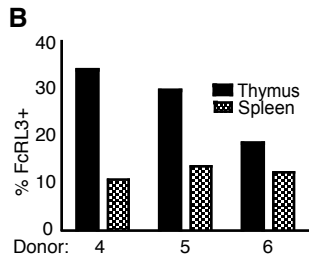
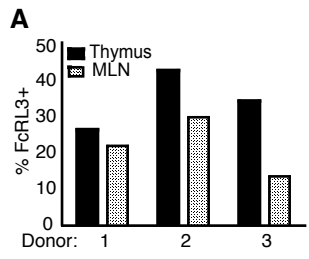
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4

