## Supplemental Methods

### Isolation of splenic Tregs

Murine spleens were harvested aseptically and used to generate a single-cell suspension. Red blood cells were lysed with ACK lysis buffer (0.15M $NH_4Cl$, 10mM $KHCO_3$, 0.1mM EDTA) prior to magnetic-activated cell sorting of $CD4^+CD25^+$ and $CD4^+CD25^-$ cells using the $CD4^+CD25^+$ Regulatory T Cell Isolation Kit (Miltenyi Biotec) protocol. Briefly, $CD4^-$ cells were magnetically labeled and the unlabeled population of $CD4^+$ cells was collected. This population was then magnetically labeled for CD25, and both $CD25^+$ and $CD25^-$ cells were isolated. $CD4^+CD25^+$ cells were sorted further using a FACSAria Cell Sorter (BD Biosciences) to collect $CD25^{high}$ cells. This resulting cell population was used immediately in experiments as naive Tregs.

### Details on Flow cytometry analysis

Tregs from *in vitro* expanded cultures and from peripheral blood cells isolated after adoptive transfer experiments in mice were characterized by flow cytometry. *In vitro* expanded cells were stained with CD4, CD25, Foxp3 markers to characterize $CD4^+CD25^+Foxp3^+$ Tregs. Peripheral blood lymphocytes from mice receiving adoptively transferred Tregs were analyzed in a similar fashion. First, live cells were gated, with CD4+ cells defined as the T-cell population. Next, Tregs were identified as live CD4+CD25+Foxp3+ cells. Live $CD4^+CD25^+Foxp3^+$ Tregs were then gated via the CD45.2 marker for exogenous Tregs and via CD45.1 marker for endogenous Tregs. Lastly, CTLA-4 expression was used to measure percent activation of exogenous $CD45.2^+$ Tregs and endogenous $CD45.1^+$ Tregs.

### Statistical Analysis

In all figures, data are presented as the mean ± standard deviation. Statistical comparisons involving two groups were performed using the two-tailed Student's t-test. Groups are assumed to have a normal distribution, using mean and standard deviation to calculate p-values. Comparisons between more than two groups at a single time point were performed using one-way Analysis of Variance (ANOVA[34]). P-values were calculated using between-group variation and within-group variation. Comparisons between two or more groups at multiple time points was calculated using repeated measures ANOVA. Similar to one-way ANOVA, repeated measures ANOVA compares variance between groups relative to within-group variation, while also comparing variance of groups over time. P-values $< 0.05$ are considered statistically significant (*), while p-values $<0.01$ are considered very statistically significant (**).