

SUPPLEMENTAL MATERIAL

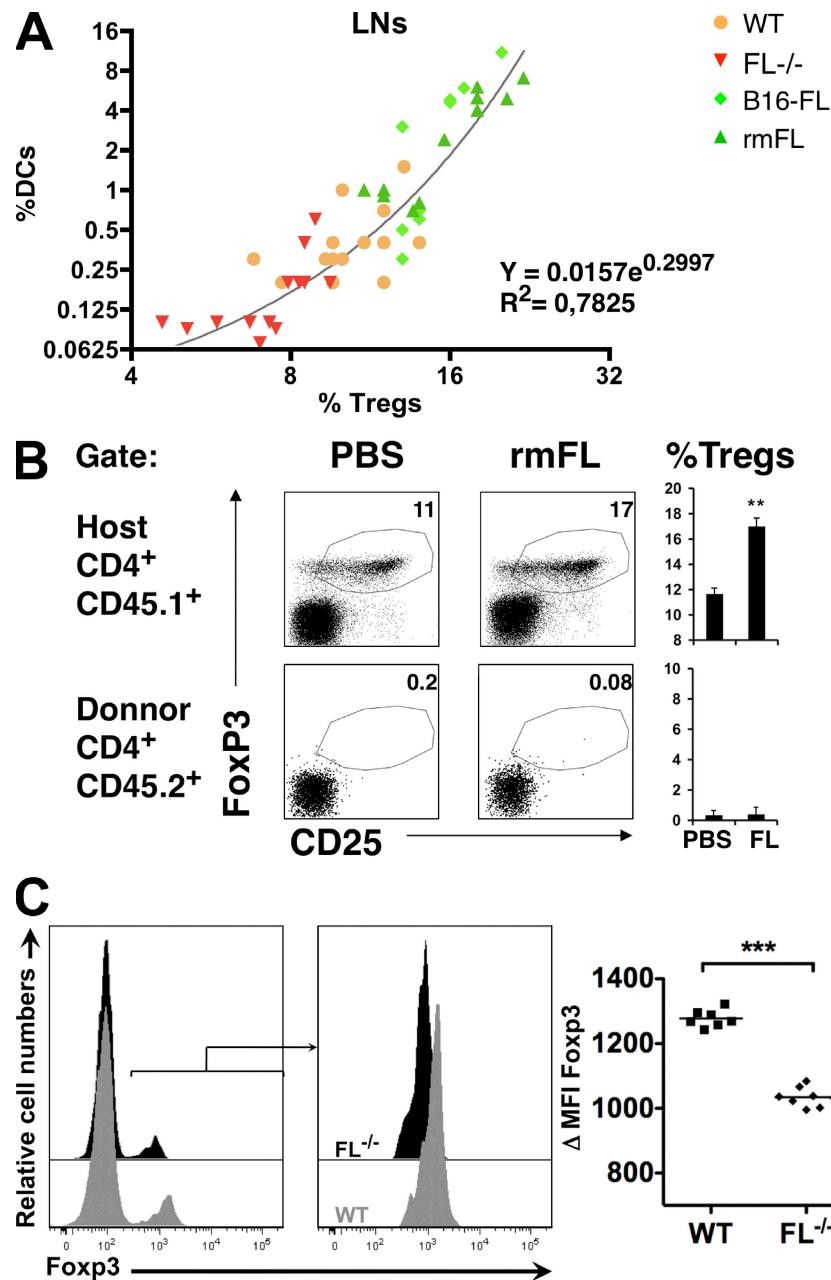
Darrasse-Jèze et al., <http://www.jem.org/cgi/content/full/jem.20090746/DC1>

Figure S1. Characterization of T reg cell homeostasis in mice overexpressing or deficient for FL. (A) Levels of DCs and T reg cells are tightly correlated in LN. Graph shows nonlinear regression analysis of percentages of conventional CD11c^{hi}MHCII⁺ DCs (cDCs; y axis) versus Foxp3⁺CD25⁺ cells among CD3⁺CD4⁺ T cells (T reg cells; x axis) in peripheral LN (mix of brachial, axillary, inguinal, popliteal, cervical pancreatic, and paraaortic LN). C57BL/6 mice were untreated (WT), injected with B16-FL tumor (B16FL), treated for 12 d with rmFL, or untreated FL^{-/-} mice ($n = 4-12$ mice in five experiments). (B) CD4⁺Foxp3⁻ T cells are not converted to Foxp3⁺CD25⁺ T reg cells by treatment with FL. 10^7 CD45.2⁺CD4⁺Foxp3^{GFP}⁻ Th cells were transferred on day 0 into CD45.1⁺ C57BL/6 mice treated with rmFL every 2 d from day -4 to day 12. Representative dot plots show CD25 and Foxp3 expression by host (top) and donor (bottom) CD4⁺ splenocytes. Histograms summarize the percentages of host and donor Foxp3⁺CD25⁺ T reg cells among CD3⁺CD4⁺ T cells in PBS- and rmFL-treated mice ($n = 3$ mice per group in two experiments). (C) Foxp3 expression is decreased in splenic T reg cells from FL^{-/-} mice. Foxp3 expression profile of CD4⁺ T cells (left) and T reg cells (middle) from FL^{-/-} (black) and WT B6 control (gray) mice. Scatter plot (right) summarizes Foxp3 intensity of expression in T reg cells as measured by the mean fluorescence index difference (Δ MFI) between T reg cells and CD4⁺Foxp3⁻ cells \pm SD. Each dot represents one mouse ($n = 7-8$ mice per group in three experiments). The horizontal bars represent means. **, $P < 0.01$; ***, $P < 0.001$.

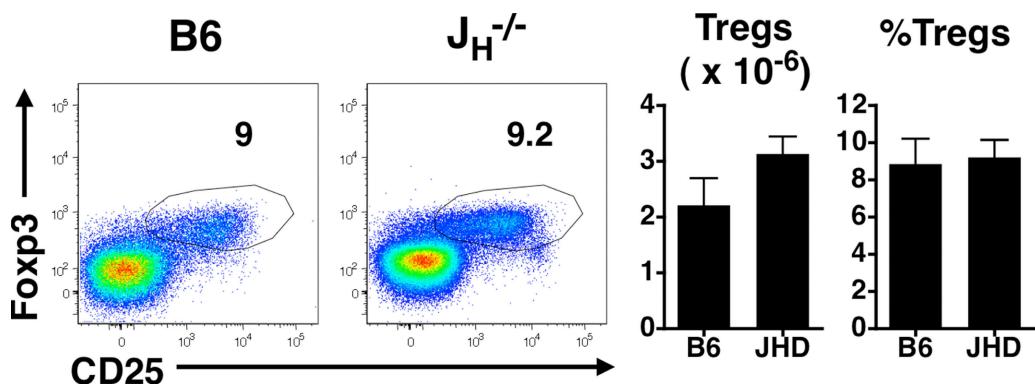


Figure S2. Normal numbers of T reg cells in the spleens of B cell–deficient mice. Dot plots (left) show CD25 versus Foxp3 staining on CD4⁺ T cell splenocytes from B6 control and $J_H^{-/-}$ (JHD) B cell–deficient mice. Histograms (right) summarize mean percentages \pm SD ($n = 4$ mice per group in two experiments).

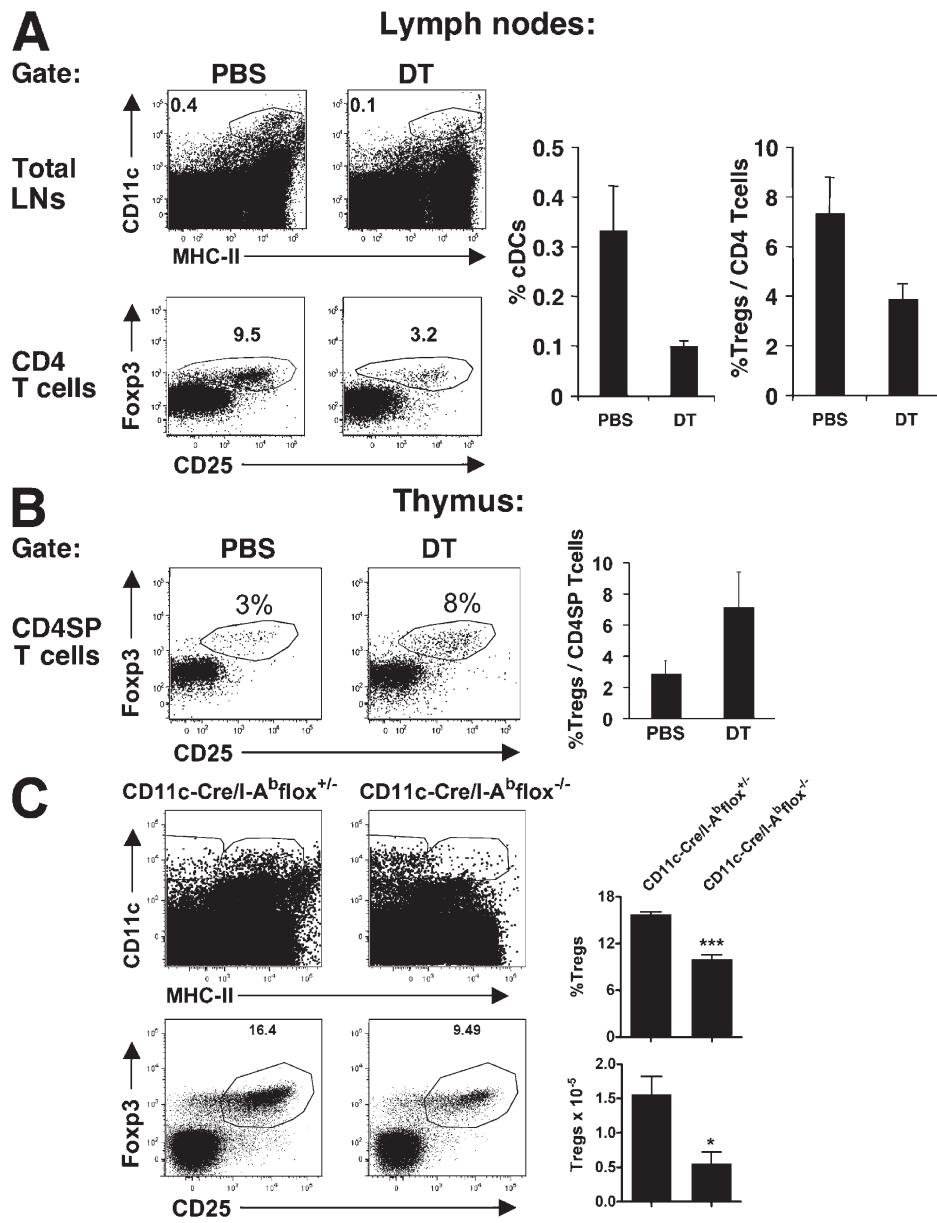


Figure S3. T reg cell homeostasis in lymphoid organs of CD11c-DTR and CD11c-Cre/IAbflox mice. (A) Dot plots show the percentages of CD-11chⁱMHCII⁺ DCs (cDCs; top) and Foxp3⁺CD25⁺ T reg cells among CD3⁺CD4⁺ T cells (bottom) in the LN of CD11cDTR→WT chimeric mice measured after 12 d of treatment with PBS or DT. Numbers in panels indicate percentages. Histograms show the corresponding means ± SD of the percentages of cDCs and T reg cells ($n = 6$ mice in one representative out of five experiments). (B) Dot plots show T reg cells among CD3⁺CD4⁺CD8⁻ single-positive cells in the thymus of CD11cDTR→WT chimeric mice treated with PBS or DT for 12 d. Histograms summarize the absolute numbers and percentages of T reg cells ± SD ($n = 3$ mice per group in four experiments). (C) MHCII expression by endogenous DCs (top) and the percentage of T reg cells (middle) in the LN of CD11c-Cre/IAbflox^{+/−} and CD11c-Cre/IAbflox^{−/−} mice. Histograms show the means ± SD of the percentages and absolute numbers of the indicated populations. One representative out of two experiments is shown. *, P < 0.05; ***, P < 0.001.

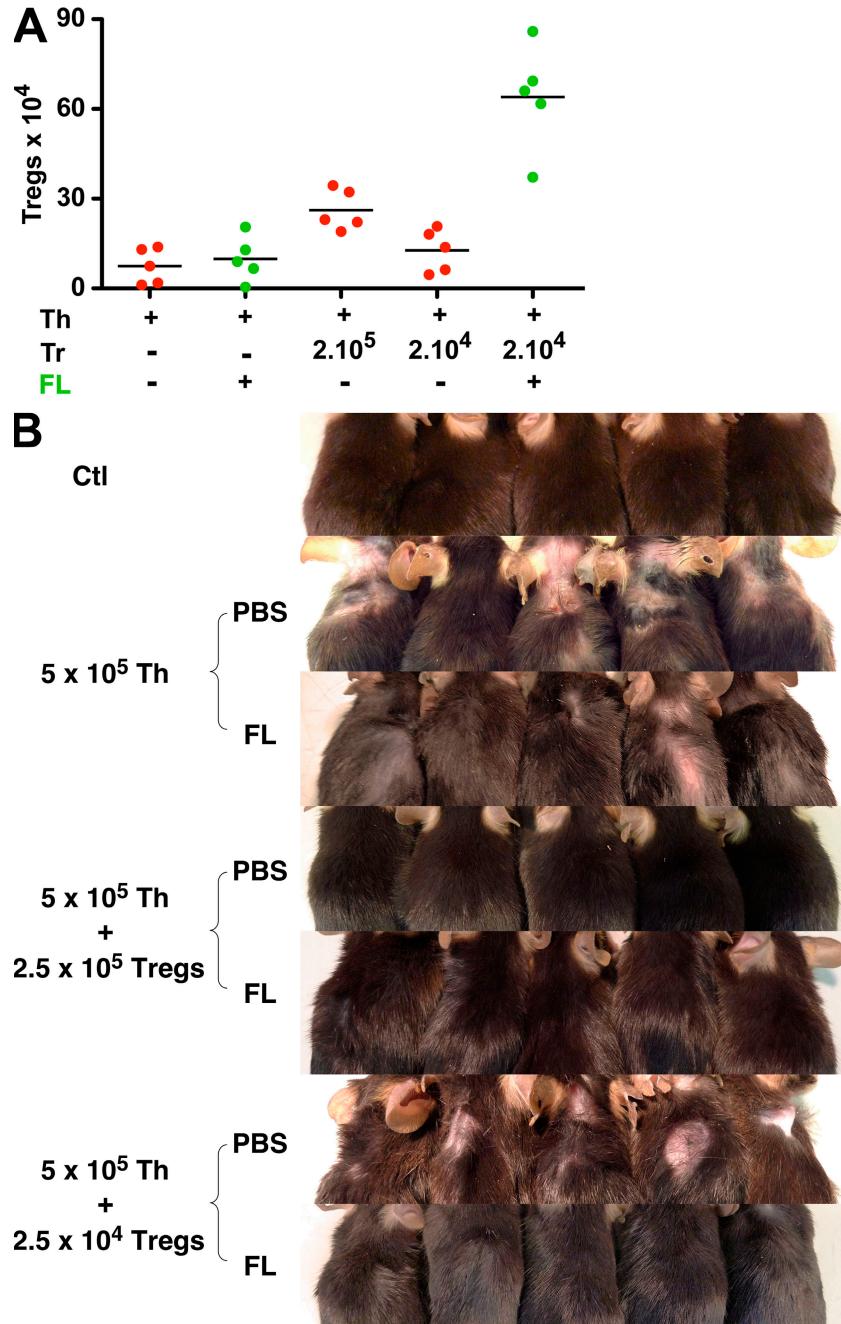


Figure S4. *RAG^{-/-}* mice treated with FL after T cell transfer show increased levels of T reg cells and are protected from hair loss. (A) Scatter plots show absolute numbers of CD4⁺CD25⁺Foxp3⁺ T reg cells in spleens from the *RAG^{-/-}* mice sacrificed 8 wk after adoptive transfer and FL treatment (individual data and means are presented; $n = 5$ mice in one representative out of two experiments). Horizontal bars represent means. (B) PBS- or FL-treated *RAG^{-/-}* mice adoptively transferred with the indicated T cells were examined for loss of fur at 8 wk.