## **Supporting Information**

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**Fig. S1.** Gating scheme for detection of transferred congenically marked cells. Sorted naïve ( $CD44^{lo/mid} CD62L^{hi}$ ) GFP<sup>+</sup> recent thymic emigrants (RTEs) and GFP<sup>-</sup> mature naïve (MN) T cells were mixed at an ~1:1 ratio (*Left*) and injected into lymphoreplete hosts (1–1.5 × 10<sup>6</sup> of each population per recipient). At analysis time points, donor and host cell populations were resolved using congenic markers (*Center*), with cells from uninjected mice serving as gating controls (*Right*).



**Fig. S2.** Thymocyte populations are normal in percent and number in mice transgenic for GFP under control of the recombination activating gene 2 promoter (RAG2p-GFP Tg mice). Thymocytes from four RAG2p-GFP Tg and three non-Tg age- and sex-matched mice were surface stained for CD4, CD8, CD25, CD44, TCR $\beta$ , and CD69 to identify double-negative, double-positive, and CD4 and CD8 single-positive GFP<sup>+</sup> T-cell receptor  $\beta$ -positive (TCR $\beta^+$ ) thymocyte populations. preRTEs were the most mature CD69<sup>lo</sup> subset of CD4 and CD8 single-positive GFP<sup>+</sup> TCR $\beta^+$  thymocytes. (A) Total cell numbers in each thymocyte population. (B) Percentages of total thymocytes.



Fig. S3. Naïve and activated CD4 and CD8 T-cell populations are normal in percent and number in RAG2p-GFP Tg mice. Splenocytes from four RAG2p-GFP Tg and three non-Tg age- and sex-matched mice were surface stained for CD4, CD8, and CD44. (A) Total cell numbers in each splenocyte population. (B) Percentages of total splenocytes.



**Fig. S4.** GFP does not serve as a transplantation antigen in the RAG2p-GFP Tg system. Naïve CD4 (*Left*) or CD8 (*Right*) RTEs and MN T cells were coinjected into lymphoreplete RAG2p-GFP Tg (GFP<sup>+</sup>) or GFP<sup>-</sup> WT mice primed with GFP<sup>+</sup> splenocytes (GFP 1°), and the splenic RTE:MN ratio relative to input was calculated 3 wk posttransfer. n = 3 mice per group; data are representative of three independent experiments. Error bars represent SEM. Differences were not statistically significant (N.S) (P = 0.64 and P = 0.91 for CD4 and CD8 RTE:MN, respectively, using an unpaired Student's t test).