

**Figure S1. Decreased accumulation of DNMA ML as compared to WT B6 CD4<sup>+</sup> T cells in the intestine of irradiated BALB/c recipients.**

T cell depleted bone marrow from B6-CD45.1 mice ( $5 \times 10^6$ ) was transplanted into lethally irradiated BALB/c recipients (850 Gy) together with  $2 \times 10^6$  CD4<sup>+</sup> T cells from control wild type B6 mice (WT), DNMA ML B6 mice, or a 1:1 mixture of WT and DNMA ML CD4<sup>+</sup> T cells ( $10^6$  cells each). Lamina propria lymphocytes were isolated from the small intestine at day 11 after transplantation, as described in Material and Methods.

(a) Flow cytometric analysis of representative samples from WT or DNMA ML T cell recipients (each n=5). Decreased numbers of donor-derived CD45.2<sup>+</sup> H-2Kb<sup>+</sup> DNMA ML CD4<sup>+</sup> T cells were recovered as compared to WT CD4<sup>+</sup> T cells.

(b) Representative analysis of a BALB/c recipient of mixed WT and DNMA ML B6 CD4<sup>+</sup> T cells (1:1, n=5). DNMA ML-GFP<sup>+</sup> T cells were recovered at a ratio slightly higher than the expected 50%, indicating that accumulation of DNMA ML T cells in the gut was rescued in the presence of WT T cells.

**Figure S2. Enhanced suppression does not mediate the decreased pathogenicity of DNMA ML CD4<sup>+</sup> T cells.**

(a) Experimental design. T cell depleted bone marrow (TCD BM) was transplanted into lethally irradiated BALB/c recipients, with or without  $2 \times 10^6$  MACS-purified CD4<sup>+</sup> T cells from control wild type B6 mice (WT), DNMA ML B6 mice, or a 1:1 mixture of WT

and DNMAAML CD4<sup>+</sup> T cells. **(b)** Tracking of donor-derived T cells at day 5 in the group receiving the 1:1 mixture of WT and DNMAAML cells. Close to 50% of donor-derived CD4<sup>+</sup> T cells expressed DNMAAMLGFP, consistent with similar *in vivo* expansion. **(c)** Overall survival after transplantation. Similarly to recipients of WT CD4<sup>+</sup> T cells (n=5), high-grade lethality was observed in the group receiving both WT and DNMAAML CD4<sup>+</sup> T cells (n=6), indicating that enhanced immune suppression by DNMAAML CD4<sup>+</sup> T cells did not account by itself for the reduced pathogenicity of these cells.

**Figure S3. Preserved proliferation and expansion, but defective function of alloreactive CSL/RBP-Jk-deficient CD4<sup>+</sup> T cells.**

CFSE-labeled CD4<sup>+</sup> T cells were tracked after transplantation of 2x10<sup>6</sup> cells from WT B6 or *Cd4-Cre Rbpj<sup>fl/fl</sup>* donors (KO, lacking CSL/RBP-Jk) into lethally irradiated MHC-mismatched BALB/c mice (900 rads). **(a)** Tracking and CFSE dilution profile of donor-derived CD4<sup>+</sup> T cells in the spleen at day 5 after transplantation. Absolute numbers of donor-derived CD4<sup>+</sup> T cells recovered from the spleen at day 5 (mean ± SD, n=3). **(b)** Decreased expression of CD25 in donor-derived *Cd4-Cre Rbpj<sup>fl/fl</sup>* T cells at day 5. Bar graphs show the percentage of cells expressing CD25 and the CD25 mean fluorescence intensity (MFI) among CFSE<sup>10</sup> donor-derived CD4<sup>+</sup> T cells (mean ± SD, n=3). **(c)** Intracellular staining for IFN $\gamma$  and TNF $\alpha$  as well as IL-2 **(d)** after *ex vivo* restimulation with plate-bound anti-CD3 antibody. Data are representative of three experiments. **(e)** Normal early activation of CSL/RBP-Jk-deficient CD4<sup>+</sup> T cells as assessed through upregulation of CD69, CD44 and CD25 36 hours after transplantation. \*p<0.05, \*\*p<0.01 (two-tailed unpaired Student t test).

**Figure S4. Preserved proliferation of CSL/RBP-Jk-deficient alloreactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells in unirradiated BDF1 recipients.**

*Rbpj<sup>ff</sup>Cd4-Cre<sup>+</sup>* donor B6 splenocytes (lacking CSL/RBP-Jk) were labeled with CFSE and infused into unirradiated BDF1 recipients, in comparison with CFSE-labeled splenocytes from littermate controls (40x10<sup>6</sup> each). CFSE content was assessed at day 5 among donor-derived (H-2Kb<sup>+</sup>H-2Kd<sup>-</sup>) CD4<sup>+</sup> and CD8<sup>+</sup> T cells. This alternative genetic strategy allowed us to examine CFSE dilution in Notch-deficient alloreactive T cells without interference from the DNMAmL-GFP fusion protein (compare to fig. 6).

**Figure S5. Increased FoxP3 expression among alloreactive DNMAmL CD4<sup>+</sup> T cells in unirradiated BDF1 recipients.** WT or DNMAmL CD4<sup>+</sup> T cells were labeled with CFSE and infused into unirradiated BDF1 recipients (B6xDBA/2, H-2<sup>b/d</sup>). Intracellular FoxP3 was assessed on day 5 by intracellular staining among donor-derived CD4<sup>+</sup> T cells. The presence of FoxP3 in divided CFSE<sup>low</sup> CD4<sup>+</sup> T cells is consistent with the emergence of peripheral Tregs.

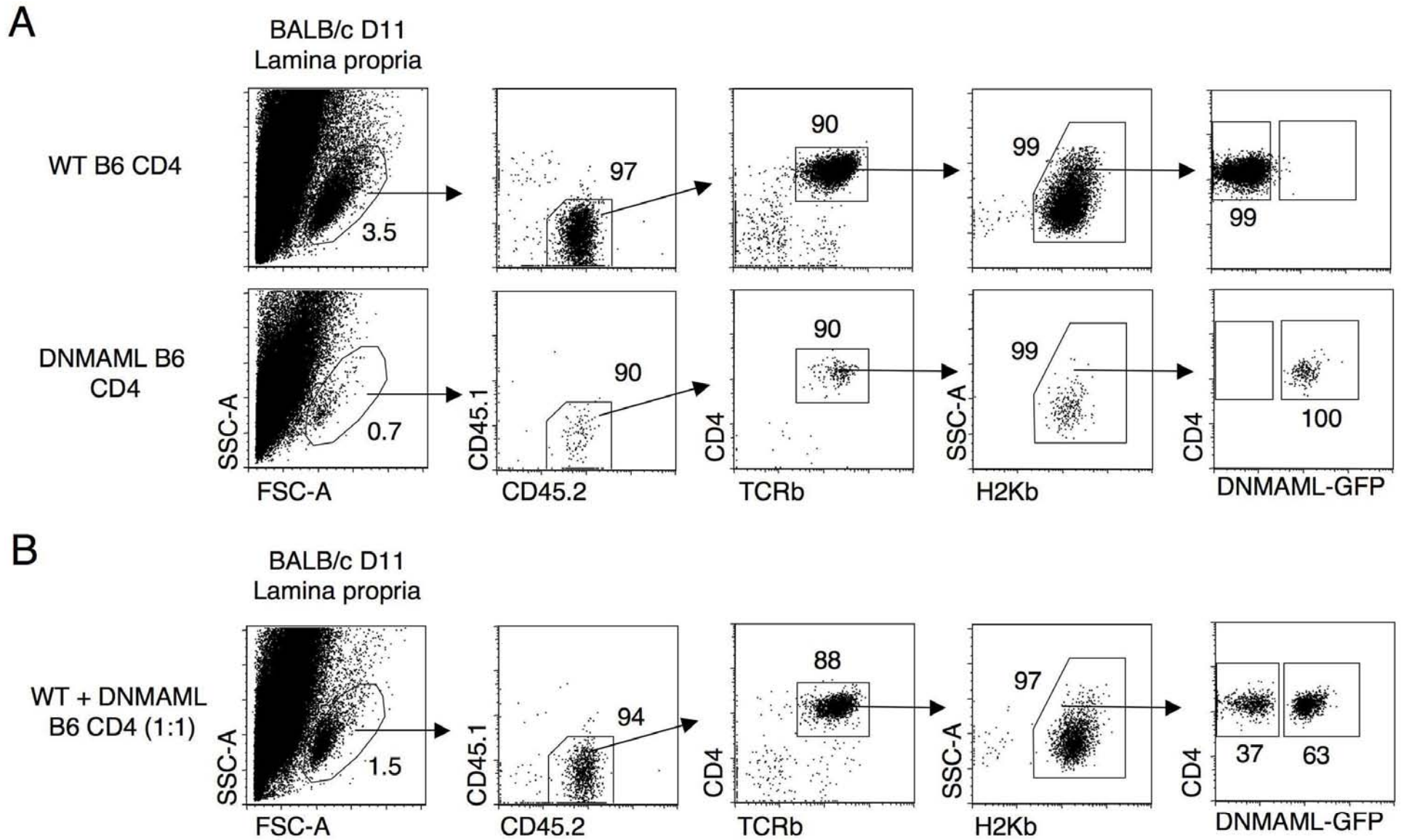


Figure S1

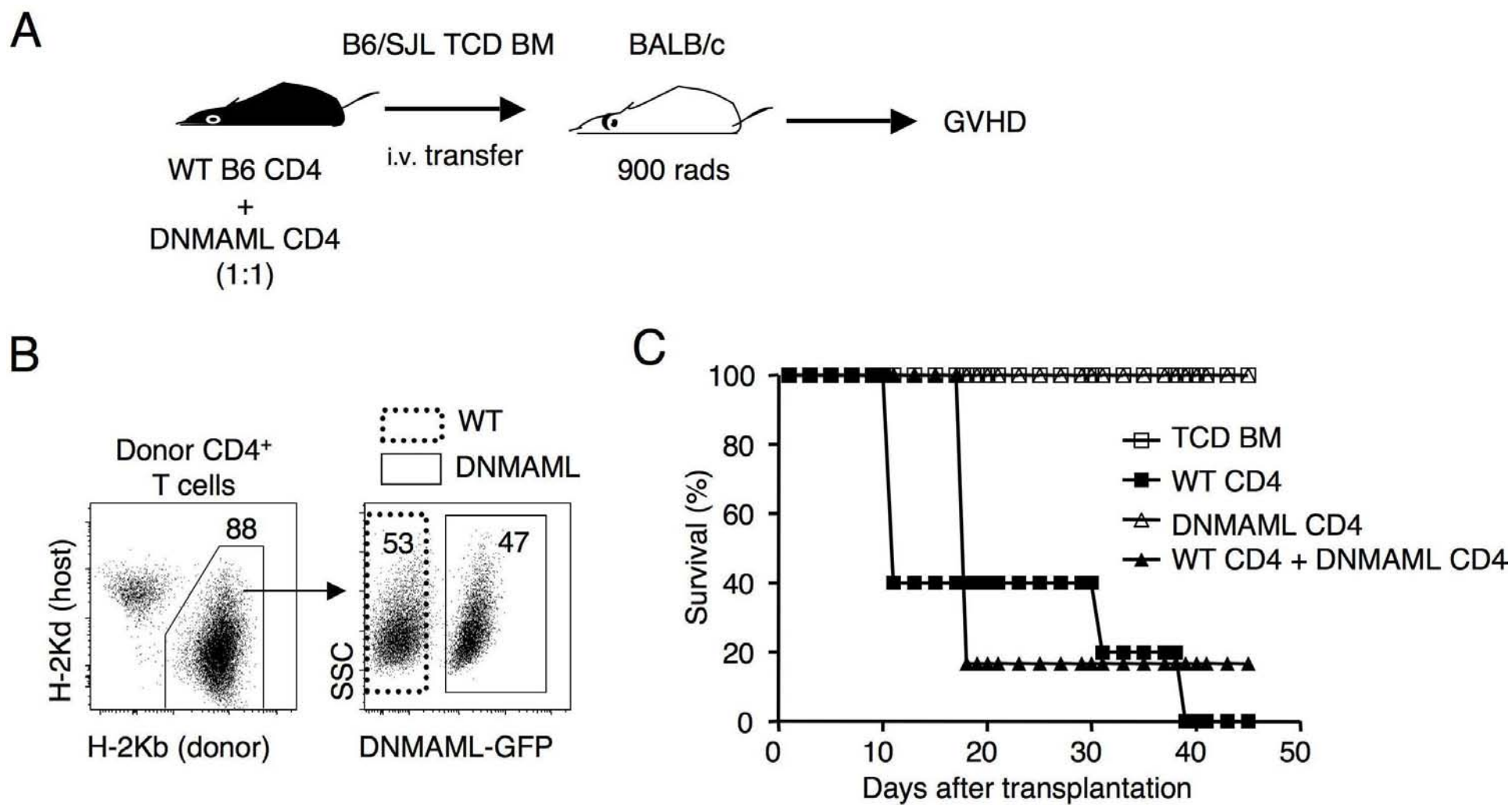


Figure S2

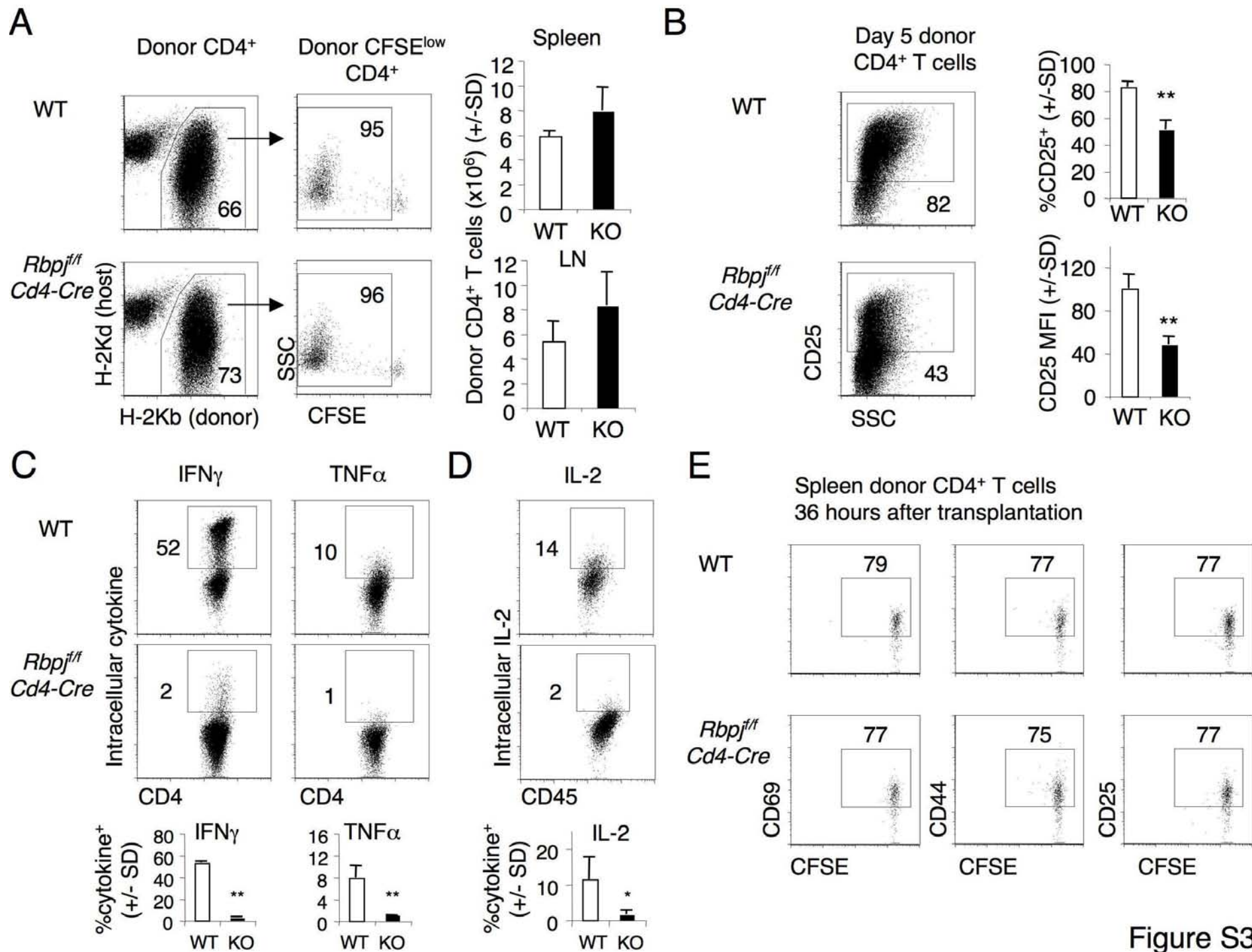


Figure S3

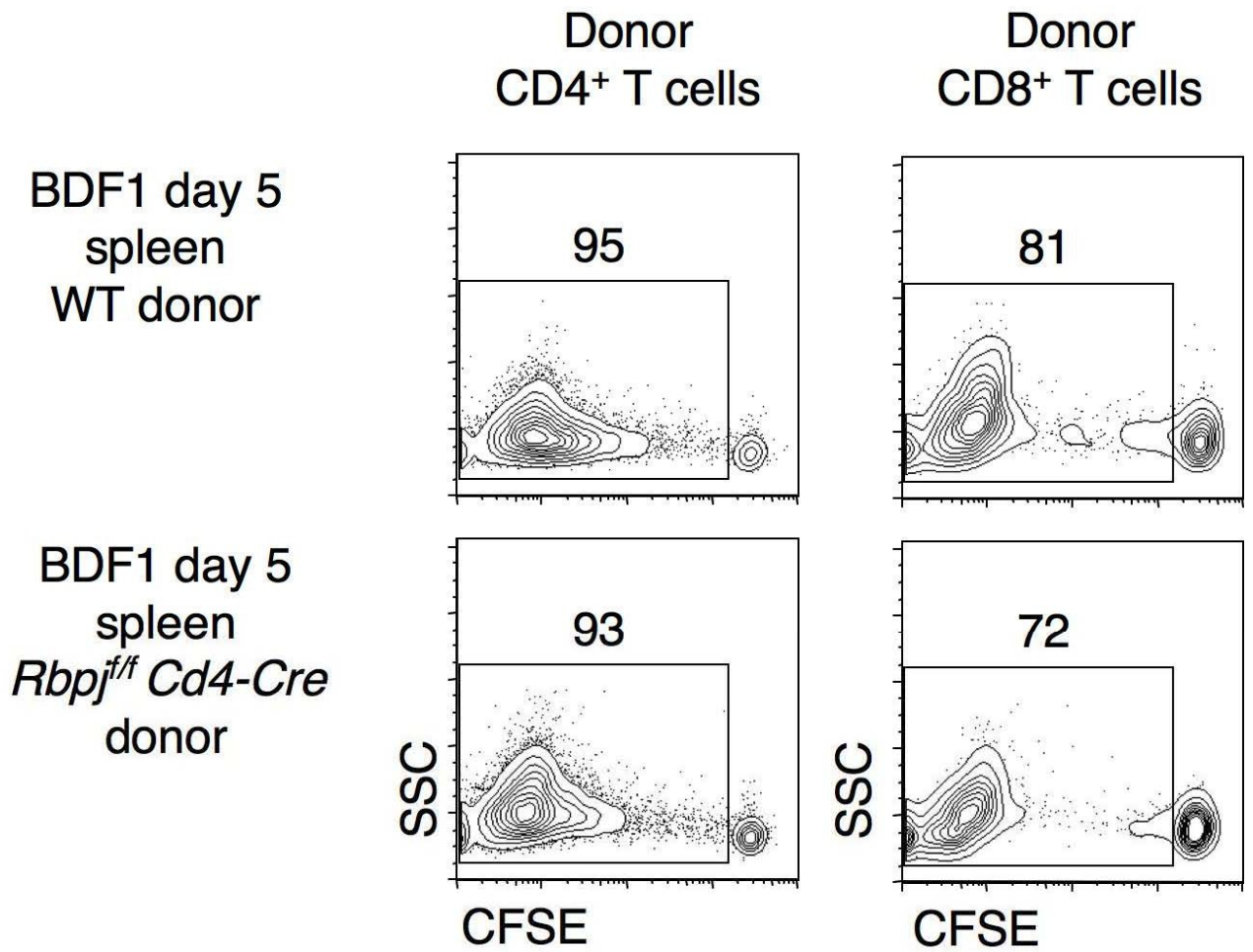


Figure S4

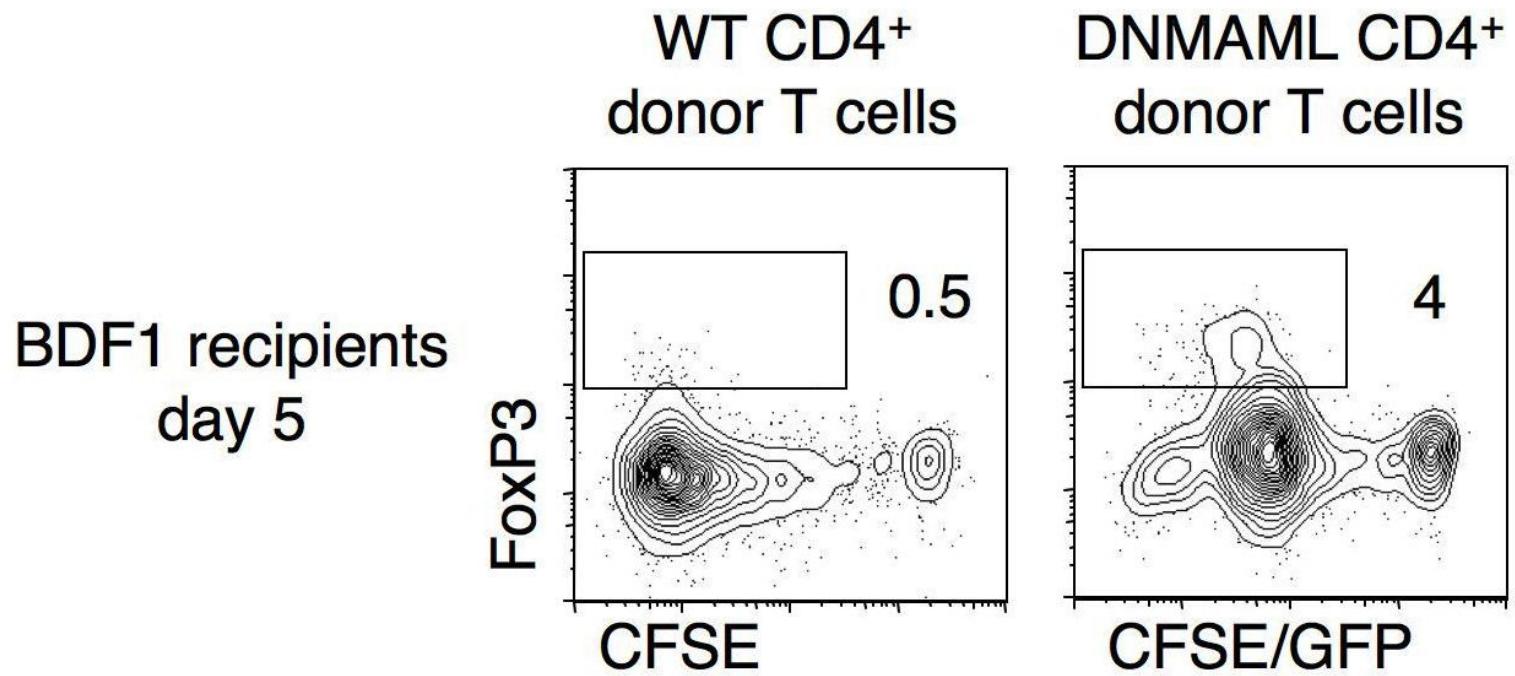


Figure S5