Supporting Information

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Fig. S1. "SignatureMatch" analysis of genes highlighted as region A in Fig. 1A. Raw expression values were normalized to 1 or 0 for spleen Treg and spleen Tconv, respectively, and displayed as a heat map where red represents the expression value of a gene at the same or a greater level than what was found for spleen Treg, whereas black represents the expression value of a gene that is at the same or a lower level than that of spleen Tconv. Reference groups were compared to in vitro activated CD4 T cells (1).

1. Hill JA, et al. (2007) Foxp3 transcription-factor-dependent and -independent regulation of the regulatory T cell transcriptional signature. Immunity 27:786–800.



Fig. S2. Fold-change–fold-change plot. Shown are genes of the activation signature (1) (red, over-expressed; blue, underrepresented). Ratios of spleen Treg vs. Tconv were plotted against the ratios of CD103⁻KLRG1⁻Fopx3⁺ (*Top*), CD103⁺KLRG1⁺Fopx3⁺ (*Middle*), and homeostatically converted cells (*Bottom*), all three divided by spleen Tconv.

1. Hill JA, et al. (2007) Foxp3 transcription-factor-dependent and -independent regulation of the regulatory T cell transcriptional signature. Immunity 27:786–800.



Fig. S3. Homeostatic conversion cannot be blocked by anti-TGF β . Foxp3-GFP negative T cells (CD4⁺CD8a⁻B220⁻CD11b/c⁻) were sorted from BDC2.5/NOD. Thy 1.1.Foxp3-GFP mice and transferred i.v. (1 × 10⁵ cells/mouse) to NOD.RAG1^{0/0} recipients. TGF β was blocked by injecting 1 mg anti-TGF β (1D11) antibody every second day until day 12. Cell were analyzed on day 14 from different organs. The TGF β signature (from Fig. 4A) is not biased in homeostatically converted cells (*Right*).



Fig. S4. Effects of anti-TGF treatment on host cells in OT-II transfer experiments. (*A*) The expression of Foxp3 and CD103 was monitored in CD4⁺, CD45.1⁺, and CD45.2⁻ host spleen cells treated with anti-TGF blocking antibody or PBS only. Summary data for several experiments are shown on the *Right* and statistics were calculated by *t* test.

Other Supporting Information Files

Table S1 (DOC) Table S2 (DOC)