Supporting Information

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SI Materials and Methods

Antibodies. Anti-Vα2-PE, anti-Vβ5.1/5.2-FITC, anti-CTLA-4(CD152)-PE (clone BN13), and anti-IL17A-PE were purchased from BD PharMingen. For intracellular cytokine staining cells were stimulated overnight with PMA (50 ng/mL) and ionomycin (500 ng/mL) with the addition of Golgi-plug (BD) for the final 4 h of incubation. For analysis, cells were fixed (1% paraformaldyde) and permeabilized (0.5% saponin). Anti-CD25-PE (clone PC-61), anti-CD4-APC.AlexaFlour750 (clone RM4–5), anti-Thy1.1(CD90.1)-PE.Cy7 (clone HIS51), and FoxP3-APC (clone FKJ-16s) were purchased from eBioscience (Insight Biotechnology). Intracellular FoxP3 staining was carried out as per the supplied protocol. Anti-glucocorticoid-induced tumor necrosis factor receptor (GITR)-PE was purchased from R&D Systems.

Retroviral Vectors. pMX-OTIIα.IRES.OTIIβ, pMP71-OTIIα.P2A.OTIIβ, and pMP71-FoxP3.F2A.OTIIα.P2A.OTIIβ were generated. pMP71 vectors were modified for optimal gene (and surface TCR) expression by codon optimization and addition of an engineered cysteine bond between the TCR chains [as previously described (1)].

Peptides and DC Pulsing. DCs were pulsed for 1–2 h in standard tissue culture media (RPMI 1640 supplemented with 10% heat inactivated Fetal bovine serum) with saturating amounts (100 μM) of synthetic peptide. DCs were washed and re-counted before stimulations. Peptides were generated by ProImmune and were: Ova323–339–ISQAVHAAHAEINEAGR (OTII-TCR cognate peptide–presented by I-Ab); NP366–379–ASNENDAM (F5-TCR cognate peptide), and Ova265–280–TEWTSSNVMEERKIKV (irrelevant peptide–presented by I-Ab).

1. Thomas S, et al. (2007) Targeting the Wilms tumor antigen 1 by TCR gene transfer: TCR variants improve tetramer binding but not the function of gene modified human T cells. *J Immunol* 179:5803–5810.

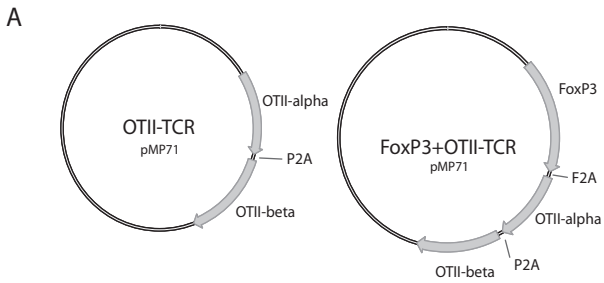

Fig. S1. Retroviral vector diagrams. Expression-optimized vectors were generated to encoded FoxP3 and the α and β chain of the OTII-TCR or the OTII-TCR α and β chains only.

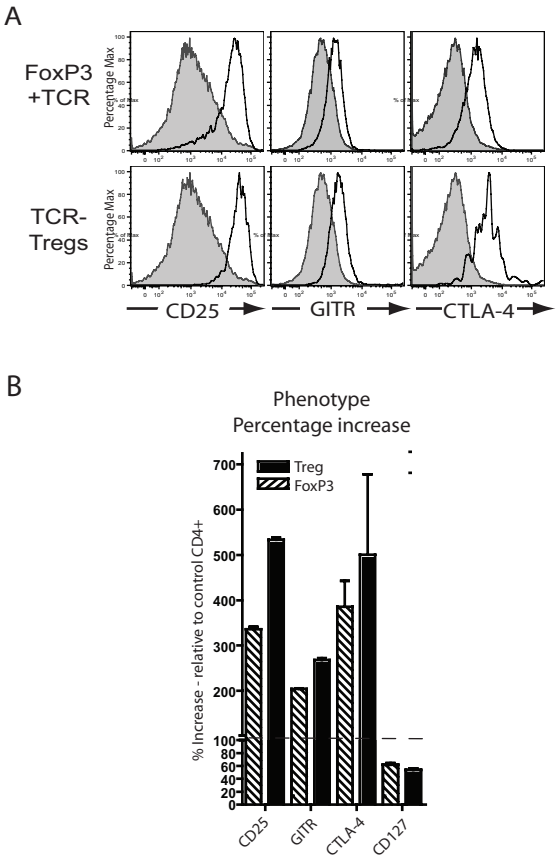

Fig. S2. CD25, CTLA-4, and GITR are upregulated on Tregs and FoxP3-converted $CD4^+$ T cells and are maintained for 6 weeks in vivo. (*A*) FoxP3+TCR-transduced $CD4^+$ T cells (top histogram, black line, gated on $FoxP3^+$ $CD4^+$), TCR-transduced Tregs (bottom histogram, black line, gated on $FoxP3^+$ $CD4^+$), and TCR only transduced control T cells (both histograms, solid gray line, gated on $V\beta5^+$ $CD4^+$) were stained 2 days after transduction for CD25, CTLA-4 (extra and intracellular) and GITR (representative of four separate experiments). (*B*) FoxP3+TCR transduced T cells, TCR transduced Tregs and TCR only transduced T cells were transferred intravenously into 6Gy-lymphodepleted C57BL/6-Thy1.2 mice. The CD25, CTLA-4, GITR, and CD127 expression of the transduced Thy1.1 cells was analyzed at week 6 (gated on Thy1.1 positive, $V\beta5$ positive). The mean fluorescence intensity was compared with the MFI of control TCR-transduced $CD4^+$ T cells. Chart represents the average + standard error from three mice per group.