SUPPLEMENTAL MATERIAL

Kendal et al., http://www.jem.org/cgi/content/full/jem.20110767/DC1

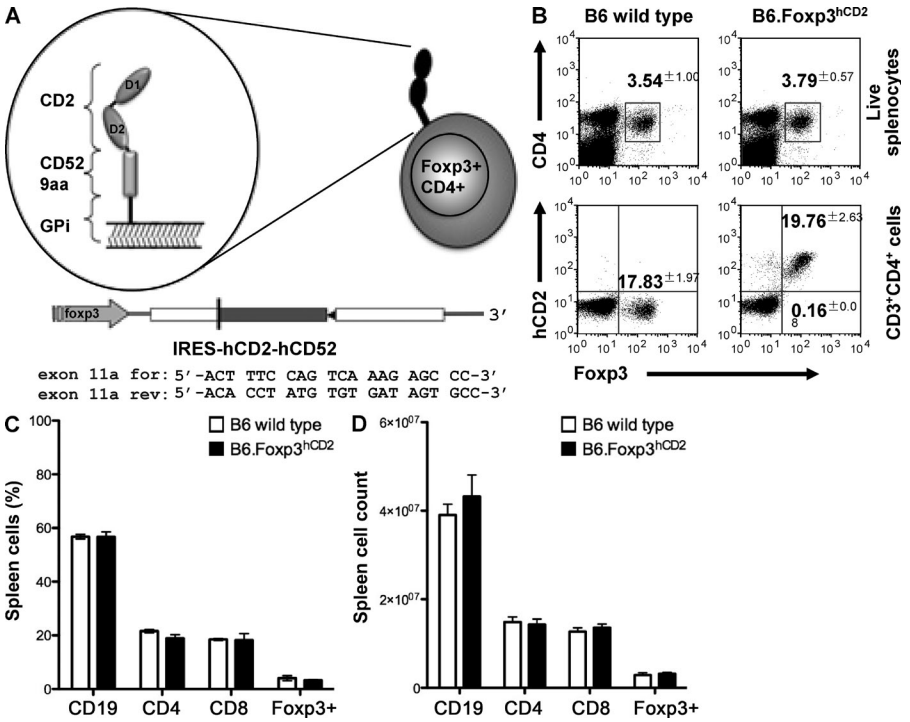

Figure S1. Nature and cellular expression of the CD52–hCD2 reporter system for Foxp3 expression. (A) B6.Foxp3^{hCD2} mice contain a sequence coding for a GPi-linked human CD52_CD2 fusion protein in the 3′ UTR of the X-linked *foxp3* gene. (B) PE-conjugated anti-hCD2 mAb stains >98% of Foxp3^{+}CD4^{+} T cells. (C) Flow cytometry analysis of lymphocyte composition of B6.Foxp3^{hCD2} and B6 WT mice. (D) There is no significant difference in the percentage and absolute cell counts of CD4^{+}, CD8^{+}, CD19^{+}, and CD4^{+}Foxp3^{+} lymphocytes between the two strains. All error bars represent SEM.

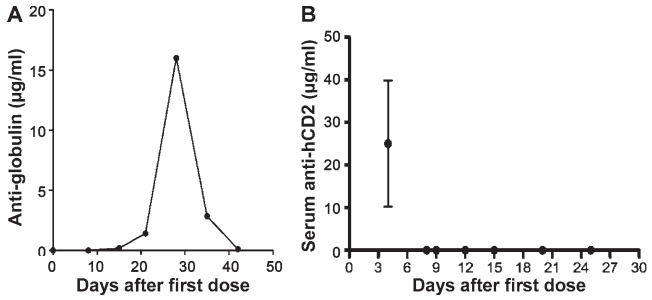

Figure S2. Ablation occurred independently of the host anti-globulin response. (A) Anti-globulin immune response of mice to the treatment antibody is detectable 2 wk after a single dose of 1 mg YTH655 rat IgG2b anti-hCD2 by double capture IOC ELISA (Cobbold et al., 1990). The anti-globulin response peaks at 4 wk and is undetectable after 6 wk. (B) Flow cytometry of fluorescently conjugated anti–rat IgG2b mAb was compared with a standard curve to detect residual anti-hCD2 in the serum of treated mice (Fig. 1 B). Serum anti-hCD2 mAb is not detectable with this method beyond 7 d after the first dose. All error bars represent SEM.

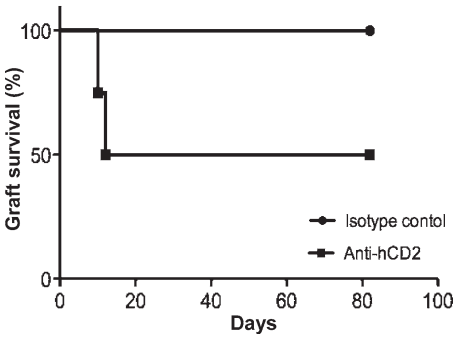

Figure S3. Transplantation tolerance in mice tolerating grafts mismatched for multiple minor antigens was abrogated by ablation of Foxp3⁺ T cells in vivo. Male (CBAxB6^{hCD2})F1 recipient mice (H-2^{b} × H-2^{k}) tolerated a Balb/K (H-2^{k}) after nondepleting anti-CD4, CD8, and CD40L mAb (3 mg × 3 over 7 d). A second challenge Balb/K graft was transplanted on day 60 and survived long term. Ablation of hCD2⁺Foxp3⁺ cells by anti-hCD2 (250 µg × 7) resulted in rejection of half the challenge Balb/K grafts (squares, *n* = 8) compared with control group (circles, *n* = 7; P = 0.0359).

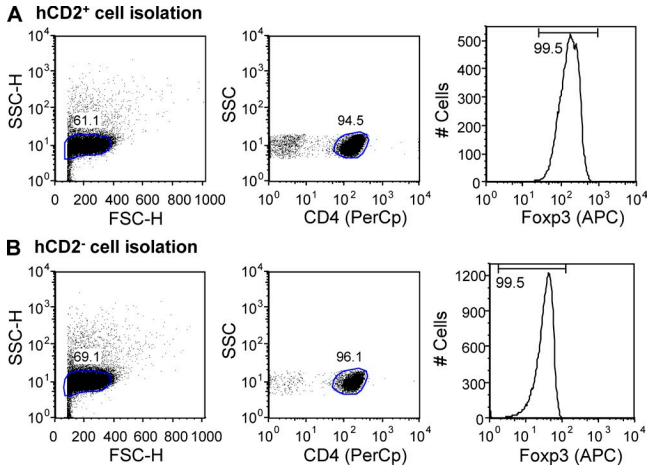

Figure S4. Induction and isolation of Foxp3⁺ iTreg cells in vitro. (A) FACS analysis of MoFlow-sorted female CD3⁺CD4⁺hCD2⁺$^{/pos}$ RAG$^{-/-}$ Marilyn.Foxp3^{hCD2} DBYT cells and (B) CD3⁺CD4⁺hCD2$^{-/neg}$ RAG$^{-/-}$ Marilyn.Foxp3^{hCD2} DBYT cells after 7 d of in vitro culture with TGF-β, dendritic cells, and male H-Y antigen before transfer into male B6 RAG$^{-/-}$ recipients (see Fig. 4).

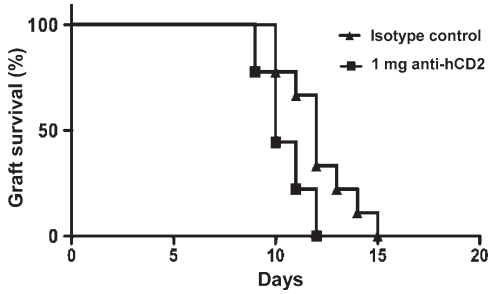

Figure S5. Evidence for a small but significant suppressive effect within central lymphoid cells derived from tolerant TCR transgenic mice. Female RAG$^{-/-}$ mice received male B6.RAG$^{-/-}$ skin in conjunction with 2.5 × 10^{6} central lymphoid cells from anti-CD4 mAb–treated female RAG$^{-/-}$ Marilyn.Foxp3^{hCD2} mice that had accepted a male B6.RAG$^{-/-}$ skin graft for >60 d. Treatment with 1 mg anti-CD2 mAb (squares, *n* = 9) resulted in significant decrease in graft survival compared with control mice (triangles, *n* = 9; P = 0.0313).

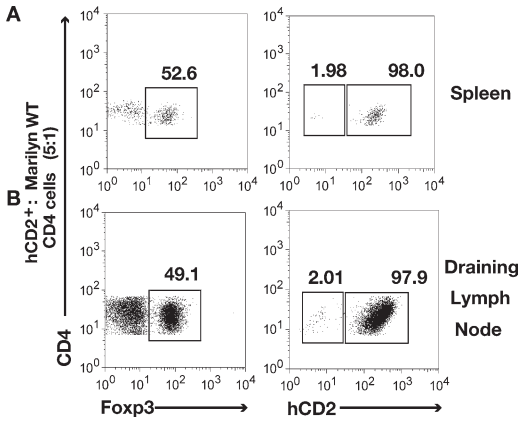

Figure S6. Few newly converted Foxp3⁺ T cells were found in draining lymph nodes of mice where hCD2⁺ (Foxp3⁺) T cells had failed to suppress rejection. FACS analysis of female B6.RAG$^{-/-}$ mice that rejected male CBA.RAG$^{-/-}$ skin grafs when infused with a 5:1 mixture of female hCD2⁺ RAG$^{-/-}$Marilyn.Foxp3^{hCD2} T cell DBYT TGF-induced T cells together with 1×10^5 naive Marilyn^{WT} CD4⁺ T cells. (A and B) 1.98% of FoxP3⁺ spleen cells (A) and 2.01% of FoxP3⁺ cells (B) in the draining lymph node were hCD2 negative compared with 24.6 and 18.2%, respectively, in similar mice that accepted the male graft (Fig. 6 C).