SUPPLEMENTAL MATERIAL

Sela et al., http://www.jem.org/cgi/content/full/jem.20110466/DC1

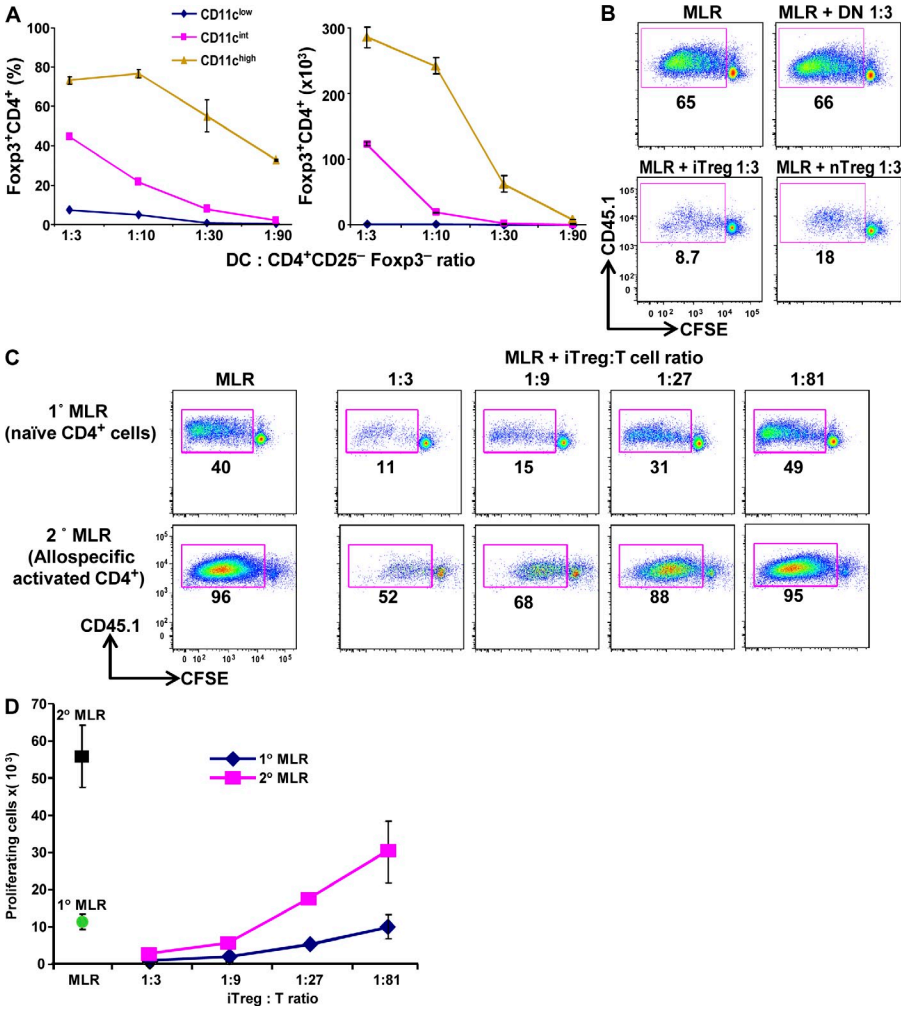

Figure S1. DCs generate de novo suppressive CD4+CD25+Foxp3+ (RFP+) from a polyclonal T cell repertoire in the MLR. (A) Sorted C57BL/6 CD4+CD25−Foxp3− (RFP−) spleen cells were cultured 5 d with BALB/c splenic CD11c+ DCs that had been sorted into CD11c-high, -intermediate, and -low fractions, together with 20 ng/ml TGF-β and ATRA. The frequency (left) and total numbers (right) of induced CD4+CD25+Foxp3+ (RFP+) iT reg cells by FACS analysis are shown on day 5. (B) iT reg cells were induced as in A, and CD25−Foxp3− (RFP−; double negative [DN]) cells were isolated from a separate MLR with BALB/c splenic CD11c+ DCs at day 5. These were added to a fresh MLR that contained CFSE-labeled CD45.1 C57BL/6 CD4+ T cells and BALB/c DCs to detect MLR suppression (reduced CFSE dilution) 5 d later. We also compared foxp3+ nT reg cells from B6 mice that had been expanded in MLR with IL-2 to iT reg cells induced as in A. (C) FACS plots (top row) of CFSE-labeled naive B6 CD45.1+CD4+ T cells (1° MLR), or sorted activated/effector allospecific CD4+CD25+ cells generated from CD4+CD25− cells in 1° MLR (2° MLR) that were incubated with BALB/c DCs with or without sorted B6 anti-BALB/c iT reg cells at the indicated ratios of iT reg cells to CD4+ T cells. 5 d after addition of the FACS-sorted iT reg cells, the suppression of either primary MLR (naive CD4+, top row) or secondary MLR (1° MLR activated/effector, bottom row) was compared by analyzing dilution of CFSE-labeled cells (FACS plot in C and enumeration of absolute proliferating cell number in graph D). Results are each one representative experiment of three. Error bars denote mean ± SD.

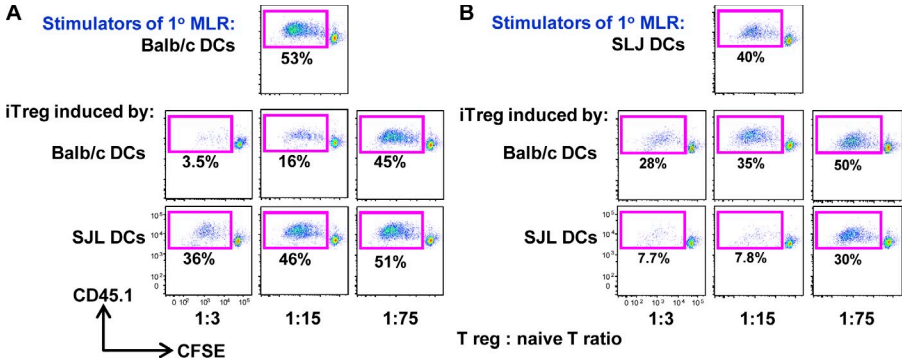

Figure S2. Polyclonal CD4⁺CD25⁺Foxp3⁺ iT reg cells are specific for antigens on the inducing DC. Sorted B6 CD4⁺CD25⁻Foxp3⁻ (RFP⁻) cells were incubated 5 d with either BALB/c or SJL, and splenic CD11c⁺ DCs along with TGF-β and ATRA to induce B6 iT reg cells that were either anti-BALB/c or anti-SJL. Then the two different iT reg cells (CD4⁺CD25⁺Foxp3⁺RFP⁺; Suppressors) were sorted and compared for their suppressive activity of an MLR that was stimulated with either BALB/c (left) or SJL (right) CD11c⁺ DCs (Stimulators). Results are one representative experiment of four.

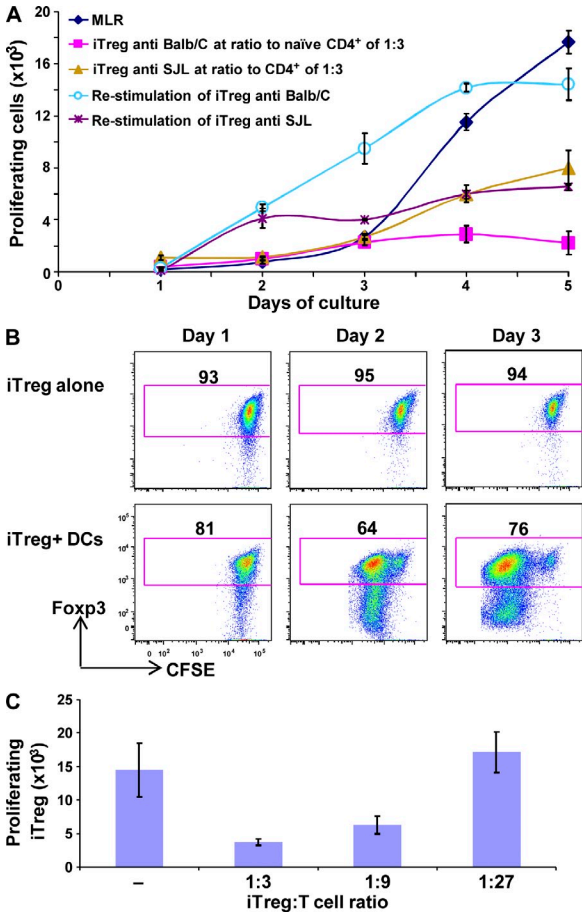

Figure S3. Splenic DCs efficiently restimulate allo-iT reg cells that maintain their suppressive activity. (A) CFSE-labeled iT reg cells were induced with either BALB/c (anti BALB/c, line 4) or SJL (anti SJL, line 5) DCs and were restimulated with BALB/c DCs. CFSE-labeled CD45.1⁺ C57BL/6 CD4⁺ T cells were incubated with BALB/c DCs alone (line 1) or with either anti-Balb (line 2) or anti-SJL (line 3) iT reg cells. The dilution of CFSE was measured with FACS for 5 d. (B) CFSE-labeled iT reg cells were incubated either alone (top) or restimulated in a secondary culture with DCs (bottom), and the percentage of Foxp3⁺ cells was measured over 3 d (as in Fig. 3 C). (C) Fresh BALB/c DCs and CFSE-labeled CD45.1 C57BL/6 CD4⁺ T cells were added to a 3-d culture of iT reg cells restimulated with BALB/c DCs to show that the restimulated iT reg cells retained suppressive activity (reduced CFSE dilution). Results are one representative experiment of three. Error bars denote mean ± SD.

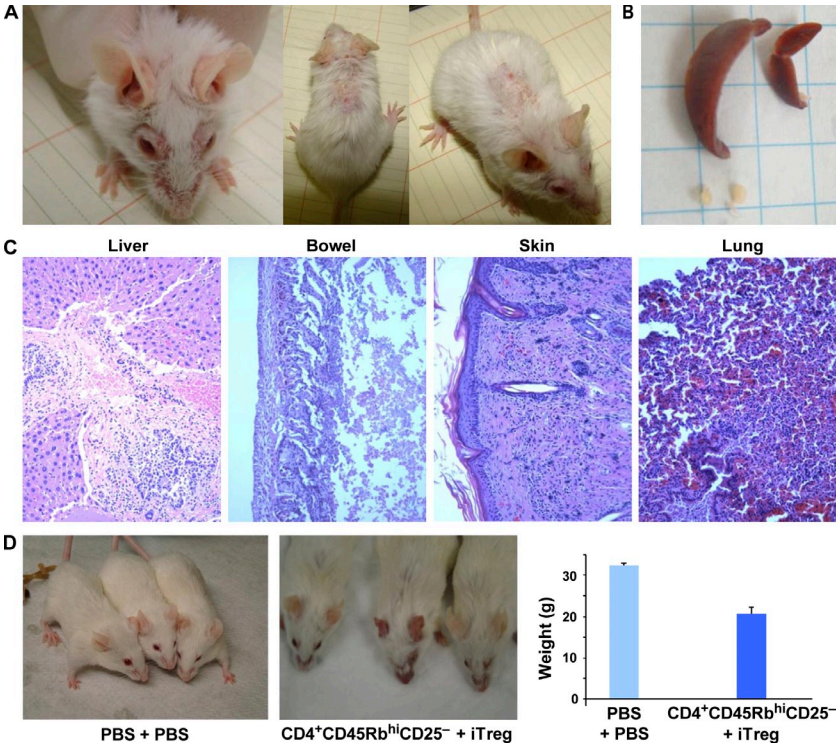

Figure S4. iT reg cells induced by DCs suppress GVHD in vivo. (A) C.B-17 scid (BALB/c) mice were adoptively transferred with allogeneic (B6) $CD4^+CD45RB^{hi}CD25^-CD45.1^+$ cells along with PBS and, 2 mo later, the mice were evaluated for skin manifestations of GVHD. (B) The group described in A (left) was compared with control (PBS only) mice (right) for changes in the size of the spleen (top) and lymph nodes (bottom). (C) H&E staining of various organs 2 mo after adoptive transfer with $CD4^+CD45RB^{hi}CD25^-$ cells + PBS to induce GVHD. (D) C.B-17 scid (BALB/c) mice were treated with PBS only (left) or adoptively transferred with allogeneic C57BL/6 $CD4^+CD45RB^{hi}CD25^-CD45.1^+$ cells (to induce GVHD) along with iT reg cells ($CD4^+CD25^+foxp3^+$) to suppress GVHD (middle). Skin manifestations (left and middle) or weight (right) of mice was measured 6 mo later. Results are one representative experiment of four. Error bars denote mean ± SD.

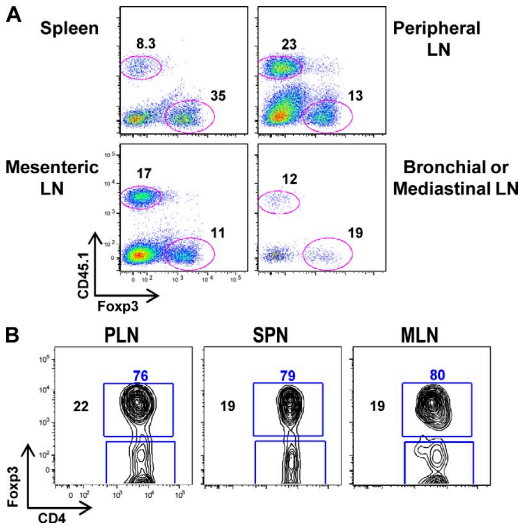

Figure S5. iT reg cells induced by DCs persist in vivo. (A) C.B-17 scid (BALB/c) mice were adoptively transferred with B6 $CD45.1^+CD4^+CD45RB^{hi}CD25^-$ T cells (to induce GVHD) or B6 $CD45.1^+CD4^+CD45RB^{hi}CD25^-$ T cells and iT reg cells, induced by BALB/c DCs in a 5-d MLR supplemented with TGF-β and ATRA. The indicated lymphoid tissues were compared 2.5 mo later for expression of Foxp3 in CD45.1-positive or -negative cells. (B) As in A, but 1.5 mo after adoptive transfer, iT reg cells were evaluated by FACS for rate of conversion to $CD4^+Foxp3^-$ (RFP^-). The dot plot is gated on live $CD3^+$ cells after exclusion of CD45.1 (disease inducing) cells. Results are one representative experiment of three.