SUPPLEMENTAL DATA

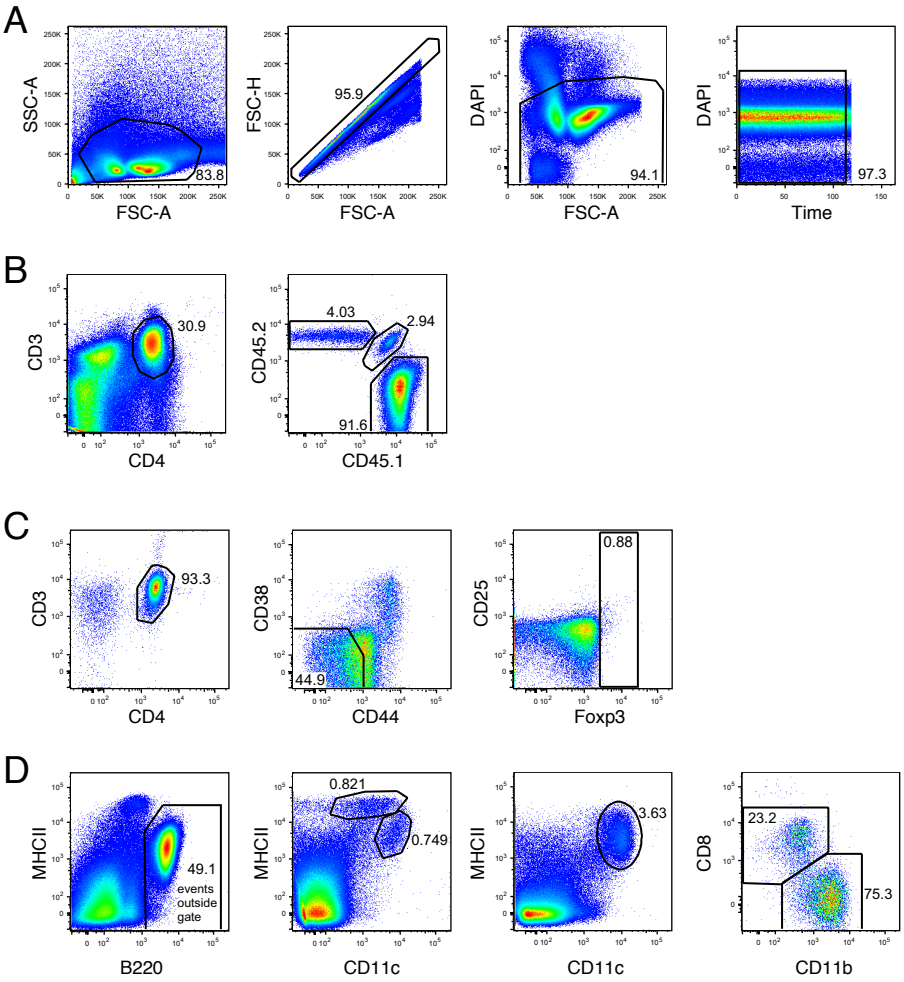

Figure S1. Flow cytometric gating strategy for Tregs, T-cells and dendritic cells. A) Viable cells were identified by first placing a size gate based on forward scatter-area (FSC-A) v side scatter-area (SSC-A). Doublets were then excluded using a FSC-A v FSC-height (FSC-H) gate. For all surface stains dead cells were excluded based on DAPI staining. A time gate was placed in order to exclude clogs or debris at the end of the run. B) For identification of T-cells and Tregs, cells were first gated for size and time as described for A. When identification of adoptively transferred T-cells or Tregs was required a CD45 allelic difference between host mice and donor mice was used. C) For T-cell reconstitution experiments the $CD4^{+}CD25^{-}$ T-cell fraction following magnetic separation was flow sorted for CD4 expression, then the absence of expression of CD38 and CD44, resulting in a population of naïve T cells with minimal Foxp3-expressing cells. D) For DC analysis cells were first gated according to A, then B220-expressing cells excluded. DC subsets were then identified based on expression of CD11c and MHCII. In the pLN and mLN two major subsets of DCs were present: migratory DCs ($MHCII^{hi}$ $CD11c^{int}$) and blood-derived ($MHCII^{int}$ $CD11c^{hi}$). Spleen contained only the blood-derived subset, which was further subdivided based on differential expression of CD8 and CD11b.

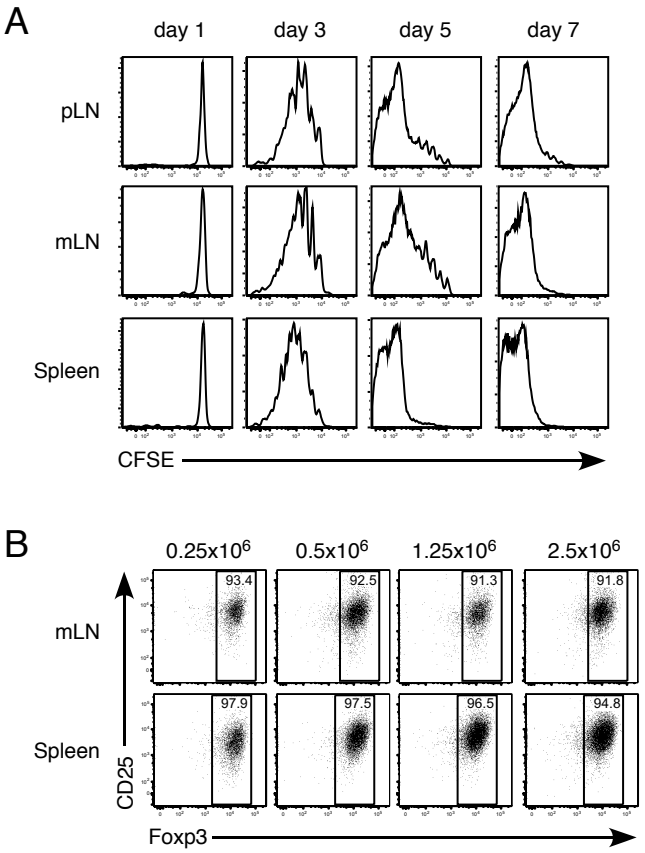

Figure S2. Proliferation of reconstituting Treg cells in lymphopenic host mice. $2.5x10^6$ CFSE-labeled $CD4^+CD25^+$ Tregs were adoptively transferred into lymphopenic *Rag*$^{-/-}$ mice and treated with IL-2/JES6-1 second daily. Treg proliferation was assessed on days 1, 3, 5, and 7. Donor cells were identified based on a CD45 allelic difference from the host, then gated based on expression of CD4 and Foxp3. CFSE division profiles of donor $CD4^+FoxP3^+$ donor cells from the pLN, mLN and spleen of Treg-reconstituted mice are shown. Plots are from a single experiment. B) Expression of CD25 and FoxP3 on donor $CD4^+$ T cells in the mLN and spleen of *Rag*$^{-/-}$ mice reconstituted with the specified number of Treg (as described in Figure 1A). Data are representative plots from a single experiment with n=4 per group.

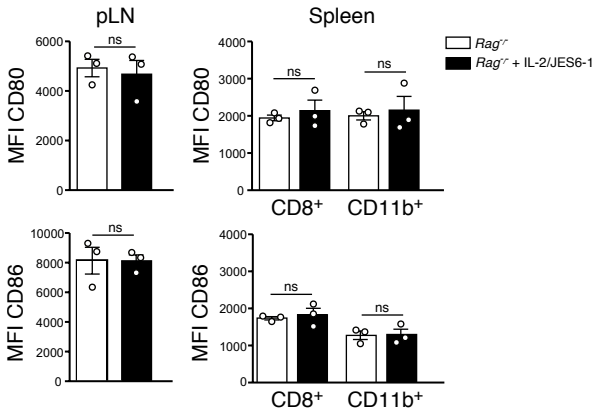

Figure S3. Administration of IL-2/JES6-1 complexes to immunodeficient mice does not affect DC expression of co-stimulatory molecules. *Rag*$^{-/-}$ mice were treated with IL-2/JES6-1 on days 0, 2, 4 and 6, and lymphoid organs harvested on day 7. MFI of CD80 (upper panel) and CD86 expression (lower panel) by migratory DCs from the pLN, and CD8^{+} and CD11b^{+} splenic DCs in IL-2/JES6-1-treated versus untreated *Rag*$^{-/-}$ mice was examined (n=3/group). Data are from a single experiment. Statistical analysis was performed using two-tailed unpaired t tests. Bars represent mean ± SEM with individual values indicated by the open circles. ns=not significant.

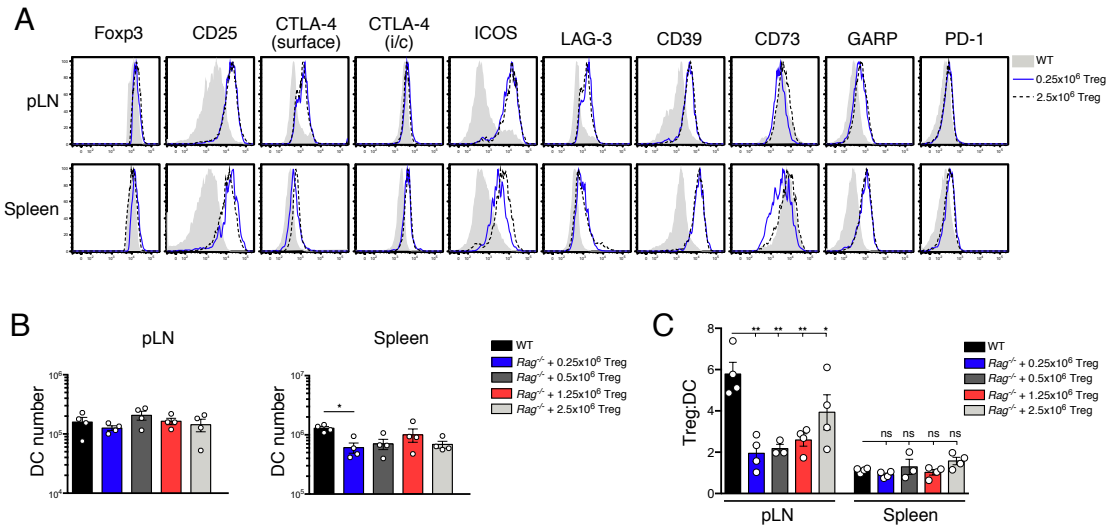

Figure S4. Comparison of Treg expression of activation markers and Treg:DC ratio in the pLN and spleen of Treg-reconstituted mice. *Rag*$^{-/-}$ mice were reconstituted with either 0.25x10^6 or 2.5x10^6 purified Treg as described in Figure 1. Expression of a panel of Treg surface markers including Foxp3, CD25, CTLA-4, ICOS, LAG-3, CD39, CD73, GARP and PD-1 was examined on donor CD4$^+$Foxp3$^+$ cells 7 days post-Treg transfer. Intracellular staining for CTLA-4 expression was also performed. Expression levels of each marker were compared between Treg from untreated WT mice (shaded histograms), *Rag*$^{-/-}$ mice reconstituted with 0.25x10^6 Treg (unbroken line), and *Rag*$^{-/-}$ mice reconstituted with 2.5x10^6 Treg (broken line). Analysis was performed on pooled samples from 4 mice per group. B) The absolute number of DCs in Treg-reconstituted mice for the experiment described in Figure 2C-D was calculated for the pLN (including migratory DCs and blood-derived DCs) and spleen (including CD8$^+$ and CD11b$^+$ blood-derived DCs). C) The ratio of Treg to DC in the pLN and spleen was calculated for the experiment described in Fig 2C-D. Data in B-C are from a single experiment with 4 mice per group. Statistical analysis was performed using a one-way ANOVA with Newman-Keuls post-test. Bars represent mean ± SEM with individual values indicated by the open circles. ns=not significant, *=p<0.05, **=p<0.01.

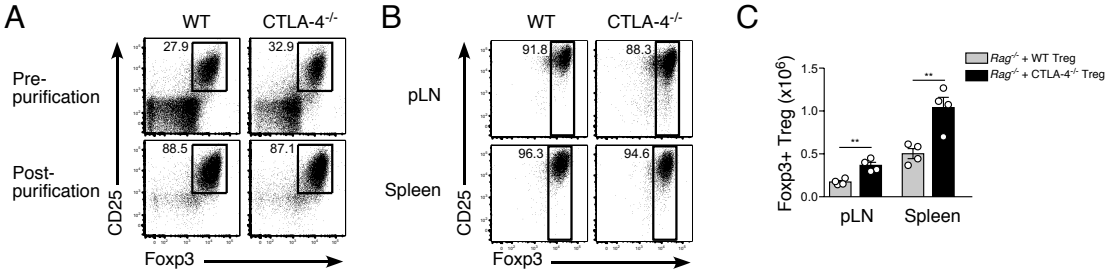

Figure S5. Treg purity and cell numbers in lymphopenic mice reconstituted with either CTLA-4-sufficient or CTLA-4-deficient Tregs. CTLA-4-deficient Tregs were isolated from mixed bone marrow chimeras, generated as described in Figure 4. A) Upper panels: expression of Foxp3 versus CD25 on $CD4^+$ T cells before flow sorting. Lower panels: sorted $CD4^+CD25^+$ Tregs were stained for Foxp3 expression to assess purity. Numbers refer to the percentage of cells within the gate. B) Representative plots of expression of Foxp3 versus CD25 on $CD4^+$ T cells from the pLN (upper panels) and spleen (lower panels) of *Rag*$^{-/-}$ mice reconstituted with either WT Treg or CTLA-4-deficient Treg (n=4/group). C) Absolute numbers of $FoxP3^+$ Treg in the pLN and spleen of *Rag*$^{-/-}$ mice reconstituted with either WT or CTLA-4-deficient Treg cells. Data in A-C are representative of two independent experiments. Statistical analysis was performed using a two-tailed unpaired t test. Bars represent mean ± SEM with individual values indicated by the open circles. **=$p<0.01$.

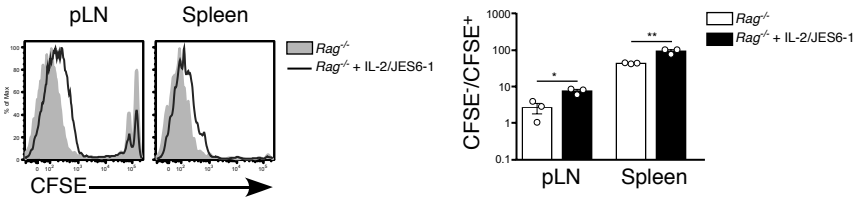

Figure S6. Conventional T cells undergo rapid division in immunodeficient hosts independent of IL-2/JES6-1 treatment. 1x10^6 CFSE-labeled CD4 T cells were adoptively transferred into *Rag*$^{-/-}$ mice, with or without second daily treatment with IL-2/JES6-1 complexes. Representative CFSE division profiles in the pLN and spleen at day 7 post-transfer from a single experiment (n=3 per group) are shown (left). The ratio of fully divided (CFSE$^-$) cells to undivided or slowly dividing T cells (CFSE$^+$) cells was calculated (right). Statistical analysis was performed using a two-tailed unpaired t test. Bars represent mean ± SEM with individual values indicated by the open circles. *=$p<0.05$, **=$p<0.01$.

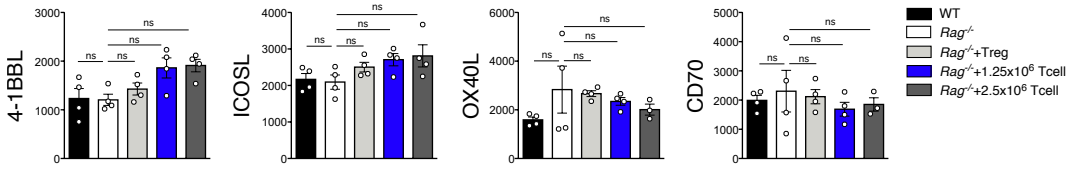

Figure S7. Expression of 4-1BBL, ICOSL, OX40L and CD70 on pLN migratory DCs from Treg-reconstituted mice. *Rag*$^{-/-}$ mice were reconstituted with 2.5x10^6 Tregs or naïve conventional T-cells at a dose of 1.25x10^6 or 2.5x10^6 as described in Figure 7 (n=4 per group). Expression of a range of co-stimulatory molecules including 4-1BBL, ICOSL, OX40L and CD70 was examined on pLN migratory DCs at day 7. Data are from a single experiment. Statistical analysis was performed using a one-way ANOVA with Newman-Keuls post-test. Bars represent mean ± SEM with individual values indicated by the open circles. ns=non-significant.

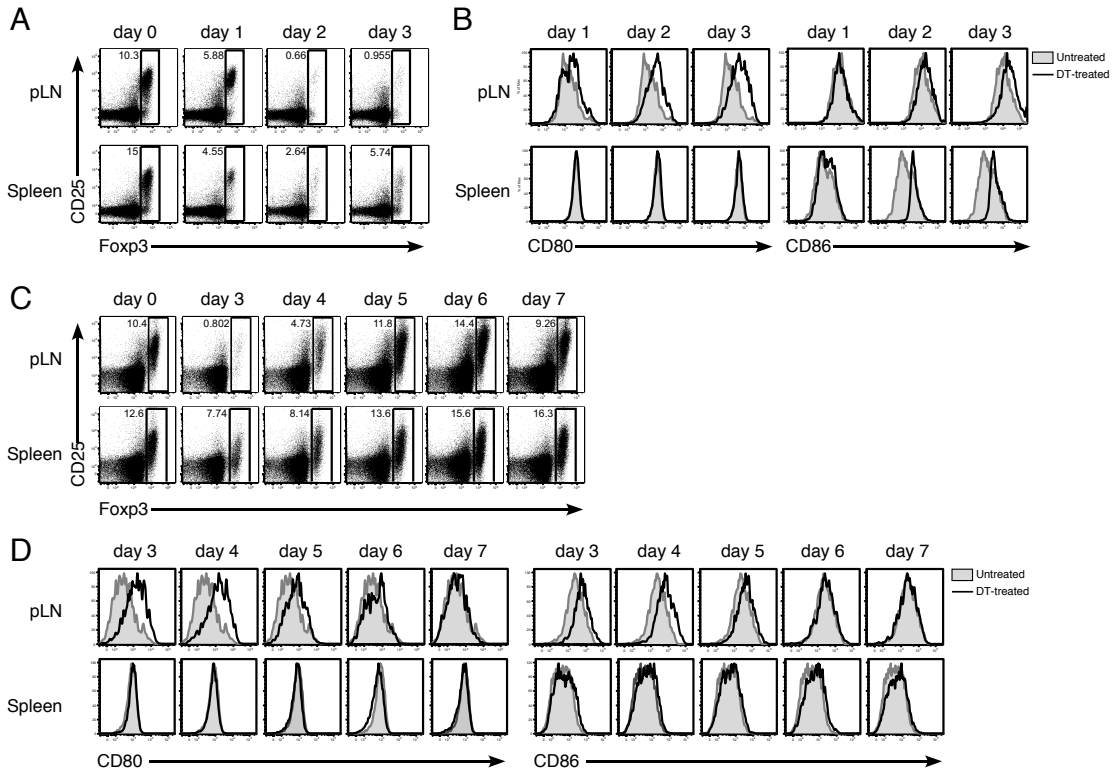

Figure S8. Phenotype of Tregs and DCs following acute Treg depletion in DEREG mice. Flow cytometric analysis of CD4 T cells and DCs following acute Treg depletion of DEREG mice, as described for Figure 8. A) Representative plots of Foxp3 versus CD25 expression on pLN or spleen cells gated for $CD4^+$. Numbers indicate the percentage of cells within the gate on days 0-3. B) Expression of CD80 (left panel) and CD86 (right panel) by pLN migratory DCs and splenic DCs at days 1-3 (open histograms) compared with untreated mice at d0 (shaded histograms). Data in A-B are from a single experiment with 3-4 mice per group. C) Representative plots of Foxp3 versus CD25 expression on pLN or spleen cells gated for $CD4^+$. Numbers indicate the percentage of cells within the gate on days 0 and 3-7. D) Expression of CD80 (left panel) and CD86 (right panel) by pLN migratory DCs and splenic DCs at days 3-7 (open histograms) compared with untreated mice at day 0 (shaded histograms). Data in C-D are from a single experiment with 3-4 mice per group.

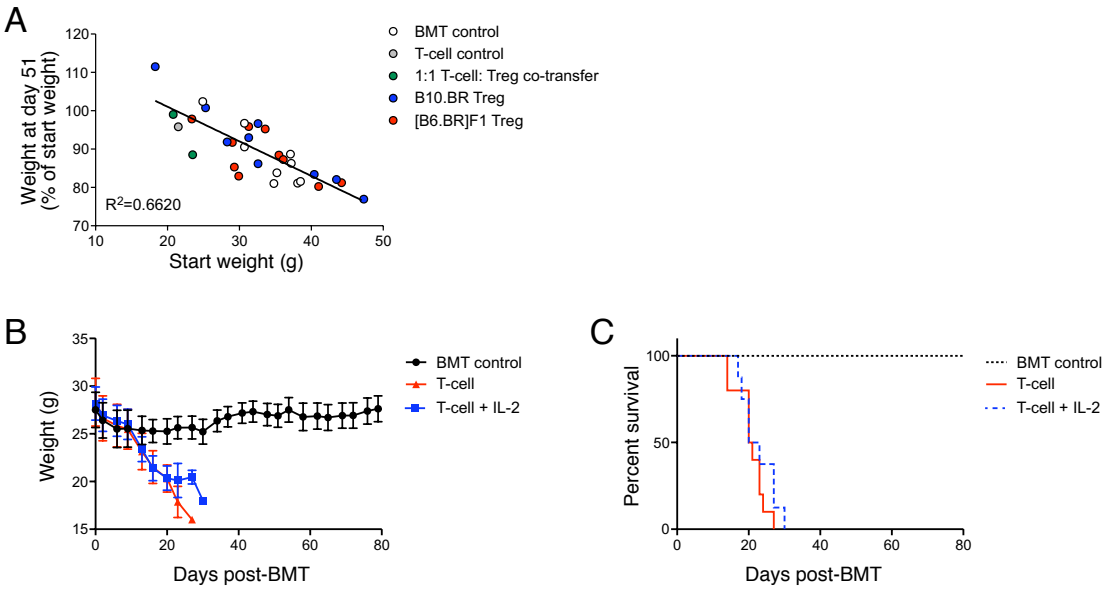

Figure S9. Effect of starting weight and IL-2/JES6-1 treatment in a GVHD model. A) Relationship between starting weight at the time of irradiation (x-axis) and the weight maintained following recovery from BMT (y-axis). Included are all mice that survived >50 days in the experiments described in Figures 10E-F (BMT group: n=9; T cell control: n=1, co-transferred Tregs: n=2; B10.BR Treg-reconstituted: n=9; [B6.BR]F1 Treg-reconstituted: n=10). The weight maintained following recovery from BMT was calculated based on weight at day 51, and is expressed as a percentage of the initial weight at time of irradiation. A line of best fit with R^2 value is shown ($p<0.0001$). Data are pooled from 2 independent experiments. B-C) Mice were irradiated and transplanted with allogeneic BM cells as described in Figure 10. Mice received either BM cells alone (n=7), BM cells with allogeneic T cells at day 7 (n=10), or BM cells with allogeneic T cells at day 7 and IL-2/JES6-1 treatment at days 0, 2, 4, 6, and 8 (n=8). Shown are weights (B) and Kaplan-Meier survival analysis (C) from a single experiment. Median survival time for T-cell group was 20.5 days, while the median survival time for IL-2-treated T cell recipients was 21.5 days. Survival significantly differed between BMT controls and both the T-cell group ($p=0.0003$) and IL-2-treated T-cell recipients ($p=0.0004$). There was no significant difference between the T-cell group and the IL-2-treated T cell group ($p=0.5313$).

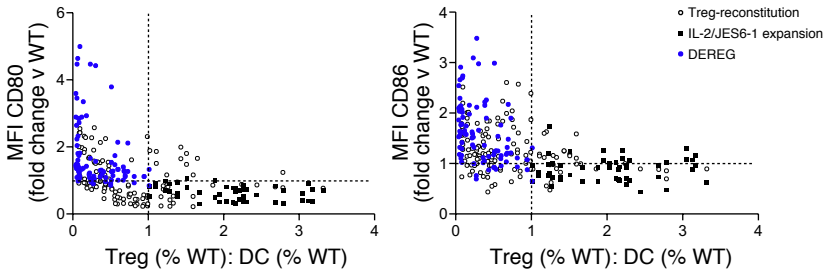

Figure S10. Relationship between Treg:DC ratio and DC expression of CD80/CD86 after Treg-reconstitution, Treg expansion, and Treg depletion. MFI of CD80 (left panel) and CD86 (right panel) expression on migratory DCs in the pLN and mLN, and blood-derived DCs in the pLN, mLN, and spleen was examined in Treg-reconstituted mice at day 7 post Treg-transfer (n=30, from 5 independent experiments including those described in Figures 2C-D, 3A, and 7A), WT mice at day 5 after IL-2/JES6-1 treatment at days 0, 1, and 2 (n=12, from 3 independent experiments including Figure 6A-C), or DEREG mice at day 1-7 following DT treatment at days 0 and 1 (n=30, from 3 independent experiments including those described in Figure 8A-B). Expression of CD80/CD86 for each subset was normalized against the mean WT expression level in the relevant experiment, and is plotted against the Treg:DC ratio in the lymphoid organ (normalized against the WT ratio). Data were fitted to a one-phase decay model (CD80:R^2=0.3444; CD86: R^2=0.2812). The WT level of CD80/CD86 and the WT Treg:DC ratio are both indicated by broken lines.