Supplemental Materials and Methods

Mice

BALB/c (H-2^d) and C57BL/6 (H-2^b) mice were purchased from the National Cancer Institute (Frederick, MD). A/J mice (H-2^a) were purchased from the Jackson Laboratory (JAX). PD-$L1^{-/-}$ BALB/c breeders were provided by Dr. Lieping Chen (Yale University, New Haven, CT, USA). PD-$L1^{-/-}$ C57BL/6 breeders were provided by Dr. Haidong Dong (Mayo Clinic, Rochester, Minnesota, USA). Congenic CD45.1^+ C57BL/6 mice, $CD80^{-/-}$ C57BL/6 breeders and IFN-$\gamma^{-/-}$ C57BL/6 breeders were purchased from JAX Lab. $Rag2^{-/-}$ BALB/c mice were purchased Taconic Farms (Germantown, NY). NSG mice were provided by the Animal Tumor Model Core (City of Hope, Duarte, CA). All mice were maintained in a pathogen-free room in the City of Hope Animal Research Center. All animal protocols were approved by the City of Hope Institutional Animal Care and Use Committee (IACUC).

Induction and assessment of GVHD

BALB/c recipients were exposed to 850 cGy total body irradiation (TBI) with the use of a [^{137}Cs] source 8-10 hours before HCT, and then injected intravenously (i.v.) with C57BL/6 donor spleen cells ($2.5x10^6$ or 5.0×10^6),Thy1.2^+ cells (1×10^6), sorted $CD8^+$ T (1×10^6) or $CD4^+$ T (0.075×10^6) cells and T cell-depleted BM (TCD-BM) cells (2.5×10^6). C57BL/6 recipients were exposed to 1100 cGy TBI and then injected i.v. with A/J donor spleen cells ($10x10^6$, 20×10^6 or 40×10^6) or $CD8^+$ TCD spleen and BM cells (10×10^6). NSG recipients were injected i.p. with human PBMC (20×10^6) from healthy donors. For secondary transplantation, $Rag2^{-/-}$ BALB/c recipients were exposed to 200 cGy TBI 24h before HCT and were injected i.v. with sorted $CD8^+$ T cells ($1x10^6$) isolated from the liver of anti-CD4 or rat-IgG-treated primary recipients together with primary recipient strain TCD-BM (5×10^6). T cell depletion from the bone marrow was

accomplished by using biotin-conjugated anti-CD4 and anti-CD8 mAbs, and streptavidin Microbeads (Miltenyi Biotec, Germany), followed by passage through an autoMACS Pro cell sorter (Miltenyi Biotec, Germany). Enrichment of Thy1.2^{+} cells from spleen was accomplished by using mouse anti-CD90.2 microbeads (Miltenyi Biotec, Germany). The purity of enrichment was >98%, whereas the purity after depletion was >99%.The assessment and scoring of clinical acute signs of GVHD and clinical cutaneous GVHD has been described previously (1, 2).

Isolation of cells from GVHD target tissues

Liver samples were mashed through a 70μm cell strainer, and MNC were isolated from the cell suspensions with Lymphocyte M. Digestion buffer [RPMI containing 5% fetal bovine,10mM HEPES,10U heparin, collagenase D (1mg/ml), and DNase I (1000U/ml)] was carefully injected into lung lobes, and specimens were incubated at 37°C for 45 min. After a second cycle of digestion, lung tissue were mashed through a 70μm cell strainer, and MNC were isolated from cell suspensions with Lymphocyte M. Colon specimens were washed in PBS, cut into 0.5 mm pieces and suspended in PBS containing 1% Bovine serum and 0.002M EDTA, vortexed for 10 min., passed through 70μm strainer and glass wool, and centrifuged for 5 min at 2000 rpm to isolate epithelial cells and lymphocyte.

Antibodies, FACS analysis and FACS sorting

Purified depleting anti-mouse CD4 mAb (GK1.5), blocking anti-mouse PD-L1 (10F.9G2), and neutralizing anti-IL-2 (JES6-1A12) for *in vivo* treatment was purchased from Bio X Cell (West Lebanon, NH). Depleting anti-human CD4 mAb (IT1208) for *in vivo* treatment was provided by Dr. Ito (IDAC Theranostics, Inc. Tokyo, JAPAN). 43H12 hybridoma that produces murine PD-L1-specific mAb was provided by Dr. Lieping Chen (Yale University, New Haven) and produced by Bio X Cell under MTA contract. ChromPure Rat IgG (012-000-003) were purchased from

Jackson ImmunoResearch Laboratories, Inc (West Grove, PA, USA). H-2Kb (AF6-88.5), α4β7 (DATK32), Ly51 (6C3) and FITC Annexin V were purchased from BD Pharmingen (San Diego, CA). mAbs to TCRβ (H57-597), H-2K^b (AF6-88.5), CD3(UCHT1), CD4 (RM4-5), CD8a(SK1), CD8a (53-6.7), CD45 (30-F11), CD11b(M1/70), CD11c(N418), Gr-1(RB6-8C5), B7H1 (H1M5), PD-1 (RMP1-30), CD44 (IM7), CD62L (MEL-14), EpCAM (G8.8), FASL (MFL3), IL7Rα (A7R34), TIM3 (RMT3-23), IFN-γ (XMG1.2), EOMES (Dan 11 mag) and Foxp3 (FJK-16s) were purchased from eBioscience (San Diego, CA). mAbs to CCR9 (Clone 242503) and IL-22R (Clone 496514) were purchased from R&D Systems (Minneapolis, MN). Anti-CXCR3 mAb and anti-T-bet (4B10) were purchased from Biolegend (San Diego, CA). Polyclonal Rabbit Anti-Human Lysozyme EC 3.2.1.17 was purchased from DAKO (Carpinteria, CA). Anti-RNF128:FITC (GRAIL) mAb (ARP43311_T100) were purchased AVIVA SYSTEMS BIOLOGY(San Diego, CA). Anti-Cytokeratin mAb was purchased from Sigma-Aldrich (Louis, MO). mAb to Ulex europaeus agglutinin 1 (UEA-1) was purchased from Vector Laboratories (Burlingame, CA). Flow cytometry analyses were performed with a CyAn Immunocytometry system (DAKO Cytomation, Fort Collins, CO) and BD LSRFortessa (Franklin Lakes, NJ), the resulting data were analyzed with FlowJo software (Tree Star, Ashland, OR). T cell sorting was performed with a BD FACS Aria SORP sorter at the City of Hope FACS facility. The sorted cells were used for transplantation and real-time RT-PCR.

In vivo BrdU labeling and Annexin V staining

On day 7 or day 10 after HCT, T cell proliferation was measured with a single i.p. injection of BrdU (2.5 mg/mouse, 100 mg/g) 3h before tissue harvesting. Analysis of donor CD8$^+$ T cells for BrdU incorporation was performed according to the manufacturer's instructions (BD Pharmingen). For Annexin V staining, the percentage of Annexin V$^+$ cells among donor CD8$^+$ T

cells was assessed by flow cytometry according to the manufacturer's instructions (eBioscience, San Diego, CA).

Real-time RT-PCR: Real-time RT-PCR analysis of mRNA for CCL25, CXCL9, CXCL10, CXCL11 was performed as described in our previous publications (1, 6). Primers used are as follows:

CCL25:

Forward: 5'-TTACCAGCACAGGATCAAATGG-3'

Reverse: 5'-CGGAAGTAGAATCTCACAGCAC-3'

CXCL9:

Forward: 5'-TCCTTTTGGGCATCATCTTC-3'

Reverse: 5'-TTCCCCCTCTTTTGCTTTTT-3'

CXCL10:

Forward: 5'-CGATGACGGGCCAGTGAGAATG-3'

Reverse: 5'-TCAACACGTGGGCAGGATAGGCT-3'

CXCL11:

Forward: 5'-AGTAACGGCTGCGACAAAGT-3'

Reverse: 5'-GCATGTTCCAAGACAGCAGA-3'

GAPDH:

Forward: 5'-TCACCACCATGGAGAAGGC-3'

Reverse: 5'-GCTAAGCAGTTGGTGGTGCA-3'

Relative expression levels of genes were normalized within each sample to the housekeeping gene GAPDH.

Measurement of cytokines and liver function in serum

Cytokines in serum were measured by enzyme-linked immune sorbent assay (ELISA). The ELISA kits for IFN-γ, TNF-α and IL-2 were purchased from R&D Systems (Minneapolis, MN). ELISA kit for mouse IL-27 was purchased from Biolegend (San Diego, Ca). Measurements of liver function (AST, ALT and ALB) were performed by the Charles River Clinical Pathology Laboratory (Wilmington, MA). Serum AST levels during GVL experiments was measured with Aspartate Aminotransferase activity assay kit purchased from abcam (Cambridge, MA).

Histopathology

Tissue specimens were fixed in formalin before embedding in paraffin blocks, sectioned and stained with H&E. Slides were examined at 200X or 400X magnification and visualized with an Olympus and a Pixera (600CL) cooled charge-coupled device camera (Pixera, Los Gatos, CA). Tissue damage was blindly assessed according to a defined scoring system, as described previously.(1, 2) In brief, skin GVHD was scored by tissue damage in the epidermis and dermis and by loss of subcutaneous fat; the maximum score is 9. Salivary GVHD was scored by mononuclear cell infiltration and follicular destruction; the maximum score is 8. Liver GVHD was scored by the severity of lymphocytic infiltrate, number of involved tracts and severity of liver cell necrosis; the maximum score is 9. Lung GVHD was scored by periluminal infiltrates, pneumonitis, and the severity of lung tissue damage; the maximum score is 9. Gut GVHD was scored by mononuclear cell infiltration and morphological aberrations (e.g. hyperplasia and crypt loss), with a maximum score of 8.

Histo-immunofluorescent staining of intestinal Paneth cells, epithelial cells.

Small Intestine and colon tissues were harvested, formalin-fixed and paraffin embedded. Small intestines were stained with rat-anti-mouse IL-22Rα antibody (R&D Systems) and polyclonal

rabbit anti-human lysozyme (Dakocytomation). Colon tissues were stained with anti-cytokeratin-Pan (Sigma).

TUNEL assay of hepatocyte apoptosis

Paraffin sections were stained with DAPI and TUNEL according to the manufacturer's instructions (Roche, Indianapolis, IN) and imaged with the use of an Olympus IX81 Automated Inverted Microscope. Images were taken with a 400x objective and analyzed using Image-Pro Premier.

Bioluminescent imaging

Mice were injected with luciferase$^+$ BCL1 cells (BCL1/ Luc$^+$) i.p. *In vivo* imaging of tumor growth has been previously described (7). Briefly, mice were injected with 200µl firefly luciferin i.p. (Caliper Life Sciences, Hopkinton, MA), anesthetized, and imaged by using an IVIS100 (Xenogen) and AmiX (Spectral) imaging system**.** Data were analyzed by using Igor Pro 4.09A software purchased from WaveMetrics (Lake Oswego, OR) and Amiview software purchased from Spectral Instruments Imaging (New York, NY).

Production of mouse B7H1-Fc

B7H1-Fc–expressing plasmid was a kind gift from Dr. Lieping Chen (Yale University School of Medicine). The DNA plasmid contained the coding sequence for the murine B7H1 extracellular domain that was fused with the CH2-CH3 region of human IgG1 heavy chain. B7H1-Fc fusion protein was expressed transiently in Chinese Hamster Ovary Suspension (CHO-S) cell line using Thermo Fisher Freestyle CHO expression system as manufacture protocol. The supernatant of the transiently transfected CHO-S was collected after 7 days and passed through the protein G agarose beads (GenScript) packed column that had been equilibrated in 1X PBS

pH.7.4. B7H1-Fc bound protein was washed with 1XPBS pH7.4, eluted with 0.1M Glycine pH2.5, dialyzed in 1XPBS pH 7.4 and concentrated into 1.0 mg/ml aliquots before freezing in -80oC until further use.

Cell lines

Luciference transfected BCL-1 cell line were provided by Dr. Christopher Contag at Stanford University (Stanford, CA). BC-CML cell line were provided by Dr. Warren Shlomchik at Pittsburgh University (Pittsburgh, PA). Raji cell line were provided by Dr. Stephen Forman (City of Hope, Duarte, CA)

Statistics

Data were displayed as mean ± SEM. Bodyweight, diarrhea, cutaneous damage scoring, GVHD and survival in different groups were compared by using the rank sum test or log-rank test. Comparison of three means was analyzed using one-way ANOVA multiple comparisons, while comparison of two means was analyzed by using an unpaired two-tailed Student t-test (Prism, version 6.0; GraphPad Software), P less than 0.05 was considered as significant. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

Supplemental figures and figure legends

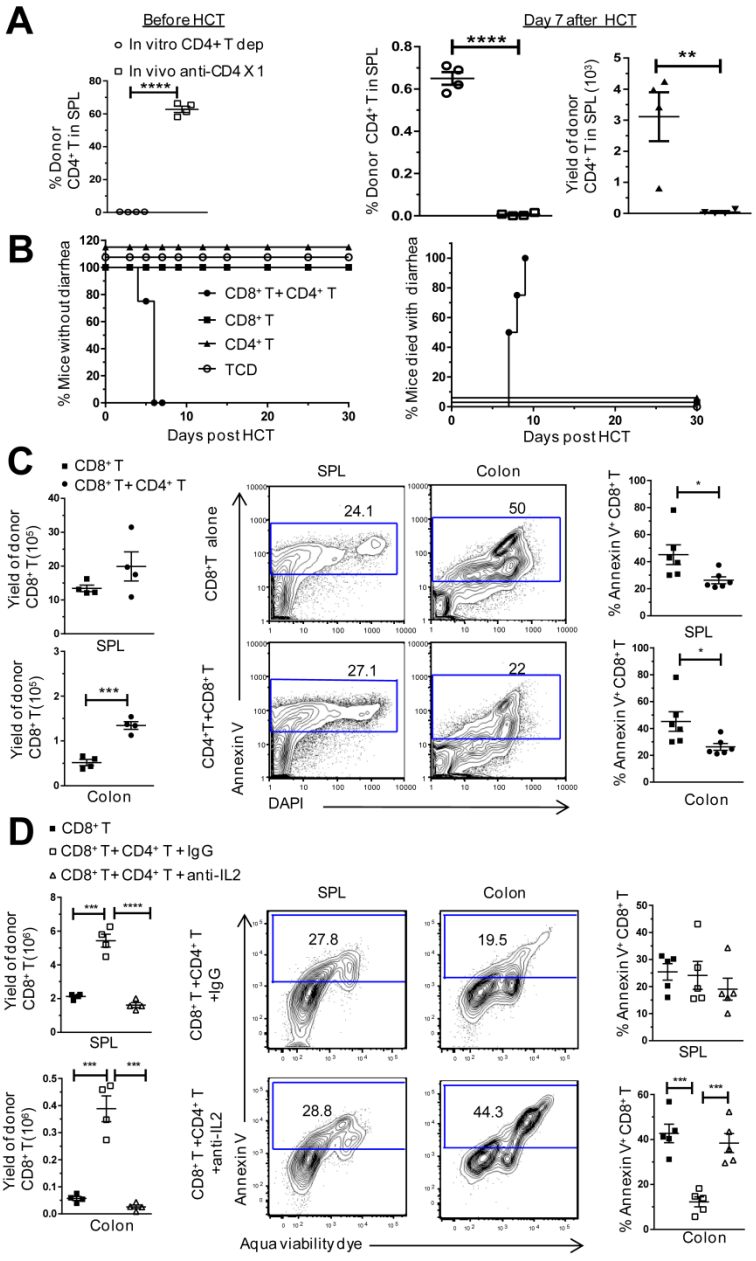

Figure S1: Small numbers of donor CD4$^+$ T cells augment survival of donor CD8$^+$ T cells in GVHD target tissues in an IL-2 dependent manner. (A) Lethally irradiated BALB/c recipients were transplanted with C57BL/6 TCD-BM (2.5x10^6) together with either splenocytes (5x10^6) or ex vivo CD4$^+$ T cell-depleted splenocytes that contained the same number of CD8$^+$ T

cells as present in $5x10^6$ whole spleen cells. The recipients of whole spleen cells were injected with depleting anti-CD4 mAb (500ug/mouse) at the time of HCT to in vivo deplete donor $CD4^+$ T cells. The donor splenocytes before HCT and splenocytes from the recipients 7 days after HCT were analyzed for the percentage and yield of donor $CD4^+$ T cells. Representative patterns and means ± SEM of the percentage and yield of donor $CD4^+$ T cells in the spleen are shown. Mean ± SEM; n=4 per group. **(B)** Lethally irradiated BALB/c recipients were injected with TCD-BM ($2.5x10^6$) alone, TCD-BM plus flow cytometry-sorted $CD4^+$ T cells (0.075×10^6) alone, TCD-BM plus sorted $CD8^+$ T cells (1×10^6) alone, or TCD-BM plus both $CD4^+$ and $CD8^+$ T cells. Recipients were monitored for clinical signs of GVHD such as diarrhea and survival. Percentage of mice without diarrhea and percent mice died in association with diarrhea are shown; n=6-8 per group combined from two replicate experiments. **(C)** Yield of donor $CD8^+$ T cells and percentage of Annexin V^+ donor $CD8^+$ T cells in the spleen and colon in recipients transplanted with $CD8^+$ T cells alone or with $CD4^+$ T cells. N=4-6 per group. **(D)** Lethally irradiated BALB/c recipients were injected with $CD8^+$ T ($1x10^6$) and $CD4^+$ T cells ($0.075x10^6$) and TCD-BM, and then injected IP with control rat IgG or anti-IL-2 (500 μg/mouse) on days 0, 2, 4 and 6 after HCT. On day 7, spleen and colon tissue infiltrating $CD8^+$ T cells were analyzed for apoptosis. N=4-5. Data represent mean ± SE combined from two replicate experiments. P values were calculated by unpaired two-tailed Student t tests (A, C and D) or log-rank test (B) (*$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$, **** $p < 0.0001$).

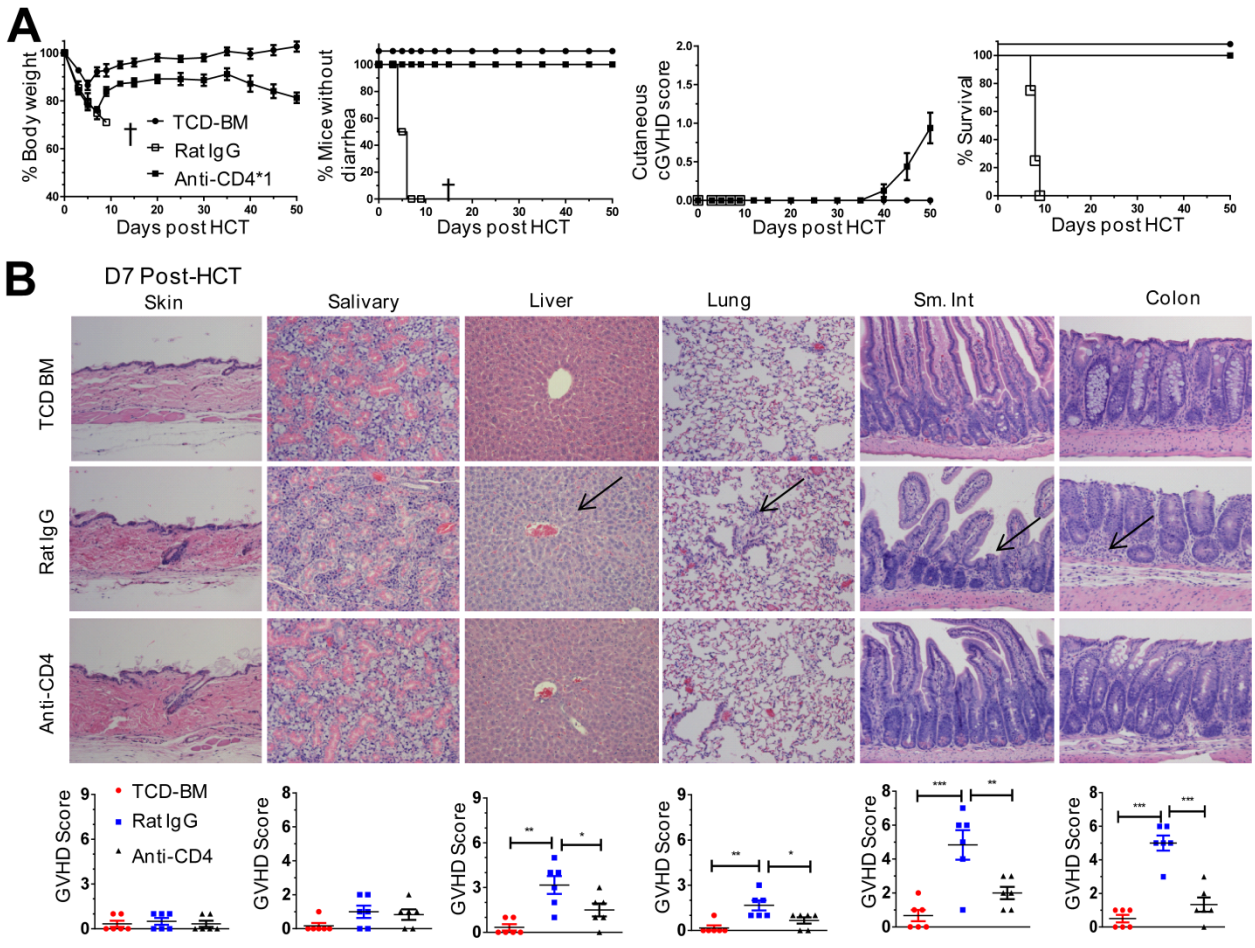

Figure S2: A single injection of anti-CD4 mAb after HCT prevents acute but not chronic GVHD, with C57BL/6 donors and BALB/C recipients. Lethally irradiated BALB/c recipients transplanted with splenocytes ($5x10^6$) and TCD-BM ($2.5x10^6$) from C57BL/6 donors were given a single i.v. injection of rat-IgG or anti-CD4 mAb (500μg/mouse) at the time of HCT. Recipients given TCD-BM ($2.5x10^6$) alone were used as controls. Recipients were monitored for clinical signs of GVHD, including body weight change, diarrhea, hair loss, and survival († indicates death of all recipients in a group). **(A)** Percentage of body weight change, percentage of recipients without diarrhea, clinical cutaneous GVHD score, and percentage of surviving recipients. n=8 per group combined from two replicate experiments. **(B)** At 7 days after HCT, skin, salivary gland, lung, liver, small intestine (Sm. Int) and colon tissues were evaluated for histopathologic evidence of GVHD . Representative photomicrographs are shown (original

magnification 200x), n=6 per group. Arrows indicate the changes in GVHD recipients as compared with control recipients. Data represent mean ± SE combined from two replicate experiments. P values were calculated by unpaired two-tailed Student t tests (B) or log-rank test (A) (*p<0.05, ** p<0.01, *** p<0.001).

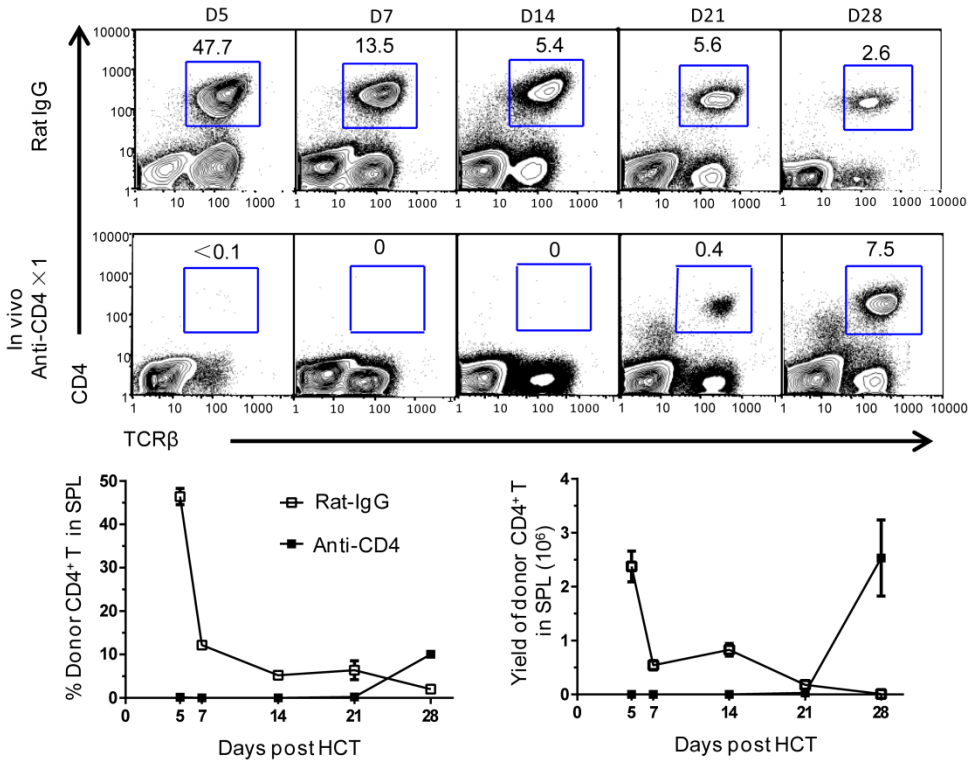

Figure S3: Recovery kinetics of CD4$^+$ T cells after a single anti-CD4 mAb treatment.

Lethally irradiated BALB/c recipients transplanted with splenocytes (2.5x10^6) and TCD-BM (2.5x10^6) from C57BL/6 donors were given a single i.v. injection of rat-IgG or anti-CD4 mAb (500μg/mouse) at the time of HCT. On days 5, 7, 14, 21 and 28 after HCT, splenocytes from recipients were analyzed for CD4$^+$ T cells expansion and recovery. Representative patterns and means ± SEM of the percentage and yield of CD4$^+$ T cells in the spleen are shown. Mean ± SEM; n=4 for each group at each time point.

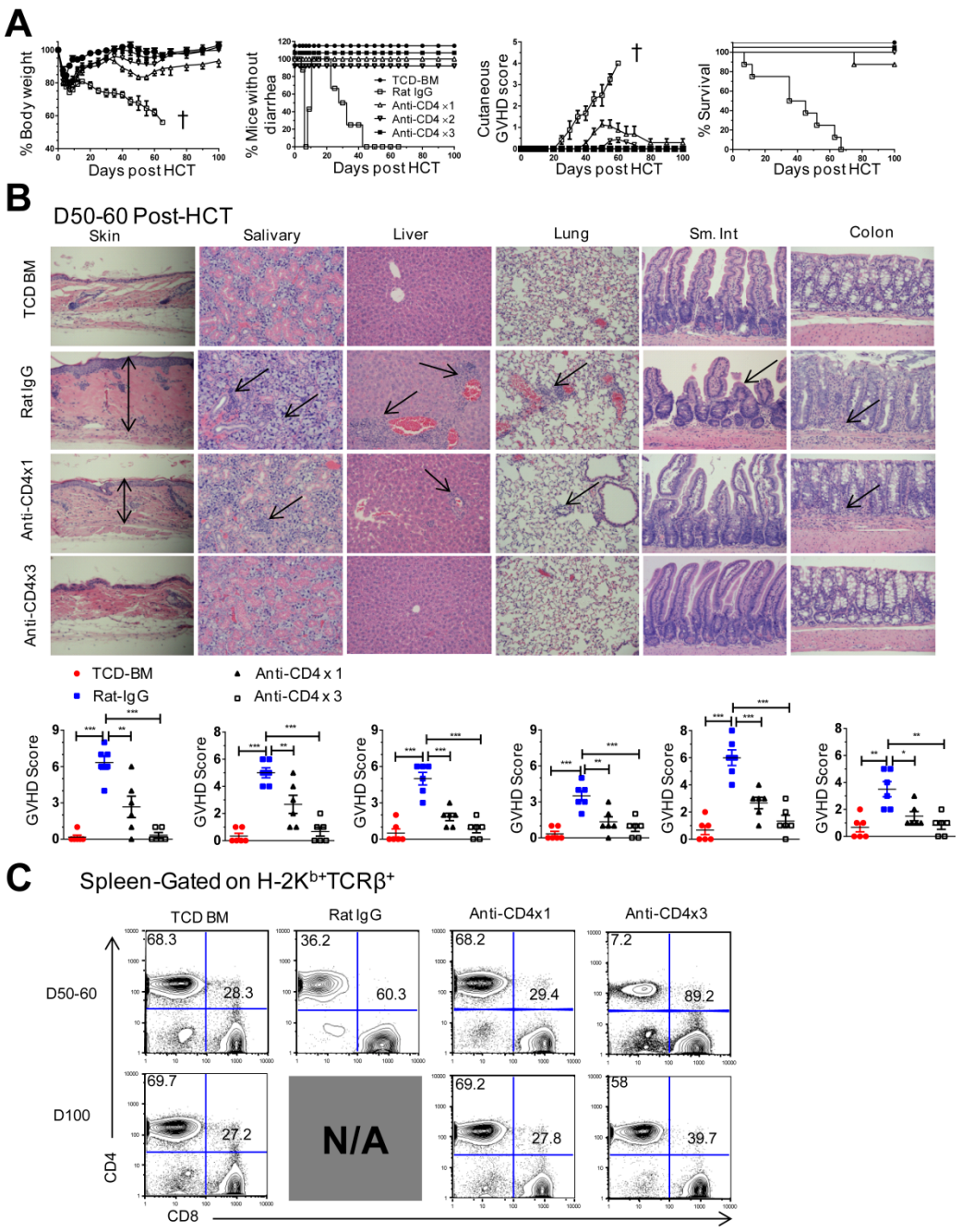

Figure S4: Three injections of anti-CD4 mAb prevent both acute and chronic GVHD.

Lethally irradiated BALB/c recipients transplanted with splenocytes ($2.5x10^6$) and TCD-BM ($2.5x10^6$) from C57BL/6 donors were given from 1 to 3 i.v. injections of rat-IgG or anti-CD4 mAb (500μg/mouse) at days 0, 14 and 28 after HCT. Recipients given TCD-BM ($2.5x10^6$) alone were used as controls. Mice were monitored for clinical signs of GVHD and survival. **(A)** Percentage

of body-weight change, percentage of recipients without diarrhea, clinical cutaneous GVHD score, and percent survival; n=8 per group combined from two replicate experiments. **(B)** At 50-60 days after HCT, histopathology of skin, salivary gland, lung, liver, small intestine and colon was evaluated. A representative photomicrograph (original magnification x 200) and mean ± SEM of histopathology scores are shown; n=6 per group. Arrows indicate the changes in GVHD recipients as compared with control recipients. **(C)** At day 50-60 and day100 after HCT, spleens were harvested from recipients, stained with anti-H-2K^{b}, TCRβ, CD4 and CD8 mAbs and analyzed for CD4^{+} T cell recovery after anti-CD4 mAb treatment. A representative panel from 1 of 4 recipients in each group is shown. Data represent mean ± SE combined from two replicate experiments. P values were calculated by unpaired two-tailed Student t tests (B) or log-rank test (A) (*p<0.05, ** p<0.01, *** p<0.001).

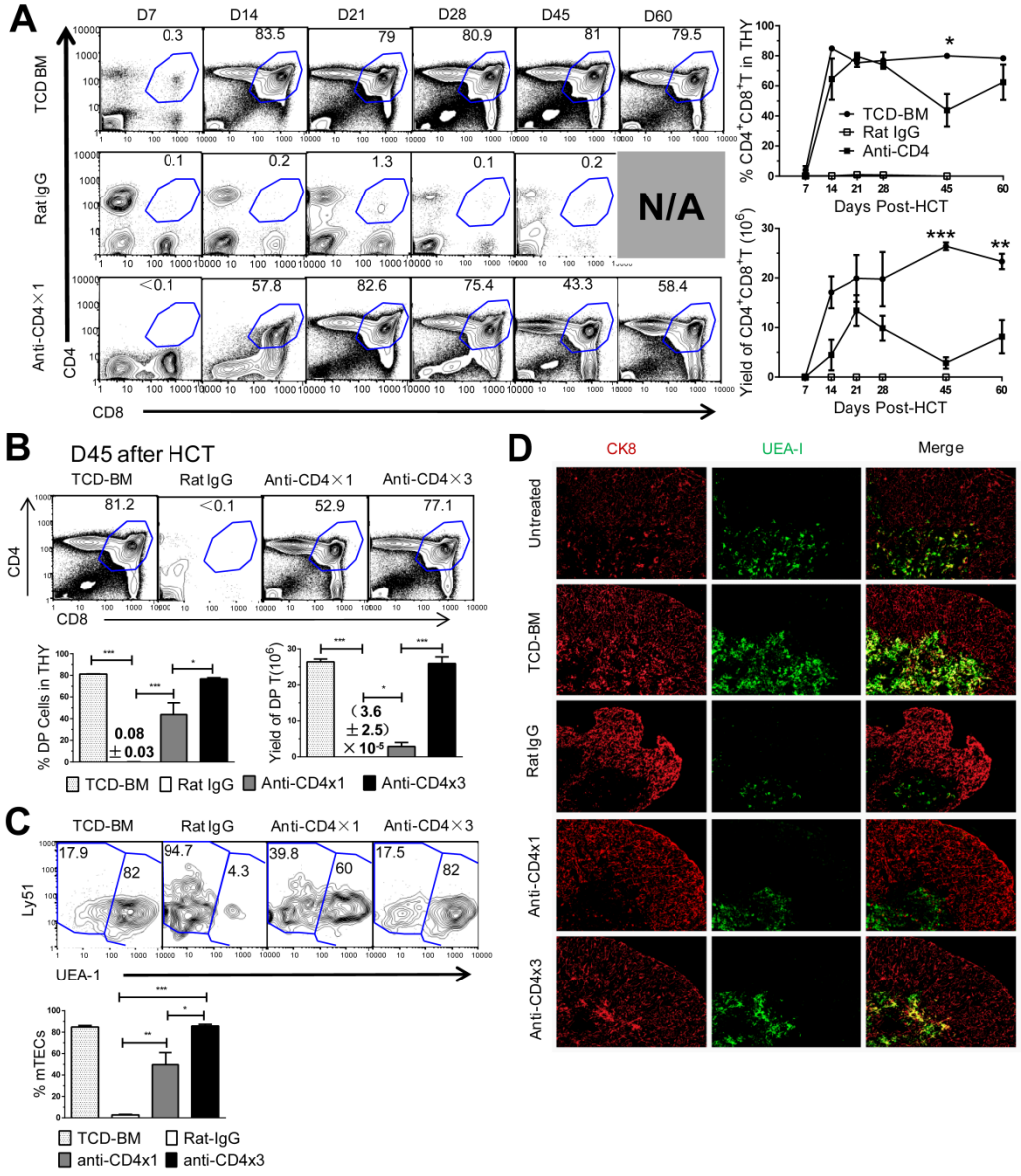

Figure S5: Depletion of donor CD4$^+$ T cells allows thymic epithelial cell regeneration.

Lethally irradiated BALB/c recipients received HCT and anti-CD4 or rat-IgG treatment as described in Figure 2. Recipients given TCD-BM were used as controls. The percentage and yield of CD4$^+$CD8$^+$ (DP) thymocytes were kinetically measured on days 7, 14, 21, 28, 45 and 60 days after HCT. Percentage, yield and histoimmunofluoresent staining of mTEC were measured on day 45. **(A)** Kinetic analysis of DP thymocytes. A representative flow cytometry pattern is shown from 1 of 4 replicate experiments; Mean ± SE of DP percentage among total thymocytes

and yield is shown. **(B)** On day 45 after HCT, percentage and yield of CD4+CD8+ thymocytes were measured and compared via flow cytometry analysis. (**C)** On day 45 after HCT, percentage of mTEC were measured and compared via flow cytometry analysis. Representative patterns and Mean ± SE (N=4) are shown (*p< 0.05, ** p< 0.01, ***p< 0.001). **(D)** Histoimmunofluorescent staining of mTEC and cTEC, using cytokeratin 8 (red, cTEC) and UEA-I (green, mTEC). A representative photomicrograph from each group is shown from 1 of 4 replicate experiments (original magnification x 200).

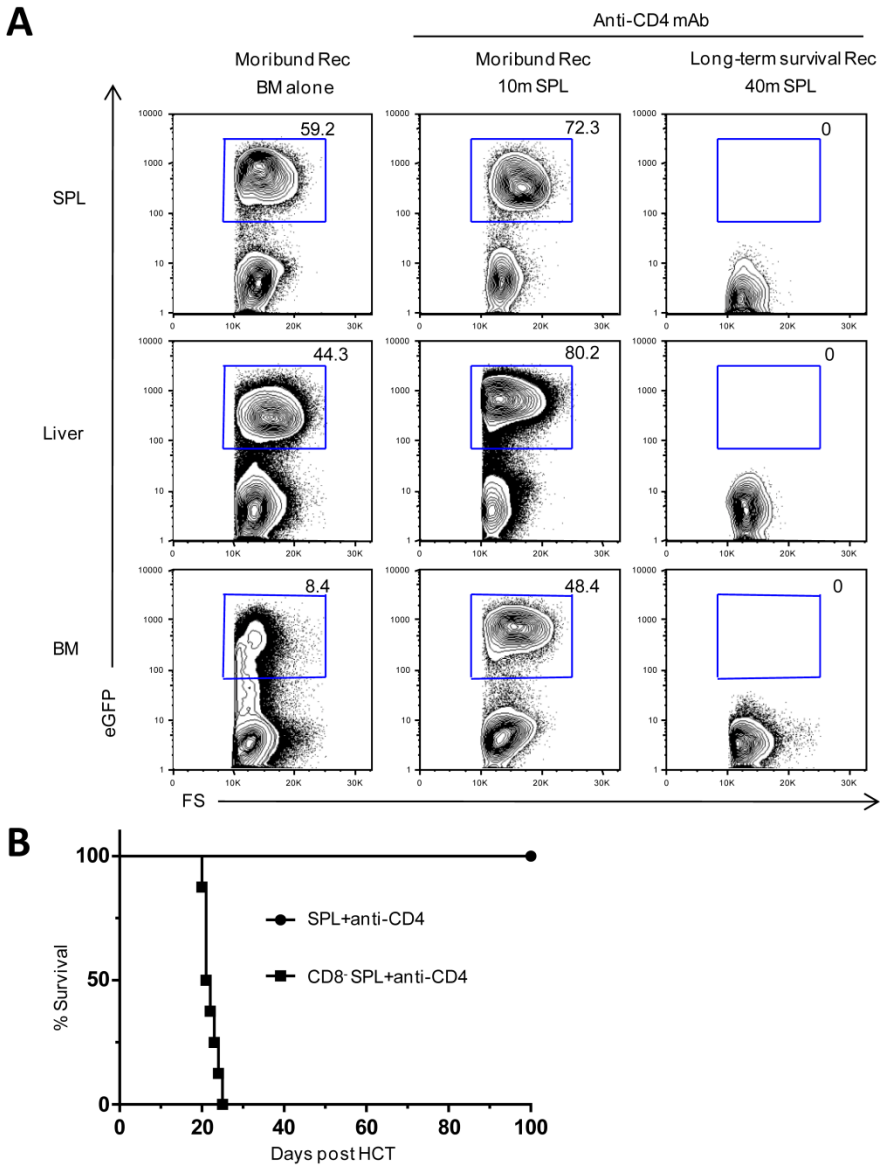

Figure S6: Depletion of donor CD4$^+$ T cells early after HCT preserves GVL in C57BL/6 recipients after transplantation from A/J donors and challenge with GVL-resistant BC-CML cells----A supplemental figure to Fig. 2. (A) Lethally irradiated C57BL/6 mice were transplanted and treated as in Fig. 2. Spleen, liver and bone marrow were harvested from recipients when they were moribund ($10x10^6$ BM alone or with $10x10^6$ spleen) or 100 days after

HCT ($40x10^6$ spleen with $10x10^6$ BM). Percentages of $eGFP^+$ BC-CML cell are shown. n=6-12 per group. **(B)** Lethally irradiated C57BL/6 mice were transplanted with $10x10^6$ BM with $40x10^6$ spleen or $CD8^+$ T cells-depleted spleen contained the same number of mononuclear cells as $40x10^6$ spleen from A/J donor, i.v.anti-CD4 mAb (500μg/mouse) on day0, 7,14,28,45 and 60 after HCT. Recipients were monitored for tumor growth and survival. Percentages of survival are shown. n=8 per group combined from two replicate experiments.

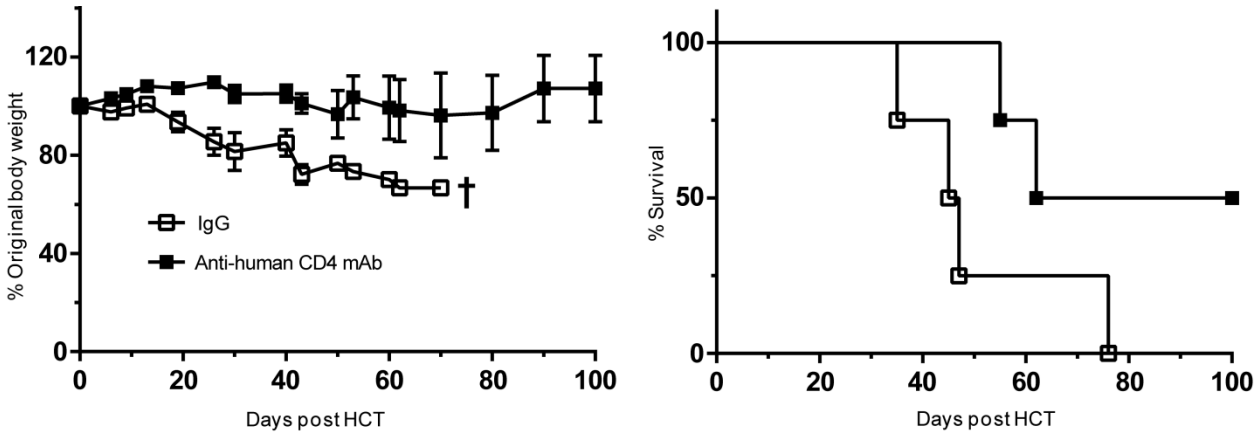

Figure S7: With 1 of 4 human PBMC donors, anti-CD4 mAb treatment only partially prevented xenogeneic GVHD. NSG recipients were transplanted with human PBMC as in Figure 3. Recipients were monitored for clinical GVHD and survival. Percentage of bodyweight change and percentage of survival is shown. n=4 per group.

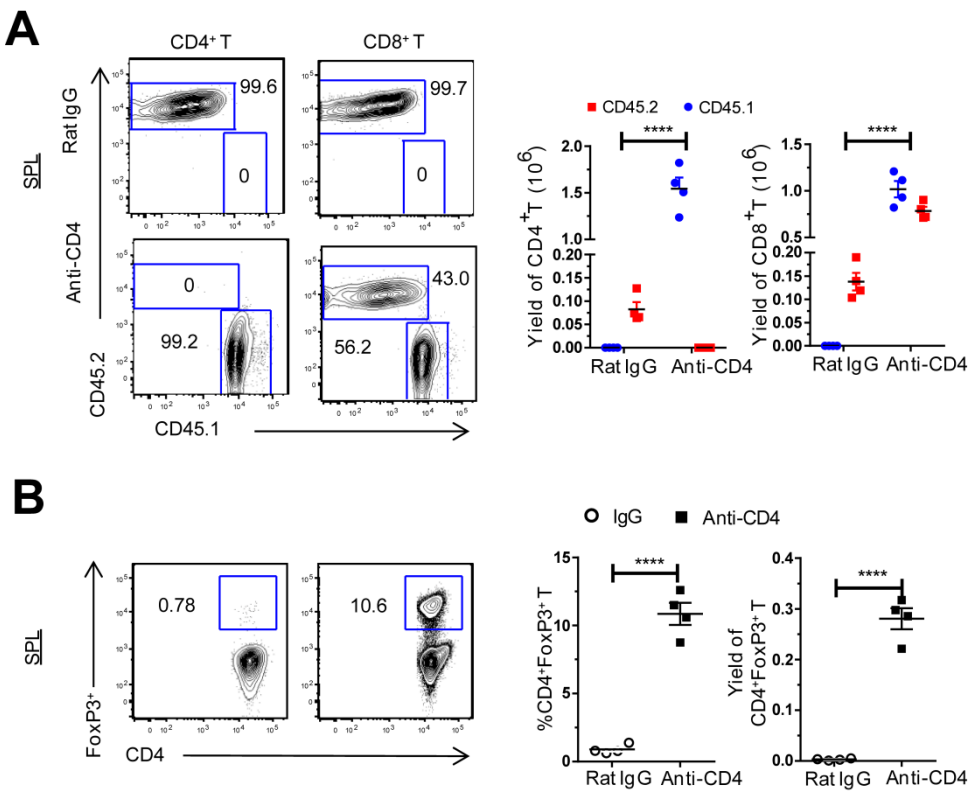

Figure S8: injected and de novo-generated T cells in IgG- or anti-CD4-treated recipients at 28 days after HCT. A supplement to Figure 4. Purified thy1.2^{+}CD45.2^{+} T cells (1x10^{6}) and CD45.1^{+}TCD-BM cells (2.5x10^{6}) were transplanted into lethally irradiated BALB/c recipients. 28 days after HCT, spleen T cells were analyzed with flow cytometry for CD45.2, CD45.1, and Foxp3. One representative pattern is shown for two replicate experiments. N=4. Data represent mean ± SE combined from two replicate experiments. P values were calculated by unpaired two-tailed Student t tests (****p< 0.0001).

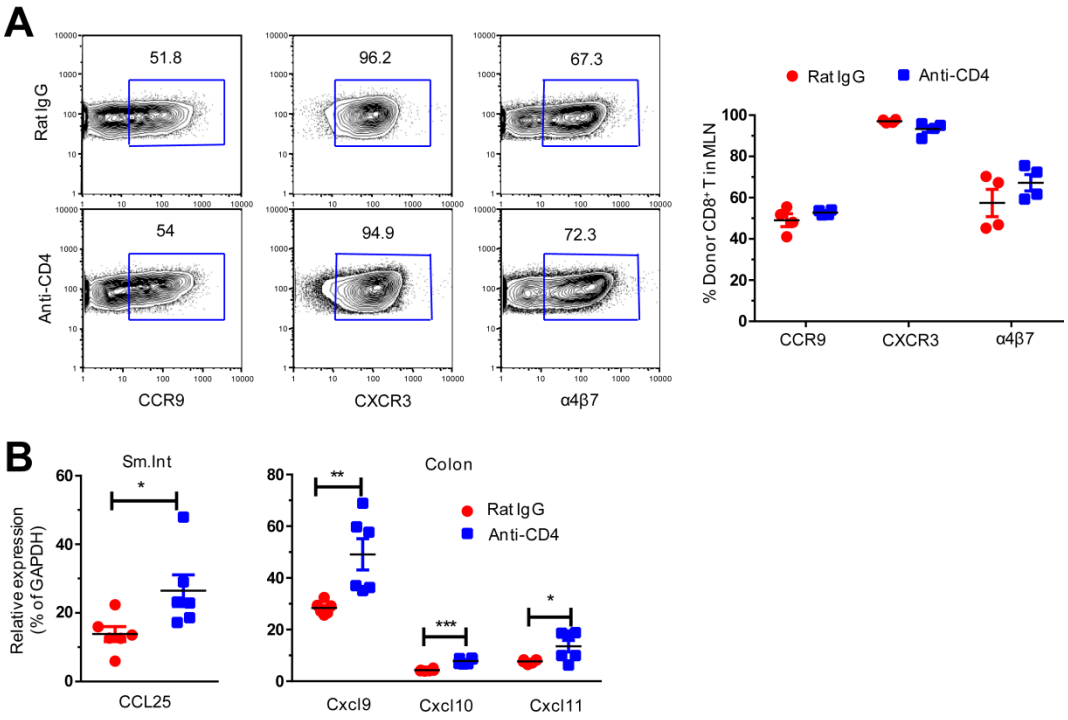

Figure S9: In vivo depletion of CD4$^+$ T cells does not affect donor CD8$^+$ T cell homing and chemokine receptor expression. Lethally irradiated BALB/c recipients transplanted with splenocytes ($2.5x10^6$) and TCD-BM ($2.5x10^6$) from C57BL/6 donors were given one i.v. injection of rat-IgG or anti-CD4 mAb (500µg/mouse) at the time of HCT. At day 7 after HCT, spleen, mesenteric lymph nodes (MLN), small intestine (Sm. Int) and colon of recipients were harvested. **(A)** Expression of CCR9, CXCR3 and α4β7 on donor CD8$^+$T cells of MLN. Mean ± SEM; n=4 per group from 2 replicate experiments. **(B)** Expression of chemokine mRNA in Sm. Int and colon was measured by real-time RT-PCR. Expression of CCL25 in Sm. Int. tissue and CXCL9, CXCL10, CXCL11 in colon tissue relative to the house keeping gene GAPDH are shown. n=6 per group. Data represent mean ± SE combined from two replicate experiments. P values were calculated by unpaired two-tailed Student t tests (*$p < 0.05$, ** $p < 0.01$).

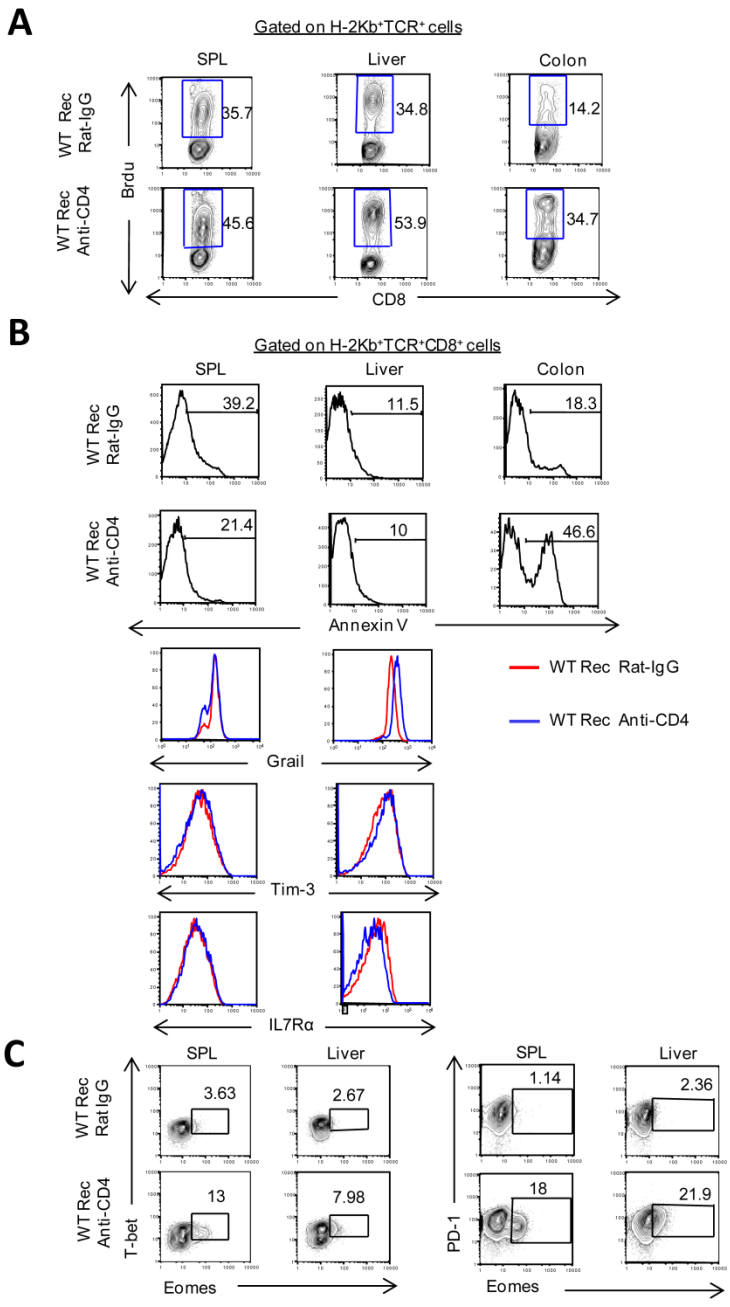

Figure S10: A supplement to Figure 6 showing representative flow cytometry patterns.

A representative panel from 1 of 4 recipients in each group is shown.

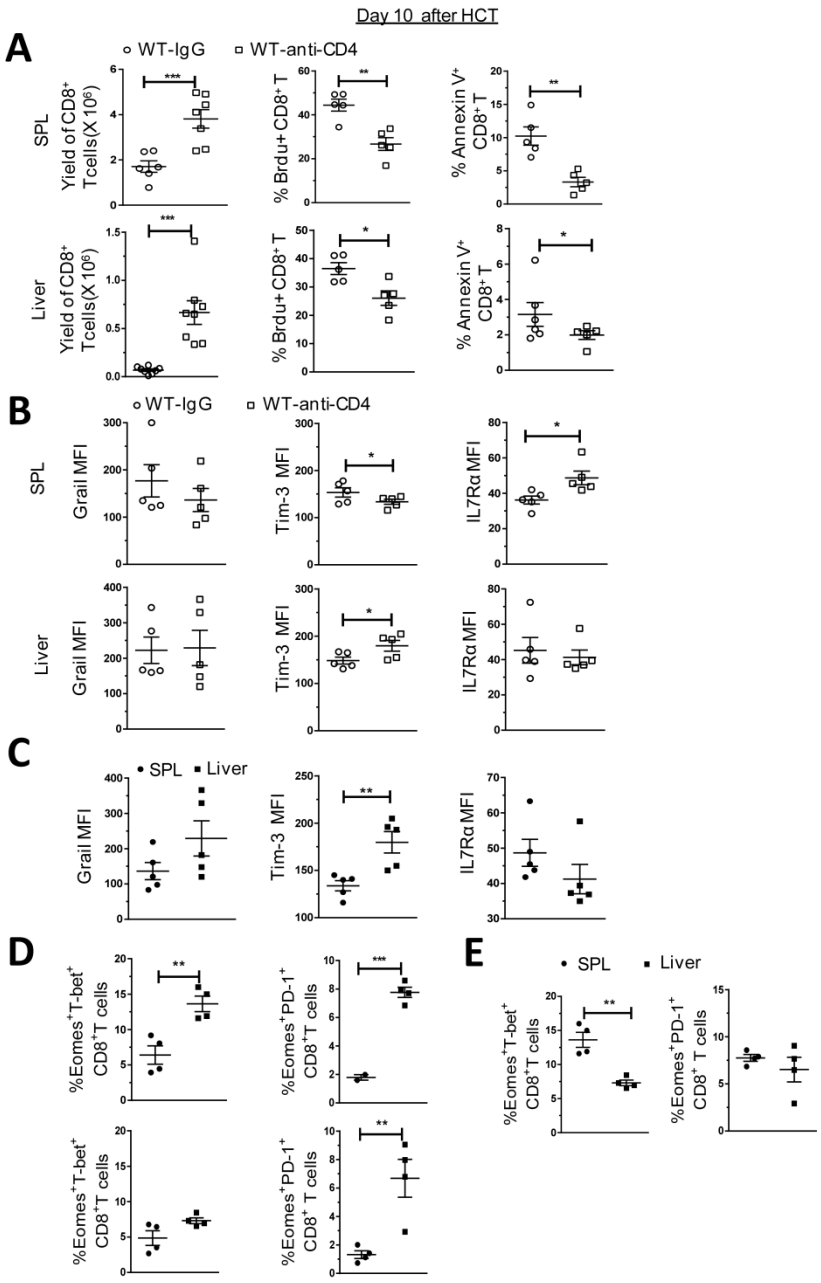

Figure S11: Depletion of donor CD4$^+$ T cells augments donor CD8$^+$ T anergy/exhaustion in the liver, but not in the spleen on day 10 after HCT. Lethally irradiated WT BALB/c mice were transplanted and treated at day 0 with IgG or anti-CD4 mAb as in Figure 5. On day 10 after HCT, spleen and liver from recipients were harvested. **(A)** Yield and BrdU and Annexin V staining of CD8$^+$ T cells in spleen and liver. (n=4-8 for each group from 2 replicate experiments) **(B-C)** Grail, Tim3 and IL7Rα expression by donor CD8$^+$ T cells in spleen and liver (n=5 for each

group from 2 replicate experiments). **(D-E)** Percentages of $Eomes^{+}T\text{-}bet^{+}$ and $Eomes^{+}PD1^{+}$ donor $CD8^{+}$ T cells in spleen and liver (n=4 for each group). Data represent mean ± SE combined from two replicate experiments. P values were calculated by unpaired two-tailed Student t tests (*p<0.05, ** p<0.01, *** p<0.001).

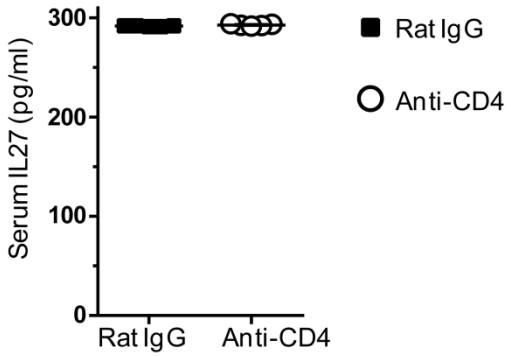

Figure S12: Serum IL-27 concentrations in Rat IgG- or anti-CD4-treated recipients. A supplement to Figure 4. HCT was performed as in Figure 4, and 7 days after HCT, serum IL-27 concentrations were measured by ELISA. Mean ± SE of 4 replicate experiments is shown.

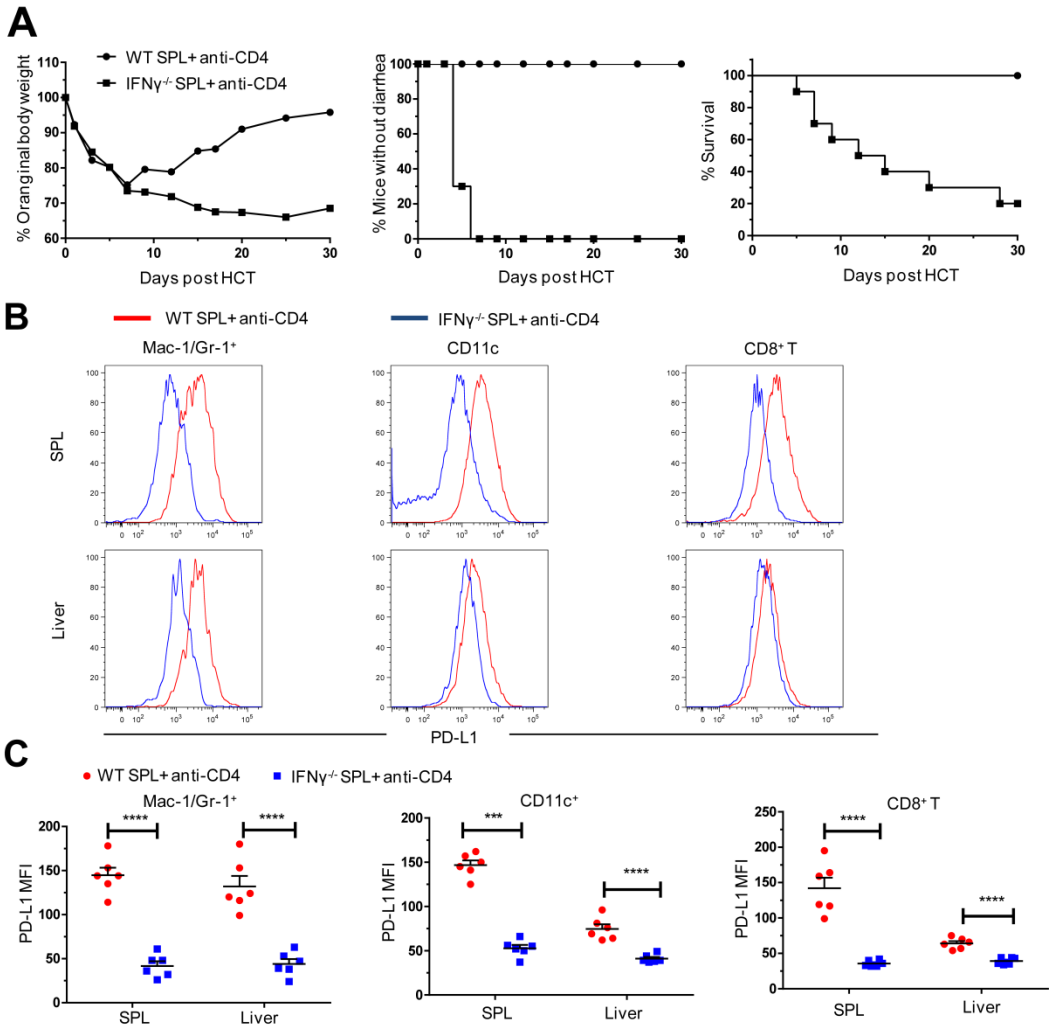

Figure S13: Anti-CD4 treatment failed to prevent acute GVHD in recipients given IFN-γ$^{-/-}$ donor transplants. Lethally irradiated BALB/c recipients transplanted with splenocytes (5x10^6) and TCD-BM (2.5x10^6) from wild-type or IFN-γ$^{-/-}$ C57BL/6 donors, and then given a single i.v. injection of anti-CD4 mAb (500μg/mouse) at the time of HCT. Recipients were monitored for clinical signs of GVHD, including body weight change, diarrhea, hair loss, and survival. **(A)** Percentage of body weight change, percentage of recipients without diarrhea, clinical cutaneous GVHD score, and percentage of surviving recipients. n=10 per group combined from two replicate experiments. **(B)** 7 days after HCT, spleen and liver CD8$^+$ T, CD11c$^+$ DC and Mac-1/Gr-1$^+$ myeloid cells were analyzed for surface PD-L1. One representative pattern is shown of

6 replicate experiments. **(C)** Mean ± SE of PD-L1 MFI, n=6. Data represent mean ± SE combined from two replicate experiments. P values were calculated by unpaired two-tailed Student t tests. (*p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

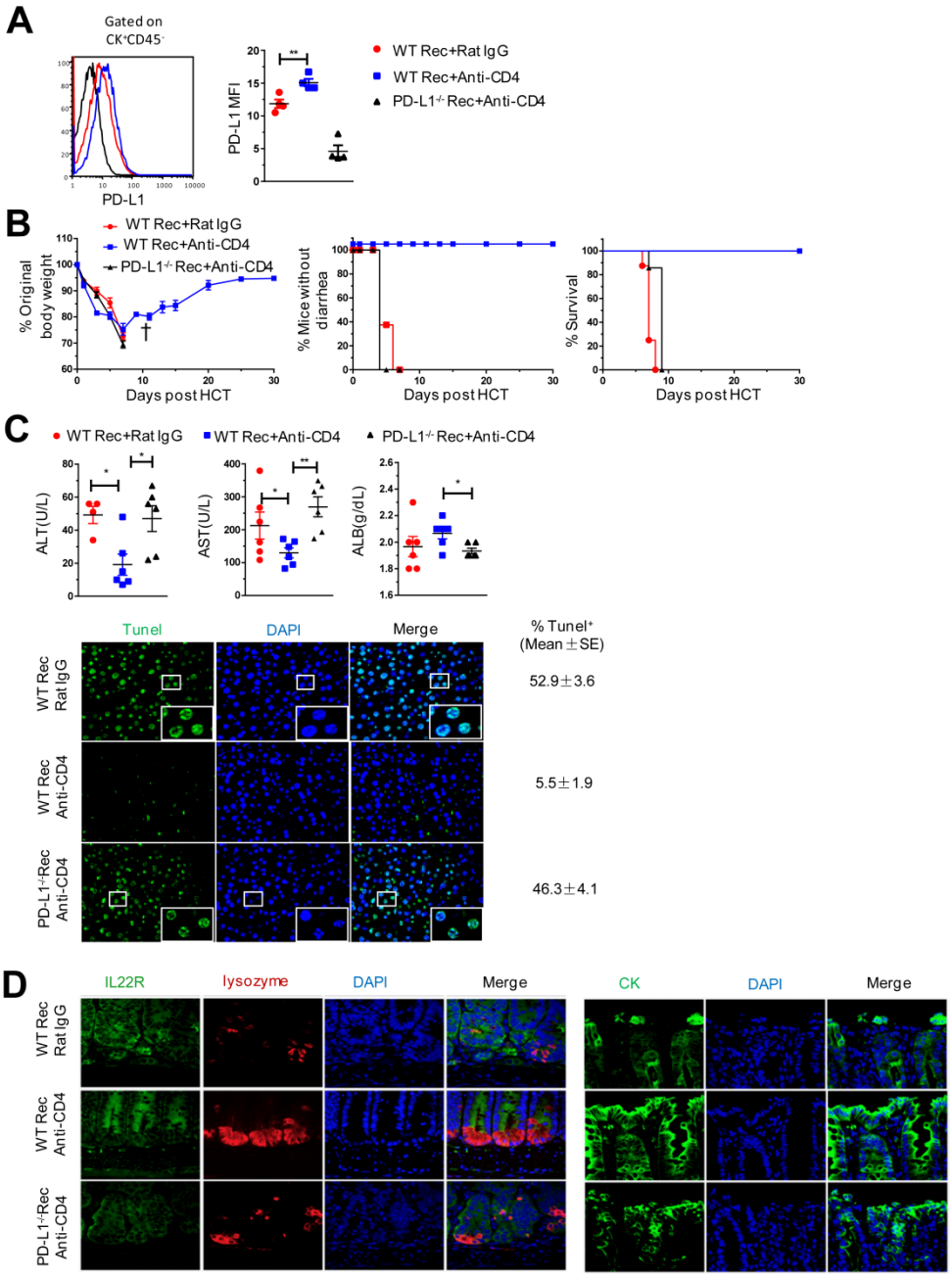

Figure S14: Depletion of donor CD4^{+} T cells prevents liver damage and protects Paneth cells and colon epithelial cells through a mechanism that depends on PD-L1 expression in host tissue. WT or PD-L1$^{-/-}$ BALB/c recipients transplanted with splenocytes (5x10^{6}) and TCD-BM cells from C57BL/6 donor were injected with anti-CD4 mAb (500µg/mouse) on day 0; as a control, WT BALB/c recipients were injected with rat-IgG (500µg/mouse) on day 0 and

transplanted with splenocytes and TCD-BM. Recipients were monitored for clinical signs of acute GVHD and survival († indicates death of all recipients in a group). **(A)** Colon epithelial cells from recipients 7 days after HCT were stained with anti-CK, CD45 and PD-L1 mAbs; representative pattern of PD-L1 expression on $CK^{+}CD45^{-}$ colonial epithelial cells and MFI of PD-L1 are shown (n=4 per group, Mean ± SEM). **(B)** Percentage of body weight change, percentage of recipients without diarrhea and percentages of surviving recipients. n= 8 per group from two replicate experiments**. (C)** Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and albumin (ALB) concentrations on day 7 in WT and PD-$L1^{-/-}$ recipients treated with anti-CD4 mAb (n=6 per group from 3 experiments, Mean±SEM); Tunel staining for hepatocyte apoptosis assay. A representative Immunofluorescent photomicrograph (original magnification x400) is shown (n=4 per group; Mean ± SEM). **(D)** Immunofluorescent staining was performed on small intestine and colon as described in Figure 5. A representative photomicrograph (original magnification x400) is shown (n=4 per group). The same representative imagines for rat-IgG-treated WT-recipients and anti-CD4-treated recipients shown in Figure 5 are shown again in comparison with representative imagines for anti-CD4-treated PD-$L1^{-/-}$ recipients. Data represent mean ± SE combined from 2-3 independent experiments. P values were calculated by unpaired two-tailed Student t tests (*p<0.05, ** p<0.01, ***p<0.001).

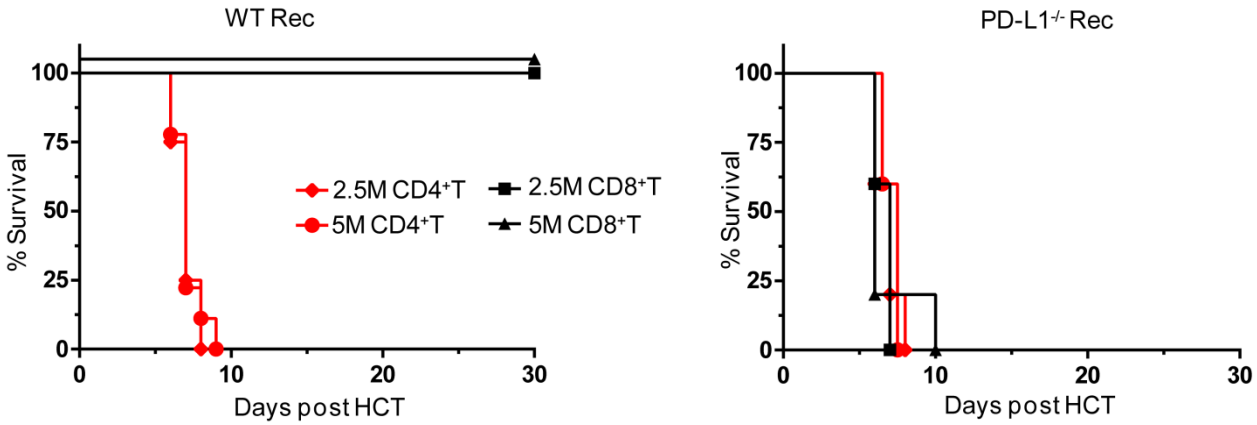

Figure S15: Similar to CD4$^+$ T cells, CD8$^+$ T cells induced lethal GVHD in PD-L1$^{-/-}$ recipients. Lethally irradiated WT and PD-L1$^{-/-}$ BALB/c recipients were injected with purified CD4$^+$ or CD8$^+$ T cells (2.5 and 5x10^6) and TCD-BM (2.5x10^6) from C57BL/6 donors. Recipient survival was compared for up to 30 days. N=8 combined from two replicate experiments.

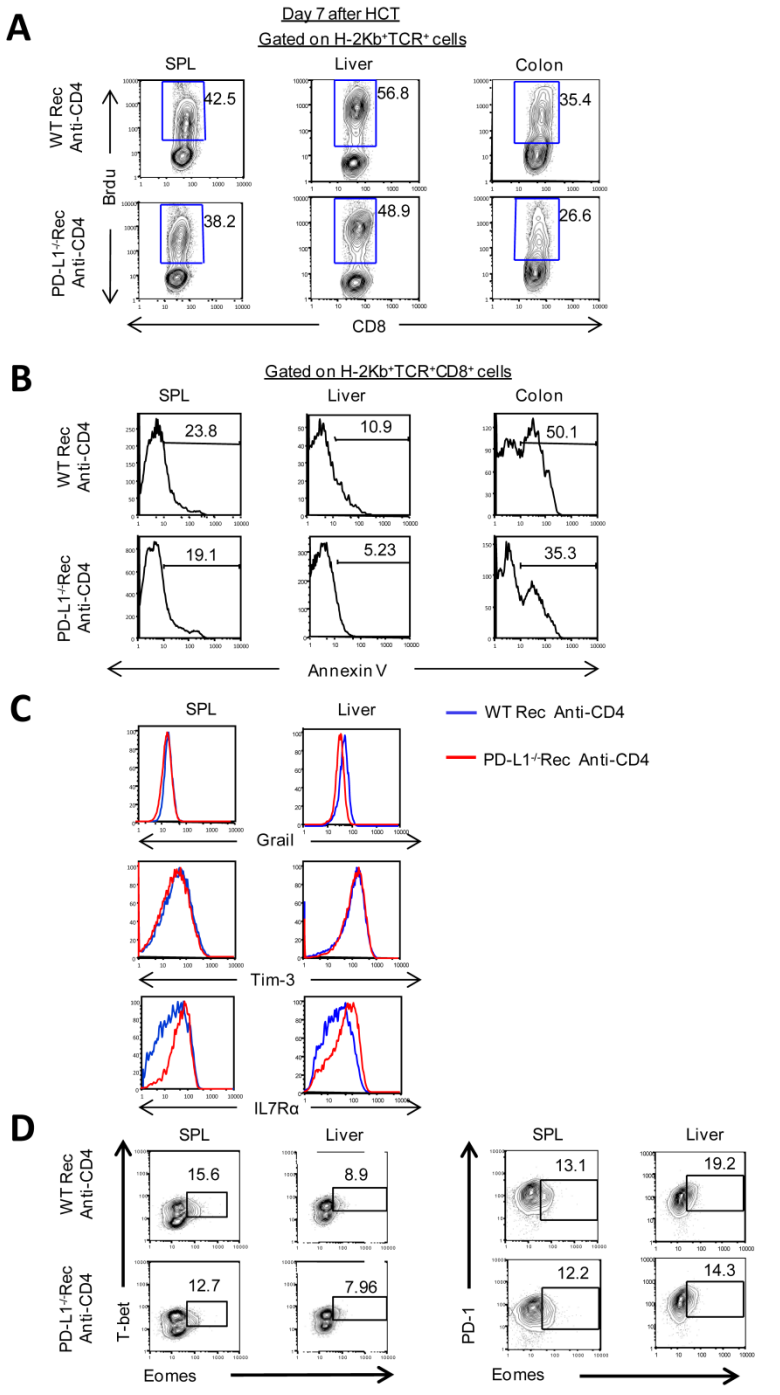

Figure S16: a supplement to Figure 7 showing representative flow cytometry patterns.

A representative panel from 1 of 4-6 recipients in each group is shown.

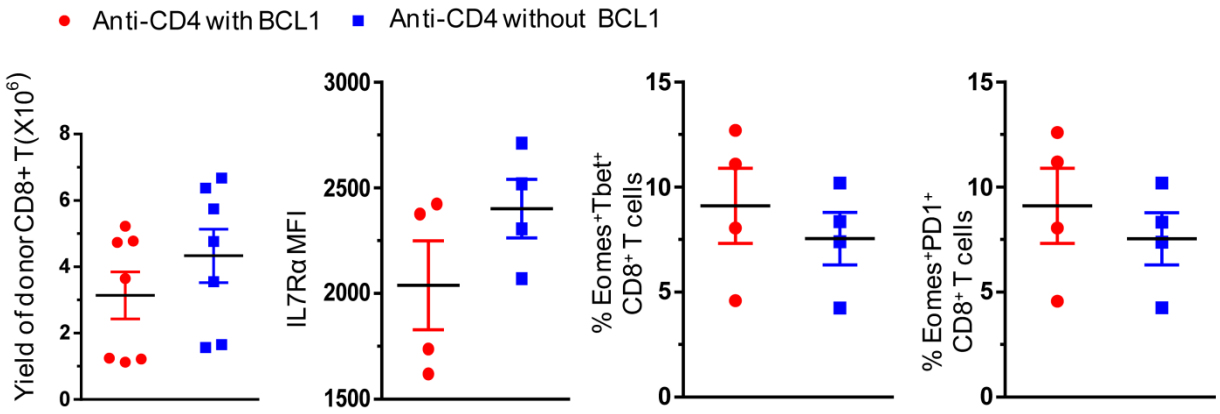

Figure S17: A supplement to Fig. 1. HCT was set up as described in Fig. 1. Seven days after HCT, $CD8^+$ T cells from the liver were analyzed for their expression of IL-7Rα, Eomes, T-bet, and PD-1. Mean ± SE of MFI is shown for 4 replicate experiments.

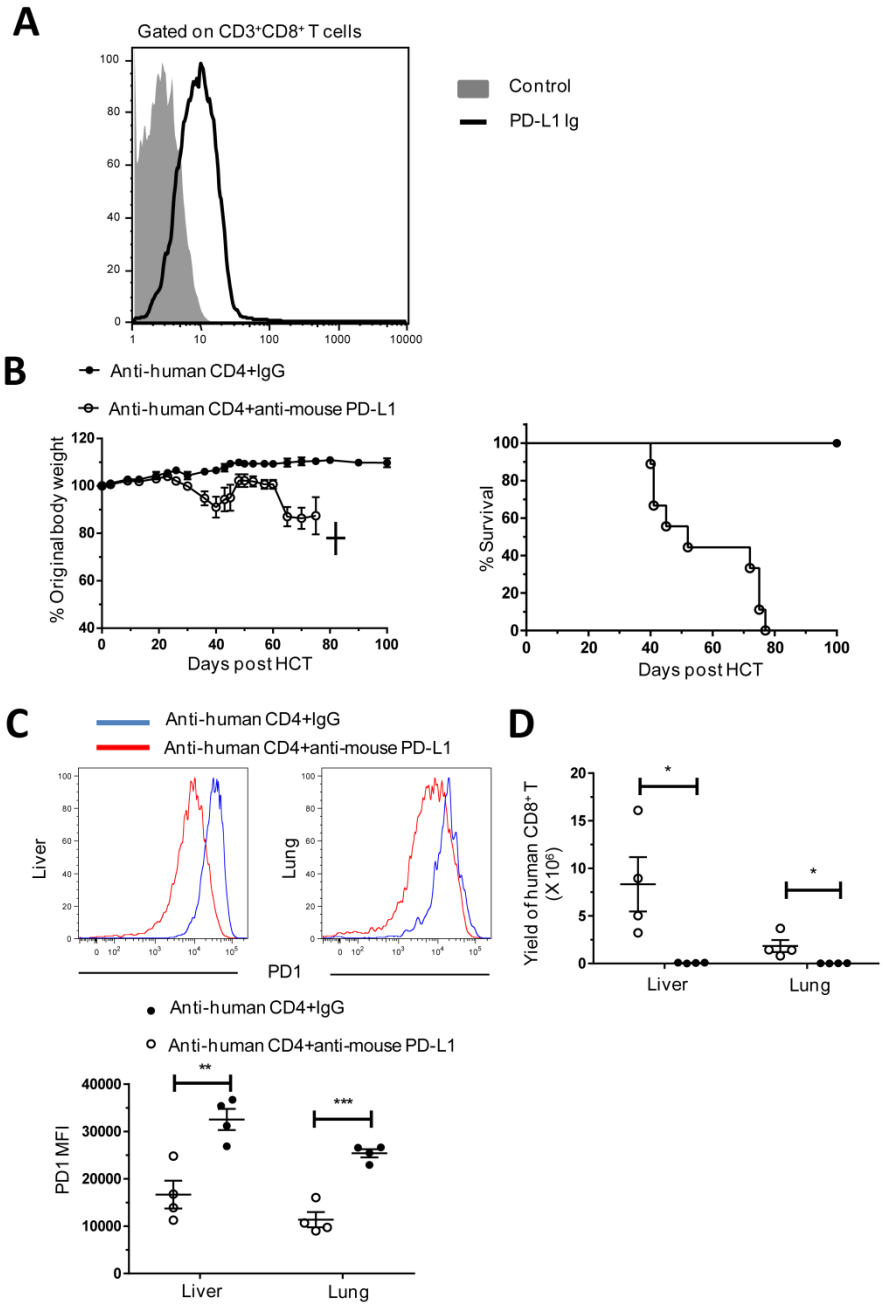

Figure S18: Blocking anti-PD-L1 treatment led to xenogeneic GVHD in anti-CD4-treated NSG mice. **(A)** Human $CD8^+$ T cells bind to mouse PD-L1-Ig. **(B)** PBMC from 3 healthy donors were distributed into 15 NSG mice with 5 mice/donor and $20x10^6$ PBMC/mouse. All mice were treated with anti-human CD4 (200µg/mouse, twice weekly for 4 weeks), and 9 mice (groups of 3 mice given cells from each of the 3 donors) were treated with anti-mouse PD-L1

(5μg/g body weight, twice weekly for 4 weeks), and the remaining 6 mice (groups of 2 mice given cells from each of the 3 donors) were treated with control IgG. Mice were monitored for clinical signs of GVHD, bodyweight and survival. All anti-PD-L1-treated mice showed bodyweight loss and died by 80 days after HCT, while control mice showed no signs of GVHD. **(C and D)** 60 days after HCT, moribund GVHD mice and control GVHD-free mice were analyzed for $CD8^+$ T cell percentage and yield in the liver and lung as well as $CD8^+$ T expression of PD-1. A representative flow cytometry patterns of 1 of 4 mice in each group and Mean ± SE of MFI and yield of $CD8^+$ T cells from the liver and lung are shown. P values were calculated by unpaired two-tailed Student t tests (*p<0.05, ** p<0.01, ***p<0.001).

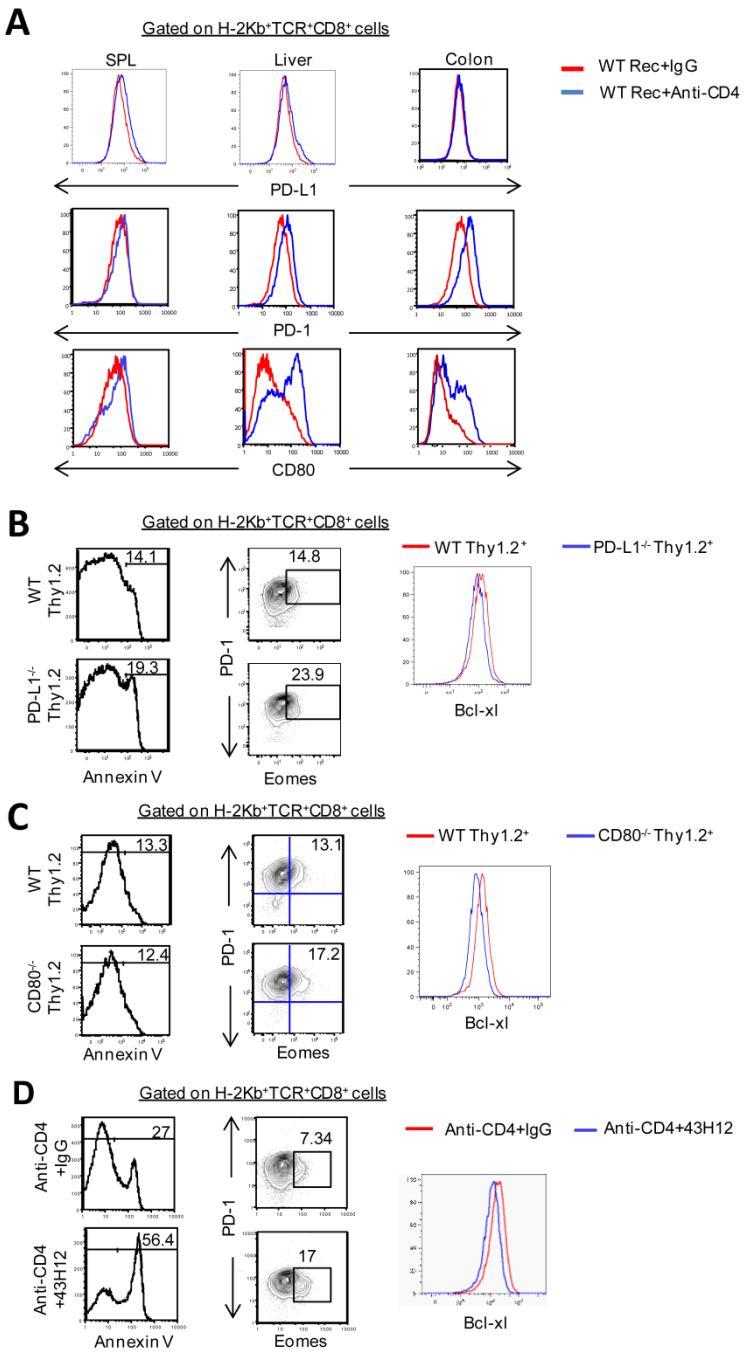

Figure S19: a supplement to Figure 8 showing representative flow cytometry patterns.

A representative panel from 1 of 4-6 recipients in each group is shown.

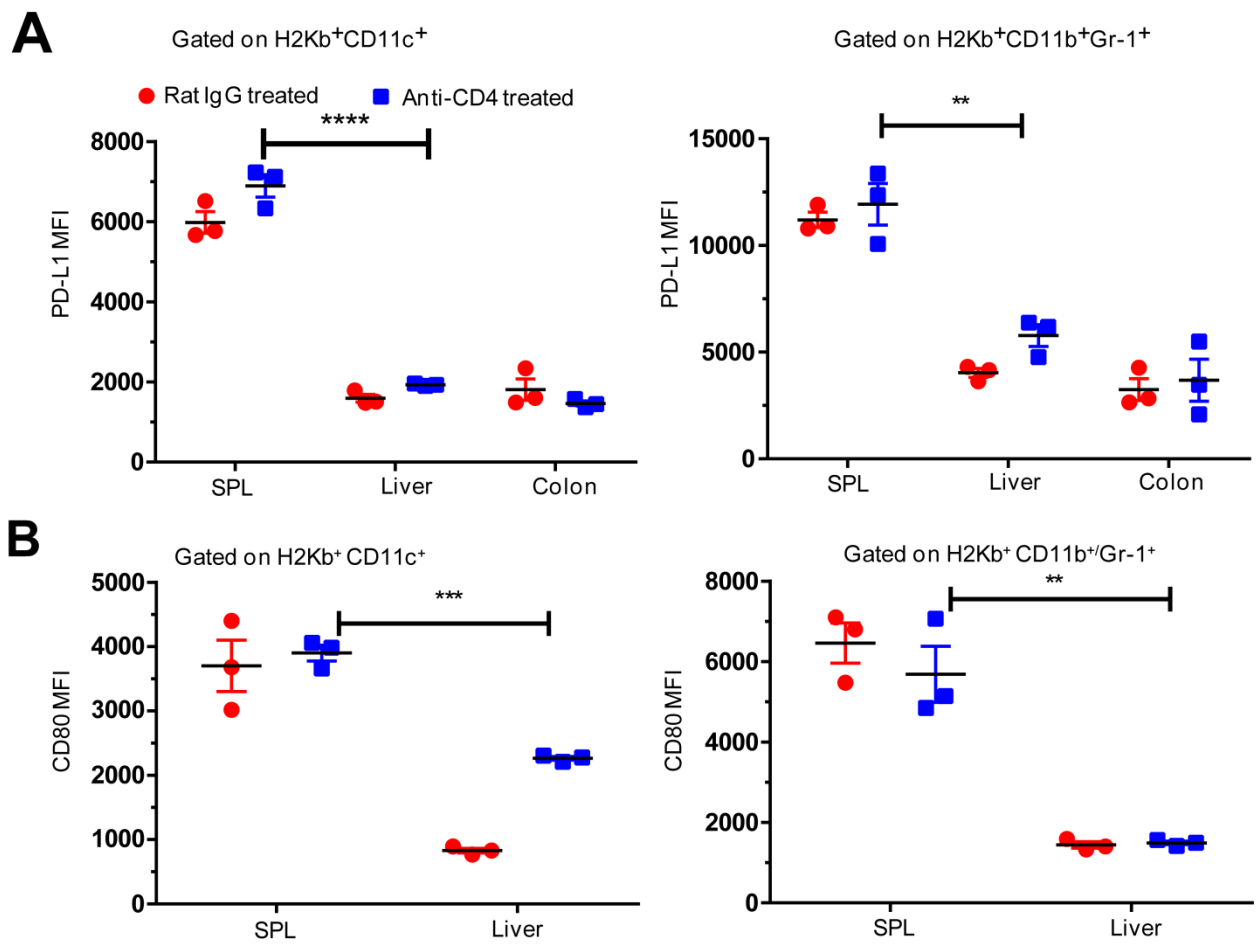

Figure S20: A supplement to Figure 6----non-T hematopoietic cell expression of PD-L1 and CD80. Seven days after HCT, donor-type CD11c$^+$ DCs and Mac-1/Gr-1$^+$ myeloid cells from the spleen, liver and colon were analyzed for expression of PD-L1 and CD80. Mean ± SE of MFI combined from 3 replicate experiments is shown. P values were calculated by unpaired two-tailed Student t tests (*p<0.05, ** p<0.01, ***p<0.001, ****p<0.0001).