

Figure S1

Figure S1. WT vs. IFN- γ R^{-/-} donor T-cells have elevated expression of PD-1 and PD-L1 during GVHD. (A and B) Lethally irradiated BALB/c recipients were infused with 10⁷ WT B6 BM cells plus 6×10⁶ WT B6 or IFN- γ R^{-/-} splenocytes. Mice were sacrificed on d5 post-BMT (n = 5–6/group) and splenic donor T-cells were analyzed by flow cytometry for PD-1 (A) or PD-L1 (B) expression. (C) Lethally irradiated B6 or BALB/c recipients were infused with 10⁷ WT B6 BM cells plus 6×10⁶ B6 Ly5.2 (CD45.1⁺) splenocytes. Mice were sacrificed on d5 post-BMT (n = 5/group) and splenic donor T-cells were analyzed by flow cytometry for PD-L2 expression. Naive B6 Ly5.2 mice (n = 5) splenocytes were also analyzed by flow cytometry to detect the percentage of T-cells expressing PD-L2. (A–C) Data are representative of 2 independent experiments. Data represent mean ± SEM and *p* values were calculated by 2-tailed *t*-test (A–C). * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

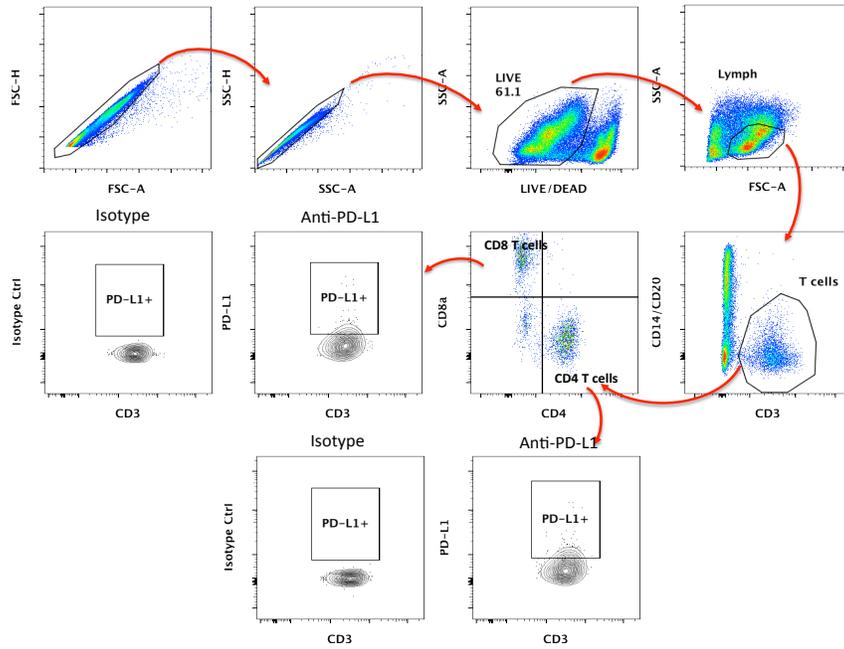
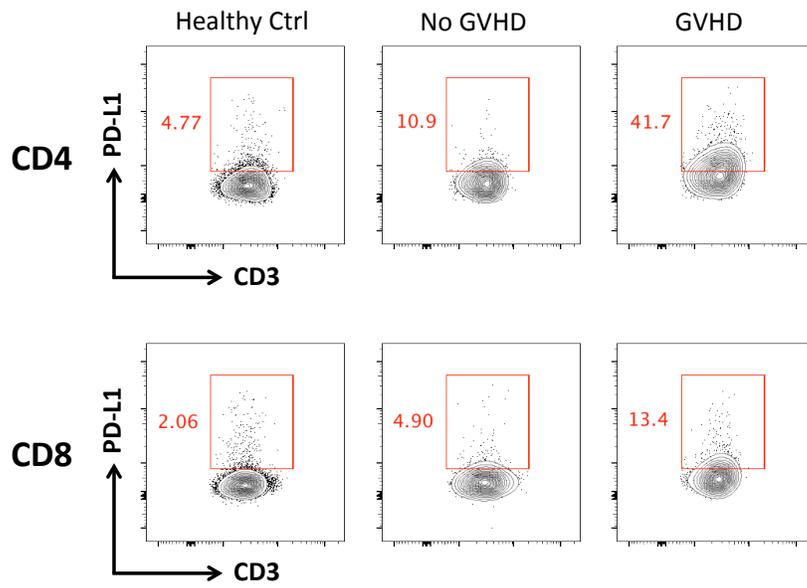
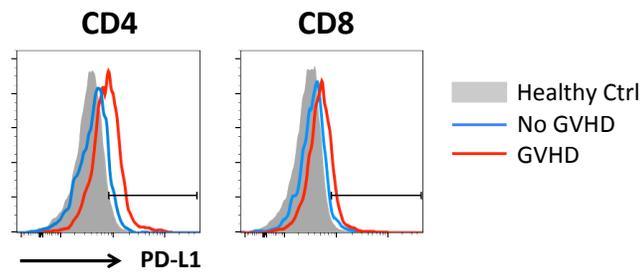
A**B****C**

Figure S2

Figure S2. T-cells have elevated expression of PD-L1 during GVHD. (A) Gating strategy for measuring PD-L1 expression on human CD4 and CD8 T-cells. PBMC were collected from healthy volunteers (Healthy Ctrl) and from patients after allogeneic BMT with or without GVHD at the time of collection (Supplemental Table 1). Cells were then stained for PD-L1 expression as described in *Methods*. (B) PD-L1⁺ gate are shown in red and numbers indicate the percentages of PD-L1⁺ cells within corresponding CD4 and CD8 T-cell gates. (C) The histograms show PD-L1 expression on CD4 or CD8 T-cells.

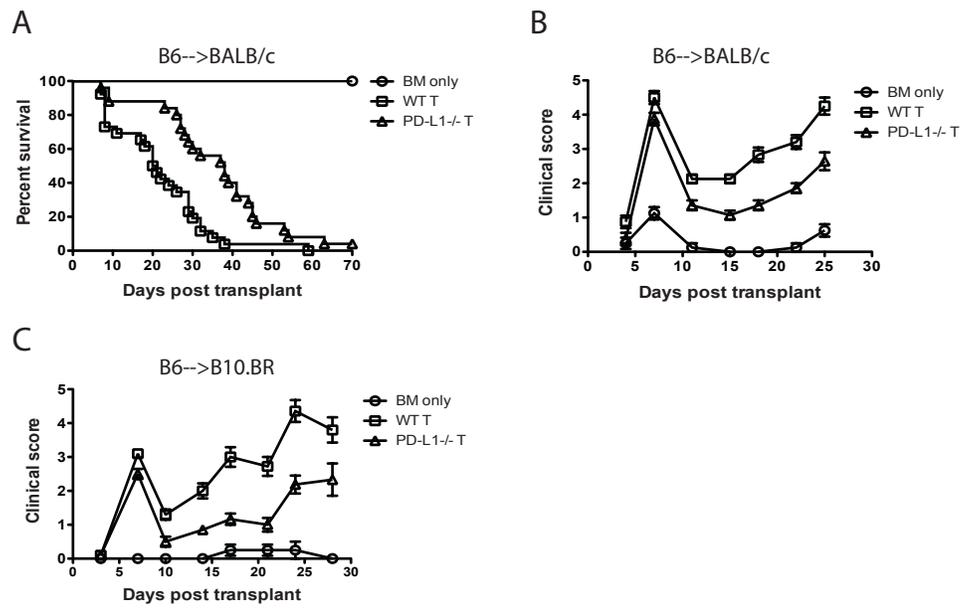


Figure S3

Figure S3. GVHD-induced PD-L1 up-regulation on donor T cells contributes to lethality. (A and B) Lethally irradiated BALB/c recipients were infused with 10^7 WT B6 BM cells alone or with 2×10^6 WT B6 or PD-L1^{-/-} purified T-cells. (A) Kaplan-Meier survival plot represents pooled data from 3 independent experiments (n = 24–26/group; recipients of WT vs. PD-L1^{-/-} donor T cells, P = 0.0005). (B) Transplanted mice were evaluated for clinical GVHD (n = 8/group). Recipients of WT vs. PD-L1^{-/-} donor T cells, P < 0.05 on d7; P < 0.01 on d11 and d25; P < 0.001 on d15, d18 and d22. Data are representative of 2 independent experiments. (C) Lethally irradiated B10.BR mice were infused with 10^7 WT B6 BM cells alone or with 3×10^6 WT B6 or PD-L1^{-/-} purified T-cells. Transplanted mice were evaluated for clinical GVHD (n = 8/group). Recipients of WT vs. PD-L1^{-/-} donor T-cells, P < 0.05 on d28; P < 0.01 on d7 and d10; P < 0.001 on d14, d17, d21 and d24. Data are representative of 2 independent experiments. Data represent mean \pm SEM (B and C), and *p* values were calculated by Log-rank test (A) or 2-tailed *t*-test (B and C).

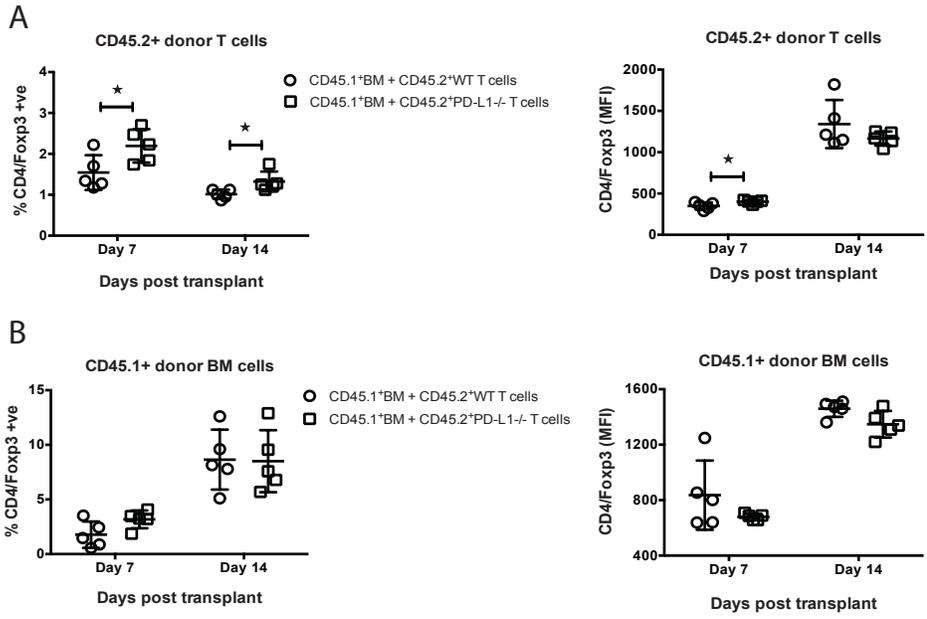


Figure S4

Figure S4. Treg cell reconstitution after allogeneic BMT. Lethally irradiated BALB/c recipients were infused with 10^7 B6 Ly5.2 (CD45.1⁺) BM cells plus CD25-depleted 1×10^6 WT B6 (CD45.2⁺) or PD-L1^{-/-} (CD45.2⁺) purified T-cells. The frequencies and mean fluorescence intensity (MFI) of splenic CD4⁺Foxp3⁺ cells derived from donor T-cells (**A**) or donor BM cells (**B**) are shown (n = 5 mice/group). (**A** and **B**) Data were obtained from one experiment. Data represent mean \pm SEM and *p* values were calculated by 2-tailed *t*-test. * *P* < 0.05.

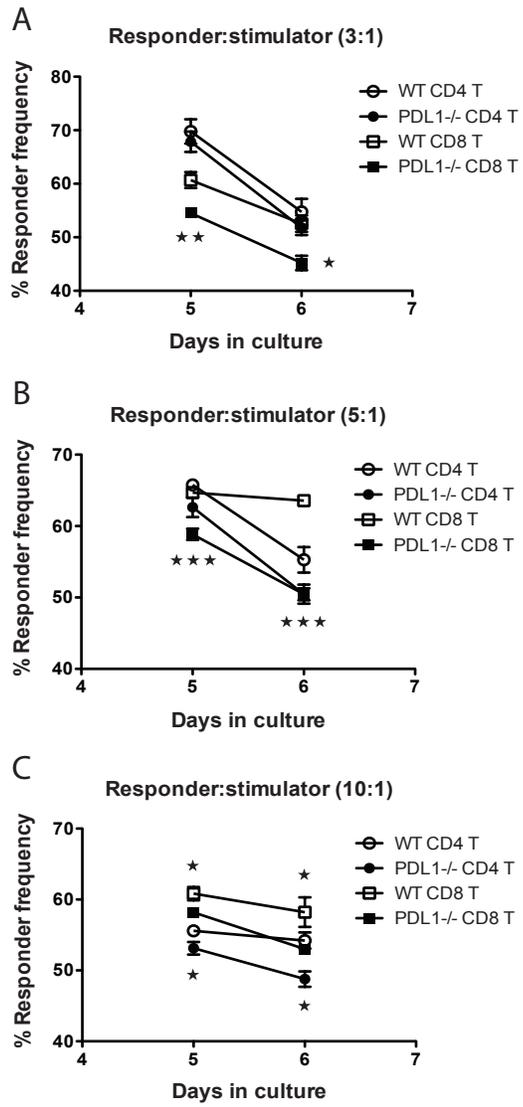


Figure S5

Figure S5. WT vs. PD-L1^{-/-} donor T-cells have increased proliferation in vitro.

(A–C) DCs were generated from BM of BALB/c mice and on d7 of culture DCs were harvested, irradiated and used as stimulators. Purified T-cells from WT B6 or PD-L1^{-/-} mice were labeled with CFSE and used as responder cells. MLR was performed by co-culturing responder and stimulator cells at the indicated ratios. Cells were analyzed by flow cytometry for CFSE dilution on day 5 and day 6 of culture. Data are representative of two independent experiments. (A) $P < 0.01$, d5 WT vs. PD-L1^{-/-} CD8 T; $P < 0.05$, d6 WT vs. PD-L1^{-/-} CD8 T. (B) $P < 0.001$, d5 WT vs. PD-L1^{-/-} CD8 T; $P < 0.001$, d6 WT vs. PD-L1^{-/-} CD8 T. (C) $P < 0.05$, d5 WT vs. PD-L1^{-/-} CD4 and CD8 T; $P < 0.05$, d6 WT vs. PD-L1^{-/-} CD4 and CD8 T. Data represent mean \pm SEM and p values were calculated by 2-tailed t -test (A–C). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Supplemental Table 1: Patients characteristics

| ID | Age | Gender | Donor source | Disease | Conditioning | GVHD prophylaxis | aGVHD onset | GVHD grade | CMV status (R/D) | CMV reactivation (Y/N) |
|-------------------|-----|--------|----------------------|-----------------|------------------|------------------|-------------|--------------------------|------------------|------------------------|
| GVHD cases | | | | | | | | | | |
| 026 | 41 | M | Haplo brother BM | AML CR2 | RIC (Flu/Cy/TBI) | PTCy/Tac/MMF | +15 | Grade III (LGI III only) | +/- | N |
| 031 | 43 | M | MUD Male PBPC | AML CR2 | RIC (Flu/Bu) | ATG/Tac/MTX | +41 | Grade II (skin III only) | (-/-) | N |
| 041 | 49 | F | MUD Male PBPC | MDS | RIC (Flu/Bu) | ATG/Tac/MTX | +21 | Grade II (skin III only) | (+/-) | Y (day +38) |
| 043 | 57 | M | Haplo Son BM | CLL | RIC (Flu/Cy/TBI) | PTCy/Tac/MMF | +26 | Grade II (UGI I/Skin I) | (-/-) | N |
| 063 | 57 | F | MUD Male PBPC | AML CR1 | RIC (Flu/Bu) | ATG/Tac/MTX | +33 | Grade III (LGI II) | (-/-) | N |
| Controls | | | | | | | | | | |
| 016 | 63 | F | HLA ID Sib Male PBPC | AML CR1 | RIC (Flu/Bu) | Tac/MTX | +34 | N/A | (+/+) | N |
| 019 | 63 | M | MUD Male PBPC | CLL transformed | RIC (Flu/Bu) | Tac/MTX | +26 | N/A | (-/-) | N |
| 021 | 68 | M | HLA ID Sib Male PBPC | T-cell NHL CR3 | RIC (Flu/Bu) | Tac/MTX | +14 | N/A | (+/+) | N |
| 023 | 59 | F | MUD Male PBPC | Ph+ ALL CR1 | RIC (Flu/Bu) | PTCy/Tac/MMF | +28 | N/A | (+/+) | N |
| 036 | 34 | M | HLA-ID Sib Male PBPC | AML CR1 | MA (Flu/Bu) | Tac/MTX | +22 | N/A | (-/-) | N |
| 040 | 40 | F | HLA-ID Sib Male PBPC | AML CR1 | MA (Flu/Bu) | Tac/MTX | +15 | N/A | (-/-) | N |
| 042 | 53 | F | MUD Male BM | AML CR1 | MA (Flu/Bu) | Tac/MTX/ATG | +20 | N/A | (-/-) | N |
| 059 | 70 | F | MUD Male BM | AML CR1 | MA (Cy/TBI) | Tac/MTX/ATG | +31 | N/A | (+/-) | Y (day + 38) |
| 067 | 64 | F | MUD Male PBPC | AML CR1 | RIC (Flu/Bu) | Tac/MTX/ATG | +29 | N/A | (-/-) | N |

Abbreviations: MUD: (10/10 matched unrelated donor); Sib: sibling; PBPC : (G-CSF mobilized peripheral blood); BM: Bone marrow; AML: acute myeloid leukemia; CLL: chronic lymphocytic leukemia; ALL: acute lymphoblastic leukemia; NHL: Non Hodgkin lymphoma; Ph+: Philadelphia chromosome positive; CR: complete remission; RIC: reduced intensity conditioning; MA: myeloablative conditioning; Flu: fludarabine; Bu: busulfan; Tac: tacrolimus; MTX: methotrexate; ATG: antithymocyte globulin; MMF: mycophenolate mofetil; PTCy: post transplant cyclophosphamide; LGI: lower gastrointestinal tract; UGI: upper gastrointestinal tract; M; male; F: female; Y: yes; N:no