SUPPLEMENTAL DATA

Table S1. Characteristics of pediatric patients whose PB DCs were analyzed

	0-I GVHD (n=11)	II-IV GVHD (n=8)	P value
Recipient age in years, median (range)	4.9 (0.7-17.1)	3.7 (1.5-10.8)	0.46
Recipient sex male, *%*	7 (63.6)	5 (62.5)	1.00
Diagnosis			
Malignancy, *n*	5	8	0.04
Bone Marrow Failure, *n*	3	0	
Immune deficiency, *n*	3	0	
Graft Source			
PB stem cells, *n*	3	2	0.23
BM stem cells, *n*	3	5	
CB stem cells, *n*	5	1	
Donor type			
Related, *n*	4	5	0.37
Unrelated, *n*	7	3	
HLA matching			
≥8/10, *n*	9	2	0.02
5/10~7/10, *n*	2	6	
Conditioning intensity			
Non-ablative, n	3	0	0.23
Myeloablative, n	8	8	
GVHD prophylaxis regimen			
Cyclosporine plus other, *n*	10	6	0.55
Tacrolimus plus other, *n*	1	2	
Post-transplant ATG and Cyclophosphamide treatment	0	0	
$CD34^+$ Cells Dose, median (range), $\times 10^6$/kg	1.18 (0.15-6.9)	5.80× (0.34~11.96)	0.19
Relapse, *n* (%)	0	1 (12.5%)	0.42

Comparisons of patient ages, $CD34^+$ cells dose were analyzed using Mann-Whitney U test. Comparisons of patient characteristics (categorical variables) between GVHD groups were analyzed using Fisher exact test.

Table S2. Characteristics of adult patients whose PB DCs were analyzed

	0-I GVHD (n=16)	II-IV GVHD (n=11)	HD (n=11)	P value
Recipient age in years, mean (range)	52.28 (25.1-67.6)	59.62 (37.5-77.3)	57.14 (37.1-62.9)	0.53
Recipient sex male, *%*	7 (43.8)	7 (63.6)	6 (54.54)	0.44
Diagnosis				
AML, *n*	13	9	N/A	
MDS, *n*	1	1	N/A	
Hodgkin lymphoma, *n*	1	0	N/A	0.90
Myelofibrosis, *n*		1	N/A	
Mantle cell lymphoma, *n*	1	0	N/A	
Graft Source				
PB stem cells, *n*	15	11	N/A	1.00
BM stem cells, *n*	1	0	N/A	
Donor type				
Related, *n*	4	3	N/A	1.00
Unrelated, *n*	8	4	N/A	
HLA matching				
8/8, *n*	13	11	N/A	0.25
< 8/8, *n*	3	0	N/A	
Conditioning intensity				
Non-ablative, n	9	7	N/A	0.68
Myeloablative, n	7	3	N/A	
GVHD prophylaxis regimen				
Cyclosporine plus other, *n*	1	0	N/A	1.00
Tacrolimus plus other, *n*	15	11	N/A	
Relapse, *n* (%)	3 (18.8)	2 (18.2)	N/A	1.00

Comparisons of patient ages were analyzed using Kruskal-Wallis test. Comparisons of patient characteristics (categorical variables) between GVHD groups were analyzed using Fisher exact test.

Table S3. Characteristics of pediatric patients whose BM progenitors were analyzed

	0-I GVHD (n=8)	II-IV GVHD (n=9)	HD (n=12)
Recipient age in years, median (range)	4.5 (0.8-5.9)	3.3 (1.3-13.9)	31.1 (10.9-42.3)
Recipient sex male, *%*	5 (62.5)	7 (77.7)	4 (33)
Diagnosis			
Malignancy, *n*	5	8	N/A
Bone Marrow Failure, *n*	3	1	N/A
Graft Source			
PB stem cells, *n*	3	9	N/A
BM stem cells, *n*	4	0	N/A
CB stem cells, *n*	1	0	N/A
Donor type			
Related, *n*	6	9	N/A
Unrelated, *n*	2	0	N/A
HLA matching			
≥8/10, *n*	4	0	N/A
5/10~6/10, *n*	4	9	N/A
Conditioning intensity			
Non-ablative, n	5	8	N/A
Myeloablative, n	3	1	N/A
GVHD prophylaxis regimen			
Cyclosporine plus other, *n*	6	7	N/A
Tacrolimus plus other, *n*	2	2	N/A
Post-transplant ATG and Cyclophosphamide treatment	0	0	N/A
$CD34^+$ Cells Dose, median (range), $\times 10^6$/kg	5.32×(0.19-11.39)	7.09 (2.2-9.54)	
Relapse, *n* (%)	2	0	

Comparisons of patient ages, $CD34^+$ cells dose were analyzed using Mann-Whitney U test. Comparisons of patient characteristics (categorical variables) between GVHD groups were analyzed using Fisher exact test.

Table S4. Antibodies

Antibodies	Source	Catalog number
CD11c Hamster anti-Mouse, PE-Cy7	Biolegend	117318
Brilliant Violet 421™ anti-mouse I-A/I-E Antibody	Biolegend	107631
FITC anti mouse CD45R/B220 antibody	Biolegend	103206
Brilliant Violet 605™ anti-mouse/human CD45R/B220 Antibody	Biolegend	103243
BUV395 Rat Anti-Mouse CD11b	BD	563553
APC anti-mouse CD86 Antibody	Biolegend	105011
FITC anti-mouse CD80 Antibody	Biolegend	104705
PE anti-mouse CD274 (B7-H1, PD-L1) Antibody	Biolegend	155403
PE anti-mouse Siglec H Antibody	Biolegend	129605
APC anti-mouse CD317 (BST2, PDCA-1) Antibody	Biolegend	127015
PE anti-mouse CD199 (CCR9) Antibody	Biolegend	128709
FITC anti-mouse CD48 Antibody	Biolegend	103403
APC anti-mouse CD150 Antibody	Biolegend	115909
PE anti-mouse CD135 Antibody	Biolegend	135305
Alexa Fluor® 700 anti-mouse Ly-6A/E (Sca-1) Antibody	Biolegend	108141
PE/Cy7 anti-mouse CD117 (c-Kit) Antibody	Biolegend	105813
Alexa Fluor® 647 anti-mouse CD85k (gp49 Receptor) Antibody	Biolegend	144905
FITC anti-BrdU Antibody	Biolegend	364103
FITC anti-mouse CD45.2 Antibody	Biolegend	109805
APC-cy7 anti-H2Kd mouse Antibody	Biolegend	116630
FITC anti-mouse CD4 Antibody	Biolegend	100406
Alexa Fluor® 647 anti-mouse FOXP3 Antibody	Biolegend	126407
anti-mouse IFN-g PE/CY7	Biolegend	505825
Biotin anti-mouse CD3 Antibody	Biolegend	100244
Biotin anti-mouse/human CD45R/B220 Antibody	Biolegend	103204
Biotin anti-mouse Ly-6G/Ly-6C (Gr-1) Antibody	Biolegend	108404
Biotin anti-mouse/human CD11b Antibody	Biolegend	101204
Biotin anti-mouse TER-119/Erythroid Cells Antibody	Biolegend	116204
APC/Cy7 Streptavidin	Biolegend	405208
LEAF™ Purified anti-mouse IFNAR-1 Antibody	Biolegend	127303
LEAF™ Purified anti-mouse IFN-γ Antibody	Biolegend	505706
LEAF™ Purified anti-mouse TNF-α Antibody	Biolegend	510804

CD85k (Gp49b) Monoclonal Antibody (H1.1), Functional Grade	eBioscience	16-5784-85
LEAF purified anti-mouse CD3	Biolegend	100223
Hamster anti- mouse CD28	BD	553294
FITC anti-human CD3 Antibody	Biolegend	300306
FITC anti-human CD20 Antibody	Biolegend	302304
FITC anti-human CD19 Antibody	Biolegend	302206
FITC anti-human CD16 Antibody	Biolegend	302006
FITC anti-human CD15 (SSEA-1) Antibody	Biolegend	323004
FITC anti-human CD14 Antibody	Biolegend	325604
FITC anti-human CD56 (NCAM) Antibody	Biolegend	318304
BV421 Mouse Anti-Human HLA-DR	BD	562804
PE Mouse Anti-Human CD123	BD	554529
PE/Cyanine7 anti-human CD1c Antibody	Biolegend	331516
APC Mouse Anti-Human CD34	BD	555824
CD38 Monoclonal Antibody (HB7), FITC	eBioscience	11-0388-42
CD45RA Antibody, APC-eFluor® 780	eBioscience	47-0458-42
PE/Cyanine7 anti-human CD10 Antibody	Biolegend	312214
CD115 (c-fms) Monoclonal Antibody (12-3A3-1B10), Super Bright436	eBioscience	62-1559-41

Table S5. Primers for real-time RT-PCR

Gene name	Primer sequence	
18s	Forward	5′-CGGCTACCACATCCAAGG-3′
	Reverse	5′-GCTGCTGGCACCAGACTT-3′
Tcf4	Forward	5′-TTTGCCGTCTTCAGTCTACG-3′
	Reverse	5′-GCATGAAGAAGGAGCTAGGG-3′
Irf8	Forward	5′-TACAATCAGGAGGTGGATGC-3′
	Reverse	5′-TTCAGAGCACAGCGTAACCT-3′
Flt3	Forward	5′-CCCTACTTTCCAGGCACATT-3′
	Reverse	5′-CATTGAACCCTGAGAGCTGA-3′
p16^{Ink4a}	Forward	5′-GTGTGCATGACGTGCGGG-3′
	Reverse	5′-GCAGTTCGAATCTGCACCGTAG-3′
Spi1	Forward	5′-CGCAAGAAGATGACCTACCA-3′
	Reverse	5′-ACTTTCTTCACCTCGCCTGT-3′
Ifna	Forward	5′-AAGCCATCCTTGTGCTAAGAGA-3′
	Reverse	5′-AGCAAGTTGGTTGAGGAAGAG-3′
Ifnb	Forward	5′-TGGGTGGAATGAGACTATTGTT-3′
	Reverse	5′-CTCCCACGTCAATCAATCTTTCCTC-3′
Pdl1	Forward	5′-GCTCCAAAGGACTTGTACGTG-3′
	Reverse	5′-TGATCTGAAGGGCAGCATTTC-3′
Lilrb4	Forward	5′-ATGGGCACAAAAAGAAGGCTAA-3′
	Reverse	5′-GGCATAGGTTACATCCTGGGTC-3′
Apoe	Forward	5′-CTGACAGGATGCCTAGCCG-3′
	Reverse	5′-CGCAGGTAATCCCAGAAGC-3′
Ido	Forward	5′-CAAGACCTGAAAGCATTGGA-3′
	Reverse	5′-CACAAAGTCACGCATCCTCT-3′

SUPPLEMENTAL FIGURES

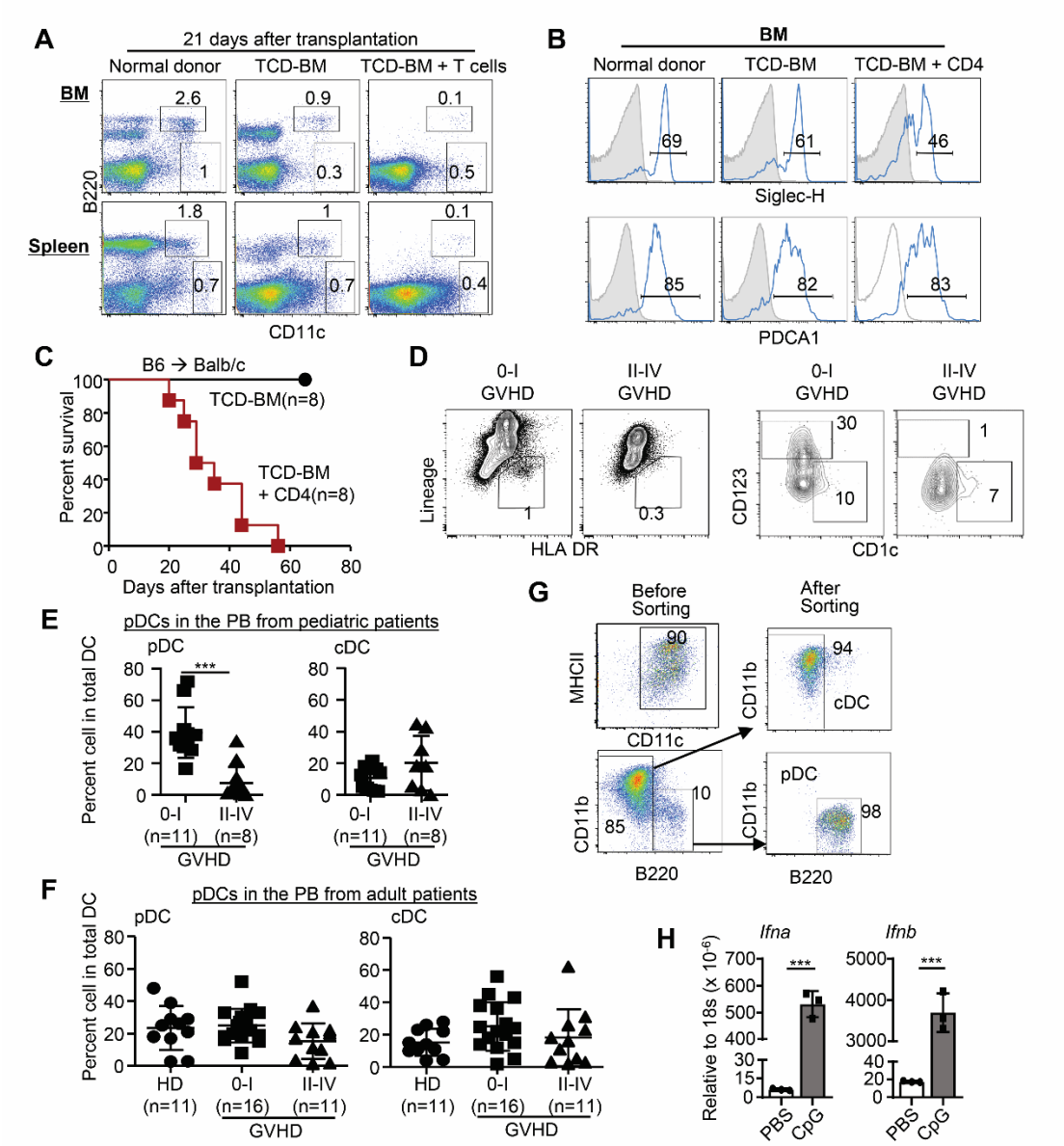


Figure S1. DC gating strategy and characterization. (A-C) B6 mouse-derived T cell-depleted bone marrow (TCD-BM) ($5x10^6$) was transplanted, with or without CD4 T cells ($0.5x10^6$), into lethally irradiated BALB/c mice to induce GVHD. **(A)** Plots show pDCs and cDCs in the BM and spleen at day 21 after transplantation. (**B**) Histograms show Siglec H and PDCA1 expression on $B220^+CD11c^+$ pDCs. (**C**) Survival of transplant recipient mice were monitored over time. **(D)** Plots show the fraction of pDCs and cDCs in PB obtained from patients undergoing allo-HSCT. Samples were collected at the time of GVHD onset (between 21 and 50 days after transplantation). (**E,F**) Plots show the percentage of pDCs and cDCs from human samples derived from the PB of healthy donors (HD) and patients undergoing allo-HSCT that are indicated in Table S1 and Table S2. **(G)** Donor-type pDCs were ex vivo generated from B6 BM, activated by TLR4 (LPS)- and TLR7/8(R8484)-agonists overnight, and sorted using FACS cell sorter. **(H)** pDCs were ex vivo generated from B6 BM, activated by TLR9-agonist (CpG) and FACS-sorted. mRNA levels of *Ifna* and *Ifnb* were measured in PBS or CpG stimulated pDCs. Two group comparisons by Mann-Whitney U test (E), multiple comparisons by Kruskal-Wallis test with Dunne's multiple comparison test (F), and two group comparisons by unpaired *t* test (two-tailed) (H). ***, P<0.001.

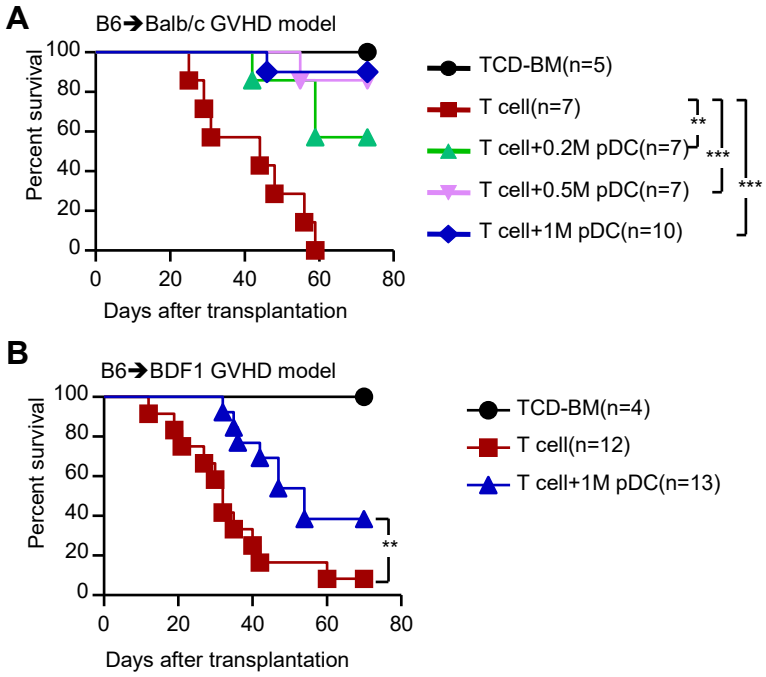


Figure S2. Donor pDC therapy reduces GVHD in mice. (**A**) B6 TCD-BM ($5x10^6$) was transplanted, with or without $CD4^+$ T cells ($0.5x10^6$), into lethally irradiated (8.5Gys) Balb/c mice, followed with or without injection of 0.2 $x10^6$ (0.2M), 0.5 $x10^6$ (0.5M) or $1x10^6$ (1M) B6 pDCs at day 0, 1 and 2. Survival was monitored. (**B**) B6 TCD-BM ($5x10^6$) was transplanted, with or without $CD4^+$ T cells ($1.0x10^6$) plus $CD8^+$ T cells ($1.0x10^6$), into lethally irradiated BDF1 mice to induce GVHD, followed with or without injection of B6 pDCs ($1x10^6$) at day 0, 1 and 2. Survival was monitored and comparisons by Log-rank test. **, $P<0.01$; ***, $P<0.001$.

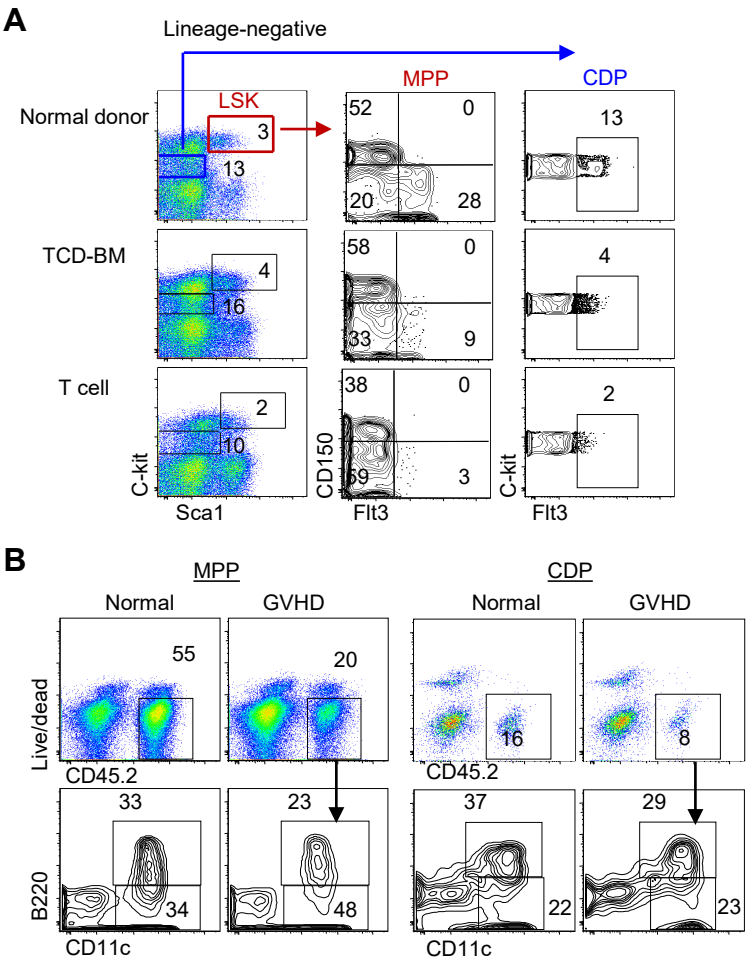


Figure S3. GVHD causes loss of DC progenitors. (A) Plots show the phenotype of lineage$^-$Scal-1$^+$c-kit$^+$ (LSK cells), MPPs and CDPs in the BM from normal, TCD-BM and T cell recipients 21 days post transplantation. **(B-C)** MPPs and CDPs were FSCS-sorted from normal and GVHD mice (CD45.2$^+$) and cultured with feeder cells (BM from B6/SJL mice, CD45.1$^+$) in the presence of Flt3L + SCF. MPPs were cultured for 9 days. CDPs were cultured for 3 days.

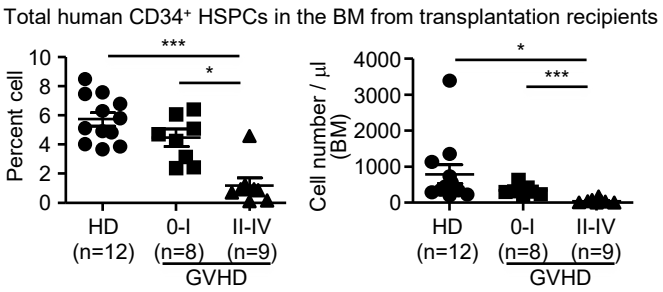


Figure S4. Decreases of HSCs during GVHD. Plots and graphs show the percentage and number of human CD34$^+$ HSPCs from HD (n=12), grade 0-I GVHD patients (n=8) and grade II-IV GVHD patients (n=9). The demographic characteristics of these pediatric patients is indicated in Table S3. Multiple comparisons by Kruskal-Wallis test with Dunn's multiple comparisons test. *, P<0.05; **, P<0.01; ***, P<0.001.

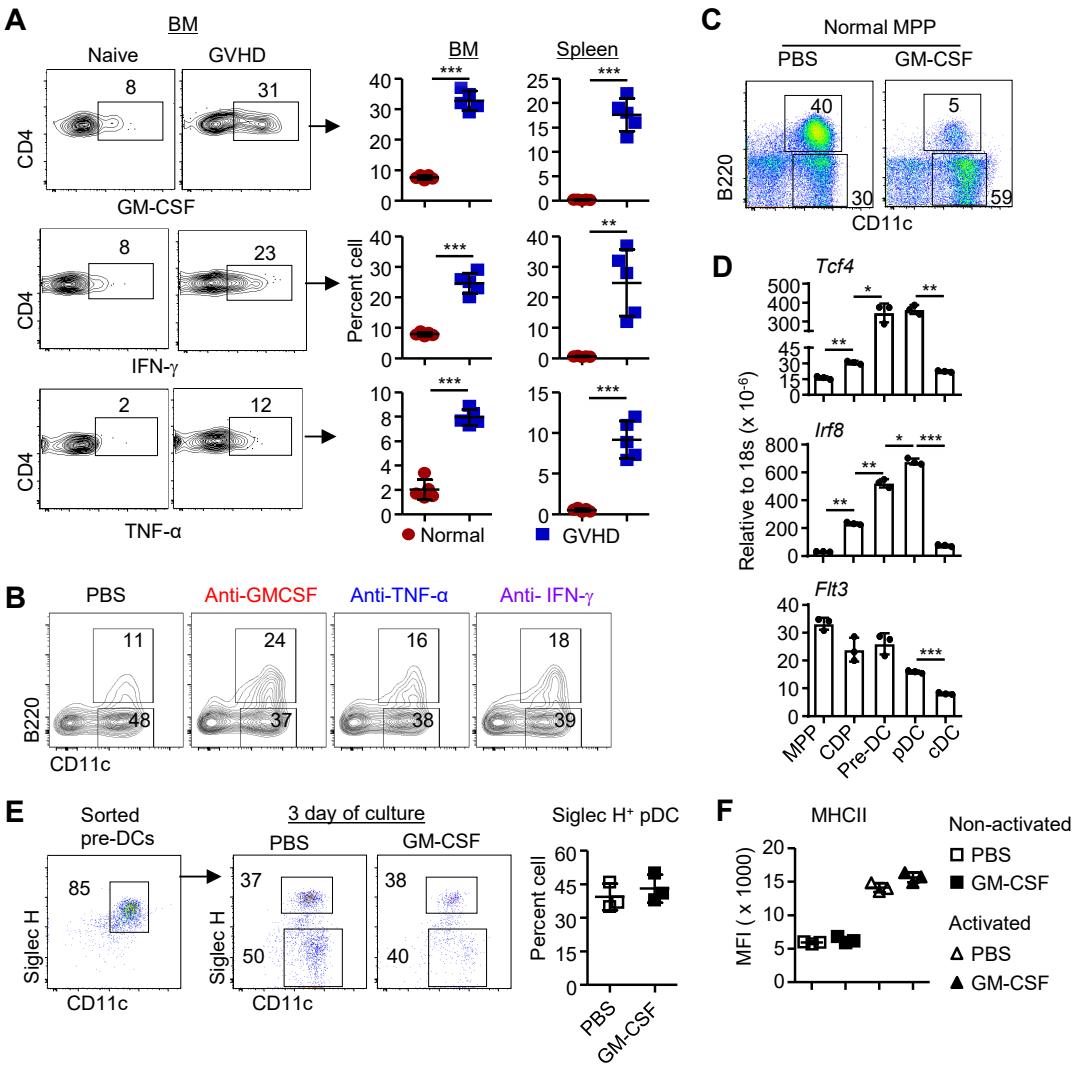


Figure S5. Cytokine expression of alloreactive T cells and their inhibition effect on pDC generation in vitro. (A) B6 TCD-BM (5 x 10^6) was transplanted with CD4 T cells (5 x 10^5) into lethally irradiated BALB/c mice. Plots and graphs show representative cytokine expression of T cells from BM and spleen of naïve and GVHD mice 21 days after transplantation (n=5). (**B**) Neutralizing Abs specific to each cytokine were added to the transwell plate that contained c-kit$^+$ HSPCs and activated GVHD T cells as mentioned above. Plots show pDC generation 9 days later. **(C)** GM-CSF (5ng/ml) was added into c-kit$^+$ HSPCs culture in the presence of Flt3L and SCF. Plots show pDC generation 9 days after culture. (**D**) Real-time RT-PCR shows gene expression in highly purified cell subsets. (**E**) Siglec H$^+$ pre-DCs were purified and cultured in the presence or absence of GM-CSF. Three days later, cells were assessed for mature pDCs. (**F**) Expression of MHC-II on the surface of matured pDCs. Results shown are representative of at least two independent experiments. Two group comparisons by unpaired *t* test (two-tailed) (A, E), multiple comparisons by Kruskal-Wallis test with Dunn's multiple comparison test (D), and One-way ANOVA with Boferroni's multiple comparison test (F). **, P<0.01; ***, P<0.001.

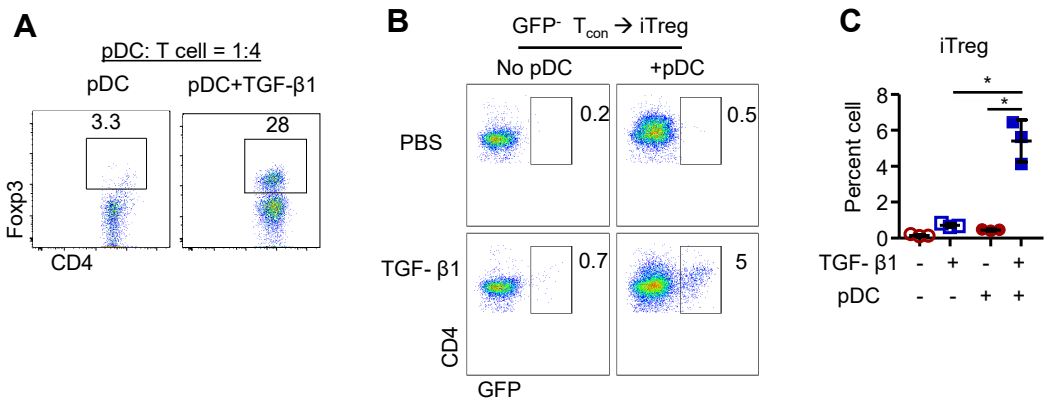


Figure S6. The impact of donor pDCs on Treg expansion and induction. (A) B6 T cells were activated by anti-CD3Ab/CD28Ab, in the presence of B6 pDCs at 1:4 ratio of DC:T cells, with or without adding TGF-β1(5ng/ml). Foxp3^{+} CD4 T cells were examined at day 7 of culture. **(B-C)** GFP^{-}CD4^{+} T cells were sorted from Foxp3^{EGFP} mice that co-express EGFP and Foxp3 under the control of the endogenous promoter. GFP^{-}CD4^{+} T cells were termed conventional T cells (Tcon). GFP^{-}CD4^{+} Tcon were cultured in the presence or absence of pDCs, with or without adding TGF-β1(5ng/ml), for 5 days. Cells were collected to measure the generation of inducible Tregs (iTregs) using flow cytometry analysis. Multiple comparisons by One-way ANOVA with Boferroni's multiple comparison test (C). **, P<0.01.

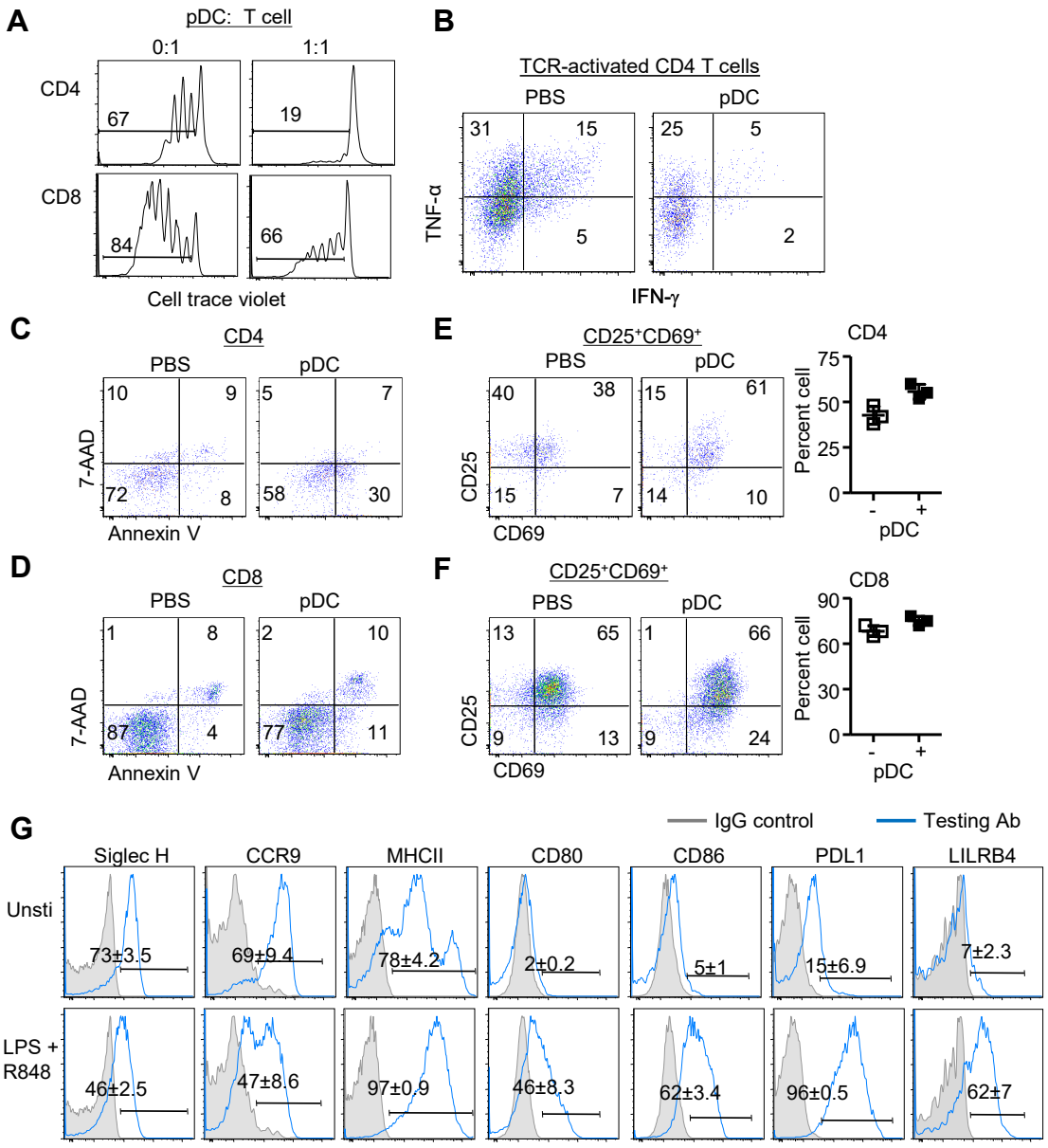


Figure S7. pDCs are potent suppressors of TCR-activated T cells. (A-F) Naïve CD4 or CD8 T cells from B6 mice were activated by anti-CD3Ab/CD28Ab with addition of B6 pDCs at a 1:1 ratio. Four days later, cells were tested for their proliferation (**A**), cytokine production (**B**), annexin V-positivity in activated CD4 and CD8 T cell culture (**C-D**) and expression of activation markers on the surface of CD4 T cells (**E-F**). **(G)** Histograms show surface expression of tested molecules on the surface of ex vivo generated B6 pDCs before and after activation of TLR4 and TLR7/8. Results shown are representative of 3 to 4 independent experiments.

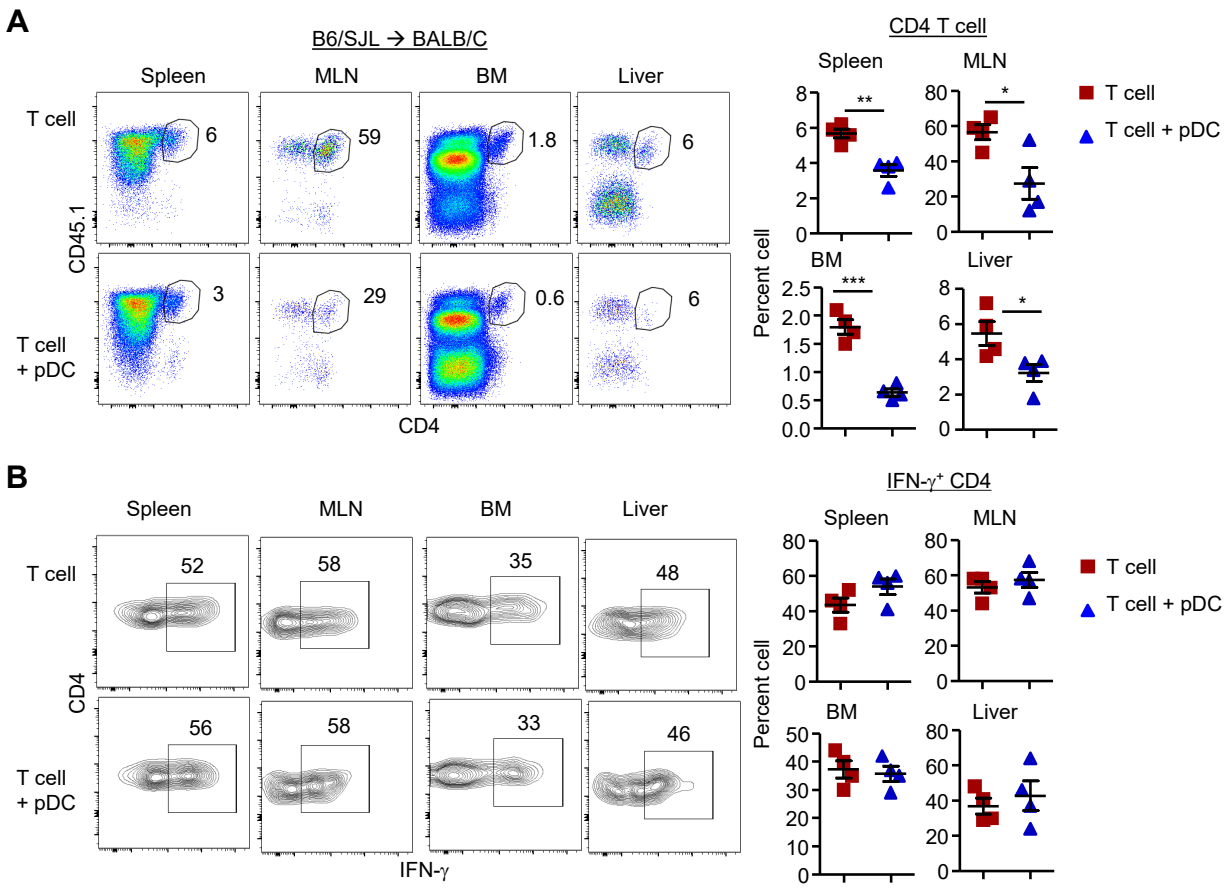
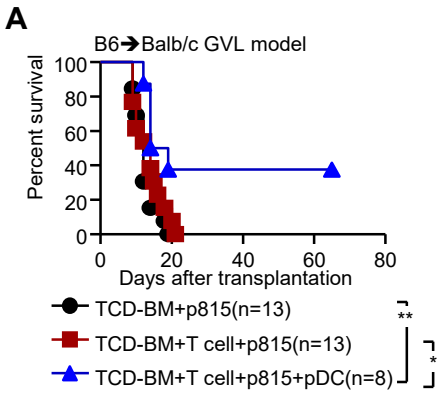
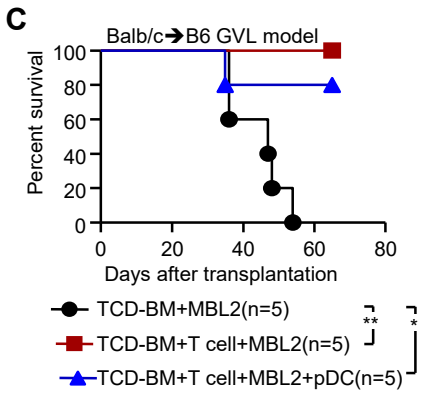


Figure S8. Cytokine expression of T cells 7 days post HSCT. (A) B6/SJL TCD-BM + CD4 T cells were transferred to lethally irradiated BALB/c mice (n=4, each group), followed by injection of B6 pDCs at days 0, 1 and 2 or no treatment. Donor cells were collected at day 7. Plots and graphs show fractions of CD4 T cells in donor cells and cytokine expression from the BM, spleen, liver and MLN. **(B)** Plots and graphs show the fraction of IFN-γ-producing cells. Two group comparisons by unpaired *t* test (two-tailed). *, P<0.05; **, P<0.01; ***, P<0.001.



B **GVHD death vs. leukemia death**

	Tumor death	GVHD death	Survival time Mean±SD
TCD-BM+p815	13/13	0/13	12.54±3.1
TCD-BM+T cell+p815	13/13	4/13	12.69±3.0
TCD-BM+T cell +p815+pDC	5/8	0/8	33.50±26.2



D **GVHD death vs. leukemia death**

	Tumor death	GVHD death	Survival time Mean±SD
TCD-BM+MBL2	5/5	0/5	44.20±8.0
TCD-BM+T cell+MBL2	0/5	0/5	65±0
TCD-BM+T cell +MBL2+pDC	1/5	0/5	59.00±13.4

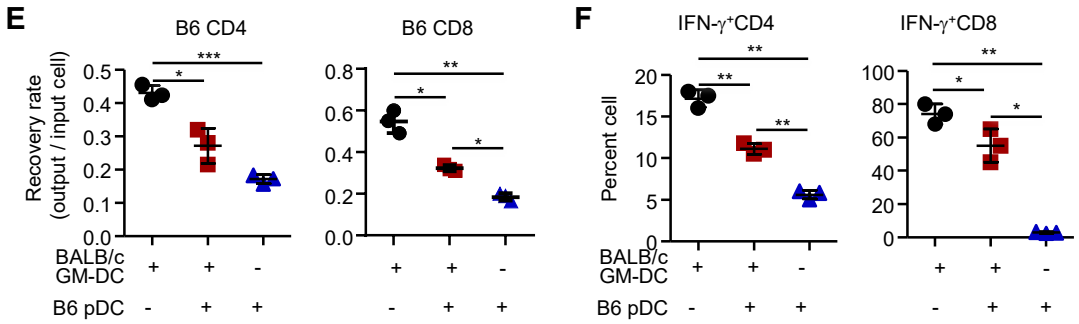


Figure S9. Donor pDC therapy preserves anti-leukemia activity of donor T cells in mice undergoing allo-HSCT. (**A-B**) Lethally irradiated BALB/c recipients (8.5 Gy) received B6 TCD-BM (5 x10^6), with or without $CD4^+$ cells (0.5 x 10^6) and challenged with P815 mastocytoma cells (3 x10^3). B6 pDCs (1x10^6) were administered to these recipients at days 0, 1 and 2. Survival, leukemia death and GVHD death (histological examination) were monitored. (**C-D**) Lethally irradiated B6 recipients (10.0 Gy) received BALB/c TCD-BM (5 x10^6), with or without $CD4^+$ T cells (0.5 x 10^6) and $CD8^+$ T cells (0.5 x 10^6), and challenged with MBL2 AML cells (3x10^4). B6 pDCs (1x10^6) were administered to these recipients at days 0, 1 and 2. Survival, leukemia death and GVHD death (histological examination) were monitored. (**E-F**) B6 $CD4^+$ T cells and $CD8^+$ T cells were cultured in the presence or absence of BALB/c DCs derived from cultured BM cells at a T cell:DC ratio of 4:1. B6 pDCs were added to the culture at a ratio of T cell:pDC of 2:1. Five days later, cells were collected, counted and examined for their production of IFN-γ. Data shown in **E-F** are representative of 2 independent experiments. Survival comparisons by Log-rank test (A, C), and multiple comparisons by One-way ANOVA with Tukey's multiple comparison test (E, F). *P, <0.05; **P , <0.01; ***P , <0.001.