Supplemental Materials and Methods

Sample processing

Blood samples were centrifuged for 10 minutes at 2000 rpm to collect plasma. Samples were then resuspended with PBS and peripheral blood mononuclear cells were isolated using the Ficoll-Hypaque (GE Healthcare) based on density gradient centrifugation.

Flow Cytometry

PBMCs from patients were blocked with human FcR blocking reagent (Miltanyi Biotec) for 10 minutes and then stained with mixtures of antibodies against cell surface antigens for 15 minutes. PBMCs were stained with surface antibodies, to assess transcription factors and then permeabilized to measure intracellular proteins with commercial kits (Foxp3/Transcription Factor Staining Buffer Set, eBioscience). For cytokines, PBMCs were stimulated with PMA (100ng/ml) and ionomycin (1µg/ml) in the presence of Golgistop for 6 hours. Cells were fixed and permeabilized with a BD Cytofix kit and antibodies. The stained samples were then measured in the Fortessa and data was analyzed with FlowJo 10 (UMAP, DownSampleV3 and FlowSOM plugin). Briefly, equal number of $CD4^+$ T cells from the peripheral blood of cGVHD and No-GVHD were down-sampled first and then auto-cluster with “UMAP” which further indicated 9 clusters for human $CD4^+$ T cells and 9 clusters for mouse $CD4^+$ T cells with FlowSOM to generate the heatmap for Figure 2A & B, Figure 3B-D. Log transformed average mean fluorescence intensity (MFI) of helper T cell-related markers was also shown as heatmap in Figure 2I by Morpheus.

Anti-human antibodies used were the following: anti-CD3(UCHT1 or OKT3), anti-CD4(SK3), anti-CD45RA (HI100), anti-CD45RO(UCHL1), anti-PD1(EH12.2H7), anti-CXCR5(MU5UBEE), anti-ICOS(C398.4A), anti-CD127 (eBioRDR5), anti-HLA-DR (L243), anti-CCR7(150503),anti-

CCR2 (K036C2), anti-PSGL1(KPL-1), anti-CD19(SJ25C1), anti-CD27(M-T271), anti-CD38(HIT2), anti-CD138 (44F9), anti-IgD(IA6-2), anti-BCL6 (7D1), anti-IRF4(3E4), anti-MAF (sym0F1), anti-Blimp1(#646702), anti-IFN-γ(4S.B3), anti-IL-4(8D4-8), anti-IL-21 (eBio3A3-N2 (3A3-N2)), anti-IL-2 (MQ1-17H12), anti-CXCL13(#IC801R,R&D).

The antibodies used to stain murine cells included anti-CD4 (RM4-5), anti-CD4 (GK1.5), anti-CD3(17A2),anti-CD8 (53-6.7), anti-TCRβ(H57-597), anti-H-2Kb(AF6-88.5), anti-CD45.1 (A20),anti-CD45.2(#104), anti-CD62L(MEL-14), anti-PSGL1(2PH1), anti-CD69 (H1.2F3), anti-PD1(29F.1A12), anti-ICOS (7E.17G9), anti-CXCR5 (SPRCL5), anti-CD44 (IM7), anti-CD40L (MR1),anti-SLAMF6 (13-G3), anti-FR4(eBio12A5),anti-TIGIT (Vstm3), anti-CD160 (7H1), anti-Lag3 (eBioC9B7W (C9B7W)), anti-2B4 (m2B4 (B6)458.1),anti-I-Ab (AF6-120.1), anti-CCR6 (29-2L17), anti-CXCR3(CXCR3-173), anti-CCR2 (SA203G11), anti-TCR Va2.2 (B20.1), anti-TCR Vb3(KJ25), anti-TCR-Vb8.3 (IB3.3), anti-IL21R (4A9),anti-CD19(1D3), anti-CD138 (281-2), anti-IgD(IA6-2), anti-IgM(RMM-1), anti-PD-L2(TY25), anti-CD80(16-10A1), anti-CD73 (TY/11.8),anti-GL7 (GL-7 (GL7)), anti-Fas (15A7), anti-mouse Bcl6 (Bcl-DWN), anti-mouse Blimp1(5E7), anti-IRF4 (3E4), anti-T-bet (eBio4B10 (4B10)),anti-mouse Maf (sym0F1), anti-IFNγ(XMG1.2) and anti-IL21 (FFA21).

T-B cell co-cultures

Total lymphocytes were isolated from the blood of no-GVHD or cGVHD patients. Sorted $CD3^+CD4^+CD45RA^-PD1^{hi}CXCR5^+$ Tfh cells from patients with no GVHD and $CD3^+CD4^+$ $CD45RA^-PD1^{hi}CXCR5^-$ Tph cells from cGVHD patients were cultured with allogeneic $5x10^4$ naïve B ($CD19^+$ IgD^+ $CD27^-$) or memory B cells ($CD19^+IgD^{lo/-}$ $CD27^+$) at a 1:5 ratio in the RPMI/10% FBS medium with the presence of LPS (5μg/ml) and SEB (1μg/ml). To test IL-21 signaling in the co-cultures, 20 μg/ml IL-21R Fc (R&D system) was added as reported[1]. Cells were harvested after 7 days of culture and plasma cells were measured by flow cytometry. Total

IgG concentrations in the culture supernatant were measured by ELISA kit according to the manufacturer protocol (Invitrogen).

Animal

CD4-cre-BCL6$^{f/f}$ mice were bred at the Research Animal Center of the City of Hope.

Isolation of mononuclear cells from tissues

cGVHD target tissues from mouse recipients of BM plus T cells were harvested for mononuclear cell isolation. Briefly, the spleen and thymus meshed through a 70μm nylon cell strainer and cells were collected. Liver tissues also meshed through a 20μm strainer and cells were collected and washed with PBS with 2% BSA. Liver mononuclear cells were further enriched with 40% and 70% Percoll gradient (Sigma-Aldrich). Lung tissues were cut into pieces and digested with Collagenase VIII (Sigma-Aldrich) and DNase I (Sigma-Aldrich) in the RPMI-1640/10%FBS complete medium. Digested tissues then meshed through a 20μm strainer and cells were collected and washed. Lymphocytes were further isolated with mouse Lympholyte-M (Cedarlane). Lymphocytes from the blood of mice were also enriched with mouse Lympholyte-M (Cedarlane). Isolated Lymphocytes were washed and counted with a Cellometer Auto 2000 Cell Viability Counter (Nextcelom).

Pathology

Spleen, liver, lung and skin from cGVHD mice were harvested, fixed in formalin and embedded into the paraffin blocks. Samples were then cut into 5~6μm sections and stained with hematoxylin, eosin (HE) and Masson's trichrome at the Pathology-Solid Tumor Core at the City of Hope. HE slides were scanned or taken images with Zeiss Observer II at the City of Hope Light Microscopy Core.

Imaging Mass Cytometry (IMC)

Formalin-fixed, paraffin-embedded (FFPE) sections of lung tissues from mice with cGVHD were mounted on white slides, dewaxed with fresh xylene and rehydrated with serial dilutions of alcohol, and antigen was retrieved in a Biocare Medical decloaking chamber at 95°C for 65 min in Tris-EDTA (pH=9). The slides were cooled to room temperature, then blocked with SuperBlock T20 (PBS) Blocking Buffer (Thermo Scientific) for 30 minutes and incubated overnight at 4°C in primary antibody mixtures shown below. Slides were washed with PBS-TB (PBS supplemented with 0.05% Tween 20 and 1%BSA) 3 times and stained with a nuclear intercalator solution containing iridium 193 and 191 isotopes for 10 min at room temperature. Finally, the slides were washed in distilled water and dried at room temperature for data acquisition using a Hyperion imaging mass cytometer (Fluidigm). All data were stored as MCD files and txt files. Software MCD viewer and Fiji ImageJ were used for image processing.

Metal	Antibody	Clone	Company
149Sm	Anti-CD11b	EPR1344	Fludigm
152Sm	Anti-CD11c	D1V9Y	Cell Signaling
153Eu	Anti-CD4	4SM95	Thermo Scientific
158Gd	Anti-E-cadherin	Polyclonal	Fludigm
163Dy	Anti-CD162	4RA10	BD Pharmingen
164Dy	Anti-CD8a	4SM15	Thermo Scientific
166Er	Anti-B220	RA3-6B2	Thermo Scientific
168Er	Anti-Ki67	B56	Fludigm
169Tm	Anti-Collagen I	Polyclonal	Fludigm
170Er	anti-CD3	Poly C-Terminal	Fludigm
171Dy	Histone	D1H1	Fludigm
175Lu	Anti-CD69	polyclonal	Thermo Scientific

Immunofluorescence staining

FFPE sections were processed as described above. Sections were stained with rabbit anti-mouse CD4 (ab183685, Abcam) and rat anti-mouse CD162 (Clone#4RA10, BD) at 4°C overnight. Slides were washed with PBS-TB and stained with Alexa Fluor 555 conjugated donkey anti-rabbit IgG and Alexa Fluor 647 conjugated chicken anti-rat IgG for 1 hour at room temperature. Slides were washed and stained with Alexa Fluor 488-Rat anti-mouse B220 (#RA3-6B2) for 2 hours at room temperature and DAPI for 10 min. After washing, images were taken with a Zeiss LSM 700 confocal microscope at 200X magnification.

For evaluation of IgG deposition, liver, lung and skin tissues were harvested and fixed with OCT and then cut into 5 μm sections at the Molecular Pathology Core. Cryosections were then stained with Alexa Fluro 488-labeled goat anti-mouse IgG overnight, and after washing, images were taken with a Zeiss LSM700 confocal microscope at 200X magnification.

ELISA

To measure total IgG concentrations in serum samples from mice, high-binding 96-well plates were coated with purified anti-mouse IgG overnight and samples were then added to the plate. Secondary antibody goat anti-mouse IgG HRP/ TMB solution was then applied to measure the IgG concentration by TecScan. To measure anti-dsDNA IgG autoantibodies, commercial mouse anti-dsDNA ELISA kit (Signosis, Santa Clara) was used. To measure serum cytokines production, we used the commercial mouse Th1/Th2/Th17 CBA kit (BD Biosciences).

Adoptive Transfer

To evaluate whether Trh cells can recirculate into the blood. Irradiated BALB/c recipients were receiving 2.5 x 10^6 T cell-depleted bone marrow cells and 0.2 x 10^6 bead-enriched CD90.2$^+$ spleen T cells from CD45.1$^+$ C57BL/6 donors to establish cGVHD. CD45.1$^+$ Trh cells from the

liver and lung were then sorted through Aria SORP on day 30 after HCT. 1.0 x 10^6 sorted CD45.1$^+$ Trh cells were then injected intravenously into adoptive BALB/c recipients that were given TCD-BM cells from CD45.2$^+$ C57BL/6 donors 14 days ago. Tissue distribution of the injected Trh cells was analyzed 14 days after adoptive transfer.

To evaluate whether Tph cells can enter tissues and develop into Trh cells, irradiated BALB/c recipients were engrafted with 2.5 x 10^6 T cell-depleted bone marrow cells and 0.2 x 10^6 bead-enriched Thy1.2$^+$ spleen T cells from CD45.2$^+$ C57BL/6 donors to establish cGVHD. CD45.2$^+$ Tph cells from the blood of cGVHD mice were isolated by sorting on day 30 after HCT. 5 x 10^3 sorted CD45.2$^+$ Tph cells were then injected into secondary cGVHD recipients that had been engrafted 14 days earlier with CD45.1$^+$ TCD-BM plus Thy1.2$^+$ T cells. The tissue distribution of the injected Tph cells was analyzed 14 days after adoptive transfer.

TCR-sequencing and analysis

For TCR-CDR3 sequencing, TCR-seq libraries were prepared by using a SMARTer-mouse TCR α/β Profiling Kit (Takara USA Inc., Mountain View, CA) according to the manufacturer's protocols. Briefly, the reverse transcription reaction adds non-template nucleotides to the first strand of the cDNA used as template nucleotides for posterior PCR oligonucleotides. Afterward, a semi-nested PCR with the first PCR amplification of the full-length variable region of the TCR is performed, followed by a subsequent round of PCR to further amplify full-length sequences of V(D)J variable regions of TCRα or TCRβ subunits and incorporate adapter sequences for Illumina sequencing. The cDNA libraries were sequenced on an Illumina MiSeq (2 × 300 nt). The quality of sequencing reads was assessed using FastQC. To extract CDR3 sequences, identify V, D and J genes, and assemble clonotypes by CDR3 sequences, the raw sequencing reads were processed by using MiXCR v2.1.9 software[2]. TRB and TRA repertoires were further analyzed with R packages “scatterplot” and “Immunarch" to determine the repertoire overlap

index. The TCR-sequencing data can be accessed in the NCBI's Gene Expression Omnibus database (GSE200520).

RNA-seq analysis

RNA-seq data is Public RNA-sequencing data obtained from our previous publication (GEO, GSE157566)[1]. Heatmap of differentially expressed transcriptional factors was generated with edgeR and Morpheus.

Statistical analysis

Results are combined from more than two replicate experiments and data are presented as the means ± SEM. Statistical comparisons were performed as indicated in the figure legends. $P<0.05$ was considered statistically significant. All the statistical analyses were performed using GraphPad Prism Software 8.

References:

1. Kong X, Zeng D, Wu X, et al. Tissue-resident PSGL1loCD4+ T cells promote B cell differentiation and chronic graft-versus-host disease-associated autoimmunity. *J Clin Invest*. 2021;131(1):e135468.
2. Bolotin DA, Poslavsky S, Mitrophanov I, et al. MiXCR: software for comprehensive adaptive immunity profiling. *Nat Methods*. 2015;12(5):380-381.

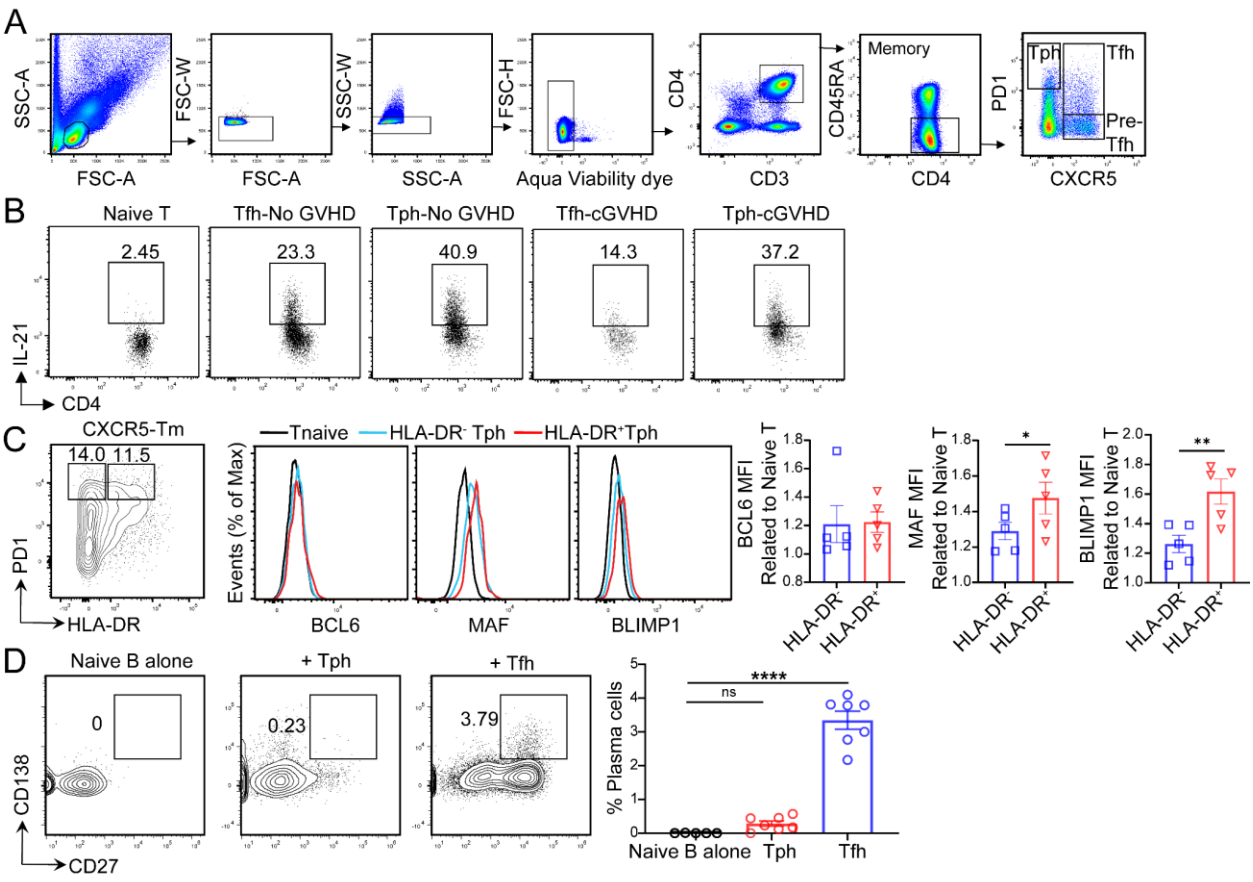
Supplemental Table 1. Clinical characteristics of HCT patients

		No cGVHD N=7	cGVHD N=23
Age, y	Average	53	45
	Range	48~63	22~73
Sex (%)	F	5	8
	M	2	15
Conditioning Regimen (%)	MAC	3	10
	RIC	4	13
Donor type (%)	MRD	1	5
	MUD	3	4
	mmRD	1	3
	mmUD	2	11
Graft source (%)	PBSC	6	19
	CBT	1	1
	BM	0	3
GVHD Prophylaxis (%)	Tac+Sir	4	14
	Tac+Sir+MMF	1	1
	CSP+MMF	1	3
	Tac+Mtx	1	4
	Tac+MMF	0	1
Initial Disease (%)	AML/AML from MDS	3	7
	ALL	1	5
	CML	1	1
	PLL	0	1
	MDS	1	0
	NHL	0	3
	MM	0	3
	AA	0	2
	Other	1	1
Mo from HSCT to sample collection		12 months	39 months

Note: Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BM, bone marrow; CBT, cord blood transplantation; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; F, female; M, male; MAC, myeloablative conditioning; MDS, myelodysplastic syndrome; MM, multiple myeloma; mmRD, mismatched related donor; mmUD, mismatched unrelated donor; Mo, months; MRD, matched related donor; MUD, matched unrelated donor; NHL, non-Hodgkin lymphoma; PBSC, peripheral blood stem cell; RIC, reduced-intensity conditioning; SAA, severe aplastic anemia; Tac-MTX, tacrolimus-methotrexate; Tac-Sir, tacrolimus-sirolimus; Tac-Sir-MTX, tacrolimus-sirolimus-methotrexate; Y, year.

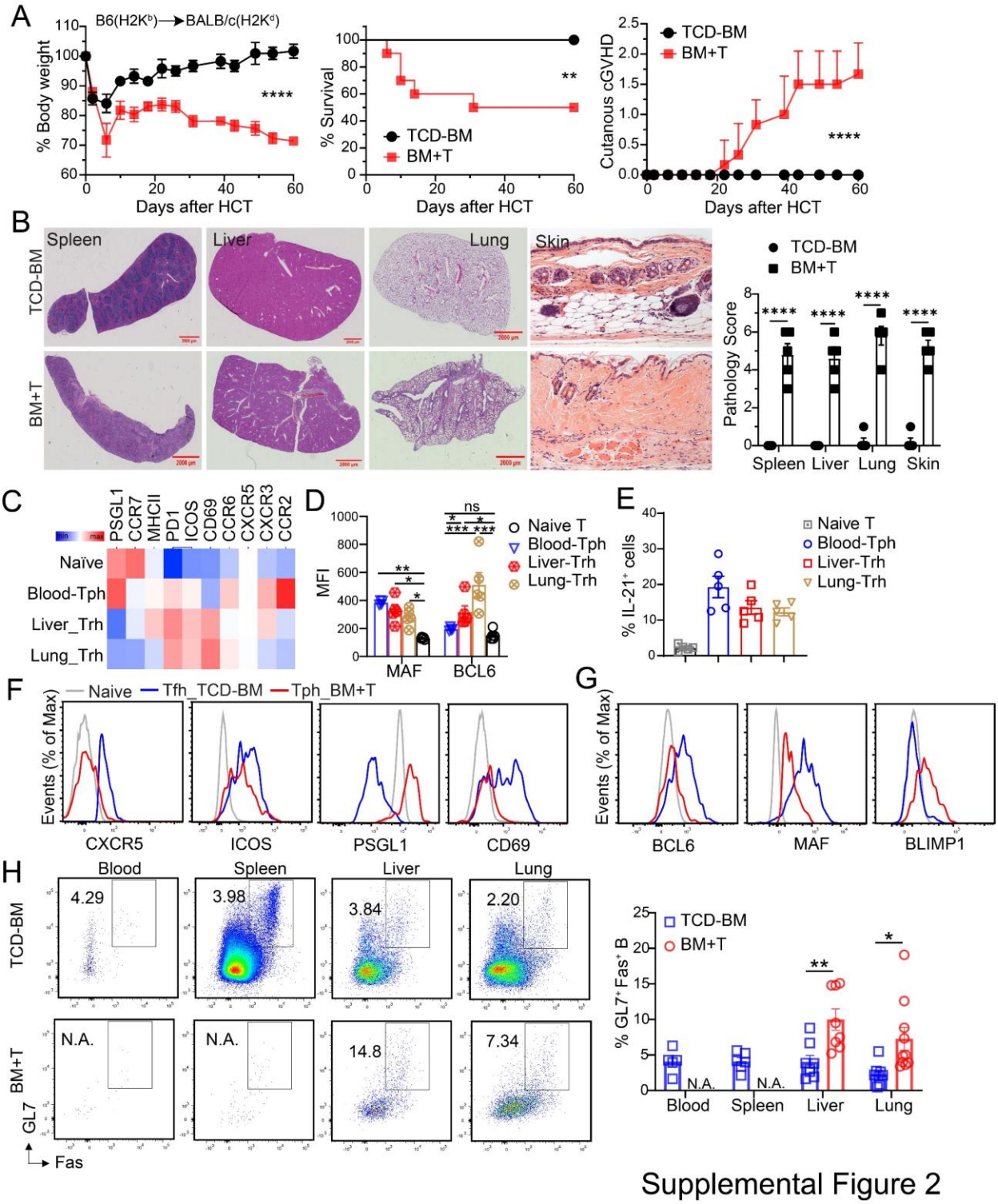
Supplemental Table 2. Clinical characteristics of cGVHD patients

cGVHD severity	Mild N=7	Moderate N=8	Severe N=8
Active cGVHD	3/7	7/8	7/8
Prior aGVHD	0/7	4/8	4/8
Organ manifestations			
Mucosal, oral and/or genital	4/7	6/8	4/8
Eye	4/7	4/8	1/8
Skin	2/7	5/8	8/8
Lung	0/7	2/8	1/8
Liver	1/7	3/8	3/8
GI	0/7	1/8	2/8



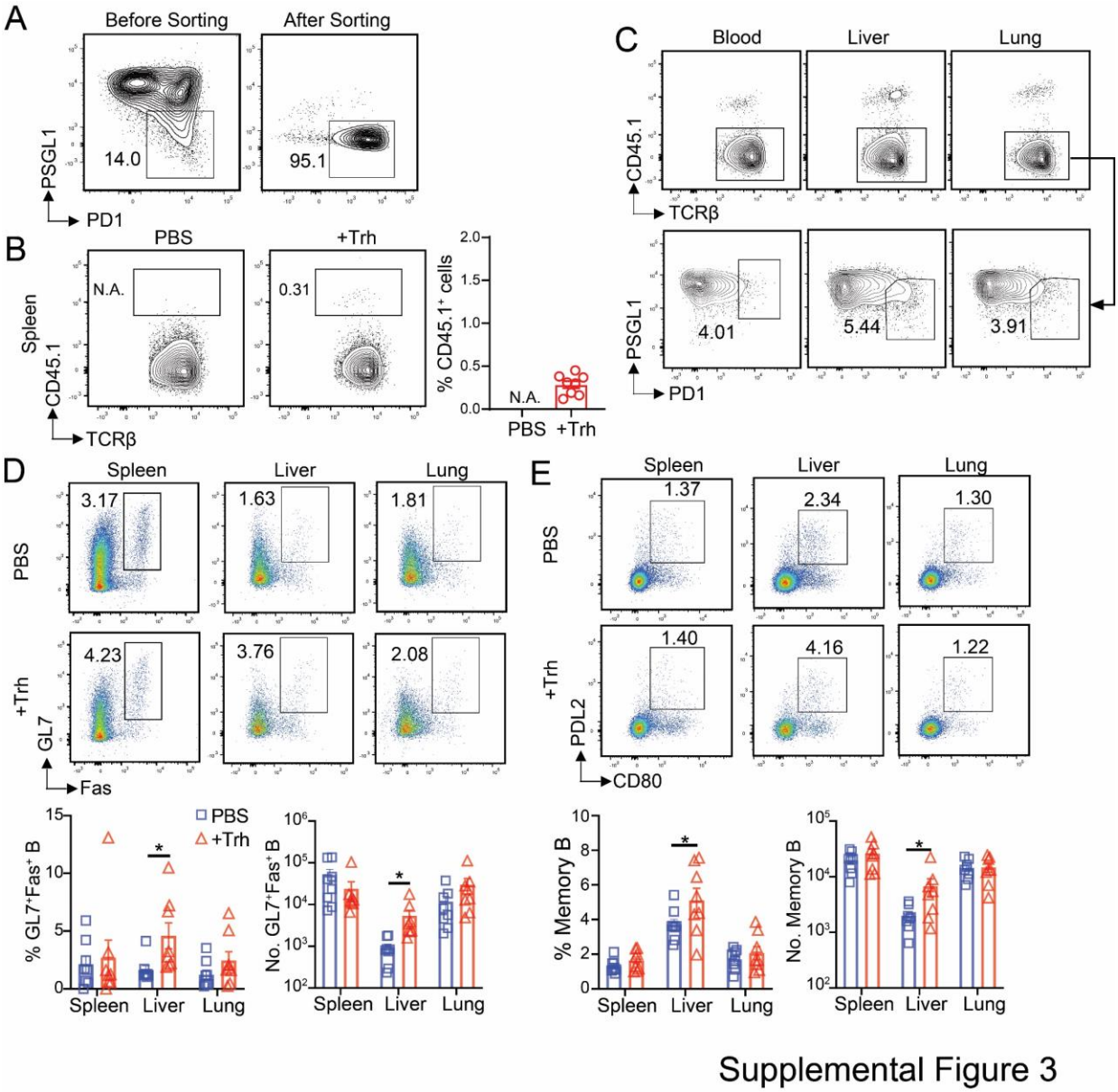
Supplemental Figure 1

Supplemental Figure 1. Pathogenic expansion of Tph cells in cGVHD patients. (A) Gating strategy to identify circulating Tph cells, Tfh and pre-Tfh cells of one representative No-GVHD patient. **(B)** Blood MNCs were stimulated with PMA plus ionomycin, and IL-21 expression among Tfh cells and Tph cells was measured. **(C)** Intracellular expression of BCL6, MAF and BLIMP1 were compared between HLA-DR^{-}PD1^{hi} CXCR5^{-} Tph cells and HLA-DR^{+}PD1^{hi}CXCR5^{-} Tph cells in cGVHD patients. **(D)** Naïve B cells were co-cultured with Tph cells or Tfh cells, and CD27^{+}CD138^{+} plasma cells among live CD19^{+} B cells were examined after culture for 7 days. Representative staining patterns and percentage (Mean ± SEM, N=5~7) of plasma cells among CD19^{+} B cells are shown. Data were combined from two replicate experiments. *P-value* was calculated by unpaired Student t-test **(C)** and One-way ANOVA with Holm-Sadik test **(D)**. ****p<0.0001.



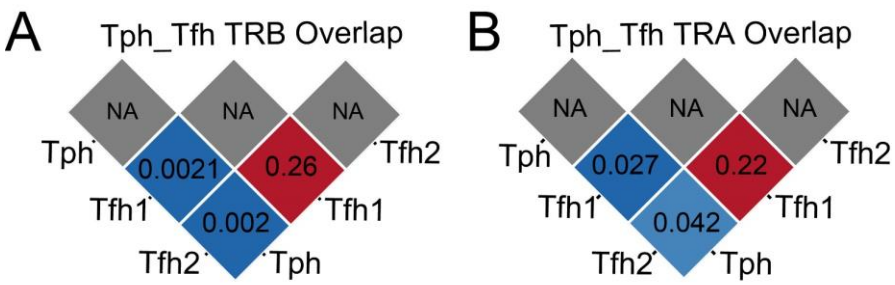
Supplemental Figure 2

Supplemental Figure 2. Tph, Trh cells and GCB-like cells are expanded in mice with cGVHD. Irradiated BALB/c recipients were engrafted with $2.5x10^6$ TCD-BM alone (no GVHD) or TCD-BM plus $1x10^6$ whole spleen cells or $0.2x10^6$ Thy1.2$^+$ T cells (BM+T) to establish the murine model of cGVHD. MNCs from the peripheral blood, spleen, liver and lung of cGVHD recipients were collected on day 60 after HCT. **(A)** Plots of %body weight, %survival and cutaneous cGVHD are shown (N=10). **(B)** Representative HE stains of spleen, liver, lung and skin are shown together with pathology scores (N=5). Skin original magnification X200. **(C)** Surface expression levels of PSGL1, CCR7, MHCII, PD1, ICOS, CD69, CCR6, CXCR5, CXCR3 and CCR2 were examined, and average MFI is shown as heatmap, N=6**. (D)** Intracellular expression of MAF and BCL6 was also measured. **(E)** Tph cells and Trh cells were stimulated, and intracellular expression of IL-21 was measured. **(F)** Surface expression levels of CXCR5, ICOS, PSGL1 and CD69 were compared between Tph from cGVHD mice and spleen Tfh cells from No GVHD mice. **(G)** Intracellular BCL6, MAF and BLIMP1 expression levels were also compared. **(H)** %GL7$^+$Fas$^+$ B cells among total CD19$^+$ B cells in the blood, spleen, liver and lung of no-GVHD and cGVHD mice were compared. Mean ± SEM, N=8. *P-values* were calculated by nonlinear regression with comparison of fits and Log-rank test for survival **(A)** and two-way ANOVA with Holm-Sadik test **(B, D, H)**. *p<0.05; **p<0.01; ***p<0.001,****p<0.0001.



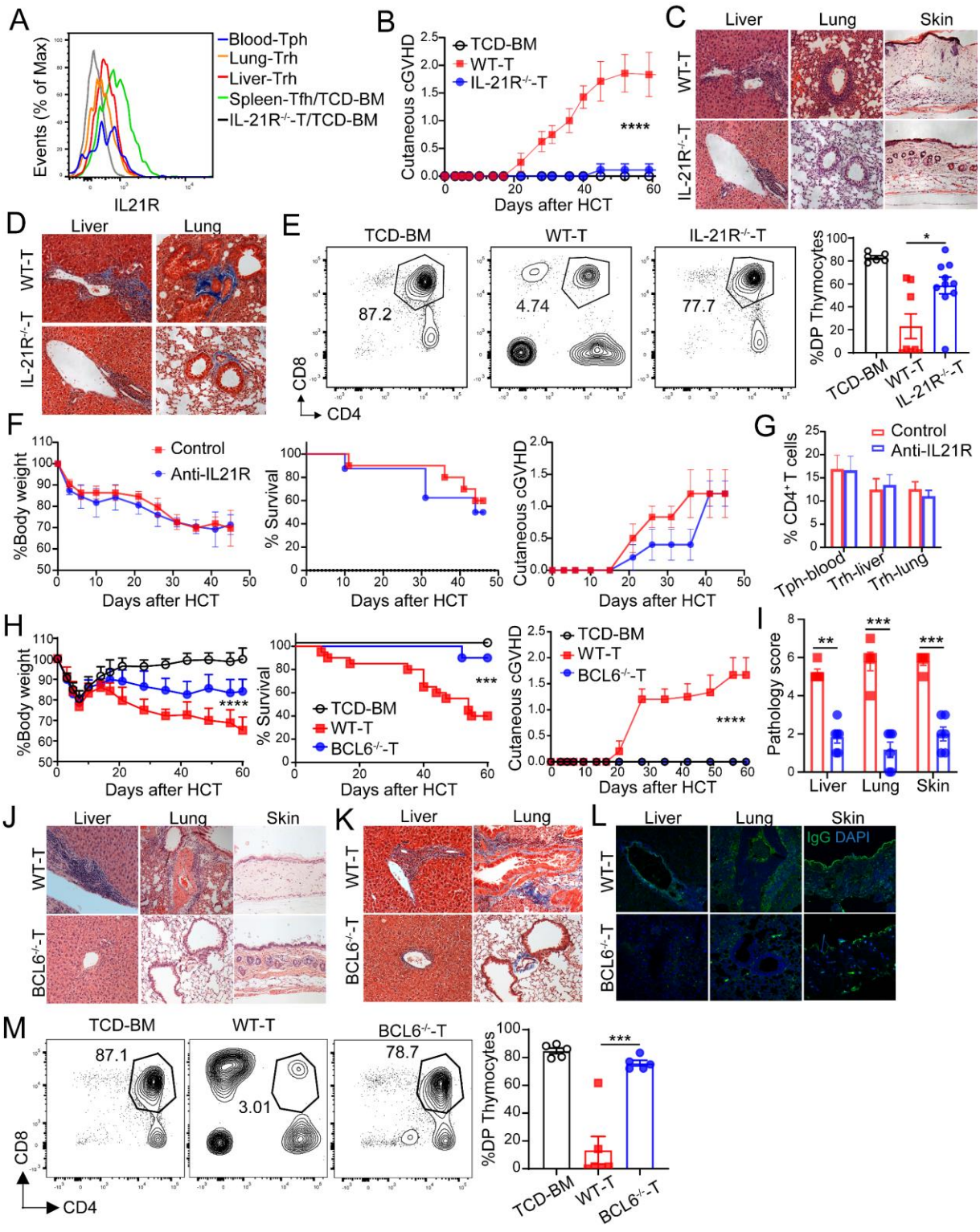
Supplemental Figure 3

Supplemental Figure 3. Tph cells could circulate with Trh cells in the cGVHD target tissue. $CD45.1^+$ Trh cells from the liver and lung of cGVHD mice were sorted and adoptively transferred into congenic $CD45.2^+$ No GVHD mice at day 14 after HCT with TCD-BM alone. $CD45.1^+$ T cells were tracked by flow cytometry 14 days after the adoptive transfer. **(A)** Trh cell purity was checked before and after sorting. **(B)** Percentage of $CD45.1^+$ T cells among total $CD4^+$T cells in the spleen after adoptive transfer into $CD45.2^+$ recipients. Representative staining pattern and Mean ± SEM are shown; N=8. **(C)** Representative panel of Tph cells in the blood and Trh cells in the liver and lung from adoptive transfer No GVHD recipients are shown. **(D)** Representative staining patterns of Fas^+GL7^+ B cells among total B cells and **(E)** $PDL2^+CD80^+$ memory B cells among IgD^- B cells in the spleen, liver and lung were measured. Data represents as Mean ± SEM. *P-values* were calculated by Two-way ANOVA with Holm-Sadik test **(D & E).** *p<0.05; **p<0.01.



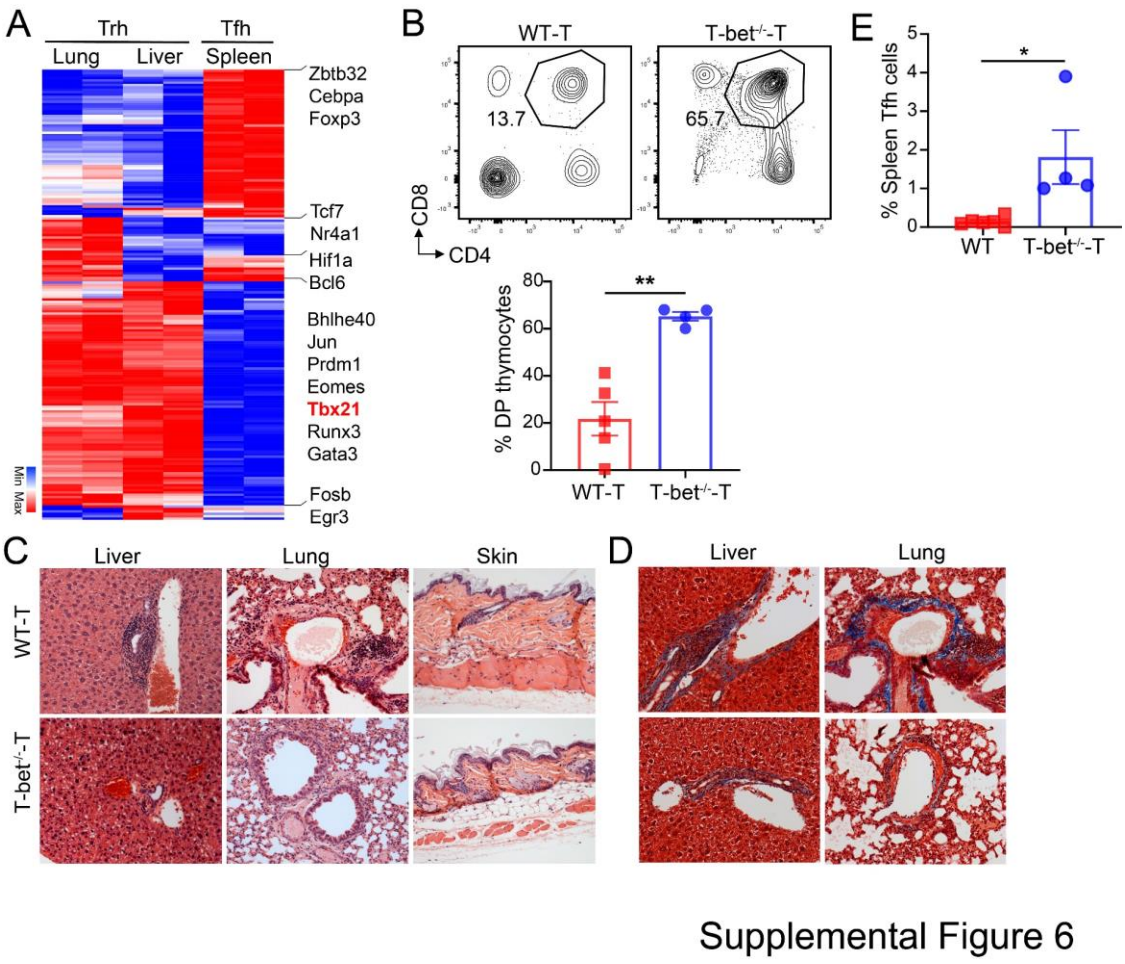
Supplemental Figure 4

Supplemental Figure 4. Tph cells in the blood of cGVHD mouse have little overlapping repertoires with Tfh cells from the spleen of syngeneic No-GVHD mouse. (A) TRB repertoires and **(B)** TRA repertoires of Tph cells sorted from pooled peripheral blood lymphocytes of cGVHD recipients (N=15) and two batches of Tfh cells sorted from pooled spleen cells of No GVHD recipients (N=3) were compared with “Morisita’s overlap index” and are shown in heatmaps.



Supplemental Figure 5

Supplemental Figure 5. Absence of IL-21R or Bcl6 in donor T cells attenuates cGVHD pathology. This is a supplement to Figure 6. **(A)** Comparison of surface expression levels of IL-21R among Tph cells from blood and Trh cells from liver and lung of cGVHD mice. Additional controls include spleen Tfh cells of No GVHD mice, and $CD4^+$ T cells from the liver of IL-$21R^{-/-}$ No GVHD mice at day 60 after HCT. **(B-E)** Supplement to Fig. 6A-C, Comparison of recipients given WT or IL-$21R^{-/-}$ T cells. **(B)** Plot of cutaneous cGVHD score. **(C)** Representative liver, lung and skin pathology via HE stains, **(D)** representative liver and lung pathology via trichrome stains, original magnification X200. **(E)** %$CD4^+CD8^+$ double-positive were also measured. Irradiated BALB/c recipients were engrafted with $2.5x10^6$ TCD-BM plus $1x10^6$ splenocytes from C57BL/6 donors and were treated with 200µg anti-IL-21R (N=8) or PBS control (N=10) i.p. twice weekly starting from the day before HCT until day 45 afterward. **(F)** Plots of % body weight, %survival and cutaneous cGVHD scores. **(G)** Tph cells from blood and Trh cells from liver and lung were compared between control and anti-IL-21R antibody-treated groups on day 46 after HCT. **(H-M)** Supplement to Fig. 6G-I. Irradiated BALB/c recipients were given TCD-BM cells with WT-T cells or $BCL6^{-/-}$-T cells. **(H)** Plots of %body weight, %survival and cutaneous cGVHD score. **(I)** Pathology scores of the liver, lung and skin; (**J)** Representative patterns of HE stains of the liver, lung and skin; **(K)** representative patterns of trichrome stains of liver and lung on day 60 after HCT. **(L)** IgG deposition in representative liver, lung and skin sections is shown. Original magnification X200. **(M)** %$CD4^+CD8^+$ double-positive thymocytes at day 60 after HCT. Mean ± SEM data are combined from three replicate experiments: N=6-10. *P-values* were calculated by nonlinear regression with comparison of fits and Log-rank test for survival **(B & H)**, One-way ANOVA with Holm-Sadik test **(E & M)** and Two-way ANOVA with Holm-Sadik test **(I)**. *p<0.05; **p<0.01; ***p<0.001, ****p<0.0001.



Supplemental Figure 6

Supplemental Figure 6. Deficiency of T-bet in donor T cells attenuates cGVHD pathology. This is supplemental to Figure 7. **(A)** Spleen Tfh cells from No GVHD mice and Trh cells from cGVHD mice were sorted out for RNA-seq and transcriptional factor expression levels were compared in heatmap. **(B)** Percentages of $CD4^+CD8^+$ double-positive thymocytes were compared between mice engrafted with WT-T cells or T-$bet^{-/-}$-T cells. **(C)** Representative HE stains of liver, lung and skin are shown. **(D)** Representative trichrome stains of liver and lung are shown. **(E)** Percentage of Tfh cells among $CD4^+$ T cells in the spleen are also examined. Original magnification X200. Mean ± SEM is shown, N=4-6. *P-values* were calculated with unpaired Student t-test **(B & E)**. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.