Figure S1. Related to Figure 1

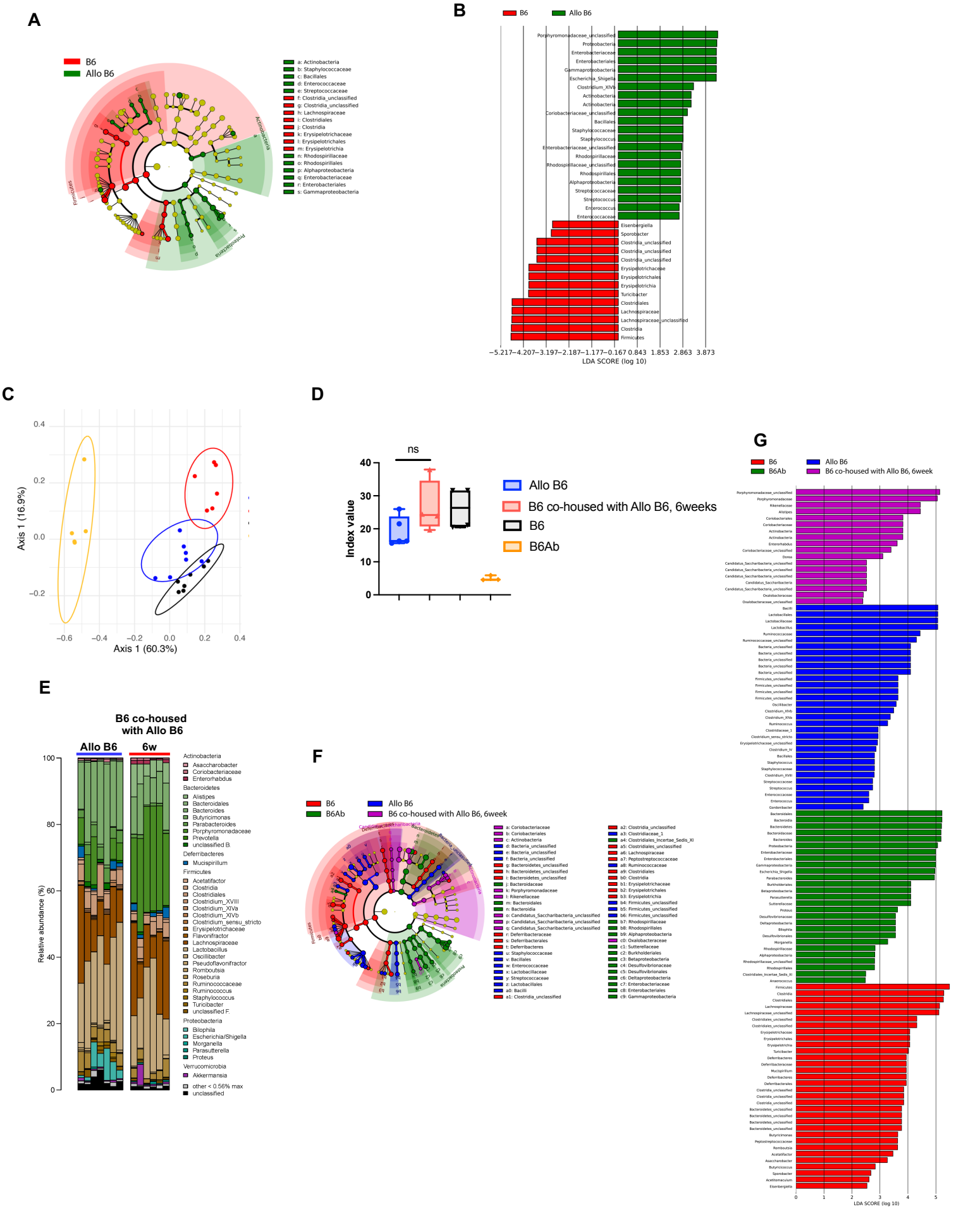


Figure S1. The microbiome composition of stool from B6 and dysbiotic B6 mice.

The details of the experimental design were shown in Fig.1A. (A and B) Stool from B6 and Allo B6 2weeks after BMT were analyzed by 16S rRNA gene sequencing. A cladogram (A) and LDA scores (B) are shown for taxa differentially abundant by LEfSe analysis. (C to G) Stool from Allo B6 before co-house and B6 co-housed with Allo B6 (2 weeks and 6 weeks after co-house) analyzed by 16S rRNA gene sequencing. PCoA (C), inverse Simpson alpha diversity index of microbiome composition (D), microbiome composition (E), and taxa differentially abundant by LEfSe analysis (F & G) are shown (C, Allo B6:N=7, B6 co-housed with Allo B6, 6week:N=6, B6 : N=5, B6Ab; N=5) (B, Allo B6:N=5, B6 co-housed with Allo B6, 6week:N=4, B6 : N=4, B6Ab; N=3) The horizontal line in box (D) represents the median with the box bounding the interquartile range. The ends of the whisker lines represent the minimum and maximum values. One-way ANOVA analysis with Tukey post hoc test (D) was used to determine significance.

Figure S2. Related to Figure 1

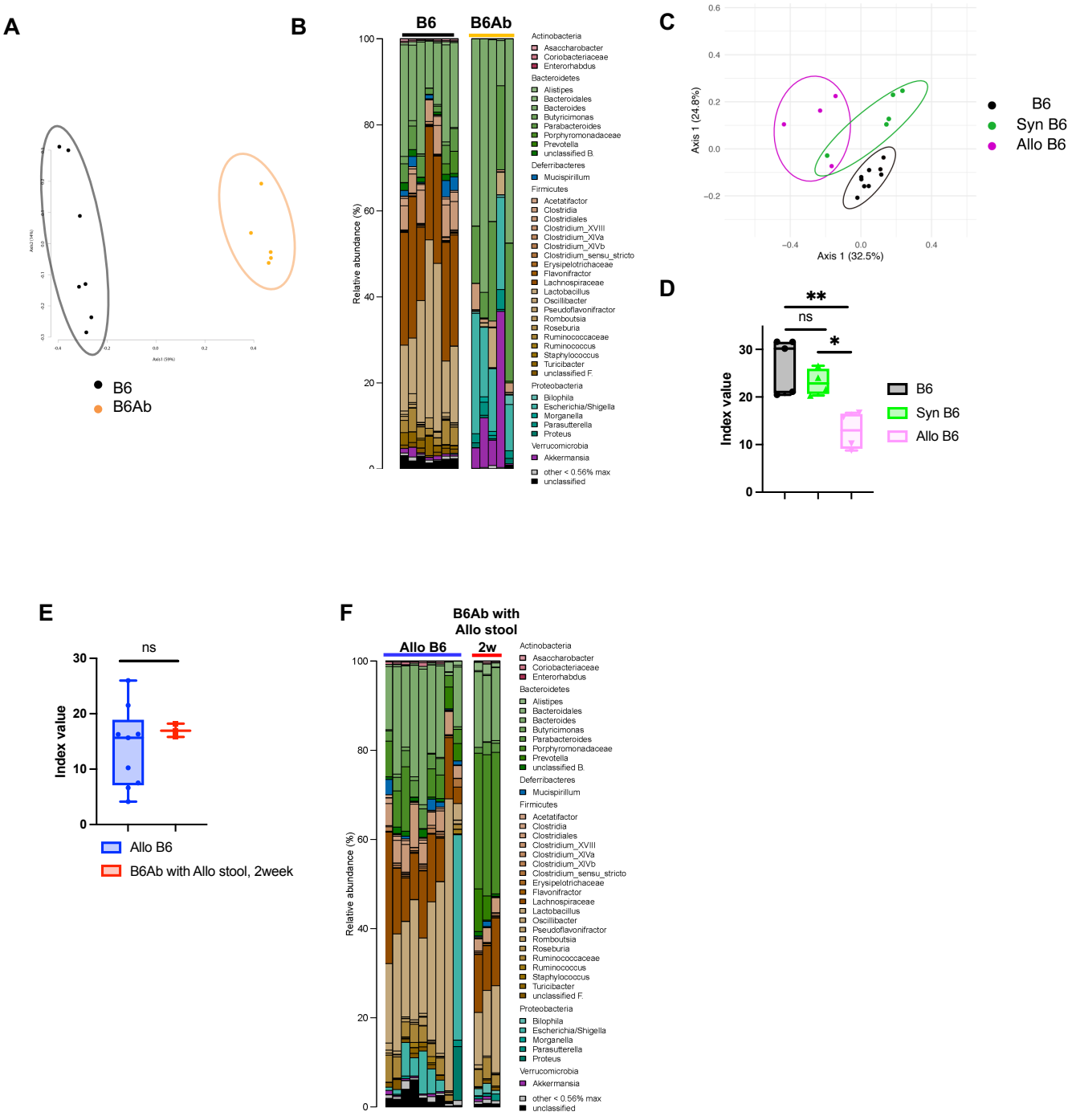
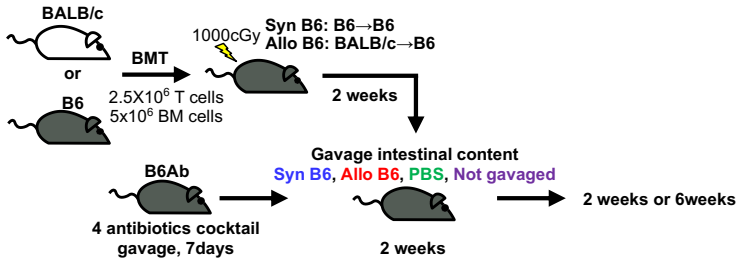
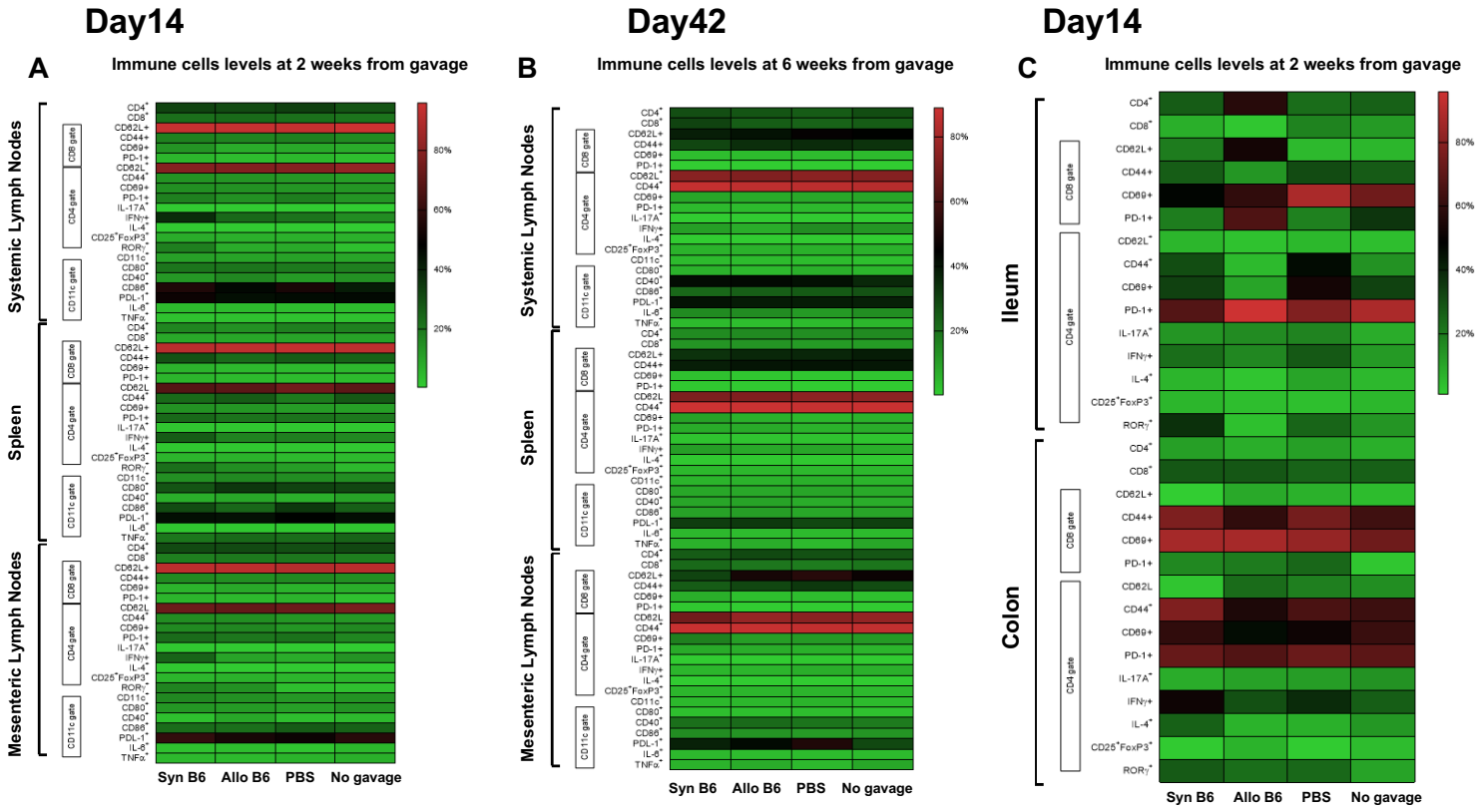


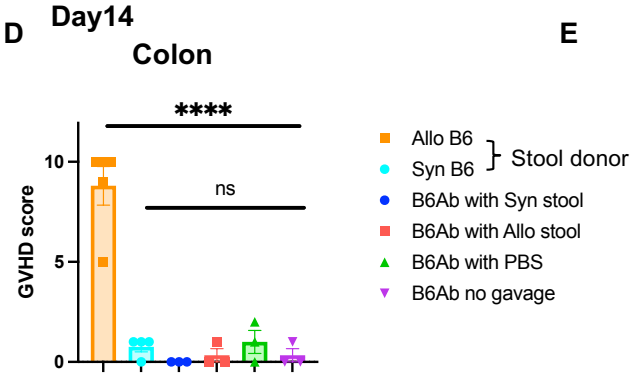
Figure S2. The microbiome composition of B6Ab and B6Ab gavaged Allo B6 stool.

B6 mice were treated for 2 weeks with 4 antibiotics cocktail (B6Ab, ampicillin 1 mg/ml, neomycin 1mg/ml, metronidazole 1mg/ml and vancomycin 0.5mg/ml). Stool from B6Ab and B6 were analyzed by 16S rRNA gene sequencing. (A and B) PCoA (A), and microbiome composition (B) in stool from B6 and B6Ab were shown (B6N: N=7, B6Ab, N=5). (C and D) B6 received BMT from B6 (Syn) or BALB/c (Allo) donor. Stool from B6, Syn B6, and Allo B6 day7 after BMT were analyzed by 16S rRNA gene sequencing. PCoA (C) and inverse Simpson alpha diversity index of microbiome composition (D) in stool were shown. (B6: N=9, Syn B6: N=5, Allo B6: N=4) (E and F) The details of the experimental design were shown in Fig.1M. The inverse Simpson alpha diversity index of microbiome composition (E), microbiome composition (F) of stool from Allo B6 2weeks after BMT and B6Ab with Allo stool (2week) are shown (Allo B6: N=9, B6Ab with Allo stool, 2week: N=3). The horizontal line in box (D, E) represents the median with the box bounding the interquartile range. The ends of the whisker lines represent the minimum and maximum values. One-way ANOVA analysis with Tukey post hoc test (D) and two-tailed unpaired t-test (E) were used to determine significance. *P<0.05, **P<0.01.

Figure S3. Related to Figure 2







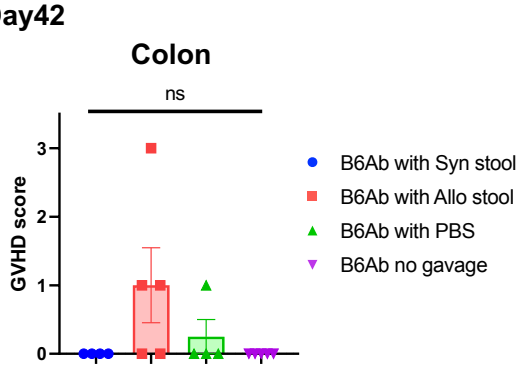


Figure S3. Systematic phenotyping of T cells and dendritic cells in lymphoid tissue and intestine.

B6 mice were treated for 2 weeks with 4 antibiotics cocktail (ampicillin 1 mg/ml, neomycin 1mg/ml, metronidazole 1mg/ml and vancomycin 0.5mg/ml), followed by 10 doses of intestinal content gavage from BMT recipient mice 2 weeks after BMT. Each gavage day one mouse whole intestinal content was collected and homogenized in sterile PBS. (A and B) Immune profiles of systemic lymph node, spleen, and mesenteric lymph node from mice at day 14 (A) and day 42 (B) after stool gavage are shown. (C) The immune profiles of T cells from colon and ileum of mice at day 14 after stool gavage. (D and E) The pathological GVHD score of colon from stool donor mice day14 after BMT and mice at day 14 (D) and day 42(E) after stool gavage (Allo B6: N=5, Syn B6: N=4, B6Ab with Syn stool, Day14: N=3, Day42: N=4, B6Ab with Allo stool, Day14: N=3, Day42: N=5, B6Ab with PBS, Day14: N=3, Day42: N=4, B6Ab no Gavage, Day14: N=3, Day42: N=5). One-way ANOVA analysis with Tukey post hoc test (D, E) was used to determine significance (mean ± s.e.m.). ****P<0.0001.

Figure S4. Related to Figure 4

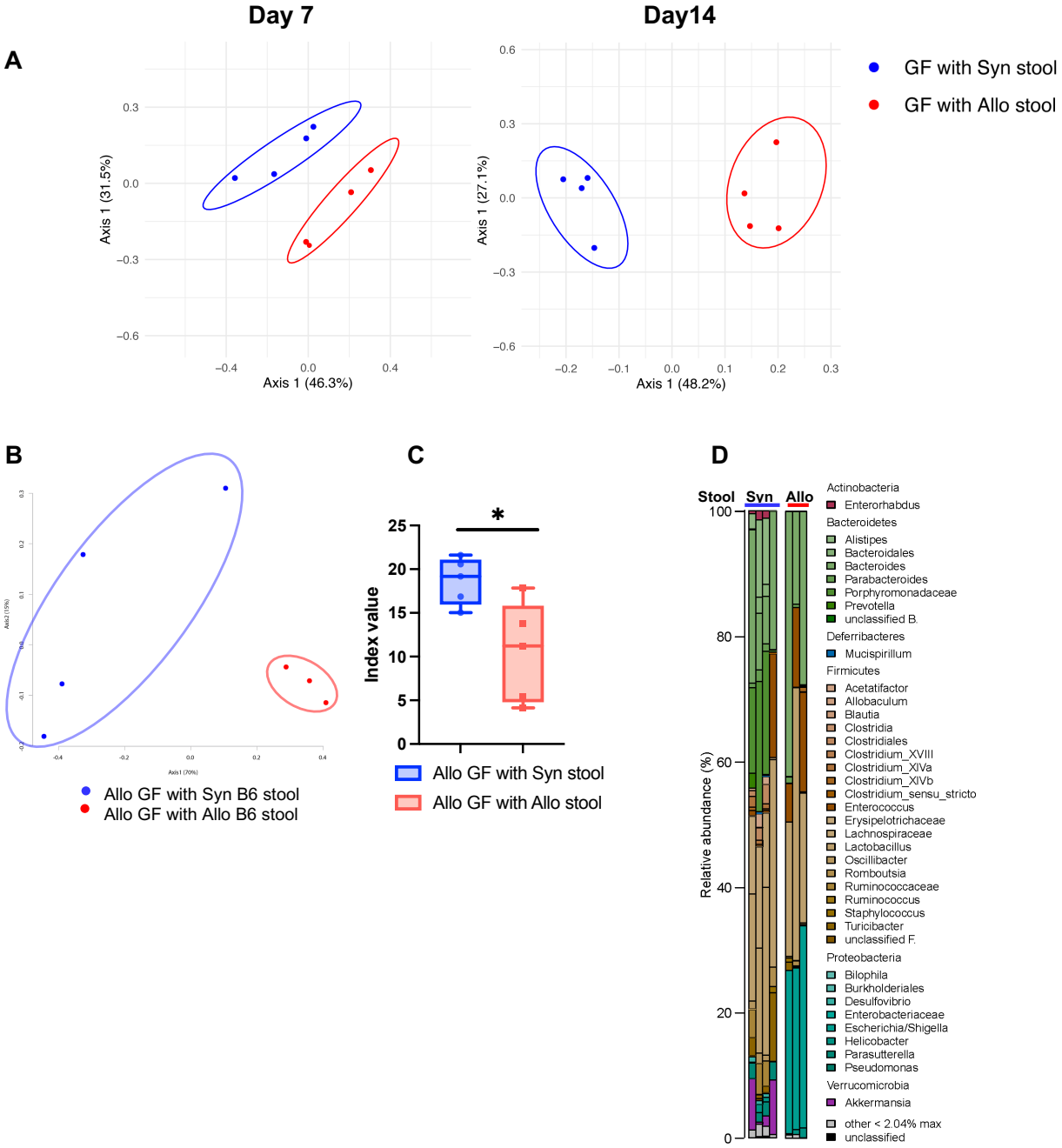


Figure S4. The microbiome composition of allogeneic GF mice with Syn or Allo B6 stool.

(A) The details of the experimental design were shown in Fig.4A to C. Stool from colon and ileum from GF with Syn or Allo B6 stool at day7 (left) and day14 (right) after stool gavage analyzed by 16S rRNA gene sequencing. PCoA were shown(n=4). (B to D) The details of the experimental design were shown in Fig. 4G. Stool from Allo GF with Syn or Allo B6 stool 2weeks after intestinal content gavage analyzed by 16S rRNA gene sequencing. (B to D) PCoA (B), inverse Simpson alpha diversity index of microbiome composition (C), and microbiome composition (D) are shown (Allo GF with Syn B6 stool: N=4, Allo GF with Allo B6 stool: (B) N=3, (C) N=5). Two-tailed unpaired t-test (C) (mean ± s.e.m.) was used to determine significance. *P<0.05

Figure S5. Related to Figure 5

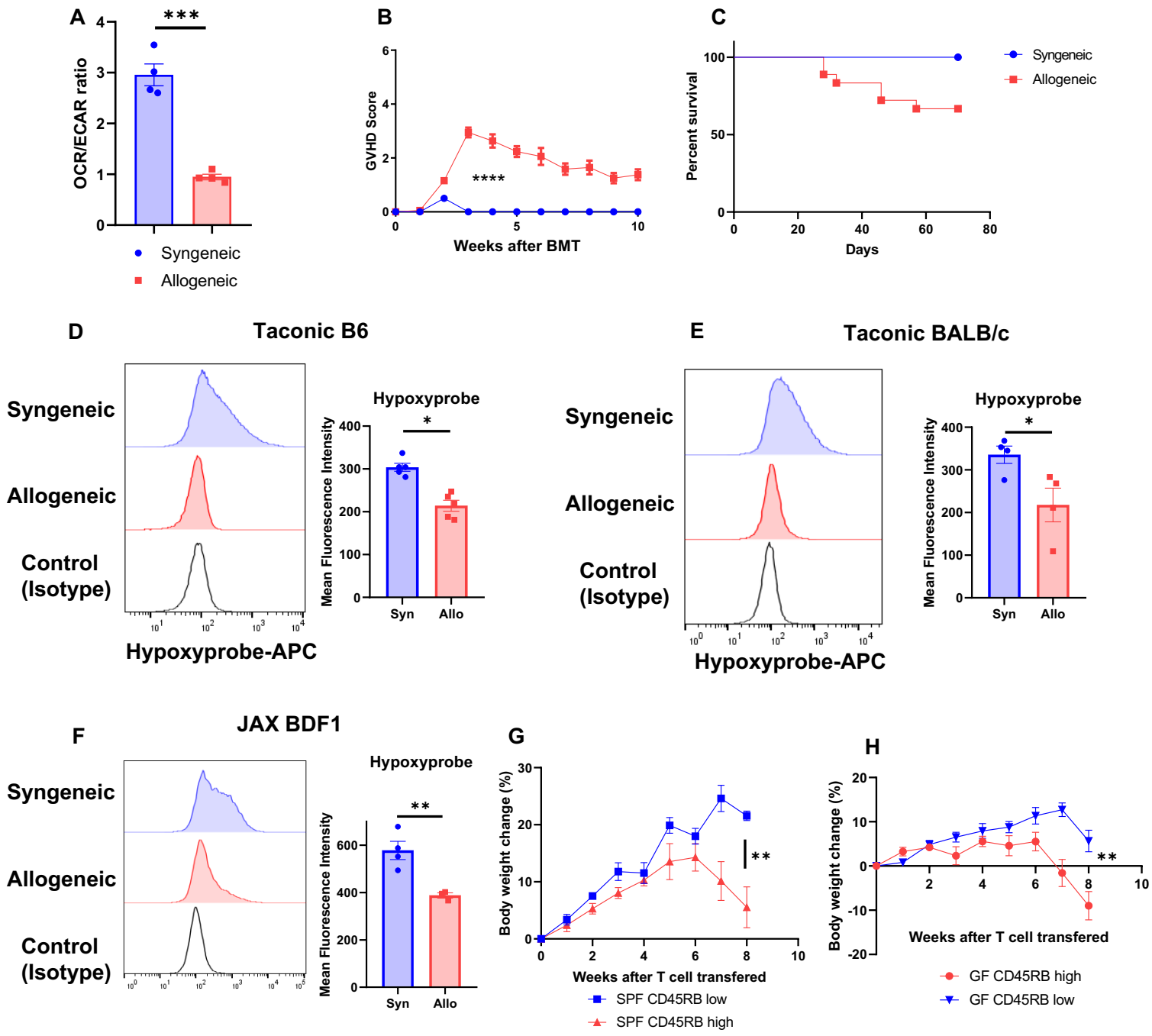


Figure S5. The defect of O2 utilization in intestine after allo-HSCT.

(A) Maximum OCR/ extracellular acidification rate (ECAR) ratio of isolated colonic IECs from syngeneic and allogeneic mice (BALB/c→ B6) at day21 after BMT are shown (N=4). (B and C) The details of experimental design is in Fig.5D. Clinical GVHD score (B) and survival rate (C) were shown (Syngeneic: N=6, Allogeneic: N=19). (D to F) Taconic B6 (D), Taconic BALB/c (E), and JAX BDF1(F) mice received BMT as described in Methods. Representative image of flowcytometry with Hypoxyprobe-APC and mean fluorescent intensity in syngeneic and allogeneic mice at day7 after BMT were shown. (G and H) The details of the experimental design were shown in Fig. 5G and H. Body weigh change of SPF mice (G) and GF mice(H) were shown (SPF CD45RB low: N=4, SPF CD45RB high: N=6, GF CD45RB low: N=4, GF CD45RB high: N=6). Two-tailed unpaired t-test (A, D to H) and two-tailed Mann-Whitney test (B) (mean ± s.e.m.) were used to determine significance. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Figure S6. Related to Figure 7

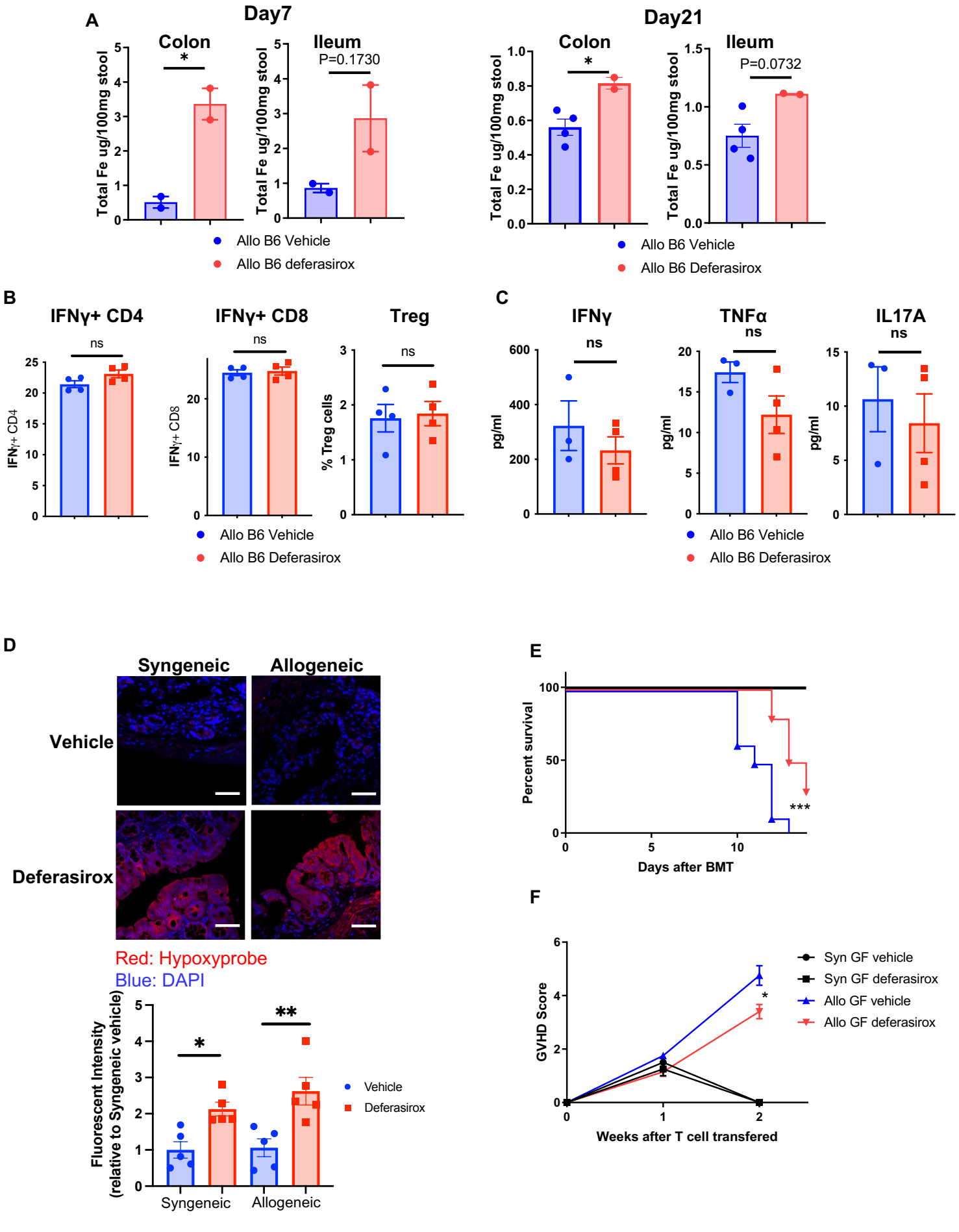


Figure S6. Iron chelator treatment does not alter T cells function.

(A) The details of the experimental design were shown in Fig7G to L. Quantification of iron in stool from colon and ileum in Allo B6 and Allo B6 deferasirox at 7day and 21day after BMT were shown (Day7, N=2, Day21, Vehicle: N=4, Deferasirox: N=2). (B) The percent of IFNγ+ CD4, IFNγ+ CD8, and Treg cells in spleen from recipients at day7 after BMT were shown (N=4). (C) The cytokine levels of IFNγ, TNFα, and IL17A in serum from recipients at day7 after BMT were shown (Vehicle: N=3, Deferasirox: N=4). (D to F) GF mice received BMT from BALB/c donor mice. BMT recipients were orally treated with deferasirox (20mg/kg) and vehicle every day. (D) Hypoxyprobe staining and relative fluorescent intensity in colon from recipients 21days after BMT (scale bar= 50μm). Three independent experiments were performed. Survival rate (E) and clinical GVHD score (F) of BMT recipients (Syn GF vehicle: N=2, Syn GF deferasirox: N= 2, Allo GF vehicle: N=8, Allo GF deferasirox: N=10). Two-tailed unpaired t-test (A, B, C, D), log-rank test (E), or two-tailed Mann-Whitney test (F) was used to determine significance (mean ± s.e.m.). *P < 0.05, **P<0.01, ***P<0.001.

Table S1. HCT model. Related to STAR Methods

HCT model			**Day0: The day of transplantation**		
MHC-mismatched model	Recipient	Donor	Conditioning	T cells, cells	TCD-BM, cells
	C57BL/6	Allo: BALB/c or Syn: C57BL/6	10Gy Total-body irradiation(TBI), day-1	CD90.2+, 2.5 × 10^6	5 × 10^6
	GF B6	Allo: BALB/c or Syn: C57BL/6	10Gy TBI, day-1	CD90.2+, 2.5 × 10^6	5 × 10^6
	Hif1afl/fl Vil1-cre	Allo: BALB/c or Syn:C57BL/6	10Gy TBI, day-1	CD90.2+, 2.5 × 10^6	5 × 10^6
	129	C57BL/6, BM +/- T cells	10Gy TBI, day-1	CD5+, 2 × 10^6	5 × 10^6
No-conditioning model	B6D2F1	Syn: B6D2F1 or Allo:C57BL/6	No conditioning	10 × 10^7 splenocytes	No bone marrow
Chemotherapy conditioning model	C57BL/6	Allo: BALB/c or Syn:C57BL/6	Busulfan (B2635, 25 mg kg^{-1} from day -7 to -4; Sigma-Aldrich), Cyclophosphamide (C7397, 100 mg kg^{-1} from day -3 to -2; Sigma-Aldrich) Intraperitoneal injection	CD90.2+, 1 × 10^7	1 × 10^7

Table S2. Clinical GVHD score. Related to STAR Methods

Clinical GVHD score				
Grade 0	Grade 0.5	Grade 1	Grade 1.5	Grade 2
<10%	N/A	10-25%	N/A	>25%
No hunch	Slight hunch, straightens when walks	Animals stay hunched when walk	Animals does not straighten ou	Animals tend s to stand on rear toes
Very mobile, hard to chach	Slower than naïve mice, easier to catch	Not moving, but will move when poked	Not moving, will move slightly when poked	Not moving, will not move if poked
No redness, abrasions, lesion or scaling present	Redness in one area only	Abrasions in 1area, or mild abrasions in 2 areas	Bad abrasions in 2 areas	Extremely bad abrasion, cracking skin, dried blood etc.
No fur pathology	Ridging on the side of belly or nape of neck	Ridging across or the side of belly plus neck	Unkempt matted and ruffled fur	Badly matted fur on belly, and on top