Supplementary Materials for

Reduced dose of PTCy followed by adjuvant α-galactosylceramide enhances GVL effect without sacrificing GVHD suppression

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Supplemental Method

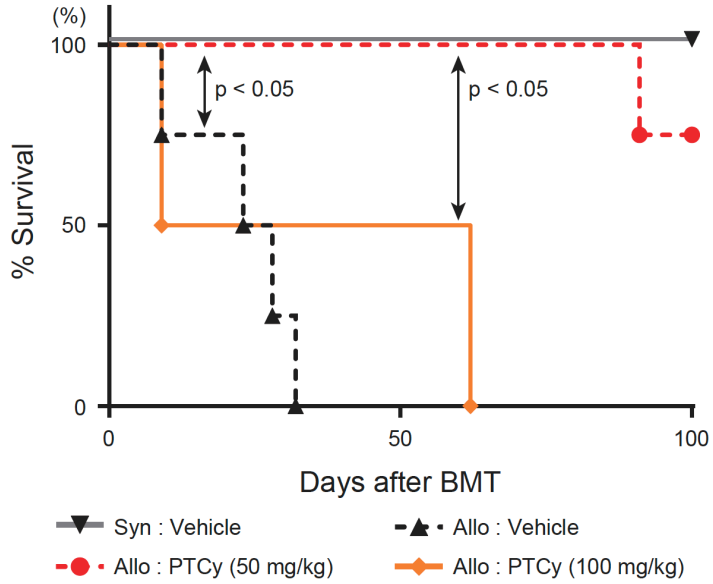
Antibodies for flow cytometry

The following mAbs used in this study were purchased from BD Biosciences (San Jose, CA, USA), BioLegend (San Diego, CA, USA), eBioscience (San Diego, CA, USA) or R&D Systems (Minneapolis, MN, USA): eFluor450-conjugated anti-B220 (RA3-6B2), eFluor450-conjugated anti-CD4 (GK1.5), PB-conjugated anti-TCRβ (H57-597), FITC-conjugated anti-CD4 (RM4-5), FITC-conjugated anti-H-2Kd (SF1-1.1.1), PE-conjugated anti-H-2Kd (SF1-1.1.1), PE-conjugated anti-CD45.1 (A20), PE-Cy7-conjugated anti-CD25 (PC61.5), PE-Cy7-conjugated anti-CD45.1 (A20), APC-conjugated anti-CD11c (N418), APC-conjugated anti-CD19 (MB19-1), APC-eFluor780-conjugated anti-CD8a (53-6.7), APC-eFluor780-conjugated anti-CD11b (M1/70), APC-eFluor780-conjugated anti-CD19 (1D3), APC-Cy7-conjugated anti-CD3 (17A2), PE-Cy7-conjugated anti-PLZF (9E12), APC-conjugated anti-Foxp3 (FJK-16s), and Alexa Fluor647-conjugated anti-RORγt (Q31-378).

Evaluation of the GVL effect by bioluminescence imaging.

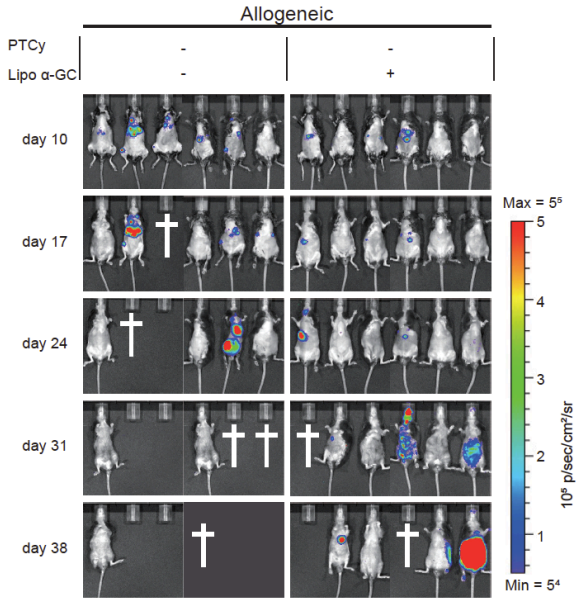
In a GVL model, 3 mg of D-Luciferin (OZ Biosciences USA, San Diego, CA, USA) were injected intraperitoneally into each mouse, and the tumor burden was assessed by bioluminescence imaging (BLI) on days 17, 24, 31, 38, and 45. BLI was performed with an IVIS (IVIS Lumina, Xenogen, Alameda, CA, USA). Images were analyzed with Living Image Software 3.2 (Xenogen).

Supplemental Fig. 1



5×10^6 TCD-BM cells and 10×10^6 splenocytes from B6 donor mice were administered to lethally irradiated (12 Gy TBI) B6D2F1 recipient mice. Two different doses of cyclophosphamide (50 mg/kg or 100 mg/kg) or a control vehicle was administered on day 3 after BMT. PTCy at a dose of 50 mg/kg significantly protected recipients from acute GVHD, while a dose of 100 mg/kg failed to do so. Overall survival rates were compared among Syn: Vehicle (n = 4), Allo: Vehicle (n = 4), Allo: 50 mg/kg PTCy (n = 4) or Allo: 100 mg/kg PTCy (n = 4). Data is obtained from one experiment. P values by log-rank test and the Holm's adjustment for multiple comparison.

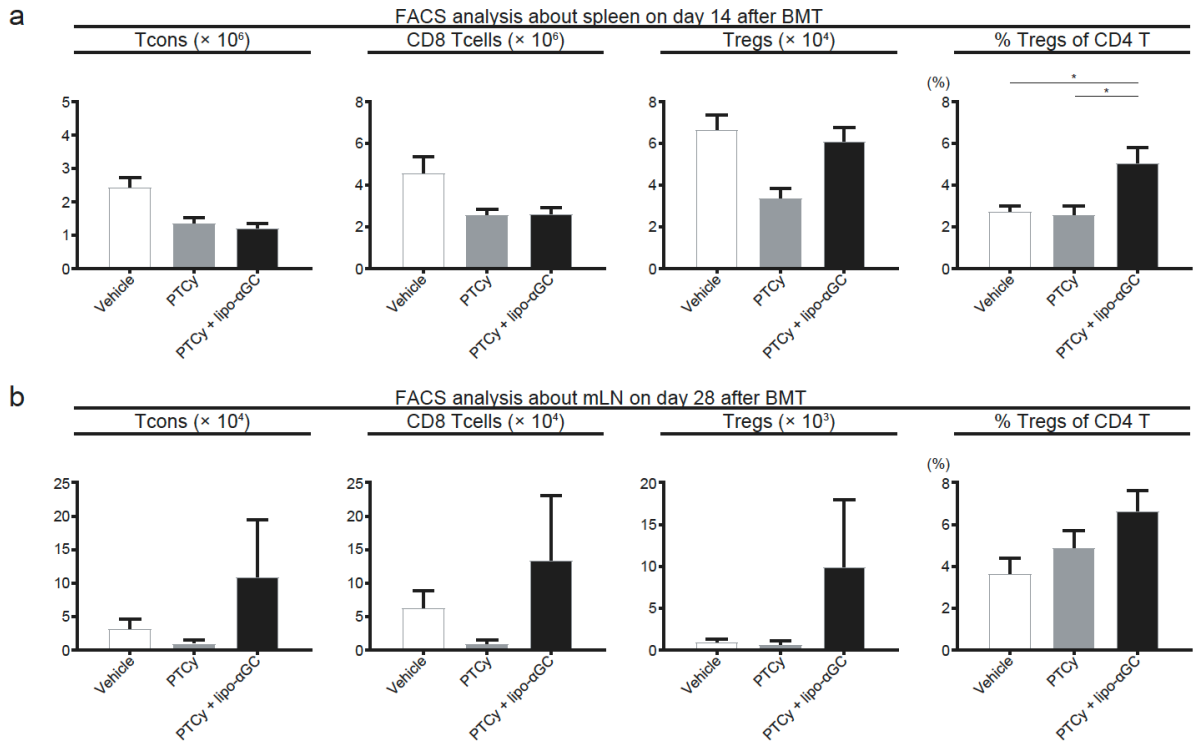
Supplemental Fig. 2



IVIS study of the allogeneic cohorts without PTCy.

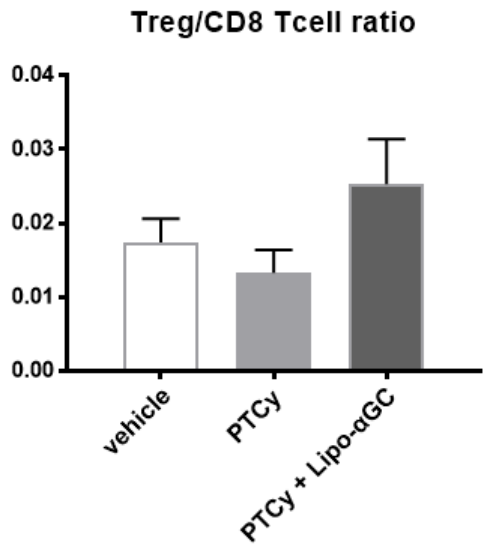
The results from groups with or without lipo α-GC (n = 6 in each group) at serial time points are shown.

Supplemental Fig. 3



(a) FACS analysis of splenocytes on day 14 after BMT revealed the absolute number of Tcons, CD8 T cells and Tregs and percentages of Tregs. (b) FACS analysis of mLN cells on day 28 after BMT revealed the absolute number of Tcons, CD8 T cells and Tregs and percentages of Tregs. P values by one-way ANOVA and the Tukey's adjustment for multiple comparison. * $P < .05$.

Supplemental Fig. 4



Flow cytometric analysis of mLN cells on day 14 after BMT revealed the Treg to $CD8^+$ T cells ratio in each group.