

Supplementary information for

**Donor Treg expansion by liposomal  $\alpha$ -galactosylceramide modulates T<sub>fh</sub> cells and prevents sclerodermatous chronic graft-versus-host disease**

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**This file includes:**

Supplemental figures 1-8

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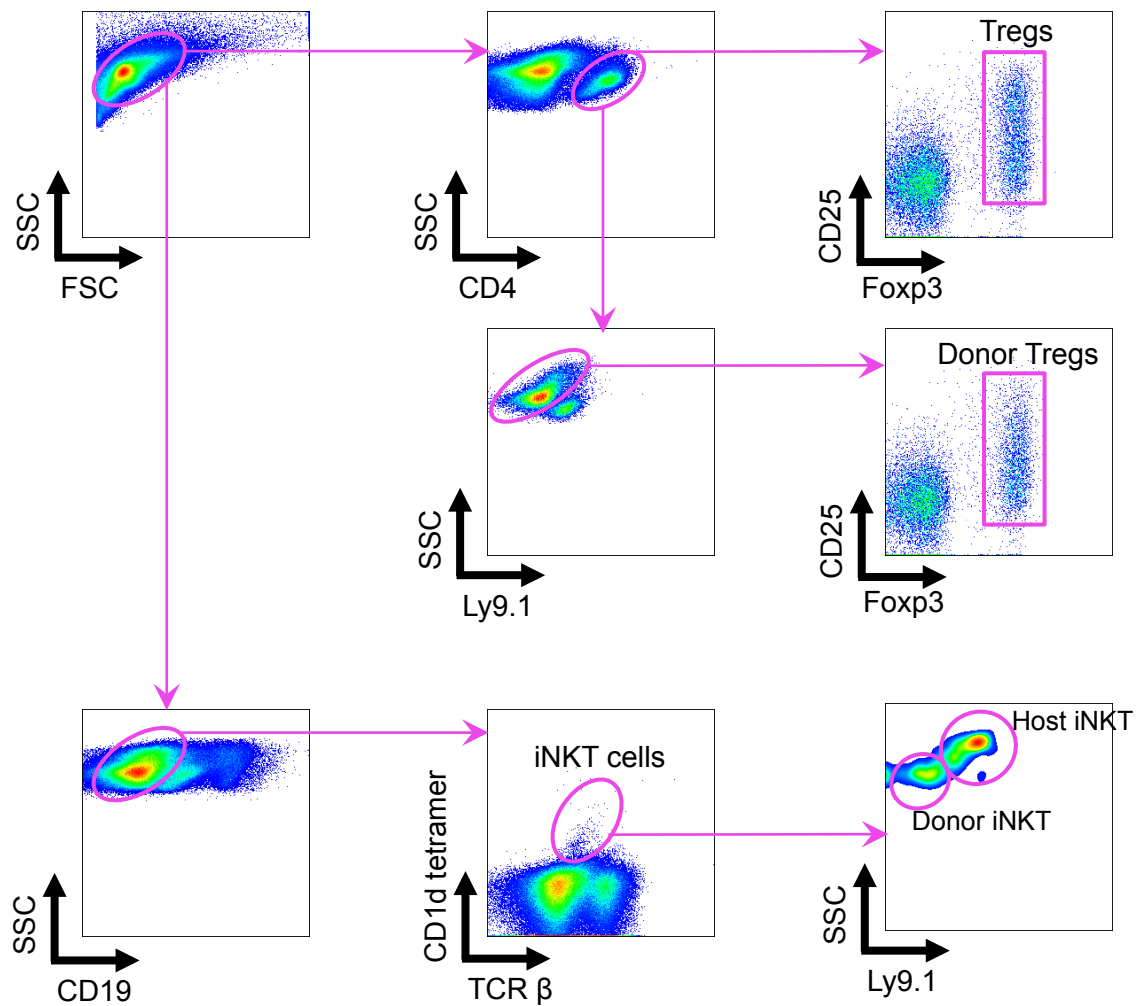
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Supplemental figure1.

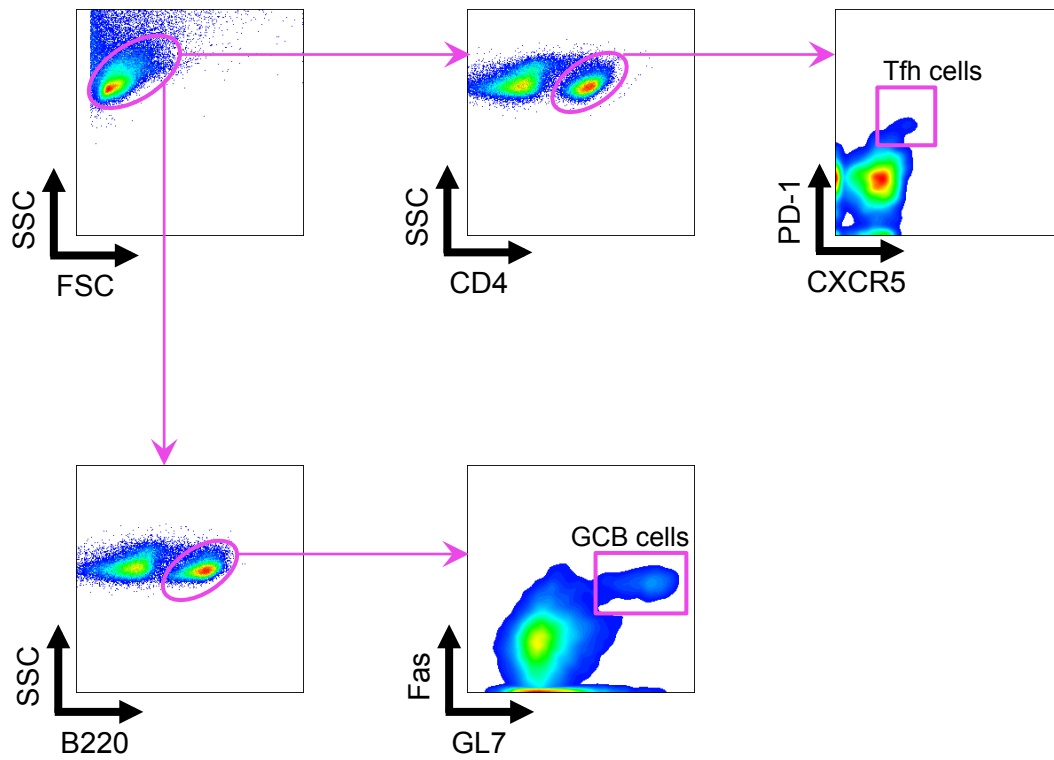


**Gating strategy for Tregs and iNKT cells.**

Tregs are identified as  $CD4^+CD25^{med-high}Foxp3^+$ . Donor-derived  $CD4^+$  T cells and Tregs are identified by the expression pattern of Ly9.1.

iNKT cells are identified as  $CD19^-TCR\beta^-CD1d-tetramer^+$  and further divided into donor-derived and host-derived subsets by the expression pattern of Ly9.1.

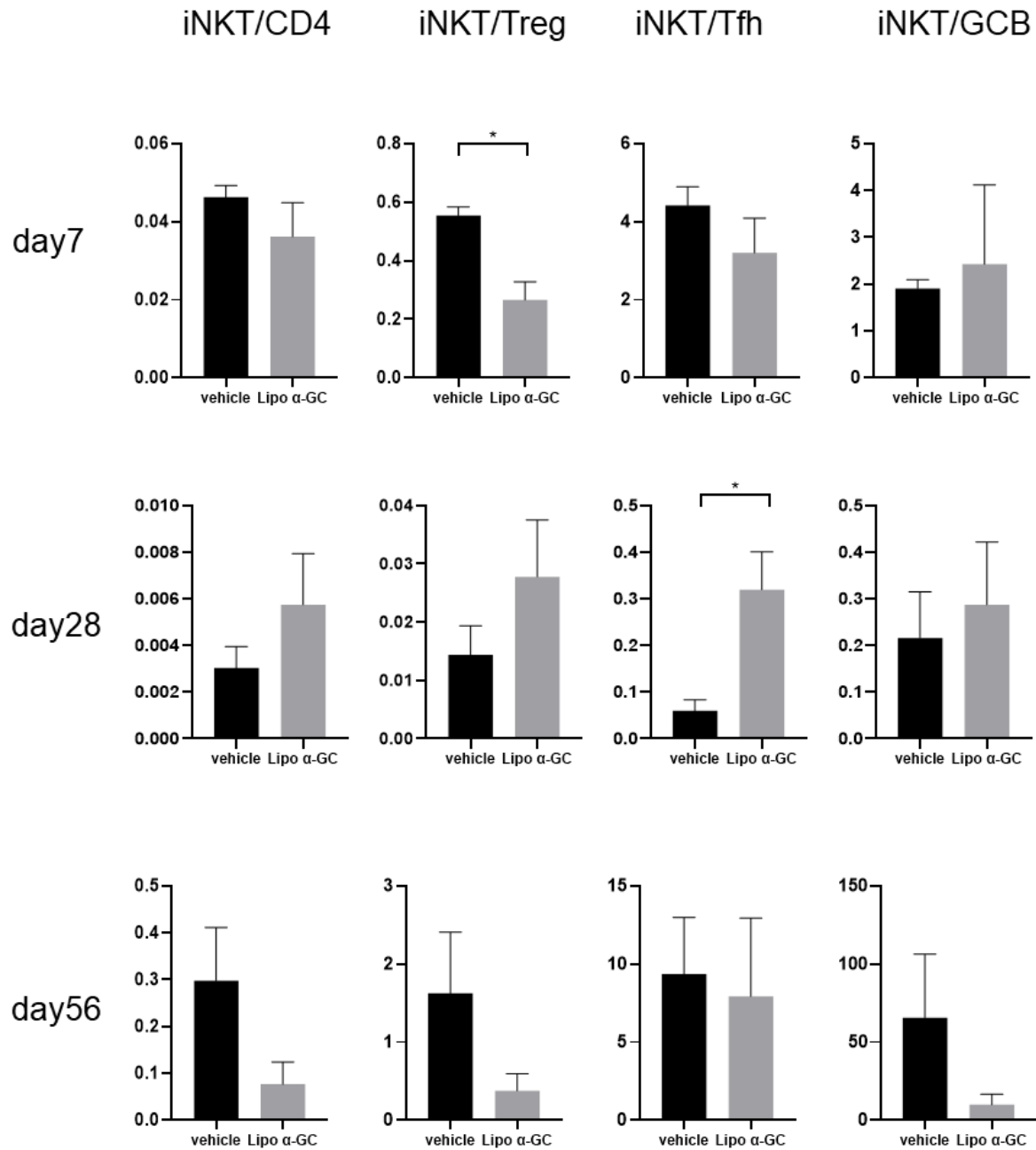
Supplemental figure2.



**Gating strategy for Tfh cells and GCB cells.**

T follicular helper cells (Tfh cells) are identified as  $CD4^+CXCR5^{med-high}PD-1^+$ . GCB cells are identified as  $B220^+GL7^+Fas^+$ .

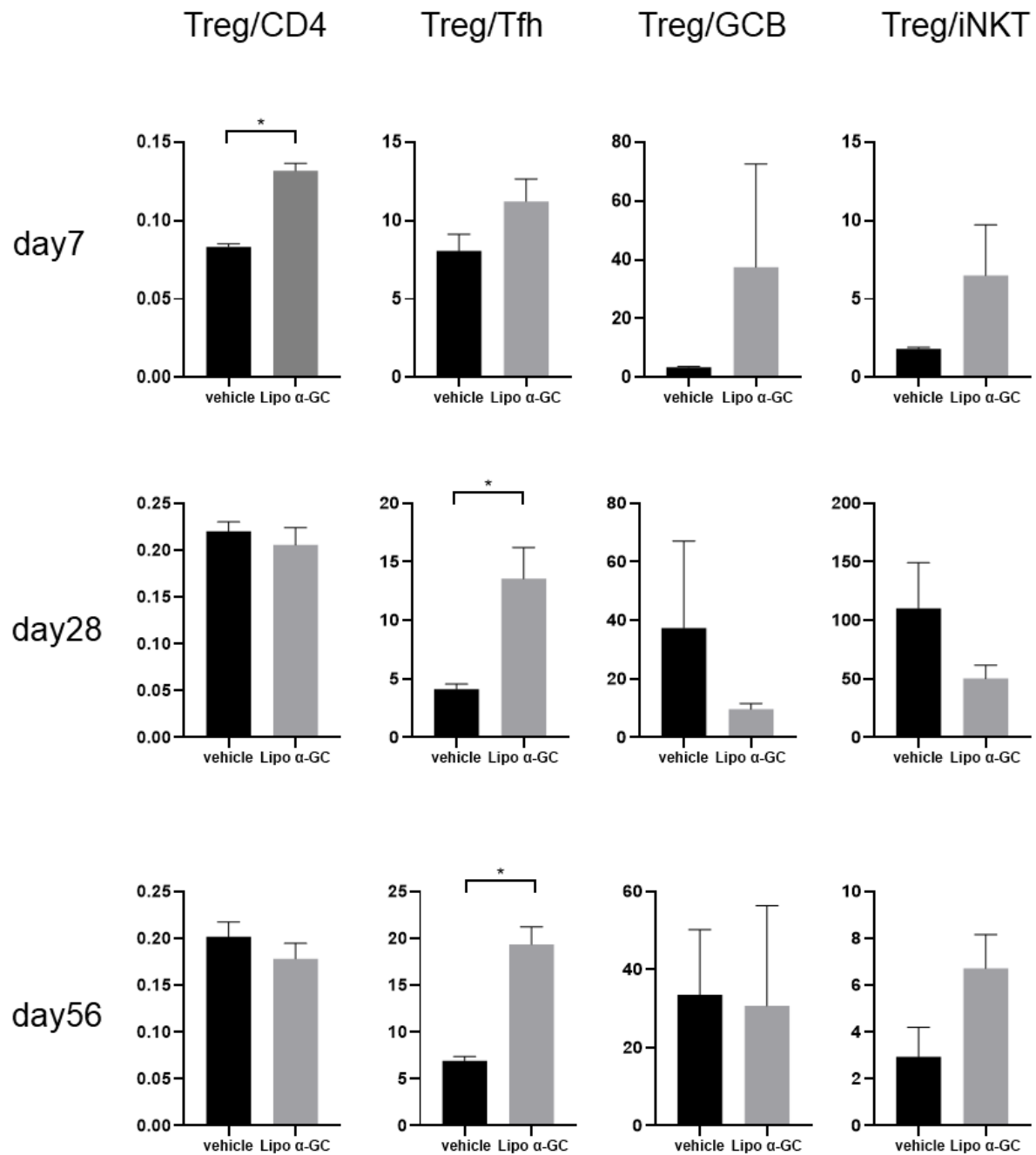
Supplemental figure 3.



**The relative proportion of iNKT to CD4, Treg, Tfh and GCB in spleen on day7, 28, and 56.**

The relative proportion of iNKT to Treg in spleen on day7 decreased in lipo  $\alpha$ -GC group significantly, and the relative proportion of iNKT to Tfh in spleen on day28 decreased in vehicle group significantly. Statistical analysis was performed by Student's t-test.\*P<0.05

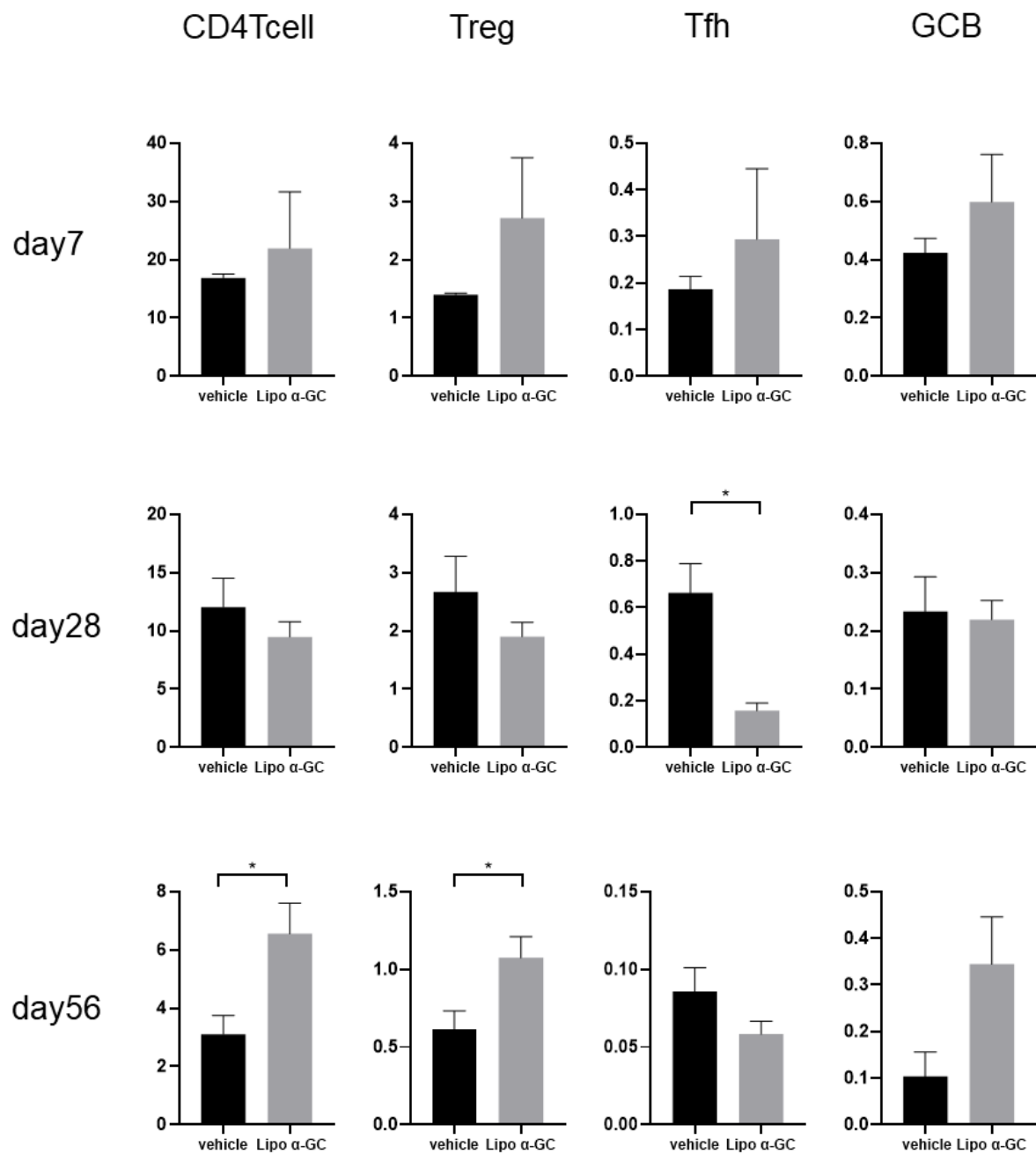
Supplemental figure 4.



**The relative proportion of Treg to CD4, Tfh, GCB and iNKT in spleen on day7, 28, and 56.**

The relative proportion of Treg to CD4 increased in lipo  $\alpha$ -GC group significantly on day7 and the relative proportion of Treg to Tfh in spleen on day28, 56 increased in lipo  $\alpha$ -GC group significantly. Statistical analysis was performed by Student's t-test.\*P<0.05

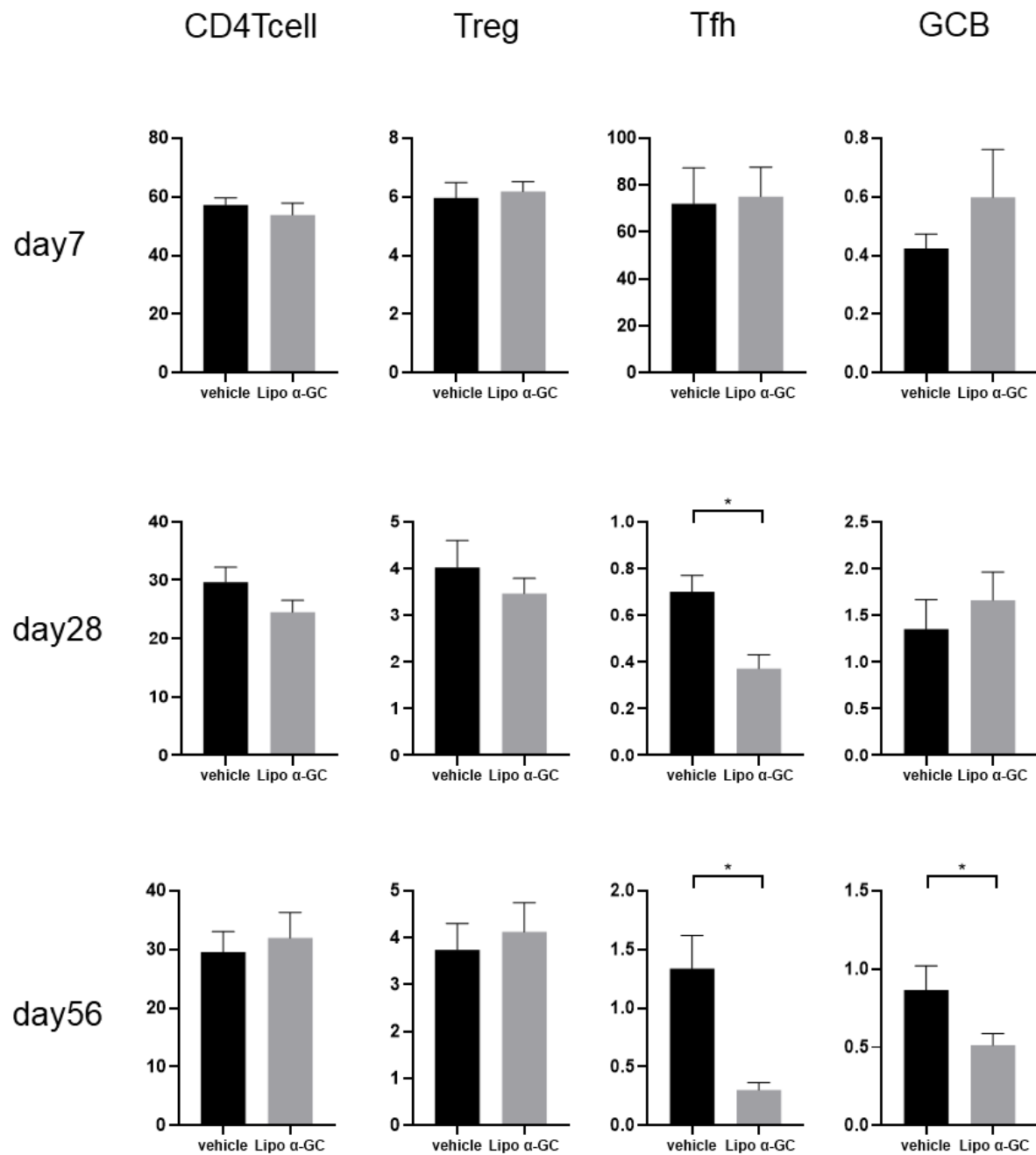
Supplemental figure 5.



**The relative proportion of CD4, Treg, Tfh and GCB to total lymphocyte on day 7, 28, and 56 in spleen.**

The relative proportion of CD4 and Treg increased in spleen on day 56 in lipo α-GC group significantly and the relative proportion of Tfh on day 28 decreased in lipo α-GC group significantly. Statistical analysis was performed by Student's t-test. \*P < 0.05

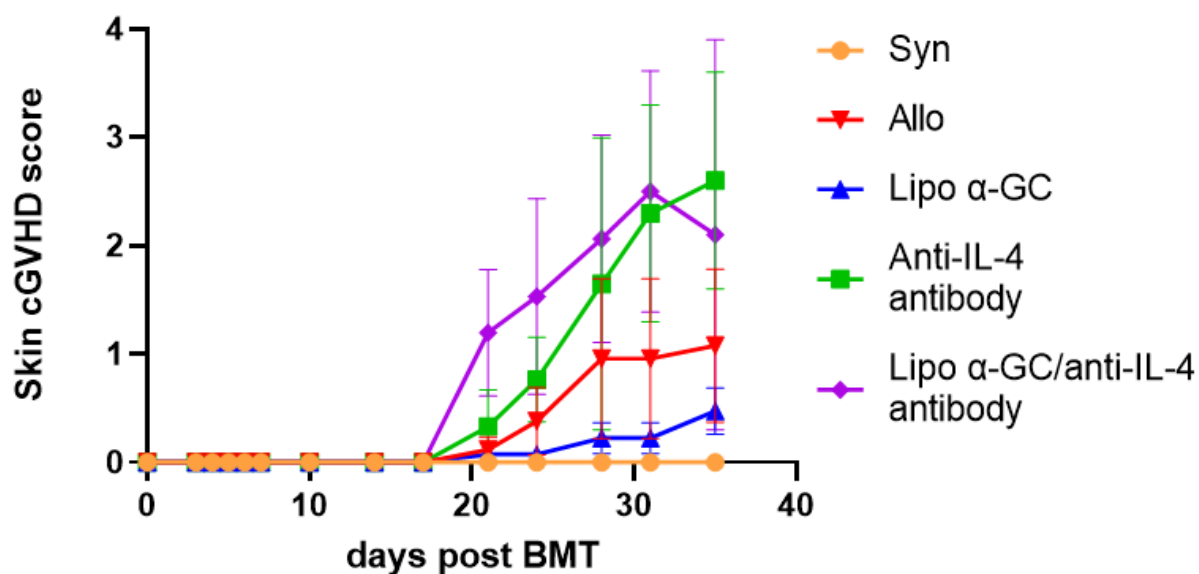
Supplemental figure 6.



**The relative proportion of CD4, Treg, Tfh and GCB to total lymphocyte on day7, 28, and 56 in MLN.**

The relative proportion of Tfh on day28, 56 and GCB on day56 in MLNs decreased in lipo α-GC group significantly. Statistical analysis was performed by Student's t-test. \*P<0.05

Supplemental Figure 7.

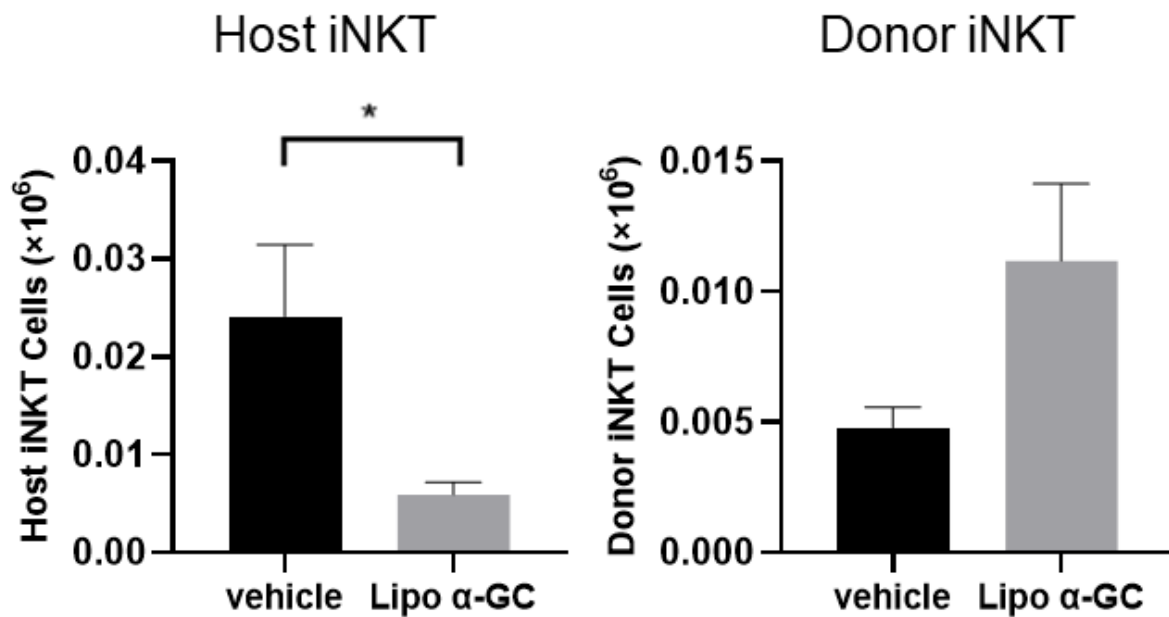


**Preventive effect of lipo  $\alpha$ -GC against cGVHD was cancelled by anti-IL-4 antibody.**

BALB/c recipient mice received 6 Gy of TBI; then,  $8 \times 10^6$  BM cells and  $10 \times 10^6$  spleen cells were administered from syngeneic Balb/c mice or allogeneic B10.D2 donor mice. Lipo  $\alpha$ -GC was administered at a dose of 1  $\mu$ g/kg via tail vein injection on days 0, 3, and 5 after BMT. Anti-IL4 antibody was administered intraperitoneally on day 0 (2mg/body), and day 3 and 5 (1mg/body) after BMT. Time courses of skin cGVHD score after BMT in syngeneic (n = 3), allogeneic (n = 10), allogeneic/lipo  $\alpha$ -GC (n = 10), allogeneic/anti-IL-4 antibody (n = 6), and allogeneic/lipo  $\alpha$ -GC/anti-IL-4 antibody (n = 6) groups. Data are expressed as the means  $\pm$  SEM.



Supplemental Figure 8.



**Lipo α-GC increases donor iNKT cells and decreases host iNKT cells in lymph nodes in an early phase after BMT.**

iNKT cells were defined as CD19<sup>-</sup>TCR-β<sup>+</sup>CD1d tetramer<sup>+</sup> cells. Chimerism was evaluated in the host (Ly9.1<sup>+</sup>) or donor (Ly9.1<sup>-</sup>). The number of donor iNKT cells and host iNKT cells in MLNs after BMT in vehicle (n = 4) and lipo α-GC (n = 4) mice on day 7. Data are expressed as the means ± SEM. P-values were determined using an unpaired, two-tailed Student's t-test. \*P < 0.05.