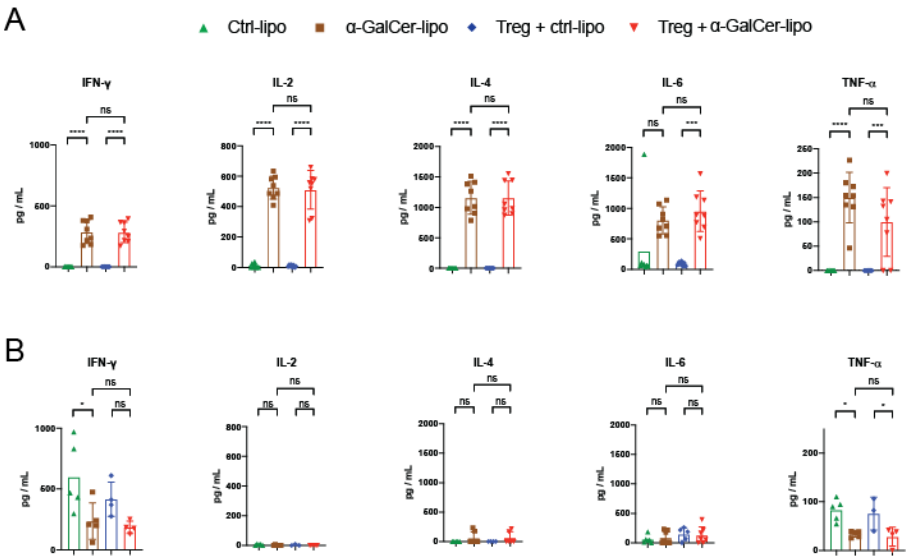
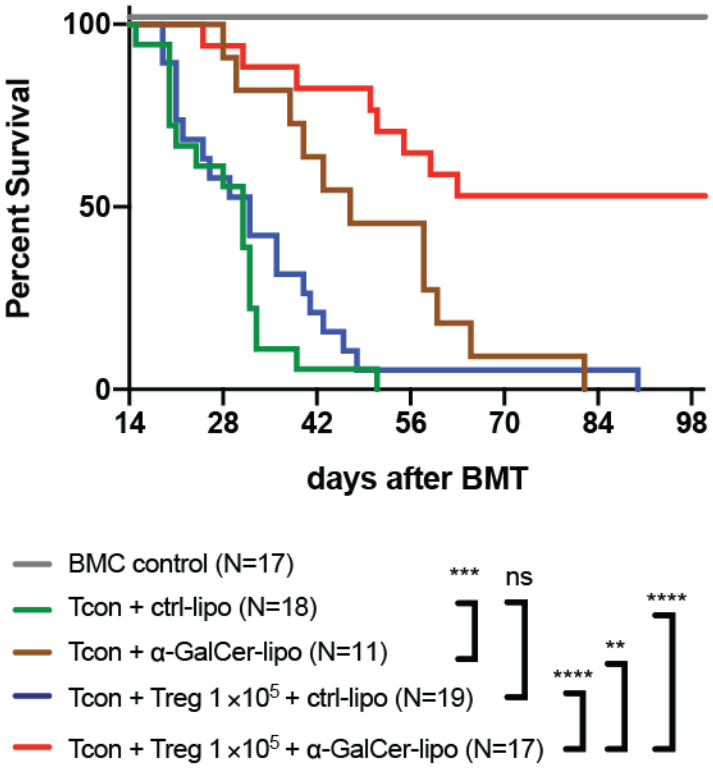
Supplementary figures



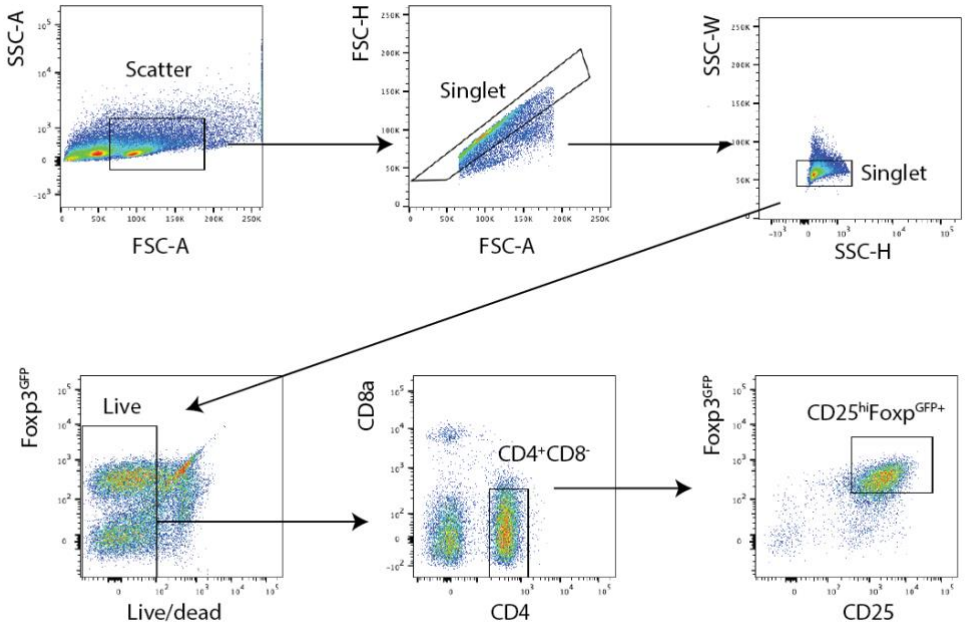
Sup figure 1. Serum cytokine level at 2 hr and 10 day after bone marrow transplantation

Recipient BALB/c mice were irradiated 8.8-Gy and transferred C57BL/6 bone marrow cells plus Tcons with or without 1.0×10^5 sorted $Foxp3^{GFP+}$ Tregs followed by administration of ctrl-lipo or α-GalCer-lipo. Serum samples were collected at 2 hr (A) and 10 day (B) after BMT. Mean ± SD from pooled data of two independent experiments. P-value *<0.05, **<0.01, ***<0.001, ****<0.0001 calculated by ordinary one way ANOVA with Holm-Sidak correction. ns: not significant.



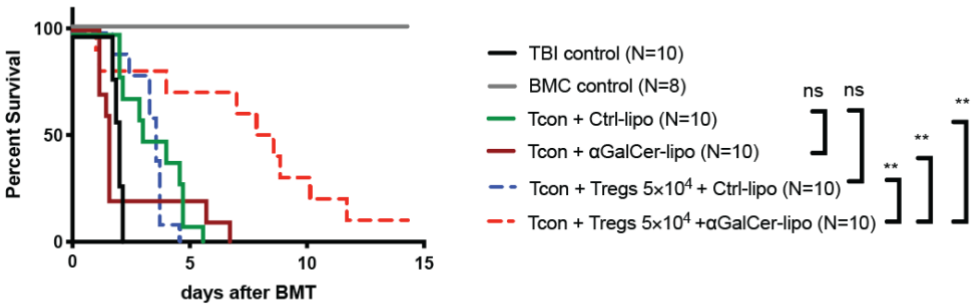
Sup figure 2. Murine survival curve from day 14 after BMT.

The mice that survived beyond day 14 were implemented into the analysis. P-value **<0.01, ***<0.001, ****<0.0001 calculated by Log-rank test between indicated two groups. Pooled data of four independent experiments is shown. ns: not significant.



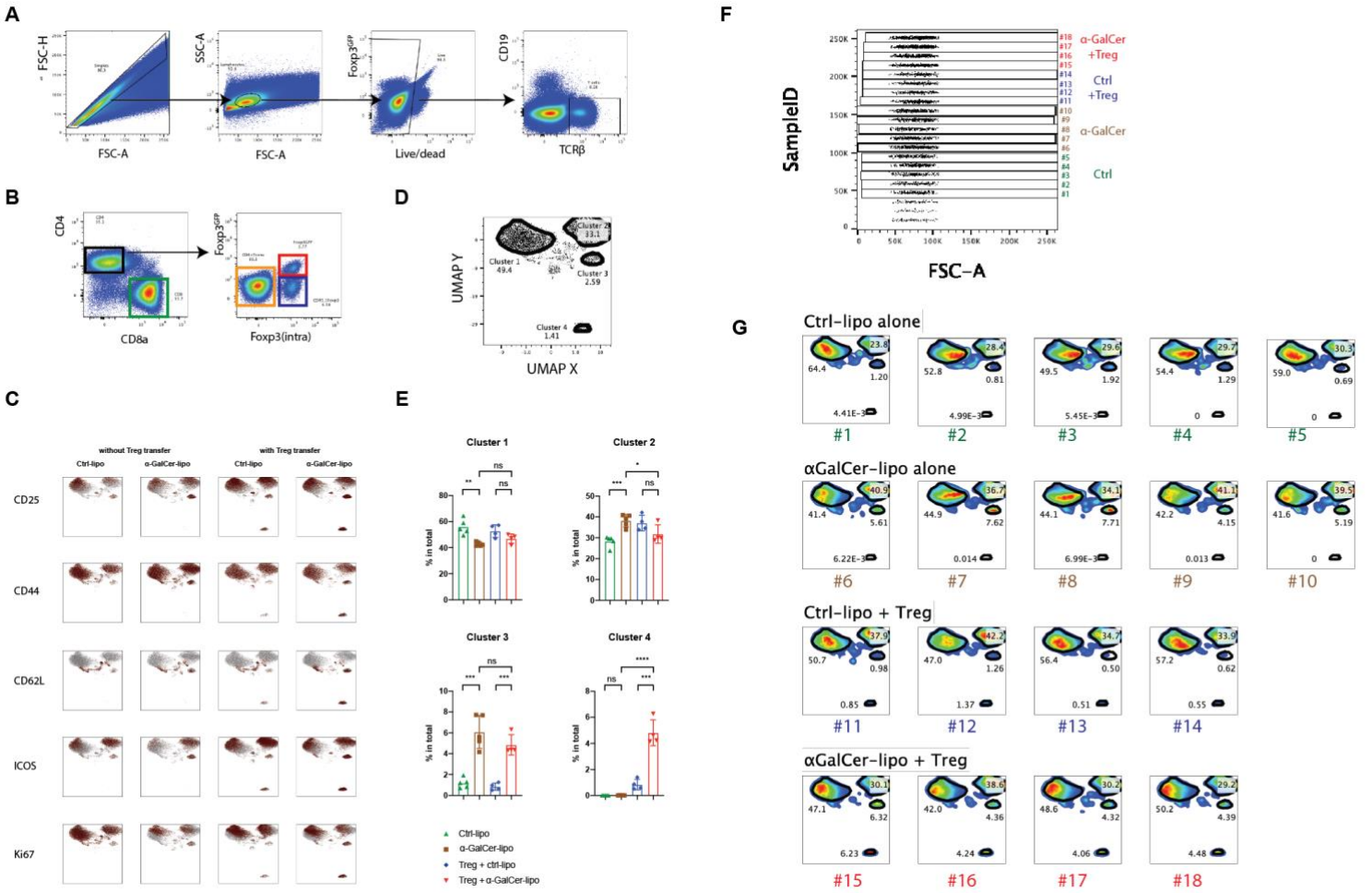
Sup figure 3. Sorting strategy for $CD4^{+}Foxp3^{GFP+}$ Tregs.

$CD25^{+}$ cells were magnetic bead-enriched from single cell suspension obtained from secondary lymphoid tissues of B6-$Foxp3^{GFP+}$ mice, and further stained with CD4, CD8, and LIVE/DEAD Aquamine. $CD4^{+}CD^{-}CD25^{hi}Foxp3^{GFP+}$ cells were gated and acquired by flow cell sorter.



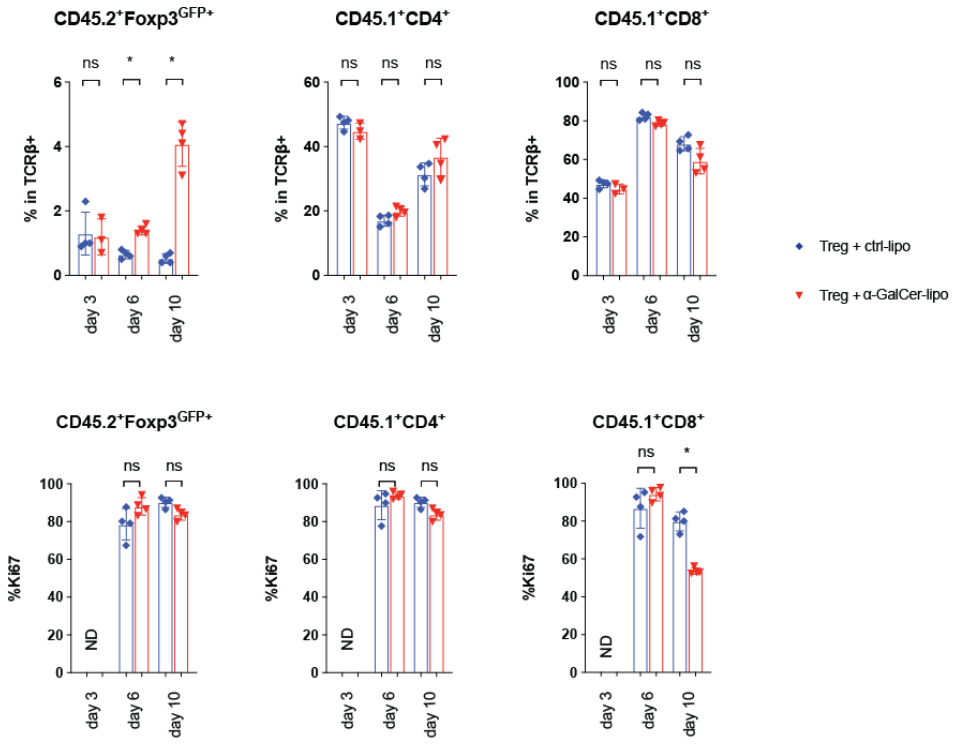
Sup figure 4. Administration of α-GalCer-lipo significantly improved murine survival when combined with 5×10^4 Treg transfer

Flow sorted $Foxp3^{GFP+}$ Tregs were injected into recipient mice at 1:20 Treg:Tcon ratio at the time of bone marrow transplantation. P-value ***<0.001, ****<0.0001 calculated by Log-rank test between indicated two groups. Pooled data of two independent experiments was shown.


A
FSC-H
FSC-A
SSC-A
FSC-A
Foxp3GFP
Live/dead
CD19
TCRβ
B
CD4
CD8a
Foxp3GFP
Foxp3(intra)
C
without Treg transfer
with Treg transfer
Ctrl-lipo
α-GalCer-lipo
Ctrl-lipo
α-GalCer-lipo
CD25
CD44
CD62L
ICOS
Ki67
D
UMAP Y
UMAP X
Cluster 1
49.4
Cluster 2
33.1
Cluster 3
2.59
Cluster 4
1.41
E
Cluster 1
Cluster 2
Cluster 3
Cluster 4
% in total
ns
Ctrl-lipo
α-GalCer-lipo
Treg + ctrl-lipo
Treg + α-GalCer-lipo
F
SampleID
FSC-A
α-GalCer +Treg
Ctrl +Treg
α-GalCer
Ctrl
G
Ctrl-lipo alone
αGalCer-lipo alone
Ctrl-lipo + Treg
αGalCer-lipo + Treg

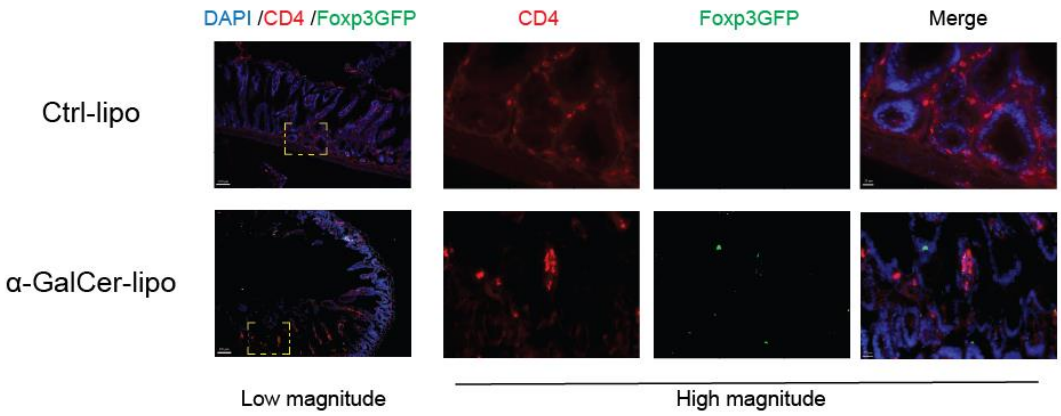
Sup figure 5. Gating strategy for UMAP plot.

(A) All data from one representative experiment were concatenated. Singlet-living lymphocytes were gated and $TCR\beta^{+}CD19^{-}$ gated cells were implemented for downstream analysis. UMAP was calculated according to expression level of CD4, CD8a, CD45.1, CD45.2, Foxp3 (intracellular stained), $Foxp3^{GFP}$, CD25, CD44, CD62L, ICOS, and Ki67. (B) $CD4^{+}Foxp3^{-}$ cells (orange box: $CD4^{+}$ Tcons), $CD8^{+}$ cells (green box: $CD8^{+}$ Tcons), $CD4^{+}Foxp3^{+}Foxp^{GFP-}$ cells (blue box: Tcon-derived Tregs), and $CD4^{+}Foxp3^{GFP+}$ cells (red box: transferred Tregs) were gated, and overlaid on UMAP plot (main figure 2E). (C) $CD25^{+}$, $CD44^{+}$, $CD62L^{+}$, $ICOS^{+}$, and $Ki67^{+}$ cells (brown dots) that were overlaid on representative UMAP plot of each group. (D) Four major clusters (1 – 4) identified and gated for the following analysis. (E) The proportion of cluster 1 – 4 in each treatment group. P-value **<0.01, ***<0.001, ****<0.0001 calculated by student t-test between indicated two groups. (F) Concatenated data was divided into an individual mouse by gating on each sample number; #1-5: Ctrl-lipo, #6-10: α-GalCer-lipo, #11-14: Ctrl-lipo + Treg, #15-18: α-GalCer-lipo + Treg. (G) UMAP plots gated on individual mouse. The data from one representative of two individual experiment. ns: not significant.



Sup figure 6. T cell phenotype on day 3, day 6, and day 10.

Splenocytes from ctrl- and α-GalCer-lipo treated recipients were obtained on day 3, day 6, day 10 after BMT and analyzed by Flow cytometry. The proportion (above) and Ki67 expression level (bottom) of $CD4^{+}Foxp3^{GFP+}$ Tregs, $CD45.1^{+}CD4^{+}$ Tcons, and $CD45.1^{+}CD8^{+}$ Tcons at the indicated time points.



Sup figure 7. Immunohistochemistry of small intestine

Images of immunofluorescent staining for CD4 (red), Foxp3GFP (Green), and DAPI (Blue). Left; low magnification (10x, white bar indicates 100 µm), right; high magnification of inset region (40x, white bar indicates 20 µm). One representative image of four independent mice.