Supplementary Figure Legends

Supplemental Figure 1: Naïve T cells express Siglec-G.

Splenocytes were isolated from WT B6 or Siglec-G$^{-/-}$ animals that have not been transplanted (n=3 per group) and analyzed for Siglec-G expression utilizing a monoclonal antibody to Siglec-G (clone SH2.1). (a) isotype control, (b) B220^{+} B cells, (c) Siglec-G$^{-/-}$ T cells, (d) 1/100 dilution, (e) 1/200 dilution, (f) 1/400 dilution, (g) 1/1000 dilution. Data shown are representative of one of three similar experiments.

Supplemental Figure 2: Phenotypic analysis of various T cell subsets and activation markers in naïve Siglec-G$^{-/-}$ and WT B6 animals.

Splenocytes were isolated from WT B6 or Siglec-G$^{-/-}$ animals that have not been transplanted (n=5-6 per group) and analyzed for absolute total splenocyte numbers (a), the percentages and absolute numbers of CD4^{+} and CD8^{+} T cells (b), naïve (CD44^{-}CD62L^{+}), effector memory (EM: CD44^{+}CD62^{-}), central memory (CM: CD44^{+}CD62L^{+}) subsets(c), activation marker expression (CD25 and CD69) in CD4^{+} or CD8^{+} T cells (d-g), and regulatory T cells (Treg: CD4^{+}CD25^{+}Foxp3^{+})(h). Error bars show the mean ± SEM.

Supplemental Figure 3: Siglec-G in T cells represses its responses in the presence of DAMP.

(a) Isolated splenic CD90.2^{+} T cells from either B6-WT or Siglec-G$^{-/-}$ animals were incubated with anti-CD3 (2ug/ml) and anti-CD28 (1ug/ml) antibodies in the presence or absence of HMGB1 (10µg/ml) for 48 hours and analyzed for proliferation following ^{3}H-thymidine incorporation during the last 6 hours of incubation. Representative data from one of three independent experiments are shown. Unpaired *t* test, ***p<0.001. (b) Protein expression of phosphorylate (p)-STAT3, total STAT3 at 6 hour (STAT-3) was evaluated by western blot. Representative data from one of two independent experiments are shown. Representative image and quantification data are shown on top and bottom, respectively.

(c) Phosphorylated LCK levels were analyzed by FACS at 6 hours after stimulation. Combined data from 4 different experiments are shown. (d) Protein analyses of phosphorylate (p)-SHP2, total SHP-2 by western blot were performed at 48 hours after stimulation. Representative data from one of two independent experiments are shown. A representative image and quantification data are shown in the top and bottom, respectively. (e-f) TIGIT (e) and Lag3 (f) expression were analyzed by FACS at 24 or 48 hours after stimulation. Pooled data from three independent experiments are shown. The bar shows the mean ± SEM.

Supplemental Figure 4: Syngeneic Siglec-G$^{-/-}$ T cells showed equivalent donor T cell expansion and activation in allo-BMT.

(a-c) B6 Ly5.2 animals received 10Gy on day -1 and were transplanted with 0.75x10^{6} CD90.2^{+} splenic T cells from syngeneic either B6-WT or B6-Siglec-G$^{-/-}$ animals along with 5x10^{6} TCD-BM from B6 donors. (a) Donor T cell (CD45.2^{+}CD4^{+}CD8^{+}) expansion in the spleen and intraepithelial lymphocytes (IEL) at days 7 after BMT (n=3 per group). (b-c) CD69^{+} expression of donor T cells in spleen (b) or IEL at day 7 after BMT (n=3 per group). The bar shows the mean ± SEM.

Supplemental Figure 5: Donor Siglec-G$^{-/-}$ T cells exacerbate GVHD in experimental model.

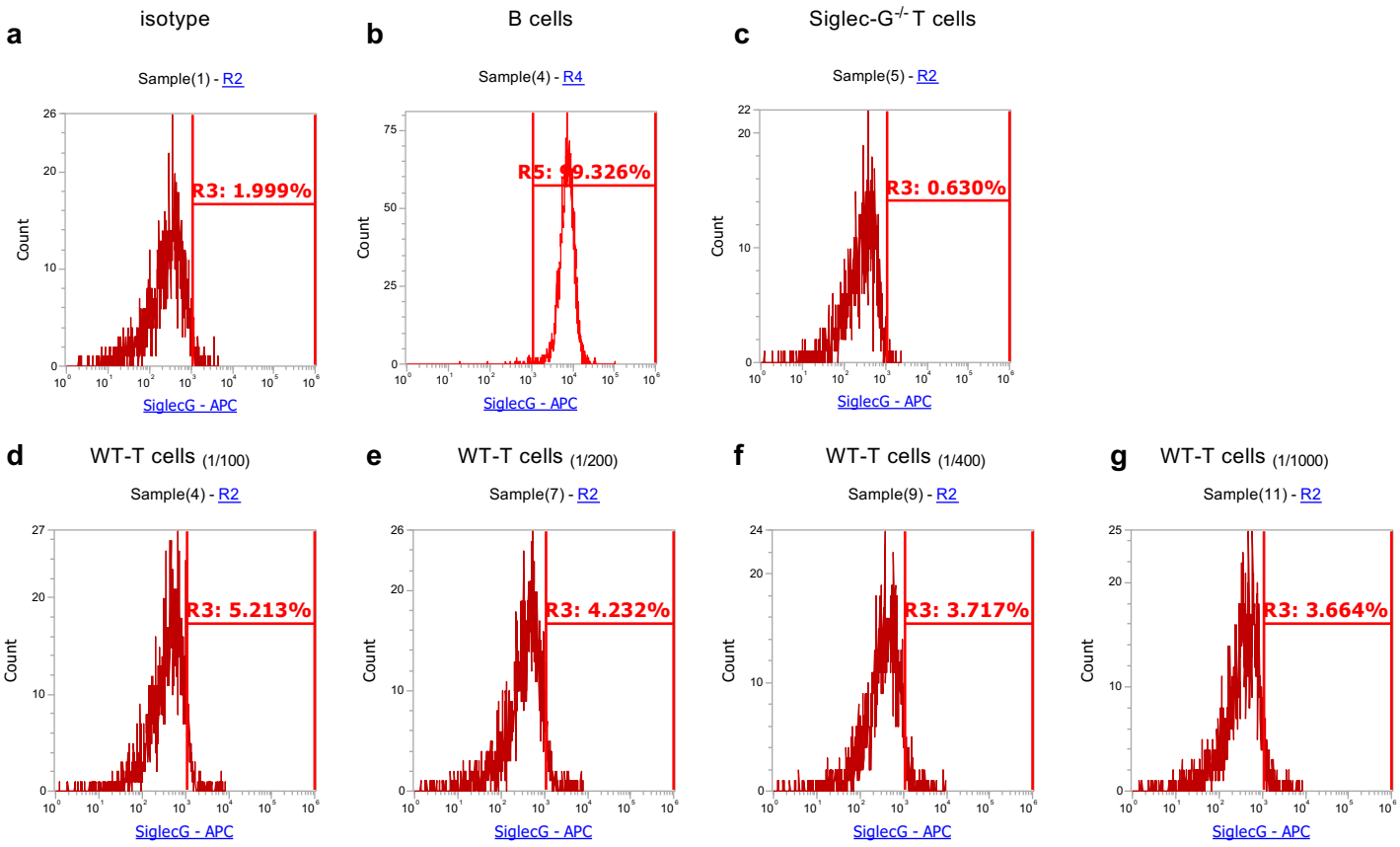
(a-d) BALB/c animals received 8.5Gy on day -1 and were transplanted with 0.75x10^{6} CD90.2^{+} splenic T cells from either syngeneic BALB/c or allogeneic MHC-mismatched B6-WT or B6-Siglec-G$^{-/-}$ animals along with 5x10^{6} TCD-BM from either BALB/c or B6 donors. (a-b) Donor CXCR3^{+} T cell expansion in the spleen (a) and CD69^{+} T cell in the liver (b) at day 14 after allo-BMT (n=7-8 per group, pooled from two experiments). Unpaired *t* test, *p<0.05, **p<0.01. The bar shows the mean ± SEM. (c) Serum IL-17A

levels at day 14 after allo-BMT (n=8-10 per group, pooled from three experiments). (d) The representative figures in the lung at day 14 after allo-BMT (n=2-6 per group, pooled from two experiments).

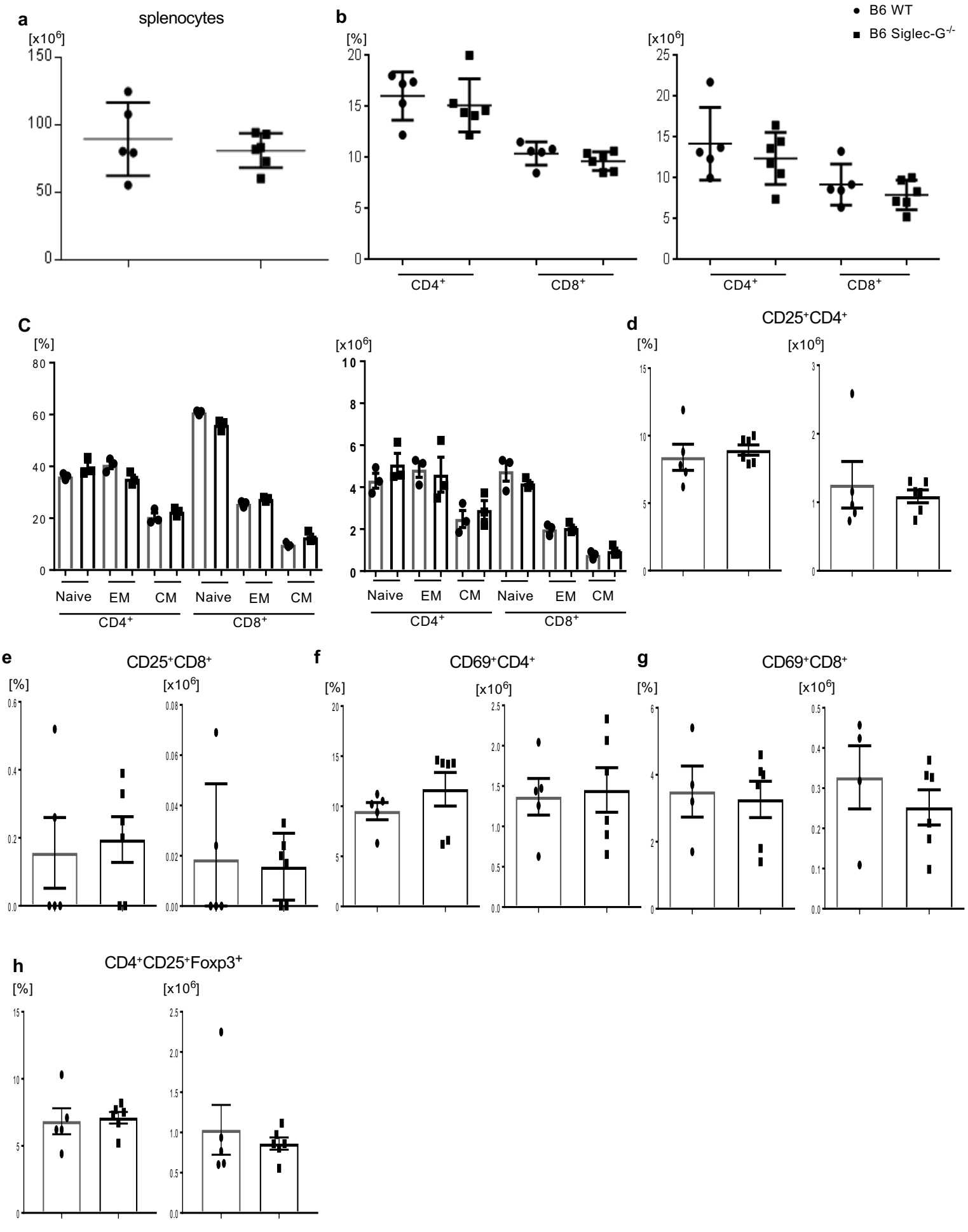
Supplemental Figure 6: Enhancing Siglec-G-CD24 axis by CD24Fc attenuates GVHD severity.

BALB/c-WT animals were lethally irradiated with 8.5Gy and infused with $0.75x10^6$ $CD90^+$ T cells along with $5x10^6$ TCD-BM cells from either syngeneic BALB/c-WT or allogeneic MHC-mismatched B6 donors. The recipients were injected with the CD24Fc (5mg/kg) or diluent control on day 0 before allo-BMT. (a) Overall survival and (b) GVHD clinical score, n=6-15 per group, pooled from three experiments. Unpaired *t* test $p<0.006$ is considered significant following value Bonferroni correction ***$p<0.0003$, **$p<0.001$, *$p<0.006$ when control vs. CD24Fc treatment groups were compared. The bar shows the mean ± SEM.

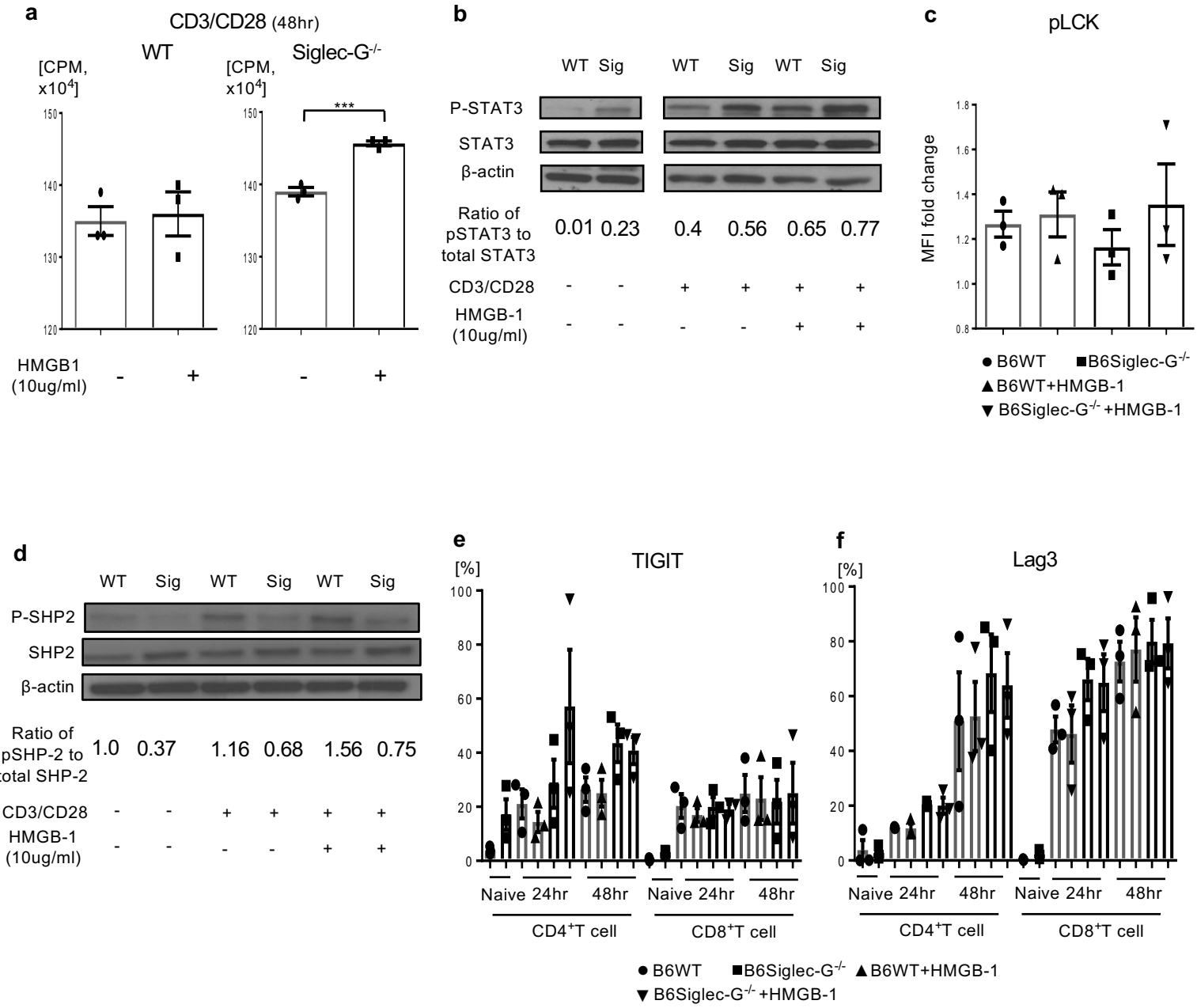
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



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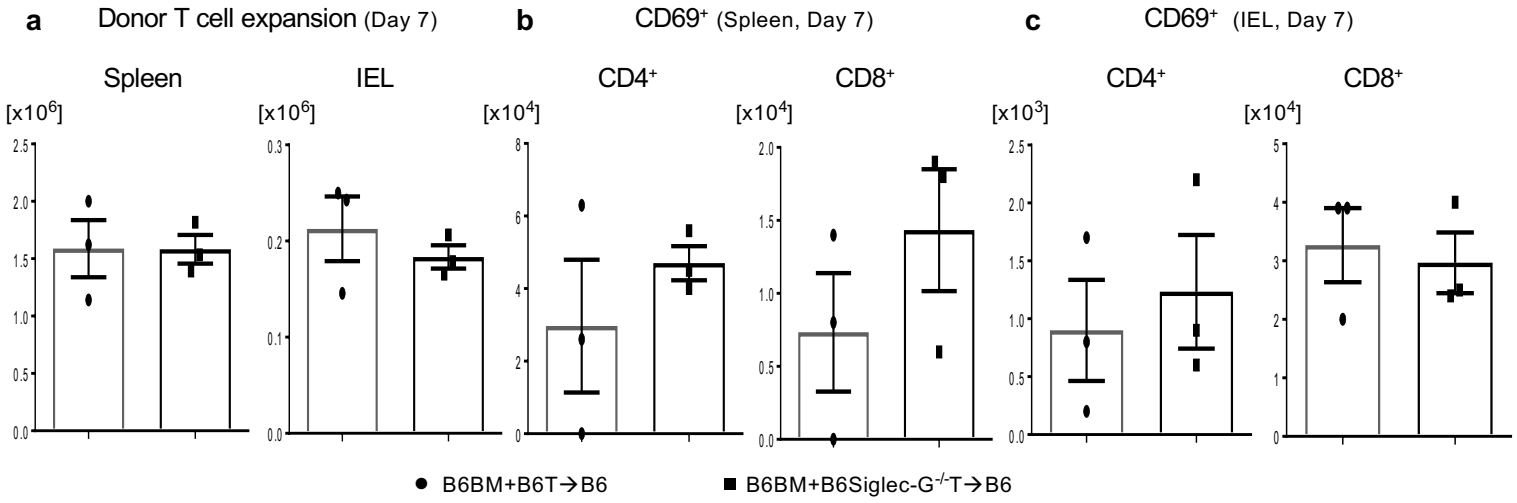
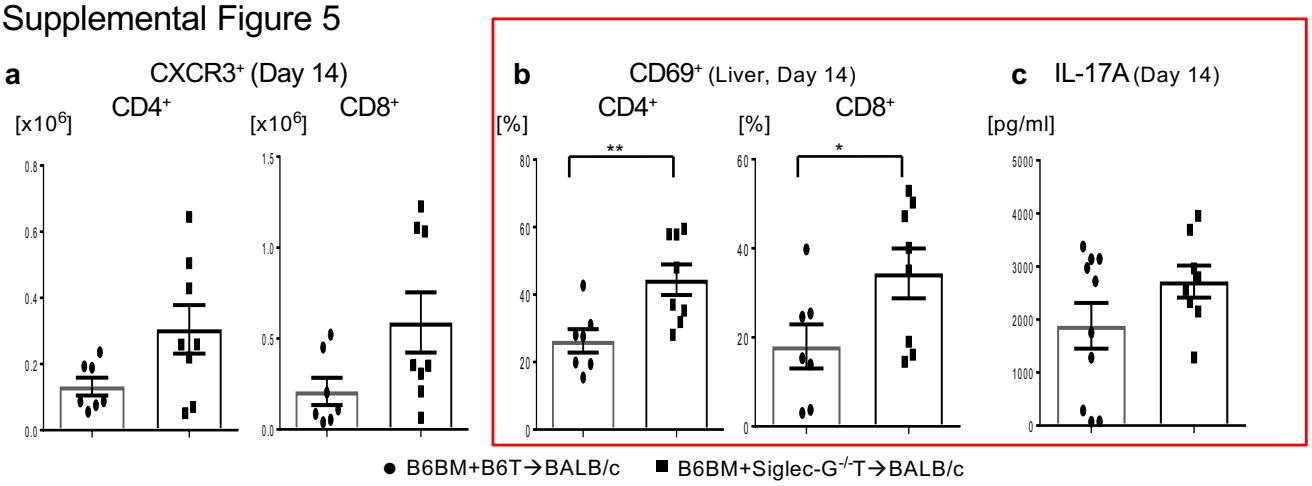
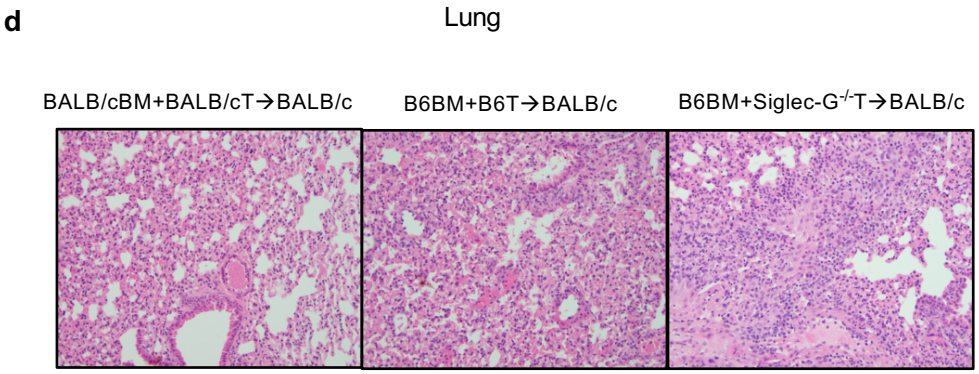
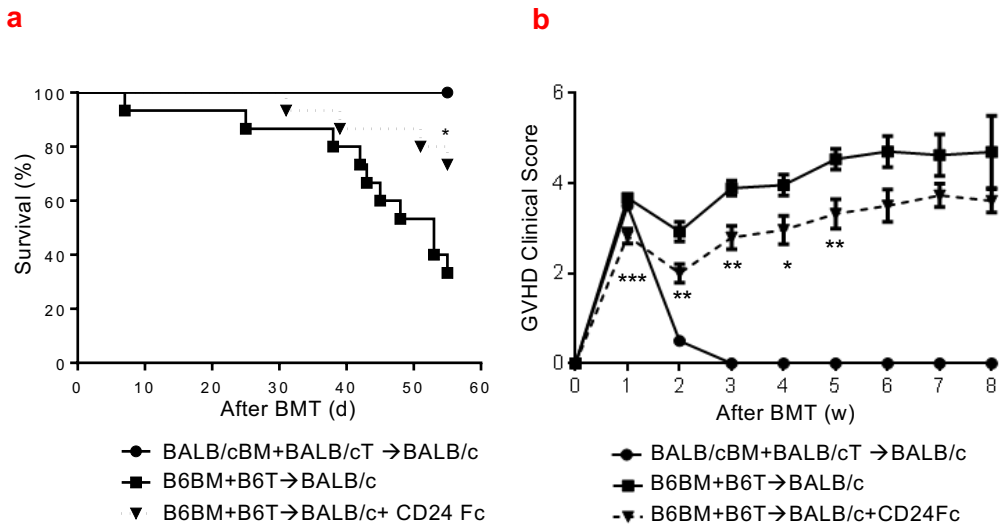


Supplemental Figure 5





Supplemental Figure 6



Supplemental Table 1. Role of Siglec-G-CD24 axis and the results of CD24Fc treatment in BMT models.

Donor T cells	Host APCs	Treatment	GVHD
B6-WT	BALB/c-WT	(-)	↑↑
B6-WT	BALB/c-WT	CD24Fc	↓
B6-WT	BALB/c-CD24$^{-/-}$	(-)	↑↑
B6-WT	BALB/c-CD24$^{-/-}$	CD24Fc	↓
B6-Siglec-G$^{-/-}$	B6-CD24$^{-/-}$	(-)	↑↑↑
B6-Siglec-G$^{-/-}$	B6-CD24$^{-/-}$	CD24Fc	↑↑↑
B6-CD24$^{-/-}$	BALB/c-CD24$^{-/-}$	(-)	↑↑↑
B6-CD24$^{-/-}$	BALB/c-CD24$^{-/-}$	CD24Fc	↓↓