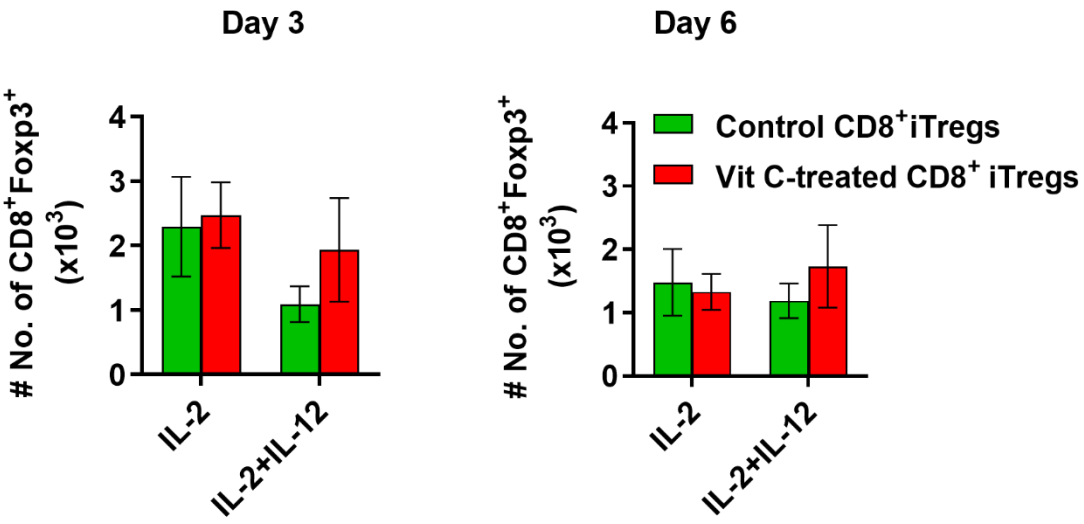
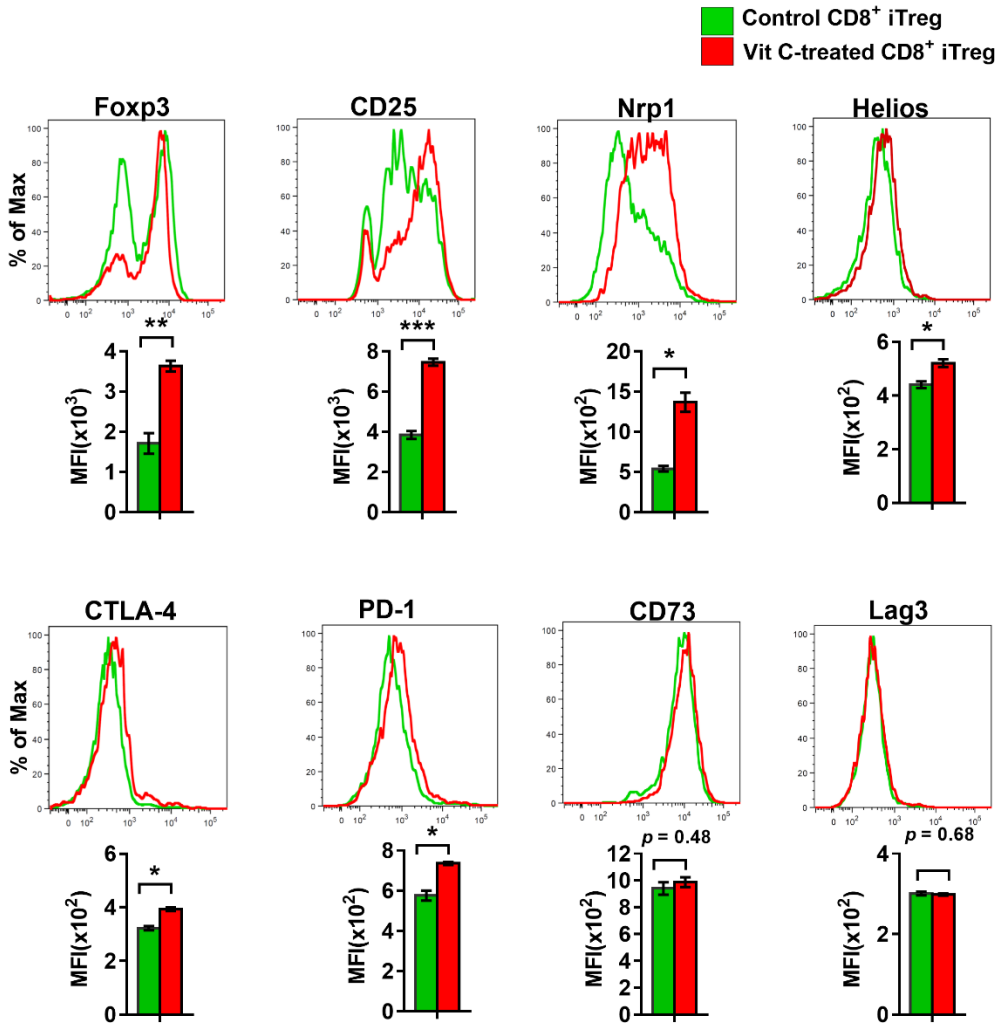
Supplementary Figure 1



Supplemental Figure 1. Absolute numbers of CD8⁺ Foxp3⁺ cells after culture for 3 and 6 days.

On day 5 after CD8⁺iTregs generation, control or Vit C-treated CD8⁺ iTregs were harvested, enriched with CD25 microbeads and co-cultured in the presence of IL-2 alone or IL-2+IL-12 recombinant cytokines (n=3/group) for 3 days and 6 days. The absolute number of Foxp3⁺ cells was calculated on day 3 or day 6. Data are representative of two independent experiments. *Student's t-test* was used for statistical analysis.

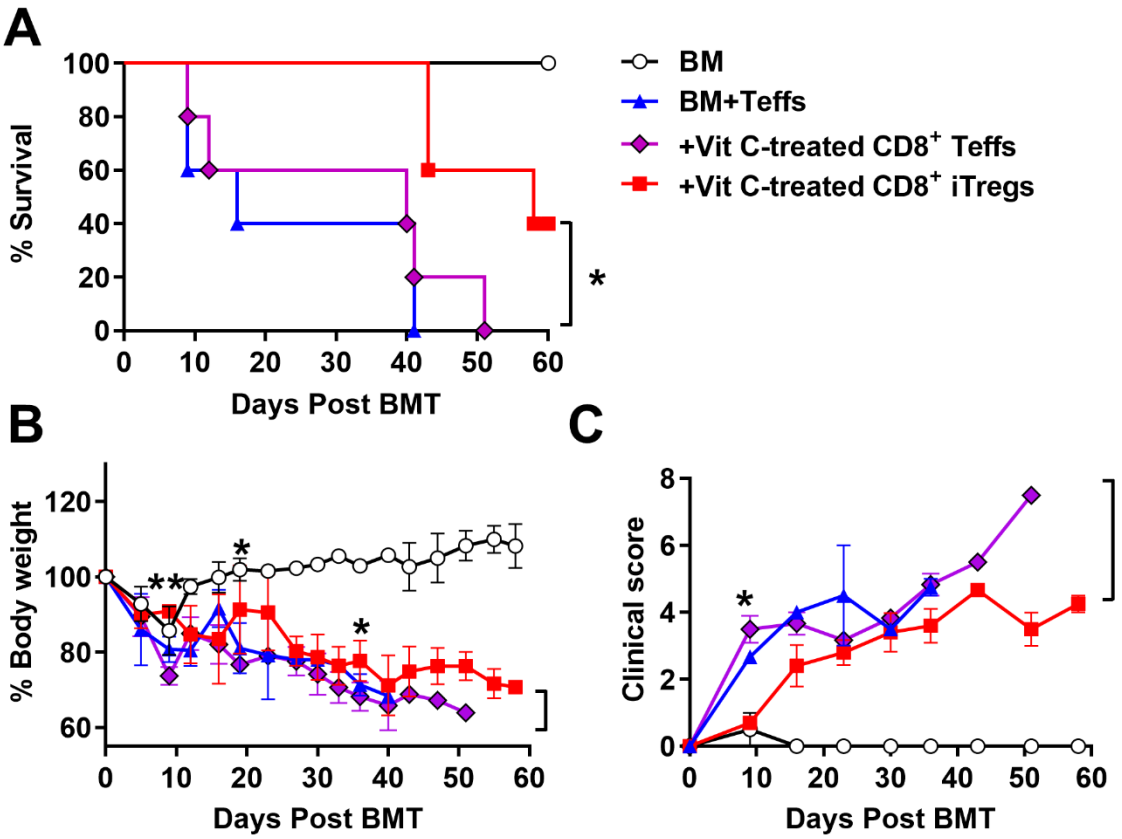
Supplementary Figure 2



Supplemental Figure 2. Treg canonical makers expressions of control or Vitamin C-treated CD8⁺ iTregs on day 5 after *in vitro* generation.

On day 5 after *in vitro* CD8⁺iTregs generation, control or VitC-treated CD8⁺ iTregs were harvested and analyzed for Foxp3, CD25, Nrp1, Helios, CTLA-4, PD-1, CD73, and Lag3 expression. Data are representative of three independent experiments. *Student's t-test* was used for statistical analysis. *p ≤ 0.05, **p ≤ 0.01 and, ***p ≤ 0.001.

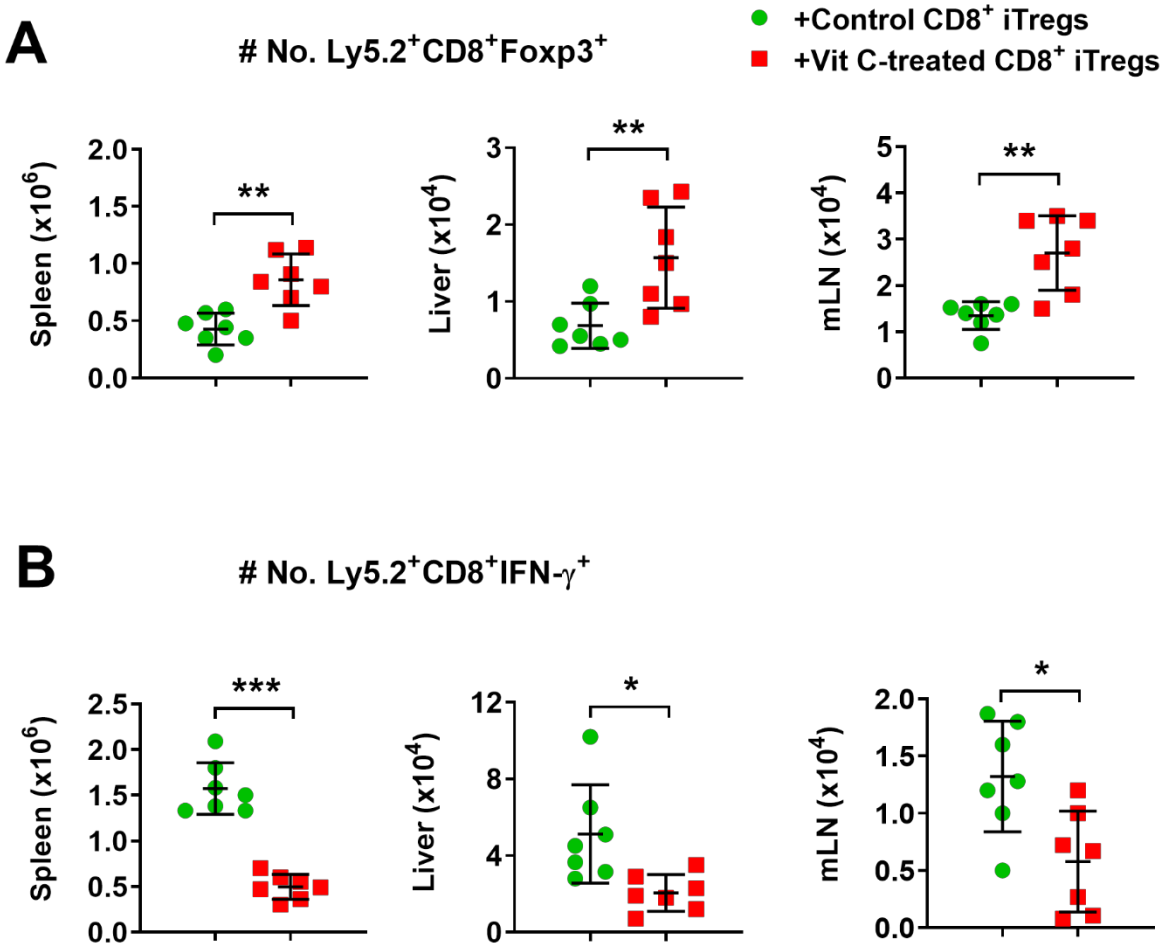
Supplementary Figure 3



Supplemental Figure 3. Effect of Vitamin C-treated CD8⁺ effector T cells on aGVHD.

Lethally irradiated BALB/c mice were transplanted with $5x10^6$ BM plus either $1x10^6$ Vit C-treated CD8⁺Teffs or CD8⁺iTregs. Three days later, $0.7x10^6$ CD25-depleted T-cells were i.v injected to induce aGVHD. Recipients were monitored for survival rate (A), body weight loss (B), and GVHD clinical signs (C) until day 60 (n=5/group). Log-rank (Mantel-Cox) test was used for statistical analysis of the survival. *Student's t-test* was used for statistic of body weight and GVHD clinical scores. *$p \leq 0.05$ and **$p \leq 0.01$.

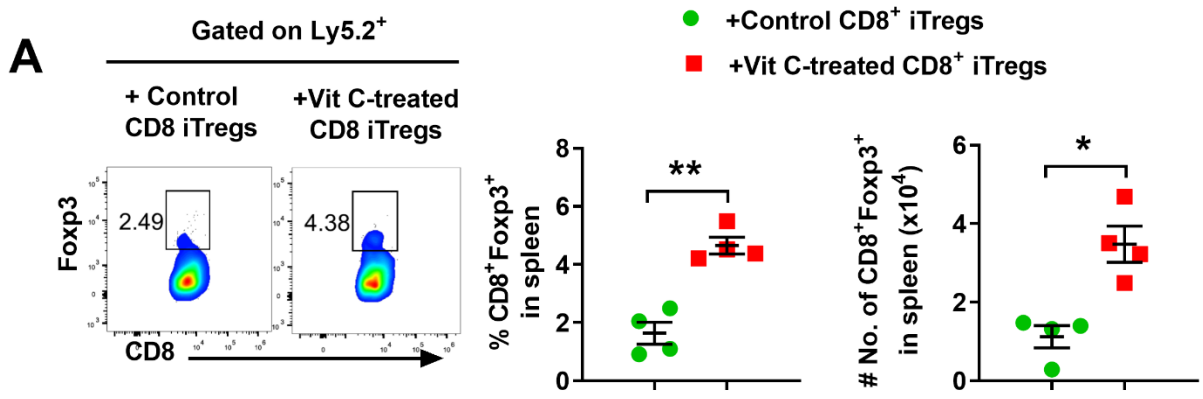
Supplementary Figure 4



Supplemental Figure 4. Absolute numbers of control and Vit C CD8⁺ iTregs (Ly5.2⁺) 7 days after being transferred in the recipients.

Lethally irradiated BALB/c mice were transferred with $5x10^6$ $Rag1^{-/-}$ BM and $1x10^6$ $CD8^+$ iTregs ($Ly5.2^+$). Three days later, $2x10^6$ CD25-depleted T-cells from C57BL/6 ($Ly5.1^+$) congenic mice were i.v. injected to induce GVHD. At day 7 after allo-BMT, spleen, liver and mesenteric lymph nodes (mLNs) were harvested and analyzed. (A) Absolute numbers of transferred control or Vit C-treated $CD8^+$Tregs that expressed Foxp3 in recipient spleen, liver or mLN are shown. (B) Absolute numbers of transferred control or Vit C-treated $CD8^+$Tregs that secreted IFN-γ are shown. Data are combined from two independent experiments (n=7/group). *Student's t-test* was used for statistical analysis. *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$.

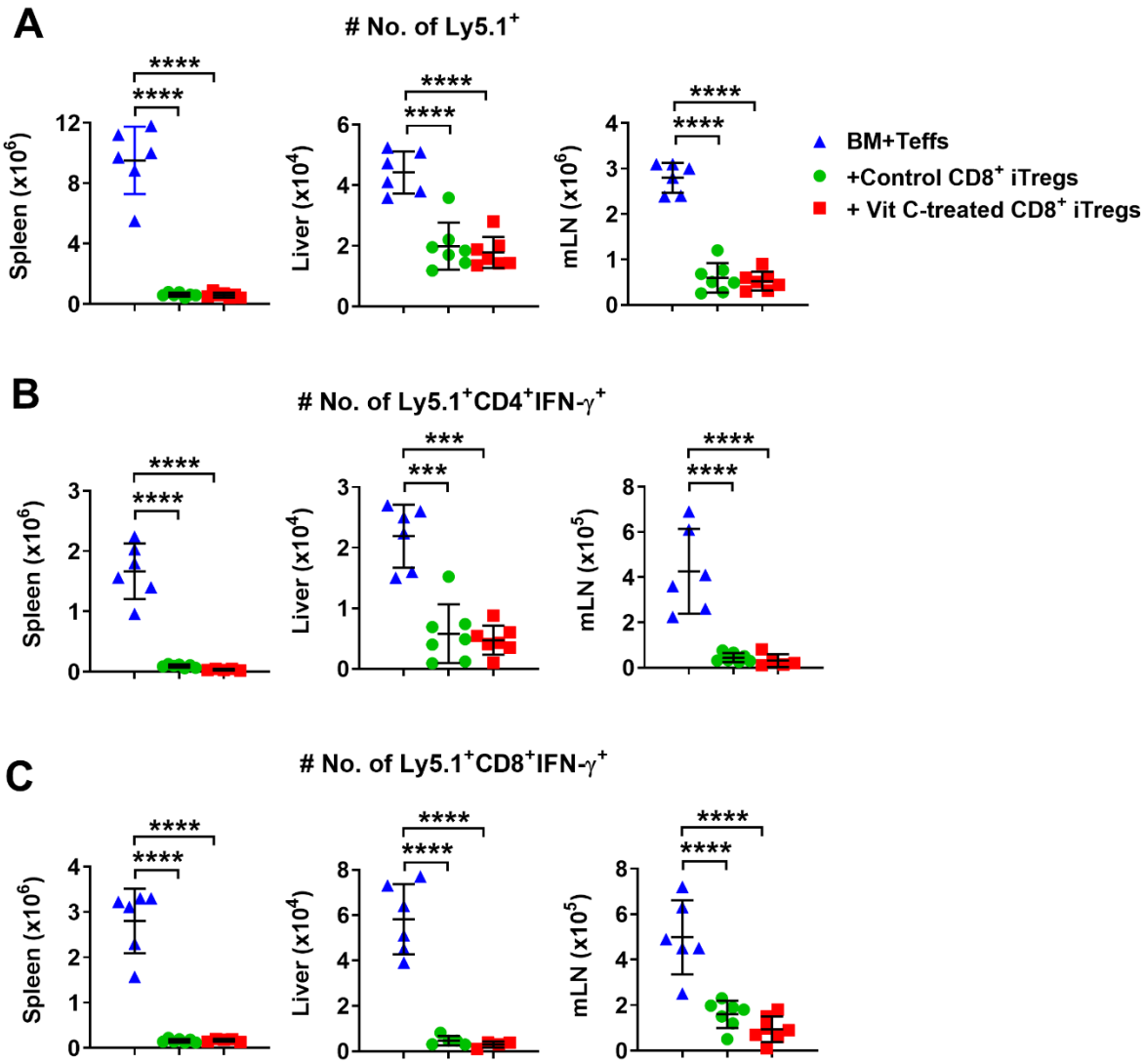
Supplementary Figure 5



Supplemental Figure 5. Percentages and absolute numbers of transferred control or Vit C-treated CD8⁺ iTregs that maintained Foxp3 expression 14 days in the recipients.

Lethally irradiated BALB/c mice were transferred with $5x10^6$ $Rag1^{-/-}$ BM and $1x10^6$ $CD8^+$ iTregs ($Ly5.2^+$). Three days later, $0.7x10^6$ CD25-depleted T-cells from C57BL/6 ($Ly5.1^+$) congenic mice were i.v injected to induce GVHD. On day 14 after allo-BMT, the recipient spleens were harvested and analyzed. (A) Frequencies and absolute numbers of Foxp3 ($Ly5.2^+CD8^+Foxp3^+$) retention among transferred control or Vit C-treated $CD8^+$ iTregs are shown (n=4/group). *Student's t-test* was used for statistical analysis. *$p \leq 0.05$ and **$p \leq 0.01$.

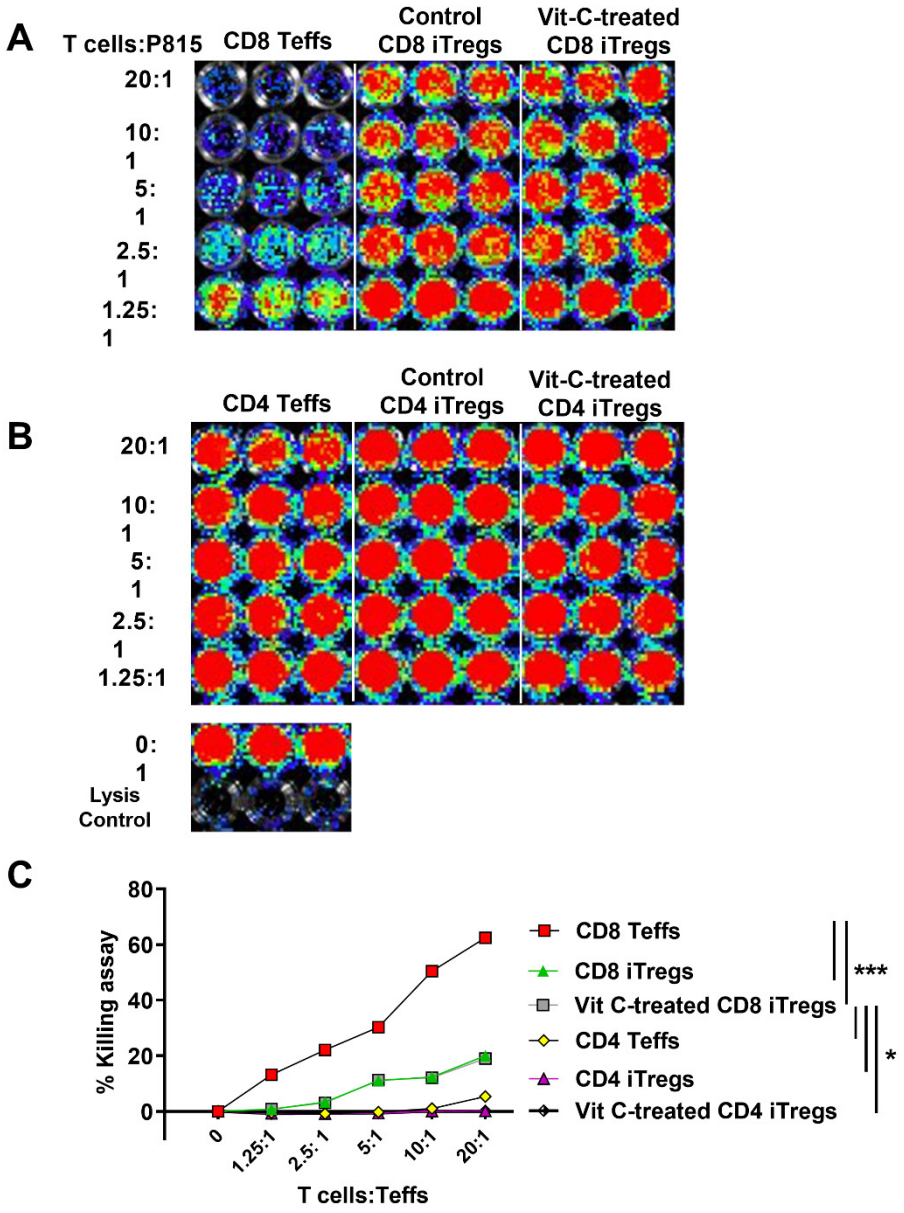
Supplementary Figure 6



Supplemental Figure 6. Absolute numbers of T effector cells that produced IFN-γ in the recipients.

Lethally irradiated BALB/c mice were transplanted with $5x10^6$ $Rag1^{-/-}$ BM and $1x10^6$ of either control or Vit C-treated $CD8^+$ iTregs ($Ly5.2^+$). Three days later, $2x10^6$ CD25-depleted T-cells from C57BL/6 ($Ly5.1^+$) congenic mice were i.v injected to induce GVHD. On day 7 after allo-BMT, spleen, liver and mesenteric lymph nodes (mLNs) were harvested and analyzed. (A) Absolute numbers of total Teff cells ($Ly5.1^+$) from spleen, liver and mLN are shown. (B-C) Absolute numbers of IFN-γ-producing either $CD4^+$ (B) or $CD8^+$ Teff cells (C) are shown. Data are combined from two independent experiments (n=7/group). One-way ANOVA was used for statistical analysis. ***$p \leq 0.001$ and ****$p \leq 0.0001$.

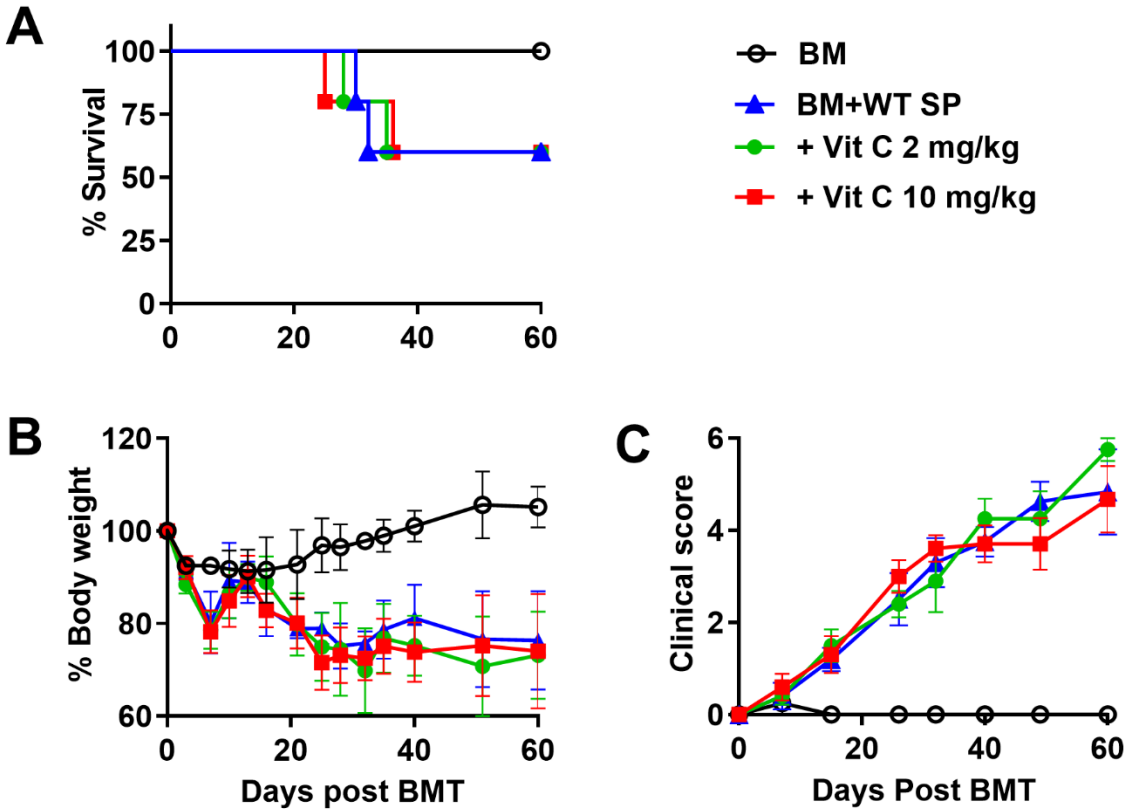
Supplementary Figure 7



Supplemental Figure 7. Cytotoxic activity of control or VitC-treated CD8⁺ iTregs against P815 expressing luciferase.

Allogeneic CD4 or CD8 Teffs or control CD8⁺iTregs or VitC-treated CD8⁺iTregs (effector;E) were co-cultured with P815-expressing luciferase (T cell : target ratio) 20:1, 10:1, 5:1, 2.5:1 and 1.25:1 at 37°C for 4 hrs. Each ratio was seeded in triplicate wells. After incubation, luciferin was added into the culture. Bioluminescent imaging data were analyzed and quantified using living imager software. (A-B) Luminescence images of 96-well plates show viable P815 tumor cells in red signal. P815-expressing luciferase incubated with lysis buffer was used as a positive control represented lysis activity. (C) Graph depicting the percentages of cytotoxic activity in different E:T ratio are shown (n=3/group). One-way ANOVA was used for statistical analysis. *$p \leq 0.05$ and ***$p \leq 0.001$.

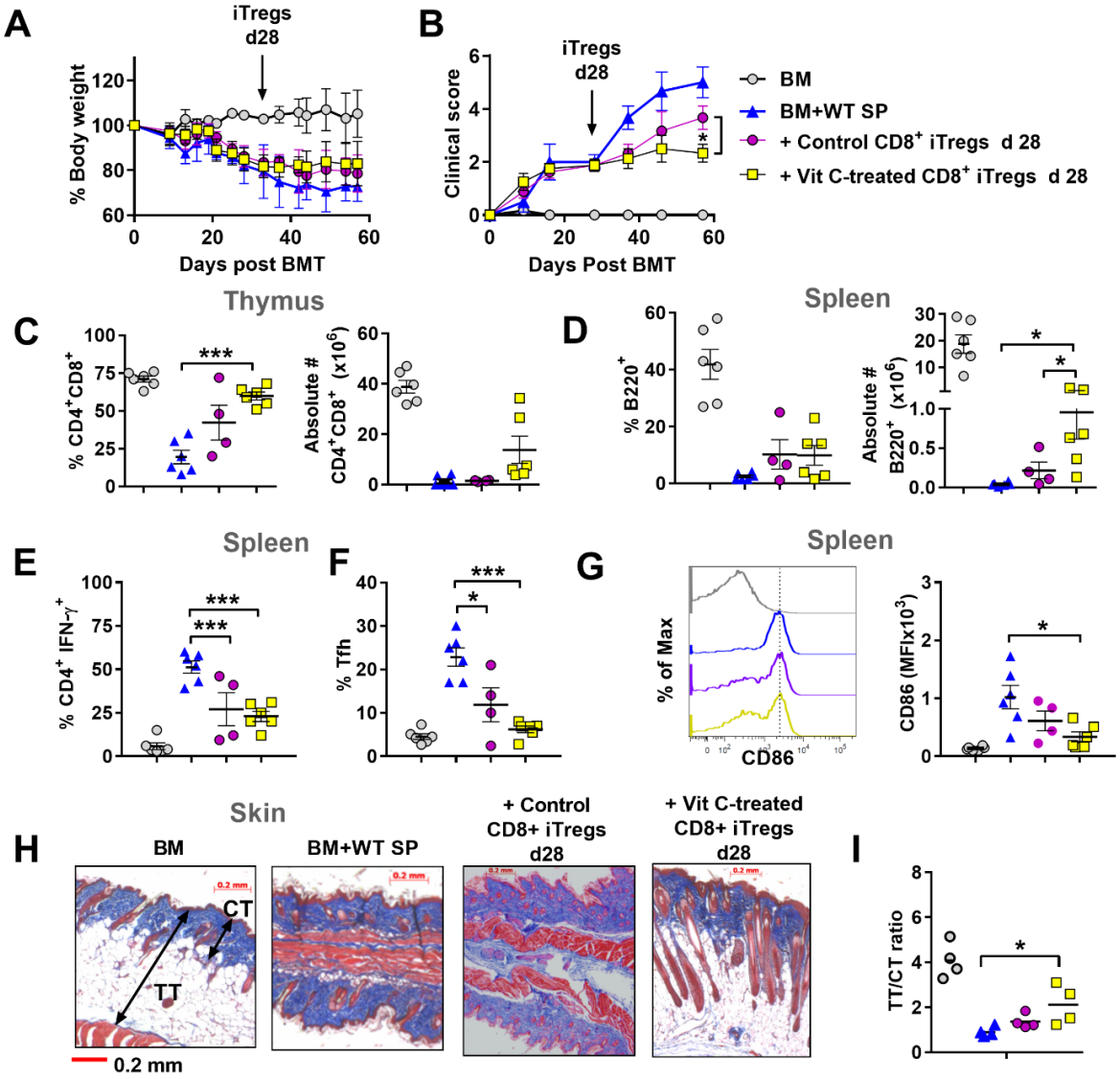
Supplementary Figure 8



Supplemental Figure 8. The effect of oral supplement with Vitamin C on cGVHD.

Lethally irradiated BALB/c recipients were transplanted with TCD-BM ($5x10^6$/mouse) and $0.5x10^6$/mouse of whole splenocytes from WT B6. The recipients were daily oral gavaged with 2 or 10 mg/kg of Vit C for 21 days. Survival rate (A), body weight loss (B), and GVHD clinical signs (C) were monitored until day 60 (n=5/group). Log-rank (Mantel-Cox) test was used for statistical analysis of mice survival. *Student's t-test* was used for statistic of body weight loss and GVHD clinical scores.

Supplementary Figure 9



Supplement Figure 9. Vitamin C-treated CD8⁺ iTreg treatment reverses established chronic GVHD.

(A-B) Lethally irradiated BALB/c mice were transplanted with TCD-BM 5x10^6/mouse and 0.35x10^6/mouse of whole splenocytes from WT B6 donors. The recipients without iTreg transfer serve as a cGVHD control group (WT SP). Tregs were transferred 0.35x10^6 /mouse at day 28 post-BMT (d28). All recipient mice were monitored for survival rate (data not shown), body weight **(A)** and cGVHD clinical scores **(B)**. Experiment was ended on day 50-55 and recipient thymus and spleen were analyzed. Percentages and total number of CD4$^+$CD8$^+$ in thymus **(C)** and B -cell reconstitution **(D)** in spleen are shown on gated donor cells. **(E-F)** Percentages of CD4$^+$ IFN-γ$^+$ and Tfh (CD4$^+$Foxp3$^-$PD^{hi}CXCR5$^+$) are shown in spleen. **(G)** Histogram and percentage of Mean Fluorescent Intensity (MFI) of CD86 expression are shown. **(H)** Recipient skin was stained by Masson's Trichrome stain (X5 magnification). **(I)** Collagen deposition in skin was calculated by dividing total thickness (TT, from epidermis to sub-cutaneous muscle layer) by collagen thickness (CT). Data are combined from two independent experiments (n = 4-6/group). One-way ANOVA was used for statistical analysis. *p ≤ 0.05 and ***p ≤ 0.001.