

Supplementary Material

Donor $\gamma\delta$ T cells promote GVL effect and mitigate aGVHD in allogeneic hematopoietic stem cell transplantation

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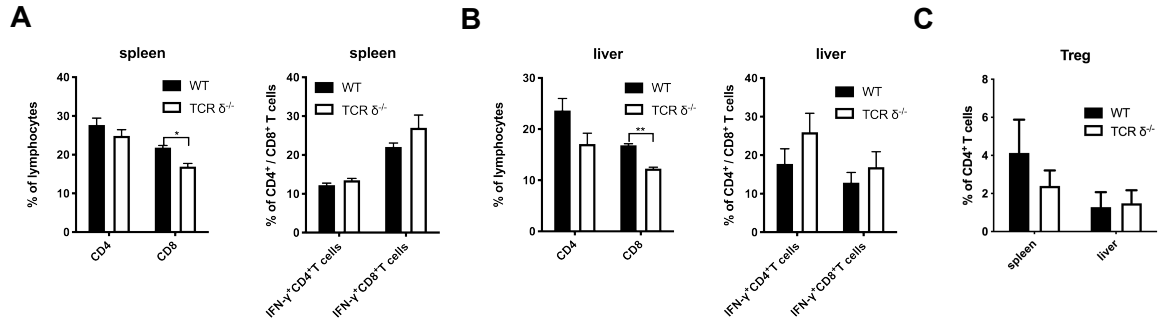
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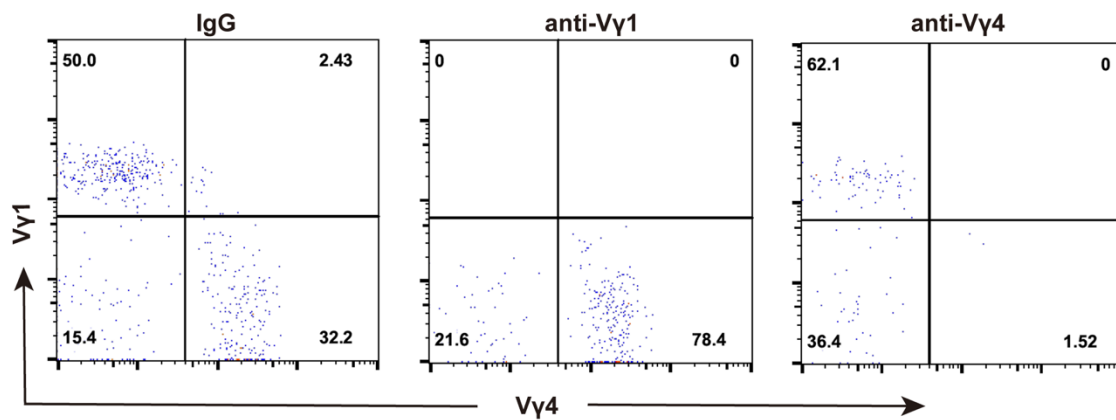
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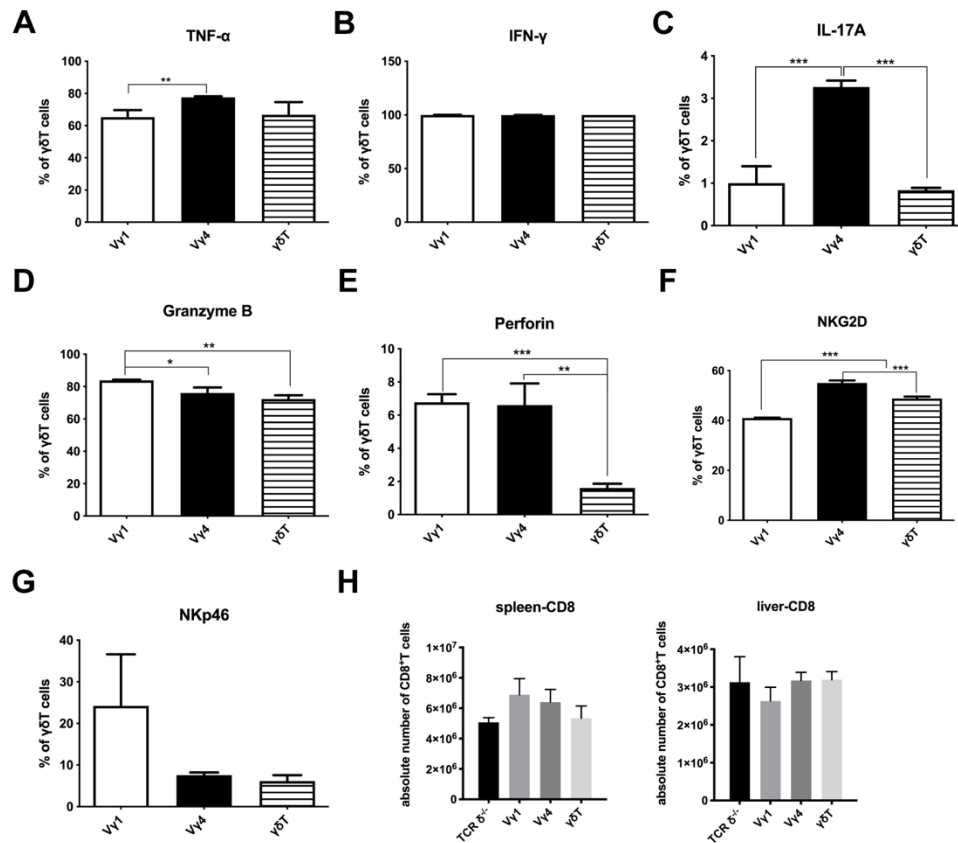
Supplemental Figure 1

Supplemental Figure 1. IFN- γ production by CD4⁺ and CD8⁺ T cells in naïve WT and TCR- $\delta^{-/-}$ C57BL/6 mice. The percentages of CD4⁺ and CD8⁺ T cells and the percentage of IFN- γ -producing CD4⁺ and CD8⁺ T cells from the spleen (A) and liver (B) of naïve WT or TCR- $\delta^{-/-}$ mouse. (C) The percentage of Treg cells in the spleen and liver from the recipients. Recipient mice (BALB/c) were lethally irradiated and received A20 lymphoma cells (1×10^6 /mouse, *iv.*) plus BM cells (5×10^6 /mouse, *iv.*) from WT or TCR $\delta^{-/-}$ mice. 10 days later, the percentage of Treg cells in the spleen and liver of hosts was detected by flow cytometry. Data shown are the representative of two independent experiments. All graphs displayed mean \pm SD. * $p < 0.05$, ** $p < 0.01$ determined by two-tailed unpaired Student's t-test.



Supplemental Figure 2

Supplemental Figure 2. The depletion of V γ 1 or V γ 4 cells *in vivo*. WT mice were treated with IgG, anti-mouse-V γ 1 or anti-mouse V γ 4 antibody (100ug/200ul/mouse, *iv.*). After 1 week, the percentages of V γ 1 and V γ 4 cells in the blood were measured by flow cytometry. Data shown are the representative of two independent experiments.



Supplemental Figure 3

Supplemental Figure 3. The phenotypes of expanded V γ 1, V γ 4 and $\gamma\delta$ T cells *in vitro*. V γ 1, V γ 4 and $\gamma\delta$ T cells were cultured from the spleen TCR- $\beta^{-/-}$ mouse. The productions of TNF α (A), IFN- γ (B), IL-17A (C), granzyme B (D) and perforin (E) were detected by intracellular staining. The expressions of NKG2D (F) and NKp46 (G) were also examined by flow cytometry (n=3). (H) The absolute number of CD8⁺T cells in the spleen and liver from the recipient mice. Recipient mice were lethally irradiated and received A20 lymphoma cells (1×10^6 /mouse, *iv.*) plus BM cells (5×10^6 /mouse, *iv.*) from TCR $\delta^{-/-}$ mice. V γ 1, V γ 4, or total $\gamma\delta$ T cells (1×10^7 cells/mouse, *iv.*) from CD45.1-TCR $\beta^{-/-}$ mouse were adoptively transferred into the recipients on day 0. On day7 post allo-HSCT, the absolute number of CD8⁺T cells from the spleen and liver of recipient mice was measured (n=5). Data shown are the representative of at least three experiments. All graphs displayed mean \pm SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ determined by one-way ANOVA followed by Bonferroni posttest.