

1 **Supplemental Figure 1. DPT express high levels of CD8 α , CD8 β and intermediate levels**
2 **of CD4.**

3 (A) Representative dot plot of human T cells gated on panHLA and CD3 taken from the spleen
4 of a GVHD mouse transplanted with PB-MNC. DP T cell are highlighted in red, CD4 T cells in
5 green and CD8 T cells in blue. (B-D) Histograms of CD8 α (B), CD8 β (C) and CD4 (D)
6 expression on each indicated T cell population.
7

8 **Supplemental Figure 2. DPT are present in xenogeneic GVHD target organs.**

9 (A-E) Representative dot plots and quantification of non-GVHD mice (top row) and GVHD mice
10 (bottom row) of the spleen (A), liver (B), bone marrow (C) and lungs (D) taken at time of
11 euthanasia. Location and percentage of DP T cells are shown in the red box. Each dot
12 represents an individual mouse taken from five independent experiments. * p<0.05, ** p<.01, ***
13 p<0.001
14

15 **Supplemental Figure 3. DPT are not activated through the STAT pathway**

16 (A-D) Representative histograms of the indicated T cell population taken from GVHD mice of
17 pSTAT1 (A), pSTAT3 (B), pSTAT4 (C), pSTAT6 (D) and further quantified in (E). ** p<.01
18

19 **Supplemental Figure 4. Expression of CD4 in DPT is not permanent but is conserved**
20 **over time.**

21 (A) Schematic depicting two experimental conditions to measure DPT growth and persistence:
22 ex vivo stimulation with γ -irradiated PB-MNC, PHA, IL-2 and IL-7 or re-transplantation into a
23 naïve NSG mouse. (B-C) Dot plots of FACS DP T cells at day 5, 13 and 21 after ex-vivo
24 stimulation (B). (C) Analysis of co-stimulatory proteins 21 days after ex vivo stimulation. (D-F)
25 Dot plots of FACS DP T cells re-transplanted into NSG mice after 10, 21, 42 and 63 days (D).
26 The percentage (E) and number (F) of DP T cells in the blood of re-transplanted mice is shown.
27 Each dot represents an individual mouse from two independent experiments.
28

29 **Supplemental Figure 5. DPT are a healthy, TH1 skewed population.**

30 (A-B) Histogram and quantification of T-bet, GATA3 and ROR γ t expression across DP, CD4
31 and CD8 T cells from a GVHD mouse compared to an isotype control. (C) BCL2 expression in
32 the indicated T cell populations. (D) Co-stimulatory protein analysis as in Figure 3F for the
33 indicated human T cells populations analyzed from the spleen at time of euthanasia.
34

35 **Supplemental Figure 6. Both cytolytic and modulatory cytokines are expressed by DPT.**

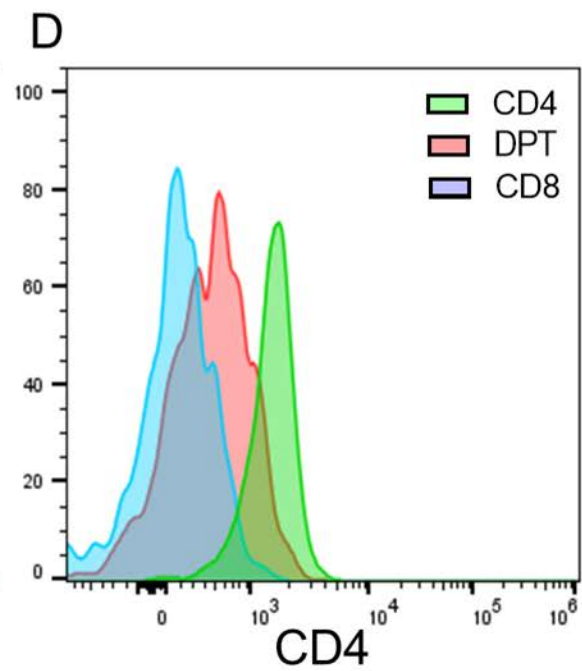
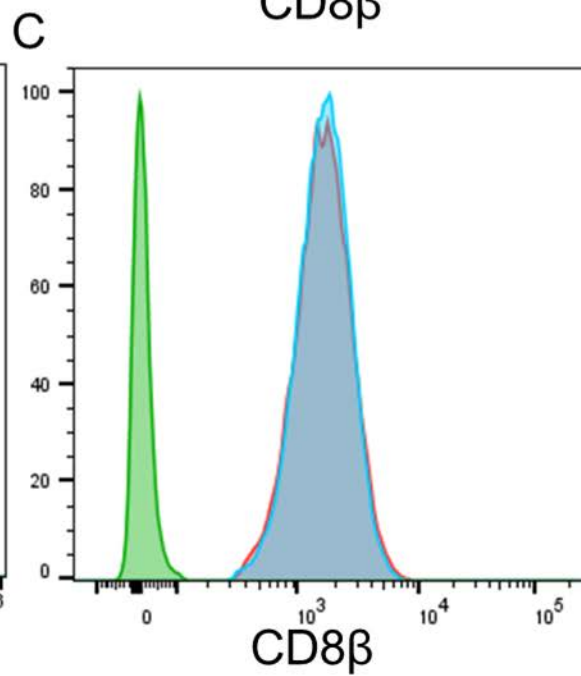
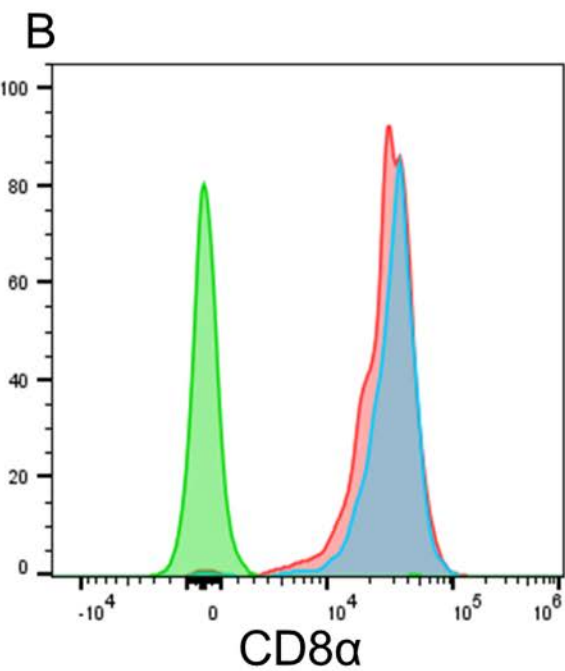
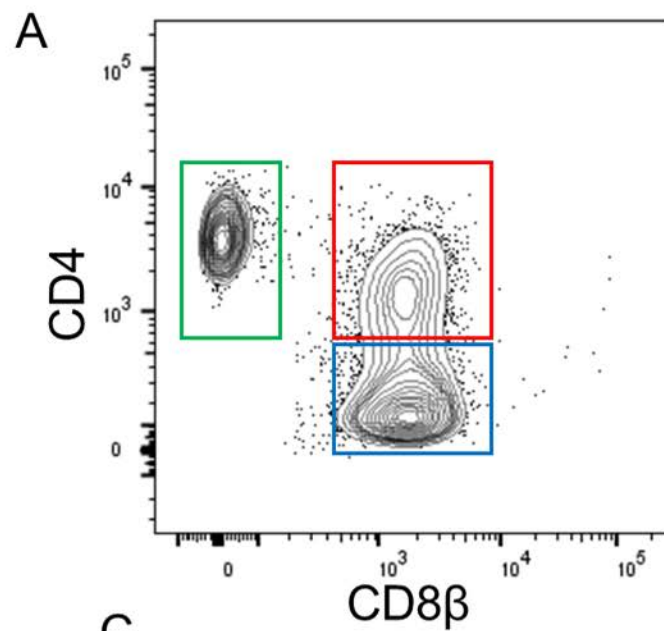
36 Representative flow plots of CD4 (top row), DPT (middle row) and CD8 (bottom row) T cells
37 cultured overnight with brefeldin prior to staining for the indicated cytokine (columns). Green,
38 red and blue boxes represent the gating strategy for each T cell population.

39

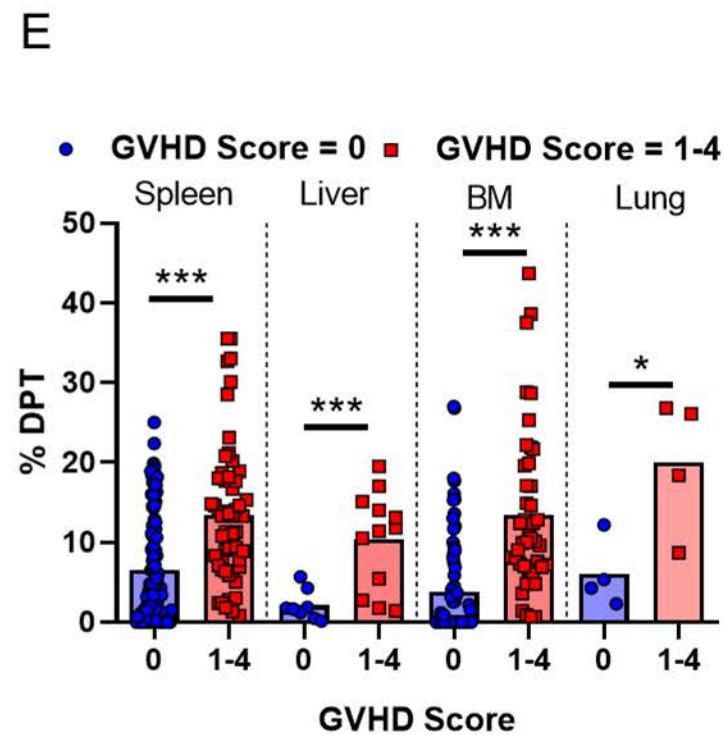
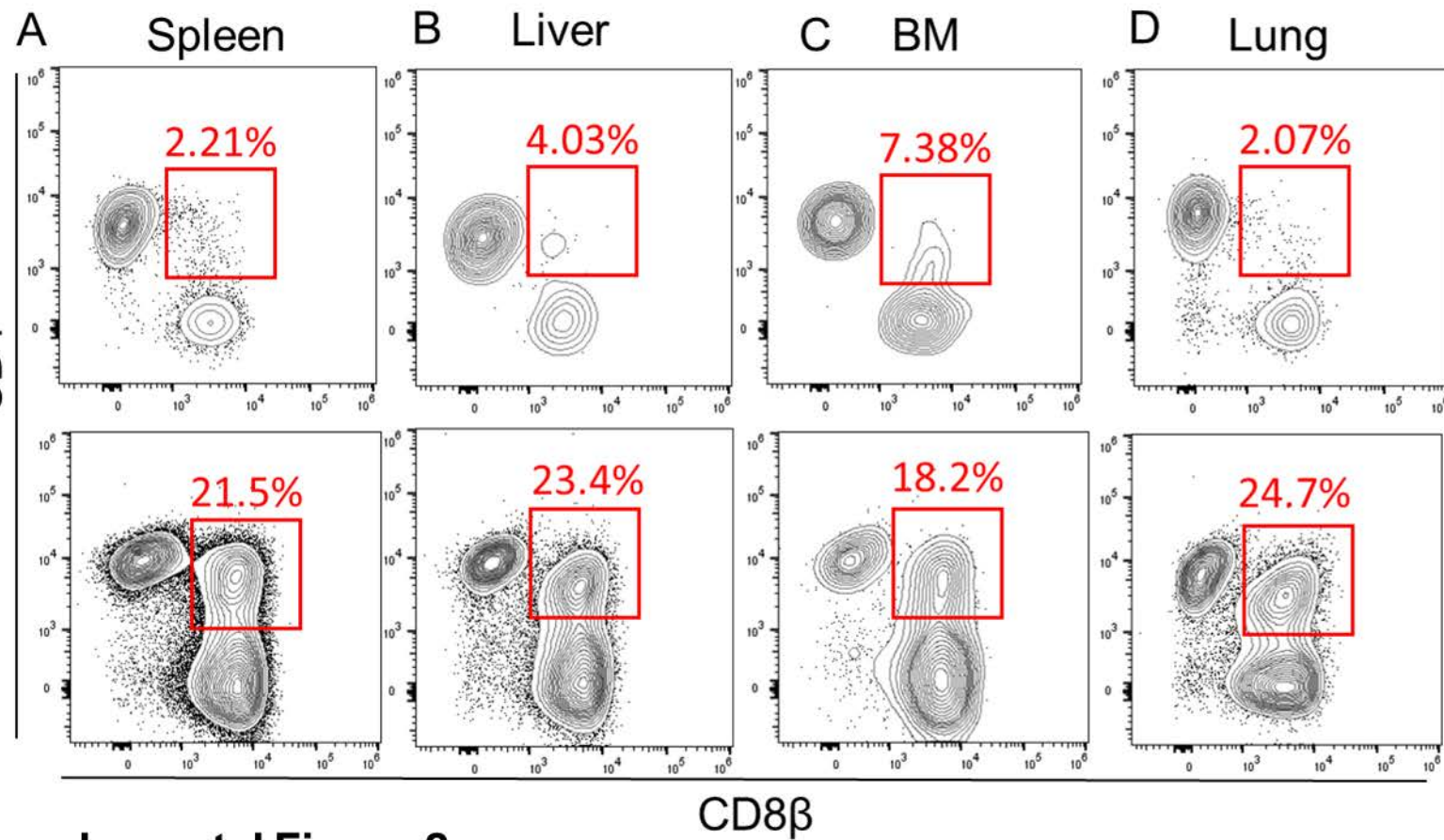
40 **Supplemental Figure 7. T cells from GVHD mice have limited GVL activity.**

41 (A-E) Mice were transplanted as described in Figure 6 with the NALM-6 cell line and the
42 indicated T cell population sorted from GVHD mice. Mice were monitored for survival (A),
43 leukemic growth using IVIS imaging (B-C) and leukemia/T cell expansion using flow cytometry
44 (D-E). (F-H) Experiment was repeated using the RS4;11 cell line and monitored for survival (F),
45 pathology score (G), leukemia/T cell expansion using flow cytometry (H-I) and the concentration
46 of IFN γ in the serum of mice (H).

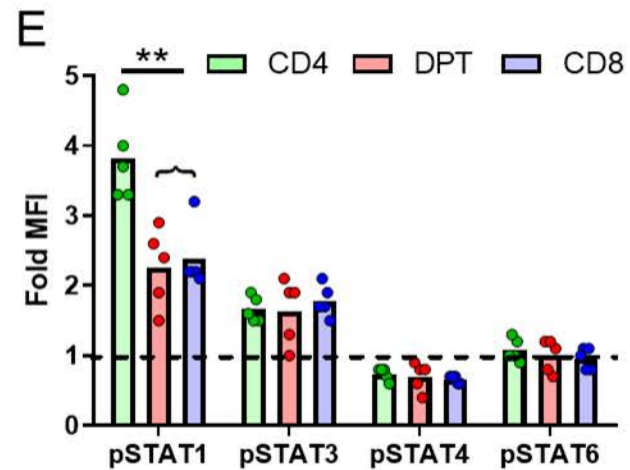
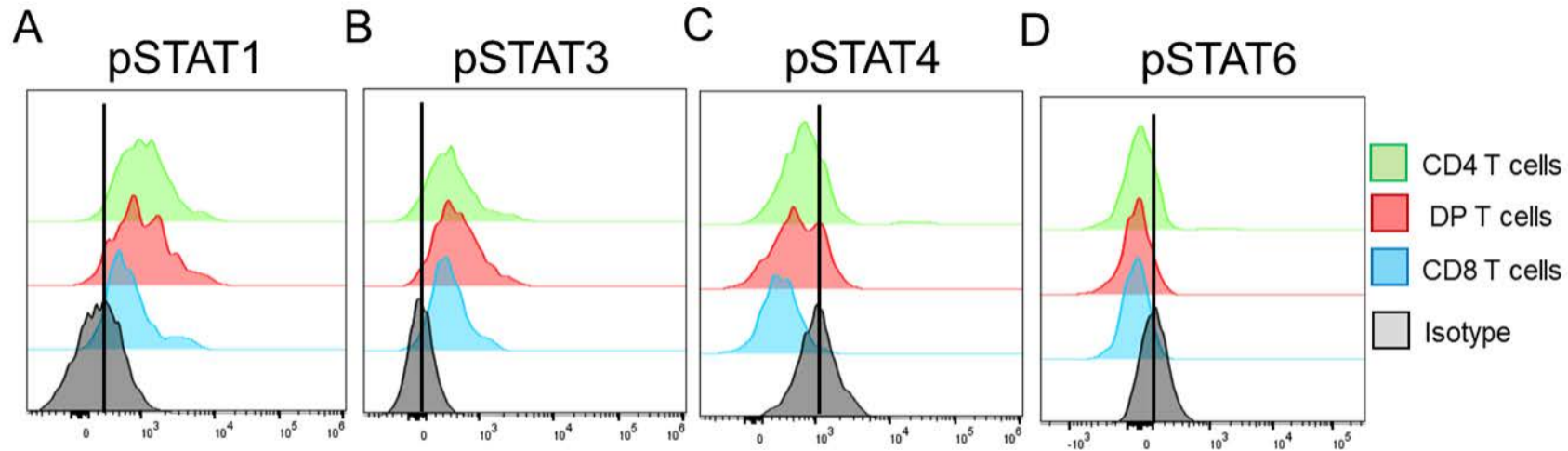
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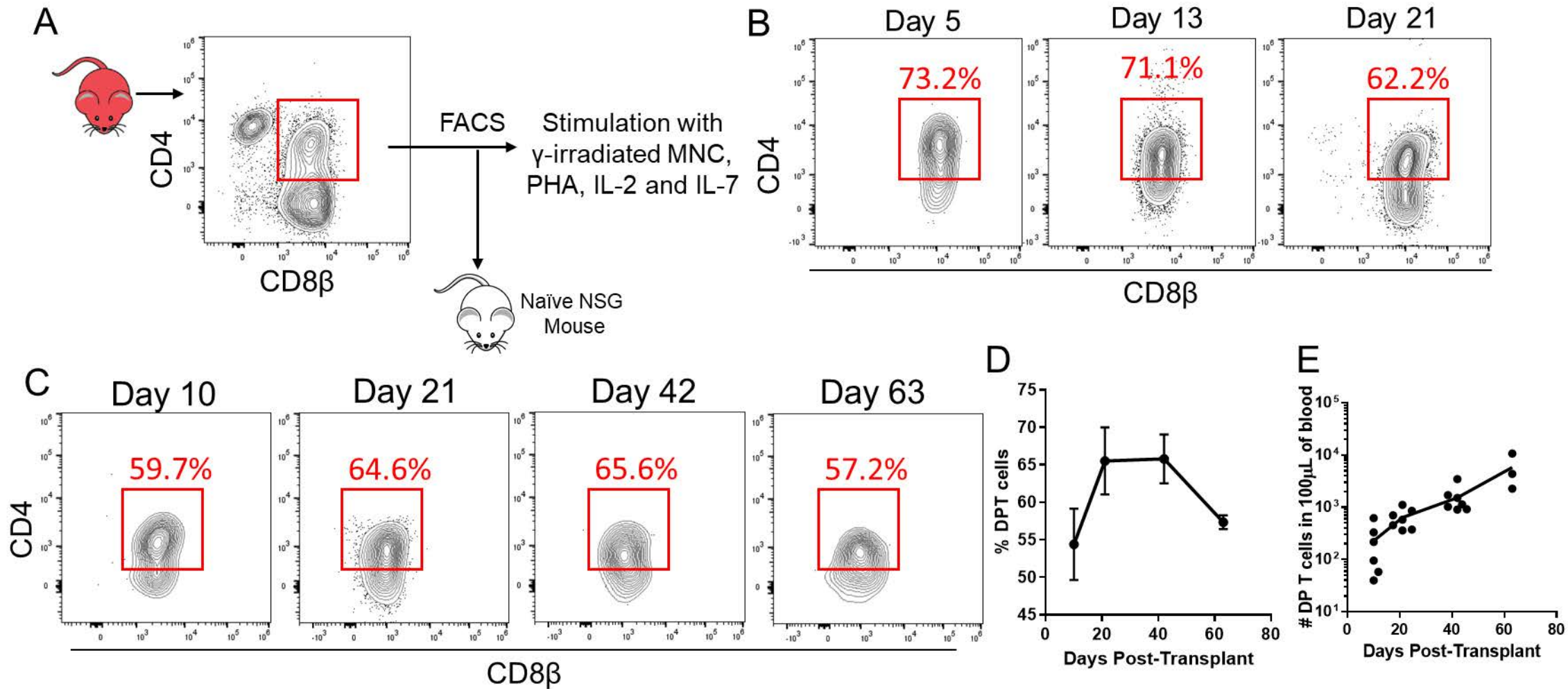
Supplemental Figure 1



Supplemental Figure 2

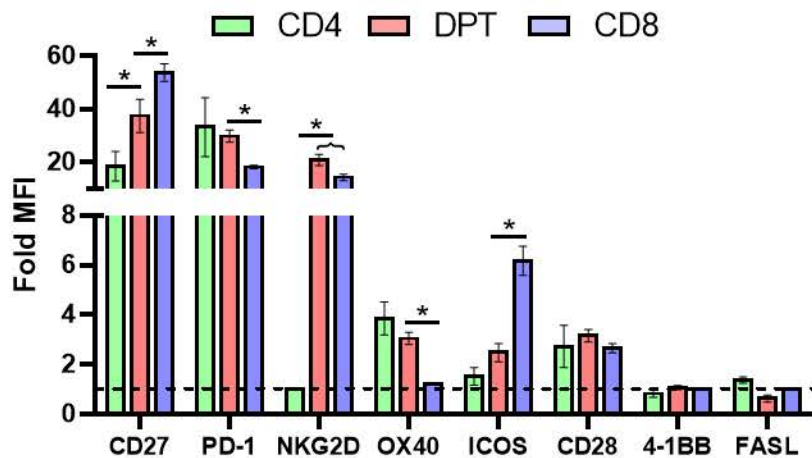


Supplemental Figure 3

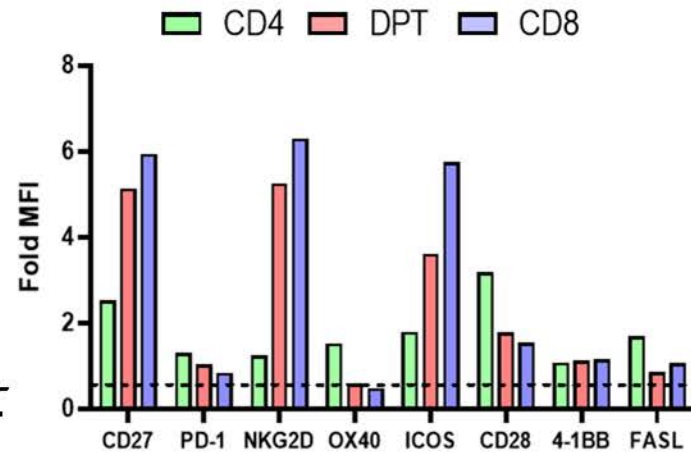


Supplemental Figure 4

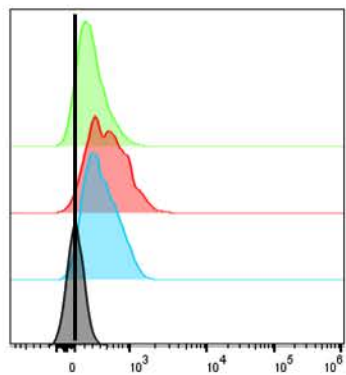
A Spleen



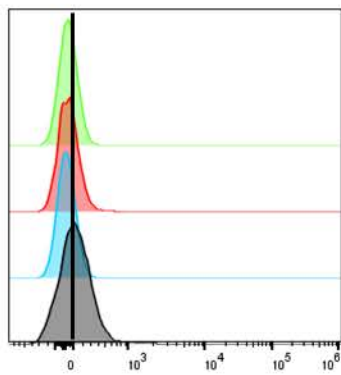
B Ex vivo Stimulation



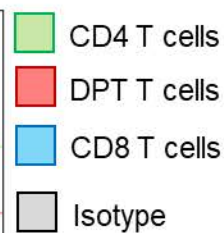
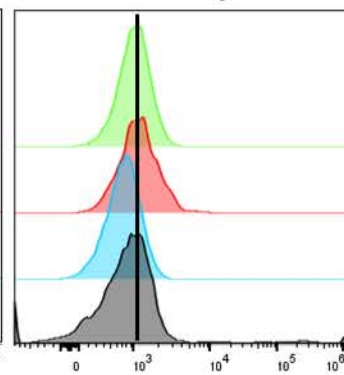
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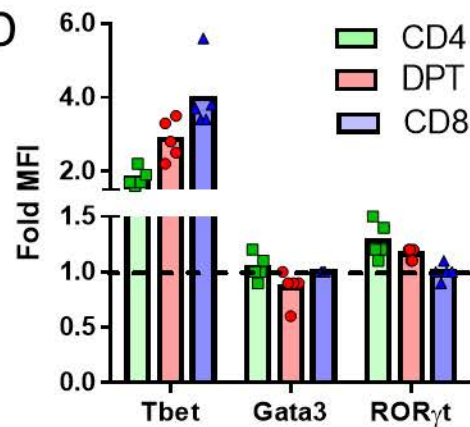
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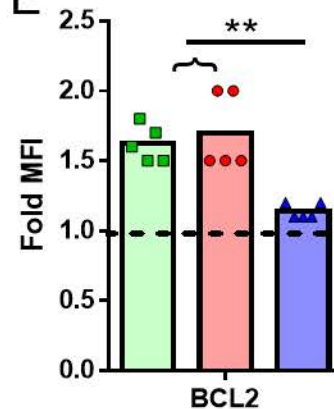
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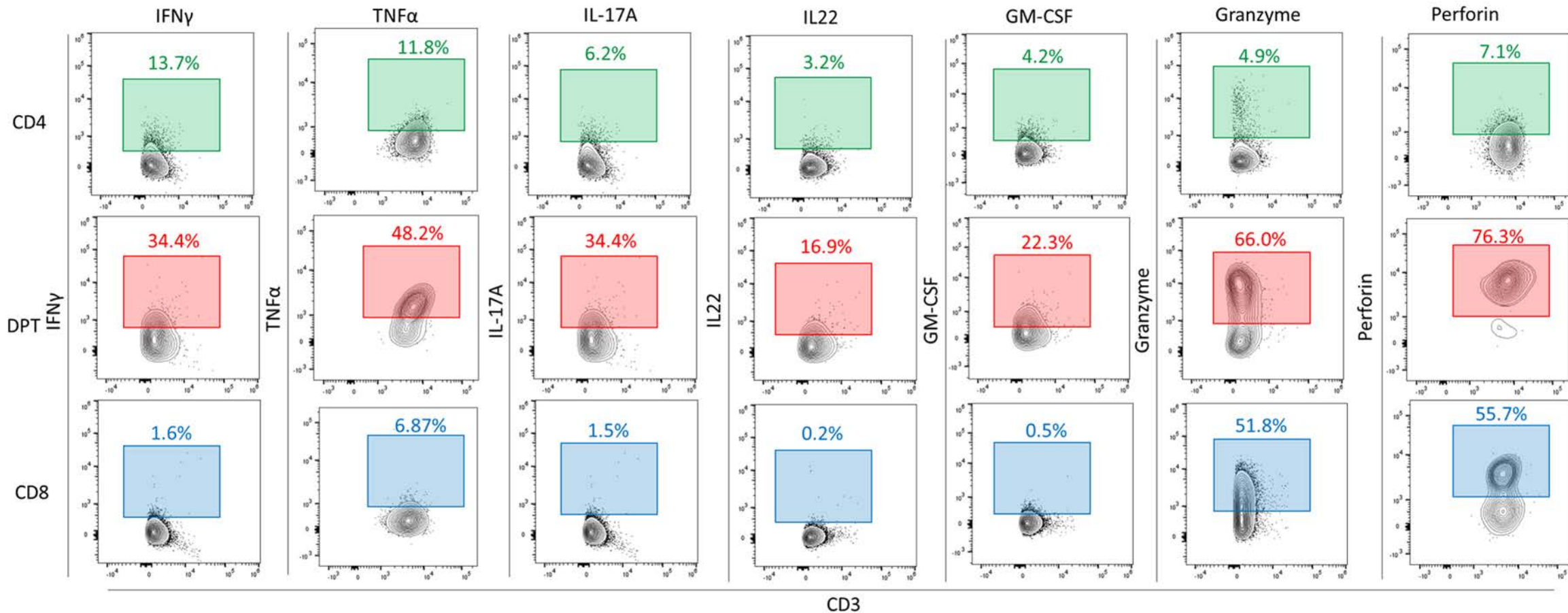


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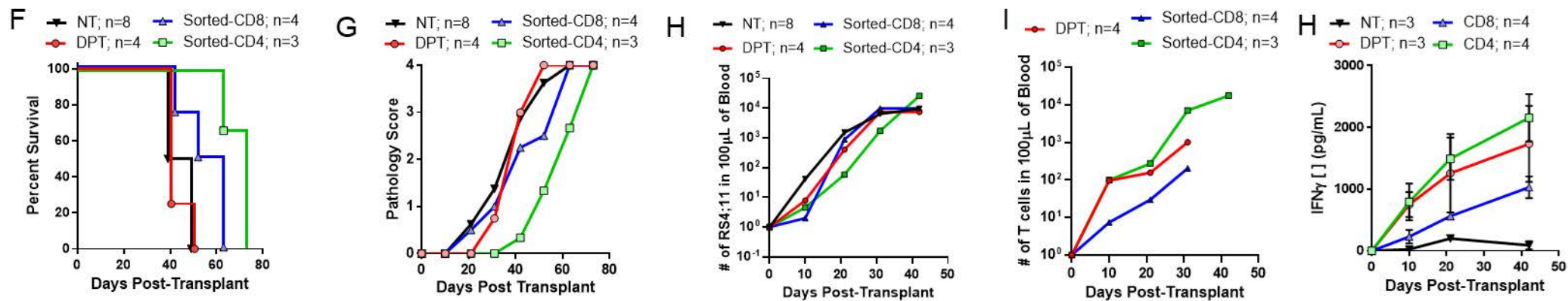
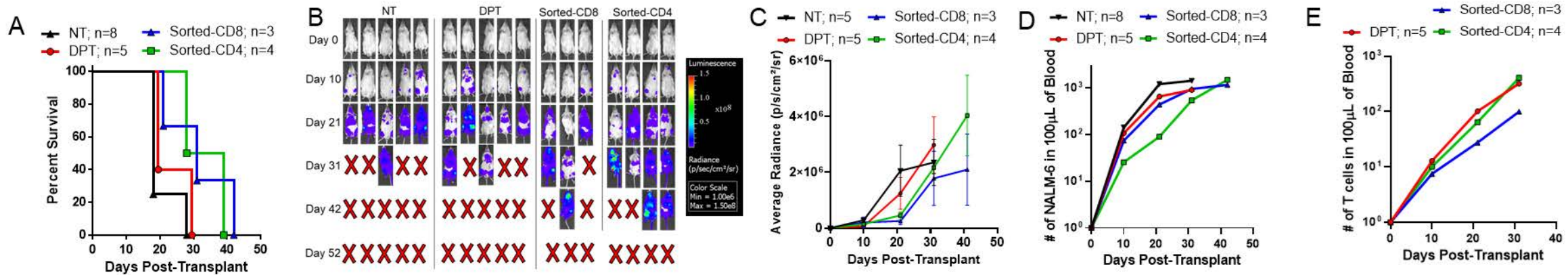


E





Supplemental Figure 6



Supplemental Figure 7