Supplementary Data for

Optimized Timing of Post-Transplantation Cyclophosphamide in MHC-Haploidentical Murine Hematopoietic Cell Transplantation

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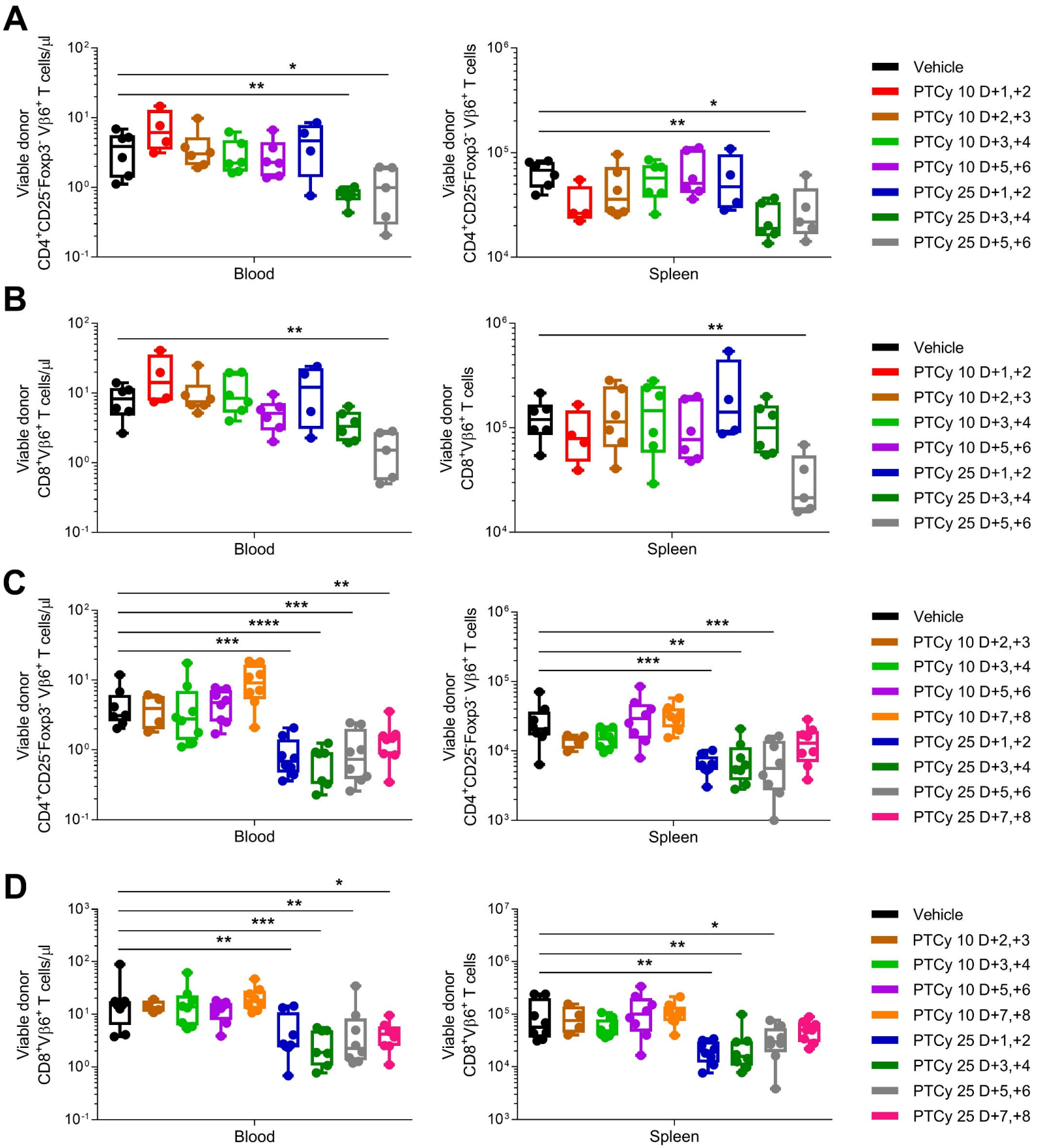

Figure S1. Absolute numbers of alloreactive effector T cells at days +7 and +21. For days **(A-B)** +7 and **(C-D)** +21, absolute numbers of **(A,C)** $CD4^+CD25^-Foxp3^-V\beta6^+$ T cells or **(B,D)** $CD8^+V\beta6^+$ T cells are shown corresponding to the data shown in Figure 6A-B. Total numbers were calculated for each sample by taking the total number of gated events divided by the total number of flow cytometrically determined viable ($LIVE/DEAD^-$) events and then multiplying by the total number of viable cells as determined by hematocytometric counting using trypan blue exclusion. *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001 on one-way ANOVA followed by the Holm-Sidak post hoc test using the vehicle-treated group as the control.

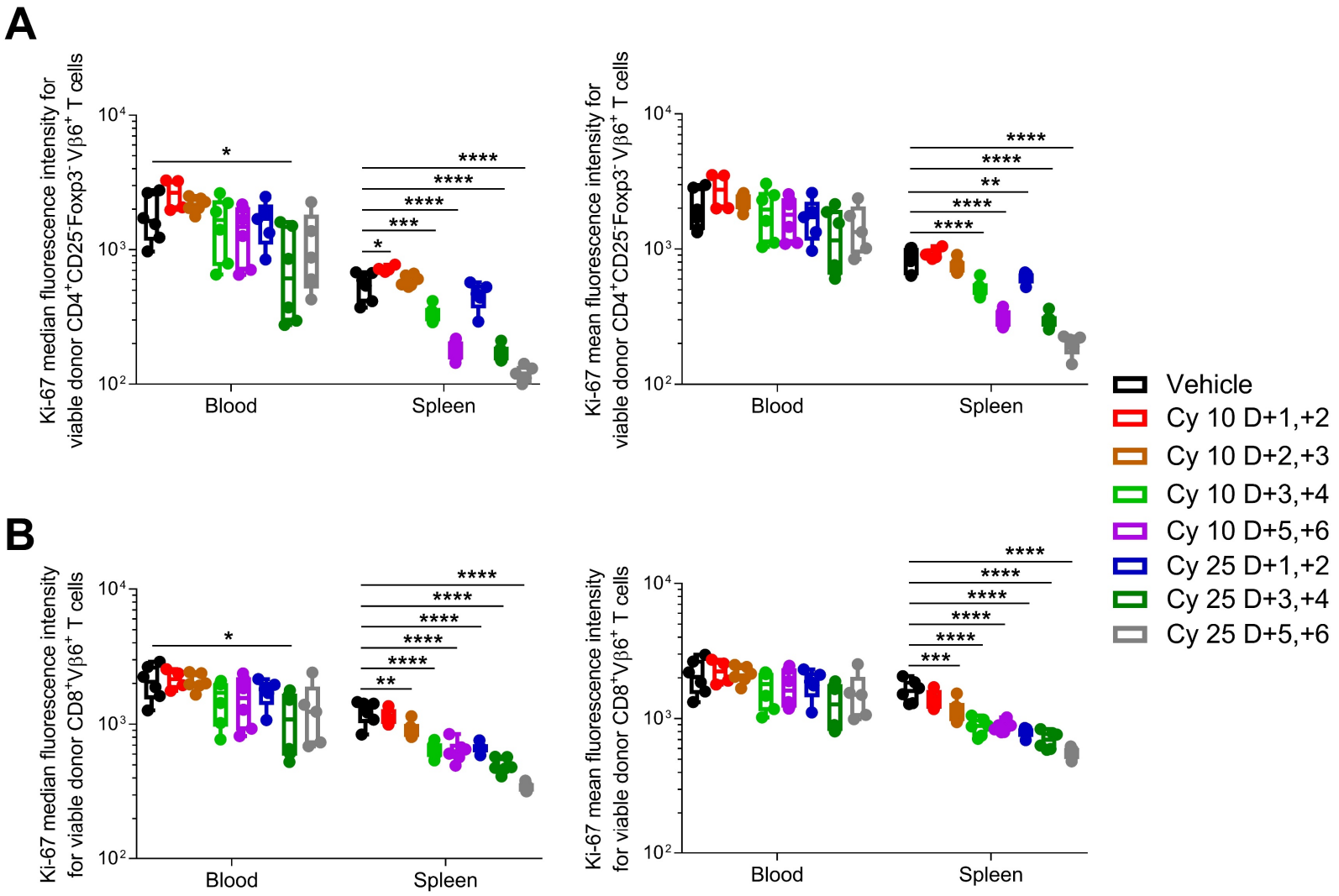

Figure S2. Median and mean fluorescence intensities of Ki-67 for alloreactive effector T cells at day +7. (Left) median fluorescence intensities and **(Right)** mean fluorescence intensities of Ki-67 within **(A)** $CD4^+CD25^-Foxp3^-V\beta6^+$ T cells or **(B)** $CD8^+V\beta6^+$ T cells are shown. These data correspond to the data shown in Figure 6C and show overall similar results. The median fluorescence intensities of the fluorescence-minus-one (FMO) controls for the two experiments were -64 and -46 for $CD4^+CD25^-Foxp3^-V\beta6^+$ T cells and -26 and 20 for $CD8^+V\beta6^+$ T cells. The mean fluorescence intensities of the FMO controls for the two experiments were -73 and -53 for $CD4^+CD25^-Foxp3^-V\beta6^+$ T cells and -37 and 24 for $CD8^+V\beta6^+$ T cells. *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001 on one-way ANOVA followed by the Holm-Sidak post hoc test using the vehicle-treated group as the control.

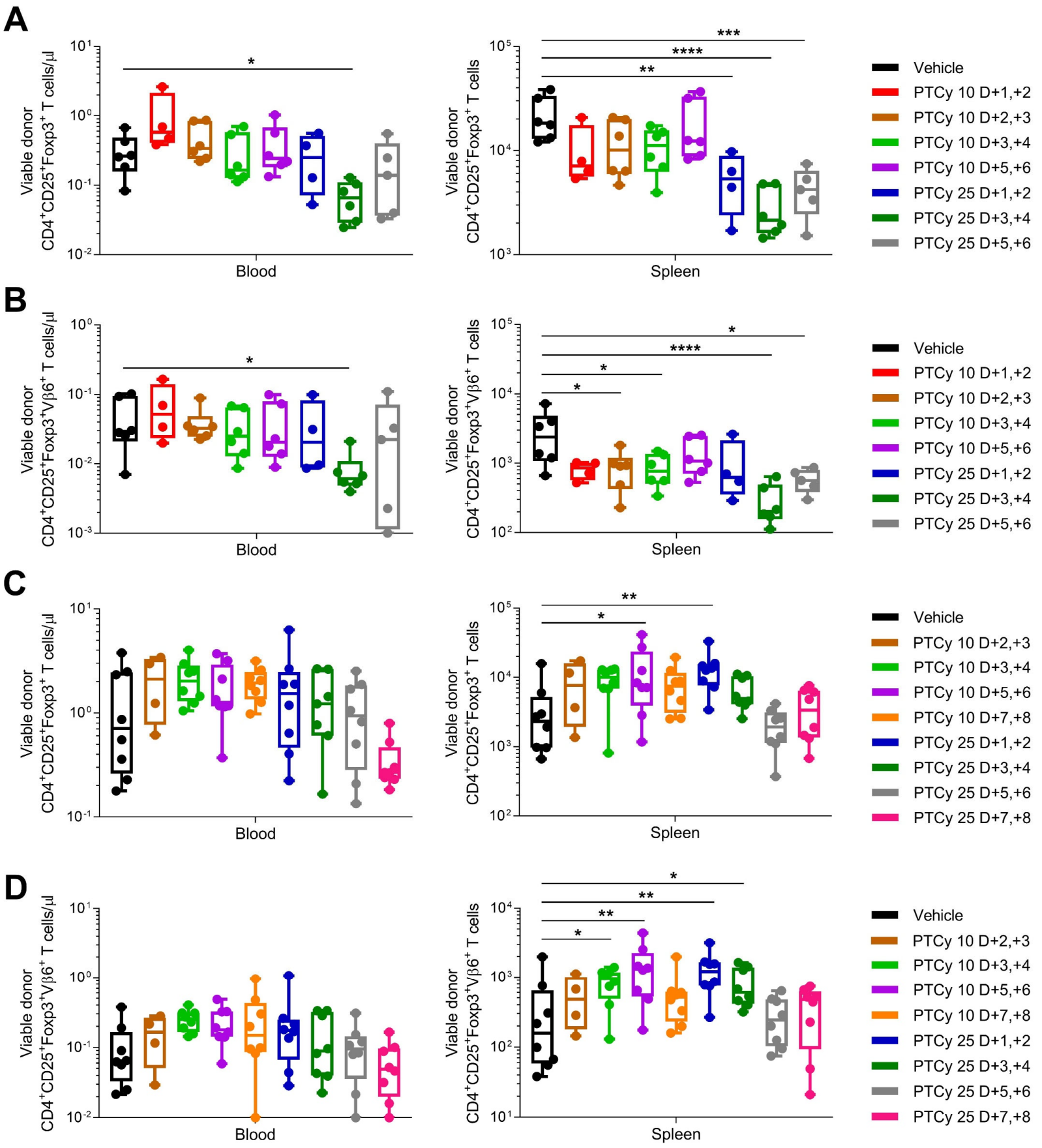

Figure S3. Absolute numbers of CD4⁺CD25⁺Foxp3⁺ regulatory T cells at days +7 and +21. For days **(A-B)** +7 and **(C-D)** +21, absolute numbers of **(A,C)** total CD4⁺CD25⁺Foxp3⁺ T cells or **(B,D)** CD4⁺CD25⁺Foxp3⁺Vβ6⁺ T cells are shown corresponding to the data shown in Figure 7A-D. Total numbers were calculated for each sample by taking the total number of gated events divided by the total number of flow cytometrically determined viable (LIVE/DEAD⁻) events and then multiplying by the total number of viable cells as determined by hematocytometric counting using trypan blue exclusion. *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001 on one-way ANOVA followed by the Holm-Sidak post hoc test using the vehicle-treated group as the control.