Supplemental Data

Table of Contents

Supplemental Methods

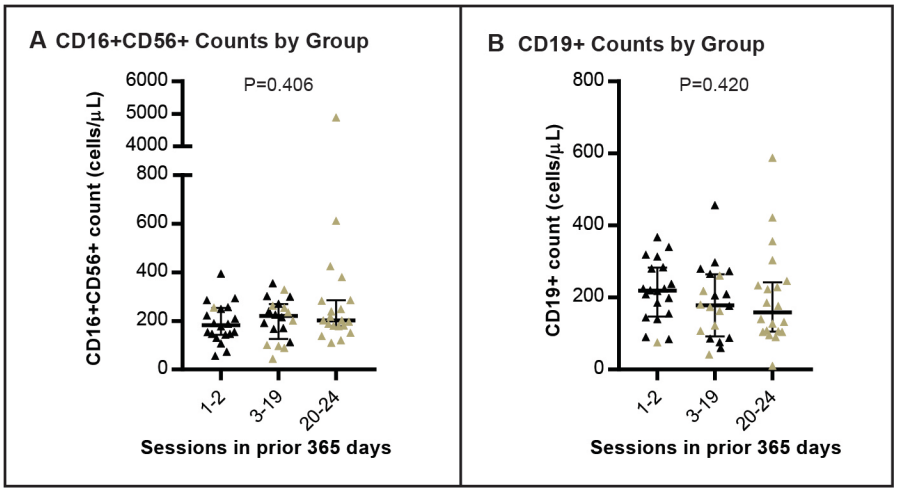
Detailed flow cytometry

Mononuclear cells were isolated using BD Vacutainer™ Glass Mononuclear Cell Preparation (CPT) Tubes (BD 362761). These cells were frozen using Cell Freezing Medium-DMSO (Sigma C6164) at the Brigham and Women's Hospital Center for Clinical Investigation and aliquots stored in liquid nitrogen until use. Cryopreserved peripheral blood mononuclear cells (PBMCs) were thawed into warm RPMI (Gibco 11875-093)/10% fetal calf serum (FCS) (Gemini 100-106), washed once in cold RPMI/10% FCS, and stained in PBS (Hyclone SH30013.03)/1% FCS in the presence of Fc blocking antibody (eBioscience/ThermoFisher 14-9161-71). MR1 tetramer staining was performed at room temperature for 30 minutes. Then, the remaining antibodies were added, and the cells were incubated on ice for 30 minutes. Cells were washed twice in cold PBS/1% FCS and passed through a 70-µm filter (Component Supply U-CMN-70). Data were acquired on a BD LSRFortessa flow cytometer and analyzed using FlowJo 10.4.2. The samples were run in three batches on consecutive days, with samples from all three donation groups in each batch. All samples were analyzed together, with a single set of gates applied to all samples.

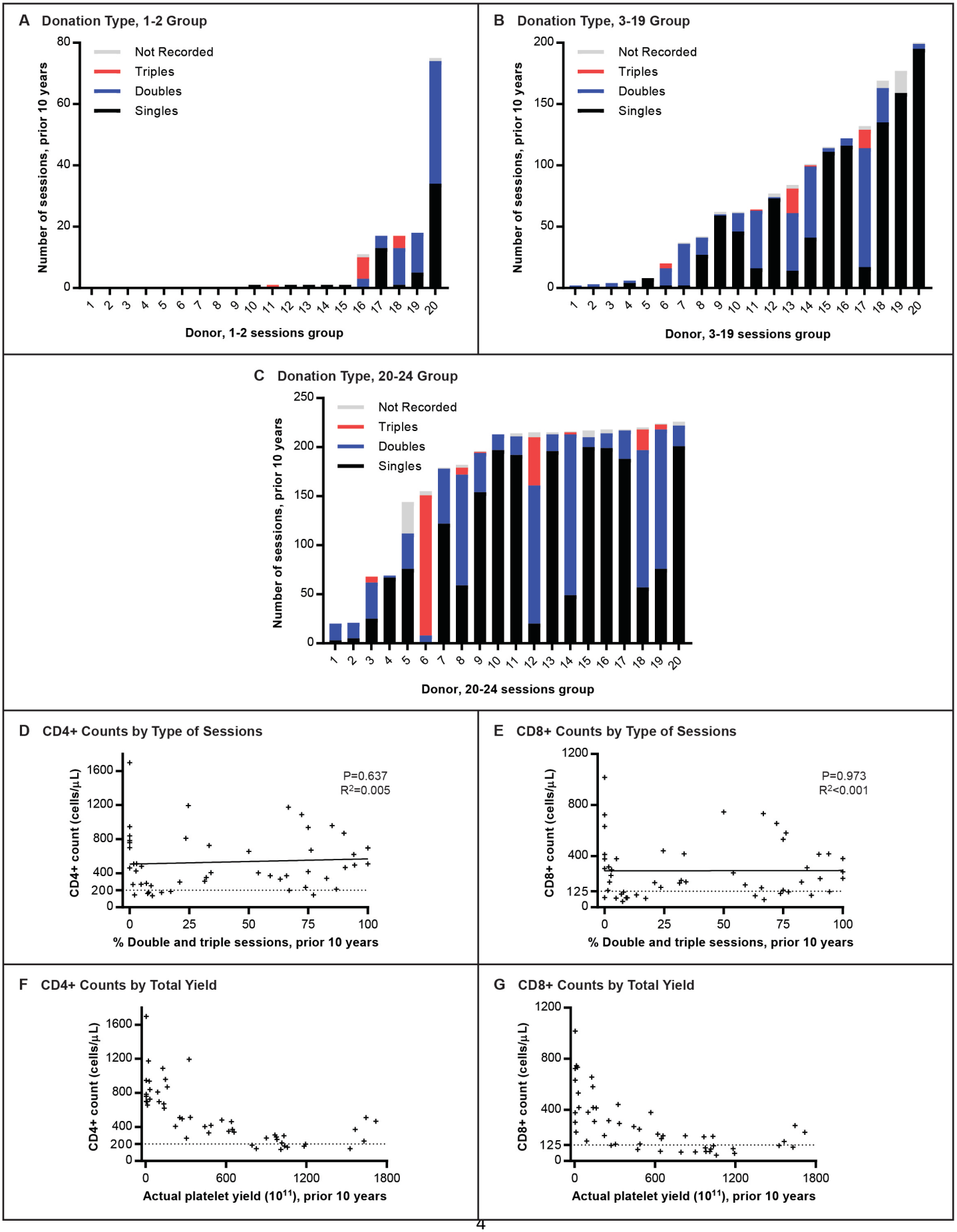
T-cell differentiation subsets were gated based on expression of CCR7 and CD45RA: Tcm (CCR7+CD45RA-), Tem (CCR7-CD45RA-), and TEMRA (CCR7-CD45RA+). Naive T-cells were gated as CCR7+CD45RA+CD27+Fas-. Tscm were gated as CCR7+CD45RA+CD27+Fas+. T helper subsets were gated based on expression of CXCR3 and CCR6: Th1 (CXCR3+CCR6-), Th2 (CXCR3-CCR6-), and Th17 (CXCR3-CCR6+). Tregs were identified as CD25+CD127+. In the case of Tregs and CD38+HLA-DR+CD8+ T-cells, MR1-tetramer+ T-cells (MAIT cells) were excluded prior to gating on CD4+ and CD8+ T-cells, respectively. Absolute numbers were calculated by multiplying the frequency among CD4+ or CD8+ T-cell populations by the absolute number of CD4+ or CD8+ T-cells obtained from the Brigham and Women's Hospital clinical laboratory. Tcm = T central memory, Tem = T effector memory, Tscm = T stem cell-like memory, Tfh = T follicular helper, TEMRA = T effector memory CD45RA+.

For supplemental Figure 6, peripheral blood from a cohort of non-inflammatory control patients was collected under a separate research protocol approved by the Partners HealthCare Institutional Review Board (2014P002558). The cohort comprised nine individuals age 60 years or older (median age 63, range 61-74) without a history of autoimmune disease or infection with human immunodeficiency virus, hepatitis B virus, or hepatitis C virus. One individual had a history of breast cancer. PBMCs were isolated by density centrifugation using Ficoll-Paque PLUS (GE Healthcare 17-1440-03) and stored in liquid nitrogen until use. PBMC samples from Cryopreserved PBMCs were thawed and stained in three batches as described above, in parallel with the PBMC samples obtained from the patients undergoing plateletpheresis. Data from these control samples were acquired and analyzed in parallel with the donor samples.

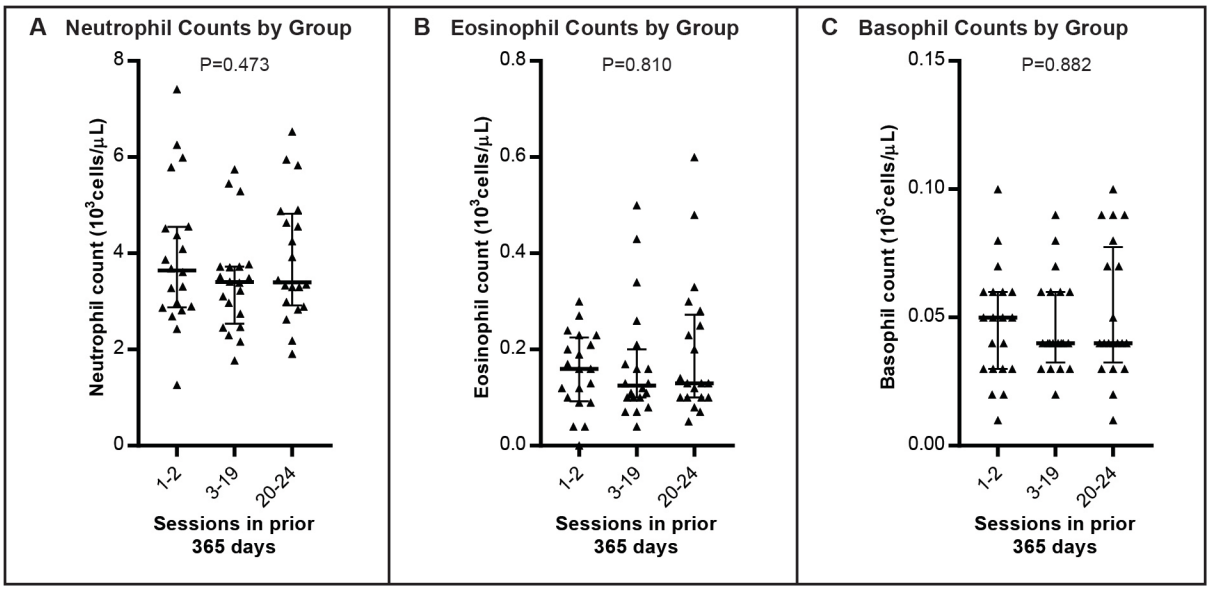
Supplemental Figures



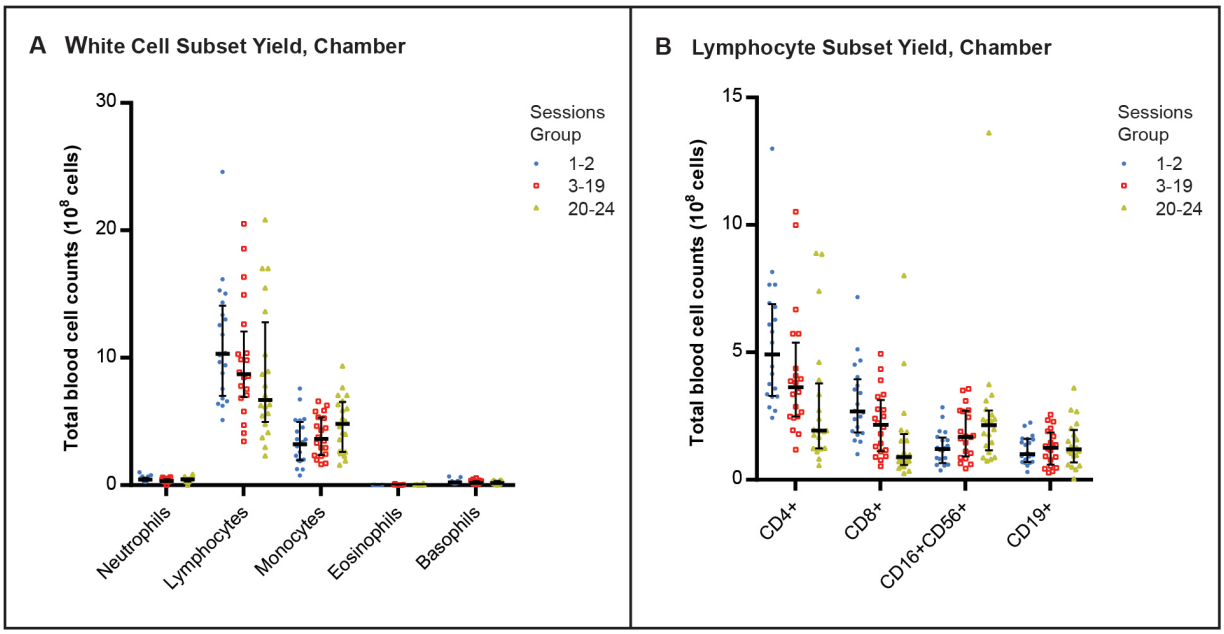
Supplemental Figure 1. Select Lymphocyte Subset Counts. Panel A shows the absolute CD16+CD56+ counts for the three groups. Panel B shows the absolute CD19+ counts for the three groups. Donors who have undergone 20-24 successful plateletpheresis sessions at any point in the prior 20-year period are indicated by gold symbols. Horizontal lines indicate the median and interquartile range.


A Donation Type, 1-2 Group
Not Recorded
Triples
Doubles
Singles
Number of sessions, prior 10 years
Donor, 1-2 sessions group
B Donation Type, 3-19 Group
Not Recorded
Triples
Doubles
Singles
Number of sessions, prior 10 years
Donor, 3-19 sessions group
C Donation Type, 20-24 Group
Not Recorded
Triples
Doubles
Singles
Number of sessions, prior 10 years
Donor, 20-24 sessions group
D CD4+ Counts by Type of Sessions
P=0.637
R²=0.005
CD4+ count (cells/µL)
% Double and triple sessions, prior 10 years
E CD8+ Counts by Type of Sessions
P=0.973
R²<0.001
CD8+ count (cells/µL)
% Double and triple sessions, prior 10 years
F CD4+ Counts by Total Yield
CD4+ count (cells/µL)
Actual platelet yield (10¹¹), prior 10 years
G CD8+ Counts by Total Yield
CD8+ count (cells/µL)
Actual platelet yield (10¹¹), prior 10 years

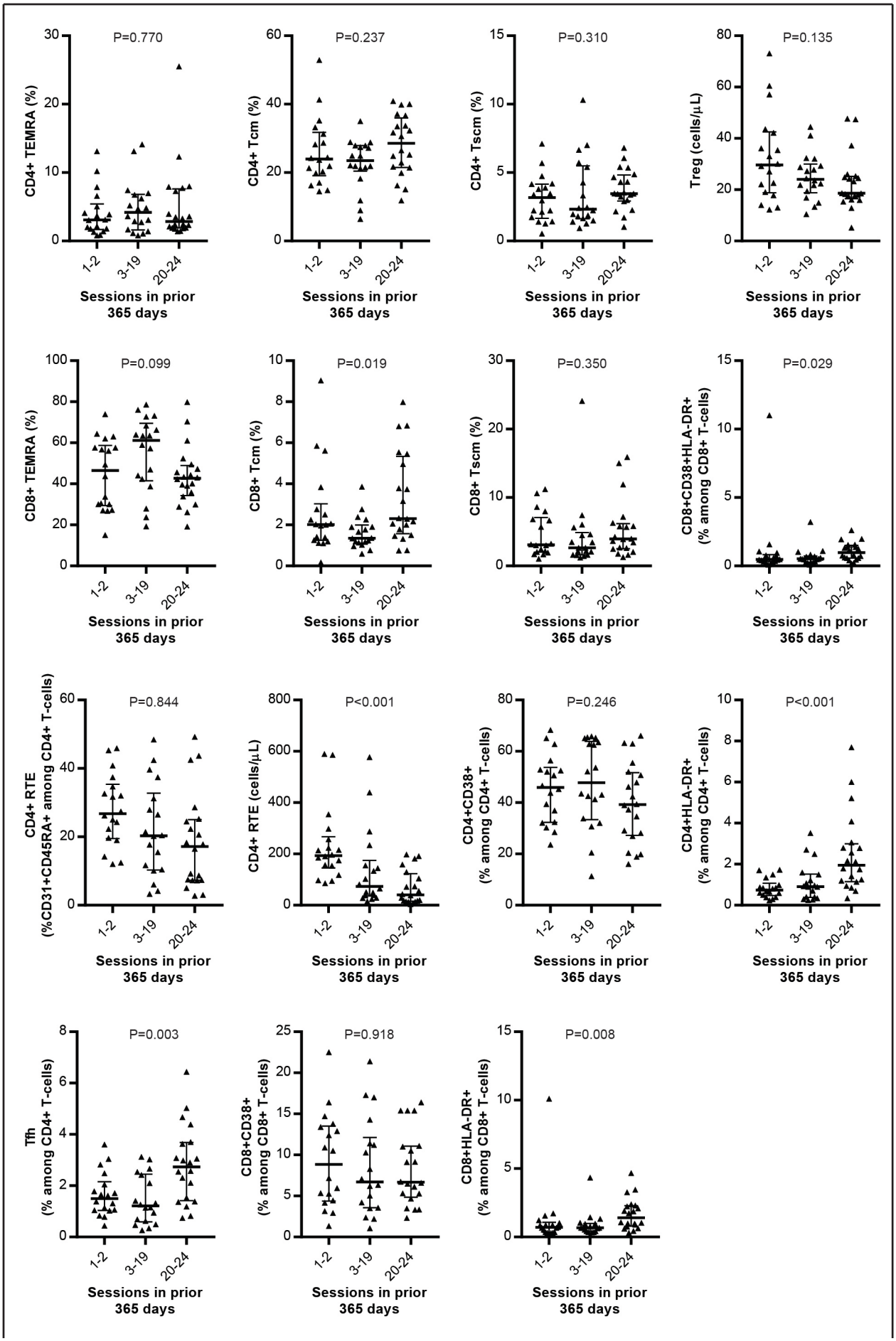
Supplemental Figure 2. Donation Types and Platelet Yields by Donor with Relationship to CD4+ and CD8+ Counts. Panels A-C show platelet donation types over the prior 10 years for the three groups. Panels D-E show the relationship between CD4+ and CD8+ counts and the percentage of double and triple sessions over the prior 10 years. For these panels, only 51 donors are included because 9 donors in the 1-2 sessions group had not undergone plateletpheresis prior to the date of study participation and CD4+ and CD8+ counts were measured pre-plateletpheresis. Panels F-G show CD4+ and CD8+ counts relative to actual platelet yields. Yields from the day of study participation were not included in any of these analyses since comparator blood counts were drawn pre-plateletpheresis. Horizontal dashed lines indicate 200 cells/μL for CD4+ T-cell plots and 125 cells/μL for CD8+ T-cell plots. Solid lines are linear regression lines.

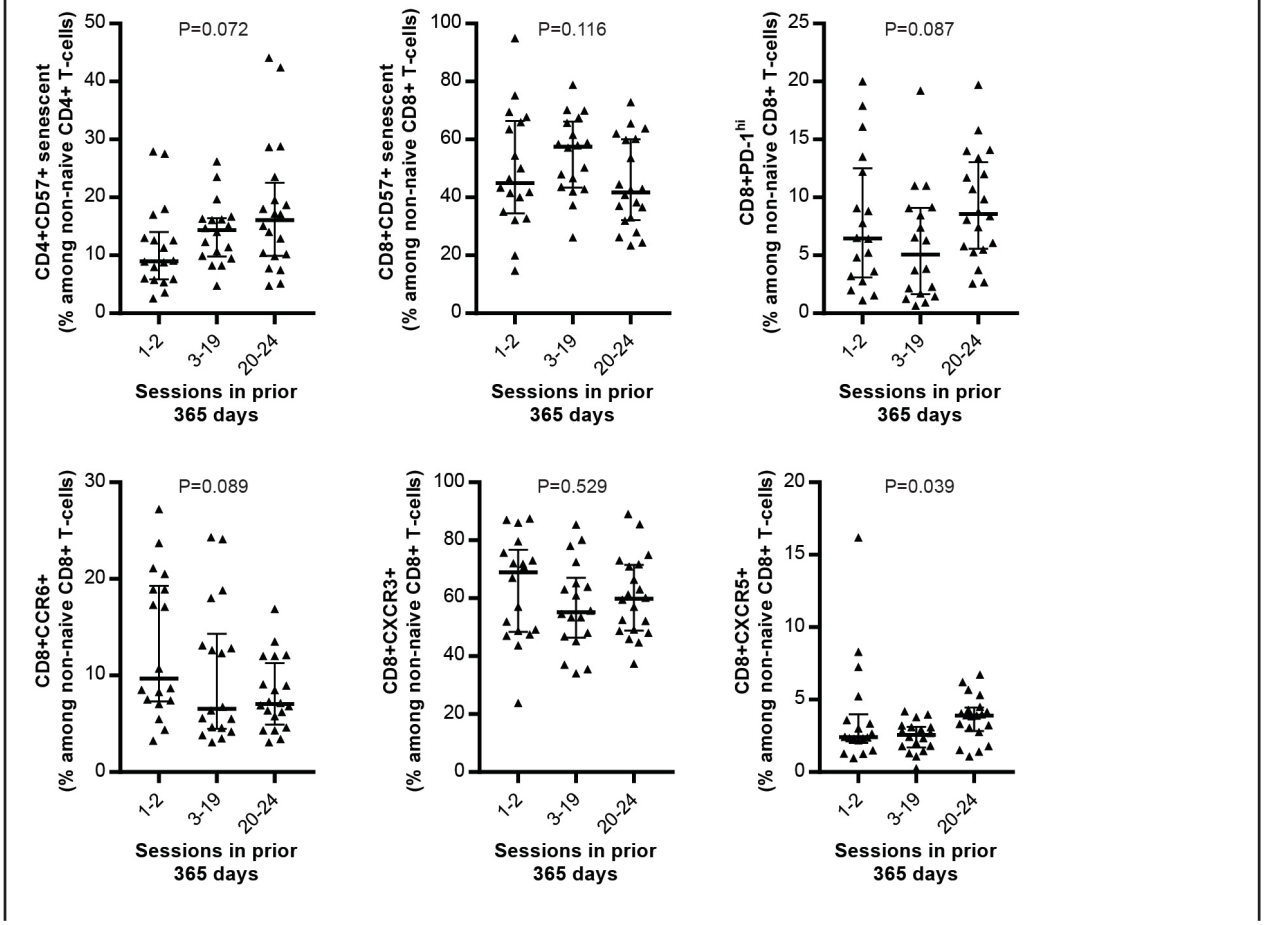


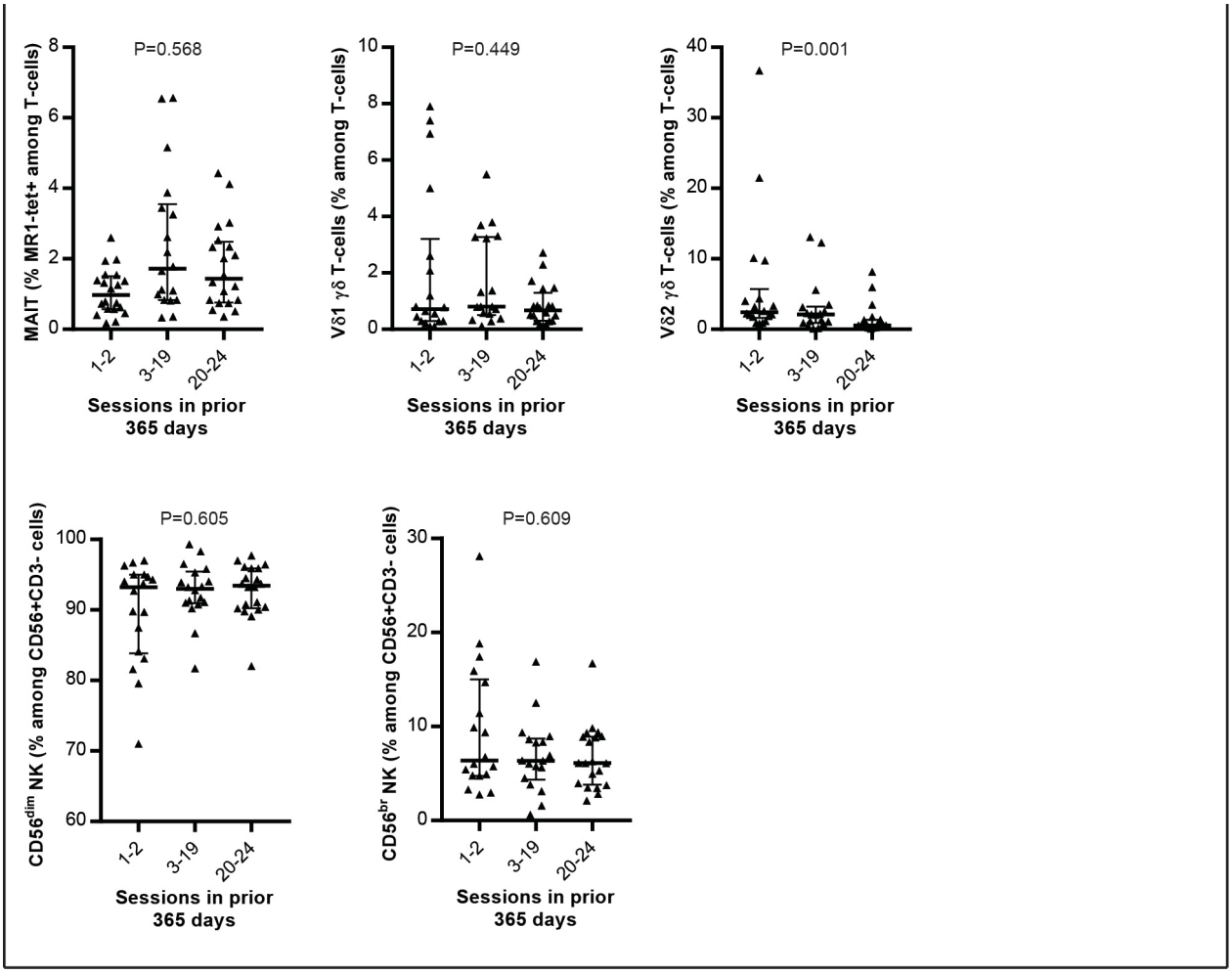
Supplemental Figure 3. Select White Blood Cell Subset Counts. Panel A shows the absolute neutrophil counts for the three groups. Panel B shows the absolute eosinophil counts for the three groups. Panel C shows the absolute basophil counts for the three groups. Horizontal lines indicate the median and interquartile range.



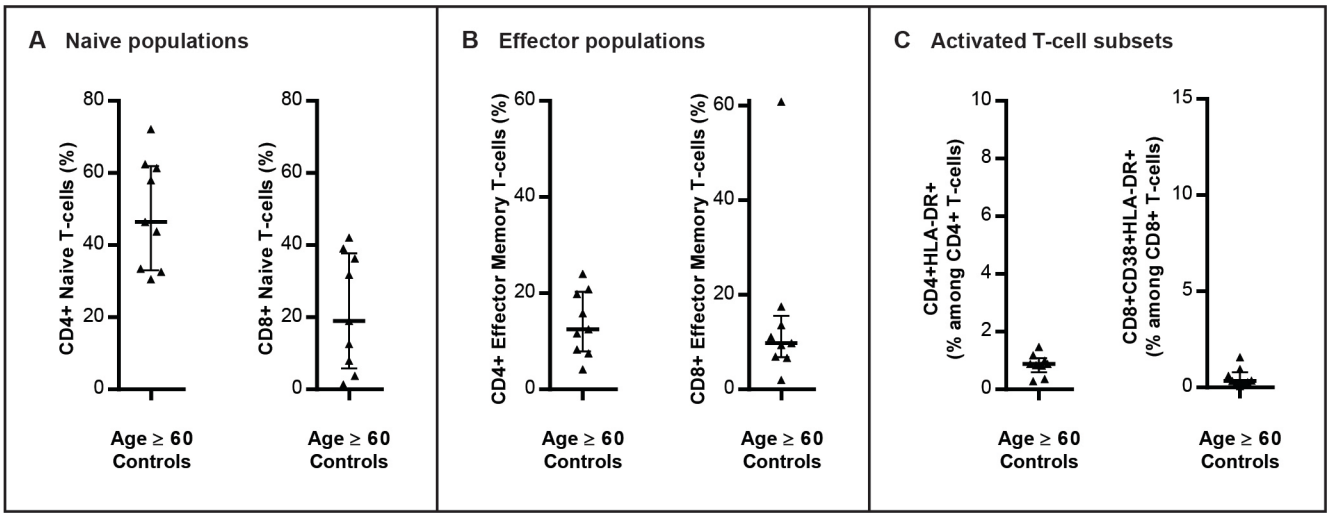
Supplemental Figure 4. Yield, Leukoreduction System Chambers. Panel A shows total numbers of white cell subsets. Panel B shows total numbers of lymphocyte subsets. The numbers were calculated assuming a leukoreduction system chamber volume of 11.35 mL, as specified by the manufacturer. Horizontal lines indicate the median and interquartile range.


P=0.770
CD4+ TEMRA (%)
P=0.237
CD4+ Tcm (%)
P=0.310
CD4+ Tscm (%)
P=0.135
Treg (cells/μL)
P=0.099
CD8+ TEMRA (%)
P=0.019
CD8+ Tcm (%)
P=0.350
CD8+ Tscm (%)
P=0.029
CD8+CD38+HLA-DR+ (% among CD8+ T-cells)
P=0.844
CD4+ RTE (%CD31+CD45RA+ among CD4+ T-cells)
P<0.001
CD4+ RTE (cells/μL)
P=0.246
CD4+CD38+ (% among CD4+ T-cells)
P<0.001
CD4+HLA-DR+ (% among CD4+ T-cells)
P=0.003
Tfh (% among CD4+ T-cells)
P=0.918
CD8+CD38+ (% among CD8+ T-cells)
P=0.008
CD8+HLA-DR+ (% among CD8+ T-cells)
1-2
3-19
20-24
Sessions in prior 365 days

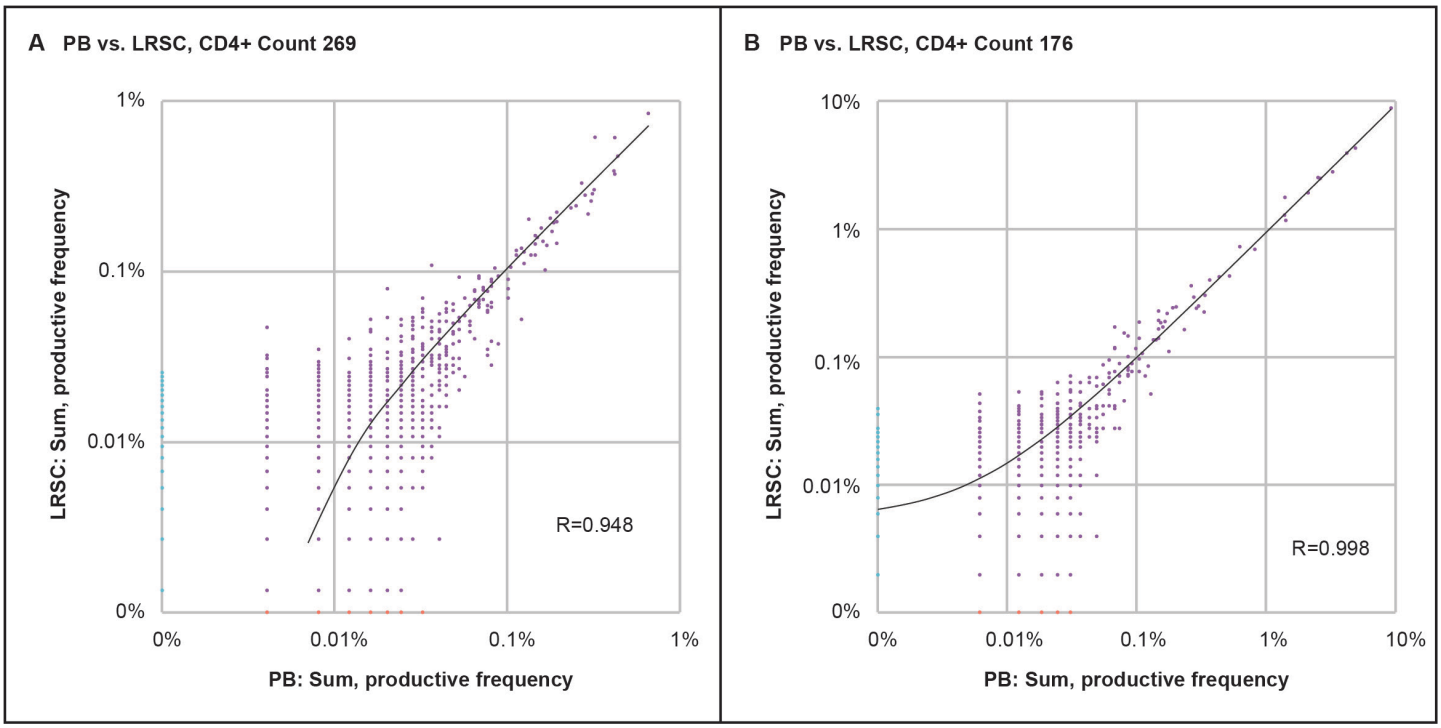

CD4+CD57+ senescent (% among non-naive CD4+ T-cells)
P=0.072
CD8+CD57+ senescent (% among non-naive CD8+ T-cells)
P=0.116
CD8+PD-1hi (% among non-naive CD8+ T-cells)
P=0.087
CD8+CCR6+ (% among non-naive CD8+ T-cells)
P=0.089
CD8+CXCR3+ (% among non-naive CD8+ T-cells)
P=0.529
CD8+CXCR5+ (% among non-naive CD8+ T-cells)
P=0.039
1-2
3-19
20-24
Sessions in prior 365 days



Supplemental Figure 5. Select Detailed Lymphocyte Immunophenotyping Plots. Non-naive refers to CD8+ T-cells or CD4+ T-cells are that are not CCR7+CD45RA+. Tfh cells were gated on as PD-1^{hi}CXCR5+CD4+ T-cells. Treg refers to T regulatory cells, RTE to recent thymic emigrant, MAIT to mucosal-associated invariant T-cells, Vd1 to gamma-delta T-cells expressing the Vδ1 T-cell receptor, Vδ2 to gamma-delta T-cells expressing the Vδ2 T-cell receptor, and NK to natural killer cells. Horizontal lines indicate the median and interquartile range. For RTE, Treg, HLA-DR, and CD38 T-cell populations, MR1-tet+ cells (MAIT cells) were gated out.



Supplemental Figure 6. Select T-cell populations in a control cohort age 60 years or older. Panel A shows percentages of naive CD4+ and CD8+ T-cells. Panel B shows percentages of effector memory CD4+ and CD8+ T-cells. Panel C shows two activated T-cell subsets. PBMCs for these samples were isolated using Ficoll-Paque PLUS rather than CPT tubes but were stained and analyzed by flow cytometry in parallel with the PBMC samples from the platelet donors depicted in Figure 3 and supplemental Figure 5. Population gating was performed as indicated in Figure 3 and supplemental Figure 5. Horizontal lines indicate the median and interquartile range.



Supplemental Figure 7. Correlation between productive clones identified in the leukoreduction system chamber compared to peripheral blood. Panel A shows the correlation between productive clones identified in the LRSC compared to PB in a subject with a CD4+ T-lymphocyte count of 269 cells/µL. Panel B shows the correlation between productive clones identified in the LRSC compared to PB in a subject with a CD4+ T-lymphocyte count of 176 cells/µL. Matches were made using amino acid sequences. Purple dots indicate clones that are shared between the two samples. Blue dots indicate clones that are unique to the LRSC sample. Orange dots indicate clones that are unique to the PB sample. The line indicates the linear regression line, generated on a linear scale but plotted here on a logarithmic scale. R-values for the set of common rearrangements are shown. LRSC refers to leukoreduction system chamber and PB to peripheral blood.

Supplemental Tables

Supplemental Table 1. Antibodies and other reagents used for detailed flow cytometry.

Antibody target (or tetramer/ reagent name)	Fluorochrome	Clone	Vendor/ Source
CD185 (CXCR5)	BV421	J252D4	Biolegend
CD3	BV510	UCHT1	Biolegend
CD45RA	BV605	HI100	Biolegend
CD4	BV650	OKT4	Biolegend
CD196 (CCR6)	BV711	G034E3	Biolegend
CD28	BV785	CD28.2	Biolegend
CD197 (CCR7)	PerCP Cy5.5	G043H7	Biolegend
CD95 (Fas)	PE	DX2	Biolegend
CD183 (CXCR3)	PE Cy7	G025H7	Biolegend
CD279 (PD-1)	APC	EH12.2H7	Biolegend
CD8a	Alexa fluor 700	RPA-T8	Biolegend
CD27	APC Cy7	M-T271	Biolegend
CD127 (IL-7Rα)	BV650	A019D5	Biolegend
TCR Vδ2	BV711	B6	Biolegend
HLA-DR	BV785	L243	Biolegend
CD38	PerCP Cy5.5	HIT2	Biolegend
CD25	PE	M-A251	Biolegend
CD4	PerCP Cy5.5	OKT4	Biolegend
CD56 (NCAM)	APC Cy7	5.1H11	Biolegend
CD31	BV421	WM59	Biolegend
Fixable Viability Dye eFluor 455UV	eFluor 455UV	n/a	eBioscience
CD57	FITC	TB01	eBioscience
TCR Vδ1	FITC	REA173	Miltenyi Biotec
MR1 tetramer loaded with 5-OP-RU	APC	n/a	NIH Tetramer Core Facility*

*The MR1 tetramer technology was developed jointly by Dr. James McCluskey, Dr. Jamie Rossjohn, and Dr. David Fairlie, and the material was produced by the NIH Tetramer Core Facility as permitted to be distributed by the University of Melbourne.

Supplemental Table 2. Linear regression model of platelet donation history in past 20 years and age for CD4+ and CD8+ T-cells.

Model	CD4+ T-cells		CD8+ T-cells	
	Estimate (cells/μL)	P-Value	Estimate	P-Value (cells/μL)
20-year donations only				
Constant	839		498	
50 or more donations	-491	<0.001	-329	<0.001
Age only				
Constant	570		279	
Age 50-59	-221	0.048	-113	0.043
Age 60-69	-255	0.011	-132	0.009
Age 70 or older	-294	0.005	-139	0.007
All variables				
Constant	878		552	
50 or more donations	-384	<0.001	-204	<0.001
Age 50-59	-136	0.135	-211	<0.001
Age 60-69	-139	0.094	-204	<0.001
Age 70 or older	-234	0.014	-217	<0.001

Supplemental Table 3. White blood cell counts in the collected platelet product. Results are stratified by the number of plateletpheresis sessions in the prior 365 days including the day of study participation. A "unit" refers to a blood component unit, in this case the platelet unit collected.

Sessions Group	Median (10^6cells/unit)	Range (10^6cells/unit)	First Quartile (10^6cells/unit)	Third Quartile (10^6cells/unit)	Interquartile Range (10^6cells/unit)
1-2	0.14	0 to 4.77	0.0825	0.2275	0.145
3-19	0.15	0 to 1.2	0.0275	0.325	0.2975
20-24	0.27	0 to >5*	0.1	0.4975	0.3975

*If the single >5 value is excluded, then the range is 0 to 0.64.

Supplemental Table 4. Leukoreduction system chamber cell counts and yields. Median values and ranges are shown. For yield calculations, a chamber volume of 11.35 mL was assumed based on manufacturer specifications.

Measure	1-2 Sessions Group (N = 20)	3-19 Sessions Group (N = 20)	20-24 Sessions Group (N = 20)
WBC count (x 10^3/μL)	121.1 (70.6-293.5)	108.1 (64-245.4)	107.8 (44.7-241.6)
WBC yield (x 10^9)	1.37	1.23	1.22
Neutrophil count (x 10^3/μL)	3.9 (2.0-9.0)	3.1 (0.1-5.7)	4 (0.0-7.6)
Neutrophil yield (x 10^8)	0.5	0.4	0.5
Lymphocyte count (x 10^3/μL)	90.8 (45.1-216.6)	76.8 (30.3-180.8)	58.9 (20.4-183.7)
Lymphocyte yield (x 10^8)	10.3	8.7	6.7
Monocyte count (x 10^3/μL)	28.4 (6.8-66.8)	32.0 (14.3-58)	42.5 (14.0-82.5)
Monocyte yield (x 10^8)	3.2	3.6	4.8
CD4+ count (x 10^3/μL)	43.3 (21.4-114.5)	32.0 (10.4-92.7)	17.0 (5.0-78.4)
CD4+ yield (x 10^8)	4.9	3.6	1.9
CD8+ count (x 10^3/μL)	23.6 (8.8-63.1)	19.0 (4.7-43.6)	7.9 (2.3-70.7)
CD8+ yield (x 10^8)	2.7	2.2	0.9
CD16+CD56+ count (x 10^3/μL)	10.6 (3.3-25.1)	14.8 (3.9-31.4)	18.9 (6.6-120.0)
CD16+CD56+ yield (x 10^8)	1.2	1.7	2.1
CD19+ count (x 10^3/μL)	8.9 (2.7-19.8)	11.1 (2.4-22.5)	10.5 (0.4-31.8)
CD19+ yield (x 10^8)	0.9	2.1	1.2