**Supplemental information**

Multimodal analysis for human *ex vivo*

studies shows extensive molecular changes

from delays in blood processing

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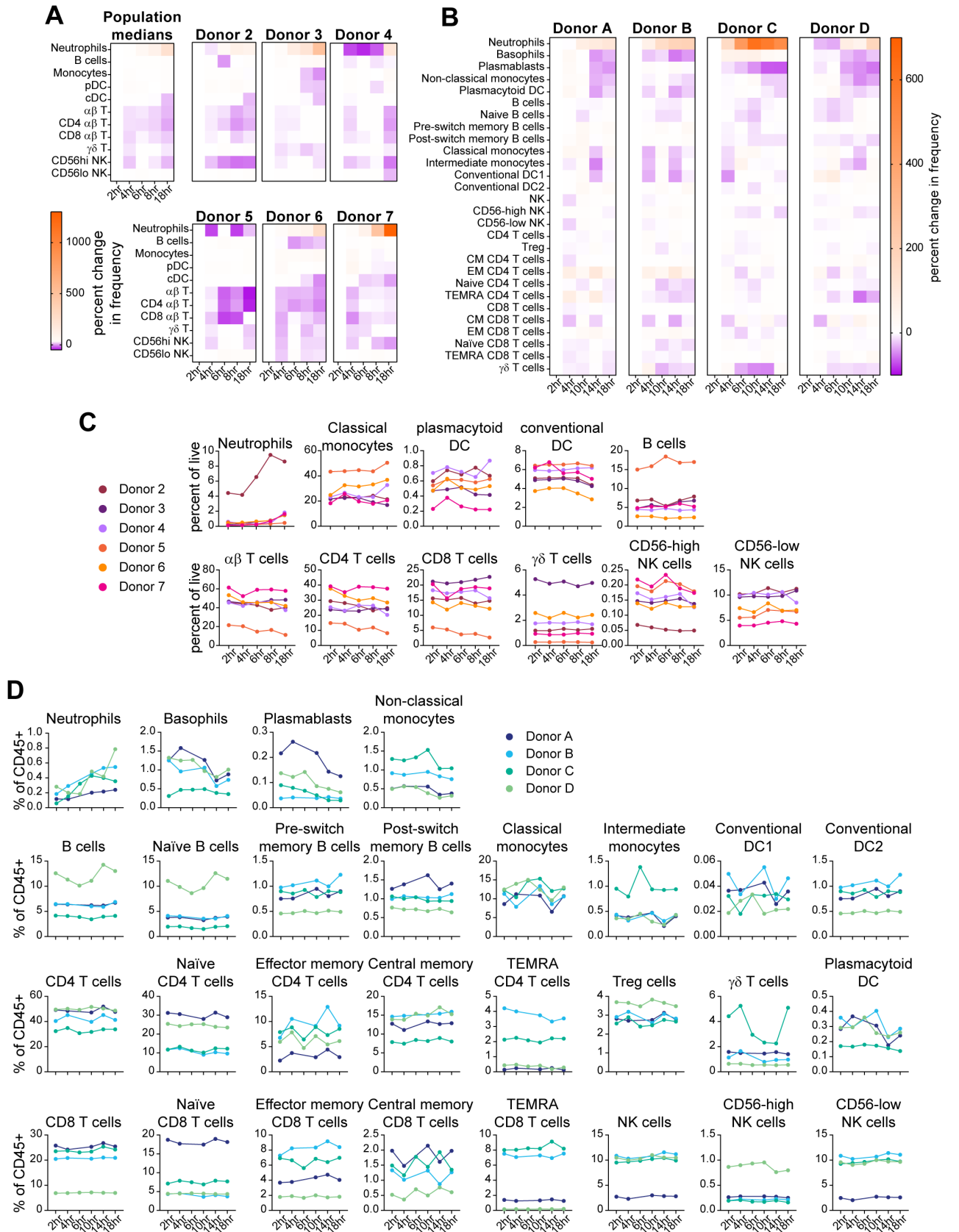
Supplemental Figure S1
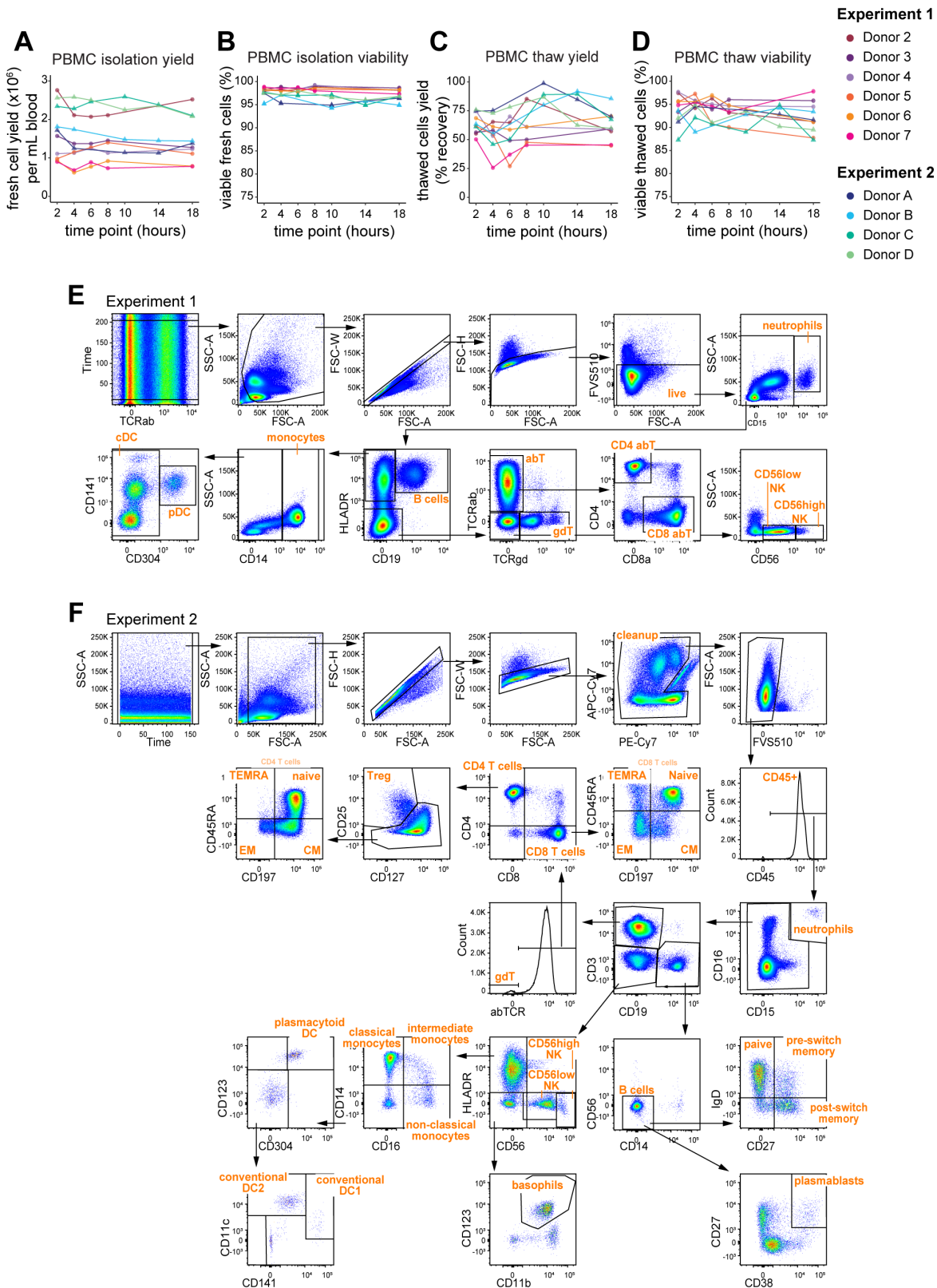
Supplemental Figure S1, Related to Figure 1 – PBMC yield and viability, flow cytometry gating

(A-B) PBMC were isolated from whole blood by ficoll separation at the indicated time points after blood draw and assessed for yield (A) and viability (B).

(C-D) 5×10^6 PBMC isolated as in panels A-B and frozen in liquid nitrogen were thawed and assessed for yield (C) and viability (D) prior to downstream assays. "% recovery" was calculated as the recovery of live PBMC divided by 5×10^6.

(E-F) Flow cytometry gating schemes for Experiment 1 (E) and Experiment 2 (F). Orange labels indicate gates used to determine population frequencies. The gate labeled "cleanup" in Experiment 2 was used to remove dye aggregates.
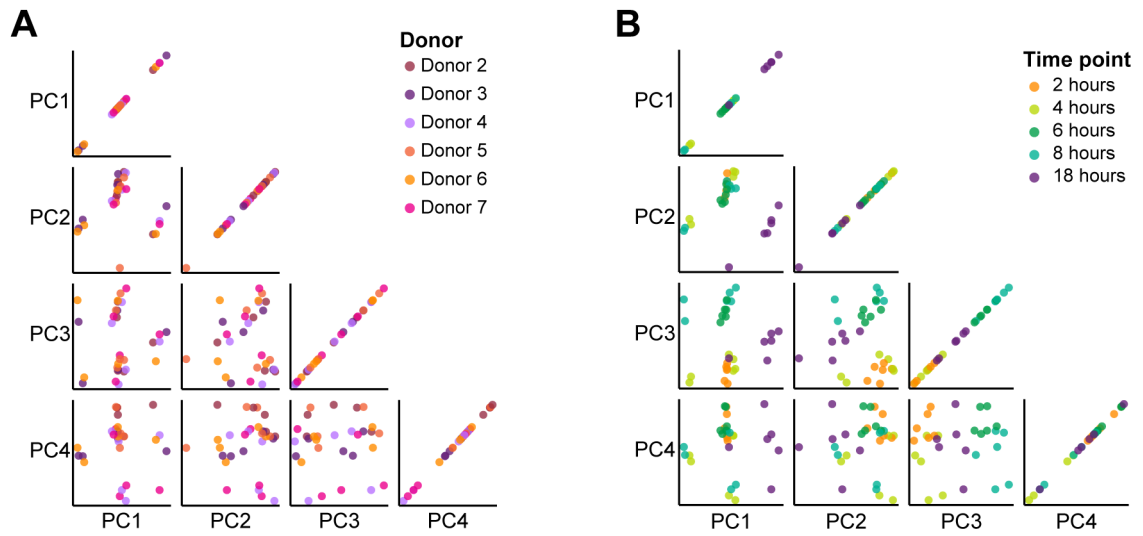
Supplemental Figure S2

Supplemental Figure S2, Related to Figure 1 – Flow cytometry frequencies

Flow cytometry data were gated by traditional methods for major PBMC populations, as in Supplemental Figure 1. Because of technical artifacts, the 6-hour time points of donors A and B were excluded from analysis.

(A-B) The percent change in frequency was calculated for each population relative to the 2-hour PBMC processing time point and population medians were calculated. Data are displayed as a heat map for Experiment 1 (A) or Experiment 2 (B).

(C-D) Population frequencies are shown per donor as a percent of live cells in Experiment 1 (C) or percent of CD45+ cells in Experiment 2 (D).
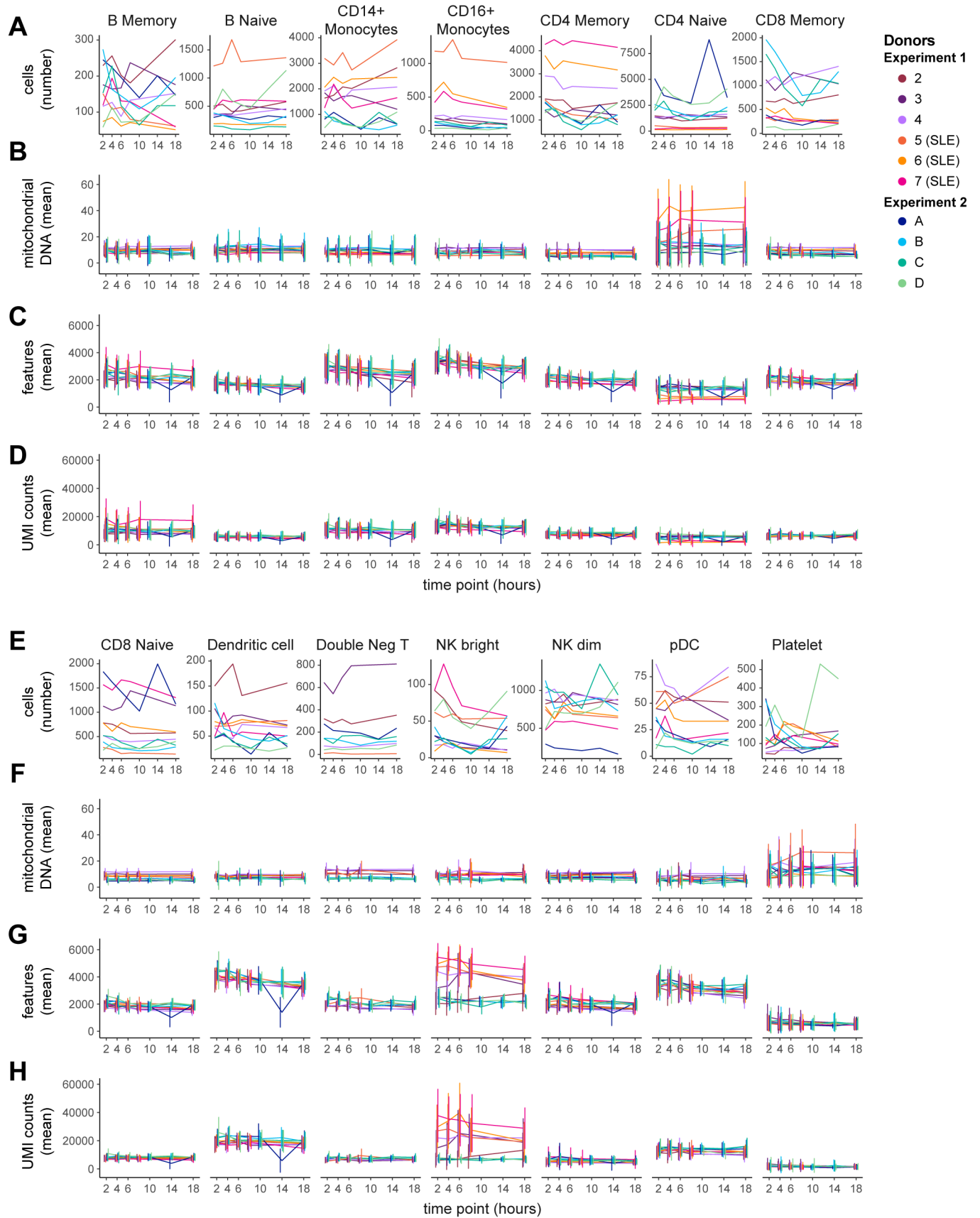
Supplemental Figure S3

Supplemental Figure S3, Related to Figure 2 – Nanostring bulk transcriptomics principle components analysis

PBMC in Experiment 1 were prepared from whole blood at various times after blood draw and assayed by targeted transcriptomics in bulk using Nanostring nCounter. The normalized counts were analyzed by principle components analysis and colored by donor (A) or by time point (B).
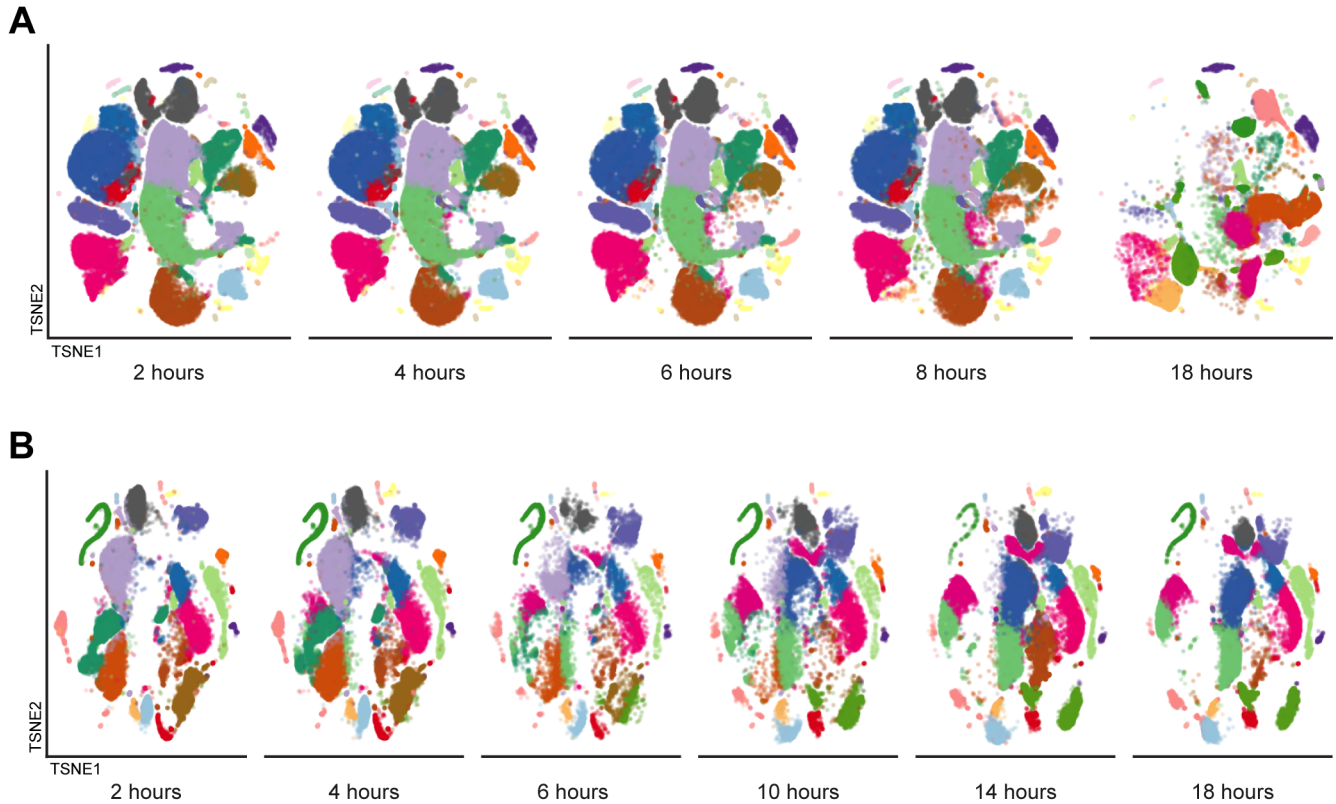
Supplemental Figure S4

Supplemental Figure S4, Related to Figure 4 – Single-cell RNA sequencing, technical metrics

Single-cell RNA-sequencing technical metrics were compiled for Experiment 1 (A-D) and Experiment 2 (E-H) and categorized by cell type, as assigned by Seurat-based reference alignment (see STAR Methods for details).

The number of quality singlets (A, E), mean mitochondrial DNA (B, F), features (C, G), and UMI (D, H) were quantified per cell type and colored by donor.

Supplemental Figure S5

A

B

Supplemental Figure S5, Related to Figure 4 – Single-cell RNA sequencing, tSNE individually by time

The single-cell RNA-sequencing normalized gene expression matrices from each sample were identified for donor and time point and aggregated per experiment. The multidimensional data was displayed in two dimensions using tSNE, split out by time post-blood draw for Experiment 1 (A) and Experiment 2 (B), and colored by Louvain cluster. Additional details can be found in the STAR Methods.

Supplemental Figure S6

A Differentially expressed genes - Experiment 1 Heatlhy Donors
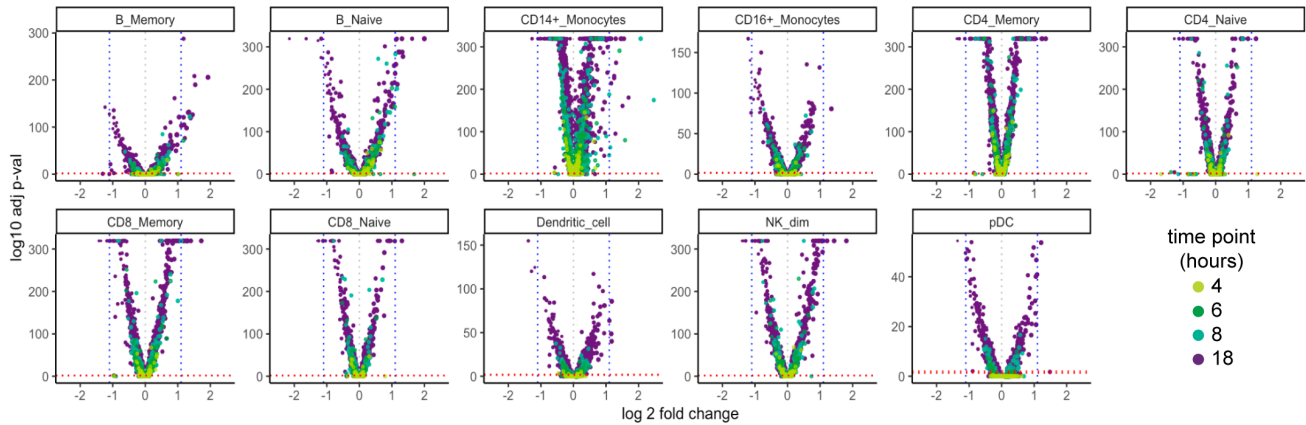
B Differentially expressed genes - Experiment 1 SLE Donors

C Differentially expressed genes - Experiment 2 Healthy Donors

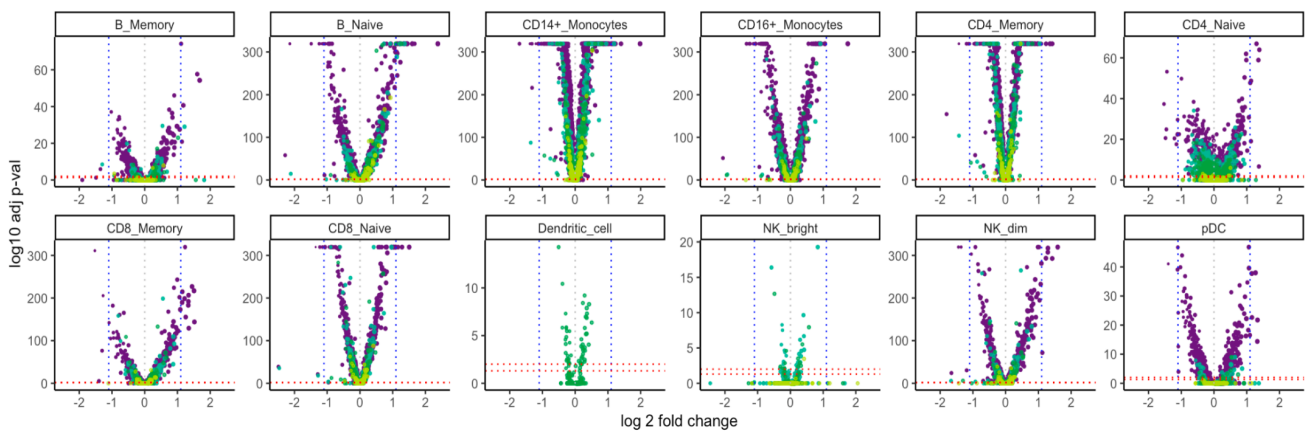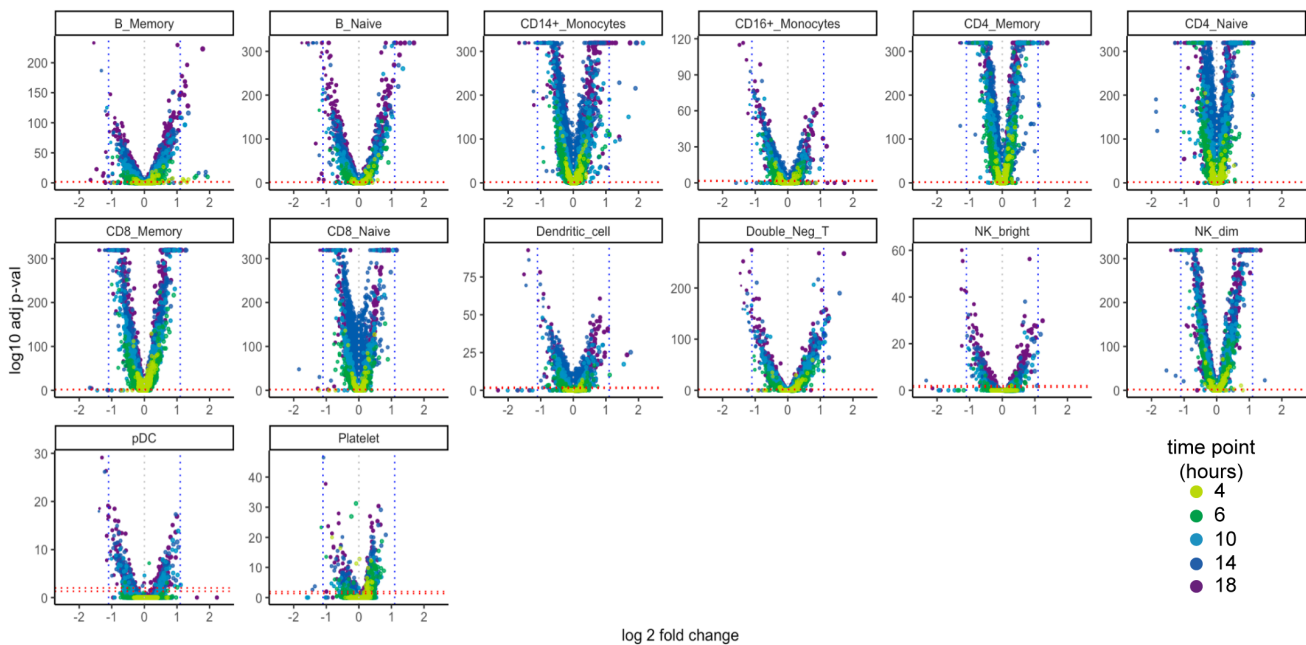**Supplemental Figure S6, Related to Figure 5 – Single-cell RNA sequencing, differentially expressed genes**

Differential expression in genes at each time point relative to 2 hours post-draw are represented as log2 fold change (x-axis) vs. log10 adjusted p-value (y-axis) (see the STAR Methods for details). Blue dashed lines indicate log2 fold change of |1.1| and red dashed lines indicate adjusted p-values of 0.05 and 0.01. For visualization purposes, adjusted p-values at zero are set to the non-zero minimum of all other adjusted p-values and divided by two. Cell types not represented did not have differentially expressed genes (after p-value adjustment).

(A-C) Differential expression in healthy (A) and SLE (B) donors of Experiment 1 and healthy donors of Experiment 2 (C). The legend in panel (A) also applies to panel (B).

Supplemental Figure S7
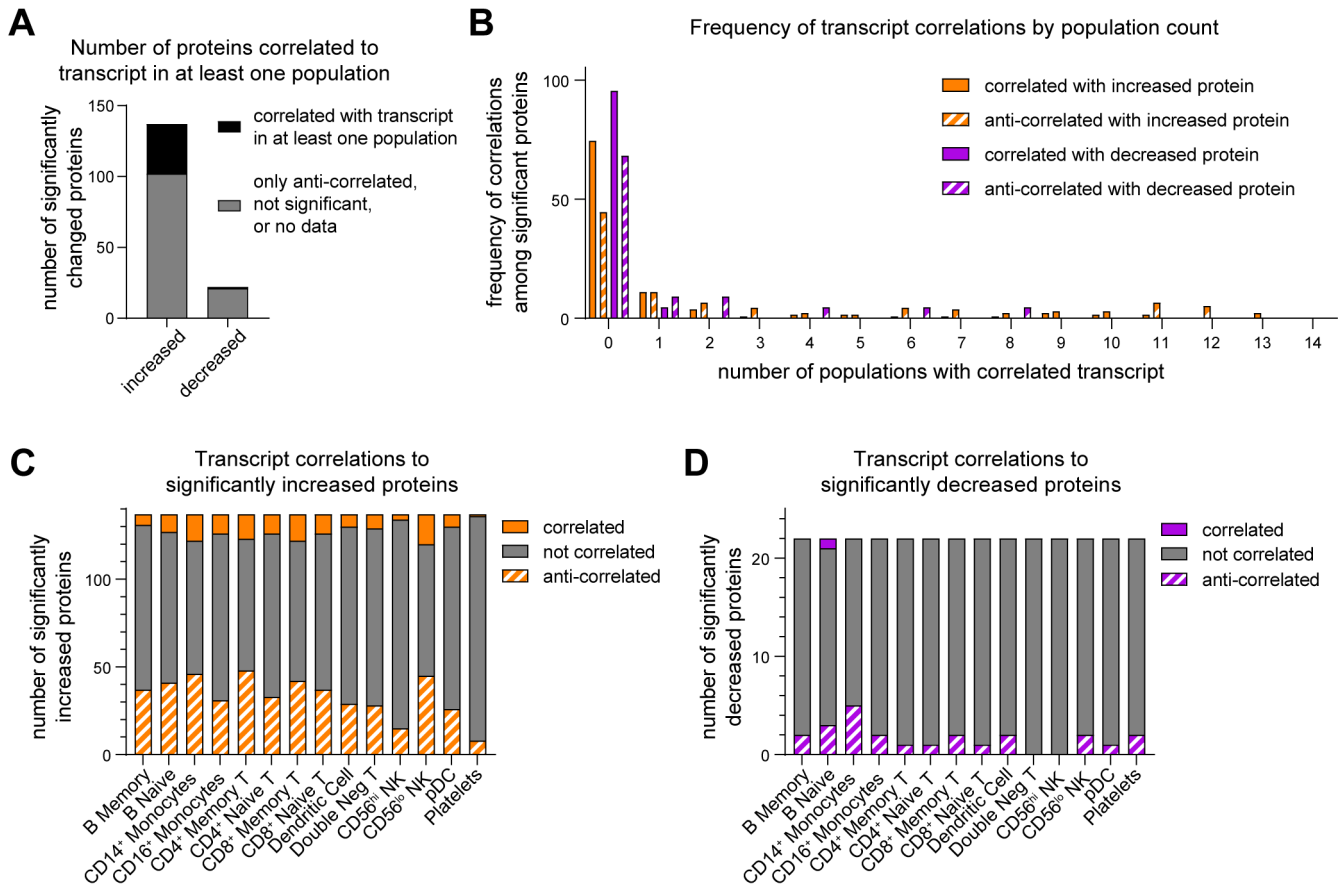
Supplemental Figure S7, Related to Figure 7 – Protein-transcript correlations

Proteins identified in Figure 3 were filtered for significance and directionality across the entire time course and the corresponding transcripts were similarly assessed by cell type.

(A) Proteins of increasing and decreasing abundance were scored for correlation (black) with the corresponding transcript in a least one cell type. Protein-transcript pairs that were not correlated (gray) could be anti-correlated, not significant, or have missing transcript data (for example, from drop-outs in the single-cell RNA-sequencing data).

(B) To assess whether protein-transcript correlations were common to many cell types or typically restricted to a limited number of cell types, increasing (orange) and decreasing (purple) proteins were scored for correlation (solid color) or anti-correlation (hashed color) with their corresponding transcript in each cell type and the count of each correlation was tallied across the cell types. Because proteins are scored if they are correlated or anti-correlated in at least one of fourteen populations, a single protein could be scored as both.

(C-D) To assess whether specific cell types had a propensity or bias in their correlations, correlated (solid color), anti-correlated (hashed color), and not correlated proteins (gray) were tallied by cell type for proteins increased (C, orange) and decreased (D, purple) over the time course.

Supplemental Figure S8
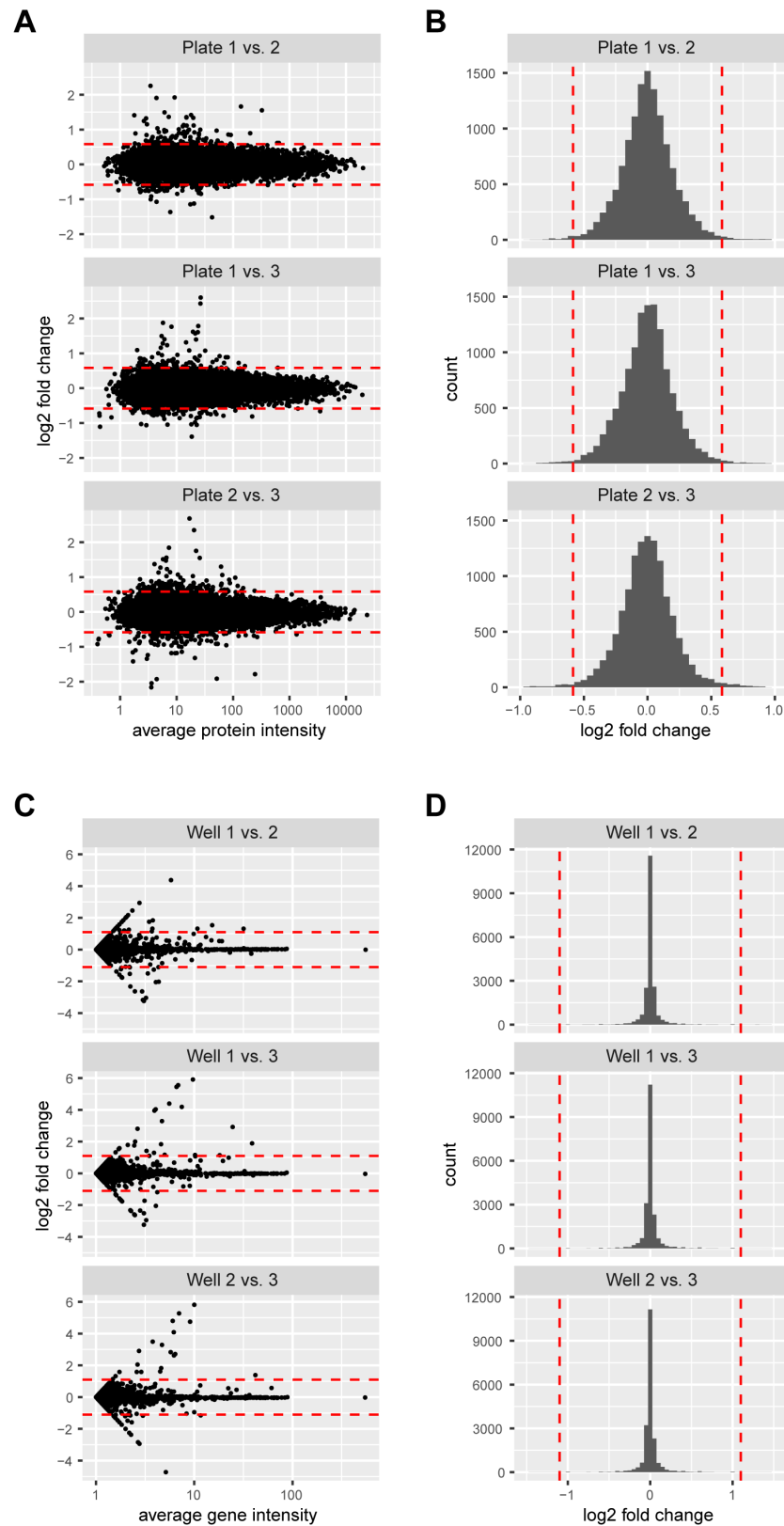
Supplemental Figure S8, Related to Figure 4 – Analysis of technical variance in proteomics and single-cell RNA-sequencing assays

(A-B) To assess the technical variance of the plasma protein assay, plasma samples from six donors from both 2 hours and 6 hours post-blood draw were compared across three different assay plates. The data are shown as MA plots (A) and the distributions of fold changes (B) in plate-to-plate comparisons. The red dash lines indicate the log2 fold change cutoff (0.585) used in the study.

(C-D) To assess the technical variance of the single-cell RNA-sequencing assay, replicate aliquots of PBMC from a non-study sample were assayed in three different wells (see STAR Methods/Single Cell Transcriptomics). Average transcript intensities were calculated from cells with non-zero counts and fold changes between replicates were calculated. The data are shown as MA plots (A) and the distributions of fold changes (B) in well-to-well comparisons. The red dash lines indicate the log2 fold change cutoff (1.1) used in the study.

Supplemental Table S7, Related to Figure 1 - Donor information, Experiment 1

Experiment 1

	Sample ID Donor Type	2 Healthy	3 Healthy	4 Healthy	5 SLE	6 SLE	7 SLE
Demo-graphics	Age	25	25	27	30	25	33
	Sex	male	male	male	female	female	female
	Race (self-reported)	White, Caucasian	White, Caucasian	White, Caucasian	White, Caucasian	White, Caucasian, Native Hawaiian, Other Pacific Islander	White, Caucasian
	Hispanic/Latino Ethnicity (self-reported)	no	no	no	no	no	no
CBC RESULTS	Absolute Basophils (cells/uL)	47	50	50	22	31	11
	Absolute Eosinophils (cells/uL)	265	99	140	81	220	69
	Absolute Lymphocytes (cells/uL)	3050	1981	1751	988	1488	1341
	Absolute Monocytes (cells/uL)	577	341	351	510	604	286
	Absolute Neutrophils (cells/uL)	3861	4629	2210	1099	3758	3593
	Hematocrit (%)	42.3	43.1	41.1	29.5	41	37
	Hemoglobin (g/dL)	14.6	14.8	14.6	9.7	14.1	12.2
	Mch (pg)	31.7	29.1	31.7	28.2	31.8	29.3
	Mchc (g/dL)	34.5	34.3	35.5	32.9	34.4	33
	Mcv (fL)	92	84.7	89.3	85.8	92.6	88.9
	Percent Basophils	0.6	0.7	1.1	0.8	0.5	0.2
	Percent Eosinophils	3.4	1.4	3.1	3	3.6	1.3
	Percent Lymphocytes	39.1	27.9	38.9	36.6	24.4	25.3
	Percent Monocytes	7.4	4.8	7.8	18.9	9.9	5.4
	Platelet Count (thousand/uL)	250	316	223	269	279	245
	Rdw Rbc Distribution Width (%)	12.2	12.7	12.3	13	11.5	11.7
	Red Blood Cell Count (millions/uL)	4.6	5.09	4.6	3.44	4.43	4.16
	White Blood Cell Count (thousand/uL)	7.8	7.1	4.5	2.7	6.1	5.3
	Smoking History	[no data]	[no data]	[no data]	Never Smoker	Former Smoker	Never Smoker
	Duration of SLE (years)	[not applicable]	[not applicable]	[not applicable]	8.3	5.8	4
	Notes at time of draw	[none]	[none]	[none]	Stable lupus	Active symptoms - pleurisy, joint stiffness (hip, lower extremities), rash, fatigue	Not flaring -doing well
Treatment #1	Medication #1 Name	[not applicable]	[not applicable]	[not applicable]	Hydroxy-chloroquine	Hydroxy-chloroquine	Hydroxy-chloroquine
	Dosage	[not applicable]	[not applicable]	[not applicable]	200 mg	200 mg	200 mg
	Frequency	[not applicable]	[not applicable]	[not applicable]	2x a day	2x a day	2x a day
	Duration of treatment	[not applicable]	[not applicable]	[not applicable]	8 years	5.65 years	3.87 years
Treatment #2	Medication #2 Name	[not applicable]	[not applicable]	[not applicable]	[none]	Methotrexate	[none]
	Dosage	[not applicable]	[not applicable]	[not applicable]	[none]	7.5 mg	[none]
	Frequency	[not applicable]	[not applicable]	[not applicable]	[none]	1x a week	[none]
	Duration of treatment	[not applicable]	[not applicable]	[not applicable]	[none]	7 months	[none]

Supplemental Table S8, Related to Figure 1 - Donor information, Experiment 2

Experiment 2

Sample ID Donor Type	A Health	B Health	C Health	D Health
Age	33	51	26	51
Sex	female	male	male	female
Race (self-reported)	[no answer]	[no answer]	[no answer]	White
Hispanic/Latino Ethnicity (self-reported)	Caucasian	Caucasian	White	Non-hispanic
Smoking status	no	no	no	no
LYM (%)	28.2	33.1	36.3	33.4
LYM (10^3/mm3)	1.6	1.9	2.3	2.6
MON (%)	6	4.8	5.3	4.6
MON (10^3/mm3)	0.3	0.2	0.3	0.3
GRA (%)	65.8	62.1	58.4	62
GRA (10^3/mm3)	3.9	3.8	3.9	5.1
WBC (10^3/mm3)	5.8	5.9	6.5	8
RBC (10^6/mm3)	3.9	4.9	4.5	4.8
HGB (g/dL)	12.6	14.8	14.7	13.2
HCT (%)	37.8	44.4	43.1	41.4
PLT (10^3/mm3)	287	232	237	351
MCV (um3)	96	91	95	86
MCH (pg)	31.9	30.3	32.5	27.3
MCHC (g/dL)	33.2	33.4	34.2	32
RDW (%)	13.5	13.5	13	16
MPV (um3)	7	7.8	7.7	6.7