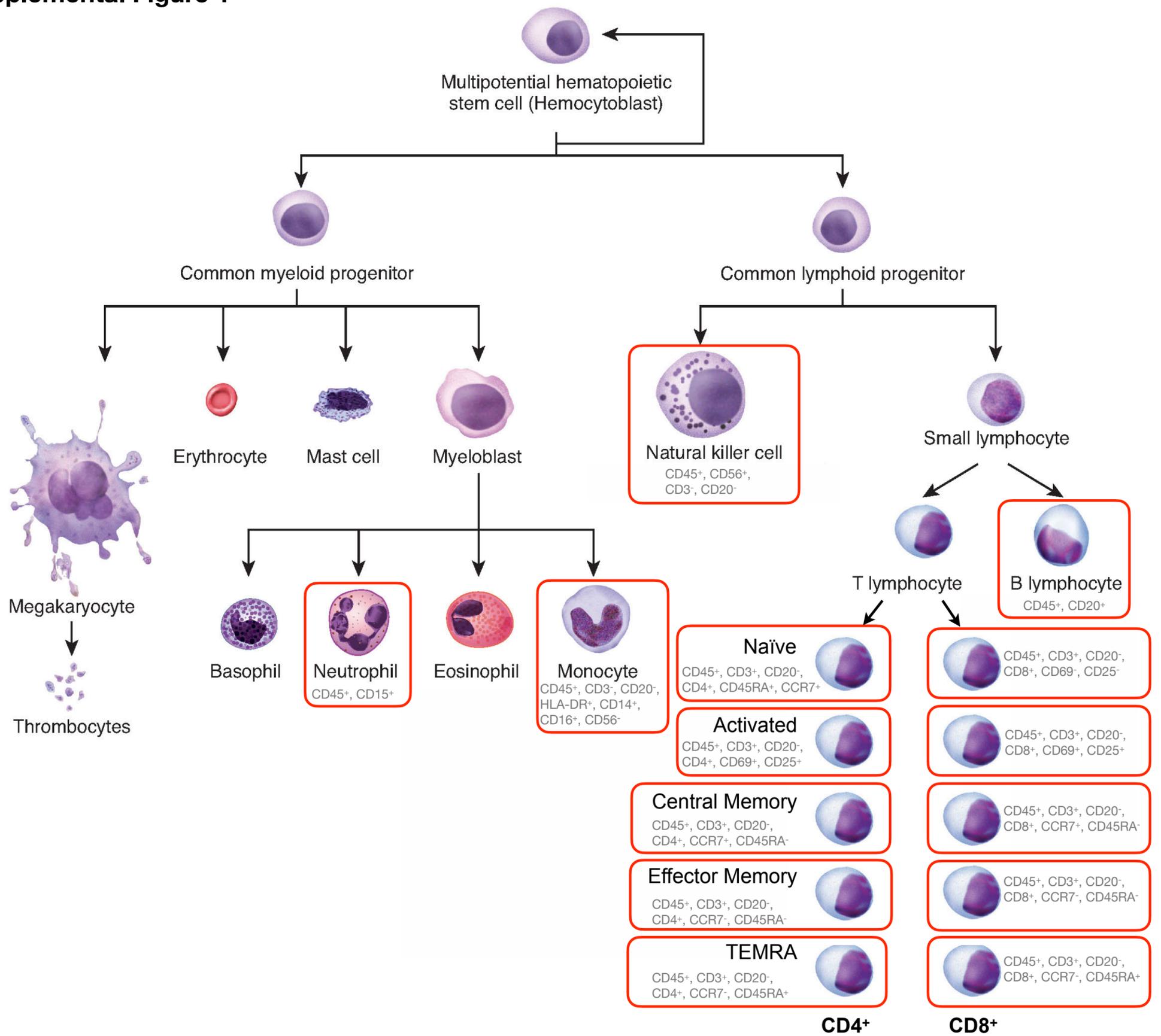


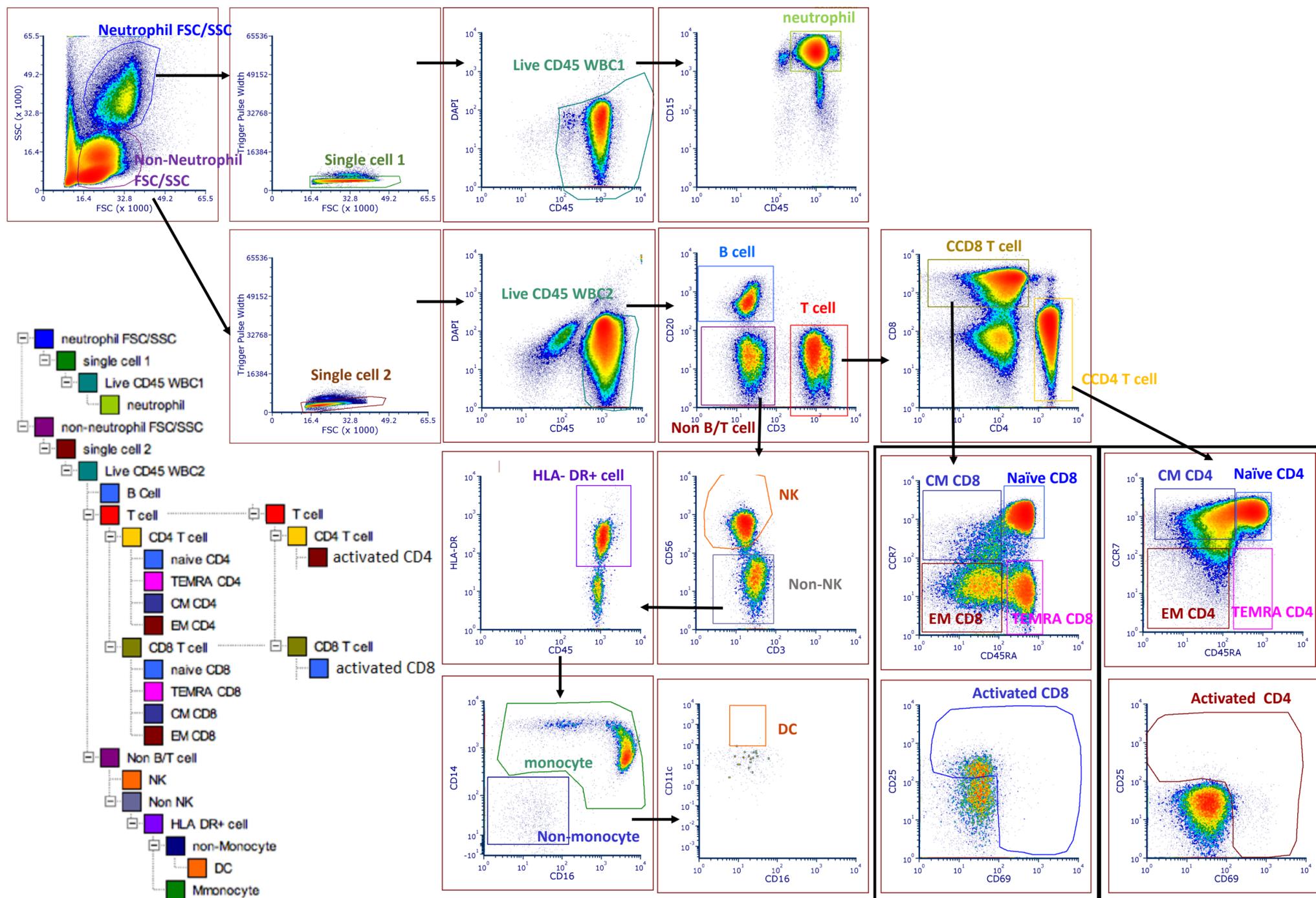
# Supplemental Figure 1



**Supplemental Figure 1 – Diagram of leukocyte cell lineages.**

Cells analyzed in this study are circled in red. The cell surface markers used to define sub-populations are indicated below each cell subtype. Diagram adapted from: [https://oerpub.github.io/epubjs-demo-book/content/m46036.xhtml#fig-ch03\\_06\\_01](https://oerpub.github.io/epubjs-demo-book/content/m46036.xhtml#fig-ch03_06_01).

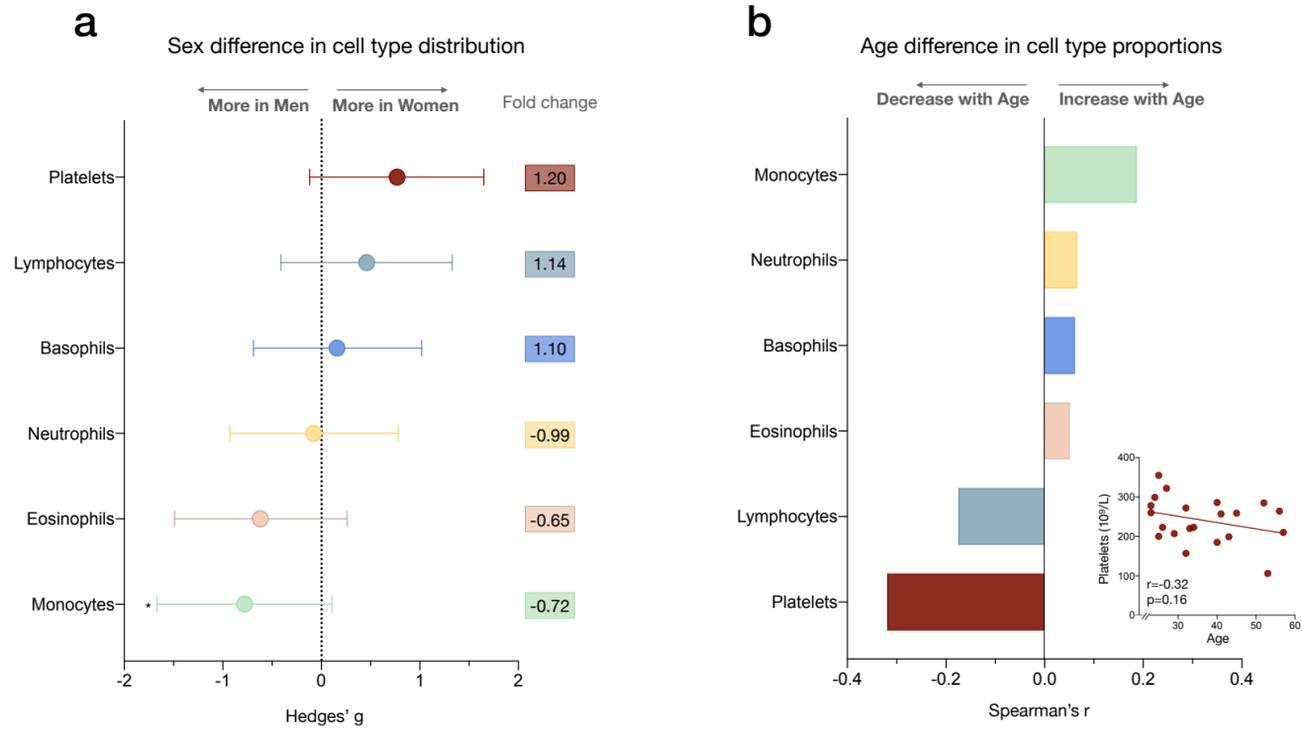
## Supplemental Figure 2



### Supplemental Figure 2 – Gating strategy to quantify all cell subtypes and sorting major cell subtypes for mitochondrial phenotyping.

See the methods section for details of labeling cocktails and cell sorter parameters. An initial run of 2M cells was used to establish the six most abundant cell subtypes (targets) for each participant, followed by FACS to obtain at least one 5M aliquot of each target cell subtype. Up to five 5M aliquots were collected per cell subtype, per participant, to establish technical variability in downstream assays.

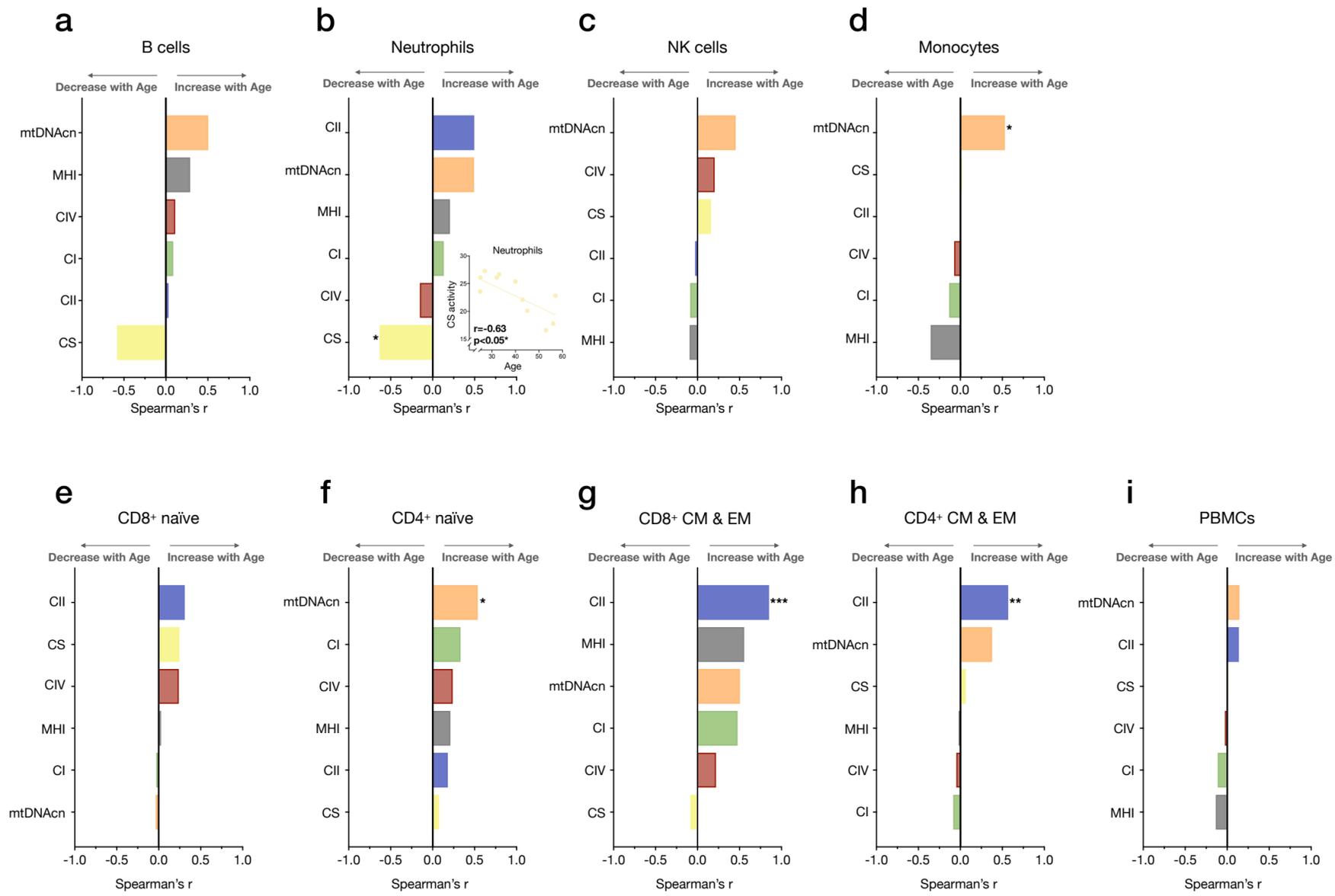
## Supplemental Figure 3



### Supplemental Figure 3 – Sex differences and age correlations with leukocyte abundance measured by complete blood count.

(a) Forest plot illustrating the effect size (g) of the sex differences in cell proportion derived from the complete blood count (CBC) results (n=21). Fold changes in the raw values are also shown. P-values from non-parametric Mann-Whitney T test. Error bars reflect the 95% C.I. on the effect size. (b) Correlation (Spearman's r) between age and cell proportion derived from the complete blood count. n=21, p<0.05\*.

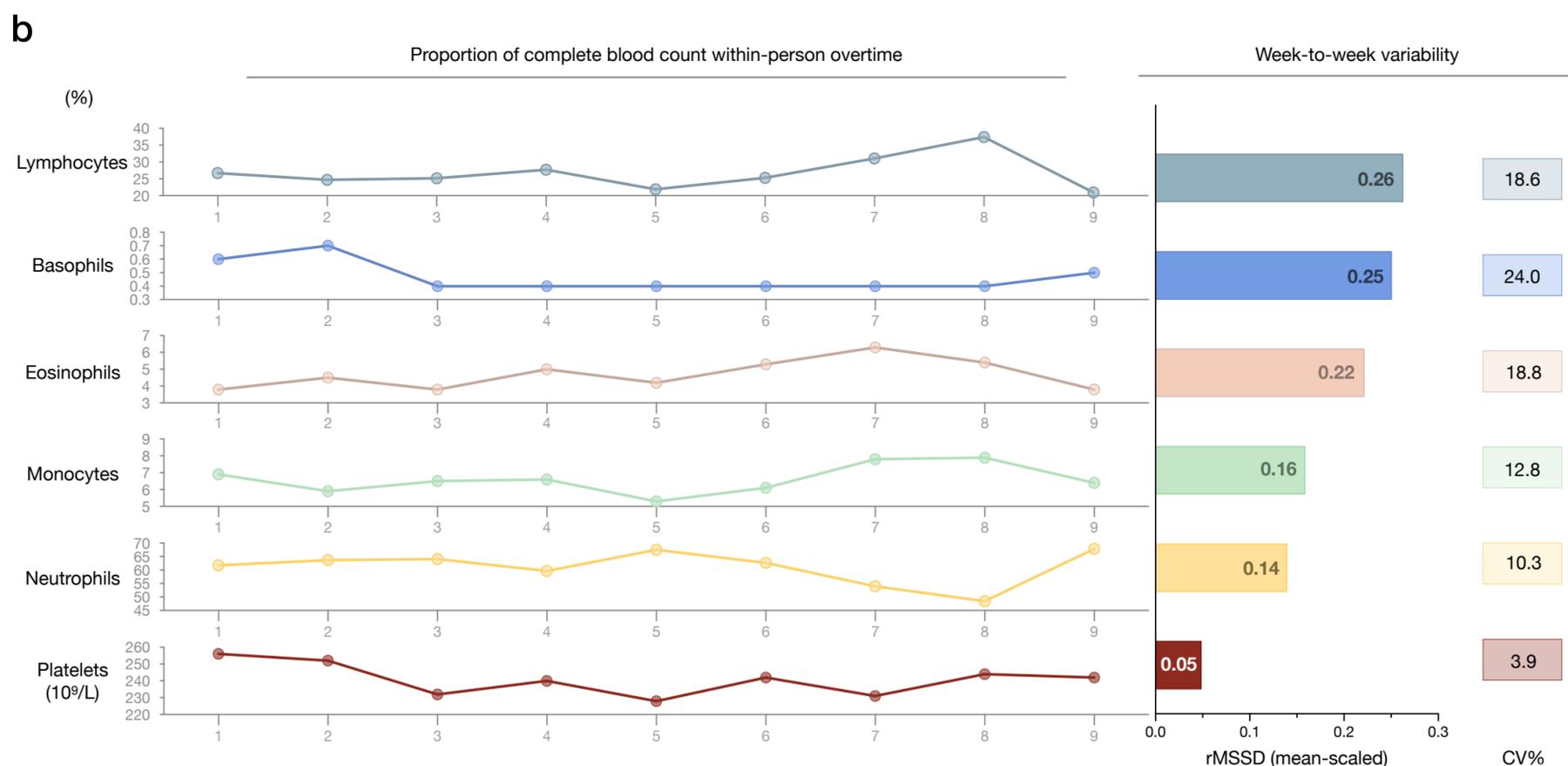
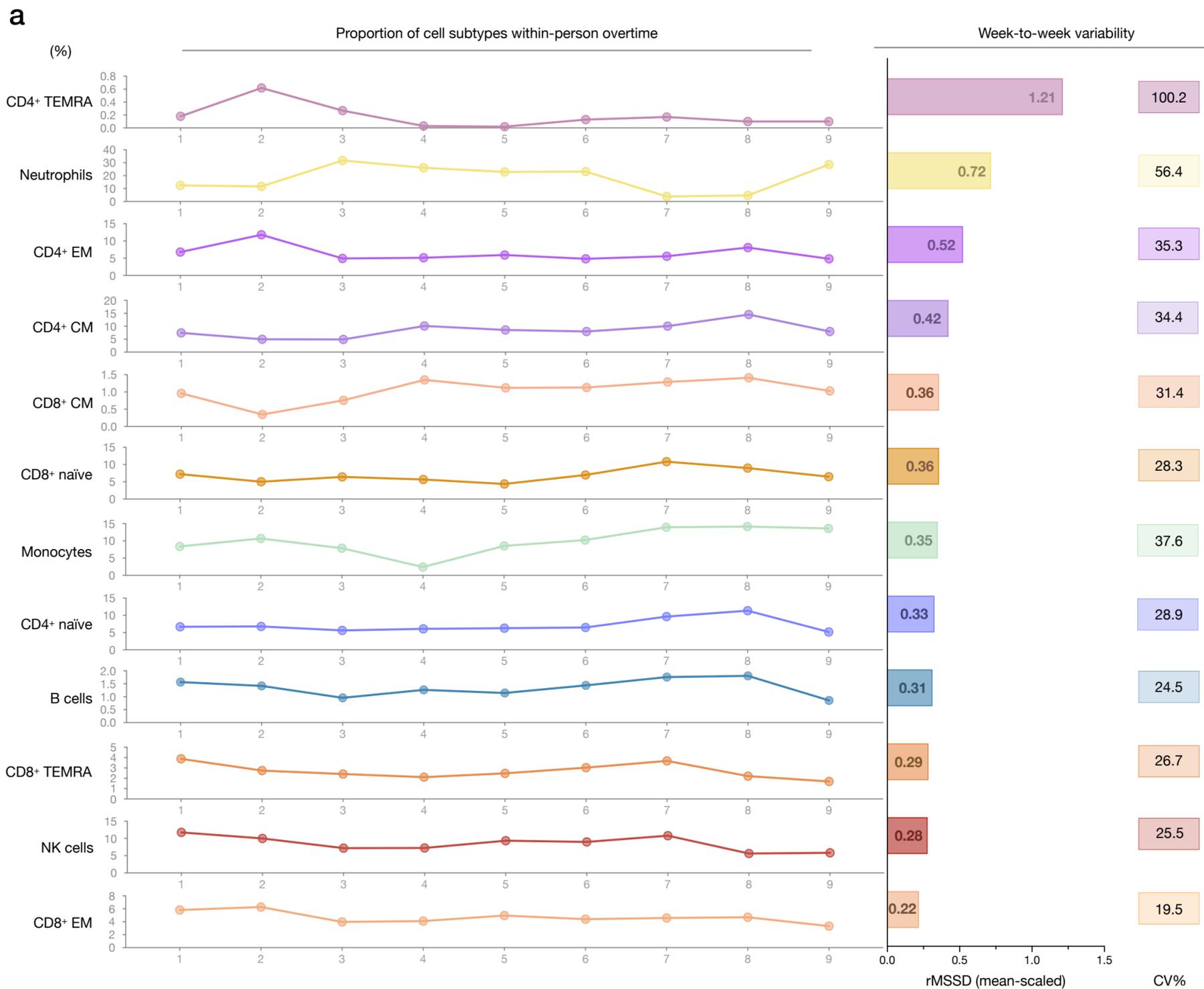
# Supplemental Figure 4



**Supplemental Figure 4 – Association between age and cell subtype mitochondrial features.**

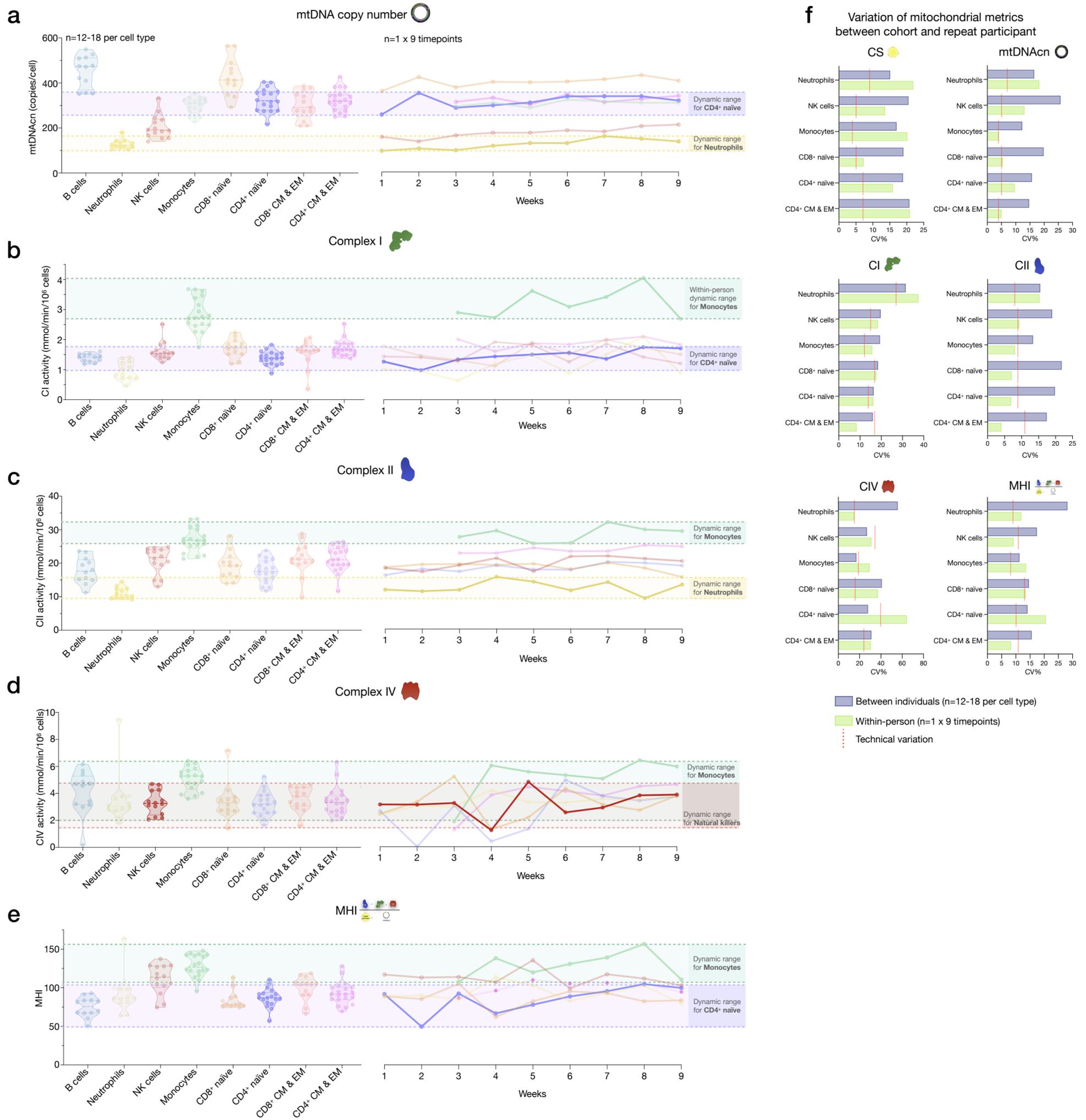
(a-i) Correlations between mitochondria features and age. This figure presents the same data as main Figure 4, but organized by cell subtypes rather than mitochondrial feature to facilitate the visualization of subtype-level effects. Spearman's r,  $p < 0.05^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001^{***}$ .

# Supplemental Figure 5



**Supplemental Figure 5 – Within-person variability of cell subtype proportions overtime.** (a) Within-person variation of cell type proportions across 9 weeks. The FACS-derived raw cell proportions (% of total cells) are shown on the left. Root mean square of successive differences (rMSSD) illustrating the magnitude of variability between successive weeks, and C.V. illustrating the magnitude of variability across the total 9 weeks are provided on the right for each cell subtype, by mitochondrial feature. (b) Same as a, but based on CBC results.

# Supplemental Figure 6



**Supplemental Figure 6 – Variability of mitochondrial features across cell subtypes between the cohort and the repeat participant.**

(a-e) Side-by-side comparison of mitochondrial features between the cohort (n=12-18 per cell type) and the repeat participant (n=1 x 9 timepoints) across cell subtypes. This figure shows the same data as in main Figure 5h, but for all mitochondrial features. (f) Summary of a-e illustrated by a bar graph showing observed variation (C.V.) of mitochondrial features between the cohort and the repeat participant. The technical variation established on a subset of samples and likely represents a conservative overestimation of noise is shown by red lines.

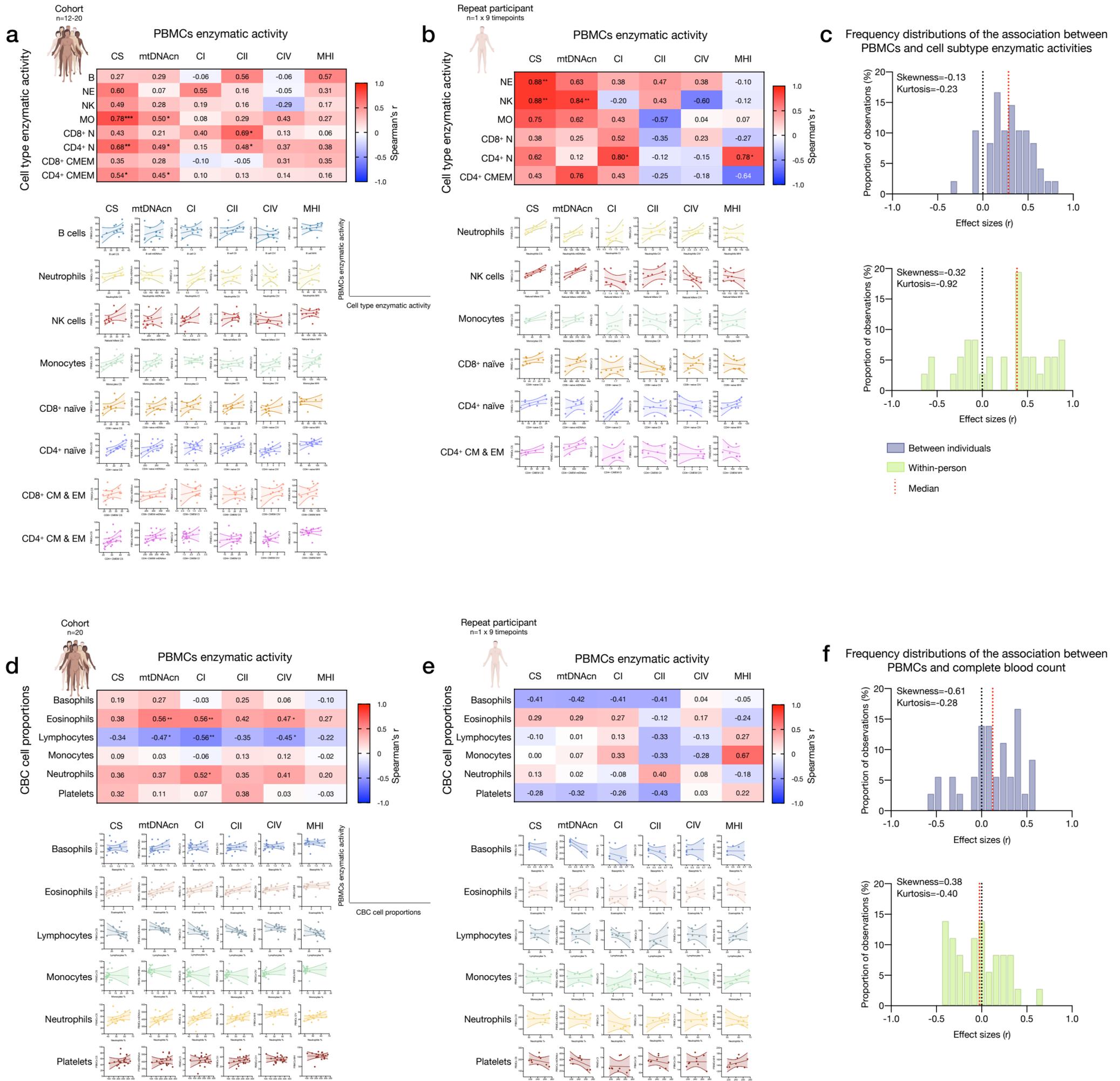
## Supplemental Figure 7

Mitotype	Interpretation	Index
Complex I activity (CI)/CS Complex II activity (CII)/CS Complex IV activity (CIV)/CS	RC enzymatic activity per unit of mito content	RC enzyme activity per CS
mtDNAcn/CS	Mito genome density	
CI/CII CI/CIV CIV/CII	RC enzymatic activity ratios	RC enzyme ratios
CI/mtDNAcn CII/mtDNAcn CIV/mtDNAcn	RC enzymatic activity per unit of genome	RC enzyme per mtDNA
CI / (mtDNAcn/CS) CII / (mtDNAcn/CS) CIV / (mtDNAcn/CS)	RC enzymatic activity per unit of mtDNA density	RC enzyme per mtDNA density
(CI/mtDNAcn) / (mtDNAcn/CS) (CII/mtDNAcn) / (mtDNAcn/CS) (CIV/mtDNAcn) / (mtDNAcn/CS)	RC enzymatic activity per genome in relation to mtDNA density	RC enzyme per mtDNA rel. to mtDNA density

### Supplemental Figure 7 – Operationalization and categorization of mitotypes.

Chart illustrating mitotype ratios and their simple interpretations.

# Supplemental Figure 8



## Supplemental Figure 8 – Associations between subtype-specific enzymatic activities and CBC cell proportions with mitochondrial features measured in PBMCs.

(a) Correlations of the mitochondrial features measured in each cell subtype and the same mitochondrial feature measured in PBMCs for the cohort and (b) repeat participant. Heatmaps for the cohort (n=12-20) and the repeat participant (n=1 x 9 timepoints) show to what extent PBMCs-based measures reflect activities in various immunologically-defined cell subtypes. This data integrates data presented in Figures 3 and 5, here focused on PBMCs. (c) Frequency distributions of effect sizes for association between PBMC and cell subtype mitochondrial features for the cohort and the repeat participant (total correlation pairs=36, for both), showing a predominance of positive correlations. (d) Correlations between CBC-based cell type abundance (% of total leukocytes) and PBMCs mitochondrial features for the cohort (n=20) the (e) repeat participant (n=1 x 9 timepoints). (f) Frequency distribution of effect sizes.

## Supplemental Table 1

Cell Type	Biological function	Markers
<b>B cells</b>	Present antigens as well as provide co-stimulation and cytokines to T cells, in turn producing antigen specific antibodies (Hoffman, Lakkis et al. 2016).	CD45 <sup>+</sup> , CD20 <sup>+</sup>
<b>Neutrophils</b>	Recruited to sites of infection, having the ability to recognize and phagocytose microbes, and then kill pathogens through a combination of cytotoxic mechanisms (Mayadas, Cullere et al. 2014).	CD45 <sup>+</sup> , CD15 <sup>+</sup>
<b>NK cells</b> Natural killer cells	Effector lymphocytes in the innate immune response with cytotoxicity and cytokine producing functions (Vivier, Tomasello et al. 2008).	CD45 <sup>+</sup> , CD56 <sup>+</sup> , CD3 <sup>-</sup> , CD20 <sup>-</sup>
<b>Monocytes</b>	In response to infection or injury, monocytes can phagocytose and present antigens, secrete chemokine and proliferate. Once monocytes are recruited to tissues they are capable of differentiating into macrophages and dendritic cells (Chiu and Bharat 2016).	CD45 <sup>+</sup> , CD3 <sup>-</sup> , CD20 <sup>-</sup> , HLA-DR <sup>+</sup> , CD14 <sup>+</sup> , CD16 <sup>+</sup> , CD56 <sup>-</sup>
<b>CD4<sup>+</sup> naïve T cells</b>	Mature naïve CD4 <sup>+</sup> T cells are deployed to secondary lymphoid organs where they continuously survey for pMHC II molecules, for antigen recognition (Luckheeram, Zhou et al. 2012)	CD45 <sup>+</sup> , CD3 <sup>+</sup> , CD20 <sup>-</sup> , CD4 <sup>+</sup> , CD45RA <sup>+</sup> , CCR7 <sup>+</sup> ,
<b>CD4<sup>+</sup> activated T cells</b>	Mediate immune response through secretion of specific cytokines, activate other immune cells, and play a critical role in the suppression of immune reaction. Often referred to as helper T cells (Luckheeram, Zhou et al. 2012).	CD45 <sup>+</sup> , CD3 <sup>+</sup> , CD20 <sup>-</sup> , CD4 <sup>+</sup> , CD69 <sup>+</sup> , CD25 <sup>+</sup>
<b>CD4<sup>+</sup> CM</b> CD4 <sup>+</sup> central memory T cells	Express the chemokine receptor CCR7, allowing them to circulate lymphoid organs and have a strong proliferative capacity but little effector functions (Stubbe, Vanderheyde et al. 2006, Gasper, Tejera et al. 2014).	CD45 <sup>+</sup> , CD3 <sup>+</sup> , CD20 <sup>-</sup> , CD4 <sup>+</sup> , CCR7 <sup>+</sup> , CD45RA <sup>-</sup>
<b>CD4<sup>+</sup> EM</b> CD4 <sup>+</sup> effector memory T cells	Do not express CCR7 and thus reside in the peripheral tissues. CD4 EM cells produce effector cytokines and have limited proliferative capacity (Stubbe, Vanderheyde et al. 2006, Gasper, Tejera et al. 2014).	CD45 <sup>+</sup> , CD3 <sup>+</sup> , CD20 <sup>-</sup> , CD4 <sup>+</sup> , CCR7 <sup>-</sup> , CD45RA <sup>-</sup>
<b>CD4<sup>+</sup> TEMRA</b> CD4 <sup>+</sup> terminally differentiated effector memory cells re-expressing CD45RA	Exhibit enhanced expression of effector molecules, providing protective immunity against reoccurring infections (Tian, Babor et al. 2017).	CD45 <sup>+</sup> , CD3 <sup>+</sup> , CD20 <sup>-</sup> , CD4 <sup>+</sup> , CCR7 <sup>-</sup> , CD45RA <sup>+</sup>
<b>CD8<sup>+</sup> naïve T cells</b>	Mature naïve CD8 <sup>+</sup> T cells are deployed to secondary lymphoid organs where they continuously survey for pMHC I molecules (found on all nucleated cells) for antigen recognition (Zhang and Bevan 2011).	CD45 <sup>+</sup> , CD3 <sup>+</sup> , CD20 <sup>-</sup> , CD8 <sup>+</sup> , CD69 <sup>-</sup> , CD25 <sup>-</sup>
<b>CD8<sup>+</sup> activated T cells</b>	Recognize antigens and kill infected or malignant cells by secreting cytokines and cytotoxic granules. Often referred to as cytotoxic T cells (Mahnke, Brodie et al. 2013).	CD45 <sup>+</sup> , CD3 <sup>+</sup> , CD20 <sup>-</sup> , CD8 <sup>+</sup> , CD69 <sup>+</sup> , CD25 <sup>+</sup>
<b>CD8<sup>+</sup> CM</b> CD8 <sup>+</sup> central memory T cells	Specialized response to systematic infections due to their centralized location in secondary lymphoid organs and superior proliferative abilities (Martin and Badovinac 2018).	CD45 <sup>+</sup> , CD3 <sup>+</sup> , CD20 <sup>-</sup> , CD8 <sup>+</sup> , CCR7 <sup>+</sup> , CD45RA <sup>-</sup>
<b>CD8<sup>+</sup> EM</b> CD8 <sup>+</sup> effector memory T cells	Specialized response to infections in peripheral tissues due to their cytotoxicity and ability to localize to tissues (Martin and Badovinac 2018).	CD45 <sup>+</sup> , CD3 <sup>+</sup> , CD20 <sup>-</sup> , CD8 <sup>+</sup> , CCR7 <sup>-</sup> , CD45RA <sup>-</sup>
<b>CD8<sup>+</sup> TEMRA</b> CD8 <sup>+</sup> terminally differentiated effector memory cells re-expressing CD45RA	Exhibit enhanced expression of effector molecules, providing protective immunity against reoccurring infections (Tian, Babor et al. 2017).	CD45 <sup>+</sup> , CD3 <sup>+</sup> , CD20 <sup>-</sup> , CD8 <sup>+</sup> , CCR7 <sup>-</sup> , CD45RA <sup>+</sup>

### Supplemental Table 1 – Leukocyte subtypes included in the study.

Immune cell subtypes included in this study, including a brief summary of their functions and cell surface markers used for immunolabeling and FACS.

#### Additional references to Supplemental Table 1:

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- Zhang, N. and M. J. Bevan (2011). "CD8(+) T cells: foot soldiers of the immune system." *Immunity* 35(2): 161-168.

## Supplemental Table 2

Subject ID	Age	Sex	Ethnicity	CBC-based cell proportions						FACS-based cell proportions												
				Basophils (%)	Eosinophils (%)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Platelets (10 <sup>9</sup> /L)	B cells (%)	Neutrophils (%)	NK cells (%)	Monocytes (%)	CD8 <sup>+</sup> naïve cells (%)	CD4 <sup>+</sup> naïve cells (%)	CD8 <sup>+</sup> CM cells (%)	CD8 <sup>+</sup> EM cells (%)	CD8 <sup>+</sup> TEMRA cells (%)	CD4 <sup>+</sup> CM cells (%)	CD4 <sup>+</sup> EM cells (%)	CD4 <sup>+</sup> TEMRA cells (%)	
<b>Females</b>																						
1	23	F	Asian	0.3	0.5	34.0	8.3	56.6	278	7.52	6.50	2.79	7.59	7.05	9.00	0.91	8.09	6.40	12.19	3.25	0.01	
2	23	F	Asian	1.2	1.3	42.4	7.8	47.1	260	7.45	21.24	7.45	10.54	7.38	12.57	1.08	6.04	0.60	5.65	3.63	0.14	
3	25	F	Asian	0.6	1.9	53.4	9.9	34	355	15.33	1.38	2.73	2.21	10.81	12.24	0.94	9.44	1.76	7.13	5.32	0.11	
4	27	F	White	0.5	1.4	38.6	9.1	50.2	322	6.93	7.31	3.98	10.27	9.34	19.66	1.88	4.86	0.75	15.34	2.37	0.04	
5	32	F	White	1.1	3.1	28.7	8.0	58.8	272	11.74	0.93	5.84	14.88	5.44	17.06	1.80	4.77	3.25	10.50	3.73	0.15	
6	32	F	White	0.4	5.4	32.2	7.0	54.6	157	5.36	10.47	12.31	7.41	5.75	17.83	1.15	3.95	0.62	9.23	3.68	0.05	
7	40	F	White	0.8	1.1	23.3	7.9	66.6	286	8.60	14.84	5.99	11.26	3.27	12.90	1.00	5.21	1.89	10.14	6.00	0.17	
8	41	F	White	0.5	0.9	40.6	8.1	49.4	257	3.59	1.91	7.01	0.88	5.97	3.22	1.18	3.43	1.01	31.03	3.64	0.01	
9	43	F	African American	0.8	0.4	46.8	6.5	45.1	199	<0.01	3.73	7.82	6.65	3.05	9.20	2.41	5.15	3.37	11.73	11.43	0.57	
10	52	F	White	1.4	3.7	34.7	8.7	51.3	285	7.99	5.31	5.73	16.30	6.65	25.83	1.43	3.22	0.74	8.37	3.14	0.07	
11	57	F	Asian	0.3	0.6	47.5	7.5	43.8	210	6.31	11.89	11.43	8.46	5.51	14.78	1.18	6.79	4.87	5.46	8.02	1.12	
<b>Males</b>																						
12	24	M	Asian	1.6	3.4	36.4	7.6	51	299	4.19	0.67	18.44	9.98	16.79	12.03	0.93	3.09	1.57	8.36	3.65	0.01	
13	25	M	White	0.4	1.1	42.6	9.7	45.4	200	5.80	7.24	5.68	10.24	5.30	8.99	2.44	11.18	6.95	11.79	5.33	0.20	
14	26	M	Asian	0.4	2.2	32.6	6.3	58.1	223	5.76	2.75	8.18	8.38	8.46	13.05	1.03	7.74	4.89	9.22	9.16	0.29	
15	29	M	African American	0.2	1.0	49.8	9.6	39.2	207	5.24	0.95	1.91	13.38	7.44	6.04	1.76	2.36	27.94	15.96	3.96	0.01	
16	33	M	White	0.5	4.7	22.1	11.8	60.5	220	9.79	10.78	13.48	22.82	4.27	3.19	0.71	1.76	0.62	13.80	3.33	0.01	
17	34	M	White	0.4	5.7	37.8	10.0	46.1	223	11.81	2.48	10.91	13.39	9.77	16.68	1.83	2.92	2.19	11.70	2.68	0.03	
18	40	M	White	0.6	2.9	36.0	11.7	48.4	185	5.44	1.15	12.45	13.39	7.44	13.53	1.42	4.43	1.19	14.34	3.43	0.04	
19	45	M	White	1.1	2.2	31.5	9.2	55.6	259	6.64	15.16	11.42	4.10	5.01	8.14	2.74	3.89	0.42	11.46	6.04	0.05	
20	53	M	White	0.4	3.4	10.7	26.2	58.9	106	<0.01	18.80	3.59	49.14	2.26	0.57	0.83	1.50	0.50	5.61	4.03	0.05	
21	56	M	Asian	0.9	1.8	37.1	10.0	49.8	264	3.75	6.94	16.24	12.05	1.30	2.49	3.22	5.82	6.44	10.43	7.83	0.08	
<b>Repeat Participant</b>																						
Repeat-01	34	M	White	0.6	3.8	26.7	6.9	61.8	256	1.57	12.53	11.76	8.38	6.69	7.24	0.96	5.83	3.88	7.46	6.78	0.18	
Repeat-02	34	M	White	0.7	4.5	24.7	5.9	63.7	252	1.42	11.71	10.01	10.70	6.78	5.02	0.35	6.28	2.75	4.98	11.81	0.62	
Repeat-03	34	M	White	0.4	3.8	25.2	6.5	64.1	232	0.96	31.83	7.21	7.90	5.62	6.47	0.76	3.97	2.41	4.89	4.96	0.27	
Repeat-04	34	M	White	0.4	5.0	27.7	6.6	59.7	240	1.27	26.09	7.23	2.44	6.12	5.70	1.35	4.10	2.12	10.12	5.15	0.03	
Repeat-05	34	M	White	0.4	4.2	21.9	5.3	67.5	228	1.15	22.93	9.38	8.55	6.29	4.37	1.12	4.95	2.49	8.60	5.92	0.02	
Repeat-06	34	M	White	0.4	5.3	25.3	6.1	62.7	242	1.44	23.19	9.03	10.28	6.50	7.02	1.13	4.39	3.03	7.97	4.85	0.13	
Repeat-07	34	M	White	0.4	6.3	31.1	7.8	54	231	1.76	3.97	10.81	13.95	9.66	10.84	1.29	4.60	3.68	10.05	5.61	0.17	
Repeat-08	34	M	White	0.4	5.4	37.4	7.9	48.5	244	1.81	4.76	5.65	14.18	11.36	9.02	1.41	4.69	2.22	14.53	8.09	0.10	
Repeat-09	34	M	White	0.5	3.8	21.0	6.4	67.9	242	0.86	28.71	5.82	13.62	5.21	6.52	1.03	3.34	1.69	7.98	4.86	0.10	

### Supplemental Table 2 – CBC- and FACS-based cell proportions for all study participants and time points.

Participants are ordered by age. CBC measurements were performed using a Sysmex XN-9000™ instrument, and FACS-based cell proportions were determined using a BD™ Influx cell sorter. (See methods for details).

## Supplemental Table 3

	Neutrophils	NK cells	Monocytes	CD8+ naïve	CD4+ naïve	CD8+ CM & EM	CD4+ CM & EM	PBMCs	Average
<b>Measured parameters</b>									
<b>Citrate synthase (CS)</b>	9.1%	5.5%	4.1%	4.6%	6.7%	6.6%	6.9%	7.1%	<b>6.2%</b>
<b>mtDNAcn</b>	6.6%	4.6%	4.2%	5.4%	4.9%	6.0%	4.0%	4.8%	<b>5.1%</b>
<b>Complex I</b>	27.3%	15.4%	12.1%	16.9%	14.2%	12.9%	17.2%	16.6%	<b>16.6%</b>
<b>Complex II</b>	7.6%	8.9%	9.2%	8.9%	8.7%	14.2%	11.0%	6.0%	<b>9.8%</b>
<b>Complex IV</b>	14.5%	33.6%	19.4%	18.1%	40.3%	24.1%	23.6%	13.8%	<b>24.8%</b>
<b>Calculated parameters</b>									
<b>MHI</b>	9.0%	10.6%	7.7%	12.7%	10.4%	10.8%	11.4%	6.1%	<b>10.4%</b>

### Supplemental Table 3 – Technical variation for each mitochondrial assay and calculated MHI by cell type.

Coefficients of variation (C.V.s) across 2-5 biological replicates (different 5M cell pellet isolated from the same blood draw) for each cell subtype and PBMCs (See *Methods* for details).

## Supplemental Table 4

**Counting cocktail** contains markers for activated T cells and was used to label  $2 \times 10^6$  cells to determine the abundance of each cell subtype prior to sorting.  
**Sorting cocktail** contains markers for memory T cells, used to isolate subpopulations by FACS.

Cocktail		Marker	Fluorophore	Volume	Catalog number
Counting cocktail	1	CD20	A700	2.5ul	BioLegend 302322
	2	CD3	FITC	2.5ul	BioLegend 300406
	3	CD4	BV650	2.5ul	BioLegend 317435
	4	CD8	BV711	2.5ul	BioLegend 301043
	5	CD56	PE-Dazzle 594	2.5ul	BioLegend 362543
	6	CD16	APC-Cy7	2.5ul	BioLegend 302017
	7	CD14	PE-Cy7	2.5ul	BioLegend 367111
	8	HLA-DR	PerCP-Cy5.5	2.5ul	BioLegend 307629
	9	CD45	BV510	2.5ul	BioLegend 368525
	10	CD15	BV786	2.5ul	BioLegend 323043
	11	CD11c	BV605	2.5ul	BioLegend 301636
	12	CD69	PE	2.5ul	BioLegend 310906
	13	CD25	APC	2.5ul	BioLegend 356110
	14	Viability Dye	DAPI	2ug/l	BD 564907
Sorting cocktail	1	CD20	A700	25ul	BioLegend 302322
	2	CD3	FITC	25ul	BioLegend 300406
	3	CD4	BV650	25ul	BioLegend 317435
	4	CD8	BV711	25ul	BioLegend 301043
	5	CD56	PE-Dazzle 594	25ul	BioLegend 362543
	6	CD16	APC-Cy7	25ul	BioLegend 302017
	7	CD14	PE-Cy7	25ul	BioLegend 367111
	8	HLA-DR	PerCP-Cy5.5	25ul	BioLegend 307629
	9	CD45	BV510	25ul	BioLegend 368525
	10	CD15	BV786	25ul	BioLegend 323043
	11	CCR7	PE	25ul	BioLegend 353203
	12	CD45RA	APC	25ul	BioLegend 304111
	13	Viability Dye	DAPI	2ug/l	BD 564907

All tubes are 5 ml Polypropylene (ThermoFisher #352063)

**Supplemental Table 4 – Recipes for antibody cocktails used to detect cell surface markers for FACS-based cell proportions and sorting.**  
 (See *Methods* for details).