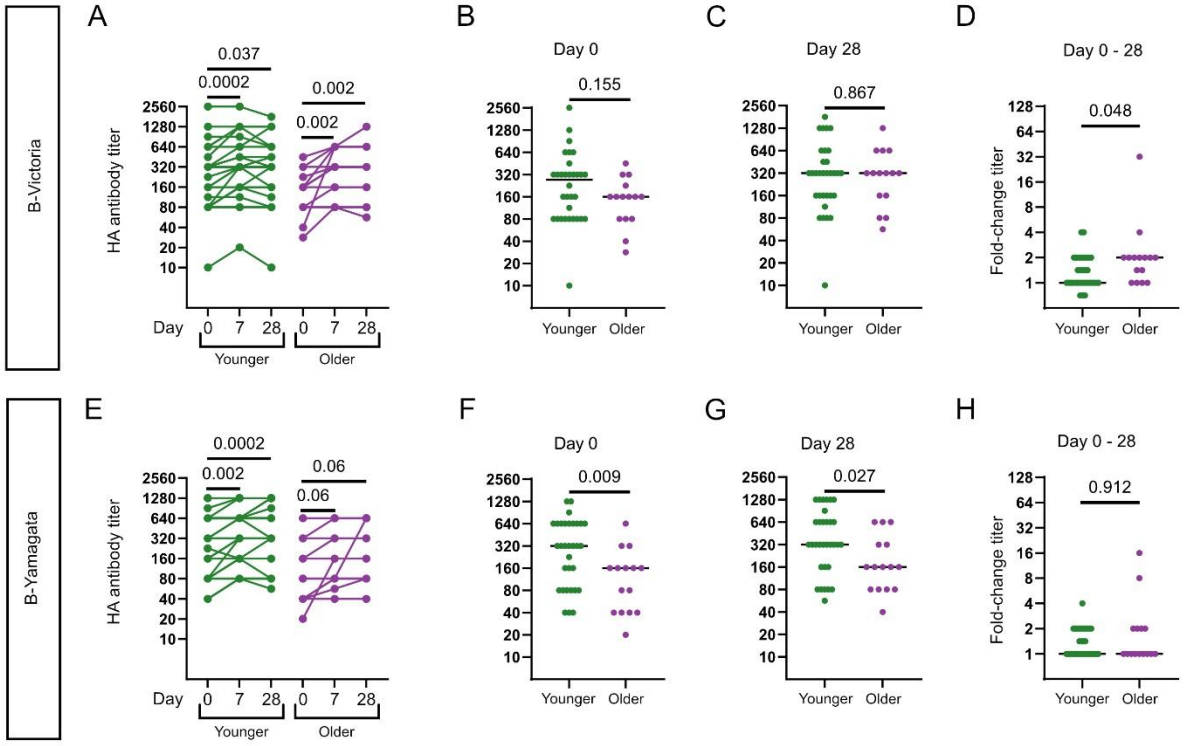
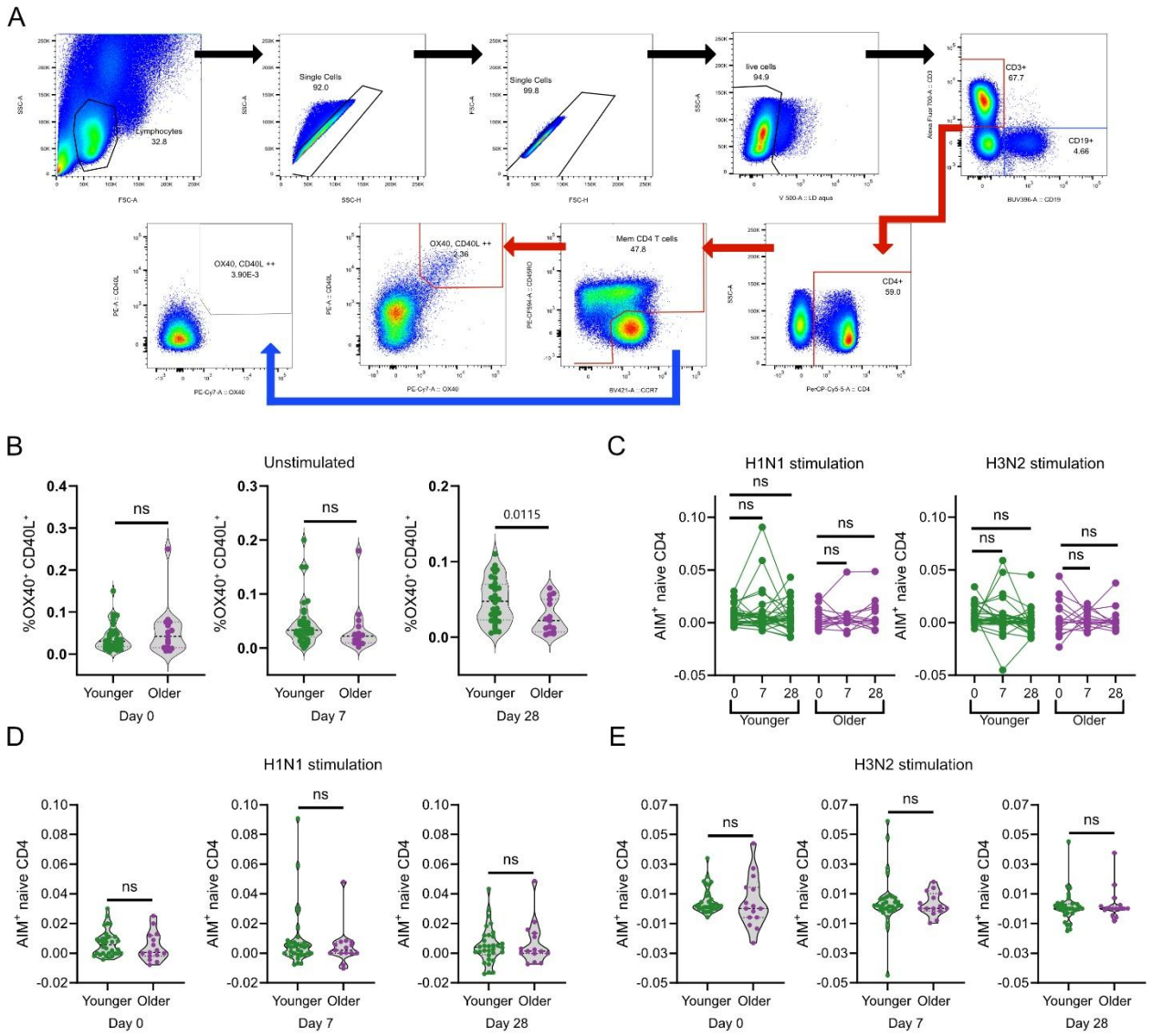
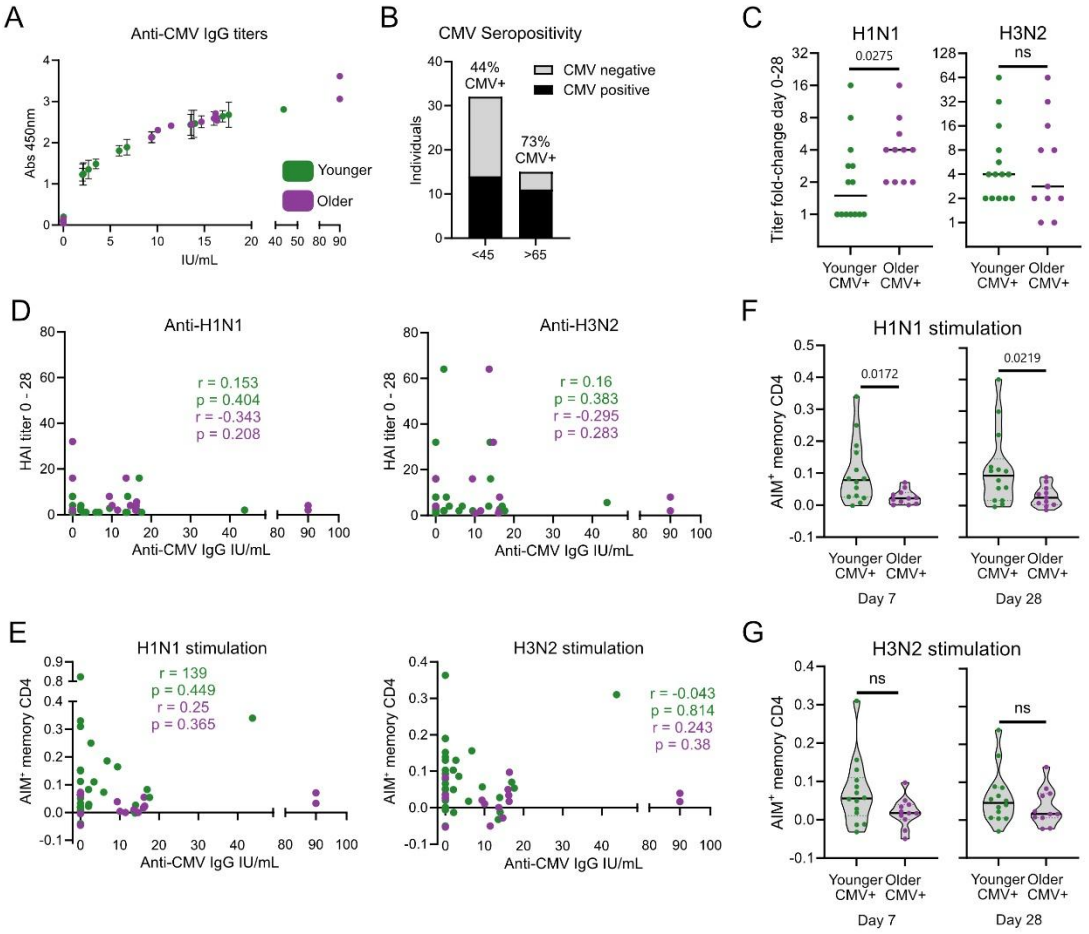

B-Victoria
A
HA antibody titer
0.037
0.0002
0.002
0.002
2560
1280
640
320
160
80
40
20
10
Day 0 7 28 0 7 28
Younger
Older
B
Day 0
0.155
Younger Older
C
Day 28
0.867
Younger Older
D
Day 0 - 28
Fold-change titer
0.048
128
64
32
16
8
4
2
1
Younger Older
B-Yamagata
E
HA antibody titer
0.0002
0.002
0.06
0.06
Day 0 7 28 0 7 28
Younger
Older
F
Day 0
0.009
Younger Older
G
Day 28
0.027
Younger Older
H
Day 0 - 28
Fold-change titer
0.912
Younger Older

Supplemental Figure 1. HAI titers to influenza B differ between older and younger adults. (A-D) HAI antibody responses to the B-Victoria vaccine component, and (E-H) HAI antibody responses to the B-Yamagata vaccine component. (A, H) Statistical comparisons represent two-sided paired Wilcoxon rank tests with Bonferroni correction, while (B-D, F-H) represent a two-sided Mann-Whitney U statistical test. For statistical tests, ns indicates not significant. Independent biological samples include younger adults (n = 32) and older adults (n = 15).

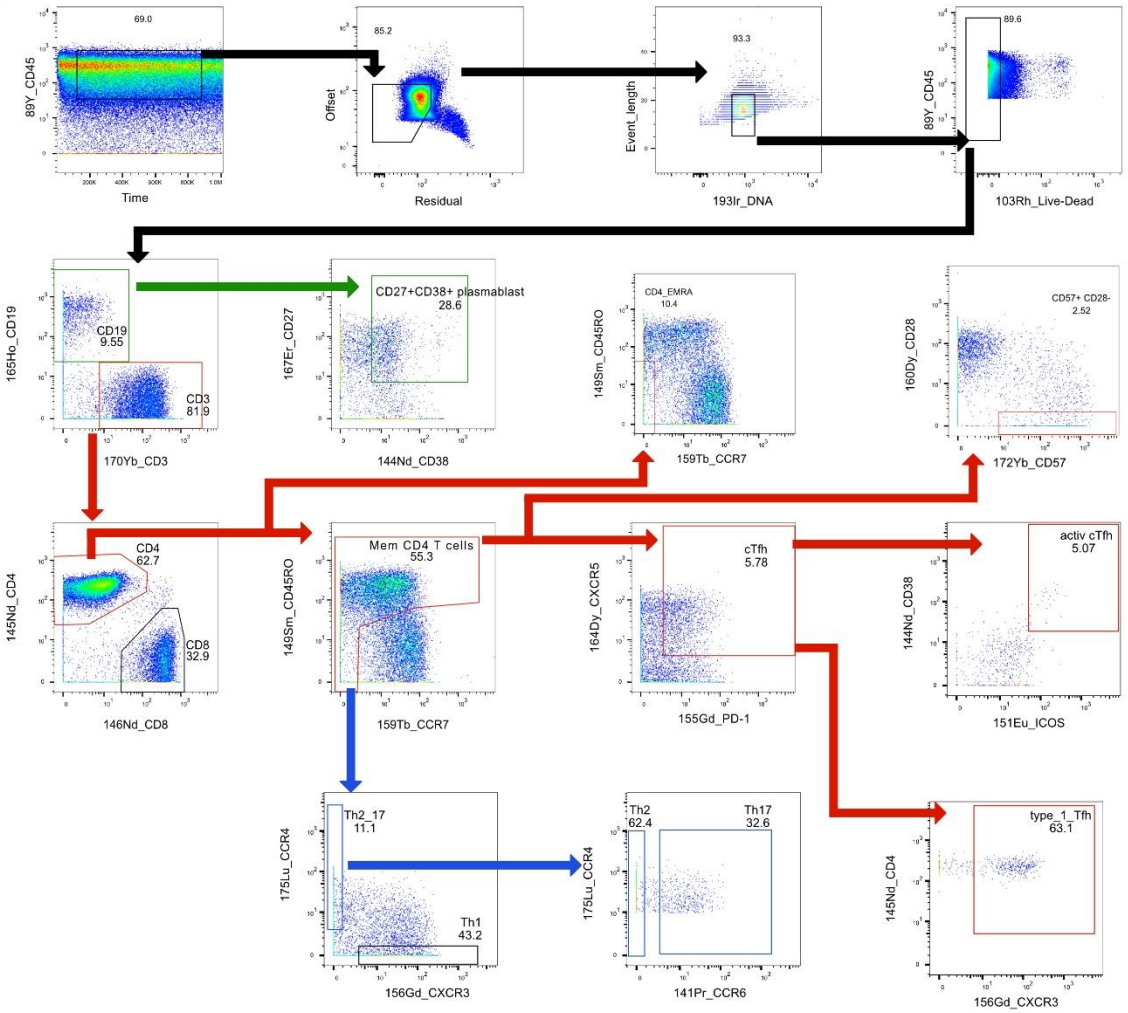

A
Lymphocytes
32.8
Single Cells
92.0
Single Cells
99.8
live cells
94.9
CD3+
67.7
CD19+
4.66
CD4+
59.0
Mem CD4 T cells
47.8
OX40, CD40L ++
2.36
OX40, CD40L ++
3.90E-3
B
Unstimulated
%OX40+ CD40L+
ns
0.0115
Younger
Older
Day 0
Day 7
Day 28
C
H1N1 stimulation
H3N2 stimulation
AIM+ naive CD4
ns
0
7
28
Younger
Older
D
H1N1 stimulation
AIM+ naive CD4
ns
Younger
Older
Day 0
Day 7
Day 28
E
H3N2 stimulation
AIM+ naive CD4
ns
Younger
Older
Day 0
Day 7
Day 28

Supplemental Figure 2. Flow Cyometry AIM assay gating strategy and naïve CD4 T cell activation. (A) Gating strategy for AIM assay with colored arrows representing population lineages. (B) Frequency of activated memory $CD4^+$ T cells in the unstimulated condition comparing older and younger adults at day 0 (left), day 7 (center), and day 28 (right) using a two-sided Mann-Whitney test. (C) Comparison of antigen-reactive naïve $CD4^+$ T cells at 0-, 7-, or 28-days post-vaccination in older (purple) and younger (green) adults assessed by two-sided paired Wilcoxon rank tests with Bonferroni correction. (D-E) Comparison of antigen-reactive naïve $CD4^+$ T cells between older and younger adults analyzed by a two-sided Mann-Whitney U tests. Percentages of activated cells without stimulation have been subtracted from the stimulated conditions for each antigen. For statistical tests, ns indicates not significant. Independent biological samples include younger adults (n = 32) and older adults (n = 15).

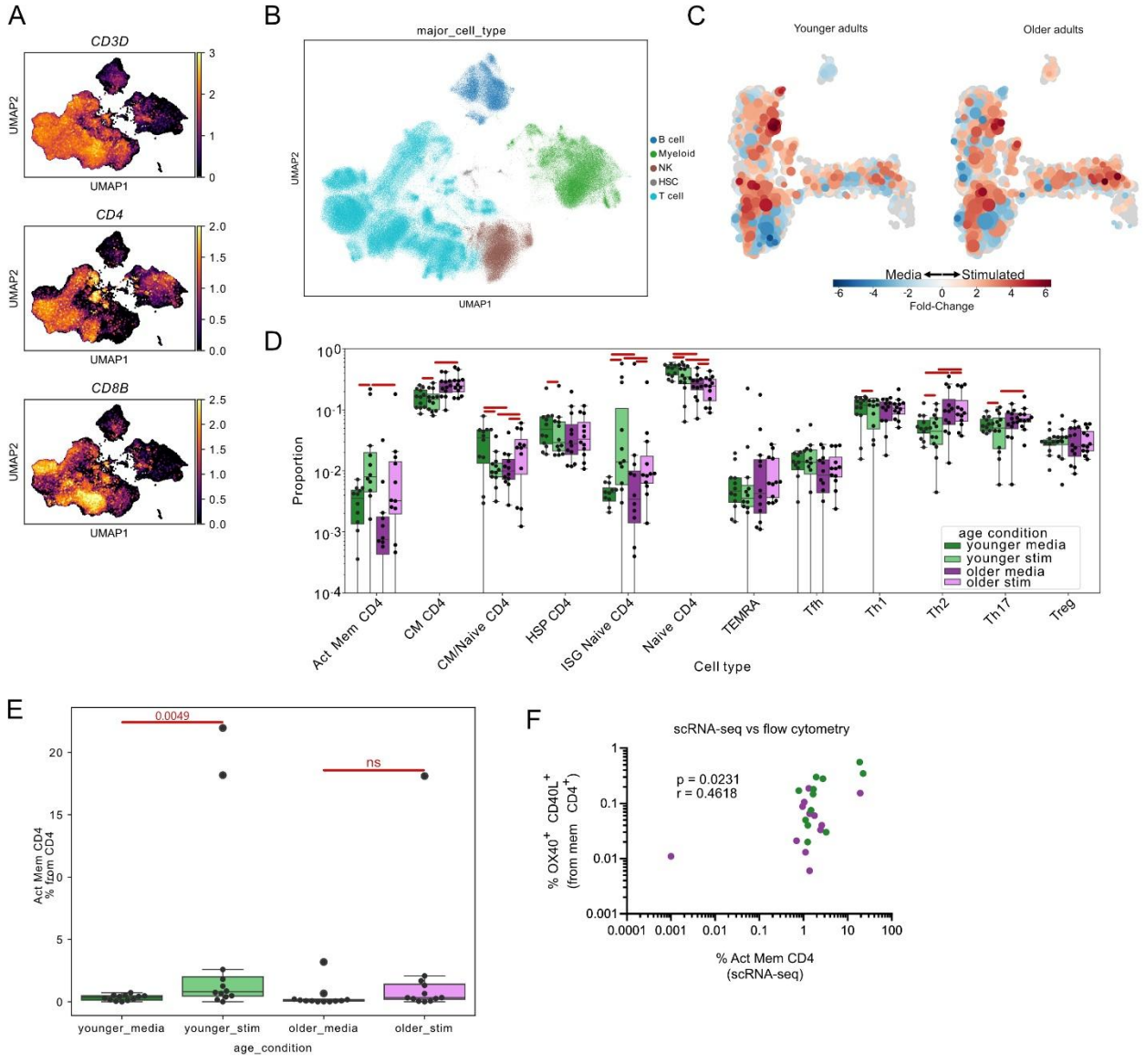

A
Anti-CMV IgG titers
Abs 450nm
IU/mL
Younger
Older
B
CMV Seropositivity
Individuals
44% CMV+
73% CMV+
CMV negative
CMV positive
<45
>65
C
H1N1
H3N2
0.0275
ns
Titer fold-change day 0-28
Younger CMV+
Older CMV+
D
Anti-H1N1
Anti-H3N2
HAI titer 0 - 28
r = 0.153
p = 0.404
r = -0.343
p = 0.208
r = 0.16
p = 0.383
r = -0.295
p = 0.283
Anti-CMV IgG IU/mL
E
H1N1 stimulation
H3N2 stimulation
AIM+ memory CD4
r = 139
p = 0.449
r = 0.25
p = 0.365
r = -0.043
p = 0.814
r = 0.243
p = 0.38
Anti-CMV IgG IU/mL
F
H1N1 stimulation
0.0172
0.0219
Day 7
Day 28
G
H3N2 stimulation
ns

Supplemental Figure 3. CMV seroprevalence has no impact on vaccine responses. (A) Measurement of CMV antibody titers with younger adults colored in green and older adults colored in purple. (B) CMV serostatus by age group, with CMV-positive individuals in black and CMV-negative individuals in grey. (C) Comparison of H1N1 and H3N2 HAI titer fold change in CMV positive younger and older adults by a two-sided Mann-Whitney test. (D-E) Spearman correlations between anti-CMV antibody titer and (D) HAI fold-change at day 28 or (E) influenza-specific memory $CD4^+$ T cells at day 7 post-vaccination from flow cytometry AIM data. Data points and statistics are colored green for younger adults and purple for older adults. (F-G) Frequencies of flow cytometry AIM positive memory CD4 T cells at (left) day 7 or (right) day 28 post vaccination against an (F) H1N1 stimulus or (G) H3N2 stimulus by a two-sided Mann-Whitney test. For statistical tests, ns indicates not significant. Independent biological samples include (A-B, D-E) younger adults (n = 32) and older adults (n = 15), and (C, F, G) younger adults (n = 14) and older adults (n = 11).

A

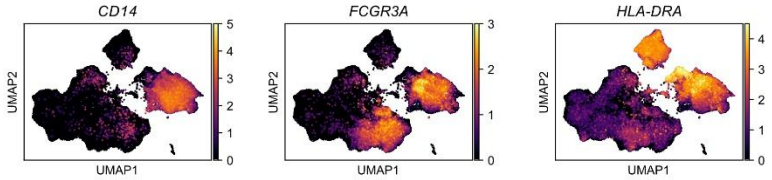

89Y_CD45
69.0
Time
Offset
85.2
Residual
Event_length
93.3
193Ir_DNA
89Y_CD45
89.6
103Rh_Live-Dead
165Ho_CD19
CD19
9.55
CD3
81.3
170Yb_CD3
167Er_CD27
CD27+CD38+ plasmablast
28.6
144Nd_CD38
149Sm_CD45RO
CD4_EMRA
10.4
159Tb_CCR7
160Dy_CD28
CD57+ CD28-
2.52
172Yb_CD57
145Nd_CD4
CD4
62.7
CD8
32.9
146Nd_CD8
149Sm_CD45RO
Mem CD4 T cells
55.3
159Tb_CCR7
164Dy_CXCR5
cTfh
5.78
155Gd_PD-1
144Nd_CD38
activ cTfh
5.07
151Eu_ICOS
175Lu_CCR4
Th2_17
11.1
Th1
43.2
156Gd_CXCR3
175Lu_CCR4
Th2
62.4
Th17
32.6
141Pr_CCR6
145Nd_CD4
type_1_Tfh
63.1
156Gd_CXCR3

Supplemental Figure 4. Mass cytometry immunophenotyping gating. (A) Gating strategy with colored arrows representing lineages.

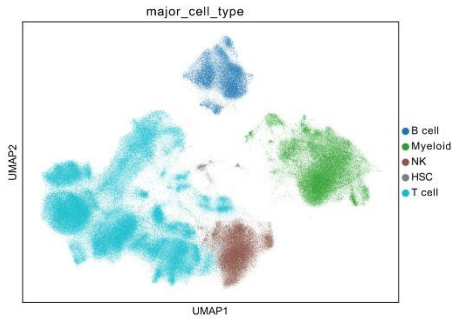

A
CD3D
CD4
CD8B
UMAP1
UMAP2
B
major_cell_type
B cell
Myeloid
NK
HSC
T cell
C
Younger adults
Older adults
Media
Stimulated
Fold-Change
D
Proportion
age condition
younger media
younger stim
older media
older stim
Act Mem CD4
CM CD4
CM/Naive CD4
HSP CD4
ISG Naive CD4
Naive CD4
TEMRA
Tfh
Th1
Th2
Th17
Treg
Cell type
E
0.0049
ns
Act Mem CD4 % from CD4
younger_media
younger_stim
older_media
older_stim
age_condition
F
scRNA-seq vs flow cytometry
p = 0.0231
r = 0.4618
% OX40+ CD40L+ (from mem CD4+)
% Act Mem CD4 (scRNA-seq)

Supplementary Figure 5. Identification of $CD4^+$ T cells and changes with stimulation. (A) Gene transcript abundance of *CD3D*, *CD4*, and *CD8B* is overlaid on UMAP and contains all PBMC. Gene counts are transformed using a shifted log. (B) Identification of major cell subsets before splitting by cell type and integration. (C) Nearest-neighbor search to calculate fold-change between media and stimulated cells in younger and older adult CD4 T cells. (D) CD4 T cell composition proportions on a $\log_{10}$ scale comparing populations using a Bayesian model. Significant population changes are denoted by a red bar. (E) Frequency of activated memory $CD4^+$ T cells split by age and condition using a paired a two-sided Wilcoxon rank test. (F) Comparison of activated cell frequencies by AIM assay and the activated memory CD4 population by scRNA-seq by Spearman correlation for all individuals. Independent biological samples include younger adults (n = 32) and older adults (n = 15).

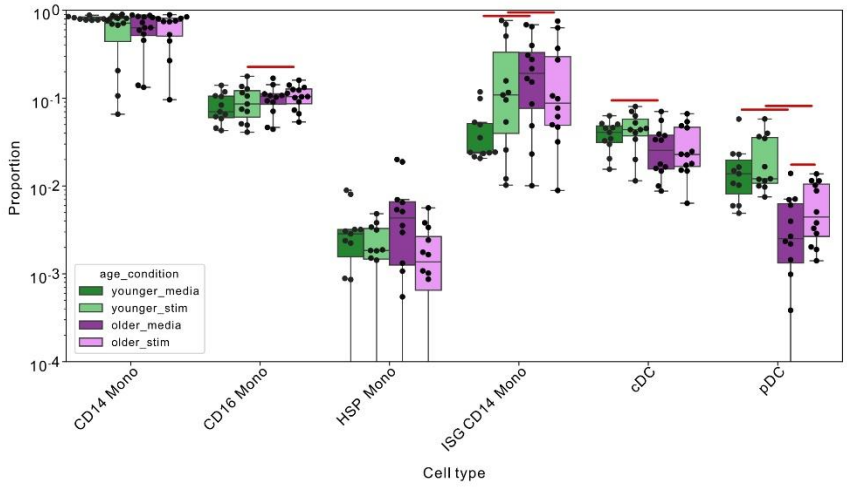
A


CD14
FCGR3A
HLA-DRA
UMAP1
UMAP2

B


major_cell_type
B cell
Myeloid
NK
HSC
T cell
UMAP1
UMAP2

C


Proportion
age_condition
younger_media
younger_stim
older_media
older_stim
CD14 Mono
CD16 Mono
HSP Mono
ISG CD14 Mono
cDC
pDC
Cell type

Supplemental Figure 6. Identification of myeloid cells in scRNA-seq dataset. (A) Gene transcript abundance of CD14, CD16(FCGR3A), and HLA-DR overlayed on UMAP containing all cells. Gene counts are transformed using a shifted log. (B) Identification of major cell subsets before splitting by cell type and integration. (C) Myeloid cell composition proportions on a $\log_{10}$ scale comparing populations using a Bayesian model. Significant population changes are denoted by a red bar. Independent biological samples include younger adults (n = 32) and older adults (n = 15).