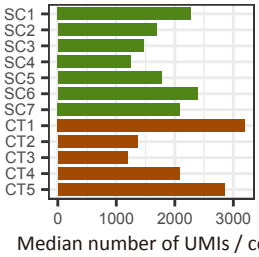
Figure S1

A

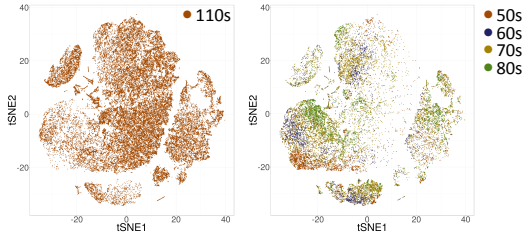
ID	Age	Sex	Type	RNA-Seq	FACS1	FACS2	Name
SC1	110s	F	-	**O**	O	O	RKNS001
SC2	110s	M	-	**O**	O	O	RKNS002
SC3	110s	F	-	**O**	O	-	RKNS003
SC4	110s	F	-	**O**	O	-	RKNS005
SC5	110s	F	-	**O**	-	O	RKNS007
SC6	110s	F	-	**O**	-	O	RKNS014
SC7	110s	M	-	**O**	-	O	RKNS016
CT1	50s	M	Un	**O**	O	-	RKNS000
CT2	70s	F	Off	**O**	O	-	RKNS004
CT3	60s	F	Un	**O**	O	-	RKNS006
CT4	70s	F	Un	**O**	-	O	RKNS012
CT5	80s	M	Off	**O**	-	O	RKNS013
SC8	100s	M	-	-	-	O	RKNS008
SC9	110s	F	-	-	-	O	RKNS017
SC10	110s	F	-	-	-	O	RKNS011
CT6	80s	M	Off	-	-	O	RKNS009
CT7	80s	F	Un	-	-	O	RKNS010
CT8	80s	M	Off	-	-	O	RKNS015
CT9	40s	M	Un	-	-	O	RKNS000

Off: offspring, Un: unrelated

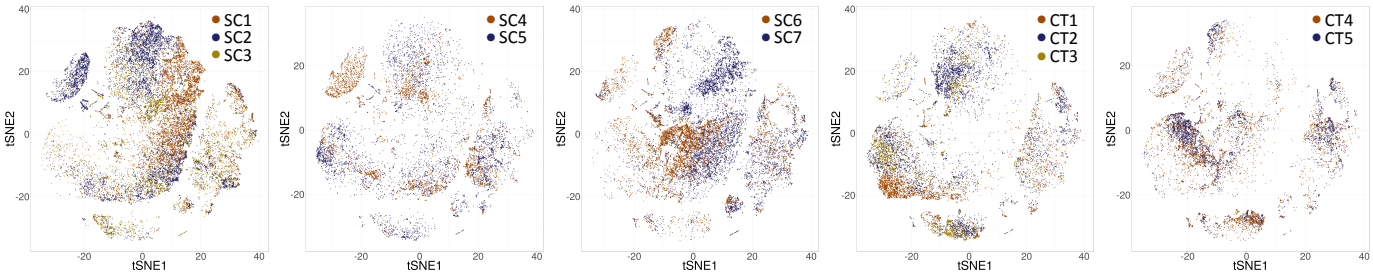
B



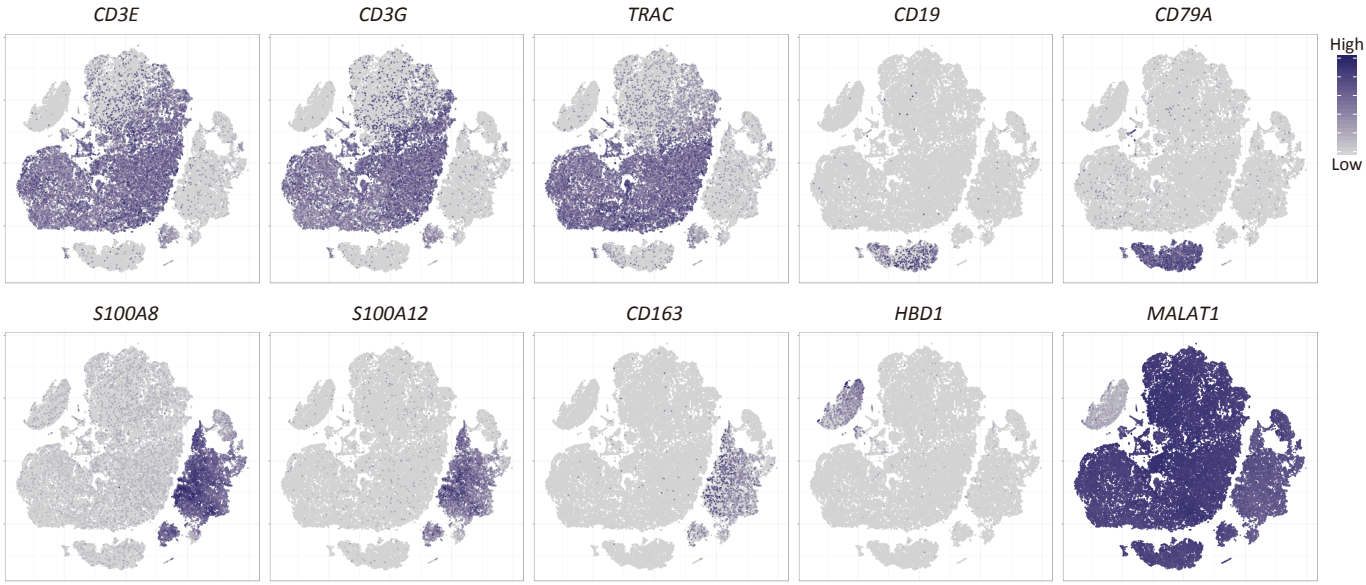
C



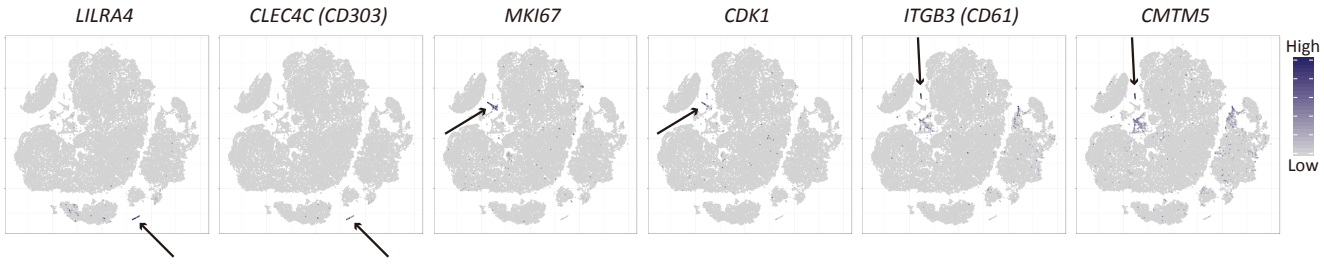
D



E



F



www.pnas.org/cgi/doi/10.1073/pnas.1907883116

Figure S2

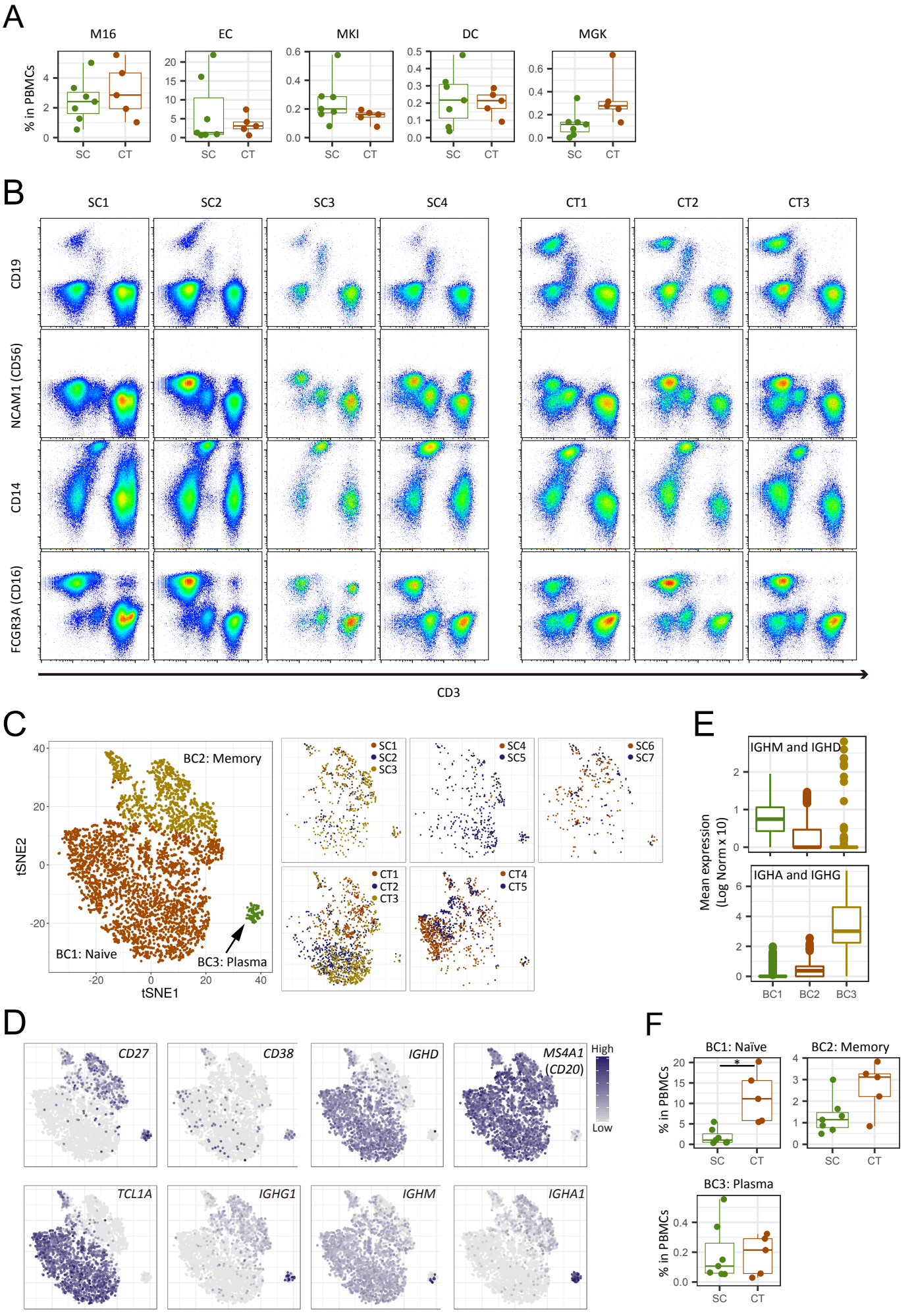
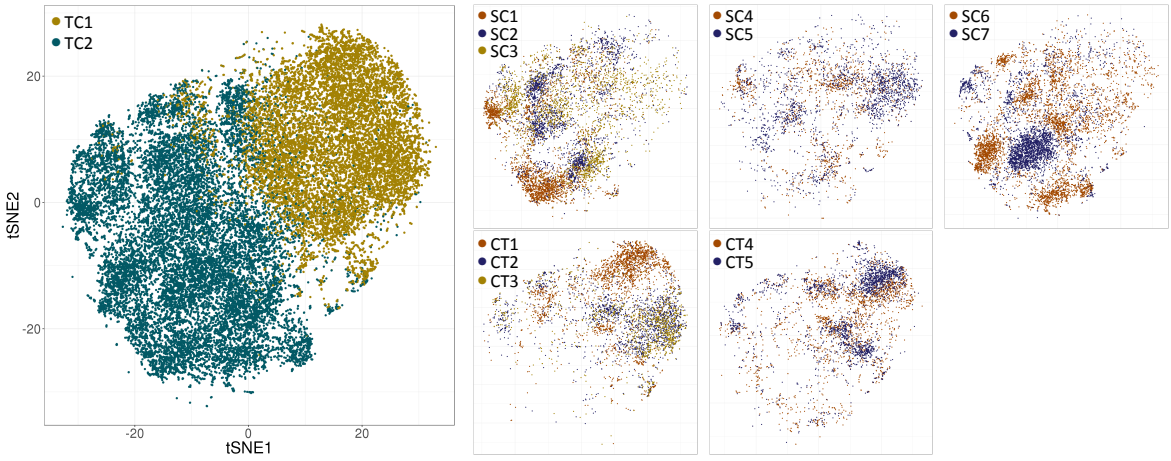
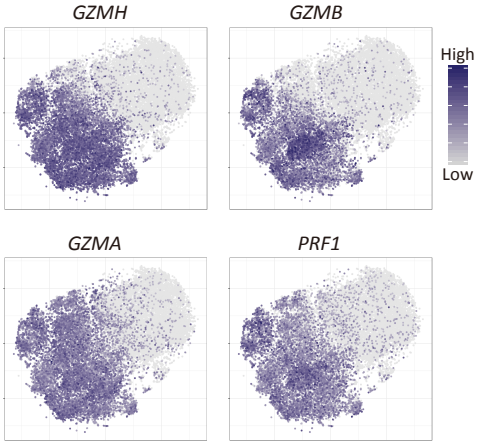

A
M16
EC
MKI
DC
MGK
% in PBMCs
SC
CT
B
SC1
SC2
SC3
SC4
CT1
CT2
CT3
CD19
NCAM1 (CD56)
CD14
FCGR3A (CD16)
CD3
C
BC2: Memory
BC1: Naive
BC3: Plasma
tSNE1
tSNE2
SC1
SC2
SC3
SC4
SC5
SC6
SC7
CT1
CT2
CT3
CT4
CT5
D
CD27
CD38
IGHD
MS4A1 (CD20)
High
Low
TCL1A
IGHG1
IGHM
IGHA1
E
IGHM and IGHD
IGHA and IGHG
Mean expression (Log Norm x 10)
BC1
BC2
BC3
F
BC1: Naïve
BC2: Memory
BC3: Plasma
% in PBMCs
SC
CT
*

Figure S3

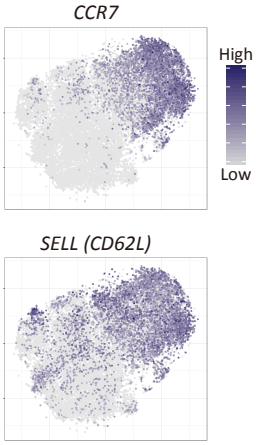
A



B

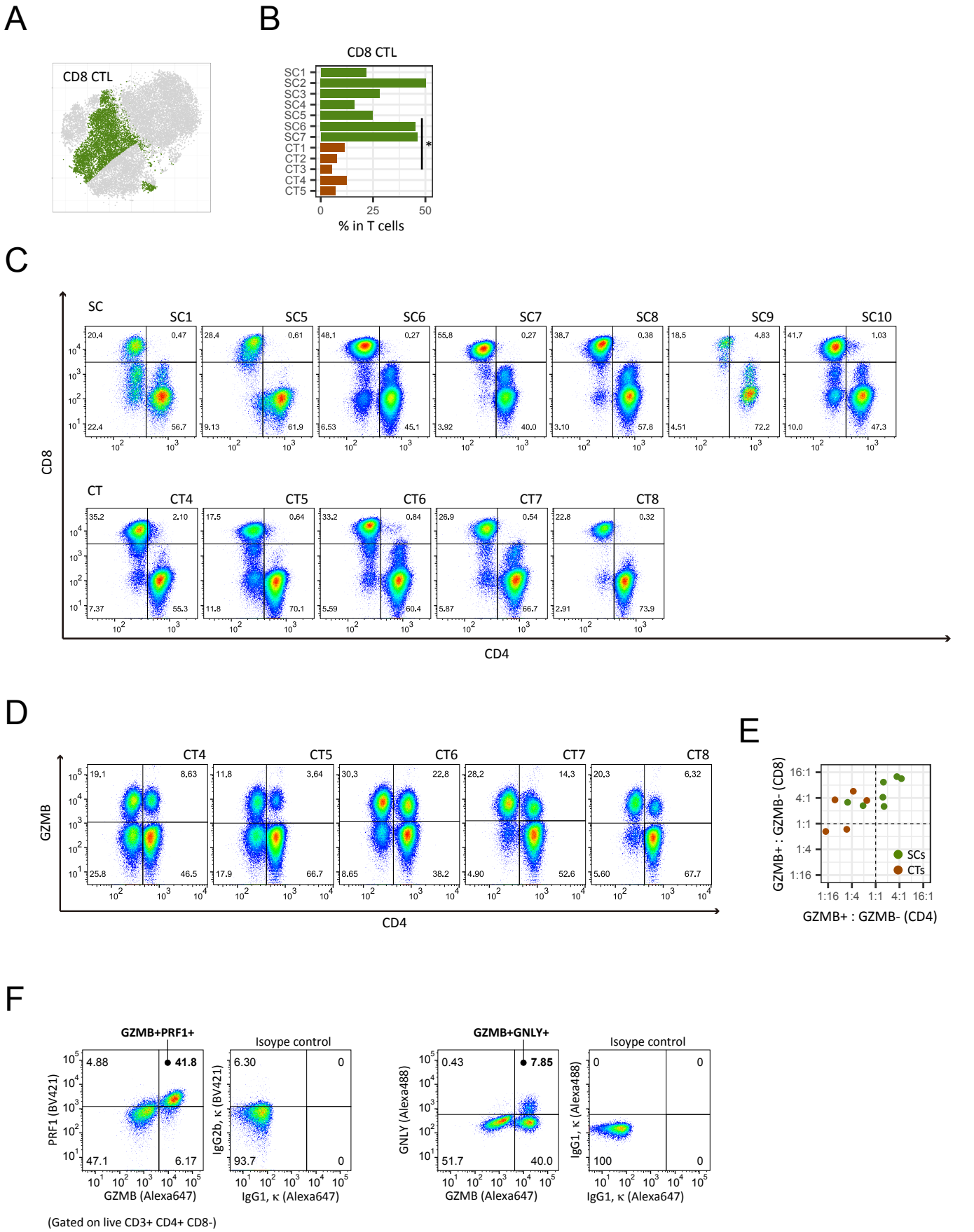


C



D

Figure S4



(Gated on live CD3+ CD4+ CD8-)

Figure S5

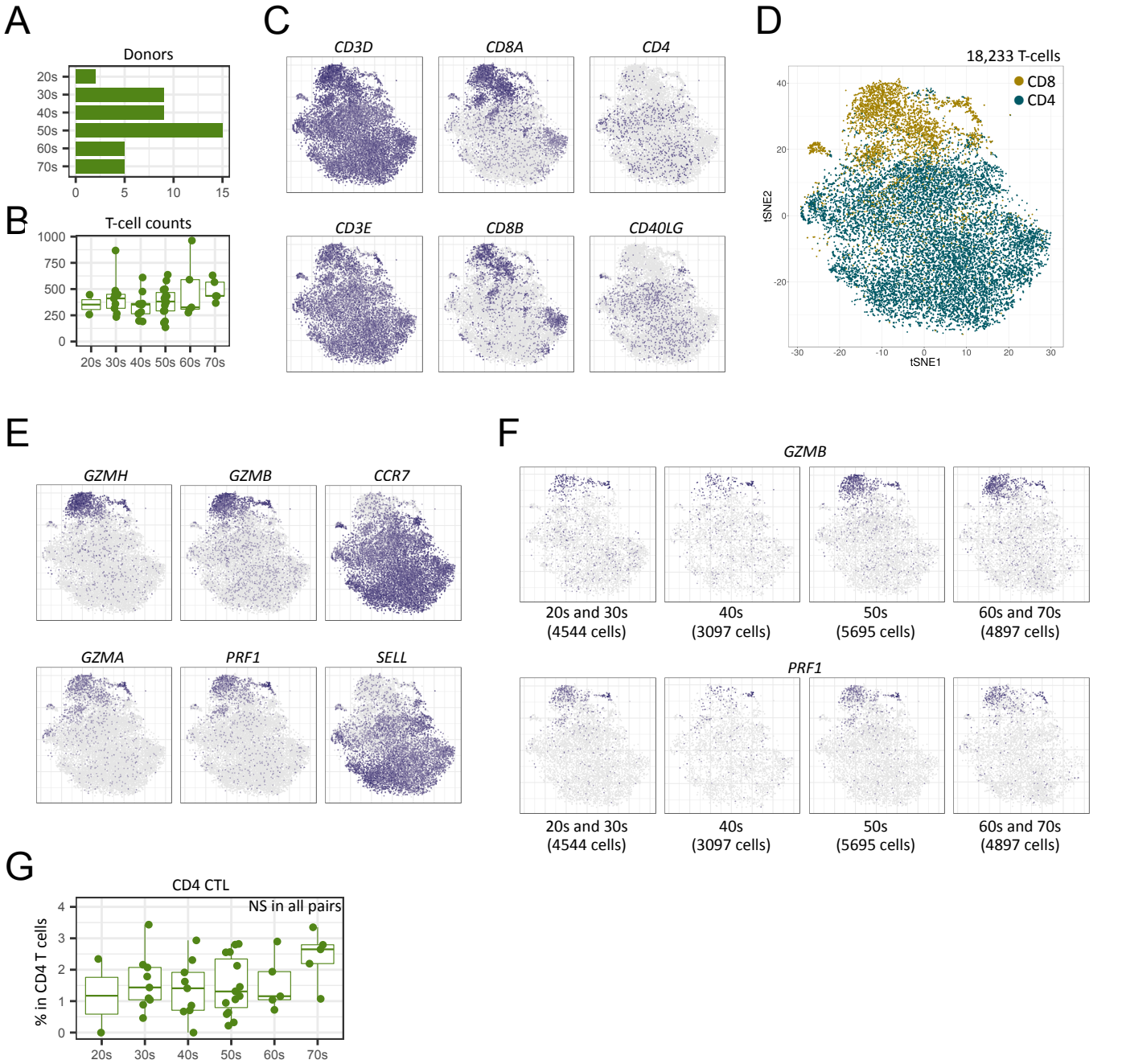

A
Donors
20s
30s
40s
50s
60s
70s
0
5
10
15
B
T-cell counts
1000
750
500
250
0
20s 30s 40s 50s 60s 70s
C
CD3D
CD8A
CD4
CD3E
CD8B
CD40LG
D
18,233 T-cells
CD8
CD4
tSNE2
tSNE1
E
GZMH
GZMB
CCR7
GZMA
PRF1
SELL
F
GZMB
20s and 30s
(4544 cells)
40s
(3097 cells)
50s
(5695 cells)
60s and 70s
(4897 cells)
PRF1
20s and 30s
(4544 cells)
40s
(3097 cells)
50s
(5695 cells)
60s and 70s
(4897 cells)
G
CD4 CTL
NS in all pairs
% in CD4 T cells
20s
30s
40s
50s
60s
70s

Figure S6

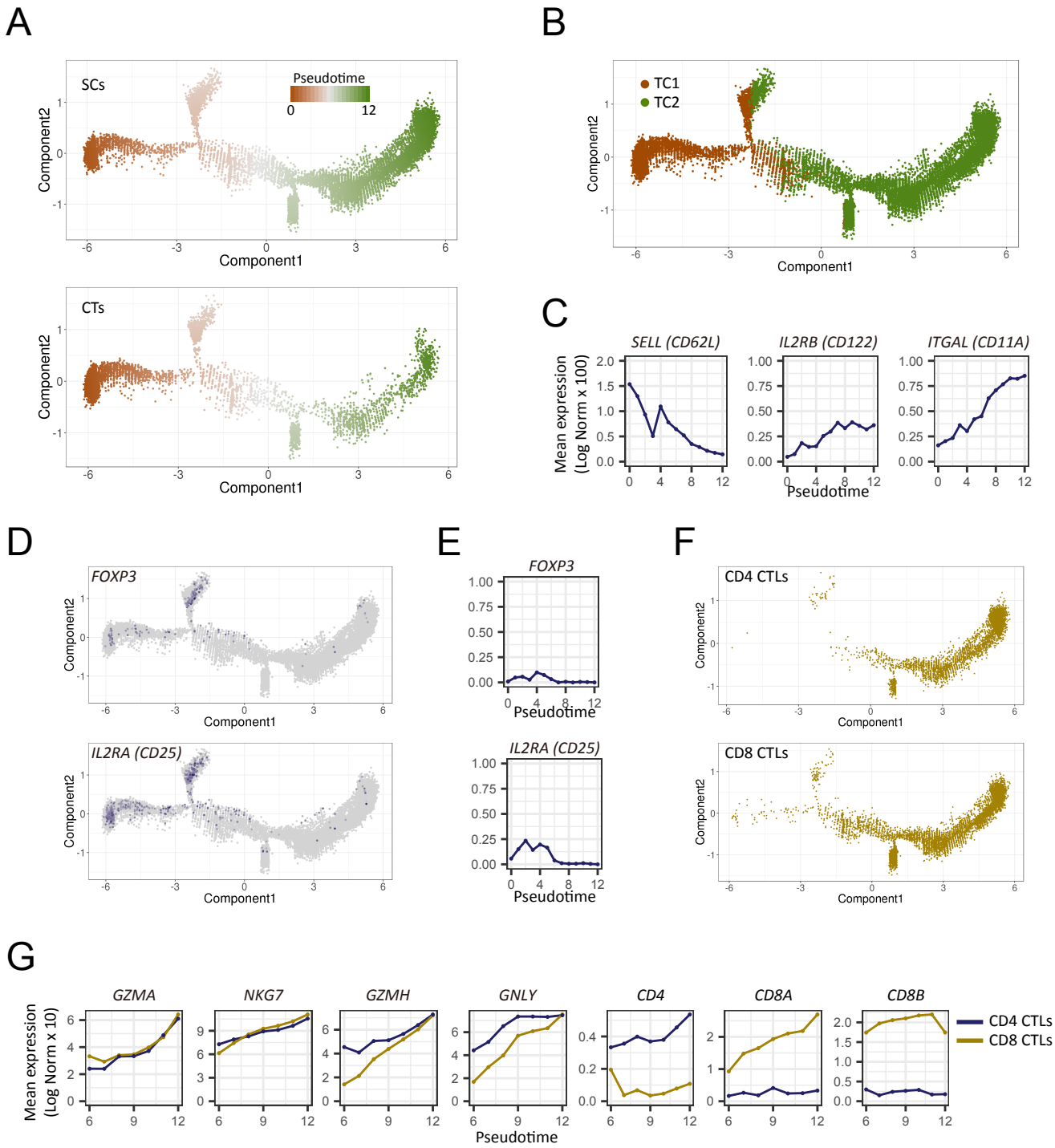

A
SCs
Pseudotime
0
12
Component2
Component1
CTs
B
TC1
TC2
C
SELL (CD62L)
IL2RB (CD122)
ITGAL (CD11A)
Mean expression (Log Norm x 100)
Pseudotime
D
FOXP3
IL2RA (CD25)
E
F
CD4 CTLs
CD8 CTLs
G
GZMA
NKG7
GZMH
GNLY
CD4
CD8A
CD8B
Mean expression (Log Norm x 10)
CD4 CTLs
CD8 CTLs

Figure S7

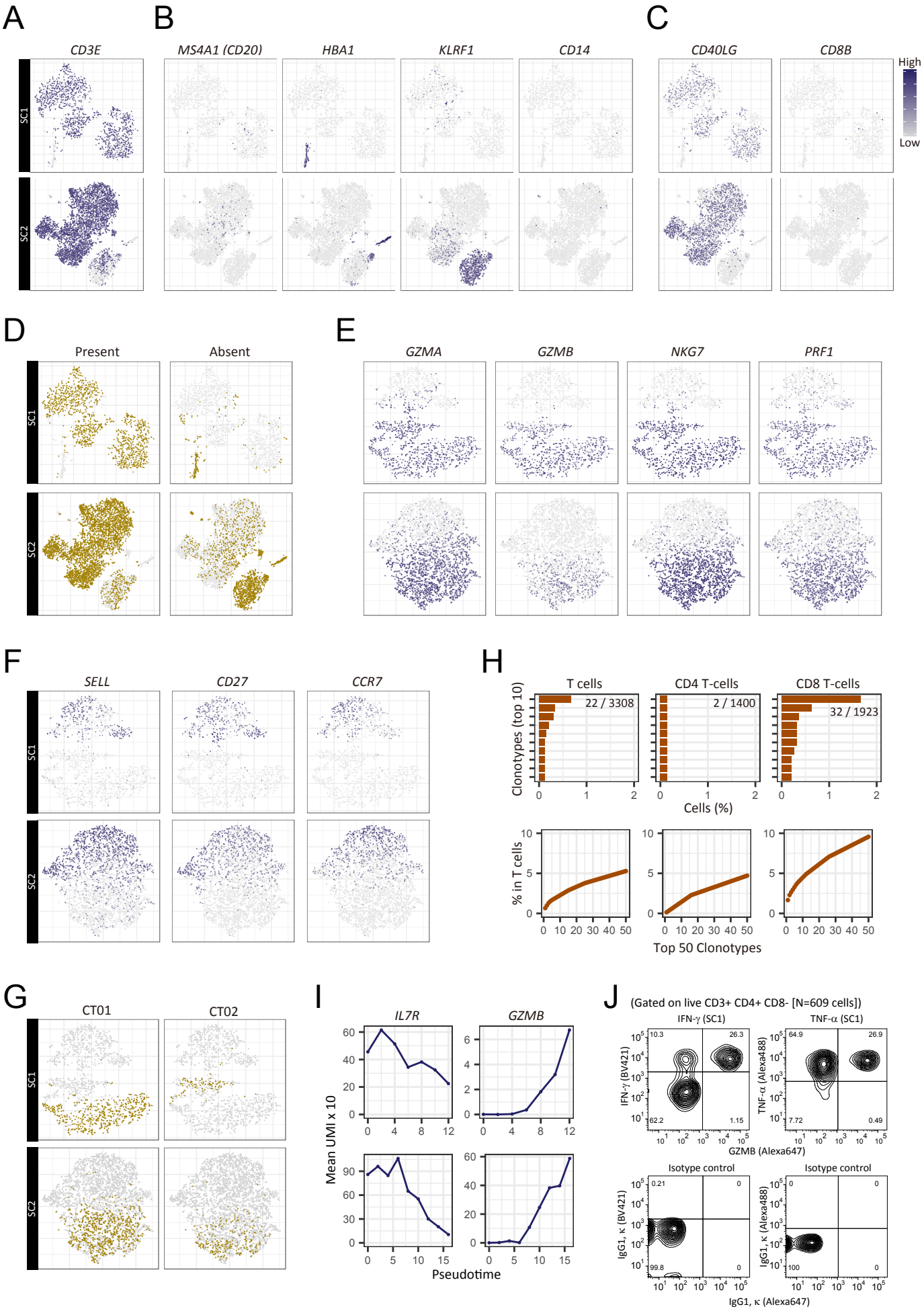

A
B
C
CD3E
MS4A1 (CD20)
HBA1
KLRF1
CD14
CD40LG
CD8B
High
Low
SC1
SC2
D
Present
Absent
E
GZMA
GZMB
NKG7
PRF1
F
SELL
CD27
CCR7
H
T cells
CD4 T-cells
CD8 T-cells
22 / 3308
2 / 1400
32 / 1923
Clonotypes (top 10)
Cells (%)
% in T cells
Top 50 Clonotypes
G
CT01
CT02
I
IL7R
GZMB
Mean UMI x 10
Pseudotime
J
(Gated on live CD3+ CD4+ CD8- [N=609 cells])
IFN-γ (SC1)
TNF-α (SC1)
IFN-γ (BV421)
TNF-α (Alexa488)
GZMB (Alexa647)
Isotype control
IgG1, κ (BV421)
IgG1, κ (Alexa488)
IgG1, κ (Alexa647)

Figure S1. Single-cell transcriptome profile of PBMCs. **a.** Samples used for single-cell RNA-Seq and FACS analyses. FACS1 used antibodies against major cell-type markers: CD3, CD19, CD14, CD16, and NCAM1 (CD56), whereas FACS2 used antibodies against T-cell subtype markers: CD3, CD4, CD8, and GZMB. **b.** Median numbers of UMI counts per cell for each donor **c.** Two-dimensional tSNE visualization, separated into supercentenarians and controls. Different colors in the right panel represent 4 age groups. **d.** Two-dimensional tSNE visualization, separated into two or three donor groups. **e.** Expression of marker genes for cell-type markers and *MALAT1* as the highest expressed gene; cell positions are from the t-SNE plots in Fig. 1c. **f.** Expression of marker genes used to define three small clusters by k-means clustering (i.e., MKI67+ proliferating cells [MKI], dendritic cells [DC], and megakaryocytes [MGK]).

Figure S2. Reduction in the number of B cells in supercentenarians. **a.** Boxplots of the percentage of each indicated cell type in PBMCs. **b.** FACS plots for four supercentenarians (SC1–SC4) and three controls (CT1–CT3) profiled using CD3, CD19, NCAM1 (CD56), CD14, and FCGR3A (CD16). **c.** Two-dimensional tSNE visualization of B cells for all 12 donors or separately for two or three donor groups. **d.** Expression of markers for B-cell subtypes. **e.** Expression of immunoglobulin heavy chains in each B-cell subtype. Upper panel shows total expression of *IGHM* and *IGHD*, which are used before the class switch. Lower panel shows total expression of *IGHA1*, *IGHA2*, *IGHG1*, *IGHG2*, *IGHG3*, and *IGHG4*, which are used after the class switch. **f.** Boxplots of the percentage of each B-cell subtype (defined by k-means clustering) in PBMCs of seven supercentenarians (SC1–SC7) and five controls (CT1–CT5). *, $P < 0.05$ (Wilcoxon rank sum test); no asterisk means not significant.

Figure S3. Expansion of cytotoxic T-cell populations in supercentenarians. **a.** Two-dimensional tSNE visualization of T cells using the Seurat R package. Different colors represent the original TC1 and TC2 clusters; 86.3% of the original TC1 is clustered into Seurat_TC1; 97.6% of the original TC2 is clustered into Seurat_TC2. **b–d.** Expression of cytotoxic genes and lymph node homing markers; cell positions are from the t-SNE plot in **a.**

Figure S4. Expansion of cytotoxic CD4 T-cell populations in supercentenarians. **a.** CD8 CTLs defined based on the expression of *CD8A* and *CD8B*; cell positions are from the t-SNE plot in Fig. 4a. **b.** Percentages of CD8 T-cells among the total T-cells. *, $P < 0.05$ (Wilcoxon rank sum test). **c.** FACS profiles of supercentenarians (SC1, SC5–SC7, SC9, and SC10) and one semi-supercentenarian (SC8), and controls (CT4–CT8). Cells gated on CD3+ were profiled using CD4 (x-axis) and CD8 (y-axis). Cells in lower right and upper left corners are classified as CD4 and CD8 T-cells, respectively. **d.** FACS profiles of five controls (CT4–CT8). Cells gated on CD3+

were profiled using CD4 (x-axis) and GZMB (y-axis). **e.** Ratio between the percentage of GZMB+ and GZMB− cells in CD4 (x-axis) and CD8 (y-axis) T-cells. Ratio "1:1" indicates that the percentage of GZMB+ cells equals that of GZMB− cells. **f.** FACS profiles of one supercentenarian (SC2). Cells gated on live CD3+ CD4+ CD8- were profiled using GZMB or IgG1 κ (x-axis) and PRF1, GNLY, or IgG2b κ (y-axis).

Figure S5. Expressions of cytotoxic genes in T-cells from young donors. **a.** The numbers of donors used in the analysis for each decade of age. **b.** The numbers of T-cells per donor in each decade of age. **c.** Expression of the indicated marker genes. **d.** Two-dimensional tSNE visualization of 18,233 T cells using the Seurat R package. Different colors represent two subtypes defend by the original paper. **e.** Expression of the indicated marker genes. **f.** Expression of *GZMB* and *PRF1* in each age group. **g.** Percentages of estimated CD4 CTLs in CD4 T-cells. NS means not significant (Wilcoxon rank sum test).

Figure S6. The differentiation state of T cells in supercentenarians. **a.** Pseudotime trajectory of T cells, shown separately for supercentenarians (SC) and controls (CT). **b.** Pseudotime trajectory of T cells colored by TC1 and TC2. **c.** Expression transition of differentiation-associated genes. **d.** Expression of *FOXP3* and *IL2RA* (*CD25*) mapped in pseudotime. **e.** Expression transition of *FOXP3* and *IL2RA* (*CD25*) along the pseudotime. **f.** Distribution of CD4 and CD8 CTLs mapped in pseudotime. **g.** Expression transition of selected genes shown separately for CD4 and CD8 CTLs.

Figure S7. Single-cell 5′ transcriptome and TCR profiles for two supercentenarians (SC1, upper panels and SC2, lower panels). **a.** Expression of a marker gene for T-cells. **b.** Expression of marker genes for B-cells (*MS4A1*), erythrocytes (*HBA1*), NK cells (*KLRF1*), and monocytes (*CD14*). **c.** Expression of marker genes for CD4 T-cells (*CD40LG*) and CD8 T-cells (*CD8B*). **d.** Presence or absence of cells in TCR libraries. **e.** Expression of marker genes for cytotoxic T-cells. **f.** Expression of marker genes for T-cell differentiation. **g.** Distribution of the top 2 most abundant clonotypes, CD01 and CT02, on the t-SNE map. **h.** Diversity of TCRs in publicly available datasets released by another group. **i.** Transient expression of genes associated with T-cell differentiation. **j.** FACS profiles of one supercentenarian (SC1) upon PMA/ionomycin stimulation. Cells gated on live CD3+ CD4+ CD8- were profiled using GZMB or IgG1 κ (x-axis) and IFN-γ, TNF-α, or IgG1 κ (y-axis). The graph is shown in contour plot due to the small number of cells (609 cells) available.