ACCSCIENCE PUBLISHING

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ORIGINAL RESEARCH ARTICLE

An exploratory study on establishing reference intervals for circulating immune cells and an immune age prediction model in healthy young and middle-aged adults

Supplementary File

Table S1. Antibody marker of circulating immune cells

Phenotype	Antibody marker
CD45^{+} cells	CD45^{+}
CD45^{+}LIN^{-}cells	CD45^{+}LIN^{-}
T cells	CD3^{+}
B cells	CD19^{+}CD20^{+}
Natural killer cells	CD3^{-}CD56^{+}
CD8^{+} T cells	CD3^{+}CD8^{+}CD4^{-}
Naive CD8^{+} T cells	CD3^{+}CD8^{+}CD4^{-}CD45RA^{+}
CD8^{+} T effector memory cells	CD3^{+}CD8^{+}CD4^{-}CD45RA^{-}CD197^{-}
CD8^{+} T central memory cells	CD3^{+}CD8^{+}CD4^{-}CD45RA^{-}CD197^{+}
HLA-DR^{+}CD8^{+} T effector memory cells	CD3^{+}CD8^{+}CD4^{-}CD45RA^{-}CD197^{-}CD38^{+}HLA-DR^{+}
HLA-DR^{+}CD8^{+} T central memory cells	CD3^{+}CD8^{+}CD4^{-}CD45RA^{-}CD197^{+}CD38^{+}HLA-DR^{+}
CD8^{+}CD39^{+} T cells	CD3^{+}CD8^{+}CD39^{+}
CD8^{+}PD-1^{+} T cells	CD3^{+}CD8^{+}PD-1^{+}
CD8^{+}CD122^{+} T cells	CD3^{+}CD8^{+}CD122^{+}
CD8^{+}CD28^{+} T cells	CD3^{+}CD8^{+}CD28^{+}
CD8^{+}CD27^{+} T cells	CD3^{+}CD8^{+}CD27^{+}
CD8^{+}CD27^{-}CD28^{-} T cells	CD3^{+}CD8^{+}CD27^{-}CD28^{-}
CD8^{+}CX3CR1^{+} T cells	CD3^{+}CD8^{+}CX3CR1^{+}
CD8^{+}CX3CR1^{-}CD27^{-} T cells	CD3^{+}CD8^{+}CX3CR1^{-}CD27^{-}
CD8^{+}CX3CR1^{-}CD27^{+} T cells	CD3^{+}CD8^{+}CX3CR1^{-}CD27^{+}
CD8^{+}CD137^{+} T cells	CD3^{+}CD8^{+}CD137^{+}
CD8^{+}CXCR5^{+} T cells	CD3^{+}CD8^{+}CXCR5^{+}
CD4^{+}T cells	CD3^{+}CD4^{+}CD8^{-}
Naive CD4^{+} T cells	CD3^{+}CD4^{+}CD8^{-}CD45RA^{+}
CD4^{+} T effector memory cells	CD3^{+}CD4^{+}CD8^{-}CD45RA^{-}CD197^{-}
CD4^{+} T central memory cells	CD3^{+}CD4^{+}CD8^{-}CD45RA^{-}CD197^{+}
HLA-DR^{+}CD4^{+} T effector memory cells	CD3^{+}CD4^{+}CD8^{-}CD45RA^{-}CD197^{-}CD38^{+}HLA-DR^{+}

(Cont'd...)

Table S1. (*Continued*)

Phenotype	Antibody marker
HLA-DR^{+}CD4^{+} T central memory cells	CD3^{+}CD4^{+}CD8^{-}CD45RA^{-}CD197^{+}CD38^{+}HLA-DR^{+}
Treg	CD3^{+}CD4^{+}CD25^{+}CD127^{+}
CD4^{+}CD278^{+} T cells	CD3^{+}CD4^{+}CD278^{+}
CD4^{+}CXCR3^{+} T cells	CD3^{+}CD4^{+}CXCR3^{+}
CD4^{+}CXCR5^{+} T cells	CD3^{+}CD4^{+}CXCR5^{+}
Classical monocytes cells	CD45^{+}CD3^{-}CD19^{-}CD14^{+}CD16^{-}HLA-DR^{high}
Monocyte-like myeloid cells	CD45^{+}CD3^{-}CD19^{-}CD16^{-}CD11b^{+}HLA-DR^{-}CD14^{+}
Polymorphonuclear myeloid cells	CD45^{+}CD3^{-}CD19^{-}CD16^{-}CD11b^{+}HLA-DR^{-}CD14^{-}
CD14^{+}CD40 cells	CD14^{+}CD40^{+}

Table S2. Baseline characteristics of the study participants

Characteristics	Young group (*n* = 67)	Middle-aged group (*n* = 57)
Age (years), median (range)	31.63 (21–40)	47.39 (41–57)
Sex, *n* (%)		
Male	33 (49.3)	29 (50.9)
Female	34 (50.7)	28 (49.1)
Chronic diseases, *n* (%)		
Yes	0 (0)	0 (0)
No	67 (100)	57 (100)
Malignant tumors, *n* (%)		
Yes	0 (0)	0 (0)
No	67 (100)	57 (100)
Autoimmune diseases, *n* (%)		
Yes	0 (0)	0 (0)
No	67 (100)	57 (100)
Smoking, *n* (%)		
Yes	6 (9)	13 (22.8)
No	61 (91)	44 (77.2)

Table S3. Reference interval for circulating immune cells

Phenotype	Mean ± SD	Median (25–75%)	Reference Intervals	
			Lower (95%CI)	Upper (95% CI)
T cells (%)	–	69.70 (63.78–74.08)	50.76 (49.57–56.55)	80.31 (79.3–81.47)
T cells (cells/µL)	–	1,265.50 (1,087.75–1,567.00)	687.43 (554.70–849.55)	1,889.60 (1,798.05–2,167.10)
B cells (%)	–	10.90 (8.63–12.78)	5.5 (5.00–6.40)	16.4 (15.80–17.00)
B cells (cells/µL)	–	204.00 (163.25–262.25)	99 (96–119)	347 (323–357)
Natural killer cells (%)	–	17.25 (13.23–24.20)	8.31 (4.50–9.00)	37.76 (31.8–39.6)
Natural killer cells (cells/µL)	–	330.50 (235.00–490.75)	135.03 (81.78–160.73)	721.35 (618.90–808.50)
Classical monocyte cells (%)	–	7.17 (6.24–8.42)	4.43 (4.15–5.12)	10.89 (9.66–11.09)
Classical monocyte cells (cells/µL)	–	305.74 (238.10–370.71)	177.86 (152.86–194.68)	500.43 (475.95–551.44)
Monocyte-like myeloid cells (%)）	–	0.11 (0.50–0.17)	0	0.31 (0.23–0.32)
Monocyte-like myeloid cells (cells/µL)	–	5.50 (2.57–9.70)	0	17.19 (12.31–19.07)
Polymorphonuclear myeloid cells (%)	–	5.29 (1.32–34.64)	0.14 (0.12–0.29)	70.28 (61.05–73.58)
Polymorphonuclear myeloid cells (cells/µL)	–	220.18 (50.17–1384.34)	6.86 (5.56–12.21)	2,801.41 (2,327.06–3,016.78)
CD14$^+$CD40$^+$ cells (%)	–	1.64 (0.82–2.88)	0.03 (0.02–0.12)	4.95 (4.22–5.31)
CD14$^+$CD40$^+$ cells (cells/µL)	–	4.15 (2.30–9.02)	0.13 (0.04–0.38)	14.04 (12.72–17.10)
CD45$^+$LIN$^-$ (%)	77.37 ± 8.51	77.16 (71.40–83.46)	60.82 (57.90–66.29)	92.98 (89.08–99.26)
CD45$^+$LIN$^-$ (cells/µL)	–	3,987.41 (3,556.53–4,953.59)	2,517.79 (1,976.71–2,896.72)	6,365.28 (6,048.40–6,554.51)
CD45$^+$ (cells/µL)	–	5,282 (4,652.57–6,399.26)	3,396.03 (3,129–3,857.46)	7,759.34 (7,143.69–8,361.03)

Note: Data are presented as absolute count (cells/µL) and percentage (%).
Abbreviations: CI: Confidence interval; LIN: Lineage markers; SD: Standard deviation.

Table S4. Reference intervals for CD8$^+$ T lymphocyte subsets

Phenotype	Mean ± SD	Median (25–75%)	Reference intervals	
			Lower (95% CI)	Upper (95% CI)
CD8$^+$ T cells (%)	33.3 ± 10.0	–	15.81 (13.2–17.48)	53.9 (47.79–56.8)
CD8$^+$ T cells (cells/µL)	–	640.5 (477.0–793.0)	240 (170–324)	1,067 (1,006–1,148)
Naive CD8$^+$ T cells (%)	31.5 ± 14.1	–	6.86 (5.40–10.13)	59.66 (52.97–63.47)
Naive CD8$^+$ T cells (cells/µL)	–	169.6 (110.3–289.1)	49.37 (39.16–68.77)	454.05 (401.4–529.23)
CD8$^+$ T effector memory cells (%)	–	24.7 (19.7–32.2)	7.40 (6.81–9.58)	47.22 (41.18–49.30)
CD8$^+$ T effector memory cells (cells/µL)	–	159.9 (92.4–229.2)	42.05 (37.41–56.21)	388.34 (335.71–416.754)

(*Cont'd...*)

Table S4. (*Continued*)

Phenotype	**Mean ± SD**	**Median (25–75%)**	**Reference intervals**	
			Lower (95% CI)	**Upper (95% CI)**
CD8$^+$ T central memory cells (%)	–	10.2 (6.7–15.2)	3.91 (3.30–4.60)	22.79 (20.30–24.00)
CD8$^+$ T central memory cells (cells/μL)	–	62.0 (40.2–83.8)	27.18 (19.11–32.84)	137.33 (125.38–143.74)
HLA-DR$^+$CD8$^+$ T effector memory cells (%)	–	2.2 (1.6–4.8)	0.40 (0.00–0.69)	7.71 (7.00–8.43)
HLA-DR$^+$CD8$^+$ T effector memory cells (cells/μL)	–	3.5 (2.0–6.2)	0.64 (0.24–1.14)	9.97 (8.22–11.73)
HLA-DR$^+$CD8$^+$ T central memory cells (%)	–	2.7 (1.2–4.3)	0.30 (0.00–0.58)	7.86 (5.59–8.36)
HLA-DR$^+$CD8$^+$ T central memory cells (cells/μL)	–	1.6 (0.9–2.8)	0.19 (0–0.30)	4.67 (3.85–5.53)
CD8$^+$CD39$^+$ T cells (%)	–	1.5 (0.4–4.8)	0.10 (0.10–0.20)	9.70 (8.40–11.20)
CD8$^+$CD39$^+$ T cells (cells/μL)	–	8.7 (2.9–29.2)	0.71 (0.33–1.04)	52.47 (42.78–59.76)
CD8+PD-1+ T cells (%)	11.7 ± 6.7	–	3.440 (2.81–4.20)	22.97 (19.30–24.80)
CD8$^+$ PD-1$^+$ T cells (cells/μL)	72.8 ± 41.4	–	18.83 (13.95–28.20)	424.84 (401.31–465.67)
CD8$^+$CD28$^+$ T cells (%)	54.6 ± 15.5	–	25.71 (15.20–32.70)	82.15 (78.48–82.60)
CD8$^+$CD28$^+$ T cells (cells/μL)	–	322.7 (233.3–426.2)	145.64 (118.86–186.57)	577.33 (529.21–637.52)
CD8$^+$CD122$^+$ T cells (%)	0.02 ± 0.06	–	0	0
CD8$^+$CD122$^+$ T cells (cells/μL)	0.15 ± 0.47	–	0	0
CD8$^+$CD27$^+$ T cells (%)	–	68.8 (52.2–78.3)	34.86 (31.80–41.93)	82.15 (78.48–82.60)
CD8$^+$CD27$^+$ T cells (cells/μL)	–	391.9 (287.4–523.4)	201.54 (119.34–217.75)	751.01 (665.04–757.88)
CD8$^+$CD27$^-$CD28$^-$ T cells (%)	–	29.2 (20.0–45.6)	7.05 (3.50–9.55)	62.93 (59.24–64.3)
CD8$^+$CD27$^-$CD28$^-$ T cells (cells/μL)	–	200.0 (93.8–319.3)	26.67 (24.75–37.74)	536.41 (450.07–588.64)
CD8$^+$CX3CR1$^+$ T cells (%)	26.6 ± 16.3	–	4.01 (2.60–6.93)	58.46 (49.97–68.50)
CD8$^+$CX3CR1$^+$ T cells (cells/μL)	189.1 ± 161.3	–	18.56 (13.94–28.20)	159.25 (137.73–163.98)
CD8$^+$CX3CR1$^-$CD27$^-$ T cells (%)	–	12.6 (7.5–18.9)	2.63 (2.20–3.62)	31.36 (27.30–35.10)
CD8$^+$CX3CR1$^-$CD27$^-$ T cells (cells/μL)	–	70.7 (40.8–132.1)	13.45 (7.94–19.35)	238.21 (200.61–254.02)
CD8$^+$CX3CR1$^-$CD27$^+$ T cells (%)	–	60.1 (46.3–73.9)	26.73 (19.00V31.86)	90.05 (83.51–92.30)
CD8$^+$CX3CR1$^-$CD27$^+$ T cells (cells/μL)	–	342.0 (258.9–484.0)	146.33 (99.96–185.03)	671.44 (621.08–711.69)
CD8$^+$CD137$^+$ T cells (%)	–	0.06 (0.00–0.18)	0	0.4 (0.30–0.41)
CD8$^+$CD137$^+$ T cells (cells/μL)	–	0.36 (0.00–1.24)	0	2.57 (2.18;2.74)
CD8$^+$CXCR5$^+$ T cells (%)	–	0.58 (0.27–1.10)	0	1.84 (1.65;2.12)
CD8$^+$CXCR5 $^+$T cells (cells/μL)	–	3.8 (1.76–6.58)	0	11.36 (9.85;12.43)

Note: Data are presented as absolute count (cells/μL) and percentage (%).
Abbreviations: CI: Confidence interval; SD: Standard deviation.

Table S5. Reference intervals for CD4$^+$ T lymphocyte subsets

Phenotype	**Mean ± SD**	**Median (25–75%)**	**Reference intervals**	
			Lower (95% CI)	**Upper (95% CI)**
CD4$^+$ T cells (%)	34.4 ± 7.3	–	20.81 (19.90–26.00)	48.06 (45.3–53.27)
CD4$^+$ T cells (cells/μL)	–	669.5 (535.3–766.5)	341.95 (226.48–411.13)	974.03 (914.6–1,017.28)
Naive CD4$^+$ T cells (%)	44.3 ± 11.4	–	24.62 (23.30–26.70)	65.55 (59.67–67.90)
Naive CD4$^+$ T cells (cells/μL)	–	296.7 (200.5–380.8)	96.74 (73.73–134.04)	515.46 (445.86–554.23)
CD4$^+$ T effector memory cells (%)）	–	13.9 (10.5–19.0)	5.06 (4.60–6.90)	26.48 (24.52–30.10)
CD4$^+$ T effector memory cells (cells/μL)	–	91.3 (70.8–120.6)	36.81 (29.74–41.71)	177.97 (152.10–181.82)
CD4$^+$ T central memory cells (%)	35.6 ± 8.8	–	20.95 (18.80–24.31)	54.10 (51.40–55.40)
CD4$^+$ T central memory cells (cells/μL)	–	233.0 (173.8–284.1)	97.60 (80.97–139.02)	406.31 (335.65–426.97)
HLA-DR$^+$CD4$^+$ T effector memory cells (%)	–	1.2 (0.8–2.1)	0.20 (0.00–0.30)	3.01 (2.70–3.51)
HLA-DR$^+$CD4$^+$ T effector memory cells (cells/μL)	–	1.2 (0.7–1.8)	0.17 (0–0.29)	2.87 (2.43–2.96)
HLA-DR$^+$CD4$^+$ T central memory cells (%)	–	0.60 (0.4–1.0)	0.10 (0.10–0.20)	1.60 (1.40–1.70)
HLA-DR$^+$CD4$^+$ T central memory cells (cells/μL)	–	1.5 (0.9–2.3)	0.21 (0.14–0.42)	3.60 (3.34–3.94)
Treg cells (%)	–	6.2 (5.4–7.1)	3.89 (3.19–4.19)	9.00 (7.90–9.12)
Treg cells (cells/μL)	–	40.7 (30.7–51.8)	19.57 (9.08–22.76)	76.57 (64.94–79.35)
CD4$^+$CD278$^+$ T cells (%)	–	6.2 (4.0–8.9)	1.76 (1.68–2.58)	14.01 (12.78–15.04)
CD4$^+$CD278$^+$ T cells (cells/μL)	48.3 ± 42.1	–	10.10 (7.90–16.88)	91.33 (82.81–98.53)
CD4$^+$CXCR3$^+$ T cells (%)	–	33.1(27.9–38.5)	19.74 (17.49–23.37)	51.61 (46.34–52.63)
CD4$^+$CXCR3$^+$ T cells (cells/μL)	–	215.6 (166.9–280.1)	97.63 (71.4–129.71)	395.69 (351.86–431.48)
CD4$^+$CXCR5$^+$ T cells (%)	16.0 ± 5.2	–	8.42 (5.71–9.66)	26.71 (23.41–27.15)
CD4$^+$CXCR5$^+$ T cells (cells/μL)	–	99.4 (74.7–130.8)	44.72 (39.56–55.02)	194.37 (176.56–201.68)

Note: Data are presented as absolute count (cells/μL) and percentage (%).
Abbreviations: CI: Confidence interval; SD: Standard deviation.

Table S6. Model performance comparison across algorithms

Model	**BestParams**	**MAE**	R^2
Gradient boosting regressor	{learning_rate: 0.1, max_depth: 5, min_samples_split: 4, n_estimators: 100, subsample: 0.8}	6.295	0.491
Random forest	{max_depth: 10, min_samples_split: 5, n_estimators: 100}	6.365	0.488
Linear regression	None	6.432	0.393
Ridge	{alpha: 10.0}	6.529	0.391
Support vector regression	{C: 1, epsilon: 0.01, kernel: linear}	6.552	0.408
K-nearest neighbors	{n_neighbors: 9, weights: distance}	6.930	0.418

Abbreviations: MAE: Mean absolute error; R^2: Coefficient of determination.

Table S7. Comparison of percentages and absolute counts of 36 detected circulating immune cells between genders

Phenotype	Mean ± SD (male)	Mean ± SD (female)	*p*-value
T cells (%)	65.0 ± 11.9	69.8 ± 6.9	0.025
T cells (cells/μL)	1359.9 ± 458.1	1327.1 ± 348.8	0.778
B cells (%)	11.1 ± 4.2	11.2 ± 2.7	0.234
B cells (cells/μL)	225.8 ± 91.6	213.4 ± 79.5	0.412
Natural killer cells (%)	22.0 ± 11.0	17.0 ± 7.6	0.005
Natural killer cells (cells/μL)	453.2 ± 261.9	336.7 ± 228.8	<0.001
$CD8^+$ T cells (%)	33.5 ± 11.6	33.4 ± 8.1	0.937
$CD8^+$ T cells (cells/μL)	708.9 ± 350.8	627.9 ± 191.0	0.113
Naive $CD8^+$ T cells (%)	30.1 ± 13.6	32.9 ± 14.5	0.272
Naive $CD8^+$ T cells (cells/μL)	200.8 ± 128.2	210.7 ± 119.5	0.589
$CD8^+$ T effector memory cells (%)	27.5 ± 12.1	25.8 ± 11.9	0.214
$CD8^+$ T effector memory cells (cells/μL)	193.2 ± 132.8	162.6 ± 95.8	0.187
$CD8^+$ T central memory cells (%)	11.6 ± 6.0	11.2 ± 6.1	0.687
$CD8^+$ T central memory cells (cells/μL)	78.9 ± 62.1	64.5 ± 25.9	0.928
$HLA\text{-}DR^+CD8^+$ T effector memory cells (%)	3.3 ± 2.8	3.6 ± 3.8	0.968
$HLA\text{-}DR^+CD8^+$ T effector memory cells (cells/μL)	21.2 ± 20.3	21.6 ± 20.5	0.678
$HLA\text{-}DR^+CD8^+$ T central memory cells (%)	3.9 ± 3.6	3.1 ± 2.8	0.173
$HLA\text{-}DR^+CD8^+$ T central memory cells (cells/μL)	26.4 ± 27.2	19.7 ± 20.8	0.166
$CD8^+CD39^+$ T cells (%)	3.3 ± 3.8	2.8 ± 3.1	0.635
$CD8^+CD39^+$ T cells (cells/μL)	19.1 ± 20.6	15.6 ± 16.3	0.483
$CD8^+PD\text{-}1^+$ T cells (%)	12.8 ± 7.2	10.6 ± 6.0	0.082
$CD8^+PD\text{-}1^+$ T cells (cells/μL)	81.4 ± 43.7	64.2 ± 37.3	0.016
$CD8^+CD122^+$ T cells (%)	0.018 ± 0.046	0.031 ± 0.067	0.247
$CD8^+CD122^+$ T cells (cells/μL)	0.10 ± 0.30	0.21 ± 0.58	0.210
$CD8^+CD28^+$ T cells (%)	54.5 ± 16.0	54.8 ± 15.1	0.938
$CD8^+CD28^+$ T cells (cells/μL)	363.3 ± 172.9	339.5 ± 132.6	0.614
$CD8^+CD27^+$ T cells (%)	64.4 ± 14.9	67.4 ± 16.8	0.308
$CD8^+CD27^+$ T cells (cells/μL)	430.8 ± 190.6	421.9 ± 170.3	0.849
$CD8^+CD27^-CD28^-$ T cells (%)	34.1 ± 16.1	30.7 ± 16.7	0.292
$CD8^+CD27^-CD28^-$ T cells (cells/μL)	270.2 ± 234.7	195.9 ± 125.5	0.114
$CD8^+CX3CR1^+$ T cells (%)	30.8 ± 16.4	22.4 ± 15.3	0.002
$CD8^+CX3CR1^+$ T cells (cells/μL)	233.9 ± 191.1	144.2 ± 108.8	0.008
$CD8^+CX3CR1^-CD27^-$ T cells (%)	13.2 ± 8.5	15.3 ± 9.4	0.167

(*Cont'd...*)

Table S7. (*Continued*)

Phenotype	Mean ± SD (male)	Mean ± SD (female)	*p*-value
CD8$^+$CX3CR1$^-$CD27$^-$ T cells (cells/μL)	102.3 ± 105.7	94.0 ± 62.5	0.418
CD8$^+$CX3CR1$^-$CD27$^+$ T cells (%)	56.1 ± 16.4	62.5 ± 17.8	0.045
CD8$^+$CX3CR1$^-$CD27$^+$ T cells (cells/μL)	370.2 ± 172.2	391.0 ± 165.7	0.506
CD8$^+$CD137$^+$ T cells (%)	0.135 ± 0.201	0.194 ± 0.312	0.512
CD8$^+$CD137$^+$ T cells (cells/μL)	0.86 ± 1.20	1.02 ± 1.48	0.522
CD8$^+$CXCR5$^+$ T cells (%)	1.01 ± 1.38	0.83 ± 0.82	0.891
CD8$^+$CXCR5$^+$ T cells (cells/μL)	5.9 ± 6.2	4.7 ± 4.3	0.531
CD4$^+$ T cells (%)	31.9 ± 7.4	36.9 ± 6.4	<0.001
CD4$^+$ T cells (cells/μL)	659.8 ± 212.7	707.8 ± 230.6	0.326
Naive CD4$^+$ T cells (%)	43.0 ± 11.5	45.5 ± 11.2	0.269
Naive CD4$^+$ T cells (cells/μL)	291.0 ± 140.3	328.2 ± 150.8	0.184
CD4$^+$ T effector memory cells (%)	15.3 ± 6.7	15.1 ± 6.2	0.875
CD4$^+$ T effector memory cells (cells/μL)	95.9 ± 42.3	103.7 ± 55.2	0.519
CD4$^+$ T central memory cells (%)	37.1 ± 8.8	34.1 ± 8.6	0.059
CD4$^+$ T central memory cells (cells/μL)	242.9 ± 97.4	237.2 ± 84.4	0.972
HLA-DR$^+$CD4$^+$ T effector memory cells (%)	1.57 ± 1.30	1.49 ± 1.07	0.958
HLA-DR$^+$CD4$^+$ T effector memory cells (cells/μL)	9.87 ± 7.66	10.40 ± 8.87	0.859
HLA-DR$^+$CD4$^+$ T central memory cells (%)	0.73 ± 0.47	0.73 ± 0.46	0.950
HLA-DR$^+$CD4$^+$ T central memory cells (cells/μL)	4.68 ± 3.04	5.04 ± 3.52	0.666
Treg (%)	6.36 ± 1.49	6.20 ± 1.76	0.173
Treg (cells/μL)	42.5 ± 17.3	43.3 ± 16.7	0.791
CD4$^+$CD278$^+$ T cells (%)	7.00 ± 3.60	7.46 ± 6.24	0.930
CD4$^+$CD278$^+$ T cells (cells/μL)	44.5 ± 24.67	52.0 ± 54.2	0.693
CD4$^+$CXCR3$^+$ T cells (%)	32.6 ± 7.7	35.6 ± 8.85	0.035
CD4$^+$CXCR3$^+$ T cells (cells/μL)	214.48 ± 82.45	244.15 ± 79.8	0.026
CD4$^+$CXCR5$^+$ T cells (%)	16.16 ± 5.08	15.8 ± 5.28	0.626
CD4$^+$CXCR5$^+$ T cells (cells/μL)	104.35 ± 40.0	109.28 ± 44.34	0.814
Classical monocyte cells (%)	7.69 ± 1.71	7.41 ± 2.31	0.150
Classical monocyte cells (cells/μL)	433.50 ± 115.41	390.00 ± 142.00	0.005
Monocyte-like myeloid cells (%)	0.163 ± 0.155	0.154 ± 0.213	0.086
Monocyte-like myeloid cells (cells/μL)	9.49 ± 10.25	9.56 ± 18.32	0.047
Polymorphonuclear myeloid cells (%)	21.46 ± 23.10	14.30 ± 21.61	0.010
Polymorphonuclear myeloid cells (cells/μL)	1,217.42 ± 1,366.43	832.30 ± 1389.77	0.006
CD14$^+$CD40 cells (%)	2.48 ± 4.07	2.59 ± 2.75	0.397
CD14$^+$CD40 cells (cells/μL)	140.13 ± 234.54	137.55 ± 156.73	0.712
CD45$^+$LIN$^-$ cells (%)	77.24 ± 9.64	77.36 ± 7.28	0.935
CD45$^+$LIN$^-$ cells (cells/μL)	4,438.89 ± 1,192.72	4,133.00 ± 1,188.09	0.053
CD45$^+$ cells (cells/μL)	5,708.04 ± 1,167.37	5322.36 ± 1,343.08	0.027

Abbreviation: SD: Standard deviation.

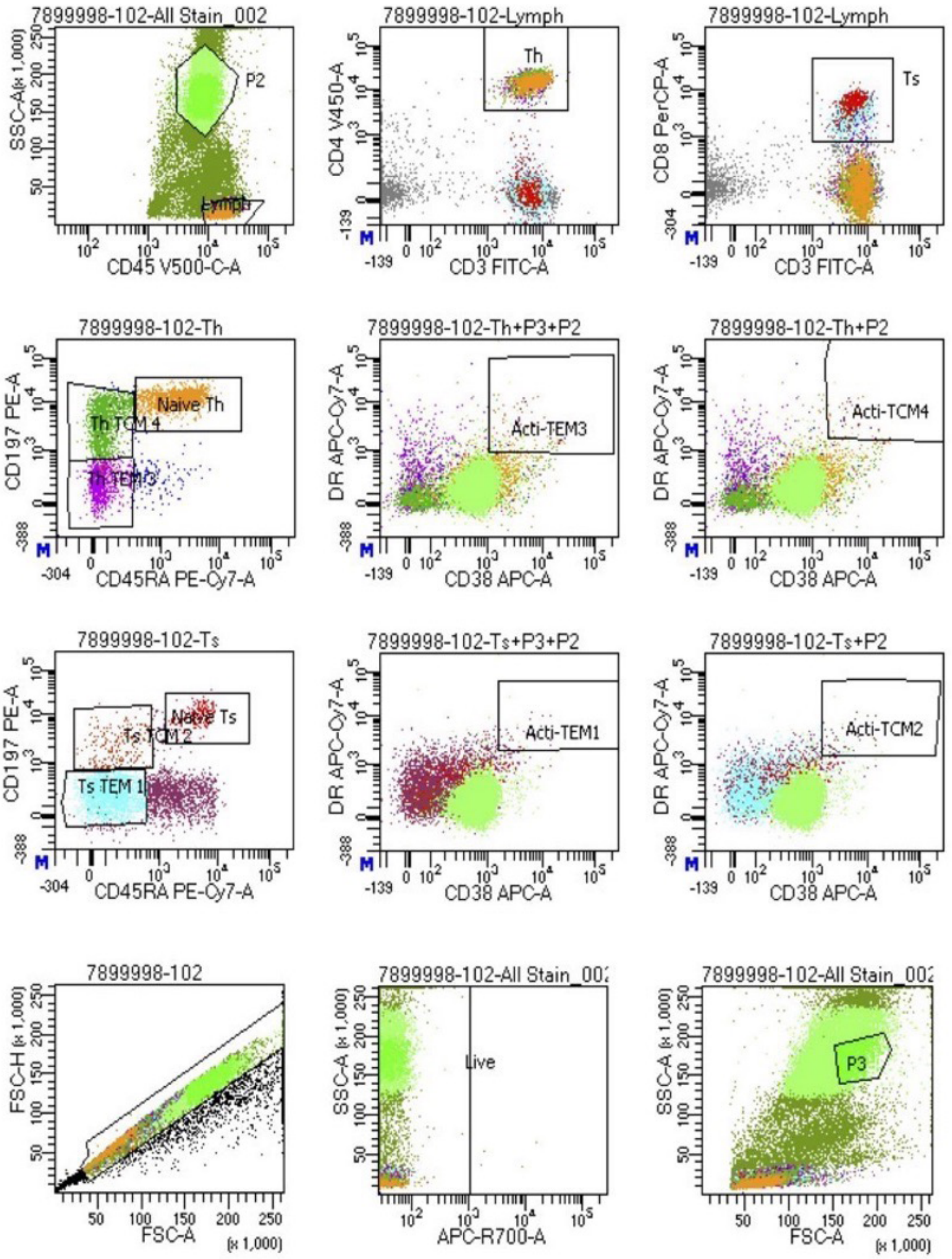

Figure S1. Gating strategy for T-cell differentiation and memory phenotypes. Within the CD3⁺ T cell population, CD8⁺ T cells (labeled as Ts in the figure) and CD4⁺T cells (labeled as Th in the figure) were first identified. T cell differentiation subsets were then preliminarily distinguished using CD45RA and CD197 (CCR7) expression. Taking CD3⁺CD4⁺ T cells as an example, the subsets were defined as follows: naive T cells (TN) as CD45RA⁺, effector memory T cells (TEM) as CD45RA–CD197⁻, and central memory T cells (TCM) as CD45RA⁻CD197⁺. Following the identification of TCM and TEM populations, activated T-cell subsets were further delineated using the activation markers HLA-DR and CD38.

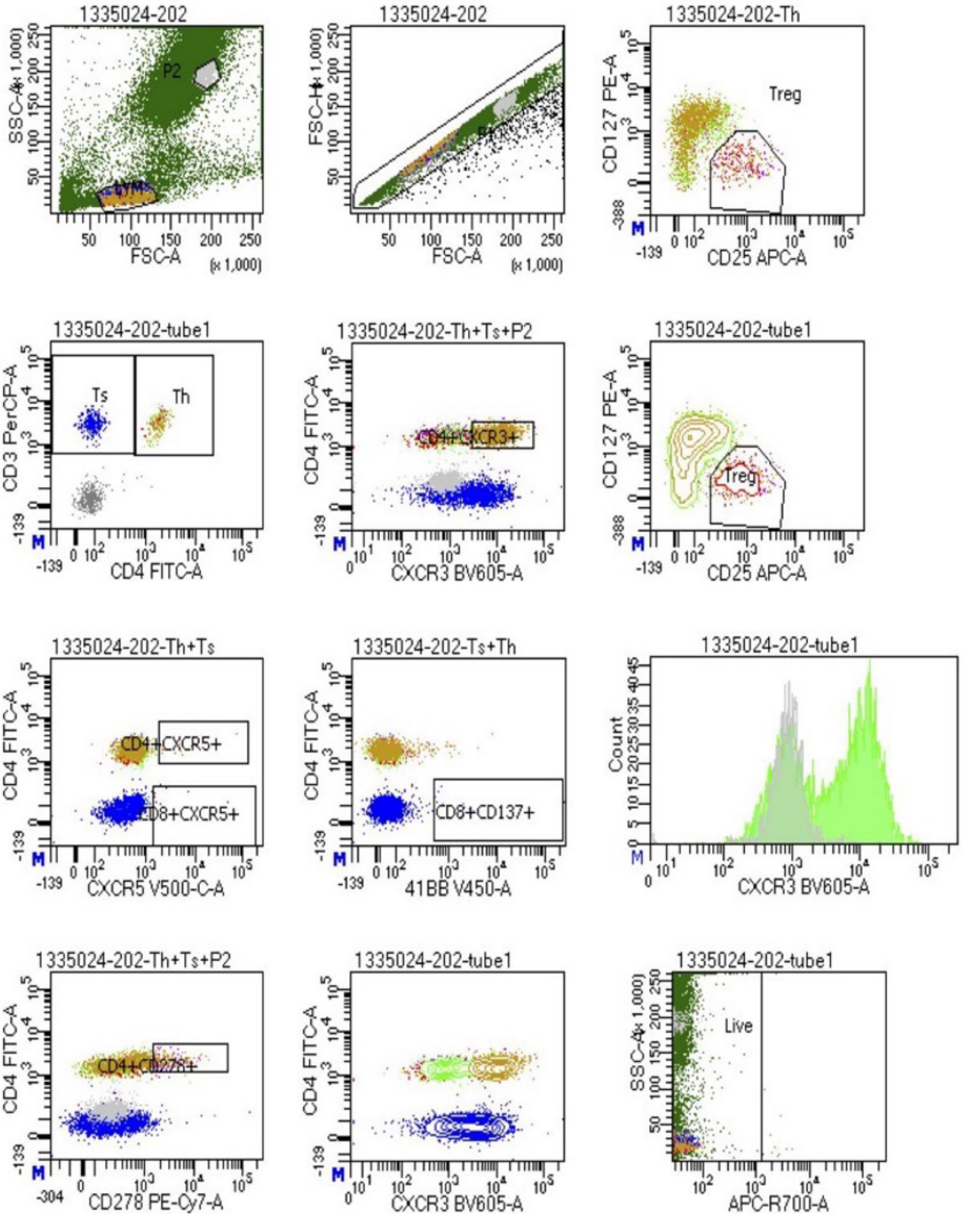

Figure S2. Gating strategy for regulatory T cells (Tregs) and other T-cell subsets. Within the CD3⁺ T cell population, CD3⁺CD4⁺ T cells (Th) and CD3⁺CD8⁺ T cells (Ts) were identified. Taking the CD4⁺ population as a representative example, regulatory T cells (Tregs) were defined by the CD4⁺CD25⁺CD127⁻ phenotype. Additionally, various specific T-cell subsets were further delineated using functional markers such as CD137 and CXCR5.

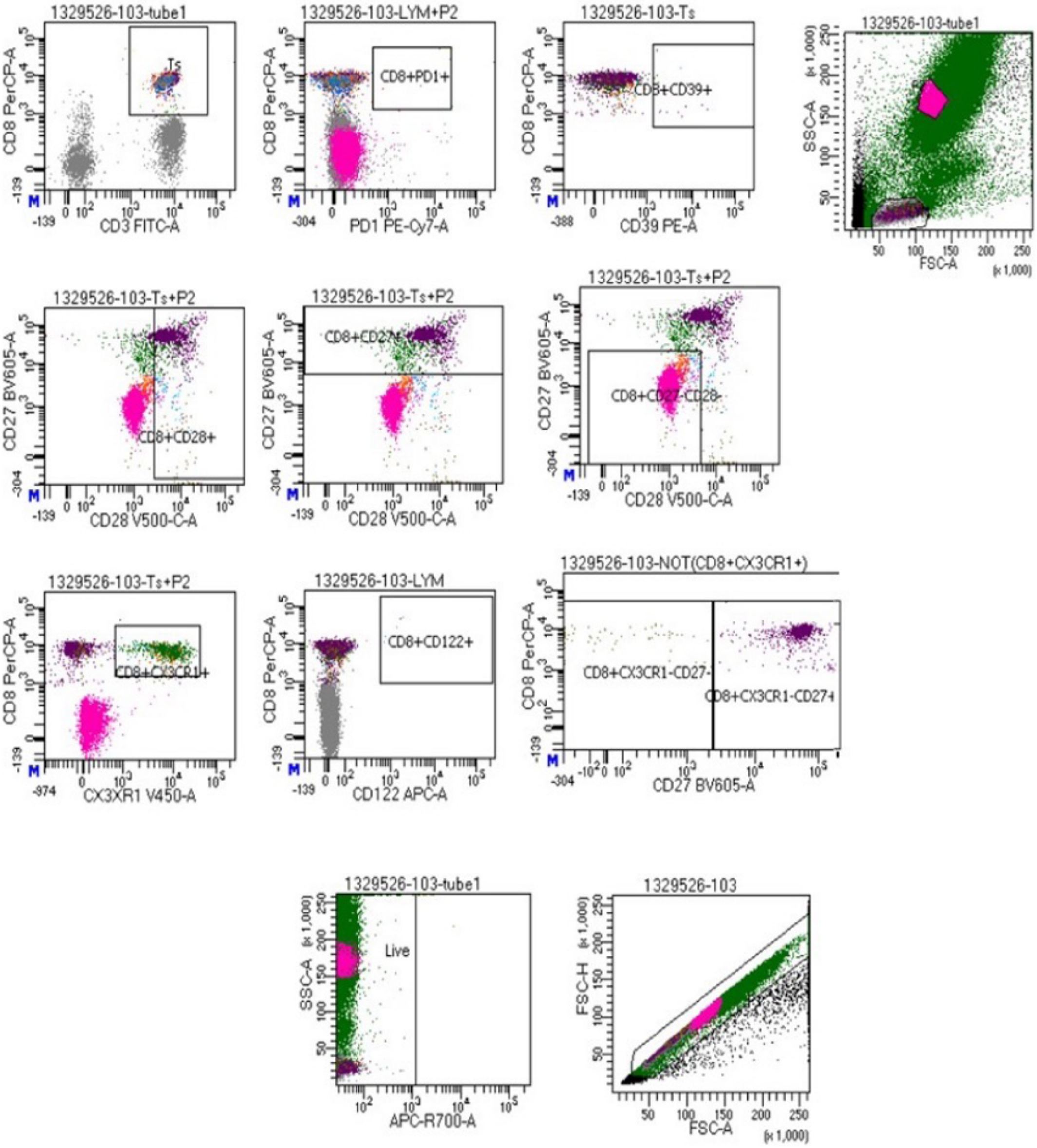

Figure S3. Gating strategy for specialized T cell subsets. Within the CD3$^+$T cell population, CD3$^+$CD8$^+$T cells (Ts) were first identified. Various functional subsets were then delineated based on the positive expression of CD39, PD-1, CD122, and CX3CR1, respectively. Concurrently, differentiation states were characterized using CD27 and CD28 to define CD8$^+$CD28$^+$ and CD8$^+$CD27$^+$ populations, as well as the terminally differentiated CD8$^+$CD28$^-$CD27$^-$ subset. Furthermore, specialized subpopulations were subfractionated by combining CX3CR1 and CD27 expression to isolate CD8$^+$CX3CR1$^-$CD27$^-$ and CD8$^+$CX3CR1$^-$CD27$^+$ T cells.

Figure S4. Correlation between peripheral immune parameters and chronological age. The horizontal bar chart illustrates the Pearson correlation coefficients for 62 immune-cell measurements in relation to age. Red bars indicate positive correlations, while blue bars indicate negative correlations. All *p*-values were adjusted for multiple comparisons using the Benjamini–Hochberg (false discovery rate [FDR]) method. Significance levels after FDR correction are indicated by asterisks: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.