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CD4:8 ratio  $>5$  is associated with a dominant naive t-cell phenotype and impaired physical functioning in CMV-seropositive very elderly people : results from the BELFRAIL study

**Reference:**

Adriaensen Wim, Derhovanesian Evelynna, Vaes Bert, Van Pottelbergh Gijs, Degryse Jean-Marie, Pawelec Graham, Hamprecht Klaus, Theeten Heidi, Matheï Catharina.- CD4:8 ratio  $>5$  is associated with a dominant naive t-cell phenotype and impaired physical functioning in CMV-seropositive very elderly people : results from the BELFRAIL study  
Journals of gerontology : series A : biological sciences and medical sciences - ISSN 1079-5006 - 70:2(2015), p. 143-154  
Full text (Publishers DOI): <http://dx.doi.org/doi:10.1093/gerona/glu018>  
To cite this reference: <http://hdl.handle.net/10067/1249980151162165141>

**CD4:8 ratio > 5 is associated with a dominant naïve T-cell phenotype and impaired physical functioning in CMV-seropositive very elderly: results from the BELFRAIL study.**

<b>Journal:</b>	Journal of Gerontology: Biological Sciences
<b>Manuscript ID:</b>	JGBS-2013-123.R3
<b>Manuscript Type:</b>	Original Article
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<b>Keywords:</b>	CD4:8 ratio, T-cell subsets, physical functioning, very elderly, Cytomegalovirus
<b>Subject:</b>	Immunosenescence, CMV, Biogerontology, T-cells, Phenotype

























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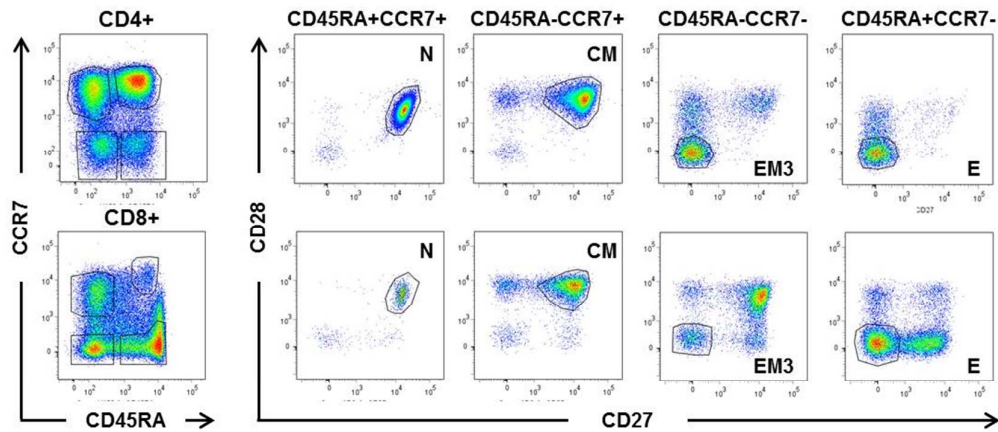




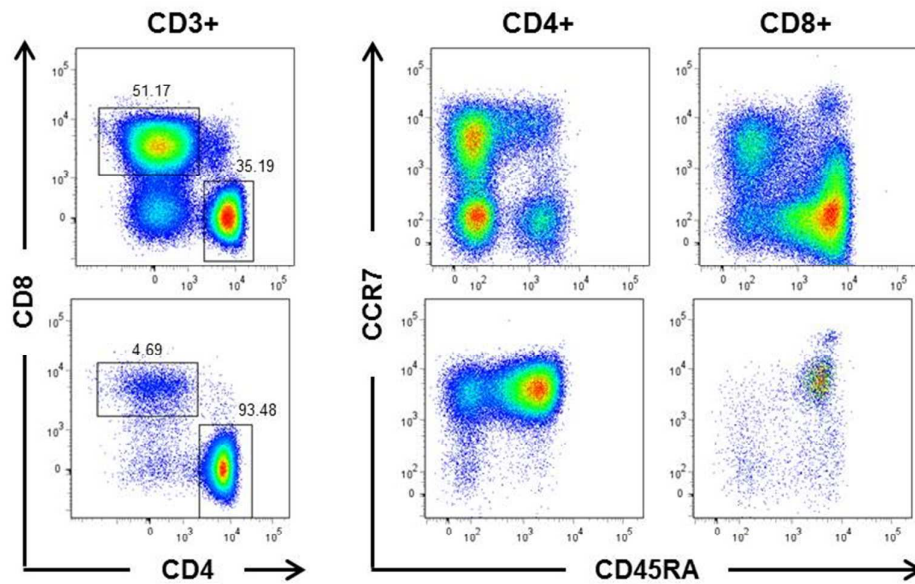






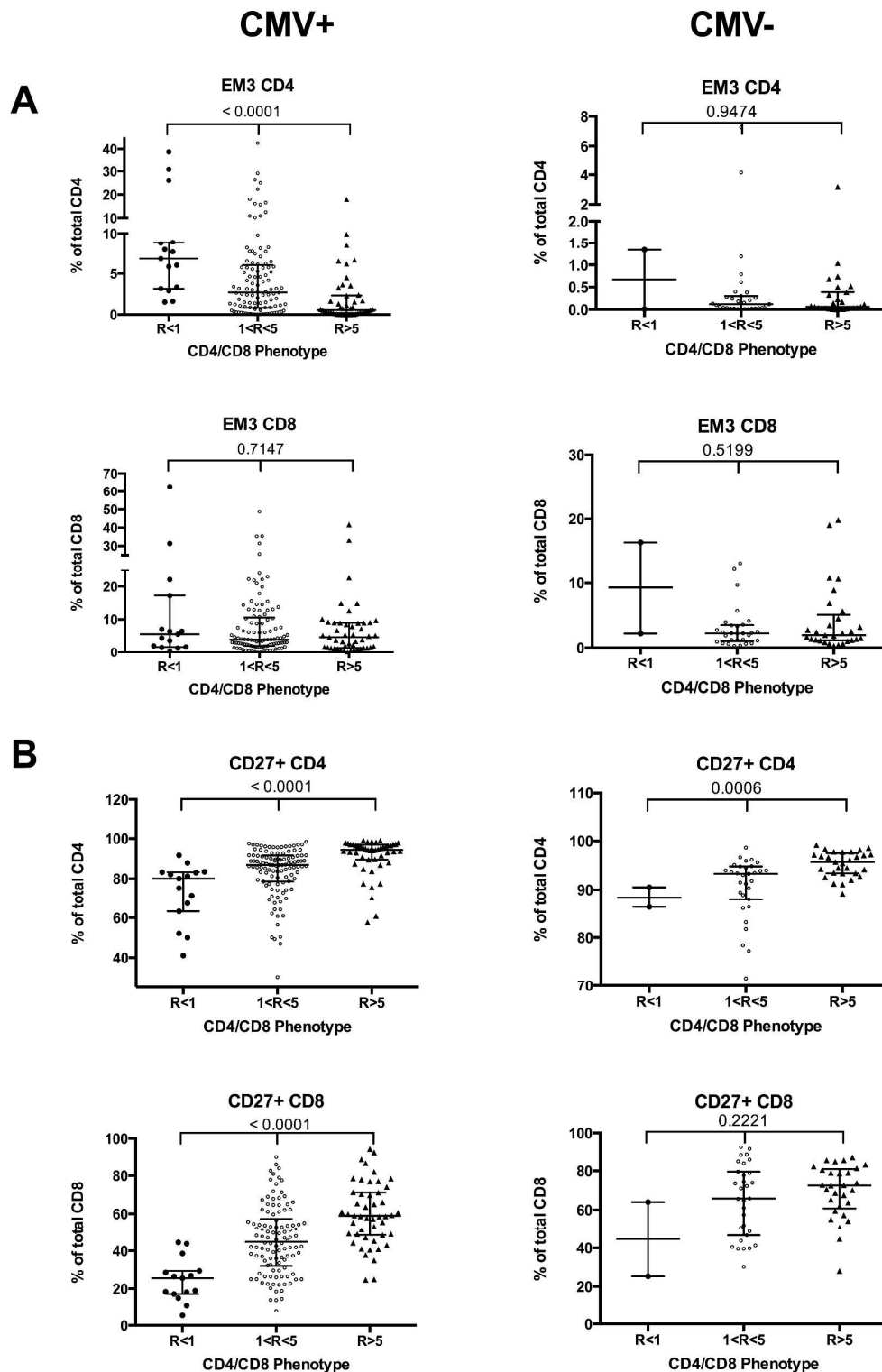


**Figure S2.** Gating strategy for characterization of the different T-cell phenotypes in peripheral blood. CD45RA expression was first plotted against CCR7 on CD4+ (upper row) and CD8+ (lower row) cells (left panel). Cells carrying both, only one or none of these markers were then analyzed for surface expression of CD27 and CD28 (right block). Naïve cells (N) were characterized as CD45RA+CCR7+CD27+CD28+, central memory cells (CM) as CD45RA-CCR7+CD27+CD28+, effector memory 3 (EM3) population as CD45RA-CCR7-CD27-CD28- and effector cells (E) as CD45RA+CCR7-CD27-CD28-. Labels above each plot represent the gated population.

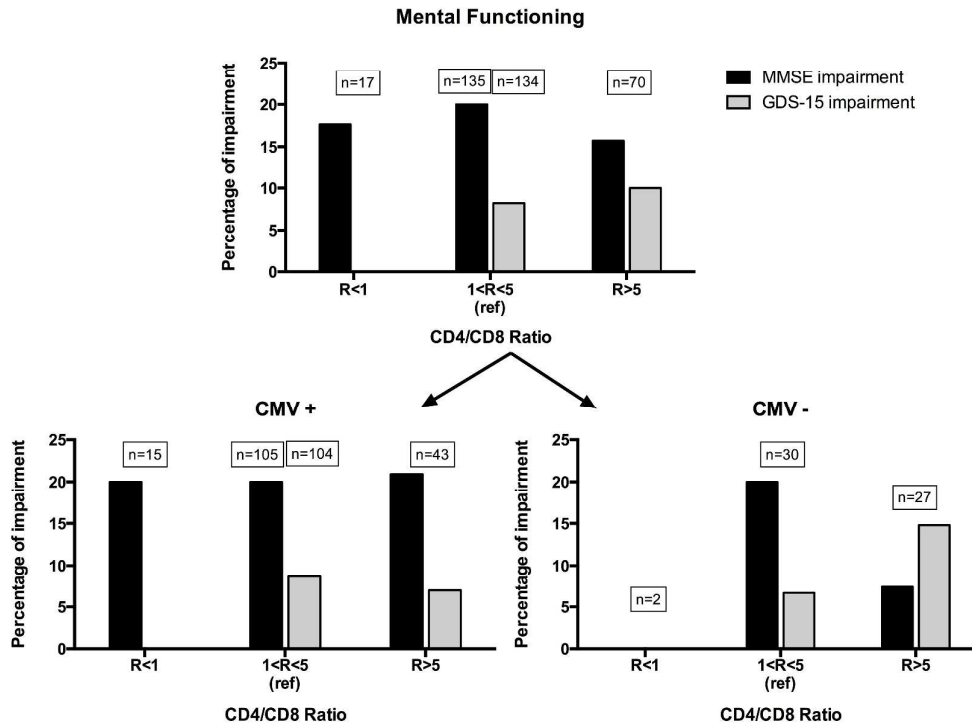


**Figure S3.** Representative FACS plots of a donors with  $R < 1$  (upper row) and  $R > 5$  (lower row). The distribution of different T-cell phenotypes based on the surface expression of CD45RA and CCR7 are shown in the right block.

Labels above each plot represent the gated population.



**Figure S4.** Expression dot plots (median and IQR) of effector-memory 3 (CD45RA-CCR7-CD27-CD28-) T-cells (A) and co-stimulatory maker CD27 (B), relative to CMV serostatus.  $p$ -value from Kruskal-Wallis test to compare between three phenotypes.



**Figure S5.** Percentage of mental impairment (MMSE and GDS-15) according to their CD4:8 ratio phenotype and CMV serostatus. (\*p<0.05)

**Table 1:** Demographics of the study and control population, based on their CMV serostatus

Old	General	CMV +	CMV -	<i>p-value</i>
	( <i>n</i> =235)	( <i>n</i> =173)	( <i>n</i> =62)	
<b>Age, mean ± SD</b>	86.7 ± 3.8	86.8 ± 3.8	86.4 ± 3.9	0.530
<b>Women, n (%)</b>	158 (67.2)	123 (71.1)	35 (56.5)	0.035
<b>High Education, n (%)</b>	27 (11.5)	16 (9.3)	11 (17.7)	0.075
<b>BMI, mean ± SD</b>	21.7 ± 3.6	21 ± 3.38	22.4 ± 4.1	0.090
<b>Ever smoked, n (%)</b>	64 (27.23)	43 (24.9)	21 (33.9)	0.171
<b>Non-CV comorbidity, median (IQR)</b>	1 (1-2)	1 (1-2)	1 (1-2)	0.200
<b>CV comorbidity, median (IQR)</b>	3 (2-4)	2 (2-3)	3 (2-4)	0.306
<b>Institutionalized, n (%)</b>	21 (8.94)	17 (9.83)	4 (6.45)	0.424
Young	General	CMV +	CMV -	<i>p-value</i>
	( <i>n</i> =25)	( <i>n</i> =3)	( <i>n</i> =22)	
<b>Age, mean ± SD</b>	28.5 ± 6.1	37.8 ± 11.6	27.2 ± 0.9	0.003
<b>Women, n (%)</b>	15 (60)	2 (66.7)	13 (59.1)	1

**Table 2:** CD4:8 ratio distribution in older persons

<b>Variables</b>	<b><i>R</i> &lt; 1</b>	<b>1 &gt; <i>R</i> &lt; 5</b>	<b><i>R</i> &gt; 5</b>	<b><i>P</i>-value</b>
	<i>(n=17, 7,2%)</i>	<i>(n=141, 60%)</i>	<i>(n=77, 32,8%)</i>	
<b>Age, mean ± SD</b>	87.7 ± 3.4	86.8 ± 3.9	86.4 ± 3.7	0.24
<b>Women, n (%)</b>	12 (70.6)	96 (68.1)	50 (65)	0.85
<b>CMV IgG+, n (%)</b>	15 (88.2)	110 (78)	48 (62.3)	0.016
<b>CD4+ /CD3, median (IQR)</b>	64.3 (45-80.11)	45.3 (29.5-78)	53.7 (39-74.5)	0.24
<b>N /CD4</b>	24.5 (10.9-37.8)	34.4 (24.3-46.2)	55.7 (40.3-64.9)	<0.001
<b>EM3 /CD4</b>	6.04 (2.9-8.7)	1.49 (0.24-5.3)	0.27 (0.04-1.04)	<0.001
<b>E /CD4</b>	0.62 (0.19-4.6)	0.4 (0.03-1.51)	0.08 (0.01-0.44)	0.003
<b>CD8+ /CD3, median (IQR)</b>	39.5 (24.8-46.1)	11.6 (7.2-18.3)	5.49 (3.58-8.15)	<0.001
<b>N /CD8</b>	0.78 (0.44-2.58)	3.67 (1.6-8.4)	10.4 (4.9-22.3)	<0.001
<b>EM3 /CD8</b>	5.55 (2-16.4)	3.36 (1.8-8.79)	2.98 (1.34-8.7)	0.276
<b>E /CD8</b>	50.4 (37.8-60.3)	30.7 (16-42.1)	14.8 (8.3-26.4)	<0.001

*R*: CD4/CD8 ratio ; *N*: Naive ; *CM*: Central memory ; *EM3*: Effector memory 3; *E*: Effector

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**Table 3:** Odds ratios of multivariate logistic regression analysis between physical functioning and high CD4:8 ratio phenotype, stratified for CMV infection.

CMV+	Unadjusted model			Model 1			Model 2		
	OR (CI)	C-stat	P-value	OR (CI)	C-stat	P-value	OR (CI)	C-stat	P-value
<b>SPPB impairment</b>	2.17 (1.03-4.57)	0.58	0.042	2.42 (1.12-5.26)	0.71	0.025	2.33 (1.05-5.18)	0.75	0.038
Age cat				2.41 (1.52-3.81)		<0.001	2.24 (1.37-3.66)		0.001
Gender				0.68 (0.29-1.6)		0.374	0.8 (0.32-1.99)		0.632
Batch							0.98 (0.91-1.06)		0.674
Number of CV							1.05 (0.84-1.32)		0.638
Number of non CV							1.4 (1.05-1.88)		0.024
<b>ADL impairment</b>	2.67 (1.27-5.8)	0.61	0.013	2.9 (1.3-6.47)	0.69	0.009	2.72 (1.2-6.18)	0.72	0.016
Age cat				2 (1.26-3.17)		0.003	1.89 (1.16-3.09)		0.011
Gender				0.95 (0.4-2.24)		0.901	1.06 (0.42-2.66)		0.897
Batch							0.98 (0.9-1.06)		0.633
Number of CV							1.12 (0.89-1.4)		0.331
Number of non CV							1.26 (0.95-1.68)		0.112
<b>CMV-</b>									
<b>SPPB impairment</b>	1.36 (0.44-4.26)	0.54	0.595	1.69 (0.48-5.88)	0.75	0.412	1.41 (0.38-5.24)	0.78	0.610
Age cat				2.82 (1.16-6.84)		0.022	2.87 (1.18-6.98)		0.02
Gender				1.48 (0.41-5.37)		0.555	1.83 (0.47-7.18)		0.386
Batch							1.06 (0.93-1.2)		0.416
Number of CV							1.37 (1.02-1.84)		0.04
Number of non CV							1.08 (0.62-1.9)		0.783
<b>ADL impairment</b>	1.02 (0.33-3.18)	0.50	0.970	1.22 (0.36-4.15)	0.74	0.750	1.01 (0.29-3.5)	0.77	0.984
Age cat				2.68 (1.16-6.21)		0.022	2.69 (1.2-6.02)		0.016
Gender				1.05 (0.3-3.73)		0.938	1.2 (0.31-4.64)		0.792
Batch							1.05 (0.93-1.19)		0.416
Number of CV							1.3 (0.94-1.78)		0.109
Number of non CV							1.05 (0.6-1.81)		0.876

OR: Odds Ratio ; CI: Confidence interval

Model 1: age categories, gender

Model 2: age categories, gender, batch, number of cardiovascular diseases and number of non-cardiovascular diseases

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**Table S1:** Comparison between the general and study population of the BELFRAIL study at baseline.

	<b>General</b>	<b>Study</b>	
	(n=567)	(n=235)	p-value
<b>Age</b> , mean $\pm$ SD	84.7 $\pm$ 3.7	84.8 $\pm$ 3.7	0.723
<b>Women</b> , n(%)	356 (62.8)	158 (67.2)	0.232
<b>Low Education</b> , n(%)	391 (69.7)	160 (68.38)	0.713
<b>BMI</b> , mean + SD	27.4 $\pm$ 4.9	27.6 $\pm$ 4.4	0.341
<b>Ever smoked</b> , n(%)	179 (31.8)	64 (27.2)	0.202
<b>Non-CV morbidity</b> , median (IQR)	1 (1-3)	1 (1-2)	0.003
<b>CV morbidity</b> , median (IQR)	3 (2-4)	2 (2-4)	0.253
<b>Institutionalized</b> , n (%)	57 (10)	21 (8.9)	0.627
<b>ADL</b> , mean $\pm$ SD	23.5 $\pm$ 5.5	23.9 $\pm$ 5.1	0.519
<b>SPPB</b> , mean $\pm$ SD	8.35 $\pm$ 3.6	7.9 $\pm$ 3.6	0.085
<b>MMSE</b> , mean $\pm$ SD	26.5 $\pm$ 3.9	26.9 $\pm$ 3.3	0.144
<b>GDS-15</b> , mean $\pm$ SD	2.9 $\pm$ 2.3	2.5 $\pm$ 2.2	0.006

**Table S2:** Distribution of T-cell phenotypes in study and control groups, in relation to CMV serostatus

T-cell subsets	OLD				YOUNG			
	General (n=235)	CMV + (n=173)	CMV - (n=62)	p-value	General (n=25)	CMV + (n=3)	CMV - (n=22)	p-value
<i>ratio , median (IQR)</i>								
<b>CD4:8</b>	3.34 (2.09-5.82)	3.08 (1.96-5.15)	4.68 (2.89-7.36)	<0.001	2.3 (2.08-2.59)	2.3 (0.9-3.23)	2.31 (2.08-2.59)	1.000
<b>Differentiation Index CD8</b>	6.36 (1.89-22.5)	8.09 (2.58-28)	2.7 (0.74-9.63)	<0.001	0.08 (0.03-0.12)	0.3 (0.12-4.07)	0.07 (0.03-0.12)	0.029
<b>Differentiation Index CD4</b>	0.04 (0.01-0.2)	0.1 (0.02-0.31)	0 (0-0.01)	<0.001	0 (0-0)	0.04 (0.01-0.09)	0 (0-0)	<0.001
<i>Percentage, median (IQR)</i>								
<b>CD4 /CD3</b>	51.9 (33.5-78)	50.3 (32-77.6)	54.8 (35.9-78.7)	0.420	84.8 (83.3-86.8)	84.2 (80.8-84.9)	85.2 (83.3-86.8)	0.320
<b>CD27 /CD4</b>	90.5 (83.3-95.1)	88.3 (80-94.3)	93.9 (91.2-96.2)	<0.001	96.7 (94.8-97.5)	93.9 (89.4-95.3)	97.1 (95.3-97.7)	0.03
<b>CD28 /CD4</b>	97.6 (91.7-99.2)	95.5 (89.4-98.6)	99.2 (98.6-99.7)	<0.001	99.8 (99.6-99.8)	97.2 (94.6-98.8)	99.8 (99.7-99.9)	0.006
<b>CD57 /CD4</b>	1.66 (0.39-5.76)	3.24 (0.95-7.52)	0.29 (0.12-0.65)	<0.001	0.12 (0.08-0.3)	1.73 (1.15-4.99)	0.11 (0.06-0.18)	0.008
<b>N /CD4</b>	39 (27.8-55.7)	38.5 (28.5-52.6)	44.3 (25.8-58.3)	0.410	59.6 (48.8-67.5)	43.6 (42.4-59.6)	60.8 (50.4-69)	0.094
<b>CM /CD4</b>	30.5 (22.4-41.1)	30.2 (22.3-39.5)	31.4 (24.3-47.3)	0.057	27.1 (21.4-34)	39.3 (24.5-41)	26.9 (19-30.8)	0.094

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<b>EM3 /CD4</b>	0.99 (0.15-4.54)	2.4 (0.4-5.86)	0.1 (0.03-0.4)	<0.001	0.02 (0.01-0.09)	0.68 (0.56-3)	0.01 (0-0.05)	0.005
<b>E /CD4</b>	0.2 (0.02-1.15)	0.51 (0.05-2.16)	0.02 (0.01-0.13)	<0.001	0.01 (0-0.04)	0.94 (0.15-1.15)	0.005 (0-0.01)	0.006
<i>Percentage, median (IQR)</i>								
<b>CD8 /CD3</b>	9.26 (5.42-17.04)	10.1 (5.78-18)	7.8 (5.32-13.6)	0.054	23.8 (21.1-25.5)	24.5 (18.1-41.6)	23.5 (21.1-25.5)	0.680
<b>CD27 /CD8</b>	53 (38.4-69.2)	47.7 (33.6-61.1)	70.3 (54.3-79.6)	<0.001	90.8 (88.2-95.5)	79.2 (36-91.9)	91 (89.1-96)	0.094
<b>CD28 /CD8</b>	45.2 (28.6-64.1)	41.7 (26-60.6)	60.5 (42.3-72.5)	<0.001	81.6 (76.7-89.5)	71.3 (30.8-82.8)	82 (77.5-89.8)	0.110
<b>CD57 /CD8</b>	26.72 (16.7-40.3)	28.6 (18-41.5)	22.9 (13.7-36.1)	0.008	10.3 (3.68-14.8)	21.7 (6.38-61.2)	8.95 (3.23-14.7)	0.094
<b>N /CD8</b>	4.95 (1.9-11.1)	4.13 (1.73-11.1)	6.35 (2.24-11.6)	0.190	54.5 (48.9-64.8)	39.9 (12.5-53.1)	55.1 (51.4-70)	0.066
<b>CM /CD8</b>	10.2 (5.4-17.7)	9.1 (4.51-15.9)	16.4 (8.5-23.6)	<0.001	5.57 (4.93-6.48)	6.03 (4.72-17.7)	5.57 (4.93-6.48)	0.620
<b>EM3 /CD8</b>	3.37 (1.65-8.91)	3.96 (1.82-9.22)	2.23 (1.18-3.77)	<0.001	0.68 (0.36-1.4)	3.46 (0.86-9.71)	0.64 (0.22-1.35)	0.040
<b>E /CD8</b>	24.3 (11.4-39.5)	30.5 (16.4-42.3)	10.5 (5.64-24.1)	<0.001	3.44 (1.64-5.39)	12.2 (3.73-41.1)	2.99 (1.56-4.95)	0.045

N: CD45RA+CCR7+CD27+CD28+ ; CM: CD45RA-CCR7+CD27+CD28+ ; EM3: CD45RA-CCR7-CD27-CD28- ; E: CD45RA+CCR7-CD27-CD28-