

**Peripheral blood T-cell reference values from high-resolution
immune phenotyping of $\gamma\delta$ and $\alpha\beta$ T-cells in younger and older
subjects in the Berlin Aging Study II**

Kilian Wistuba-Hamprecht, Karin Haehnel, Nicole Janssen, Ilja Demuth, Graham Pawelec

Supplementary Material

Table S1: Results of Mann-Whitney comparisons of the $\gamma\delta$ T-cell compartments and subsets.

p-values and median frequencies are displayed for young CMV-seropositive (yCMV+), young CMV-seronegative (yCMV-), old CMV-seropositive (oCMV+) and old CMV-seronegative (oCMV-).

phenotype		yCMV- vs oCMV-	yCMV+ vs oCMV+	yCMV- vs yCMV+	oCMV- vs oCMV+	yCMV- vs oCMV+	oCMV- vs yCMV+
$\gamma\delta$ T-cells/CD3+	p-value	<0.0001	0.4865	0.2179	0.353	0.0057	0.156
	median frequencies	3.84	2.13	2.58	2.31	3.84	2.31
V82+/ $\gamma\delta$ T-cells	p-value	0.1438	0.0691	0.2976	<0.0001	<0.0001	0.726
	median frequencies	71.49	66.30	68.61	37.88	71.49	68.61
CD27+CD28+CD45RA+CD16+/V82+	p-value	0.9871	0.2988	0.2801	0.824	0.8249	0.2921
	median frequencies	12.83	12.43	8.43	11.96	12.83	11.96
CD27+CD28+CD45RA-CD16+/V82+	p-value	0.0493	0.1733	0.5912	0.2567	0.0004	0.5261
	median frequencies	22.71	30.07	25.76	36.10	22.71	36.10
CD27+CD28-CD45RA+CD16+/V82+	p-value	0.0207	0.5185	0.0054	0.204	0.0015	0.087
	median frequencies	0.11	0.05	0.01	0.04	0.11	0.04
CD27+CD28-CD45RA+CD16-/V82+	p-value	<0.0001	0.0903	0.0022	0.0603	<0.0001	0.7623
	median frequencies	28.17	17.46	17.49	12.59	28.17	12.59
CD27+CD28-CD45RA-CD16+/V82+	p-value	0.2856	0.1739	0.0742	0.0194	0.2977	0.0243
	median frequencies	5.43	4.20	7.88	6.03	5.43	6.03
CD27+CD28+CD45RA+CD16-/V82+	p-value	0.1272	0.369	0.2187	0.3279	0.8108	0.9489
	median frequencies	1.27	1.56	1.55	1.18	1.27	1.18
CD27+CD28+CD45RA-CD16-/V82+	p-value	0.0005	0.4009	0.3303	0.9271	0.0087	0.446
	median frequencies	2.76	4.64	4.48	5.49	2.76	5.49
CD27-CD28-CD45RA+CD16+/V82+	p-value	0.6109	0.3201	0.4664	0.4664	0.7125	0.3198
	median frequencies	0.14	0.18	0.08	0.15	0.14	0.15
CD27-CD28-CD45RA+CD16-/V82+	p-value	0.9834	0.0768	0.7749	0.6014	0.327	0.8777
	median frequencies	15.60	14.95	11.47	10.76	15.60	10.76
CD27-CD28-CD45RA-CD16+/V82+	p-value	0.7188	0.6153	0.0392	0.0392	0.0447	0.0601
	median frequencies	2.62	2.68	3.71	4.27	2.62	4.27
V81+/ $\gamma\delta$ T-cells	p-value	0.8003	0.1125	0.1487	<0.0001	<0.0001	0.1822
	median frequencies	12.32	11.46	11.31	32.93	11.46	32.93
CD27+CD28+CD45RA+CD16+/V81+	p-value	0.1619	0.5522	0.4549	0.2453	0.8474	0.8696
	median frequencies	0.03	0.05	0.07	0.02	0.03	0.02
CD27+CD28+CD45RA+CD16-/V81+	p-value	<0.0001	0.0391	0.0122	0.0045	<0.0001	0.842
	median frequencies	29.14	17.70	16.35	6.33	29.14	6.33
CD27+CD28+CD45RA-CD16+/V81+	p-value	0.9352	0.6664	0.0797	0.0102	0.0015	0.1424
	median frequencies	5.11	5.89	4.22	2.15	5.11	2.15
CD27+CD28-CD45RA+CD16+/V81+	p-value	0.3458	0.5323	0.4977	0.4287	0.7999	0.9314
	median frequencies	0.06	0.03	0.04	0.05	0.06	0.05
CD27+CD28-CD45RA+CD16-/V81+	p-value	0.1011	0.0288	0.0048	<0.0001	<0.0001	0.056
	median frequencies	37.81	32.81	24.26	17.77	37.81	17.77
CD27+CD28-CD45RA-CD16+/V81+	p-value	0.056	0.0173	0.7954	0.0022	<0.0001	0.4411
	median frequencies	1.80	1.31	1.56	0.69	1.80	0.69
CD27-CD28-CD45RA+CD16+/V81+	p-value	0.0159	0.6875	0.2981	0.871	0.0174	0.7255
	median frequencies	0.34	0.65	0.46	0.74	0.34	0.74
CD27-CD28-CD45RA+CD16-/V81+	p-value	<0.0001	0.0275	0.0018	<0.0001	<0.0001	0.3885
	median frequencies	18.65	37.02	46.87	65.15	18.65	65.15
CD27-CD28-CD45RA-CD16+/V81+	p-value	0.4103	0.6057	0.6705	0.6242	0.2102	0.8439
	median frequencies	0.06	0.02	0.03	0.01	0.06	0.01
CD27-CD28-CD45RA-CD16-/V81+	p-value	0.8439	0.992	0.5574	0.5574	0.6291	0.5604
	median frequencies	0.93	1.38	1.44	0.97	0.93	0.97
V81-/ $\gamma\delta$ T-cells	p-value	0.1563	0.555	0.3605	0.3028	0.063	0.9269
	median frequencies	14.94	18.35	17.17	18.91	14.94	18.91
CD27+CD28+CD45RA+CD16+/V81-V82-	p-value	0.2828	0.0145	0.1457	0.3771	0.0359	0.0855
	median frequencies	0.04	0.0	0.14	0.0	0.04	0.0
CD27+CD28+CD45RA+CD16-/V81-V82-	p-value	<0.0001	0.0462	0.2247	0.1554	<0.0001	0.1136
	median frequencies	29.26	18.57	23.75	14.31	29.26	23.75
CD27+CD28-CD45RA-CD16+/V81-V82-	p-value	0.2628	0.0145	0.1457	0.3771	0.0359	0.0855
	median frequencies	14.94	18.35	17.17	18.91	14.94	18.91
CD27+CD28+CD45RA-CD16-/V81-V82-	p-value	0.0282	0.0145	0.1457	0.3771	0.0359	0.0855
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CD27+CD28-CD45RA-CD16+/V81-V82-	p-value	<0.0001	0.0462	0.2247	0.1554	<0.0001	0.1136
	median frequencies	29.26	18.57	23.75	14.31	29.26	14.31
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	median frequencies	14.94	18.35	17.17	18.91	14.94	18.91
CD27+CD28-CD45RA-CD16-/V81-V82-	p-value	0.2628	0.0145	0.1457	0.3771	0.0359	0.0855
	median frequencies	14.94	18.35	17.17	18.91	14.94	18.91
CD27+CD28-CD45RA-CD16+/V81-V82-	p-value	0.2628	0.0145	0.1457	0.3771	0.0359	0.0855
	median frequencies	14.94	18.35	17.17	18.91	14.94	18.91
CD27+CD28-CD45RA-CD16-/V81-V82-	p-value	0.2628	0.0145	0.1457	0.3771	0.0359	0.0855
	median frequencies	14.94	18.35	17.17	18.91	14.94	18.91
CD27+CD28-CD45RA-CD16+/V81-V82-	p-value	0.2628	0.0145	0.1457	0.3771	0.0359	0.0855
	median frequencies	14.94	18.35	17.17	18.91	14.94	18.91
CD27+CD28-CD45RA-CD16-/V81-V82-	p-value	0.2628	0.0145	0.1457	0.3771	0.0359	0.0855
	median frequencies	14.94	18.35	17.17	18.91	14.94	18.91
CD27+CD28-CD45RA-CD16+/V81-V82-	p-value	0.2628	0.0145	0.1457	0.3771	0.0359	0.0855
	median frequencies	14.94	18.35	17.17	18.91	14.94	18.91
CD27+CD28-CD45RA-CD16-/V81-V82-	p-value	0.2628	0.0145	0.1457	0.3771	0.0359	0.0855
	median frequencies	14.94	18.35	17.17	18.91	14.94	18.91
CD27+CD28-CD45RA-CD16+/V81-V82-	p-value	0.2628	0.0145	0.1457	0.		

Table S2: Results of Mann-Whitney comparisons of the CD8+ $\gamma\delta$ T-cell compartments and subsets.

p-values and median frequencies are displayed for young CMV-seropositive (yCMV+), young CMV-seronegative (yCMV-), old CMV-seropositive (oCMV+) and old CMV-seronegative (oCMV-).

phenotype		oCMV+ vs oCMV-	yCMV+ vs yCMV-	oCMV+ vs yCMV+	oCMV- vs yCMV+	oCMV+ vs yCMV-	oCMV- vs yCMV+
CD8+/ $\gamma\delta$ T-cells	p -value	0.0008	0.3605	0.1672	0.5323	0.0002	0.6131
	median frequencies	10 5.2	7.1 4.9	10 7.1	5.2 4.9	10 4.9	5.2 7.1
V82+/ $\gamma\delta$ T-cells	p -value	0.0026	0.0676	0.9293	0.2563	0.01	0.0295
	median frequencies	1.5 6.9	2.5 4.6	1.5 2.5	6.9 4.6	1.5 4.6	6.9 2.5
CD27-CD28-/V82+				n.a.			
CD27-CD28+/V82+				n.a.			
CD27+CD28-/V82+				n.a.			
CD27+CD28+/V82+				n.a.			
V81+/ $\gamma\delta$ T-cells	p -value	0.0062	0.2062	0.1221	0.0245	< 0.0001	0.9023
	median frequencies	65.5 55.6	54.6 47.5	65.5 54.6	55.6 47.5	65.5 47.5	55.6 54.6
CD27-CD28-/V81+	p -value	0.0002	0.0029	0.2143	0.0217	< 0.0001	0.2519
	median frequencies	73.0 60.0	63.5 47.9	73.0 63.5	60.0 47.9	73.0 47.9	60.0 63.5
CD27-CD28+/V81+	p -value	0.6671	0.7437	0.8928	0.8323	0.7417	0.5812
	median frequencies	1.5 1.6	1.6 1.6	1.5 1.6	1.6 1.6	1.5 1.6	1.6 1.6
CD27+CD28-/V81+	p -value	0.0162	0.0324	0.327	0.0058	< 0.0001	0.5319
	median frequencies	15.4 24.8	17.8 32.6	15.4 17.8	24.8 32.6	15.4 32.6	24.8 17.8
CD27+CD28+/V81+	p -value	0.0002	0.014	0.2136	0.001	< 0.0001	0.4634
	median frequencies	3.0 5.7	5.3 9.3	3.0 5.3	5.7 9.3	3.0 9.3	5.7 5.3
V81-V82-/ $\gamma\delta$ T-cells	p -value	0.7348	0.5247	0.0453	0.0002	0.0005	0.0358
	median frequencies	22.4 23.5	32.6 36.0	22.4 32.6	23.5 36.0	22.4 36.0	23.5 32.6
CD27-CD28-/V81-V82-	p -value	0.0357	0.006	0.0328	< 0.0001	< 0.0001	0.7036
	median frequencies	53.2 29.3	17.5 7.5	53.2 17.5	29.3 7.5	53.2 7.5	29.3 17.5
CD27-CD28+/V81-V82-	p -value	0.2367	0.0567	0.5949	< 0.0001	0.0027	0.1376
	median frequencies	1.3 1.6	1.0 0.7	1.3 1.0	1.6 1.0	1.3 0.7	1.6 1.0
CD27+CD28-/V81-V82-	p -value	0.0513	0.6587	0.4447	0.1732	0.2582	0.6149
	median frequencies	8.5 12.6	13.7 9.7	8.5 13.7	12.6 9.7	8.5 9.7	12.6 13.7
CD27+CD28+/V81-V82-	p -value	0.1561	0.0744	0.001	< 0.0001	< 0.0001	0.0222
	median frequencies	21.6 37.6	57.4 70.0	21.6 57.4	37.6 70.0	21.6 70.0	37.6 57.4

Bonferroni correction: p-values ≤ 0.0083 were considered significant

Table S3: Results of Mann-Whitney comparisons of the $\alpha\beta$ T-cell compartments and subsets.

$\alpha\beta$ T-cells are identified as CD3+ $\gamma\delta$ TCR-. p-values and median frequencies are displayed for young CMV-seropositive (yCMV+), young CMV-seronegative (yCMV-), old CMV-seropositive (oCMV+) and old CMV-seronegative (oCMV-).

phenotype		yCMV- vs oCMV-	yCMV+ vs oCMV+	yCMV- vs yCMV+	oCMV- vs oCMV+	yCMV- vs oCMV+	oCMV- vs yCMV+
y δ TCR-/CD3+	p -value	< 0.0001	0.5357	0.1989	0.2706	0.0076	0.1883
	median frequencies	95.6 97.4	96.9 97.1	95.6 96.9	97.4 97.1	95.6 97.1	97.4 96.9
CD4+/y δ TCR-	p -value	< 0.0001	0.0260	0.4939	< 0.0001	0.0002	< 0.0001
	median frequencies	69.8 81.1	66.2 74.9	69.8 66.2	81.1 74.9	69.8 74.9	81.1 66.2
CD27+CD28+CD45RA+CD16-/CD4+	p -value	< 0.0001	0.0120	0.0363	0.0034	< 0.0001	0.2520
	median frequencies	68.7 57.9	61.6 52.1	68.7 61.6	57.9 52.1	68.7 52.1	57.9 61.6
CD27+CD28+CD45RA-CD16-/CD4+	p -value	< 0.0001	0.0243	0.2630	0.484	< 0.0001	0.0521
	median frequencies	28.2 38.2	32.2 39.2	28.2 32.2	38.2 39.2	28.2 39.2	38.2 32.2
CD27+CD28-CD45RA+CD16-/CD4+	p -value	0.1934	0.3510	0.0201	< 0.0001	< 0.0001	0.1460
	median frequencies	0.3 0.4	0.6 1.0	0.3 0.6	0.4 1.0	0.3 1.0	0.4 0.6
CD27+CD28-CD45RA-CD16-/CD4+	p -value	0.0001	0.0173	0.0292	< 0.0001	< 0.0001	0.8338
	median frequencies	0.07 0.13	0.13 0.25	0.07 0.13	0.13 0.25	0.07 0.25	0.13 0.013
CD27-CD28+CD45RA+CD16-/CD4+	p -value	0.5235	0.9740	0.5544	0.1150	0.3923	0.2847
	median frequencies	0.10 0.10	0.13 0.12	0.10 0.13	0.10 0.13	0.10 0.12	0.10 0.13
CD27-CD28+CD45RA-CD16-/CD4+	p -value	0.0314	0.8988	0.0120	0.0143	0.0001	0.0956
	median frequencies	2.57 2.83	3.7 3.98	2.57 3.7	2.83 3.98	2.57 3.98	2.83 3.7
CD27-CD28-CD45RA-CD16-/CD4+	p -value	0.0029	0.0053	0.0002	< 0.0001	< 0.0001	0.0138
	median frequencies	0.04 0.06	0.22 1.12	0.04 0.22	0.06 1.12	0.04 1.12	0.06 2.22
CD27-CD28-CD45RA+CD16-/CD4+	p -value	0.0150	0.0290	0.0295	< 0.0001	< 0.0001	0.1425
	median frequencies	0.02 0.05	0.11 0.56	0.02 0.11	0.05 0.56	0.02 0.56	0.05 0.11
CD8+/y δ TCR-	p -value	< 0.0001	0.0583	0.7376	< 0.0001	0.0003	< 0.0001
	median frequencies	27.10 16.23	30.42 21.54	27.10 30.42	16.23 21.54	27.10 21.54	16.23 30.42
CD27+CD28+CD45RA+CD16-/CD8+	p -value	< 0.0001	0.0001	0.0029	0.0005	< 0.0001	0.0115
	median frequencies	76.54 46.10	65.20 33.93	76.54 65.20	46.10 33.93	76.54 33.93	46.10 65.20
CD27+CD28+CD45RA-CD16-/CD8+	p -value	< 0.0001	0.0057	0.8875	0.0009	< 0.0001	< 0.0001
	median frequencies	11.23 24.39	12.27 16.66	11.23 12,2700	24.39 16.66	11.23 16.66	24.39 12.27
CD27+CD28-CD45RA+CD16-/CD8+	p -value	0.0031	0.3548	0.1087	0.0121	0.7876	0.4089
	median frequencies	4.19 6.44	5.32 4.27	4.19 5.32	6.44 4.27	4.19 4.27	6.44 5.32
CD27+CD28-CD45RA-CD16-/CD8+	p -value	0.0352	0.4358	0.2286	0.0334	0.8229	0.7555
	median frequencies	1.21 1.69	1.99 1.17	1.21 1.99	1.69 1.17	1.21 1.17	1.69 1.99
CD27-CD28+CD45RA+CD16-/CD8+	p -value	< 0.0001	0.0035	0.1996	0.0243	< 0.0001	0.0599
	median frequencies	0.45 1.07	0.80 1.43	0.45 0.80	1.07 1.43	0.45 1.43	1.07 0.80
CD27-CD28+CD45RA-CD16-/CD8+	p -value	< 0.0001	0.0033	0.3786	0.1564	< 0.0001	0.0002
	median frequencies	0.73 1.93	0.81 1.83	0.73 0.81	1.93 1.83	0.73 1.83	1.93 0.81
CD27-CD28-CD45RA+CD16-/CD8+	p -value	< 0.0001	0.0113	< 0.0001	< 0.0001	< 0.0001	0.0140
	median frequencies	2.52 6.85	12.55 25.32	2.52 12.55	6.85 25.32	2.52 25.32	6.85 12.55
CD27-CD28-CD45RA-CD16-/CD8+	p -value	0.0204	0.7475	0.0018	0.0001	< 0.0001	0.0415
	median frequencies	0.55 0.96	3.01 2.05	0.55 3.01	0.96 2.05	0.55 2.05	0.96 3.01
CD4/CD8	p -value	< 0.0001	0.0497	0.6148	< 0.0001	0.0003	< 0.0001
	median	2.59 5.01	2.21 3.43	2.59 2.21	5.01 3.43	2.59 3.43	5.01 2.21

Bonferroni correction: p-values ≤0.0083 were considered significant

Table S4: Results of Mann-Whitney comparisons of the absolute counts for all T-cell compartments.

p-values and median counts are displayed for young CMV-seropositive (yCMV+), young CMV-seronegative (yCMV-), old CMV-seropositive (oCMV+) and old CMV-seronegative (oCMV-).

phenotype		yCMV- vs oCMV-	yCMV+ vs oCMV+	yCMV- vs yCMV+	oCMV- vs oCMV+	yCMV- vs oCMV+	oCMV- vs yCMV+
Lymphocytes	p -value	0.0002	0.9798	0.1230	0.1504	0.1045	0.3460
	median counts	1815 1540	1700 1670	1815 1700	1540 1670	1815 1670	1540 1700
T-cells	p -value	< 0.0001	0.8504	0.1255	0.0585	0.0488	0.1247
	median counts	1449 1119	1354 1323	1449 1354	1119 1323	1449 1323	1119 1354
$\alpha\beta$ T-cells	p -value	< 0.0001	0.8504	0.1808	0.0708	0.0856	0.1617
	median counts	1400 1099	1295 1276	1400 1295	1099 1276	1400 1276	1099 1295
CD4+ T-cells	p -value	0.0708	0.4497	0.1803	0.6640	0.6227	0.6701
	median counts	960 900	848 914	960 848	900 914	960 914	900 848
CD8+ T-cells	p -value	< 0.0001	0.0758	0.5311	< 0.0001	0.0003	< 0.0001
	median counts	378 171	246 299	378 246	171 299	378 299	171 246
CD4/CD8	p -value	< 0.0001	0.0497	0.6859	< 0.0001	0.0002	< 0.0001
	median	2.58 5.07	2.21 3.43	2.58 2.21	5.07 3.43	2.58 3.43	5.07 2.21
<hr/>							
$\gamma\delta$ T-cells/CD3+	p -value	< 0.0001	0.4063	0.1034	0.1185	0.0006	0.0687
	median counts	51 25	40 30	51 40	25 30	51 30	25 40
V82+ T-cells	p -value	< 0.0001	0.0742	0.0267	0.0623	< 0.0001	0.4821
	median counts	37 14	24 10	37 24	14 10	37 10	14 24
V81+ T-cells	p -value	< 0.0001	0.6345	0.8939	< 0.0001	0.1349	0.0049
	median counts	6 2	5 8	6 5	2 8	6 8	2 5
V81-V82- T-cells	p -value	< 0.0001	0.5672	0.2588	0.0051	0.0181	0.0197
	median counts	7 4	6 5	7 6	4 5	7 5	4 6
<hr/>							
V81+/V82+	p -value	0.6293	0.0641	0.1496	< 0.0001	< 0.0001	0.2794
	median	0.18 0.17	0.20 0.83	0.18 0.20	0.17 0.83	0.18 0.83	0.17 0.20

Bonferroni correction: p-values ≤ 0.0083 were considered significant

Figure S1

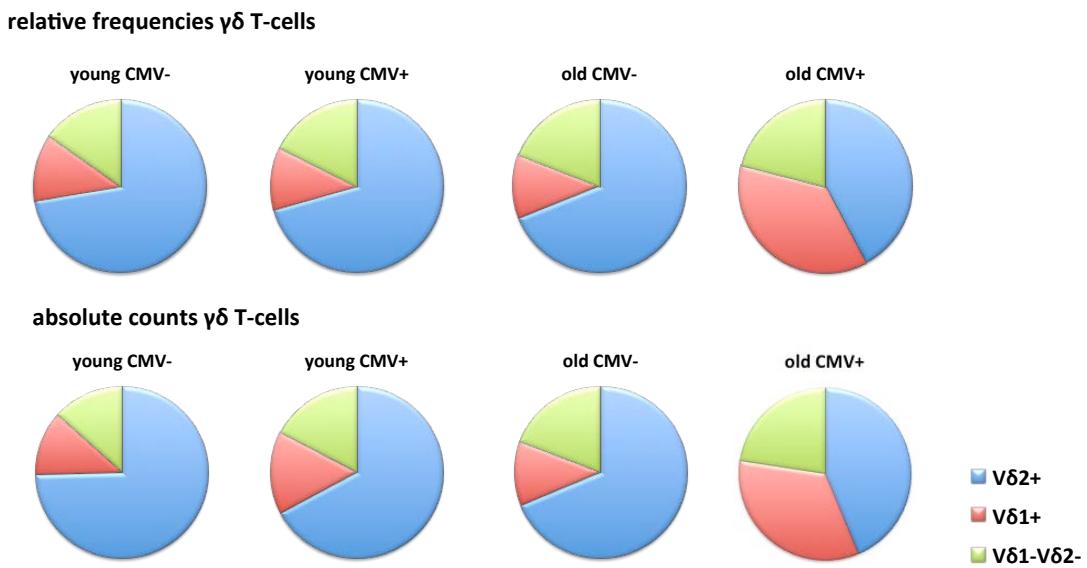


Figure S1: Median frequencies and absolute counts of the $\gamma\delta$ T-cell compartments in young and old CMV-seropositive (CMV+) and seronegative (CMV-) individuals.

Figure S2

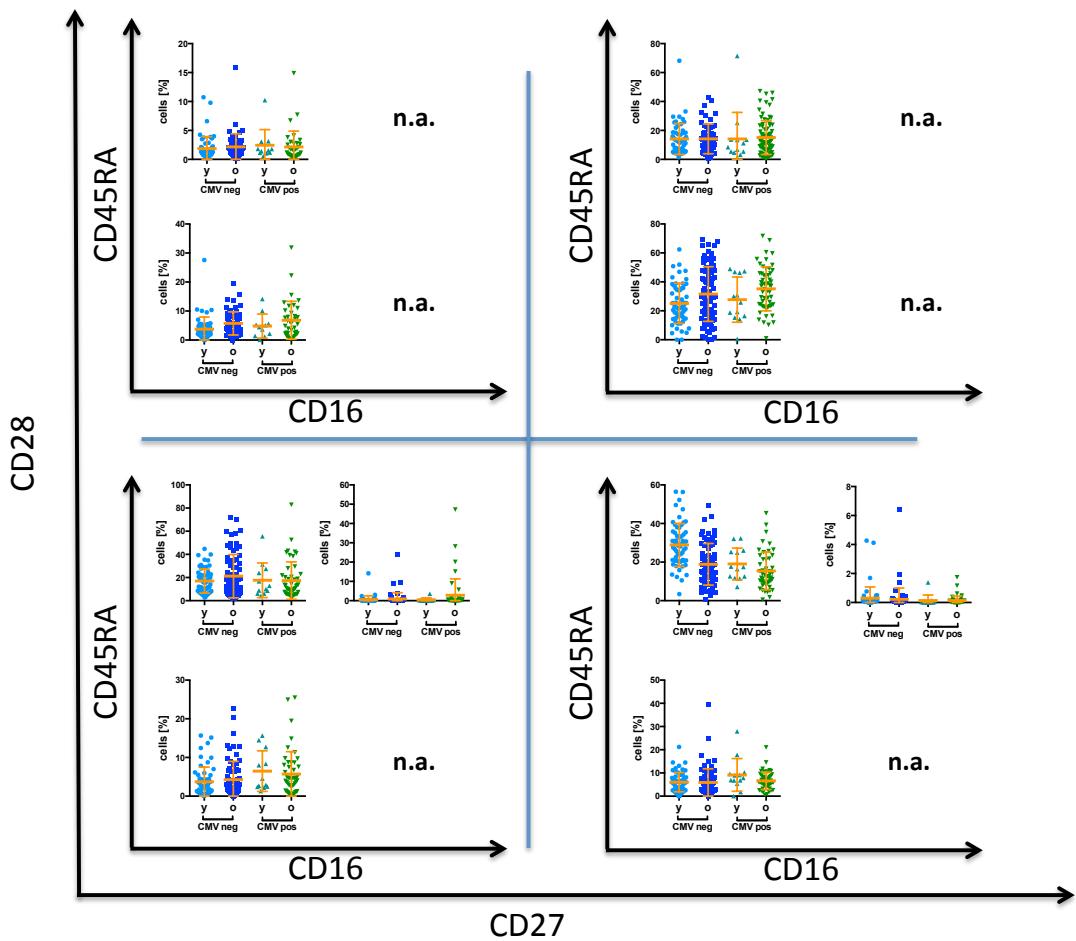


Figure S2: Differentiation phenotypes of the V δ 2+ compartment

The differentiation statuses were identified by CD27, CD28, CD45RA and CD16 in young (y) and old (o) CMV seropositive (CMV pos) and seronegative (CMV neg) individuals.

Figure S3

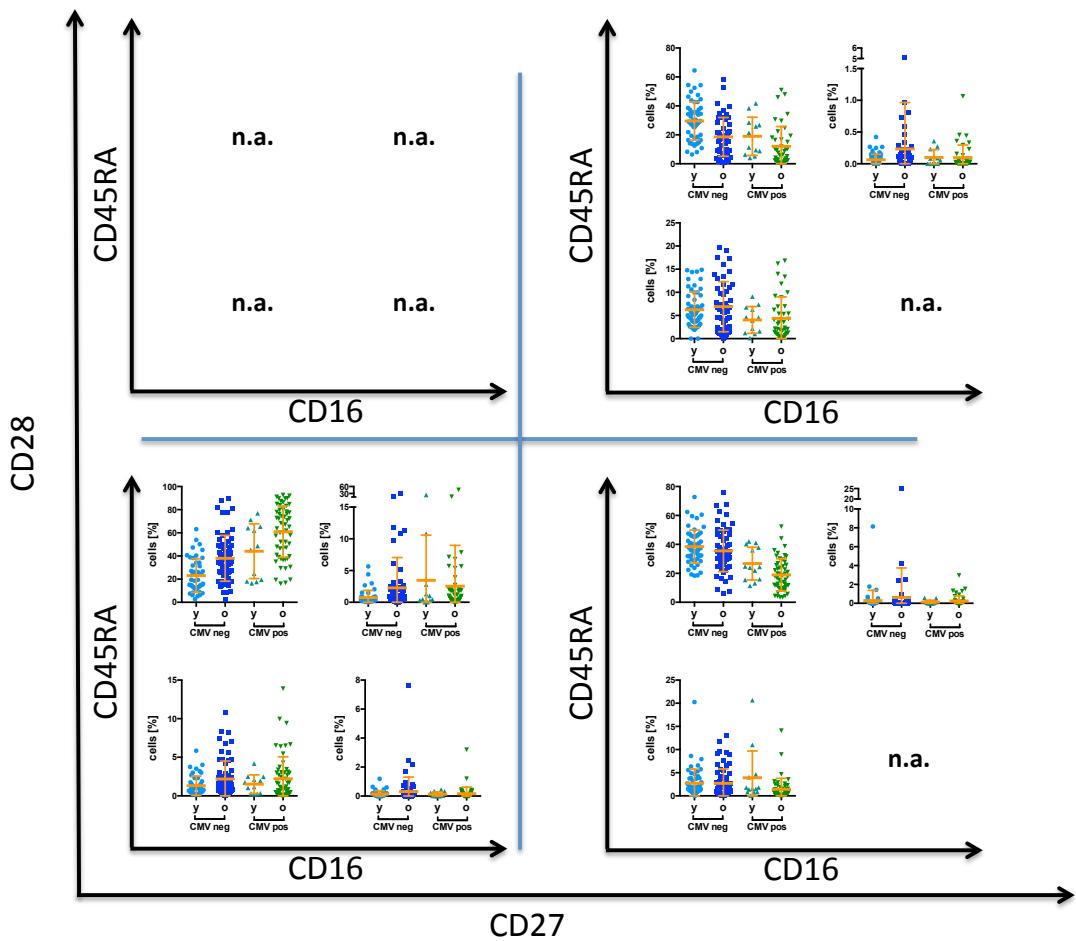


Figure S3: Differentiation phenotypes of the V δ 1+ compartment

The differentiation statuses were identified by CD27, CD28, CD45RA and CD16 in young (y) and old (o) CMV seropositive (CMV pos) and seronegative (CMV neg) individuals.

Figure S4

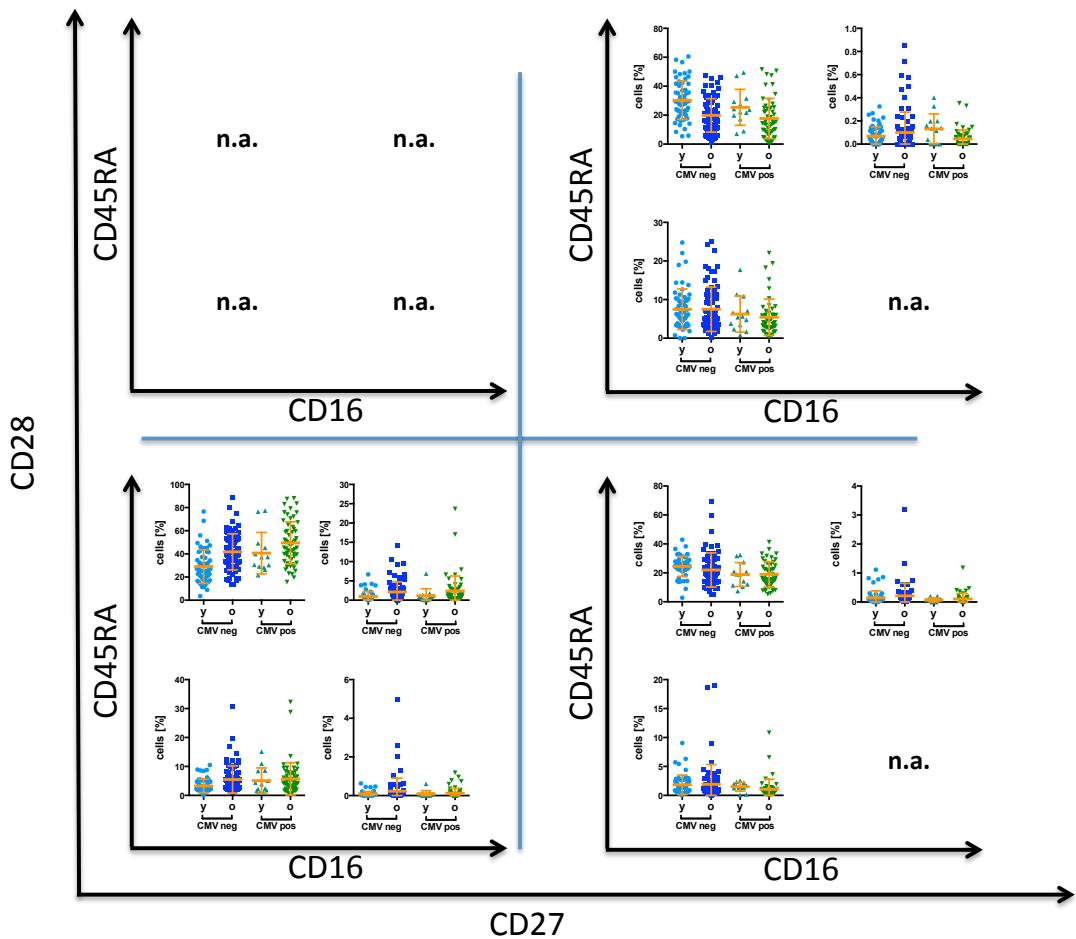


Figure S4: Differentiation phenotypes of the V δ 1-V δ 2- compartment

The differentiation statuses were identified by CD27, CD28, CD45RA and CD16 in young (y) and old (o) CMV seropositive (CMV pos) and seronegative (CMV neg) individuals.

Figure S5

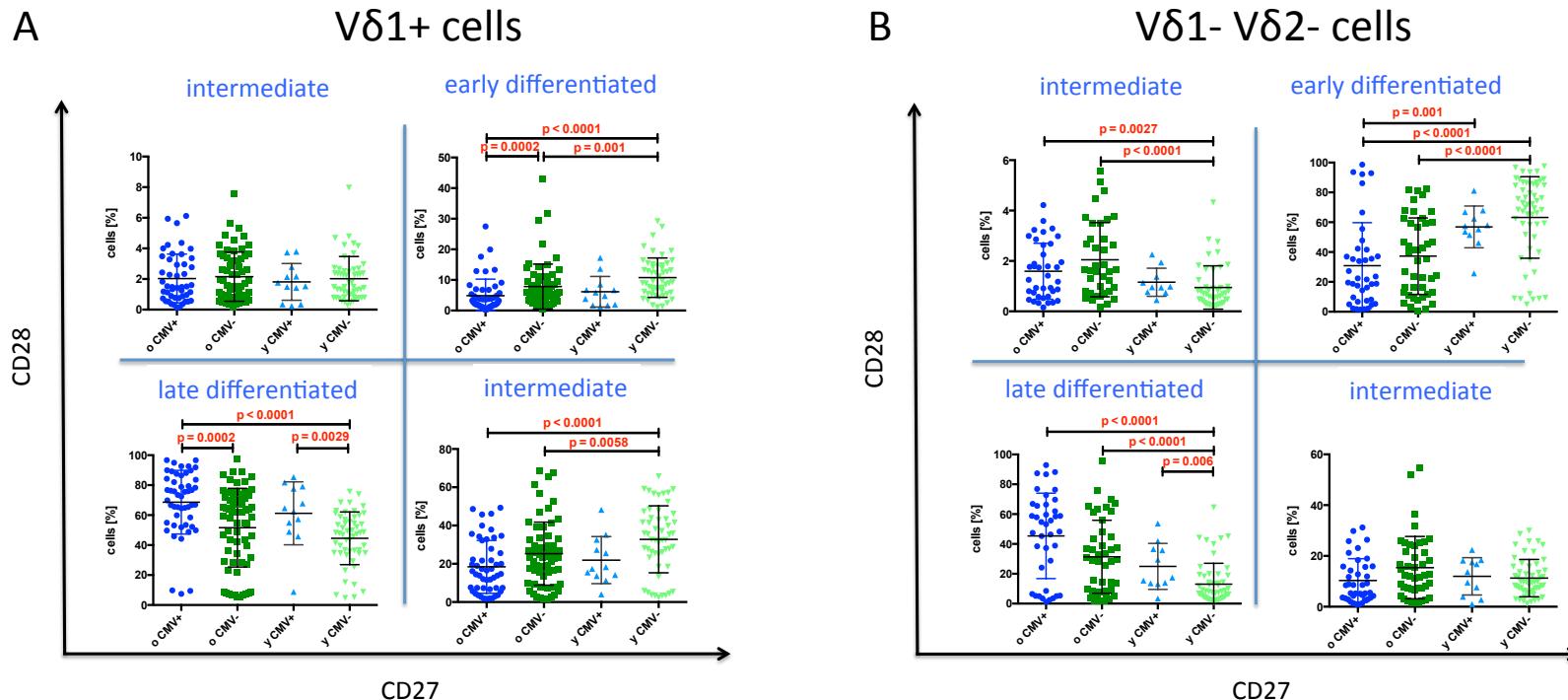


Figure S5: Differentiation phenotypes of the CD8+ $\gamma\delta$ T-cells

The differentiation statuses of the **A** V δ 1+ and **B** V δ 1- V δ 2- compartment were identified by CD27 and CD28 in young (y) and old (o) CMV seropositive (CMV+) and seronegative (CMV-) individuals.

Mann Whitney U test was used for the statistical comparison of groups. Bonferroni correction adjusted $p \leq 0.0083$ as the level of significance.

Figure S6

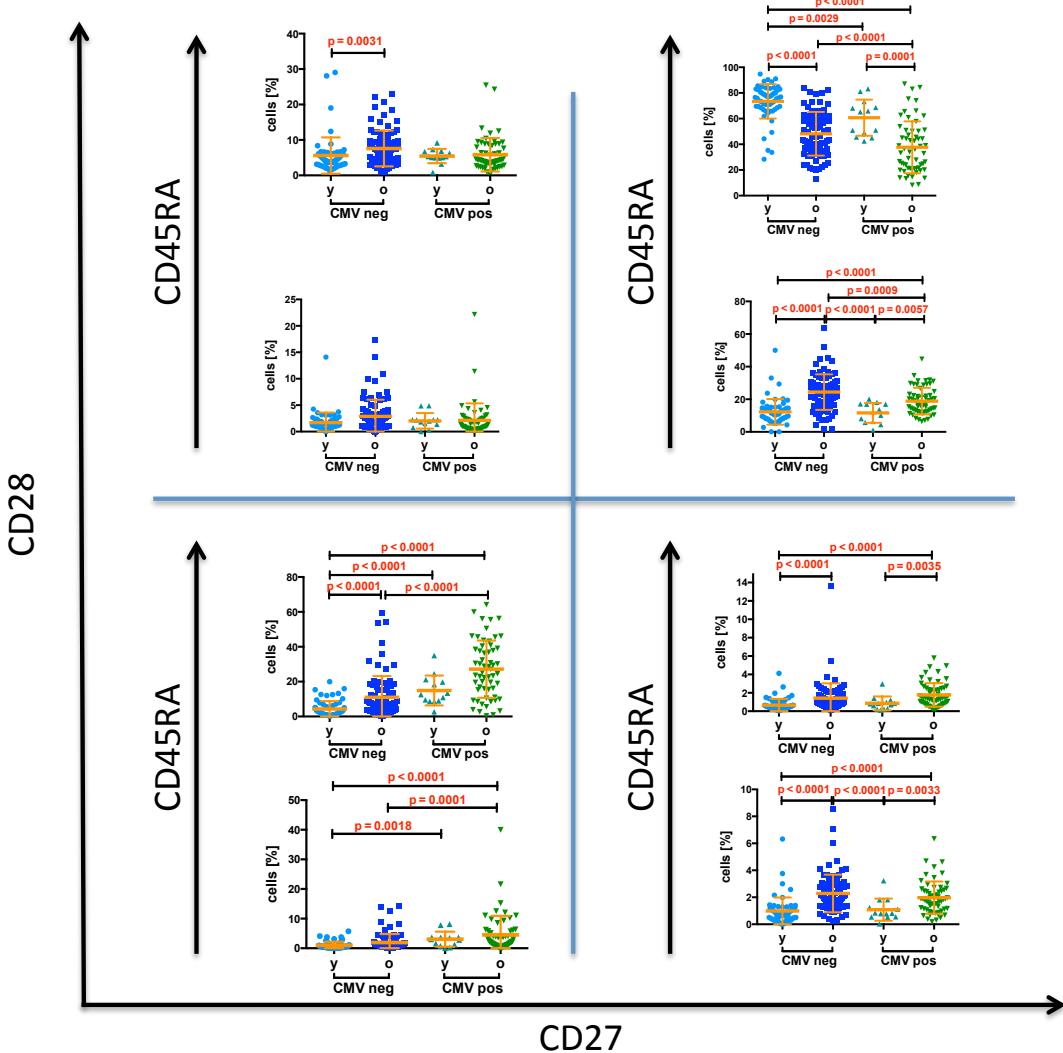


Figure S6: Differentiation phenotypes of the CD8+ $\alpha\beta$ T-cells

The differentiation compartments were identified by CD27, CD28, CD45RA and were all CD16 negative in young (y) and old (o) CMV seropositive (CMV pos) and seronegative (CMV neg) individuals.

Mann Whitney U test was used for the statistical comparison of groups.

Bonferroni correction adjusted $p \leq 0.0083$ as the level of significance.

Figure S7

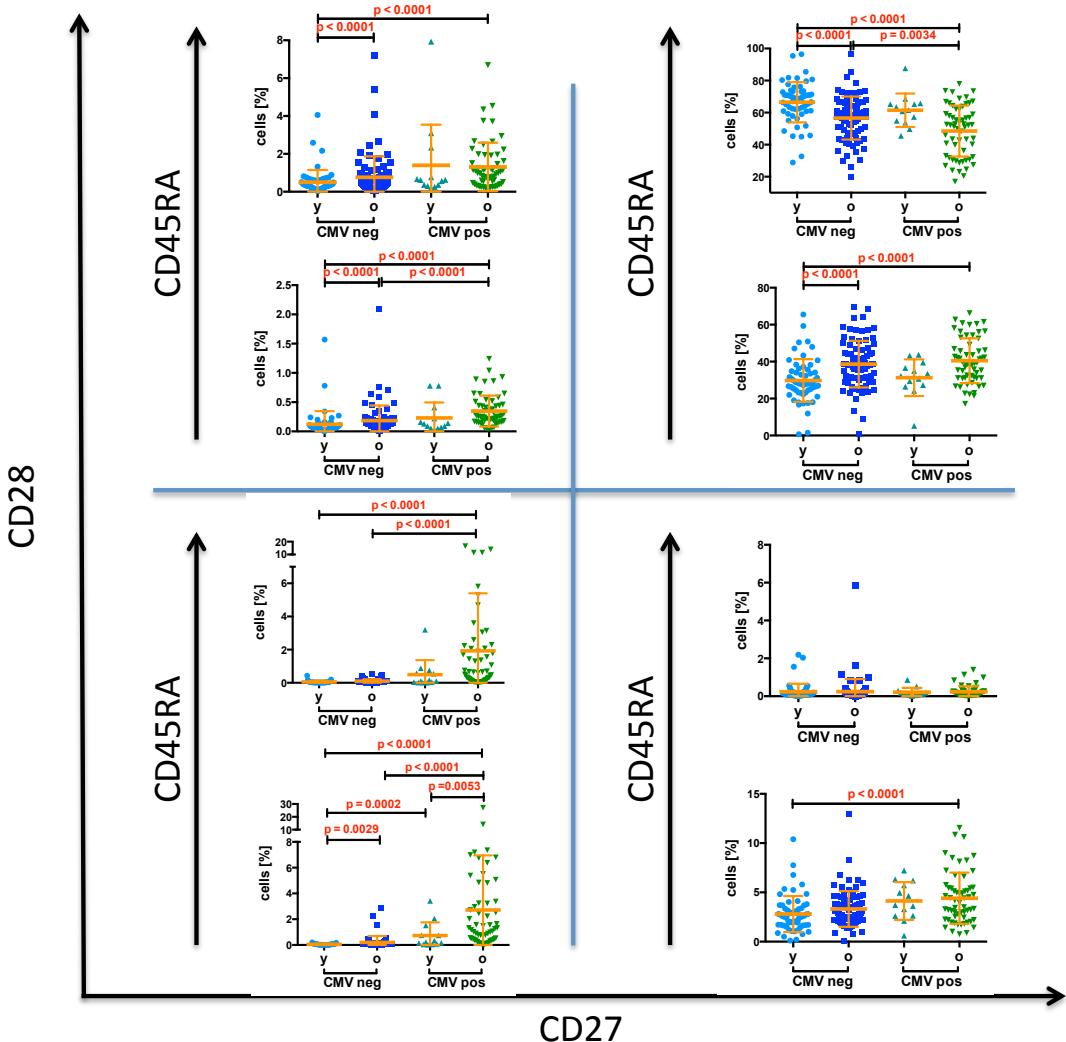


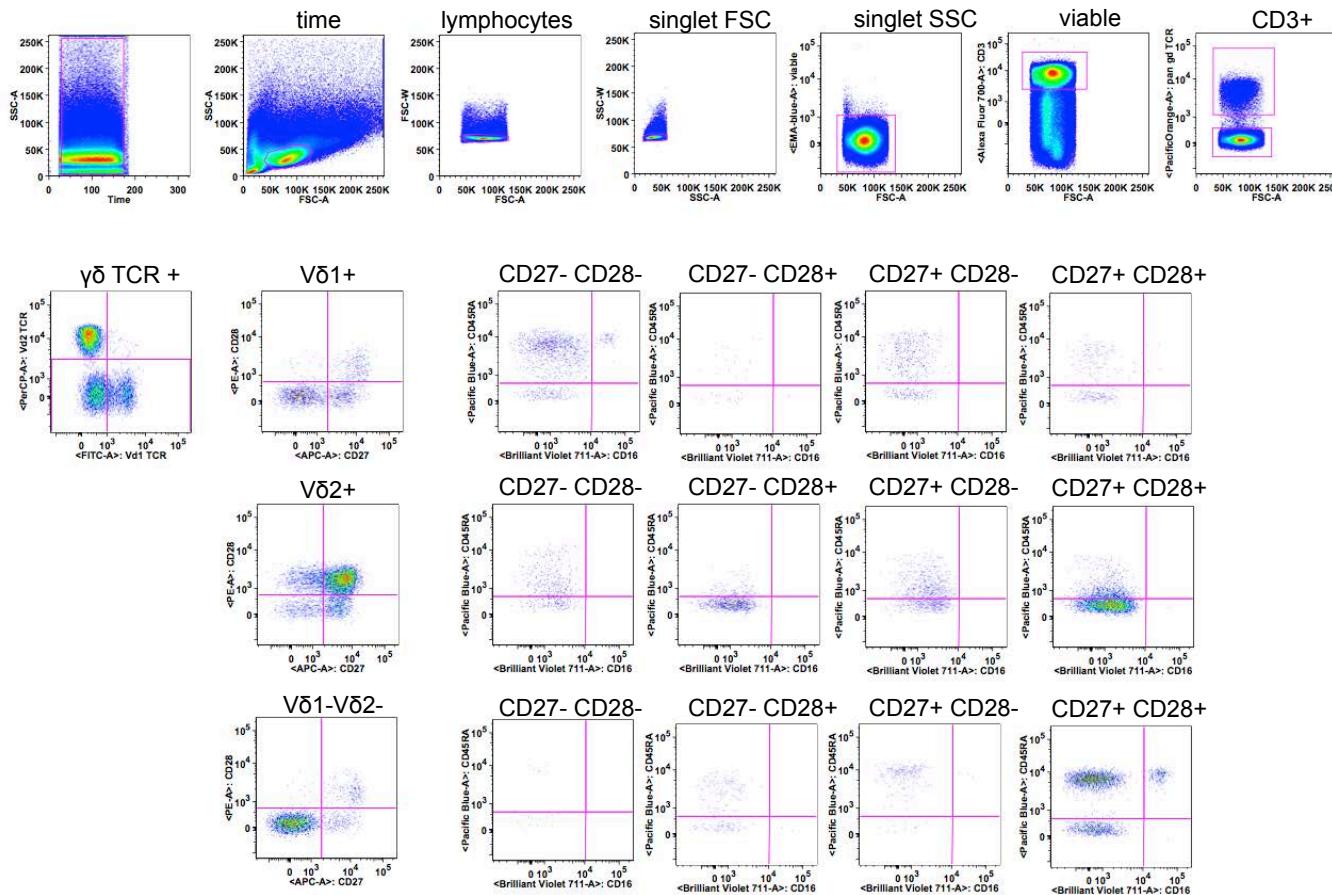
Figure S7: Differentiation phenotypes of the CD4+ $\alpha\beta$ T-cells

The differentiation compartments were identified by CD27, CD28, CD45RA and were all CD16 negative in young (y) and old (o) CMV seropositive (CMV pos) and seronegative (CMV neg) individuals.

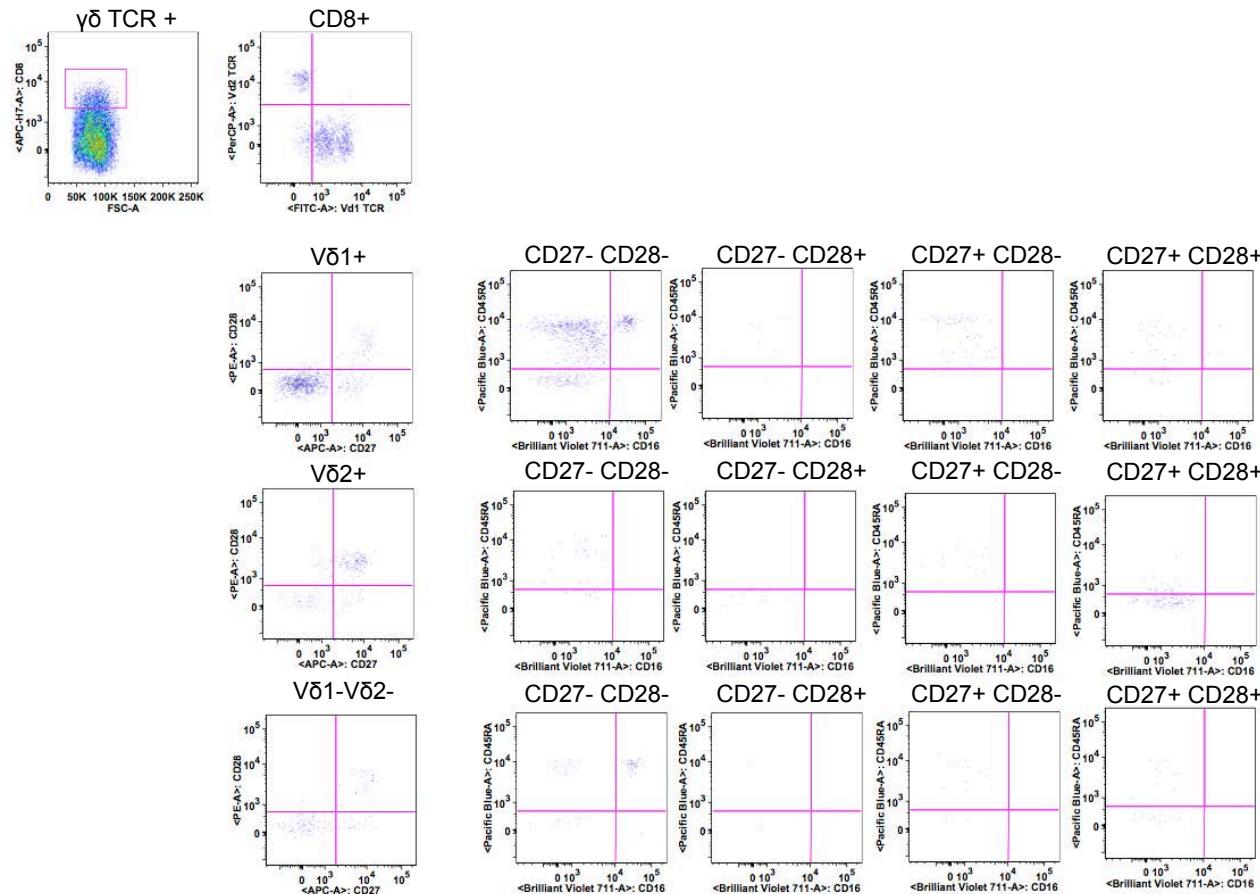
Mann Whitney U test was used for the statistical comparison of groups. Bonferroni correction adjusted $p \leq 0.0083$ as the level of significance.

Figure S8: Gating strategy

Gating strategy – part 1



Gating strategy – part 2



Gating strategy – part 3

