

CCR6+CCR4+CRTH2+	0.55 ± 0.78	1.77 ± 2.48	ns
CCR6+CCR4+CRTH2-	98.93 ± 2.35	85.38 ± 6.99	0.03468
CCR6+CCR4+CRTH2+	-	-	-
CCR6+CCR4+CRTH2+	-	-	-
CCR6+CCR4+CRTH2+	-	-	-

EFFECTOR MEMORY CD3+

% of CD3+ EFFECTOR MEMORY CD3+			
CCR6+CCR4+CRTH2+	50.42 ± 15.82	49.15 ± 13.24	ns
CCR6+CCR4+CRTH2-	4.41 ± 8.37	3.39 ± 8.11	ns
CCR6+CCR4+CRTH2+	0.38 ± 0.92	0.22 ± 0.99	ns
CCR6+CCR4+CRTH2-	40.31 ± 18.02	41.59 ± 19.08	ns
CCR6+CCR4+CRTH2+	1.59 ± 0.19	2.91 ± 4.62	-
CCR6+CCR4+CRTH2+	-	0.028 ± 0.06	-
CCR6+CCR4+CRTH2+	-	0.001 ± 0.004	-
CCR6+CCR4+CRTH2+	5.38 ± 1.87	4.81 ± 6.51	ns

EFFECTOR MEMORY CD28-

% of CD3+ EFFECTOR MEMORY CD28-			
CCR6+CCR4+CRTH2+	10.18 ± 7.87	13.41 ± 8.44	ns
CCR6+CCR4+CRTH2-	0.11 ± 0.41	0.04 ± 0.19	ns
CCR6+CCR4+CRTH2+	0.87 ± 0.17	0.92 ± 0.11	ns
CCR6+CCR4+CRTH2-	79.67 ± 14.66	81.68 ± 9.96	ns
CCR6+CCR4+CRTH2+	-	-	-
CCR6+CCR4+CRTH2+	-	-	-
CCR6+CCR4+CRTH2+	-	-	-

TEMRA

% of CD3+ TEMRA CD3+			
CCR6+CCR4+CRTH2+	25.99 ± 16.79	25.88 ± 13.58	ns
CCR6+CCR4+CRTH2-	5.28 ± 8.28	2.99 ± 7.72	ns
CCR6+CCR4+CRTH2+	0.62 ± 1.59	0.22 ± 0.75	ns
CCR6+CCR4+CRTH2-	67.28 ± 18.28	69.88 ± 15.17	ns
CCR6+CCR4+CRTH2+	-	-	-
CCR6+CCR4+CRTH2+	-	-	-
CCR6+CCR4+CRTH2+	-	-	-

TEMRA

% of CD3+ TEMRA CD28-			
CCR6+CCR4+CRTH2+	8.43 ± 14.65	6.88 ± 7.91	ns
CCR6+CCR4+CRTH2-	-	0.08 ± 0.04	-
CCR6+CCR4+CRTH2+	-	0.98 ± 0.33	-
CCR6+CCR4+CRTH2-	90.13 ± 14.54	92.16 ± 7.35	ns
CCR6+CCR4+CRTH2+	-	-	-
CCR6+CCR4+CRTH2+	-	-	-
CCR6+CCR4+CRTH2+	-	-	-

p < 0.05
p < 0.01

CCR6+CCR4+CRTH2+	0.81 ± 1.38	0.96 ± 1.37	ns	10.62 ± 19.81	5.29 ± 20.88	ns
CCR6+CCR4+CRTH2-	69.21 ± 22.11	77.68 ± 14.62	ns	833.41 ± 537.85	379.8 ± 246.23	0.04182
CCR6+CCR4+CRTH2+	0.69 ± 0.73	1.88 ± 2.76	ns	0.62 ± 2.35	2.44 ± 8.03	ns
CCR6+CCR4+CRTH2+	-	-	-	0 ± 0	0 ± 0	ns
CCR6+CCR4+CRTH2+	0.04 ± 0.09	0.32 ± 0.47	ns	0 ± 0	446.07 ± 2738.39	ns
CCR6+CCR4+CRTH2+	43.08 ± 27.44	34.35 ± 8.71	ns	252.68 ± 508.69	46.83 ± 700.37	0.04927

EFFECTOR MEMORY CD3+

% of CD3+ EFFECTOR MEMORY CD3+						
CCR6+CCR4+CRTH2+	16.43 ± 8.89	17.45 ± 8.38	ns	8486.29 ± 14093.31	4769.23 ± 6650.88	ns
CCR6+CCR4+CRTH2-	4.41 ± 8.04	3.68 ± 6.39	ns	2089.49 ± 5999.79	807.19 ± 2414.54	ns
CCR6+CCR4+CRTH2+	0.47 ± 0.63	0.38 ± 0.57	ns	305.15 ± 673.40	319.89 ± 1513.35	ns
CCR6+CCR4+CRTH2-	41.33 ± 16.19	43.22 ± 16.73	ns	1805.15 ± 1976.72	1125.69 ± 9376.27	0.02759
CCR6+CCR4+CRTH2+	1.38 ± 1.69	1.45 ± 1.18	ns	418.81 ± 405.19	433.35 ± 719.97	ns
CCR6+CCR4+CRTH2+	0.21 ± 0.44	0.23 ± 0.50	ns	60.79 ± 82.14	74.07 ± 220.97	ns
CCR6+CCR4+CRTH2+	0.01 ± 0.01	0.02 ± 0.04	ns	0.55 ± 0.38	189.36 ± 1347.03	ns
CCR6+CCR4+CRTH2+	35.89 ± 22.87	34.04 ± 18.11	ns	1265.68 ± 1992.54	935.42 ± 10916.85	ns

EFFECTOR MEMORY CD28-

% of CD3+ EFFECTOR MEMORY CD28-						
CCR6+CCR4+CRTH2+	1.86 ± 2.11	2.84 ± 2.70	ns	473.68 ± 702.68	473.51 ± 673.47	ns
CCR6+CCR4+CRTH2-	0.02 ± 0.04	0.02 ± 0.06	ns	9.07 ± 8.24	3.22 ± 6.45	0.034204
CCR6+CCR4+CRTH2+	0.95 ± 0.07	0.96 ± 0.11	ns	18.65 ± 32.4	5.2 ± 10.07	ns
CCR6+CCR4+CRTH2-	95.85 ± 4.89	95.69 ± 3.71	ns	28600.84 ± 91919.54	18634.16 ± 17266.52	0.00282
CCR6+CCR4+CRTH2+	0.15 ± 0.17	0.13 ± 0.14	ns	2.1 ± 7.02	3.63 ± 10.95	ns
CCR6+CCR4+CRTH2+	-	-	-	0 ± 0	0 ± 0	ns
CCR6+CCR4+CRTH2+	-	-	-	0 ± 0	62.29 ± 384.42	ns
CCR6+CCR4+CRTH2+	8.83 ± 2.81	3.37 ± 2.54	0.01220	326.42 ± 810.86	68.8 ± 153.64	ns

TEMRA

% of CD3+ TEMRA CD3+						
CCR6+CCR4+CRTH2+	9.99 ± 10.84	6.1 ± 6.04	ns	249.09 ± 318.81	420.34 ± 1039.64	ns
CCR6+CCR4+CRTH2-	2.13 ± 2.72	2.16 ± 6.2	ns	106.05 ± 207.66	68.38 ± 243.55	ns
CCR6+CCR4+CRTH2+	0.68 ± 1.29	0.38 ± 1.12	ns	26.30 ± 42.99	19.16 ± 70.87	ns
CCR6+CCR4+CRTH2-	76.81 ± 20.18	79.41 ± 19.08	ns	2657.51 ± 2102.1	4534.83 ± 1343.51	ns
CCR6+CCR4+CRTH2+	0.41 ± 0.54	0.37 ± 0.42	ns	1.81 ± 4.29	5.71 ± 30.4	ns
CCR6+CCR4+CRTH2-	-	0.01 ± 0.04	-	0 ± 0	0.49 ± 2.87	ns
CCR6+CCR4+CRTH2+	-	-	-	0 ± 0	206.62 ± 1302.7	ns
CCR6+CCR4+CRTH2+	29.81 ± 37.02	15.91 ± 16.02	ns	384.43 ± 724.22	433.94 ± 787.88	ns

TEMRA

% of CD3+ TEMRA CD28-						
CCR6+CCR4+CRTH2+	1.18 ± 1.59	1.87 ± 2.37	-	352.05 ± 503.94	1202.21 ± 4036.96	ns
CCR6+CCR4+CRTH2-	-	0.005 ± 0.016	-	0 ± 0	2.19 ± 8.55	ns
CCR6+CCR4+CRTH2+	0.83 ± 0.96	0.03 ± 0.04	ns	16.22 ± 16.38	65.31 ± 36.33	ns
CCR6+CCR4+CRTH2-	98.73 ± 1.62	97.93 ± 2.43	ns	43128.14 ± 30342.42	41835.95 ± 49154.47	ns
CCR6+CCR4+CRTH2+	-	-	-	0 ± 0	0 ± 0	ns
CCR6+CCR4+CRTH2+	-	-	-	0 ± 0	4789.26 ± 26887.19	ns
CCR6+CCR4+CRTH2+	-	-	-	0 ± 0	20.47 ± 73.21	ns

p < 0.05

p < 0.05
p < 0.001

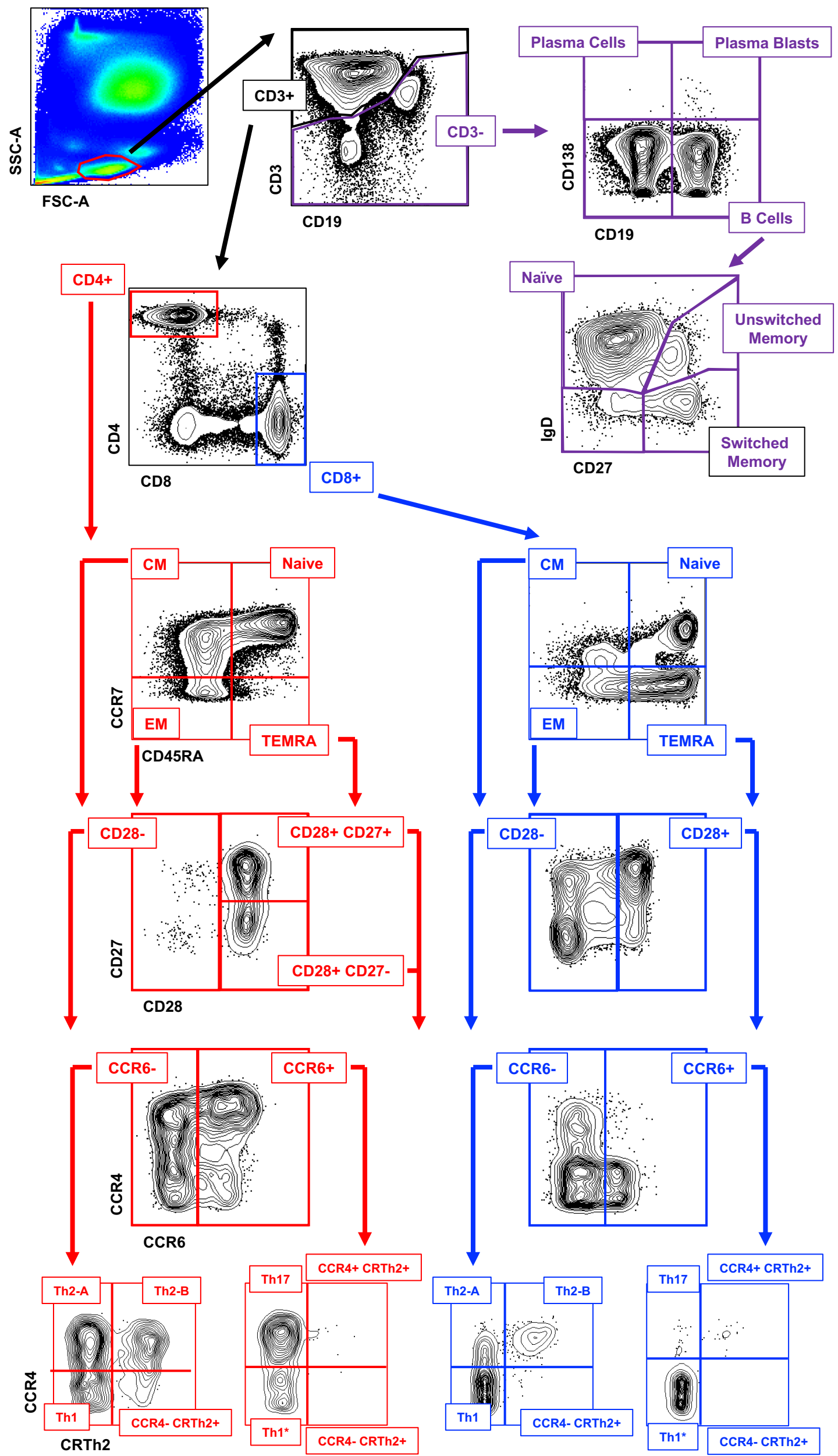
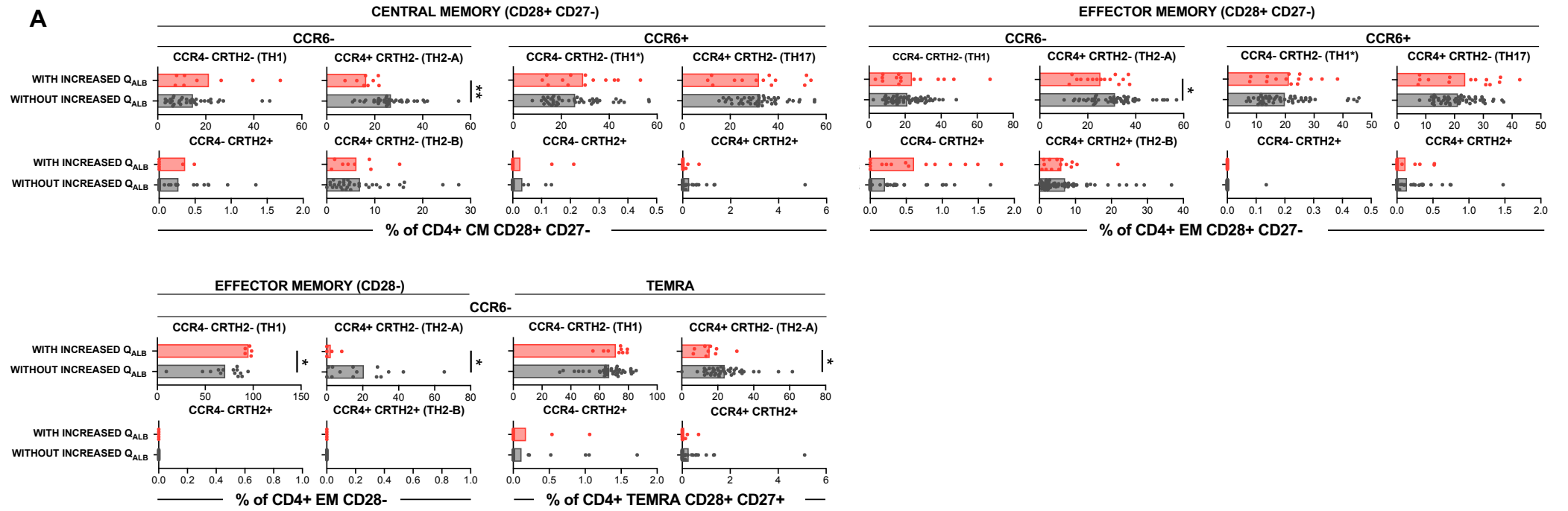
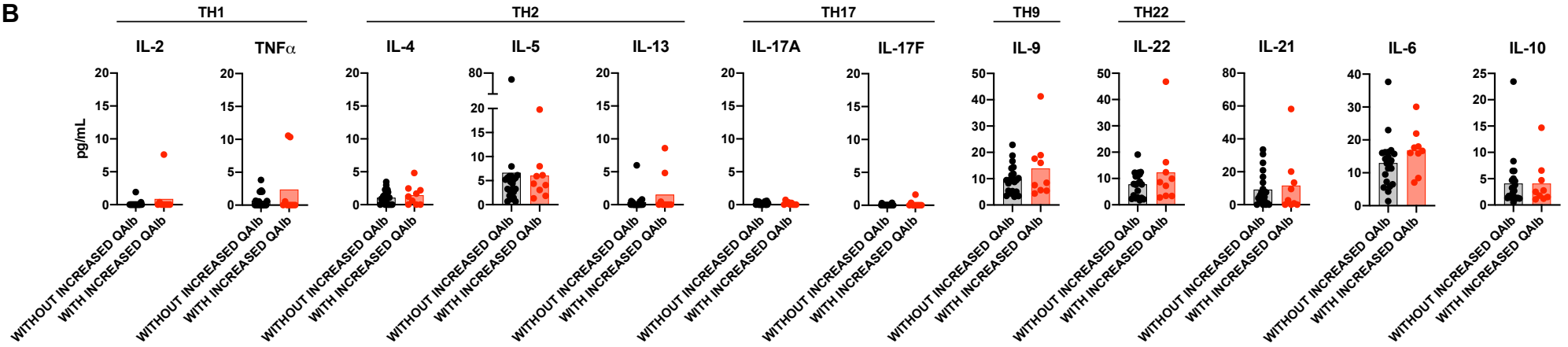


Figure S1. Gating strategy. First doublets are excluded, followed by identification of lymphocytes by size. Next, CD3- are identified and among them, plasma cells (CD19- CD138+), plasma blasts (CD19+ CD138+), B cells (CD19+ CD138-) and CD19- CD138- cells. Among B cells, naïve (IgD+ CD27-), unswitched memory (IgD+ CD27+), switched memory (IgD- CD27+) and double negative (IgD- CD27-) B cell subsets are also identified. In CD3+ T cells, CD3+ CD4+ and CD3+ CD8+ cells are first identified and then separated in CM (CCR7+ CD45RA-), EM (CCR7- CD45RA-), TEMRA (CCR7- CD45RA+) and naïve (CCR7+ CD45RA+). CM, EM and TEMRA CD8+ T cells are then separated in CD28+ and CD28- while CM, EM and TEMRA CD4+ T cells in CD28+ CD27+, CD28+ CD27- and CD28-. Each one of these CD4+ and CD8+ T cells are separated first in CCR6- and CCR6+ and then in Th1 (CCR6- CCR4- CRTH2-), Th2-A (CCR6- CCR4+ CRTH2-), Th2-B (CCR6- CCR4+ CRTH2+), CCR6- CCR4- CRTH2+, Th1* (CCR6+ CCR4- CRTH2-), Th17 (CCR6- CCR4+ CRTH2-), CCR6+ CCR4+ CRTH2+ and CCR6+ CCR4- CRTH2+ cells.

Antibodies: anti-CD3 AF700, anti-CD4 PE TR, anti-CD8 BV510, anti-CD45RA BV711, anti-CCR7 BV421, anti-CD27 APC Cy7, anti-CD28 PE Cy7, anti-CCR4 APC, anti-CRTh2 PE, anti-CCR6 BV785, anti-CD19 PerCPCy5.5, anti-IgD BV605 and anti-CD138 FITC.

A**B**

C

PERIPHERAL BLOOD

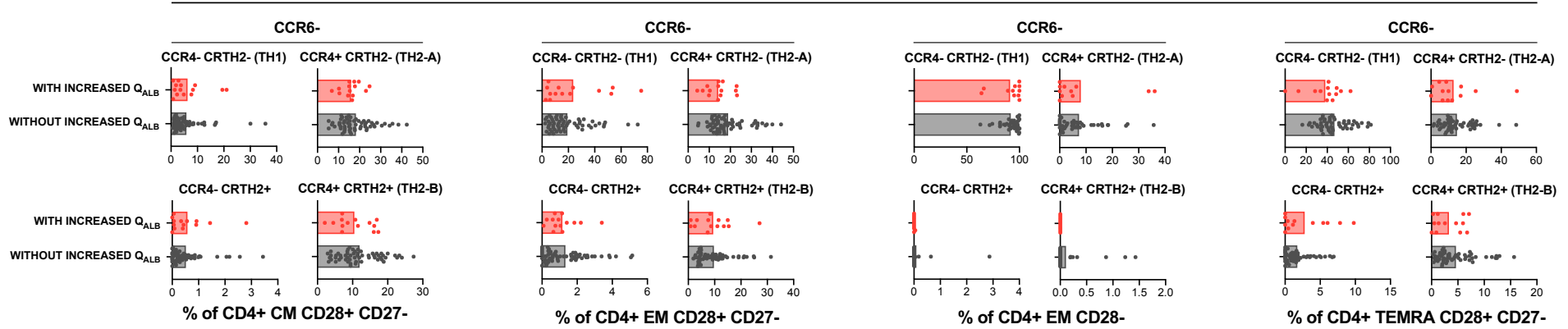


Figure S2. Immunophenotyping/cytokines in CSF/peripheral blood. A. Comparison between patients with (red) and without (black) increased Q_{Alb} of frequencies of Th1 (CCR6- CCR4- CRTH2-), Th2-A (CCR6- CCR4+ CRTH2-), Th2-B (CCR6- CCR4+ CRTH2+), CCR6- CCR4- CRTH2+, Th1* (CCR6+ CCR4- CRTH2-), Th17 (CCR6+ CCR4- CRTH2-), CCR6+ CCR4+ CRTH2+ and CCR6- CCR4- CRTH2+ cells in CM CD28+ CD27-, EM CD28+ CD27-, EM CD28- and TEMRA CD28+ CD27+ CD4+ CSF-infiltrating T cells. **B.** Comparison between patients with (and without increased Q_{Alb} of the amount of cytokines expressed as pg/ml present in the CSF of 30 patients. **C.** Comparison between patients with and without increased Q_{Alb} of the frequency of Th1 (CCR6- CCR4- CRTH2-), Th2-A (CCR6- CCR4+ CRTH2-), Th2-B (CCR6- CCR4+ CRTH2+) and CCR6- CCR4- CRTH2+ in CD4+ CM CD28+ CD27-, CD4+ EM CD28+ CD27-, CD4+ EM CD28- and CD4+ TEMRA CD28+ CD27- obtained by x-vivo immunophenotyping of peripheral blood cells. Ex-vivo immunophenotyping has been done in subcohort-1 consisting in 79 MS patients. In all graphs each dot corresponds to one patient and bars show the mean. T test applying the Bonferroni correction was used to compared patients with and without increased Q_{Alb} . Statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$) is shown.