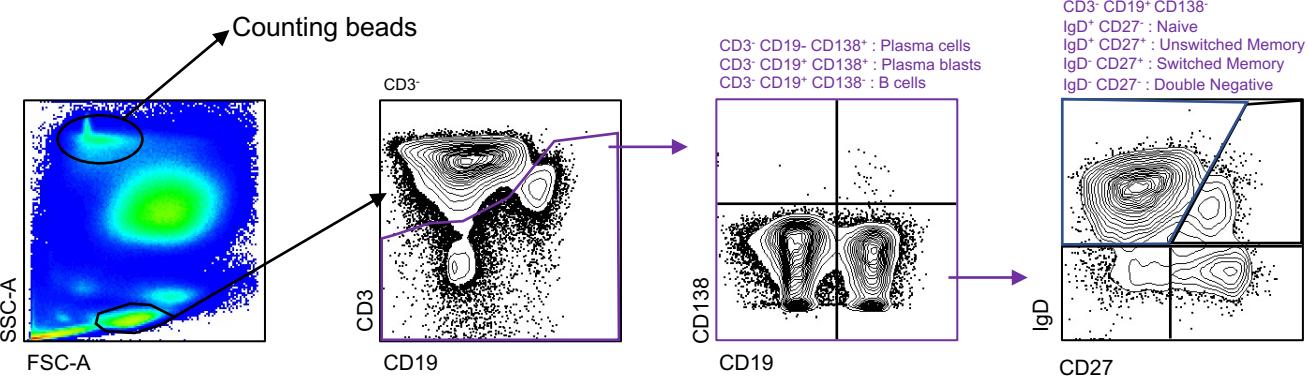
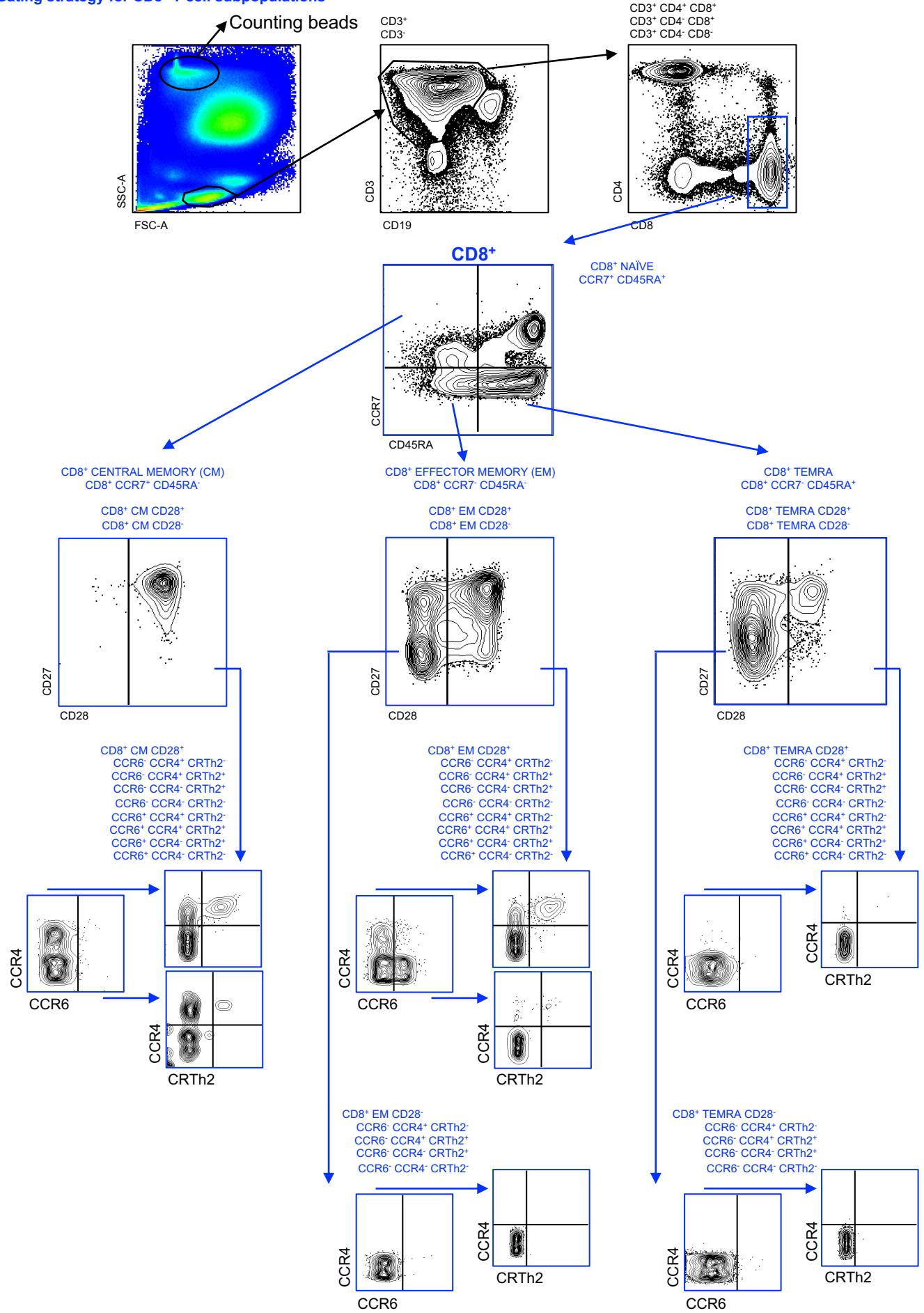


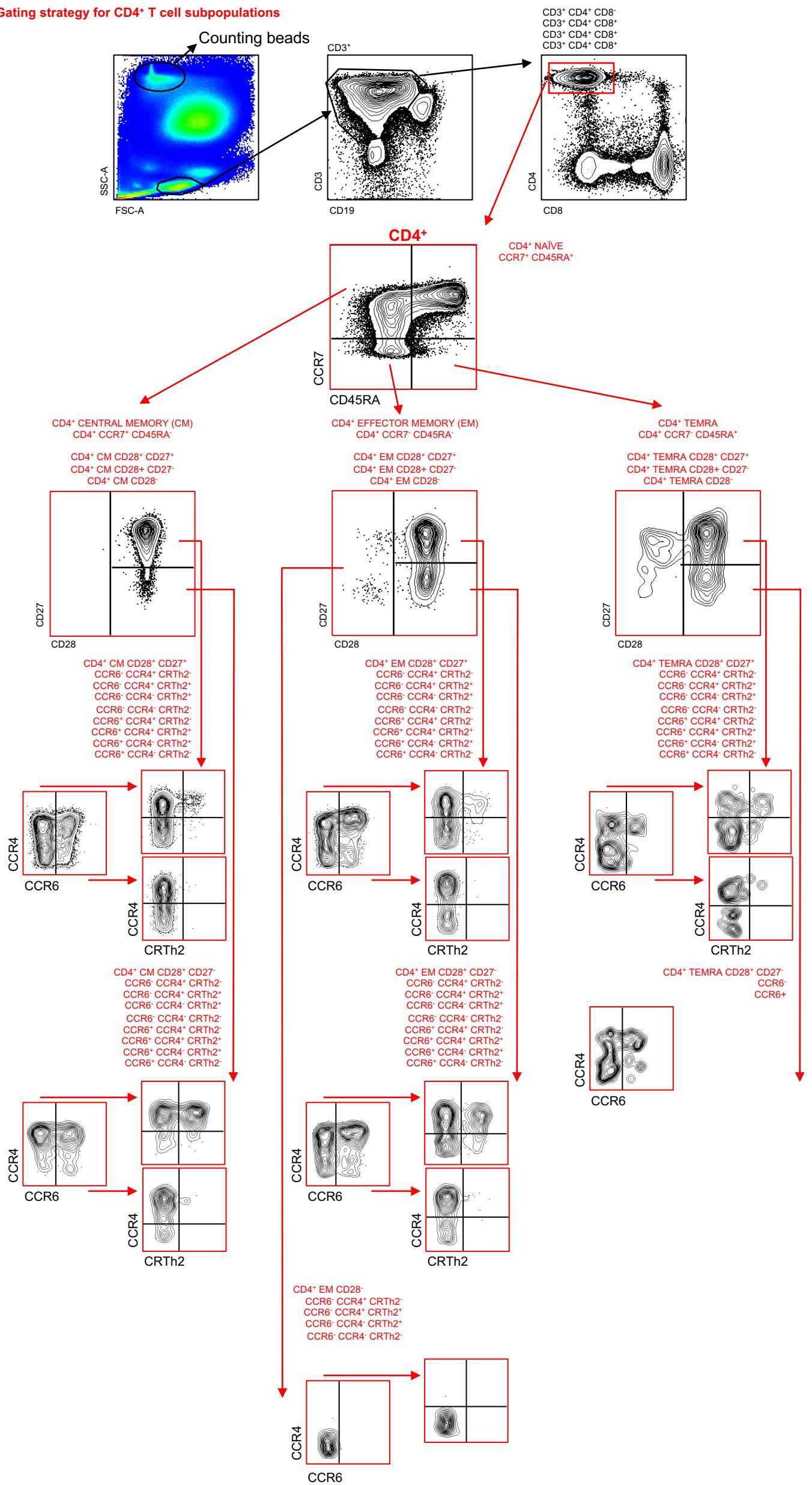
### A. Gating strategy for B cell subpopulations



### B. Gating strategy for CD8<sup>+</sup> T cell subpopulations

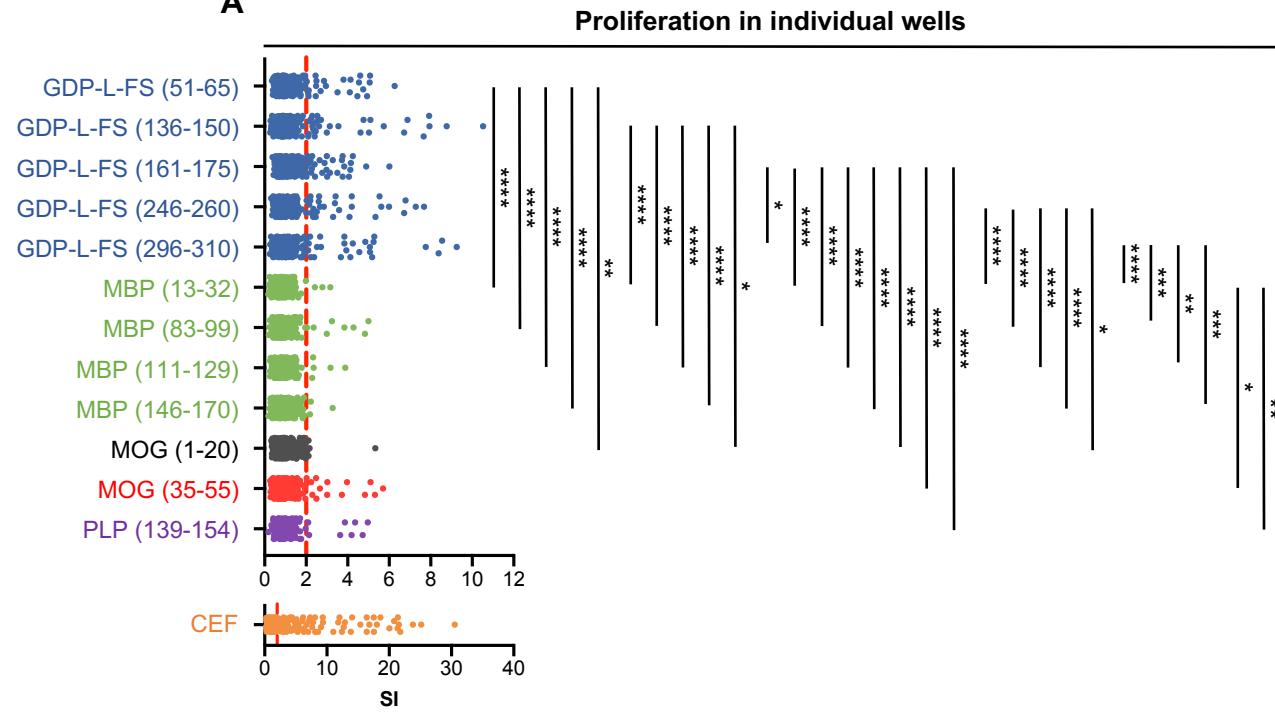
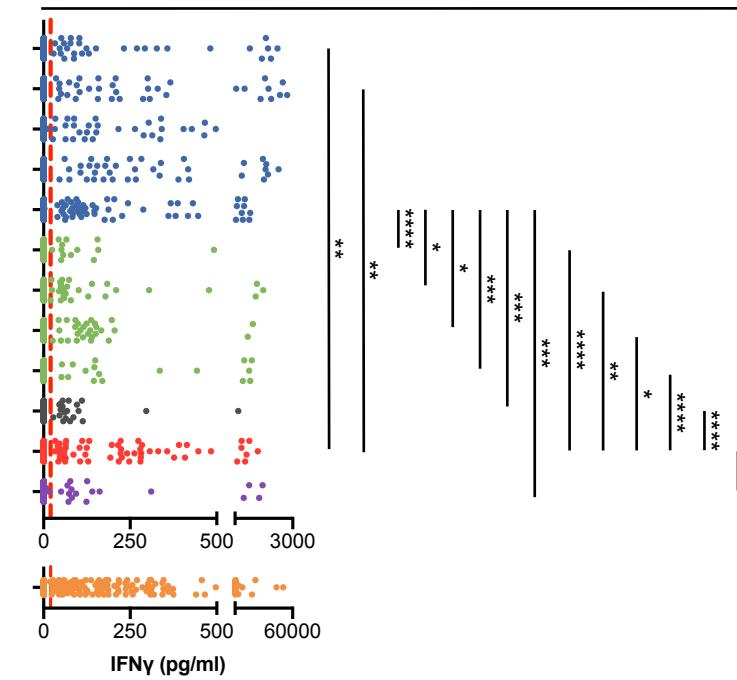
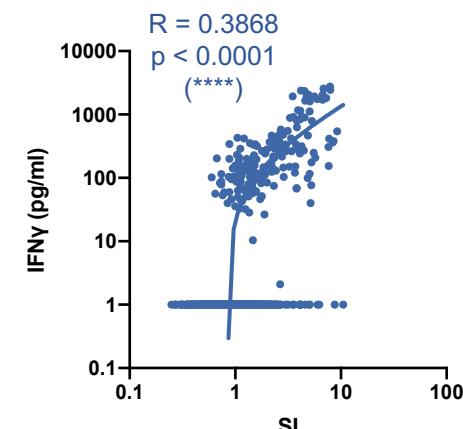
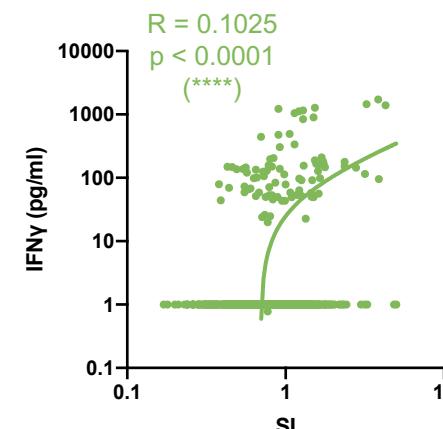
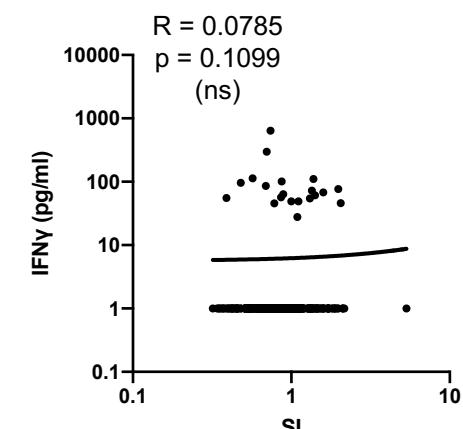
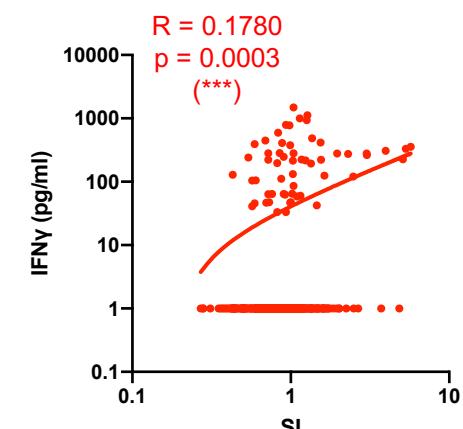
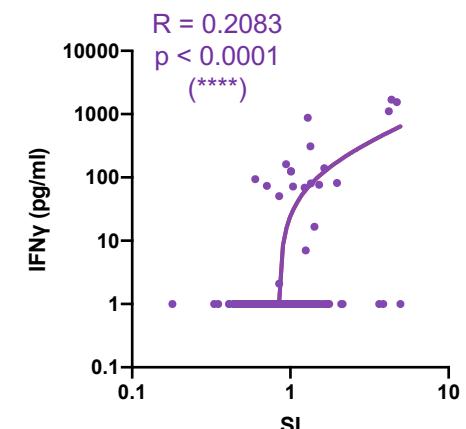


### C. Gating strategy for CD4<sup>+</sup> T cell subpopulations



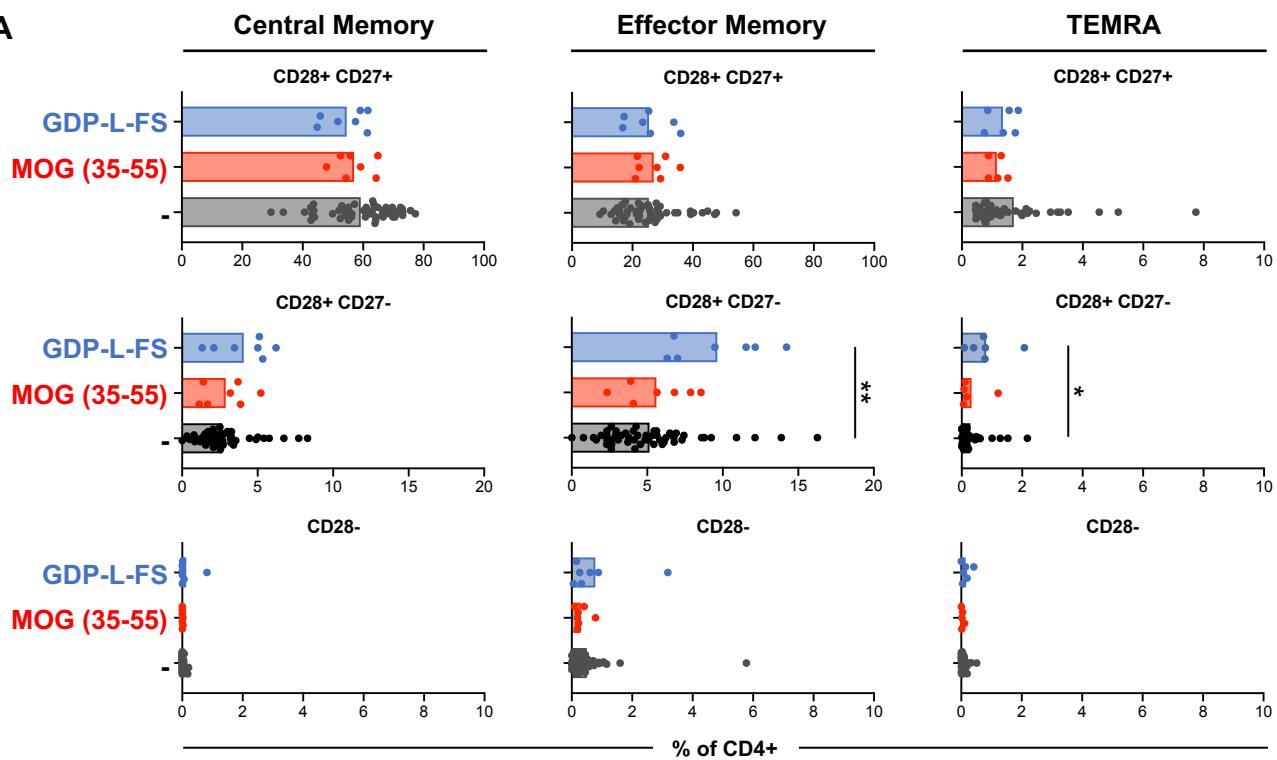
**Figure S1. Gating strategy. (A-C)** First doublets are excluded, followed by identification of lymphocytes by size. **A.** CD3- are identified and among them, plasma cells (CD19- CD138+), plasma blasts (CD19+ CD138+), B cells (CD19+ CD138-) and CD19- CD138- cells. Among B cells, naïve (IgD+ CD27-), unswitched memory (IgD+CD27+), switched memory (IgD- CD27+) and double negative (IgD- CD27-) B cell subsets are also identified. **B.** In CD3+ T cells, CD3+ CD8+ cells are first identified and then separated in CM (CCR7+ CD45RA-), EM (CCR7- CD45RA-), TEMRA (CCR7- CD45RA+) and naive (CCR7+ CD45RA+). CM, EM and TEMRA CD8+ T cells are then separated in CD28+ and CD28-. Each one of these 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. **C.** In CD3+ T cells, CD3+ CD4+ cells are first identified and then separated in CM (CCR7+ CD45RA-), EM (CCR7- CD45RA-), TEMRA (CCR7- CD45RA+) and naive (CCR7+ CD45RA+). CM, EM and TEMRA CD4+ T cells are then separated in CD28+, CD27+, CD28+ CD27- and CD28-. Each one of these CD4+ 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. SPHEROTM AccuCount Particles have been used to determine absolute counts.

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 PerCP Cy5.5, anti-IgD BV605 and anti-CD138 FITC.

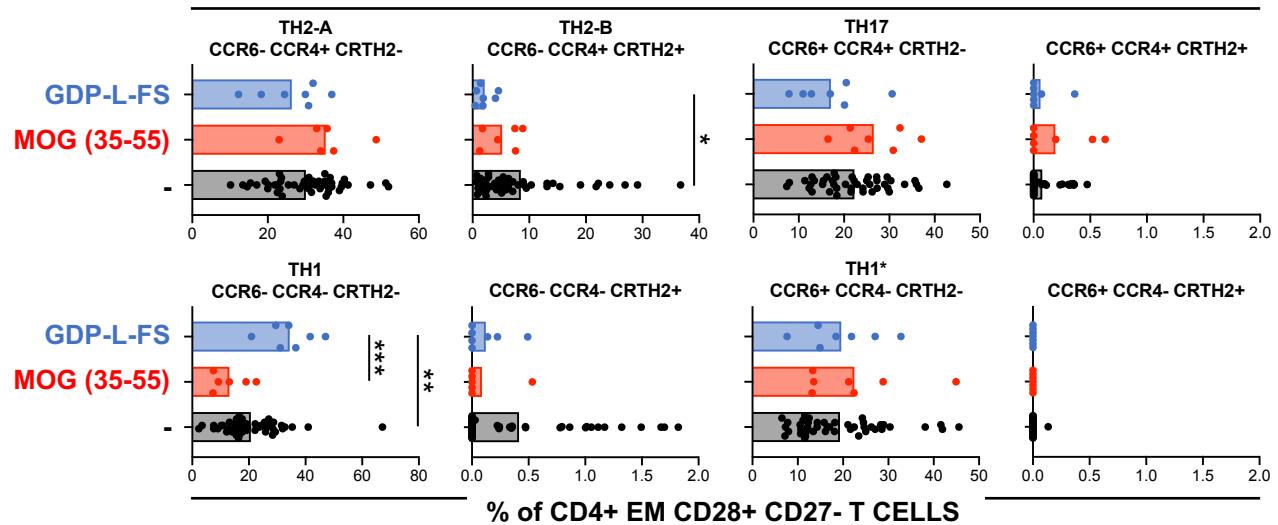
**A****IFN $\gamma$  release in individual wells****B****C****GDP-L-fucose synthase****MBP****MOG (1-20)****MOG (35-55)****PLP (139-154) peptide**

**Figure S2. Recognition of GDP-L-FS and myelin derived peptides by CSF-infiltrating CD4+ T cells from patients with MS.** **A.** Proliferative responses expressed as stimulation indexes (SI) and IFN- $\gamma$  release expressed as (pg/ml) of PHA-expanded CSF-infiltrating CD4+ T cells against GDP-L-FS (blue), myelin (MBP (green), MOG(1-20) (black), MOG(35-55) (red), PLP (violet) and CEF (orange) peptides presented by autologous PBMCs. Each dot represents one well. Each peptide has been tested in quadruplicates (4 wells) in 105 MS patients (420 wells in total per peptide). Red dotted line shows the threshold for positivity (SI  $\geq$  2 for proliferation and pg/ml of IFN- $\gamma$   $\geq$  20 for IFN- $\gamma$  release). We used Kruskal-Wallis test to compared peptide responses. Statistical significance of all comparisons (\* p<0.05, \*\* p<0.01, \*\*\* p<0.001 and \*\*\*\* p<0.0001) is shown. **B.** Ratio of % of positive wells using IFN- $\gamma$  release and proliferation for each peptide. **C.** Correlation between SIs and IFN- $\gamma$  release (pg/ml) for GDP-L-FS, MBP, MOG(1-20), MOG(35-55) and PLP(139-154) peptides. We used Spearman r to test linear correlation between variables. R as well as p values are shown.

### CSF-INFILTRATING CELLS

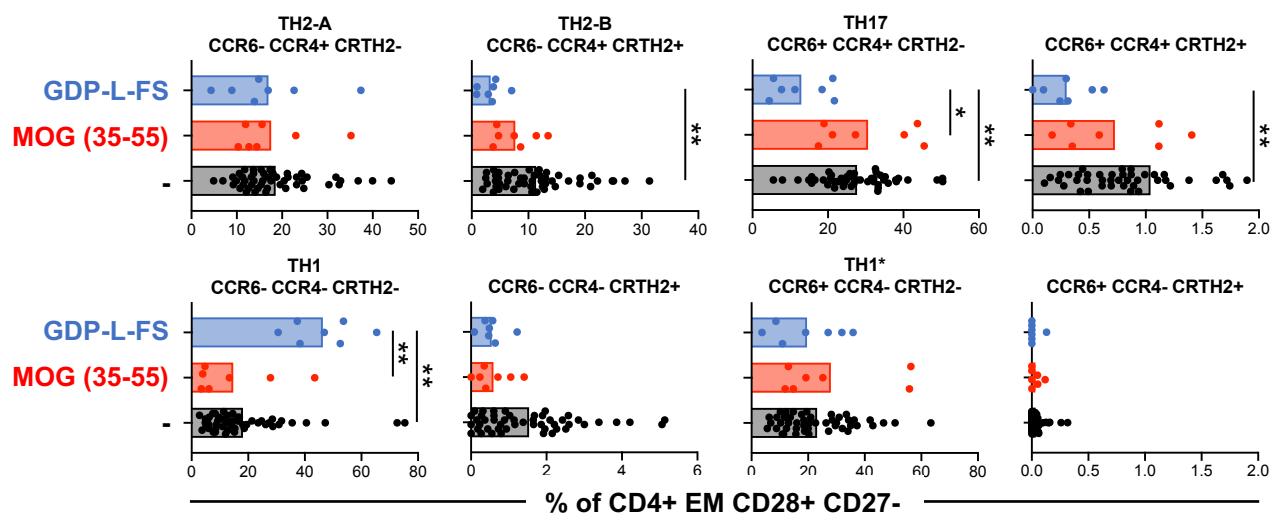
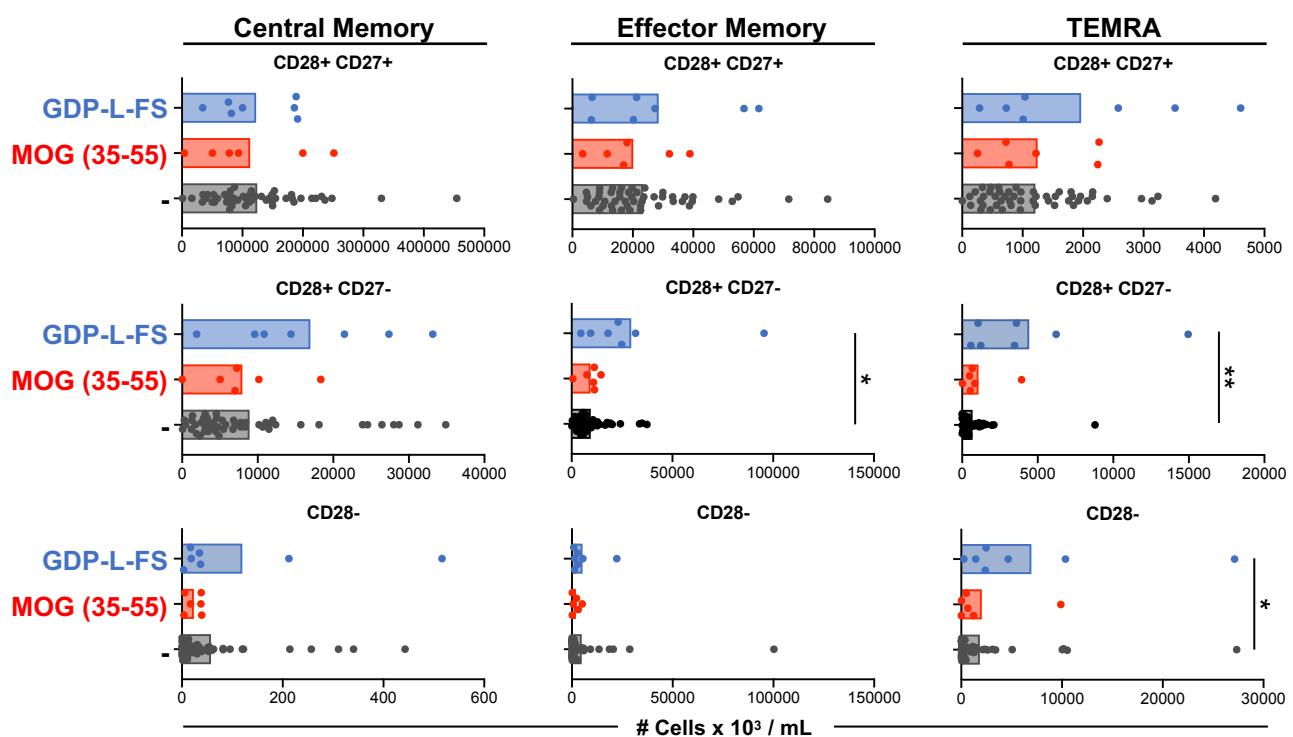
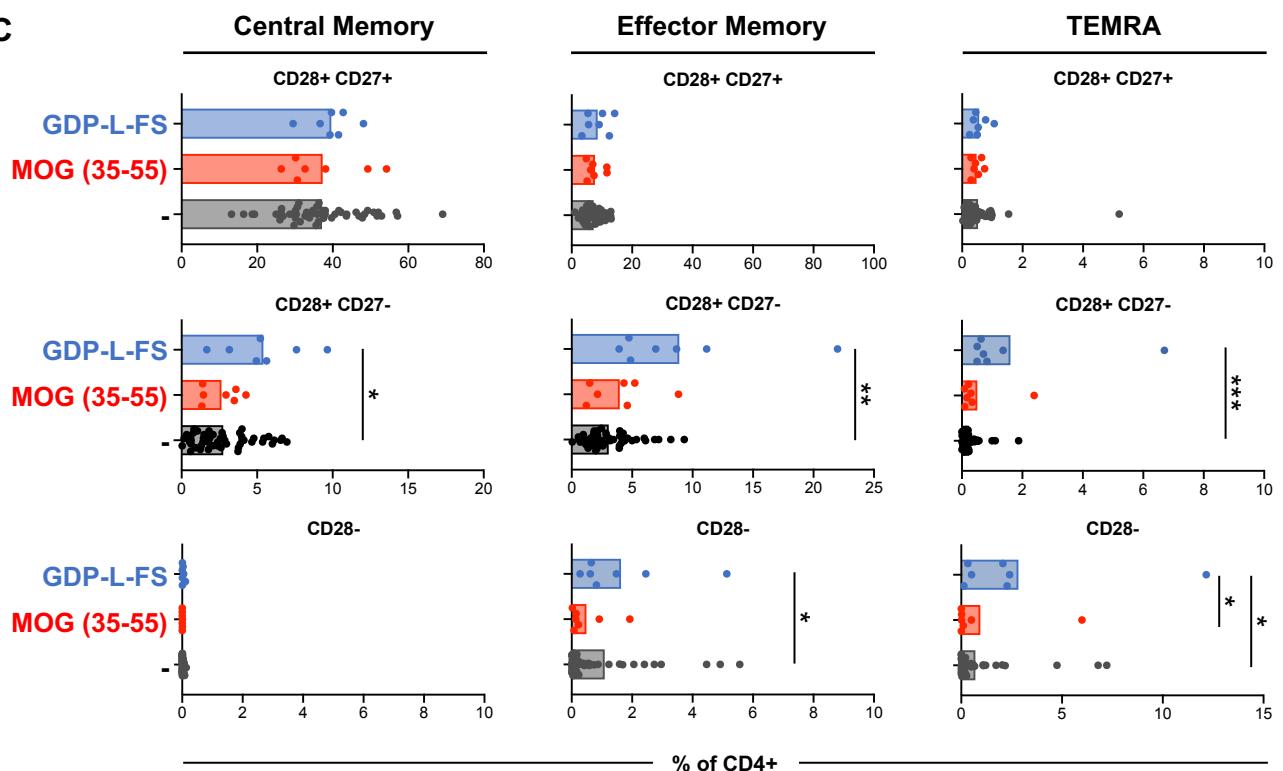
**A**

**B**

### CSF-INFILTRATING CELLS

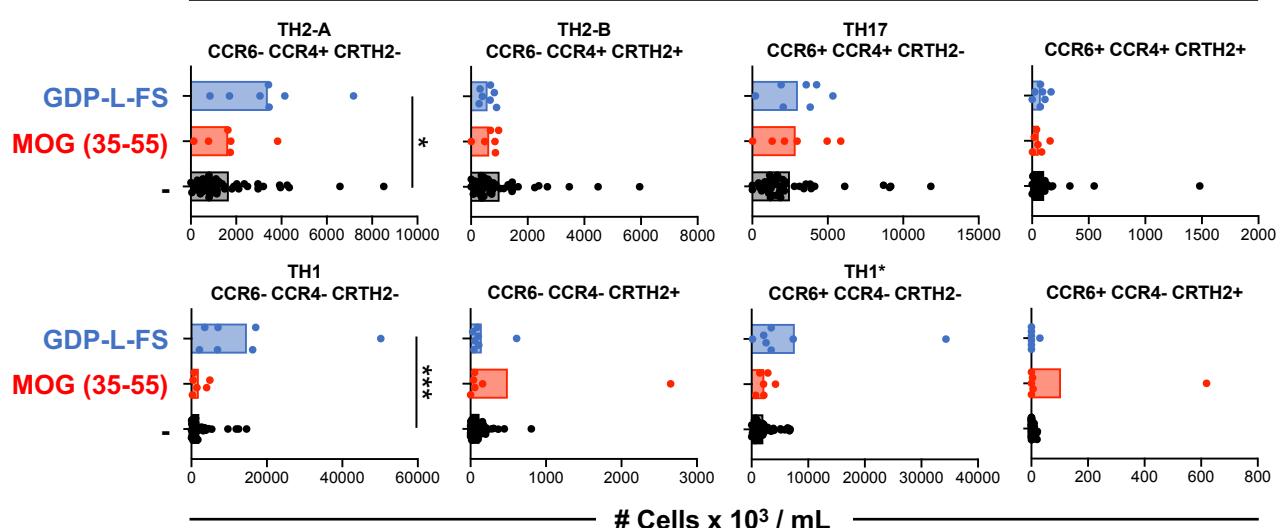


# PERIPHERAL BLOOD CELLS

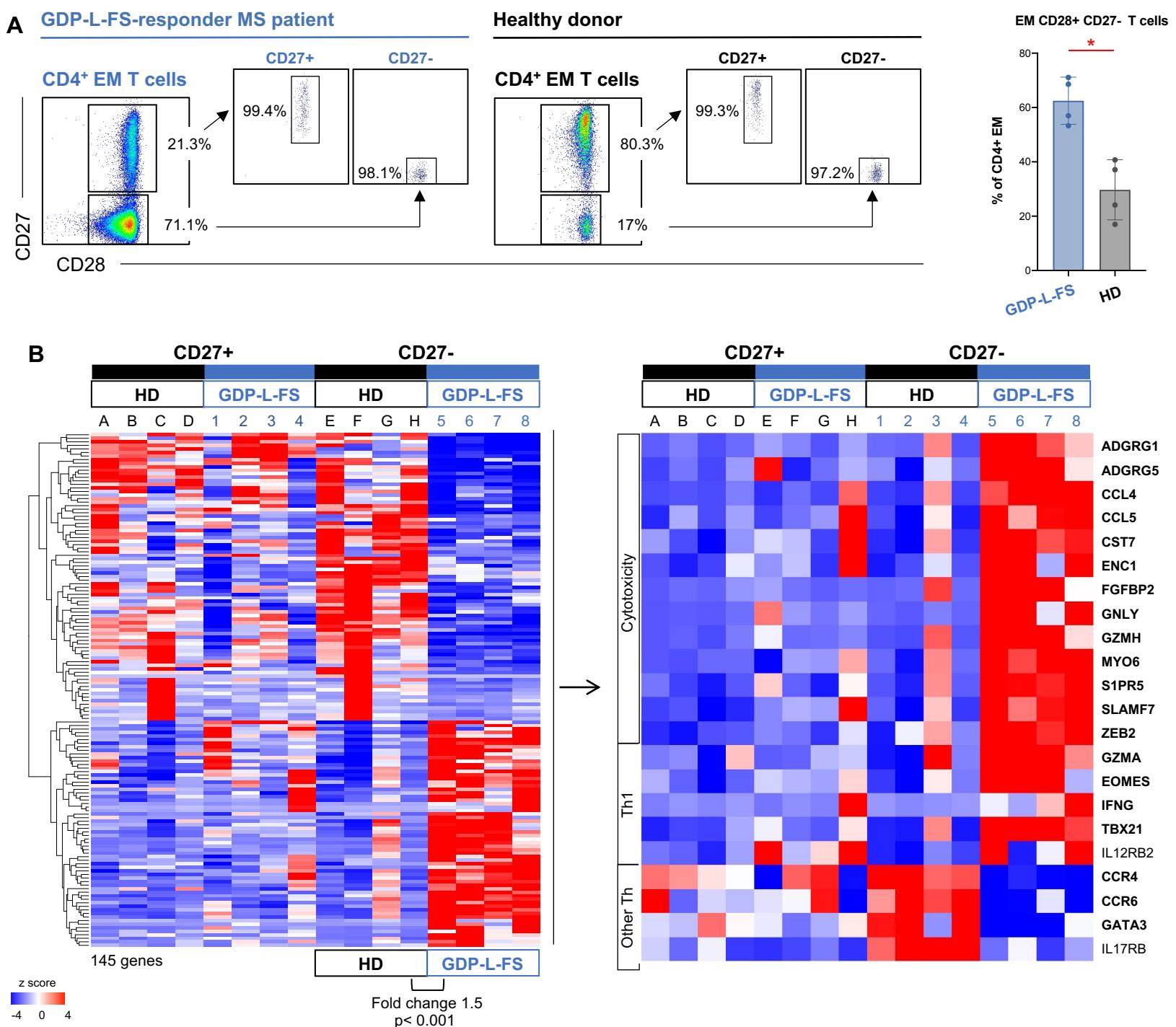
**C**



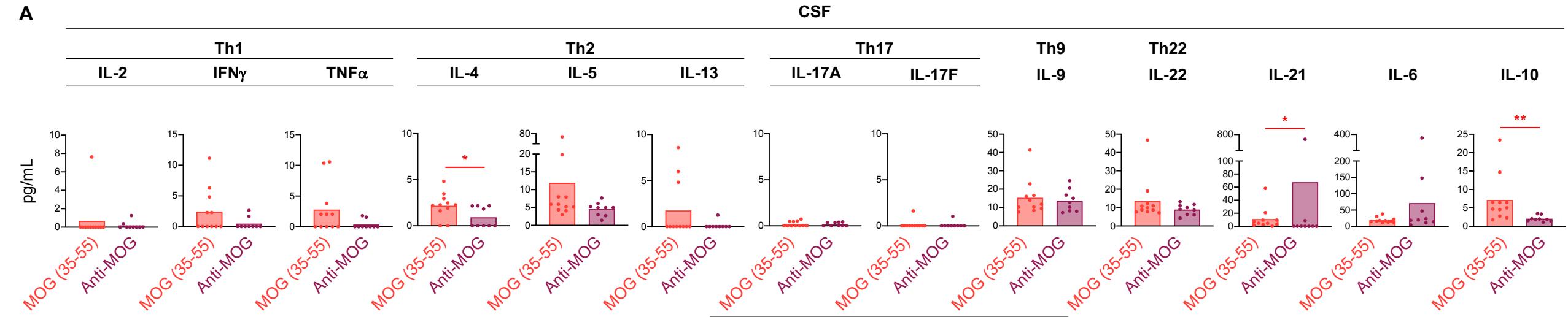
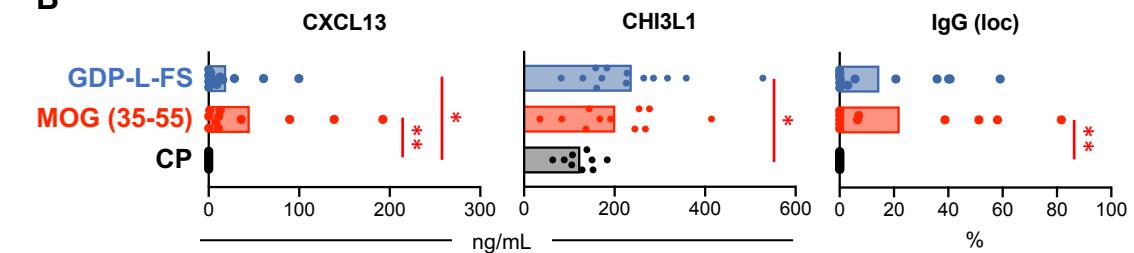
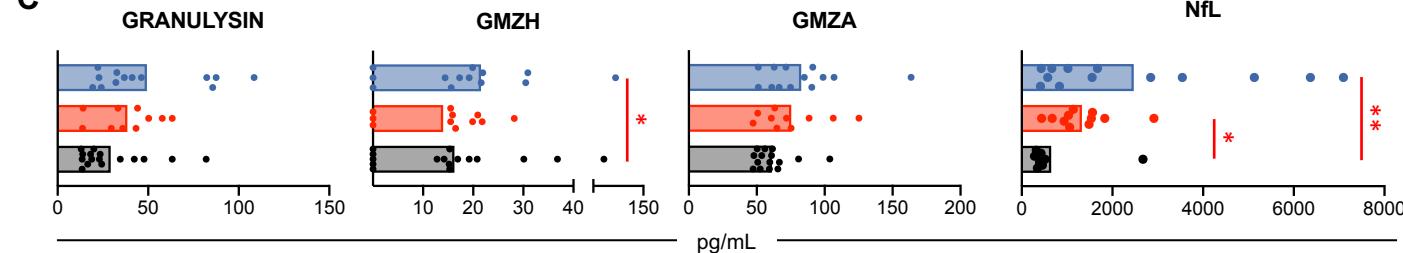
## PERIPHERAL BLOOD CELLS



**Figure S3. Ex-vivo flowcytometry immunophenotyping of CSF-infiltrating and circulating T lymphocytes.** CSF-infiltrating (A-B) and peripheral circulating (C) frequencies as well as real counts of central memory (CM, CCR7+ CD45RA-), effector memory (EM, CCR7- CD45RA-) and TEMRA (CCR7- CD45RA+) CD4+ T cell subsets expressing CD28+CD27+, CD28+CD27- and CD28-, as well as EM CD28+ CD27- CD4+ T cells with the following functional phenotypes based on the chemokine receptor expression: Th2A (CCR6- CCR4+ CRTh2-), Th2B (CCR6- CCR4+ CRTh2+), CCR6- CCR4- CRTh2+, Th1 (CCR6- CCR4- CRTh2-), Th17 (CCR6+ CCR4+ CRTh2-), CCR6+ CCR4+ CRTh2+, CCR6+ CCR4- CRTh2+ and Th1\* (CCR6+ CCR4- CRTh2-). Each dot in the graphs correspond to a single patient and the bars show the mean. GDP-L-FS patients are shown in blue, MOG(35-55) in red and non-responder in black. Cell counts have been determined using SPHEROTM AccuCount Particles. Kruskal-Wallis test was used to compared GDP-L-FS, MOG 35-55 and non-responder patients. Statistical significance (\* p<0.05, \*\* p<0.01, \*\*\* p<0.001 and \*\*\*\* p<0.0001) is shown.



**Figure S4. Purification and transcriptome analysis of EM CD27+/CD27- cells.** **A.** Gating strategy used to isolate EM CD28+ CD4+ T cells expressing or not CD27 from four GDP-L-FS-responder MS patients and four HD. The frequencies of EM CD28+ CD4+ T cells expressing or not CD27 before and after cell sorting from one representative GDP-L-FS-responder MS patient and one HD are shown. Mann-Whitney test has been used to compare the frequency of EM CD27 cells in GDP-L-FS-responder patients and HD and statistical significance (\* p<0.05) is shown. **B.** Heat map showing row-wise z scores of 145 differentially expressed transcripts identified by RNA-seq analysis and pairwise comparison of EM CD27- from GDP-L-FS responders (columns 5-8) versus EM CD27- from HD (columns E-H) (Log2Ratio > 0.5, p< 0.001). Heat map also shows the row-wise z scores of these 145 transcripts in EM CD27+ cells from GDP-L-FS-responders (columns 1-4) and HD (columns A-D). Row-wise z scores of selected transcripts associated with cytotoxicity, Th1 and other Th subsets are shown in detail. In bold are genes that also were identified as differentially expressed by RNA-seq analysis and pairwise comparison of EM CD27- versus EM CD27+ in GDP-L-FS patients (Log2Ratio > 0.5, p< 0.001).

**A****B****C**

**Figure S5. Additional characterization of CSF from patients and controls.** **A.** Cytokines present in the CSF of MOG(35-55)-responders (red) and MOGAD patients. Cytokine release is expressed as pg/ml. **B-C.** Intrathecal abundance in GDP-L-FS- and MOG(35-55)-responders and CP of: **B**, CXCL13, Chitinase-3-like protein 1 (CHI3L1) and intrathecal IgG synthesis IgG(loc); **C**, granulysin, granzyme H (GZMH), granzyme A (GZMA) and Neurofilament Light Chain (NfL). Patient color code, GDP-L-FS-responders (blue), MOG(35-55)-responders (red) and CP (black). Each dot in the graphs correspond to a single patient and the bars show the mean. Mann-Whitney test was used to compared MOG(35-55)-responders and anti-MOG patients and Kruskal-Wallis to compared GDP-L-FS-responders, MOG(35-55)-responders and CP. Statistical significance (\* p<0.05, \*\* p<0.01, \*\*\* p<0.001 and \*\*\*\* p<0.0001) is shown.

**Table S1.** Demographic and clinical features of patients and controls.

	MS PATIENTS							
	MAIN COHORT				SUB-COHORT			
	All	RIS/CIS	RRMS	PMS	All	RIS/CIS	RRMS	PMS
<b># of Patients</b>	105	14	82	9	66	12	50	4
<b>Female/Male Ratio</b>	1.9	1.8	1.9	2.0	2.1	1.4	2.3	3.0
<b>Age (y)**</b>	35.7 ± 9.8	30.6 ± 10.3	35.3 ± 9.0	46.9 ± 8.0	35.6 ± 10.0	30.0 ± 8.4	35.9 ± 9.6	49.5 ± 4.0

MS PATIENTS PREVIOUSLY TREATED				
Previous treatment	Steroids (Not during the last 4 weeks prior to enrolment)	IFN-beta-1a (Not during the last 3 months prior to enrolment)	Glatiramer acetate (Not during the last 3 months prior to enrolment)	Dimethyl fumarate (Not during the last 3 months prior to enrolment)
<b># of Patients</b>	14	1	3	3

	CONTROL PATIENTS			
	UWMA*	Pseudotumors	MOGAD PATIENTS	HEALTHY DONORS
<b># of Patients</b>	6	5	10	4
<b>Female/Male Ratio</b>	1.5		4	1.5
<b>Age (y)**</b>	$33.4 \pm 6.9$		$36.2 \pm 16.1$	$27.4 \pm 6.3$

\* Mean  $\pm$  standard deviation is shown, \*\* PMS, primary progressive and secondary progressive MS, \*\*\* undetectable white matter abnormalities.

**Table S2. GDP-L-FS, myelin and CEF Peptides**

<b>GDP-L-fucose synthase</b>	
GDP-L-fucose synthase 51-65	TAQTRALFEKVQPTH
GDP-L-fucose synthase 136-150	PHNSNFGYSYAKRMI
GDP-L-fucose synthase 161-175	YGCTFTAVIPTNVFG
GDP-L-fucose synthase 246-260	EEDEVSIKEAAEAVV
GDP-L-fucose synthase 296-310	FRFTPQFKQAVKETCA
<b>Myelin</b>	
MBP 13-32	KYLATASTMDHARHGFLPRH
MBP 83-99	ENPVVHFFKNIVTPRT
MBP 111-129	LSRFSWGAEGQRPGFGYGG
MBP 146-170	AQGTLISKIFKLGGRDSRGSPMARR
MOG 1-20	GQFRVIGPRHPIRALVGDEV
MOG 35-55	MEVGWYRPPFSRVVHLYRNGK
PLP 139-154	HCLGKWLGHPDKFVGI
<b>CEFT2 Mix (CEF)</b>	
Influenza A	FVFTLTVPSEN
Influenza A	SGPLKAEIAQRLEDV
Influenza A	YDVPDYASLRSILVASS
Influenza B	PYYTGEHAKAIGN
Tetanus	GQIGNDPNRDIL
Influenza A	PKYVKQNTLKLA
Influenza A	PKYVKQNTLKLAT
Influenza A	DRLRRDQKS
EBV	AGLTLSSLVICSYLFISRG
Tetanus	QYIKANSKFIGITEL
Tetanus	QYIKANSKFIGITE
Tetanus	FNNFTVSFWLRVPKVSASHLE
EBV	TSLYNLRRGTALA
Tetanus	KFIIKRYTPNNEIDSF
Tetanus	VSIDKFRIFKALNPK
EBV	VPGLYSPCRAFFNKEELL
HCMV	DKREMWMACIKELH
EBV	TGHGARTSTEPTTDY
EBV	KELKRQYEKKLRQ
Influenza A	RGYFKMRTGKSSIMRS
EBV	TVFYNIPPMPL
EBV	AEGLRALLARSHVER
EBV	PGPLRESIVCYFMVFLQTHI



EFFECTOR MEMORY		% of CCR+ EFFECTOR MEMORY CCR+									
		CD2+									
CCR+ CCR4+ CTRH+		14.23 ± 1.61	13.34 ± 1.22	13.44 ± 0.72	na						
CCR+ CCR4- CTRH+		0.23 ± 0.01	0.05 ± 0.00	0.05 ± 0.20	na						
CCR- CCR4+ CTRH+		0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.13	na						
CCR- CCR4- CTRH+		78.23 ± 12.89	80.05 ± 10.08	80.07 ± 11.14	na						
CCR4+ CCR4+ CTRH+		-	-	-	na						
CCR4+ CCR4- CTRH+		-	-	-	na						
CCR4- CCR4+ CTRH+		-	-	-	na						
CCR4- CCR4- CTRH+		-	-	-	na						
CCR4+ CCR4+ CTRH-		-	-	-	na						
CCR4+ CCR4- CTRH-		-	-	-	na						
CCR4- CCR4+ CTRH-		-	-	-	na						
CCR4- CCR4- CTRH-		-	-	-	na						
TEMRA											
		CD2+									
S+HCHO TEMRA CCR+		25.04 ± 15.35	21.54 ± 24.47	27.09 ± 12.75	na						
CCR+ CCR4+ CTRH+		0.59 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	na						
CCR+ CCR4- CTRH+		0.74 ± 1.95	0.05 ± 0.00	0.28 ± 0.82	na						
CCR- CCR4+ CTRH+		80.71 ± 15.89	77.86 ± 24.45	67.88 ± 14.41	na						
CCR- CCR4- CTRH+		-	-	-	na						
CCR4+ CCR4+ CTRH+		-	-	-	na						
CCR4+ CCR4- CTRH+		-	-	-	na						
CCR4- CCR4+ CTRH+		-	-	-	na						
CCR4- CCR4- CTRH+		-	-	-	na						
TEMRA											
		CD2+									
S+HCHO TEMRA CCR+		7.50 ± 3.95	10.23 ± 13.19	8.80 ± 6.58	na						
CCR+ CCR4+ CTRH+		0.20 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	na						
CCR+ CCR4- CTRH+		0.26 ± 0.46	0.10 ± 0.05	0.45 ± 1.17	na						
CCR- CCR4+ CTRH+		81.88 ± 15.57	78.88 ± 13.43	78.82 ± 13.43	na						
CCR- CCR4- CTRH+		0.23 ± 0.15	0.43 ± 0.47	0.32 ± 0.28	na						
CCR4+ CCR4+ CTRH+		-	-	-	na						
CCR4+ CCR4- CTRH+		-	-	-	na						
CCR4- CCR4+ CTRH+		-	-	-	na						
CCR4- CCR4- CTRH+		-	-	-	na						
TEMRA											
		CD2+									
S+HCHO TEMRA CCR+		2.19 ± 1.52	1.54 ± 1.72	1.83 ± 2.40	na						
CCR+ CCR4+ CTRH+		0.02 ± 0.04	0.00 ± 0.00	0.00 ± 0.01	na						
CCR+ CCR4- CTRH+		0.03 ± 0.00	0.00 ± 0.00	0.00 ± 0.04	na						
CCR- CCR4+ CTRH+		96.14 ± 3.08	85.47 ± 13.72	90.32 ± 9.83	na						
CCR- CCR4- CTRH+		-	-	-	na						
CCR4+ CCR4+ CTRH+		-	-	-	na						
CCR4+ CCR4- CTRH+		-	-	-	na						
CCR4- CCR4+ CTRH+		-	-	-	na						
CCR4- CCR4- CTRH+		-	-	-	na						

Ordinary one-way ANOVA  
 0.001 < p < 0.01  
 0.001 < p < 0.01  
 0.001 < p < 0.001

Table S4. Anti-MOG antibodies in CSF and paired serum samples

PATIENT ID	GDP-L-fucose synthase	MBP	MOG 1-20	MOG 35-55	PLP 139-154	CEF	CSF		SERUM	
							anti-MOG IgG	anti-MOG IgM	anti-MOG IgG	anti-MOG IgM
131							0		0	
718							0		0	
776							0		20	160
816							0		20	80
830							0		0	
1081							0		80	80
1129										
1173							0		0	
1440							0		0	
1479							0		0	
1489							0		0	
1493							0		20	40
1604							0		0	
1841							0		0	
<b>GPL-F RESPONDERS</b>										
702							0		0	
818							0		40	80
973							0		0	
1125							0		0	
<b>MOG(35-55) RESPONDERS</b>										
468							0		20	80
748							0		0	
1097							0		0	
1188							0		160	80
1283							0		0	
1303							0		0	
1444							0		20	80
1474							0		0	
1517							0		0	
1560							0		0	
1673							0		0	
<b>NON-RESPONDERS</b>										
66							0		0	
103							0		0	
127							0		0	
172							0		0	
204							0		80	40
247										
373							0		0	
381							0		0	
474							0		0	
697							0		0	
740							0		0	
780							0		0	
800							0		40	20
817							0		0	
866							0		0	
897							0		0	
936							0		20	40
956							0		0	
959							0		0	
971							0		0	
996							2	0	0	
1013							0		0	
1036							0		0	
1111							0		0	
1143							0		0	
1205							0		0	
1206							0		0	
1254							0		0	
1290							0		160	40
1292							0		0	
1300							0		0	
1326							0		20	40
1332							0		20	80
1346							0		20	80
1358							0		40	40
1371							0		0	
1435							2	0	160	80
1453							0		40	20
1458							0		0	
1460							0		0	
1467							0		0	
1515							0		0	
1533							0		0	
1542							0		0	
1571							0		0	
1586							0		0	
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1617										
1619							0		0	
1640							0		0	
1662							0		80	40
1664							0		0	
1690							0		0	
1693							0		0	
1701							0		0	
1702							0		0	
1712							0		80	40
1717							0		0	
1727							0		0	80

1753					0		20	80
1754					0		0	
1755					0		0	
1760					0		0	
1761					0		0	
1791					0		20	160
1799					0		0	
1823					0		0	
1824					0		0	
1840					0		20	160
1858					0		0	
1876					0		80	80
1883					0		20	160
1908					0		20	160
1911					0		0	
1915					0		0	
1916					0		0	

MOGAD-PT1	32	0	10240	1280
MOGAD-PT2	0		320	320
MOGAD-PT3	16	0	5120	40
MOGAD-PT4	1	0	640	20
MOGAD-PT5	1	0	640	40
MOGAD-PT6	256	0	20480	0
MOGAD-PT7	4	0	10240	160
MOGAD-PT9	512	0	20480	80
MOGAD-PT10	2	0	5120	40
MOGAD-PT11			640	

 Positive anti-MOG IgG titers ( $\geq 320$  for serum and  $\geq 1$  for CSF)



