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Supplementary Information for

High-dimensional immune profile elucidates response to dimethyl fumarate in multiple sclerosis

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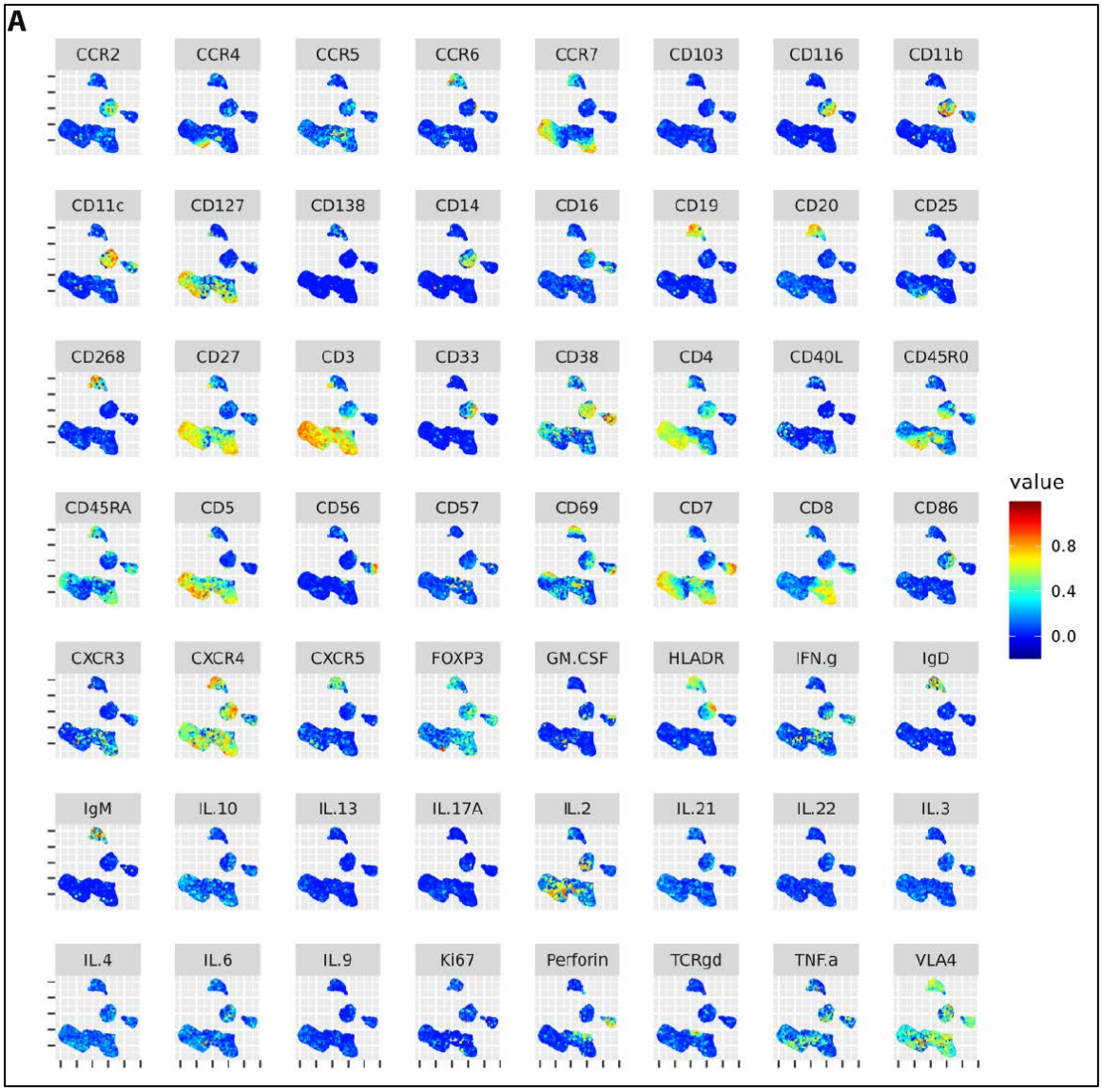

Fig. S1. Expression of mass cytometry marker in the multipanel UMAP. (A) Expression for each marker in the combined multipanel UMAP containing all PBMC by heatmap.

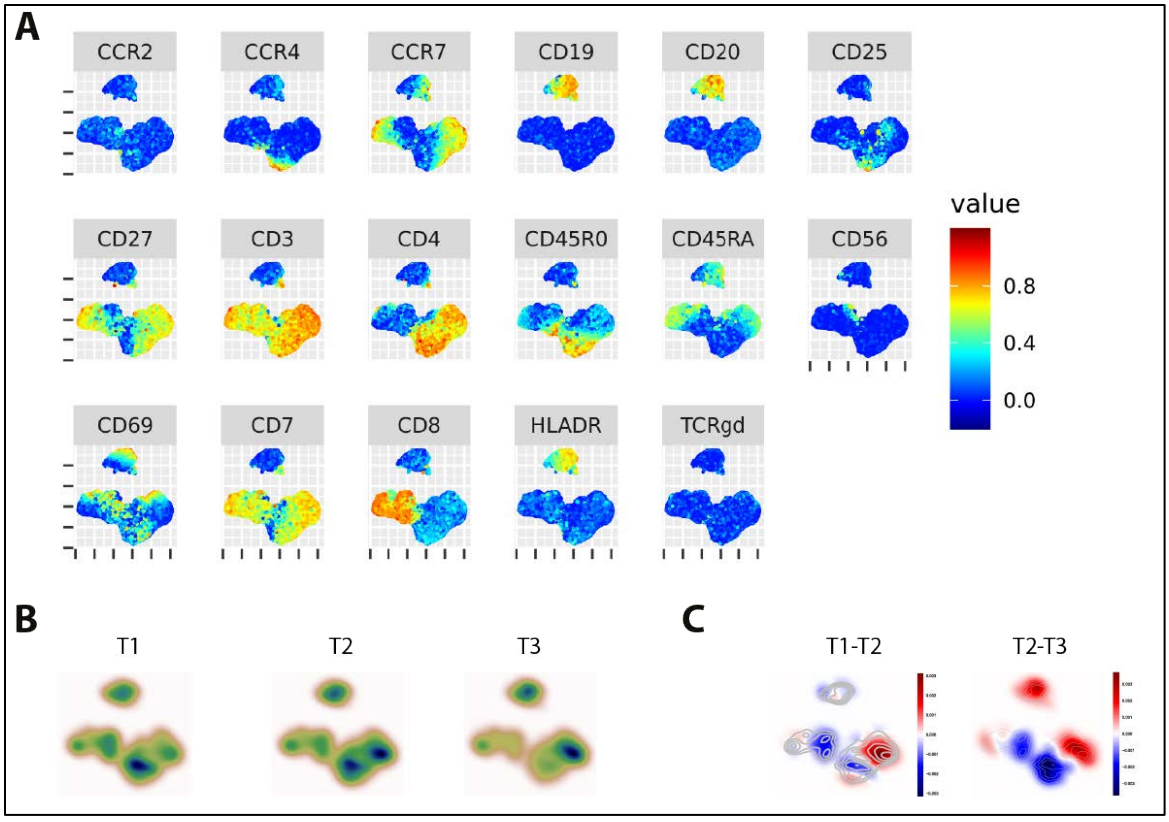

Fig. S2. Longitudinal density changes in the multipanel T and B cell UMAP. (A) Expression for each of the overlapping markers (between stimulation and conventional panel) in the reduced multipanel UMAP containing all B cells, Th and Tc cells by heatmap. (B) Cell density within the UMAP representation was computed via a Gaussian kernel density estimation for each timepoint T1 and T3. (C)The change of density within the UMAP representation was computed for change between T1 vs. T2 and T2 vs. T3, respectively.

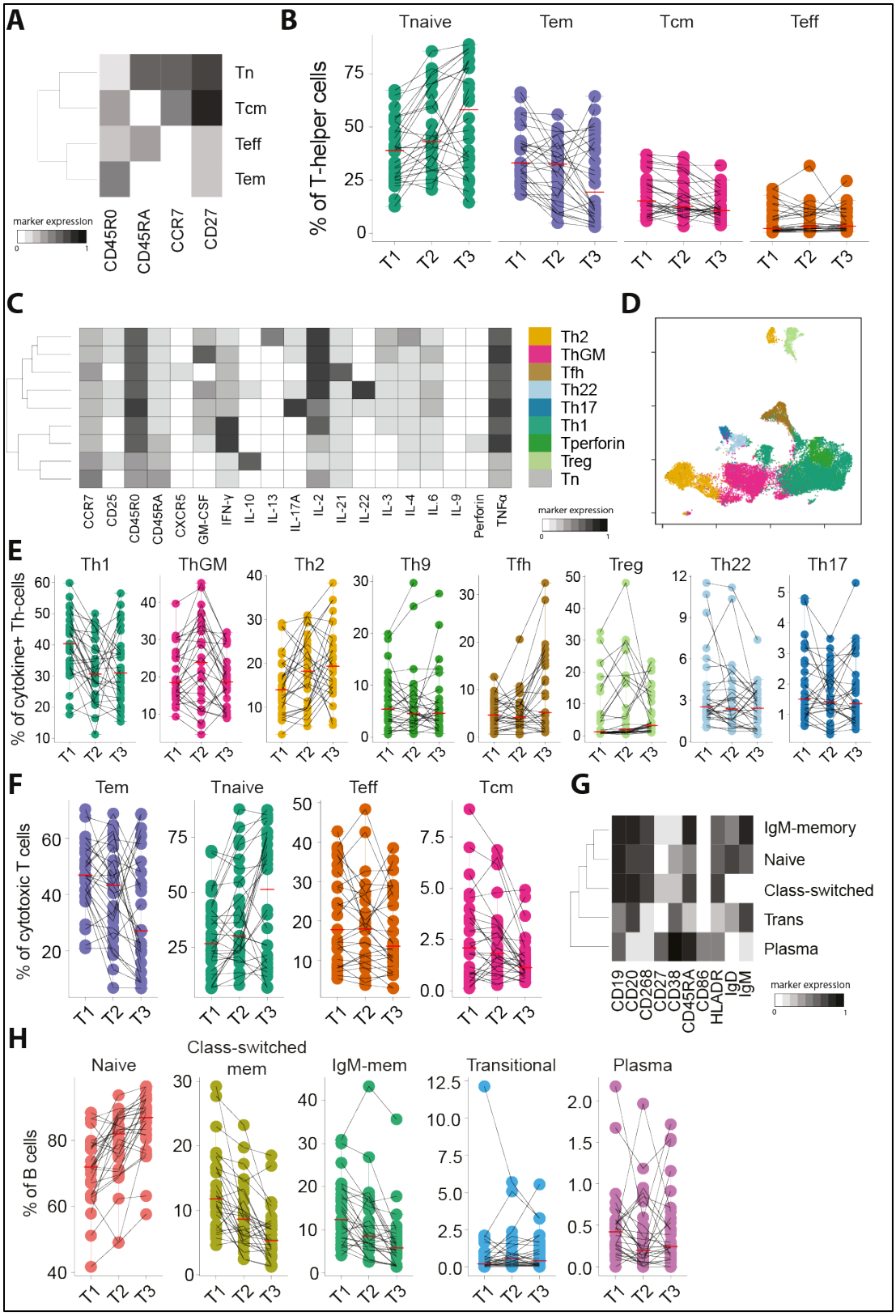

Fig. S3. Longitudinal changes of T and B cell subsets. (A) Th cells from MS patients were subdivided into naïve, effector, effector memory and central memory cells based on FlowSOM-

defined nodes. Mean population expression levels of the indicated differentiation markers. (B) Frequencies of Th subsets in Th cells. (C) FlowSOM was used to identify Th cell subsets based on their cytokine production profile. Clusters were manually annotated based on this profile. Shown are mean expression of surface and cytokine markers by the respective Th cell subsets. (D) UMAP representation of Th subsets with overlaid color code as categorized by FlowSOM-defined clusters. (E) Frequencies of Th subsets at different timepoints. A red horizontal line represents the median. Every point represents one individual sampling and each line connects the baseline and follow-up samples of individual patients. (F) Frequencies of Th subsets in cytotoxic cells. (G) B cells from MS patients were subdivided into naïve, class switched-memory, IgM-memory, transitional and plasma cells based on FlowSOM-defined nodes. Mean population expression levels of the indicated differentiation markers. (H) Frequencies of B subsets at all three timepoints.

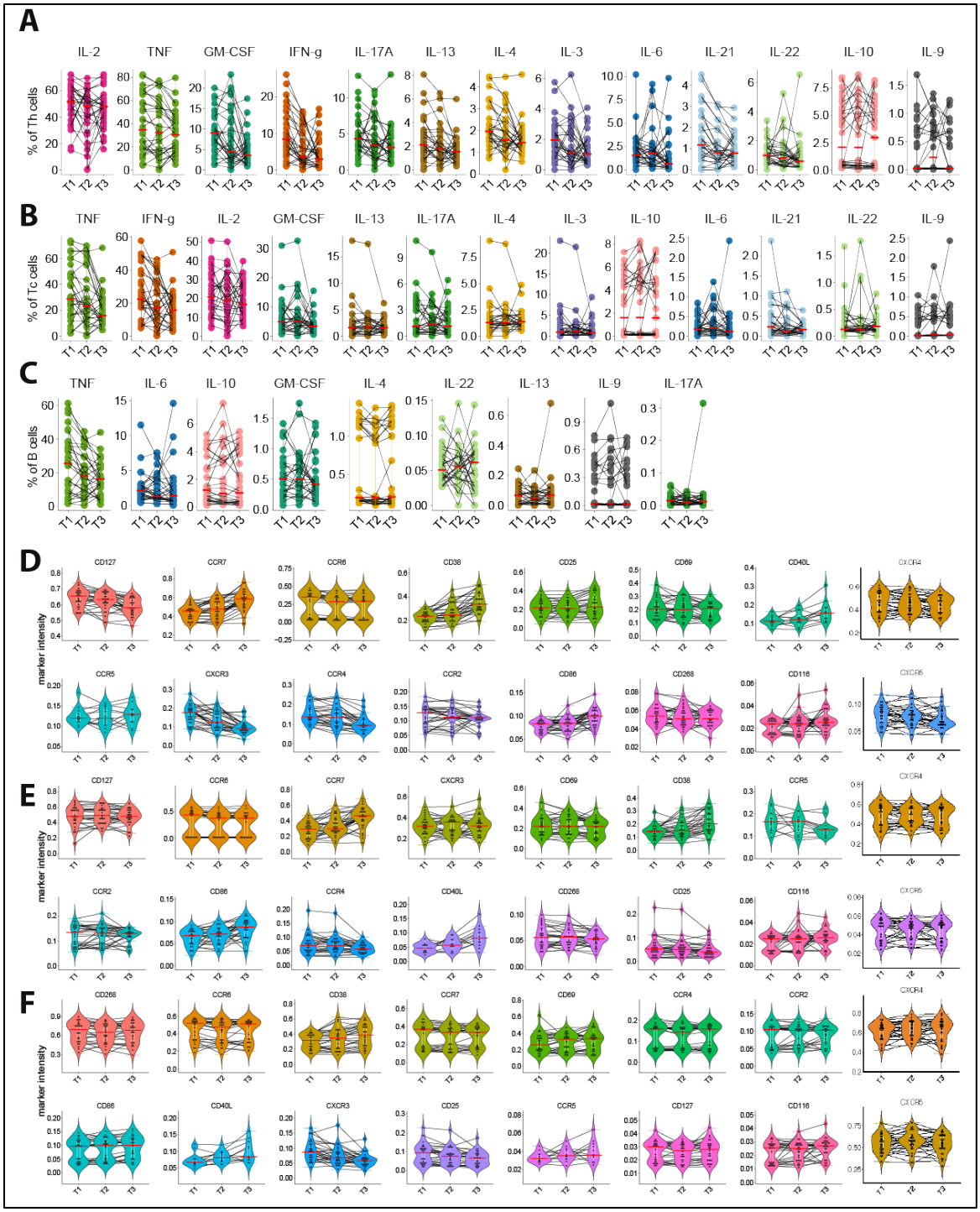

Fig S4. Longitudinal changes of cytokine and cytokine receptor expression in T and B cells. (A) Frequencies of cytokines in Th cells. (B) Frequencies of cytokines in Tc cells. (C) Frequencies of cytokines in B cells. (D) Intensity of cytokine/chemokine receptors in Th cells. (E) Intensity of cytokine/chemokine receptors in Tc cells. (F) Intensity of cytokine/chemokine receptors in B cells.

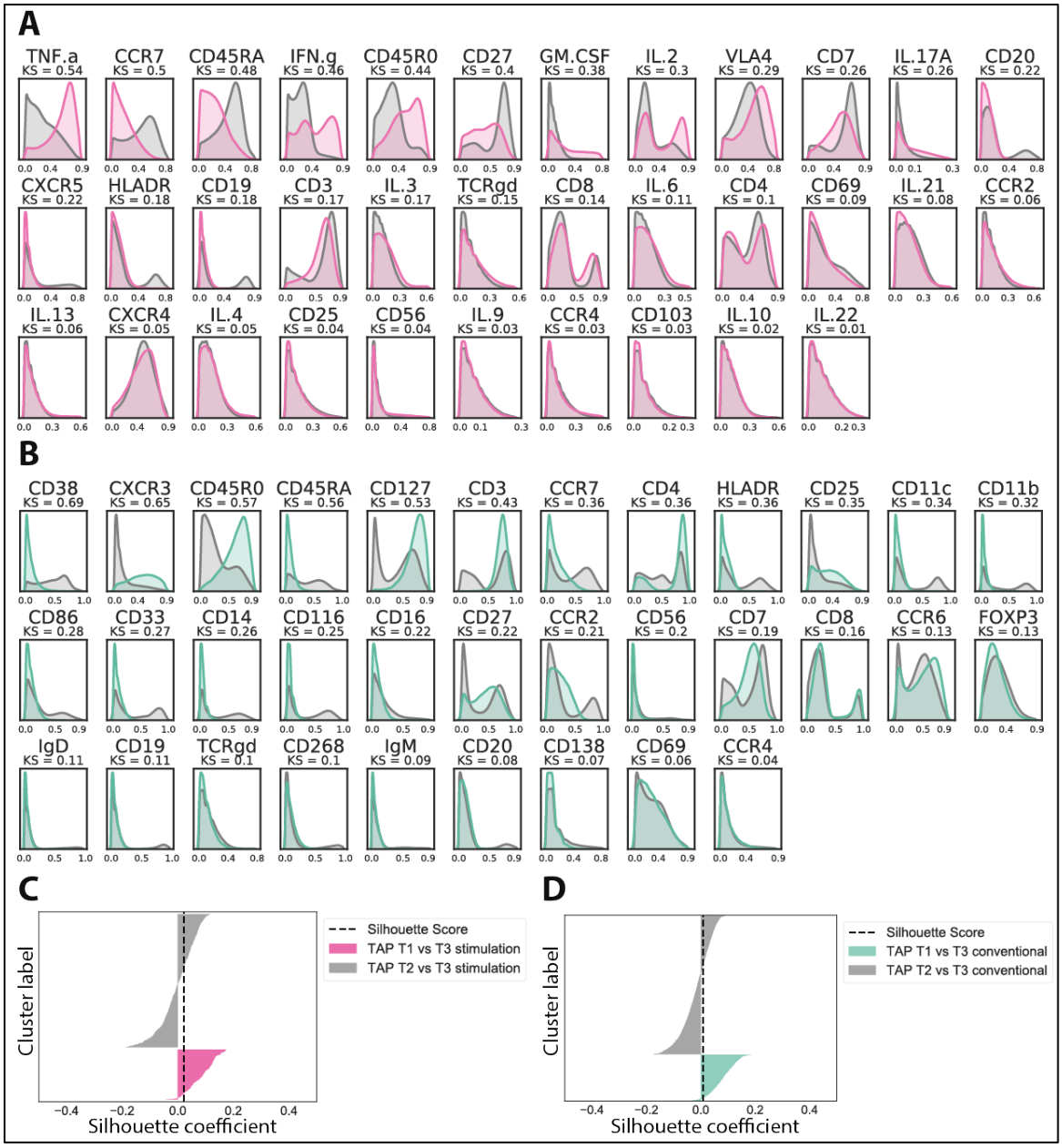

Fig. S5. Details of therapy associated phenotype CellCNN analysis determined the core immune features between T 1 vs T 3 and T 2 vs T 3 from our patient's cohort, termed as therapy associated phenotype (TAP). (A) Expression patterns of all markers from the stimulation panel between the treatment associated phenotype and the reference cell population for the stimulated panel Markers are ordered by their rank in Kolmogorov Smirnov test. (B) Expression patterns of all markers from the conventional panel between the treatment associated phenotype and the reference cell population for the stimulated panel Markers are ordered by their rank in Kolmogorov Smirnov test (C) The overlap between both cell filters identified for comparison T 1 vs T 3 and T 2 vs T 3 in the stimulation panel is estimated using the silhouette analysis. The black dashed line represents the silhouette score. (D) The overlap between both cell filters identified for comparison T 1 vs T 3 and T 2 vs T 3 in the conventional panel is estimated using the silhouette analysis. The black dashed line represents the silhouette score.

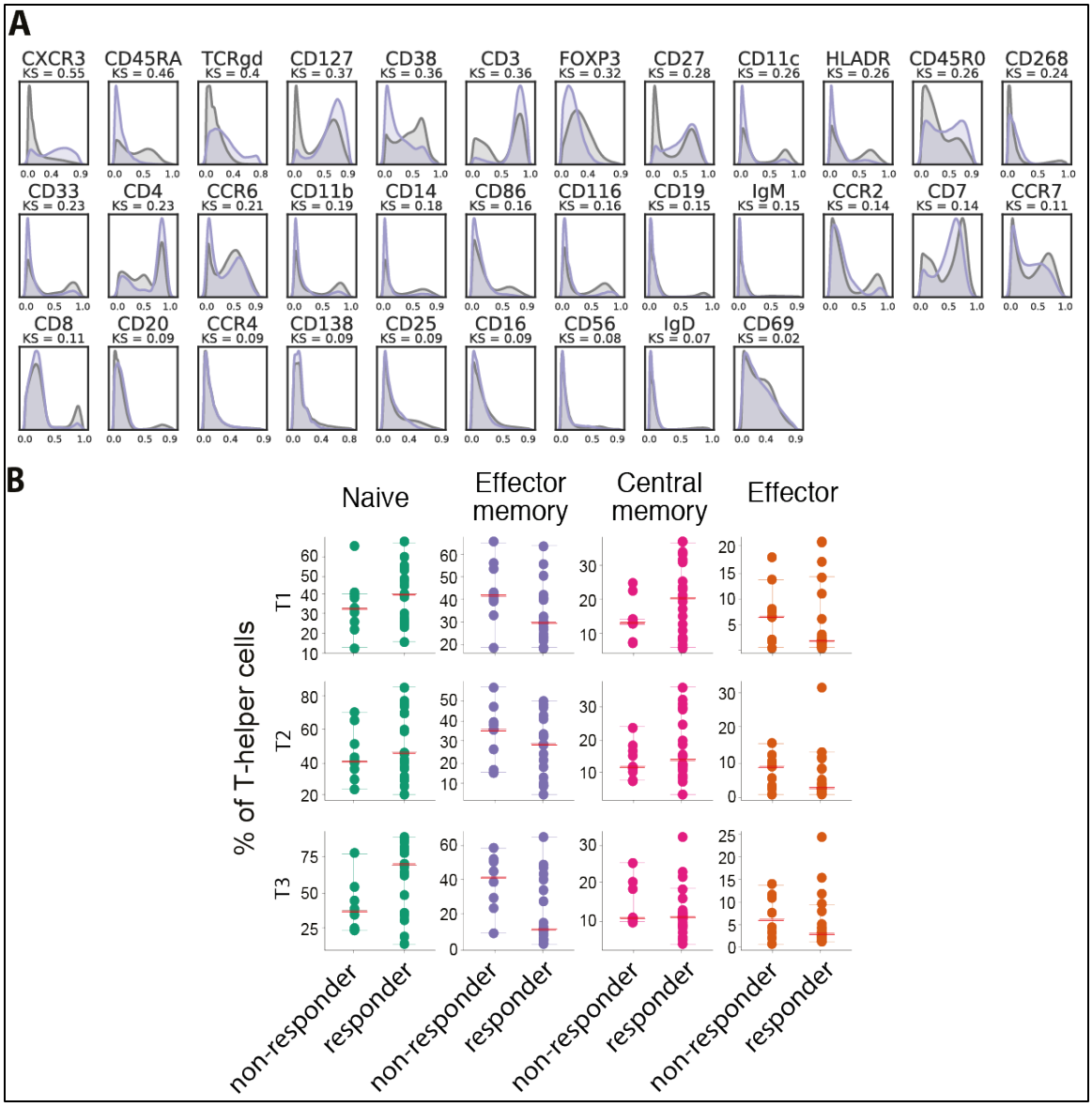

Fig. S6. Details of response associated phenotype. (A) Expression patterns of all markers between the response associated phenotype and the reference cell population for the stimulated panel. Markers are ordered by their rank in Kolmogorov Smirnov test. (B) Relative frequency of Th cell subsets at timepoints T1 - T3 between patients with/without signs of disease activity during the follow up period.

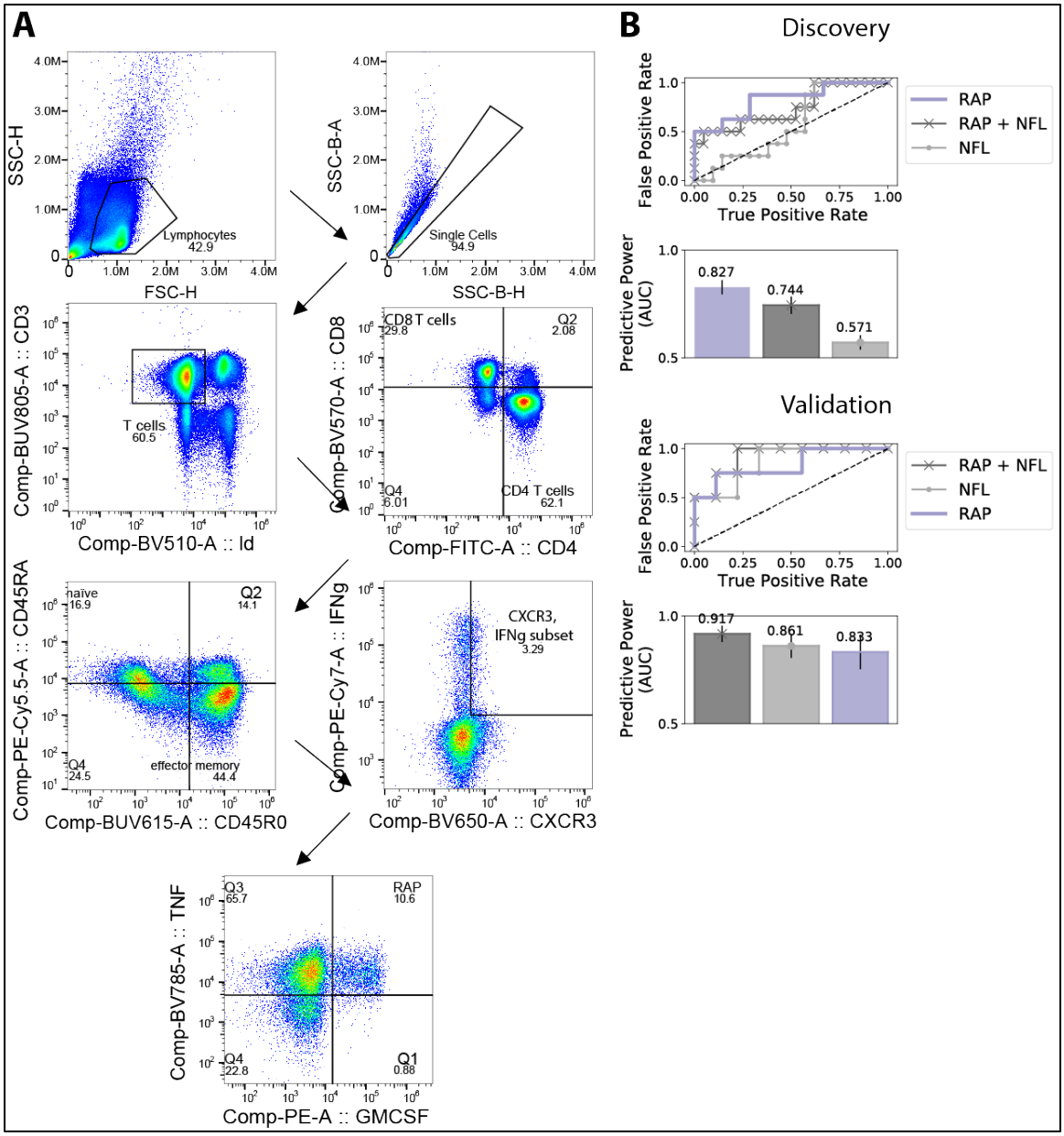

Fig. S7. Details of validation cohorts. (A) Gating strategy for the response associated immune phenotype (in flow cytometry samples). (B) ROC curve depicting the accuracy of predicting disease activity at T3 by RAP frequency at T3 and NfL z-scores at T3 and by combining the two markers (RAP z-score, Nfl z-score). Bars illustrate the predictive accuracy and standard deviation at intragroup cross-validation.

Patient ID	overall summary	mean responder	mean non-responder	p-value	4031-6181	4031-6188	4031-5558	4031-6203	4031-5781	4031-6215, 2(4031-6220	4031-6232	4031-6212	4031-6206	4031-6275	4031-6276	4031-5611	4031-5687	4031-6308	4031-5392	4031-6335	4031-6354	4031-6374	4031-6377	4031-5670	4031-6393	4031-6422	4031-6271	4031-6273	4031-6248	4031-6289	4031-6205	4031-6327	4031-5622	4031-6390
BL Characteristics																																		
sex (female = 1, male =2)	17:14	14:9	3:5	0.2602	2	2	1	1	1	2	1	1	1	1	2	1	2	1	2	1	2	2	1	1	1	1	2	2	2	1	2	2	2	1	1
age at enrolement	40.63	42.98	33.88	0.0531	36	38	45	59	54	43	50	25	57	40	42	37	44	47	36	27	58	51	26	45	55	41	33	32	23	49	38	25	34	50	19
first manifestation					01.11.2014	01.03.2014	01.06.2011	01.11.2009	01.01.1997	01.11.2008	01.01.1999	01.01.2007	01.07.2013	01.06.2006	01.05.2015	01.04.2008	01.06.2005	01.02.2010	01.02.2000	01.08.2008	01.05.1990	01.02.2000	01.12.2015	01.02.2016	01.06.2005	01.01.2009	01.04.2015	01.12.2012	01.07.2005	01.01.2006	01.09.2015	01.11.2014	01.05.2011	01.07.2005	01.04.2016
first diagnosed					01.11.2014	01.12.2014	01.06.2011	01.11.2011	01.03.2007	01.01.2013	01.09.2003	01.08.2009	01.08.2014	01.04.2015	01.06.2015	15.04.2008	15.06.2005	01.12.2010	01.10.2005	01.08.2009	01.05.1995	01.06.2005	01.04.2016	01.04.2016	01.04.2006	01.06.2016	01.10.2015	01.04.2015	01.06.2015	01.10.2015	01.10.2015	01.01.2016	01.01.2015	15.07.2005	15.04.2016
					97	78	0	1242	2962	854	4267	2133	335	93	80	2689	3733	1741	3758	2375	7589	3954	39	40	3695	118	371	133	74	47	74	14	382	3876	61
disease duration (years)	4.27	5.20	1.60	0.316	0.3	0.2	3.8	3.4	8.1	2.3	11.7	5.8	0.9	0.3	0.2	7.4	10.2	4.8	10.3	6.5	20.8	10.8	0.1	0.1	10.1	0.3	1.0	0.4	0.2	0.1	0.2	0.0	1.0	10.6	0.2
relapses/2a before start	1.00	1.04	0.88	0.9102	0	2	0	0	0	0	0	3	2	1	2	1	0	1	0	4	0	1	2	1	1	1	2	1	1	1	1	2	0	0	1
Treatment																																			
treatment start					08.02.2015	19.02.2015	18.03.2015	29.03.2015	12.04.2015	07.05.2015	01.07.2015	06.06.2015	04.07.2015	20.12.2015	25.08.2015	28.08.2015	07.09.2015	10.09.2015	22.01.2016	01.02.2016	01.03.2016	02.04.2016	20.05.2016	12.05.2016	20.05.2016	30.09.2016	15.10.2016	20.08.2015	14.08.2015	23.11.2015	22.12.2015	22.01.2016	18.01.2016	01.03.2016	25.06.2016
previous DMT (0 = no, 1 = yes)	58%	61%	50%	0.819	0	0	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	0	1	1	1	0
last medication (name)							daclizumab	fingolimod	glatirameract	teriflunomic	interferon b	interferon beta-1b				aubagio	fingolimod	interferon b	interferon b	teriflunomic	interferon b	glatirameracetate				interferon beta-1a			glatirameracetate		interferon b	glatirameracetate	glatirameracetate	
date stopped							19.02.2015	15.12.2014	31.03.2015	15.03.2015	01.01.2014	01.05.2013				15.06.2015	15.05.2015	01.09.2015	05.01.2016	01.12.2015	01.02.2016	01.12.2015				01.10.2016			07.11.2015		21.01.2016	01.01.2016	25.02.2016	
high-dose cortisol (date)														20.04.2015	26.06.2015			08.06.2015					15.04.2016	10.03.2016		30.07.2016			29.06.2015					15.05.2016
latency from last medication							27	104	12	53	546	766		244	60	74	115	9	17	62	29	123	35	63		62	14		46	16		1	17	5	41
Clinical course																																			
EDSS at T1	2.29	2.46	1.81	0.1538	1.0	2.0	2.0	3.5	3.0	2.0	2.5	2.5	3.0	1.5	3.0	1.5	4.0	2.5	3.0	3.0	2.0	4.5	1.5	2.5	2.0	2.0	2.0	1.0	2.0	1.5	2.0	2.0	0.0	1.0	5.0
EDSS at T2	2.11	2.43	1.19	0.0026	1.5	1.5	2.0	3.5	3.0	2.0	3.0	2.0	3.0	1.0	3.0	1.0	4.0	2.0	3.0	3.0	2.5	4.0	2.0	2.5	2.0	2.0	2.5	1.0	1.0	3.0	2.0	0.0	0.0	1.0	1.5
EDSS at T3	2.35	2.43	2.13	0.5251	1.0	1.0	2.0	4.5	3.0	2.5	2.5	2.0	3.0	1.0	3.0	1.0	3.5	2.0	4.0	3.0	2.0	4.0	1.5	2.5	2.0	2.5	2.5	2.0	1.0	4.0	2.5	2.0	0.0	1.0	4.5
relapse (0 = no, 1 = yes)					0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0
relapse date																												04.01.2016		01.08.2016		15.11.2016			
MRI outcome																																			
signs of activity at T3 (0 = no, 1 = yes)					0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
new lesions																												1	1	1	3	16	3	1	1
Gd-enhancing lesions																												1	0	0	0	6	0	0	0
Outcome																																			
Activity (MRI+relapse)					0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	2	1	2	1	1	1

Table S1. Patient characteristics discovery cohort. All patient characteristics regarding baseline characteristics, treatment, clinical disease course, laboratory and imaging outcomes are shown for each patient.

Isotope	Metal	Antigen	Clone	Supplier
89	Y	BC89	HI30	Fluidigm
91	Nb	BC91	HI30	Biolegend
104	Pd	BC104	HI30	Biolegend
105	Pd	BC105	HI30	Biolegend
106	Pd	BC106	HI30	Biolegend
108	Pd	BC108	HI30	Biolegend
110	Pd	BC110	HI30	Biolegend
113	In	BC113	HI30	Biolegend
115	In	BC115	HI30	Biolegend
141	Pr	VLA4	G034E3	Fluidigm
142	Nd	CD19	MP4-25D2	Fluidigm
143	Nd	CCR2	K036C2	Biolegend
144	Nd	IL-4	MP4-25D2	Biolegend
145	Nd	CD4	RPA-T4/OKT4	Fluidigm/Biolegend
146	Nd	CD8	RPA-T8/SK1	Fluidigm
147	Sm	IL-2	NCAM16.2	BD/Biolegend
148	Nd	IL-17A	BL168	Fluidigm
149	Sm	IL-3	BVD8-3G11	Biolegend
150	Nd	IL-22	22URTI	Fluidigm
151	Eu	CD103	Ber-ACT8	Fluidigm
152	Sm	TCRgd	11F2	Fluidigm
153	Eu	CD25	M-A251	Biolegend
154	Sm	IL-6	MQ2-13A5	Biolegend
155	Gd	IL-9	MH9A4	Biolegend
156	Gd	IL-13	JES10-5A2	Fluidigm
158	Gd	CCR4	205410	Fluidigm
159	Tb	GM-CSF	BVD2-21C11	Biolegend
160	Gd	CD69	FN50	Biolegend
161	Dy	CD20	2H7	Biolegend
162	Dy	CD27	O323	Biolegend
163	Dy	CD7	M5E2/6B7	Fluidigm
164	Dy	CD45R0	UCHL1	Fluidigm
165	Ho	IFN-g	B27	Fluidigm
166	Er	IL-10	JES3-9D7	Fluidigm
167	Er	CCR7	G043H7	Biolegend
168	Er	TNF	MAb11	Fluidigm
169	Tm	CD45RA	HI100	Fluidigm
170	Er	CD3	UCHT-1	Fluidigm
171	Yb	CXCR5	51505	Fluidigm
172	Yb	IL-21	3A3-N2	Fluidigm
173	Yb	CXCR4	12G5	Biolegend/Fluidigm
174	Yb	HLADR	L243	Fluidigm
175	Lu	Perforin/CD14	M5E2	Biolegend
176	Yb	CD56	MQ1-17H12	Biolegend
191	Ir	DNA1		Fluidigm
193	Ir	DNA2		Fluidigm
195	Pt	Live/Dead		Sigma
181	Ta	BC181	HI30	Biolegend
209	Bi	CD16/CD11b	3G8	Fluidigm

Table S2. Mass cytometry panel (stimulation). Antigens and clones, conjugated isotopes and metals as well as the supplier are provided for each antibody.

Isotope	Metal	Antigen	Clone	Supplier
89	Y	BC89	HI30	Fluidigm
91	Nb	BC91	HI30	Biolegend
104	Pd	BC104	HI30	Biolegend
105	Pd	BC105	HI30	Biolegend
106	Pd	BC106	HI30	Biolegend
108	Pd	BC108	HI30	Biolegend
110	Pd	BC110	HI30	Biolegend
113	In	BC113	HI30	Biolegend
115	In	BC115	HI30	Biolegend
141	Pr	CCR6	G034E3	Fluidigm
142	Nd	CD19	MP4-25D2	Fluidigm
143	Nd	CCR2	K036C2	Biolegend
144	Nd	CD57/CCR5	CD57/MP4-25I	Biolegend
145	Nd	CD4/CD4	RPA-T4/OKT4	Fluidigm/Biolegend
146	Nd	CD8a	RPA-T8/SK1	Fluidigm
147	Sm	CD11c	NCAM16.2	BD/Biolegend
148	Nd	CD16	BL168	Fluidigm
149	Sm	CD25	BVD8-3G11	Biolegend
150	Nd	CD27	22URTI	Fluidigm
151	Eu	CD38	Ber-ACT8	Fluidigm
152	Sm	TCRγδ	11F2	Fluidigm
153	Eu	CD45RA	M-A251	Biolegend
154	Sm	CD3	MQ2-13A5	Biolegend
155	Gd	CD268	11C1	Biolegend
156	Gd	CXCR3	JES10-5A2	Fluidigm
158	Gd	CCR4	205410	Fluidigm
159	Tb	CD116	BVD2-21C11	Biolegend
160	Gd	CD69	FN50	Biolegend
161	Dy	CD20	2H7	Biolegend
162	Dy	FOXP3	O323	Biolegend
163	Dy	CD7	M5E2/6B7	Fluidigm
164	Dy	CD45R0	UCHL1	Fluidigm
165	Ho	CD127	B27	Fluidigm
166	Er	CD86	JES3-9D7	Fluidigm
167	Er	CCR7	G043H7	Biolegend
168	Er	Ki67/CD40L	MAb11	Fluidigm
169	Tm	CD33	HI100	Fluidigm
170	Er	IgD	UCHT-1	Fluidigm
171	Yb	CD138	51505	Fluidigm
172	Yb	IgM	3A3-N2	Fluidigm
173	Yb	CD56/CXCR4	12G5	Biolegend/Fluidigm
174	Yb	HLADR	L243	Fluidigm
175	Lu	CD14	M5E2	Biolegend
176	Yb	CD5	MQ1-17H12	Biolegend
191	Ir	DNA1		Fluidigm
193	Ir	DNA2		Fluidigm
195	Pt	Live/Dead		Sigma
181	Ta	BC181	HI30	Biolegend
209	Bi	CD11b	3G8	Fluidigm

Table S3. Mass cytometry panel (conventional). Antigens and clones, conjugated isotopes and metals as well as the supplier are provided for each antibody.

Patient ID	overall summary	mean responder	mean non-responder	p-value	4031-6202	4031-6092	4031-5593	4031-6594	4031-5894	4031-6707	4031-6715	4031-6723	4031-6744	4031-5518	4031-6425	4031-6709	4031-6737
BL Characteristics																	
sex (female = 1, male =2)	8:5	5:4	3:1	0.5463	2	1	2	1	2	1	2	2	1	1	2	1	1
age at enrolement	31.37	31.72	30.60	0.7696	28	36	48	27	31	30	27	31	29	25	45	23	29
first manifestation					01.12.2014	01.01.2008	Feb 10	Feb 17	Sep 12	Mär 18	01.02.2018	13.01.2018	14.05.2018	01.10.2010	Jan 16	01.01.2018	01.07.2018
first diagnosed					01.11.2016	01.01.2014	Mai 11	Sep 17	Sep 12	Apr 18	18.05.2018	04.06.2018	05.07.2018	01.09.2011	23.08.2016	15.03.2018	15.07.2018
					704.00	3320.00	2752.00	273.00	1887.00	82.00	140.00	179.00	179.00	2408.00	627.00	149.00	45.00
disease duration (years)	2.69	2.90	2.21	0.7382	1.9	9.1	7.5	0.7	5.2	0.2	0.4	0.5	0.5	6.6	1.7	0.4	0.1
relapses/2a before start	0.77	0.67	1.00	0.3774	1	0	0	1	0	1	1	1	1	2	0	1	1
Treatment																	
treatment start					01.12.2016	01.07.2017	15.08.2017	03.11.2017	01.11.2017	23.05.2018	21.06.2018	11.07.2018	15.11.2018	01.07.2017	21.09.2017	30.05.2018	20.08.2018
previous DMT (0 = no, 1 = yes	54%	56%	50%	0.8675	1	1	1	0	1	0	0	1	0	1	1	0	0
last medication (name)					interferon b	interferon b	daclizumab		ocrelizumab			glatirameracetate		fingolimod	fingolimod		
date stopped					15.08.2016	15.08.2015	27.06.2017		09.04.2015			01.07.2018		01.11.2016	01.05.2017		
high-dose cortisol (date)										22.04.2018	15.02.2018		24.08.2018			18.03.2018	24.07.2018
latency from last medication					108	686	49		937	31	126	10	83	242	143	73	27
Clinical course																	
EDSS at T1	1.58	1.56	1.63	0.8263	1.0	2.0	2.0	1.0	2.0	1.0	1.0	2.0	2.0	2.0	1.0	1.5	2.0
EDSS at T2	1.69	1.56	2.00	0.283	0.0	1.5	2.5	1.0	1.5	2.0	1.5	2.0	2.0	2.0	1.5	2.5	2.0
EDSS at T3	1.73	1.61	2.00	0.4464	0.0	2.0	2.5	1.0	2.0	2.0	2.0	1.0	2.0	3.0	1.0	1.5	2.5
relapse (0 = no, 1 = yes)					0	0	0	0	0	0	0	0	0	1	1	1	1
relapse date														26.09.2017	20.12.2017	07.09.2018	04.09.2018
MRI outcome																	
signs of activity					0	0	0	0	0	0	0	0	0	1	1	1	0
new lesions														3	3	1	
Gd-enhancing lesions														3	1	0	
Outcome																	
Activity (MRI+relapse)					0	0	0	0	0	0	0	0	0	2	2	2	1

Table S4. Patient characteristics validation cohort. All patient characteristics regarding baseline characteristics, treatment, clinical disease course, laboratory and imaging outcomes are shown for each patient.

Fluorochrome	Antigen	Clone	Supplier
BV510	LD		BioLegend
BUV395	CD45	HI30	BD
BUV661	HLADR	G46-6	BD
BUV615	CD45RO	UCHL1	BD
BUV805	CD3	UCHT1	BD
BUV563	CD25	M-A251	BioLegend
PE-Cy5.5	CD45RA	HI100	BioLegend
PE-Cy5	Pangd	GL3	Ebioscience
BUV496	CD19	3G8	BD
BV480	CD27	M-T271	BD
BV421	CCR7	G043H7	BioLegend
BUV737	CD39	SJ25C1	BD
BB630	CD103	M290	BD
PerCP-eFluor® 710	KLRG1	13F12F2	ThermoScien
FITC	CD4	SKA3	BD
PE	GM-CSF	MP1-22E9 (RUO)	BD
BB790-P	CTLA4	2F1	BD
PE-Cy7	IFNg	4S.B3	BioLegend
BV570	CD8	RPA-T8	BioLegend
BV711	IL-2	MQ1-17H12	BioLegend
BV785	TNF-A	MAb11	BioLegend
A647	CXCR4	I265	BioLegend
A700	GZMB	GB11	BD
APC-C7	IL17A	BL168	BD
PE/Dazzle™ 594	CD56	NCAM16.2	BD
BV650	CXCR3	MBSA43	Ebioscience
BV605	PD1	EHI2.2H7	BioLegend

Table S5. Flow-cytometry panel. Antigens and clones, fluorochromes as well as the supplier are provided for each antibody.

						untreated			dimethyl fumarate				natalizumab			
	patient	sex	age at diagnosis (years)	type	sequence of sample acquisition	delay from diagnosis (years)	untreated period (months)	previous treatment	delay from diagnosis (years)	treatment duration (months)	disease activity (new lesions/relapses)	reason for cessation	delay from diagnosis (years)	treatment duration (months)	disease activity (new lesions/relapses)	reason for cessation reason for cessation
patient 1	1	f	25	RRMS	natalizumab > untreated > DMF	9.3	1.5	natalizumab	9.8	6	0/0	NA	8.0	80	0/0	JCV-antibody test/PML-risk
patient 2	2	f	48	RRMS	natalizumab > untreated > DMF	14.0	2.5	natalizumab	15.1	12	0/0	NA	10.7	54	0/0	JCV-antibody test/PML-risk
patient 3	3	f	49	RRMS	untreated > natalizumab > DMF	0.6	NA	naïve	6.0	7	0/0	NA	3.6	36	0/0	JCV-antibody test/PML-risk
patient 4	4	f	33	RRMS	natalizumab > DMF > untreated	7.5	12	DMF	6.6	29	0/0	side effects	1.6	18	none documented	JCV-antibody test/PML-risk
mean			38.43			7.83			9.37	13.50			5.96	47.00		

Table S6. Patient characteristics cross-treatment cohort. All patient characteristics regarding baseline characteristics, pre-treatments and treatment periods of untreated periods, DMF treatment and natalizumab treatment (with disease activity during the respective treatment period) are shown. (JCV = John Cunningham virus, PML = progressive multifocal leukoencephalopathy)