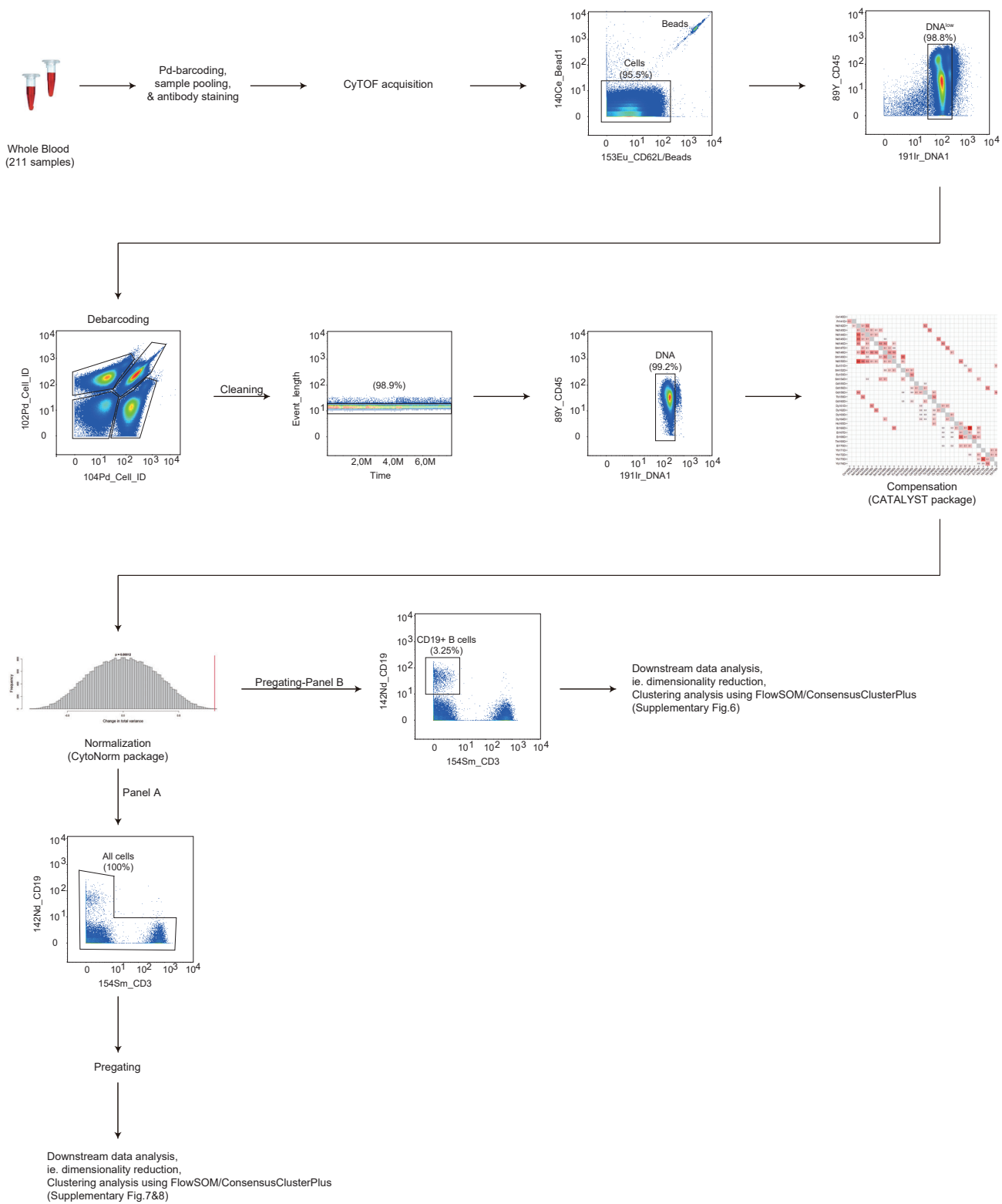


**Associations of myeloid cells with cellular and humoral responses following
vaccinations in patients with neuroimmunological diseases**

Meng Wang^{1,2,3}, Adeline Dehlinger^{1,2,3}, Camila Fernández Zapata^{1,2,3}, Maya Golan⁴,
Gerardina Gallaccio^{1,2,3}, Leif E. Sander⁵, Stephan Schlickeiser⁶, Desiree Kunkel⁷,
Tanja Schmitz-Hübsch^{1,2,3,8}, Birgit Sawitzki⁶, Arnon Karni^{4,9}, Julian Braun^{10,11}, Lucie
Loyal^{10,11}, Andreas Thiel^{10,11}, Judith Bellmann-Strobl^{1,2,3}, Friedemann Paul^{#1,2,3,8,12}, Lil
Meyer-Arndt^{#8,10,12}, Chotima Böttcher^{✉#1,2,3}

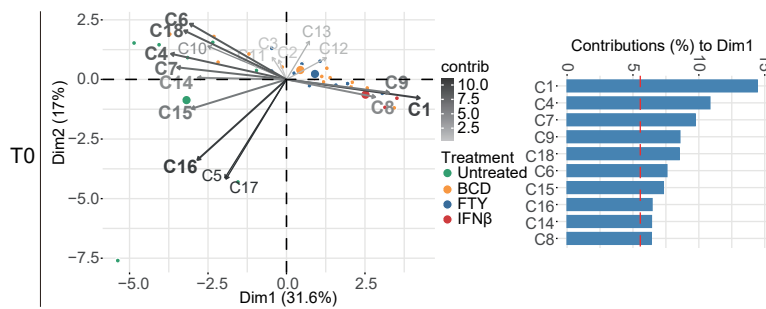
Panel A&B



Supplementary Figure 1 Schematic representation of CyTOF measurement and gating strategy from Panel A&B. A total of 211 whole blood samples were collected from MS and NMOSD patients. Whole blood samples were CD45-barcoded and pooled. Mixed samples were equally divided and stained with two panels (Panel A and B, Supplementary Tables 2 and 3) of metal-conjugated antibodies and acquired on the CyTOF instrument. Prior to pre-gating, the data were de-barcoded, compensated and normalized. In Panel A, the gating strategy for selecting single CD45⁺ cells, excluding CD3⁺CD19⁺ double positives. In Panel B, the gating strategy for cell sorting of CD19⁺ B cells.

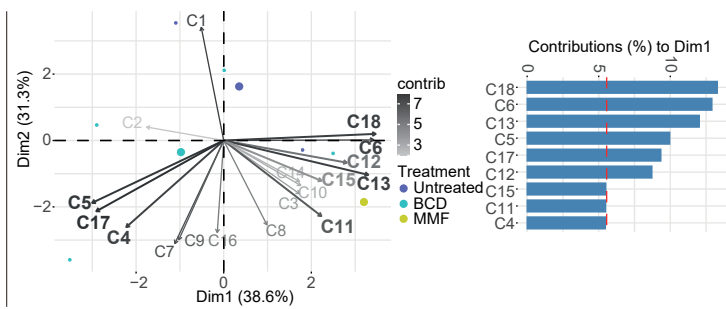
MS

A

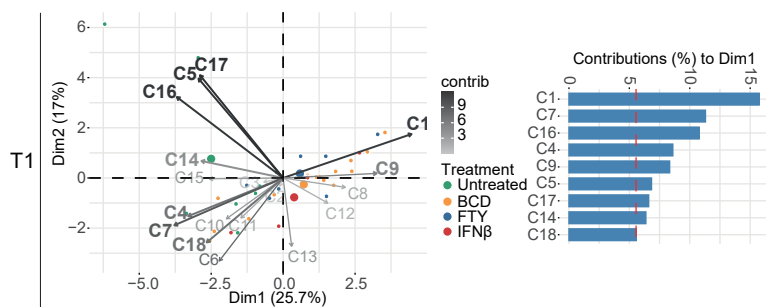


NMOSD

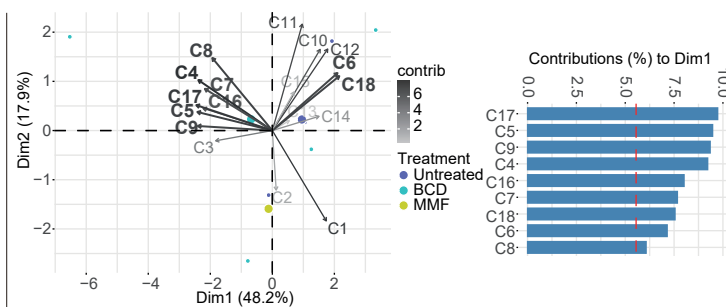
B



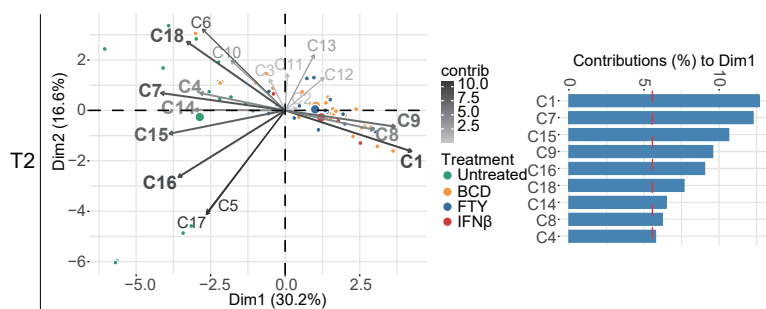
C



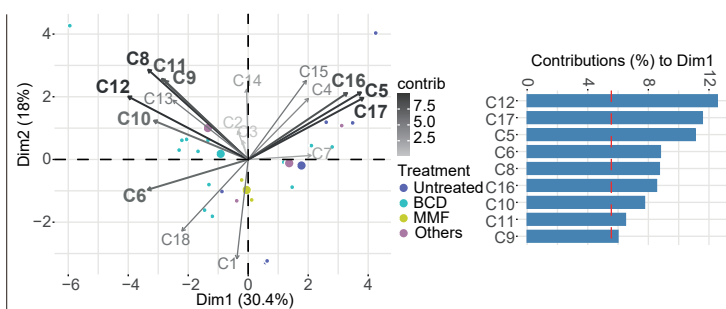
D



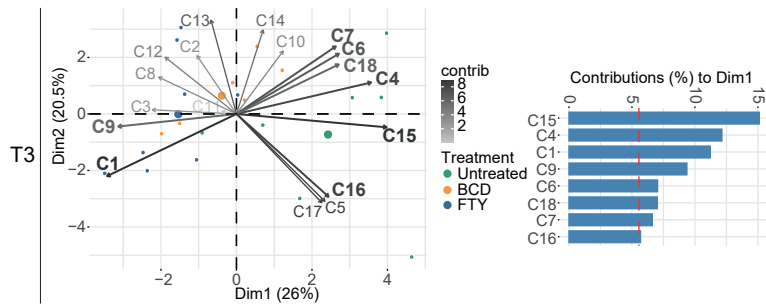
E



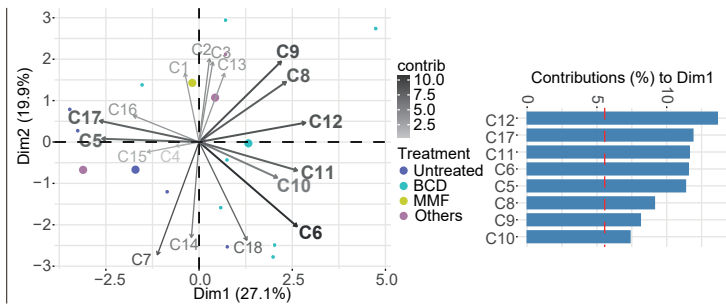
F



G



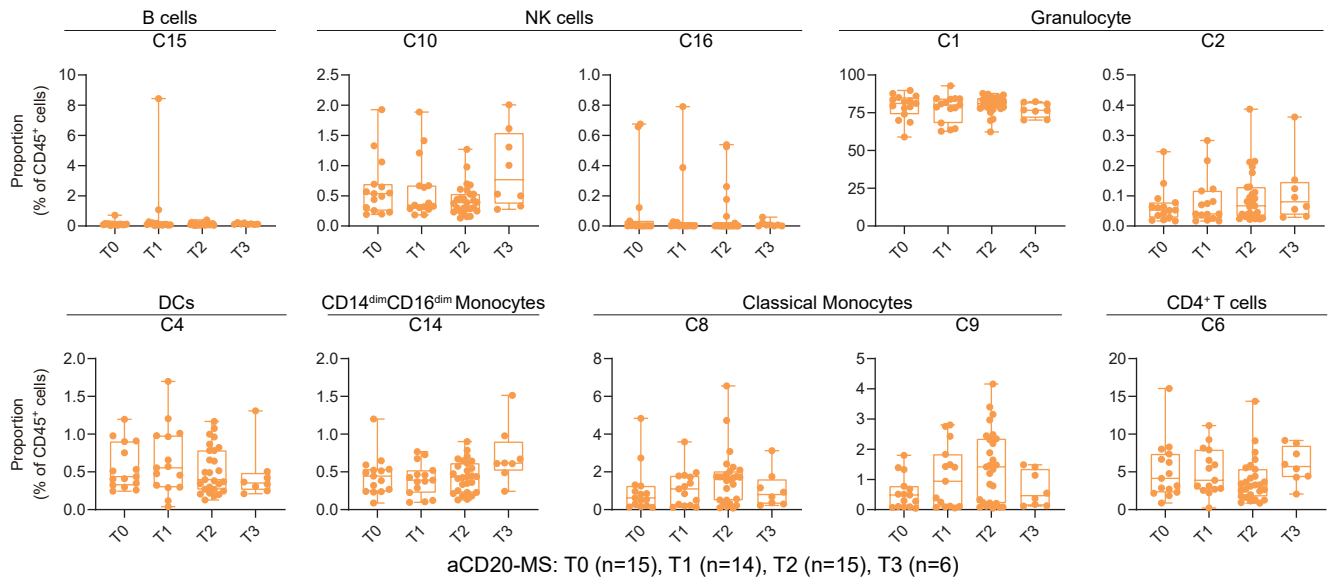
H



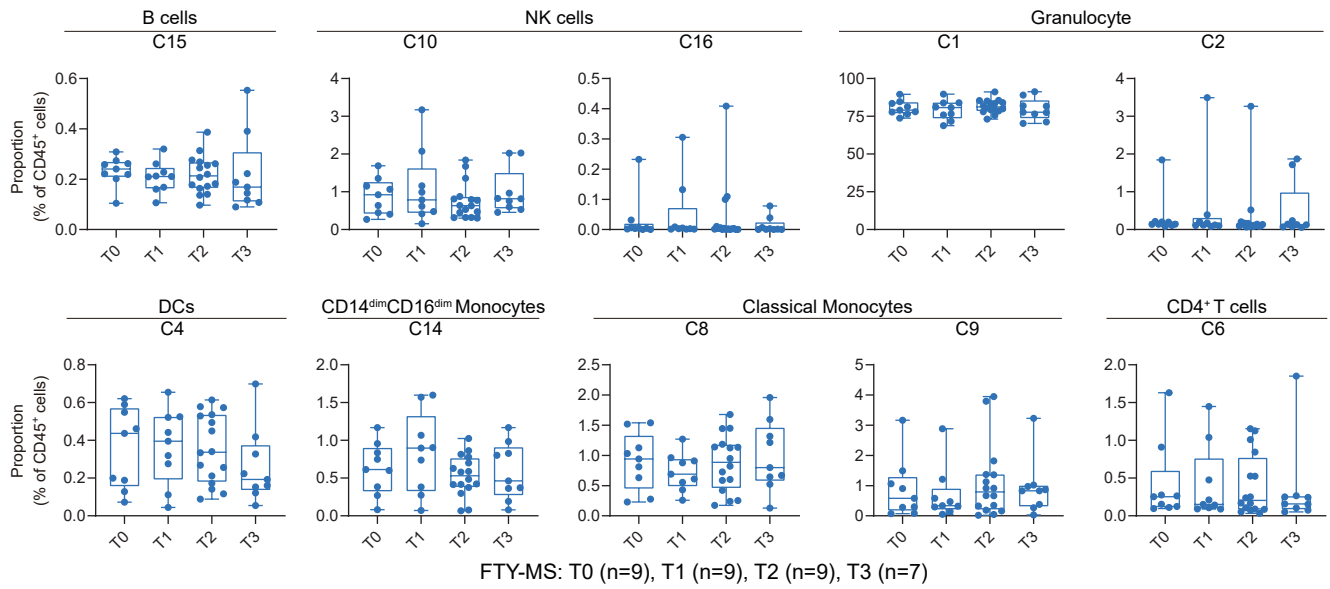
Supplementary Figure 2 Contribution of immune cell sub-populations to DMT-mediated differences in cell composition at different timepoints depicted by principal components analysis (PCA)

(A-H) PCA for the 18 identified immune clusters in untreated and DMT-treated MS (A, C, E & G) and NMOSD patients (B, D, F & H) at T0 (A & B), T1 (C & D), T2 (E & F) and T3 (G & H). Each point represents one sample's scores on the first 2 dimensions (Dim1 and Dim2). Each vector (arrow) shows the loadings of each cell cluster on the first 2 principal components. Grey scale indicates the contribution value of variables to Dim1 and Dim2. The red dashed line on the graph indicates the expected average contribution. The graph shows top variables (with a contribution larger than average) contributing to Dim1.

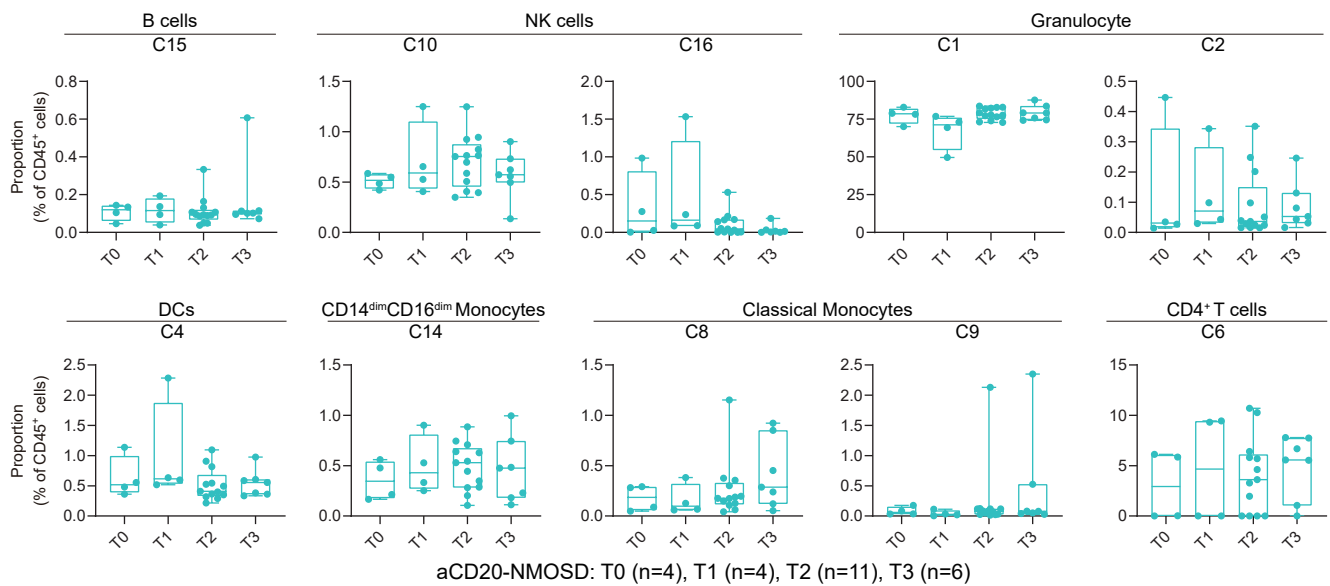
A. aCD20-MS



B. FTY-MS



C. aCD20-NMOSD



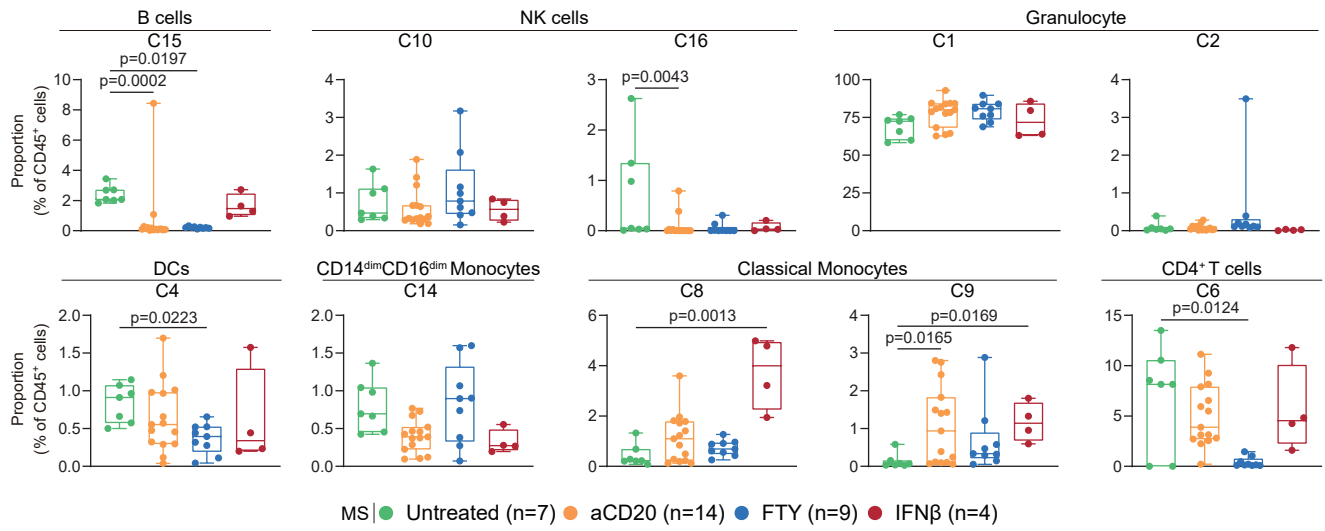
Supplementary Figure 3 Differential proportion of ten defined significant clusters (shown in Fig. 1) between four time points in DMT-treated MS and NMOSD patients.

(A) Box plots show non-significant differences of the ten defined clusters (mean \pm SD) between T0 (n=15), T1 (n=14), T2 (n=15), T3 (n=6) in aCD20-treated MS patients (Kruskal-Wallis and Dunn's multiple comparison test). Each dot represents the value of each sample. Boxes extend from the 25th to 75th percentiles. Whisker plots show the min (smallest) and max (largest) values. The line in the box denotes the median.

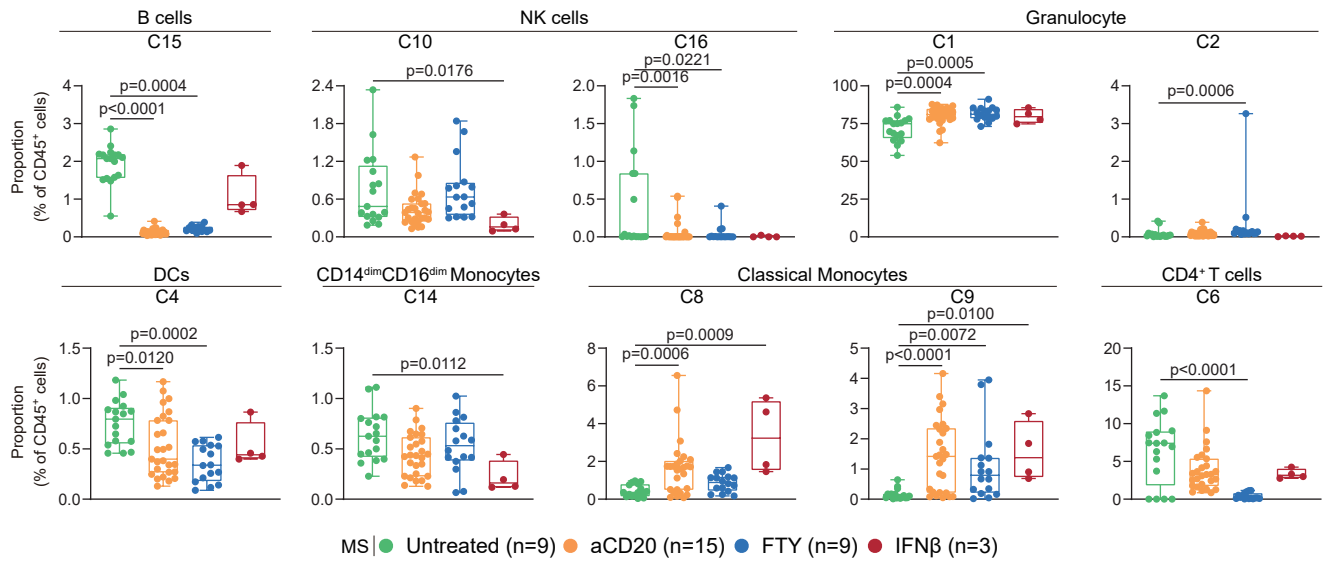
(B) Box plots show non-significant differences of the ten defined clusters (mean \pm SD) between T0 (n=9), T1 (n=9), T2 (n=9), T3 (n=7) in FTY-treated MS patients (Kruskal-Wallis and Dunn's multiple comparison test). Each dot represents the value of each sample. Boxes extend from the 25th to 75th percentiles. Whisker plots show the min (smallest) and max (largest) values. The line in the box denotes the median.

(C) Box plots show non-significant differences of the ten defined clusters (mean \pm SD) between T0 (n=4), T1 (n=4), T2 (n=11), T3 (n=6) in aCD20-treated NMOSD patients (Kruskal-Wallis and Dunn's multiple comparison test). Each dot represents the value of each sample. Boxes extend from the 25th to 75th percentiles. Whisker plots show the min (smallest) and max (largest) values. The line in the box denotes the median.

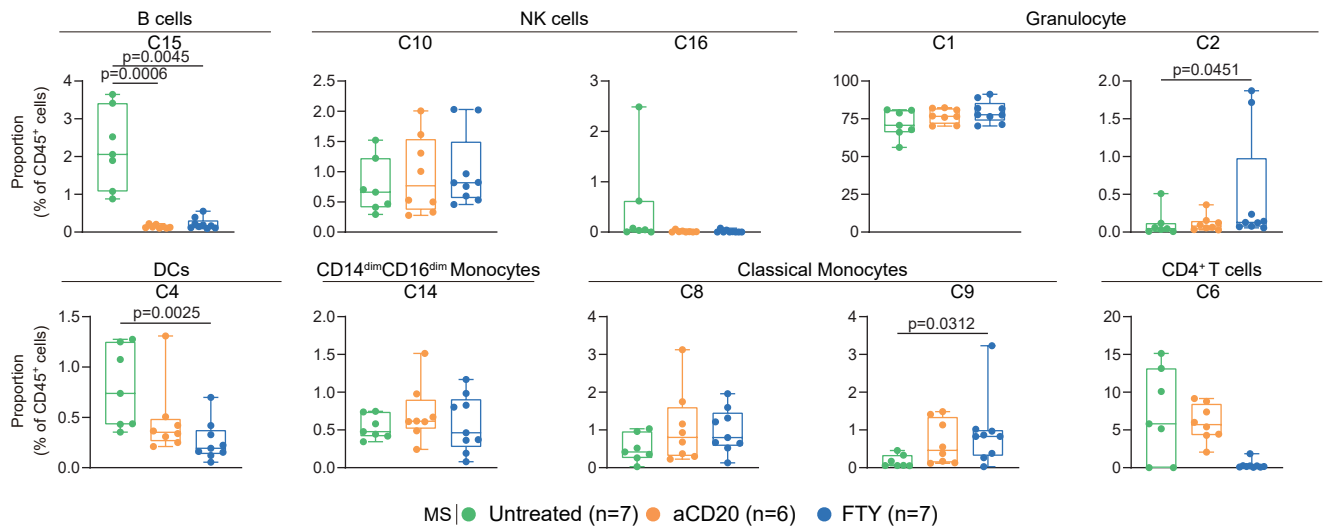
A. T1



B. T2



C. T3



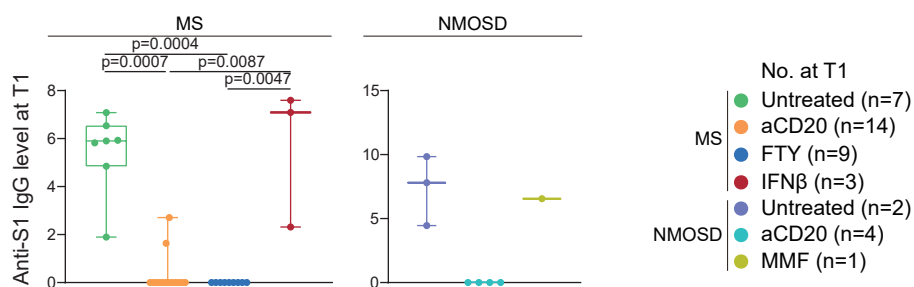
Supplementary Figure 4 Differential proportion of ten defined significant clusters (shown in Fig. 1) between different MS groups at different time points.

(A) Box plots of the ten differentially abundant clusters (mean \pm SD) between Untreated-MS (n=7), aCD20-MS (n=14), FTY-MS (n=9), IFN β -MS (n=4) at T1. Each dot represents the value of each sample. Boxes extend from the 25th to 75th percentiles. Whisker plots show the min (smallest) and max (largest) values. The line in the box denotes the median. Kruskal-Wallis and Dunn's multiple comparison test.

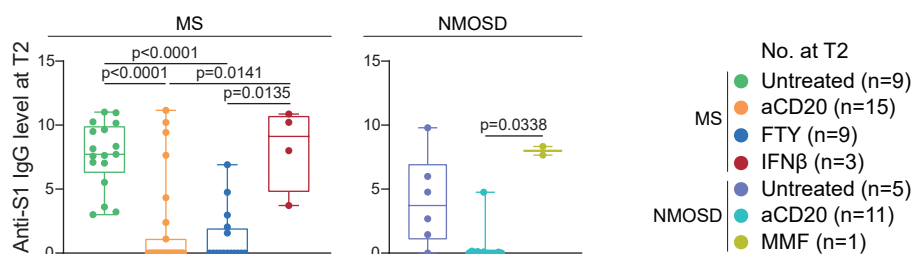
(B) Box plots of the ten differentially abundant clusters (mean \pm SD) between Untreated-MS (n=9), aCD20-MS (n=15), FTY-MS (n=9), IFN β -MS (n=3) at T2. Each dot represents the value of each sample. Boxes extend from the 25th to 75th percentiles. Whisker plots show the min (smallest) and max (largest) values. The line in the box denotes the median. Kruskal-Wallis and Dunn's multiple comparison test.

(C) Box plots of the ten differentially abundant clusters (mean \pm SD) between Untreated-MS (n=7), aCD20-MS (n=6), FTY-MS (n=7) at T3. Each dot represents the value of each sample. Boxes extend from the 25th to 75th percentiles. Whisker plots show the min (smallest) and max (largest) values. The line in the box denotes the median. Kruskal-Wallis and Dunn's multiple comparison test.

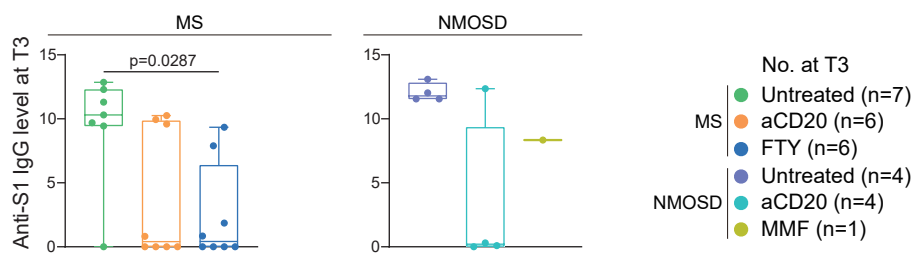
A



B



C



Supplementary Figure 5 Humoral response after SARS-CoV-2 mRNA vaccination in patients with MS and NMOSD.

(A) Anti-S1 IgG titer in MS (Untreated-MS (n=7), aCD20-MS (n=14), FTY-MS (n=9), IFN β -MS (n=3)) and NMOSD (Untreated-NMOSD (n=2), aCD20-NMOSD (n=4), MMF-NMOSD (n=1)) groups at T1. Each dot represents the value of each sample. Boxes extend from the 25th to 75th percentiles. Whisker plots show the min (smallest) and max (largest) values. The line in the box denotes the median. Kruskal-Wallis and Dunn's multiple comparison test.

(B) Anti-S1 IgG titer in MS (Untreated-MS (n=9), aCD20-MS (n=15), FTY-MS (n=9), IFN β -MS (n=3)) and NMOSD (Untreated-NMOSD (n=5), aCD20-NMOSD (n=11), MMF-NMOSD (n=1)) groups at T2. Each dot represents the value of each sample. Boxes extend from the 25th to 75th percentiles. Whisker plots show the min (smallest) and max (largest) values. The line in the box denotes the median. Kruskal-Wallis and Dunn's multiple comparison test.

(C) Anti-S1 IgG titer in MS (Untreated-MS (n=7), aCD20-MS (n=6), FTY-MS (n=6)) and NMOSD (Untreated-NMOSD (n=4), aCD20-NMOSD (n=4), MMF-NMOSD (n=1)) groups at T3. Each dot represents the value of each sample. Boxes extend from the 25th to 75th percentiles. Whisker plots show the min (smallest) and max (largest) values. The line in the box denotes the median. Kruskal-Wallis and Dunn's multiple comparison test.

Supplementary Figure 6 Association of B cell sub-population with anti-S1 IgG antibody production at T1-T3 in MS and NMOSD groups

(A) UMAP plots colored by cluster ID for 1–18 clusters of CD19+ B cells determined using the FlowSOM algorithm.

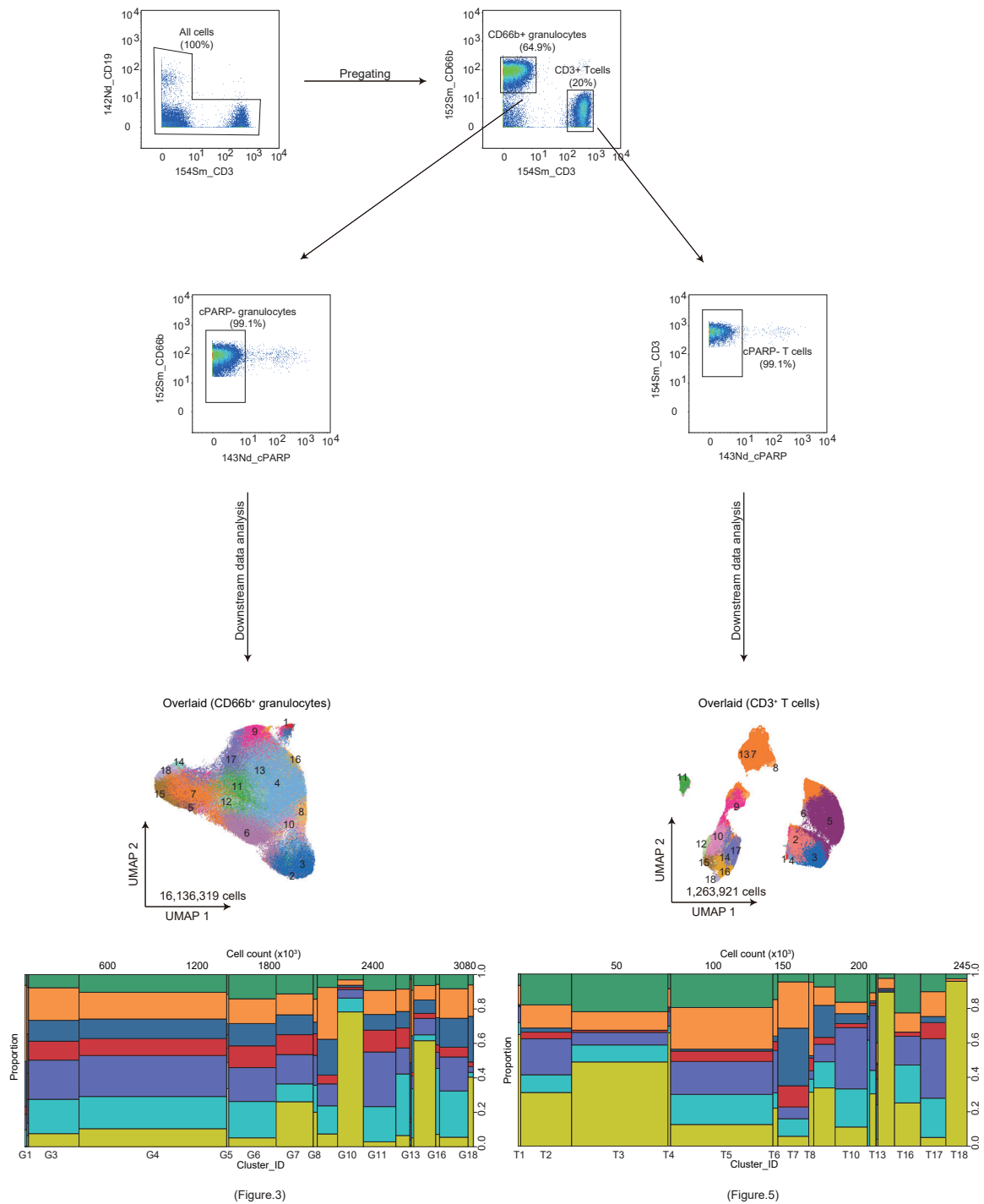
(B) Phenotypic heatmap depicting the median expression levels of selected markers per B cell cluster.

(C) Mosaic plots depicting cluster proportion and cell count per cluster in each group at T0.

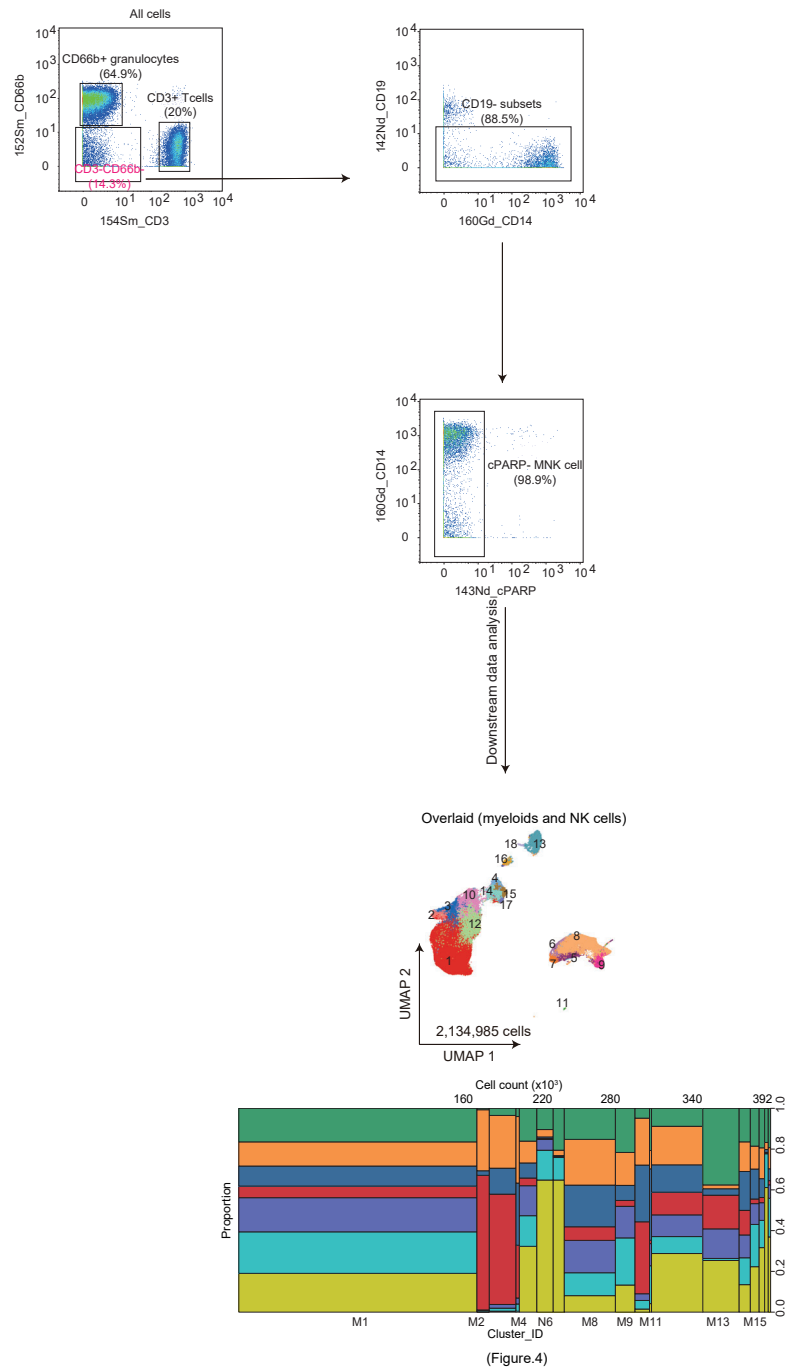
(D) Proportion of the nine differentially abundant clusters (mean \pm SD) between Untreated-MS (n=7), aCD20-MS (n=15), FTY-MS (n=9), IFN β -MS (n=3) at T0. Each dot represents the value of each sample. Boxes extend from the 25th to 75th percentiles. Whisker plots show the min (smallest) and max (largest) values. The line in the box denotes the median. Kruskal-Wallis and Dunn's multiple comparison test.

(E) Proportion of the nine clusters as in D (mean \pm SD) between Untreated-NMOSD (n=2), aCD20-NMOSD (n=4), MMF-NMOSD (n=1) at T0. Each dot represents the value of each sample. Boxes extend from the 25th to 75th percentiles. Whisker plots show the min (smallest) and max (largest) values. The line in the box denotes the median.

(F) Heatmap of the Spearman correlation coefficients between the proportion of B cell clusters and antibody titers at T1-T3 in MS groups. Nonparametric Spearman correlation test (r), two-sided.

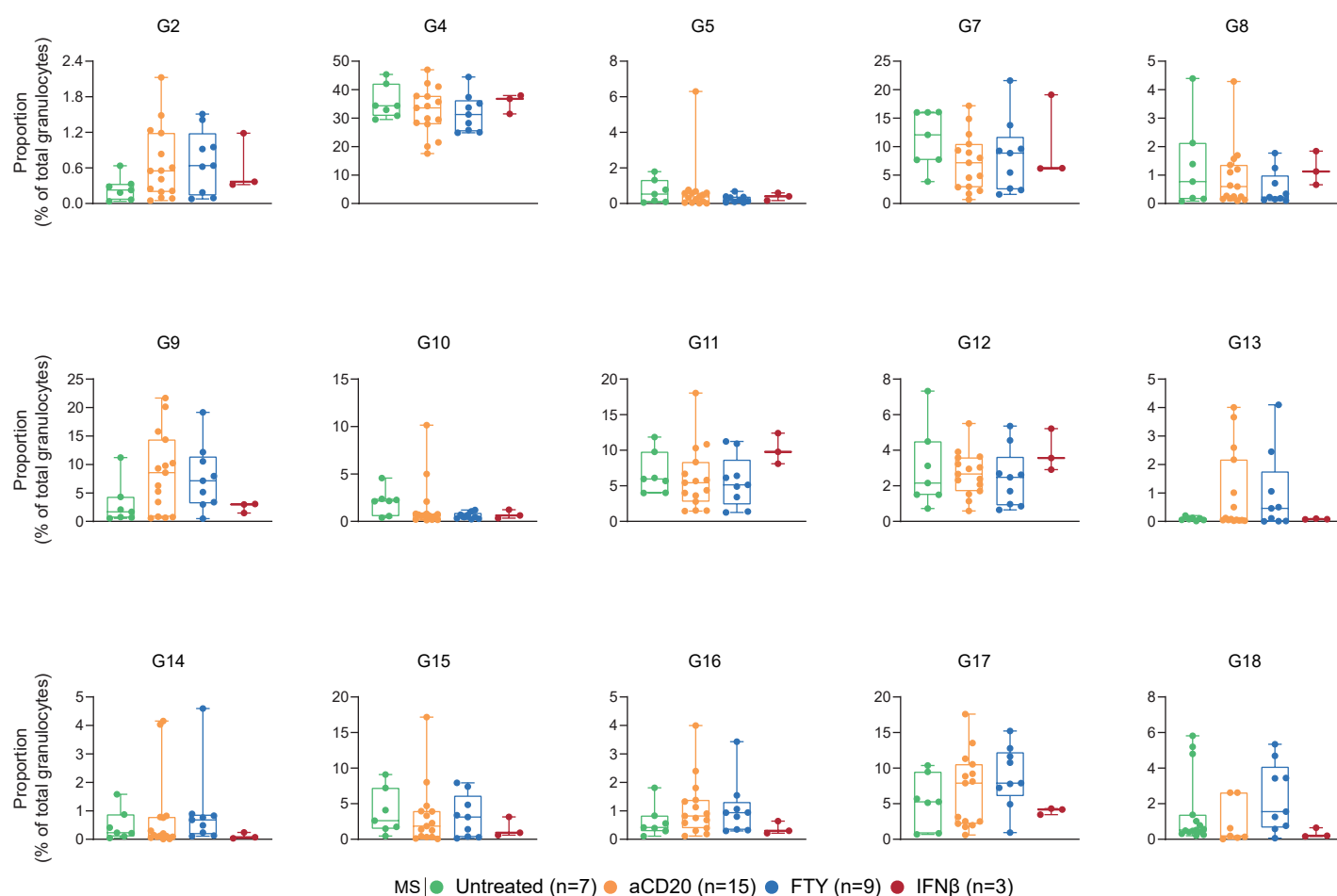


Supplementary Figure 7 Representative plots of gating strategy in Panel A, related to **Figure 3** and **5**. CD66b+ granulocytes and CD3+ T cells were sorted from all CD45+ populations.

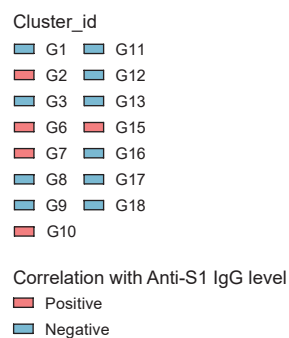
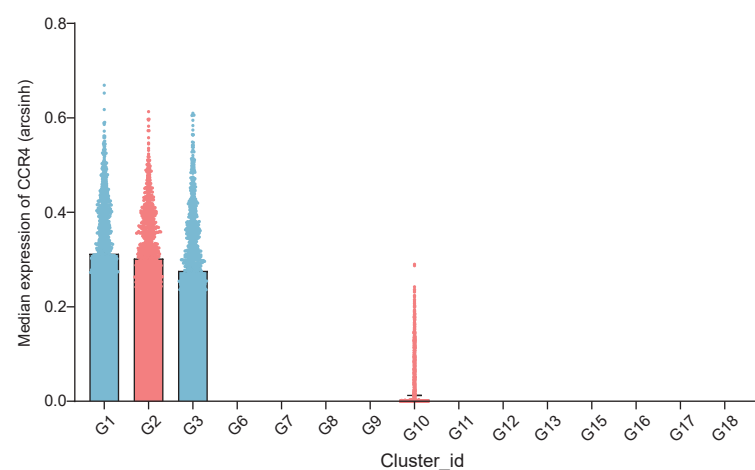
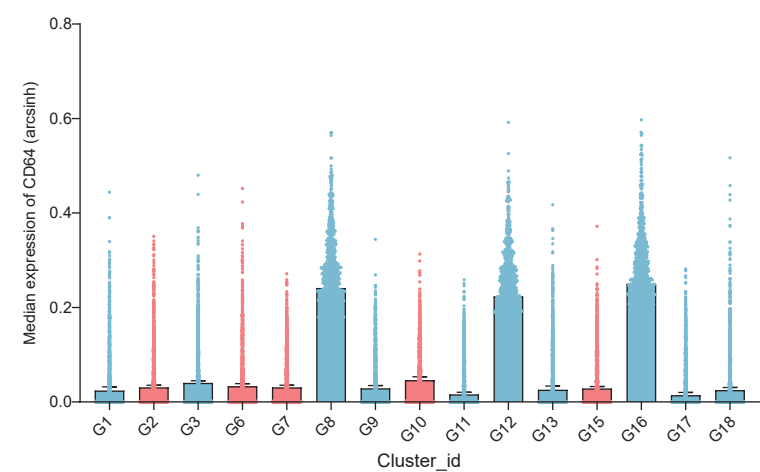
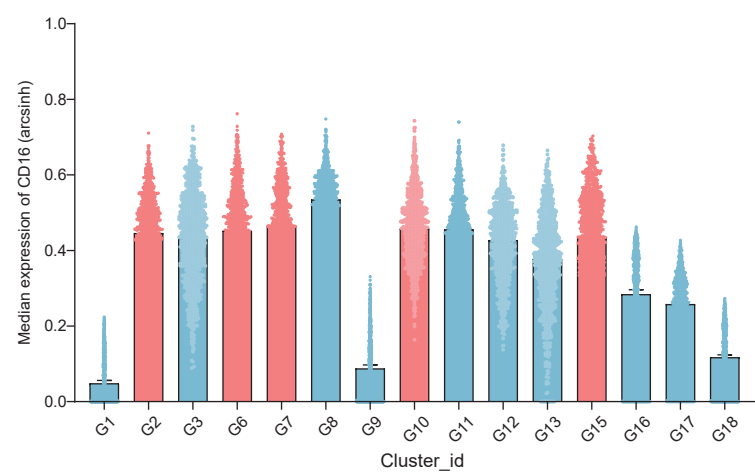
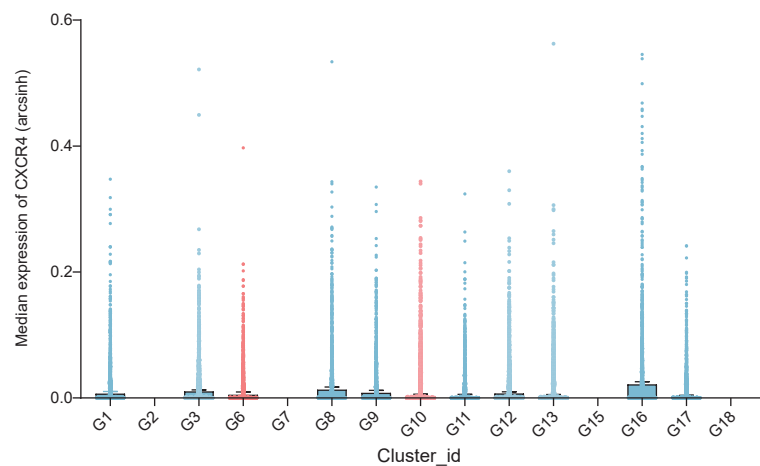
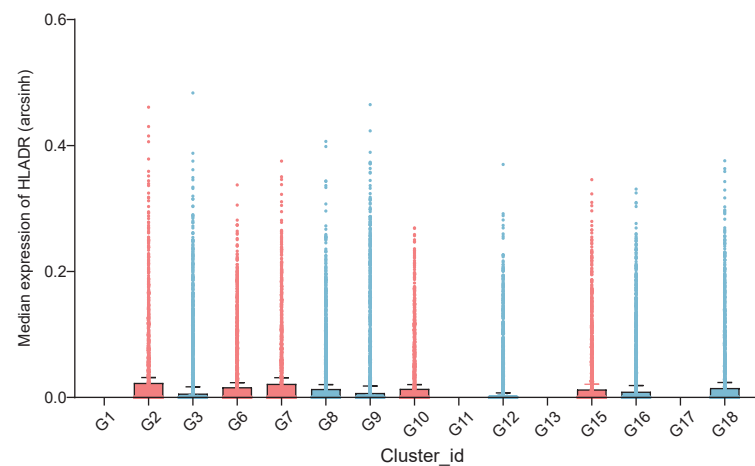


Supplementary Figure 8 Gating strategy for MNK cell in Panel A, used in **Figure 4**.

The gating of MNK cells from CD3-CD66b- populations based on marker expression of CD3, CD66b, CD19 and CD14.



Supplementary Figure 9 Proportion of the fifteen non-significant granulocyte clusters (mean \pm SD) between Untreated-MS (n=7), aCD20-MS (n=15), FTY-MS (n=9), IFN β -MS (n=3) at T0 (Kruskal-Wallis and Dunn's multiple comparison test). Each dot represents the value of each sample. Boxes extend from the 25th to 75th percentiles. Whisker plots show the min (smallest) and max (largest) values. The line in the box denotes the median.



Supplementary Figure 10 Median expression (with arcsinh transformation) of markers of granulocyte sub-clusters found correlated with anti-S1 IgG antibody levels in MS and NMOSD groups. Each dot represents the value of each single cell. Data displayed as median with 95% confidence interval. The red bars represent the clusters that show positive correlations with anti-S1 IgG antibody levels. The light blue bars represent the clusters that show negative correlations with anti-S1 IgG antibody levels.

Supplementary Table 1. Patients information

Group	MS	NMOSD
total no.	39	23
Female/Male	29/10	18/5
Age, mean\pmSD (range, years)	48 \pm 11 (28-69)	48 \pm 15 (21-78)
Therapy (no.)		
Untreated	9	5
aCD20	16	13
FTY	9	-
IFNβ	5	-
AZA	-	2
MMF	-	1
TCZ	-	1
IVIG	-	1
Timepoint (patient no./sample no.)		
T0	34/34	7/7
T1	34/35*	7/7
T2	36/64**	20/24**
T3	20/24***	13/16***
Disease duration, mean\pmSD (range, years)	20 \pm 9 (3-42)	11 \pm 7 (2-37)
Treatment duration before vaccination, mean\pmSD (range, months)	44 \pm 50 (2-233)	68 \pm 53 (17-155)
EDSS, mean\pmSD (range)		
at T0	3.1 \pm 1.8 (0-7)	3.4 \pm 1.9 (2-6)
at T1	3.3 \pm 1.4 (1.5-5)	4.8 \pm 1.8 (3.5-6)
at T2	3.1 \pm 1.6 (0-6)	2.5 \pm 1.9 (0-6.5)
at T3	3 \pm 0.5 (2.5-3.5)	1.7 \pm 0.6 (1-2)

* 2 patients have 2 samples at different timepoints after primary but before secondary vaccination.

** 31 patients have 2 or 3 samples at different timepoints but within 6 months after secondary vaccination.

*** 5 patients have 2 or 3 samples at different timepoints but within 4 months after tertiary vaccination.

Abbreviations: MS= multiple sclerosis; NMOSD= neuromyelitis optica spectrum disorders; aCD20= anti-CD20 monoclonal antibodies; FTY= fingolimod; IFN β = interferon β ; AZA= azathioprine; MMF= mycophenolate mofetil; TCZ= tocilizumab; IVIG= Intravenous immunoglobulin.

Supplementary Table 2. The *Panel A*-antibody list

Target	Isotope tag	clone	company	Dilution
Surface				
CD45	⁸⁹ Y	HI30	Fluidigm	1:200
HLA-DR	¹⁴¹ Pr	L243	Biolegend	1:100
CD19	¹⁴² Nd	HIB19	Fluidigm	1:100
CD69	¹⁴⁴ Nd	FN50	Fluidigm	1:100
CD4	¹⁴⁵ Nd	RPA-T4	Fluidigm	1:100
CD64	¹⁴⁶ Nd	10.1	Fluidigm	1:100
CD226	¹⁴⁷ Sm	REA1040	Miltenyi	1:100
CD16	¹⁴⁸ Nd	3G8	Fluidigm	1:50
CD56	¹⁴⁹ Sm	NCAM16.2	Fluidigm	1:400
ICOS	¹⁵¹ Eu	C398.4A	Fluidigm	1:50
CD66b	¹⁵² Sm	80H3	Fluidigm	1:600
CD3	¹⁵⁴ Sm	UCHT1	Fluidigm	1:200
CD11c	¹⁵⁵ Gd	Bu15	Biolegend	1:200
CCR4	¹⁵⁸ Gd	L291H4	Fluidigm	1:200
TIGIT	¹⁵⁹ Tb	MBSA43	Fluidigm	1:50
CD14	¹⁶⁰ Gd	RM052	Fluidigm	1:100
CD8	¹⁶² Dy	RPA-T8	Fluidigm	1:50
CRTH2	¹⁶³ Dy	BM16	Fluidigm	1:100
CD95	¹⁶⁴ Dy	DX2	Fluidigm	1:50
LAG3	¹⁶⁵ Ho	11C3C65	Fluidigm	1:100
CD141	¹⁶⁶ Er	M80	Fluidigm	1:50
CCR7	¹⁶⁷ Er	G043H7	Fluidigm	1:100
CD206	¹⁶⁸ Er	15-2	Fluidigm	1:50
CD33	¹⁶⁹ Tm	WM53	Fluidigm	1:100
FcεR1a	¹⁷⁰ Er	AER-37 (CRA1)	eBioscience	1:100
CD161	¹⁷¹ Yb	HP-3G10	Biolegend	1:100
CXCR4	¹⁷³ Yb	12G5	Fluidigm	1:200
CD127	¹⁷⁶ Yb	A019D5	DVS Sciences	1:100
CD47	²⁰⁹ Bi	CC2C6	Fluidigm	1:200
Intracellular				
cPARP	¹⁴³ Nd	F21-852	fluidigm	1:200
MIP1beta	¹⁵⁰ Nd	D21-1351	Fluidigm	1:100
CD68	¹⁵³ Eu	Y1/82A	Biolegend	1:100
IL6	¹⁵⁶ Gd	MQ2-13AS	Fluidigm	1:50
CTLA4	¹⁶¹ Dy	14D3	Fluidigm	1:50
OPN	¹⁷² Yb	polyclonal	LSBio	1:50
IL1beta	¹⁷⁴ Yb	CRM56	eBioscience	1:50
TNF	¹⁷⁵ Lu	MAb11	DVS Sciences	1:50

Supplementary Table 3. The *Panel B*-antibody list

Target	Isotope tag	clone	company	Dilution
Surface				
CD45	⁸⁹ Y	HI30	Fluidigm	1:200
CD49d	¹⁴¹ Pr	9F10	Fluidigm	1:100
CD19	¹⁴² Nd	HIB19	Fluidigm	1:100
HLA-DR	¹⁴³ Nd	L243	Fluidigm	1:200
CD38	¹⁴⁴ Nd	HIT2	Fluidigm	1:100
CD138	¹⁴⁵ Nd	DL-101	Fluidigm	1:200
IgD	¹⁴⁶ Nd	IA6-2	Fluidigm	1:100
CD123	¹⁴⁷ Sm	6H6	Biolegend	1:100
IgA	¹⁴⁸ Nd	polyclonal	Fluidigm	1:200
CD25	¹⁴⁹ Sm	2A3	Fluidigm	1:50
KLRG1	¹⁵⁰ Nd	14C2A07	Biolegend	1:100
IgGL	¹⁵¹ Eu	MHL-38	Fluidigm	1:200
TCRgd	¹⁵² Sm	11F2	Fluidigm	1:100
CD62L	¹⁵³ Eu	DREG-56	Fluidigm	1:100
CD3	¹⁵⁴ Sm	UCHT1	Fluidigm	1:200
CD11c	¹⁵⁵ Gd	Bu15	Biolegend	1:100
CXCR3	¹⁵⁶ Gd	G025H7	Fluidigm	1:200
CCR4	¹⁵⁸ Gd	L291H4	Fluidigm	1:200
CD1c	¹⁵⁹ Tb	L161	Biolegend	1:100
IgGK	¹⁶⁰ Gd	MHK-49	Fluidigm	1:100
CD8	¹⁶² Dy	RPA-T8	Fluidigm	1:50
CRTH2	¹⁶³ Dy	BM16	Fluidigm	1:100
CD28	¹⁶⁴ Dy	L293	BDBioscience	1:50
CD45RO	¹⁶⁵ Ho	UCHL1	Fluidigm	1:100
CD34	¹⁶⁶ Er	581	Fluidigm	1:100
CD27	¹⁶⁷ Er	O323	Fluidigm	1:50
CD24	¹⁶⁹ Tm	ML5	Fluidigm	1:50
CD20	¹⁷¹ Yb	2H7	Fluidigm	1:100
IgM	¹⁷² Yb	MHM-88	Fluidigm	1:100
CXCR4	¹⁷³ Yb	12G5	Fluidigm	1:200
PD1	¹⁷⁴ Yb	EH12.2H7	Fluidigm	1:50
CD11b	²⁰⁹ Bi	ICRF44	Fluidigm	1:200
Intracellular				
CTLA4	¹⁶¹ Dy	14D3	Fluidigm	1:50
Tbet	¹⁷⁰ Er	4B10	Biolegend	1:50
Ki67	¹⁶⁸ Er	B56	Fluidigm	1:50
TNF	¹⁷⁵ Lu	MAB11	DVS Sciences	1:50
CHI3L1	¹⁷⁶ Yb	EPR19078-157	abcam	1:100

Supplementary Table 4. Selected markers used in the clustering of All cell, T cell, granulocyte and MNK cell-*Panel A*

Target in All cells	Target in T cells	Target in granulocytes	Target in MNK cells
FceR1a	CD3	FceR1a	CD47
CCR4	CCR4	CD45	HLADR
CD11c	CD4	CD95	CCR4
CD68	CCR7	CD66b	CD11c
HLADR	CD8	CD16	CD68
CD64	CRTH2	CD64	CD14
CD14	CTLA4	CCR4	CD33
CD33	CD226	CD69	CD4
CD3	ICOS	CD161	CCR7
CD4	TIGIT	CXCR4	CD64
CD8	CD127	HLADR	CD95
CD66b	CD69	IL1beta	CD206
CD161	LAG3	CD33	CD16
CD56		CD14	OPN
CD19			CD69
			CD161
			CD56
			CD226
			TIGIT
			CD8
			CRTH2
			CD141
			IL1beta
			CD127
			TNF
			MIP1beta

Supplementary Table 5. Selected markers used in B cell subclustering-*Panel B*

Target	Isotope tag	clone	company
CD20	¹⁷¹ Yb	2H7	Fluidigm
IgM	¹⁷² Yb	MHM-88	Fluidigm
CXCR3	¹⁵⁶ Gd	G025H7	Fluidigm
CCR4	¹⁵⁸ Gd	L291H4	Fluidigm
CD45	⁸⁹ Y	HI30	Fluidigm
CD49d	¹⁴¹ Pr	9F10	Fluidigm
HLADR	¹⁴³ Nd	L243	Fluidigm
CD19	¹⁴² Nd	HIB19	Fluidigm
CD1c	¹⁵⁹ Tb	L161	Biolegend
CD38	¹⁴⁴ Nd	HIT2	Fluidigm
IgA	¹⁴⁸ Nd	polyclonal	Fluidigm
CD138	¹⁴⁵ Nd	DL-101	Fluidigm
IgD	¹⁴⁶ Nd	IA6-2	Fluidigm
IgGK	¹⁶⁰ Gd	MHK-49	Fluidigm
IgGL	¹⁵¹ Eu	MHL-38	Fluidigm
CD62L	¹⁵³ Eu	DREG-56	Fluidigm
CD24	¹⁶⁹ Tm	ML5	Fluidigm
KLRG1	¹⁵⁰ Nd	14C2A07	Biolegend
CXCR4	¹⁷³ Yb	12G5	Fluidigm
CHI3L1	¹⁷⁶ Yb	EPR19078-157	abcam
CD25	¹⁴⁹ Sm	2A3	Fluidigm
CD123	¹⁴⁷ Sm	6H6	Biolegend
CD45RO	¹⁶⁵ Ho	UCHL1	Fluidigm
TCRgd	¹⁵² Sm	11F2	Fluidigm
CD27	¹⁶⁷ Er	O323	Fluidigm
Ki67	¹⁶⁸ Er	B56	Fluidigm
CD11c	¹⁵⁵ Gd	Bu15	Biolegend
Tbet	¹⁷⁰ Er	4B10	Biolegend

Supplementary Table 6. Treatment duration in MS and NMOSD patients

MS_aCD20 (n=14, months)	MS_FTY (n=8, months)	MS_IFNs (n=3, months)	NMOSD_aCD20 (n=4, months)	NMOSD_MMF (n=1, months)
31	11	233	17	75
15	29	70	34	
11	45	34	155	
2	128		60	
30	123			
47	34			
29	10			
40	71			
25				
4				
9				
35				
17				
22				