Supplementary information

Multi-parameter immune profiling of peripheral blood mononuclear cells by multiplexed single-cell mass cytometry in patients with early multiple sclerosis

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Supplementary Table 1 Den

Demographic information

group	Ν	f/m	Age (years, mean (range))	EDSS (mean (range))
CON	11	10/1	36 (24-56)	n.a.
early MS	11	8/3	36 (26-51)	0.9 (0-2)
CD	8	5/3	38 (27-49)	n.a.
<i>p</i> value				-
early MS vs CON	-	p = 0.5865	p = 0.9529	-
CD vs CON	-	p = 0.2621	p = 0.6840	-
early MS vs CD	-	<i>p</i> = 1.0000	p = 0.5618	-

CD: Crohn's disease; CON: Healthy controls; MS: Multiple Sclerosis; CIS = Clinically Isolated Syndrome; EDSS: Expanded disability status scale

Supplementary Table 2

The list of antibodies and barcoding reagents - Panel A

isotope tag	target	clone / company
⁸⁹ Y	CD45 (live barcode)	HI30 / Fluidigm
¹¹³ In	CD45 (live barcode)	HI30 / Biolegend
¹¹⁵ ln	CD45 (live barcode)	HI30 / Biolegend
¹⁴¹ Pr	HLA-DR	L243 / Fluidigm
¹⁴² Nd	CD19	HIB19 / Fluidigm
¹⁴³ Nd	NFAT1	D43B1 / Fluidigm
¹⁴⁴ Nd	CD44	BJ18 / Biolegend
¹⁴⁵ Nd	CD4	RPA-T4 / Biolegend
¹⁴⁶ Nd	ΤΝϜα	Mab11 / Fluidigm
¹⁴⁷ Sm	CD11c	Bu15 / Fluidigm
¹⁴⁸ Nd	CD16	3G8 / Fluidigm
¹⁴⁹ Sm	CCI2	5D3-F7 / Biolegend
¹⁵⁰ Nd	CD86	IT2.2 / Biolegend
¹⁵¹ Eu	CD103	Ber-ACT8 / Fluidigm
¹⁵² Sm	CD95	DX2 / Biolegend
¹⁵³ Eu	TIM3	F38-2E2 / Biolegend
¹⁵⁴ Sm	CD3	UCHT1 / Fluidigm
¹⁵⁵ Gd	CD56	B159 / Fluidigm
¹⁵⁶ Gd	CCR5	NP-6G4 / Fluidigm
¹⁵⁸ Gd	CD101	BB27 / Fluidigm
¹⁵⁹ Tb	IRF4	3E4 / Biolegend
¹⁶⁰ Gd	CD14	RMO52 / eBioscience
¹⁶¹ Dy	EMR1 (F4/80)	A10 / Bio-Rad
¹⁶² Dy	CD8a	RPA-T8 / Fluidigm
¹⁶³ Dy	TGF-β	TW4-2F8 / Biolegend
¹⁶⁴ Dy	CD115	9-4D2-1E4 / Biolegend
¹⁶⁵ Ho	T-bet (PE)	eBio4B10/ eBioscience
¹⁶⁶ Er	IL-10	JES3-9D7 / Fluidigm
¹⁶⁷ Er	CCR7	G043H7 / Fluidigm
¹⁶⁸ Er	IFNγ	B27 / Fluidigm
¹⁶⁹ Tm	CD33	WM53 / Fluidigm
¹⁷⁰ Er	-	-
¹⁷¹ Yb	CCR2	K036C2 / Biolegend
¹⁷² Yb	CX3CR1	2A9-1 / Fluidigm
¹⁷³ Yb	CD40	5C3 / Biolegend
¹⁷⁴ Yb	CD62L	DREG-56 / Biolegend
¹⁷⁵ Lu	ADRP	polyclonal / non commercial
¹⁷⁶ Yb	IL-7R	A019D5 / Fluidigm
¹⁹⁵ Pt	CD45 (live barcode)	HI30 / Biolegend
¹⁹⁶ Pt	CD45 (live barcode)	HI30 / Biolegend
¹⁹⁸ Pt	CD45 (live barcode)	HI30 / Biolegend
¹⁹¹ lr	DNA	- / Fluidigm
¹⁹³ lr	DNA	- / Fluidigm
²⁰⁹ Bi	CD11b	ICRF44 / Fluidiam

Supplementary Table 3

The list of antibodies and barcoding reagents - Panel B

isotope tag	target	clone / company
⁸⁹ Y	CD45 (live barcode)	HI30 / Fluidigm
¹¹³ In	CD45 (live barcode)	HI30 / Biolegend
¹¹⁵ In	CD45 (live barcode)	HI30 / Biolegend
¹⁴¹ Pr	HLA-DR	L243 / Fluidigm
¹⁴² Nd	CD116	4HI / Biolegend
¹⁴³ Nd	IKZF1	Ikaros / Fluidigm
¹⁴⁴ Nd	CD38	HIT2 / Fluidigm
¹⁴⁵ Nd	CD18	TS1/18 / Fluidigm
¹⁴⁶ Nd	CD64	10.1 / Fluidigm
¹⁴⁷ Sm	CD13	WM15 / FLuidigm
¹⁴⁸ Nd	Galanin	581403 / Fluidigm
¹⁴⁹ Sm	CCI2	5D3-F7 / Biolegend
¹⁵⁰ Nd	ΜΙΡβ	D21-1351 / Biolegend
¹⁵¹ Eu	CD68	Y1/82A / Fluidigm
¹⁵² Sm	ΤΝϜα	Mab11 / Fluidigm
¹⁵³ Eu	IL-6	MQ2-13A5 / Biolegend
¹⁵⁴ Sm	CD172a	15-414 / Biolegend
¹⁵⁵ Gd	CD54 (ICAM1)	HA58 / Biolegend
¹⁵⁶ Gd	PD-L1	29E.2A3 / Fluidigm
¹⁵⁸ Gd	CD135	BB27 / Fluidigm
¹⁵⁹ Tb	GM-CSF	BVD2-21C11 / Biolegend
¹⁶⁰ Gd	CD163	GHI/61 / Biolegend
¹⁶¹ Dy	GATA6	D61E4 / Fluidigm
¹⁶² Dy	CD8a	RPA-T8 / Fluidigm
¹⁶³ Dy	CXCR3	G025H7 / Fluidigm
¹⁶⁴ Dy	Arginase-1	658922 / Fluidigm
¹⁶⁵ Ho	IL-4 (PE)	8D4-8 / Biolegend
¹⁶⁶ Er	IL-10	JES3-9D7 / Fluidigm
¹⁶⁷ Er	IRF8	7G11A45 / Biolegend
¹⁶⁸ Er	CD206	15-2 / Fluidigm
¹⁶⁹ Tm	CD33	WM53 / Fluidigm
¹⁷⁰ Er	-	-
¹⁷¹ Yb	GLUT1	195205 / R&D Systems
¹⁷² Yb	HK1	3A10 / Thermo Fisher Scientific
¹⁷³ Yb	CD141	1A4 / Biolegend
¹⁷⁴ Yb	IL-8 (FITC)	EH12.2H7 / Fluidigm
¹⁷⁵ Lu	ADRP	polyclonal / non commercial
¹⁷⁶ Yb	TREM2	237920 / R&D Systems
¹⁹⁵ Pt	CD45 (live barcode)	HI30 / Biolegend
¹⁹⁶ Pt	CD45 (live barcode)	HI30 / Biolegend
¹⁹⁸ Pt	CD45 (live barcode)	HI30 / Biolegend
¹⁹¹ lr	DNA	- / Fluidigm
¹⁹³ lr	DNA	- / Fluidigm
²⁰⁹ Bi	CD47	CC2C6 / Fluidiam



Supplementary Figure 1 Meta-cluster abundances of the myeloid cell subset (*Panel A*). (**a**) Heat map and cluster analysis of the myeloid cell population from all samples on the basis of the mean expression of analysed markers using antibody *Panel A*. Identified clusters are indicated by dendrograms. Heat colours show overall marker expression levels (red, high expression; dark blue, no expression). The bar graph shows mean frequency (all samples) of each identified cluster as a % of parent (number in black) and a % of total cells (number in gray). (**b**) The Boxplot shows distribution of cluster frequencies (of parent) of the 14 identified clusters of each individual sample in both studied groups (CON, gray; early MS, red). No significant differences in the cluster distribution was observed between the studied group.