

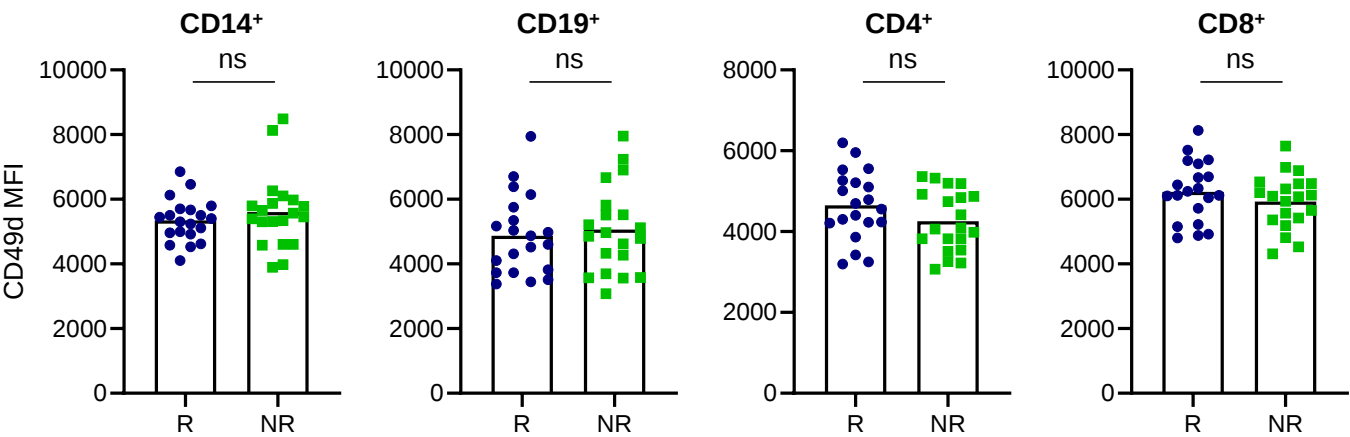


Figure S1. Expression of CD49d at the surface of PBMCs from MS patients
Measurement of CD49d ($\alpha 4$ subunit of VLA-4) Mean Intensity Fluorescence (MFI) on the surface of CD14⁺, CD19⁺, CD4⁺ and CD8⁺ cells from MS patients by flow cytometry. Bar represents the mean among the groups of responders (R) and non-responder (NR) patients and dots represent MFI of each patient. Unpaired t-tests were used for statistical evaluation (ns = $p > 0.05$).

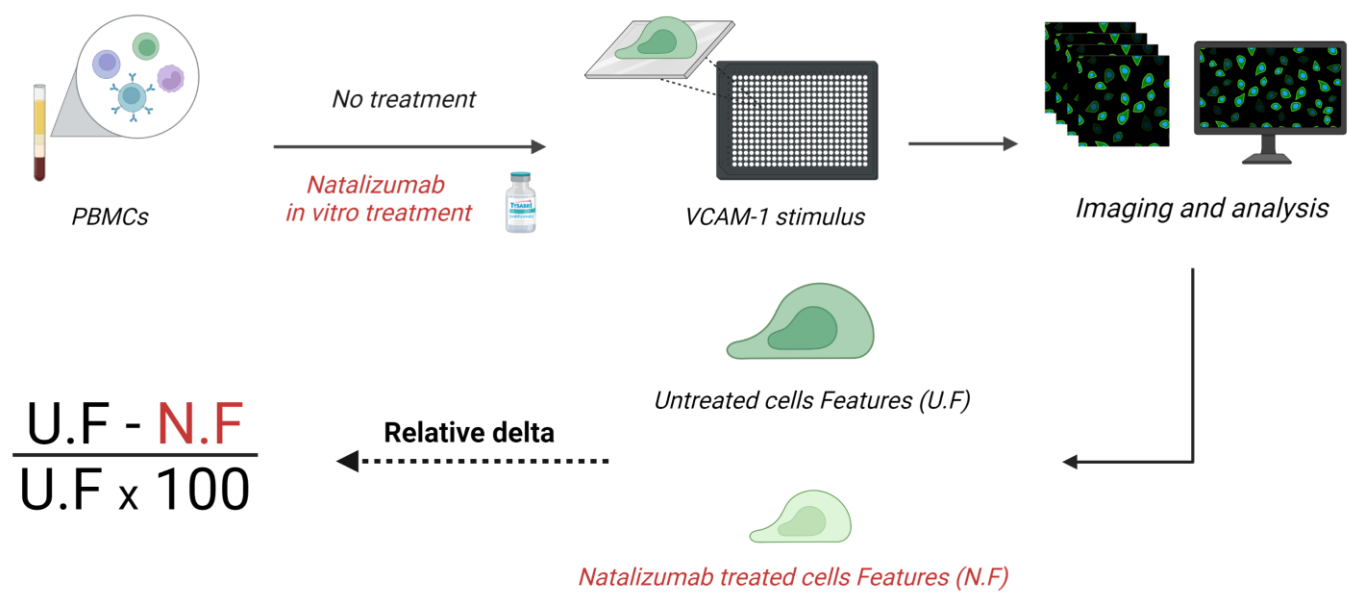


Figure S2. Calculus of the relative delta value corresponding to the impact of natalizumab on each feature
Schematic representation of how the relative delta was calculated for each of the features measured through the HCI pipeline.

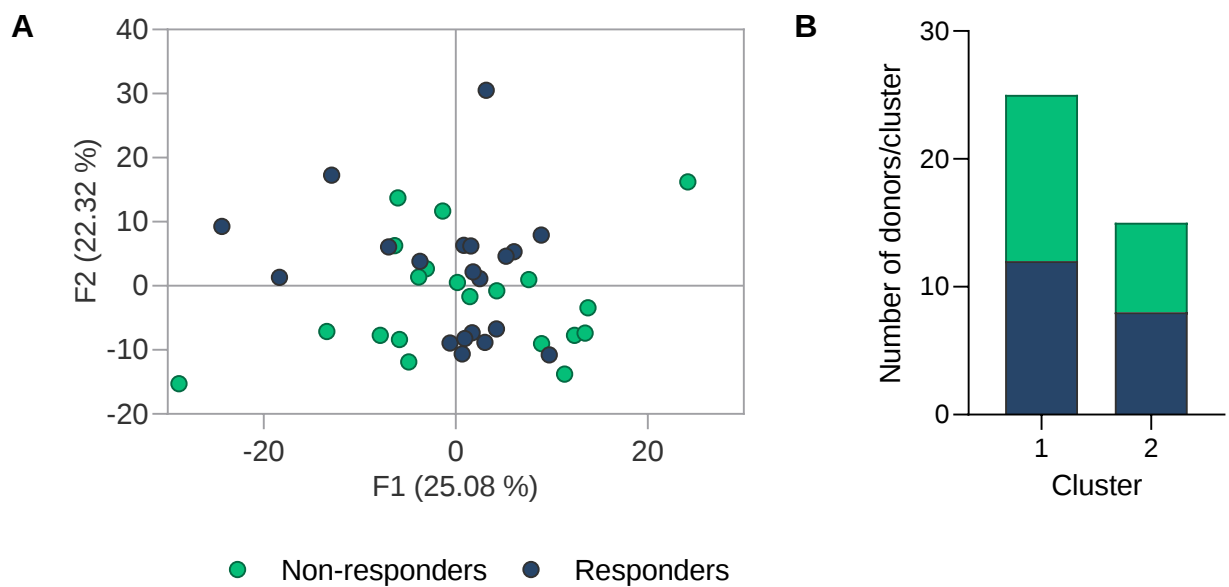


Figure S3. Morphological profiling of natalizumab exposed CD4⁺ T cells does not discriminate samples from responder and non-responder patients

(A) PCA plot of 407 features based on the average relative delta (unexposed sample – natalizumab-exposed sample) / natalizumab-exposed x 100) of cell adhesion, morphology, intensity, actin and pSLP76 distribution and texture parameters of CD4⁺ T cells from PBMCs of 40 MS patients over VCAM-1. Dots represent the mean values of three independent experiments for each donor. Dark blue dots correspond to responders' samples and green dots represent non-responders' samples.

(B) K-means clustering representation of two clusters (X axis) and the number of responder or non-responder patients CD8⁺ T cells classified in each cluster (Y axis). Dark blue represents responder patients and green non-responder patients.

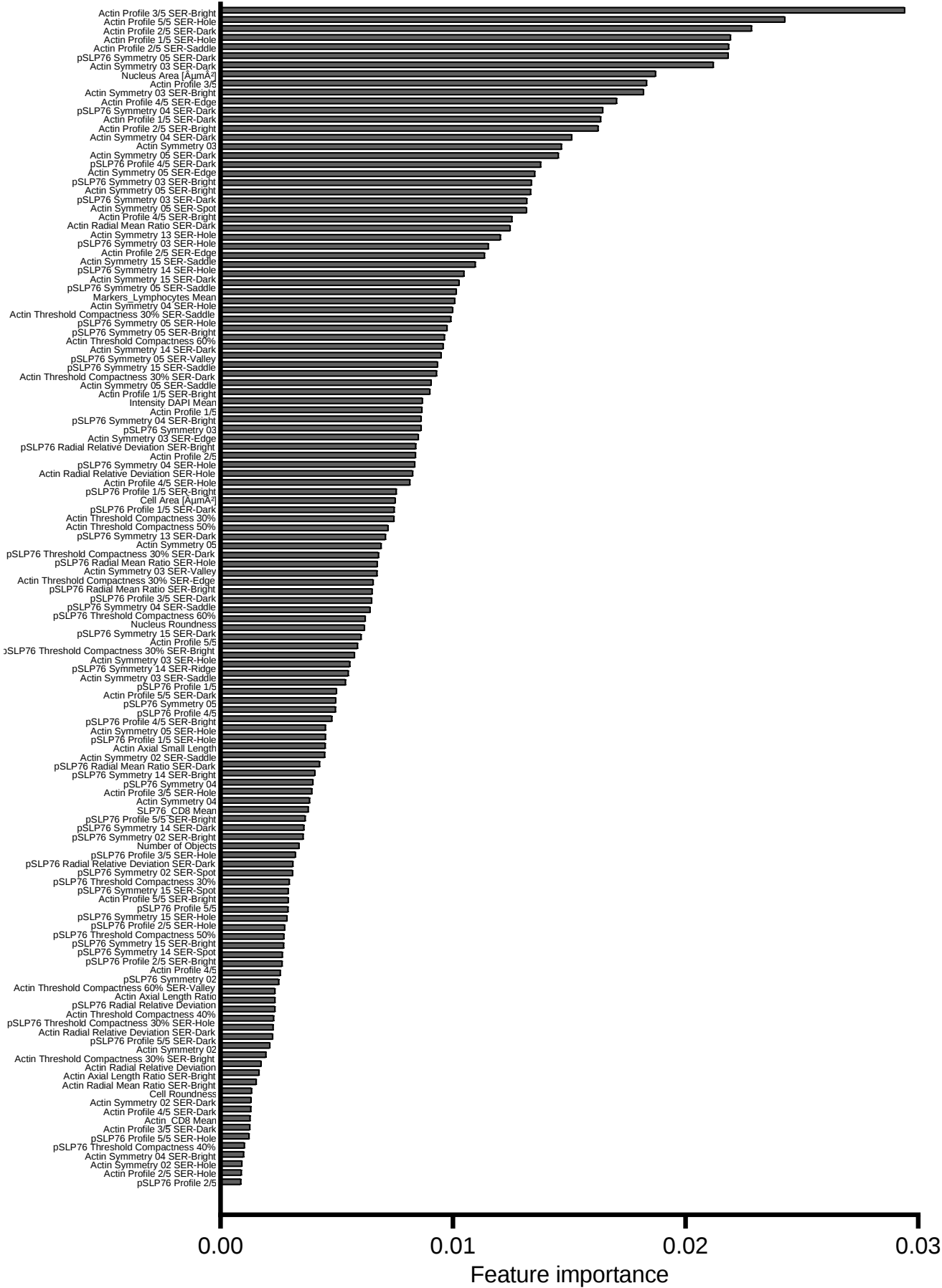


Figure S4. RF loading features most contributing to distinguish CD8⁺ T cells from responder and non-responder MS patients
Importance rank of the 133 features used in the RF analysis of the discovery cohort.

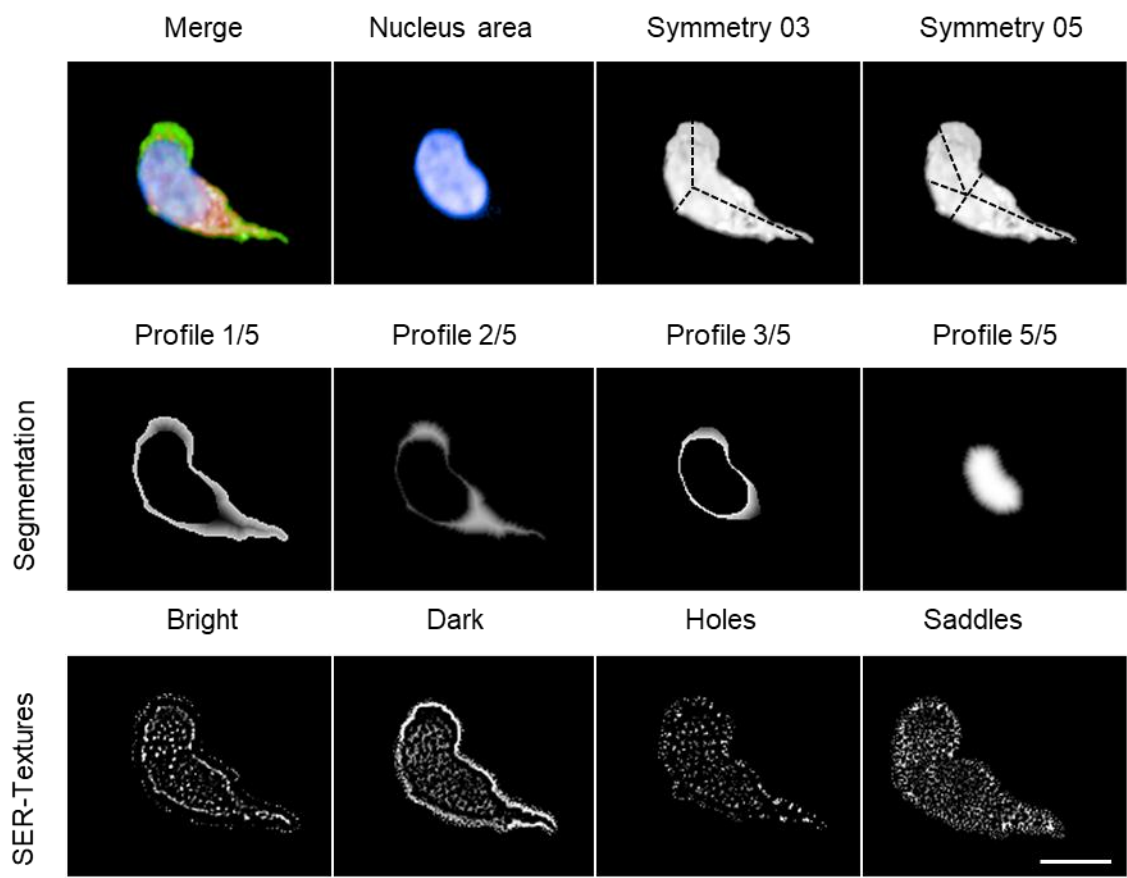


Figure S5. Description of the symmetry, profile and texture descriptors associated with the 10 most important features of the RF approach
Representative images of the 10 most relevant features (including morphology, STAR morphology and SER texture features), according to importance classification and based on the RF analysis of the discovery cohort. Green represents F-actin, red represents pSLP76 and blue represents nucleus. Scale bar: 10 μ m.

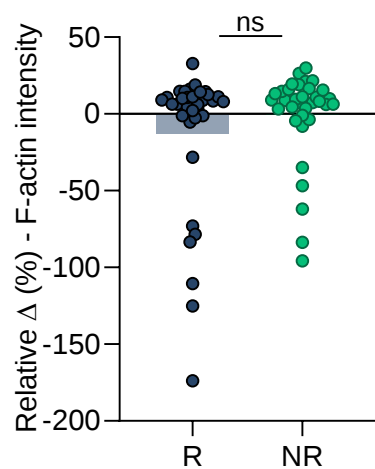


Figure S6. F-actin intensity based on relative delta does not change between responders’ and non-responder’s cells

F-actin intensity based on the average relative delta of CD8+ T cells from responder and non-responder MS patients exposed or not in vitro to natalizumab. Data represent the mean of 3 HCI experiments conducted on 68 MS patients. Bars represent the mean of the considered patient subgroup. Unpaired t-test was used for statistical evaluation (ns = $p > 0.05$).

	Features contribution (%)			
	CD4+ T cells		CD8+ T cells	
	F1	F2	F1	F2
Number of objects	23,059	17,346	12,742	36,941
Cell area	21,013	15,804	35,527	1,486
Ratio width to length	26,444	0,311	27,585	12,623
pSLP76 intensity	26,466	7,84	13,155	8,739
Actin intensity	3,018	58,698	10,991	40,211

Table S1. PCA loadings of the features most contributing to the discrimination of the effects of natalizumab *in vitro* exposure on CD4⁺ and CD8⁺ T cells from healthy donor PBMCs

PCA feature contributions relative to cell adhesion, morphology, actin and pSLP76 intensity of CD4⁺ T cells and CD8⁺ T cells from PBMCs of 10 HD stimulated with VCAM-1.

Patient ID	Sex	Age disease onset	First line treatment	Second line treatment	Time between last treatment and Natalizumab (days)	Time between last corticotherapy and Natalizumab (days)	Age at Natalizumab onset	Response to Natalizumab (R = Responder, NR = Non responder)
1	F	34	REBIF	NONE	751	37	44	R
2	M	27	AVONEX	NONE	78	144	36	R
3	F	23	AVONEX	AVONEX	69	134	28	R
4	F	54	REBIF	AVONEX	83	158	63	R
5	F	36	AVONEX	NONE	39	112	37	R
6	M	29	AVONEX	NONE	63	77	35	NR
7	F	49	AVONEX	NONE	110	124	52	NR
8	F	41	BETAHERON	NONE	40	135	47	NR
9	F	36	BETAHERON	NONE	100	100	51	NR
10	F	26	AVONEX	REBIF	44	243	33	R
11	F	46	AVONEX	NONE	48	48	47	R
12	F	28	BETAHERON	AVONEX	459	48	35	R
13	M	32	BETAHERON	NONE	38	83	33	R
14	F	18	BETAHERON	NONE	98	105	20	NR
15	F	22	COPAXONE	NONE	36	167	30	NR
16	M	36	REBIF	NONE	65	283	46	NR
17	F	29	BETAHERON	NONE	55	69	30	NR
18	F	26	REBIF	NONE	45	227	33	NR
19	F	20	AVONEX	BETAHERON	483	46	29	NR
20	M	24	AVONEX	NONE	1278	73	30	NR
21	F	31	AVONEX	NONE	222	83	42	NR
22	F	30	AVONEX	NONE	75	174	33	R
23	F	42	BETAHERON	NONE	32	95	54	R
24	M	24	REBIF	AVONEX	72	170	33	R
25	F	27	AVONEX	NONE	155	70	35	NR
26	M	38	REBIF	NONE	122	324	48	NR
27	F	18	REBIF	NONE	43	94	26	R
28	F	17	REBIF	AVONEX	200	200	19	R
29	F	27	REBIF	NONE	39	38	27	NR
30	F	18	AVONEX	NONE	56	136	31	R
31	F	20	AVONEX	NONE	239	209	23	NR
32	M	31	BETAHERON	NONE	39	384	36	NR
33	F	25	REBIF	AVONEX	41	195	46	R
34	M	29	REBIF	NONE	75	105	37	R
35	M	32	BETAHERON	NONE	38	86	49	R
36	F	20	AVONEX	NONE	80	90	32	NR
37	F	41	COPAXONE	NONE	420	225	43	NR
38	F	23	AVONEX	NONE	46	199	43	NR
39	F	29	BETAHERON	NONE	41	190	38	R
40	F	19	BETAHERON	NONE	161	109	23	R

Table S2. Clinical features of MS patients from the BIONAT discovery cohort
Clinical information relative to each MS patient from the discovery cohort.

Features contribution (%)								
	CD14+ cells		CD19+ cells		CD4+ T cells		CD8+ T cells	
	F1	F2	F1	F2	F1	F2	F1	F2
Number of objects	1,874	79,766	21,081	3,731	23,059	17,346	12,742	36,941
Cell area	28,311	0,35	10,115	28,152	21,013	15,804	35,527	1,486
Ratio width to length	22,636	7,058	4,914	57,622	26,444	0,311	27,585	12,623
pSLP76 intensity	16,601	9,618	33,204	2,574	26,466	7,84	13,155	8,739
Actin intensity	30,578	3,208	30,685	7,92	3,018	58,698	10,991	40,211

Table S3. PCA loadings of the features most contributing to the discrimination of the effects of natalizumab *in vitro* exposure on CD14⁺, CD19⁺, CD4⁺ and CD8⁺ subsets from PBMCs of MS patients
PCA feature contributions relative to cell adhesion, morphology, actin and pSLP76 intensity of CD14⁺ monocytes, CD19⁺ B cells, CD4⁺ and CD8⁺ T cells from PBMCs of 40 MS donors.

Feature	F1	F2
Actin Axial Small Length	0,638	0,012
pSLP76 Profile 1/5 SER-Saddle	0,638	0,192
Actin Axial Small Length SER-Edge	0,637	0,044
Actin Axial Small Length SER-Spot	0,634	0,064
pSLP76 Axial Small Length SER-Edge	0,634	0,042
pSLP76 Profile 1/5 SER-Edge	0,629	0,151
Actin Axial Small Length SER-Bright	0,619	0,070
pSLP76 Axial Small Length SER-Valley	0,616	0,050
Actin Axial Small Length SER-Ridge	0,613	0,069
Actin Symmetry 04 SER-Spot	0,605	0,051
pSLP76 Profile 2/5 SER-Saddle	0,603	0,110
pSLP76 Threshold Compactness 50% SER-Bright	0,601	0,002
pSLP76 Axial Small Length SER-Dark	0,598	0,048
Actin Axial Small Length SER-Valley	0,588	0,028
pSLP76 Threshold Compactness 60% SER-Bright	0,587	0,001
CD8 Width	0,585	0,028
Actin Axial Small Length SER-Saddle	0,582	0,045
pSLP76 Axial Small Length SER-Saddle	0,580	0,027
pSLP76 Profile 2/5 SER-Spot	0,574	0,197
pSLP76 Threshold Compactness 40% SER-Spot	0,572	0,010
Nucleus Area	0,572	0,050
Actin Axial Small Length SER-Dark	0,570	0,022
pSLP76 Profile 4/5 SER-Edge	0,566	0,095
pSLP76 Profile 3/5 SER-Saddle	0,566	0,107
pSLP76 Threshold Compactness 50% SER-Spot	0,563	0,000
pSLP76 Profile 3/5 SER-Ridge	0,554	0,217
pSLP76 Symmetry 05 SER-Spot	0,551	0,020
pSLP76 Profile 1/5 SER-Hole	0,549	0,317
pSLP76 Profile 2/5 SER-Bright	0,547	0,198
pSLP76 Profile 3/5 SER-Bright	0,544	0,223
pSLP76 Threshold Compactness 40% SER-Saddle	0,541	0,039
pSLP76 Threshold Compactness 50% SER-Saddle	0,540	0,012
pSLP76 Threshold Compactness 40% SER-Bright	0,535	0,012
pSLP76 Symmetry 04 SER-Bright	0,533	0,001
pSLP76 Threshold Compactness 30% SER-Spot	0,532	0,024
Actin Radial Mean	0,530	0,331
pSLP76 Threshold Compactness 60% SER-Ridge	0,524	0,033
pSLP76 Symmetry 04 SER-Spot	0,523	0,004
Actin Symmetry 03 SER-Spot	0,521	0,091
Actin Radial Mean SER-Edge	0,519	0,348
pSLP76 Radial Mean SER-Valley	0,518	0,417
pSLP76 Symmetry 05 SER-Bright	0,516	0,021
pSLP76 Threshold Compactness 60% SER-Edge	0,516	0,101
pSLP76 Threshold Compactness 50% SER-Ridge	0,514	0,030
pSLP76 Radial Mean SER-Edge	0,513	0,405
pSLP76 Threshold Compactness 30%	0,511	0,000
Actin Symmetry 14 SER-Spot	0,511	0,123
Actin Axial Small Length SER-Hole	0,510	0,027
Cell Area	0,509	0,219
CD8 Area	0,509	0,219

Table S4. List of the 50 most relevant PCA loadings of the features most contributing to the discrimination of CD8⁺ T cells from responder and non-responder MS patients

The 60 most relevant PCA features among 407 features based on the average relative delta of cell adhesion, morphology, intensity, actin and pSLP76 distribution and texture parameters pertaining to CD8⁺ T cells from PBMCs of 40 MS patients.

Patient ID	Sex	Age disease onset	First line treatment	Second line treatment	Time between last treatment and Natalizumab (days)	Time between last corticotherapy and Natalizumab (days)	Age at Natalizumab onset	Response to Natalizumab (R = Responder, NR = Non responder)
1	F	35	REBIF	NONE	60	366	38	NR
2	M	22	AVONEX	NONE	NA	81	25	R
3	F	17	REBIF	NONE	48	108	27	R
4	M	15	AVONEX	NONE	143	49	23	R
5	F	24	AVONEX	NONE	36	126	25	NR
6	F	22	REBIF	AVONEX	64	102	28	NR
7	M	23	AVONEX	NONE	NA	151	32	R
8	F	42	REBIF	NONE	41	133	45	NR
9	F	19	BETAFERON	NONE	75	684	27	NR
10	F	21	REBIF	NONE	991	39	29	NR
11	M	35	NONE	NONE	NA	36	36	NR
12	F	31	NONE	NONE	NA	61	32	R
13	F	23	NONE	NONE	NA	454	24	NR
14	F	16	BETAFERON	NONE	51	39	18	R
15	F	45	REBIF	NONE	35	247	52	R
16	F	35	NONE	NONE	NA	80	43	NR
17	M	18	REBIF	NONE	100	234	21	R
18	M	20	AVONEX	NONE	33	118	27	R
19	F	15	AVONEX	NONE	975	92	22	R
20	F	29	BETAFERON	AVONEX	43	435	44	R
21	M	13	NONE	NONE	NA	175	20	NR
22	M	15	NONE	NONE	NA	173	19	NR
23	F	32	NONE	NONE	NA	97	32	R
24	M	42	NONE	NONE	NA	170	42	NR
25	M	29	AVONEX	NONE	34	75	37	NR
26	F	56	NONE	NONE	NA	33	56	R
27	M	28	AVONEX	REBIF	47	90	37	R
28	F	16	NONE	NONE	NA	137	26	NR

Table S5. Clinical features of MS patients from the BIONAT validation cohort
Clinical information relative to each MS patient from the validation cohort.

Feature	Responders Relative Delta	Non-Responders Relative Delta	Delta difference (R-NR)	P value
Actin Symmetry 03	3,40	20,09	-16,70	0,00
Actin Symmetry 13	5,28	19,07	-13,78	0,00
Lymphocytes markers mean intensity	-3,42	4,00	-7,42	0,00
Actin Profile 2/5 SER-Valley	-2,97	8,81	-11,78	0,01
Actin Profile 2/5 SER-Saddle	-1,24	7,90	-9,13	0,01
Actin Symmetry 05 SER-Ridge	-8,86	4,42	-13,28	0,01
Actin Symmetry 15	9,41	23,73	-14,31	0,01
Actin Profile 2/5 SER-Dark	0,84	10,71	-9,87	0,01
Actin Profile 2/5 SER-Edge	-5,79	2,64	-8,44	0,01
Actin Symmetry 05	4,79	22,61	-17,82	0,02
Cell Area	14,72	7,12	7,60	0,02
Cell Width	7,17	2,28	4,89	0,03
Actin Symmetry 05 SER-Bright	-12,22	2,70	-14,93	0,03
Actin Profile 3/5 SER-Spot	0,18	9,93	-9,75	0,03
Actin Symmetry 03 SER-Edge	2,37	12,97	-10,59	0,03
pSLP76 Profile 4/5 SER-Edge	18,89	11,87	7,02	0,04
pSLP76 Symmetry 03	11,95	20,75	-8,80	0,04
Actin Symmetry 15 SER-Ridge	-3,17	9,68	-12,85	0,04
Actin Symmetry 13 SER-Dark	5,12	13,36	-8,24	0,04
Actin Symmetry 13 SER-Valley	3,99	11,84	-7,85	0,04
Actin Profile 3/5 SER-Edge	-5,61	1,01	-6,62	0,04
Actin Profile 5/5	-2,58	-8,33	5,75	0,04
pSLP76 Symmetry 04 SER-Hole	-5,43	3,81	-9,24	0,04
Actin Symmetry 13 SER-Edge	4,72	13,96	-9,24	0,05
pSLP76 Profile 4/5 SER-Valley	22,84	14,71	8,13	0,05
Actin Profile 3/5 SER-Saddle	0,17	7,25	-7,08	0,05
Nucleus Area	13,39	5,71	7,68	0,05
pSLP76 Radial Relative Deviation SER-Spot	4,61	1,36	3,26	0,05
pSLP76 Profile 4/5 SER-Dark	23,30	14,85	8,45	0,05

Table S6. Mean values of the relative delta (accounting for impact of natalizumab) for the features distinguishing samples from responder and non-responder patients

Table presenting the 29 features with p value <0.05 calculated though unpaired t-tests between the relative delta mean from responder and non-responder patients (n=34 responders and n=34 non-responders).