SUPPLEMENTAL INFORMATION FILE

Bacterial neurotoxic metabolites in Multiple Sclerosis cerebrospinal fluid and plasma.

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Table 1: Patient demographics and clinical characteristics.

Diagnosis	Age Years Mean (SD)	Sex	BMI Mean (SD)	Disease Duration Months Mean (SD)	Continued taking DMF
Controls	42.5	F: 16	27.9	N/A	N/A
n = 20	(13.88)	(80%)	(5.24)		
RRMS	43.11	F: 12	27.43	119.1	At 12months: 10 (71.4%)
n = 14	(8.59)	(86%)	(4.88)	(94.07)	At 24 months: 8 (57.1%)
SPMS	54.99	F: 9	25.39	374.1	At 12months: 9/11 (81.8%)
n =11	(6.43)	(82%)	(3.13)	(430.6)	At 24 months: 8/11 (72.7%)
Stats	ANOVA	Chi-squared	ANOVA	Welch t test	
(cx)	***P*=0.0095**	*P*=0.911	*P*=0.338	*P*=0.14	

Table showing the demographic and clinical characteristics of our patient cohort. RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; cx = cross-sectional design; SD = standard deviation; DMF = dimethyl fumarate; *n* = number of subjects

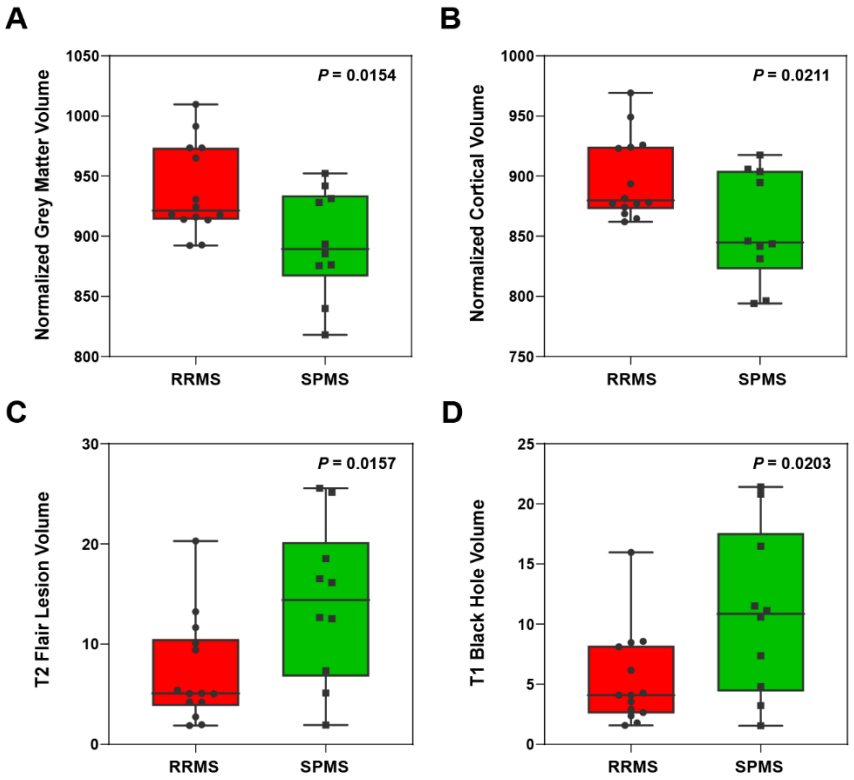

Figure S1: MRI metrics of the patients' cohort at baseline. Both normalized grey matter (A) and cortical volume (B) was significantly lower in SPMS patients compared to RRMS, as expected based on their disease phenotype. Furthermore, T2 flair lesion (C) and T1 black hole (D) volumes were higher in SPMS compared to RRMS patients. *P* values obtained with one-way ANOVA, $P < 0.05$

Table 2: Clinical data at baseline and after DMF treatment

Diagnosis	EDSS BL Median (Range)	EDSS 6m Median (Range)	EDSS 12m Median (Range)	25FTW BL Median (Range)	25FTW 12M Median (Range)	SDMT BL Median (Range)	SDMT 12M Median (Range)	PASAT BL Median (Range)	PASAT 12M Median (Range)
Controls *n* = 20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
RRMS *n* = 14	*n* =14 2 (1-4)	*n* = 14 1.5 (0-3.5)	*n* = 14 1.5 (0-5)	*n* = 14 4.65 (3.7-6.7)	*n* = 10 4.25 (3.9-6.1)	*n* = 14 62.5 (41-76)	*n* = 10 64 (42-81)	*n* = 14 47 (11-60)	*n* = 9 56 (13-66)
SPMS *n* = 11	*n* =11 3.5 (3-6.5)	*n* = 11 3.5 (2-6.5)	*n* = 9 3.5 (2-6.5)	*n* =11 7 (3.1-18.6)	*n* = 8 6.3 (4.3-20)	*n* = 11 47 (36-61)	*n* = 8 51 (26-61)	*n* = 11 35 (9-53)	*n* = 8 39 (19-49)
Stats (cx)	Welch t test ***P*=0.0002**	Welch t test ***P*=0.0004**	Welch t test ***P*=0.0050**	Welch t test ***P*=0.0298**	Welch t test *P*=0.071	Welch t test ***P*=0.0007**	Welch t test ***P*=0.0183**	Welch t test ***P*=0.0159**	Welch t test *P*=0.1231
Stats (paired) Wilcoxon signed rank test		BL-6m RRMS: *P*=0.0978 SPMS: *P*=0.2763	BL-12m **RRMS: *P*=0.0283** SPMS: *P*=0.7835		BL-12m RRMS: *P*=0.3844 SPMS: *P*=0.5281		BL-12m RRMS: *P*=0.8109 SPMS: *P*=0.6406		BL-12m **RRMS: *P*=0.0217** SPMS: *P*=0.1824

Table showing the clinical data of our cohort before and after treatment with dimethyl-fumarate (DMF). RRMS: relapsing remitting multiple sclerosis, SPMS: secondary progressive multiple sclerosis, cx: cross-sectional design, paired: paired design, SD: standard deviation, DMF: dimethyl-fumarate, *n* = number of subjects; BL = baseline; 6m = 6 months; 12m = 12 months; 24m = 24 months; EDSS = Expanded Disability Status Scale; 25FTW = 25 foot timed walk; SDMT = Symbol Digit Modalities Test; PASAT = Paced Auditory Serial Addition Test.

Table 3: Past treatments in multiple sclerosis patients.

Subject Number	MS Type	Treatment								
		None	Betaseron	Copaxone	Avonex	Tysabri	Rebif	Aubagio	Gilenya	Mycophenolate
Bio_MS-01	RRMS		Yes	Yes	Yes					
Bio_MS-02	RRMS	None								
Bio_MS-04	RRMS	None								
Bio_MS-05	RRMS	None								
Bio_MS-07	RRMS				Yes					
Bio_MS-10	RRMS	None								
Bio_MS-11	RRMS	None								
Bio_MS-13	RRMS					Yes	Yes	Yes		
Bio_MS-15	RRMS							Yes		
Bio_MS-18	RRMS				Yes					
Bio_MS-19	RRMS	None								
Bio_MS-20	RRMS				Yes					
Bio_MS-21	RRMS			Yes	Yes	Yes	Yes			
Bio_MS-31	RRMS	None								
Bio_MS-03	SPMS		Yes		Yes					
Bio_MS-06	SPMS	None								
Bio_MS-09	SPMS			Yes	Yes	Yes	Yes	Yes		
Bio_MS-12	SPMS						Yes			
Bio_MS-23	SPMS		Yes						Yes	
Bio_MS-27	SPMS			Yes						
Bio_MS-28	SPMS			Yes						
Bio_MS-29	SPMS			Yes	Yes				Yes	
Bio_MS-32	SPMS				Yes					
Bio_MS-34	SPMS				Yes	Yes		Yes		
Bio_MS-35	SPMS			Yes				Yes		Yes

Table lists all the multiple sclerosis patients with a history of previous medical treatments.

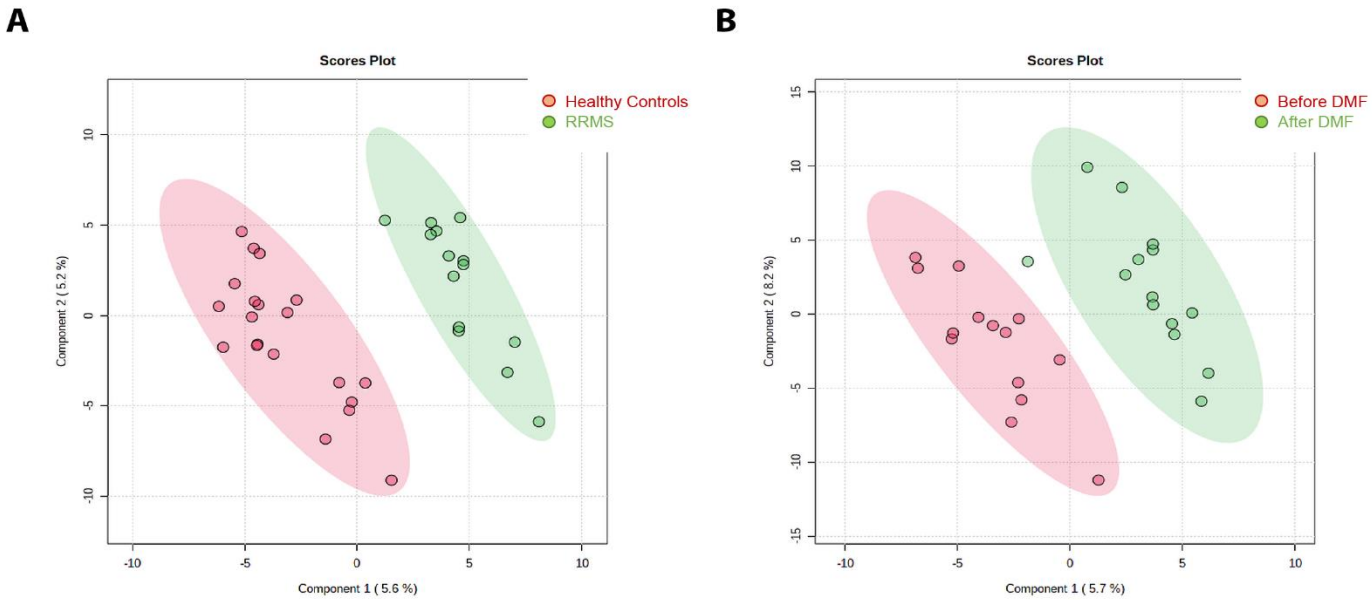

Figure S2: PLS-DA discriminates RRMS patients from Healthy Controls based on the level of metabolites in the CSF. (A) Scores from PLS-DA discrimination show separation of the metabolites between healthy controls (red) and RRMS (green) patients. (B) The plot shows separation of the metabolites between the baseline (red) and 6 months follow-up (green) after DMF treatment in RRMS patients. PLS-DA was performed using normalized levels of metabolites measured in the CSF. Healthy control, *n* = 20; RRMS-baseline, *n* = 14; RRMS-6 mos DMF, *n* = 14.

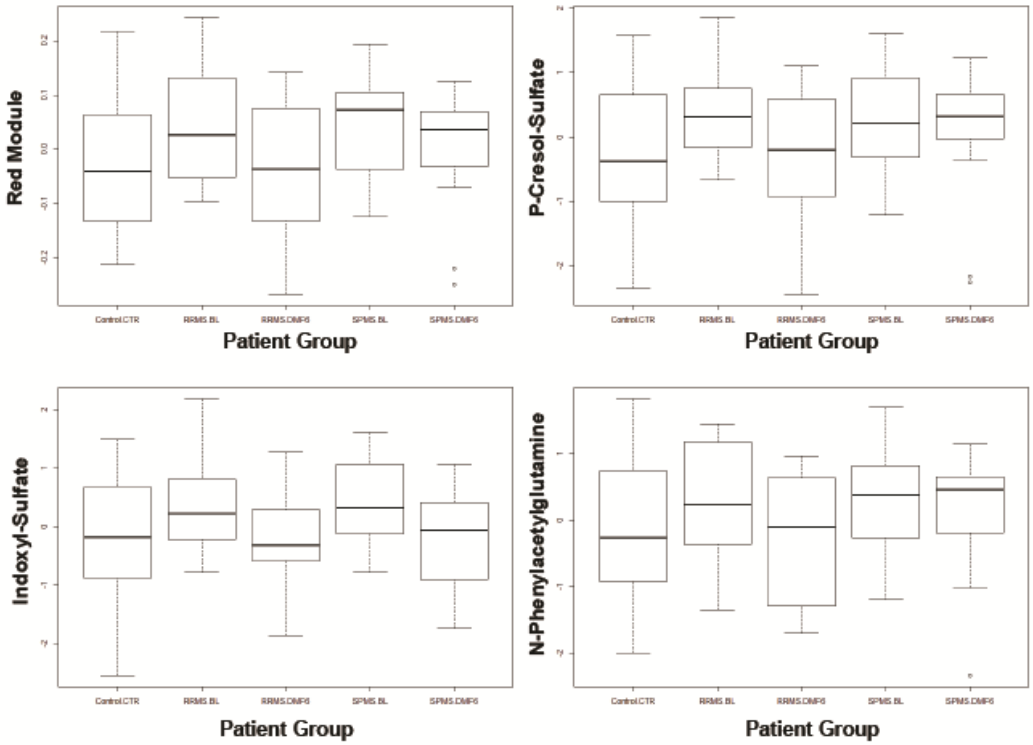

Figure S3: The cross-sectional analysis of the red module metabolites identifies higher abundance in untreated RRMS patients compared to control subjects and are lowered by DMF treatment. The eigenvector of the red module and the relative concentrations of the individual metabolites is shown across patient groups. A trend was observed towards higher levels in RRMS compared to control, which were normalized after treatment with DMF. CTR = Healthy control; BL = baseline; DMF6 = after 6 months of treatment with dimethyl fumarate (Linear regression controlling for age, P <0.05).

Table 4: Demographics of second cohort of patients.

Diagnosis	Therapy	Age Years Mean (SD)	Sex Ratio (F:M)	Previous Treatment
RRMS *n* = 11	DMF	41.45 (9.11)	9 : 2	None: 10 Terifluomide: 1
RRMS *n* = 8	antiCD20	49.38 (8.75)	5 : 3	None: 4 Terifluomide: 1 Interferon beta-1a: 1 Natalizumab: 2

Table showing the demographics of second cohort. RRMS = relapsing remitting multiple sclerosis; SD = standard deviation; DMF = dimethyl fumarate; *n* = number of subjects; F = female; M = male

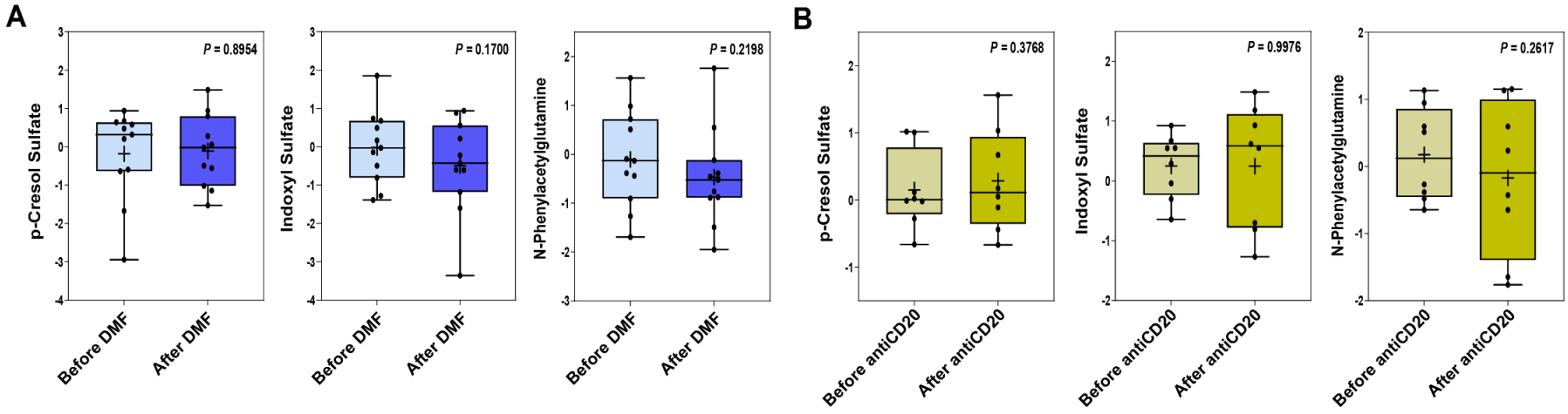

Figure S4: Decreased levels of "red module" metabolites in plasma samples of patients with disease modifying treatments affecting the microbiota. Graphs shows normalized levels of p-cresol-sulfate, indoxyl sulfate and N-phenylacetylglutamine in plasma of RRMS patients undergoing either DMF treatment (n = 11) or anti-CD20 therapy (n = 8). (A) The result shows good agreement with the result from the first cohort, with trending towards lower abundance of each metabolite after DMF treatment. (B) However, abundance of putative neurotoxic metabolites was not decreased in plasma from patients with anti-CD20 therapy, another disease-modifying therapy not affecting the microbiota. Statistical significance was calculated by paired t-test.

Table 5: Relative Levels of NFL in CSF at BL and after treatment in RRMS

Diagnosis	NFL BL Mean (SD)	NFL 6M Mean (SD)	NFL 12M Mean (SD)
RRMS *n* =14	*n* = 10 587.35 (705.23)	*n* = 10 543.7 (637.52)	*n* = 10 476.23 (315.44)
Stats (paired t-test)		BL-6M RRMS *P*=0.375	BL-12M RRMS *P*=0.6953

Table showing the relative levels of neurofilament-light chain (NFL) in the cerebrospinal fluid (CSF) of patients with relapsing remitting multiple sclerosis (RRMS) at baseline (BL) and after treatment at 6 months (6M) and 12 months (12M). SD: standard deviation, N: number of subjects. *P* values of a paired t-test between baseline and 6 months (BL-6M) and baseline and 12 months (BL-12M) are shown.

Table 6: MRI metrics at baseline and follow up

Diagnosis	Normalized Grey Matter Volume Baseline Mean (SD)	Normalized Grey Matter Volume 24 months Mean (SD)	Cortical Volume Baseline Mean (SD)	Cortical Volume 24 months Mean (SD)	Deep Grey Volume Baseline Mean (SD)	Deep Grey Volume 24 months Mean (SD)	T2 Flair Lesions Volume Baseline Mean (SD)	T2 Flair Lesion Volume 24 months Mean (SD)	T1 Black Hole Volume Baseline Mean (SD)	T1 Black Hole Volume 24 months Mean (SD)
RRMS *n* = 14	938.05 (37.32)	911.06 (60.52) *n* = 13	897.74 (34.16)	873.32 (57.8) *n* = 13	40.32 (6.4)	37.77 (9.24) *n* = 13	7.16 (5.2)	7.01 (5.3) *n* = 13	5.32 (3.9)	4.02 (3.58) *n* = 13
SPMS *n* = 10	894.21 (44.3)	853.78 (53.1) *n* = 8	857.51 (45.34)	817.96 (49.57) *n* = 8	36.73 (8.56)	35.85 (10.8) *n* = 8	14.15 (7.9)	15.65 (9.09) *n* = 8	10.89 (6.96)	11.01 (8.68) *n* = 8
Stats groups (ANOVA)	***P*=0.0154**	***P*=0.04025**	***P*=0.021**	***P*=0.03701**	*P*=0.252	*P*=0.6697	***P*=0.01574**	***P*=0.01227**	***P*=0.02026**	***P*=0.01755**
Stats paired (Wilcoxon)		BL-24M **RRMS: *P*=0.0046** **SPMS: *P*=0.0156**		BL-24M **RRMS: *P*=0.002** **SPMS: *P*=0.031**		BL-24M RRMS: *P*=0.1464 SPMS: *P*=0.109		BL-24M RRMS: *P*=0.4143 SPMS: *P*=0.156		BL-24M RRMS: *P*=0.0803 SPMS: *P*=0.687

Table showing the MRI data of our cohort at baseline (BL) and after 24 months of treatment with dimethyl-fumarate (DMF). RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; cx = cross-sectional design; paired = paired design; SD = standard deviation; DMF = dimethyl fumarate; *n* = number of subjects

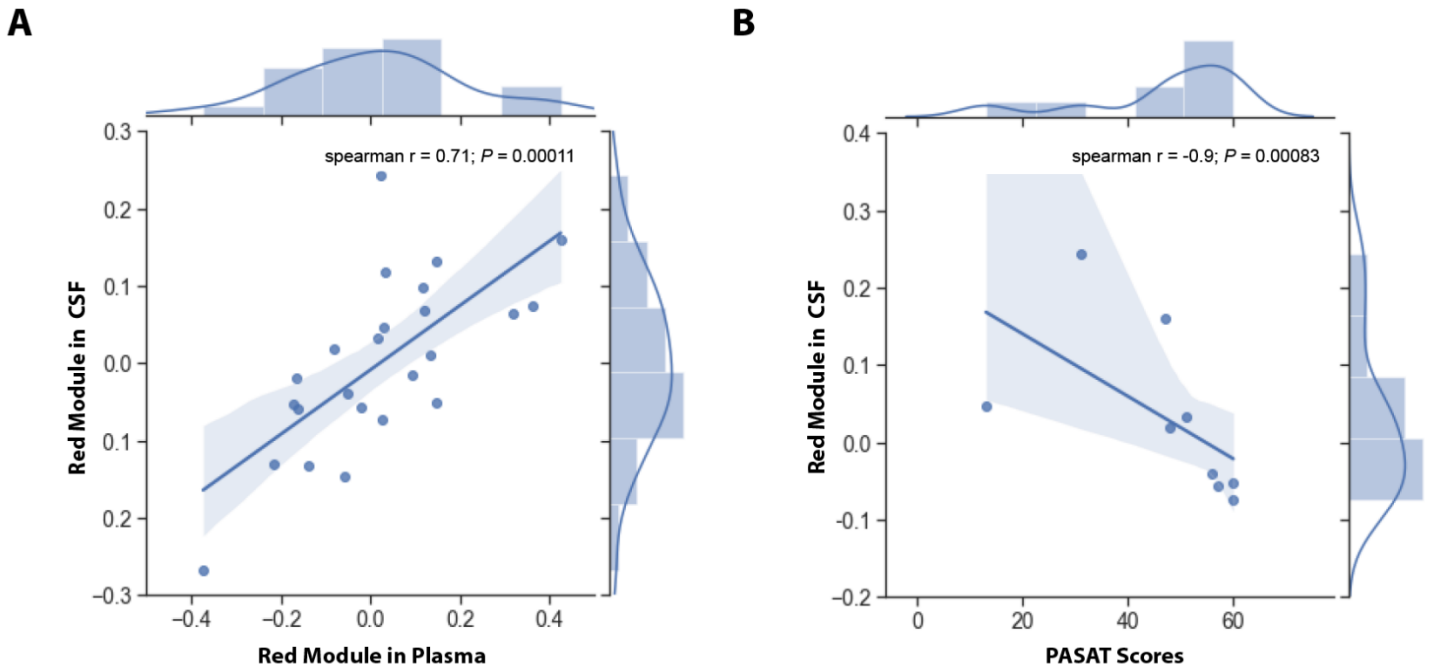

Figure S5: Clinical correlation of red module metabolites in MS patients. (A) The eigenvector of the red module metabolites in the CSF (y axis) and Plasma (x axis) is shown with a very high correlation, suggesting that these metabolites can cross the blood brain barrier. (B) The eigenvector of the red module metabolites in the CSF (y axis) significantly correlates with PASAT scores (x axis), a metric of cognitive function in multiple sclerosis patients. Spearman's correlation coefficient rho (r) and associated *P* value are indicated.