Supplementary Table 1: Multivariate regression analyses with intracellular cytokine production as dependent variables, controlling for sex and batch effects.

Supplementary Figure 1: DMF effects on absolute B-cell numbers

Absolute numbers of circulating double negative memory ($CD20^+$ $CD27^-$ IgD^-), naïve ($CD20^+$ $CD27^-$ IgD^+), class-switched memory ($CD20^+$ $CD27^+$ IgD^-) and non-class-switched memory ($CD20^+$ $CD27^+$ IgD^+) B-cells were calculated using flow cytometry (A-D). Numbers of activated ($CD80^+$) memory B-cells were also calculated (E-F). n=6-7 healthy control (71% female), 22 untreated MS (95% female), 16-17 DMF-N (71% female) and 11 DMF-L (55% female). Kruskal Wallis ANOVA with Dunn's multiple comparison test was used to compare groups. Boxplots illustrate median/interquartile range; whiskers and outliers are calculated according to Tukey's method. DMF: dimethyl fumarate; CS: class switched; nCS: non-class switched; DMF-N: non-lymphopenic DMF-treated, DMF-L: lymphopenic, DMF-treated. * p<0.05, ** p<0.01, *** p<0.001 compared to untreated MS; $^{\#}$ p<0.05, $^{\#\#}$ p<0.01, $^{\#\#\#}$ p<0.001 compared to healthy controls.

Supplementary Figure 2: DMF affects absolute numbers of functionally distinct T-cell subsets

Absolute numbers of circulating follicular T-cells ($CD4^+$ $CXCR5^+$ and $CD8^+$ $CXCR5^+$; A-B), $CD39^+$ T-regulatory cells ($CD4^+$ $CD25^+$ $Foxp3^+$, C) and mucosal invariant T-cells

(CD8$^+$ CD161$^+$; D) were calculated using flow cytometry on whole blood. n=9-10 healthy control (70% female), 21 (CD39) or 36 untreated MS (64% or 83% female) 49-50 DMF treated (70% female; 14-16 lymphopenic). Kruskal Wallis ANOVA with Dunn's multiple comparison test was used to compare groups. Boxplots illustrate median/interquartile range; whiskers and outliers are calculated according to Tukey's method. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

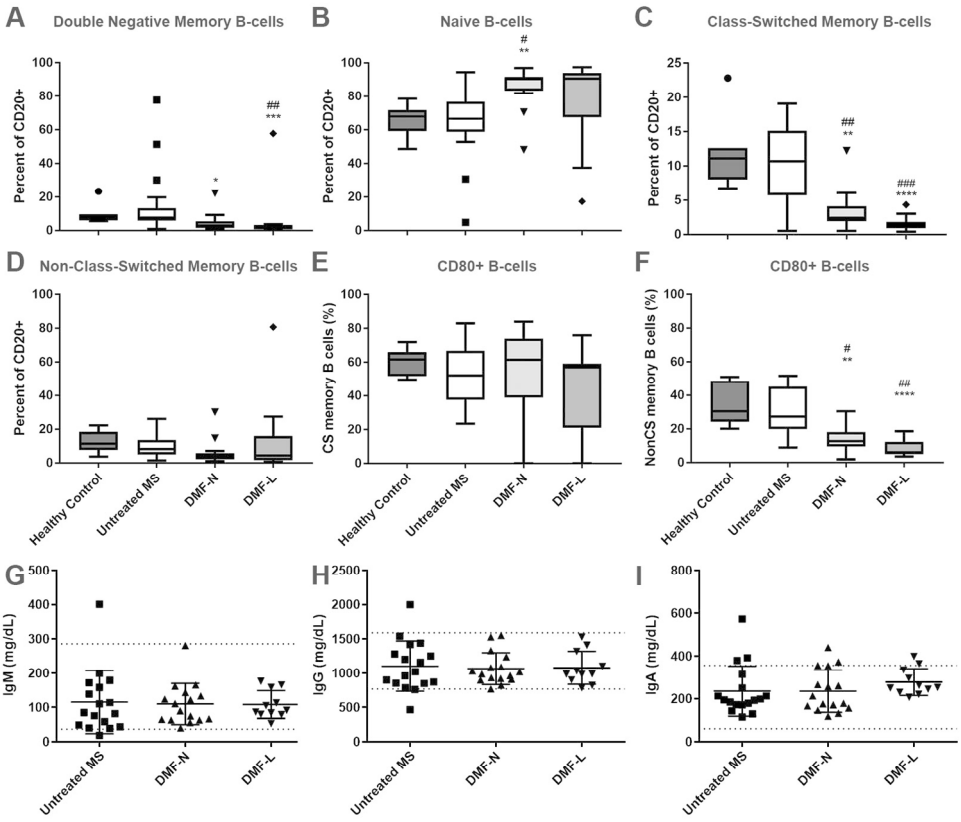

Figure 2: DMF effects on circulating B-cell phenotype and function
Double negative memory (CD20+ CD27- IgD-), naïve (CD20+ CD27- IgD+), class-switched memory (CD20+ CD27+ IgD-) and non-class-switched memory (CD20+ CD27+ IgD+) B-cells were identified using flow cytometry (A-D). The proportion of activated (CD80+) memory B-cells was also calculated (E-F). n=6-7 healthy control (71% female), 22 untreated MS (95% female), 16-17 DMF-N (71% female) and 11 DMF-L (55% female). Serum concentrations of immunoglobulins were also quantified (H-J); n=17 untreated MS, 16 DMF-N and 11 DMF-L. Kruskal Wallis ANOVA with Dunn's multiple comparison test was used to compare groups. Boxplots illustrate median/interquartile range; whiskers and outliers are calculated according to Tukey's method. Dotted lines (H-J) delineate the upper and lower limits of normal. DMF: dimethyl fumarate; CS: class switched; nCS: non-class switched; DMF-N: non-lymphopenic DMF-treated, DMF-L: lymphopenic, DMF-treated. * p<0.05, ** p<0.01, *** p<0.001 compared to untreated MS; # p<0.05, ## p<0.01, ### p<0.001 compared to healthy controls.

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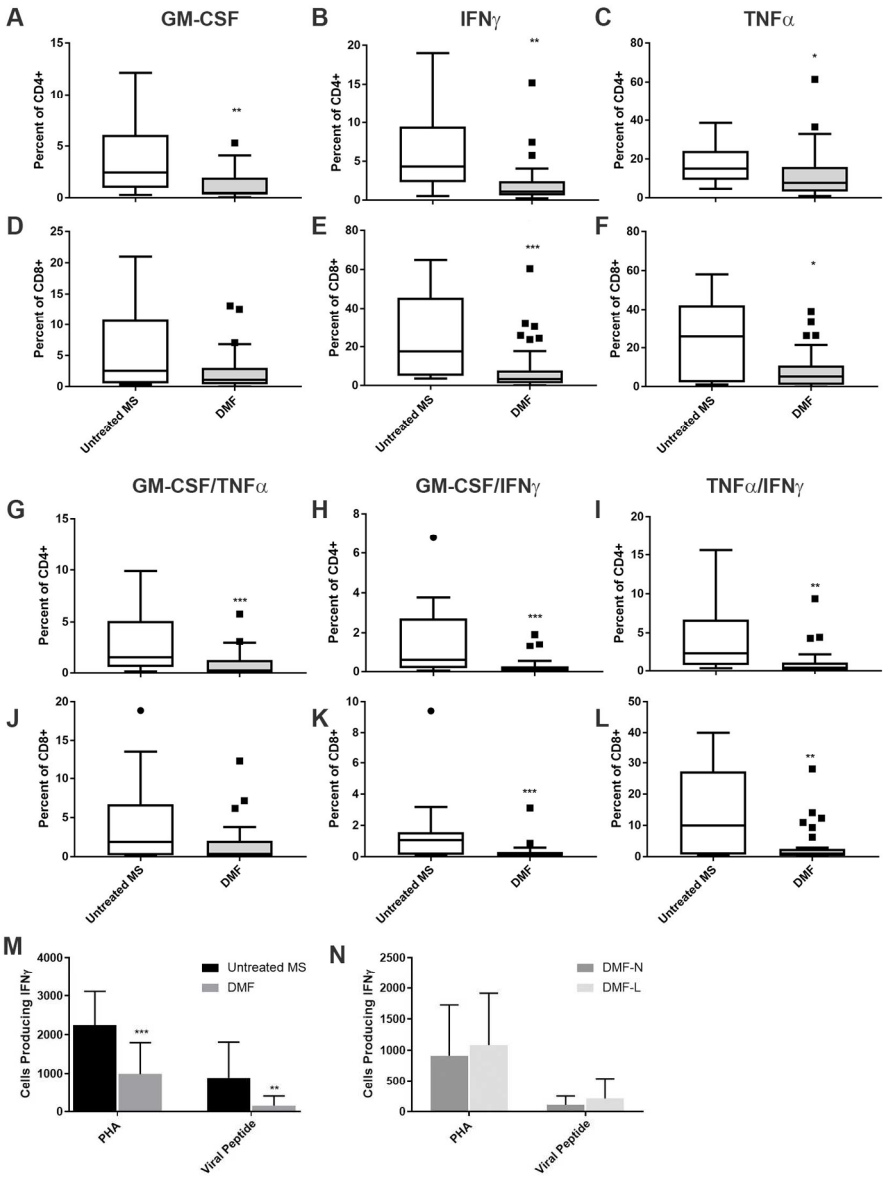

Figure 3: DMF effects on T-cell cytokine production
The proportion of CD4+ (A-C, G-I) and CD8+ (D-F, J-L) T-cells producing cytokines was evaluated using intracellular flow cytometry after exposure to antigen-independent stimulation (PMA/ionomycin). M-N: Lymphocyte production of cytokines was evaluated by ELISPOT after exposure to antigen independent (PHA) and dependent (viral peptide pool) stimuli. Numbers of cells producing IFN-γ were compared between untreated and DMF treated MS patients (M) and between non-lymphopenic and lymphopenic DMF treated MS patients (N). n=13-16 untreated MS (75% female), 29-33 DMF (14-15 lymphopenic; 70% female) for A-L. n=11 untreated MS, 22 DMF (10 lymphopenic) for M-N. Mann-Whitney U test was used to compare groups. Boxplots illustrate median/interquartile range; whiskers and outliers are calculated according to Tukey's method. DMF-N: non-lymphopenic DMF-treated, DMF-L: lymphopenic, DMF-treated, PMA: phorbol 12-myristate 13-acetate, PHA: phytohemagglutinin. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

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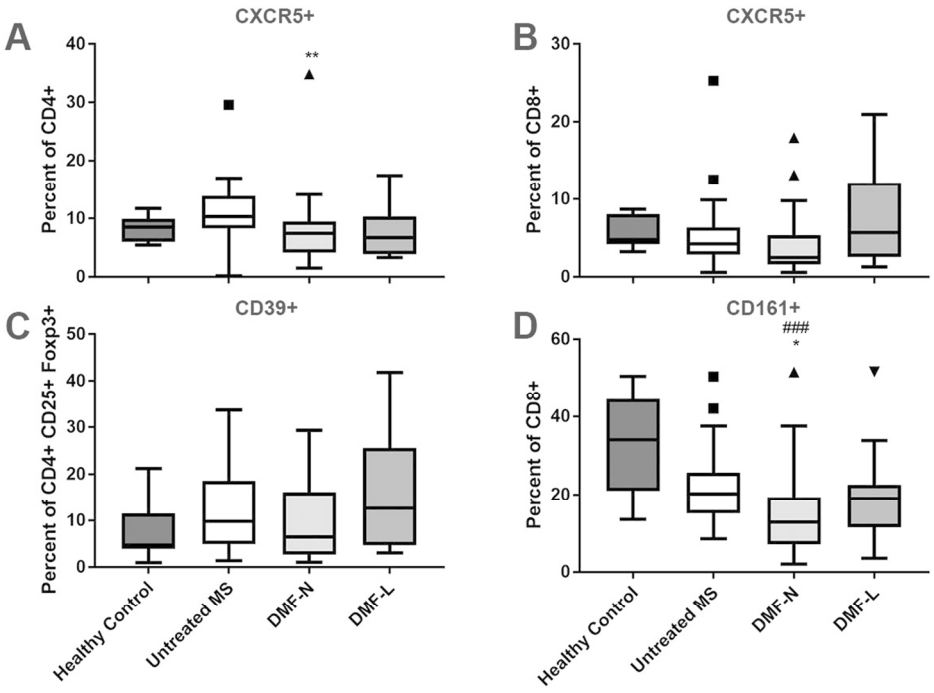

Figure 4: DMF effects on functionally distinct T-cell subsets
Proportions of circulating follicular T-cells (CD4+ CXCR5+ and CD8+ CXCR5+; A-B), CD39+ T-regulatory cells (CD4+ CD25+ Foxp3+, C) and mucosal invariant T-cells (CD8+ CD161+; D) were calculated using flow cytometry. n=9-10 healthy control (70% female), 21 (CD39) or 36 untreated MS (64% or 83% female) 49-50 DMF treated (70% female; 14-16 lymphopenic). Kruskal Wallis ANOVA with Dunn's multiple comparison test was used to compare groups. Boxplots illustrate median/interquartile range; whiskers and outliers are calculated according to Tukey's method. DMF-N: non-lymphopenic DMF-treated, DMF-L: lymphopenic, DMF-treated. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

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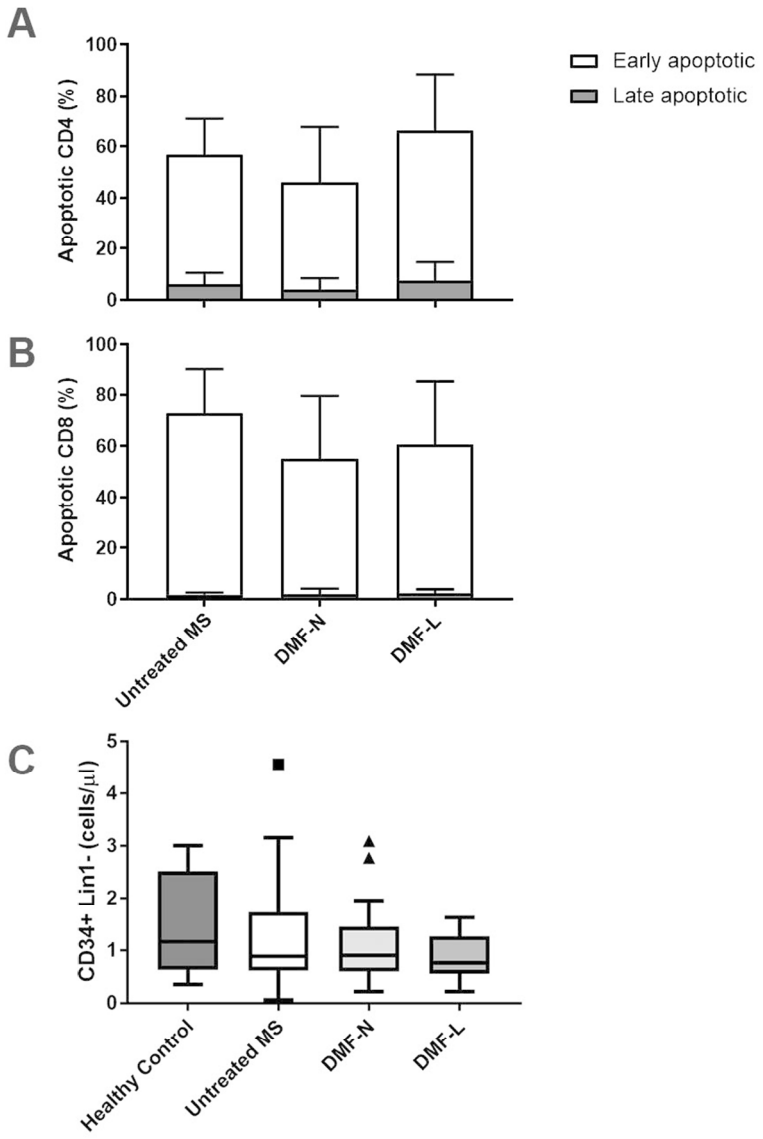

Figure 5: No significant effects of DMF on activation induced cell death or circulating hematopoietic progenitor cells

PBMC were stimulated with anti-CD3/anti-CD28 and stained for Annexin V and propidium iodide (PI) to quantify activation induced cell death (A-B). Annexin V-PE+/PI- and Annexin V-PE+/PI+ populations were considered early and late apoptotic cells, respectively. Circulating hematopoietic progenitor cells (CD34+ Lin1-) were identified using flow cytometry (C). DMF-N: non-lymphopenic DMF-treated, DMF-L: lymphopenic, DMF-treated. n=13 untreated MS (77% female), 15 DMF-N (87% female), 14 DMF-L (50% female) for activation induced cell death (A-B). n=12 healthy controls, 20 untreated MS, 26 DMF-N, and 7 DMF-L for circulating progenitors.

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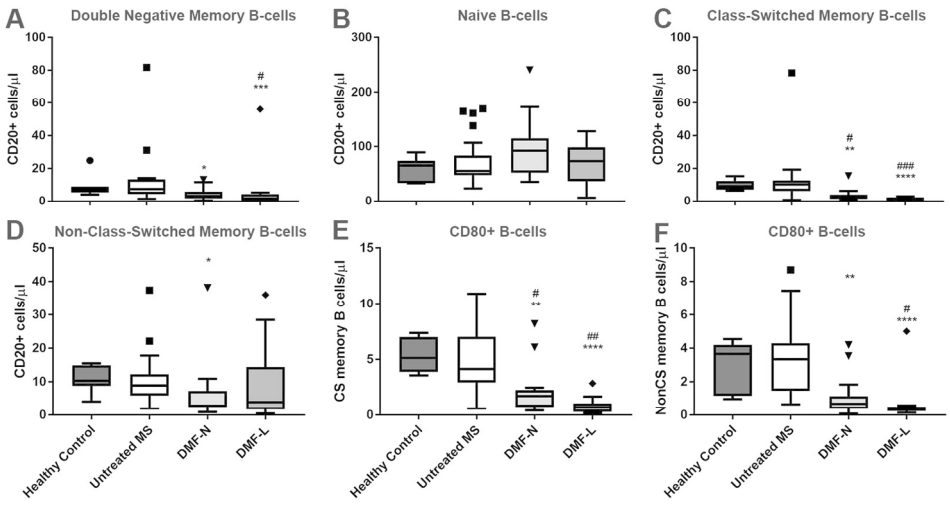
Dependent Variables	*N*	*SE of estimate*	R^2	*B for DMF*	*B for sex*	*B for batch*
CD4						
IL-10	42	0.373	0.262	-0.279*	0.181	-0.144*
IL-4	42	2.100	0.032	0.410	-0.221	-0.282
GM-CSF	42	2.442	0.249	-2.875**	-0.008	-0.149
TNF	42	12.611	0.036	-4.774	0.085	0.656
IFN	42	4.208	0.205	-4.141**	-1.179	0.087
IL-17	42	0.343	0.091	-0.211	-0.010	-0.029
GM-CSF/TNF	42	2.072	0.216	-2.231**	-0.020	0.076
GM-CSF/IFN	42	1.187	0.223	-1.287**	-0.128	-0.037
IFN/TNF	42	3.280	0.198	-3.078**	-0.842	0.065
CD8						
IL-10	49	0.457	0.183	0.170	0.291	-0.097
IL-4	49	2.822	0.223	-0.074	2.716**	-0.579
GM-CSF	49	4.459	0.243	-3.300*	4.313**	0.026
TNF	49	14.213	0.304	-16.442***	4.698	2.875
IFN	49	16.004	0.220	-14.602**	-0.681	2.931
IL-17	49	0.363	0.108	0.120	0.142	-0.069
GM-CSF/TNF	49	3.705	0.252	-2.982*	3.470**	0.154
GM-CSF/IFN	49	1.306	0.258	-1.221**	0.541	0.280*
IFN/TNF	49	9.004	0.279	-9.878**	-1.028	1.737

* $p<0.05$; ** $p<0.01$; *** $p<0.001$



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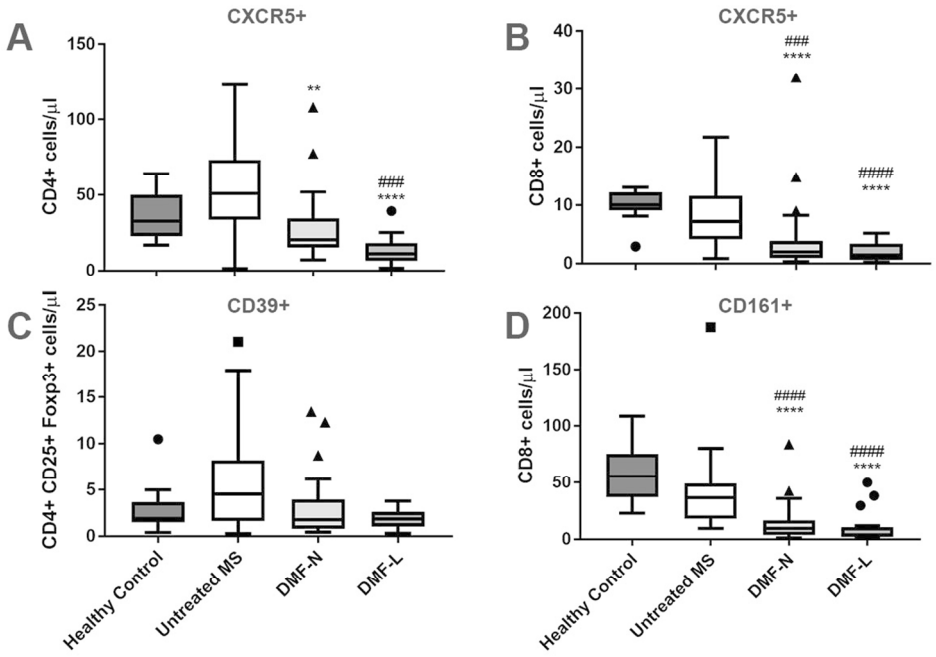

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