Supplementary data

Table 1 Antibodies used in this study

Vender	Conjugated Antibodies	Clone
Tonbo Biosciences	CD3 PE-Cy7	UCHT1
(San Diego, CA.	CD4 PE	OKT4
USA)	CD8 APC	OKT8
	CD19 PE-Cy7	HIB19
Biolegend	CD45RA APC-Cy7	H100
(San Diego, CA	CD45RO Pacific Blue	UCHL1
USA)	CCR7 APC	G043H7
	CXCR3 Brilliant Violet 421™	G025H7
	CD161-FITC	HP-3G10
	CCR6 Per CP-Cy5.5	G034E37
	CD25 Alexa Fluor® 488	BC96
	CD56 Brilliant Violet 421™	HCD56
	CD69 Brilliant Violet 510™	FN30
	CD4Brilliant Violet 510™	OKT4
	CD4 Pacific Blue	OKT4
	IL-4 APC	8D4-8
BD Biosciences	CD14 APC-Cy7	MϕP9
(San Diego, CA.	$TCR_{\alpha\beta}$ FITC	T10B9.1A-31
USA)	CCR3 Horizon™ BV510	5E8
	CCR6 PE	11A9 (RUO)
eBiosciences	Fox P3 PE	PCH101
(San Diego, CA.	Ki67 eFluor® 450	20Raj1
USA)	IL-17A PE	SHLR17
	IL-17F PE	eBio64CAP17
Thermo Fisher/Invitrogen	IFNγ FITC	B27
(Waltham, MA. USA)		

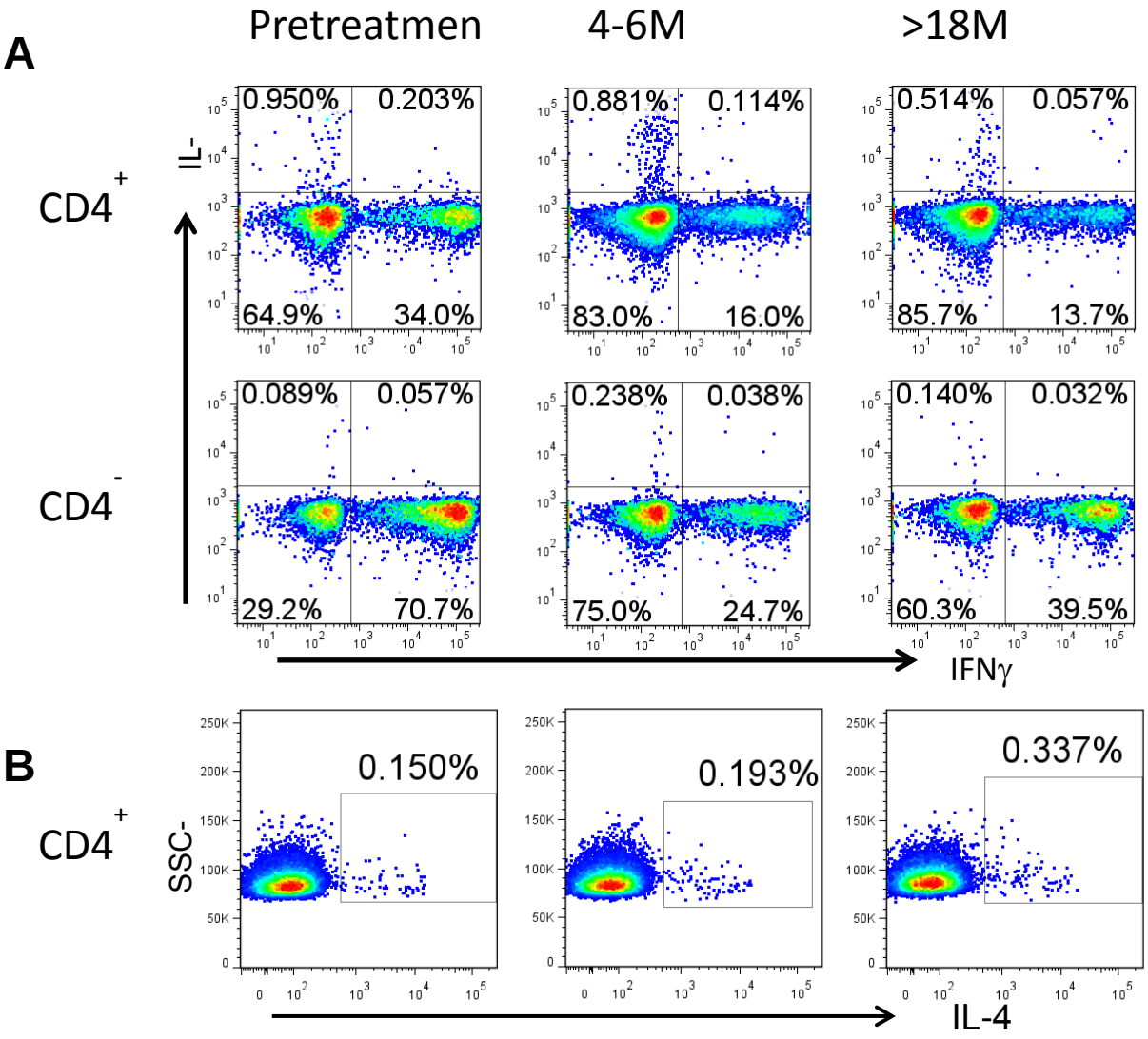

Figure 1: DMF effect on ex vivo IFNγ , IL-17 and IL-4 production by T cells (A) Representative FACS profile of IFNγ and IL-17 (IL-17A and IL-17F) expression on 6hr PMA/ ionomycin-activated $CD4^+$ (upper) and $CD4^-$ (lower) T cells from patients before (left hand), 6 month (middle) or 24 month after DMF treatment (right hand). **(B)** Representative FACS profile of IL-4 expression on ex vivo $CD4^+$ T cells from a MS patient before (left hand), 6 month (middle) and 26 month after DMF treatment (right hand).

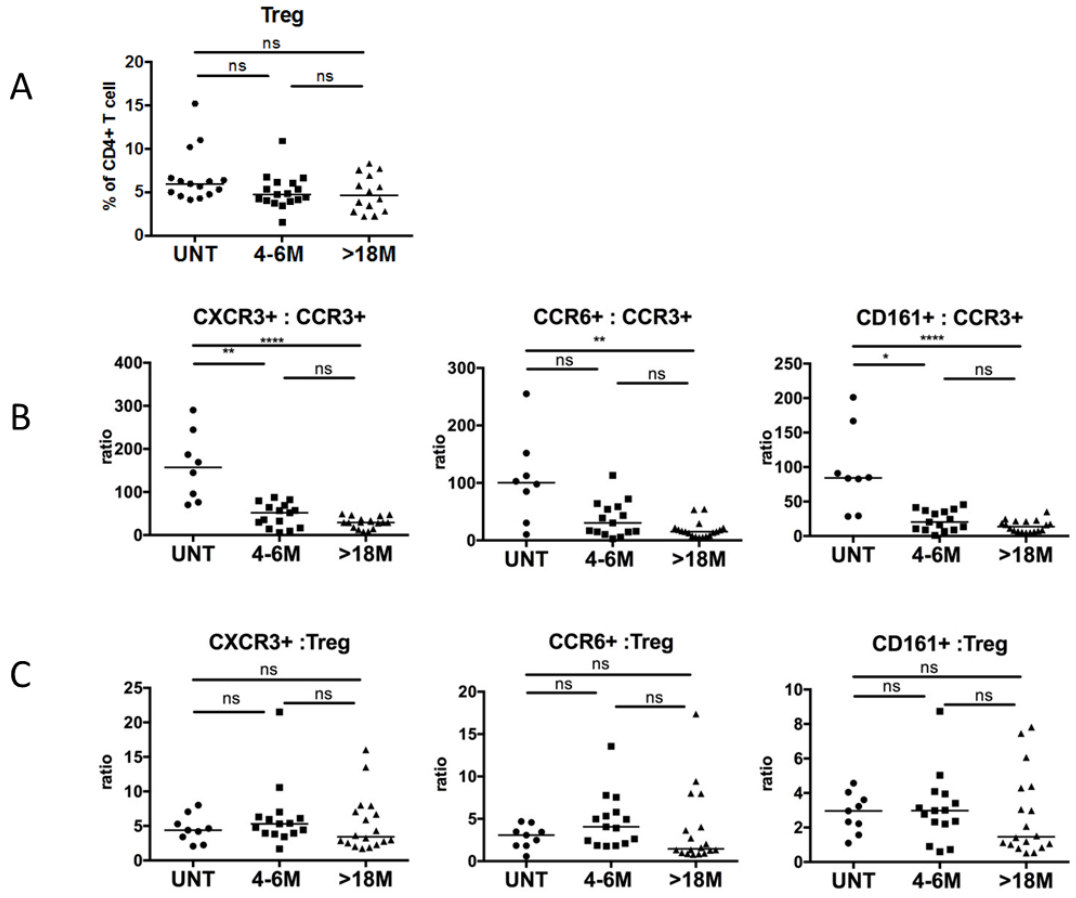

Figure 2: DMF effect on Th and Treg subsets and ratios. (A) DMF effect on Treg PBMC derived from untreated (UNT), 4-6 month DMF-treated (4-6M) and 18-26M DMF-treated (>18M) RRMS patients were stained with fluorochrome-conjugated antibodies against CD3, CD4, and CD25 followed by intracellular staining of flourochrome-conjugated anti-human FOX P3 and analyzed by flowcytometry. The Treg ($CD25^+FoxP3^+$) of total $CD4^+$ T cells were analyzed at three time point (UNT: n=15, 4-6M: n=17, >18M: n=14). **(B)** DMF effect on Th1/Th17:Th2 ratios: PBMC derived from untreated (UNT), 4-6 month DMF-treated (4-6M) and 18-26M DMF-treated (>18M) RRMS patients were stained with fluorochrome-conjugated antibodies against CD3, CD4, CXCR3, CD161, CCR6 and CCR3 and analyzed by flow cytometry. Th1:Th2 ratio was calculated using $CXCR3^+$% divided by $CCR3^+$% in CD4+ T cells (left panel,); Th17: Th2 ratio was calculated using $CCR6^+$% divided by $CCR3^+$% in CD4+ T cells (middle panel). The ratio of CD161+:Th2 was also calculated (right panel). Each dot represents the ratio from an individual patient. Upper panel is the cross-sectional analysis (UNT: n=8; 4-6M: n=15 and >18M: n=18). **(C)** DMF effect on Th1/Th17 : Treg cells ratio Th1:Treg ratio was calculated using $CXCR3^+$% divided by $CD25^+FoxP3^+$ Treg% in $CD4^+$ T cells (left lower panel); Th17: Treg ratio was calculated using CCR6+% divided by Treg% in $CD4^+$ T cells (middle lower panel). The ratio of $CD161^+$:Treg was also calculated (right lower panel). Each dot represents the ratio from an individual patient. Upper panel is the cross-sectional analysis (UNT: n=9; 4-6M: n=15 and >18M: n=18). *P*-values from Kruskal-Wallis ANOVA with Dunn's multiple comparison tests are shown above the data for each cell type group. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; ns, not significant.

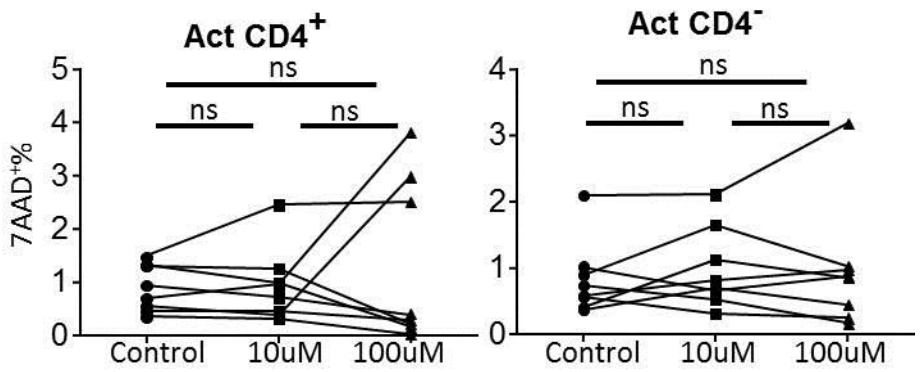

Figure 3: Cell Death of activated T cells *in vitro* PBMCs from 8 healthy donors were activated by anti-plate- coated anti-CD3 and soluble anti-CD28 in complete RPMI with or without 10μM or 100μM of DMF for 48 hours. Cells were harvested, washed and surface stained with fluorochrome-conjugated antibodies against CD3, CD8, CD4, as well as 7AAD and Annexin V and analyzed by flow cytometry. Large (FSC-A^{hi}) $CD3^+$ T cell blasts were gated as activated T cells. The percentage of dead cells measured by positive staining of 7AAD in activated $CD4^+$ T cells (left panel) and $CD4^-$ T cells (right panel) populations.