Supplementary Material

Dimethyl fumarate induces a persistent change in the composition of the innate and adaptive immune system in multiple sclerosis patients

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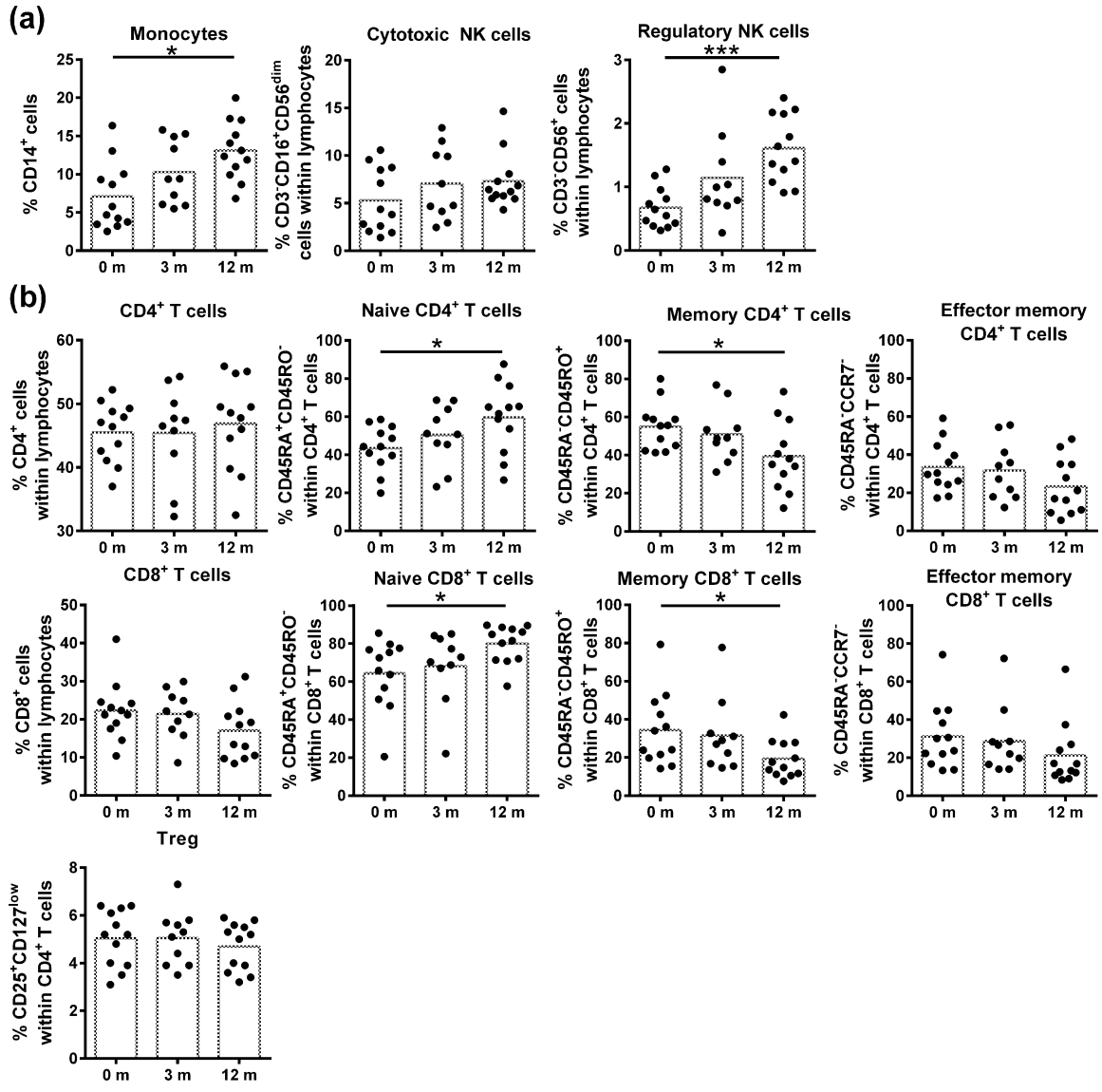
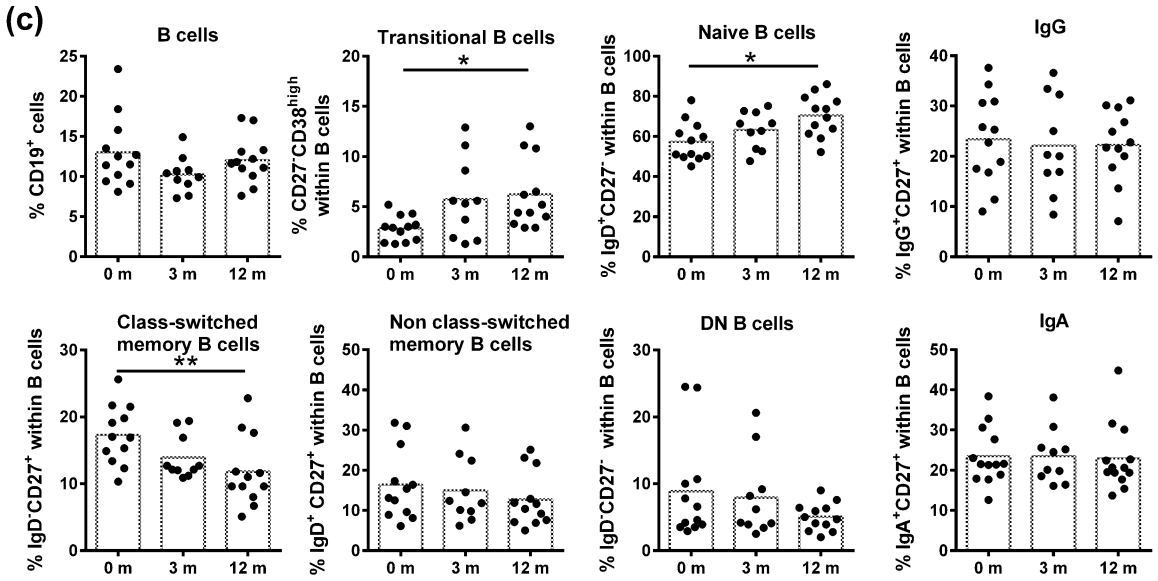
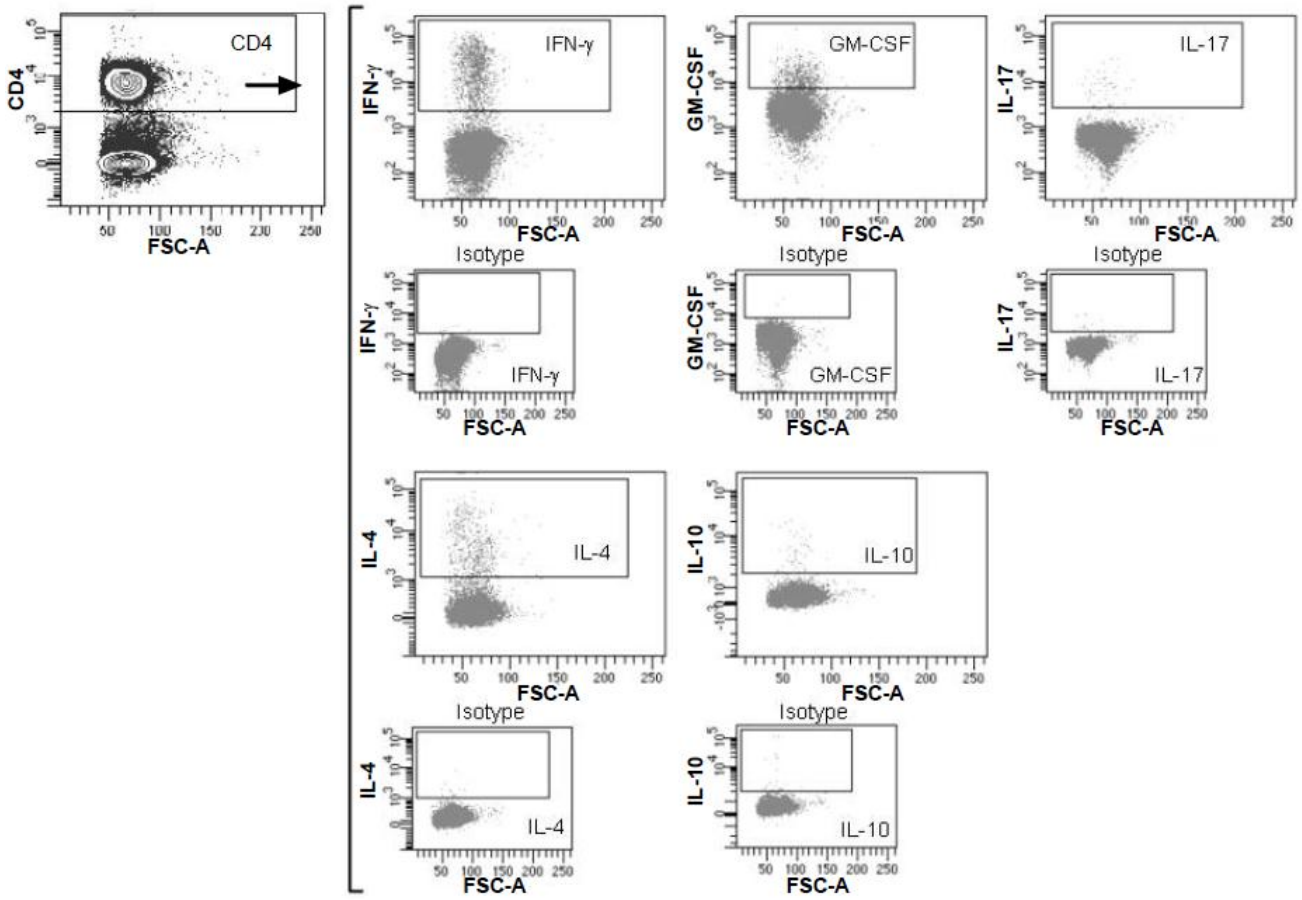


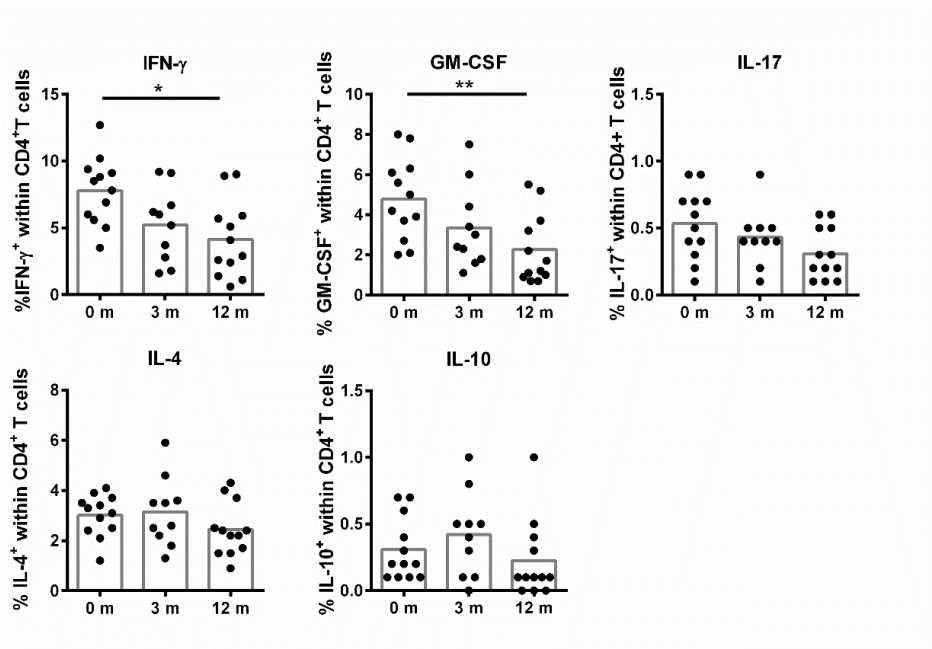


Supplementary Figure 1. DMF treatment induced redistribution of innate and adaptive immune cell subtypes after 12 m of treatment in RRMS patients, while no significant changes were detected at 3 m of DMF treatment. (a) Frequency of monocytes, NK cells, **(b)** T cell subtypes and **(c)** B cell subtypes at baseline (n = 12), after 3 m (n = 10) and after 12 m (n = 12) of DMF treatment in RRMS patients. Each dot represents an individual patient and average values are depicted as histograms. A Kruskal-Wallis one-way ANOVA test was used to determine p values. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. NK = natural killer, Treg = regulatory T cells, DN = double negative, m = months.

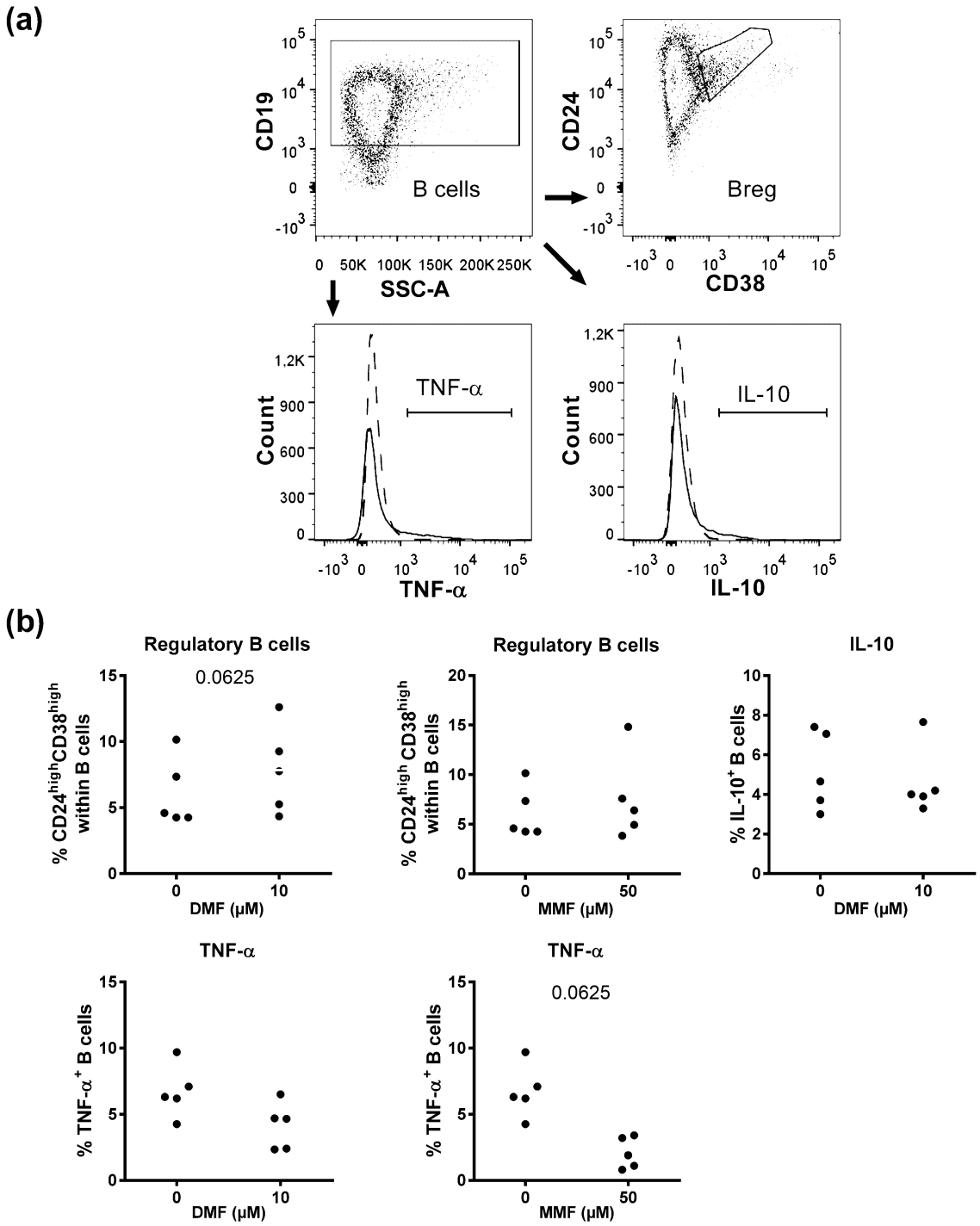
(a)


CD4
FSC-A
IFN-γ
GM-CSF
IL-17
Isotype
IL-4
IL-10

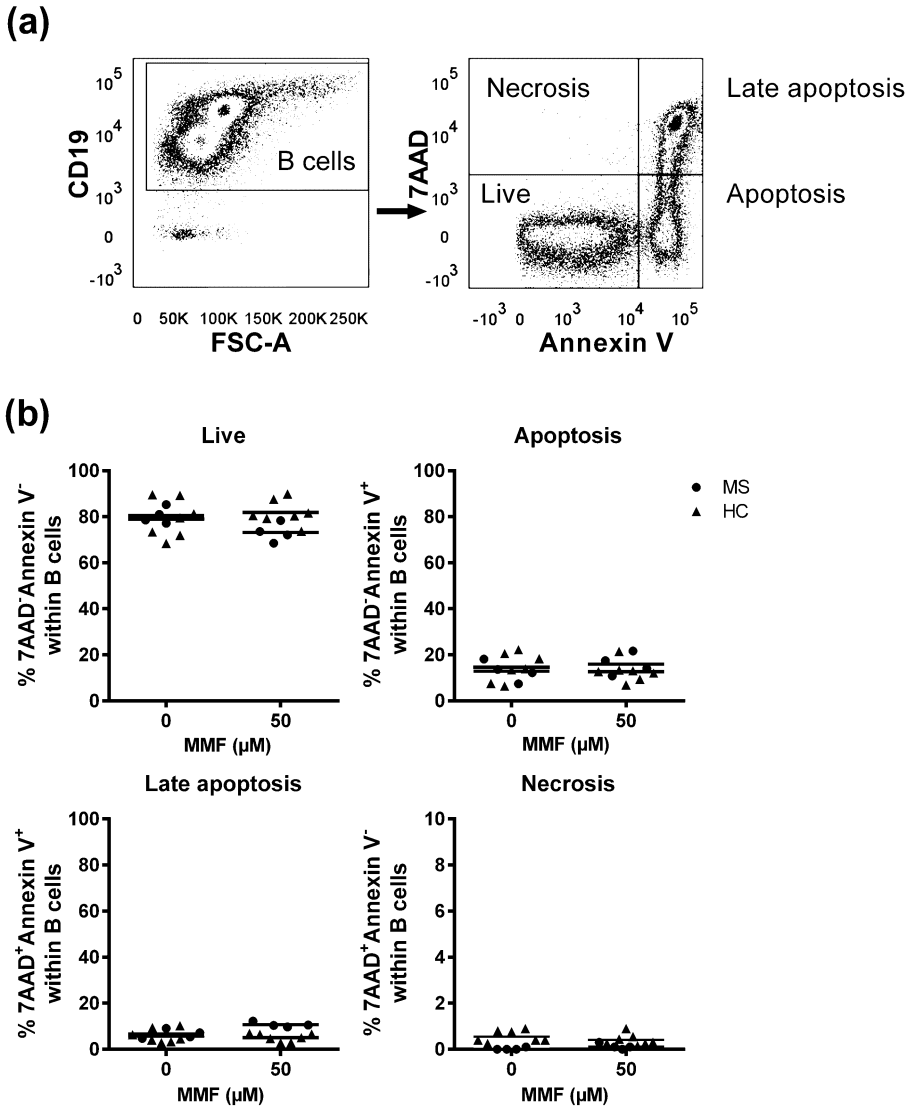
(b)


IFN-γ
GM-CSF
IL-17
IL-4
IL-10
%IFN-γ+ within CD4+T cells
% GM-CSF+ within CD4+ T cells
% IL-17+ within CD4+ T cells
% IL-4+ within CD4+ T cells
% IL-10+ within CD4+ T cells
0 m
3 m
12 m
*
**

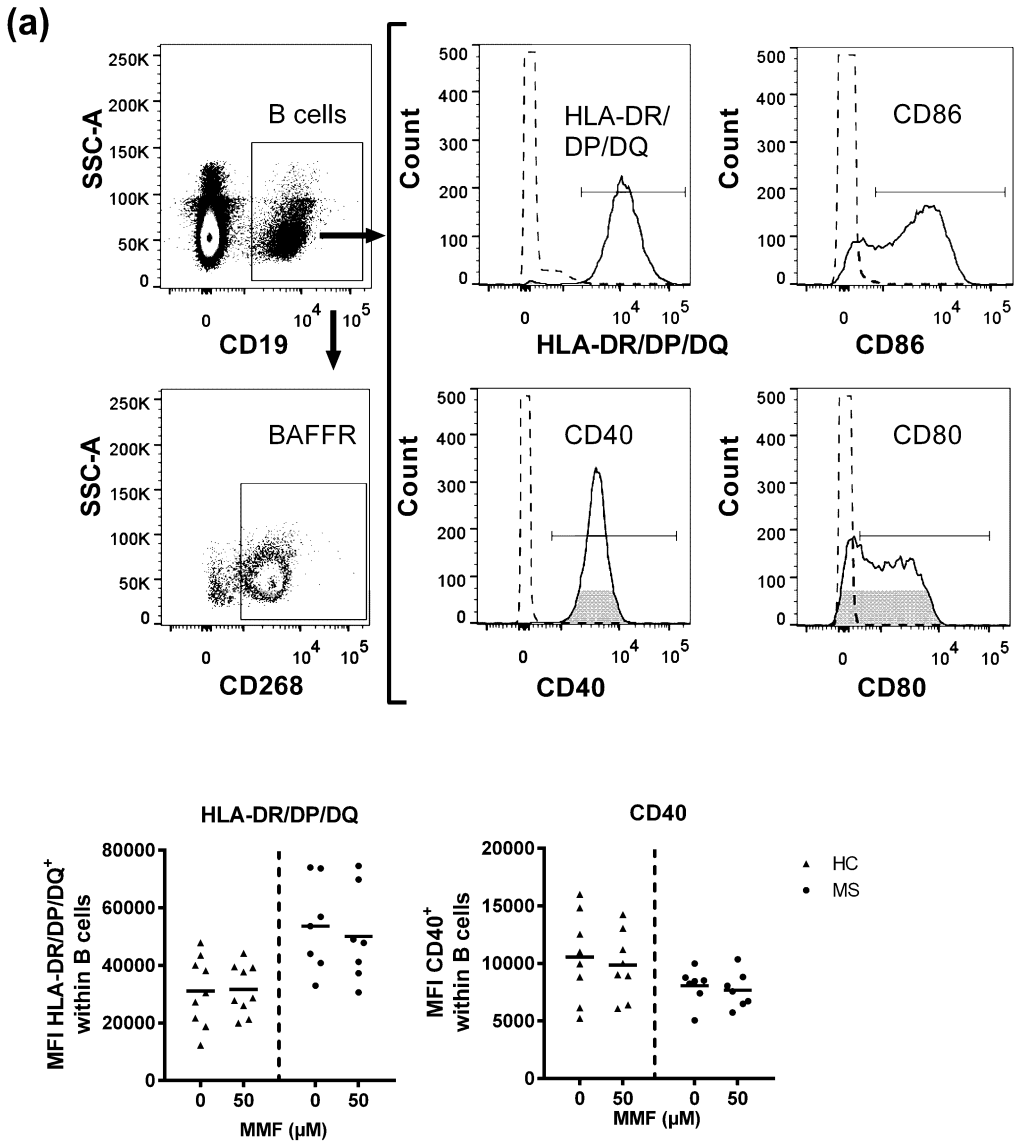
Supplementary Figure 2. DMF treatment reduced frequency of T cells expressing pro-inflammatory cytokines after 12 m, but not after 3 m of treatment. (a) A representative analysis of cytokine-expressing T cell subtypes is shown. $CD4^+$ T cells were gated from lymphocytes. IFN-γ^+, GM-CSF^+, IL-17^+, IL-10^+ and IL-4^+ cells were gated from the $CD4^+$ cell population based on isotype controls **(b)** Frequency of $CD4^+$ T cells expressing pro-inflammatory cytokines (IFN-γ, GM-CSF, IL-17) and anti-inflammatory cytokines (IL-4, IL-10) in RRMS patients at baseline (n = 12), after 3 m (n = 10) and after 12 m (n = 12) of DMF treatment. Each dot represents an individual patient and average values are depicted as histograms. A Kruskal-Wallis one-way ANOVA test was used to determine p values. * $p < 0.05$, ** $p < 0.01$. IFN-γ = interferon-γ, GM-CSF = granulocyte macrophage colony-stimulating factor, IL-17 = interleukin-17, IL-4 = interleukin-4, IL-10 = interleukin-10, m = months.



Supplementary Figure 3. A trend towards an increase in Breg is induced by DMF while MMF tends to decrease TNF-α$^+$ B cells in a direct manner. Purified B cells of untreated RRMS patients were treated *in vitro* with 10 µM DMF, 50 µM MMF or were left untreated (n = 5). (a) A representative analysis of Breg and B cell cytokines is shown. Breg ($CD24^{high}CD38^{high}$), IL-10^+ and TNF-α^+ cells were gated from the B cell population based on unstimulated controls (dashed lines). (b) Frequencies of Breg ($CD24^{high}CD38^{high}$), IL-10^+ B cells and TNF-α^+ B cells are depicted. Wilcoxon matched-pairs signed rank test was used to determine p values. DMF = dimethyl fumarate, MMF = monomethyl fumarate, TNF = tumor necrosis factor.



Supplementary Figure 4. MMF has no effect on apoptosis of B cells. Purified B cells of HC and untreated RRMS patients were treated *in vitro* with 50 µM MMF or left untreated (HC: n = 7, MS: n = 4). (a) A representative analysis of apoptosis of B cells is shown. Live (Annexin V^-7-AAD^-), apoptotic (Annexin V^+7-AAD^-), late apoptotic (Annexin V^+7-AAD^+) and necrotic (Annexin V^-7-AAD^+) cells were gated from the B cell population. (b) Frequency of live B cells (Annexin V^-7-AAD^-), apoptotic B cells (Annexin V^+7-AAD^-), late apoptotic B cells (Annexin V^+7-AAD^+) and necrotic B cells (Annexin V^-7-AAD^+) of HC and MS patients. Wilcoxon matched-pairs signed rank test was used. HC = healthy control, MS = multiple sclerosis, MMF = monomethyl fumarate.



Supplementary Figure 5. MMF has no effect on the expression of antigen presentation molecules and costimulatory molecule CD40 of B cells. Purified B cells of HC and untreated RRMS patients were treated *in vitro* with 50 µM MMF or were left untreated. (a) A representative analysis of functional molecules expressed by B cells is shown. Survival marker BAFFR, antigen presentation molecule HLA-DR/DP/DQ and costimulation molecules CD40, CD80 and CD86 were gated from the B cell population based on isotypes controls (dashed lines). (b) Expression (MFI) of HLA-DR/DP/DQ (HC: n = 9, MS: n = 7), CD40 (HC: n = 8, MS: n = 7) on B cells is depicted for HC and MS patients. A Wilcoxon matched-pairs signed rank test was used to determine p values between two groups. HLA-DR/DP/DQ = human leukocyte antigen, HC = healthy control, MS = multiple sclerosis, MMF = monomethyl fumarate, MFI = mean fluorescence intensity.