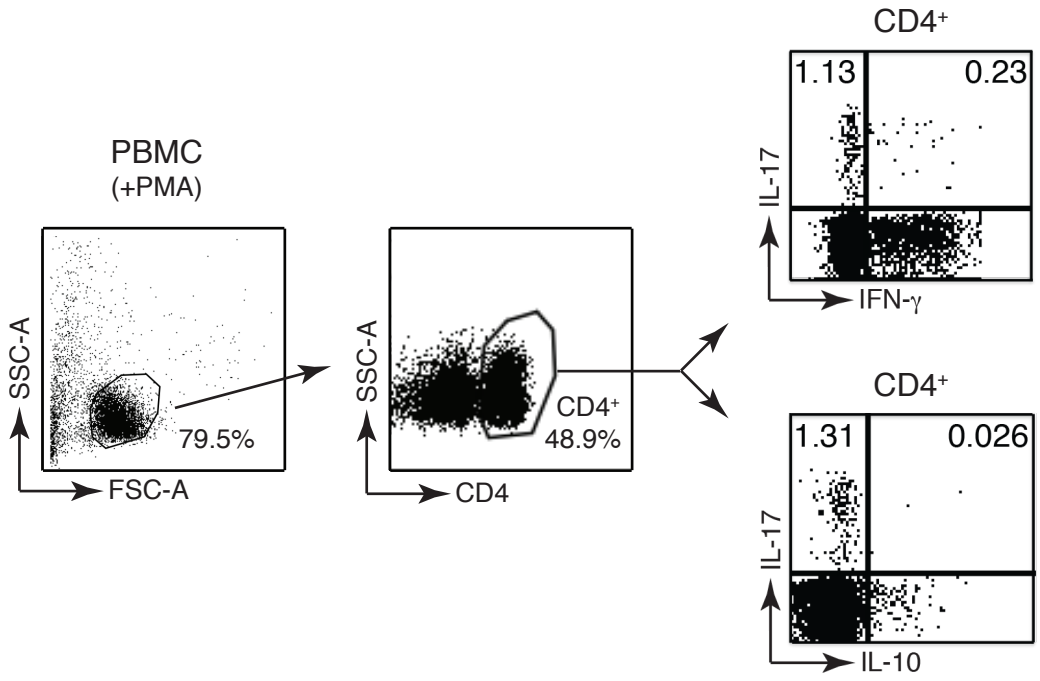
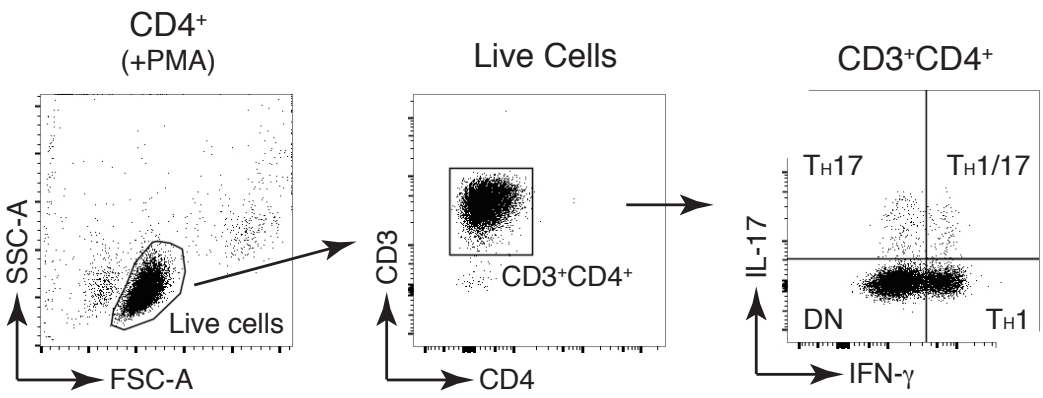


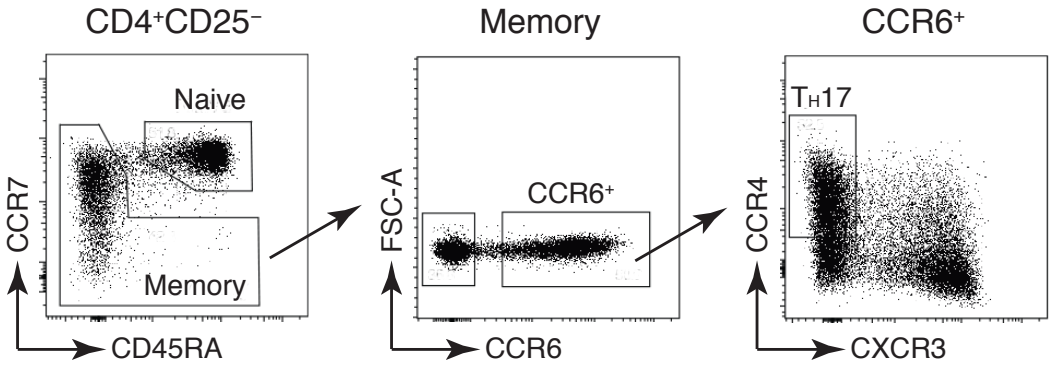
Supplementary Figure 1 Gating Strategy to determine the production of IFN-γ and IL-10 in human T_H17 cells

Isolated PBMCs were stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin for 4 hr. Production of indicated cytokines in $CD4^+$ T cells were assessed by flow cytometry with intracellular cytokine staining assay.



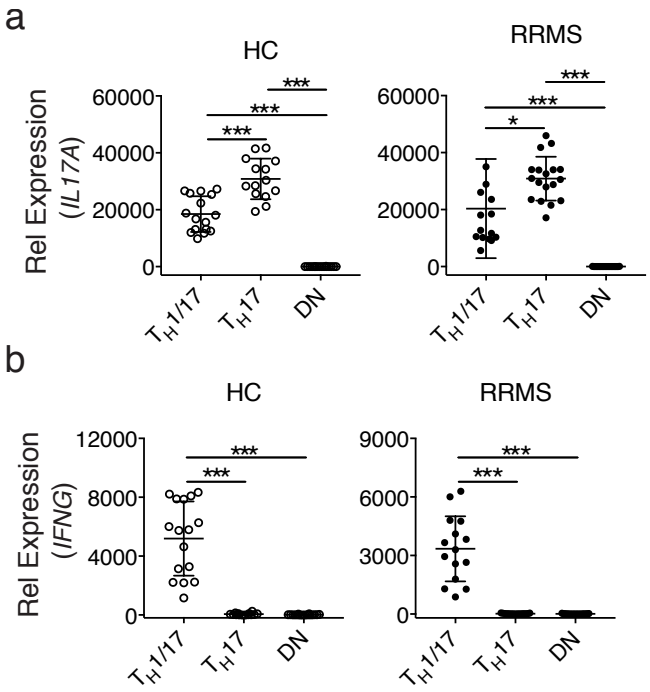
Supplementary Figure 2 Gating strategy to isolate and determine frequency of $CD4^+$ T cell subsets in total $CD4^+$ T cells in multiple sclerosis and healthy controls

$CD4^+$ T cells isolated from the peripheral blood of healthy controls (HC) and untreated patients with relapse-remitting multiple sclerosis (MS) were stimulated with PMA and ionomycin for 3-4 hr (3 hr for fresh blood samples and 4 hr for frozen PBMCs, respectively). Stimulated cells were stained with fluorescence-conjugated anti-CD3 and CD4 in combination with IFN-γ and IL-17 cytokine secretion detection kits (Miltenyi Biotec). $T_H1/17$, T_H17, T_H1 and DN cells were sorted with a FACSAria (BD Biosciences).



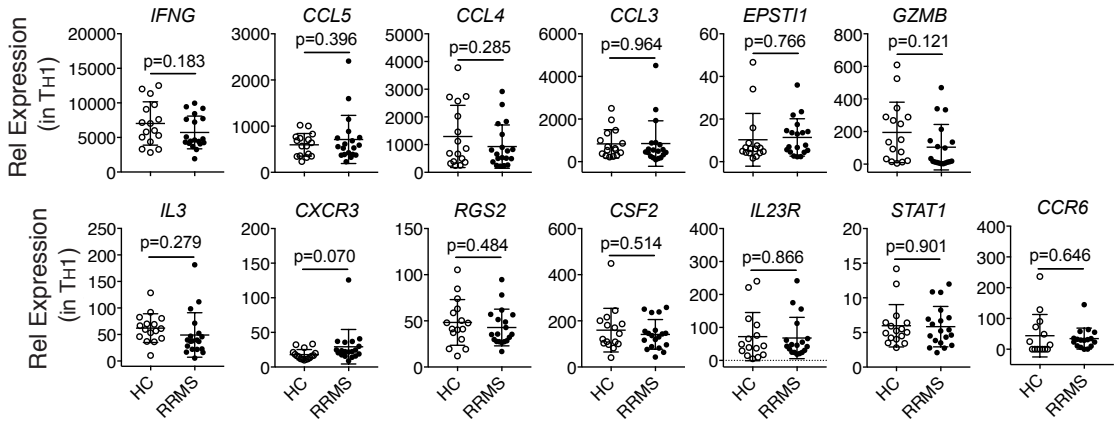
Supplementary Figure 3 Gating strategy to isolate CCR6$^+$CCR4$^+$CXCR3$^-$ memory CD4$^+$ T cells for establishing T_H17 clones

CD4$^+$ T cells isolated from PBMC were stained with anti-CD4, CD25, CCR4, CCR6, CCR7 and CXCR3 and sorted with a FACSAria (BD Biosciences) for CCR6$^+$CCR4$^+$CXCR3$^-$CD45RA$^-$CD25$^-$ memory CD4$^+$ T cells (enriched in T_H17 cells).



Supplementary Figure 4 *q*PCR analysis of *IL17A* and *IFNG* expression in $T_H1/17$, T_H17 and DN cells in MS and HC

RNA isolated from sorted $T_H1/17$, T_H17 and DN cells from the PBMC of untreated patients with relapse-remitting MS (n=19) and age- and sex-matched healthy controls (HC) (n=16) as described in Figure 5 was subjected to low-input *q*PCR analysis: a) *IL17A* and b) *IFNG*. *$p< 0.05$, ***$p<0.0001$, One-way ANOVA with Tukey's multiple comparison test (mean±s.d.).



Supplementary Figure 5 Expression of the robust PreP-signature genes of human $T_H1/17$ cells in T_H1 cells in MS and HC

RNA isolated from sorted T_H1 cells from the PBMC of untreated patients with relapse-remitting MS (n=19) and age- and sex-matched healthy controls (HC) (n=16) as described in Figure 5 was subjected to low-input *q*PCR analysis. Welch's t test *p*-values were shown (mean±s.d.).