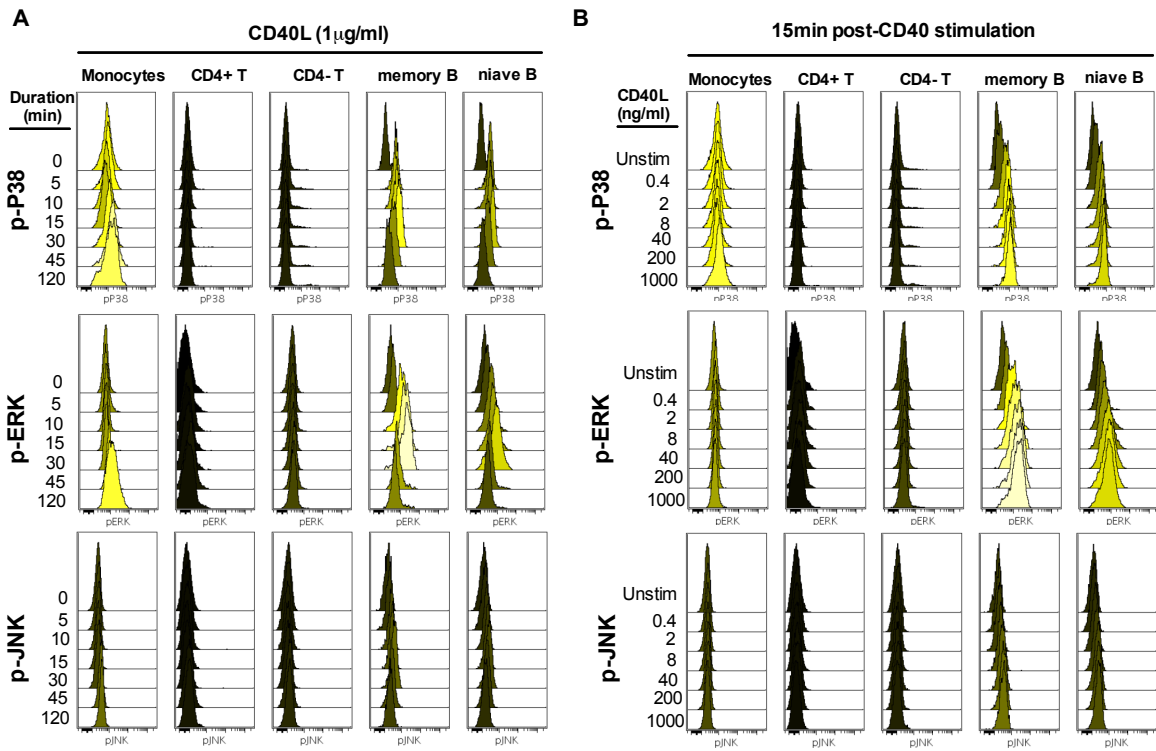
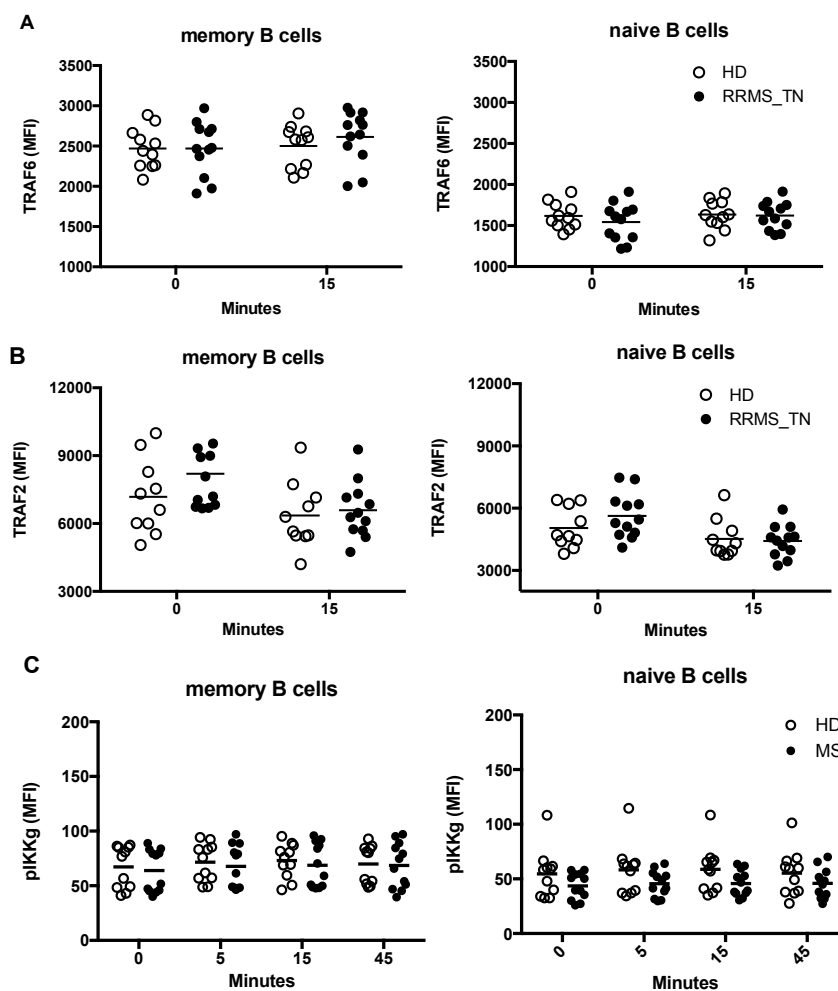


Supplemental Figure 1



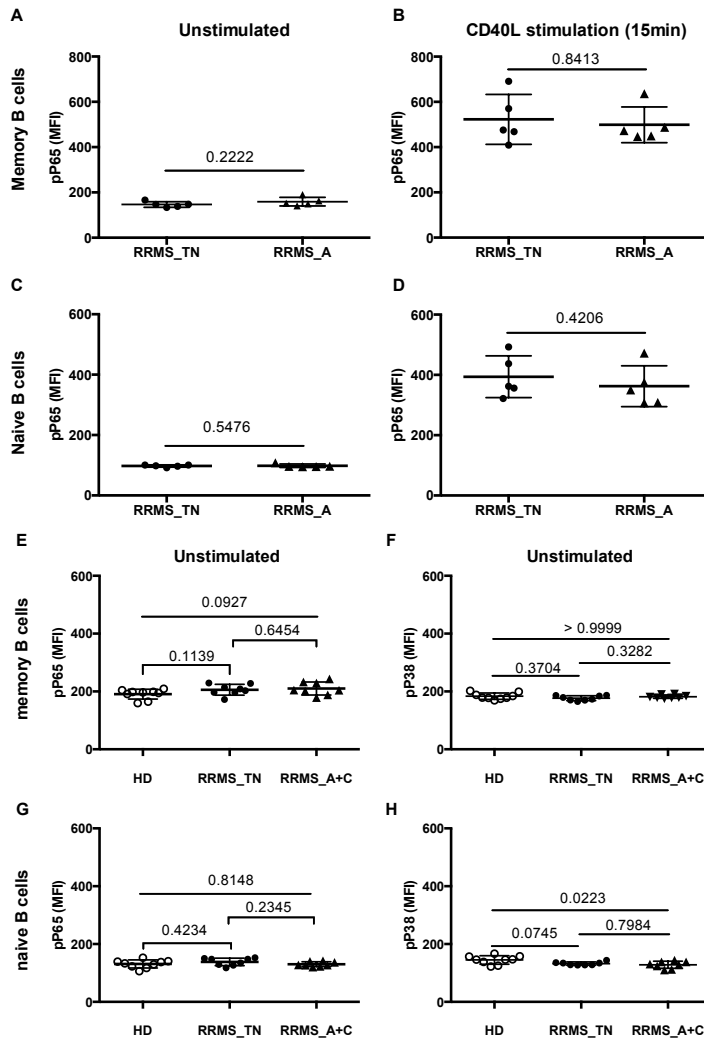
Supplemental Figure 1. Phosphorylation of P38, ERK and JNK in PBMCs in response to CD40 stimulation. The experiment was carried out as described in Figure 1 and data shown are phosphorylation of P38, ERK and JNK in indicated cell populations. Data are representative of 9 healthy individuals.

Supplemental Figure 2



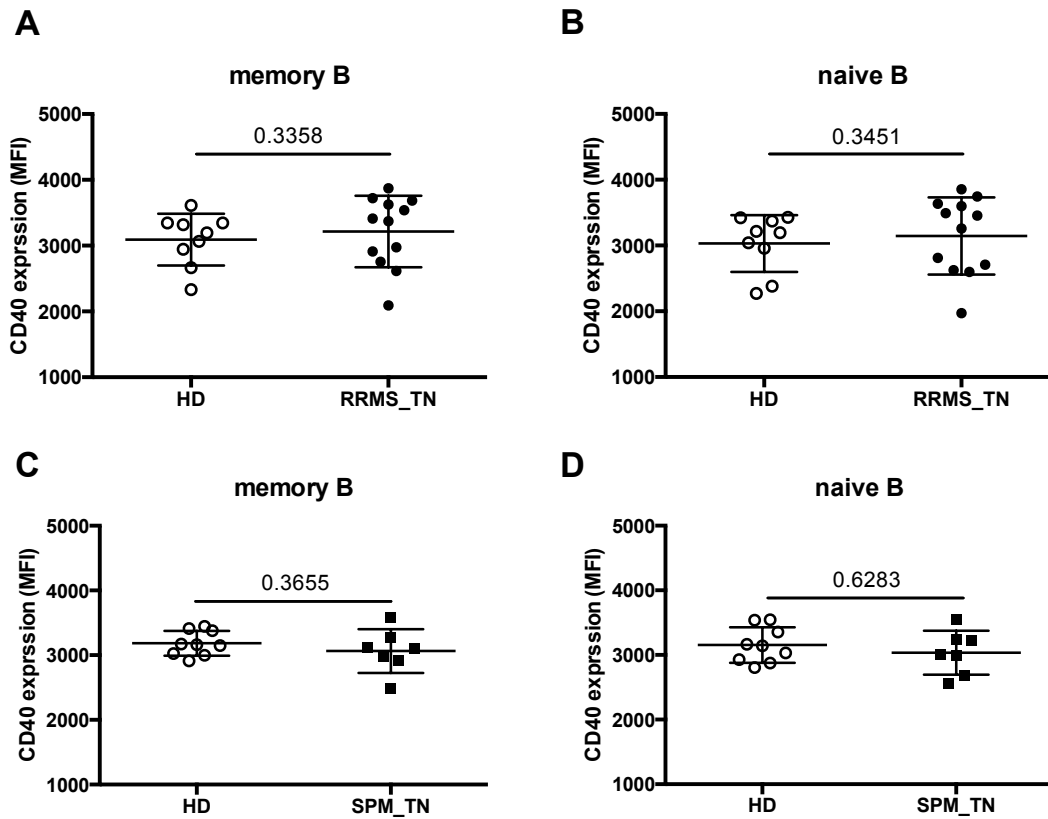
Supplemental Figure 2. Expression levels of TRAF2, TRAF6 and phosphorylated IKK γ in B cells were similar between HDs and MS patients. PBMCs from HD and treatment-naïve RRMS patients were left unstimulated (0min) or stimulated with 2ng/mL CD40L for the indicated time. Intracellular staining of TRAF2 and TRAF6 were carried out to detect the protein level of TRAF2 or TRAF6 in memory and naïve B cells. p-IKK γ level was evaluated using specific antibodies by phosflow. **A)** Expression levels of TRAF6 in 6 HDs (12 data points, open circles) and 12 RRMS_TN patients (12 data points, closed circles). **B)** Expression levels of TRAF2 in 10 HDs (open circles) and 12 RRMS_TN patients (closed circles). **C)** p-IKK γ level was evaluated in 6 HDs (12 data points, open circles) and 12 RRMS_TN patients (12 data points, closed circles).

Supplemental Figure 3



Supplemental Figure 3. Effect of IFN beta-1a alone or combination of IFN beta-1a and cellcept on basal and CD40-induced phosphorylation of P65 and P38 in B cells from RRMS patients. A-D) Basal level (unstimulated, **A** and **C**) and the level following CD40 stimulation (15min, **B** and **D**) of p-P65 in 5 RRMS patients before (RRMS_TN, closed circles) and after (RRMS_A, closed triangles) IFN beta-1a therapy were included. No significant differences between groups were observed. **E-H)** Basal levels of P65 phosphorylation (unstimulated) in HD and RRMS patients before and after the combination therapy with IFN beta-1a and cellcept (RRMS_TN vs RRMS_A+C). Combination therapy did not reduce the basal level of P65 (**E** and **G**) and P38 (**F** and **H**) phosphorylation in B cells of RRMS patients.

Supplemental Figure 4



Supplemental Figure 4. CD40 expression on B cells from treatment-naïve RRMS and SPMS patients. PBMC isolated from patients and healthy controls were analyzed by flow cytometry for CD40 expression on memory B (**A** and **C**) and naïve B cells (**B** and **D**). Results are expressed as MFI of CD40 expression. **A-B**) PBMC isolated from leukopheresis pack of healthy donors (HD, n=9) and treatment-naïve RRMS patients (RRMS_TN, n=12). **C-D**) PBMC isolated from blood of healthy donors (HD, n=9) and treatment-naïve SPMS patients (SPMS_TN, n=7).