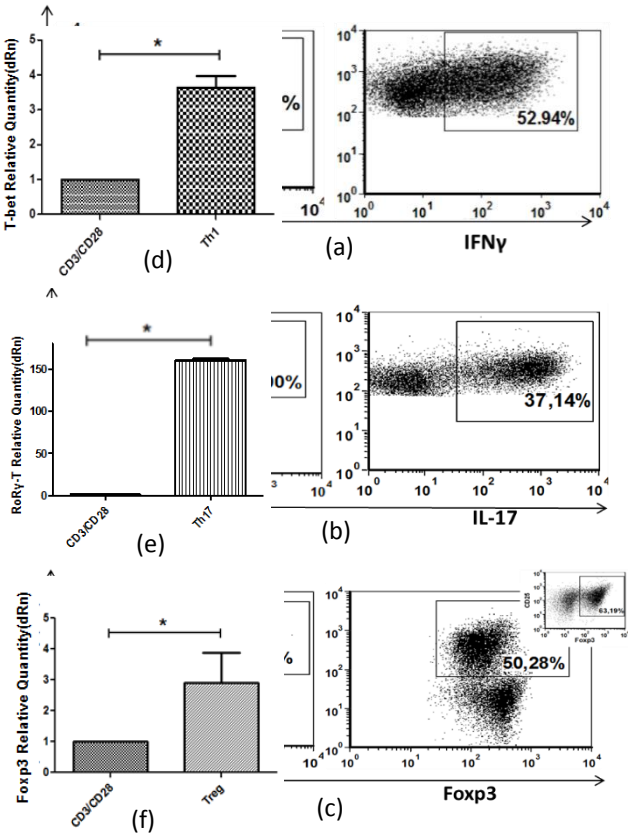
SUPPLEMENTARY METHODOLOGY

Associated to Supplementary Figure 1

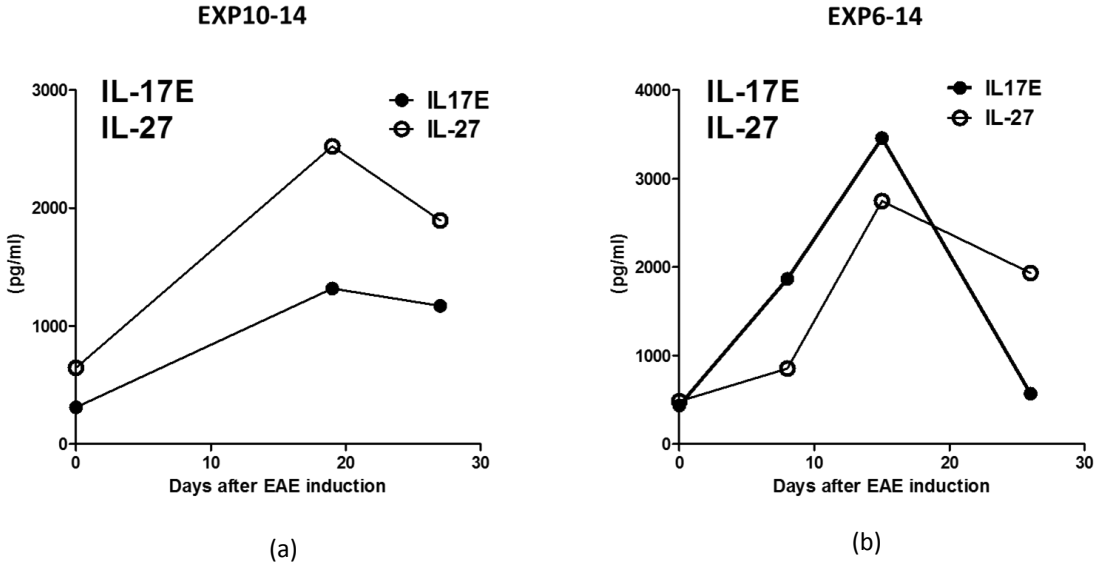
CD4+T cells were purified from splenocytes using Dynabeads Untouched Mouse Kit CD4+ T cell Isolation (Invitrogen) according to manufacturer's instructions.
For Th1, Th17 and Treg differentiation, CD4+T cells were cultured at $1x10^6$ cells/cm^2 in RPMI 1640 Glutamax, 10% FBS, 100U/ml Penicillin and 100 μg/ml Streptomycin, β-mercaptoethanol 55 μM at 37°C, 5%CO_2. CD4+ T cells was stimulated with 2.0 μg/ml anti-CD3 (BD Biosciences) and 1.5 μg/ml anti-CD28 (BD Biosciences). Skewing conditions for T helper differentiation were as follow: Th1 differentiation, 10 ng/ml IL-12 (R&D Systems, USA) and 2.5 μg/ml anti-IL-4 neutralizing antibodies (BD Biosciences). Th17 differentiation, 50 ng/ml IL-6 (R&D Systems), 5 ng/ml TGF-β1 (BioVision USA) and 2.5 μg/ml neutralizing anti-IFN-γ and anti-IL-4 antibodies (BD Biosciences). Treg differentiation, 10 ng/ml TGF-β1 (BioVision USA), 20 U/ml IL-2 (eBioscience) and 2.5 μg/ml neutralizing anti-IFN-γ and anti-IL-4 antibodies (BD Biosciences). Cultures were established for 5 days with 50% media change every 48 h. For FACS assays, T cells were re-estimulated with PMA/Ionomycin/Brefeldin-A for 4 h at 37°C before staining.

For RT-qPCR experiments, RNA extraction was carried out using the RNeasy mini kit QIAGEN (Biosonda) according to manufacturer´s instructions. The synthesis of cDNA was carried out using 2 μg of total RNA. PCR was performed using diluted cDNA (1:3) and 10 μl of primer-containing GoTaq MasterMix (Promega, 300 pmol each primer) and analyzed using Mx3000P qPCR system (Agilent Technologies). Primers used and protocol for expression levels determination are described previously in the methodology.

SUPPLEMENTARY FIGURES

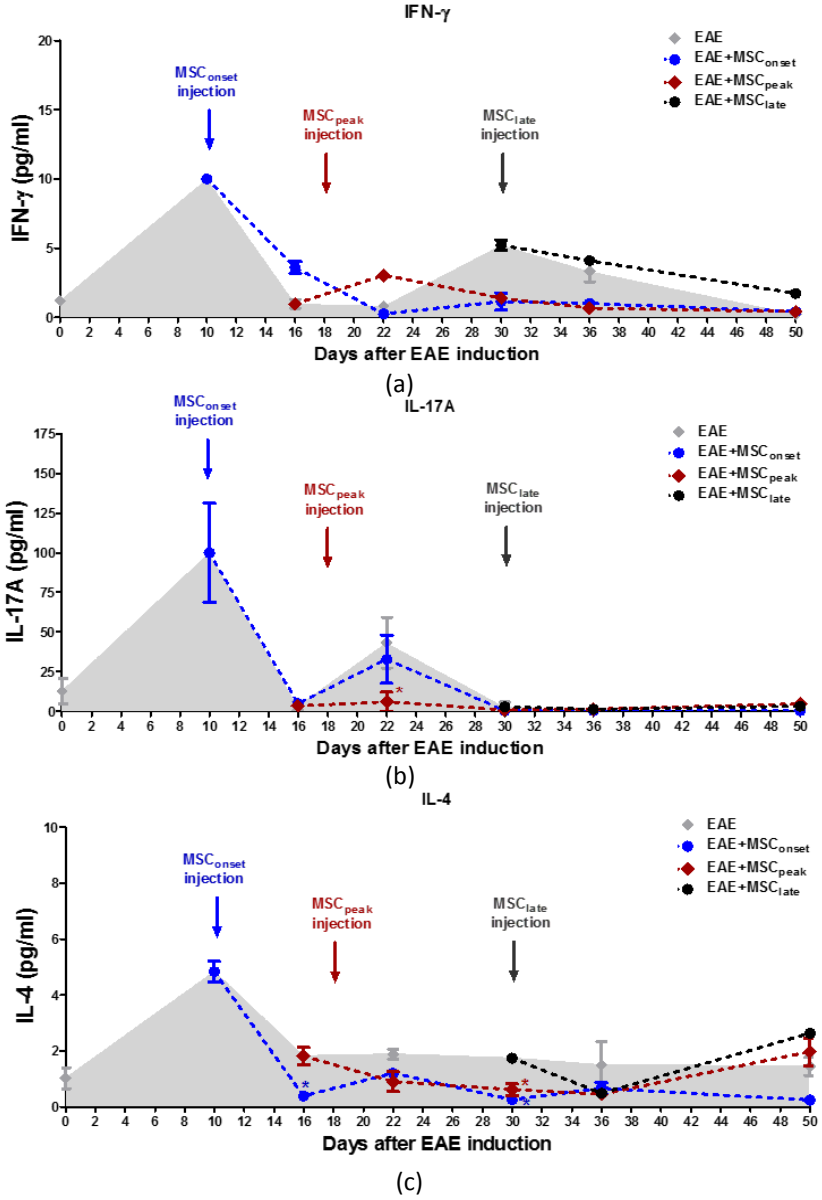


SUPPLEMENTARY FIGURE 1: Correlation of Th1, Th17 and Treg analysis by flow cytometry and RT-qPCR. Naïve CD4+ cells isolated from C57BL/6 spleens were differentiated into Th1, Th17 and Treg lineages using specific panel of cytokines (see supplementary methods). After 5 days *in vitro*, cells were stained for CD4 and either IFNγ, IL-17 or FoxP3. Flow cytometry analysis of Th1 (CD4+IFNγ+, (a), Th17 (CD4+IL17+, (b) and Treg (CD4+Foxp3+/CD4+CD25+Foxp3+, (c) populations are shown. Relative mRNA levels of the transcription factors T-bet (d), RORγT (e) and Foxp3 (f) were determined by RT-qPCR from experiments performed in parallel to the FACS assays. A positive correlation between T-bet, RORγT and Foxp3 mRNA levels and the percentage of Th1, Th17 and Treg cells was respectively detected in our differentiation assays.



(a) (b)

SUPPLEMENTARY FIGURE 2: IL-27 and IL17F plasma levels at different times of EAE progression. Blood samples from affected animals from two independent EAE experiments were taken at indicated times of EAE progression and pooled (n=7). Plasma samples were isolated by centrifugation and stored at -80°C until use. Cytokine levels of IL-27 and IL-17E were detected in triplicate using Milliplex mouse Th17 magnetic bead panel on Luminex 200TM (Merck, Millipore). a) EXP10-14 and (b) EXP6-14.



SUPPLEMENTARY FIGURE 3: Cytokine levels after MSCs injections at different times of EAE progression.

C57BL/6 mice were immunized with MOG_{35-55} to induce EAE and treated with MSCs at different time points of disease progression, i.e. at the onset (EAE+MSConset), at the peak (EAE+MSCpeak), and at the time of EAE stabilization (EAE+MSClate). Untreated EAE mice received vehicle (PBS) only on day 10. For all animals, blood samples were taken before EAE induction (day 0), at the onset of EAE (day +10), two days before the peak (day +16), after de peak (day +22), at the time of EAE stabilization (day +30), after EAE stabilization (day +36) and at the end of experiment (day +50). Blood samples were pooled (n=7) and plasma samples were isolated by centrifugation and stored at -80°C until used. Cytokine levels of INF-γ (a), IL-17A (b) and IL-4 (c) were determined using Milliplex mouse Th17 magnetic bead panel on Luminex 200TM (Merck, Millipore). The samples of each EAE group were

pooled and analyzed in triplicate. Bars represent the mean ± SEM, statistical differences were calculated using Mann–Whitney test (*, $p<0.05$).