SUPPLEMENTAL FIGURES



Supplementary Figure S1: Myeloid cells in spleen of aged mice. (A–B) Cells from blood and bone marrow compartments of aged mice were stained with anti-CD11b and anti-Gr1 antibodies and analyzed by flow cytometry. (A) Percentage of CD11b⁺Gr1⁺ cells are presented. (B) Splenocytes were collected from mice ten days after CpG-ODN+IFA or S.S treatment stained with anti-CD11b and anti-Gr1 and flow cytometry analysis was performed. Fold increase of CD11b⁺Gr1⁺ cells numbers from young and aged mice after CpG-ODN+IFA treatment related to their numbers in spleen of young or aged S.S-treated mice. Data are from three independent experiments; mean \pm SEM (n = 4 mice/group) *p < 0.05.



Supplementary Figure S2: CD124 expression in myeloid cells from young CpG-ODN+IFA-treated mice. Splenocytes were collected from young mice ten days after CpG-ODN+IFA or S.S treatment and flow cytometry analysis was performed. Mean Fluorescence Intensity (MFI) for CD124 on CD11b⁺Gr1⁺ gated cells are presented. Data are from three independent experiments; mean \pm SEM (n = 4 mice/group) *p < 0.05; p values were calculated using *t*-test.



Supplementary Figure S3: Role of IL-4 and IL-6 in arginase induction in myeloid cells from CpG-ODN+IFA-treated mice. After ten days of S.S or CpG-ODN+IFA-treatment, myeloid CD11b⁺ cells were isolated from the spleen of mice and were cultured with naïve T-cells isolated from young mice stimulated with anti-CD3 plus anti-CD28 Abs. Cytokine neutralizing antibodies were added at the beginning of the cultures: anti-mouse IL4 or/and anti-mouse IL6 After 24 h culture, cells were collected and stained with anti-CD11b and anti-Gr1 antibodies, fixed, permeabilized and then stained for arginase-1. Representative histograms of Arginase-1 expression in CD11b⁺Gr1⁺ gated cells are shown. Data are representative of one mice/age-group of one experiment of two performed.



Supplementary Figure S4: pSTAT3 levels in myeloid cells from CpG-ODN+IFA-treated mice. After ten days of CpG-ODN+IFA-treatment, myeloid CD11b⁺ cells were isolated from the spleen of young and aged mice and were cultured with naïve T-cells isolated from young mice stimulated with anti-CD3 plus anti-CD28 Abs. Anti-mouse IL6 neutralizing antibody was added at the beginning of the cultures in some wells and, after 30 min, cells were collected, fixed, permeabilized, and stained for anti-CD11b, anti-Gr1, and anti-pStat3. Representative histograms of pSTAT3 levels in CD11b⁺Gr1⁺ gated cells from young and aged mice are shown. Data are representative of one mice/age-group of one experiment of two performed.