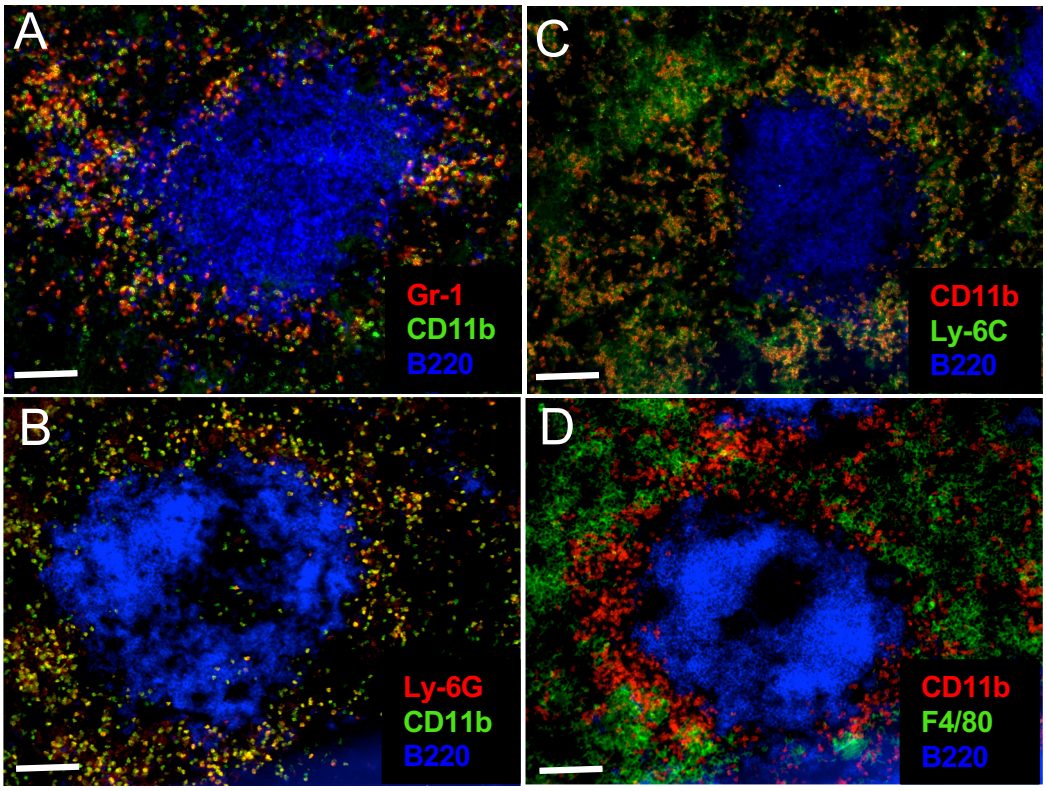
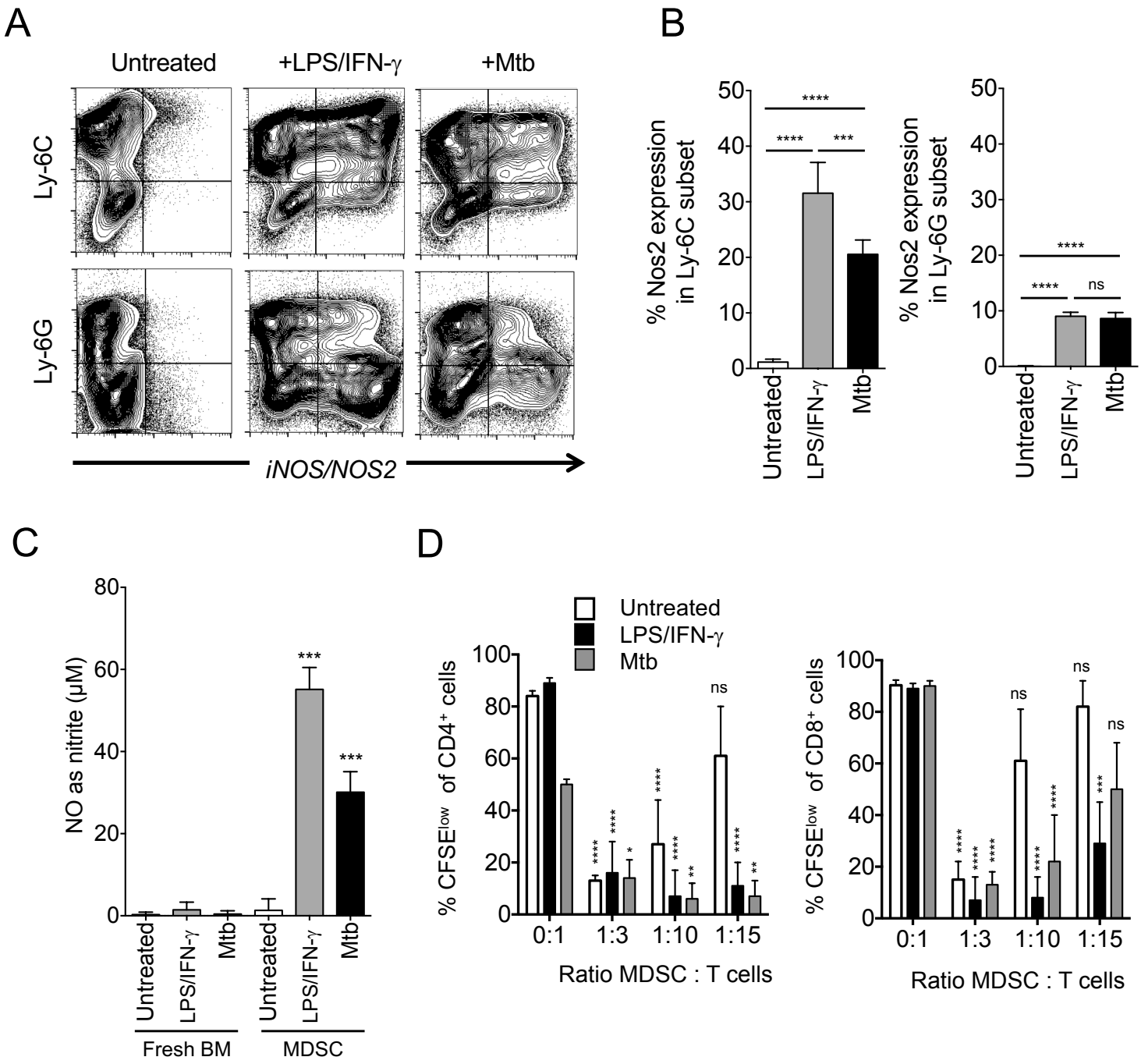
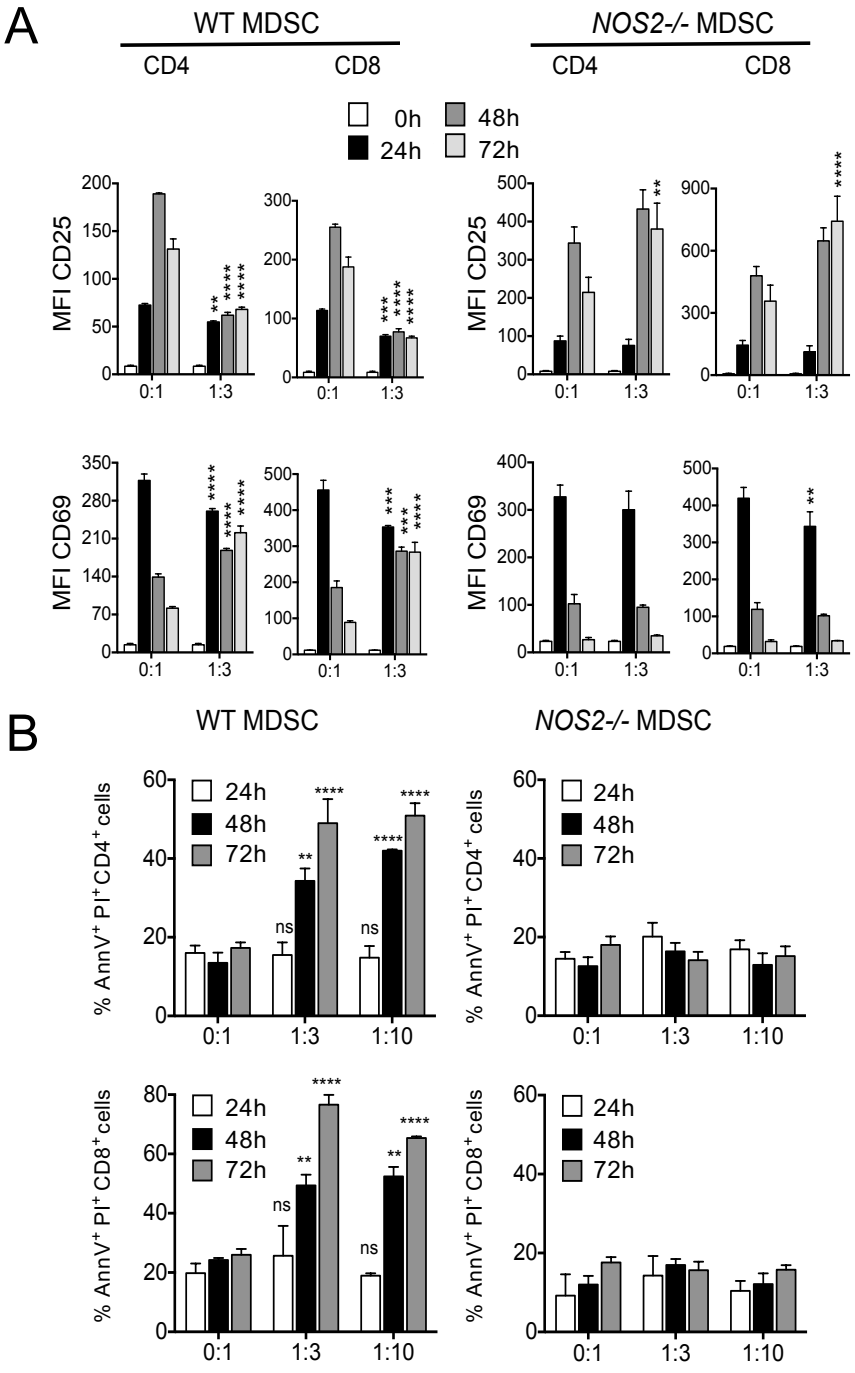


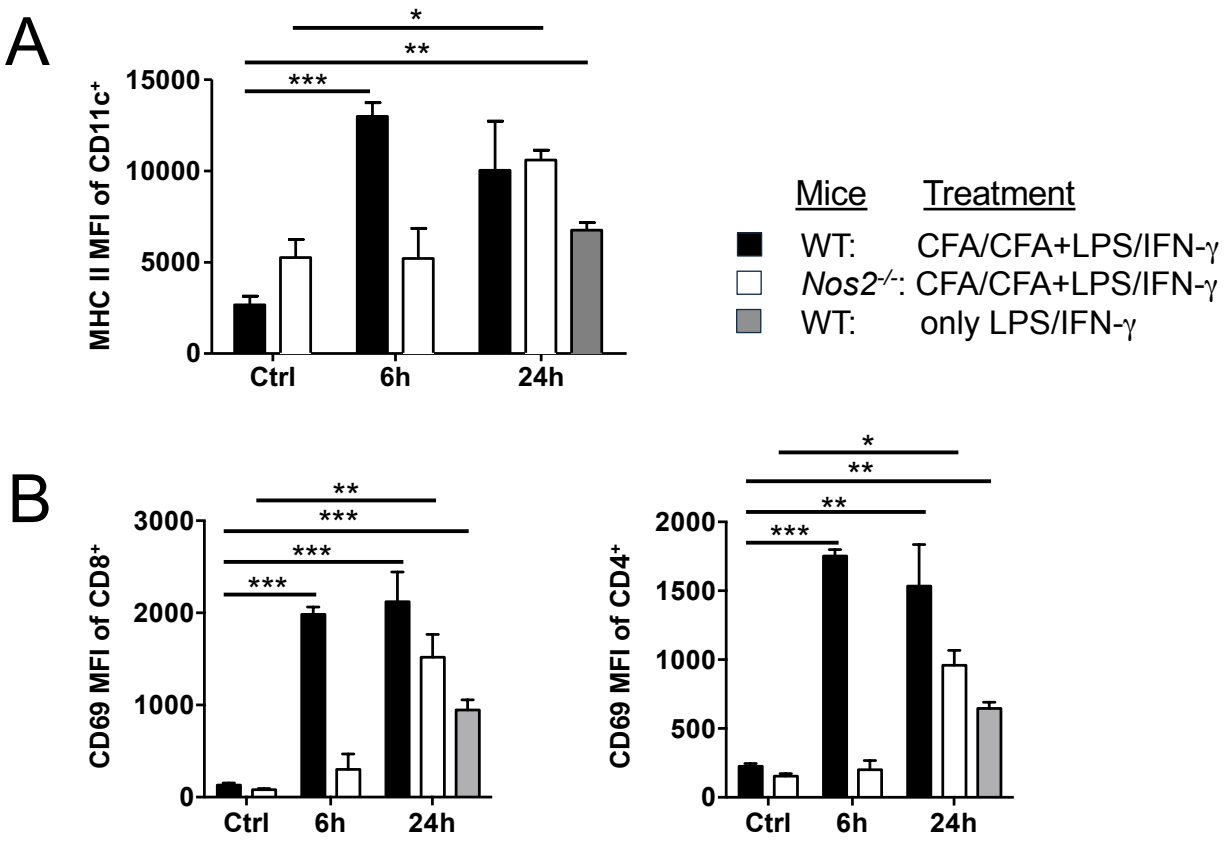
Suppl. Figure 1: MDSCs accumulate in spleens after CFA/CFA administration. (A-D) Spleens from CFA/CFA mice were collected after 15 days and cryosections stained for B220, CD11b and markers related to MDSC subsets like Gr-1 (A), Ly-6G (B), Ly-6C (C) and F4/80 (D). Microscopy data are representative of at least two to three independent experiments with n=3 mice for each group. Scale bars, 100µm. B220 staining indicates B cell zone.



Suppl. Figure 2: Stimulation of MDSCs by Mtb *in vitro* triggers iNOS/NOS2 upregulation leading to T cell suppression (A) BM-generated MDSCs were matured overnight with LPS/IFN-γ or heat-killed Mtb before intracellular detection of iNOS. Representative FACS analysis for Ly-6C$^+$ monocytic and Ly-6G$^+$ granulocytic cell subsets. (B) Quantification of A (n=5 independent experiments). (C) Supernatants from cells treated like under A were tested for NO production by Griess assay. Values correspond to NO production by $2x10^6$ cells (n=6 independent experiments). (D) T cell proliferation inhibition assay of CFSE-labeled bulk syngeneic T cells co-cultured with *in vitro* generated BM-MDSCs at different ratio and stimulated with soluble a-CD3/CD28 plus LPS/IFNγ or Mtb (n=4 independent experiments). Statistical significance was assessed by comparison of untreated versus treated cells (A, B, C) or T-cells only versus T-cells co-cultured with BM-MDSCs at different ratio (D). Statistics by one-way ANOVA with multiple comparisons and Tukey's post test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. NS, not significant.

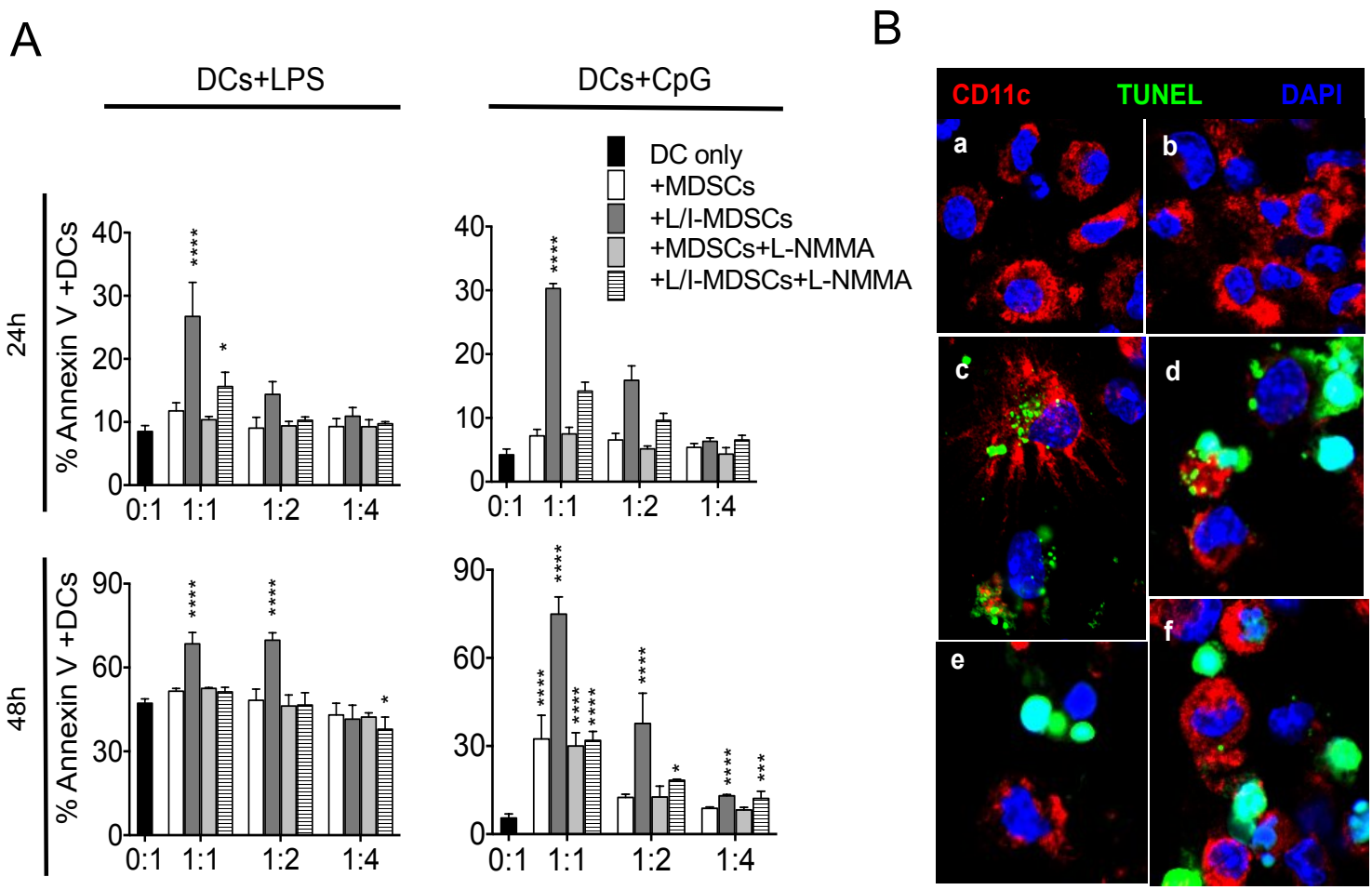


Suppl. Figure 3: NO mediates T cell suppression and apoptotis at 48h *in vitro*. Bulk T cells were stimulated with anti-CD3/CD28 and co-cultured at different ratios with BM-MDSCs from WT or *NOS2*$^{-/-}$ mice. (A) CD25 and CD69 surface expression on CD4^{+} and CD8^{+} T cells. (B) Percentage of Annexin V^{+} propidium iodide^{+} CD4^{+} and CD8^{+} dead T cells measured with FACS at indicated time points. Values are expressed as mean fluorescence intensity (MFI). Statistical significance was assessed by comparison of T cells only (R0:1) versus T cells co-cultured with BM-MDSCs at different ratios. Statistics by one-way ANOVA with multiple comparisons and Tukey's post test. **p<0.01, ***p<0.001, ****p<0.0001. NS, not significant. Values correspond to the Mean ± SD of 3 (A) or 4 (B) pooled independent experiments.



Suppl. Fig. 4. Activation of splenic DCs and T cells after injection of LPS/IFN-γ into differentially CFA immunized mice.

The spleens of the same mice as shown in figs. 4B and 4C were also analyzed at the indicated time points by FACS for (A) DC maturation by MHC II expression (as mean fluorescence intensity (MFI) on $CD11c^+$ cells and (B) the activation marker CD69 (MFI) on $CD4^+$ and $CD8^+$ T cells. Control mice remained without LPS/IFN-γ treatment. Statistics by unpaired student's T test. *p<0.05, ***p<0.001, ****p<0.0001. Values correspond to the Mean ± SD of n=3 pooled independent experiments, except Ctrl group of *NOS2*$^{-/-}$ n=2.



Suppl. Figure 5: BM-MDSCs kill mature BM-DCs by an NO-dependent mechanism in vitro. (A) *In vitro* generated BM-DCs were matured overnight with LPS or CpG and then co-cultured at different ratios with BM-MDSCs or LPS/IFN-γ-activated BM-MDSCs (L/I-MDSCs) in the presence or absence of L-NMMA. The percentage of annexin V$^+$ DCs was measured with FACS after 24h and 48h co-culture. (B) Representative pictures of TUNEL staining for apoptotic DCs after 24h (a,c,e) and 48h (b,d,f). (a,b) DCs were stimulated with either CpG (a) or LPS (b) but left without MDSCs; (c-d) CpG-stimulated DCs were cultured with L/I-MDSCs; (e-f) LPS-stimulated DCs were cultured with +L/I-MDSC. Statistical significance was assessed by comparison of DC only (R 0:1) versus DCs co-cultured with BM-MDSCs ± L-NMMA at different ratio. Statistics by one-way ANOVA with multiple comparisons and Tukey's post test. *p<0.05, ***p<0.001, ****p<0.0001. Values correspond to the Mean ± SD of 2 pooled independent experiments.