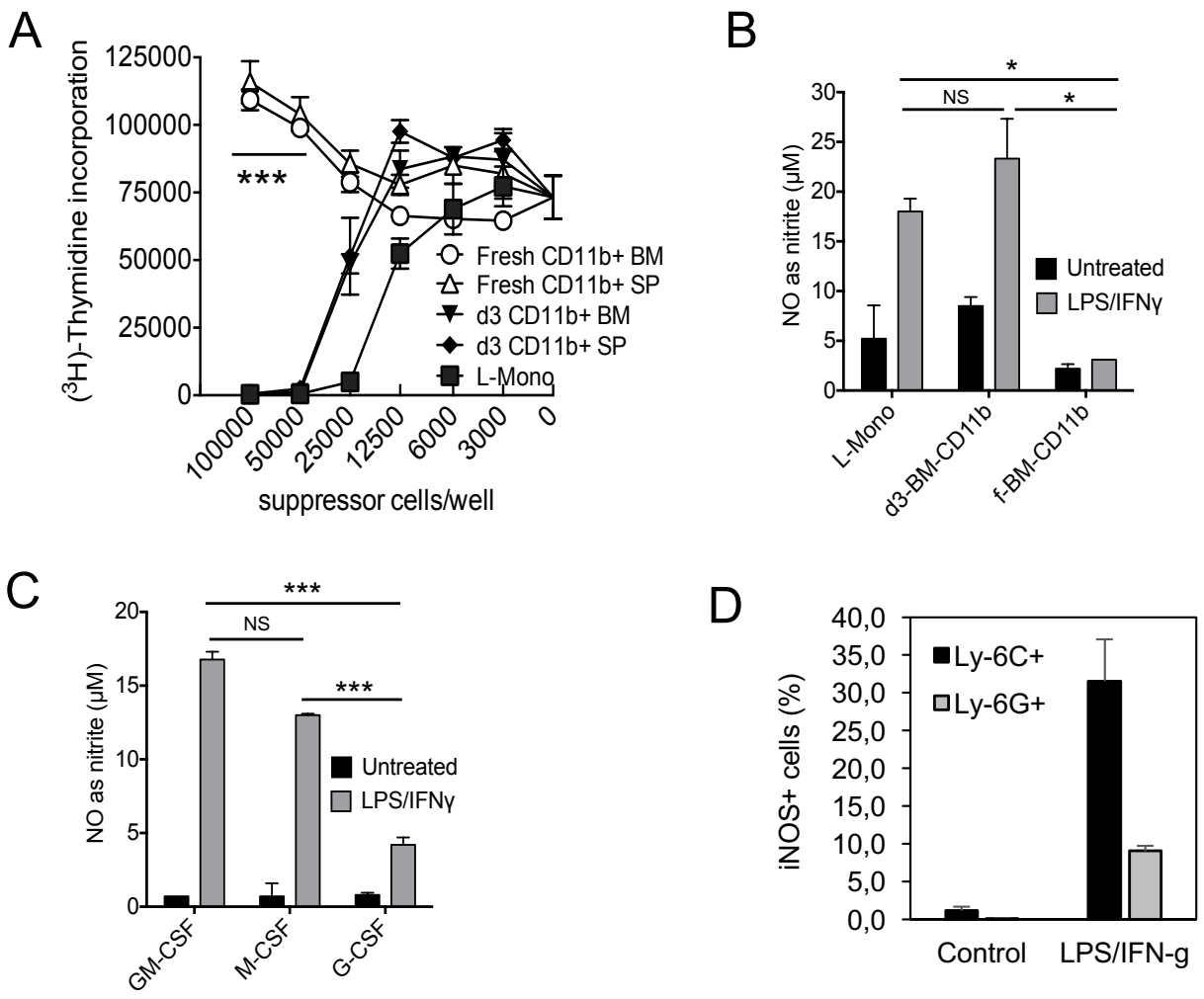


**Figure S1. Only myeloid cell cultures with GM-CSF or M-CSF can be stimulated to release NO and suppress proliferation of T cells.**

**A,** Suppressor assay in which the inhibition of T cell proliferation was tested via [$^3$H]-thymidine uptake by adding titrated amounts of either CD11b$^+$ cells freshly isolated from BM or SP or CD11b$^+$ cells cultured for 3d in GM-CSF or as a positive control bulk BM cells for 3d in GM-CSF derived by our standard protocol (termed L-Mono). One representative experiment out of 3 is shown. Statistical significance was assessed by comparison of fresh (BM or SP) versus 3d cultured CD11b$^+$ cells (BM or SP). For statistics pooled data from 3 independent experiments were used. **B,** Freshly isolated *ex vivo*-sorted CD11b$^+$ BM cells, or CD11b$^+$ BM cells cultured for 3 days in GM-CSF or L-Mono from C57BL/6 mice were treated like the cells used for Fig. 1a but stimulated overnight with LPS plus IFN-γ to test NO release with the Griess reaction. Values correspond to NO production by 2x10$^6$ cells **C,** NO production was detected like in A but from BM cells cultured in the presence of either GM-CSF, M-CSF or G-CSF at 50U/ml for 3 days. Values correspond to NO production by 2x10$^6$ cells. Statistics by one-way ANOVA with multiple comparisons and Tukey's post test. *p<0.05, ***p<0.001. NS, not significant. Values correspond to the Mean ± SD of 2 (A) or 3 (B,C) independent experiments. **D.** BM cells cultured for 3d in GM-CSF were stimulated for 16h with LPS+IFN-γ before staining for granulocytic Ly-6G+ or monocytic Ly-6C+ surface markers and intracellular iNOS and FACS analysis. Data represent mean of n=6 experiments ±SD.

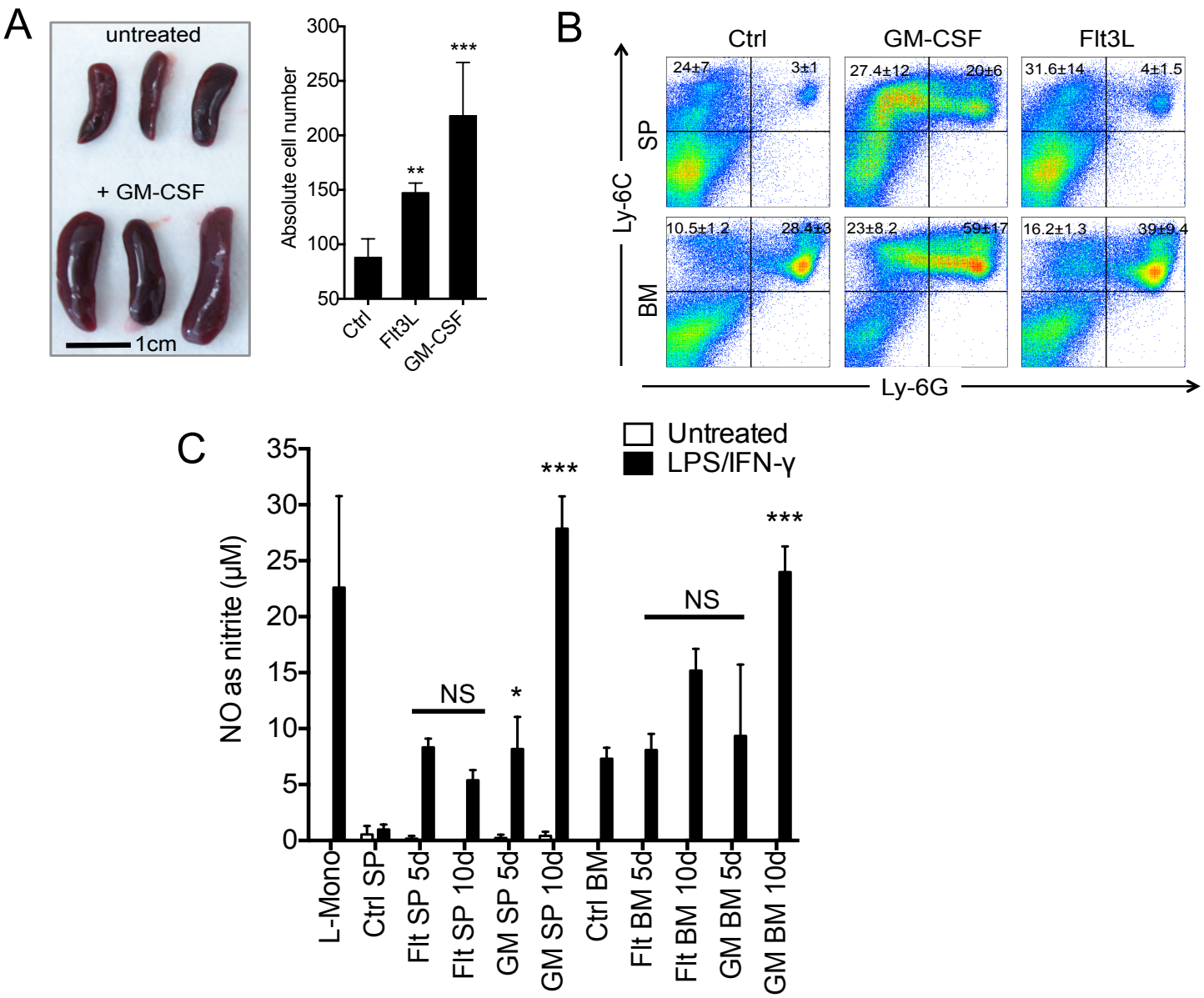


**Figure S2. GM-CSF injections increase spleen size and the myeloid compartment in BM and spleen.**

**A,** Splenomegaly in C57BL/6 mice after daily injections of GM-CSF with 2μg/day for 10 days. Values correspond to the Mean ± SD (n=3) and significance assessed by the comparison between the untreated (Ctrl) and treated mice. Statistics by unpaired Student's t-test. **p<0.01, ***p<0.001. **B,** Daily injections of either GM-CSF or Flt3L with 2μg/day over 10 days and subsequent FACS analysis of spleen and bone marrow cells demonstrated a remarkable myeloid expansion shown by the increase in the proportion of Ly-6C$^+$ Ly-6G$^+$ granulocytic and Ly-6C$^+$ Ly-6G$^-$ monocytic cells (n=4). **C,** NO production of SP or BM cells of mice injected daily for 5 or 10 days i.p. with 2 μg GM-CSF or Flt3L and stimulated in vitro by LPS/IFN-γ overnight. Values correspond to the Mean ± SD (n=6) and significance assessed by the comparison between the SP or BM from Ctrl mice +LPS/IFN-γ and the SP or BM from Flt3L or GM-CSF mice +LPS/IFN-γ. Statistics by one-way ANOVA with multiple comparisons and Tukey's post test. *p<0.05, ***p<0.001. NS, not significant.

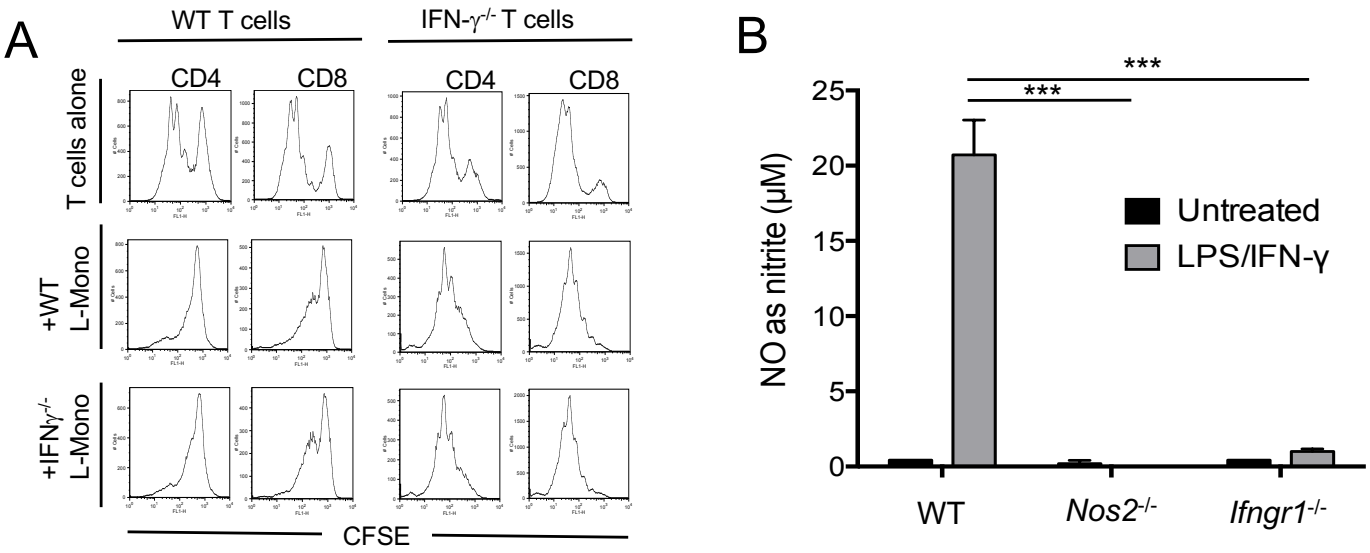


**Figure S3. IFN-γ is produced by T cells during the suppression assay which is required by L-Mono for their activation into NO-producing MDSC and suppressor function.**

**A**, WT and IFN-g-deficient T cells or L-Mono were used in a T cell supressor assay. T cells were labeled with CFSE to measure their proliferation after 3 days. One example shown (n=2). **B,** L-Mono of wild type (WT), *Nos2*$^{-/-}$ and *Ifngr1*$^{-/-}$ were tested for their potential to produce NO after stimulation with LPS plus IFN-γ and by the Griess assay. Data represent means ± SD of 4 independent experiments. Statistics by unpaired Student's t-test by comparing KO versus WT controls ***P<0.001.

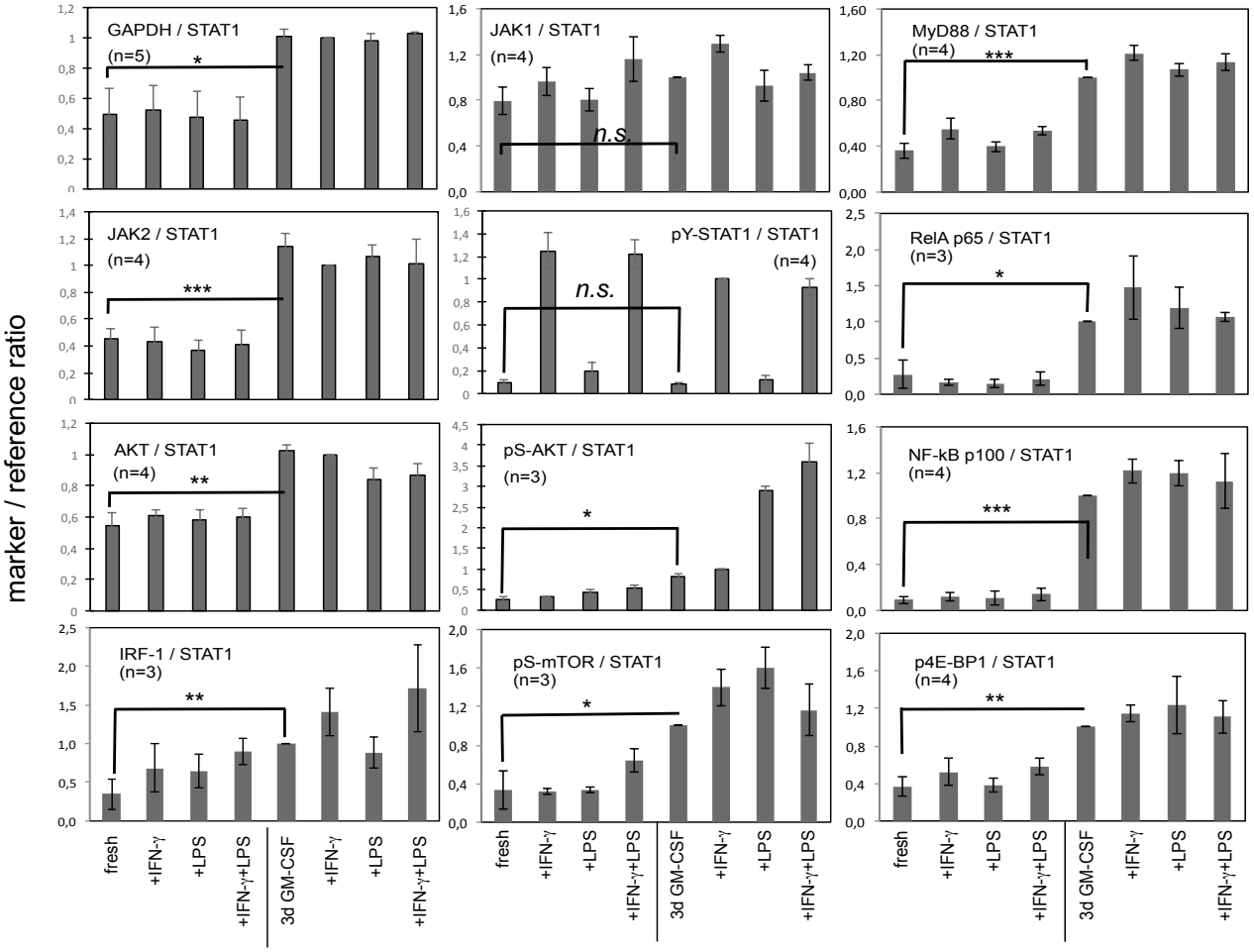


**Figure S4. Quantification of Western blot analyses.**

Western Blots were performed under the same conditions shown in figures 3A and 3B. Chemiluminescence was detected using the FluorChem Q (Protein Simple). Non-saturated exposures of each analyzed signaling molecule were quantified using the AlphaView Software and normalized to the values obtained for total STAT1 protein. Since GAPDH but not STAT1 appeared to be up-regulated after 3 days of in culture with GM-CSF (Fig. 3a and upper left panel) we used STAT1 as a reference marker for quantification. The number (n) of Western blots for quantification of each marker is indicated. Statistical significance was evaluated by paired one-tailed Student's t-test and is indicated as not significant, n.s if $p > 0.05$, or significant as * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

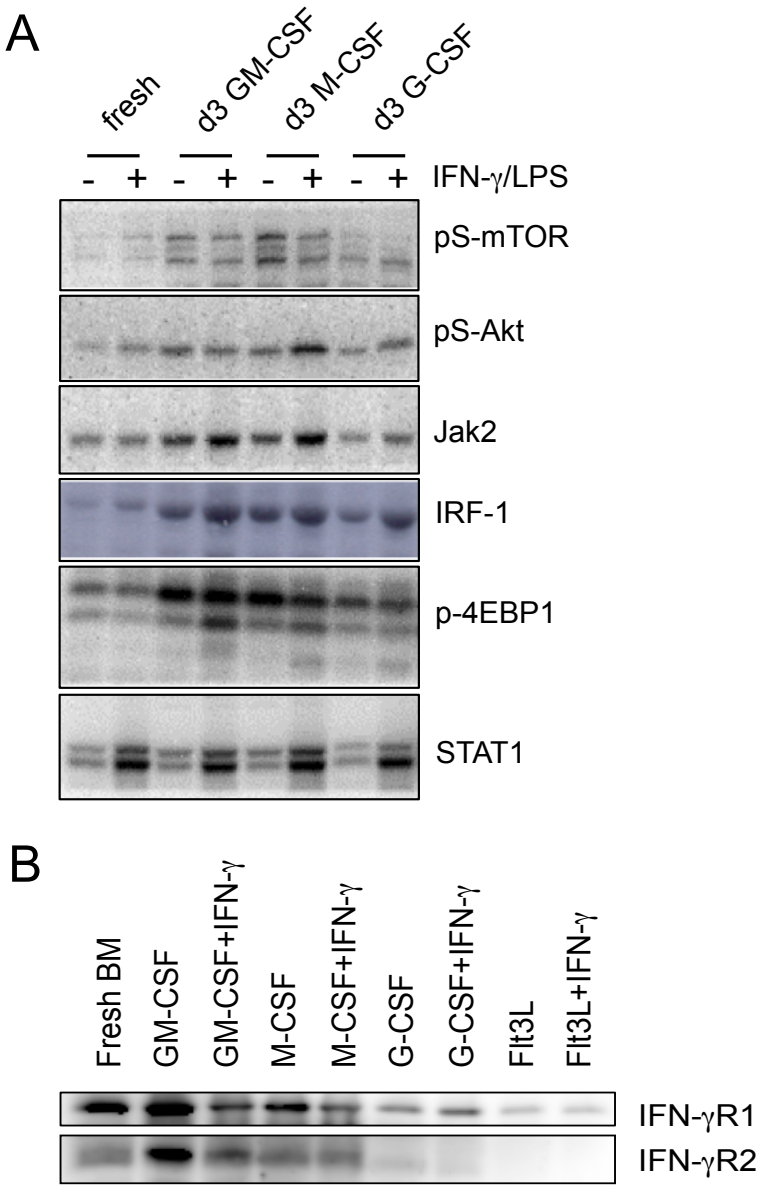


**Figure S5. IFNγR1, IFNγR2 and licensing markers are predominantly induced by GM-CSF and M-CSF**.

**A.** Western blot analyses for the indicated markers of whole cell lysates from fresh BM cells or L-Mono grown in GM-CSF, M-CSF or G-CSF for 3 days and treated or not with IFN-γ/LPS overnight (n=2). **B.** Cellular membrane extracts of indicated culture conditions for 3 days and treated or not with IFN-γ for 1h, or fresh BM cells were prepared and were further processed for Western blotting (n=2). p indicates phosphorylated forms of the indicated marker.

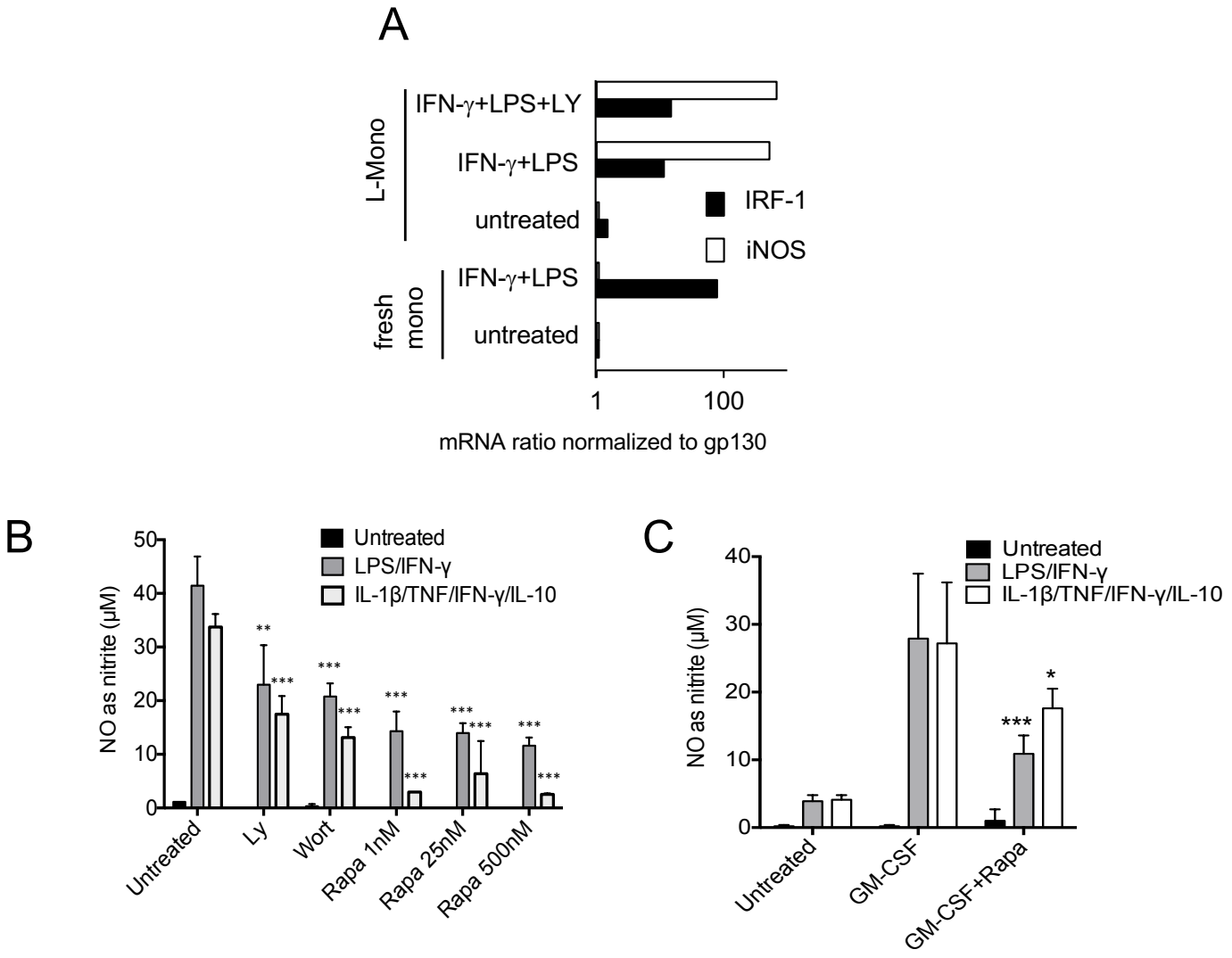


**Figure S6. Expression of murine iNOS and human IDO protein but not mRNA are inhibited by rapamycin.**

**A,** Murine fresh BM cells or L-Mono were cultured ± LY294002 and stimulated with IFN-γ/LPS for 16h as indicated. Then mRNA expression for iNOS and IRF-1 was evaluated by qPCR and compared to gp130 as a standard. **B,** Murine BM cells were cultured in GM-CSF for 3d before stimulation with IFN-g/LPS or IL-1b/TNF/ IFN-g/IL-10 for 16h. Then cell supernatants were tested for NO production by the Griess reaction. **C,** NO production measured by Griess reaction of splenic CD11b+ cells sorted from mice treated for 10d with GM-CSF only or GM-CSF + rapamycin (5 mg/kg). Values correspond to the Mean ± SD (n=4) and statistical significance assessed by the comparison of GM-CSF/rapamycin with GM-CSF treated mice. Statistics for all the panels using one-way ANOVA, with Tukey's (b,c) post-test. *p<0.05, **p<0.01, ***p<0.001. .