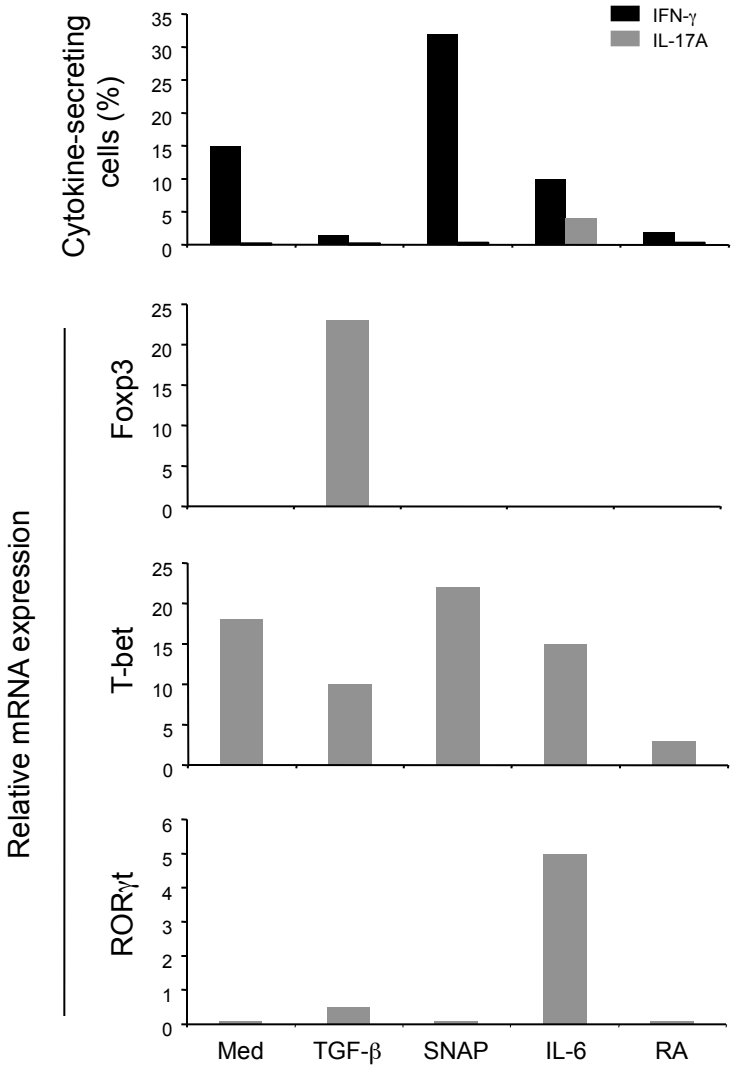
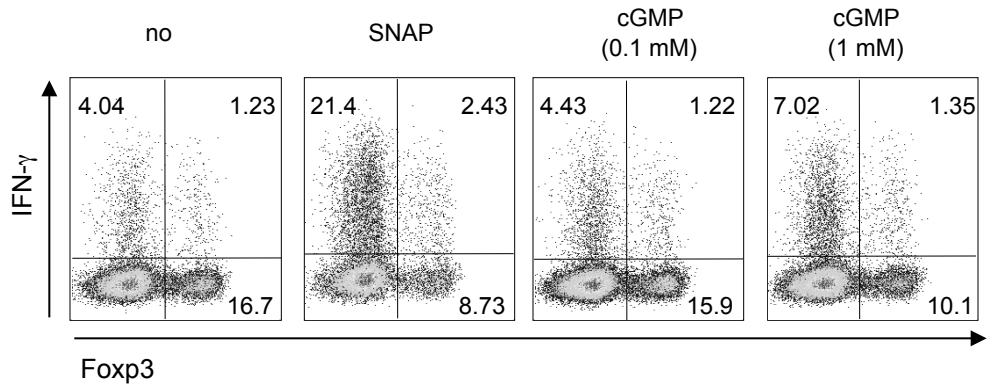
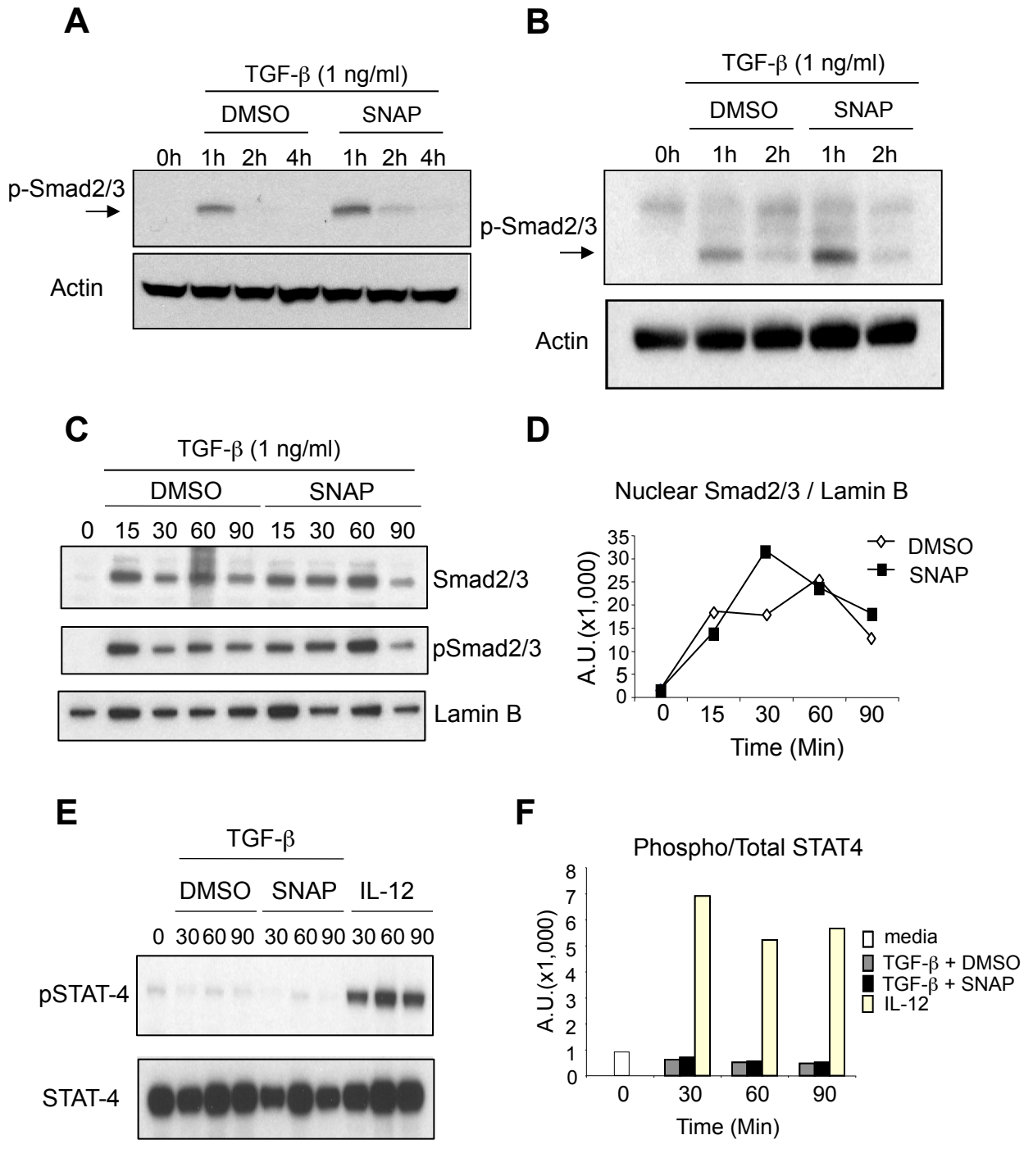
Supplementary Figure 1



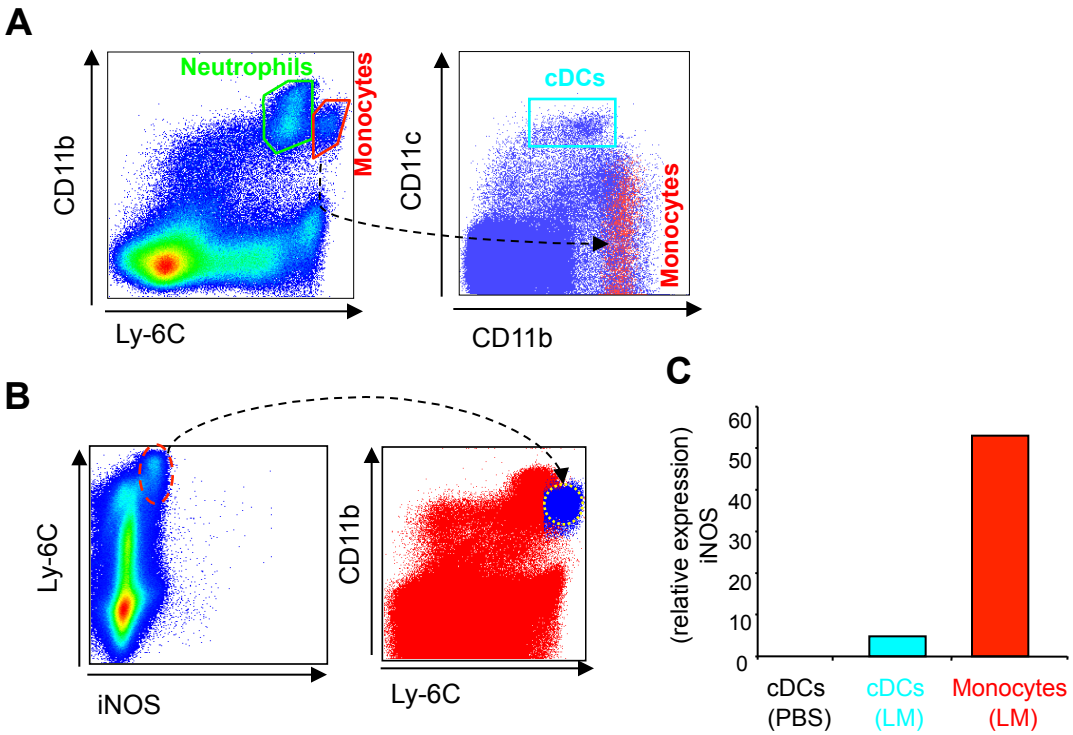
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 1. mRNA modulation by NO and exogenous cytokines related to Th differentiation. Naïve OT-II CD4 T cells were stimulated with splenic DC and OVA peptide. Where indicated, TGF-β (5 ng/ml), SNAP (100 μM), IL-6 (10 ng/ml), or RA (1 nM) alone was added at the start of culture. Expression of intracellular IL-17A vs. IFN-γ were determined after restimulation (top) and mRNA levels of Foxp3, T-bet, and RORγt were evaluated by real-time PCR without restimulation at day 4. Results are representative of three experiments.

Supplementary Figure 2. Cyclic GMP partially replicates NO in suppressing Foxp3 induction and promoting Th1 differentiation. Naïve OT-II CD4 T cells were stimulated with splenic DC and OVA peptide in the presence of TGF-β (5 ng/ml). Where indicated, SNAP (40 μM) and 8-Br-cGMP were added at the start of culture. Expression of Foxp3 and IFN-γ were determined at day 4 after restimulation. Results are representative of two experiments.

Supplementary Figure 3. NO does not modulate proximal TGF-βR signaling and STAT-4 phosphorylation in Th cells. T cell hybridomas (A, C, and D) or FACS-sorted naïve OT-II CD4 T cells (B) were stimulated with TGF-β (1 ng/ml) in the absence (A, C, and D) or presence (B) of anti-CD3/CD28 and IL-2. Where indicated, cells were treated with DMSO and SNAP (A, 100 μM; C and D, 20 μM). A-B, Phosphorylation of Smad2/3 from whole cell extracts was measured at indicated times. C, The level of total Smad2/3, phospho-Smad2/3, and Lamin B was determined in nuclear extracts. D, Normalized levels of nuclear Smad2/3 from (C) shown as arbitrary units (A.U.). A.U.

was calculated by dividing the density of total Smad2/3 by the normalized density of Lamin B. (E and F) Naive OT-II CD4 T cells were stimulated with spleen DC, OVA peptide (1 μM), and TGF-β (5 ng/ml). Where indicated the NO donor SNAP (100 μM) or IL-12 (20 ng/ml) was added. E, Western blot for pSTAT4 and total STAT4 at the indicated times (mins). F, The level of pSTAT4 versus total STAT4 was shown as arbitrary units (A.U.). Results are representative of two experiments.

Supplementary Figure 4. Identification and isolation of TipDC after *Listeria* infection. Mice were infected with *Listeria* (10,000 cfu) intravenously. A, Two days later, neutrophils (left plot, green gate), inflammatory monocytes (left plot, red gate), and cDC (right plot, cyan gate) were identified in spleens by flow staining. Monocytes gated by staining with CD11b and Ly-6C (left) were overlaid as red dots in the right FACS-plot. B, Intracellular staining of iNOS. $iNOS^{+}Ly\text{-}6C^{hi}$ populations (red circle in left plot) were identified as inflammatory monocytes shown as blue in the right plot based on CD11b, CD11c, and Ly-6C expression. C, mRNA expression of iNOS was measured by real-time PCR in purified cDC and monocytes from spleens of *Listeria*-infected mice sorted by FACS as gated in (A). cDC purified from spleens of PBS-injected mice were used as control. Results are representative of three experiments.