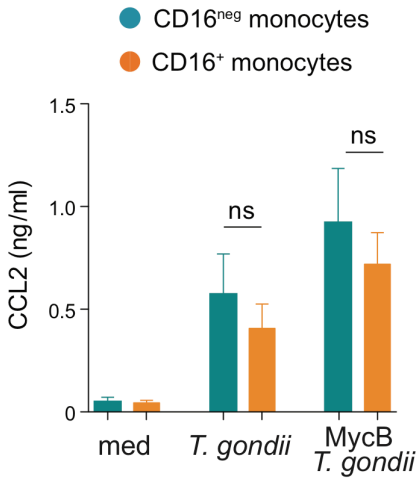
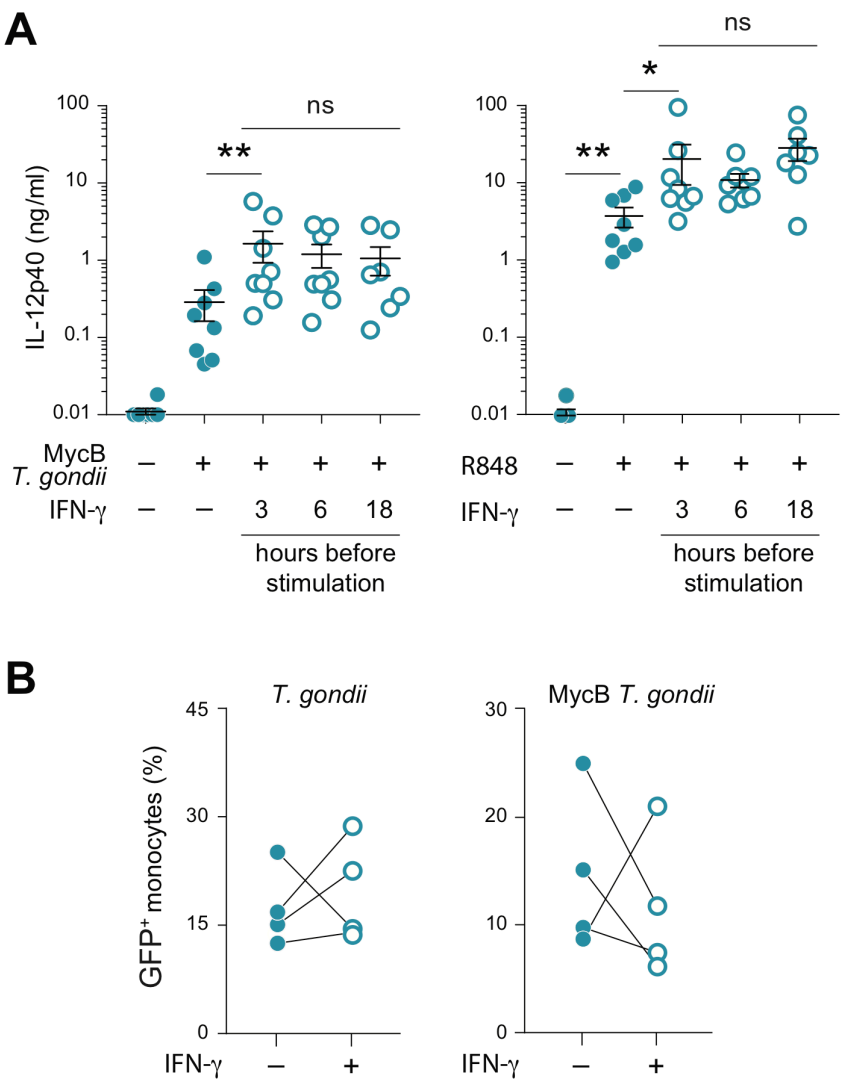


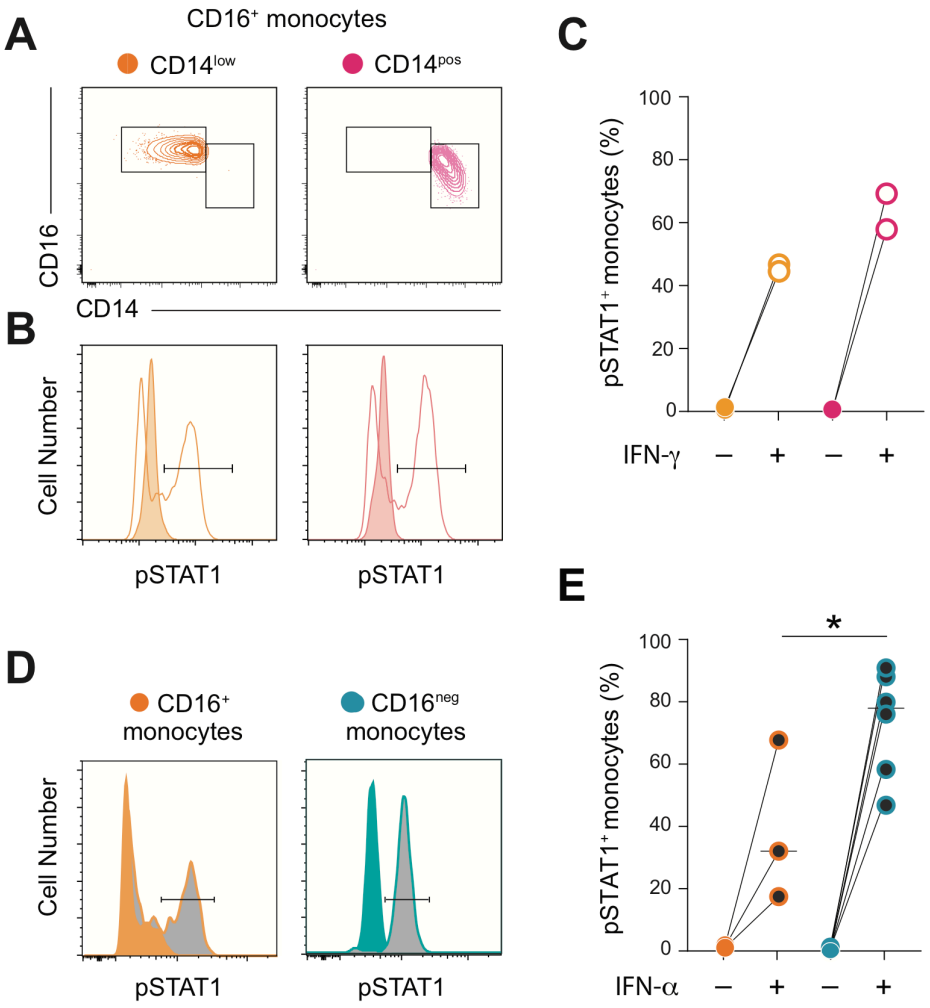
Supplemental Figure 1: Both CD16^{neg} and CD16^{+} human monocytes secrete CCL2 in response to stimulation with *T. gondii* tachyzoites

MACS-purified CD16^{neg} and CD16^{+} monocyte populations from healthy donors were cultured in medium alone or in the presence of untreated or mycalolide B (MycB) pre-treated *T. gondii* tachyzoites (MOI 1:1). After 18 hours incubation culture supernatants were collected and CCL2 was measured by ELISA. Bars represent the mean ± SEM of values obtained for individual donors (n=6).



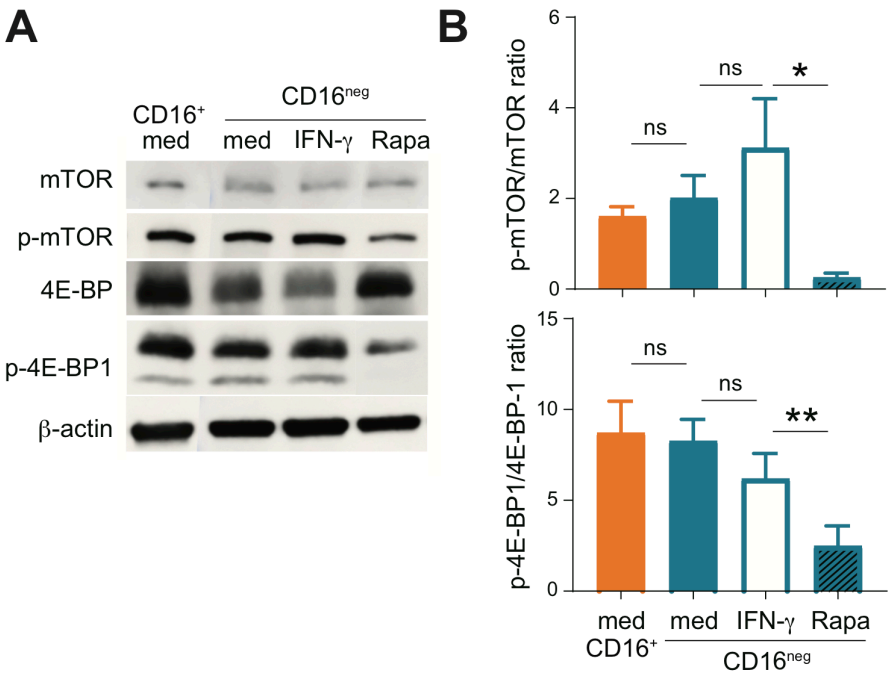
Supplemental Figure 2: Efficient IFN-γ-priming of CD16^{neg} monocytes for IL-12 secretion requires at least 3 hour pre-exposure but does not affect either parasite invasion or rate of phagocytosis of MycB-treated *T. gondii* tachyzoites

(A) MACS-purified CD16^{neg} monocytes from 8 healthy donors were primed with IFN-γ (10 ng/ml) for 3, 6, or 18 hours prior to stimulation with MycB-treated tachyzoites (left) or R848 (right). After 18 hours incubation culture supernatants were collected and IL-12p40 measured by ELISA. (B) Purified CD16^{neg} monocytes from 4 healthy individuals cultured overnight in medium alone or with IFN-γ (10 ng/ml) were exposed to untreated or MycB-treated GFP-labeled RH88 tachyzoites and the frequency of GFP+ monocytes was analyzed by flow cytometry 3 hours later. Each symbol indicates one donor. *P< 0.05, **P< 0.01.



Supplemental Figure 3: Frequency of pSTAT1⁺ cells in CD14^{low}CD16⁺ and CD14⁺CD16⁺ monocytes after IFN-γ stimulation and in the CD16⁺ and CD16^{neg} monocyte subetsbsets after exposure to IFN-α

(A) Contour plots of CD14 vs. CD16 staining pattern of FACSort-purified CD14^{low}CD16⁺ and CD14⁺CD16⁺ monocytes. (B) Representative histograms of pSTAT1-staining after 30 min stimulation with medium (filled) or IFN-γ (open) in indicated populations. (C) Frequency of pSTAT1⁺ in CD14^{low}CD16⁺ and CD14⁺CD16⁺ monocytes from two different donors. (D) Representative histogram of pSTAT1 staining for FACSort-purified CD16⁺ and CD16^{neg} monocytes cultured in medium (orange or blue filled) vs. IFN-α (10 ng/ml) (gray filled)for 30 min. (E) Graph depicts the frequency of pSTAT1⁺ CD16⁺ or CD16^{neg} monocytes from individual donors (n=3-6). *P < 0.05.



Supplemental Figure 4: mTOR activity is comparable in unstimulated CD16^{+}, CD16^{neg} and IFN-γ-primed CD16^{neg} monocytes

The levels of phosphorylated isoforms of mTOR or 4E-BP1complex was assayed by Western blot analysis of cell lysates from CD16^{+}, CD16^{neg} and IFN-γ- or rapamycin-pretreated CD16^{neg} monocytes. (A) Immunoblots of one representative donor. The protein bands were quantified and normalized to β-actin. (B) Bars represent mean ± SD values of the calculated ratios for mTOR (top) and 4E-BP1 complex (bottom) in indicated monocyte populations from 4 healthy donors. Statistical analyses were performed using Student *t*-test. *P< 0.05, **P< 0.01.