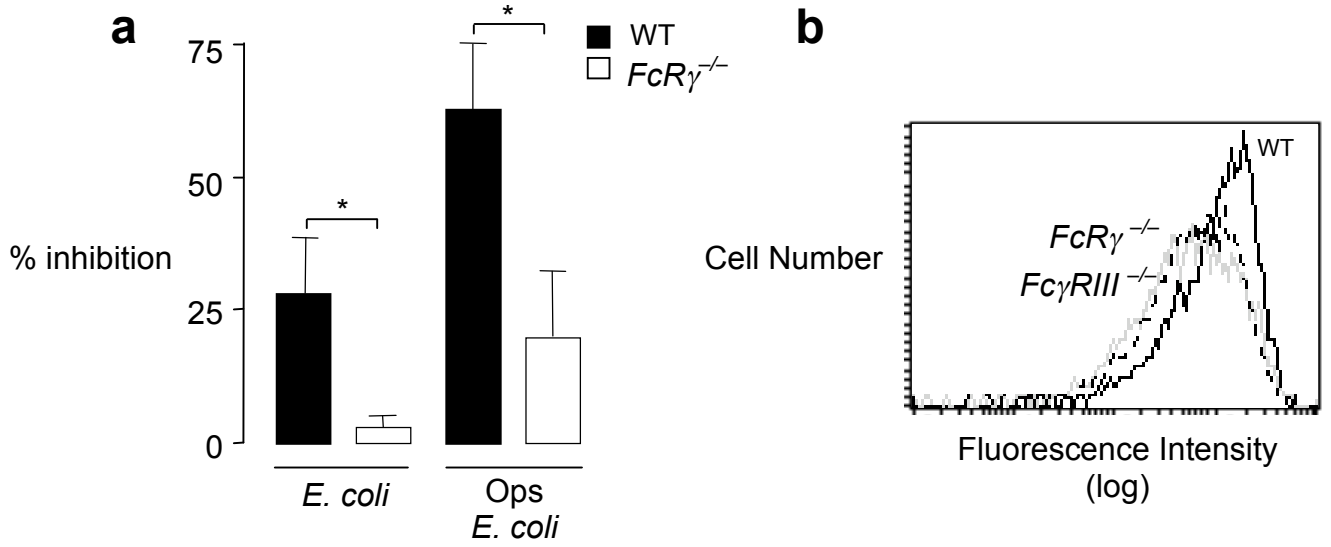
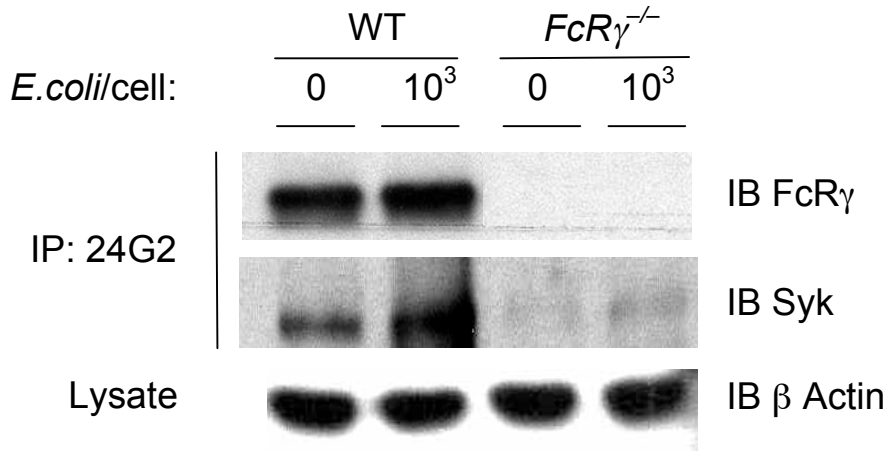
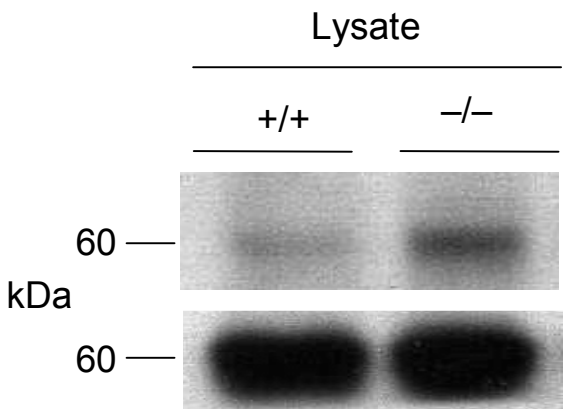
CD16 promotes *E. coli* sepsis through an FcRγ inhibitory pathway that prevents phagocytosis and facilitates inflammation. Fabiano Pinheiro da Silva, Meryem Aloulou, David Skurnik, Marc Benhamou, Antoine Andremont, Irineu T. Velasco, Murilo Chiamolera, J. Sjef Verbeek, Pierre Launay, and Renato C. Monteiro.



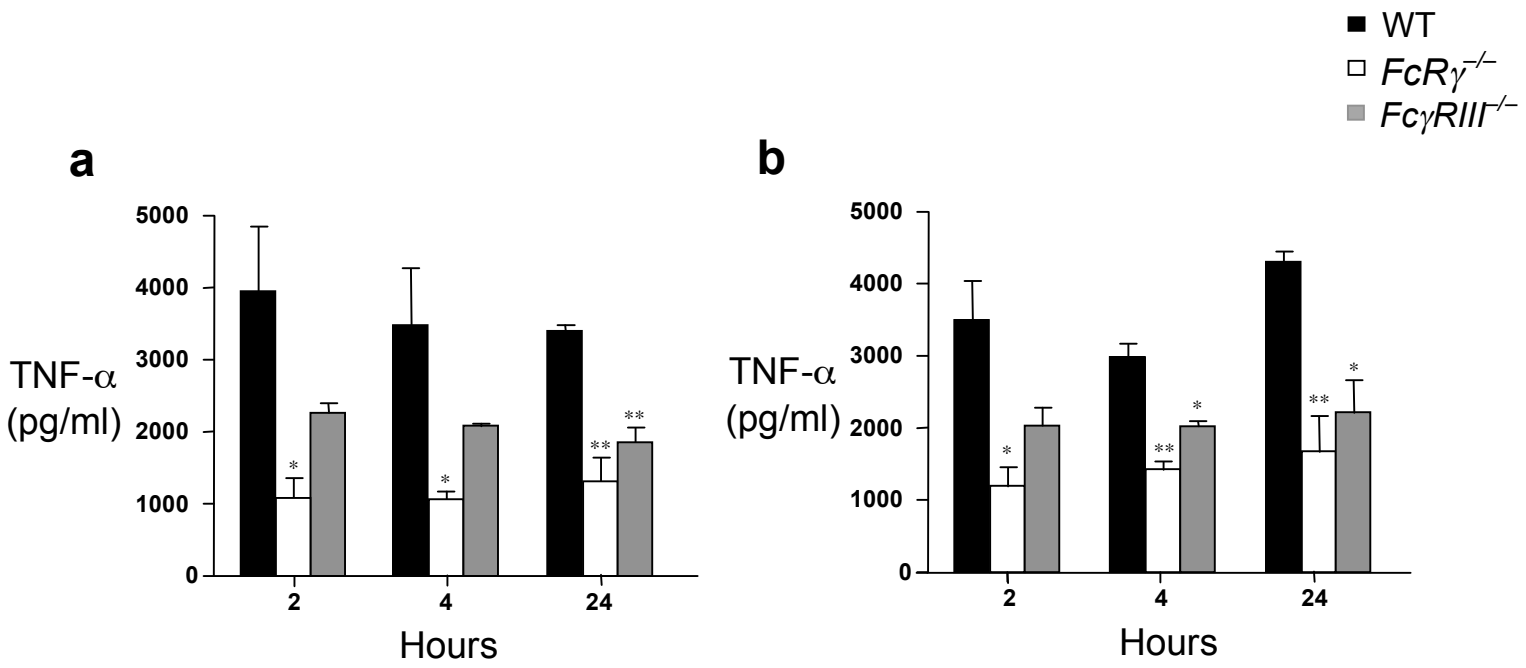
Supplementary Figure 1. 2.4G2 mAb inhibits binding of *E. coli* to macrophages. **(a)** 2.4G2 anti-FcγRII/III mAb inhibits binding of unopsonized *E. coli* to bone marrow-derived macrophages. Six-day-cultured CSF-derived BMM were preincubated with 100 μg/ml 2.4G2 or an irrevelant rat mAb (116), then washed and incubated with bacteria at 4°C for 30 min before washing and FACS analysis. Results are expressed as the percentage inhibition of *E. coli* binding by mAb 2.4G2. Mean fluorescence intensities of WT vs *FcRγ*$^{-/-}$ cells pre-incubated with irrelevant mAb 116 were: 48 ± 3.5 vs 50 ± 3 for non opsonized bacteria and 61 ± 8.4 vs 66 ± 12 for opsonized bacteria, respectively. *$P < 0.05$. Control inhibition by 2.4G2 was even stronger (>65%) with opsonized *E. coli*, confirming the critical involvement of FcγR in the binding of antibody-coated bacteria. **(b)** Decreased *E. coli* binding to peritoneal macrophages from FcRγ or CD16-deficient mice as compared to WT mice. Cells were incubated with FITC-coupled *E. coli* for 30 min on ice and then analyzed by flow cytometry.



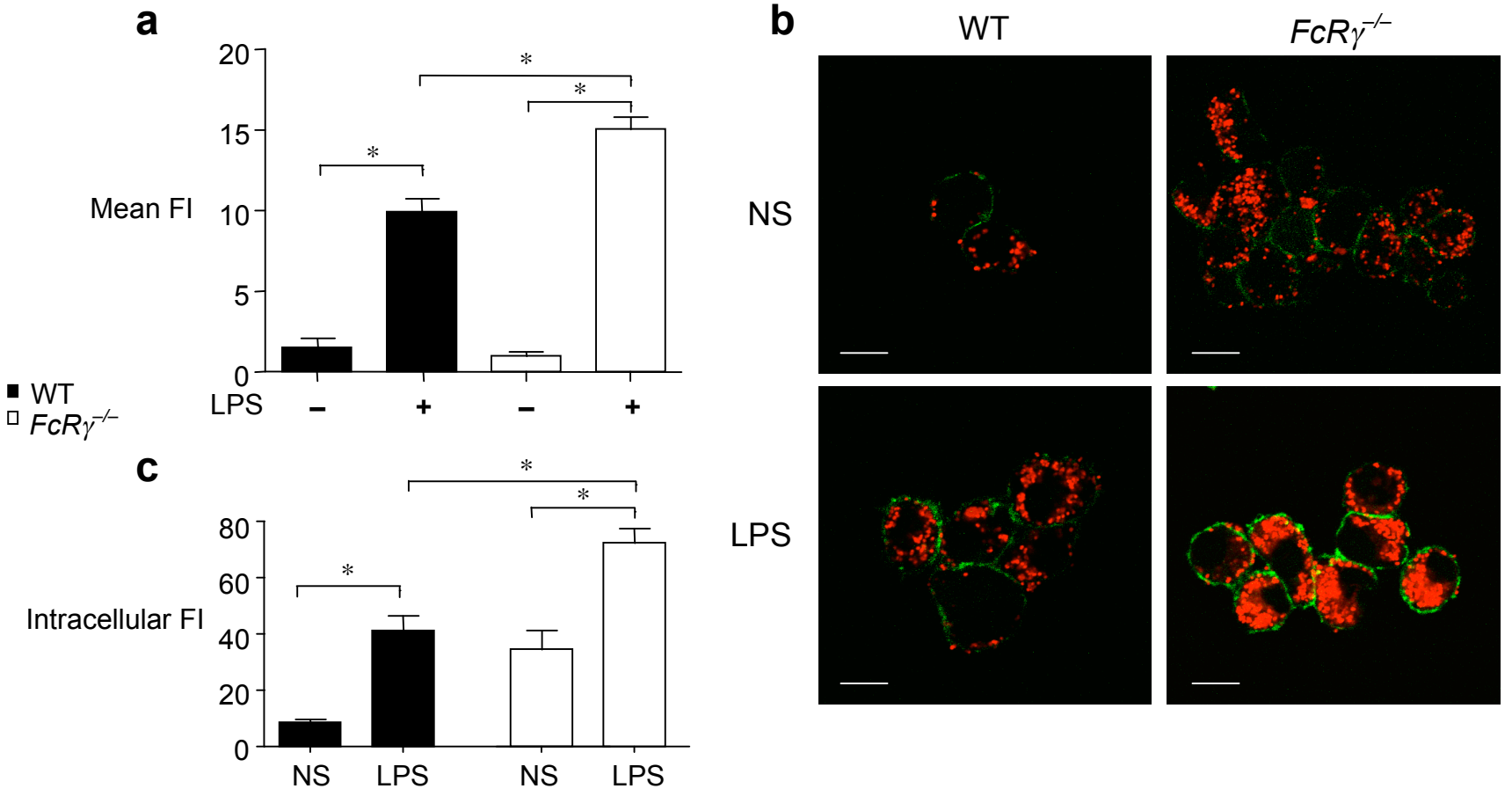
Supplementary Figure 2. *E. coli* induces recruitment of Syk. BMM from WT mice were stimulated with non-opsonized *E. coli* for 5 min at 37°C as indicated, then washed and solubilized in 1% digitonin. Lysates were immunoprecipitated with 2.4G2 or control rat IgG plus protein G Sepharose 4 Fast Flow beads. Immunoprecipitates were resolved by SDS-PAGE, transferred to a PVDF membrane and developed with anti-Syk plus a secondary goat anti-rabbit Ig Ab coupled to HRP.



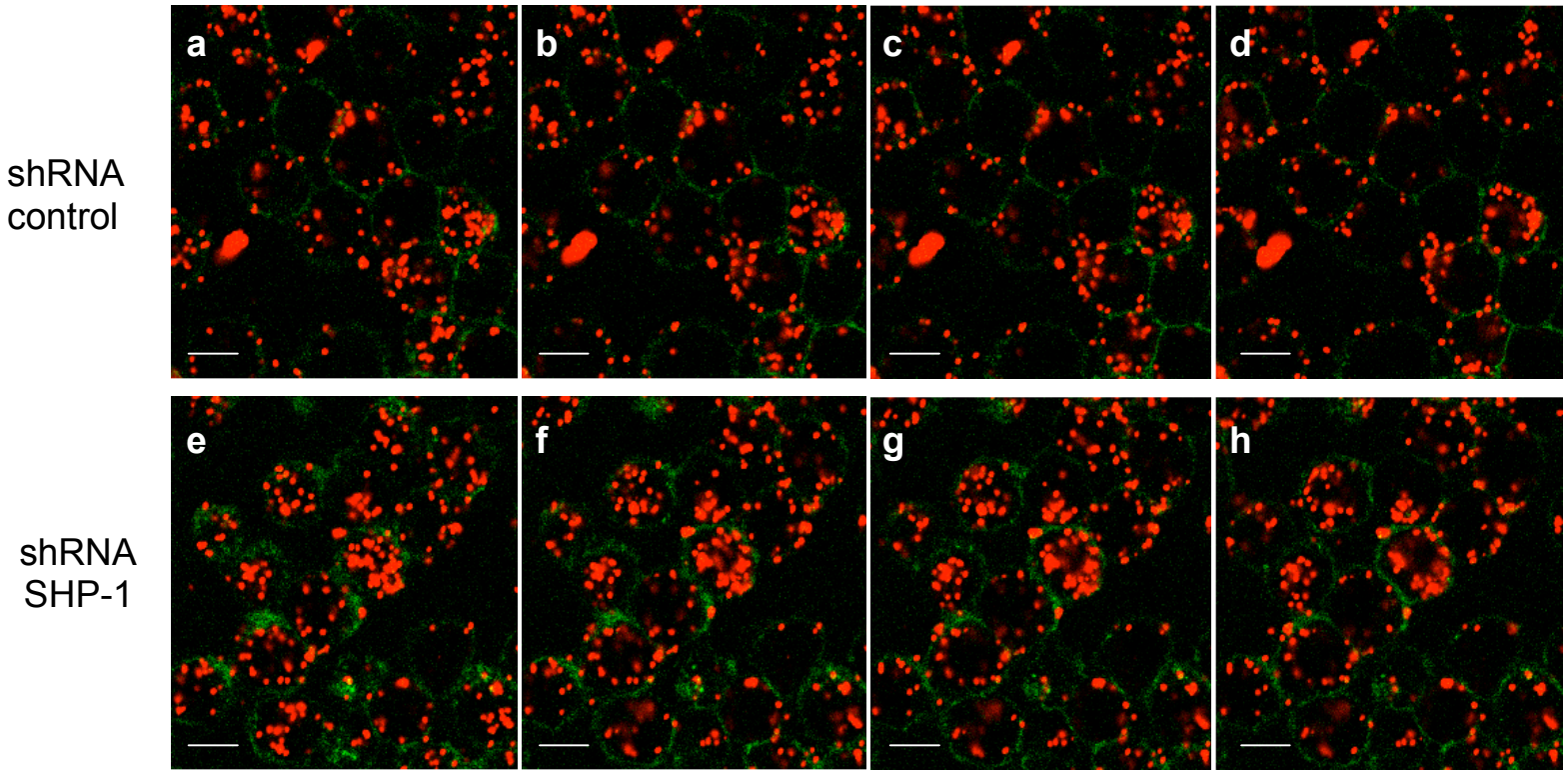
Supplementary Figure 3. Increased phosphorylation of Akt in *FcRγ*$^{-/-}$ macrophages. BMM from the indicated mouse lines were lysed as in Fig 3g, immunoprecipitated with anti-Akt Ab and analyzed in anti-PY immunoblots. Anti-Akt immunoblots were used to control for equal sample loading. Analyses were done by SDS-12% PAGE in non reducing conditions.



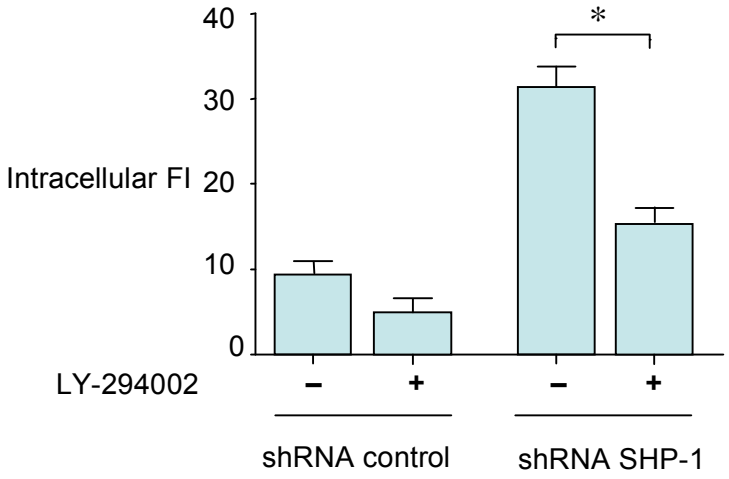
Supplementary Figure 4. FcRγ enhances TNF-α secretion following LPS stimulation. TNF-α release by BMM from *FcRγ*$^{-/-}$ and *FcγRIII*$^{-/-}$ and WT mice exposed to 20 **(a)** and 100 ng/ml **(b)** of LPS (*E. coli* derived) and for different times *in vitro*. TNF-α was measured by ELISA. The data are the means (±SD) of three independent experiments (**P* < 0.05 and ***P* < 0.01, Student's unpaired *t* test as compared to WT cells).



Supplementary Figure 5. Altered expression and function of MARCO in *FcRγ*$^{-/-}$ cells following LPS stimulation. **(a)** MARCO expression was slightly upregulated on FcRγ-deficient cells after LPS stimulation. The data are the means (±SD) of three independent experiments (*P < 0.05, Student's unpaired t test). FI: Fluorescence Intensity. **(b)** Marked upregulation of MARCO-mediated *E. coli* phagocytosis by LPS stimulation of BMM from *FcRγ*$^{-/-}$ mice. BMM were stimulated for 18 h with 100 ng/ml LPS, and phagocytosis of Texas red-labelled *E. coli* was measured as in figure 2a and b. **(c)** Quantification of phagocytosis was performed as in 2b, based on the red fluorescence intensity inside each cell. The data are the means (±SD) of three independent experiments (*P < 0.05, Student's unpaired t test). Scale Bars, 10 μm.



Supplementary Figure 6. Confocal microscopic analysis of *E. coli* phagocytosis in shRNA-treated J774 cells. Four median sections of optically sectioned cells at 0.4-μm intervals are shown. **(a–d)** shRNA control (scrambled sequence), **(e–f)** shRNA for SHP-1. Scale Bars, 10 μm



Supplementary Figure 7. SHP-1-mediated phagocytosis is PI3-K dependent. Phagocytosis of Texas-red labeled *E. coli* was measured shRNA treated J774 cells in the presence or absence of LY-294002 (25 μM) treatment for 20 min as described in Methods. Fluorescence intensity (FI) was quantified as in Fig. 2b. The data are the means (±SD) of three independent experiments (*$P < 0.05$, Student's unpaired *t* test).