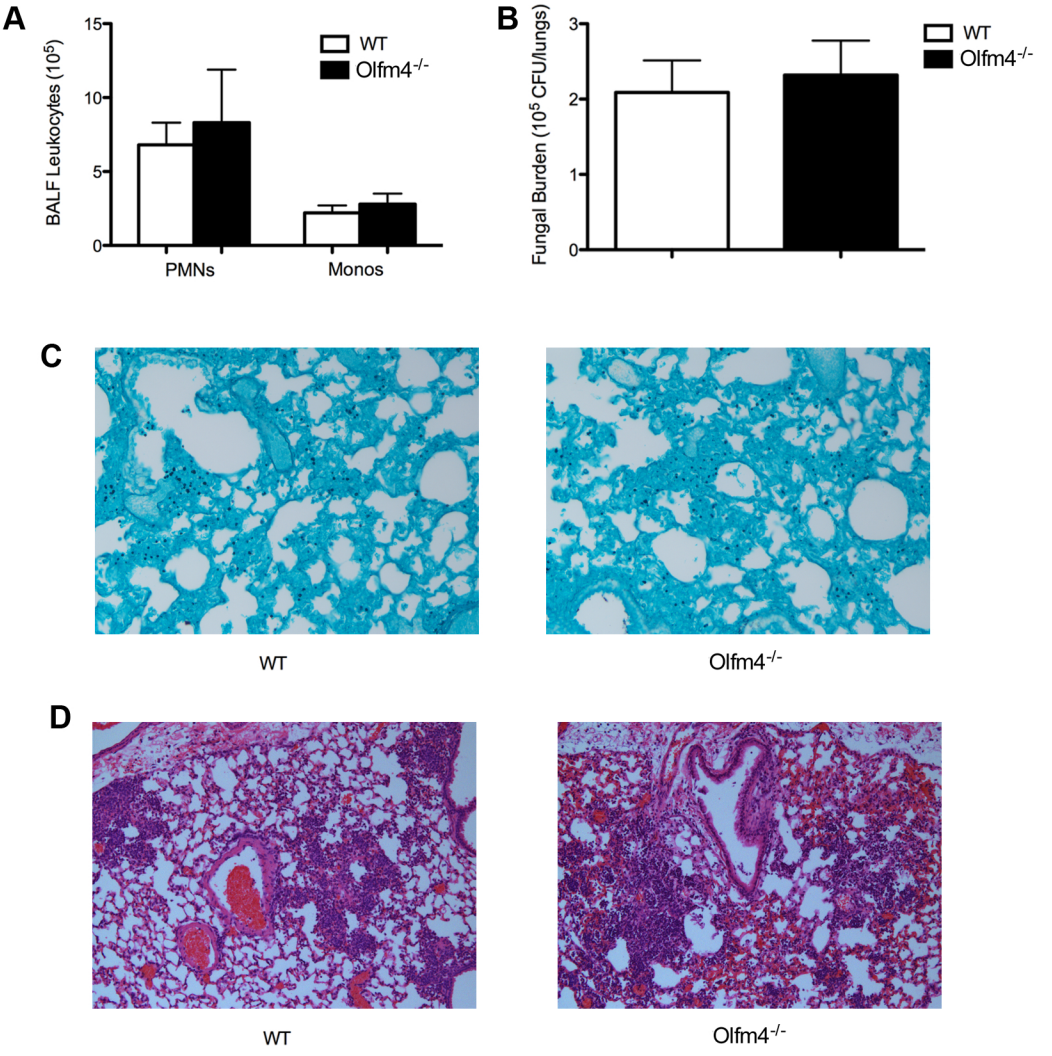
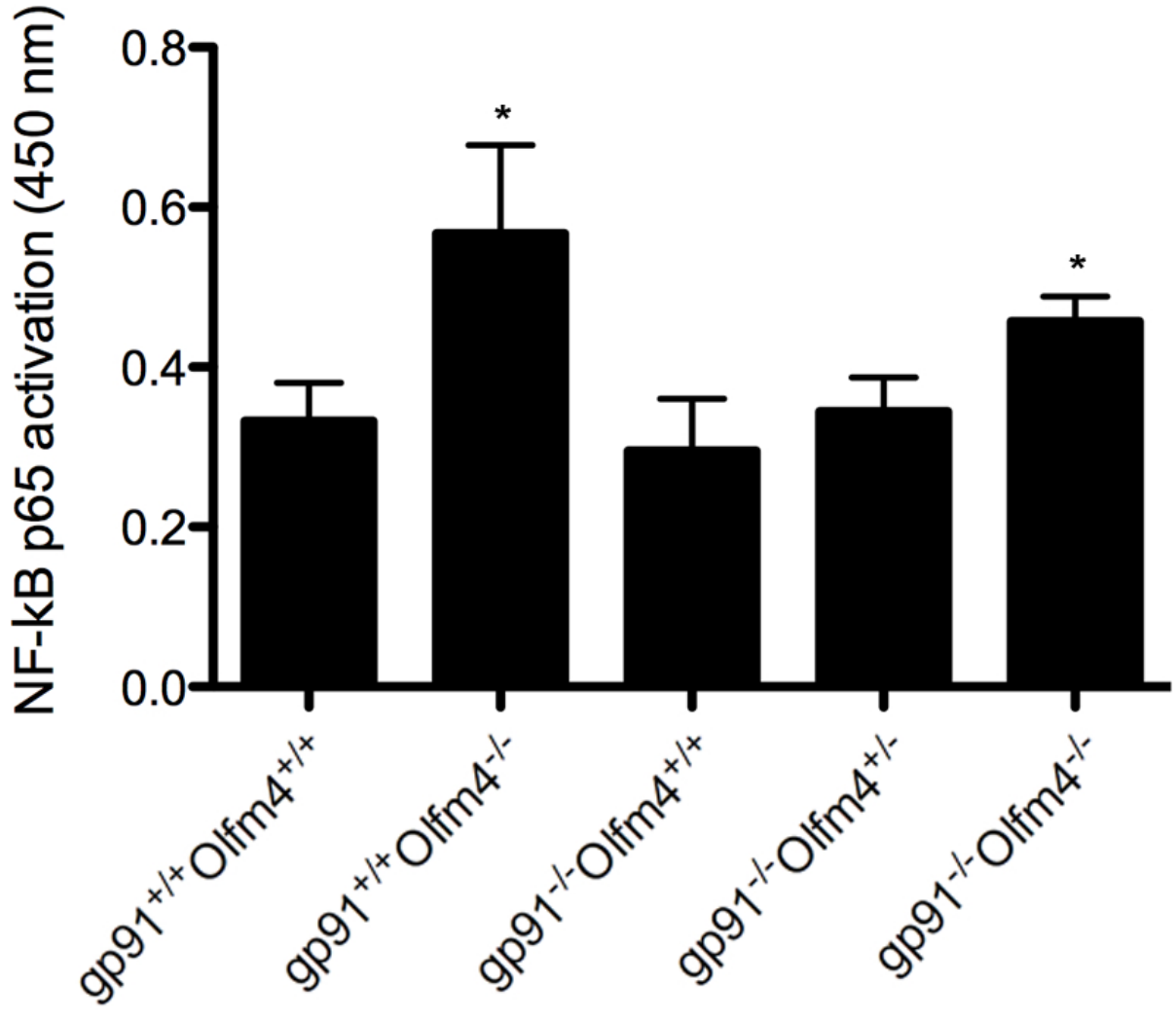
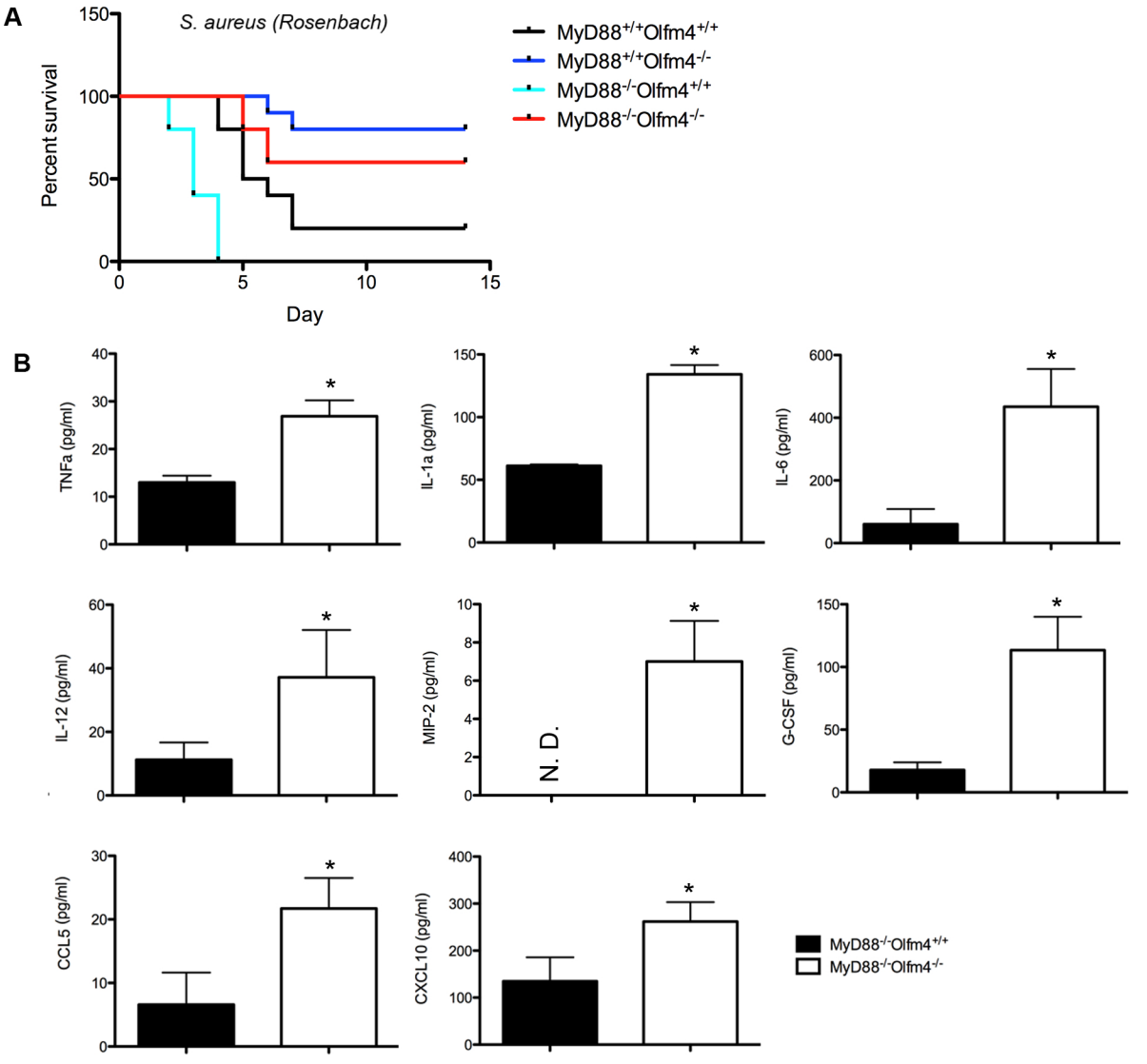


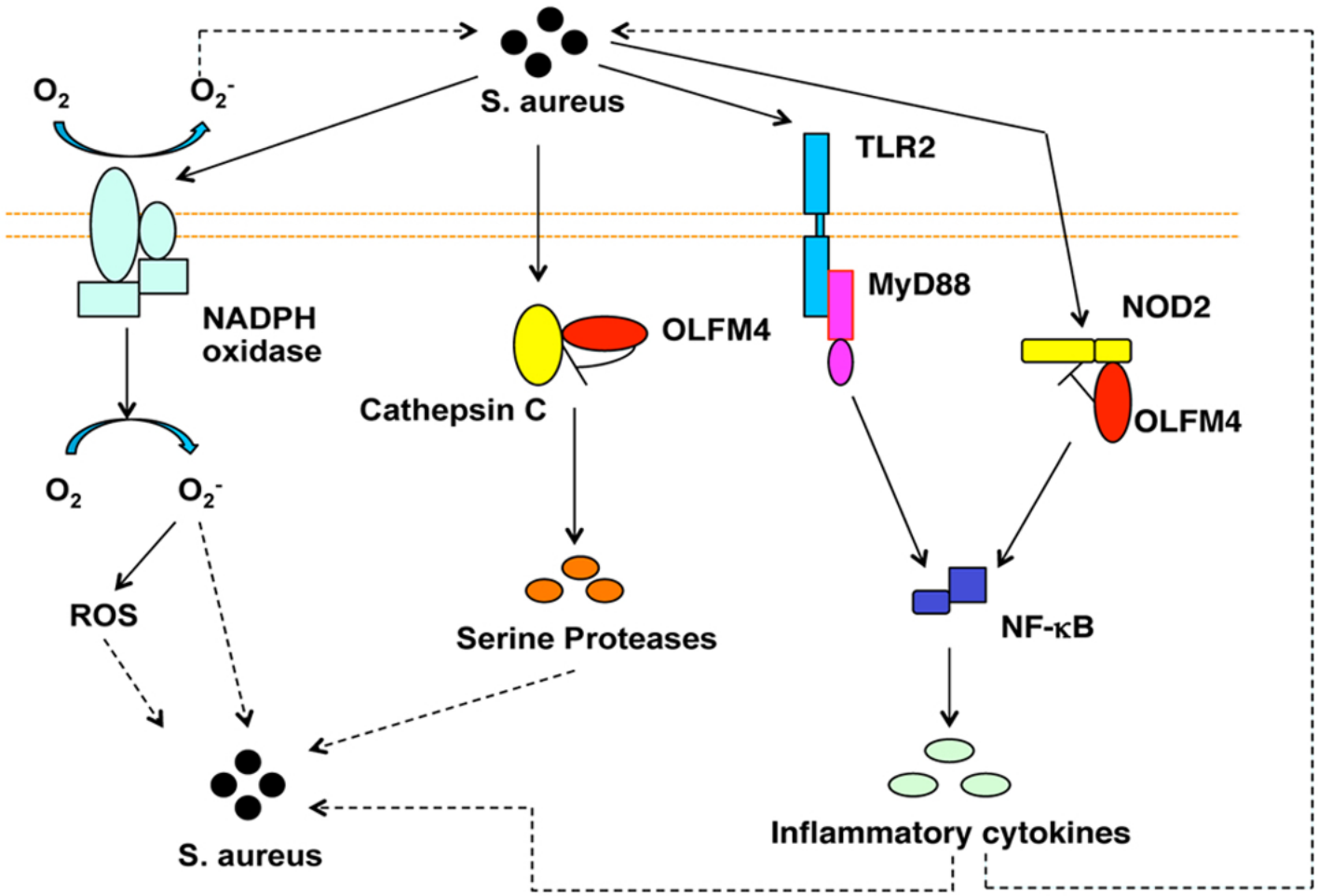
Supplemental Figure 1. Lung inflammation and histology in WT and *Olfm4*-deficient mice after *A. fumigatus* pulmonary infection. WT and *Olfm4*$^{-/-}$ mice were inoculated with *A. fumigatus* (1 x 10^7 CFU per mouse) and sacrificed after 24 hours. (A) Bronchoalveolar fluid (BALF) leukocyte recovery. Data expressed are mean ± SD (n=5). (B) Quantitative fungal cultures in lung homogenates. Data expressed are mean ± SD (n=5). (C) Representative Grocott-Gomori methenamine-silver lung staining (200x), (n=5). (D) Representative lung histology (H&E, 200x), (n=5).



Supplemental Figure 2. NF-κB activity in *Olfm4*-deficient as well as *gp91^{phox}* and *Olfm4* double-deficient mice after *S. aureus* infection. Mice were i.p. infected with *S. aureus (Rosenbach)* (5 x 10^7 CFU per mouse) and neutrophils from the mouse bone marrow were purified 6 hour after infection. NF-κB activation in the neutrophils was measured using the NF-κB p65 Transcription Factor Assay Kit from Active Motif. Data expressed are mean ± SD (n=5). * $p < 0.05$ when compared with WT (*gp91^{phox}* $^{+/+}$*Olfm4*$^{+/+}$) mice.



Supplemental Figure 3. Susceptibility and serum cytokine levels in *Olfm4*- and *MyD88*-deficient mice after *S. aureus* infection. (A) Survival probability plots (Kaplan-Meier) of experimental groups infected i.p. with *S. aureus* (*Rosenbach*) (1 x 10^7 CFU per gram of mouse), n=10. Survival was monitored every 12 hours. (B) Cytokine and chemokine levels in the serum of mice 6 hours after i.p. infection with *S. aureus* (*Rosenbach*) (5 x 10^7 CFU per mouse) were determined by high-throughput immunoassay. N. D., not detected. Data are expressed as mean ± SD (n=5). * $p < 0.05$.



Supplemental Figure 4. OLFM4 is a negative regulator of mouse innate immunity against *S. aureus* infection. *S. aureus* are killed by both NADPH oxidase- dependent and -independent pathways, including granule serine proteases and the TLR-MyD88 and NOD pathways. OLFM4 physically interacts with cathepsin C and NOD proteins and negatively regulates serine protease activity and NF-κB-mediated inflammatory cytokine production. Deletion of *Olfm4* enhances neutrophil intracellular bacterial killing and systemic defense against *S. aureus* infection in CGD mice.

Supplemental Methods

Mice

MyD88-deficient mice in the C57BL/6 background were purchased from Jackson Laboratory. *Olfm4*-deficient mice backcrossed six generations into C57BL/6 background were described previously (8). *Olfm4* and *MyD88* double-deficient mice were derived from crossing *Olfm4*-deficient and *MyD88*-deficient mice.

Nitroblue tetrazolium (NBT) assay

NBT solution was purchased from Gold Biotechnology, Inc. NBT assays were performed according to the Bruce Weinberg protocol (Medical University of South Carolina).

Oxidative burst assay

Mouse bone marrow-derived neutrophils were stimulated with PMA (100 ng/ml). Superoxide production was determined using the Diogenes Cellular Luminescence Enhancement System for Superoxide Detection according to the manufacturer's instructions (National Diagnostics).

Peritoneal bacterial clearance

The peritoneal bacterial-clearance assay was performed as described previously (8). *S. aureus* (2×10^4) was inoculated into the peritoneal cavity of mice. Six hours after i.p. injection, peritoneal exudate fluid was harvested and cultured. The number of viable bacteria (CFU) was determined using the standard-plate method.

A. fumigatus pulmonary infection

Conidial suspensions were prepared as previously described (5) and administered by oropharyngeal aspiration (16). Mice were anesthetized by isofluorane inhalation. With the tongue extended, the liquid volume (50 μl) was delivered into the distal part of the oropharynx and aspirated into the lower respiratory tract and lung.

Bronchoalveolar fluid (BALF) collection and cytospin

BALF collection was performed as previously described (17). The total number of leukocytes/ml was counted using a hemacytometer. Cells were then cytospun onto slides and stained by May-Grünwald-Giemsa, then differential cell counts assessed.

Fungal-burden assessment

Quantitative cultures of lung homogenates were performed as previously described (17). Lungs were homogenized with sterile saline. Lung homogenates were diluted and aliquots plated on malt-extract agar plates, incubated at 37ºC for 24 hours and the number of CFU determined.

Cytokine and chemokine assays

High-throughput multiplex immunoassays were performed with the Procarta cytokine assay kit from Panomics according to the manufacturer's instructions. Samples were assayed and analyzed using Bio-plex.

NF-κB activity assay

NF-κB p65 transcription factor activity was analyzed using Trans AM kit from Active Motif according to the manufacturer's instructions. Nuclear extracts were prepared with the Nuclear Extract Kit from Active Motif.