Supplementary Materials

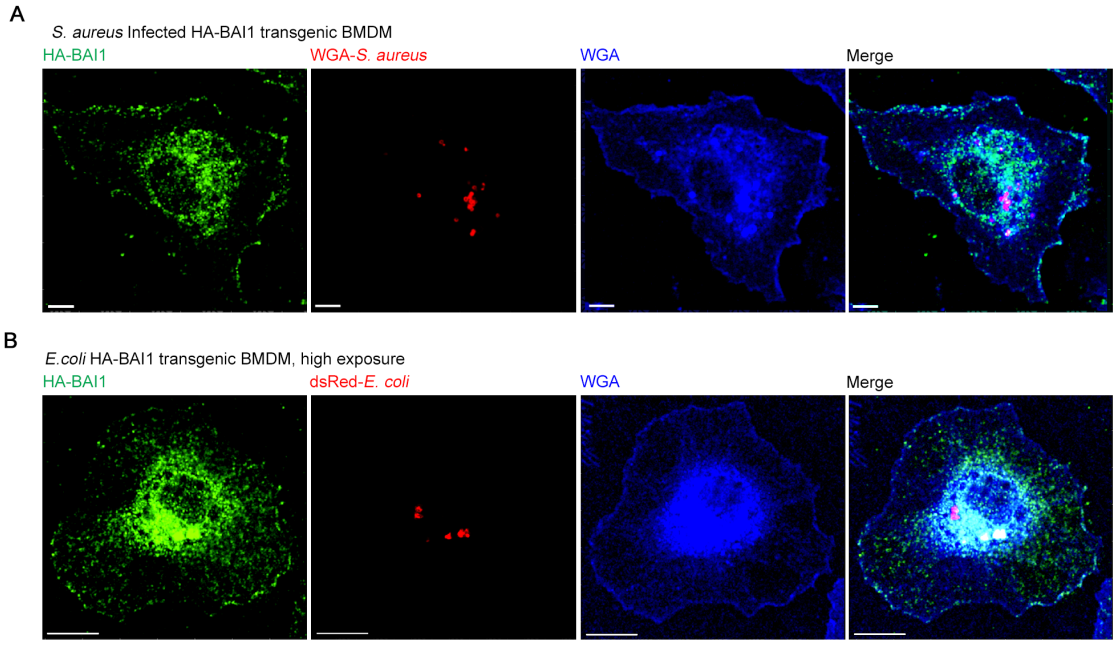

Figure S1. *BAI1 localizes to the cell periphery, the perinuclear region, and sites of bacterial cell association*

A) BMDMs expressing transgenic HA-BAI1 were infected with *S. aureus* at an MOI of 10 for 30 minutes. The image shows the single confocal section from Figure 2A with increased gain to show global cellular distribution of BAI1 after infection. Scale bar, 5μm

B) BMDMs expressing transgenic HA-BAI1 were infected with *E. coli*-DH5α at an MOI of 10 for 30 minutes. The image shows the single confocal section from Figure 2B with increased gain to show global cellular distribution of BAI1 after infection. Scale bar, 5μm

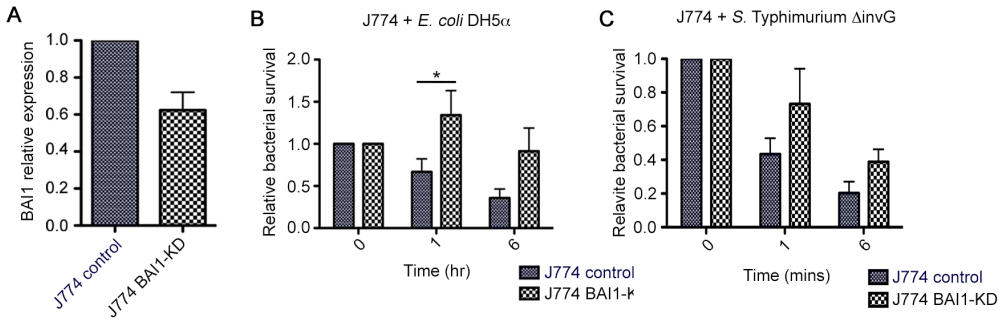

Figure S2. *BAI1 regulates cellular microbicidal activity in J774 macrophages*

A) Stable J774 macrophage cell lines were generated using lentiviral transduction with BAI1 shRNA and subsequent selection with puromycin. Scrambled shRNA was used as a control. BAI1 knockdown was quantified using qRT-PCR. Graph displays mean fold expression relative to control cells ±SEM. N=3

B) Control or BAI1-depleted J774s were infected with *E. coli*-DH5α at an MOI of 25 for 30 minutes. Bacterial killing was measured as described in Figure 3A using the Gentamicin protection assay. Data indicates relative mean survival ±SEM. Data was analyzed using Two-way ANOVA with Bonferroni post-hoc comparisons. Cell ** p<0.01, N=3

C) Assay similar to Supplemental Figure S2B but with non-invasive *S.* Typhimurium (ΔinvG). Time * p<0.05, N=3

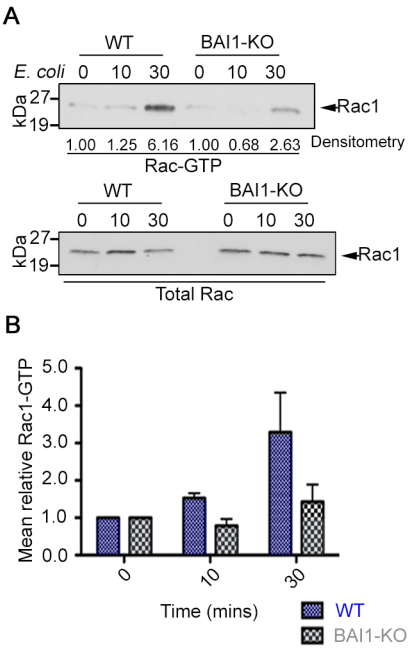

Figure S3. *Loss of BAI1 impairs Rac activation in response to E. coli in IFN-γ primed BMDMs*

A) IFN-γ primed BMDMs were incubated with *E. coli*-BW25113 for 10 or 30 minutes, and Rac activation was measured as described in Fig. 5A. Image shows representative example and densitometric quantitation.

B) Quantitation of Rac1 activation in IFN-γ primed cells, relative to uninfected cells. Graph shows mean fold Rac1-GTP activity ±SEM. Two-way ANOVA with Bonferroni post-hoc comparison was used for analysis. Cell * $p<0.05$, $N=4$

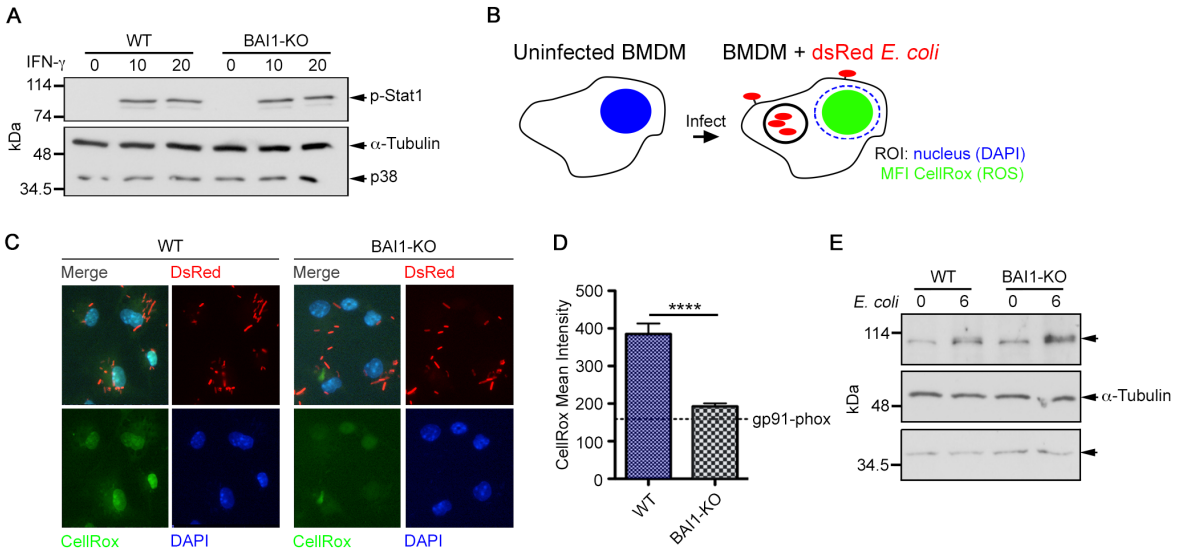

Figure S4. *Loss of BAI1 impairs intracellular ROS responses, but does not affect IFN-γ priming or iNOS induction by E. coli*

A) IFN-γ signaling was measured in cell lysates by immunoblotting for active, phospho-Stat1 in WT and BAI1-KO BMDMs treated with 20ng/ml IFN-γ for the time points shown. Image shows representative example. *N*=3

B) Schematic of CellRox analysis of ROS production in macrophages. WT and BAI1-KO BMDMs were incubated with *E. coli*-DH5α-DsRed. Cells were then incubated with 5μM CellRox Green, fixed, permeabilized and counterstained with DAPI. Cells were imaged using wide-field fluorescence microscopy. Nuclear DAPI signal defined regions of interest (ROI) to measure CellRox mean intensity.

C) Representative images of WT and BAI1-KO BMDMs after treatment with *E. coli*-DH5α and CellRox. Scale bar, 5 μm.

D) Graph shows mean fluorescence intensity ±SEM of nuclear CellRox signal. This was measured from at least 300 cells per replicate after infection. Dashed horizontal line

indicates CellRox fluorescence in gp91phox-KO macrophages. Data was analyzed using a Mann Whitney test. **** $p<0.0001$, $N=4$

E) iNOS expression was analyzed by immunoblotting lysates from WT and BAI1-KO BMDMs incubated with *E. coli*-BW25113 for 6hrs. Image shows representative example. $N=2$

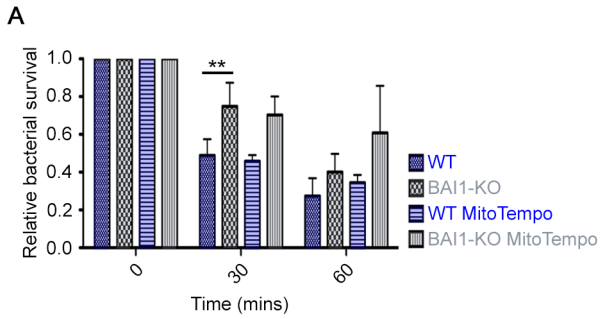

Figure S5. Mitochondrial ROS produced in response to Gram-negative bacteria is not dependent on BAI1-mediated recognition and signaling

A) Cells were pre-treated with either vehicle or the mitochondrial ROS inhibitor MitoTempo (350µM), then incubated with *E. coli*-BW25113 for the times indicated. Bactericidal activity was measured as described in Fig. 3a. Data is displayed as mean survival ±SEM. Data was analyzed using Two-way ANOVA with Bonferroni post-hoc comparisons. WT vs. BAI1-KO Time * $p<0.05$, $N=4$.

A

Disease activity analysis

Score	Posture	Activity	Haircoat	Eyes
0	Normal	Normal	Normal	Normal
1	Hunched	Slight decrease, movement after slight stimluation	Rough	Squinted or closed
2	Hunched, Head on floor	Inactive, movement after moderate stimulation	Ungroomed, hairloss	Squinted or closed with discharge
3	Prone on floor	Immobile	n/a	n/a

Animals with a cummulative score above (7.5) or a (3) in two categories were euthanized

Figure S6. Measurement of Disease Activity Analysis

A) Description of disease activity analysis based on macroscopic observation of behavior.

Movie S1. BAI1 is enriched at the phagocytic cup

A) BMDMs expressing transgenic HA-BAI1 were incubated with fluorescently conjugated anti-HA antibody (green) to label extracellular receptors, then incubated with non-invasive *Salmonella* Typhimurium (ΔinvG) expressing dsRed. Note the concentration of BAI1 in the phagocytic cup and in the nascent phagosome during bacterial uptake. Scale bar, 5 μm