

Supplementary Figure Legends

Figure S1

In vitro susceptibility of COH1 (panel A and B) and H36B (panel C and D) GBS strains and their mutants to various concentrations of erythromycin and clindamycin. WT-COH1 and WT-H36B, wild type strains; *erm*COH1 and *erm*H36B, strains carrying a constitutively activated *ermC* gene.

Figure S2

Inflammatory responses induced in BMDMs by RNA extracted from bacteria with constitutive MLS resistance. TNF- α (A) and nitric oxide (B) production in BMDMs; 5×10^5 macrophages were treated with 0.1, 1 or 10 $\mu\text{g/ml}$ concentrations of RNA extracted from WT-COH1 or *erm*COH1 strains or with LPS (100 ng/ml) and Dotap (25 $\mu\text{g/ml}$) as positive and negative controls, respectively. After 24 hours supernatants were collected and TNF- α or nitric oxide were evaluated by ELISA and Griess test, respectively. Statistical evaluation was performed by the Student's *t* test. ** $p < 0.01$; * $p < 0.05$. Data shown are means + SD of triplicate observations conducted in one experiment representative of three independent experiments.

Figure S3

Inflammatory responses in BMDMs stimulated with killed bacteria. TNF- α (A) and nitric oxide (B) production in wild type C57BL/6 BMDMs; 5×10^5 macrophages were treated with different concentrations (0.1, 1, 5, 10 or 50 $\mu\text{g/ml}$) of heat-killed WT-COH1 and *erm*COH1 bacteria or with LPS (100 ng/ml) as a positive control. TNF- α and nitric oxide were measured in supernatants collected after 24 hours. Statistical evaluation was performed by the Student's *t* test. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. Data shown are means + SD of triplicate observations conducted in one

experiment representative of three independent experiments.

Figure S4

Requirements for *in vitro* inflammatory responses to killed GBS. Nitric oxide induction in BMDCs with defects in MyD88, UNC93b1 (3d) (A), multiple (TLR7/8/9 or TLR3/7/9/11) (B) or single (TLR7, TLR8 or TLR9) TLRs (C). 5×10^5 cells were treated with 0.1, 1 or 10 $\mu\text{g/ml}$ of heat-killed WT-COH1 or *erm*COH1 bacteria or with the TLRs agonists LPS (100ng/ml), CpG B (10 $\mu\text{g/ml}$) and CL264 (25 $\mu\text{g/ml}$). NO levels were measured in supernatants collected after 24 hours. Statistical evaluation was performed by the Student's *t* test. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. Data shown are means + SD of triplicate observations conducted in one experiment and are representative of three independent experiments.

Figure S5

TLR requirements for inflammatory responses to killed GBS in BMDMs. TNF- α (A) and nitric oxide (B) induction in BMDMs with defects in multiple (TLR3/7/9/11^{-/-}) or single (TLR7^{-/-}, TLR8^{-/-} or TLR9^{-/-}) TLR: 5×10^5 cells were treated with 0.1, 1 or 10 $\mu\text{g/ml}$ concentrations of heat-killed WT-COH1 or *erm*COH1 bacteria or with the TLRs agonists LPS (100ng/ml), CpG B (10 $\mu\text{g/ml}$) and CL264 (25 $\mu\text{g/ml}$). TNF- α and nitric oxide were measured in supernatants collected after 24 hours. Statistical evaluation was performed by the Student's *t* test. *** $p < 0.001$; * $p < 0.05$. Data shown are means \pm SD of triplicate observations conducted in one experiment and are representative of three independent experiments.

Figure S6

Requirements for *in vitro* inflammatory responses to live GBS. Nitric oxide induction in BMDCs from C57BL/6 (A) or from BMDC with defects in multiple (TLR7/8/9) or single (TLR2, TLR7, TLR8 or TLR9) TLRs (B) or MyD88 and UNC93b1 (3d) (C). 5×10^5 cells were treated with 5, 10 or 20 MOIs of WT-COH1 or *erm*COH1 bacteria or with the TLR agonists LPS (100ng/ml), CpG B (10 $\mu\text{g/ml}$) and CL264 (25 $\mu\text{g/ml}$). NO levels were measured in supernatants collected after 24 hours. Statistical evaluation was performed by

the Student's *t* test. ****p* < 0.001. Data shown are means + SD of triplicate observations conducted in one experiment and are representative of three independent experiments.

Figure S7

Inflammatory responses in BMDMs stimulated with live bacteria. TNF- α (A) and nitric oxide (B) production in wild type C57BL/6 BMDMs; 5×10^5 macrophages were treated with 5, 10 or 20 MOIs of live WT-COH1 and *erm*COH1 bacteria or with LPS (100 ng/ml) as a positive control. TNF- α and nitric oxide were measured in supernatants collected after 24 hours. Data shown are means + SD of triplicate observations conducted in one experiment representative of three independent experiments.