Supplemental Figure Legends

Figure S1: Identification of TRAF6 ubiquitination upon *S*. Typhimurium infection. *Traf6*$^{+/+}$ and *Traf6*$^{-/-}$ MEFs were infected with wild-type *S.* Typhimurium at a MOI of 10 for 1 hr. The proteins of the infected cells were then separated and subjected to Western blot analysis using rabbit-anti-TRAF6 and anti-GAPDH (as loading controls).

Figure S2: Internalization of the *S*. Typhimurium effector-defective *ΔinvA* and *ΔsopB/sopE2* mutants is mediated by invasin. *Traf6*$^{+/+}$ MEFs were infected with wild-type *S.* Typhimurium (MOI=10), Δ*invA* mutant (MOI=50), Δ*invA* mutant expressing invasin (MOI=50), Δ*sopB/sopE2* mutant (MOI=50) and Δ*sopB/sopE2* mutant expressing invasin (MOI=50) for 1 hr, followed by analysis with intracellular *S.* Typhimurium numbering assays. Values are the mean (±S.D.) of three independent experiments. The asterisks indicate statistically significant (p<0.05) differences when compared to the values infected by wild type *S.* Typhimurium by using the Student's t-test.

Figure S3: TRAF6 promotes entrance of STAT3 into the nucleus and intracellular bacterial replication.

A. TRAF6 is required for phosphorylated STAT3 nucleus localization. *Traf6*$^{+/+}$ and *Traf6*$^{-/-}$ MEFs were infected with *S.* Typhimurium at an MOI of 10 for 1 hr and were fixed with paraformaldehyde after 8-hr infection. Cells were then immunostained with anti-STAT3 pY705 (green) and DAPI (blue) and visualized using fluorescence

microscopy. **B.** Representative images are shown and the percentages of STAT3-phosphorylated cells were determined. The values represent the mean (±S.D.) of three independent experiments in which at least 100 cells were quantified. The asterisks indicate statistically significant differences ($p< 0.05$) when compared to the values of *Traf6*$^{+/+}$ cells using Student's t-test. **C.** TRAF6 is required for *S.* Typhimurium intracellular replication. Serial dilution and cultivation on plates were used to determine c.f.u.s. Values are the mean (±S.D.) of three independent experiments. The asterisks indicate statistically significant differences ($p< 0.05$) when compared to the values of *Traf6*$^{+/+}$ cells using two-way ANOVA with Prism software. **D.** Bacterial intracellular replication depends on the E3 ubiquitin ligase activity of TRAF6. After transfecting *Traf6*$^{-/-}$MEFs ectopically with mock plasmids, pcDNA4-FLAG-TRAF6 or pcDNA4-FLAG-TRAF6 C70A, for 24 h before infection with *S.* Typhimurium for 8 hrs, colony-forming units were determined. Values represent the mean (±S.D.) of three independent experiments. The asterisks indicate statistically significant differences ($p< 0.05$) using Student's t-test.

Figure S4: The effect of chloramphenicol on STAT3 phosphorylation during *S.* Typhimurium infection. Infected and non-infected cells were treated with chloramphenicol (25 μg/mL) at the same time-point of (or equal to) 1 hr post-infection. STAT3 phosphorylation (pY705) was determined via Western blot using anti-GAPDH as a loading control.

Figure S5: TRAF6 catalyzes the formation of Lys63-linked polyubiquitin chains on STAT3 during *S*. Typhimurium infection. HEK293T cells were co-transfected with pcDNA4-FLAG-TRAF6 and pcDNA3-K63 ubiquitin mutant or pcDNA3-K48 ubiquitin mutant for 24 hr before *S*. Typhimurium infection for 1 hr. Samples were harvested at 8 hr post-infection and then immunoprecipitated with rabbit-anti-STAT3 and detected separately with mouse-anti-STAT3, mouse-anti-Ubiquitin, mouse-anti-Ubiquitin (K63 specific) and mouse-anti-Ubiquitin (K48 specific) antibodies via Western blots. WCEs were collected and probed with rabbit-anti-STAT3, mouse-anti-Ubiquitin, mouse-anti-Ubiquitin (K63 specific), mouse-anti-Ubiquitin (K48 specific) antibodies as well as mouse-anti-FLAG antibodies using anti-Tubulin as a loading control.

Figure S6: Quantification of mutations in STAT3 SH2 domain lysine residues on ratio of membrane to total STAT3. Quantification was performed by measuring the total and membrane wild type FLAG-STAT3 or FLAG-STAT3 K (1-6) A as shown in Figure 6D. Values represent the mean (±S.D.) of three independent experiments. Asterisks indicate statistically significant differences ($p< 0.05$) using Student's t-test.

Figure S7: The effect of the absence of TRAF6 on STAT3 membrane recruitment and phosphorylation upon *S*. Typhimurium infection. *Traf6*$^{+/+}$ and *Traf6*$^{-/-}$ MEFs were infected with *S*. Typhimurium (MOI=10). WCE and plasma membrane fractions were analyzed using rabbit-anti-STAT3, rabbit-anti-pSTAT3 (Y705), rabbit-anti-N-

Cadherin and rabbit-anti-Tubulin, respectively.

Figure S8: SopB/SopE2 on STAT3 phosphorylation upon *S*. Typhimurium infection. The effect of SopB/SopE2 on STAT3 phosphorylation were detected by Western blot (**A**) and quantification of fold activation of STAT3 pY705 (relative to uninfected cells) (**B**). The values represent the mean (±S.D.) of three independent experiments. The asterisks indicate statistically significant differences ($p< 0.05$) using Student's t-test.