Supporting Information

Helaine et al. 10.1073/pnas.1000041107

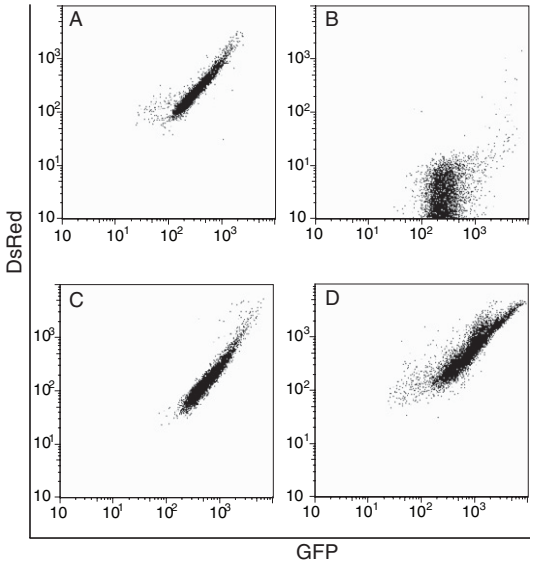


Fig. S1. FD upon replication in minimal medium. (*A–D*) Flow cytometric detection of DsRed and EGFP fluorescence in bacterial population carrying pDiGc plasmid (n = 30,000 events). (*A*) Bacteria grown overnight in minimal medium in the presence of arabinose express both fluorescent proteins. (*B*) Bacteria subcultured for 7 h in absence of arabinose lose red fluorescence. (*C*) Bacteria grown for 7 h in the presence of inducer or (*D*) when replication was blocked for 24 h by antibiotic (tetracycline 5 μg/mL) retain fluorescence.

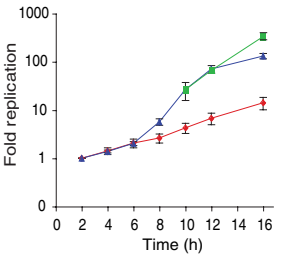


Fig. S2. Quantification of bacterial replication and net growth in RAW264.7 macrophages. Replication kinetics of WT *S.* Typhimurium determined by red FD (dark blue) and subsequent green FD (green) and that of SPI-2 mutant determined by red FD (red). Error bars (SEM) are based on three replicate experiments.

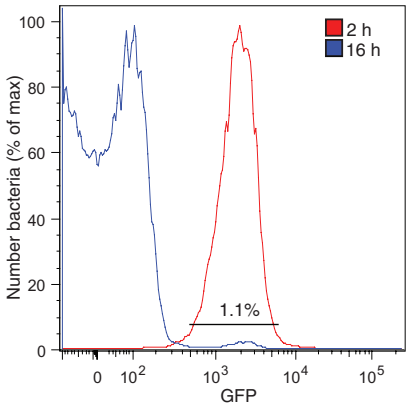


Fig. S3. Analysis of *S.* Typhimurium replication in RAW264.7 macrophages with a pFPV25-derived plasmid. Flow cytometric detection, at different time points, of EGFP fluorescence in the intracellular population of bacteria carrying a pFPV25-derived plasmid (1) encoding EGFP under the control of an arabinose-inducible promoter (P_{BAD}) and mCherry (2) under the control of a constitutive promoter (*P*rpsm; $n = 50\,000$ events). Proportion of nonreplicating bacteria at 16 h after uptake is indicated.

1. Knodler LA, et al. (2005) Cloning vectors and fluorescent proteins can significantly inhibit Salmonella enterica virulence in both epithelial cells and macrophages: implications for bacterial pathogenesis studies. *Infect Immun* 73:7027–7031.
2. Shaner NC, et al. (2004) Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nat Biotechnol* 22:1567–1572.

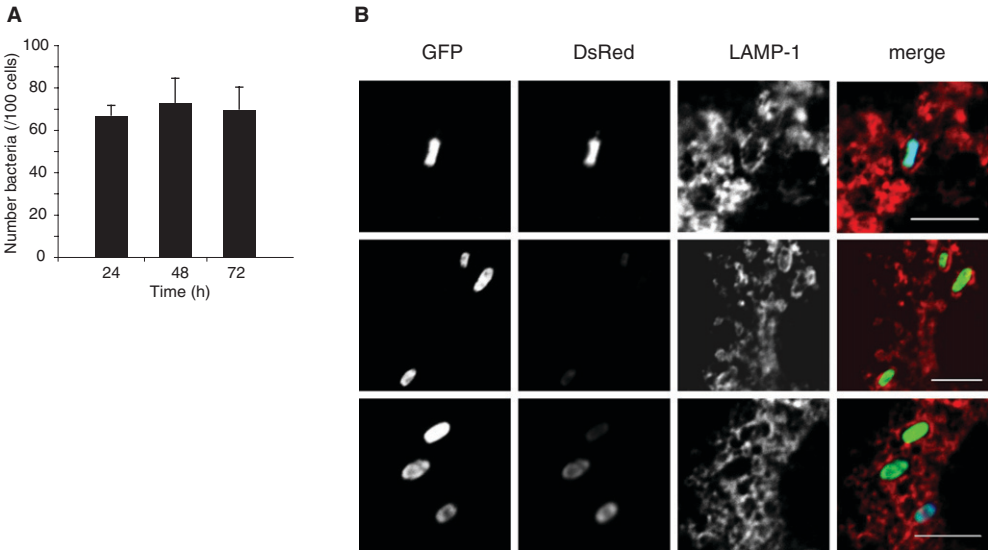
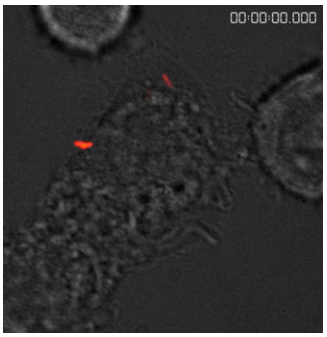


Fig. S4. Characterization of nonreplicating WT *S.* Typhimurium by microscopy. (*A*) Numbers of nonreplicating bacteria in bm macrophages (n = 500–900 macrophages examined). Error bars (SEM) are based on three replicate experiments. (*B*) Confocal microscopy of bm macrophages infected for 24 h and subsequently fixed and labeled for LAMP-1. (Scale bars: 5 μm.) False coloring applied: EGFP (green), DsRed (blue), LAMP-1 (red).



Movie S1. FD in macrophages. Time-lapse microscopy of RAW264.7 macrophages infected with bacteria expressing EGFP and DsRed from pDiGc in the absence of arabinose.

Movie S1.