Supplementary Procedures

β-Galactosidase Activity Assays

Salmonella carrying a single chromosomal copy of *fadBA-lacZ transcriptional fusion* were grown in LB or M9 supplemented or not with oleic acid. β-galactosidase activity was measured over 24 hours using a fluorimeter. Samples were taken at the indicated times, and after resuspension, assayed for β-galactosidase activity as previously described (1). β-galactosidase values were reported to the OD600 and the specific activity was expressed in Miller units (MU) (1).

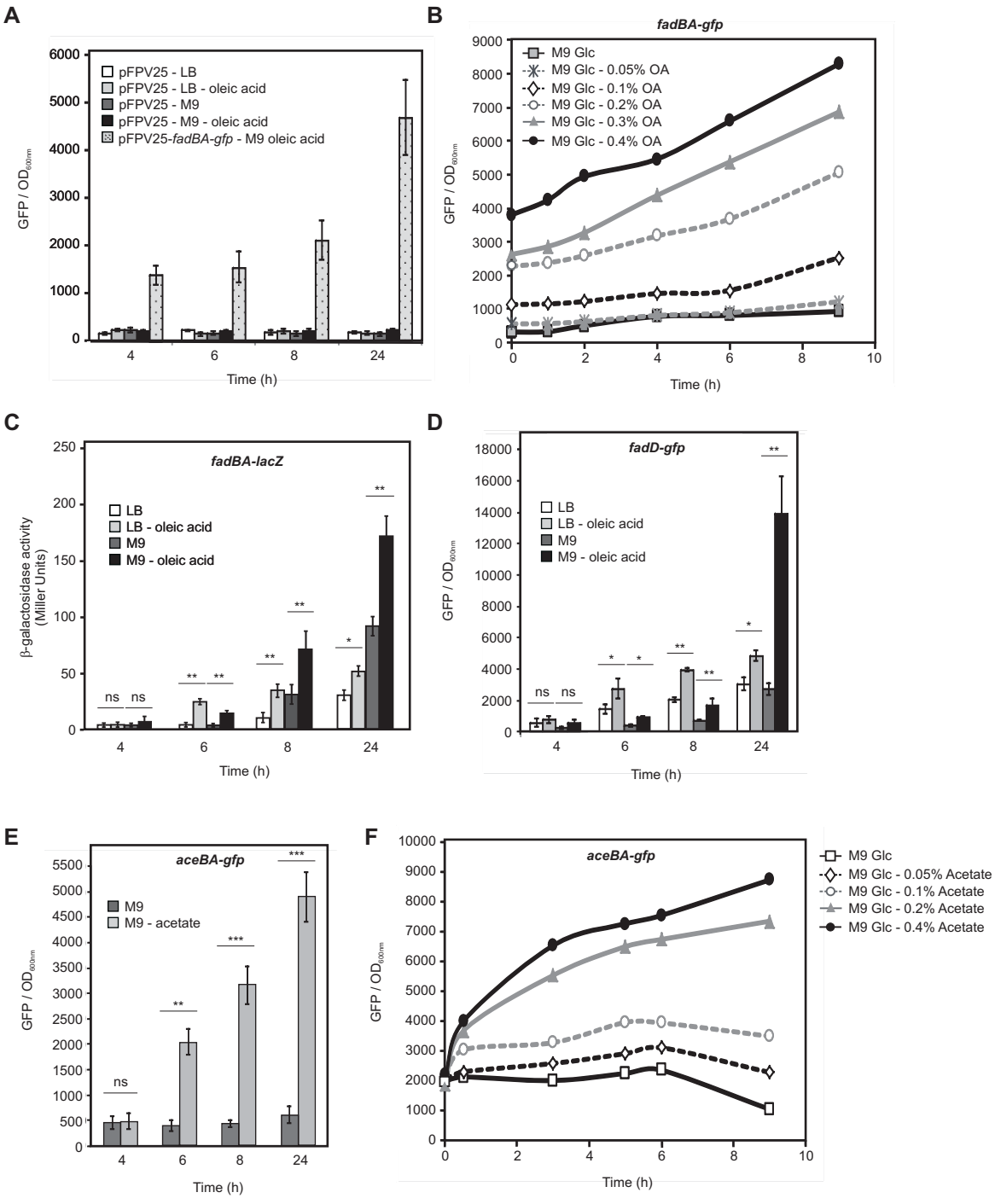
Supplementary Table 1

Strain	*Relevant genotype and/or information*	*Source or reference*
PB7963	12023 – *fadBA-lacZ*	2

References

1. Miller, J. H. (1972) *Experiments in molecular genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

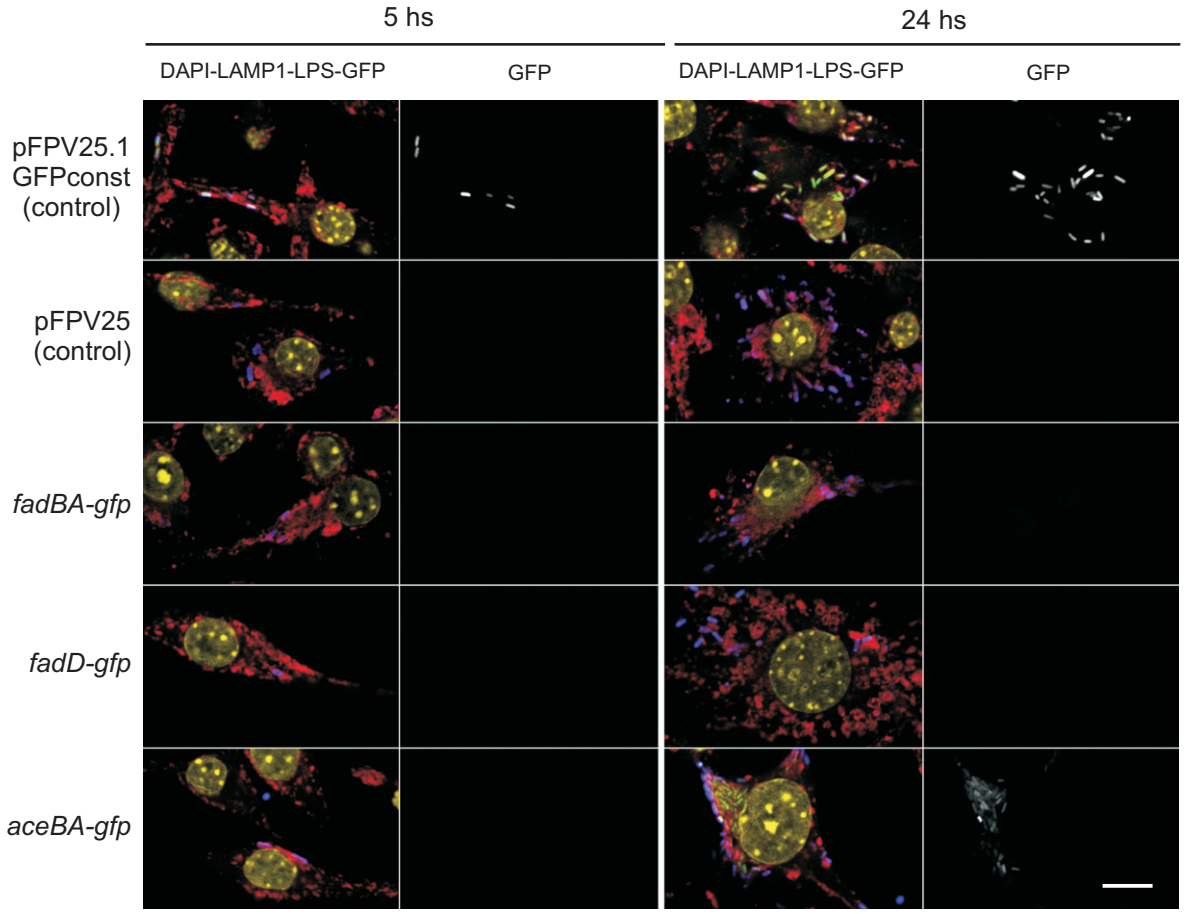
2. Viarengo G, Sciara MI, Salazar MO, Kieffer PM, Furlán RLE, Véscovi EG. Unsaturated long chain free fatty acids are input signals of the Salmonella enterica PhoP/PhoQ regulatory system. *J Biol Chem*. 2013;288(31):22346-22358.



Supplementary Fig. 1

Analysis of Salmonella β-oxidation and glyoxylate shunt pathways in axenic cultures.

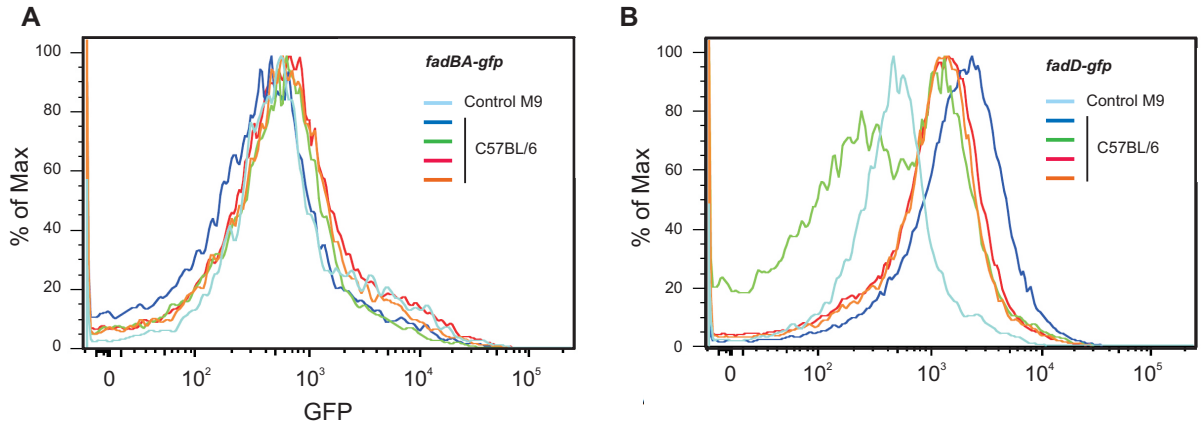
(A) Wild-type *Salmonella* (12023) carrying the plasmid pFPV25 (control) or the plasmid pFPV25 with the *fadBA-gfp* fusion was grown in LB or M9 supplemented or not with oleic acid. (B) Wild-type strains carrying the *fadBA-gfp* fusion were grown in M9 minimal medium to an OD600 of 0.4. Then, different concentrations of oleic acid were added and GFP levels were recorded over 9 hours. (C) β-galactosidase activity from *fadBA-lacZ* transcriptional fusion was measured in bacteria grown in LB or M9 supplemented or not with oleic acid. (D) Wild-type *Salmonella* (12023) carrying the *fadD-gfp* fusion was grown in LB or M9 supplemented or not with oleic acid. (E) Wild-type *Salmonella* (12023) carrying the *aceBA-gfp* fusion was grown in M9 or M9 supplemented with acetate. GFP synthesis was recorded over 24 h using a fluorimeter. The fluorescence levels shown on the graphics were calculated as the GFP values reported to the Od600. (F) Wild-type strains carrying the ace*BA-gfp* fusion were grown in M9 minimal medium to an OD600 of 0.4. Then, different concentrations of acetate were added and GFP levels were recorded over 9 hours. Values are means ± SD of 3 independent experiments. Unpaired t-test was used to determine whether two values were significantly different. *P*-values: ns, not significant; *, *P*<0.05; **, *P*<0.01; ***, *P*<0.001 .



Supplementary figure 2

Confocal analysis of β–oxidation and glyoxylate shunt probes during macrophages infection

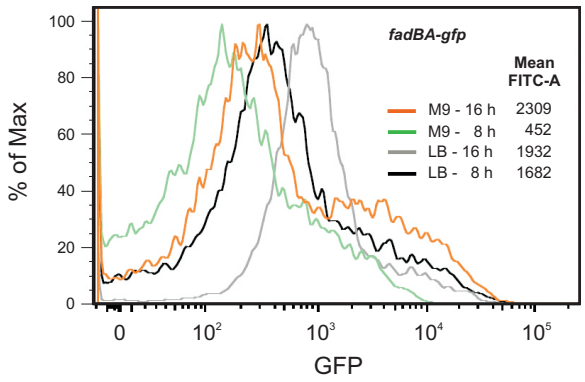
RAW 264.7 macrophages cells were infected for 5 or 24 hours with *Salmonella* strains harboring the indicated GFP fusions. Cells were fixed, immunolabeled for LAMP1 (red), DAPI (yellow), LPS (blue), and imaged by confocal microscopy for GFP (green) (scale bar, 10 μm). Strains porting the empty pFPV25 vector and pFPV25.1, expressing GFP constitutively were used as a control.



Supplementary figure 3

Cytometry analysis of Salmonella β-oxidation pathway during infections in vivo

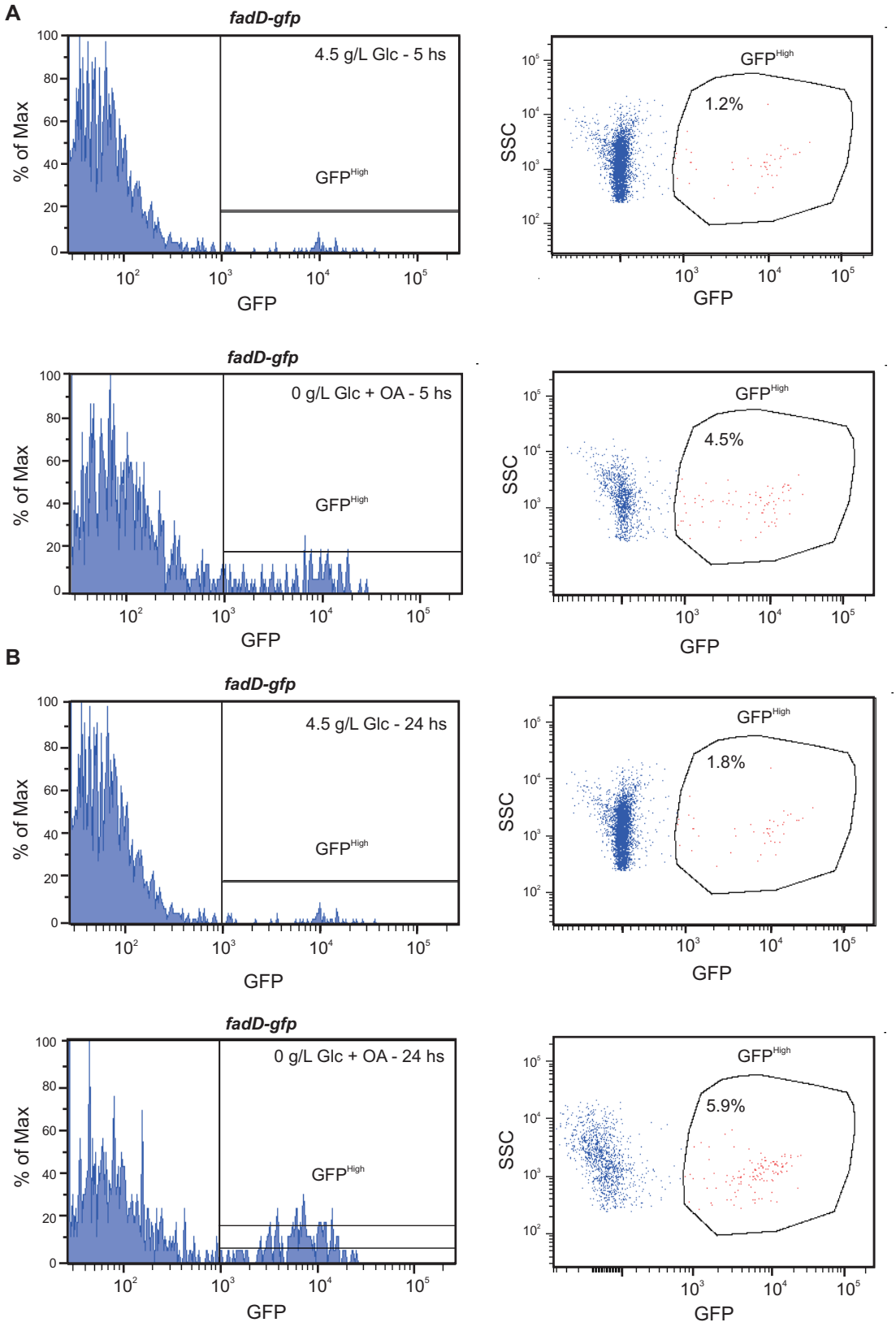
(A and B) C57BL/6 mice were infected for 48 h with wild-type *Salmonella* strains carrying the fluorescence fusions; spleens were removed and homogenized in water with TX-100. The relative fluorescence intensity of the injected bacteria and bacteria present in spleens was determined by flow cytometry. Histogram graphs that display the relative GFP fluorescence versus the number of events shows a Gaussian distribution indicating GFP expressing bacteria. Wild-type *Salmonella* carrying the *fadBA-gfp* (A) and *fadD-gfp* (B) fusions before infection (light blue, control), extracted from spleens (blue, green, red, and orange, each color correspond to a different mouse) (n = 4).



Supplementary figure 4

Cytometry analysis of Salmonella fadBA gene expression in axenic cultures

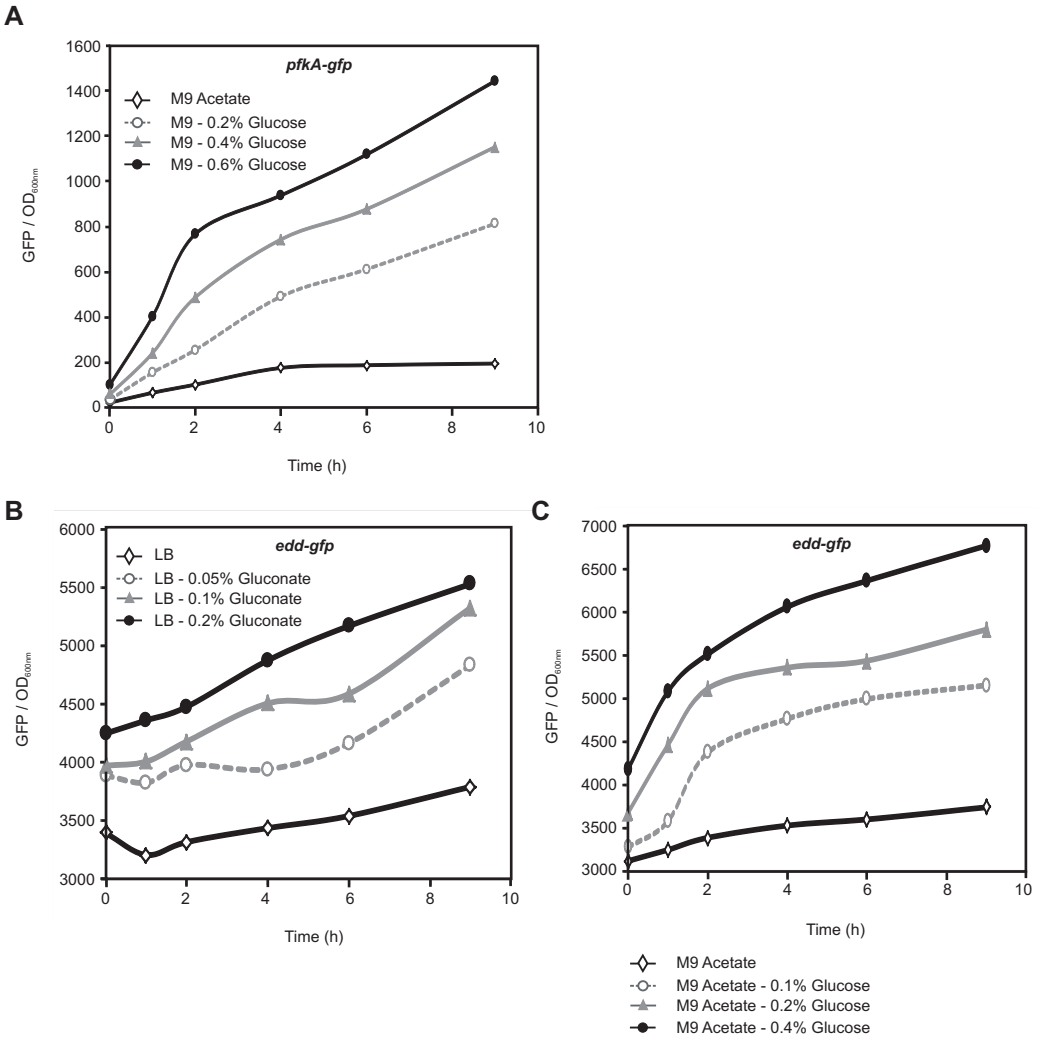
Wild-type *Salmonella* (12023) carrying the *fadBA-gfp* fusion was grown in LB or M9 media during 8 or 16 h. The relative fluorescence intensity of the bacteria was determined by flow cytometry. Histogram graphs that display the relative GFP fluorescence versus the number of events shows a Gaussian distribution indicating GFP expressing bacteria. These data are representative of at least three independent experiments.



Supplementary Fig. 5

Cytometry analysis of fadD gene expression during RAW 264.7 macrophages infections

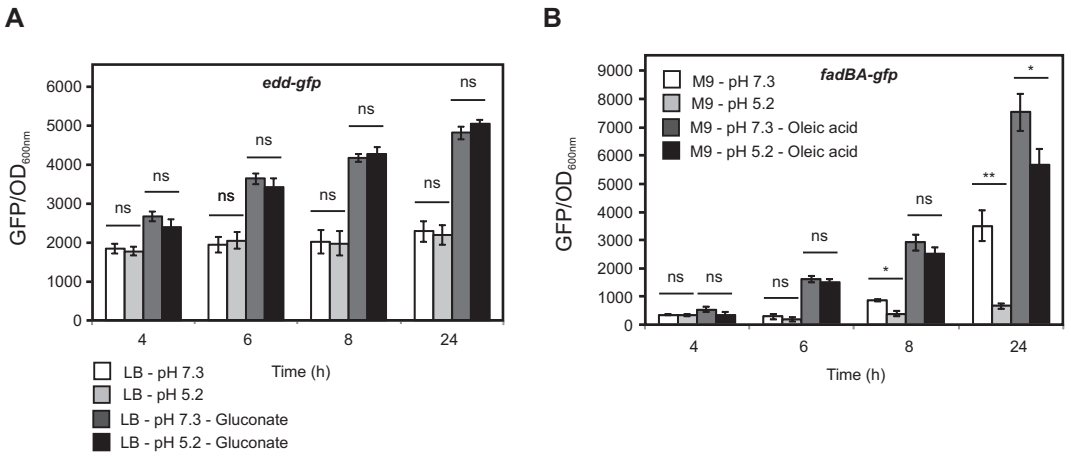
Wild-type strains carrying *fadD-gfp* fusion were cultured in M9 during 3.5 h, opsonized and phagocytized by IFN-γ activated RAW 264.7 cells, cultured in DMEM-highGlc (upper panels) or DMEM-nonGlc-OA (lower panels). 5 (A) and 24 (B) hours PI, macrophages were lysed, bacteria extracted, and the mean fluorescence and the fraction of GFP high bacteria was determined by flow cytometry. 5000 bacteria were analyzed for each sample. The left histogram graphs that display the relative GFP fluorescence versus the number of events shows a Gaussian distribution indicating GFP expressing bacteria. The rigth panels shows the GFP versus side scatter channels (SSC) analysis for wild-type *Salmonella,* carrying the *fadD-gfp* fusion extracted from macrophages. Bacteria contain a small population expressing a high level of GFP (GFP^{high}) (red and gate), compared with bacteria expressing low level of GFP (blue). *Salmonella* positive gates were drawn based on plots from samples stained with an appropriate isotype control antibody. Numbers indicate the percentage of GFP^{high} bacteria in each gate. The data presented are representative of three independent experiments.



Supplementary Fig. 6

Analysis of Salmonella glucolysis and Entner-Doudoroff pathways in axenic cultures.

(A) Wild-type *Salmonella* (12023)strains carrying the *pfkA-gfp* fusion were grown in M9 minimal medium with acetate of different concentrations of glucose were and GFP levels were recorded over 9 hours. (B) Wild-type strains carrying the *eddBA-gfp* fusion were grown in LB medium to an OD600 of 0.4. Then, different concentrations of gluconate were added and GFP levels were recorded over 9 hours. (C) Wild-type strains carrying the *eddBA-gfp* fusion were grown in M9 medium with acetate to an OD600 of 0.4. Then, different concentrations of glucose were added and GFP levels were recorded over 9 hours. GFP synthesis was recorded over using a fluorimeter. The fluorescence levels shown on the graphics were calculated as the GFP values reported to the OD600. All the results shown in these panels are representative of at least three independent experiments.



Supplementary Fig. 7

Analysis of Salmonella Entner-Doudoroff and β-oxidation pathways in axenic cultures in different pH condictions

(A) Wild-type strains carrying the *edd-gfp* fusion were grown in LB (pH 7.3) medium to an OD600 of 0.4. Then, bacteria were collected by centrifugation and grown in different media: LB pH 7.3 or LB pH 5.2, supplemented or not with gluconate. (B) Wild-type strains carrying the *fadBA-gfp* fusion were grown in M9 (pH 7.3) medium to an OD600 of 0.4. Then, bacteria were collected by centrifugation and grown in different media: M9 pH 7.3 or M9 pH 5.2 media, supplemented or not with oleic acid. GFP synthesis was recorded over 24 hours using a fluorimeter. The fluorescence levels shown on the graphics were calculated as the GFP values reported to the OD600. Values are means ± SD of 3 independent experiments. Unpaired t-test was used to determine whether two values were significantly different. *P*-values: ns, not significant; *, $P<0.05$; **, $P<0.01$.