Supplementary Material

Dynamic remodeling of the endosomal system by intracellular *Salmonella*

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Supplementary Figures

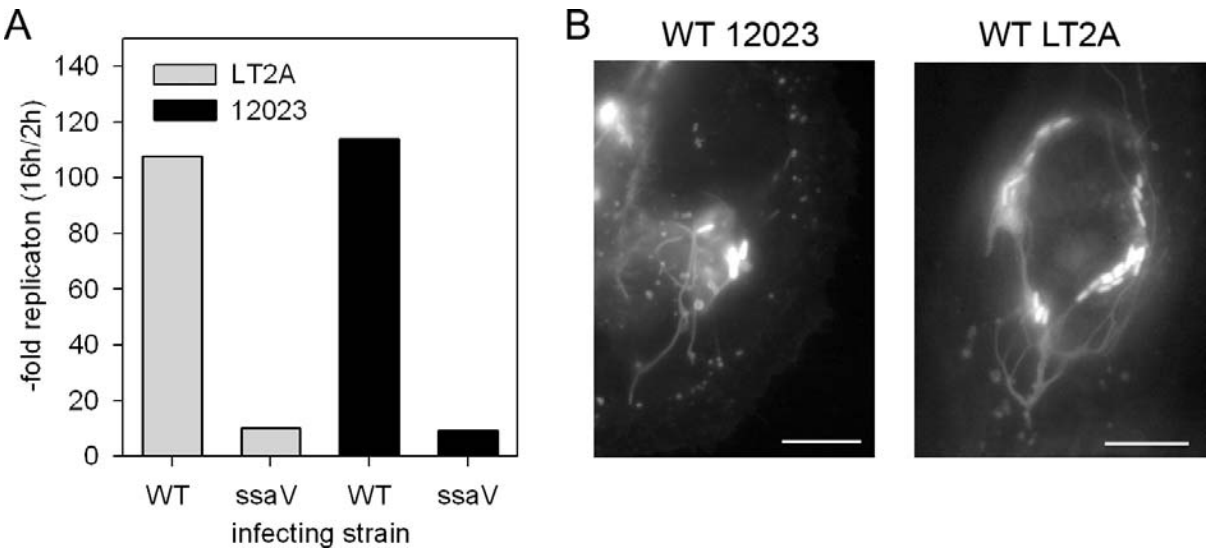


Fig. S 1. Intracellular replication of various *Salmonella enterica* strains and SIF induction. A) The intracellular replication of *S*. Typhimurium strains LT2A and 12023 was compared. HeLa cells were infected with wild-type (WT) or SPI2-deficient strains (*ssaV*) and non-internalized bacteria were killed by addition of Gentamicin. The number of viable intracellular bacteria or colony-forming units (CFU) was determined at 2 h and 16 h after infection by lysis of host cells and plating of lysates onto agar plates. The –fold intracellular replication was determined by the ratio of CFU counts at 16 h and 2 h after infection. B) LAMP-1-GFP-transfected HeLa cells were infected with GFP-expressing *Salmonella* WT 12023 or LT2A as indicated. Live cell imaging was performed 7 h after infection and representative stills of a time lapse series are shown. Scale bar, 10 μm.

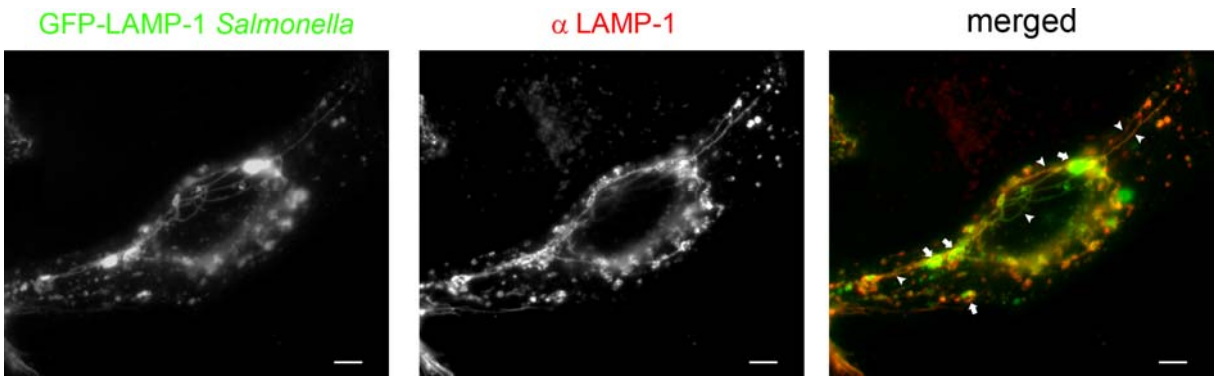


Fig. S 2. Localization of LAMP-1-GFP and endogenous LAMP-1 in *Salmonella*-infected cells. HeLa cells were transfected with the LAMP-1-GFP construct (green) and infected with *Salmonella* WT expressing GFP (green). 6 h after infection, the cells were fixed and processed for immuno-staining of LAMP-1 (red). The position of intracellular *Salmonella* and SIF is indicated in the merged image by arrows and arrowheads, respectively. Scale bar: 5 μm.

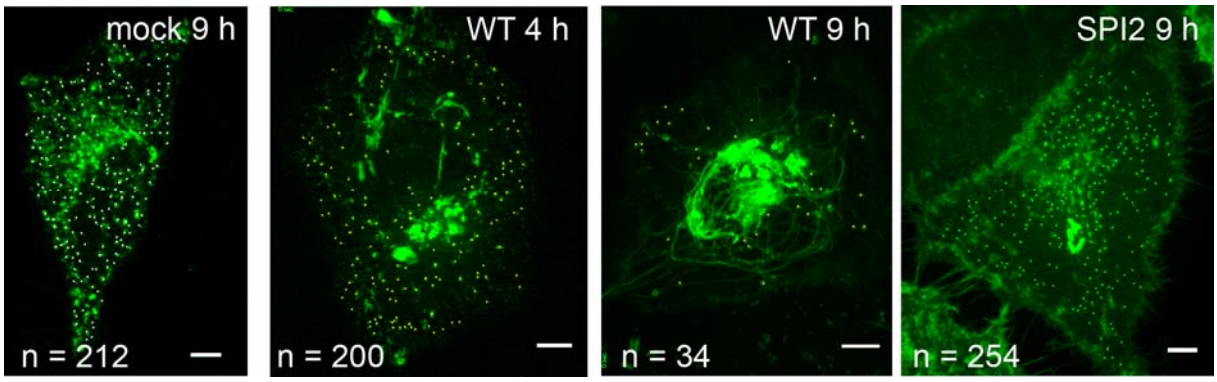


Fig. S 3. Quantification of LAMP-1-positive vesicles. HeLa cells were transfected with the LAMP-1-GFP construct. A representative still is shown from a time lapse series. The quantification of the number of spherical, LAMP-1-GFP-positive vesicles was performed manually using the Edit mode of EBML ImageJ. Scored compartments were marked by yellow dots and the total number of events is indicated. This approach was used for the quantification shown in Fig. 2B.

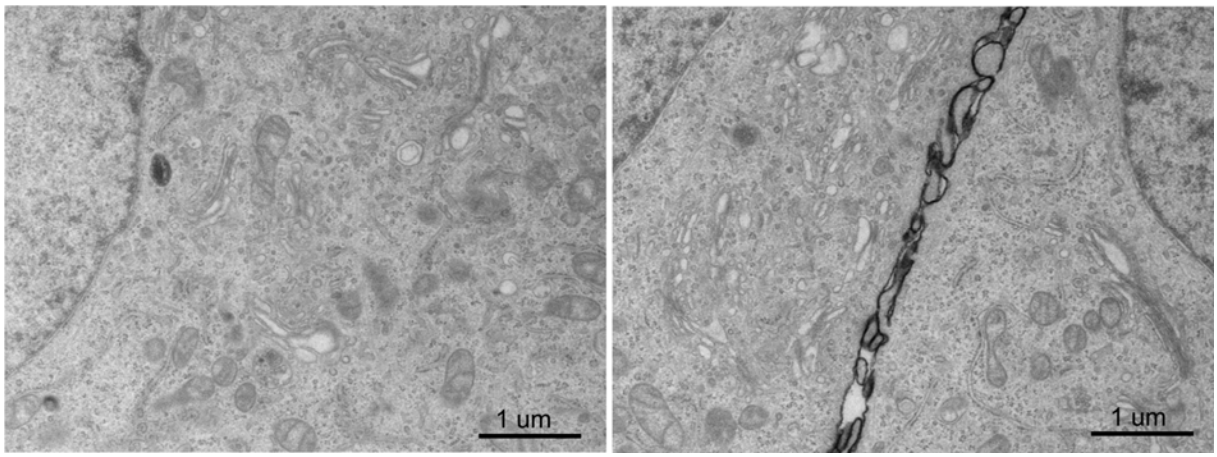


Fig. S 4. Ultrastructure of the endosomal system in mock-infected cells. HeLa cells were mock infected in parallel to infection with WT *Salmonella* and processed as described for Fig. 3C. Representative mock-infected cells are shown. Scale bars, 1 μm.

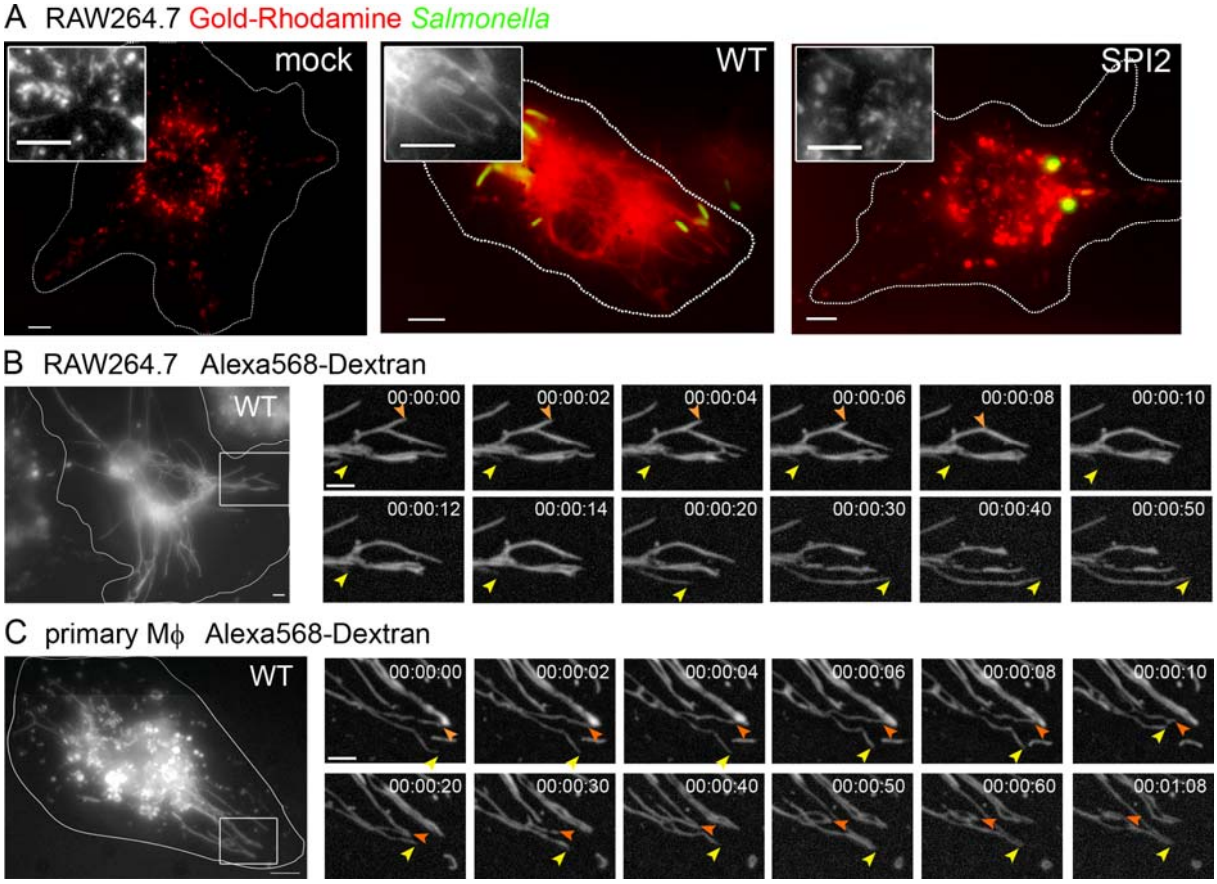
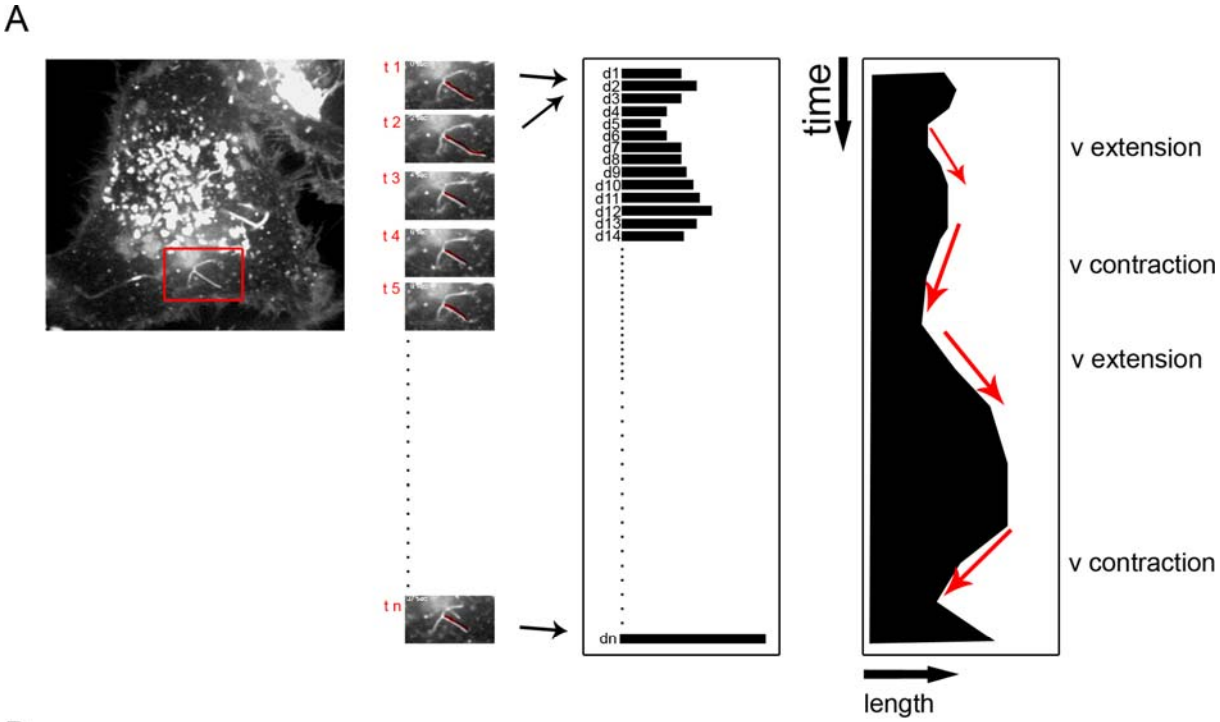


Fig. S 5. *Salmonella* induces highly dynamic SIF in various host cells types. Infection experiments were performed with the murine macrophage-like cell line RAW264.7 after activation by 5 ng/ml IFNγ (A). Cells were mock infected or infected with *Salmonella* WT or SPI2 strains expressing GFP (green). The fluid phase marker 10 nm gold-BSA-rhodamine was added 5 h after infection. Living cells were imaged 8 h after infection using a Zeiss Axiovert 200M wide field microscopy. Scale bars, 5 μm. IFNγ-activated RAW264.7 cells (B, Suppl. Movie 11) or primary murine macrophages (C, Suppl. Movie 11) were used for infection with *Salmonella* WT and the fluid phase marker Alexa568-dextran was used to follow SIF formation in living cells imaged 8 h after infection. Left panels show the overview of the infected cells (scale bars, 5 μm) and boxes indicate positions in the periphery of the cells where representative stills show the details of dynamic alternations of SIF (scale bars, 1 μm). The relative time is expressed as hh:mm:ss and SIF growth and SIF contraction is indicated by yellow and orange arrowheads, respectively.


A
t 1
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v extension
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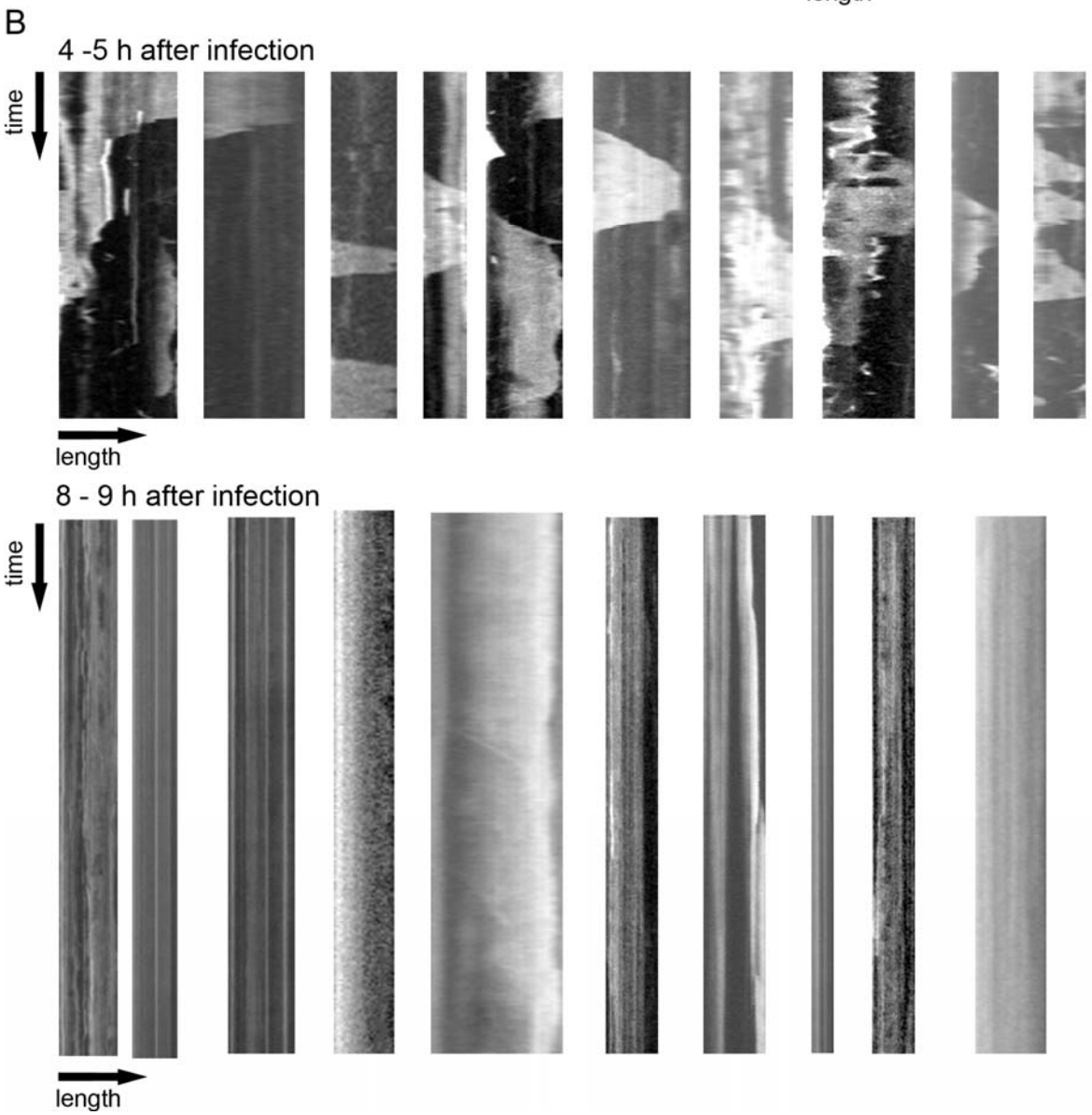
B
4 -5 h after infection
time
length
8 - 9 h after infection
time
length

Fig. S 6. Analyses of SIF dynamics by kymographs. A) Schematic representation of the analysis of SIF extension and contraction. Individual SIF were identified in time lapse series and analyzed by the EMBL ImageJ plugin Kymograph. Kymograph (also called time-space plot) is a graphical method of displaying and analysing moving structures. To create such a plot the trajectory of the moving object has to be found. Once this is defined the pixel values of this trajectory are copied to a new image. This procedure is repeated for each frame of the image stack. If the moving structure is resulting from a fluorescently labeled particle it is represented by a bright line in the kymograph. The slope of this line is proportional to the velocity of the moving particle and also dependent on the directionality of the particle. Non moving particle can be identified by vertical or horizontal lines (depending on the plotting method). Thus it is possible with this method to analyse the speed and directionality of particles.

B) Kymographs for individual SIF recorded at 4-5 h or 8-9 h after infection of LAMP-GFP-transfected HeLa cells with *Salmonella* WT. These are examples of the kymographs used to calculate the data shown in Fig. 7C.

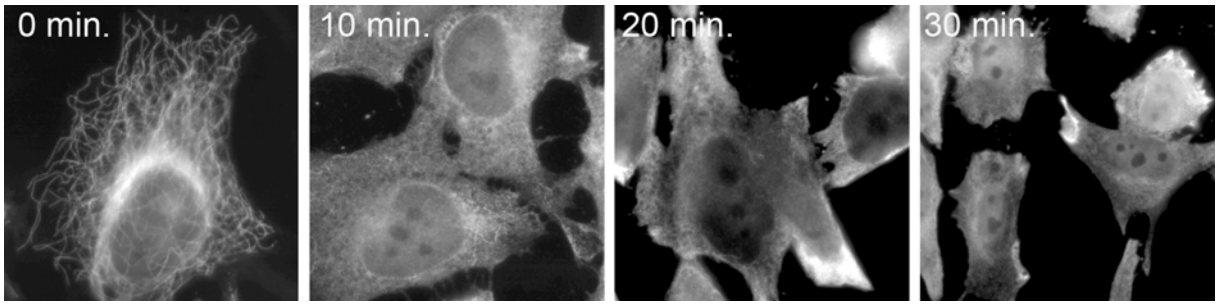

Fig. S 7. Effect of addition of Nocodazole on the microtubule cytoskeleton. HeLa cells were treated with a final concentration of 10 μg/ml Nocodazole. The cells were fixed at the indicated time points and processed for immuno-staining of β-tubulin.

Supplementary Movie Legends

Movie 1 corresponding to Fig. 2A. Movement and fusion of a LAMP-1-GFP-positive vesicle to the SCV. An overview of an infected cell is shown with a LAMP-1-GFP-positive vesicle docking to an SCV (indicated by a circle). Scale bar, 5 μm.

Movie 2. corresponding to Fig. 2B. Appearance of tubular extensions from an SCV. Scale bar, 5 μm.

Movie 3 corresponding to Fig. 4. A long time lapse from 1 h to 10 h after infection show the formation of *Salmonella* microcolonies, highly dynamic SIF at early time points after infection and the appearance of a complex SIF network later after infection. Images were taken with intervals of 3 min. Scale bar, 5 μm.

Movie 4 corresponding to Fig. 5A. Interferon γ-stimulated RAW264.7 macrophages were infected with *Salmonella* WT or a SPI2 mutant strains expressing GFP or mock-infected. The cells were pulsed with Alexa568-dextran 5 h after infection and imaged 2 h later. Detail sections are shown as indicated by the box in the overview still. Scale bar, 1 μm.

Movie 5 corresponding to Fig. 6A and Fig. 6B. Growth, branching and collapse of SIF. HeLa cells were infected with LAMP-1-GFP and infected with *Salmonella* WT expressing GFP. The event shown was observed 4 h after infection. Scale bar, 2 μm.

Movie 6 corresponding to Fig. 6D. The dynamic variations in SIF diameter were recorded a setup described for Fig. 6A.

Movie 7 corresponding to Fig. 7A. Dynamics of SIF formation shown for a representative cell at 4 h after infection.

Movie 8 corresponding to Fig. 7B. Dynamics of SIF formation shown for a representative cell at 8 h after infection.

Movie 9 corresponding to Fig. 8B. HeLa cells were transfected with the LAMP-1-GFP construct and infected with GFP-expressing *Salmonella* WT. At 4 to 5 h after infection, the solvent (mock) or inhibitors nocodazole or taxol were added and live imaging of infected cells was performed.

Movie 10 corresponding to Fig. 8D. The effect of nocodazole treatment and removal on the dynamics of vesicle and SIF movement is shown.

Movie 11 corresponding to Fig. S5 B and C. RAW264.7 cell (B) or primary murine macrophages were infected with Salmonella WT and the fluid phase marker Alexa568-detran was used to follow SIF formation in living cells.