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Supplemental Information

Dynamic Growth and Shrinkage of the *Salmonella*-Containing Vacuole Determines the Intracellular Pathogen Niche

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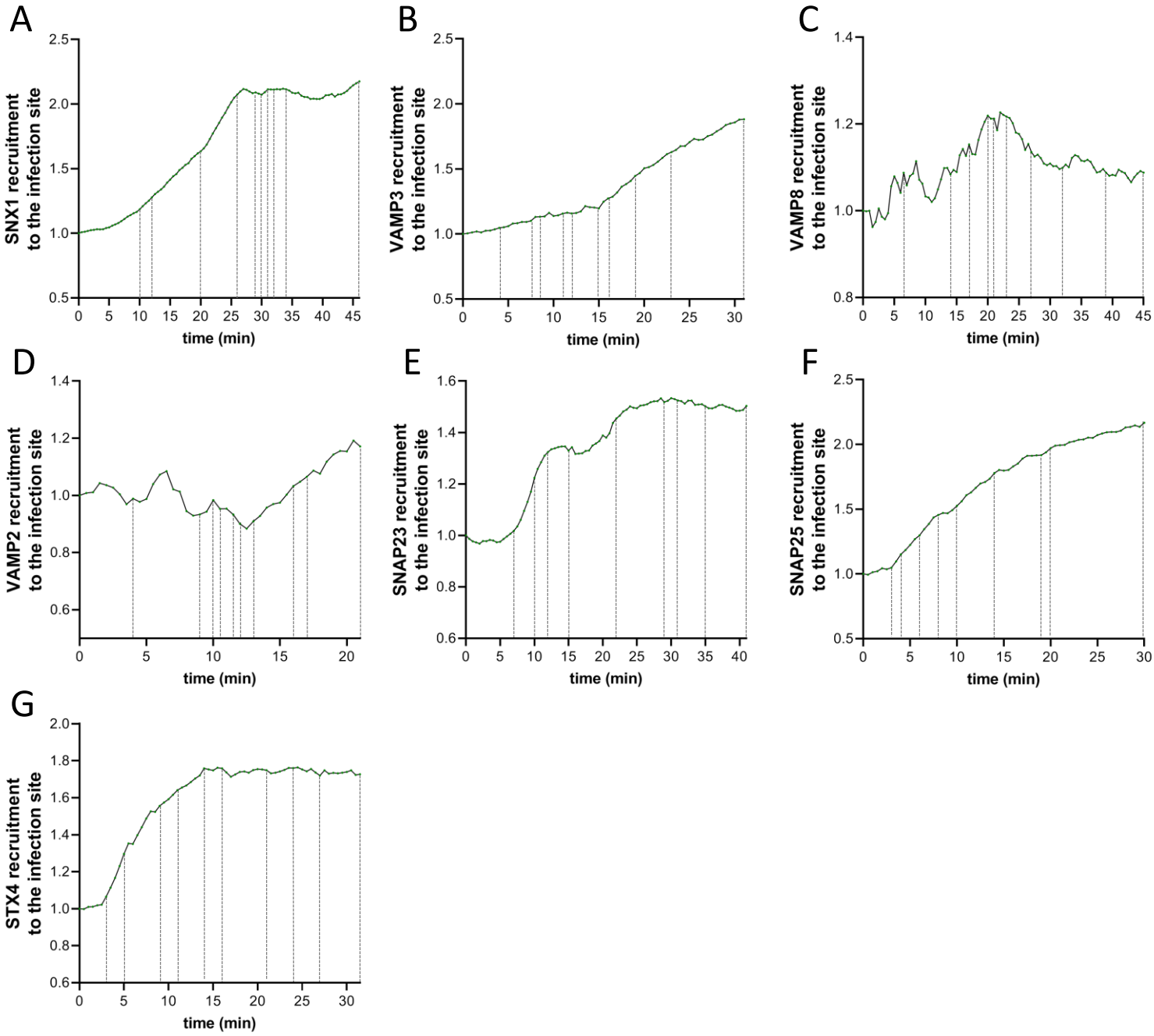

Figure S1. Quantification of the mean fluorescence intensity increases at the infection site over-time of the analyzed GFP-tagged proteins of interest. Infection sites are shown in the associated main Figures and Videos. Dashed lines correspond to time-point displayed in main Figures. The decrease of the overall fluorescence intensity due to photobleaching was corrected normalizing the values for each time-point with the fluorescence intensity outside of the site of interest. **A.** SNX1 recruitment to the infection site over time, related to Figure 1B and Video S1B. **B.** VAMP3 recruitment to the infection site over time, related to Figure 4A and Video S2A. **C.** VAMP8 recruitment to the infection site over time, related to Figure 4B and Video S2B. **D.** VAMP2 recruitment to the infection site over time, related to Figure 4C and Video S2C. **E.** SNAP23 recruitment to the infection site over time, related to Figure 4D and Video S2D. **F.** SNAP25 recruitment to the infection site over time, related to Figure 4E and Video S2E. **G.** STX4 recruitment to the infection site over time, related to Figure 6B and Video S4A.

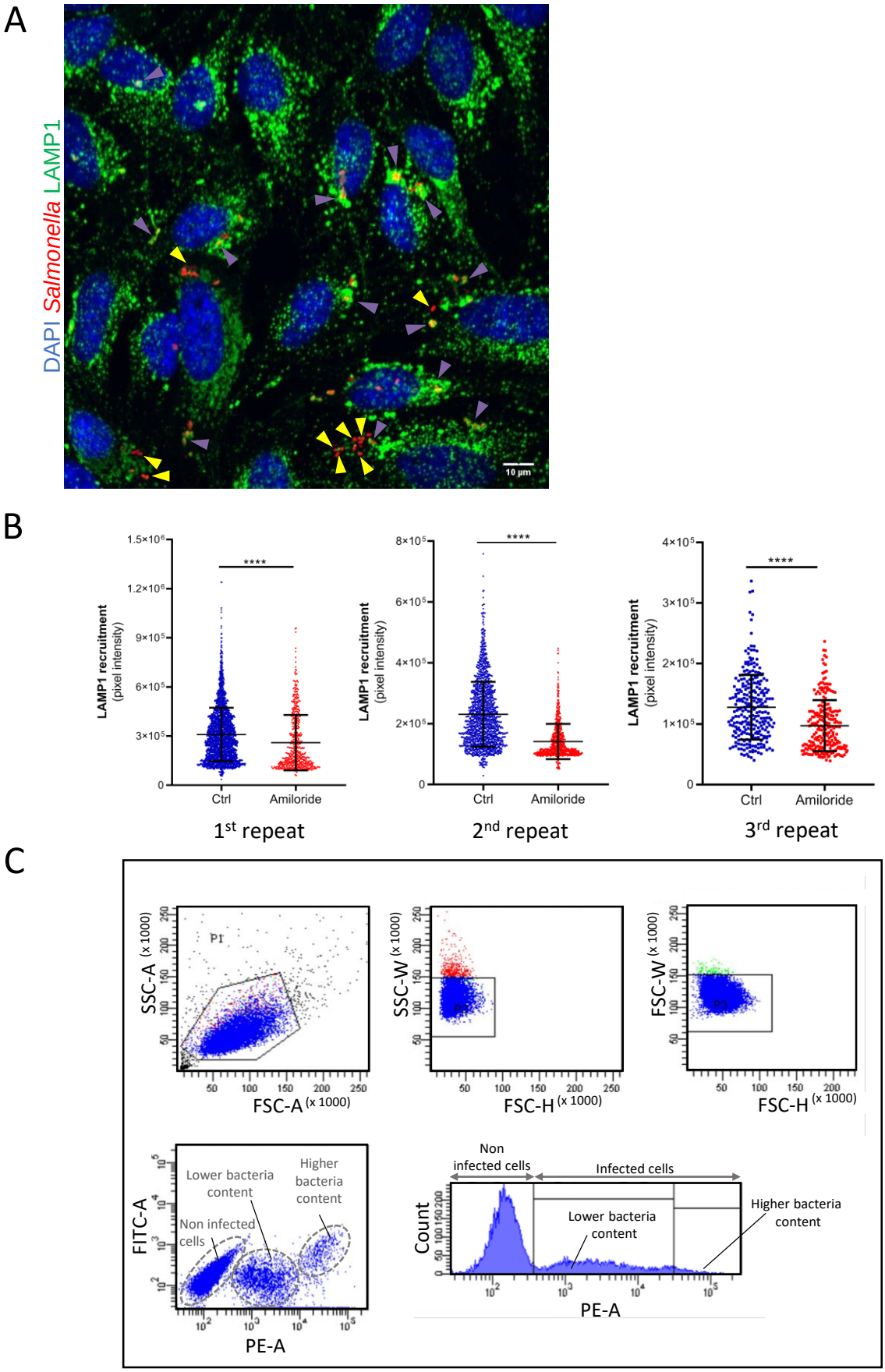

Figure S2. A-B. Related to Figure 2C. **A.** Confocal microscopy of HeLa cells infected with fluorescent *Salmonella* (in red) and fixed at 2 h pi. Samples were immunolabeled for LAMP1 (in green). Scale bars: 10 µm. Yellow arrow: *Salmonella* not surrounded by a LAMP1 coat. Purple arrow: *Salmonella* surrounded by a LAMP1 coat. **B**. Quantification of LAMP1 recruitment around *Salmonella* at 2 h pi using the Imaris software tool for 3D image analysis as represented in A. Individual dots: mean pixel intensity in the LAMP1 channel at a given bacterial localization. The graphs represent measures over 10 fields of view per conditions in 3 independent experiments plotted individually. P-values were obtained after t-test. Error-bars: +/- SD. **C.** Quantification of HeLa cells

containing hyper-replicating salmonellae by flow cytometry, related to Figure 2E. Doublets were excluded using SSC/FSC gating. Fluorescence compensation was performed using the FITC and PE channels. The populations of non-infected cells and infected cells with a lower and higher number of intracellular bacteria were distinguished using the dsRed fluorescence intensity per cell (PE channel).

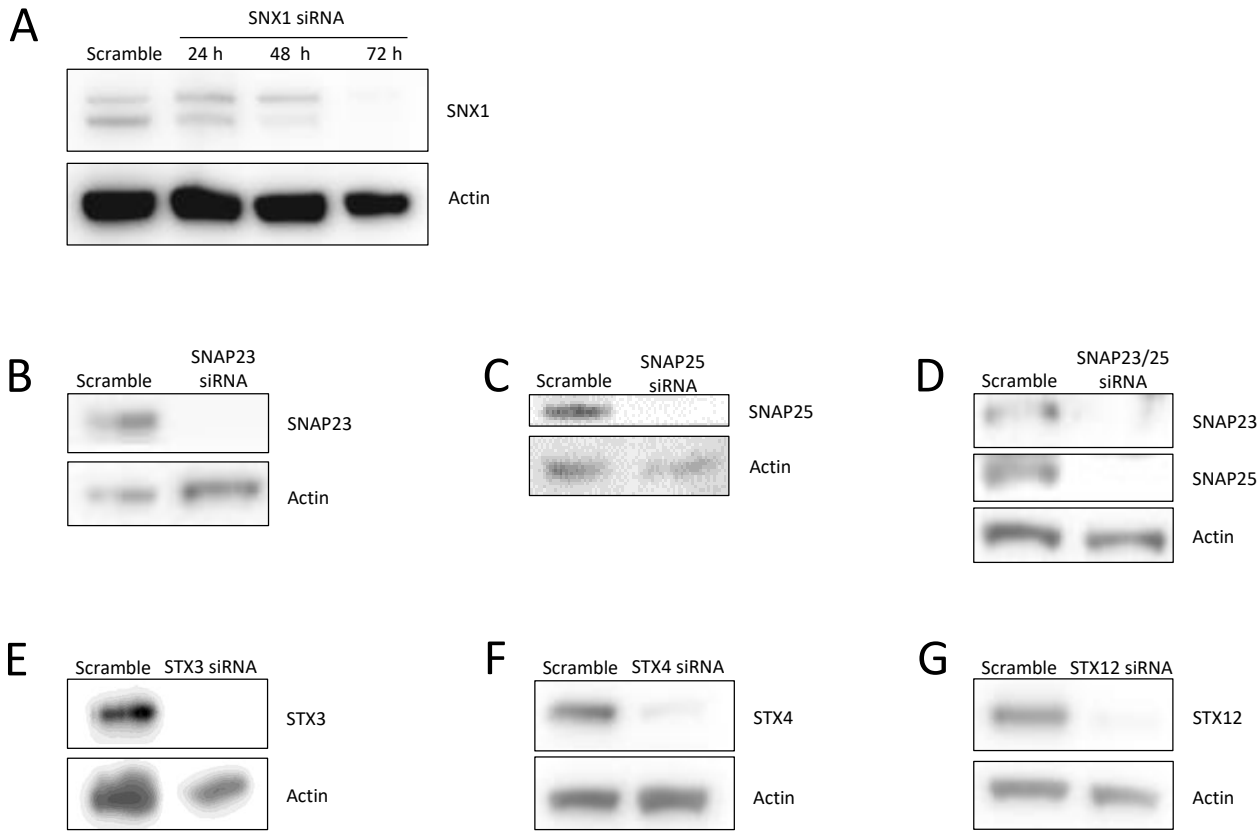

Figure S3. A. Knockdown efficiencies assessed by Western Blot of HeLa cells extract after 24, 48 and 72 h of SNX1 siRNA treatment, related to Figures 2F-G. **B-G** Knockdown efficiencies assessed by Western Blot of HeLa cells extract after 72 h of siRNA treatment. **B.** SNAP23 siRNA knockdown efficiency, related to Figure 5. **C.** SNAP25 siRNA knockdown efficiency, related to Figure 5. **D.** SNAP23 and SNAP25 double siRNA knockdown efficiency, related to Figure 5. **E.** STX3 siRNA knockdown efficiency, related to Figure 6A. **F.** STX4 siRNA knockdown efficiency, related to Figure 6. **G.** STX12 siRNA knockdown efficiency, related to Figure 6A.

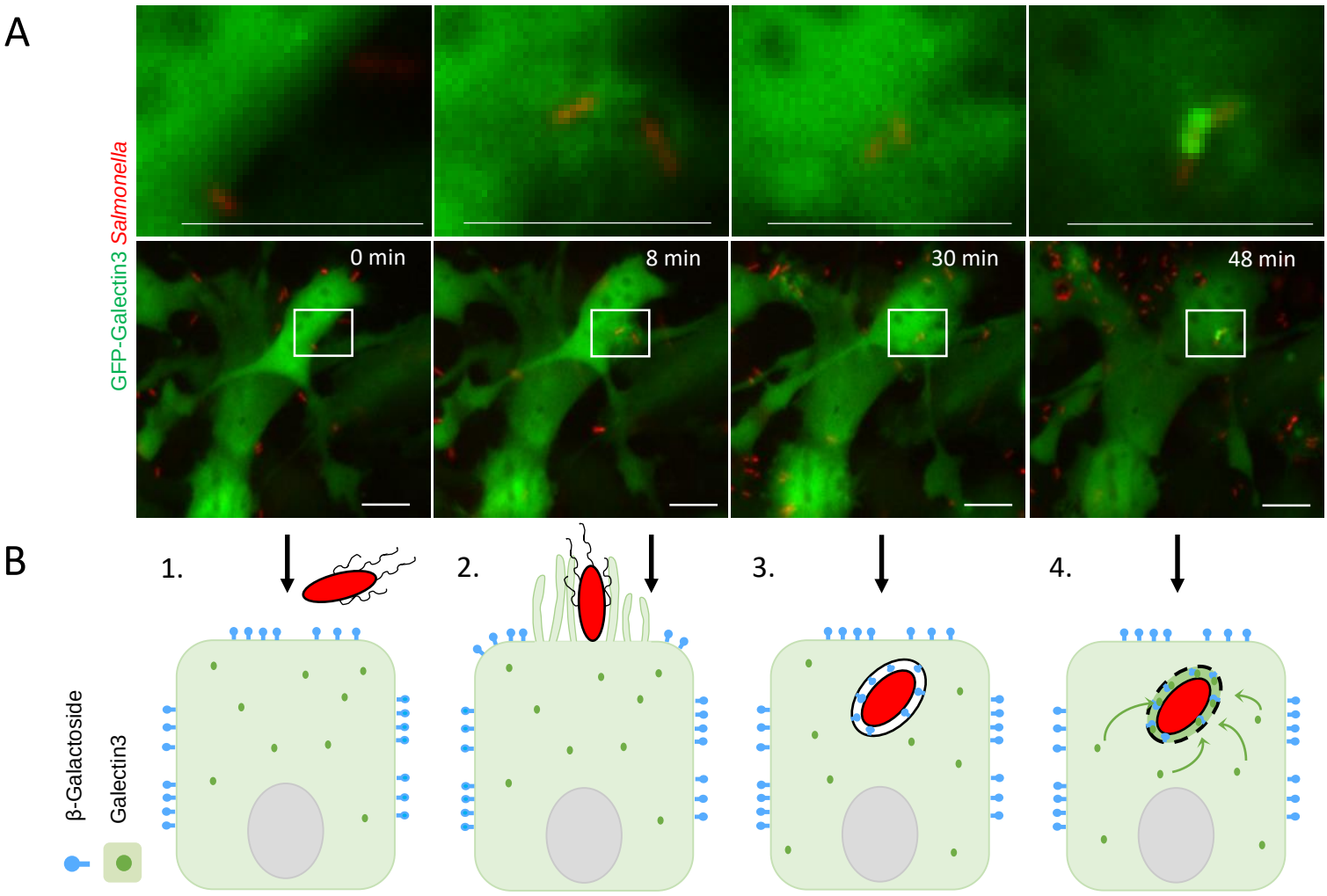

Figure S4. Related to Figures 2H and 5F, and Video S4E. Detection of SCV rupturing events using the fluorescent reporter GFP-Galectin3. **A.** Time-lapse of HeLa cells transfected with GFP-Galectin3 (in green) and infected with fluorescent *Salmonella* (in red). Scale bars: 5 µm. **B.** Scheme of the Galectin3 recruitment at the ruptured SCV over time. Step 1: HeLa cells expressing cytosolic GFP-Galectin3 are incubated with fluorescent salmonellae. β-Galactoside is present on the outside layer of the plasma membrane. Step 2: *Salmonella* infects a given host cell, inducing ruffle formation. Step 3: The bacterium is engulfed in the SCV. β-Galactoside is present on the outside layer of the plasma membrane and the inner layer of the SCV membrane. Step 4: The SCV ruptures, allowing the access of Galectin3 to the β-Galactoside attached to the rupturing SCV membrane. The accumulation of Galectin3 on the damaged membrane leads to a local increase of the detected GFP fluorescence intensity.

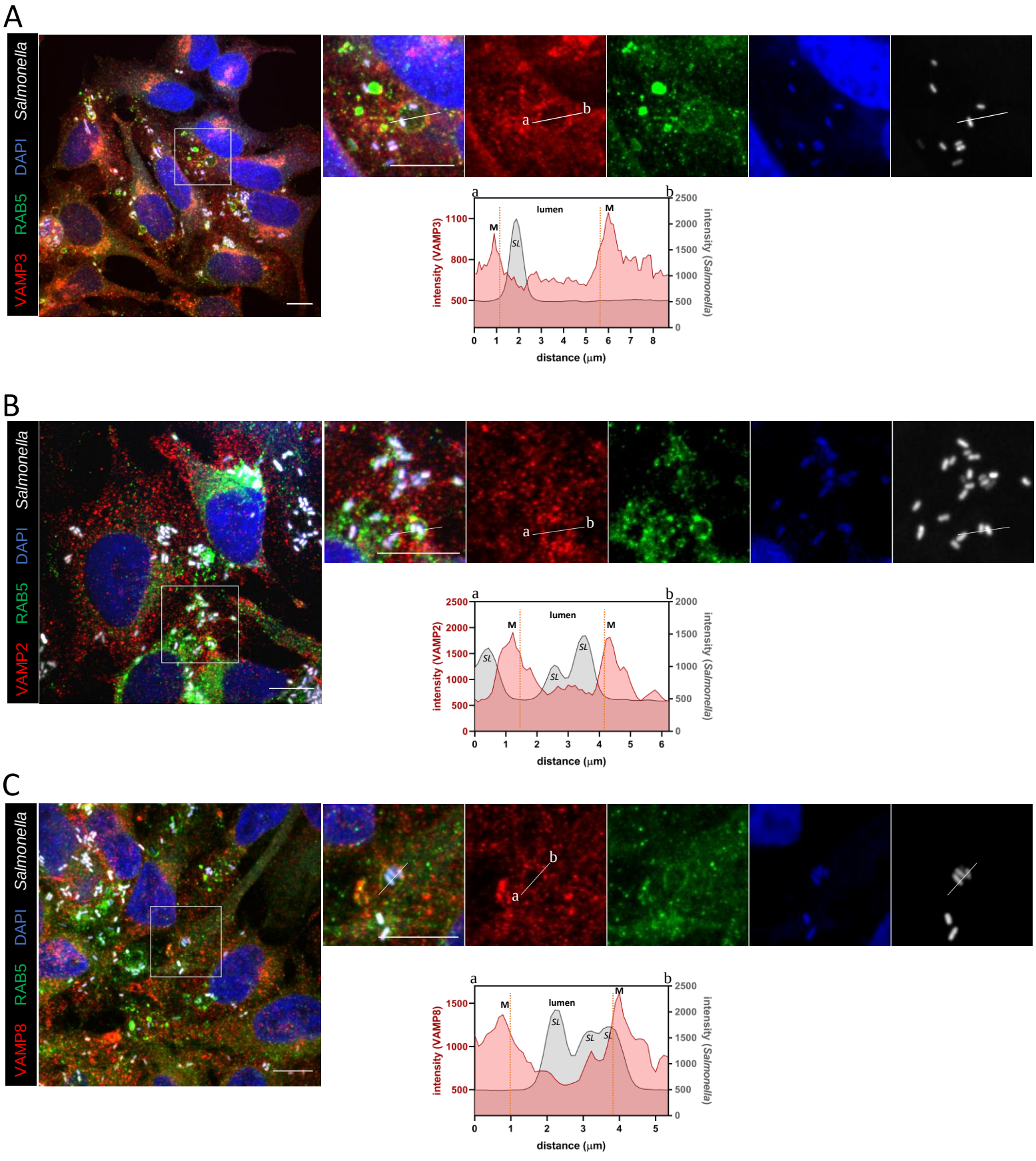

Figure S5. Confocal microscopy of a HeLa cell infected for 30 min with dsRed-expressing *Salmonella* (in white) before fixation and (immuno-)labeling with DAPI (in blue) and antibodies against RAB5 (in green) and VAMP proteins (in red). Graphs show the measured pixel intensities in the VAMP (red) and *Salmonella* (grey) channels along the white lines. “M” letters designate the localization of IAM membranes, "SL" designate the localization of salmonellae. **A.** VAMP3 localization by immunofluorescence, related to Figure 4A. **B.** VAMP2 localization by immunofluorescence, related to Figure 4C. **C.** VAMP8 localization by immunofluorescence, related to Figure 4B.

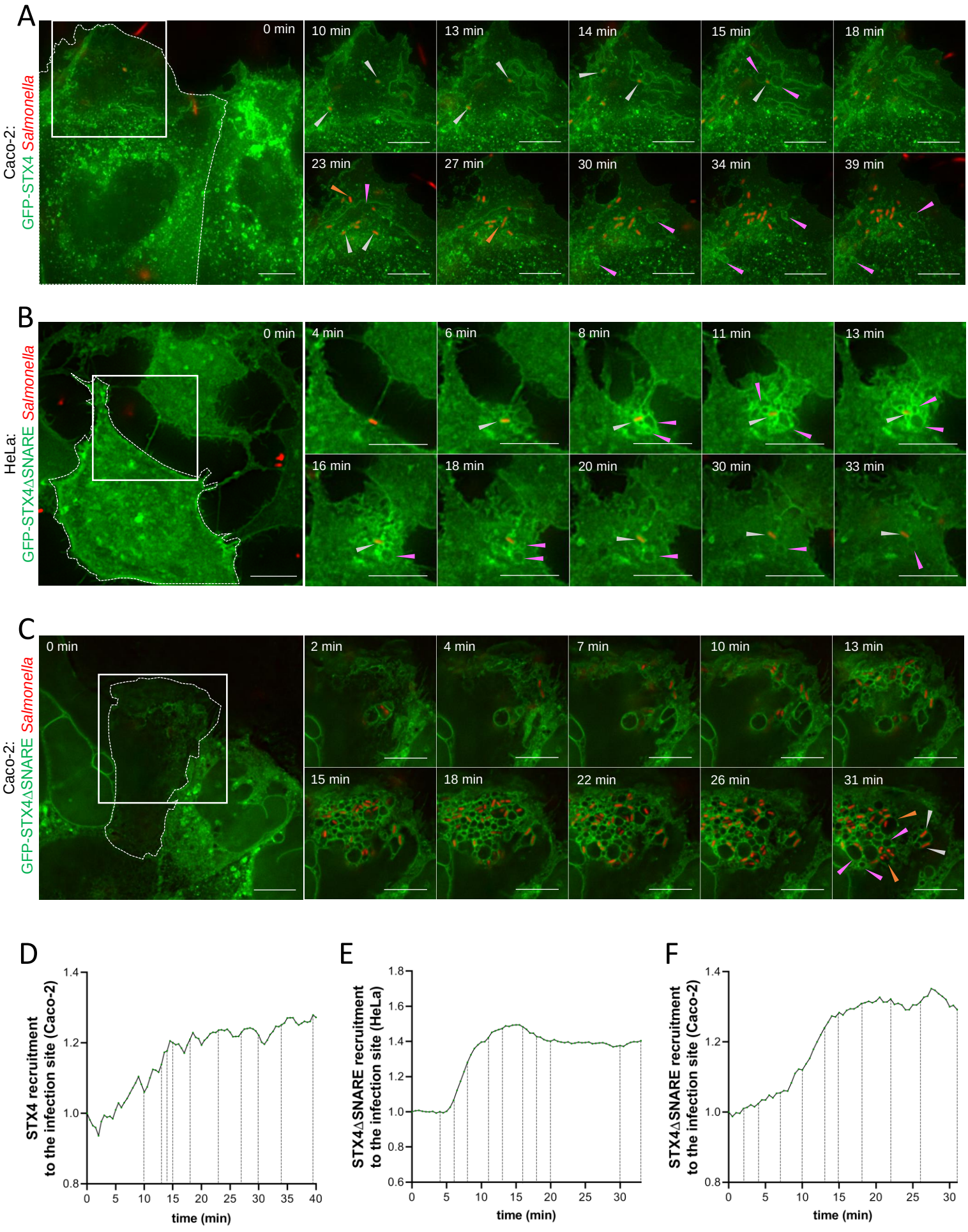

Figure S6. Related to Figure 6E. **A.** Time-lapse microscopy of Caco-2 cells transfected with GFP-tagged STX4 (in green) and infected with fluorescent *Salmonella* (in red). *See also Video S4C.* **B-C.** Time-lapse microscopy of

HeLa (**B**) and Caco-2 (**C**) cells transfected with GFP-STX4ΔSNARE (in green) and infected with fluorescent *Salmonella* (in red). *See also Video S4B&D.* **A-C.** Scale bars: 10 µm. Orange arrowheads designate large SCVs, pink arrowheads designate IAMs, white arrowheads designate tight SCVs. **D-F.** Quantification of the mean fluorescence intensity increases at the infection site over-time of GFP-tagged protein of interest. Infection sites are shown in the associated Figures and Movies. Dashed lines correspond to time-point displayed in the associated figures. The decrease of the overall fluorescence intensity due to photobleaching was corrected normalizing the values for each time-point by the fluorescence intensity outside of the site of interest. **D.** STX4 recruitment to the infection site of a Caco-2 cell over time, related to Figure S6A and Video S4C. **E.** STX4ΔSNARE recruitment to the infection site of a HeLa cell over time, related to Figure S6B and Video S4B. **F.** STX4ΔSNARE recruitment to the infection site of a Caco-2 cell over time, related to Figure S6C and Video S4D.