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Supplemental Material to:

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Role of VAMP3 and VAMP7 in the commitment of *Yersinia pseudotuberculosis* to LC3-associated pathways involving single- or double-membrane vacuoles

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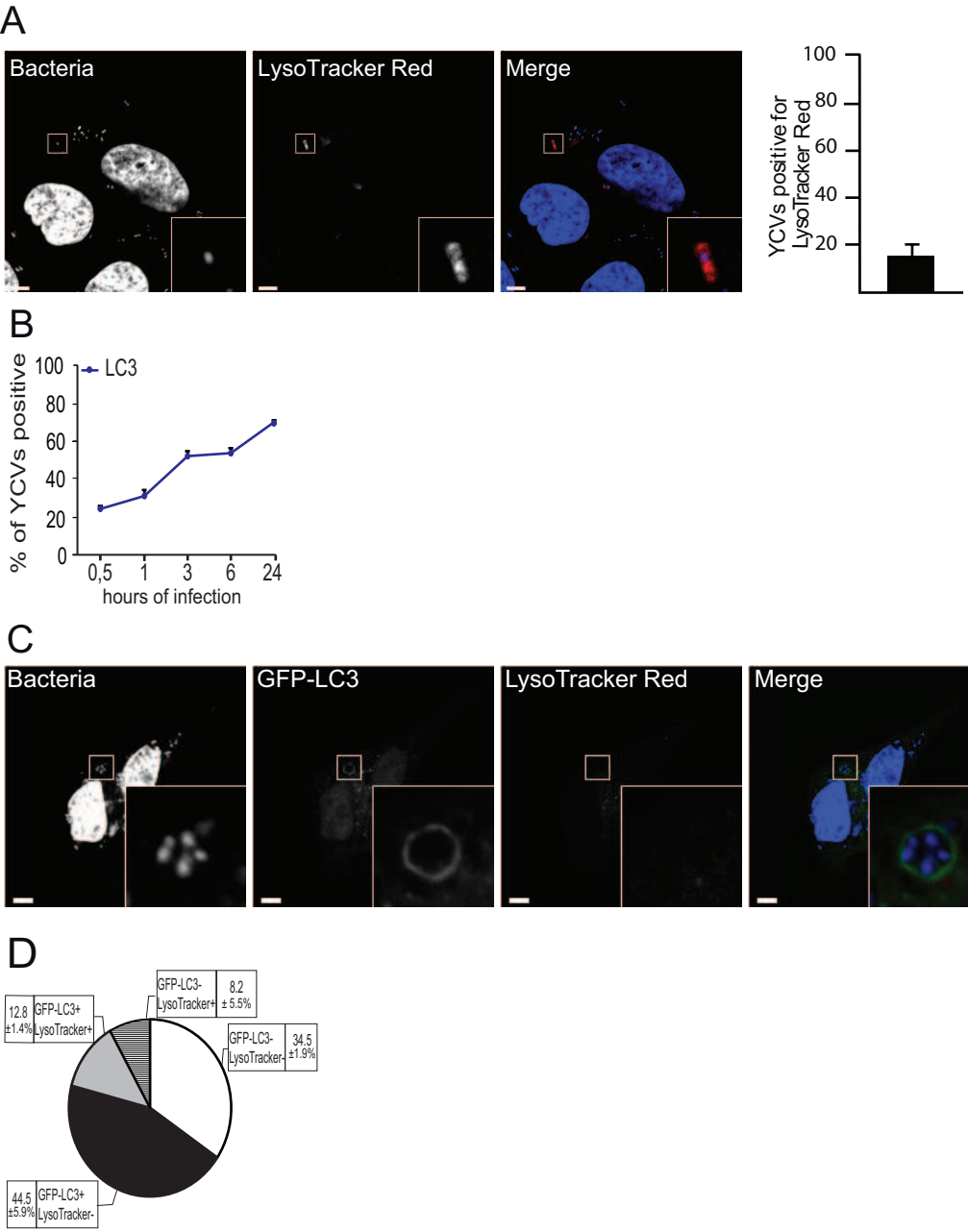

Figure S1 Ligeon *et al*.

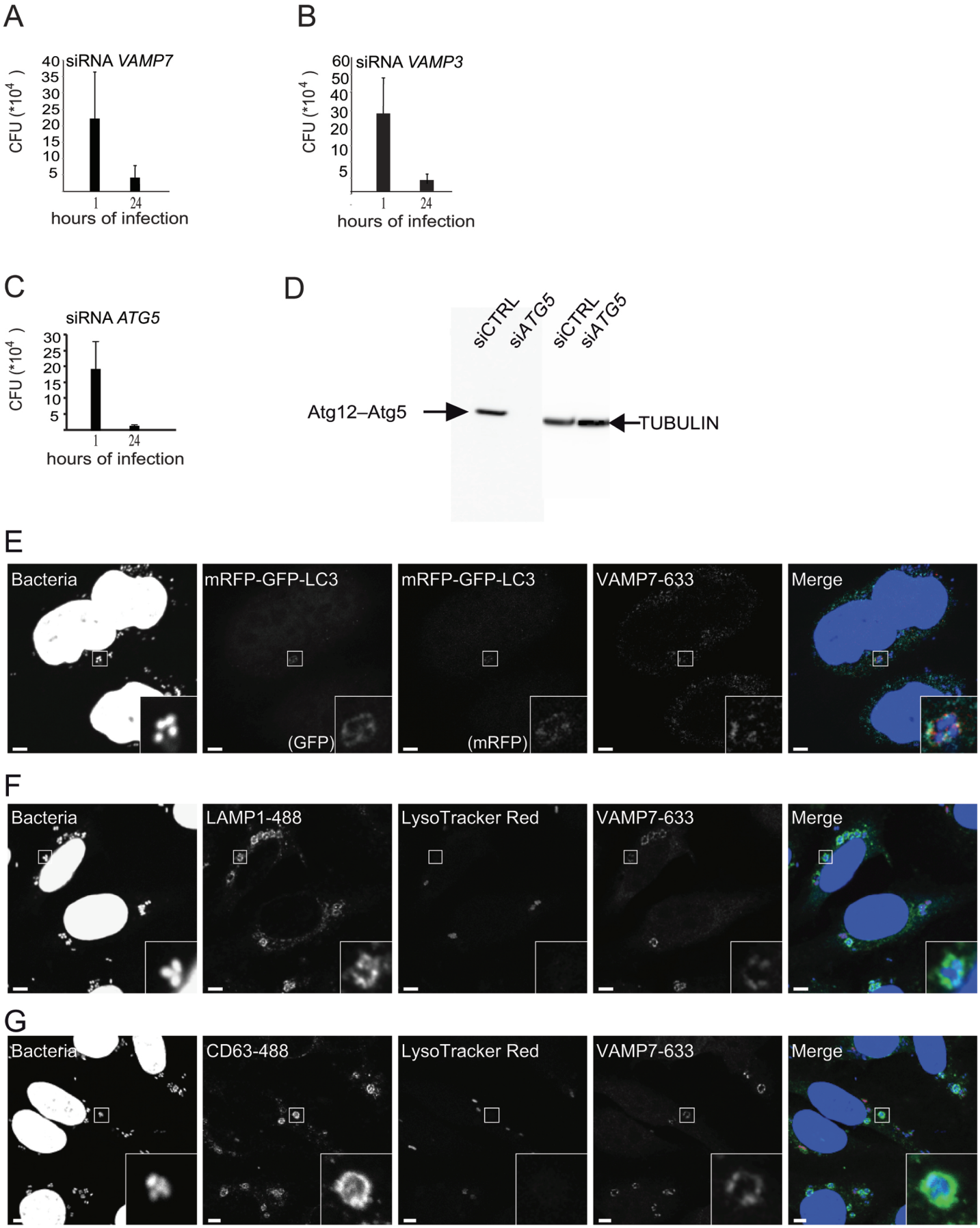

Figure S2 Ligeon *et al*.

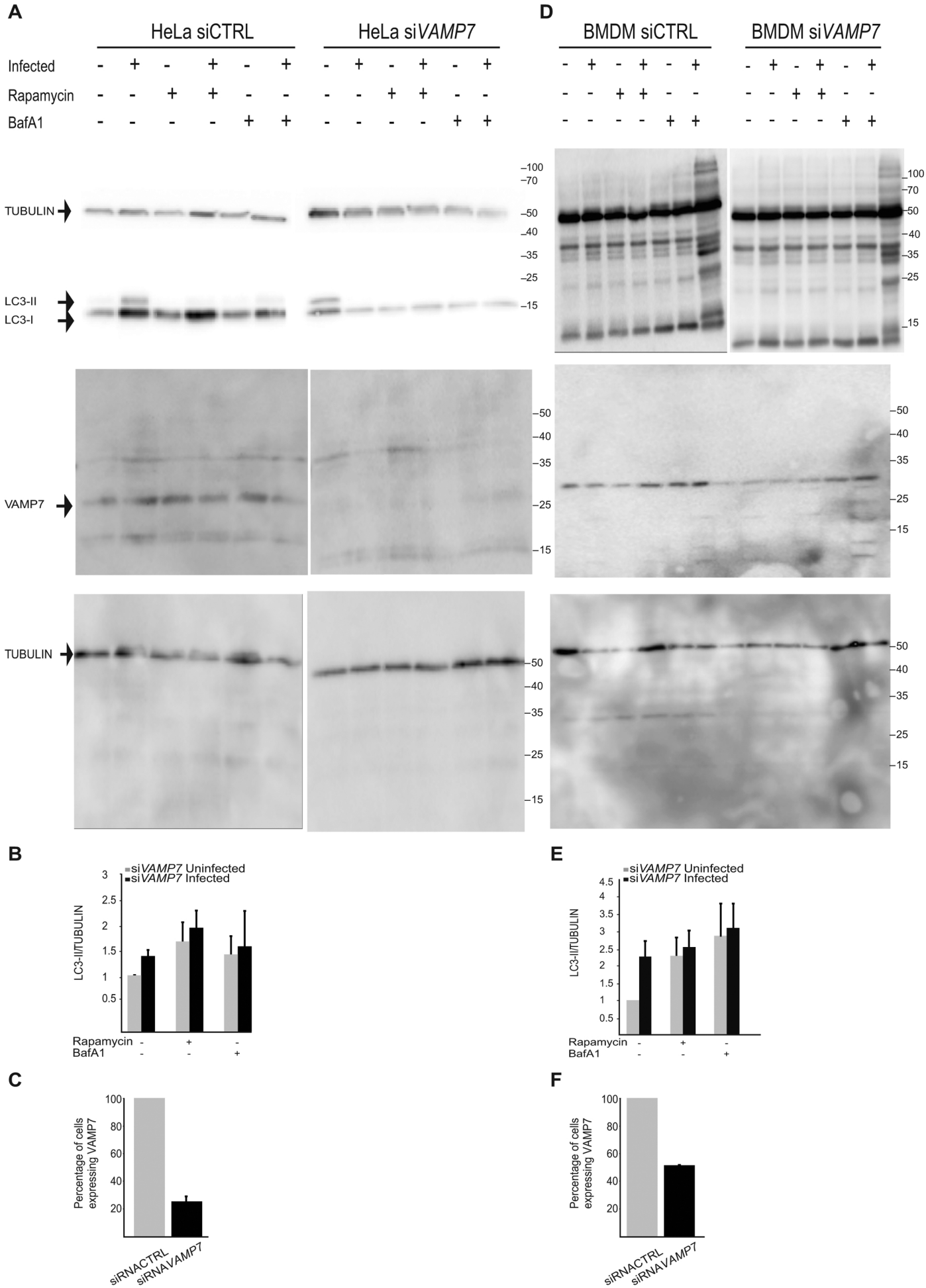

Figure S3 Ligeon *et al*.

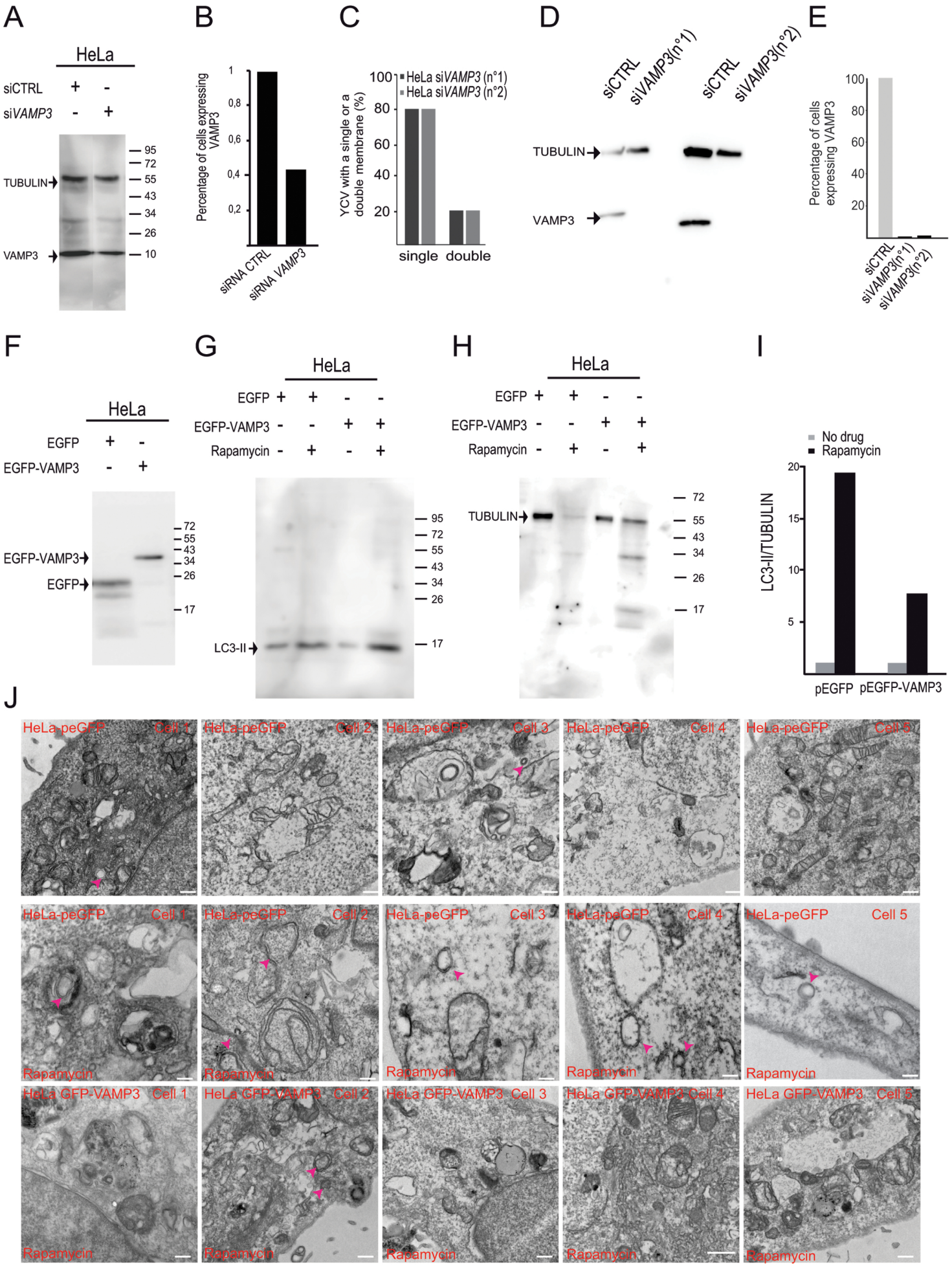

Figure S4 Ligeon *et al*.

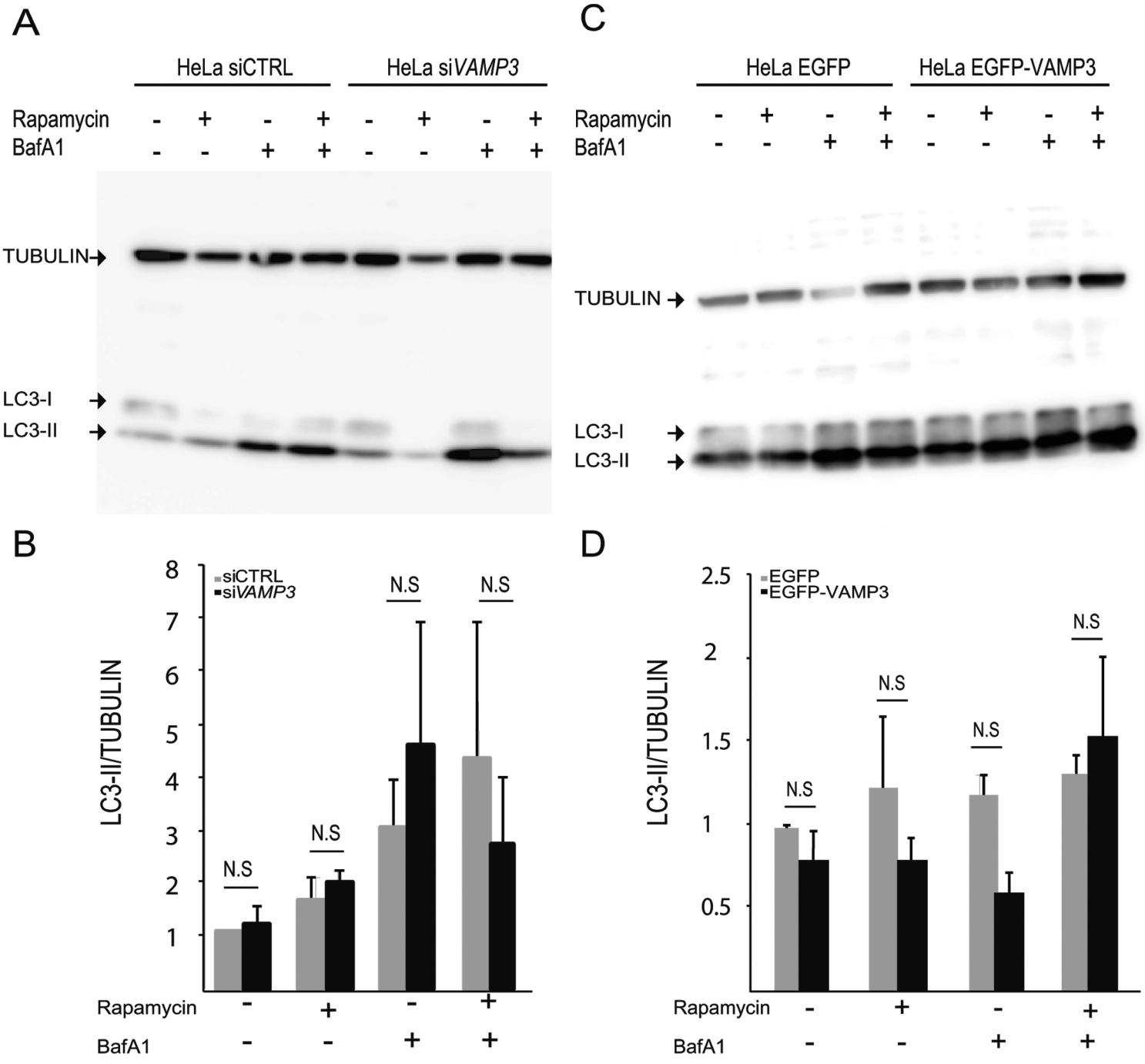

Figure S5 Ligeon *et al.*

Figure S1. A low proportion of YCVs can reach lysosomes. (**A**) HeLa cells were infected with *Y. pseudotuberculosis* for 4 h, treated with LysoTracker® Red for 2 h and processed for CLSM. Bacteria were visualized after staining with DAPI. Scale bars: 5 µm. Magnification: x5. The percentage of YCVs positive for LysoTracker® Red is indicated. In each experiment, at least 20 cells were quantified in a double-blind analysis. Values are quoted as the mean ± SEM from at least 3 independent experiments. (**B**) HeLa cells transiently expressing mRFP-LC3 were infected with *Y. pseusotuberculosis.* The graph shows the quantification of YCVs displaying mRFP-LC3 proteins at several time points of infection. Data represent average percentages of mRFP-LC3-positive YCVs in 3 independent experiments and after the analysis of at least 20 transfected and infected cells per experiment. (**C**) HeLa cells transiently expressing GFP-LC3 were infected with *Y. pseudotuberculosis* and, treated with LysoTracker® Red for 2 h. At 4 h p.i., cells were fixed and processed for CLSM. Bacteria were visualized after staining with DAPI. Scale bars: 5 µm. Magnification: x5. The distribution of bacteria is plotted as a function of GFP-LC3 and LysoTracker®Red labeling. In each experiment, at least 20 cells infected with *Y. pseudotuberculosis* were quantified. Data are displayed as the mean ± SEM from at least 2 independent experiments (**D**).

Figure S2. *Y pseudotuberculosis* uses the LAP pathway to replicate. Intracellular replication of *Y. pseudotuberculosis* inside HeLa cells treated with *VAMP7* siRNA (**A**) and *VAMP3* siRNA (**B**). Colonies were counted 1 h and 24 h after infection. Data represent the mean ± SEM of 3 independent experiments. (**C**) Intracellular replication of *Y. pseudotuberculosis* inside HeLa cells treated with *ATG5* siRNA. Colonies were counted 1 h and 24 h after infection. Data represent the mean ± SEM of 3 independent experiments. (**D**) HeLa cells were transfected with control siRNA (siCTRL) or siRNA against *ATG5* and then analyzed for ATG5 expression by immunoblotting. (**E**) HeLa cells stably expressing mRFP-GFP-LC3 were infected with *Y. pseudotuberculosis* for 4 h and processed for CLSM. Bacteria were visualized after staining with DAPI. VAMP7 proteins were detected with anti-VAMP7 rabbit antibodies and labeled with secondary antibodies coupled to Alexafluor®-633. The insert shows bacteria inside mRFP-GFP-LC3- and VAMP7-positive vacuoles. Scale bars: 5 µm. Magnification: x4. (**F**) HeLa cells were infected with *Y. pseudotuberculosis* for 4 h, treated with LysoTracker® Red for 2 h and then processed for CLSM. Bacteria were visualized after staining with DAPI. LAMP1 was detected with anti-LAMP1 mouse antibodies and labeled with secondary antibodies coupled to Alexafluor®488. VAMP7 proteins were detected with anti-VAMP7 rabbit antibodies and labeled with secondary antibodies coupled to Alexafluor®-

633. The insert shows bacteria inside vacuoles that are positive for LAMP1 and VAMP7 but negative for LysoTracker® Red. Scale bars: 5 µm. Magnification x4. (**G**) HeLa cells were infected with *Y. pseudotuberculosis* for 4 h, treated with LysoTracker® Red for 2 h and then processed for CLSM. Bacteria were visualized after staining with DAPI. CD63 was detected with anti-CD63 mouse antibodies and labeled with secondary antibodies coupled to Alexafluor®488. VAMP7 proteins were detected with anti-VAMP7 rabbit antibodies and labeled with secondary antibodies coupled to Alexafluor®633 antibodies. The insert shows bacteria inside vacuoles that are positive for CD63- and VAMP7 but negative for LysoTracker® Red. Scale bars: 5 µm. Magnification x5.

Figure S3. VAMP7 is involved in the recruitment of LC3 to YCVs. (**A**) The complete dataset for the western blots shown in Figure 5. (**B**) The LC3-II/TUBULIN ratio for HeLa cells treated with *VAMP7* siRNA (si*VAMP7*), as a function of cell treatment and infection. Data are quoted as the mean of at least 3 independent experiments and error bars correspond to the SEM. (**C**) Percentage of HeLa cells expressing VAMP7 proteins after treatment with control or *VAMP7* siRNA. Data are quoted as the mean of at least 2 independent experiments. (**D**) The complete dataset for the western blots shown in Figure 6. (**E**) The LC3-II/TUUBLIN ratio for BMDMs treated with *VAMP7* siRNA (si*VAMP7*), as a function of cell treatment or infection. Data are quoted as the mean of at least 3 independent experiments. (**F**) The percentage of BMDMs expressing VAMP7 proteins after treatment with control or *VAMP7* siRNA. Data are quoted as the mean of at least 2 independent experiments.

Figure S4. VAMP3 influences the morphology of autophagosomes. (**A**) HeLa cells were transfected with control (siCTRL) or *VAMP3* siRNA (si*VAMP3*), sorted and treated again with siCTRL or si*VAMP3*, and analyzed for VAMP3 silencing by immunoblotting. Protein loading was checked against the alpha-tubulin (TUBA) content (panel **B**). (**C**) HeLa cells were transfected with 2 different individual *VAMP4* siRNAs and analyzed by electron microscopy. The panel shows the quantification of the percentages of YCVs with limiting single and double membrane. Forty-five cells were analyzed. (**D**) HeLa cells were transfected with individual siRNA against *VAMP3* (si*VAMP3* (n°1) or si*VAMP3* (n°2)) and analyzed for VAMP3 silencing by immunoblotting. Protein loading was checked against the alpha-tubulin (TUBA) content (panel **E**). (**F**) HeLa cells transfected with pEGFP or pEGFP-VAMP3 were analyzed for protein expression by immunoblotting using an anti-GFP antibody. (**G**) HeLa cells transfected with pEGFP or pEGFP-VAMP3 were analyzed for LC3 by immunoblotting.

The cells were treated with rapamycin as indicated and DMSO was used as a solvent control. Protein loading was checked against the alpha-tubulin (TUBA) content (**H**). (**I**) The LC3-II/TUBULIN ratio for HeLa transiently expressing EGFP or EGFP-VAMP3, as a function of cell treatment. (**J**) A panel of 5 TEM images for EGFP-expressing cells treated (or not) with rapamycin and for EGFP-VAMP3-expressing cells treated with rapamycin (corresponding to the histogram in **Figure 7I**). The top panels show the profiles of HeLa cells transiently expressing EGFP; few vacuoles with double membranes were observed. The middle panels show HeLa cells transfected with EGFP and treated with rapamycin; a greater proportion of vacuoles with a double membrane were observed (pink arrows). The bottom panels display double-membrane vacuoles in HeLa cells overexpressing VAMP3 and treated with rapamycin. The pink arrows show the few observed vacuoles with a double membrane.

Figure S5. VAMP3 does not influence the level of autophagy. (**A**) HeLa cells were transfected with control siRNA (siCTRL) or siRNA against *VAMP3 (siVAMP3*) and then analyzed for LC3 by immunoblotting. The cells were treated with rapamycin and/or BafA1 The LC3-II/TUBULIN ratio as a function of the cell treatment is indicated in panel (**B**). Data are quoted as the mean of at least 3 independent experiments. (**C**) HeLa cells were transfected with pEGFP or pEGFP-VAMP3 and then analyzed for LC3 by immunoblotting. The cells were treated with rapamycin and/or BafA1. The LC3-II/TUUBLIN ratio as a function of the cell treatment is indicated in panel (**D**). Data are quoted as the mean of at least 2 independent experiments.

Movie S1. VAMP3 and VAMP7 recruitment to YCVs. HeLa cells transiently expressing EGFP-VAMP3 (green) and mRFP-VAMP7 (red) were infected with *Y. pseudotuberculosis*, stained with DAPI (DNA, blue) and directly observed by video microscopy for 3 h 33 min. The movie shows the migration of EGFP-VAMP3 and mRFP-VAMP7 proteins on a single YCV. Acquisition times are indicated in the bottom left corner (h:min). Scale bar, 5 µm.

Movie S2. VAMP7 and LC3 recruitment to YCVs. HeLa transiently expressing GFP-VAMP7 (green) and mRFP-LC3 (red) were infected with *Y. pseudotuberculosis*, stained with DAPI and then observed by video microscopy for 1h. The arrows indicate the migration of GFP-VAMP7 and mRFP-LC3 proteins on a single YCV. Acquisition times are indicated in the lower left corner (h:min). Scale bar: 5 µm.