Supplemental Figure Legends

Supplemental Figure 1. DFCP1 associates with bacteria-containing autophagosomes via its PI(3)P and ER-binding domains. (*A*) HeLa cells were co-transfected with RFP-LC3 and DFCP1-GFP and infected with *S.* Typhimurium (*Sal*) for 1 h, then cells were immunostained with a polyclonal antibody to *Sal*. Representative confocal z-slices of non-infected cells or cells with $LC3^-$ bacteria are shown. Size bar, 10 μm. HeLa cells were co-transfected with RFP-LC3 and either FYVE(C347S)-TM-GFP (*B*) or TM-GFP (*C*). Cells were infected with *S.* Typhimurium (*Sal*) and immunostained as in A. Representative epifluorescent images are shown, with the inner panels representing a higher magnification of the boxed area. Size bar, 10 μm.

Supplemental Figure 2. Autophagosomes surrounding *S.* Typhimurium do not colocalize with ER, COPII, ERGIC or COPI markers. (*A*) Cells were transfected with GFP-LC3 and infected with *S.* Typhimurium (*Sal*) for 1 h. Cells were then fixed and stained with antibodies to the indicated proteins. Calnexin (CXN), protein disulfide isomerase (PDI) and ribosomal protein S6 (S6) are ER markers; Sec31, Sec24 and Sec13 are COPII vesicle markers; ERGIC53 is an ERGIC marker; COPI and β-COP are COPI vesicle markers. Magnified images of boxed areas are shown in the upper right corners. Size bars, 5 μm. (*B*) Western blots showing knockdown of Rab1B and Atg12 expression in HeLa cells by siRNA.

Supplemental Figure 3. Live imaging of Rab1 and LC3 during *S.* Typhimurium infection. Representative still frames of Supplemental Movie l. Cells were co-transfected

with GFP-Rab1B and RFP-LC3, and infected with *S.* Typhimurium (*Sal*) labeled with a succinimidyl ester conjugated to AlexaFluor 647 (NHS-647; blue). Live imaging was performed on a spinning disk confocal microscope from the beginning of infection until 1 h post infection. Images were acquired at 1 min intervals as Z-stacks of 17 slices (each 0.5 μm thick). Asterisk indicates the bacterium being targeted by autophagy. The time post infection of each frame is indicated above the panels. Size bar, 0.5 μm.

Supplemental Figure 4. The effect of Rab1 knockdown in intracellular bacterial growth. HeLa cells were treated with either control siRNA or both isoforms of *RAB1* for 48 hours. Then cells were infected by *S.* Typhimurium expressing RFP. At indicated time points (1 h and 8 h post invasion), cells were fixed and intracellular bacterial numbers were counted (100 infected cells for each condition). The percentage of infected cells with different bacterial numbers (1-5, 6-10, 11-15, 16-20, >20) were quantified and grouped into 5 categories. Shown are the means from 3 independent experimental results and error bars represent standard deviations.

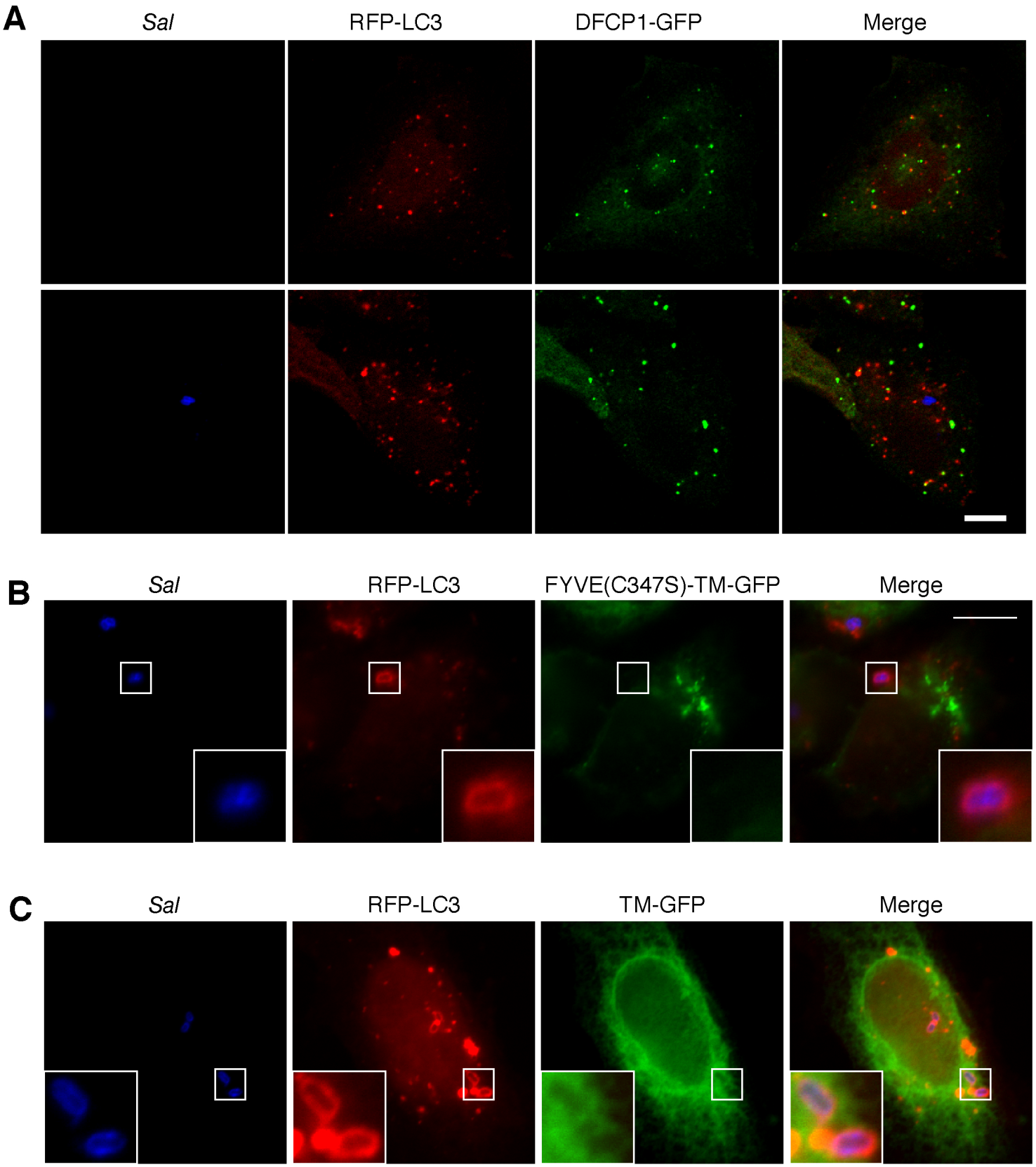
Supplemental Figure 5. Rab1 is involved in autophagy independently of its role in ER-to-Golgi transport. (*A*) HeLa cells were transfected with the indicated dominant negative CFP-GTPase constructs or treated with BFA, then fixed and stained with a monoclonal antibody to giantin to visualize the Golgi. For Rab1B(S22N), ARF1(T31N) and Sar1B(T39N) images, the transfected cells demonstrate Golgi disruption whereas neighboring non-transfected cells do not. Size bars, 5 μm. (*B*) HeLa cells were treated with the indicated siRNAs and immunostained with a mouse monoclonal antibody against GM130. Cell outlines were shown in white lines. The percentage of cells

containing intact Golgi complex was quantified in (*C*) and compared to that in control siRNA-treated cells. Asterisks indicate a significant less cells with intact Golgi complex, $p<0.05$. (*D*) Cells were co-transfected with RFP-LC3 and NleA-GFP or GFP alone, and infected with *S.* Typhimurium expressing RFP for 1 h. Boxes in the upper right corners are magnified images of RFP-LC3$^+$ bacteria. Size bars, 5 μm. (*E*) Quantification of LC3$^+$ *S.* Typhimurium in cells with or without NleA-GFP transfection.

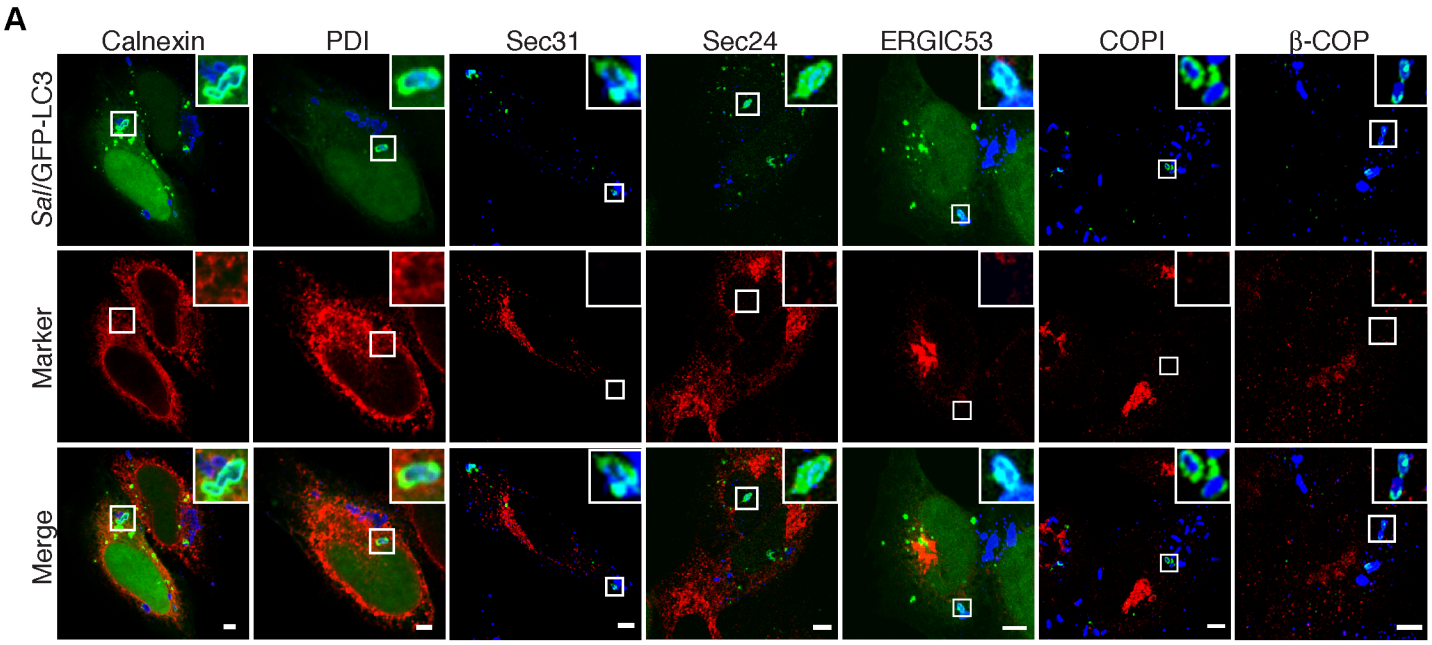
Supplemental Movie 1. Live imaging of Rab1 and LC3 during *S.* Typhimurium infection. HeLa epithelial cells were co-transfected with GFP-Rab1B and RFP-LC3, and infected with *S.* Typhimurium labeled with a succinimidyl ester conjugated to AlexaFluor 647 (NHS-647; blue). Live imaging was performed on a spinning disk confocal microscope from the beginning of infection until 1 h post infection. Images were acquired at 1 min intervals as Z-stacks of 17 slices (each 0.5 μm thick). Representative still frames of the resulting movie are shown in Supp. Figure 2.

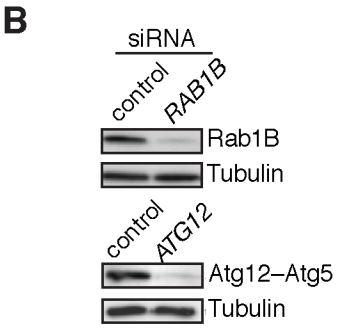
Supplemental Movie 2. 3D reconstruction of DFCP1, LC3 and Rab1B colocalization with *S.* Typhimurium. HeLa cells were co-transfected with DFCP1-GFP and RFP-LC3 and infected with *S.* Typhimurium for 1 h. Cell were immunostained for Rab1B and *S.* Typhimurium. Imaging, deconvolution and 3D reconstruction was performed using Volocity 5.0.

Supplemental Figure 1

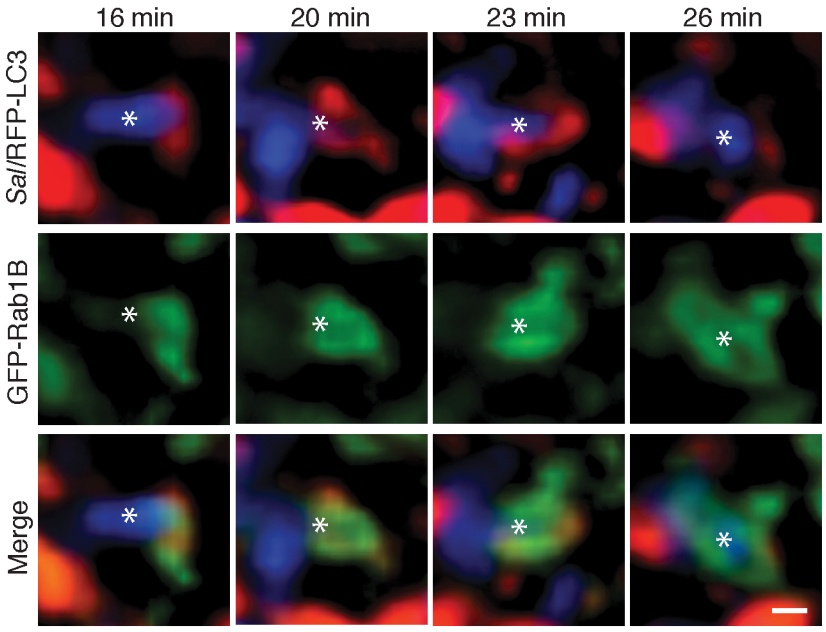


Supplemental Figure 2

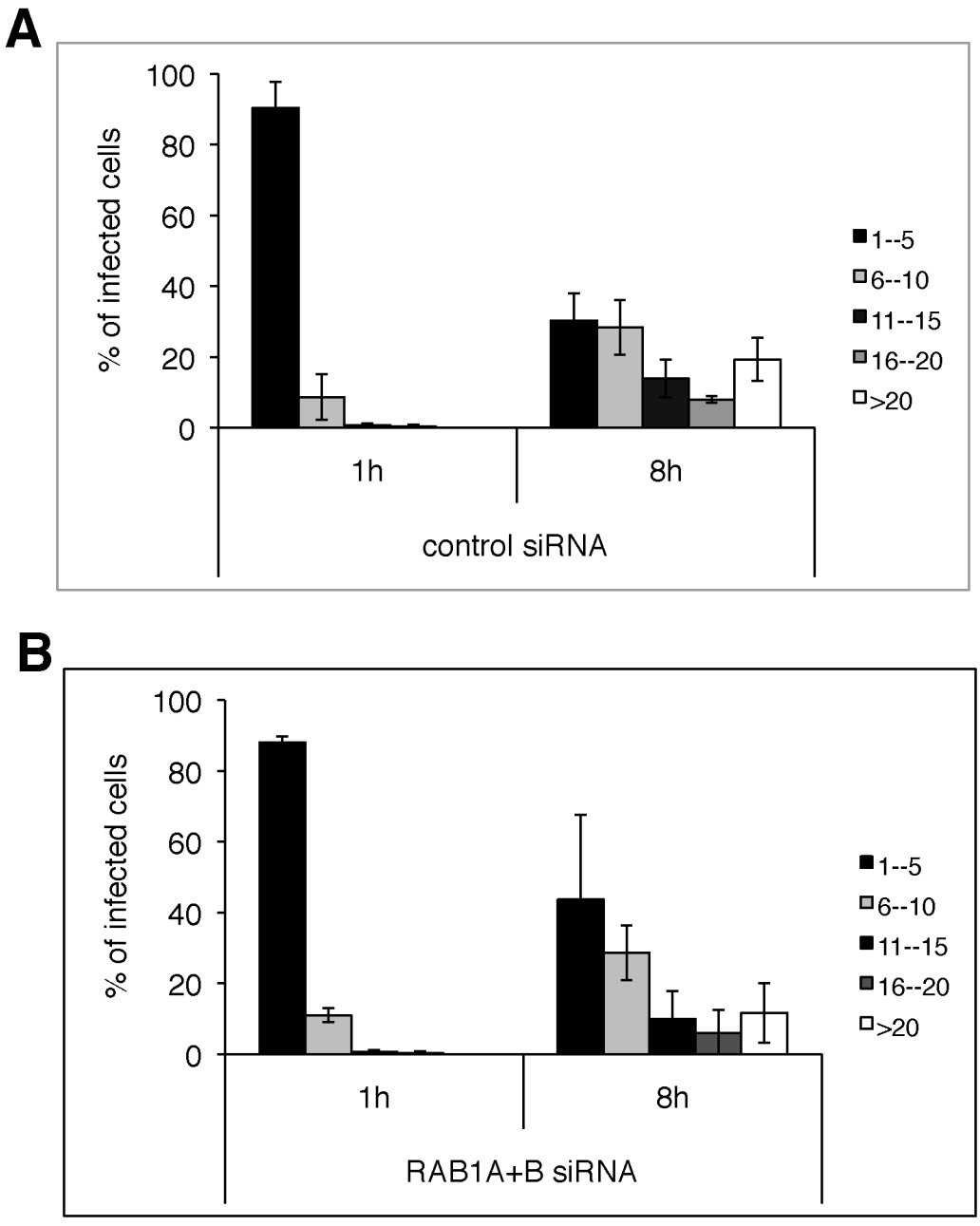




Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5