SUPPLEMENTAL FIGURE LEGENDS

Figure S1: *Listeria* affects mTOR localization and NDP52+/LAMP2+ *Listeria* are GFP-LC3+ at 1h p.i. (A) HeLa cells left unstimulated (CTR) or infected with *Listeria*–GFP for 1h or 3h, analyzed by IF using an antibody against mTOR. Scale bars, 10 μm. **(B)** HeLa cells infected with *Listeria* WT (top) or TKO (bottom) for 1h, analyzed by IF using antibodies against NDP52 and LAMP2. **(C)** HeLa cells transfected overnight with GFP-LC3 were infected with *Listeria* WT for 1h and analyzed by IF using an antibody against NDP52. Scale bars for panels B-C, 2.5 μm.

Figure S2: NDP52+ PlcA/B- *Listeria* are also GFP-LC3+. HeLa cells transfected overnight with GFP-LC3 were infected with *Listeria* PlcA/B- for 4h and analyzed by IF using an antibody against NDP52. Scale bars, 2 μm.

Figure S3: *Listeria*-induced NDP52+ granule formation requires PlcA and PlcB but not ActA or cell-to-cell spread. (A) HeLa cells infected with *Listeria* WT, PlcA- or PlcB- for 4h, analyzed by IF using an antibody against NDP52. Scale bars, 2 μm. **(B)** HeLa cells infected with *Listeria* WT for 4h, in the absence or presence of Cytochalasin D (1 μg/ml) added 60 minutes after bacterial invasion, analyzed by IF using an antibody against NDP52. **(C)** qPCR analysis of *ATF3* induction following infection of HeLa cells with *Listeria* WT or ActA- for 1h to 4h. Values are means s.e.m. n=3. **(D)** HeLa cells were infected with *Listeria* wild type (WT) or ActA- mutant strain for 0.5h to 4h, and lysates were analyzed by blotting using indicated antibodies. **(E)** HeLa cells infected with *Listeria* ActA- for 3h, analyzed by IF using antibodies against NDP52 and Galectin-8 (Gal-8). Scale bar, 2.5 μm.

Figure S4: Characterization of *Listeria*-induced NDP52+ granules. (A) HeLa cells infected with *Listeria* WT for 4h, analyzed by IF using antibodies against NDP52 and ubiquitin (Ubi). Pictures on the left and right sides show examples of NDP52+/Ubi+ and NDP52+/Ubi- granules, respectively. Scale bars, 2 μm. **(B)** HeLa cells left unstimulated (CTR) or infected with *Listeria* WT for 1-4h, or with *Listeria* TKO for 4h and with analyzed by IF using an antibody against ubiquitin. Scale bars, 5 μm. (**C**) Percentage of cells infected with *Listeria* WT for 1-4h, or with *Listeria* TKO for 4h, displaying one or several Ubiquitin+ granules. Values are means s.e.m. n=3. **(D-E)** HeLa cells infected with *Listeria* WT for 4h, analyzed by IF using antibodies against NDP52 and Galectin-8 (D) or NDP52 and LAMP-2 (E). Scale bars for panels D-E, 5 μm.

Figure S5: Detection of endogenous LC3 and DFCP-1 in *Listeria*-induced NDP52+ granules. (A-B) HeLa cells infected with *Listeria* WT for 4h, analyzed by IF using antibodies against LC3/Galectin-8 (Gal-8) (A) or DFCP-1/Gal-8 (B). Scale bars, 2.5 μm.

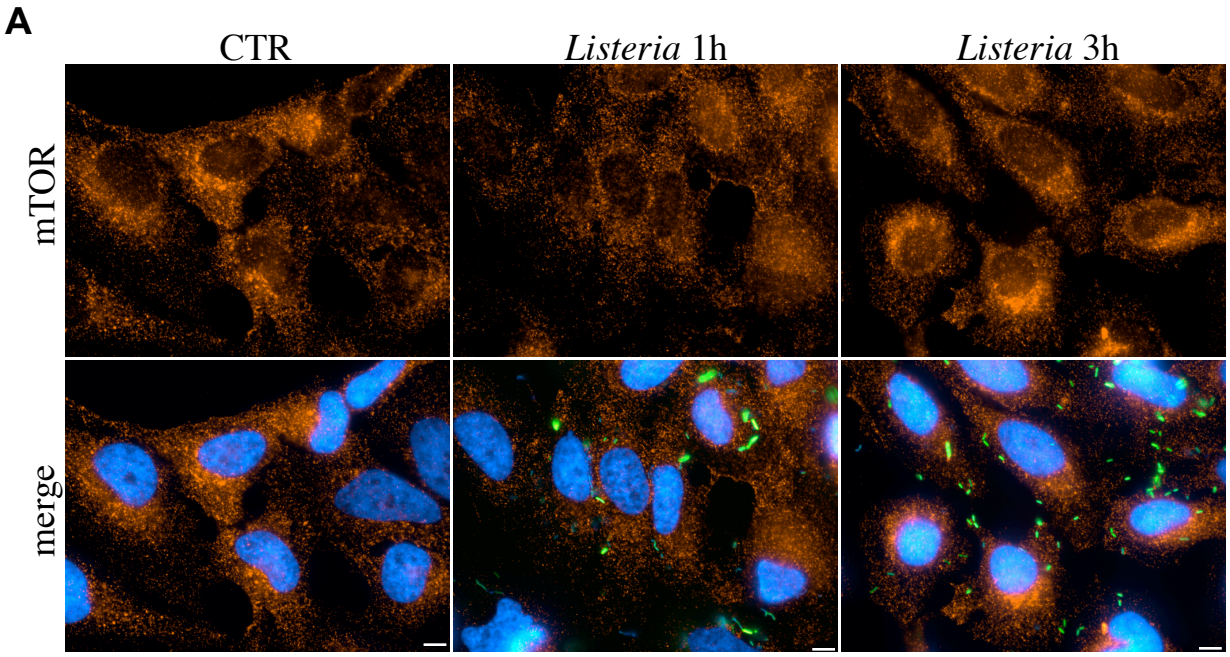
Figure S6: Detection of endogenous ATG16L1 in *Listeria*-induced NDP52+ granules. HeLa cells infected with *Listeria* WT for 1h, 2h or 4h (top) or 1h, 2h, 3h, 4h (bottom), analyzed by IF using antibodies against ATG16L1/Galectin-8 (Gal-8). Scale bars, 5 μm.

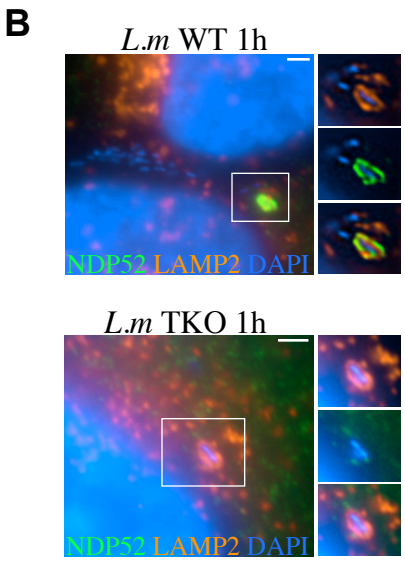
Figure S7: DFCP-1 are associated with *Listeria* autophagosomes at all stages of engulfment. HeLa cells infected with *Listeria*–GFP WT for 1h, analyzed by IF using antibodies against DFCP-1/Galectin-8 (Gal-8). Four examples are shown, highlighting the fact that DFCP-1 and Gal-8 remain associated with the structures that engulf *Listeria*, at early and late stages. In the first three examples, DFCP-1 and Gal-8 localize at a pole of *Listeria*, and the engulfment is partial. In the last example, the bacterium appears to be fully entrapped. Scale bars, 1.5 μm.

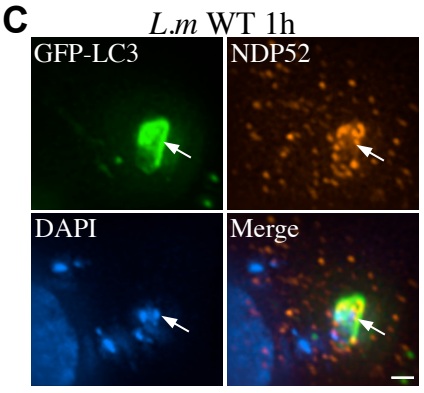
Figure S8: Functional validation of the WIPI-2 knockdown. (A) HeLa cells were transduced for 3 days with lentiviruses expressing shRNA constructs (#1 to #5) targeting human WIPI-2 or with a lentivirus control targeting a scramble (Scr.) sequence. Cell lysates were used to perform western blot against endogenous WIPI-2 or a tubulin loading control. shRNA #4 gave the best results and was used further. **(A)** HeLa cells transduced for 3 days with lentiviruses expressing shRNA WIPI-2 #4 (WIPI-2 KD) or shRNA scramble (Scramble KD) were either left unstimulated or were amino acid (AA) starved for 3h. Coverslips were analyzed by IF using antibodies against LC3 and Galectin-8 (Gal-8). Note that AA starvation induced formation of LC3+ dots in Scramble but not WIPI-2 KD cells. Scale bars, 5 μm.

Figure S9: WIPI-2 silencing inhibits LC3 but not Galectin-8 localization to *Listeria* granules. HeLa cells transduced for 3 days with lentiviruses expressing shRNA WIPI-2 #4 (WIPI-2 KD) or shRNA scramble (Scramble KD) were infected with *Listeria* wild type for 4h. Coverslips were analyzed by IF using antibodies against LC3 and Galectin-8 (Gal-8). Scale bars, 5 μm.

Figure S10: *Listeria* granules do not progress to autolysosomes. HeLa cells grown on coverslips were transfected with GFP-RFP-LC3 overnight and infected with *Listeria* for 3h. *Listeria* granules were detected in IF using an antibody against NDP52, and imaged on the Cy5 channel. The GFP and RFP fluorescence were imaged on FITC and Texas Red channels, respectively. All the NDP52+ granules were also GFP+ and RFP+, as shown on the representative image. Scale bar, 5 μm.

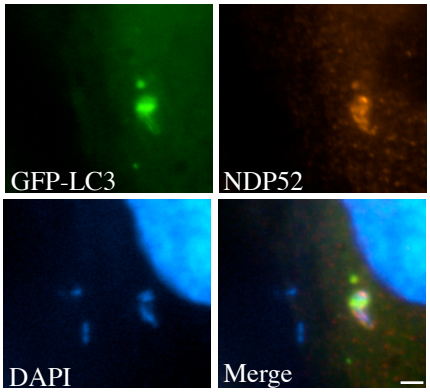




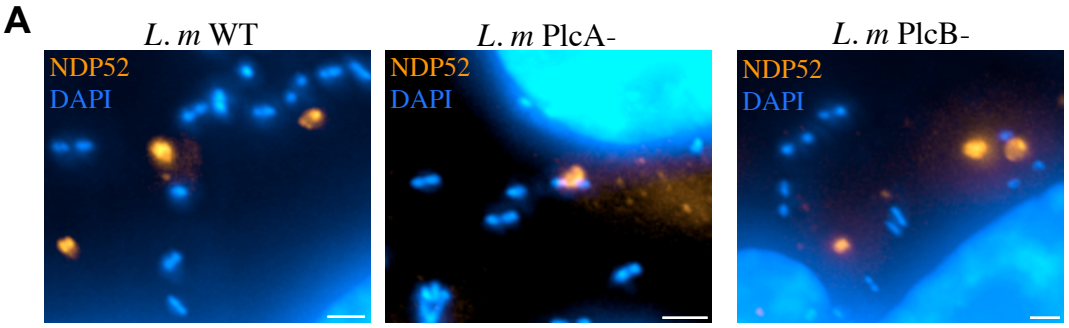


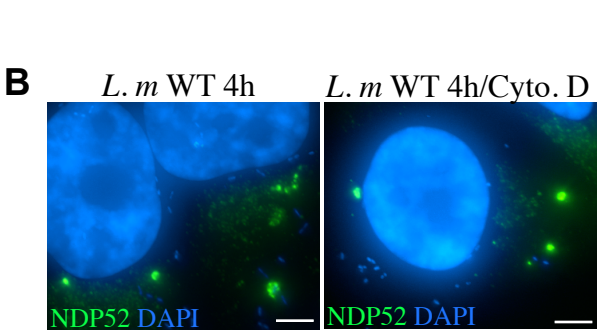
Tattoli et al. Figure S1

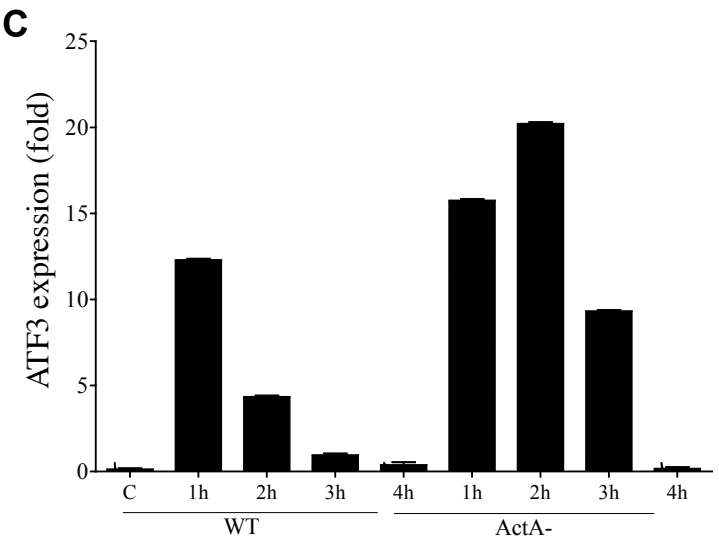
L. m PlcA/B- 4h

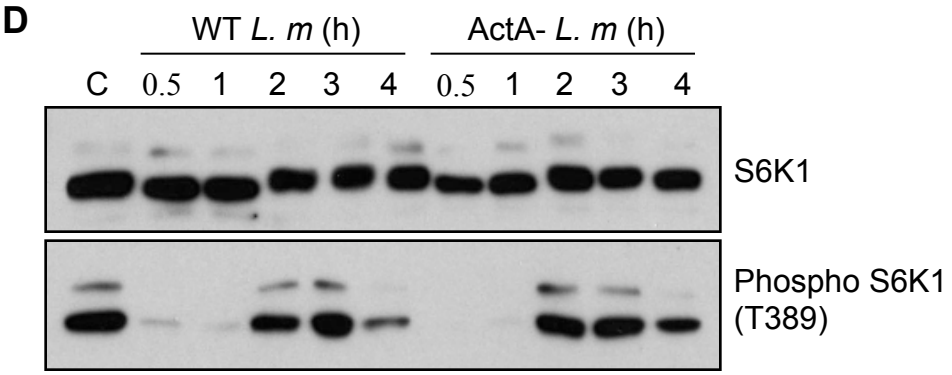


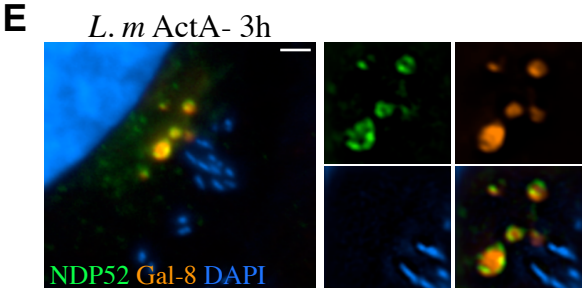
Tattoli et al. Figure S2



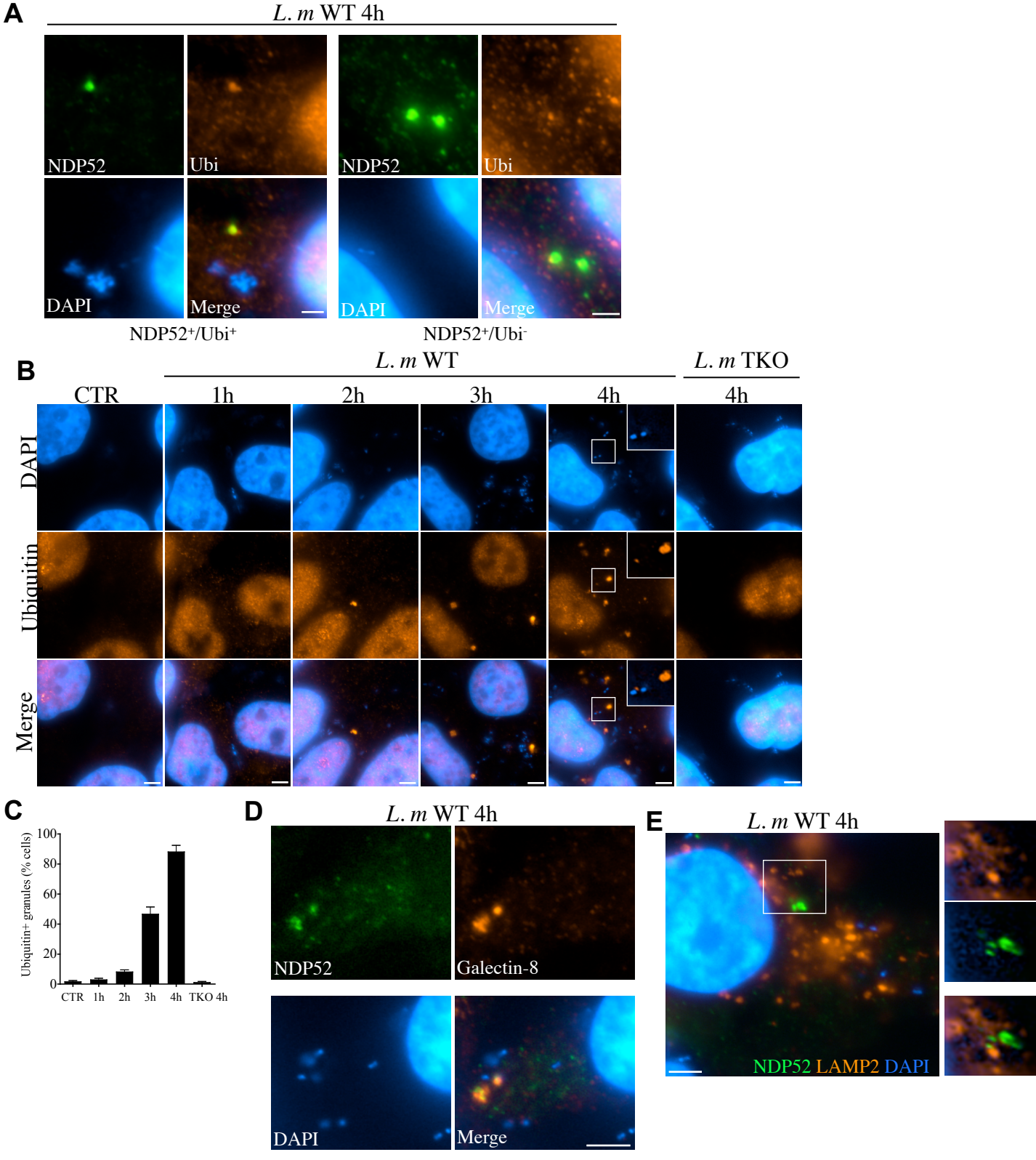








Tattoli et al. Figure S3



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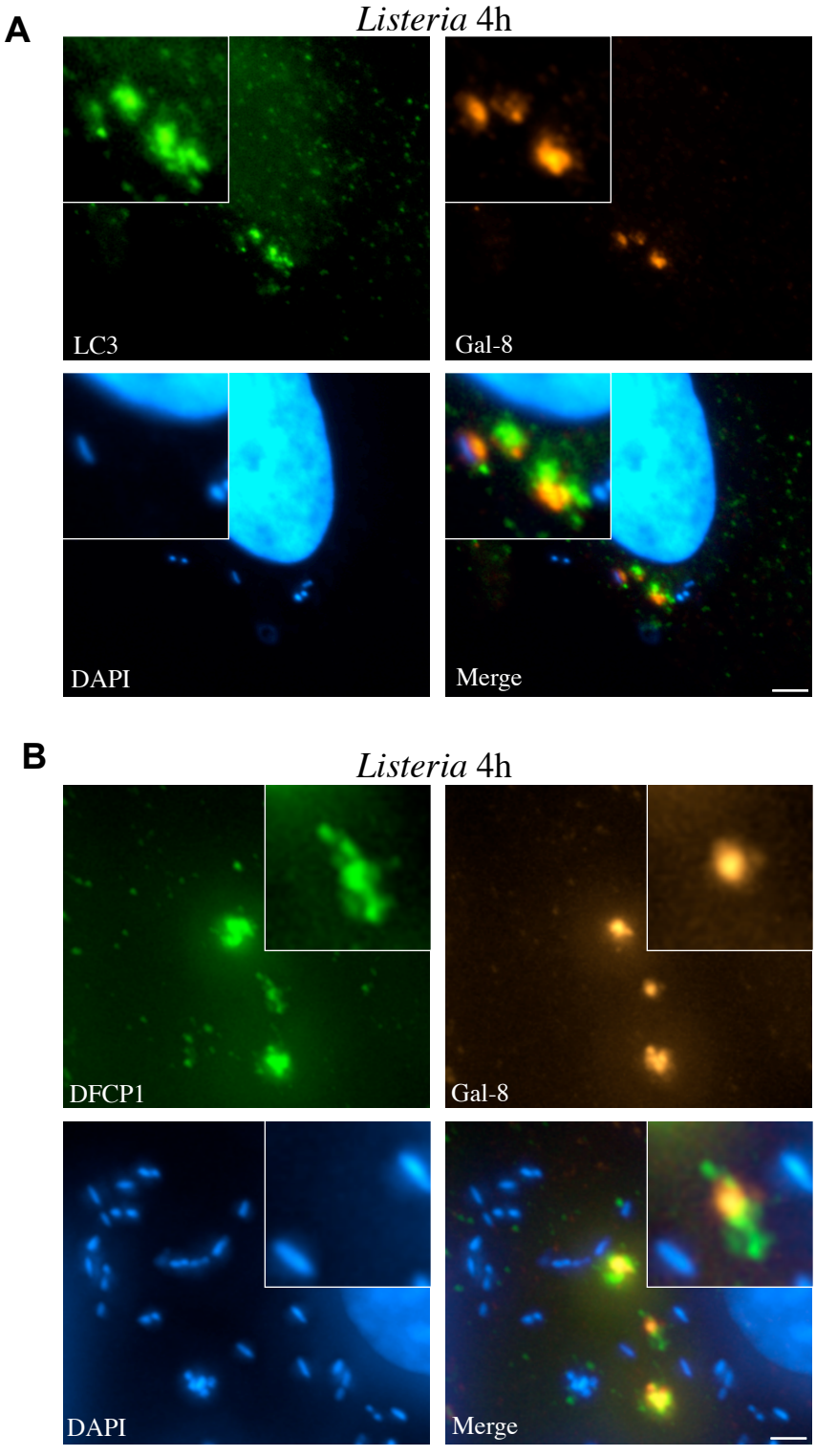

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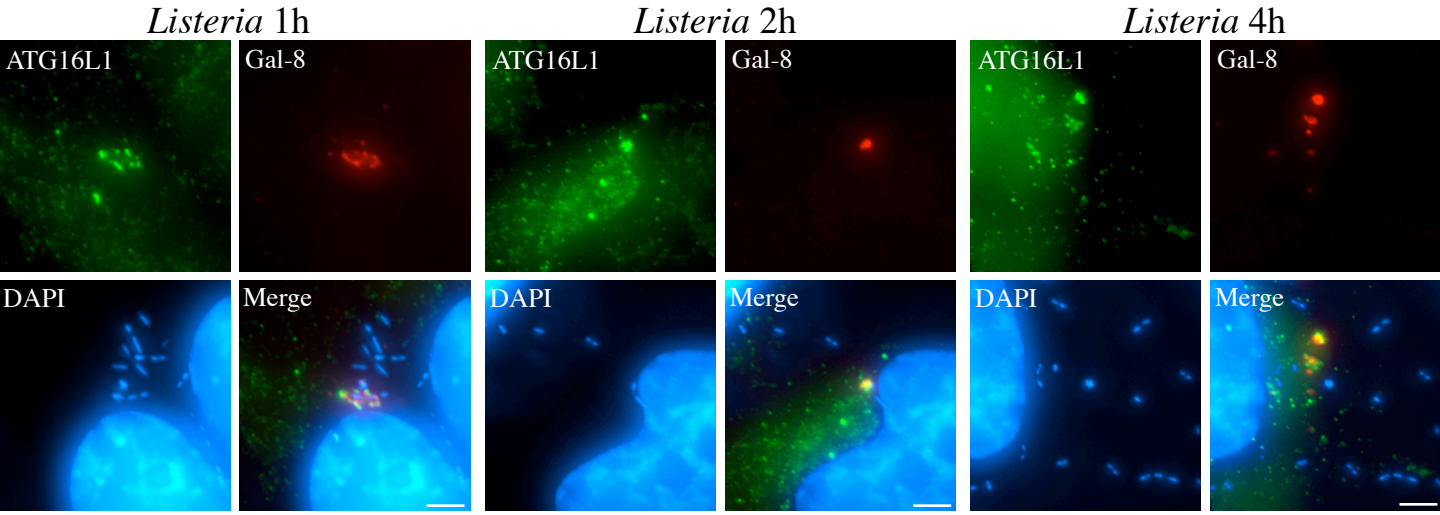
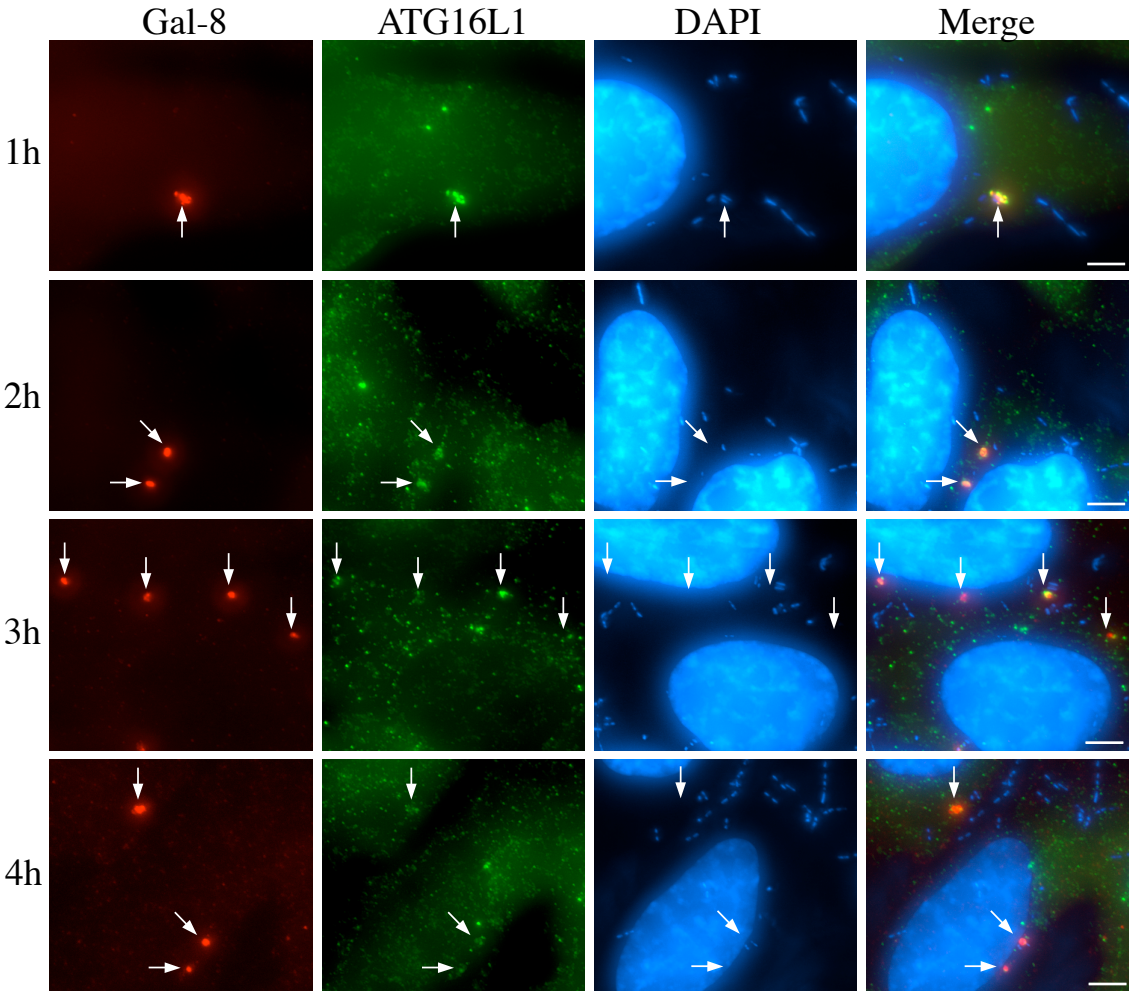
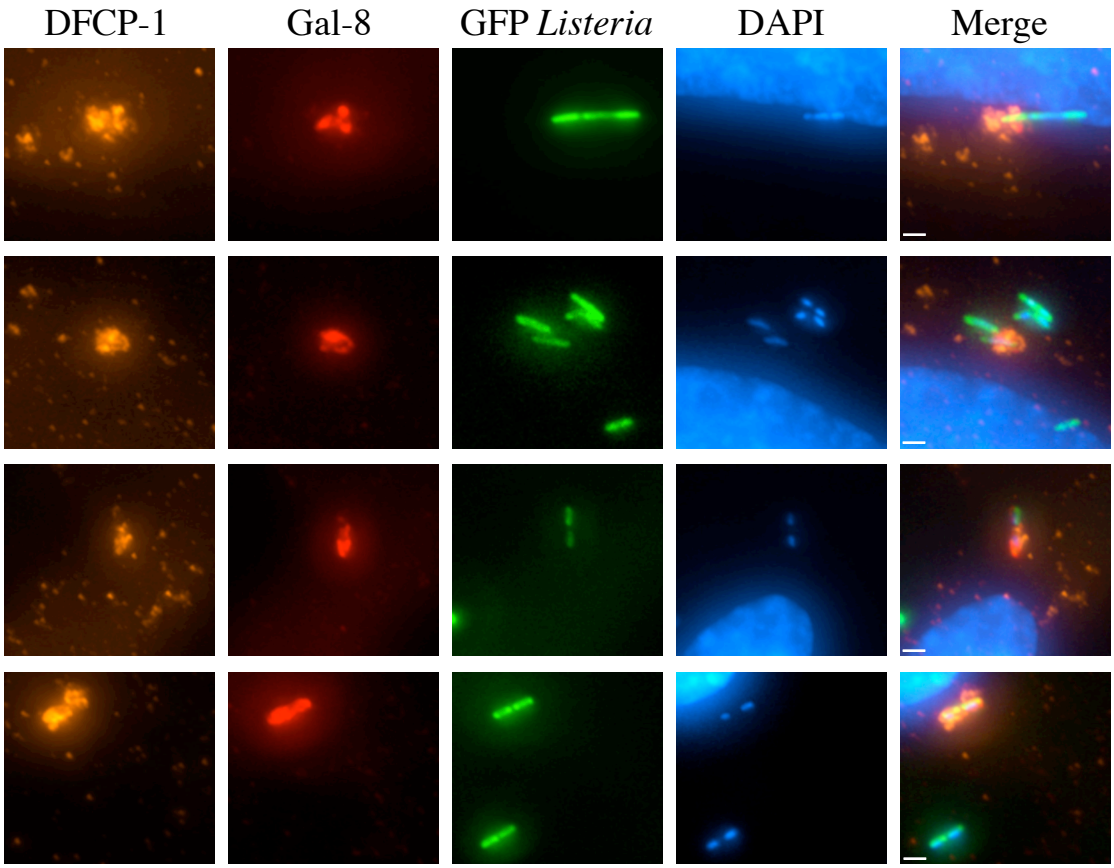


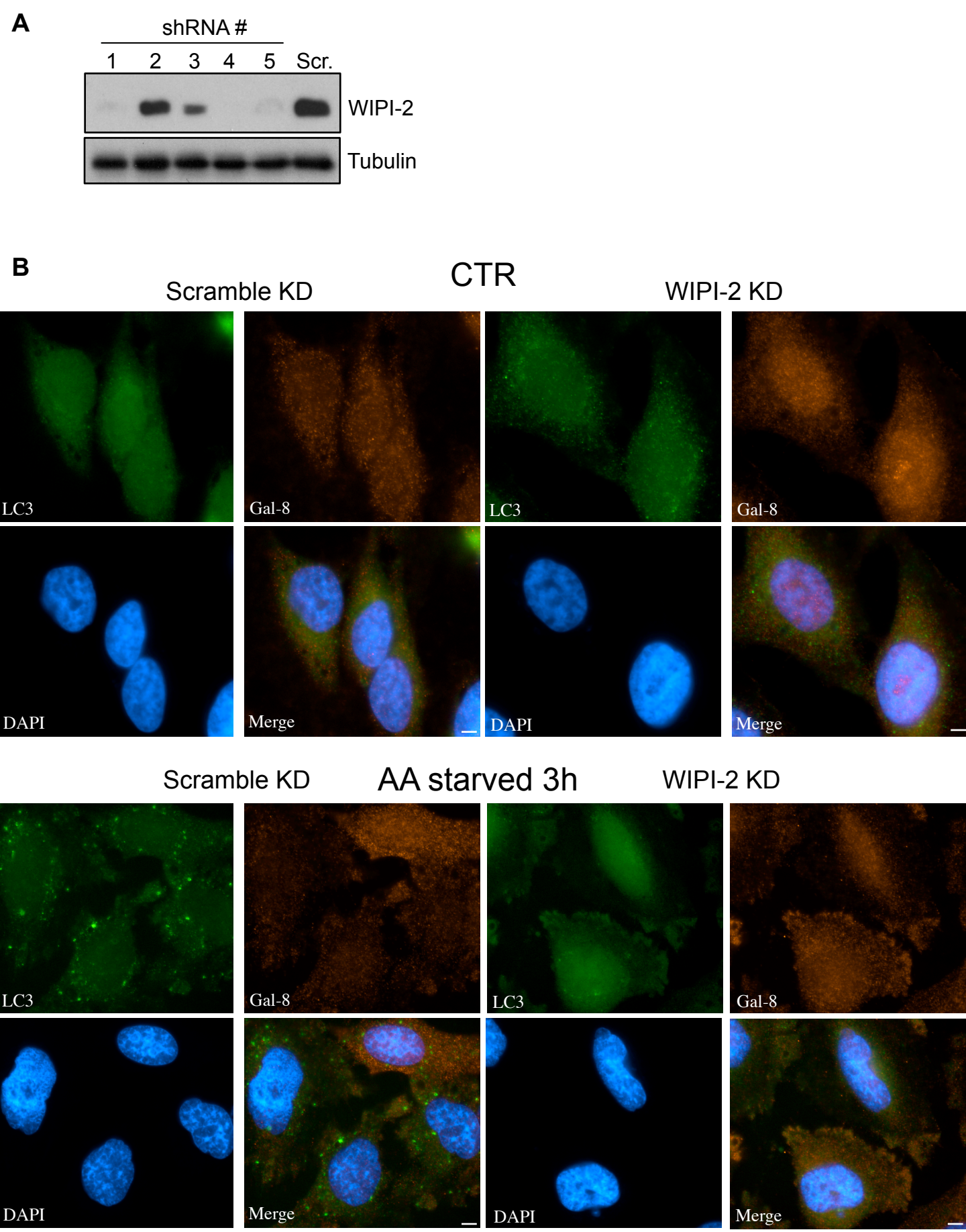


Tattoli et al. Figure S6

Listeria 1h



Tattoli et al. Figure S7



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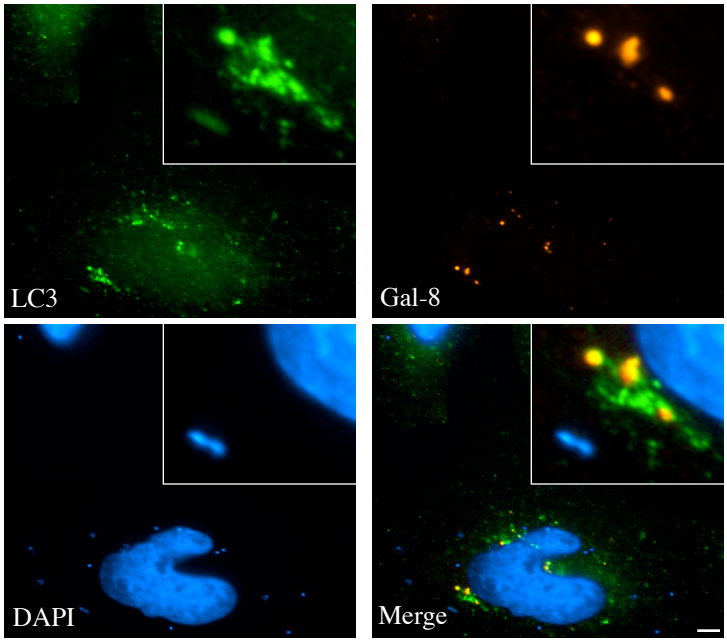
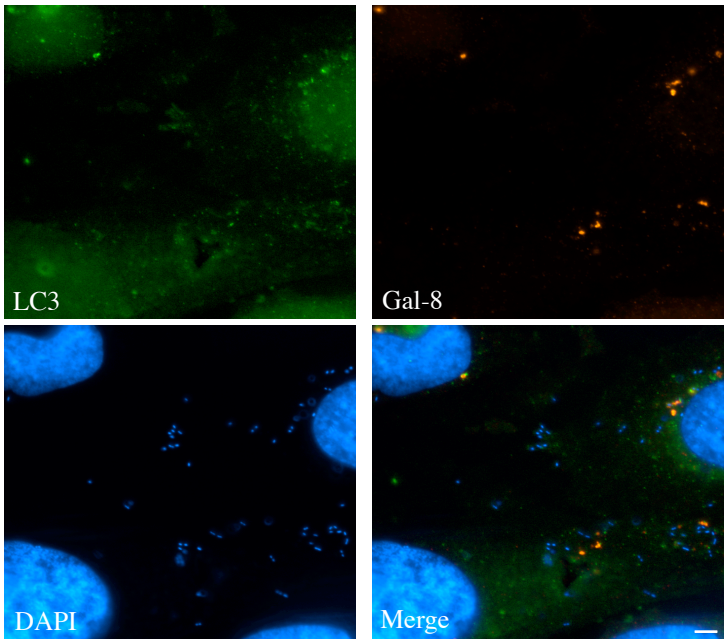
Listeria
4h

Scramble KD

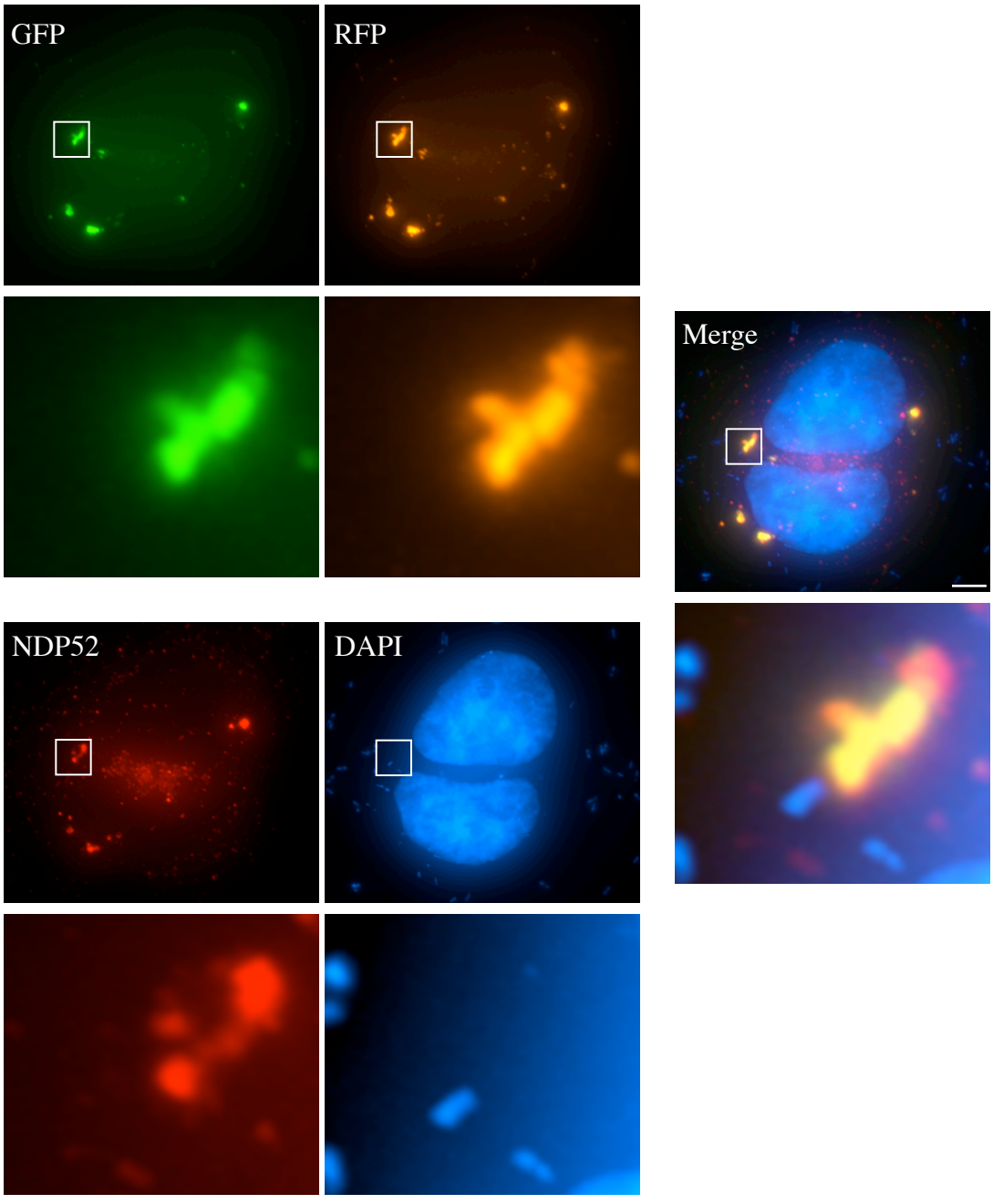


WIPI-2 KD



Tattoli et al. Figure S9

Listeria 3h



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