Expanded View Figures

Figure EV1. Infection, knockdown, and wild-type *S.* Typhimurium controls (related to Fig 1).

A Lysates from HeLa cells transfected with indicated pooled siRNAs for 72 h were analyzed by SDS–PAGE and immunoblotting. Due to low specificity of commercially available antibodies, the efficiency of RNAi-mediated depletion of NKLAM and RNF144A could not be assessed by immunoblotting. Notably, Parkin is not expressed in HeLa cells.

B HeLa cells reversely transfected with sicontrol or pooled siRNAs targeting all 14 known RBR Ub E3 ligases for 72 h were infected with wild-type cytoGFP-expressing *S.* Typhimurium for 2 h prior to fixation and immunolabeling with anti-polyUb antibody (FK2). Number of GFP-positive (GFP^+) and Ub-positive and GFP-positive (Ub^+/GFP^+) bacteria was determined using an automated quantification software and normalized to sicontrol counting on average 800 cells/sample (GFP-positive *S.* Typhimurium/cell = 3.90 ± 0.42, ubiquitylated *S.* Typhimurium [%] = 11.76 ± 2.10). Data represent mean ± SD. $n = 2$ biological replicates.

C, D *z*-scores of GFP^+ (C) and Ub^+/GFP^+ (D) bacteria from (B).

E HeLa cells transfected with sicontrol or left untreated (mock) for 72 h were infected with Δ*sifA* cytoGFP-expressing *S.* Typhimurium for 2 h prior to fixation. Number of GFP^+ bacteria in at least 250 cells/sample was determined using automated quantification. Data represent mean ± SD. Significance was determined using unpaired Student's *t*-test. ns = not significant. $n = 3$ biological replicates.

F–I Lysates from HeLa cells transfected with indicated single siRNAs for 72 h were analyzed by SDS–PAGE and immunoblotting.

J HeLa cells transfected with indicated single siRNAs for 72 h were infected as in (B) followed by fixation and confocal microscopy. Number of GFP^+ bacteria was determined by automated quantification in 250 cells/sample on average. Data represent mean ± SD. Significance was determined using one-way ANOVA. $^*P < 0.05$, $^{**}P < 0.01$. $n = 3$ biological replicates.

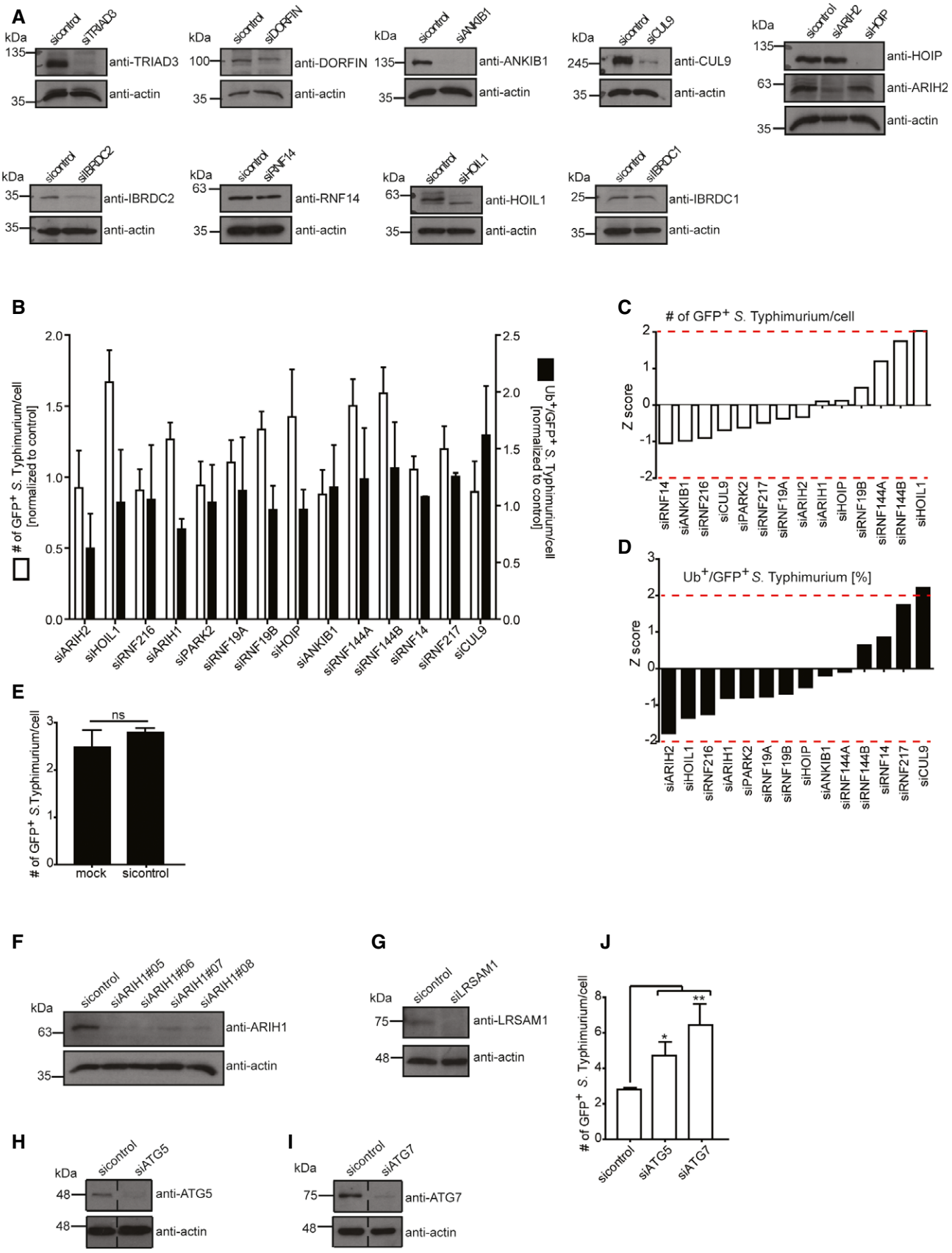

Figure EV1.

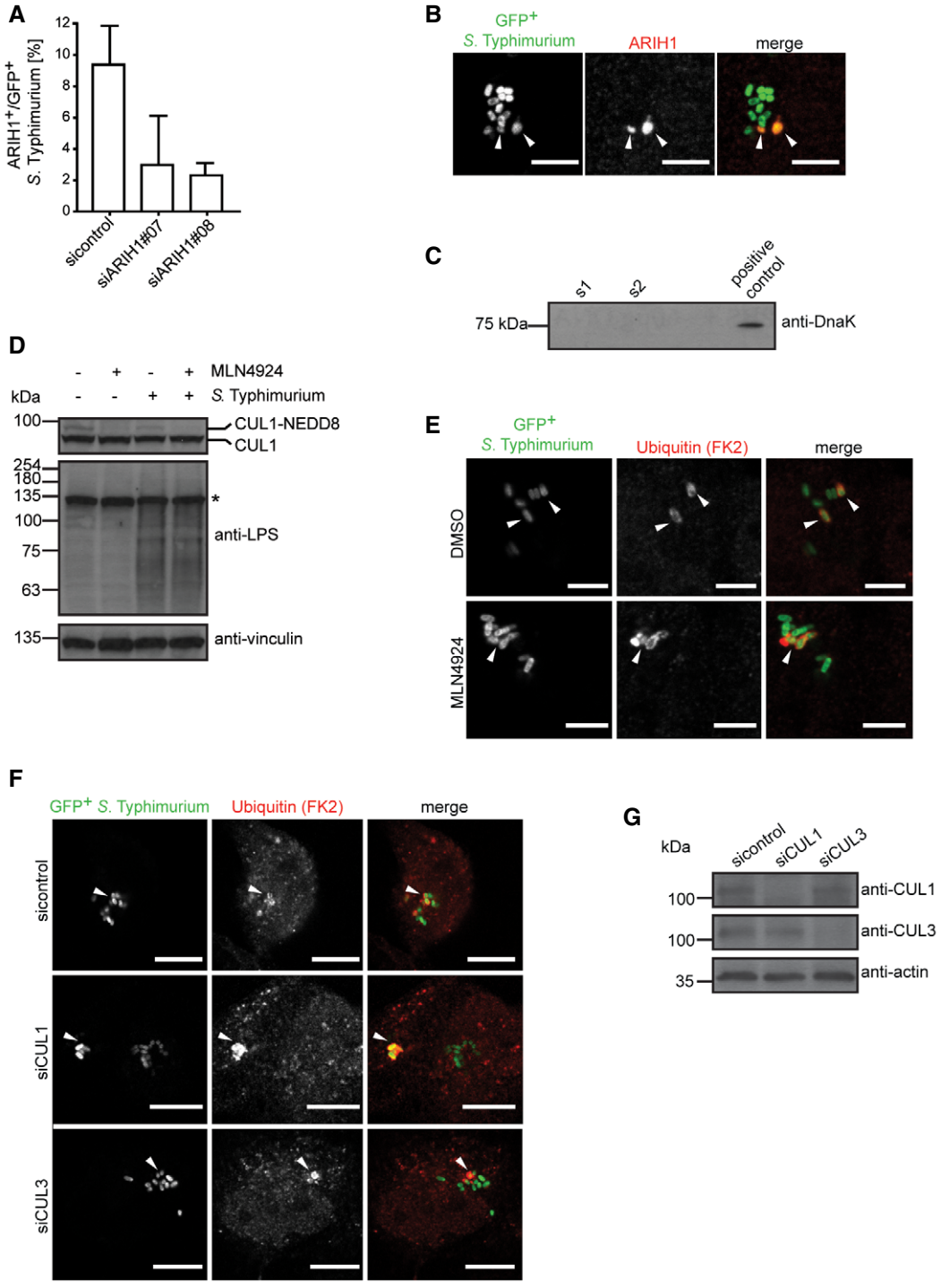

Figure EV2.

Figure EV2. Neddylated CRLs are not required for ubiquitylation of cytosolic *S.* Typhimurium (related to Figs 2–4).

A HeLa cells transfected with indicated single siRNAs for 72 h were infected with cytoGFP-expressing Δ*sifA S.* Typhimurium for 2 h followed by fixation and anti-ARIH1 immunolabeling. Number of ARIH1$^+$/GFP$^+$ bacteria in at least 250 cells/sample was determined using automated quantification. Data represent mean ± SD. n = 2 biological replicates.

B HeLa cells were infected with cytoGFP wild-type *S.* Typhimurium for 2 h followed by fixation, immunolabeling with anti-ARIH1 antibody, and confocal microscopy. Arrowheads indicate colocalization events. Scale bar: 5 μm.

C Integrity control of bacteria. While performing the *in vitro* ubiquitylation reaction, bacterial supernatants were sampled right before (s1) and immediately after (s2) the reaction. Intact bacteria were used as a positive control.

D HeLa cells infected as in (A) or left uninfected were treated with 2 μM MLN4924 or DMSO during the course of the infection prior to lysis. Lysates were analyzed by SDS–PAGE and immunoblotting.

E HeLa cells infected as in (A) were treated with MLN4924 or DMSO as in (D) prior to fixation and immunolabeling with anti-Ub antibody (FK2). Arrowheads indicate colocalization events. Scale bar: 5 μm.

F HeLa cells were transfected with indicated pooled siRNAs and infected as in (A) prior to fixation and anti-Ub (FK2) immunolabeling. Arrowheads indicate colocalization events. Scale bar: 5 μm.

G HeLa cells were reversely transfected with indicated pooled siRNAs for 72 h and lysed. Lysates were analyzed by SDS–PAGE and immunoblotting.

Figure EV3. Colocalization of xenophagy components to cytosolic *S.* Typhimurium upon ARIH1 depletion (related to Fig 4).

A Lysates from wild-type and *ATG7* CRISPR/Cas9 knockout HeLa cells were analyzed by SDS–PAGE and immunoblotting.

B–I HeLa cells transfected with indicated siRNAs for 72 h were infected with Δ*sifA* cytoGFP-expressing *S.* Typhimurium for 2 h, fixed, and immunolabeled with anti-LC3B (B), anti-NDP52 (C), anti-p62 (D), or anti-OPTN (E) antibodies. Number of GFP$^+$ bacteria that colocalized with LC3B (F), NDP52 (G), p62 (H), or OPTN (I) was determined by automated quantification in at least 100 cells/sample. Data represent mean ± SD. n = 2 biological replicates.

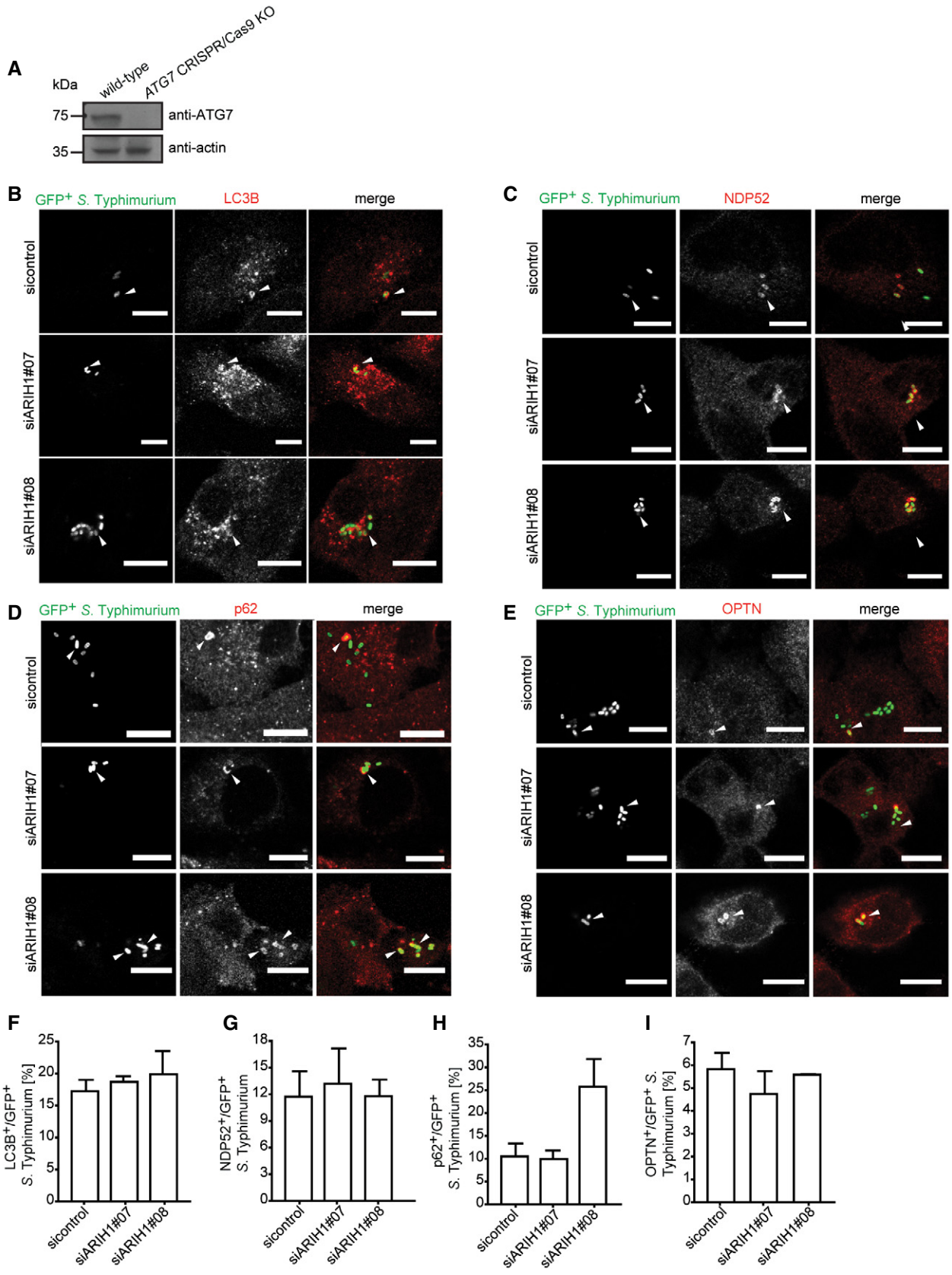

Figure EV3.

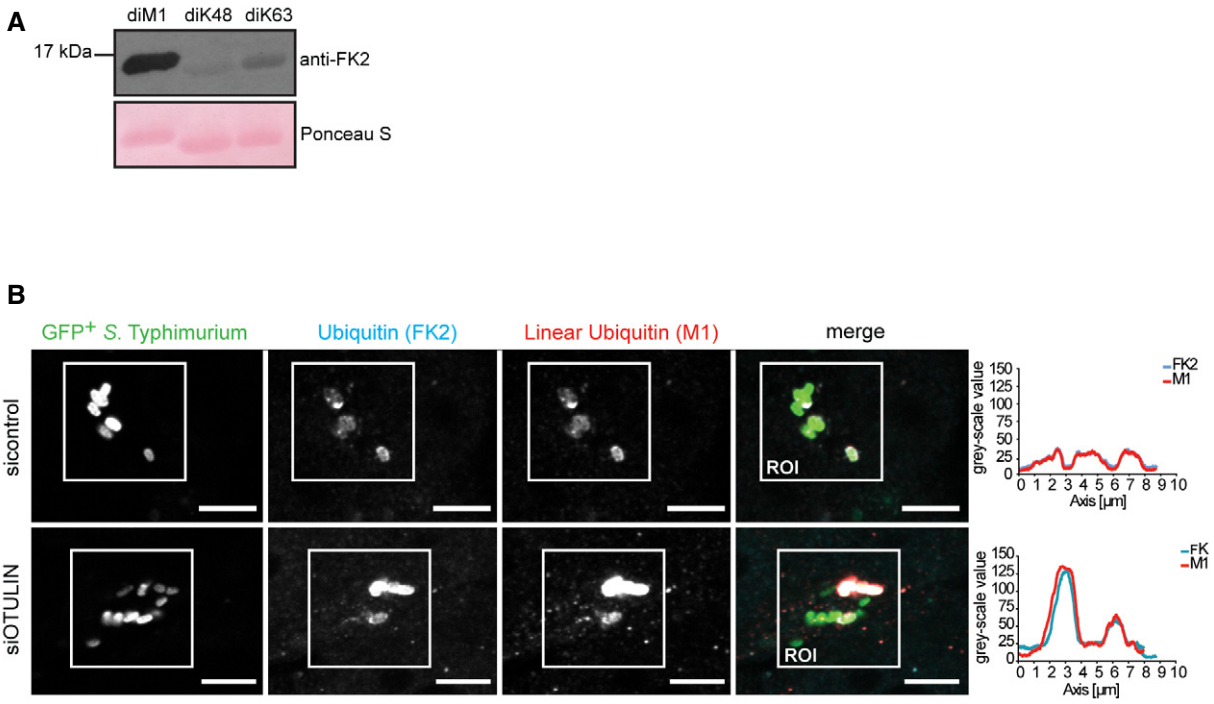

Figure EV4. Immune detection of Ub chains topologies (related to Fig 5).

A Purified linear (M1), K48-linked, and K63-linked di-Ub variants were separated by SDS–PAGE and analyzed by immunoblotting.

B HeLa cells were transfected with indicated siRNAs and infected as in (A) followed by fixation and immunolabeling with anti-polyUb antibodies (FK2 and M1). Scale bar: 5 µm. Regions of interest (ROIs) show colocalization of FK2-positive and M1-positive bacteria and a concomitant increase in fluorescence intensities.

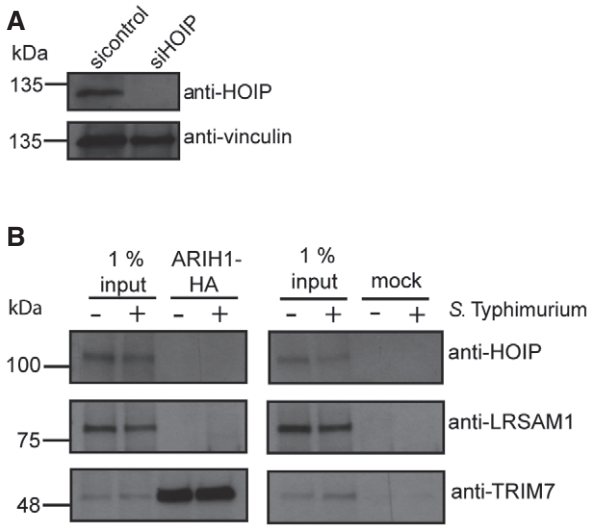

Figure EV5. ARIH1 does not bind to LRSAM1 or HOIP (related to Fig 6).

A HeLa cells were reversely transfected with indicated single siRNA for 72 h and lysed. Lysates were analyzed by SDS–PAGE and immunoblotting.

B Lysates from HeLa cells expressing C-terminally HA-tagged ARIH1 were subjected to HA immunoprecipitation and analyzed by SDS–PAGE and immunoblotting.