Supporting Information

SI Figure 6 dsRNA targeting Vps28, CG8055, Tsg101, Vps4, but not CG8743, results in the accumulation of vesicular ubiquitin. S2 cells were treated with control dsRNA (GFP) or dsRNA targeting Vps28, CG8055, Tsg101, Vps4, and CG8743. Three days after RNAi treatment, cells were fixed and stained to visualize ubiquitinated proteins. Although loss of CG8743 phenocopied depletion of the ESCRT components in S2 cells, it is likely to play a role in phagosome maturation that is distinct from that played by the ESCRT machinery, because there was no alteration in ubiquitin trafficking after depletion of CG8743. Images were acquired by using the confocal microscope.

SI Figure 7 *Drosophila* S2 cells depleted of Rab7, CG8743, or ESCRT factors exhibit diminished GFP when infected with *M. fortuitum map49::GFP*. S2 cells were treated with control dsRNA (GFP) or dsRNAs targeting Rab7, CG8743, or the ESCRT factors Vps28, CG8055, Tsg101, and Vps4 followed by infection with *M. fortuitum map49::GF*P for 2 d. The percentage of GFP-positive S2 cells was quantified. Data represent the average from six images from three replicates (± SD) as quantitated with Metamorph software.

SI Figure 8 *A* and *B* & SI Figure 8 *C* and *D* Electron microscopic (EM) appearance of *M. smegmatis* in S2 cells depleted of Tsg101. SL2 cells were untreated (*A* and *C*) or treated with dsRNA targeting Tsg101 (*B* and *D*). Cells were infected with *M. smegmatis* at a MOI <1. At 8 h (*A* and *B*), 13 h (*C* and *D*), and 24 h (data not shown) p.i., samples were fixed and processed for EM. [Samples were fixed in 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) followed by osmication and uranyl acetate staining, alcohol dehydration, and embedding in TAAB Epon. Sections (95 nm) were cut with a Leica Ultracut microtome, picked up on 100-m Formvar-coated Cu grids, stained with 0.2% lead citrate, and imaged with the Philips Technai Spirit Electron Microscope.] The number of bacteria seen in S2 cells increased >2-fold between 8 h and 13 h p.i, demonstrating that bacterial growth was occurring during this time (n > 100 S2 cells counted). In Tsg101-depleted cells, 8 h p.i. 93% of bacteria were in a vacuolar compartment (*B*), whereas 83% appeared vacuolar at 13 h (*D*). At 24 h the vacuolar number decreased to 76% (data not shown; n > 100 at all time points). Different vacuolar morphologies were seen at each time point-from compartments with very tightly juxtaposed membranes to more spacious compartments.

SI Figure 9 Four individual Tsg101 siRNAs behave similarly. RAW cells were treated with control siRNA or four different siRNAs targeting Tsg101 individually or as a pool. (*A*) All siRNAs targeting Tsg101 lead to loss of Tsg101 protein on Western analysis. (*B*) When the same extracts were analyzed for ubiquitinated proteins, all Tsg101-depleted samples had increased levels of ubiquitinated proteins, demonstrating disruption of ESCRT function. (*C*) RAW cells were depleted for Tsg101 by using individual siRNAs or pool, followed by infection with *M. smegmatis* hsp60::GFP. Whereas bacterial growth was not seen in control cells, all Tsg101-depleted samples exhibited similarly increased bacterial growth. Cells were stained with TRITC-phalloidin (shown in red), and GFP is green. [Magnification: ´ 20 (Autoscope).]

SI Figure 10 Depletion of ESCRT factors results in diminished induction from the *map49* promoter in *M. fortuitum*, not in diminished bacterial growth. S2 cells were treated with control dsRNA (GFP) or dsRNA targeting Vps28, Tsg101, Vps4, or CG8055. Cells were infected with *M. fortuitum msp12::dsRed2 map49::GFP* for 2 days. Composite images show dsRed2 in red, GFP in green, and Hoechst in blue. Confocal images are ´ 63.