Supporting Information

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SI Materials and Methods

Bacteria and Culture. *Anaplasma phagocytophilum* (*Ap*) HZ strain (1) was cultured in human promyelocytic leukemia HL-60 cells (ATCC), and *Ehrlichia chaffeensis* (Arkansas strain) (2) was cultured in human acute leukemia THP-1 cells (ATCC) in complete RPMI1640 medium supplemented with 10% (vol/vol) FBS and 2 mM L-glutamine. RF/6A cells (ATCC) and HEK293 cells (ATCC) were cultured in advanced MEM and DMEM, respectively. Cultures were incubated at 37 °C under 5% CO_2 in a humidified atmosphere. Host cell-free *Ap* was prepared by sonication as described (3). Cells were infected with *Ap* or *E. chaffeensis* at a multiplicity of infection of 100 unless otherwise indicated.

Yeast Two-Hybrid System. A DNA fragment encoding *Anaplasma* translocated substrate 1 (Ats-1; amino acids 2–376) was amplified by PCR. This fragment was cloned into the pGBKT7-BD vector (Clontech) followed by transformation into yeast strain AH109 from the Matchmaker GAL4 Two-Hybrid System 3 (Clontech) was the bait. Bait-expressing AH109 was mated with the yeast strain Y187 that had been transformed with a human bone marrow cDNA library in the pACT2-AD vector (Clontech). Mated yeast cells were first grown on low-stringency selection plates (-Leu, -Trp, and -His) and then replated onto high-stringency selection plates (-Leu, -Trp, -His, and -Ade in the presence of X-α-gal). Blue colonies were considered to express putative interacting proteins. Plasmids from positive clones were purified from yeast cells using the ChargeSwitch Plasmid Yeast Mini kit (Invitrogen). Inserts from the pACT2-AD library were transformed into *Escherichia coli* TOP10F′ competent cells (Invitrogen), purified, and sequenced.

Cloning and Cell Transfection. DNA fragments encoding Ats-1 and its mutants were amplified by PCR from *Ap* genomic DNA. The DNA fragments encoding Beclin 1 and Beclin 1(1–272), double FYVE-containing protein 1 (DFCP1), Atg14L, and the mitochondria-targeting sequence of human cytochrome *c* oxidase subunit VIIIA (Cox8A) were amplified by PCR from cDNA from human neutrophils (4). The DNA fragment encoding LC3 was amplified by PCR from pEGFP-LC3 plasmid (5). The DNA fragment encoding Rab7 was amplified by PCR from pEGFP-Rab7 plasmid. The addition of the sequence encoding the HA tag to the 5′-end of the Beclin 1-, DFCP1-, Atg14L-, and LC3-encoding sequences was performed by PCR as described (6). To generate Cox8A-Ats-1(ΔN17)-GFP, two DNA fragments that encode the mitochondria-targeting sequence of Cox8A and Ats-1(ΔN17)-GFP, respectively, were fused by PCR. To make Ats-1 mitochondria-targeting sequence (MTS) mutants, a series of forward primers encoding desired mutation was used in PCRs to create DNA sequences encoding Ats-1(Δ4R), Ats-1(+4R), and Ats-1(4-5AA). PCR products encoding Ats-1, Ats-1 mutants, HA-Beclin 1, HA-DFCP1, HA-Atg14L, HA-LC3, and HA-Rab7 were cloned into pEGFP-N1 (Clontech). All primers used for PCR amplification are listed in Table S1. Plasmids were purified using the EndoFree Plasmid Maxi Kit (Qiagen). Transfection of RF/6A endothelial cells was performed using electroporation as described (7), except that confluent cells were used. HEK293 cells were transfected using Fugene HD (Roche).

Coimmunoprecipitation. HEK293 cells cotransfected with plasmids expressing Ats-1 and HA-Beclin 1, Ats-1(90–250)-GFP and HA-Beclin 1(1–272), Ats-1(218–376)-GFP and HA-Beclin 1(1–272), Ats-1–GFP and HA-Atg14L, Ats-1(ΔN17)-GFP and HA-Atg14L, Cox8A-Ats-1(ΔN17)-GFP and HA-Atg14L, or Ats-1–GFP and Myc-UVRAG were washed two times with PBS and resuspended in immunoprecipitation (IP) buffer [50 mM Hepes (pH 7.4), 150 mM KCl, 1 mM EDTA, 1.0% (vol/vol) Triton X-100, 10% (vol/vol) glycerol, and protease inhibitor mixture (Set III; Calbiochem)]. The lysate was centrifuged at 16,000 × *g* for 10 min at 4 °C, and the supernatant was incubated with rabbit anti–Ats-1, rabbit preimmune IgG, mouse anti-HA (HA.11, clone 16B12; Covance), or mouse anti-GST (isotype control, clone B-14; Santa Cruz) for 2 h at 4 °C followed by overnight incubation with protein A or protein G agarose beads (Santa Cruz Biotechnology). The beads were washed six times with IP buffer, and the immune complexes were eluted from the beads by boiling in 2× SDS/PAGE loading buffer [100 mM Tris·HCl (pH 6.8), 4% (wt/vol) SDS, 10% (vol/vol) β-mercaptoethanol, 0.2% (wt/vol) bromophenol blue, and 20% (vol/vol) glycerol] for 5 min and then subjected to SDS/PAGE and immunoblotting. Primary antibodies for immunoblotting were mouse monoclonal anti-HA (HA.11, clone 16B12; Covance), mouse monoclonal anti-Myc (Invitrogen), and affinity-purified rabbit anti–Ats-1 (6).

Colocalization. RF/6A cells at high confluence were cotransfected with plasmids encoding Ats-1–GFP, Cox8A-Ats-1(ΔN17)-GFP, or Ats-1 with HA-Beclin 1, HA-DFCP1, HA-Atg14L, HA-LC3, or HA-Rab7. Colocalization of Ats-1 with HA-tagged proteins was determined at 1 d posttransfection (p.t.) HEK293 cells were transfected with plasmid encoding Ats-1–GFP. Colocalization of Ats-1 with endogenous lysosomal-associated membrane protein 1 (LAMP-1) was determined 2 d p.t. RF/6A cells were transfected with plasmid encoding HA-DFCP1, HA-Atg14L, or GFP-LC3 followed by infection with *Ap* at 10 h p.t. to study the colocalization at 2 d postinfection. Cells were fixed in 2% (wt/vol) paraformaldehyde in PBS at room temperature for 30 min followed by permeabilization in PBS containing 0.4% (wt/vol) BSA, 0.2% (wt/vol) gelatin, and 0.3% (wt/vol) saponin solution. Cells were labeled with rabbit anti–Ats-1, horse anti-*Ap* (8), mouse monoclonal anti-*Ap* P44 (clone 5C11) (9), or mouse monoclonal antibodies against cytochrome *c* (clone 2G8; Santa Cruz Biotechnology), Mn-Sod (clone MnS-1; Alexis), HA, or LAMP-1 (clone H4A3; Developmental Studies Hybridoma Bank). RF/6A cells were transfected with plasmid encoding Ats-1–GFP or GFP-LC3 followed by infection with mCherry-expressing *Ap* at 10 h p.t. Live-cell imaging was performed at 2 d postinfection.

Correlative Light-Electron Microscopy. Correlative light-electron microscopy was carried out as described (10). Briefly, RF/6A cells transfected with plasmid expressing Ats-1–GFP or GFP were grown on CELLocate coverslips (Eppendorf) and fixed at 1 d p.t. with 1.25% (wt/vol) paraformaldehyde, 2.5% (wt/vol) glutaraldehyde, and 0.03% (vol/vol) picric acid in 0.1 M sodium cacodylate buffer (pH 7.4) at 4 °C for 8 h. After washing with the cacodylate buffer, samples were observed under a fluorescence microscope, and the cell with the structure of interest was localized with the help of grid coordinates. Samples were then fixed in 1% (wt/vol) OsO_4 solution containing 1.5% (wt/vol) potassium ferrocyanide in 0.1 M sodium phosphate buffer (pH 7.4) for 1 h at room temperature, dehydrated, and embedded in epoxy resin, which was allowed to polymerize for 48 h, for conventional EM. After removing CELLocate coverslips, the cells were sectioned horizontally to the substrate at 80 nm. Sections were collected on copper grids covered with formvar-carbon supporting film and examined with the H7600 transmission electron microscope.

LC3 Conversion. HEK293 cells were transfected with plasmid expressing Ats-1–GFP or GFP. Bafilomycin A1 (Sigma) was added to transfected cells at a final concentration of 400 nM at 20 h p.t. and allowed to incubate with cells for 4 h. To study LC3 conversion in *Ap*-infected HL-60 cells, infected and uninfected (control) cells were harvested at 2 d postinfection (90% infected cells). Cells were lysed and subjected to Western blotting using rabbit anti-LC3 (Novus Biologicals), rabbit antiactin, mouse anti–α-tubulin (Santa Cruz Biotechnology), or mouse anti-GFP and anti-*Ap* P44.

Ats-1–GFP Turnover. RF/6A cells transfected with plasmid encoding GFP or Ats-1–GFP were treated with cycloheximide (Sigma) at the final concentration of 20 μg/mL at 1 d p.t. Cells were harvested at 0, 4, 8, 24, 48, and 72 h posttreatment and subjected to Western blot analysis using rabbit antiactin (Sigma) and mouse anti-GFP (clone B-2; Santa Cruz Biotechnology).

Localization of Ats-1 in HL-60 Cells Selectively Permeabilized with Streptolysin O. The method to selectively permeabilize plasma membranes with streptolysin O (SLO) (11) was used with some modifications. Briefly, *Ap*-infected HL-60 cells were resuspended in serum-free RPMI1640 medium and kept on ice followed by the addition of DTT-activated SLO (Sigma). After incubation on ice for 15 min, cells were washed three times with serum-free RPMI1640 to remove unbound SLO. SLO-treated cells were incubated at 37 °C for 30 min in serum-free RPMI1640 supplemented with 20 mM Hepes (pH 7.5) and 5 mM EGTA to allow oligomerization of SLO and the formation of pores on the plasma membrane. Cells were then immediately fixed in 4% (wt/vol) paraformaldehyde solution and stained with rabbit anti–Ats-1 and mouse anti-P44 followed by incubation with secondary antibodies Alexa Fluor 488-conjugated goat anti-rabbit IgG and Alexa Fluor 555-conjugated goat anti-mouse IgG (Invitrogen) in PBS supplemented with 0.5% (wt/vol) BSA. After washing with PBS, the immunostained cells were incubated with the PGS to permeabilize the membranes and labeled with mouse anti-P44 and Alexa Fluor 350-conjugated goat anti-mouse IgG.

Phosphorylated Mammalian Target of Rapamycin Level. RF/6A cells transfected with plasmid encoding GFP or Ats-1–GFP were harvested at 1 d p.t. HL-60 cells infected with host cell-free *Ap* were harvested at 4, 12, 24, 48, and 72 h postinfection. Harvested cells were subjected to Western blot analysis using antibodies against phosphorylated mammalian target of rapamycin (mTOR; S2448; Cell Signaling Technology), P44, and α-tubulin or actin followed by membrane stripping to disassociate bound antibodies using Western blot stripping buffer (Pierce); they were reprobed with anti-mTOR (Cell Signaling Technology).

Immuno-EM. The immunogold method with preembedding gold enhancement was performed as described (12). In brief, cells were fixed in 4% (wt/vol) paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) for 2 h at room temperature and permeabilized in the same buffer containing 0.25% (wt/vol) saponin for 30 min. After blocking for 30 min in the same buffer containing 0.1% (wt/vol) saponin, 10% (wt/vol) BSA, 10% (vol/vol) normal goat serum, and 0.1% (wt/vol) cold-water fish skin gelatin (blocking solution), specimens were incubated with rabbit anti–Ats-1 or mouse anti-GFP overnight followed by incubation with colloidal gold (1.4 nm in diameter; Nanoprobes) conjugated to goat anti-rabbit IgG or anti-mouse IgG in blocking solution for 2 h. After treatment with a gold enhancement kit (GoldEnhance EM; Nanoprobes) for 2 min at room temperature to intensify the signal, the specimens were postfixed in 1% (wt/vol) OsO_4 solution containing 1.5% (wt/vol) potassium ferrocyanide, dehydrated in a series of graded ethanol solutions, and embedded in epoxy resin. Ultra-thin sections were collected, stained with uranyl acetate and lead citrate, and observed with a Hitachi H7600 transmission electron microscope.

Subcellular Fractionation by OptiPrep Density Gradient Centrifugation. Subcellular fractionation was performed as described in the work by Xiong and Rikihisa (13). Briefly *Ap*-infected at 3 d postinfection and uninfected HL-60 cells were homogenized in homogenization buffer [250 mM sucrose, 20 mM Tris·HCl (pH 7.4), 1 mM EDTA] containing protease inhibitor mixture. After removing the unbroken cells and nuclei by centrifugation ($500 \times g$ for 5 min), the supernatant was loaded onto the top of an 11-mL linear 5–25% (vol/vol) OptiPrep (Sigma) gradient prepared with the homogenization buffer. The gradient was centrifuged at $200{,}000 \times g$ for 3 h in a Beckman SW41 rotor. Fractions (12 total at 1 mL each) were collected from top to bottom sequentially. Equal aliquots from each fraction were analyzed by immunoblotting using anti-P44, anti-LC3, anticalnexin (Stressgen), anti–LAMP-2 (clone H4B4; Developmental Studies Hybridoma Bank), and anti-EEA1 (BD). To examine the intactness of *Ap* inclusions in density gradient centrifugation, the seventh fraction that contains *Ap* inclusions was treated with pronase (Sigma) at 200 μg/mL for 30 min followed by Western blot analysis using anti-P44. As a positive control, flotillin-1, which was in the same fraction as *Ap* inclusions, was treated the same way and detected with antiflotillin-1 antibody (BD).

Antibody Delivery by Chariot. Chariot reagent (1 μL; Active Motif) was mixed with 100 μL PBS containing 1 μg purified rabbit anti–Ats-1 or control preimmune rabbit IgG. After incubation for 30 min at room temperature, the complex was added to 5×10^5 *Ap*-infected HL-60 cells (1 d postinfection) in 2 mL medium, and these cells were then cultured for an additional 2 d. The infectivity was then determined by Diff-Quik staining. In addition, *Ap* P44, human α-tubulin, and LC3 were analyzed by Western blotting.

Effect of Ats-1–GFP on Bacterial Growth. RF/6A cells were transfected with plasmid expressing Ats-1–GFP or GFP followed by infection with *Ap* or *E. chaffeensis* at 10 h p.t. Infected cells were harvested at 3 d postinfection and analyzed by Western blotting with rabbit anti-actin, mouse anti-P44, and anti-GFP or rabbit antibody against P28 from *E. chaffeensis* (anti-P28) (14).

***beclin 1* Heterozygous-Deficient Mice.** Five each *beclin* $1^{+/-}$ C57BL/6 mice and congenic WT mice (5- to 6-wk-old males) were inoculated i.p. with *Ap*-infected HL-60 cells (>90% cells infected; 1×10^6 cells/mouse). Blood samples were collected at 5 d after inoculation and subjected to DNA isolation using a QIAamp DNA blood mini kit; quantitative PCR was performed using *Ap* 16S rDNA and mouse glyceraldehyde 3-phosphate dehydrogenase (G3PDH) gene primers. The protocol for use of animals in this study was approved by the Institutional Laboratory Animal Care and Use Committee.

Amino Acids Supplementation with 3-Methyladenine, and Effect of Lysosomal, Proteasome Inhibitors, and Diisopropylfluorophosphate on *Ap* Infection. HL-60 cells at 3×10^5 cells were infected with host cell-free *Ap* in 1 mL complete RPMI1640 medium. The media of infected cells were supplemented with complete medium (control) or complete medium containing 5 × MEM amino acid solution (pH 7.4; Invitrogen), 3-methyladenine (final concentration = 2 mM), or 3-methyladenine (2 mM) and 5 × MEM amino acids at 1 d postinfection. The *Ap* infection at 3 d postinfection was assessed by quantitative PCR using *Ap* 16S rDNA and human G3PDH gene primers. The primer sequences for human G3PDH gene were described in the work by Zhang et al. (15). To determine the effect of different chemicals on *Ap* infection, HL-60 cells at 3×10^5 cells were infected with host cell-free *Ap* in 1 mL complete RPMI1640 medium containing 10 nM Bafilomycin A1 (Sigma), 0.1 μM MG-132 (Millipore), 0.1 μM diisopropyl-

fluorophosphate (DFP), which was added 1 d postinfection, or solvent control. To determine whether pretreated host cells or host cell-free bacteria by DFP have an effect on the later infection, HL-60 cells or host cell-free *Ap* were treated with DFP (0.1 μM) for 0.5 h on ice, washed by centrifugation to remove DFP, and then used for infection. All bacterial infectivity was assayed 3 d postinfection by Diff-Quik staining.

Imaging and Image Processing. Imaging for fixed cells was performed using an Eclipse E400 fluorescence microscope with a xenon-mercury light source (Nikon) or a FluoView FV1000 confocal laser scanning microscope (Olympus). Live-cell imaging was performed with a Deltavision Deconvolution Microscope (Applied Precision). For Western blot analysis, images were captured using a CCD camera (LAS-3000; Fujifilm), and band density was measured with Fujifilm MultiGauge software. Images were processed using Photoshop CS2 software (Adobe).

Statistical Analysis. Analyses of significant differences between means were performed using two-tailed Student *t* and ANOVA tests. Data are shown as mean values ± SDs from three independent experiments. The dot plot for the experiment of *beclin 1* heterozygous-deficient mice was drawn using GraphPad Prism 5 program (GraphPad).

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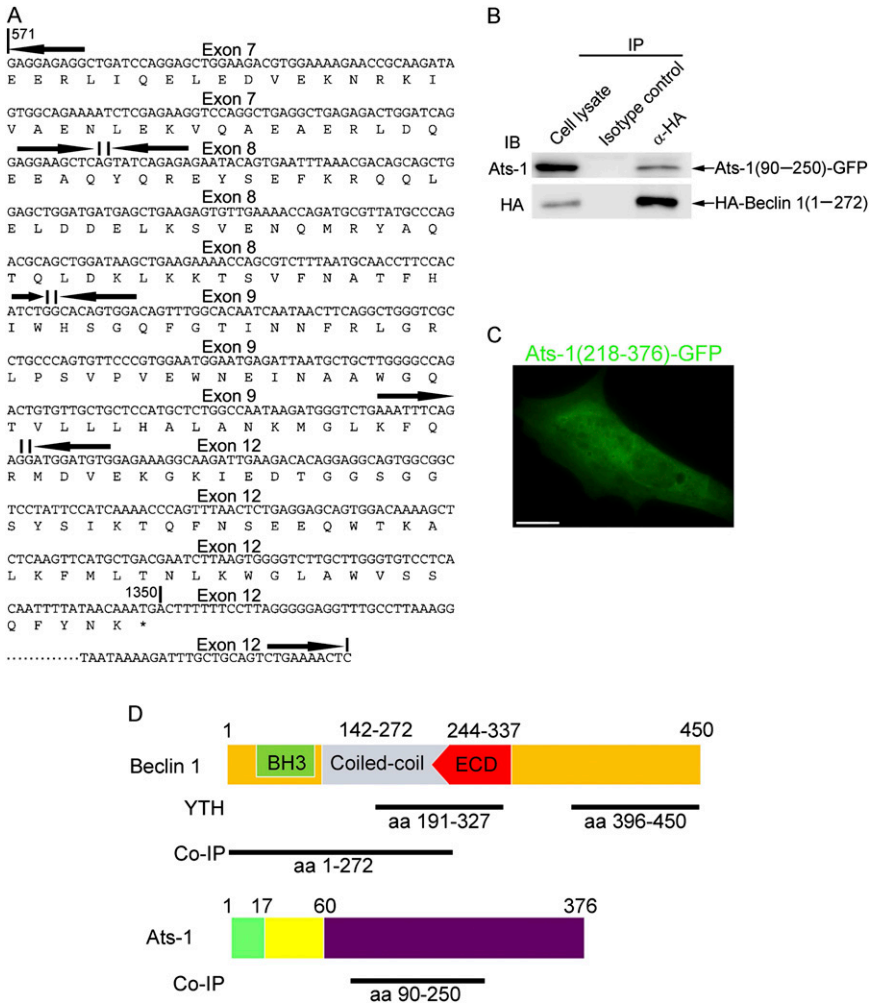


Fig. S1. Beclin 1 was identified as an Ats-1 interacting partner. (*A*) Sequencing of the DNA insert in the prey plasmid recovered from a yeast two-hybrid screening (YTH) revealed a partial human Beclin 1 gene, which contains nucleotides 571–981 and 1186–1359 of the coding sequence of *beclin 1* mRNA corresponding to exons 7–9 and 12. The boundaries of each exon are delineated with arrows and vertical lines. The predicted amino acid sequence is showed below the DNA sequence. An asterisk indicates the stop codon. Dotted line indicates the omitted nucleotides. (*B*) Reciprocal co-IP assay for protein interaction between Ats-1(90–250)-GFP and HA-Beclin 1(1–272). Cotransfected HEK293 cells were IP with isotype control or anti–(α)-HA. Precipitates were immunoblotted (IB) with anti-HA and anti–Ats-1. (*C*) The diffuse pattern of Ats-1(218-376)-GFP in transfected RF/6A cells. (Scale bar: 10 μm.) (*D*) Summary of the results of Ats-1 and Beclin 1 interaction in YTH and co-IP. Beclin 1 has three distinct domains that are a BH3 domain (amino acids 88–150), a coiled-coil domain (amino acids 144–269), and an evolutionarily conserved domain (ECD; amino acids 244–337). Truncated Beclin 1 (amino acids 191–327 and 396–450) and Beclin 1 (amino acids 1–272) were found to interact with Ats-1 in YTH and co-IP, respectively. Ats-1 has MTS in its N terminus (amino acids 1–17). Approximately 60 aa of N terminus are cleaved off when Ats-1 is imported into mitochondria. Ats-1 fragment (amino acids 90–250) was found interacting with Beclin 1 (amino acids 1–272) in co-IP.

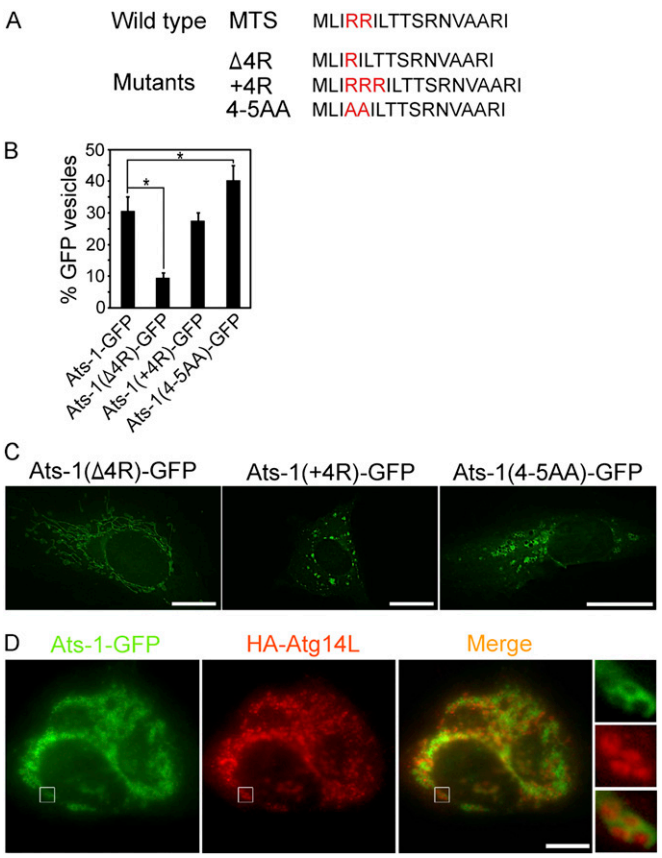


Fig. S2. Vesicle formation of Ats-1 MTS mutants and colocalization between Ats-1–GFP and HA-Atg14L. (*A*) The MTS sequence of Ats-1 and four mutants. The Ats-1 MTS is mutated by deleting the first Arginine (Δ4R), adding Arginine between fourth and fifth amino acids (+4R), or replacing the first two Arginines with two Alanine ((4-5AA). (*B*) The percentage of cells with vesicle formation in Ats-1 or Ats-1 MTS mutant-expressing cells. RF/6A cells were transfected with plasmid encoding Ats-1 or its MTS mutants, including Δ4R, +4R, and 4-5AA; 100 transfected cells in each group were subjected to fluorescence imaging, and the percentage of cells with vesicle formation was calculated. *Significantly different from Ats-1–GFP by Student *t* test ($P < 0.05$). (*C*) The selective image of Ats-1 MTS mutants to show mitochondrial targeting [Ats-1(Δ4R)-GFP] and vesicle formation [Ats-1(+4R)-GFP and Ats-1(4-5AA)]. (Scale bars: 10 μm.) (*D*) RF/6A cells were cotransfected with plasmids encoding Ats-1–GFP and HA-Atg14L and subjected to immunolabeling. Boxed areas are magnified on the right. (Scale bar: 10 μm.)

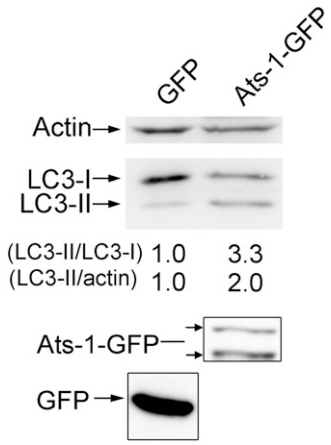


Fig. S3. Immunoblotting of lysates showing LC3 conversion in HEK293 cells expressing Ats-1–GFP or GFP (1 d p.t.). Relative band density ratios for LC3-II/LC3-I and LC3-II/actin are shown, with the ratios of those band densities from GFP set as one. The expression of GFP and Ats-1–GFP in transfected cells is shown at the bottom.

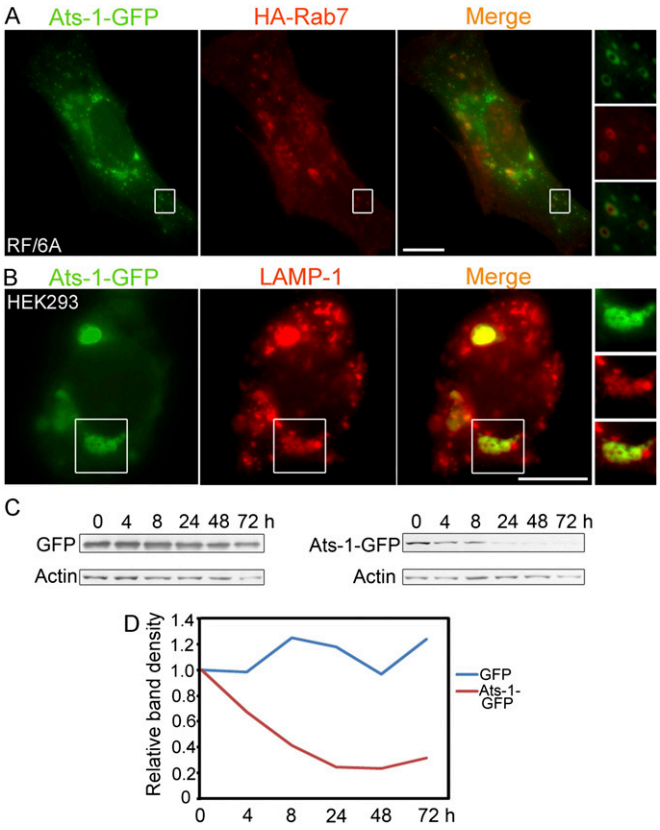


Fig. S4. The colocalization of Ats-1–GFP with HA-Rab7 and LAMP-1 and the degradation of Ats-1–GFP in transfected cells. (*A*) RF/6A cells were cotransfected with plasmids encoding Ats-1–GFP and HA-Rab7 and subjected to immunolabeling. Boxed areas are magnified on the right. (Scale bar: 10 μm.) (*B*) HEK293 cells transfected with plasmid encoding Ats-1–GFP were stained with anti–LAMP-1 antibody, which does not label LAMP-1 in monkey endothelial RF/6A cells. Boxed areas are magnified on the right. (Scale bar: 10 μm.) (*C*) Degradation of GFP and Ats-1–GFP in transfected cells. RF/6A cells transfected with plasmid encoding GFP or Ats-1–GFP were treated with cycloheximide at the final concentration of 20 μg/mL at 1 d p.t. Cells were harvested at 0, 4, 8, 24, 48, and 72 h posttreatment and subjected to Western blot analysis using antibodies against actin and GFP. (*D*) Relative band density ratio of GFP or Ats-1–GFP to actin at different time points posttreatment when GFP or the Ats-1–GFP to actin ratio at 0 h is set to one.

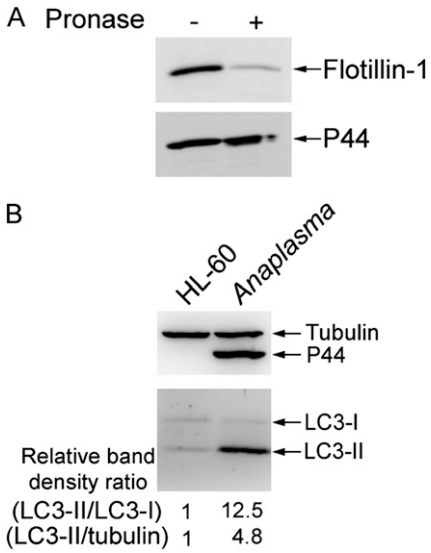


Fig. S5. Lack of digestion by pronase for *A. phagocytophilum* P44 in fractionated *A. phagocytophilum* inclusions and LC3 conversion in HL-60 cells infected with *A. phagocytophilum*. (*A*) *A. phagocytophilum*-infected cells were fractionated by OptiPrep density gradient centrifugation. A portion of fraction containing *A. phagocytophilum* and flotillin-1 was subjected to pronase treatment and Western blot analysis using antibodies against to P44 and flotillin-1. +, pronase treatment; –, no pronase treatment. (*B*) *A. phagocytophilum*-infected HL-60 cells (90% of cells infected) and uninfected HL-60 cells were harvested and subjected to Western blot analysis using mouse antitubulin, mouse anti-P44, and rabbit anti-LC3. The band density of tubulin, LC3-I, and LC3-II was measured, and the relative ratio of LC3-II/tubulin and LC3-II/LC3-I was calculated for *A. phagocytophilum*-infected HL-60 cells (*Anaplasma*), with the ratios in uninfected HL-60 cells (HL-60) set as one.

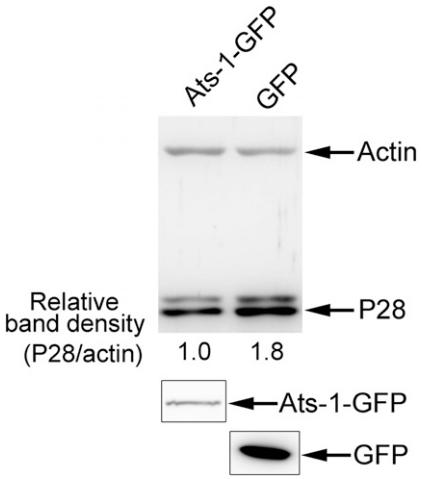


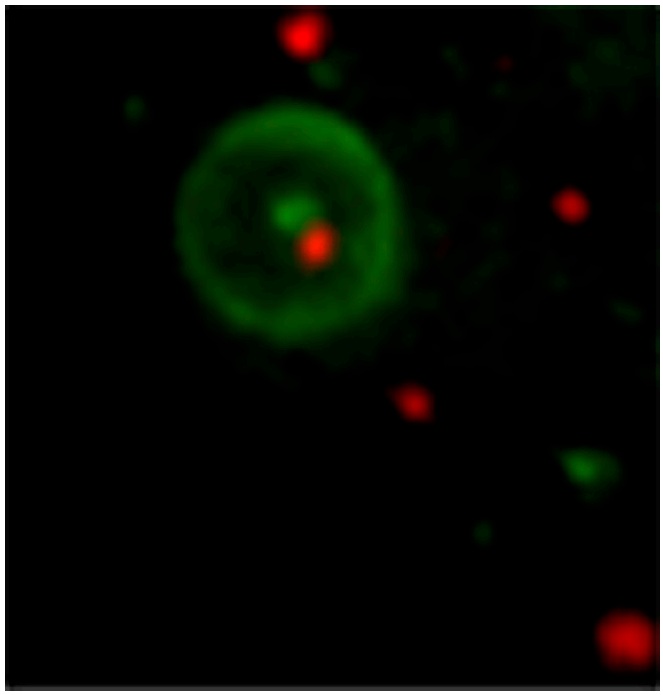
Fig. S6. Ats-1–GFP partially inhibits *E. chaffeensis* infection. RF/6A cells were transfected with Ats-1–GFP or GFP alone followed by infection with *E. chaffeensis* at 10 h posttransfection. Infected cells were harvested at 3 d postinfection and probed with rabbit anti-P28 (*E. chaffeensis* major outer membrane protein), antiactin, and anti-GFP in Western blot analysis. The values under the bands show the relative ratios of band intensities of P28 to actin, with the ratio in the Ats-1–GFP lane set as one. The expressions of GFP and Ats-1–GFP in transfected cells are shown at the bottom.

Table S1. Primers used for PCR amplification

Primer ID	Sequence	Purpose	Note
Ats-1 YTH Forward	5′-CTGCATATGCTAATAAGAAGAATTCTGACT-3′	Construct Ats-1 bait in Y2H screening	NdeI site is underlined
Ats-1 YTH Reverse	5′-CAGGTCGACTTACCTCGTACCTTTACCATG-3′	Construct Ats-1 bait in Y2H screening	SalI site is underlined
Ats-1 Forward	5′-GCAGTCGACGCCACCATGGTGCTAATAAGAAGAAT TCTGAC-3′	Construct Ats-1 and Ats-1–GFP for ectopic expression	Sal I site is underlined
Ats-1 Reverse	5′-AGTGCGGCCGCTTACCTCGTACCTTTACCATGTG-3′	Construct Ats-1 for ectopic expression	Not I site is underlined
Ats-1–GFP reverse	5′-GTGGATCCCGCCTCGTACCTTTACCATGTGT-3′	Construct Ats-1–GFP, Ats-1(Δ N17)-GFP, Cox8A-Ats-1(Δ N17)-GFP, and Ats-1(218–376)-GFP for ectopic expression	BamHI site is underlined
Ats-1(90-250)-GFP Forward	5′-GCAGTCGACGCCACCATGGGCGATGGAAGGGTAT CTGAA-3′	Construct Ats-1(90–250)-GFP for ectopic expression	Sal I site is underlined
Ats-1(90-250)-GFP Reverse	5′-GTGGATCCCGAGCGTCTGCTGATGCAGATT-3′	Construct Ats-1(90–250)-GFP for ectopic expression	BamHI site is underlined
Ats-1(218-376)-GFP Forward	5′-GCAGTCGACGCCACCATGaatgctcttgctccagtaag-3′	Construct Ats-1(218–376)-GFP for ectopic expression	Sal I site is underlined
Ats-1(Δ N17)-GFP Forward	5′-GCAGTCGACGCCACCATGGCGCGCATTGTTTCTGGATTC-3′	Construct Ats-1(Δ N17)-GFP for ectopic expression	Sal I site is underlined
Universe HA Forward 3	5′-GCAGTCGACGCCACCATG*TACCCATACGATGTTCCAGA*-3′	Add HA-encoding sequence in third step extension in PCR	Sal I site is underlined; sequence-encoding HA tag is italicized
Beclin 1 HA Forward 2	5′-ATG*TACCCATACGATGTTCCAGATTACGCT*gaaggg-3′	Add HA-encoding sequence in second step extension in HA-Beclin 1 PCR	Sequence-encoding HA tag is italicized
Beclin 1 HA Forward 1	5′-*GTTCCAGATTACGCT*gaagggtctaagacgtccaac-3′	Add HA-encoding sequence in first step extension in HA-Beclin 1 PCR	Sequence-encoding HA tag is italicized
Beclin 1 Reverse	5′-agtgcggccgctcatttgttataaaattgtgag-3′	Construct HA-Beclin 1 for ectopic expression	Not I site is underlined
Beclin 1(1-272) Reverse	5′-agtgcggccgctcatgcattaaagacgttggttttc-3′	Construct HA-Beclin 1 (1–272) for ectopic expression	Not I site is underlined
DFCP1 HA Forward 2	5′-ATG*TACCCATACGATGTTCCAGATTACGCT*agtgccc-3′	Add HA-encoding sequence in second step extension in HA-DFCP1 PCR	Sequence-encoding HA tag is italicized
DFCP1 HA Forward 1	5′-*GTTCCAGATTACGCT*agtgcccagacttccccagcag-3′	Add HA-encoding sequence in first step extension in HA-DFCP1 PCR	Sequence-encoding HA tag is italicized
DFCP1 Reverse	5′-agtgcggccgcttaaaggtcaccgggcttttta-3′	Construct HA-DFCP1 for ectopic expression	Not I site is underlined
Atg14L HA Forward 2	5′-ATG*TACCCATACGATGTTCCAGATTACGCT*gcgtctc-3′	Add HA-encoding sequence in second step extension in HA-Atg14L PCR	Sequence-encoding HA tag is italicized
Atg14L HA Forward 1	5′-*GTTCCAGATTACGCT*gcgtctcccagtgggaagggag-3′	Add HA-encoding sequence in first step extension in HA-Atg14L PCR	Sequence-encoding HA tag is italicized
Atg14L Reverse	5′-agtgcggccgcttaacggtgtccagtgtaagct-3′	Construct HA-Atg14L for ectopic expression	Not I site is underlined
LC3 HA Forward 2	5′-ATG*TACCCATACGATGTTCCAGATTACGCT*ccgtccg-3′	Add HA-encoding sequence in second step extension in HA-LC3 PCR	Sequence-encoding HA tag is italicized
LC3 HA Forward 1	5′-*GTTCCAGATTACGCT*ccgtccgagaagaccttcaaac-3′	Add HA-encoding sequence in first step extension in HA-LC3 PCR	Sequence-encoding HA tag is italicized
LC3 Reverse	5′-agtgcggccgctcacaagcatggctctcttcctg-3′	Construct HA-LC3 for ectopic expression	Not I site is underlined
HA-Rab7 Forward 1	5′- *GTTCCAGATTACGCT*ACCTCTAGGAAGAAAGTGTTGC-3′	Add HA-encoding sequence in first step extension in HA-Rab7 PCR	Sequence-encoding HA tag is italicized
HA-Rab7 Forward 2	5′- ATG*TACCCATACGATGTTCCAGATTACGCT*ACCTCT-3′	Add HA-encoding sequence in second step extension in HA-Rab7 PCR	Sequence-encoding HA tag is italicized
HA-Rab7 Reverse	5′-AGTGCGGCCGC TCAGCAACTGCAGCTTTCTGC-3	Construct HA-Rab7 for ectopic expression	Not I site is underlined
Cox8A Forward	5′-CTCCGTGCCATCATGTCCGTCCTG-3′	Amplify Cox8A gene fragment	
Cox8A Reverse	5′-ACCCCTTCACTCTGGCCTCCTGTA-3′	Amplify Cox8A gene fragment	
Cox8A-Mito Forward	5′-GCAGTCGACGCCACCATGT CCGTCCTGACGCCGCTG-3′	Amplify DNA fragment encoding MTS of Cox8A for fusion with Ats-1(Δ N17)-GFP	Sal I site is underlined

Table S1. Cont.

Primer ID	Sequence	Purpose	Note
Cox8A-Mito Reverse	5′-*TCCAGAAACCAACGAA*TGGATCTTGGCGCGCG-3′	Amplify DNA fragment encoding MTS of Cox8A for fusion with Ats-1(Δ N17)-GFP	Italicized sequences complementary to the ones encoding the N terminus of Ast-1(Δ N17)
Cox8A-Ats-1(Δ N17) Forward	5′-*GATCCATTCG TTGGT*TTCTGGA TTCACAGCTC CAG-3′	Amplify DNA fragment encoding Ats-1(Δ N17) to construct Cox8A-Ats-1(Δ N17)	Italicized sequences complementary to the ones encoding the C terminus of MTS of Cox8A
mG3PDH Forward	5′-TCAGTGGGCCCTCAGATGC-3′	Amplify mouse G3PDH gene in real-time PCR	
mG3PDH Reverse	5′-ATGGCCTTCCGTGTTCCTAC-3′	Amplify mouse G3PDH gene in real-time PCR	
16S rDNA Forward	5′-GGTGAGTAATGCATAGGAATC-3′	Amplify *A. phagocytophilum* 16S rDNA in real-time PCR	
16S rDNA Reverse	5′-GCTCATCTAATAGCGATAAATC-3′	Amplify *A. phagocytophilum* 16S rDNA in real-time PCR	
Ats-1 (Δ4R) Forward	5′-GCAGTCGACGCCACCATGCTAATA AGAATTCTGACTAC-3′	Construct Ats-1(Δ4R)-GFP for ectopic expression	Sal I site is underlined
Ats-1 (+4R) Forward	5′-GCAGTCGACGCCACCATGCTAATA AGAAGAAGAATTC TGACTAC-3′	Construct Ats-1(+4R)-GFP for ectopic expression	Sal I site is underlined
Ats-1 (4-5AA) Forward	5′-GCAGTCGACGCCACCATGCTAATA GCTGCGATTC TGACTAC-3′	Construct Ats-1(Δ4R)-GFP for ectopic expression	Sal I site is underlined



Movie S1. Dynamic interaction between GFP-LC3 and *A. phagocytophilum* inclusion. GFP-LC3–expressing cells were infected with mCherry-*A. phagocytophilum*. Green, GFP-LC3; red, mCherry-*A. phagocytophilum*.

Movie S1