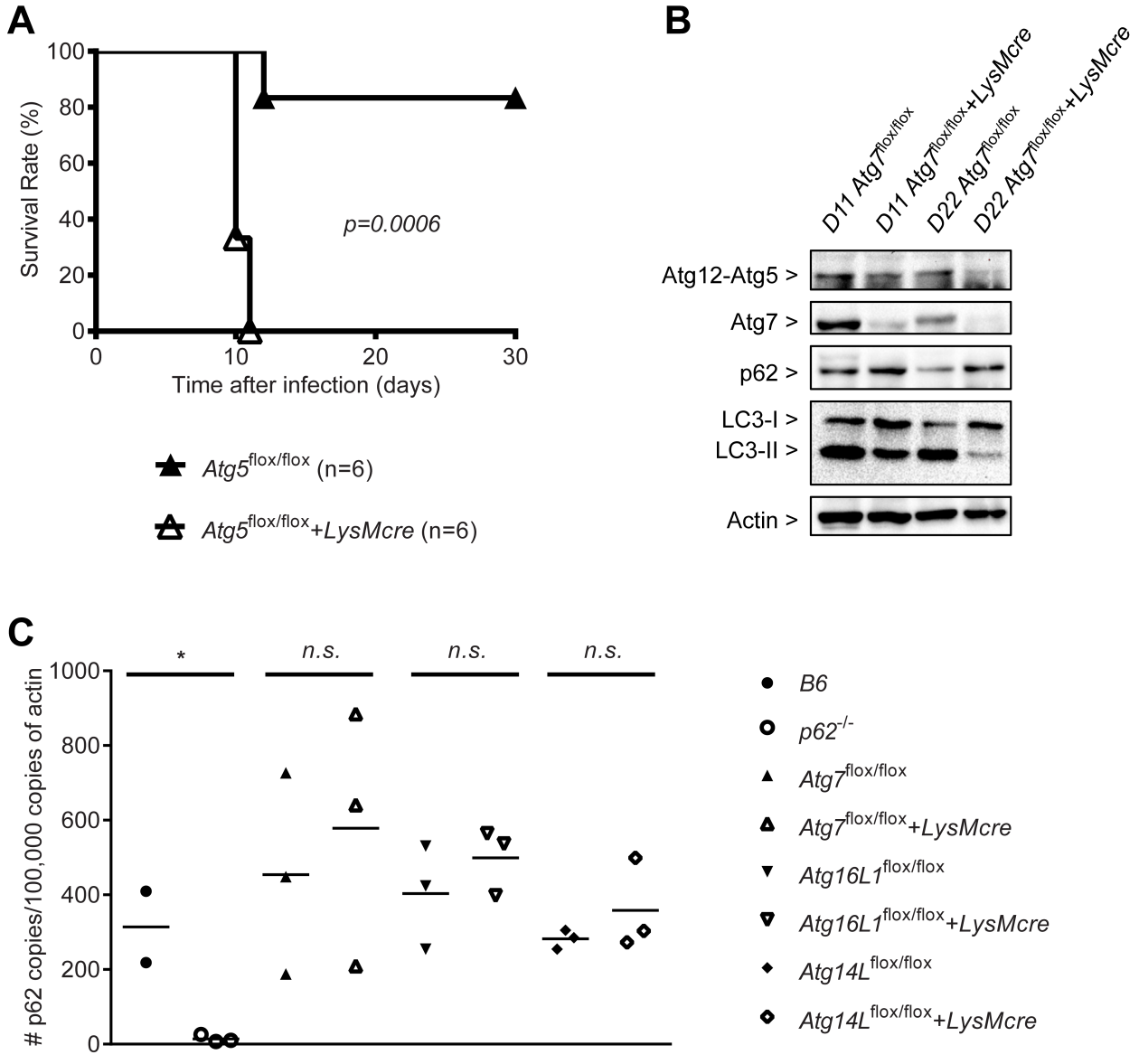
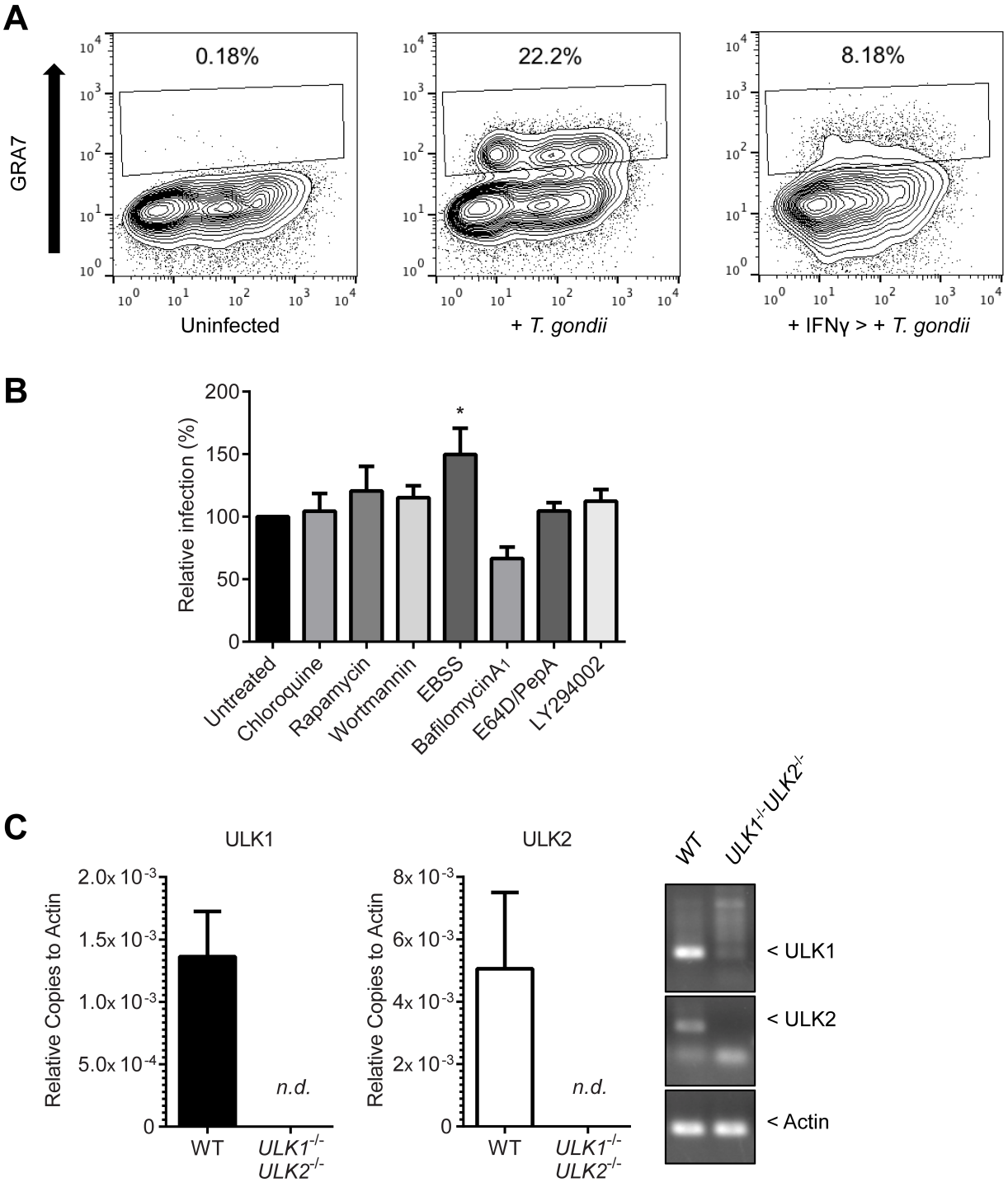


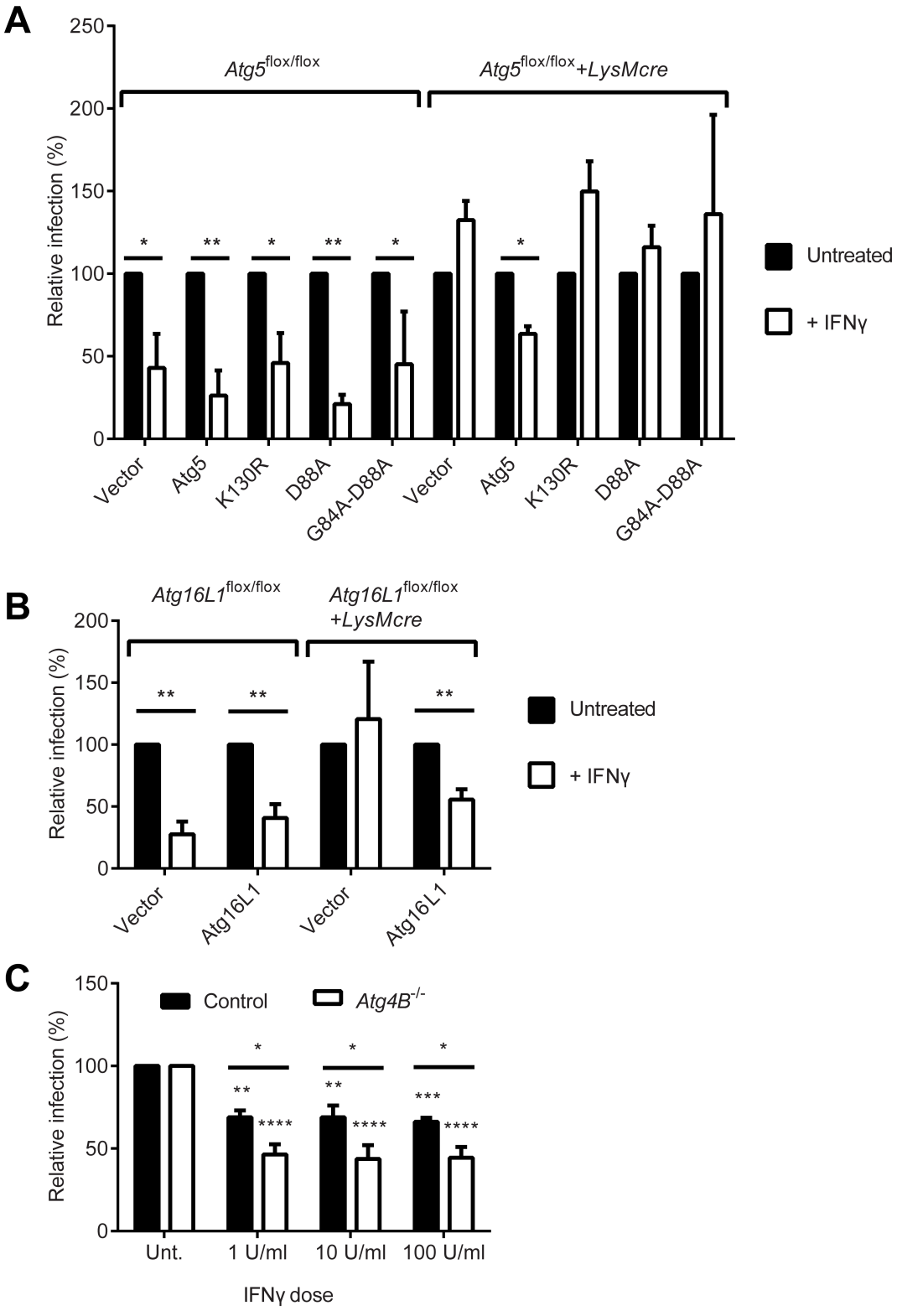

Choi et al. Figure S1

**Supplementary Figure 1. Atg12-Atg5-Atg16L1 complex is required for IFNγ to control *T. gondii* infection, related to Figure 1.** (A) Survival curves of *Atg5*$^{flox/flox}$*+/-LysMcre* mice after intraperitoneal inoculation with 200 (male) or 100 (female) of *T. gondii* per mouse. The total number of mice used is indicated in parentheses. Statistical analysis by Log-rank (Mantel-Cox) test. (B) A representative western blot analysis for the functional deletion of Atg7 in normal (11 days) and prolonged (22 days) culture of bone marrow derived macrophages (BMDM) from *Atg7*$^{flox/flox}$*+/-LysMcre* mice. Remaining Atg12-Atg5 conjugate and LC3-I to LC3-II conversion indicates the residual function of Atg7 even after prolonged culture of BMDMs from *Atg7*$^{flox/flox}$*+LysMcre* mice. (C) Quantitative analysis of p62 transcripts in the primary bone marrow derived macrophages isolated from C57BL/6 (*B6*), *p62*$^{-/-}$, *Atg7*$^{flox/flox}$*+/-LysMcre*, *Atg16L1*$^{flox/flox}$*+/-LysMcre* and *Atg14L*$^{flox/flox}$*+/-LysMcre* mice. Statistical analysis by unpaired t-test. n.s.: not significant. *: $p < 0.05$. Experiments were performed thrice and each data point with average is shown.



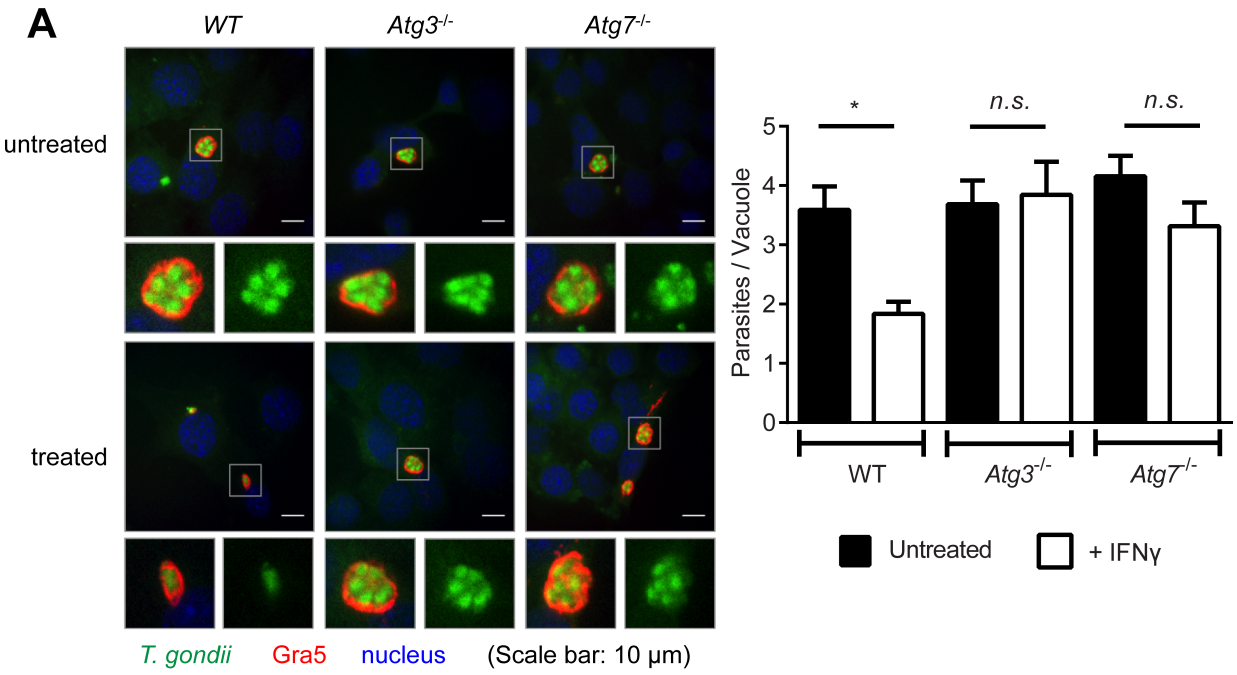

Choi et al. Figure S2

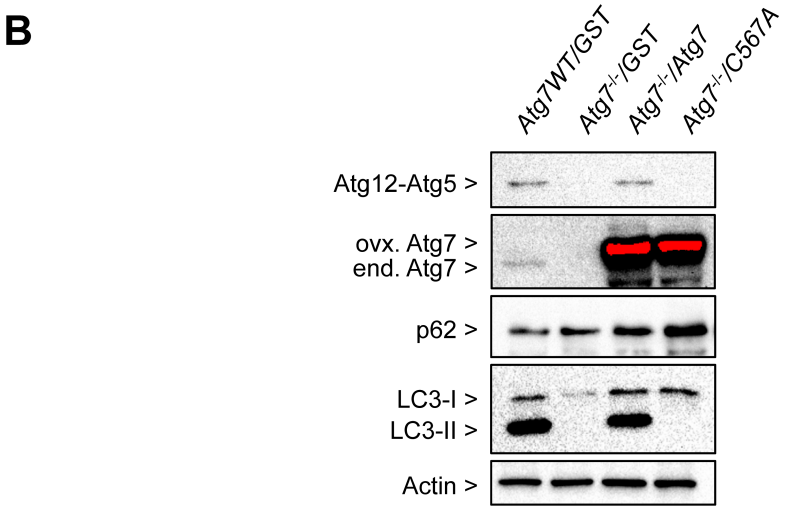
**Supplementary Figure 2. Canonical degradative autophagy is not required for IFNγ to control *T. gondii* infection, related to Figure 2.** (A) Representative flow cytometry plots for the infection of *T. gondii* and its control by IFNγ at 24 hour-post-infection (hpi). Wild type BMDMs transduced with lentiviruses expressing green fluorescence protein (GFP) were either untreated or treated with 100 U/ml of IFNγ for 24 hrs and then infected with *T. gondii* at the multiplicity of infection 1 (MOI=1). The level of *T. gondii* infection was quantified by intracellular staining for GRA7 (dense granule protein 7). (B) Flow cytometry analysis for the relative infection (%) of *T. gondii* at 24 hpi (MOI=1) in chemical-treated wild type macrophages compared to the untreated. (C) Quantitative PCR analysis for the transcript level of ULK1 (left) and ULK2 (middle). n.d.: not detected. A representative picture of agarose gel electrophoresis for PCR products. Statistical analysis by one-way analysis of variance (1-ANOVA) with Tukey post test. Differences not specifically indicated to be significant were not significant ($p > 0.05$). *: $p < 0.05$. Experiments were performed at least three times and the data were combined for presentation as average ± SEM.




Choi et al. Figure S3

**Supplementary Figure 3. Atg12-Atg5-Atg16L1 complex is required for IFNγ to control *T. gondii* infection, related to Figures 3 and 4.** (A) Immunofluorescence analysis for the relative infection (% of untreated) of *T. gondii* at 24 hpi (MOI=1) with or without 24 hr pre-treatment of 100 U/ml of IFNγ in *Atg5*$^{flox/flox}$*+/-LysMcre* BMDMs transduced with control, wild type Atg5 or its mutants: K130R - defective in Atg12 conjugation, D88A - partially defective in Atg16L1 binding, G84A-D88A – severely defective in Atg16L1 binding (Hwang et al., 2012). (B) Restoration of IFNγ-mediated control of *T. gondii* in Atg16L1-deficient macrophages by the lentiviral expression of wild type Atg16L1. Flow cytometry analysis for the relative infection (% of untreated) of *T. gondii* at 24 hpi (MOI=1) with or without 24 hr pre-treatment of 100 U/ml of IFNγ in *Atg16L1*$^{flox/flox}$*+/-LysMcre* BMDMs transduced with control or wild type Atg16L1. (C) Atg4B is not required for the control of *T. gondii* by IFNγ. Flow cytometry analysis for the relative infection (%) of *T. gondii* at 24 hpi (MOI=1) with or without 24 hr pre-treatment of 100 U/ml of IFNγ in control and Atg4B-deficient macrophages. Statistical analysis by one-way analysis of variance (1-ANOVA) with Tukey post test. Differences not specifically indicated to be significant were not significant ($p > 0.05$). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$. Experiments were performed at least three times and the data were combined for presentation as average ± SEM.




Choi et al. Figure S4

**Supplementary Figure 4. The ubiquitin-like conjugation systems of the autophagy pathway are required for the inhibition of *T. gondii* replication by IFNγ, related to Figure 7.** (A) Immunofluorescence analysis of MEFs at 20 hpi (MOI=1) of *T. gondii* infection with or without 24 hr pre-treatment of 100 U/ml of IFNγ. Representative images (left) and quantitation (right) of immunofluorescence analysis for *T. gondii* and parasitophorus vacuole (GRA5 staining) in wild type, *Atg3*$^{-/-}$ and *Atg7*$^{-/-}$ MEFs. For the quantitation, at least 100 of infected *T. gondii* were analyzed for each cell types. Statistical analysis by one-way analysis of variance (1-ANOVA) with Tukey post test. n.s.: not significant. *: $p < 0.05$. Experiments were performed twice and the data were combined for presentation as average ± SEM. (B) A representative western blot analysis for wild type and *Atg7*$^{-/-}$ MEFs transduced with control (GST), wild type Atg7, or its enzymatically inactive mutant (C567A).

## Supplemental Experimental Procedures

**Cells** Bone marrow derived macrophages (BMDMs) were generated as previously described (Hwang et al., 2012). Briefly, mouse bone marrows were isolated and cultured for 7 days in BMDM media (Dulbeco's Modified Eagle Medium, 10% fetal bovine serum, 5% horse serum, 10% CMG14-12 (Takeshita et al., 2000), 1x MEM nonessential amino acids, 1mM sodium pyruvate, 2 mM L-glutamine). For the BMDM of *Atg5*$^{flox/flox}$*+/-LysMcre*, adherent BMDMs were disaggregated and set up for experiments on day 7 and infected on day 11. For the BMDM of *Atg3*$^{flox/flox}$*+/-LysMcre, Atg7*$^{flox/flox}$*+/-LysMcre, Atg14L*$^{flox/flox}$*+/-LysMcre,* and *Atg16L1*$^{flox/flox}$*+/-LysMcre*, adherent BMDMs were detached on day 7 and again on 14, set up for experiments on day 14, and infected on day 21 to maximize the functional deletion of the genes. Day 7 or Day 14 BMDMs were plated in 24-well plates (24wp, 1 x $10^5$ cells/well) for protein analysis by western blot or immunofluorescence assay (on cover slips inside wells) and in 6-well plate (6wp, 5 x $10^5$ cells/well) for replication analysis of *T. gondii* infection by flow cytometry. *ULK1*$^{-/-}$*ULK2*$^{-/-}$ MEF and *Atg3*$^{-/-}$ MEF were kindly provided by Dr. Sharon Tooze, London Research Institute, U.K. (McAlpine et al., 2013) and Dr. Masaaki Komatsu, Tokyo Metropolitan Institute of Medical Science, Japan (Sou et al., 2008), respectively. Human Foreskin Fibroblast (HFF) and 293T cells were cultured as previously described and used for the maintenance of *T. gondii* and for production of lentiviruses and co-immunoprecipitation, respectively (Hwang et al., 2012; Selleck et al., 2013).

**Lentiviral Transduction** pCDH-MCS-T2A-copGFP-MSCV (System Biosciences; CD523A-1) (Hwang et al., 2012) and pHAGE-N-Flag-HA (Behrends et al., 2010) lentiviral vectors were used as backbones to express the genes of interest. The T2A sequence in the pCDH-MCS-T2A-

copGFP-MSCV vector allows the detection of expressed proteins with anti-2A antibody by tagging the proteins at their C-terminus and transduced cells with simultaneously produced green fluorescent protein. The pHAGE-N-Flag-HA vector is a Gateway destination vector that expresses FLAG-HA-tagged proteins and allows the selection of transduced cells with puromycin. Lentiviruses were generated in 293T cells by transfecting the lentiviral plasmids with packaging vector (psPAX2) and pseudotyping vector (pMD2.G) as previously described (Hwang et al., 2012). Filtered lentiviruses were added onto BMDMs on day 4 and again on day 5 and incubated until day 7.

**Immunofluorescence and Flow cytometry** The localization of LC3 and IFNγ effectors were assessed by indirect immunofluorescence. LC3 was stained with rabbit polyclonal anti-LC3 (MBL, PM036) at 1:1000 and DyLight™ 594 Donkey anti-rabbit IgG (BioLegend, 406405), Irga6 was stained with mouse monoclonal anti-Irga6 (5D9 or 10D7, (Papic et al., 2008; Zhao et al., 2008)) at 1:1000 and DyLight™ 649 Goat anti-mouse IgG (BioLegend, 405312), GBP1-5 were stained with mouse monoclonal anti-GBP1-5 (Santa Cruz, SC-166960) at 1:100 and DyLight™ 649 Goat anti-mouse IgG (BioLegend, 405312) in PBS/0.05% Saponin/5% normal goat serum/5% fetal bovine serum. Irgb6 was stained with goat polyclonal anti-Irgb6 (Santa Cruz, sc-11079) at 1:50 and Alexa Fluor® 647- Donkey Anti-Goat IgG (Jackson immunological Res Lab, 705-606-147) in PBS/0.05% Saponin/5% normal donkey serum. For quantification of replication of *T. gondii,* GRA5, marker for parasitophorous vacuole, was stained with mouse monoclonal anti-GRA5 (Selleck et al., 2013) at 1:1000 and DyLight™ 649 Goat anti-mouse IgG. Nucleus was stained with DAPI (4',6-diamidino-2-phenylindole) in PBS/0.05% Saponin with appropriate blocking serum, and *T. gondii* was detected with GFP expressed by PTG strain. The images were acquired using Olympus DSU Spinning Disk confocal with 60x and 100x objective lenses.

For flow cytometry, ME49-infected cells were stained with rabbit anti-dense granule protein 7 (GRA7, which accumulates within cells and co-localizes with the PVM of *T. gondii* (Fischer et al., 1998)) at 1:1000 dilution in PBS-T/1% normal goat serum/1% normal mouse serum at RT for 1 hr. Cells were then washed with PBS-T, stained with DyLight 649 Donkey anti-rabbit IgG (Biolegend; 406406) at RT for 1 hr, and washed with PBS-T before data acquisition. PTG-infected cells were just fixed and then analyzed. Flow data was acquired using FACSCalibur or FACSCanto flow cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star). Uninfected/stained samples were used as gating control and infected/stained samples without IFNγ-treatment were used as a normalization control to calculate the relative infection (%) of IFNγ-treated samples. For transduced samples, the infection with *T. gondii* was analyzed only in the transduced cells by green fluorescence or after puromycin selection.

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