

Supplemental Materials and Methods

Parasite strains. Type 1 RH parasites lacking ROP18 (3), ROP5 (1) and ROP17 (2) were described previously.

Cell Culture. HAP1 cells and a mutant lacking the GBP cluster (including GBP1, 2,3,4,5,6 and 7) were kindly provided by Dr. Masahiro Yamamoto, as described previously (4).

Intracellular clearance assay. To examine intracellular clearance, IFN γ -activated HeLa cells were infected with freshly egressed parasites, washed 3 times with PBS, and either fixed at 2 hr post infection, or returned to culture in complete medium for 24 hr. Percent of infected cells was determined by the number of parasites per number of host cells multiplied by 100. Parasites were localized with a rabbit polyclonal antibody to RH tachyzoites followed by goat anti-rabbit IgG conjugated to Alexa fluor 488. Host nuclei were localized with DAPI. Images were collected on the Cytation 3 Imager at 10X with 5 images captured per coverslip with at least 10,000 host cells captured total. One experiment with 3 technical replicates was shown. Host cell nuclei and parasites were counted using the Gen 5 Software (Biotek)

Tryptophan supplementation replication assay. To examine intracellular replication of *T. gondii*, IFN γ -activated HeLa cells were infected with parasites for 2 hr, washed 3 times with PBS and fed with fresh containing either 1mM final concentration of L-Tryptophan (Sigma) or the same volume of 0.1N NaOH. L-Tryptophan was resuspended to 100 mM in 0.1N NaOH. Cells were fixed 24 hr post infection and intracellular parasites were localized using mAb DG52 or a rabbit polyclonal antibody to RH tachyzoites. Secondary antibodies recognizing the species of primary antibody conjugated to Alexa fluor 488 or 594 were used as indicated. The number of parasites per vacuole were determined by manually counting on a fluorescence microscope. The number of parasites per vacuole was determined from at least 50 vacuoles on three individual coverslips.

Western Blot analysis. ATG16L1 knockout cells for western blot were lysed in 1% NP-40 lysis buffer (1% NP-40, 150mM NaCl, 25mM Tris, 1mM EDTA, pH 7.4) with protease inhibitors. Samples were resuspended in denaturing SDS sample buffer,

resolved on a 10% acrylamide gel, and transferred to a nitrocellulose membrane. Blots were probed with rabbit anti-ATG16L1 (Sigma), rabbit anti-LC3 (MBL) or mAb C4 against Actin (Millipore) followed by goat anti rabbit or mouse IgG conjugated to IRDye 680 or 800 as indicated (LI-COR). For LC3 western blot, proteins were transferred to a PVDF membrane. Blots were imaged on the Odyssey Infrared Imager (LI-COR). ATG7 knockout cells for western blot were lysed in RIPA buffer, resuspended in denaturing SDS sample buffer, resolved on a 10% acrylamide gel, and transferred to a nitrocellulose membrane. For LC3 western blot, proteins were transferred to a PVDF membrane. Blots were probed with rabbit anti-ATG7 (Sigma), rabbit anti-LC3 (Sigma), or mAb GAPDH-71.1 directly conjugated to HRP (Sigma). Blots were exposed to peroxidase solution, exposed to film and imaged.

Antibodies. ATG16L1 was localized with a rabbit polyclonal antibody (Sigma). Actin was localized with mAb C4 to actin. ATG7 was localized with a rabbit polyclonal antibody (Sigma). GAPDH was localized with a mouse mAb GAPDH-71.1 directly conjugated to HRP (Sigma). LC3 was detected with a rabbit polyclonal antibody (MBL or Sigma, as indicated). Secondary antibodies used for western blots include goat anti-rabbit IgG conjugated to IRDye 800 (Licor), goat anti-mouse IgG conjugated to IRDye 680 (Licor) and goat-anti rabbit IgG conjugated to HRP (Sigma).

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