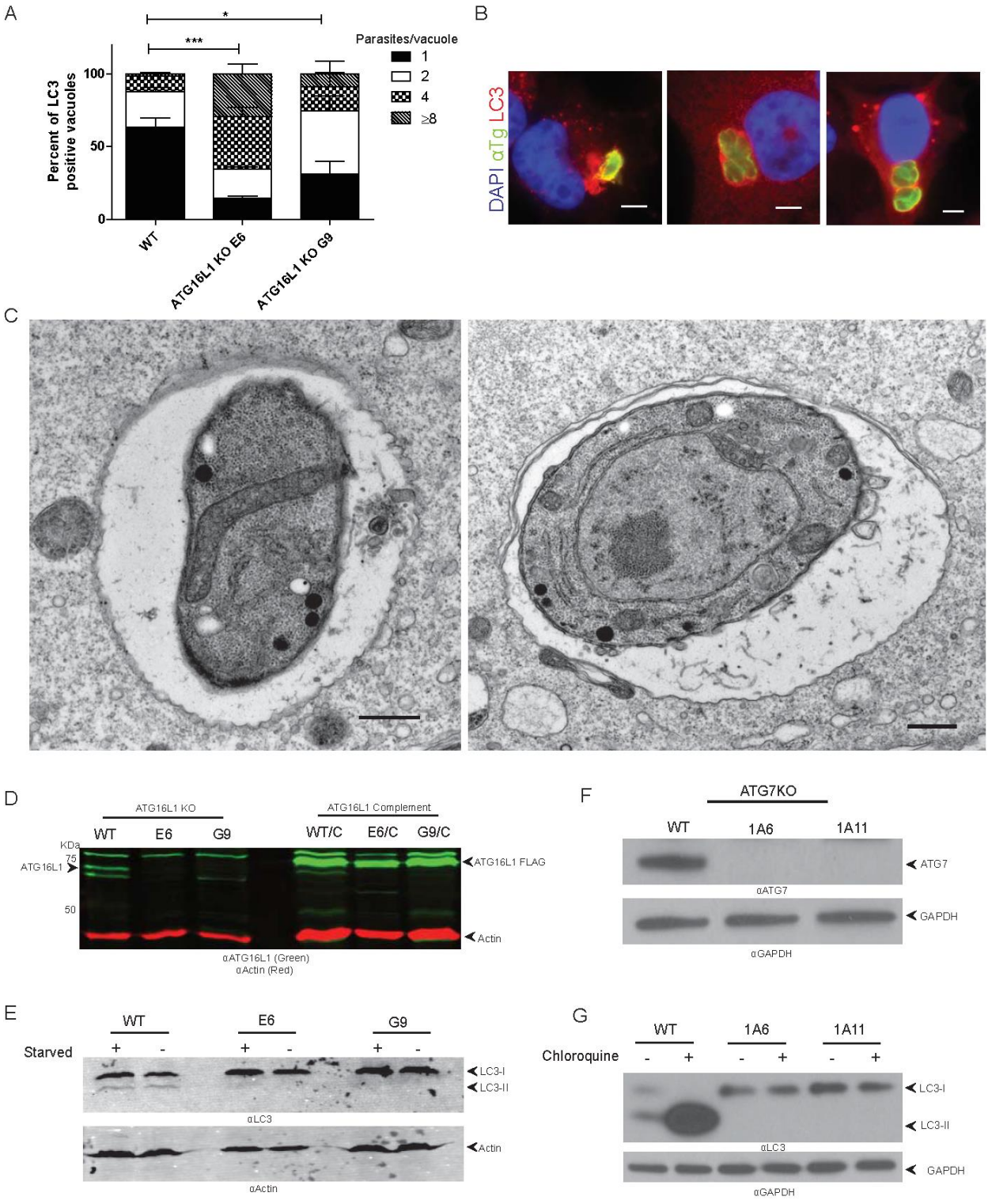
Figure S6


A
*
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Parasites/vacuole
1
2
4
≥8
Percent of LC3 positive vacuoles
100
50
0
WT
ATG16L1 KO E6
ATG16L1 KO G9
B
DAPI αTg LC3
C
D
ATG16L1 KO
ATG16L1 Complement
WT
E6
G9
WT/C
E6/C
G9/C
KDa
75
ATG16L1
50
ATG16L1 FLAG
Actin
αATG16L1 (Green)
αActin (Red)
E
WT
E6
G9
Starved
+
-
LC3-I
LC3-II
αLC3
Actin
αActin
F
ATG7KO
WT
1A6
1A11
ATG7
αATG7
GAPDH
αGAPDH
G
WT
1A6
1A11
Chloroquine
-
+
LC3-I
LC3-II
αLC3
GAPDH
αGAPDH

FIG. S6. Replication, ultrastructural analysis and western blot detection in ATG16L1 and ATG7 knockout HeLa cells. Related to FIG. 6

A) Replication of type 3 (VEG) *T. gondii* in LC3-positive PVs in IFNγ-activated ATG16L1 KO HeLa cells at 24 hr post infection. N = 3 experiments. Mean ± SEM. (***P<0.0001, 2-way ANOVA). B) Representative images of *T. gondii* vacuoles in wild type (WT) or ATG16L1 KO (clone E6 or G9) HeLa cells. LC3 was localized with a rabbit polyclonal antibody followed by goat-anti rabbit IgG conjugated to Alexa Fluor 594 (red). Parasites were localized with mAb DG52 to SAG1 followed by goat anti-mouse IgG conjugated to Alexa Fluor 488 (green). Host nuclei were localized with DAPI. Scale bar = 5 μm. C) Transmission electron microscopy reveals the structures of representative PVMs of a type 3 (VEG) parasites in IFNγ-activated ATG16L1 deficient HeLa cells at 6 hr post infection. Scale bars = 5 nm. D) Expression of ATG16L1 detected by western blotting of HeLa cell lysates of wild type (WT) and ATG16L1 knockout clones (E6, G9) with a rabbit polyclonal antibody against ATG16L1 followed by Licor IR Dye 800CW goat anti-rabbit IgG (green). Actin was used as a loading control, detected with mouse mAb C4 followed by Licor IR Dye 680CW goat anti-mouse IgG (red). Arrowheads indicate ATG16L1 or Flag-Tagged ATG16L1 protein. Nonspecific proteins recognized by ATG16L1 antibody were seen. E) LC3 lipidation in ATG16L1 knockout clones. Cells were either starved in EMEM without serum or cultured in complete media for 3 hr. LC3 was detected with a rabbit polyclonal antibody followed by Licor IR Dye 800CW goat anti-rabbit IgG. Actin was used as a loading control as above. F) Expression of ATG7 detected by western blotting of HeLa cell lysates of wild type (WT) and ATG7 knockout clones (1A6, 1A11). ATG7 was detected with a rabbit polyclonal antibody followed by goat anti-rabbit conjugated to HRP. GAPDH was used as a loading control, detected with mAb GAPDH-71.1 directly conjugated to HRP. G) LC3 lipidation in ATG7 knockout clones. Cells were treated with 40 μM choloroquine for 4 hr prior to cell harvest or left untreated. LC3 was detected with a rabbit polyclonal antibody followed by goat anti-rabbit IgG conjugated to HRP. GAPDH was used as a loading control and detected as above.