Supplemental Materials

Molecular Biology of the Cell

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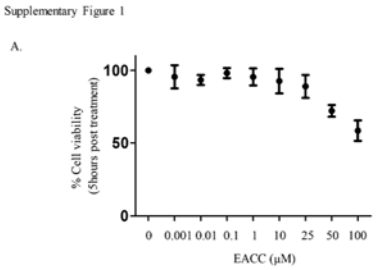

Figure S1. Cell viability assay

(A) HeLa cells were counted and equal numbers (1500 cells/well) were plated in 384 well plate in growth medium. The following day, different concentrations of EACC ranging from 100nM to 100μM were mixed in starvation media, added onto the cells and incubated for five hours. Post incubation, CellTiter-Glo Reagent was added to each well, and luminescence was measured using Varioskan Flash (Thermo Fisher Scientific). Graph represents percent cell viability five hours post EACC treatment in starvation conditions.

Supplementary Figure 2

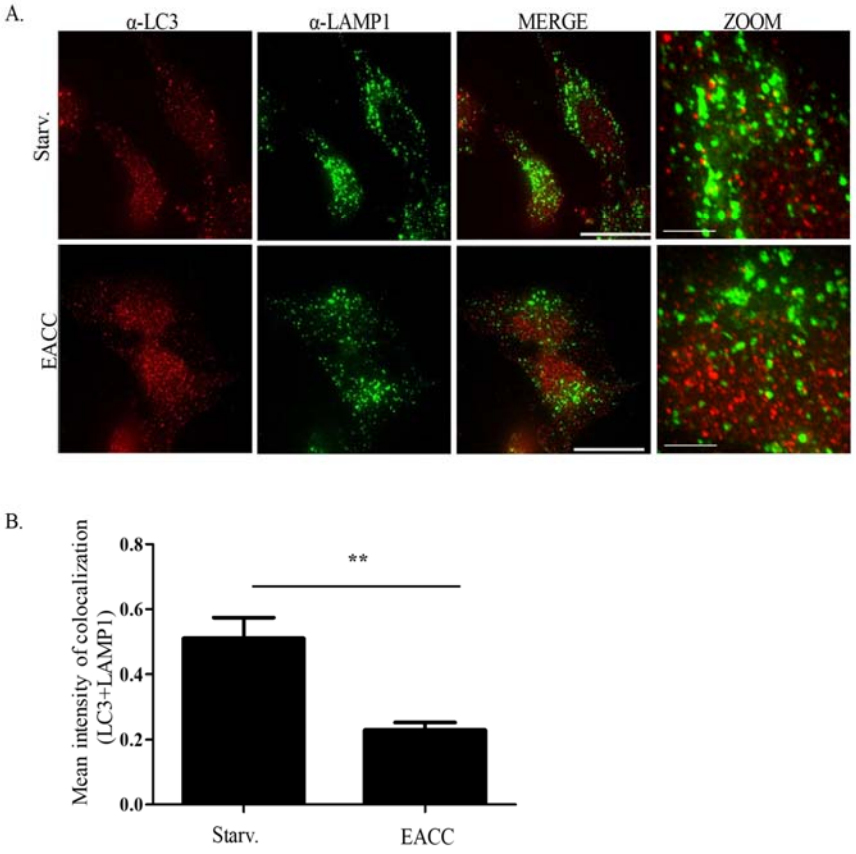

Figure S2. EACC blocks autophagosome-lysosome fusion but does not affect endo-lysosomal function

(A) HeLa cells were treated with EACC (10μM) for 2 hours in starvation conditions and immunostained with anti-LC3 and anti-LAMP1 antibody.Scale=15 μm.Graph showing the mean intensity of colocalization between LC3 and LAMP1 measured as in **1H**. Data shown here represents a minimum of 45 cells from 3 independent experiments plotted as mean ± SEM.Statistical significance was analysed by Student's unpaired t-test.***$P<$ 0.001.

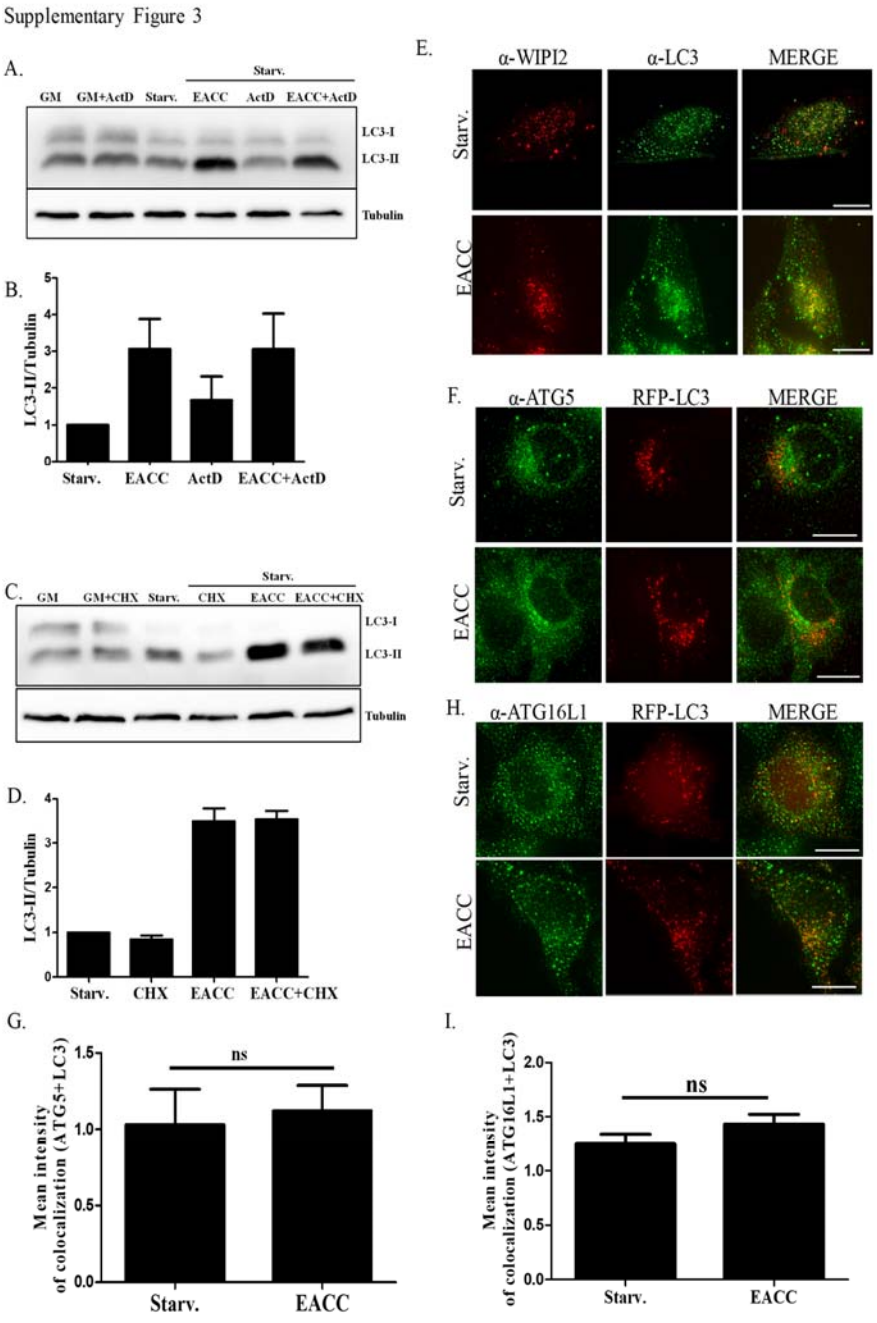

Figure S3.EACC does not affect early autophagic events

(A) HeLa cells were either left untreated or pretreated with Actinomycin D (ActD) in basal (GM) or starvation conditions (EBSS) for 1 hour in order to block transcription. This was followed by treatment with EACC (10μM) for 2 hours in presence of Actinomycin D. Samples were collected and immunoblotted for anti-LC3 and anti-β-Tubulin antibodies. **(B)**Relative levels of LC3-II: β-Tubulinin untreated versus treated samples were quantitated for 3 independent experiments.**(C)**HeLa cells were either left untreated or pretreated with cycloheximide (CHX) in basal or starvation conditions for 1 hour in order to block protein translation. This was followed by treatment with EACC (10μM) for 2 hoursin presence of cycloheximide. Samples were collected and immunoblotted for anti-LC3 and anti-β-Tubulin antibodies. **(D)** Relative levels of LC3-II: β-Tubulinin untreated versus treated samples were quantitated for 3 independent experiments.**(E)**HeLa cells were treated with EACC (10μM) for 2 hours in starvation conditions and immunostained with anti-LC3 and anti-WIPI2 antibody.Scale=15 μm**(F)**RFP-LC3 transfected HeLa cells were treated with EACC (10μM) for 2 hours in starvation conditions and immunostained with anti-ATG16L1 antibody.Scale=15 μm**(G)**Graph showing the mean intensity of colocalization between ATG16L1 and RFP-LC3 measured as in **1H**. Data shown here represents a minimum of 45 cells from 3 independent experiments plotted as mean ± SEM.Statistical significance was analysed by Student's unpaired t-test.ns=non-significant. **(H)**HeLa cells were transfected with RFP-LC3, treated with EACC and immunostained with anti-ATG5 antibody. Scale=15 μm**(I)** Graph showing the mean intensity of colocalization between ATG5 and RFP-LC3 measured as in **1H**. Data shown here represents a minimum of 45 cells from 3

independent experiments plotted as mean ± SEM.Statistical significance was analysed by Student’s unpaired t-test.ns=non-significant.