Landes Bioscience www.landesbioscience.com

Supplemental Material to:

Yingying Lu, Shichen Dong, Bai-Xia Hao, Chang Li, Kai-Yuan Zhu, Wen-Jing Guo, Qian Wang, King-Ho Cheung, Connie WM Wong, Wu-Tian Wu, Huss Markus, and Jianbo Yue

Vacuolin-1 potently and reversibly inhibits autophagosome-lysosome fusion by activating RAB5A

Autophagy 2014; 10(11)
http://dx.doi.org/10.4161/auto.32200

www.landesbioscience.com/journals/autophagy/article/32200

Figure S1

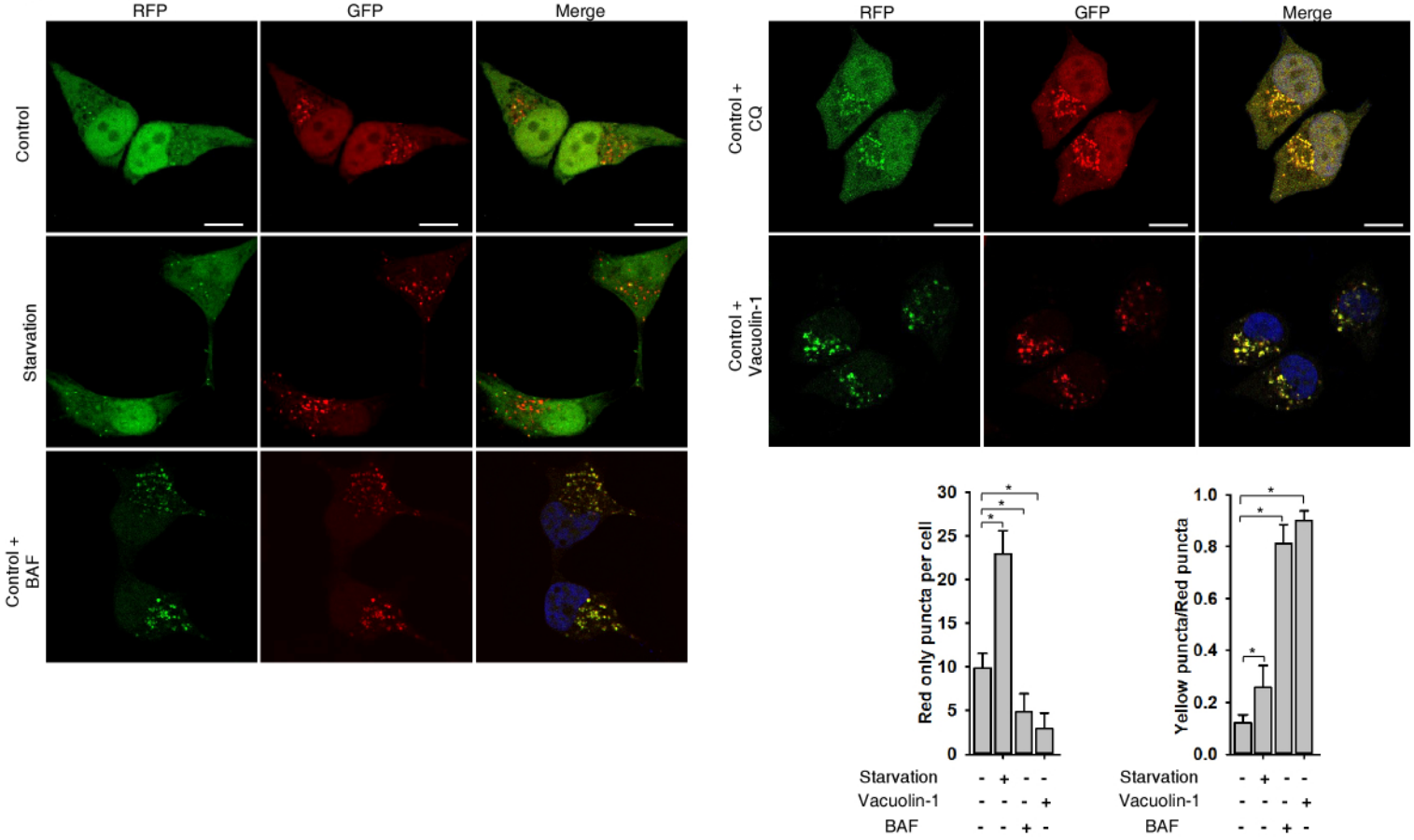


Figure S2

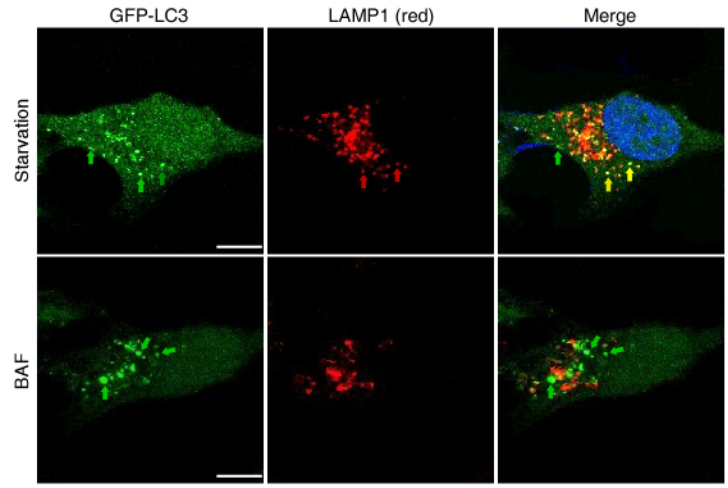


Figure S3

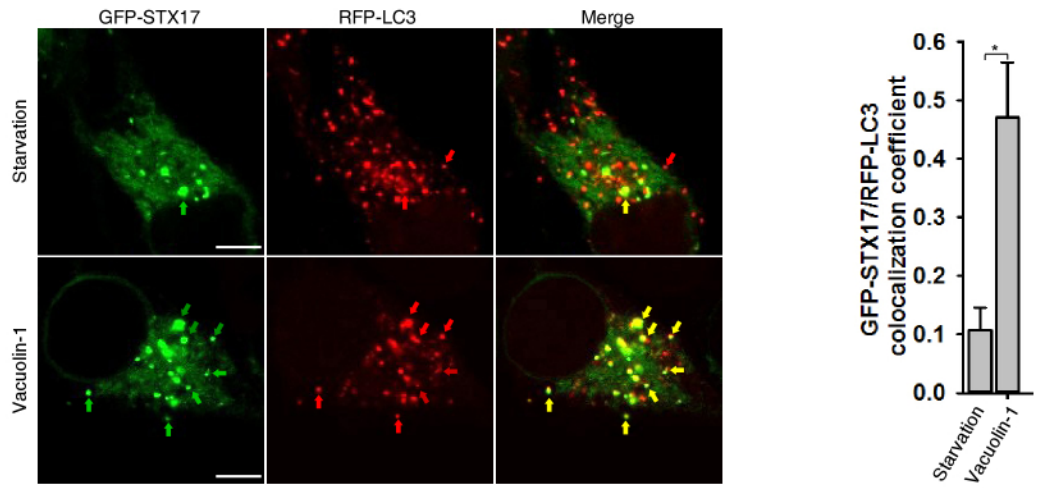


Figure S4

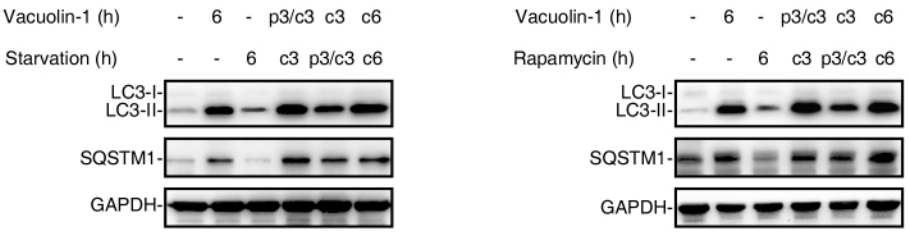


Figure S5

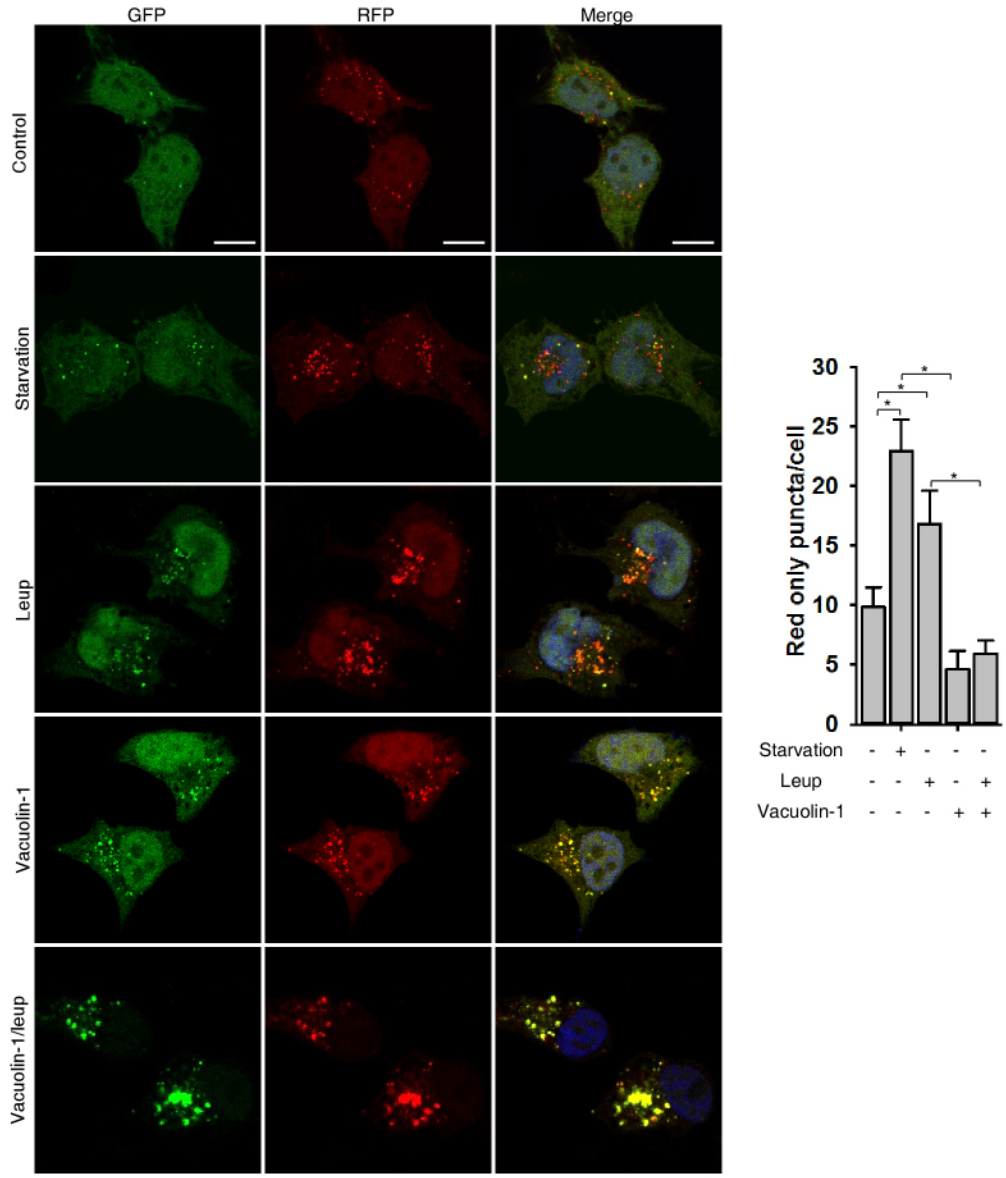


Figure S6

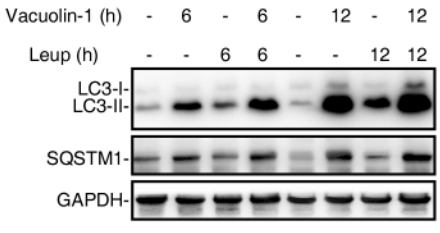


Figure S7

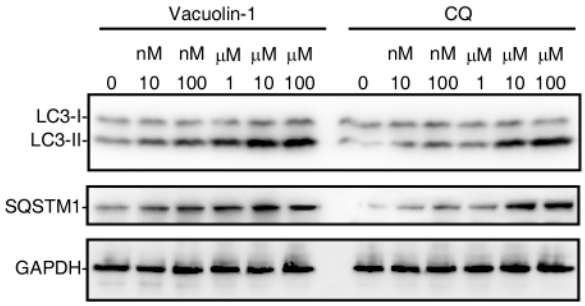
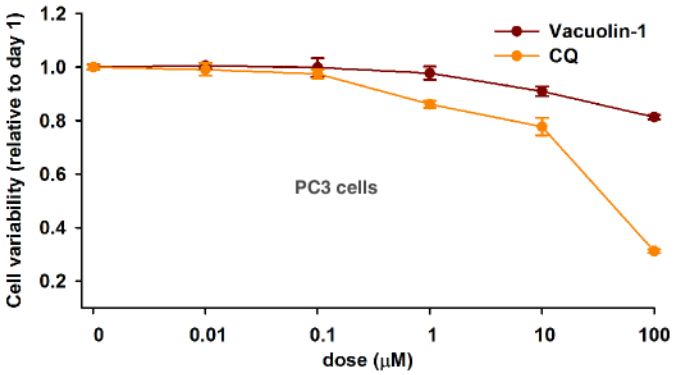
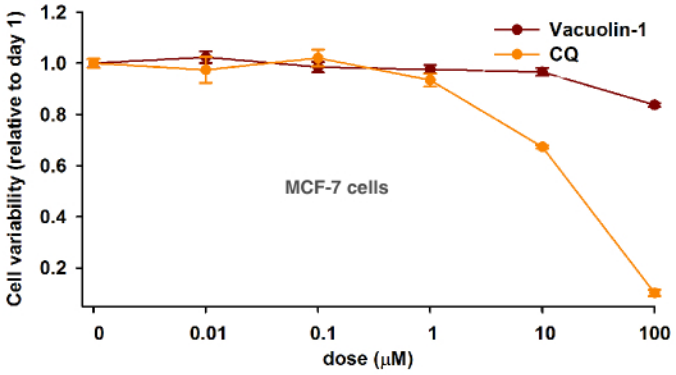
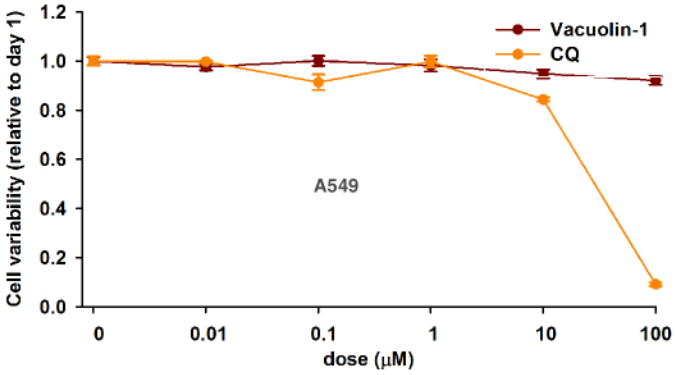


Figure S8







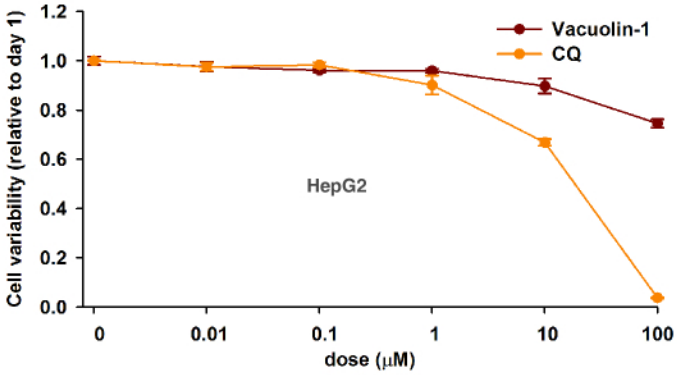


Figure S9

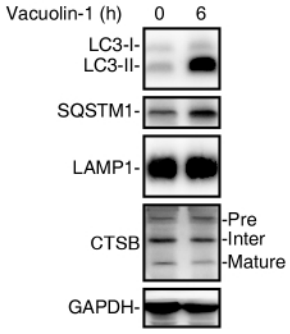
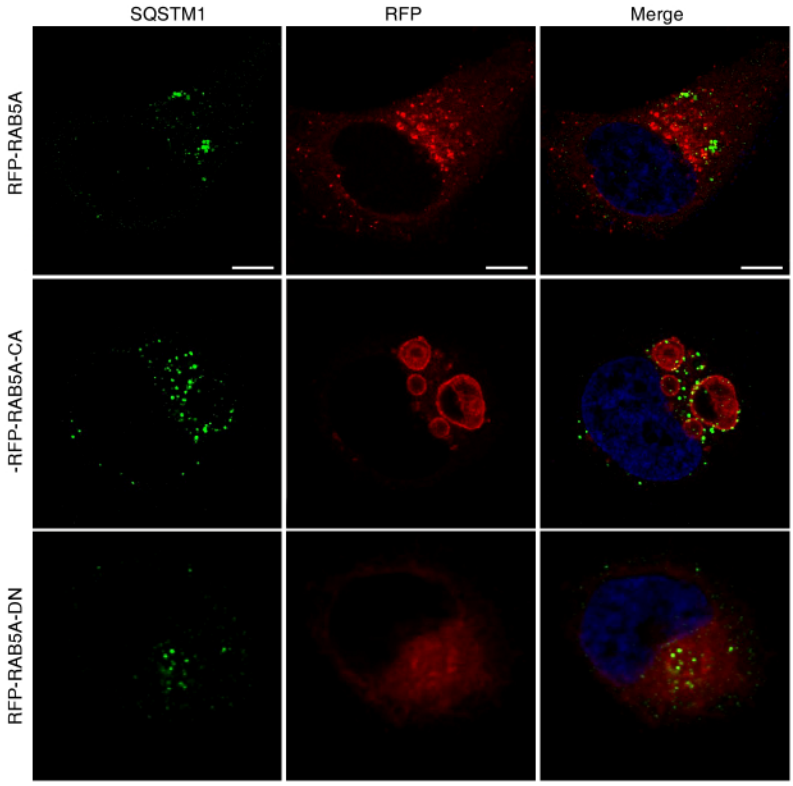


Figure S10



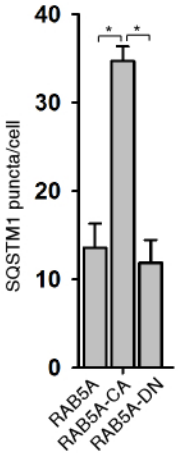


Figure S11

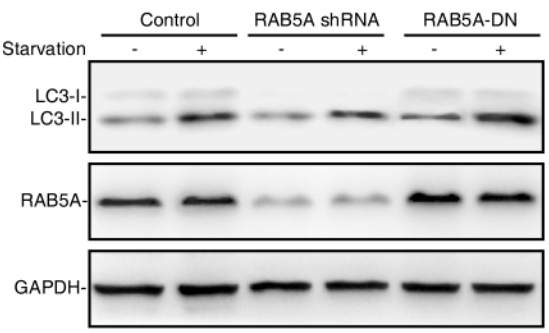
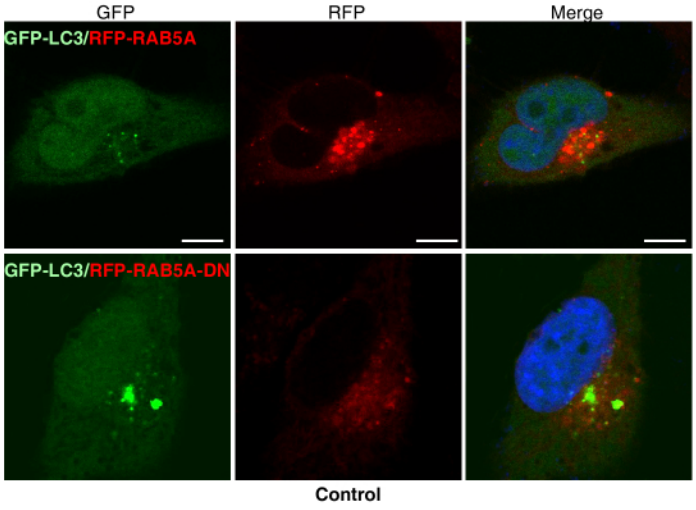


Figure S12



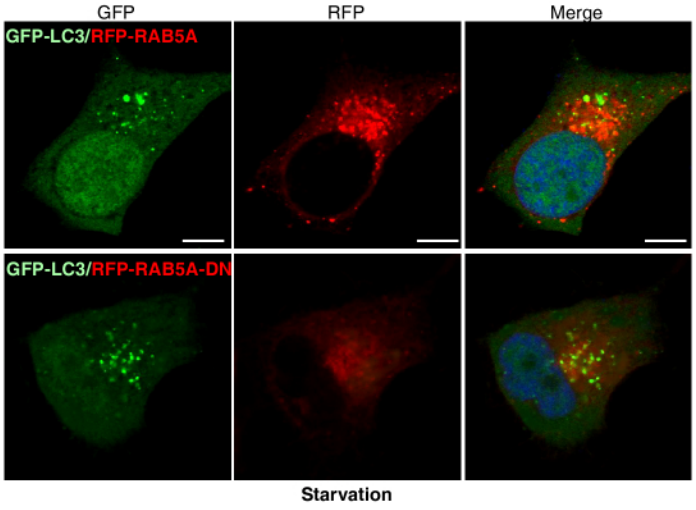


Figure S13

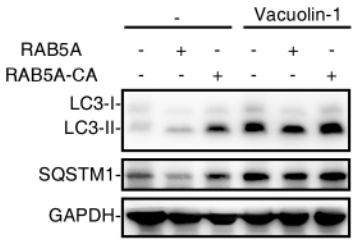


Figure S14

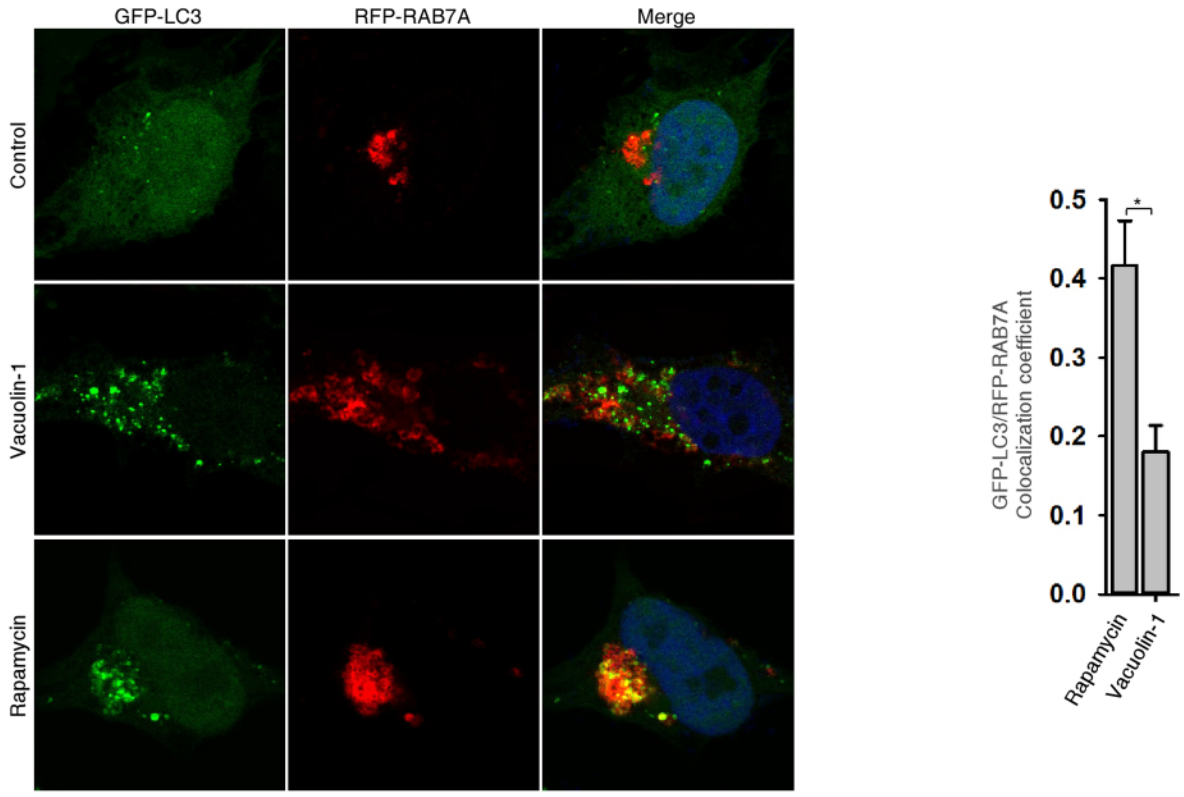
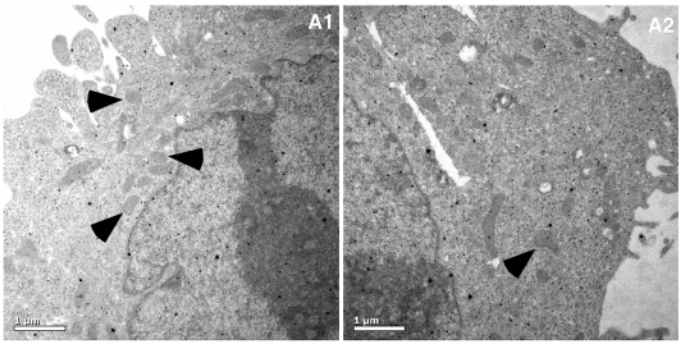
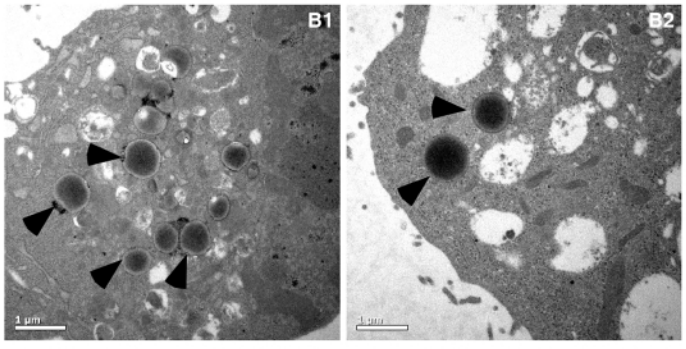


Figure S15



Control



Vacuolin-1

Figure S1. BAF (100 nM), CQ (10 μM), and vacuolin-1 (1 μM) treatment induced the accumulation of yellow LC3B-II puncta in tfLC3B-expressing HeLa cells. Quantifications of LC3B red only puncta/per cell and LC3B yellow puncta/red puncta (%) are expressed as mean ± S.E., n= ~ 80 cells of 3 independent experiments. The * symbols indicate the results of the Student *t* Test analysis, $P < 0.05$. Scale bar = 20 μM.

Figure S2. BAF (100 nM) markedly induced GFP-LC3B-II puncta in HeLa cells, which were not colocalized with RFP-LAMP1. Scale bar = 20 μM. Experiments were repeated at least 3 times.

Figure S3. Vacuolin-1 (1 μM) markedly induced RFP-LC3B puncta in HeLa cells, which were colocalized with GFP-STX17. Scale bar = 20 μM. The RFP-LC3 and GFP-STX17 colocalization coefficiency is expressed as mean ± S.E., n= ~ 50 to 60 cells of 3 independent experiments. The * symbols indicate the results of the Student *t* Test analysis, $P < 0.05$.

Figure S4. Vacuolin-1 induced the accumulation of both LC3B-II and SQSTM1 in the presence of autophagy inducer, starvation or rapamycin. HeLa cells were treated with vacuolin-1 (1 μM), starvation, or rapamycin (1 μM) alone for 6 h, or pretreated with vacuolin-1, starvation, or rapamycin for 3 h, followed by cotreatment with vacuolin-1, starvation, or rapamycin for another 3 h as indicated. Western blot analyses against LC3B and SQSTM1 were then performed and repeated 2 times respectively. p, denotes pretreatment; c, denotes cotreatment.

Figure S5. Vacuolin-1 (1 μM) markedly decreased the number of autolysosomes induced by leup in tfLC3B-expressing HeLa cells. Quantification of LC3B red only puncta/per cell is expressed as mean ± S.E., n= ~ 60 to 80 cells of 3 independent experiments. The * symbols indicate the results of the Student t Test analysis, $P < 0.05$.

Figure S6. Cotreatment with vacuolin-1 and leup only marginally increased the accumulation of LC3B-II and SQSTM1 compared to vacuolin-1 treatment alone. HeLa cells were treated with vacuolin-1 (1 μM) in the presence or absence of leup (10 μM) for 6 h or 12 h as indicated. Western blot analyses against LC3B and SQSTM1 were then performed and repeated 2 times respectively.

Figure S7. Vacuolin-1 was more potent than CQ in suppressing autophagy in PC3 cells. Cells were treated with vacuolin-1 or CQ at the indicated dose for 6 h, and western blot analyses against LC3B and SQSTM1 were then performed and repeated 3 times.

Figure S8. Vacuolin-1 showed little toxic in PC3, HepG2, MCF7, and A549 cells. Cells were treated with vacuolin-1 or CQ for the indicated doses for 48 h, and cell viability were then determined by MTT assay. All experiments were repeated three times in triplicate wells; data from a representative experiment are shown.

Figure S9. Vacuolin-1 did not change the lysosomal activity. The expression of LAMP1 and the processing of CTSB from the precursor form to its mature form in HeLa cells treated with or without vacuolin-1 (1 μM) for 6 h. Western blot analyses against LC3B, SQSTM1, LAMP1 and CTSB were then performed and repeated 3 times.

Figure S10. Expression of RAB5A-CA alone induced the accumulation of SQSTM1 as assessed by immunostaining analyses. Scale bar = 20 μM. Quantification of SQSTM1 puncta per cell is expressed as mean ± S.E., n= ~ 80 cells of 3 independent experiments. The * symbols indicate the results of the Student *t* Test analysis, $P < 0.05$.

Figure S11. RAB5A knockdown or expression of RAB5A-DN did not affect starvation induced LC3B-II in HeLa cells. Experiments were repeated 3 times.

Figure S12. Expression of RAB5A-DN did not affect starvation induced LC3 puncta in HeLa cells. This experiment was repeated 3 times.

Figure S13. Overexpression of RAB5A-RFP did not further enhance the effect of vacuolin-1 in terms of LC3 accumulation. RAB5A-RFP- or RAB5A-CA-RFP-expressing HeLa cells were treated with or without vacuolin-1 (1 μM) for 6 h. Western blot analyses against LC3B and SQSTM1 were then performed and repeated 3 times.

Figure S14. Vacuolin-1 (1 μM) treatment prevented the recruitment of RFP-RAB7A to autophagosomes in GFP-LC3-expressing HeLa cells. The RFP-RAB7A/GFP-LC3B colocalization coefficiency is expressed as mean ± S.E., n= ~ 50 to 60 cells of 3 independent experiments. The * symbols indicate the results of the Student *t* Test analysis, $P < 0.05$. Scale bar = 20 μM.

Figure S15. Vacuolin-1 treatment enlarged lysosomes in HeLa cells as shown by electron microscopy.

Table S1. List of primers.

1	*RAB5A* shRNA # 1 GCAGCCTTCCTTTCCAAAGTT #2 CCAGGAATCAGTGTTGTAGTA
2	Scrambled shRNA CCTAAGGTTAAGTCGCCCTCG