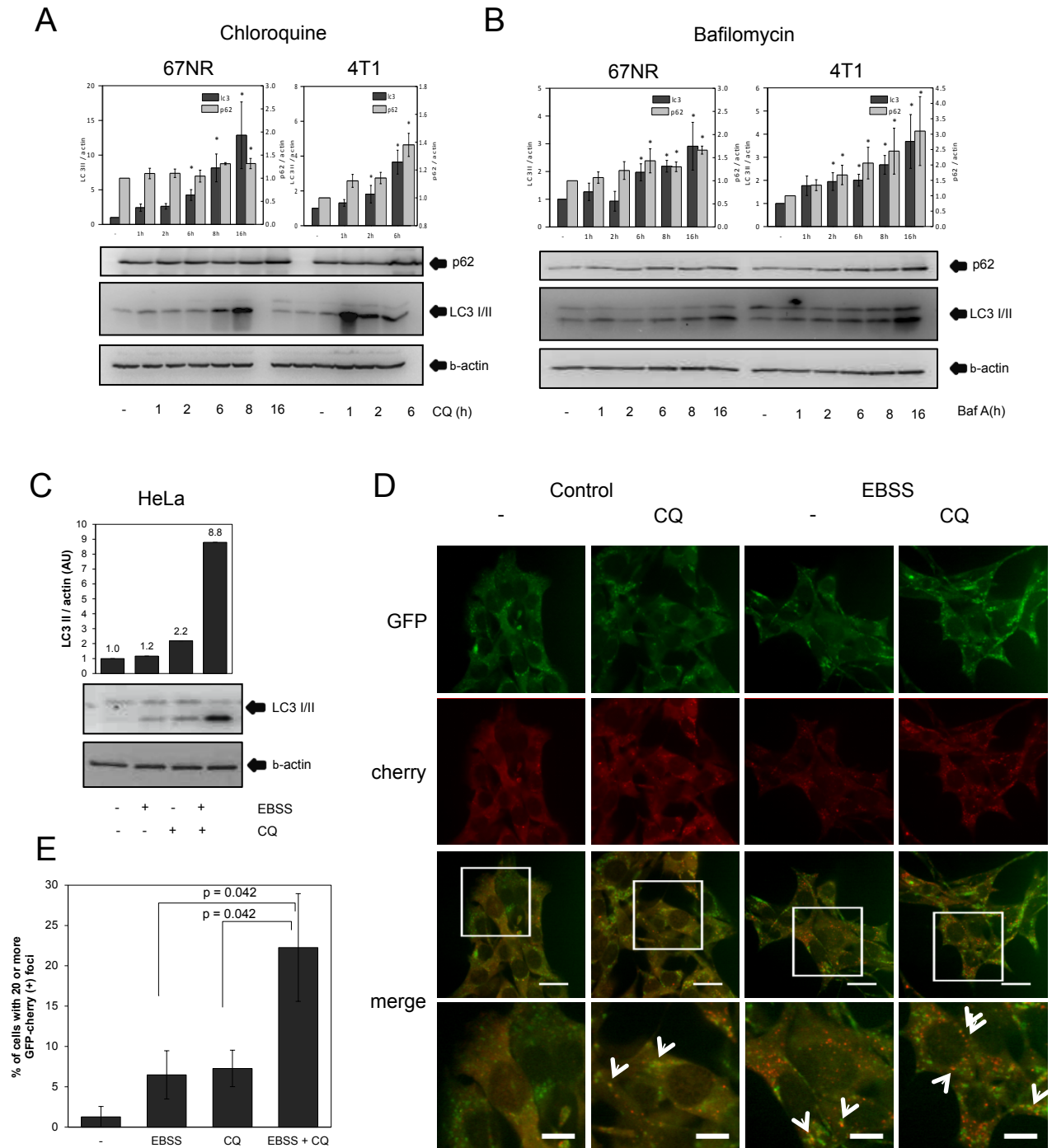


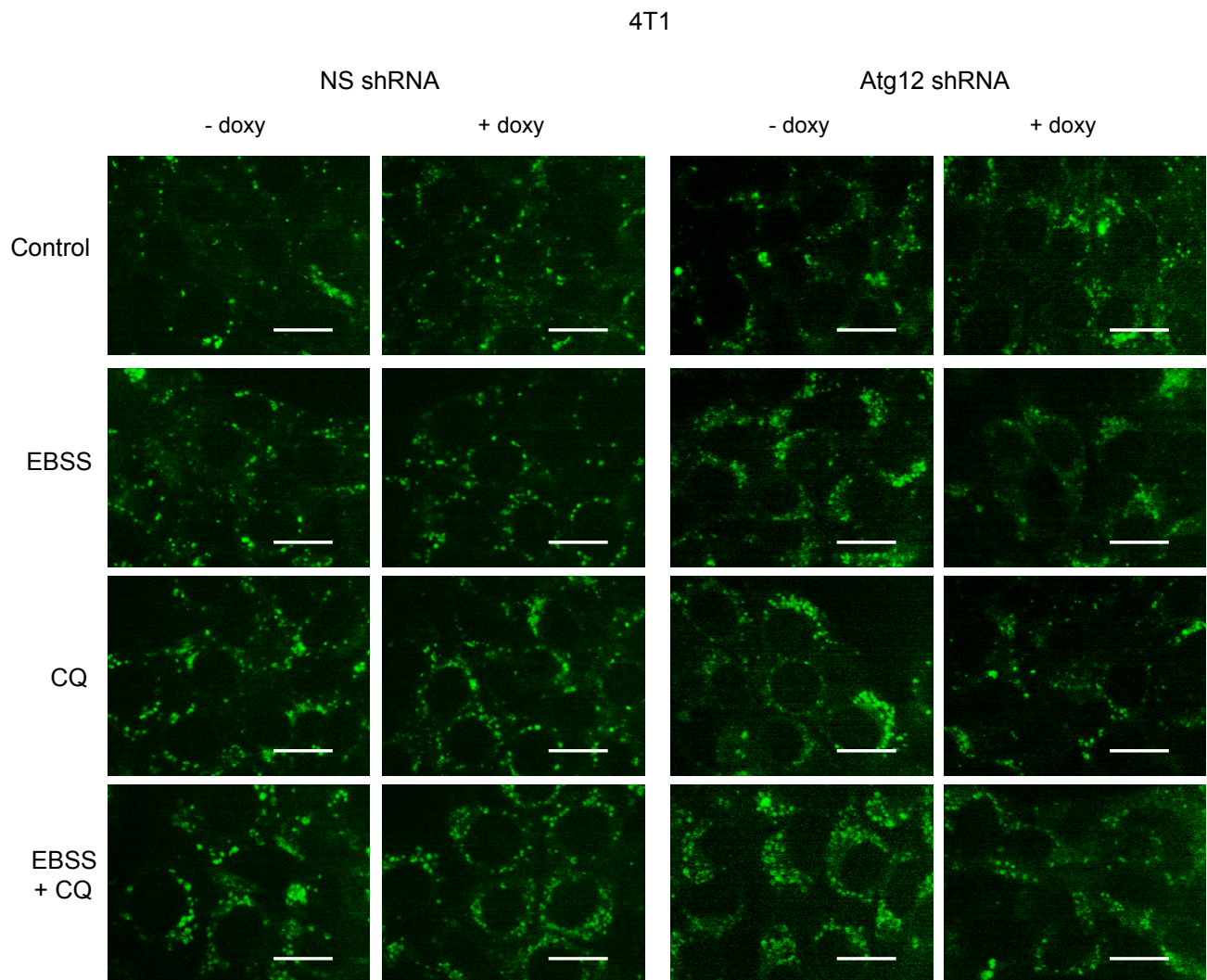


**Supplemental Material to:**  
**Maycotte P, Aryal S, Cummings CT, Thorburn J,**  
**Morgan MJ, Thorburn A**  
**Chloroquine sensitizes breast cancer cells to**  
**chemotherapy independent of autophagy**  
**Autophagy 2012; 8(2); [http://dx.doi.org/10.4161/](http://dx.doi.org/10.4161/auto.8.2.18554)**  
**[auto.8.2.18554](http://dx.doi.org/10.4161/auto.8.2.18554)**

**[www.landesbioscience.com/journals/autophagy/article/18554](http://www.landesbioscience.com/journals/autophagy/article/18554)**

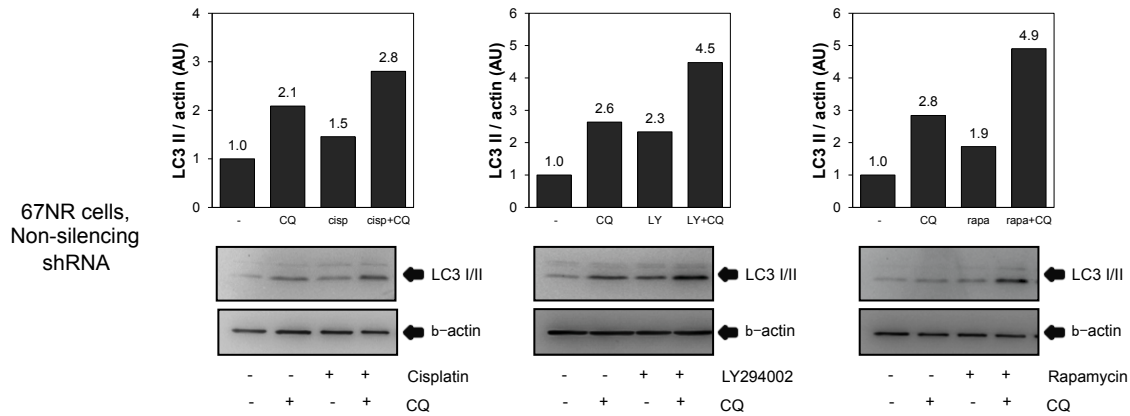


**Supplementary Figure 1.** Basal autophagy was measured by the addition of 10 mM CQ (A) or 1 nM Bafilomycin A (B) for different time points in both cell lines and evaluated by LC3II and p62 accumulation. In C, HeLa cells were starved with EBSS for 2h +/- 10 mM CQ and autophagic flux was evaluated by LC3 WB. GFP-cherry-LC3 expressing 67NR cells were imaged in a confocal microscope for autophagic flux after 3h starvation (EBSS) +/- 10 mM CQ (D) and quantification of yellow dots is shown in E. Graphs in A and B show density analysis of the mean +/- SE of 3 independent experiments. \* represents difference with  $p < 0.05$  to untreated controls (A, B). Bars in D represent 20  $\mu$ m and the ones in the insets 10  $\mu$ m. Arrows in D show examples of yellow dots.



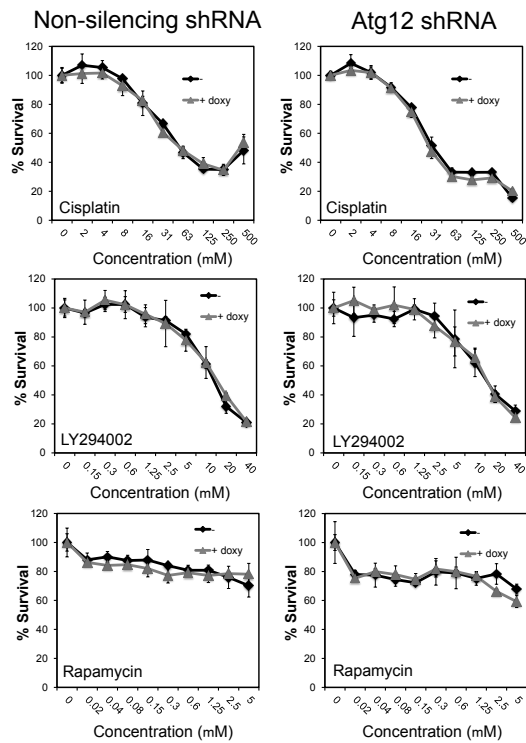
**Supplementary Figure 2. Establishment of an inducible system to manipulate autophagy.** 4T1 cells were transduced with a lentivirus containing either an inducible Atg12 shRNA or a non-silencing (NS) shRNA (see materials and methods) and then were transfected with a GFP-LC3 construct. LC3 redistribution after starvation (EBSS, 2h) was evaluated after 72 h of doxycycline (doxy) treatment +/- the addition of CQ. Scale bars represent 20  $\mu$ m.

A



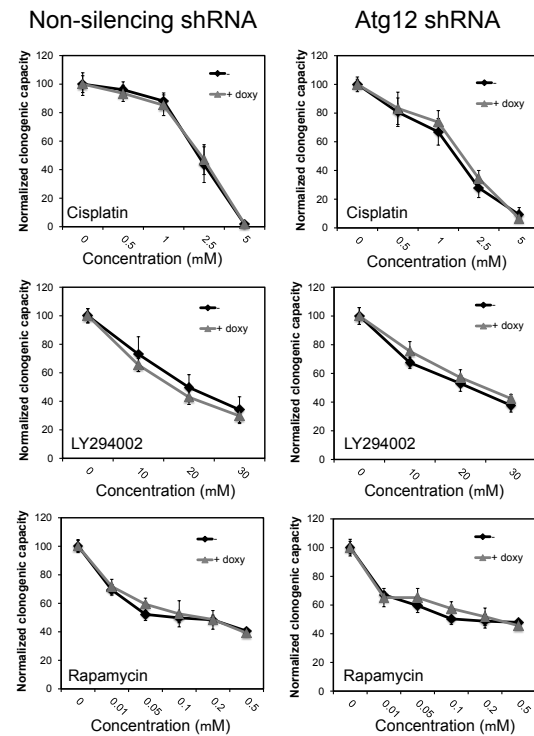
B

4T1, Short term assay

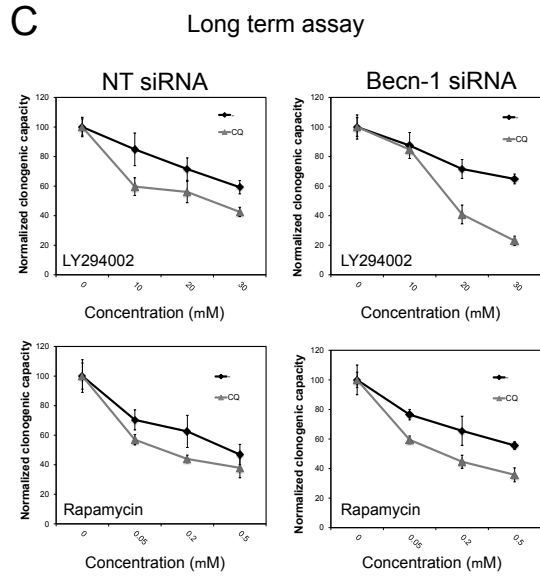
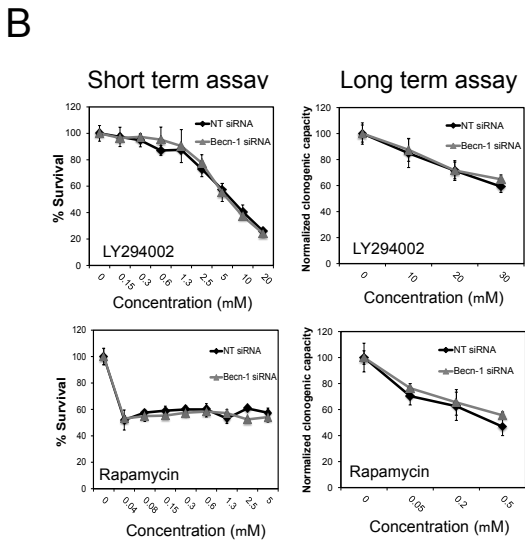
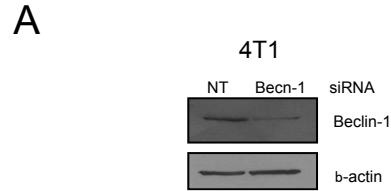


C

4T1, Long term assay



**Supplementary Figure 3. CQ induced sensitization to chemotherapy is not mimicked by Atg12 knockdown.** 67NR cells expressing an inducible non-silencing shRNA were evaluated for autophagy induction after treatment with cisplatin (1 mM, 6 h), LY294002 (20 mM, 8 h) or Rapamycin (0.2 mM, 8 h) with an LC3 Western Blot. 4T1 cells expressing either an inducible Non-silencing or an Atg12 shRNA were treated with cisplatin, LY294002 or rapamycin at the indicated concentrations and viability was evaluated with short-term (MTS) or long-term (clonogenic) assays. Graphs in A show density quantification of a representative blot. Graphs in B and C show mean  $\pm$  SE of three independent experiments.

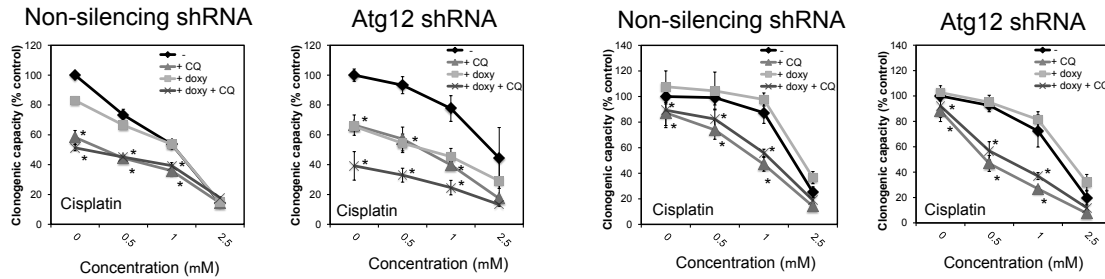


**Supplementary Figure 4. Beclin-1 knockdown has no effect on viability of 4T1 cells treated with LY294002 or rapamycin.** 4T1 cells transfected with Beclin-1 (Becn-1) or non-targeting siRNAs (NT) were analyzed for Western Blot 72 h after transfection (A), or were treated with LY294002 (48h) or rapamycin (72h) for short-term (MTS) assays or for 24h for long-term clonogenic assays and allowed to recover for 4 days (B). In C, cells were treated as in B but in the presence of CQ. Viability was evaluated in a long-term clonogenic assay. Graphs show mean  $\pm$  SD of one representative experiment from two independent experiments performed in triplicate.

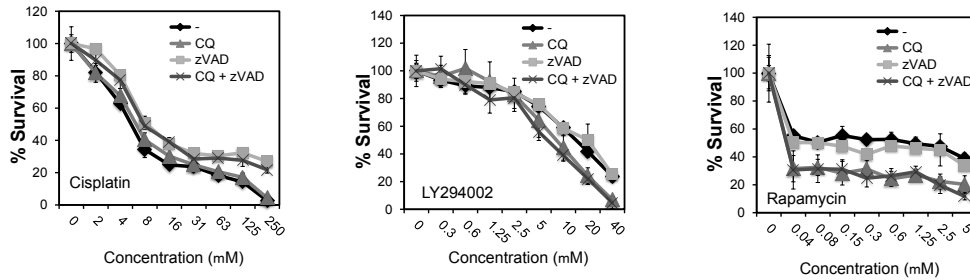
A

67NR cells, Long term assay

4T1 cells, Long term assay

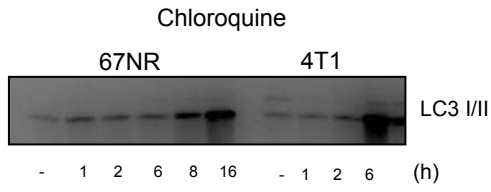


B

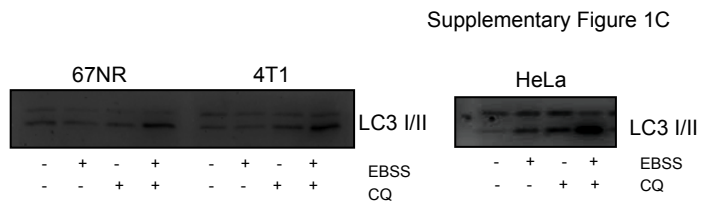


**Supplementary Figure 5.** In A, 67NR or 4T1 cells were grown in the absence or presence of doxycycline for 72h, treated for 24 h +/- doxycycline (doxy) or CQ, the treatment was washed and replaced with fresh medium +/- doxycycline and the cells were allowed to recover for 4 days for long-term (clonogenic) assays. Graphs show mean +/- standard error of three independent experiments performed in triplicate. All treatments were normalized to untreated controls. In B, 4T1 cells were treated with cisplatin (24 h), LY294002 (48 h) or rapamycin (72 h) +/- CQ or 50 mM zVAD and viability was evaluated with an MTS assay. Graphs show mean +/- standard deviation of one representative experiment performed in triplicate.

Supplementary Figure 1A

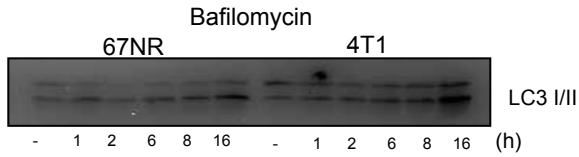


1A

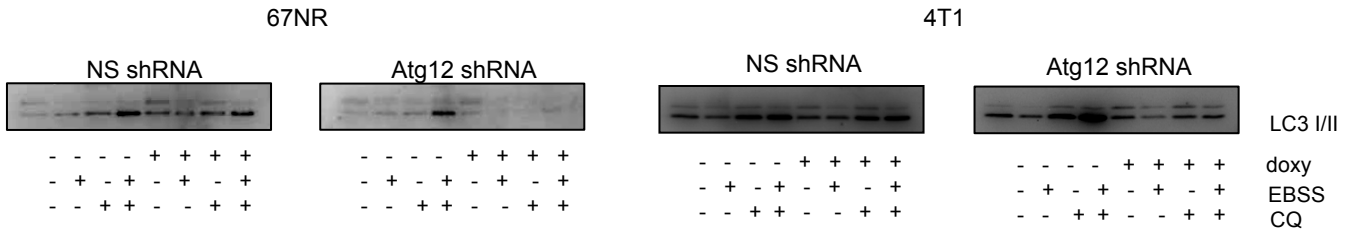


Supplementary Figure 1C

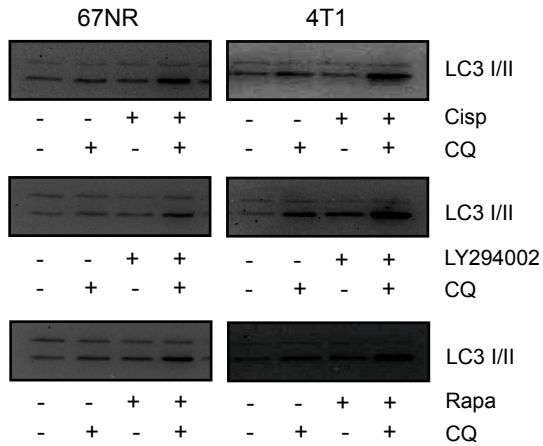
Supplementary Figure 1B



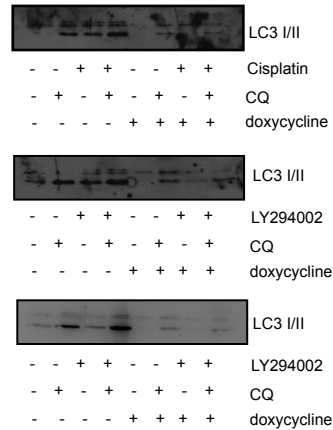
2B



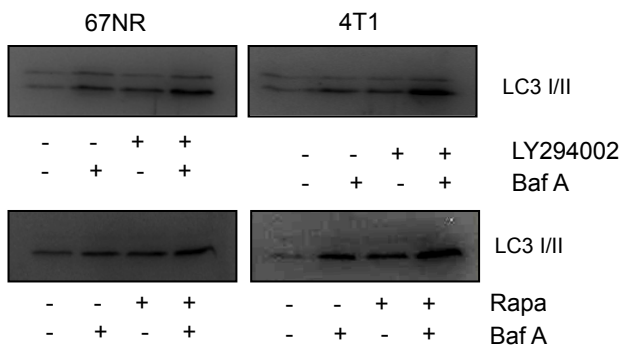
3A



5A



7A



Supplementary Figure 6. Overexposed images of LC3 Western Blots included in the paper.