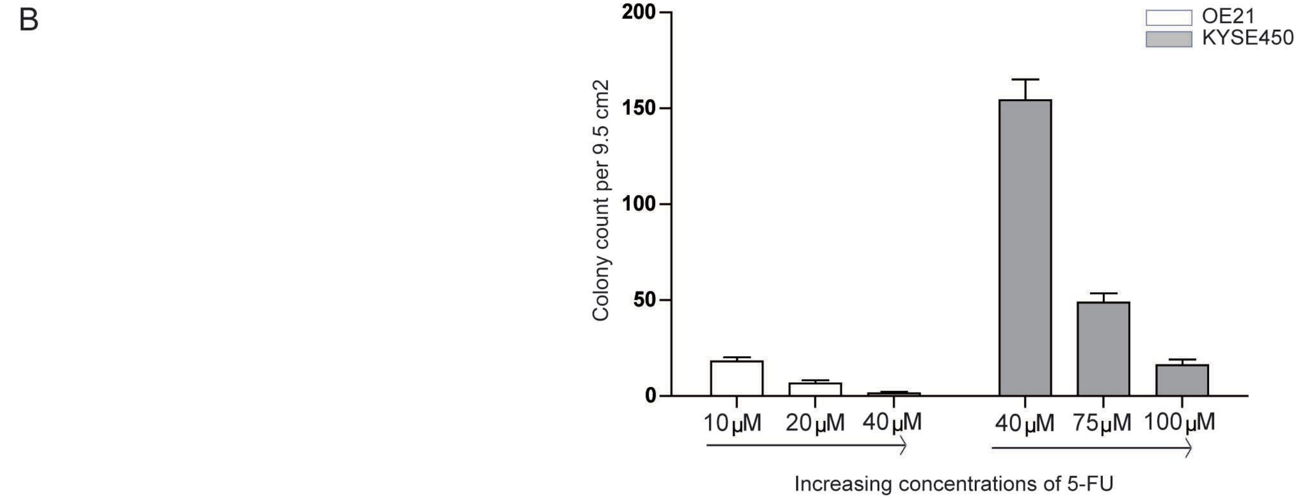
Supplementary Figure 1

A

	Untreated	1μM 5-FU	10μM 5-FU	20μM 5-FU	40μM 5-FU
OE21			18	07	01

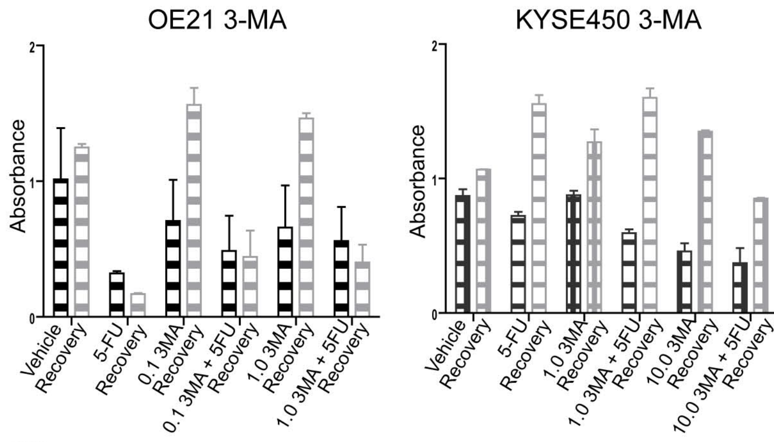
	Untreated	20μM 5-FU	40μM 5-FU	75μM 5-FU	100μM 5-FU
KYSE450			154	48	16

B

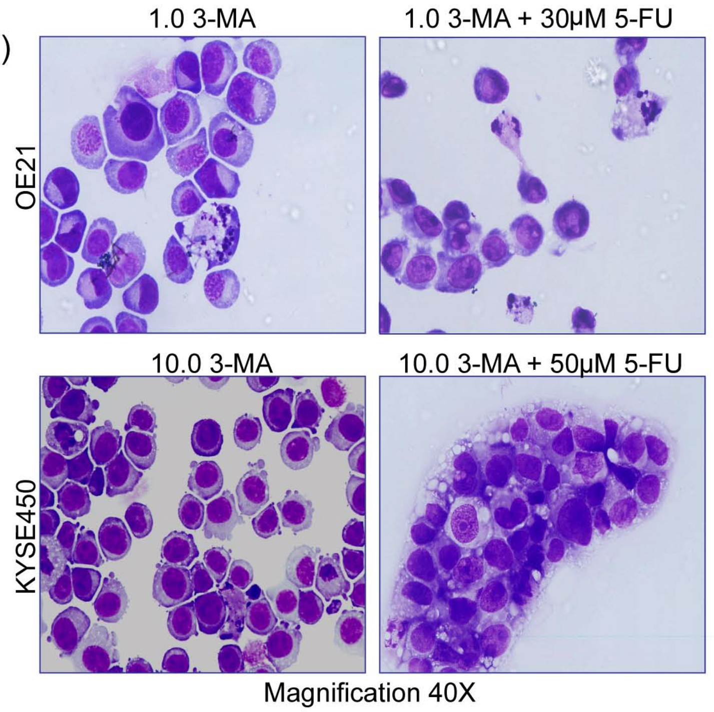


Supplementary Figure 2

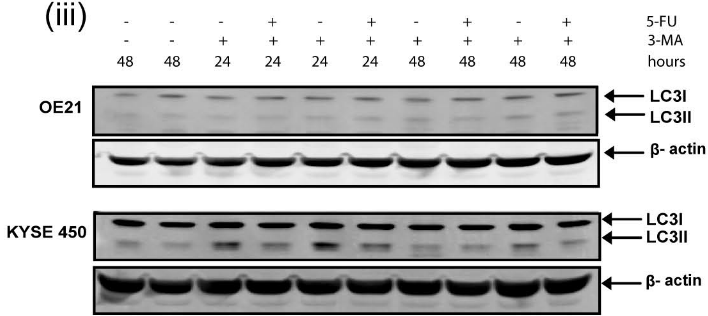
A (i)



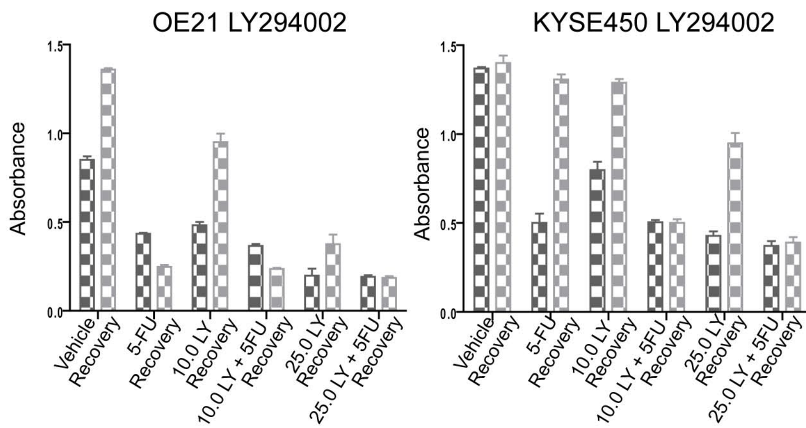
(ii)



(iii)



B (i)



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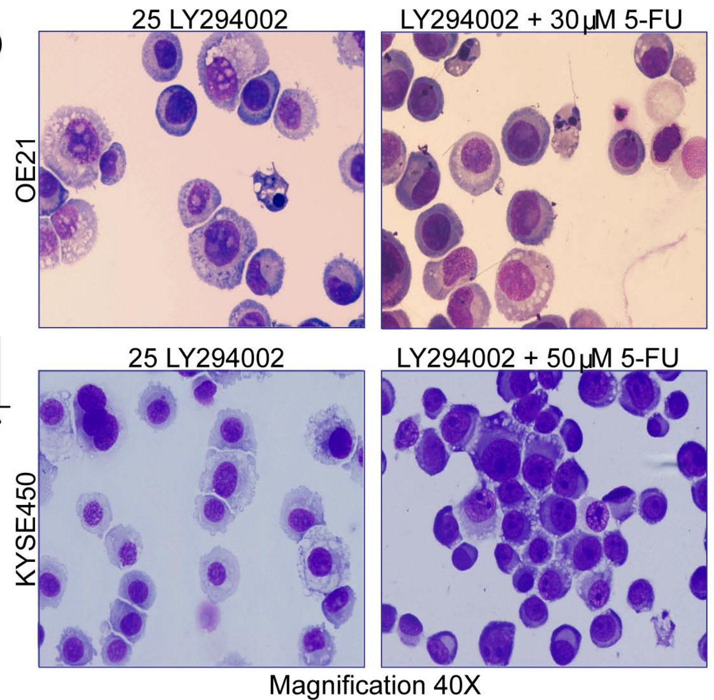
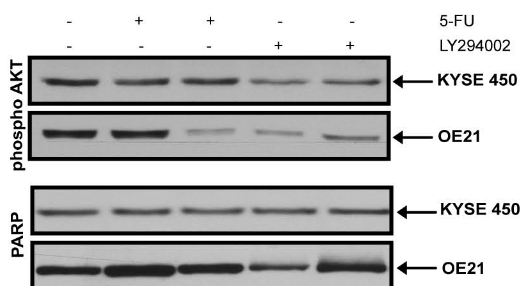
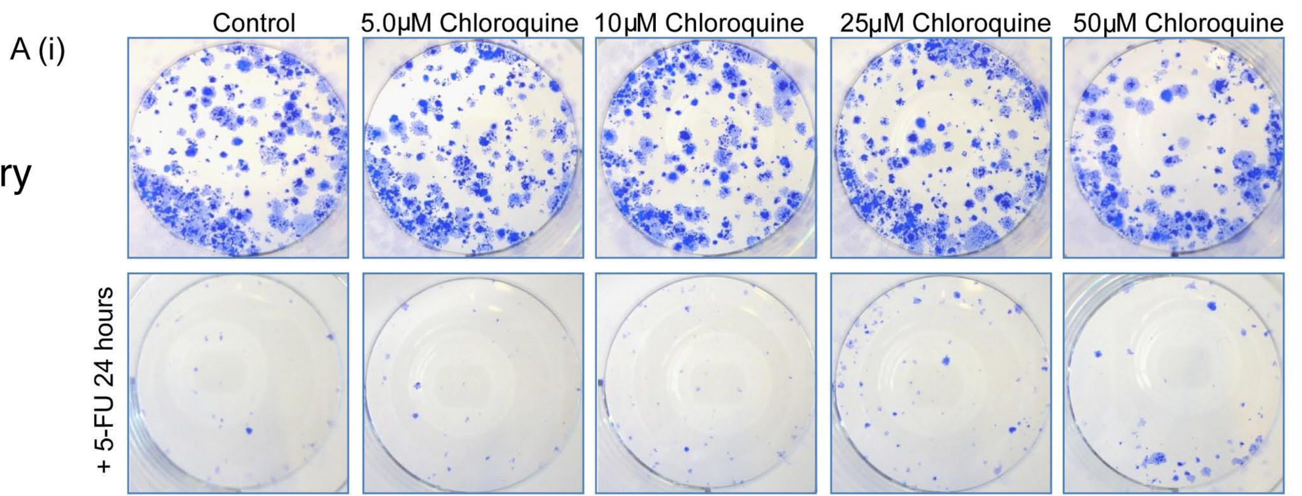


C

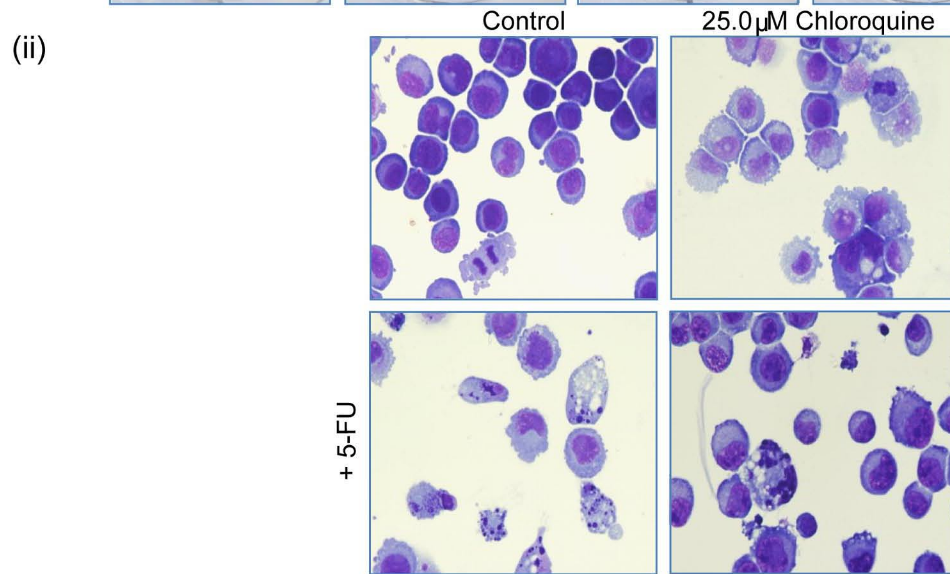


Supplementary Figure 3

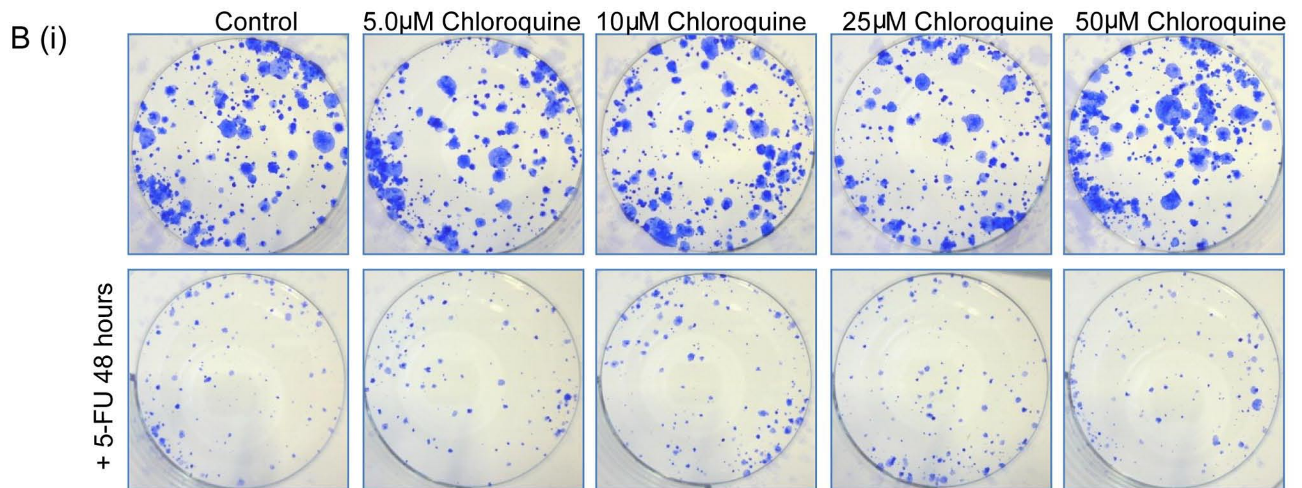
A (i)



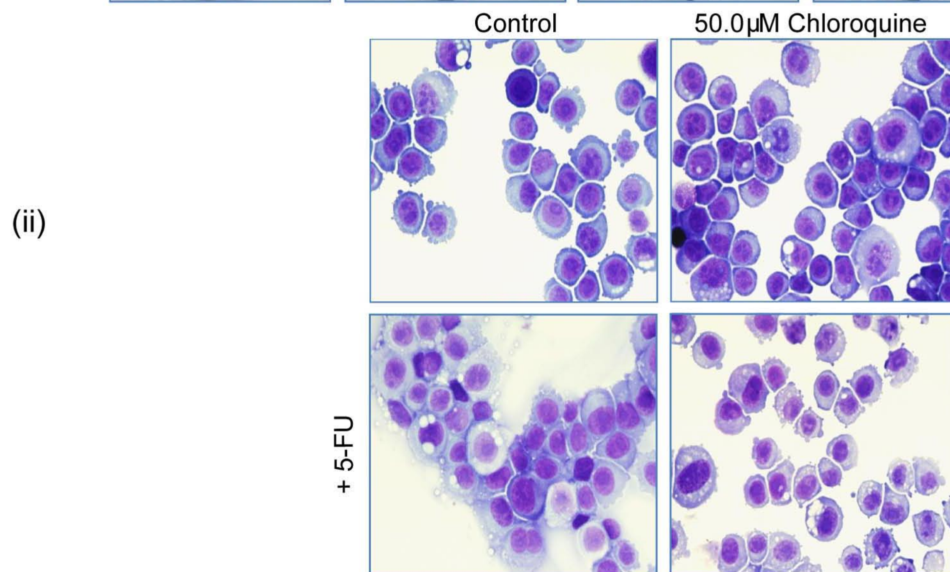
(ii)



B (i)



(ii)



Supplementary Figure Legends

Supplementary Figure 1. Differential sensitivity to 5-fluorouracil (5-FU). Sensitivity of esophageal cell lines to 5-FU was measured by examining their ability to recover following withdrawal of a range of concentrations of 5-FU, assessed with a colony formation assay. **A** Drug sensitive (OE21) and drug resistant (KYSE450) cell lines were treated with a range of concentrations of 5-FU (1 – 40 μM) and (20 – 100 μM) respectively for 48 hours. Viable, adherent cells were counted and re-seeded (1,000 cell per well) into a well of a six well plate (in triplicate), in the absence of drug. Ten to fourteen days later, colonies were fixed and stained. Each well shown is a representative image of at least fifteen similar wells (five independent experiments). **B** Stained colonies were counted (where possible) to determine the effects of the various concentrations of 5-FU on both OE21 and KYSE450 treated cells. Data is presented as mean colony count +/- SEM of three independent experiments. Comparable numbers of colonies grow in the 10 μM 5-FU treated OE21 and 100 μM 5-FU treated KYSE450 cells, highlighting a 10 fold differential in sensitivity of these esophageal cell lines, shown in **A** and quantified in **B.**

Supplementary Figure 2. Effects of PI-3 kinase inhibitors, 3-methyladenine (3-MA) and LY294002 on 5-fluorouracil (5-FU) treatment. A (i) OE21 and KYSE450 cell lines were treated with a range of concentrations of 3-MA (0.1 to 10.0 mM) without and with 5-fluorouracil (30 or 50μM respectively) for 48 hours and viability was assessed using the MTT assay. Recovery data was acquired 48 hours after drug removal. **(ii)** Morphological features of OE21 cells following 3-MA treatment without (upper left) and with 5-FU (30 μM) (upper right), following 48 hour incubation. KYSE450 cells were treated with 3-MA (10.0 mM) without (lower left) and with 5-FU (50 μM) (lower right), also for 48 hours. Treatment with

3-MA alone did not significantly affect morphology or either cell line (left panels) (magnification 40X). Pre-treatment with 3-MA induced mixed morphologies (apoptosis and Type II death) in OE21 cells (upper right) and enhancement of Type II morphologies in KYSE450 (lower right) cells. **(iii)** Expression of LC3 isoforms following 24 hour and 48 hour treatments: LC3II accumulates within 24 hours in response to 3-MA alone and when combined with 5-FU. **B (i)** OE21 and KYSE450 cells were treated with LY294002 (10 and 25 μM) alone or in combination with 5-FU (30 or 50 μM respectively), and viability assessed using the MTT assay. Recovery was measured 48 hours after drug removal. **(ii)** The morphology induced by LY294002 (25 μM) was predominantly autophagic in both OE21 (upper left) and KYSE450 (lower left), with marked cytoplasmic vacuoles in both cell lines. When combined with 5-FU, OE21 cells (30 μM) display both apoptotic and autophagic morphologies (upper right), while KYSE450 (50 μM 5-FU) cells display enhanced autophagy (lower right) in response to combination treatments (magnification 40X). **C** LY294002 reduced phosphorylation of a downstream target of PI3 Kinase Type I, Akt. KYSE450 and OE21 cells were incubated with 5-FU or LY294002 and subjected to Western blot analysis with anti-phospho Akt. Lane 1: vehicle control, lanes 2 & 3: 40 μM 5-FU (24 and 48 hours), lanes 4 & 5: LY294002 10 and 25 μM for 48 hours. Blots were probed with anti-PARP antibody to demonstrate equal loading.

Supplementary Figure 3. Effect of chloroquine in combination with 5-FU on cell viability and morphology. OE21 and KYSE450 cells were treated with chloroquine or 5-FU alone or in combination for 24 or 48 hours respectively. In combination treatments, chloroquine was added 2 hours prior to 5-FU. The ability of cell lines to recover following drug withdrawal was assessed with a colony formation assay. OE21 **A (i)** and KYSE450 **B (i)** cells were treated with a range of concentrations of chloroquine (5 μM, 10 μM, 25 μM and 50

μM) alone (upper panels) and in combination with 5-FU (40 μM) (lower panels). Viable, adherent cells were counted and re-seeded (1,000 cells per well) into a well of a six well plate (in triplicate), in the absence of drug. Cells were allowed to adhere and grow for between 10 to 14 days, following which, colonies were fixed and visualized. Each well shown is a representative image of at least 12 similar wells. Chloroquine alone did not prevent the re-growth of colonies in either cell line. When combined with 5-FU in the OE21 cells, the presence of chloroquine did not influence colony re-growth at any concentration (**A (i)**) – suggesting that there is no advancement of apoptosis, or inhibition of recovery in these cells. Morphological features of these cells were examined post treatment to assess the levels of autophagy and/or apoptosis present in single and combination treatments. OE21 cells **A (ii)** were treated with chloroquine (25 μM) (upper right panel) or 5-FU (40 μM) (lower left panel) alone, or in combination (lower right panel) for 24 hours. Analysis of morphology indicated an expanded vesicular compartment with chloroquine treatment alone and morphology (predominantly apoptosis) was similar in 5-FU treated cells without or with chloroquine (A **(ii))**.

The recovery of KYSE450 cells following 48 hour treatment with 5-FU was also unaffected by a range of concentrations of chloroquine (**B (i)**). KYSE450 cells **B (ii)** were treated with chloroquine (50 μM) (upper right panel) or 5-FU (40 μM) (lower left panel) alone, or in combination (lower right panel) for 48 hours. Chloroquine could induce vesicular accumulation, but it did not affect the morphology of 5-FU treated KYSE450 cells **(B (ii)** lower right panel).