

**Figure S1. [Pemetrexed + fingolimod] treatment inactivates mTOR, activates the AMPK and**

**ULK1 and simultaneously induces autophagosome and autolysosome formation. A. Spiky**

ovarian cancer cells were treated with vehicle control or with [pemetrexed (500 nM) + fingolimod (200 nM)] for 6h. After six h, cells were fixed in place and immunostaining performed to detect the expression and phosphorylation of the indicated proteins. Staining densities of phospho-/total-protein levels are determined in 40 cells in triplicate using software integral in the Hermes WiScan

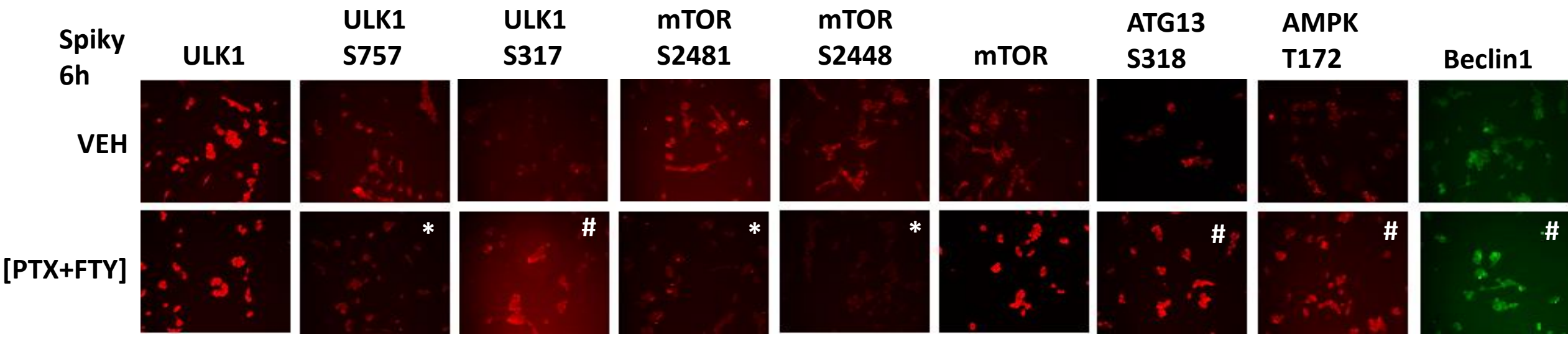
microscope (n = 120 cells +/- SEM). # p < 0.05 greater than vehicle control value; \* p < 0.05 less than vehicle control value. **B.** Spiky cells were transfected with a plasmid to express LC3-GFP-RFP.

Twenty-four h after transfection, cells were treated with vehicle control or with [pemetrexed (500 nM) + fingolimod (200 nM)] for 3h, 6h or 12h. At each time point the mean number of intense staining

GFP+ and RFP+ vesicles in the cells was determined (at least 40 cells per condition were counted) (n = 120 cells +/-SEM). # p < 0.05 greater than vehicle control value.

**Figure S2. [Pemetrexed + fingolimod] lethality requires ATG5-dependent mitochondrial dysfunction leading to apoptotic and necroptotic death processes.** Spiky ovarian cancer cells were transfected with the following plasmids to express: CMV / empty vector; c-FLIP-s; BCL-XL; dominant negative caspase 9; activated AKT; activated MEK1; activated mTOR; activated STAT3. Other Spiky cells were transfected with the following siRNA molecules to knock down the expression of: control scramble, siSCR; apoptosis inducing factor, AIF; AMPK $\alpha$  subunit; ATM; BAX; BAK; Beclin1; BID; cathepsin B; ATG5; eIF2 $\alpha$ ; NOXA; PUMA; PERK; RIP-1; ULK1. Twenty-four h after transfection cells were treated with vehicle control or with [pemetrexed (500 nM) + fingolimod (200 nM)] for 24h. Cells were then isolated and viability determined via a live/dead assay (n = 3 +/- SEM). \* p < 0.05 lower value than the corresponding value in CMV/siSCR transfected cells; \*\* p < 0.01 lower value than the corresponding value in CMV/siSCR transfected cells.

**S1A**



**S1B**

