

Figure S1. Calpeptin or rapamycin enhance autophagic flux. GFP-Lc3 embryos at 2 dpf were treated with the indicated chemicals for 24 h. Protein extracts were analyzed by SDS-PAGE and detected using anti-LC3, or anti-tubulin antibody as a loading control. The ratio of GFP-Lc3-II versus tubulin was quantified using ImageJ software (<http://rsb.info.nih.gov/ij/>) and indicated below each lane. The value for rapamycin treatment was set to 1.0 and other values were normalized. Cal, calpeptin; Rap, rapamycin; P/E, pepstatin A and E64d.

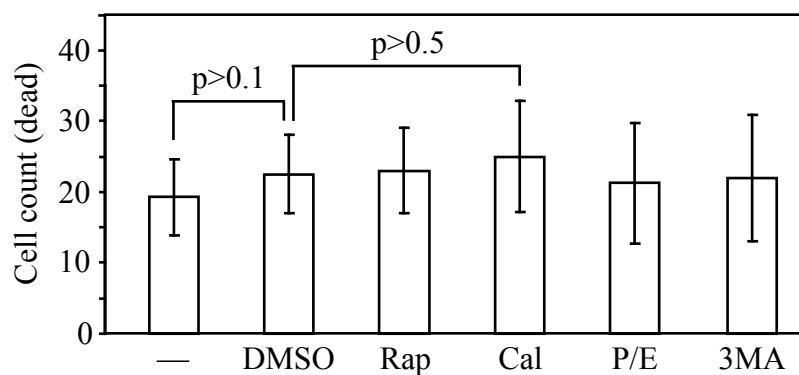


Figure S2. Quantification of cell death by acridine orange staining. 2 dpf non-transgenic wild-type embryos were raised in the embryo water with or without the indicated solvent (DMSO) or chemicals (at the concentrations mentioned in Materials and Methods) for 24 h and stained with acridine orange. Images of each embryo from the cloaca to the two-thirds distance towards the tail tip were taken by fluorescence microscopy. The number of acridine orange-stained cells was counted manually and represented as mean \pm s.d. of four embryos. Significance was calculated by a non-paired samples *t*-test. Rap, rapamycin; Cal, calpeptin; P/E, pepstatin A and E64d; 3MA, 3-methyladenine.