



Supp Figure 1. ARF localizes to mitochondria and induces autophagy

(A) Confocal microscopy of U2OS-ARF cells using antisera to ARF, MitoTracker dye, and Far Red to detect nuclei following 24 hours treatment with doxycycline. The merge of all three channels is depicted on the right.

(B) Left panel: U2OS-ARF cells treated for the indicated timepoints with doxycycline (black), 0.5 ug/mL adriamycin (hatched) or left untreated (white) and analyzed for Annexin V staining using the Guava PCA (Guava Technologies). Right panel: U2OS-ARF cells were assayed for mitochondrial depolarization using the Mitochondrial Depolarization assay and Guava PCA (Guava Technologies) following doxycycline treatment for 48 and 72 hours.

(C) Western analysis for the proteins indicated of whole cell lysate from U2OS cells 48 hours after transfection with wild type ARF or OTC-ARF, compared to untransfected cells (UNTR). Note that OTC-ARF causes accumulation of LC3 II, to levels equivalent to that induced by ARF.

(D) Electron microscopy of U2OS cells transfected for 24 hours with the plasmids indicated (vector, or OTC-ARF), compared to U2OS-ARF cells untreated or treated with doxycycline for 24 hours.