



## **Supplemental Material to:**

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**Suppression of basal autophagy reduces lung cancer  
cell proliferation and enhances caspase-dependent and  
-independent apoptosis by stimulating ROS formation**

**Autophagy 2012; 8(7)**

**<http://dx.doi.org/10.4161/auto.20123>**

**[www.landesbioscience.com/journals/autophagy/article/20123](http://www.landesbioscience.com/journals/autophagy/article/20123)**

**Supplementary Figure 1** Effects of autophagy inhibitors on cell death induced by cisplatin and etoposide. (a) Inhibition of autophagy in A549 cells attenuates etoposide-induced cell death. Cells were treated for 36h with etoposide (10  $\mu$ M) and/or 3-methyladenine (5 mM) and cell death was measured using AV/PI staining. (b) Effect of autophagy inhibition on mitochondrial membrane potential in U1810 cells treated with etoposide alone or in combination with 3-methyladenine. (c) Representative dot-plots of Annexin V/ PI staining of U1810 cells treated as described in the legend to Figure 2B. (d) Inhibitor of autophagy chloroquine (50  $\mu$ M) drastically enhances cell death induced by cisplatin (7.5  $\mu$ M).

**Supplementary Figure 2** Effect of autophagy inhibition on drug-mediated cell-cycle distribution and cell death. (a) The cell cycle distribution in etoposide-treated U1810 cells (2.5  $\mu$ M) with normal or ATG7-mediated suppressed autophagy. (b) Effect of autophagy inhibition on etoposide-mediated cell death. U1810 cells with basal or siRNA-mediated inhibited autophagy were treated with etoposide (2.5  $\mu$ M) and cell death was measured using AV/PI staining.

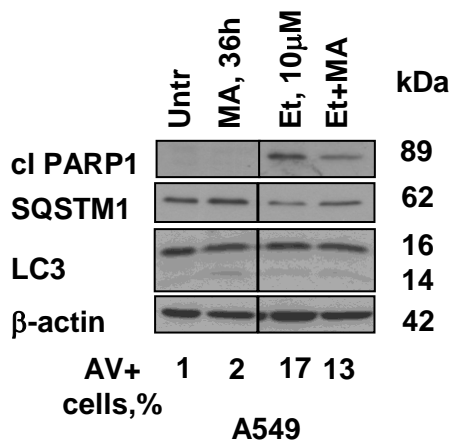
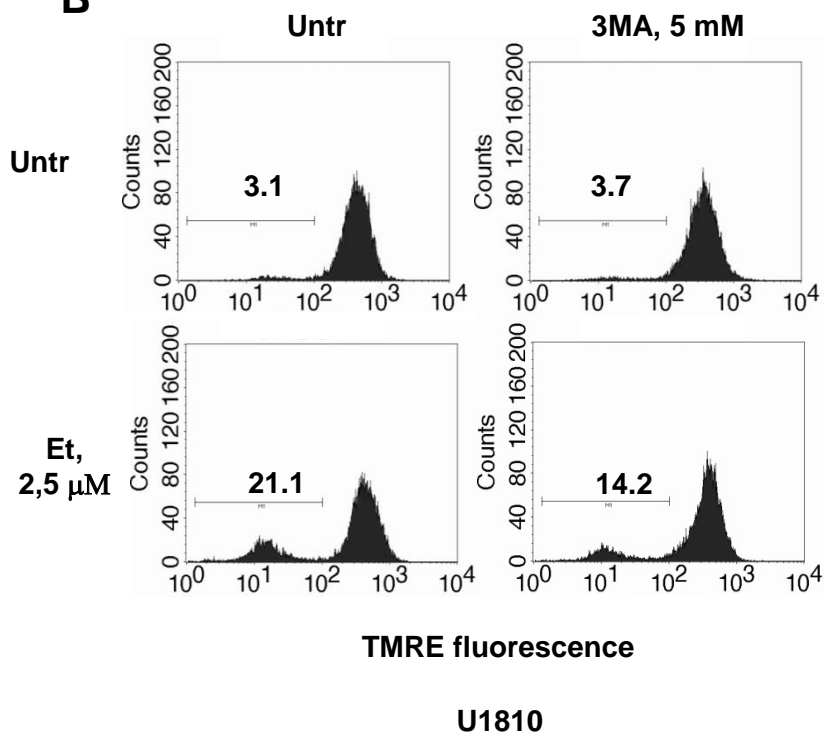
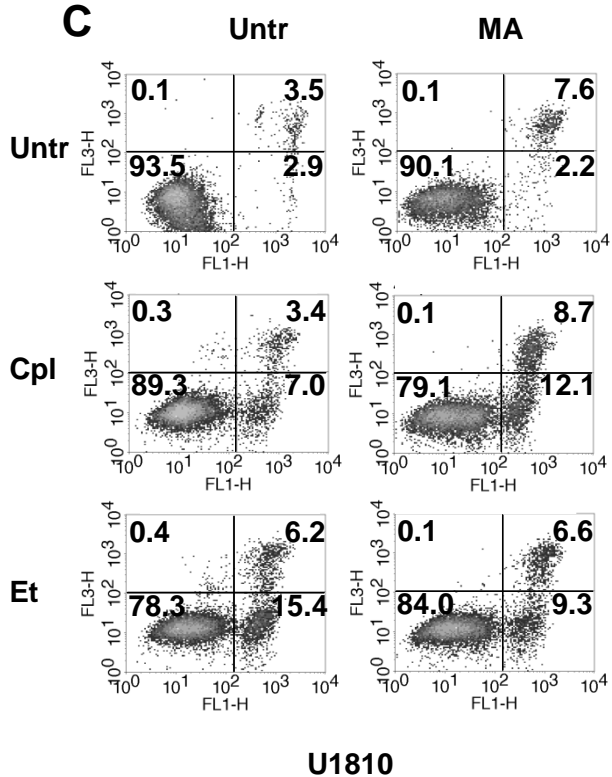
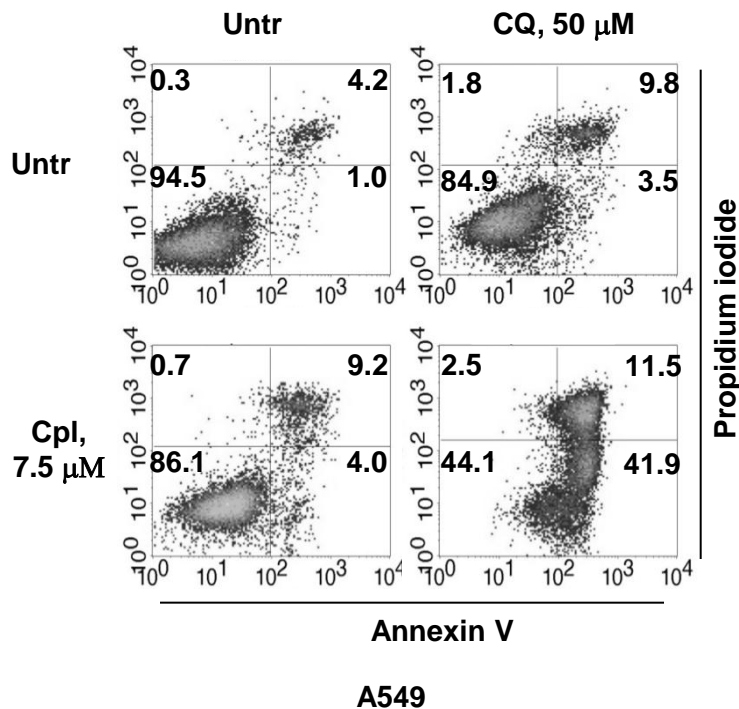
**Supplementary Figure 3** Inhibition of autophagy stimulates formation of ROS and contributes to AIF-dependent cell death in NSCLC cells. (a) Cells were co-transfected with scramble, ATG7 or AIF siRNAs and 48 h after transfection treated with cisplatin (15  $\mu$ M, 24h). (b) Downregulation of autophagy-related gene BECN1 stimulates formation of ROS in U1810 cells. ROS was measured using dihydroethidium as described in Materials and Methods.

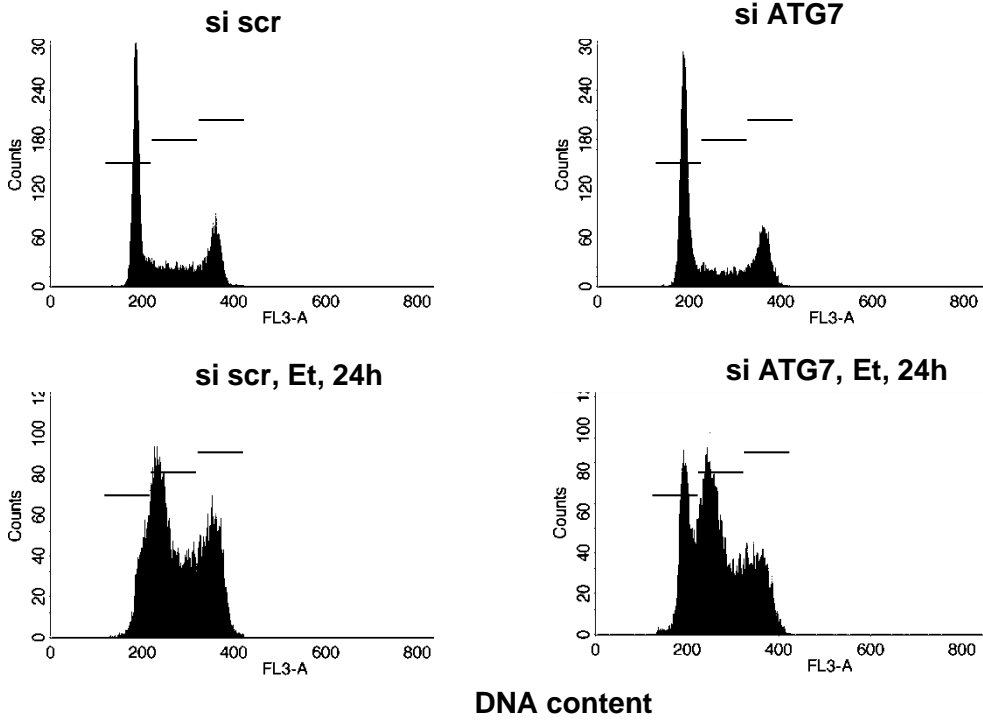
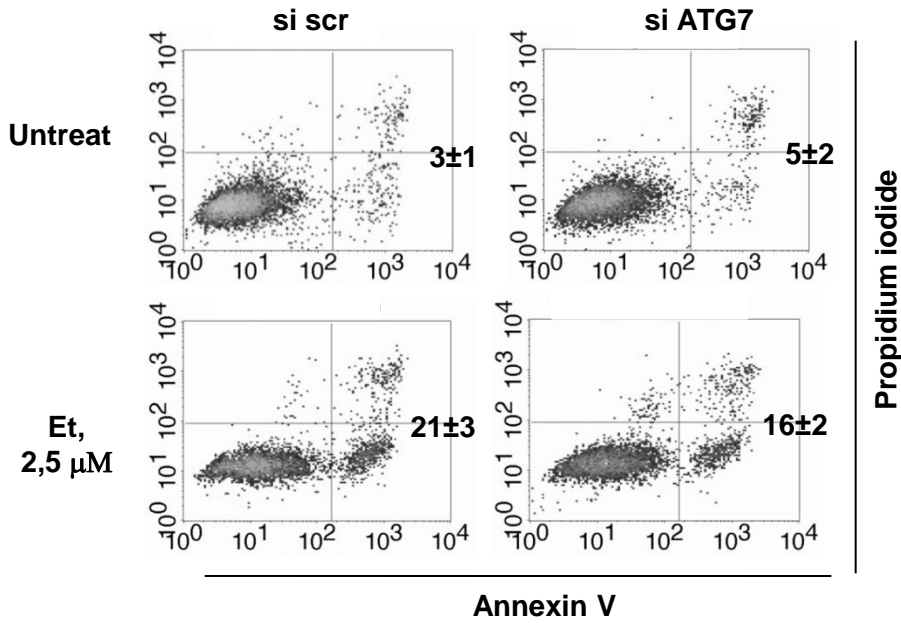
#### **Supplementary Figure 4**

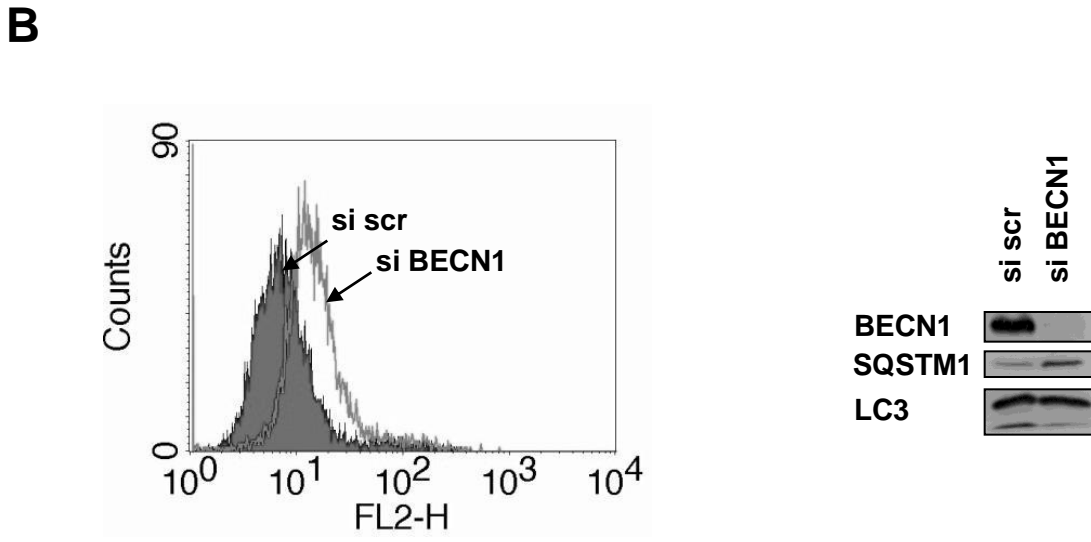
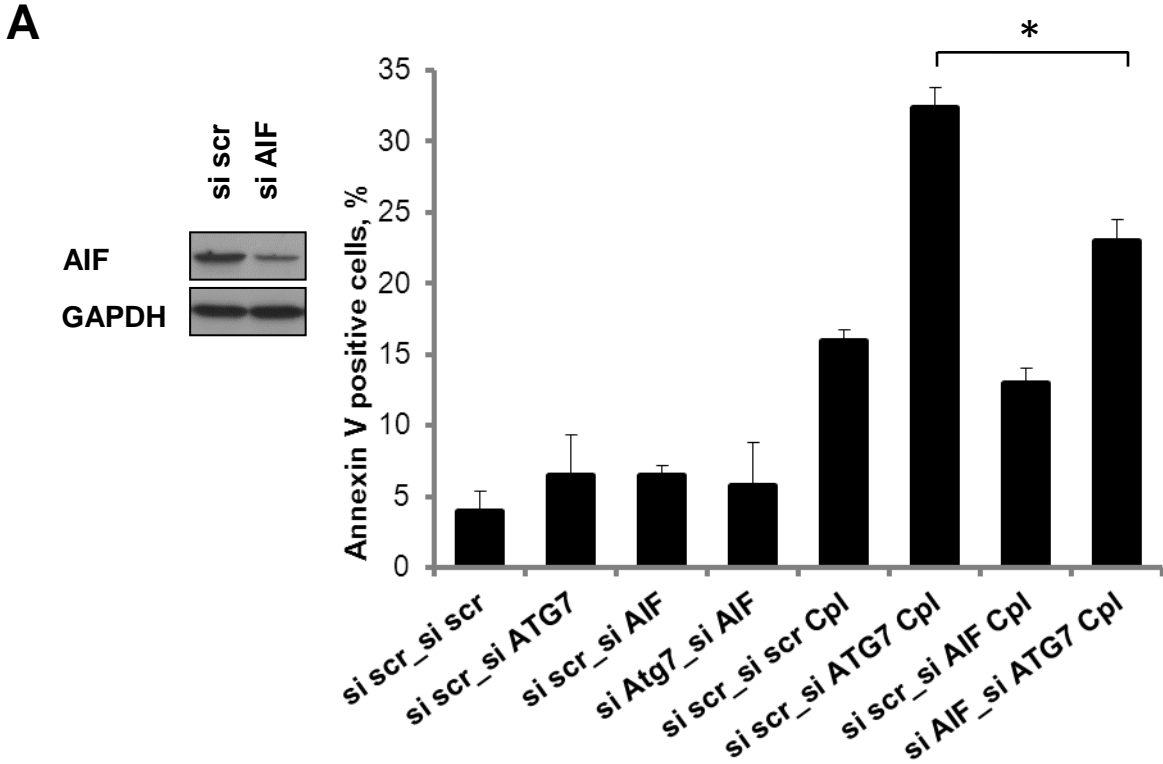
(a) Effect of antimycin A and rotenone on ROS formation in U1810 cells detected by oxidation of dihydroethidium. (b) Effect of mitochondrial ROS inducers on cisplatin-mediated cell death.

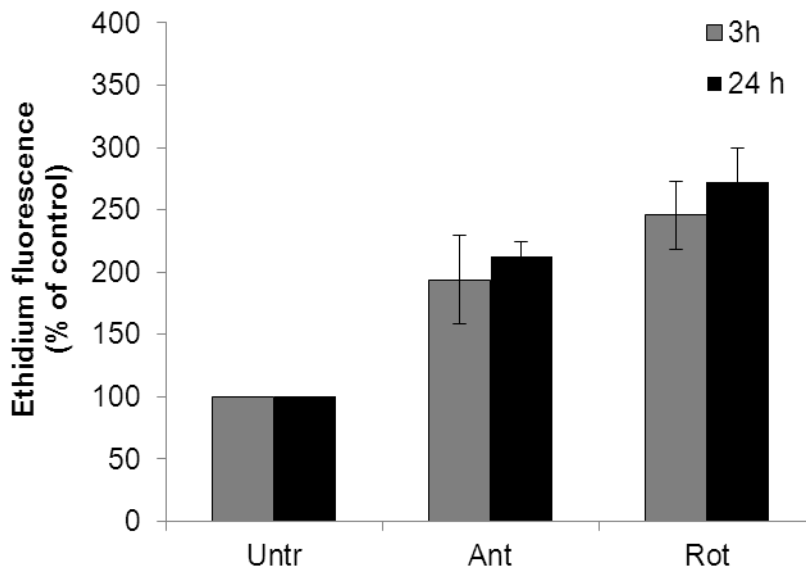
Supplementary Movie 1. Representative live-cell imaging of U1810 cells expressing pHyPer-cyto treated with cisplatin (15  $\mu\text{M}$ ) and chloroquine (50  $\mu\text{M}$ ).

Supplementary Movie 2. Representative live-cell imaging of U1810 cells expressing pHyPer-dMito treated with cisplatin (15  $\mu\text{M}$ ) and chloroquine (50  $\mu\text{M}$ ).

**A****B****C****D**

**A****B**



**A****B**