Antitumor activity of *Abrus* agglutinin through activation of apoptosis and autophagy-dependent cell death

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Abrus agglutinin (AGG), isolated from *Abrus precatorious*, a medicinal plant induces antitumor activity through activation of apoptosis and autophagy-dependent cell death. AGG effectively inhibited the cell viability of different oral squamous cell carcinoma with IC_{50} value 1-10 µg/ml. AGG selectively inhibited growth and, caused cell cycle arrest and mitochondrial apoptosis through reactive oxygen species (ROS) mediated ATM-p73 dependent pathway in FaDu cells. AGG-induced ROS accumulation was identified as chief mechanism of its effect on apoptosis, DNA damage and DNA-damage response which significantly reversed by ROS scavenger N-acetylcysteine (NAC). Moreover, AGG found to interact with mitochondrial manganese-dependent superoxide dismutase which might inhibit its activity and upshot ROS in FaDu cells. Moreover, we documented that AGG mediated Akt dephosphorylation led to ER stress resulting in the induction of autophagy-dependent cell death through the canonical pathway in cervical cancer cells. At the molecular level, AGG induced ER stress in PERK dependent pathway and inhibition of ER stress by salubrinal, eIF2α phosphatase inhibitor as well as siPERK reduced autophagic death in the presence of AGG. Further, our *in silico* and colocalization study showed that AGG interacted with pleckstrin homology (PH) domain of Akt to suppress its phosphorylation and consequent downstream mTOR dephosphorylation in HeLa cells. Moreover, administration of AGG (50 µg/kg body weight) significantly inhibited the growth of FaDu xenografts in athymic nude mice. In conclusion, we established that AGG-stimulated cell death by apoptosis and autophagy might be used as a novel tumor suppressor mechanism for cancer therapy.

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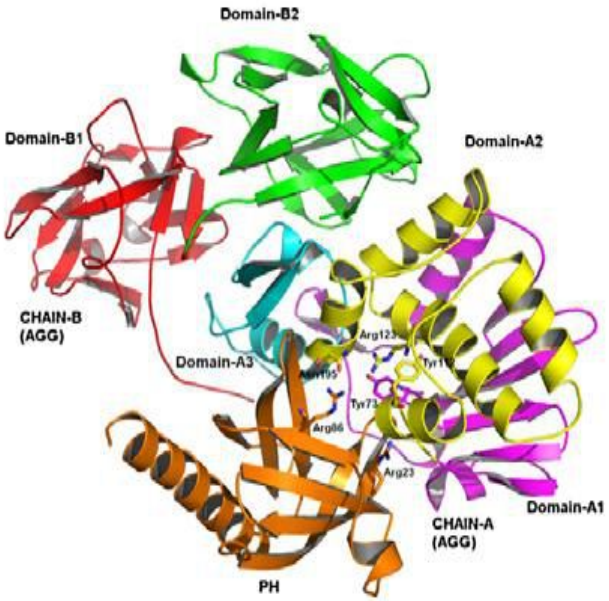
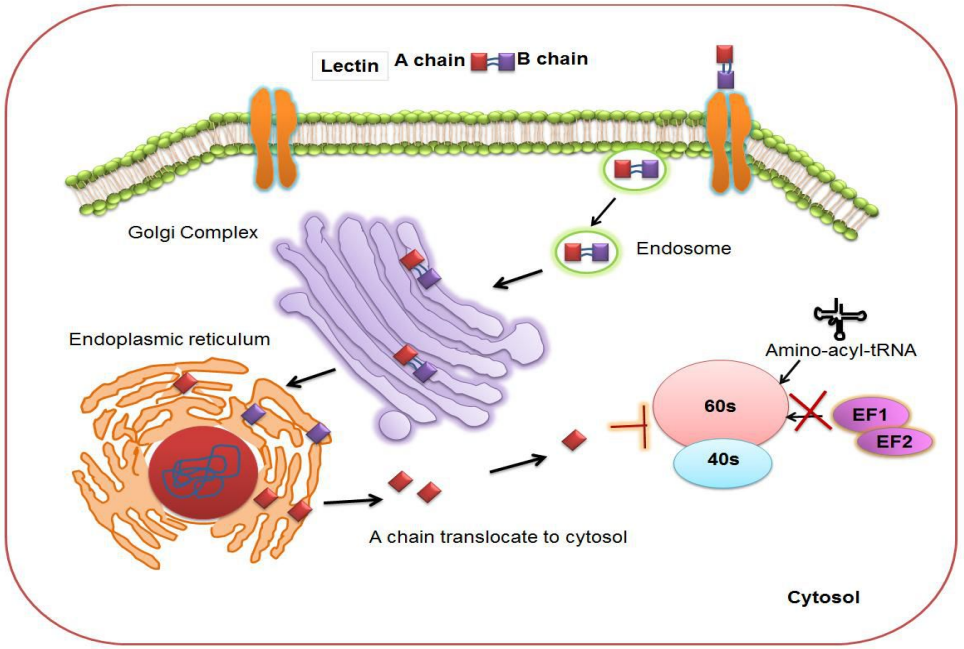
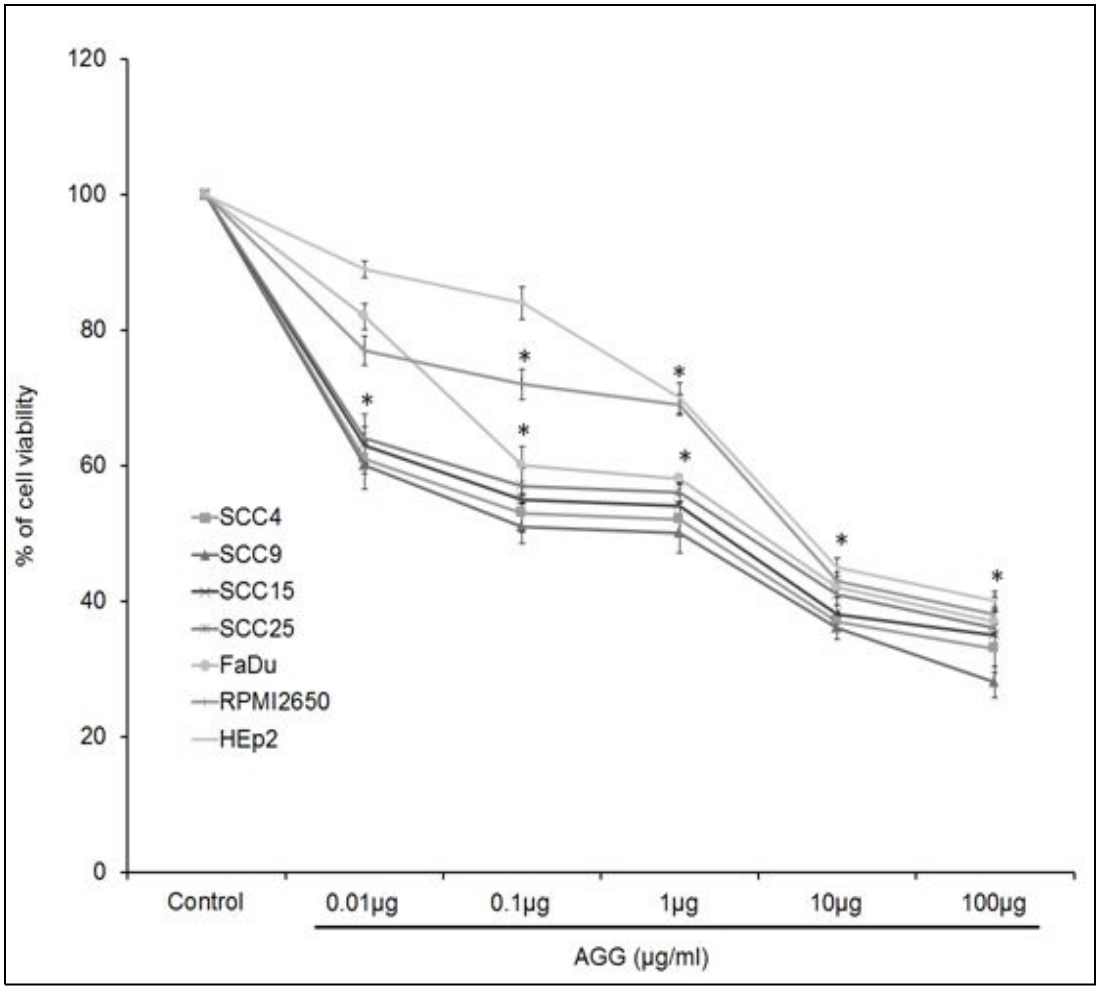
Abrus agglutinin (AGG)

- A lectin isolated from plant *Abrus precatorius* (Crab's eye)
- Two A chain (32 kDa) with N-glycosidase activity
- Two B chain (35 kDa) with carbohydrate binding activity
- RIP-2 class
- Carbohydrate specificity towards [gal(β1-3)GalNAc]
- Toxicity of the protein is (LD_{50} = 5 mg / kg body weight)





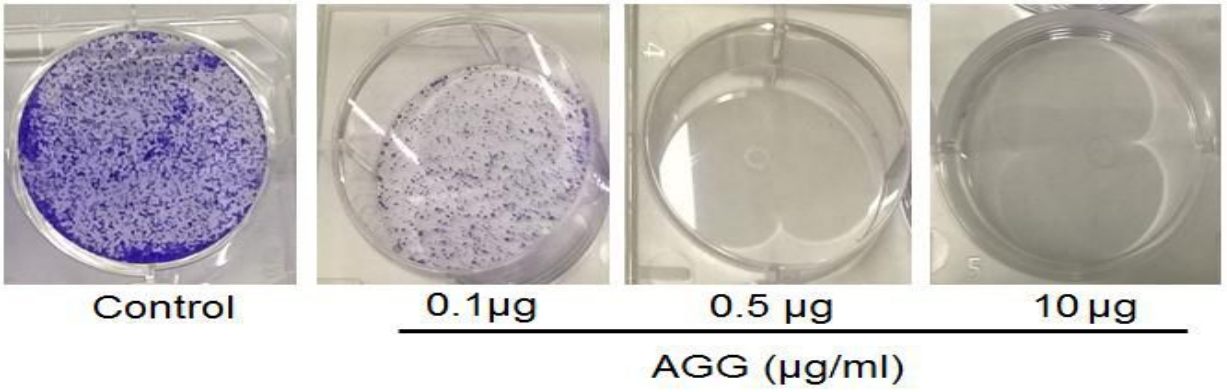
Effect of AGG on growth inhibition in Oral squamous cell carcinoma

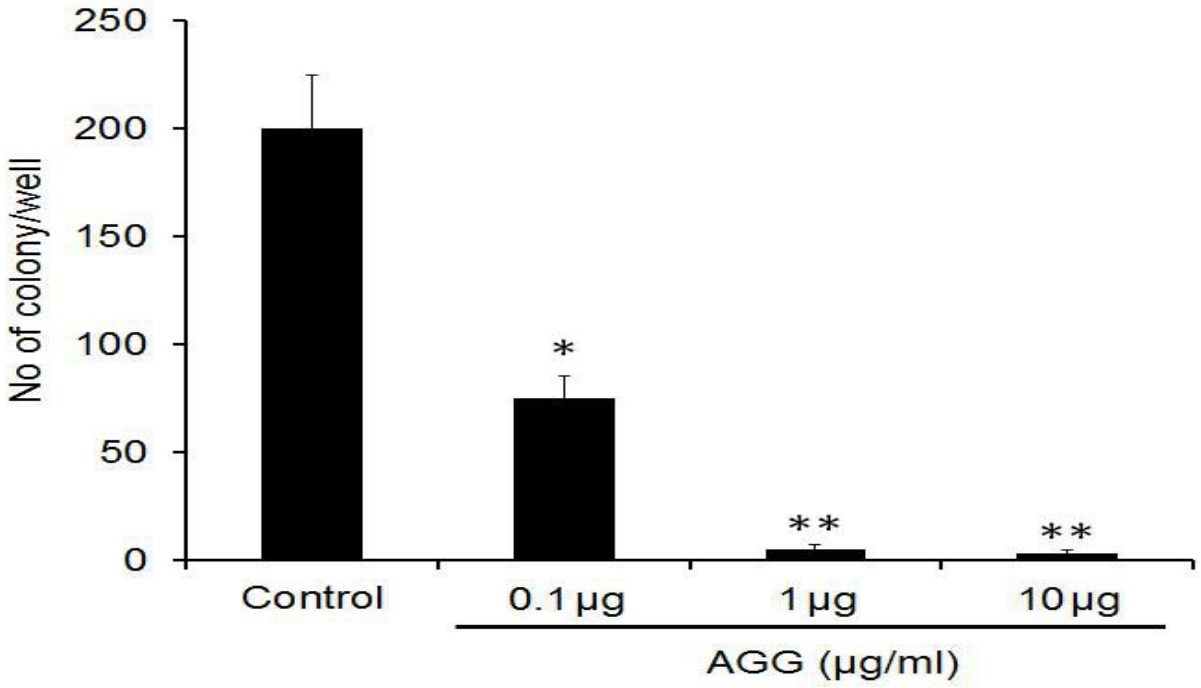


Cell lines	IC_{50} (µg/ml)
FaDu	3.2 ±0.6
HEp2	8.9±0.6
SCC4	2.1 ±1.4
SCC9	1 ±0.2
SCC15	3.2 ±0.8
SCC25	4.4 ±0.4
RPMI2650	7.8 ±0.8

INFERENCE: AGG were treated on different oral cancer cell lines for 72 h and the data showed that viability of cells declined in a dose-dependent manner. The concentrations required for inhibiting cell growth by 50% (IC_{50}) for FaDu, HEp2, SCC4, SCC9, SCC15, SCC25, and RPMI 2650 were 3.2 ± 0.6, 8.9 ± 0.6, 2.1 ± 1.4, 1.0 ± 0.2, 3.2 ± 0.8, 4.4 ± 0.4, 7.8 ± 0.8 µg/ml, respectively (Table).

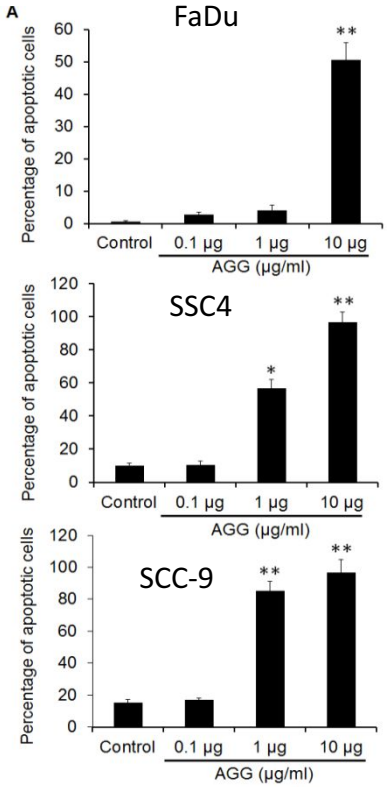
AGG inhibits colony forming potential in FaDu cells

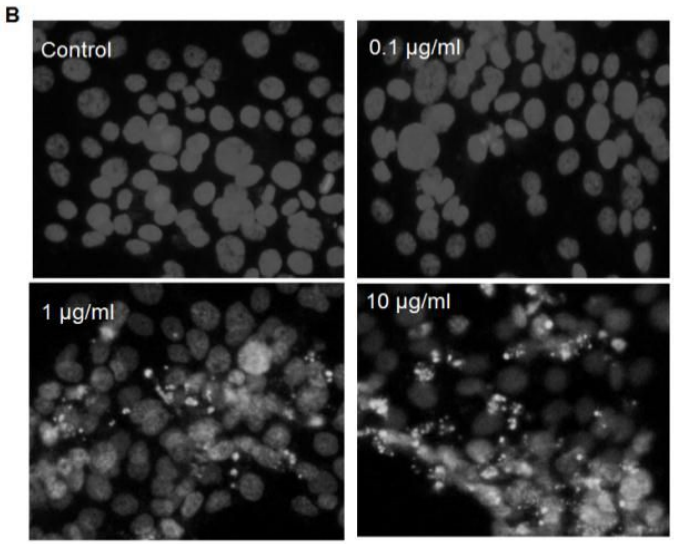


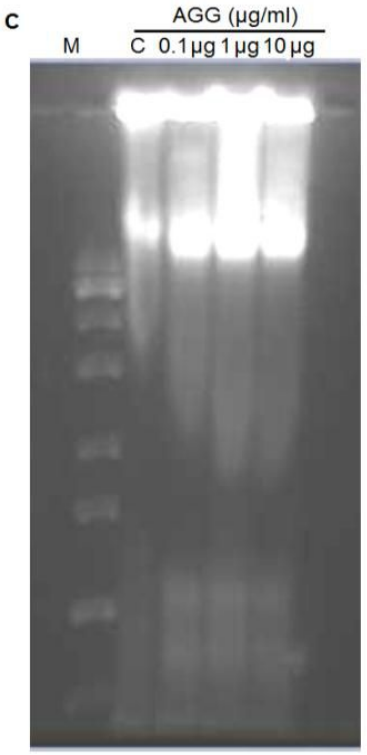


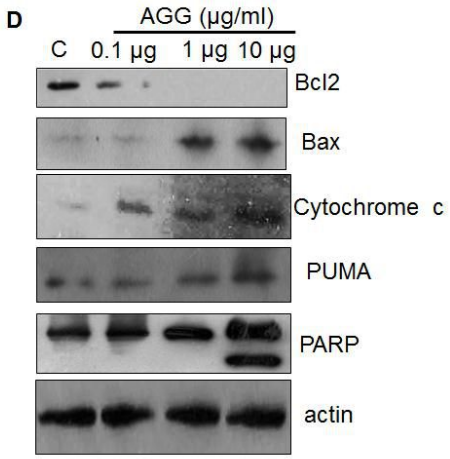
INFERENCE: AGG exhibited its long term effect by inhibiting colony forming potential in FaDu, a human pharyngeal cancer cells in a dose-dependent manner

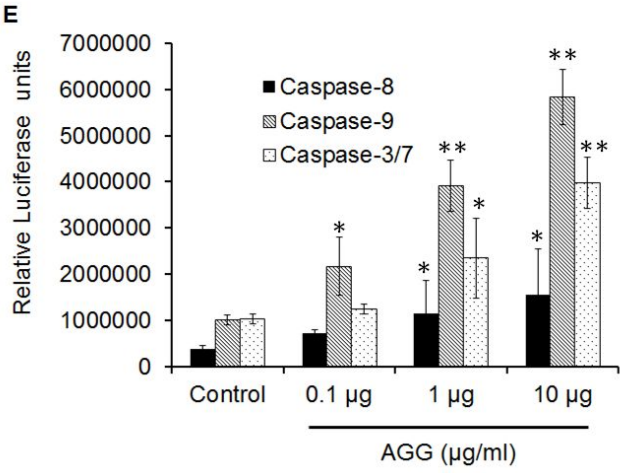
AGG induces apoptosis in oral squamous cell carcinoma



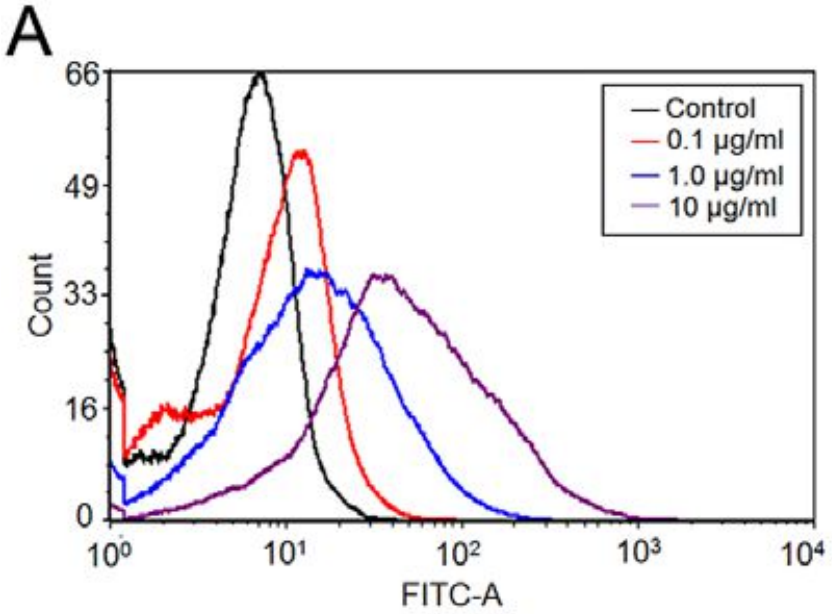


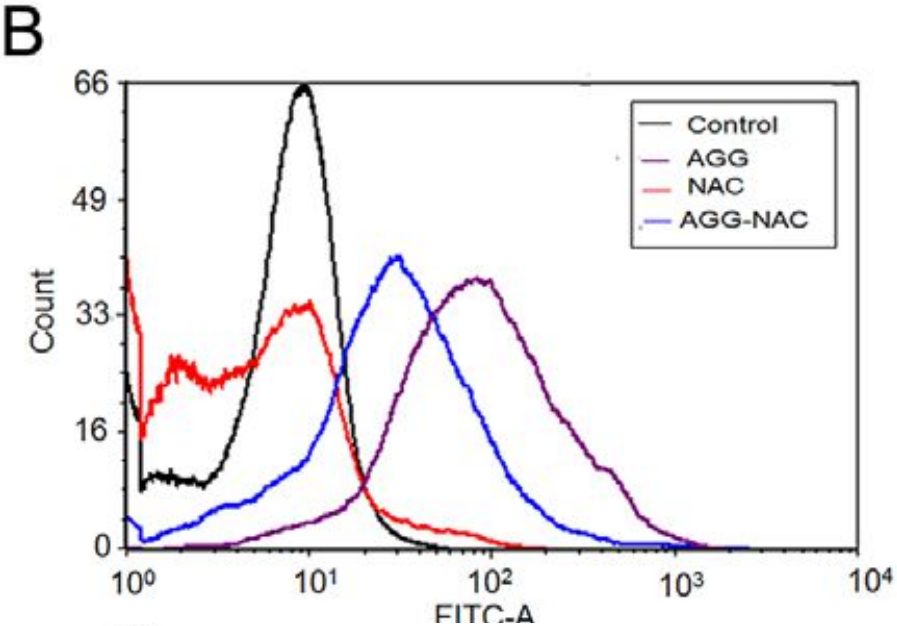


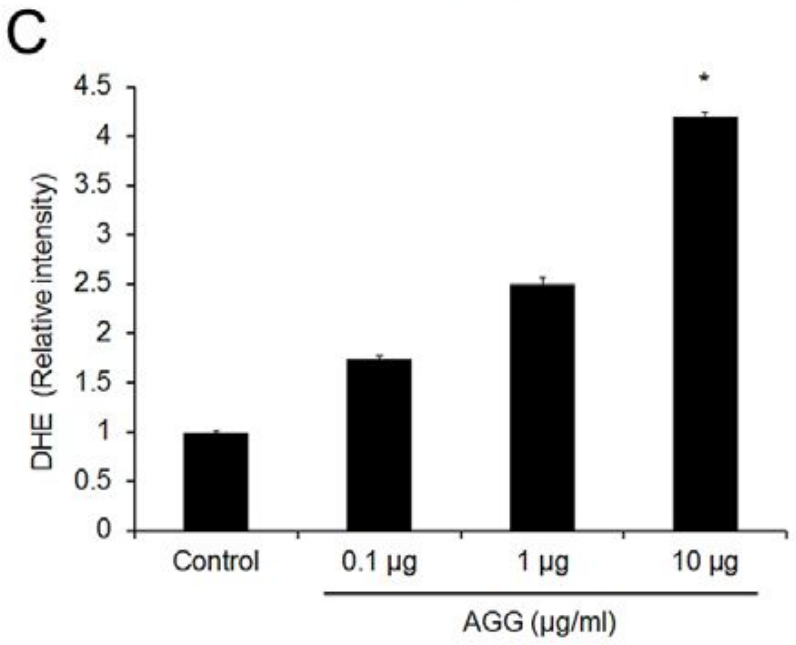


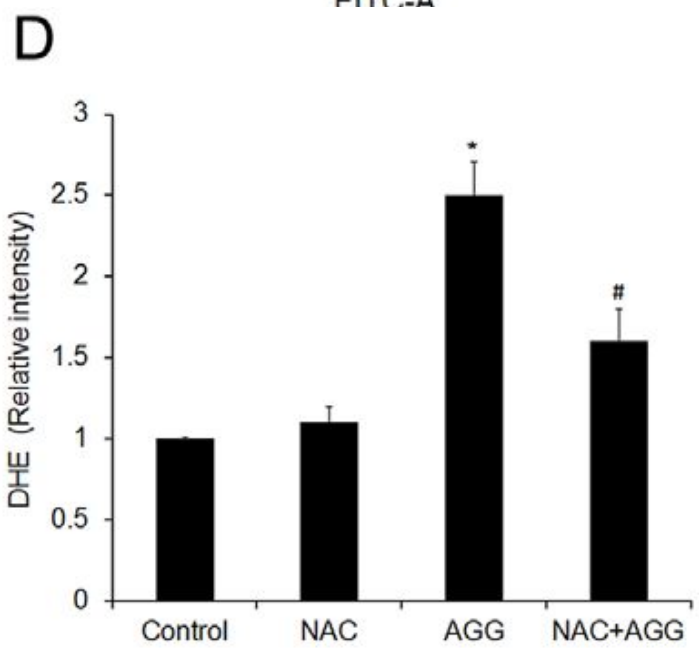


Analysis of ROS and superoxide level in exposure to AGG

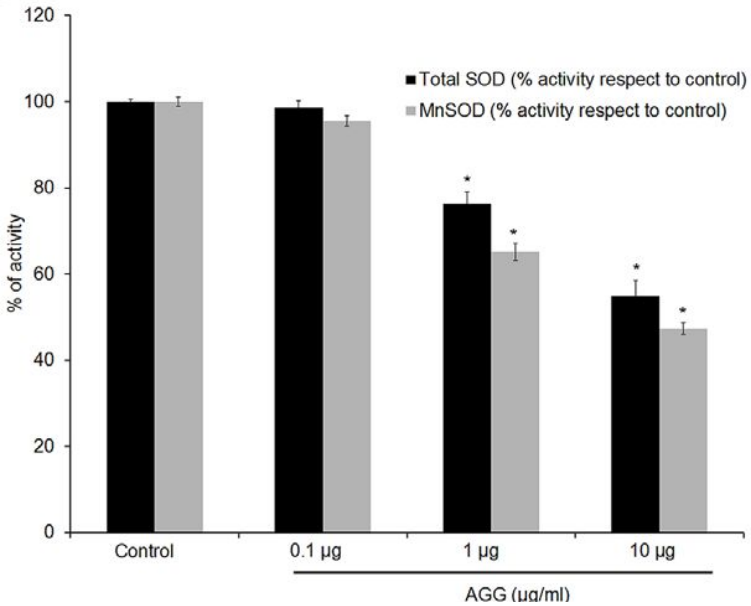


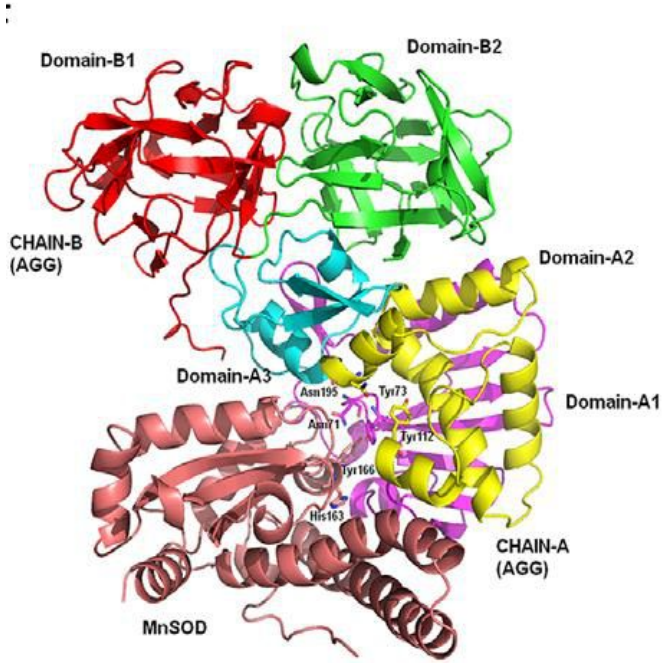


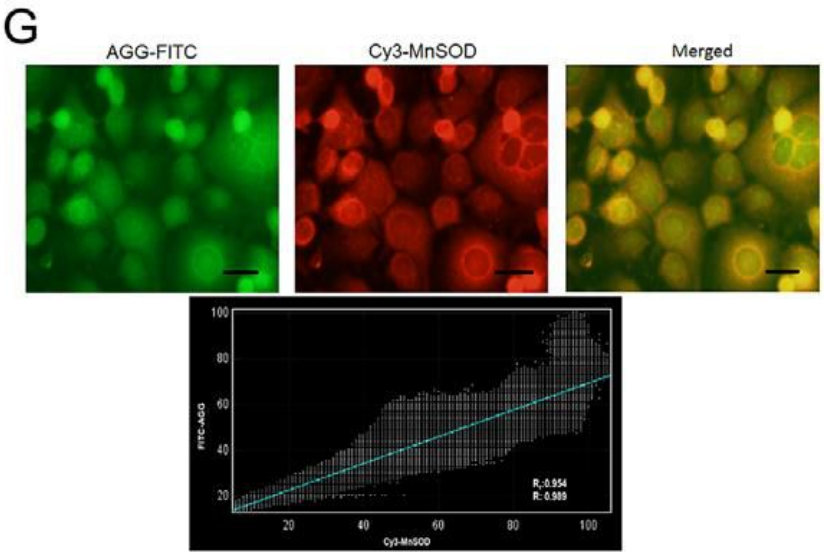




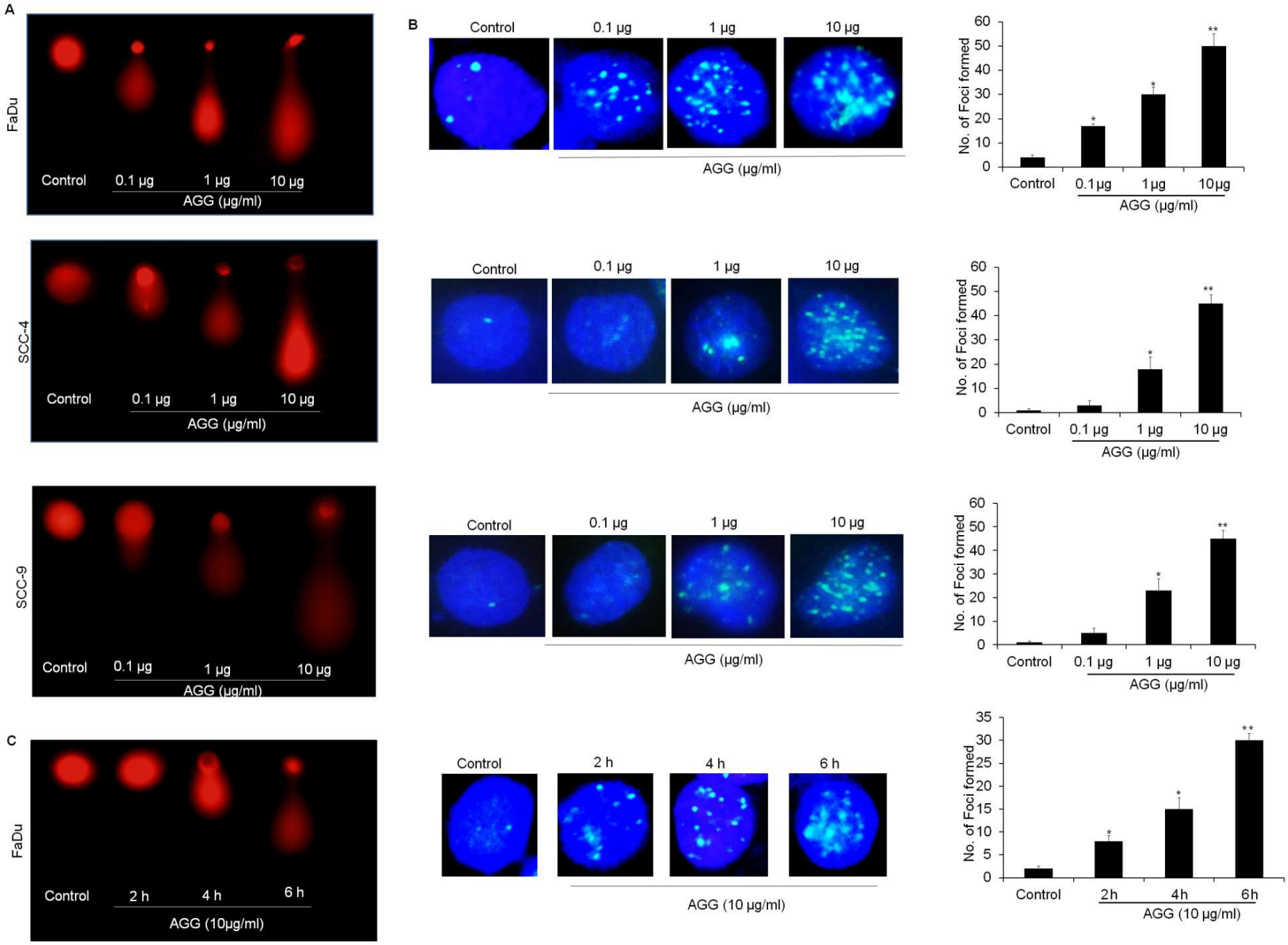
AGG inhibits superoxide dismutase activity



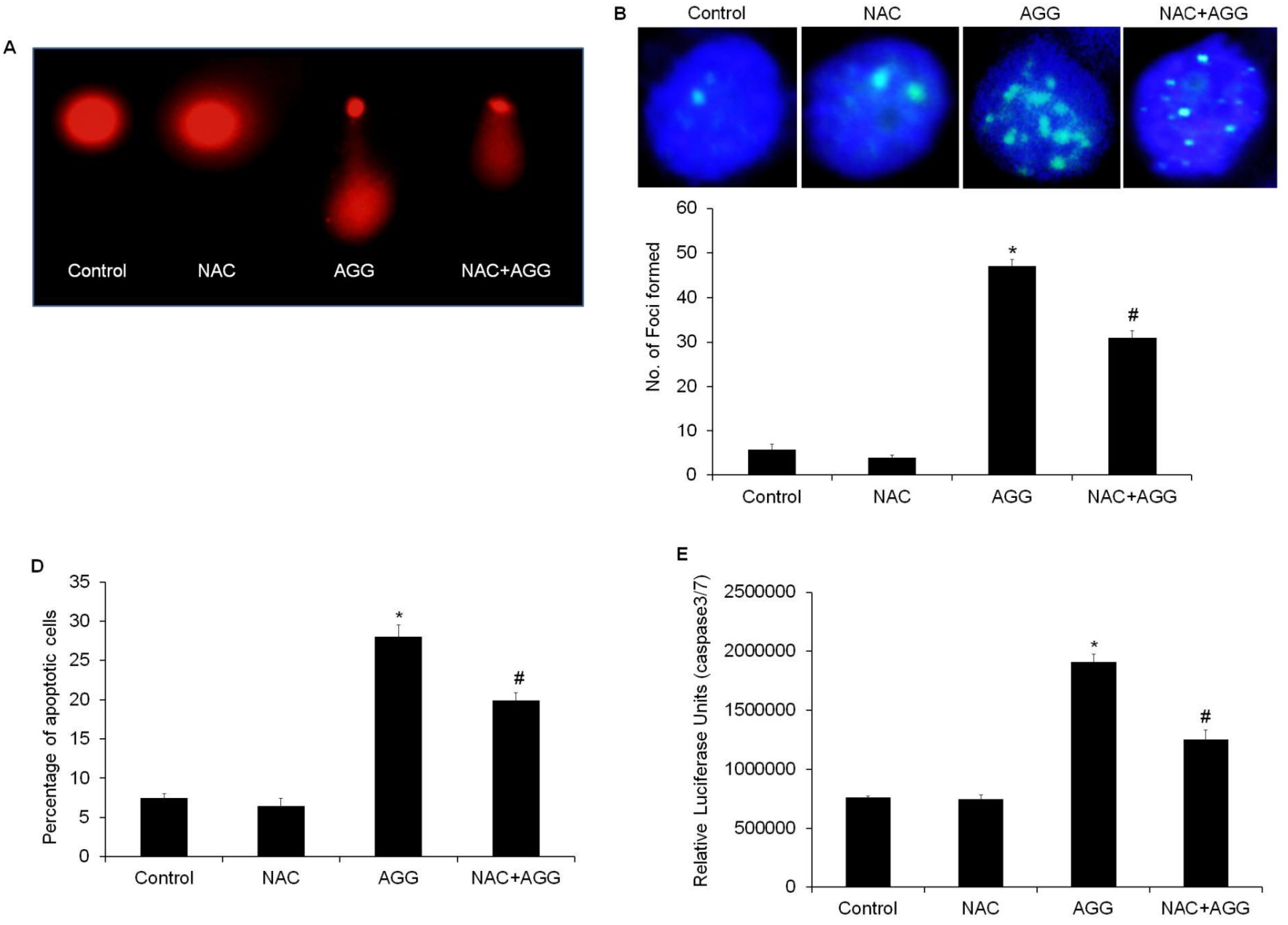




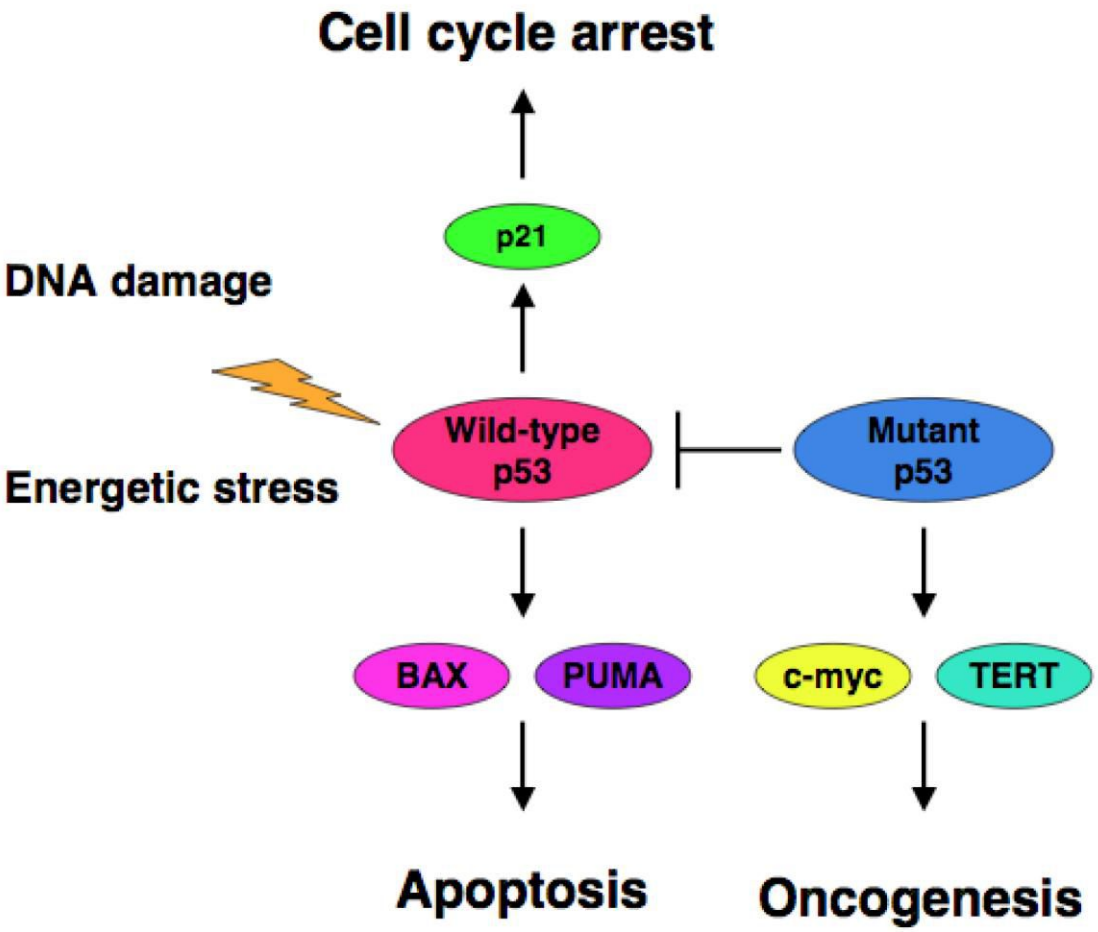
AGG facilitates DNA damage in oral cancer cells

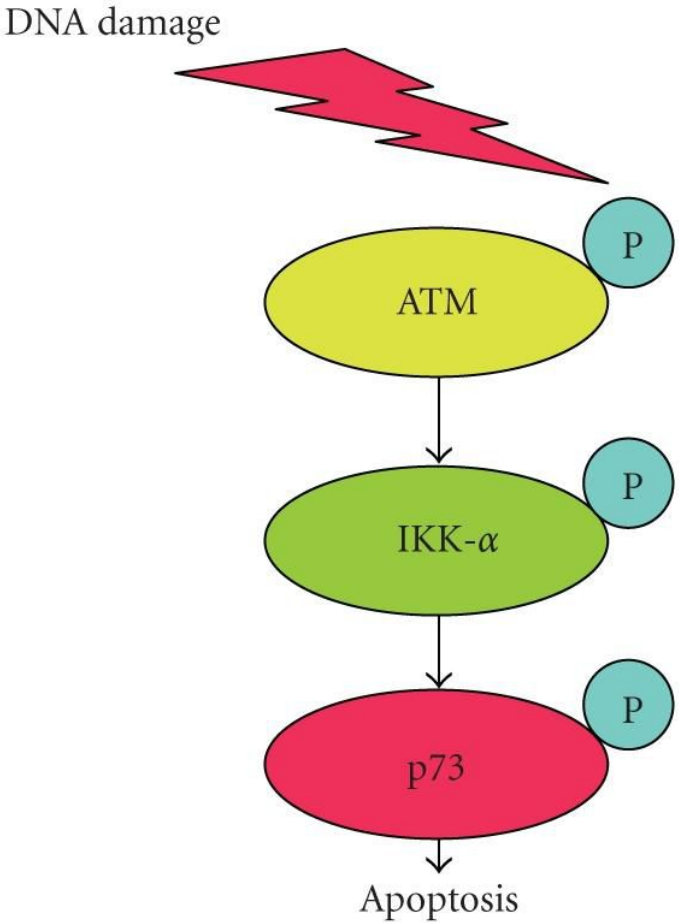


AGG-induced DNA damage regulates through ROS generation

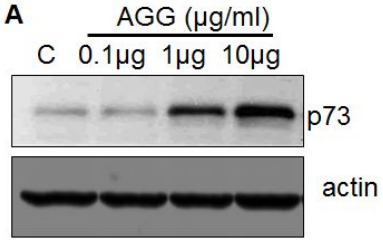


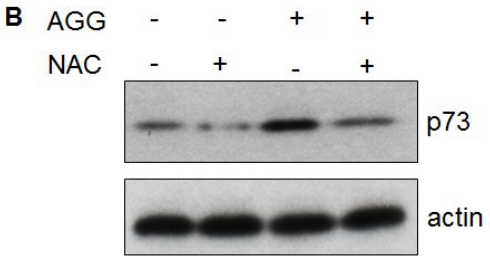
p73 activation in p53 dysfunctional cancer cells

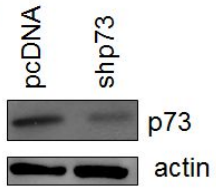


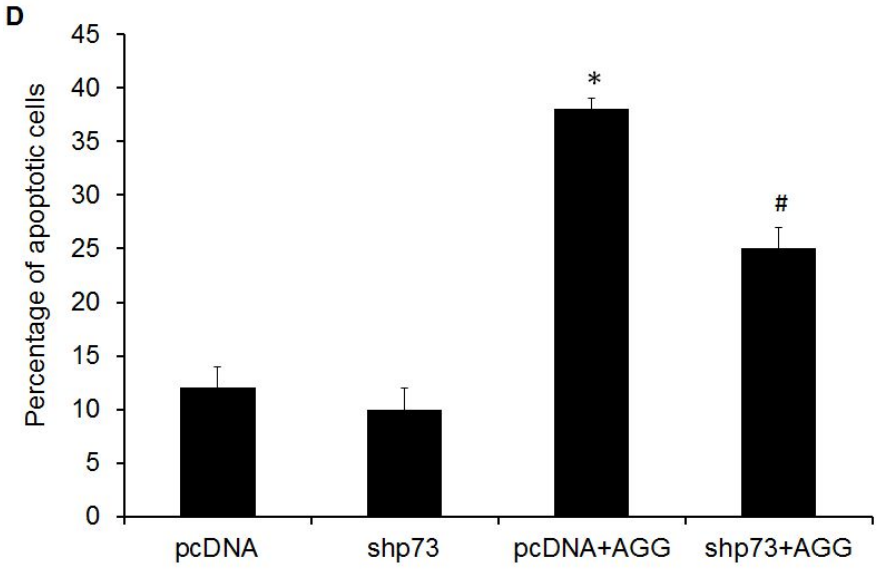


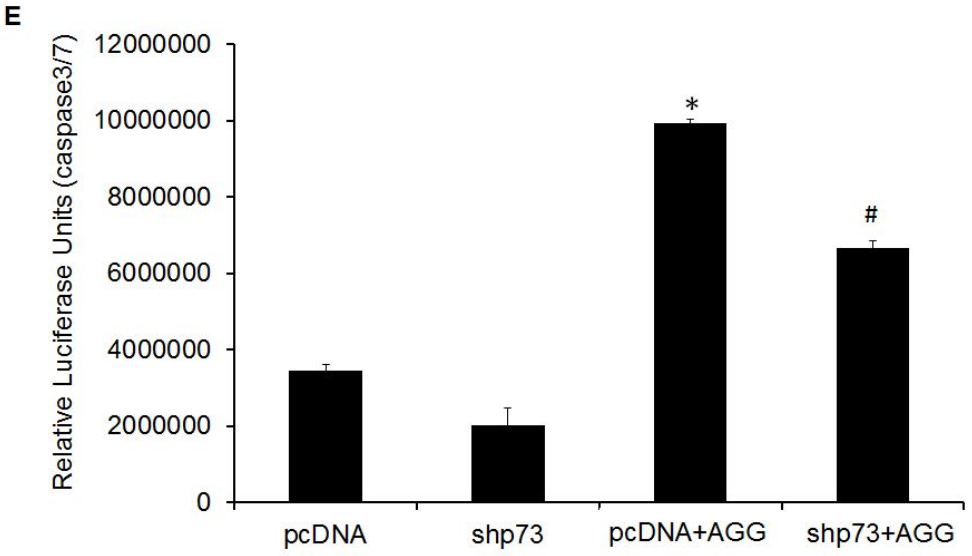
AGG induces p73-mediated apoptosis



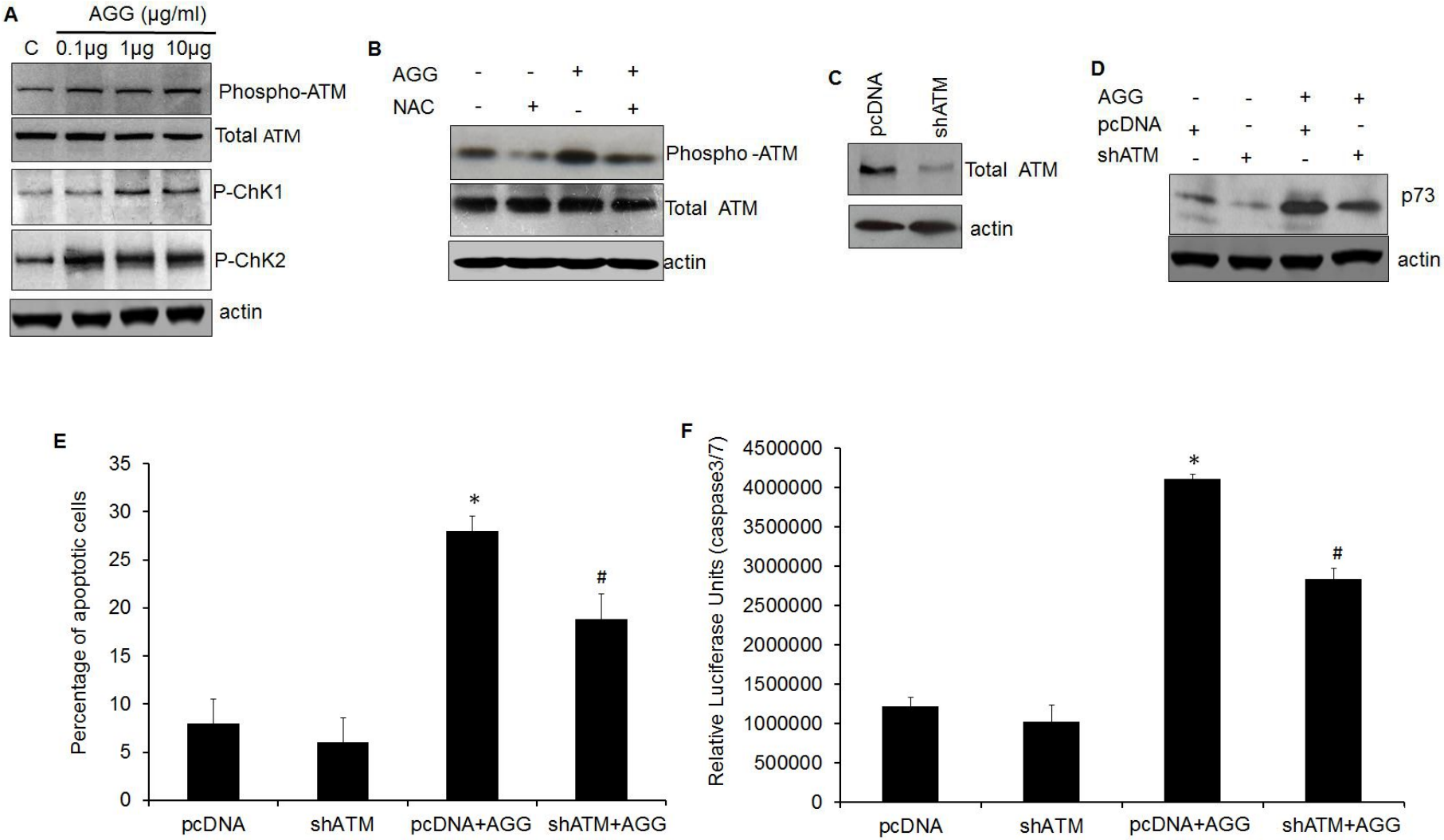




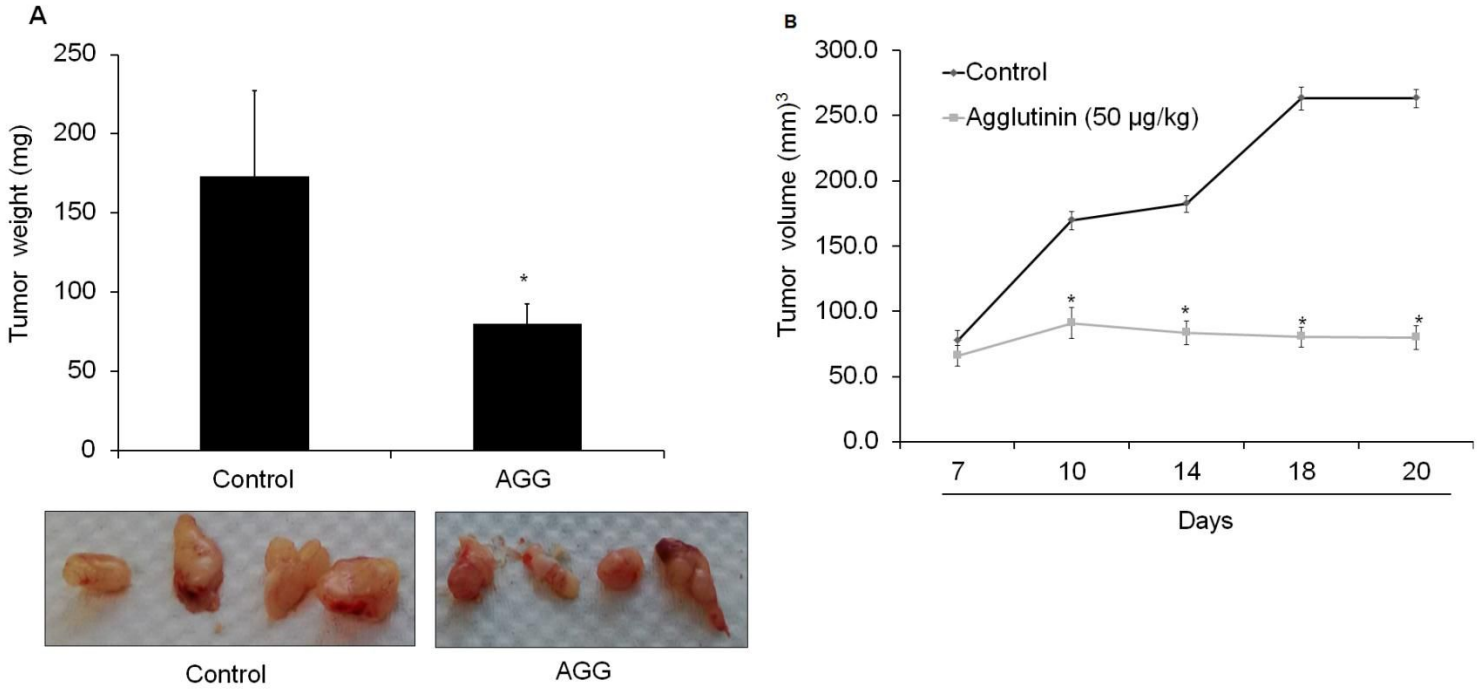


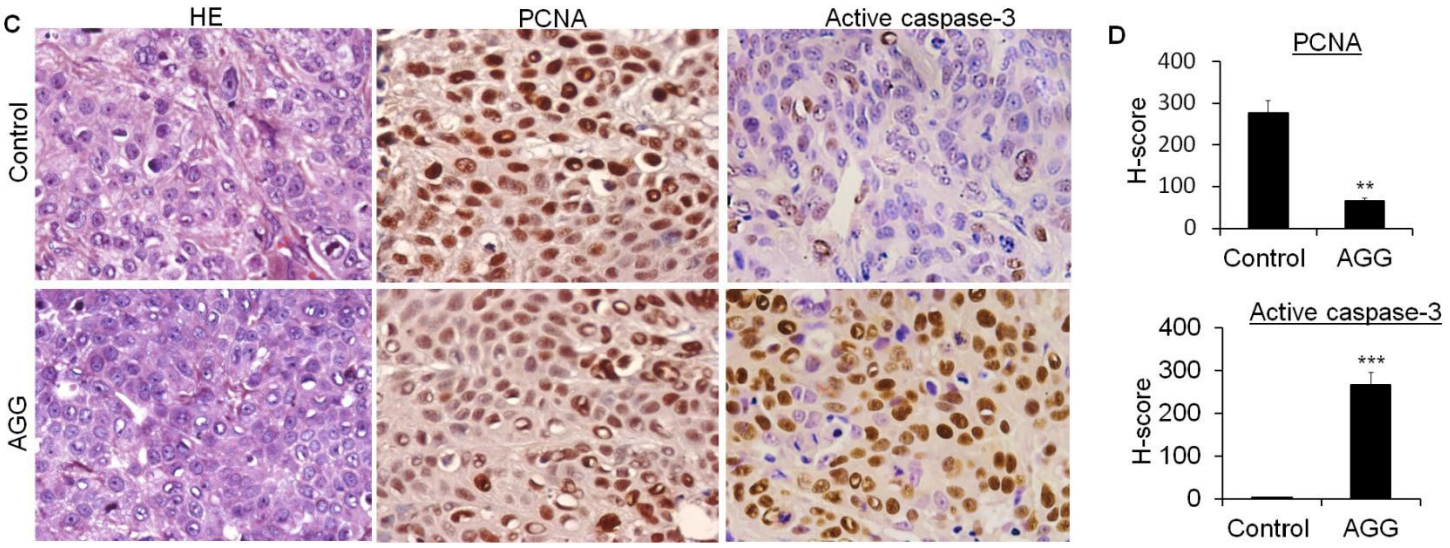


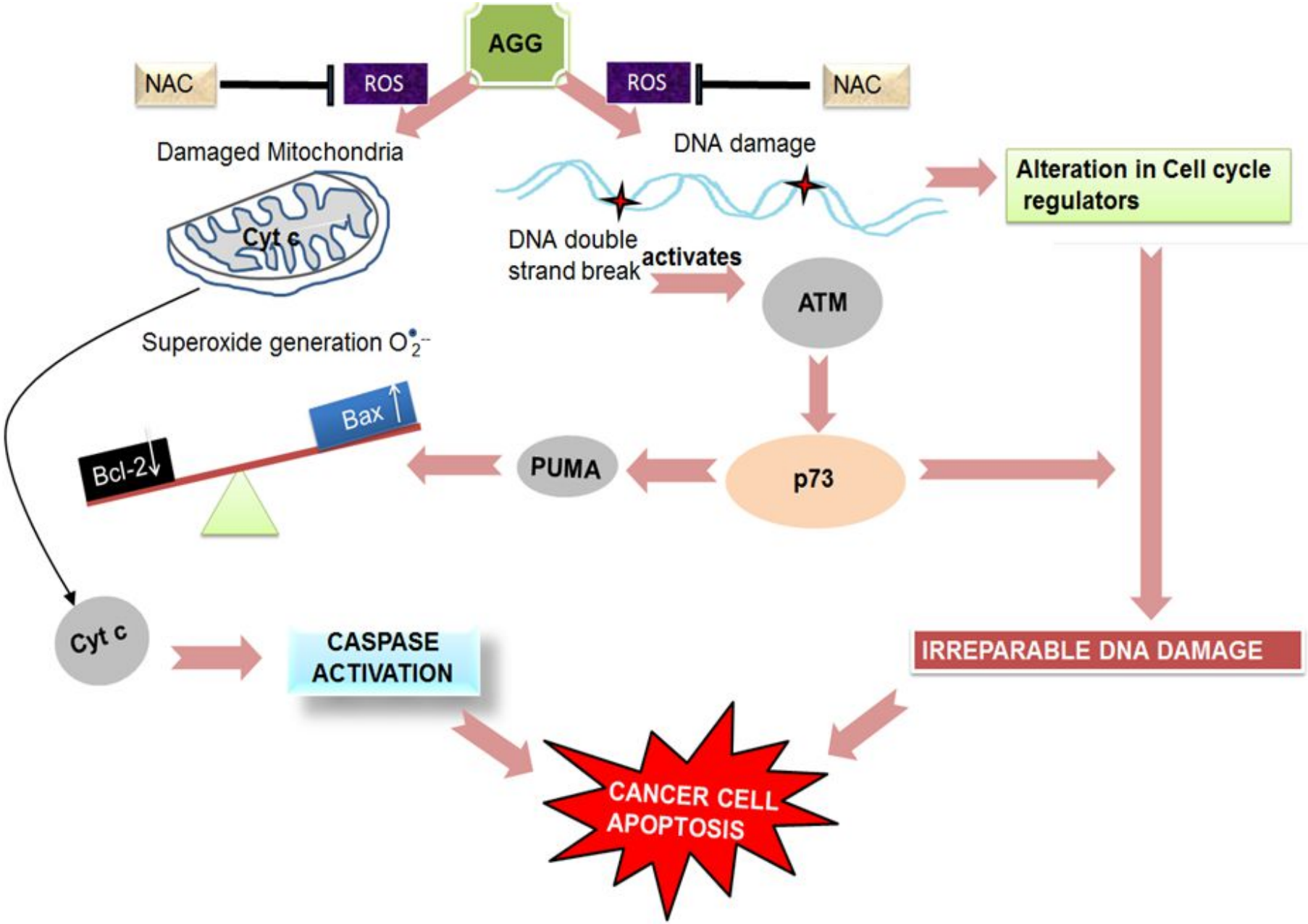
AGG-activated ATM prompts p73 dependent apoptosis in FaDu cells



AGG supresses the growth of OSCC tumor *in vivo*

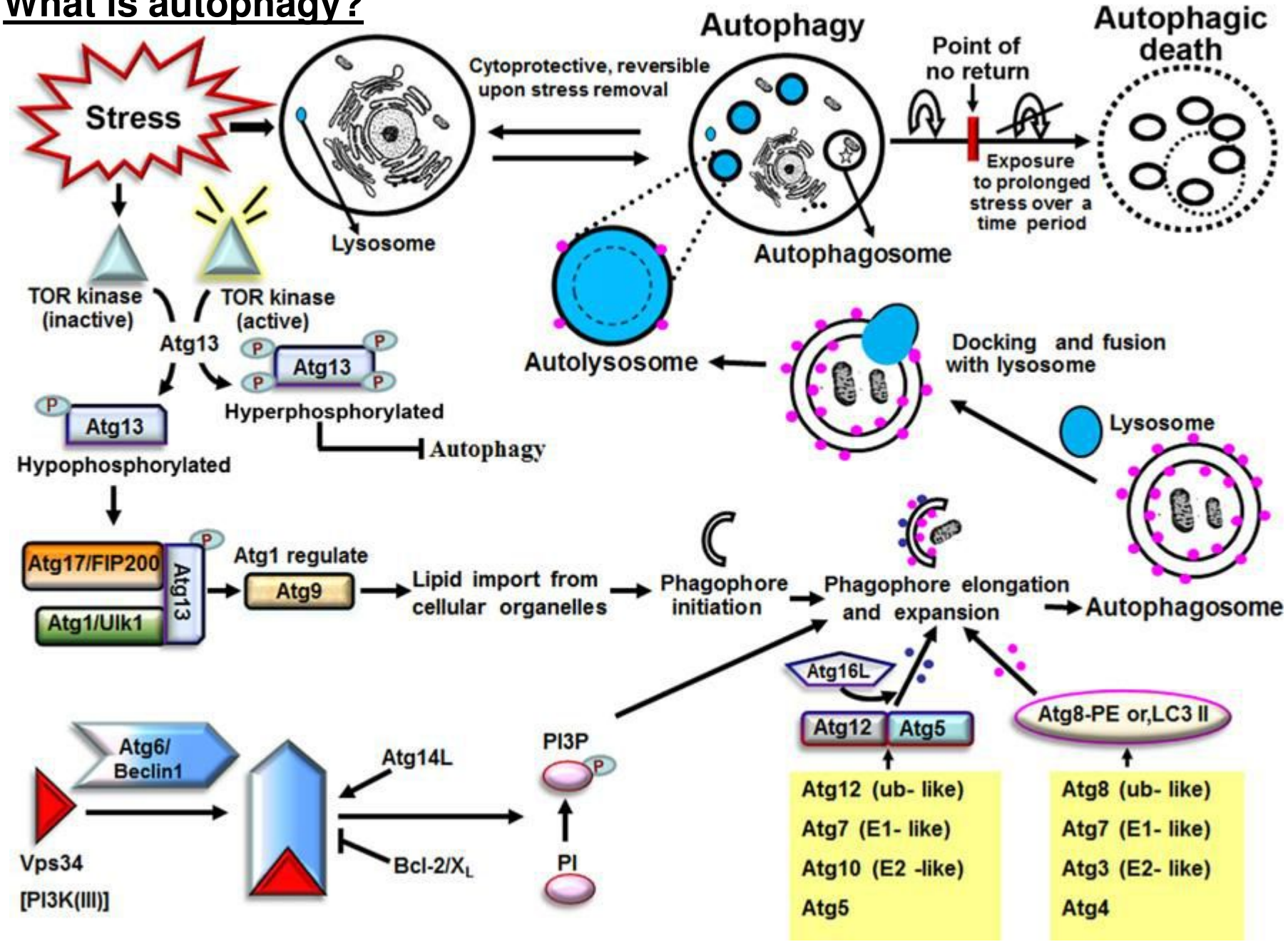






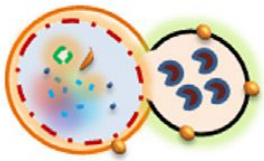
Sinha et al Mol Carcinog. 2017 ,56(11):2400-2413

What is autophagy?



Bhutia et al Adv Cancer Res. 2013;118:61-95

Pro- and anti-tumor roles of autophagy in different stages of cancer



AUTOPHAGY

PROTUMOR

? Cell survival during treatment

↑ Apoptosis resistance

↓ Anoikis

↑ Cell survival during dormancy

↑ Nutrient fuelling by stromal cells

↑ Cell survival during treatment

ANTITUMOR

↑ ATGs-Tumor Suppressor

↑ Autophagy

↓ Oxidative stress

↓ DNA damage and genomic instability

↓ Damaged mitochondria and protein aggregates

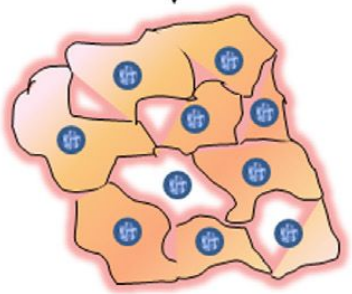
↑ Senescence

↓ Necrosis and inflammation

↑ Autophagic cell death during treatment

TRANSFORMED CELL

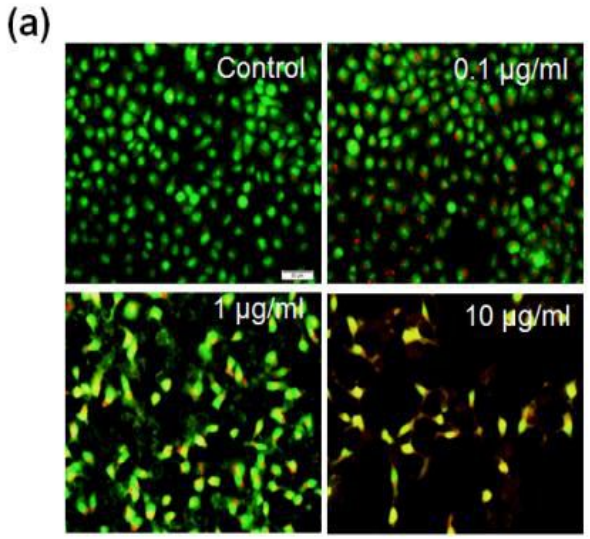
MALIGNANT CELL

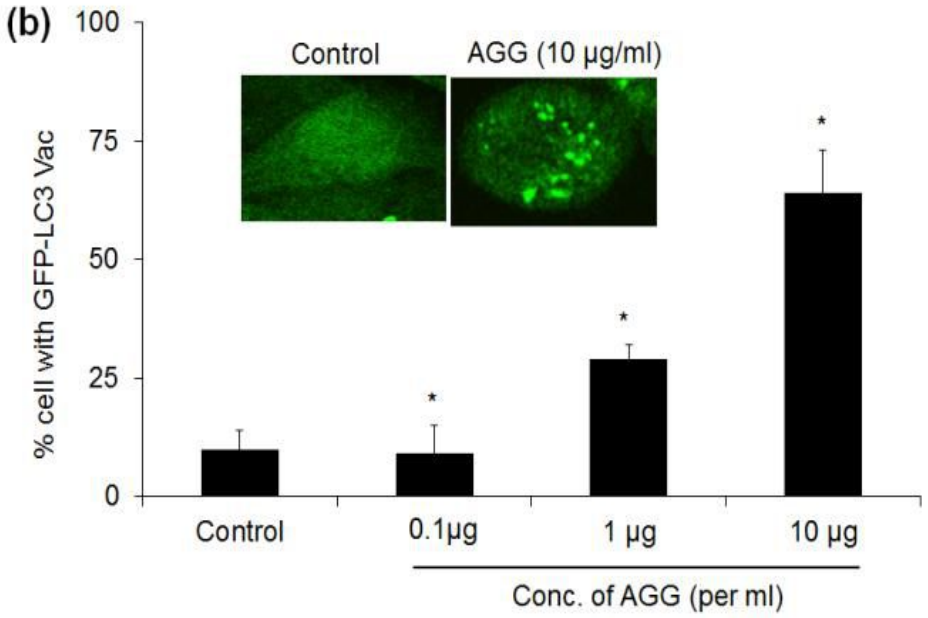


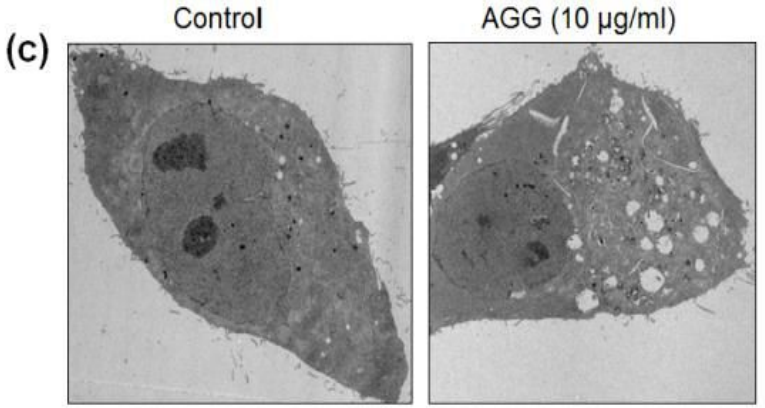
CANCER

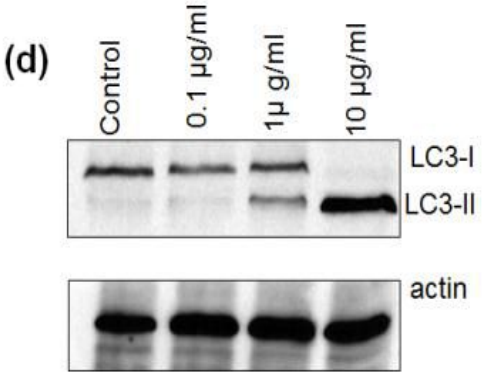
Panda *et al.*, Seminars in Cell & Developmental Biology (2015)

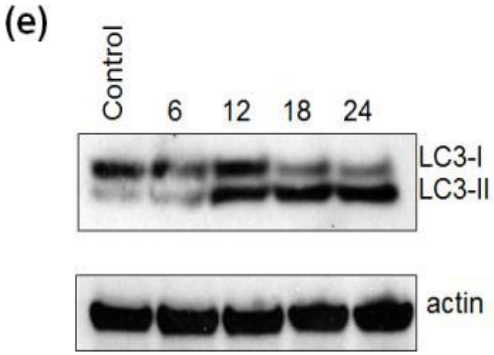
AGG induces autophagic cell death in cervical cell carcinoma



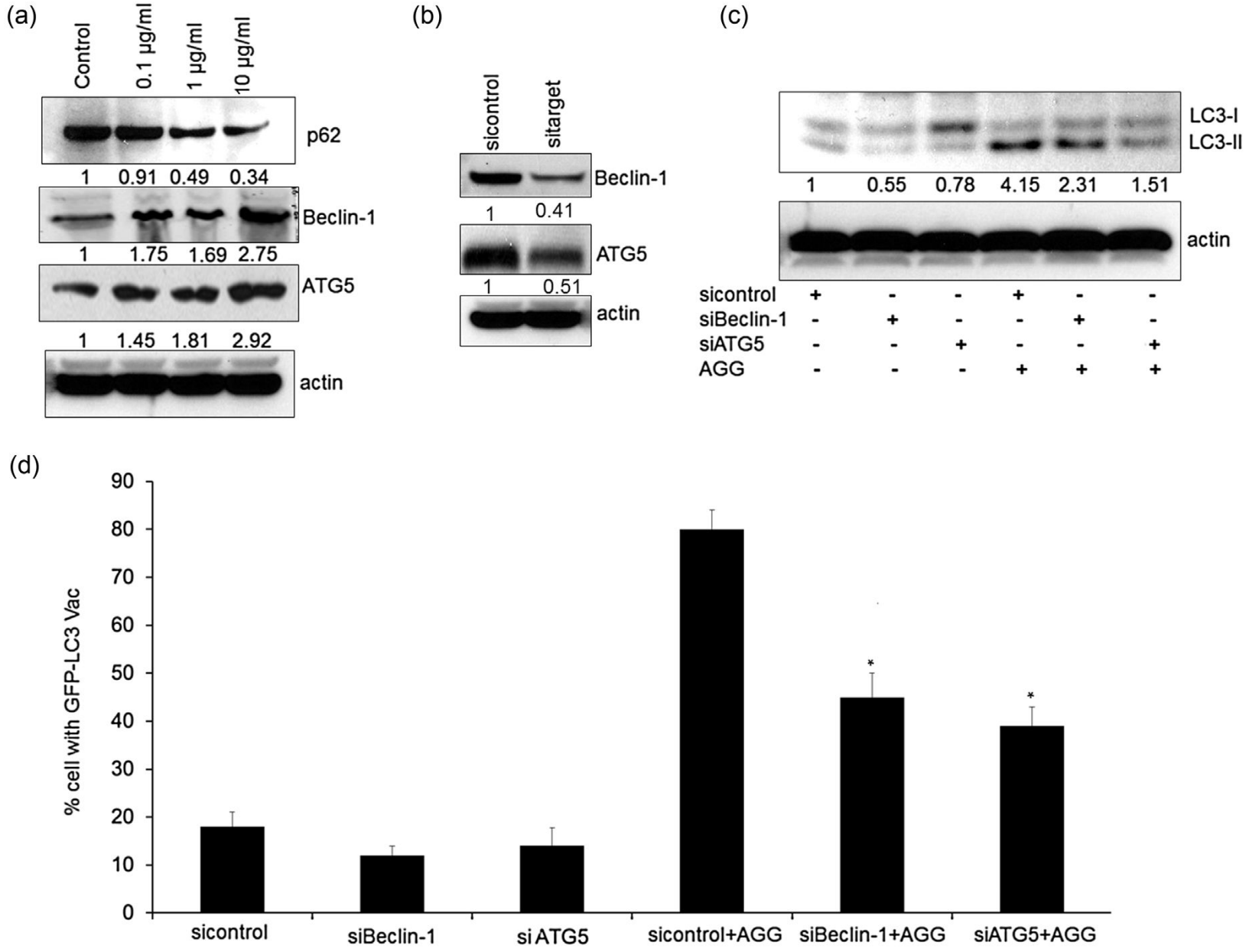




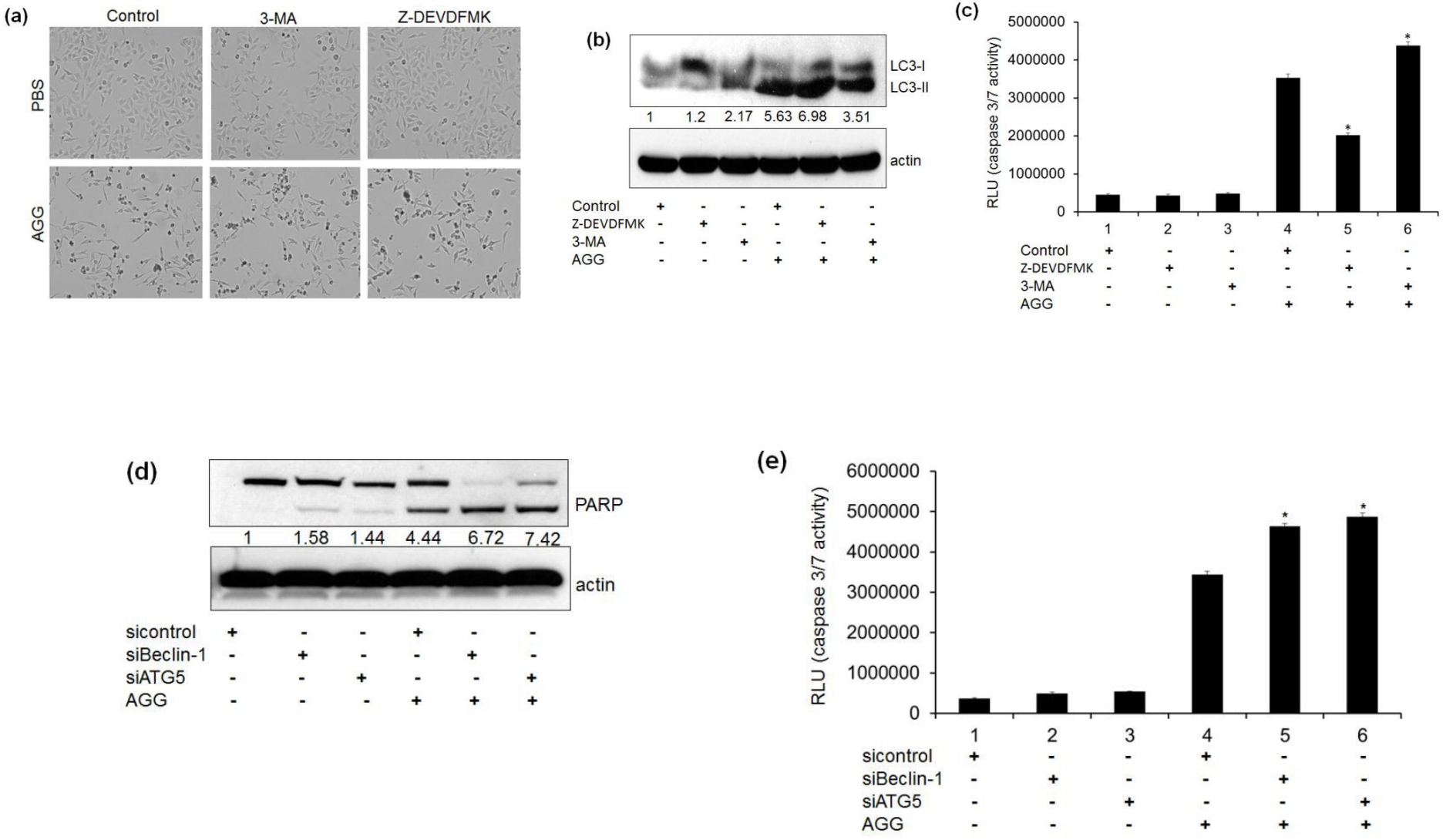




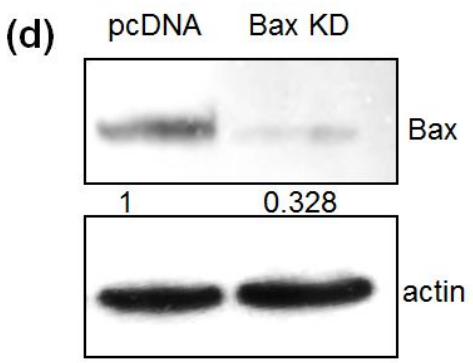
AGG-induced autophagic cell death is mediated through the canonical pathway

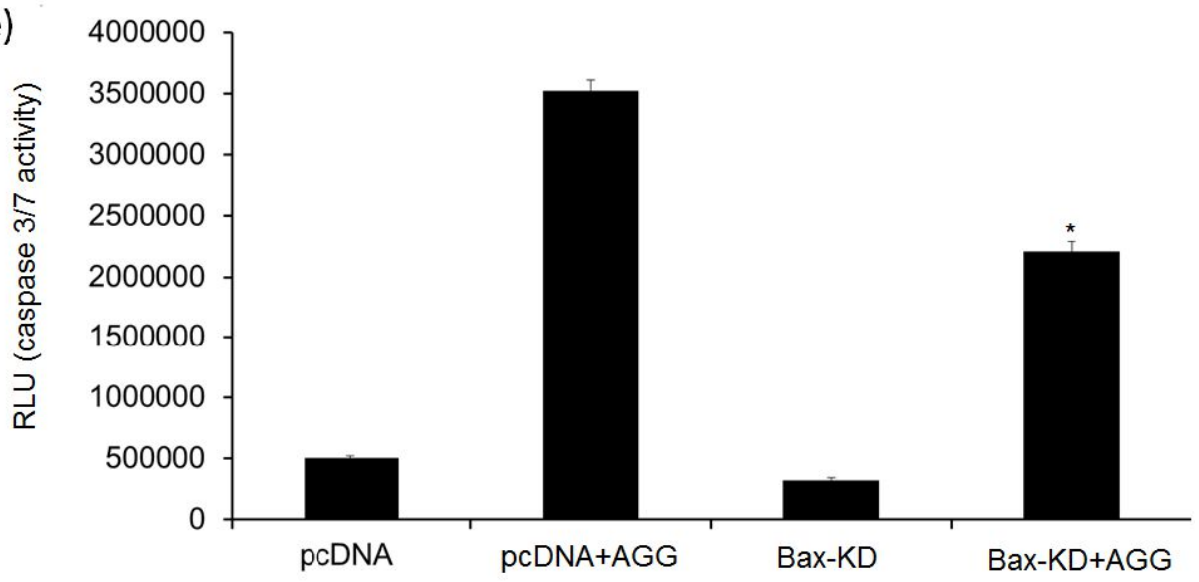


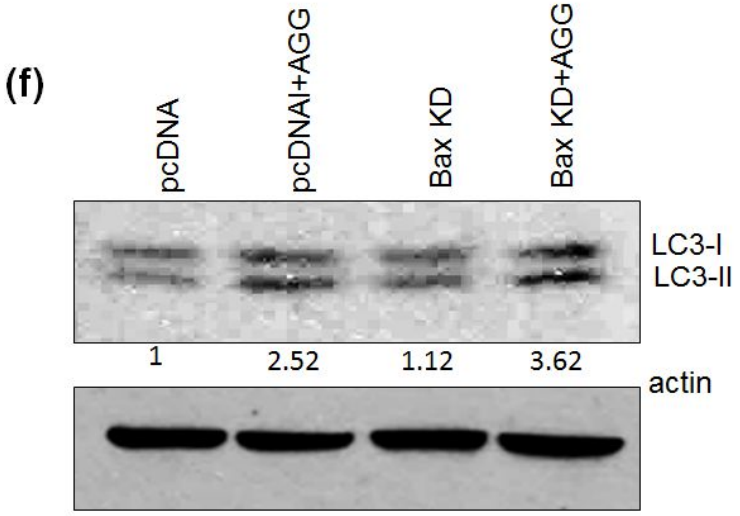
Crosstalk Between AGG-Induced Apoptosis and Autophagy-Dependent Cell Death

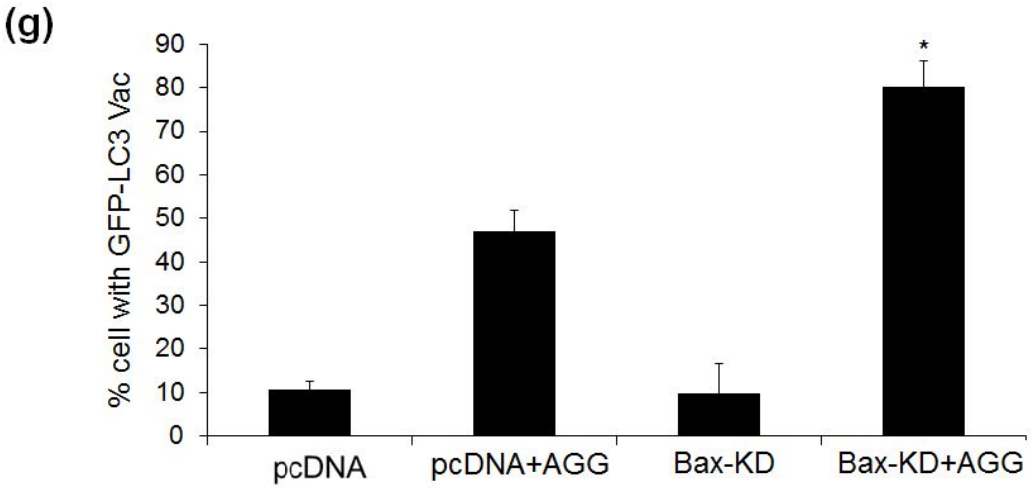


AGG-Induced Autophagic Cell Death in Apoptosis Deficient Cervical Cancer Cells

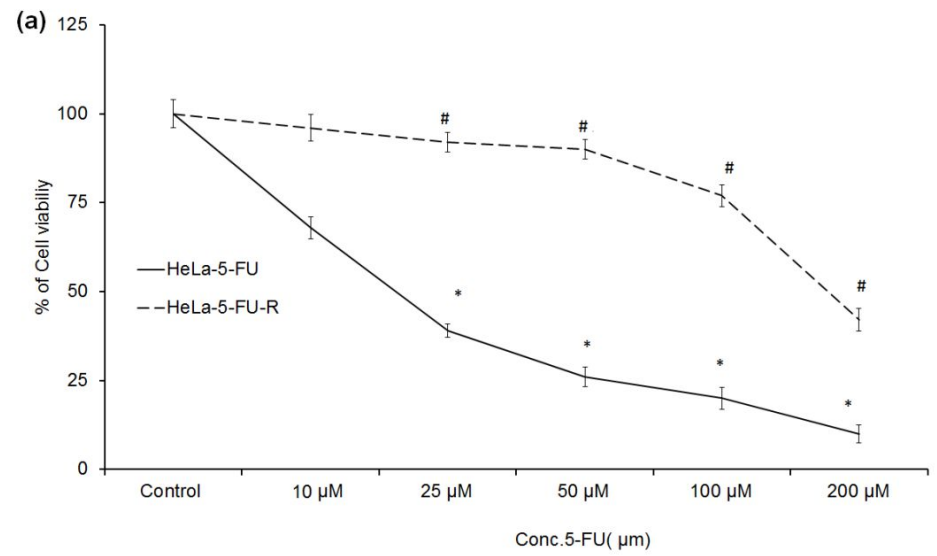


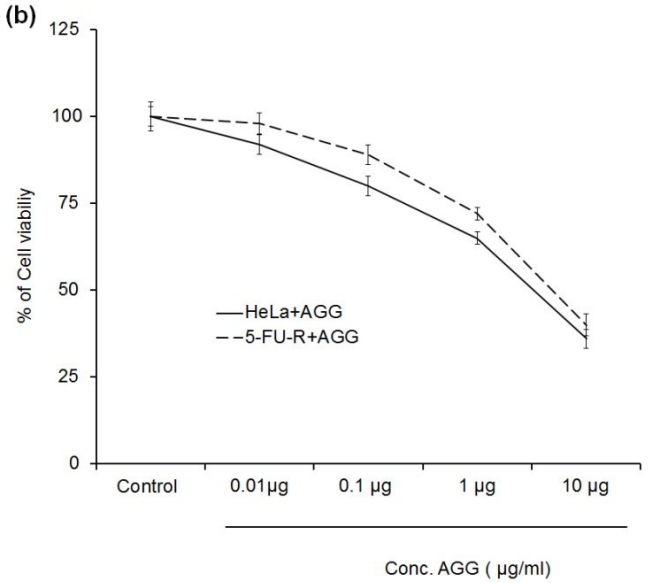


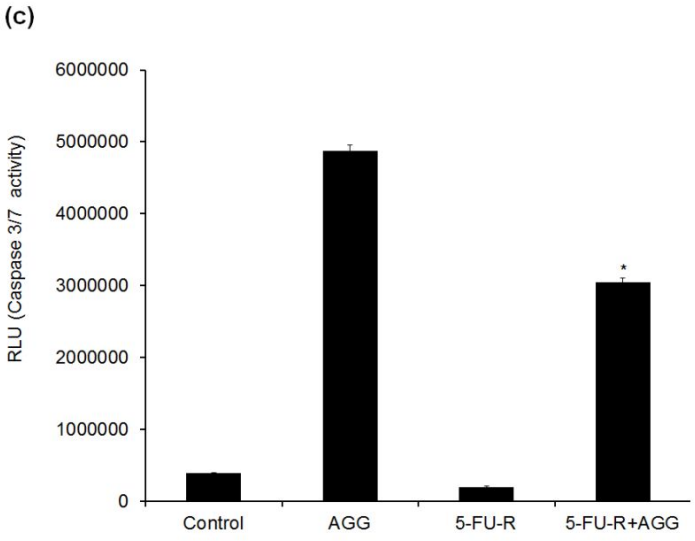


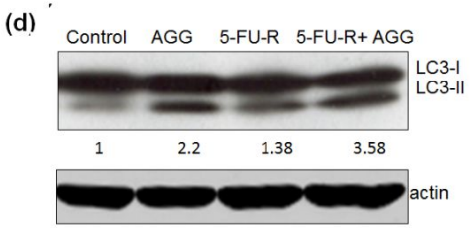


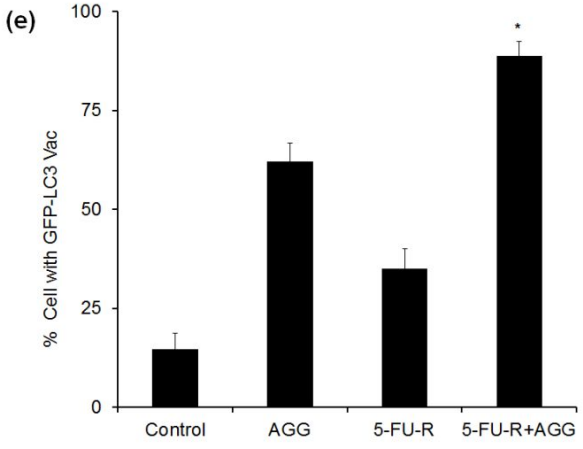
AGG-Induced Autophagic Cell Death in resistant Cervical Cancer Cells



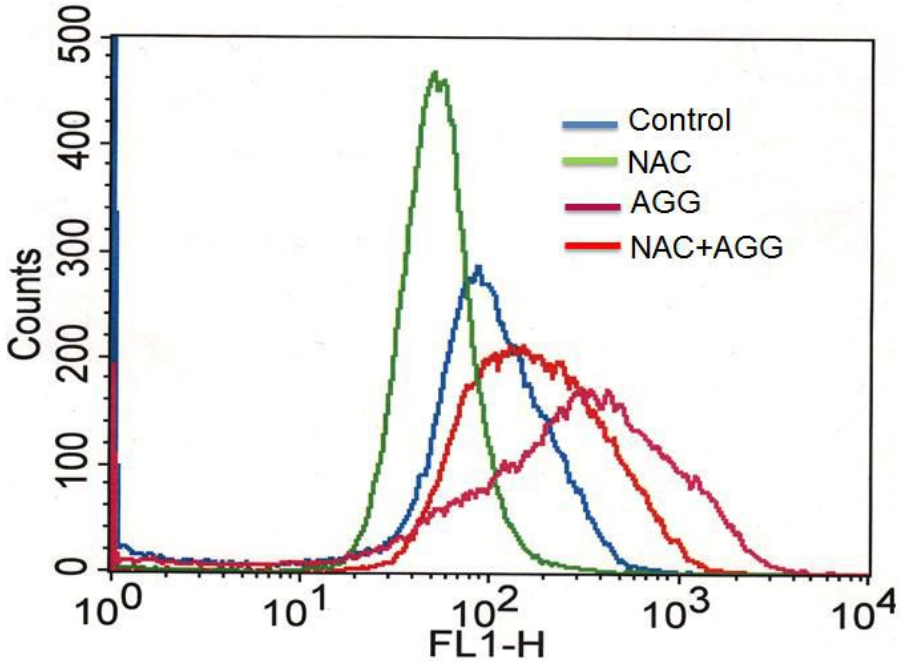


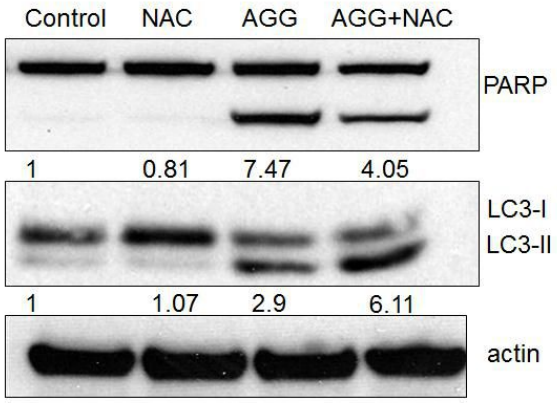


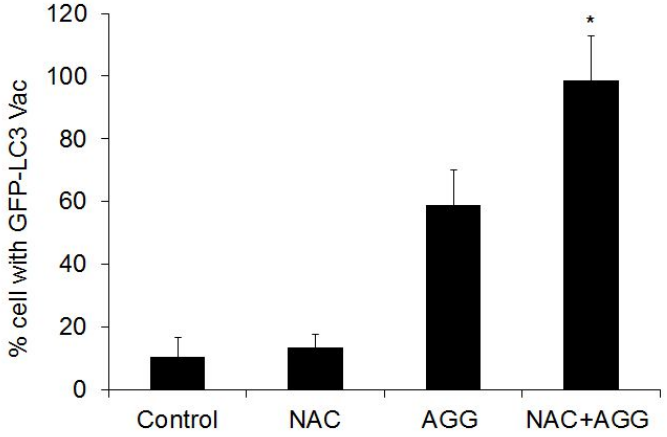




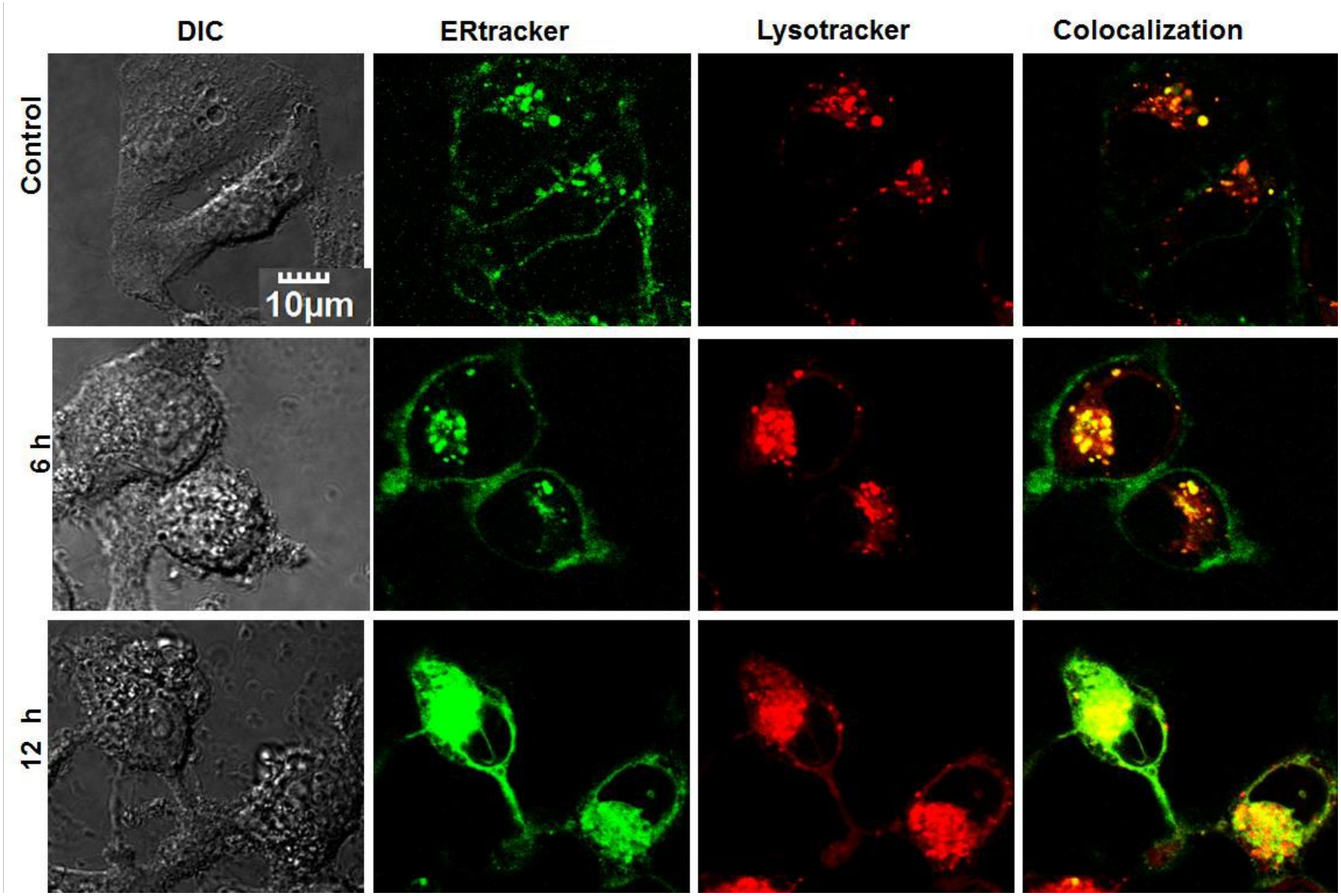
The role of reactive oxygen species in AGG-induced autophagic death



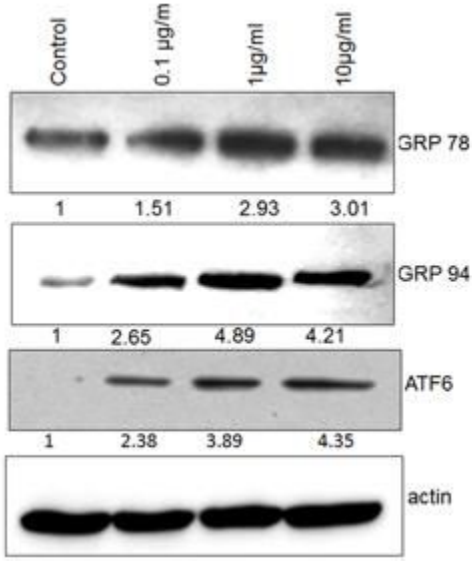


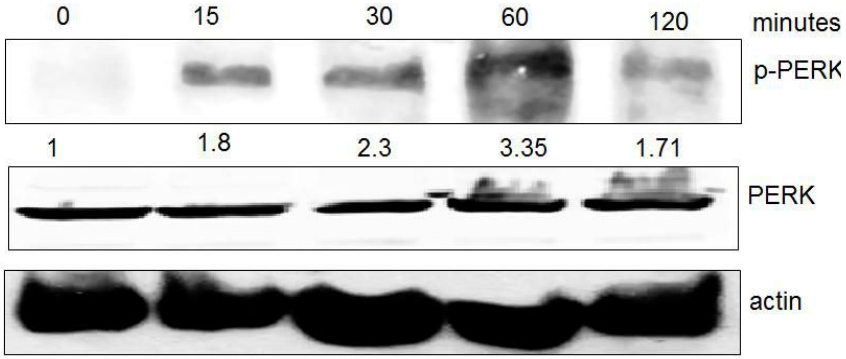


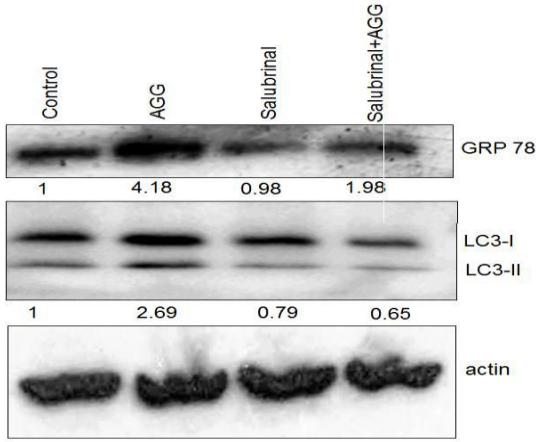
Colocalization between lysosome and autophagosome in AGG exposed cells

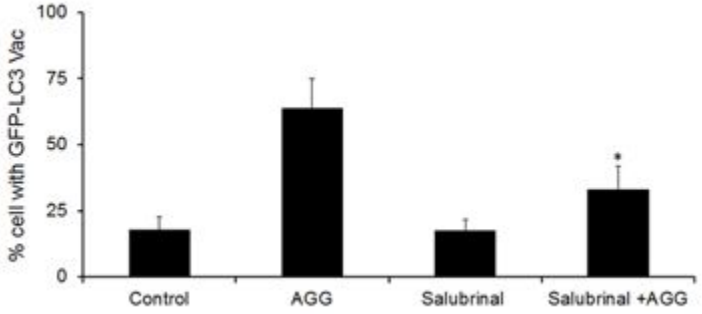


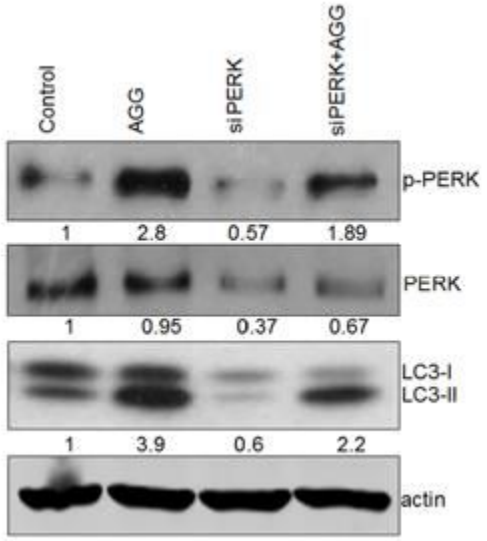
AGG-induced autophagic cell Death mediated through ER Stress

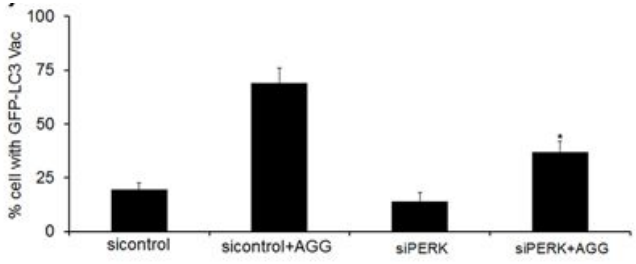




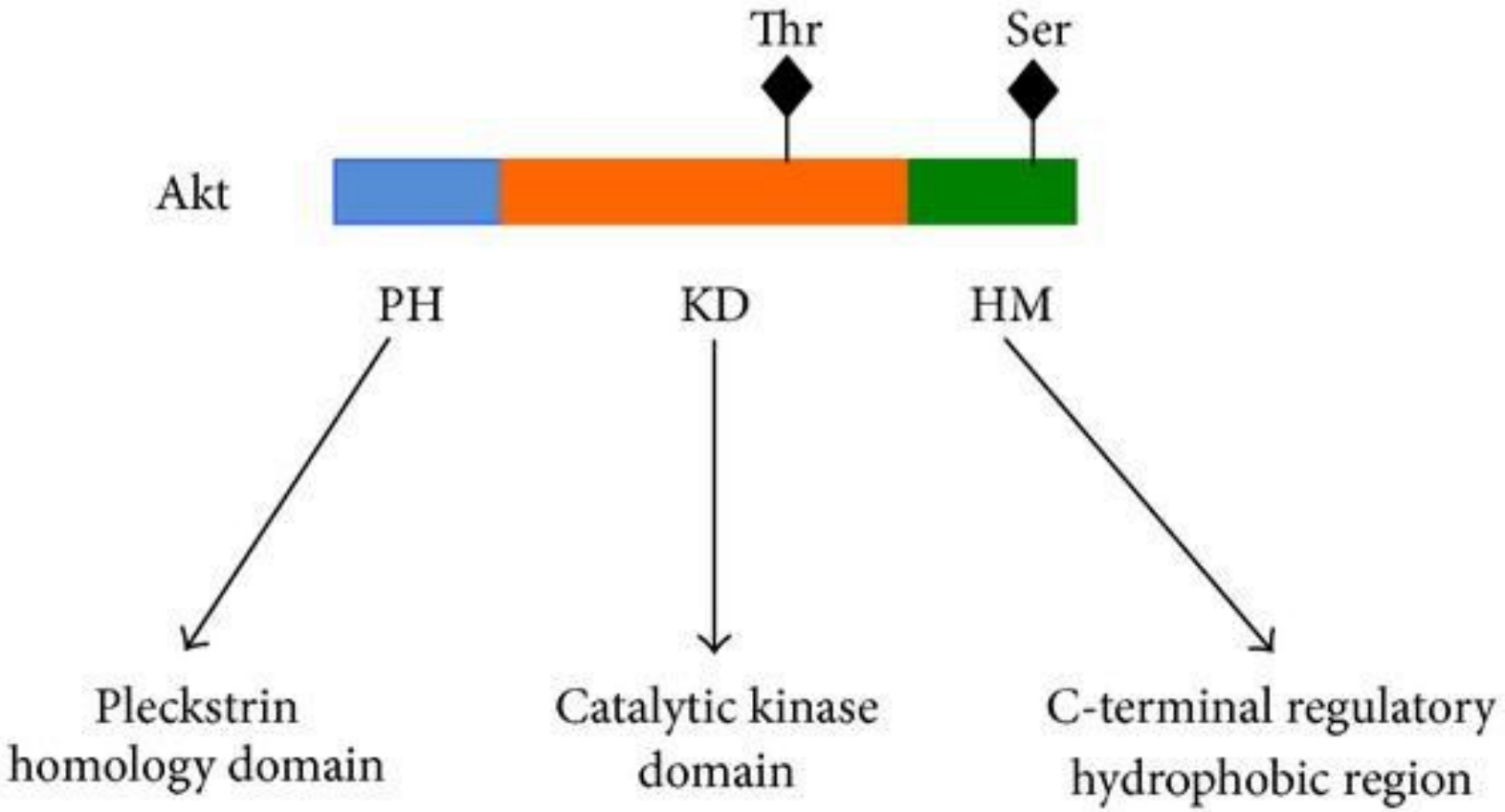






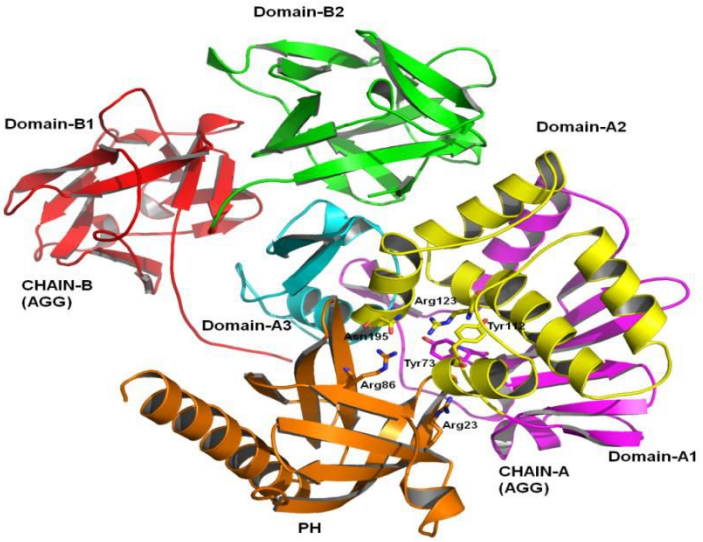


Pleckstrin Homology domain



AGG mediated inhibition of Akt/PH domain promotes ER stress-induced autophagic cell death

a



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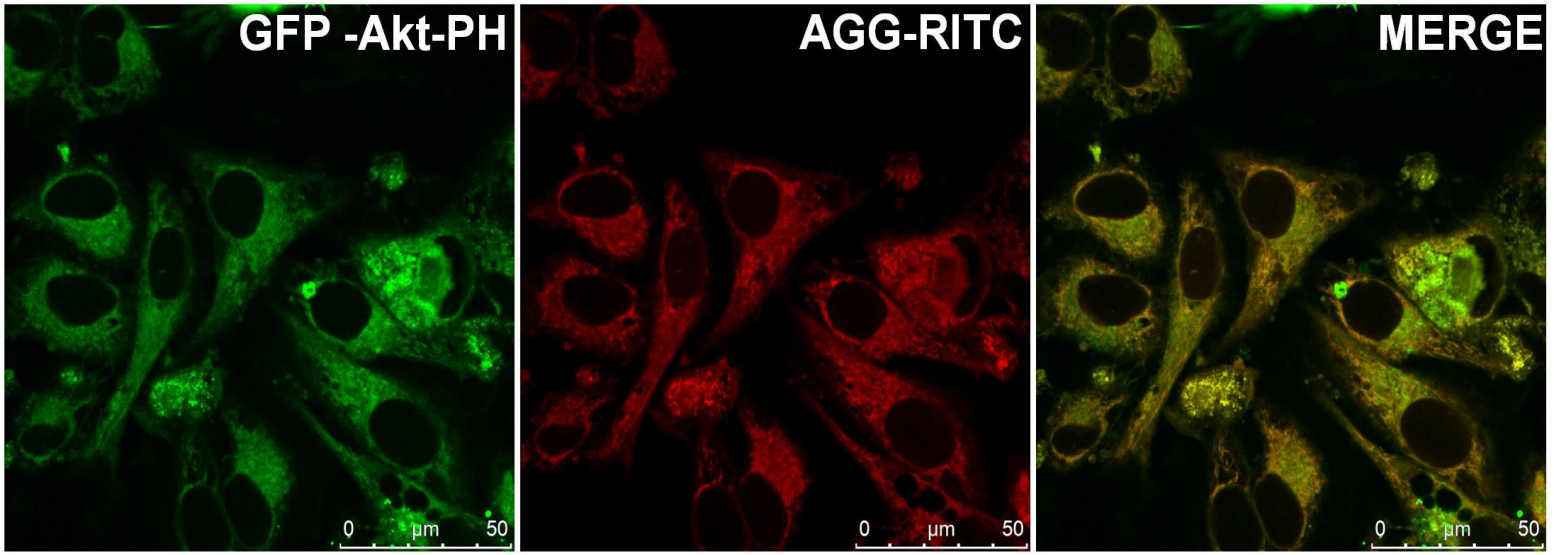
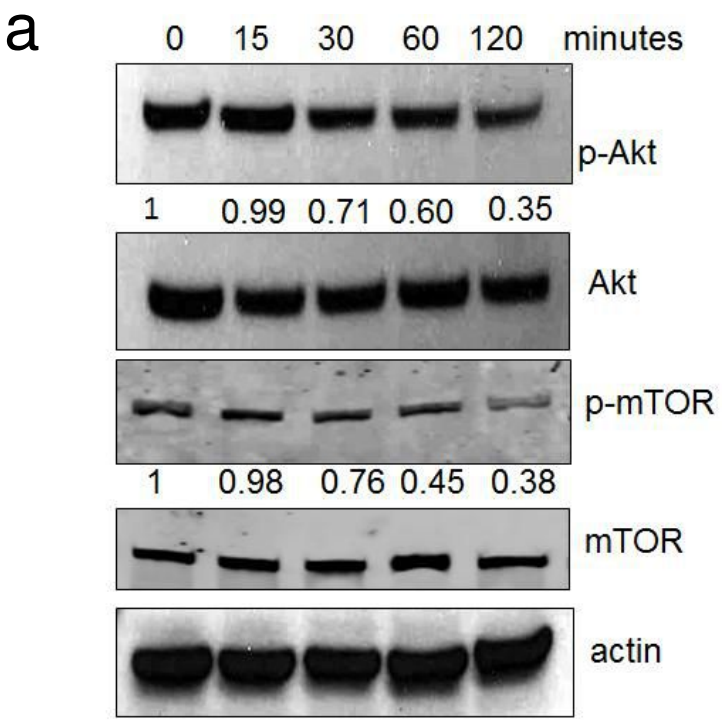
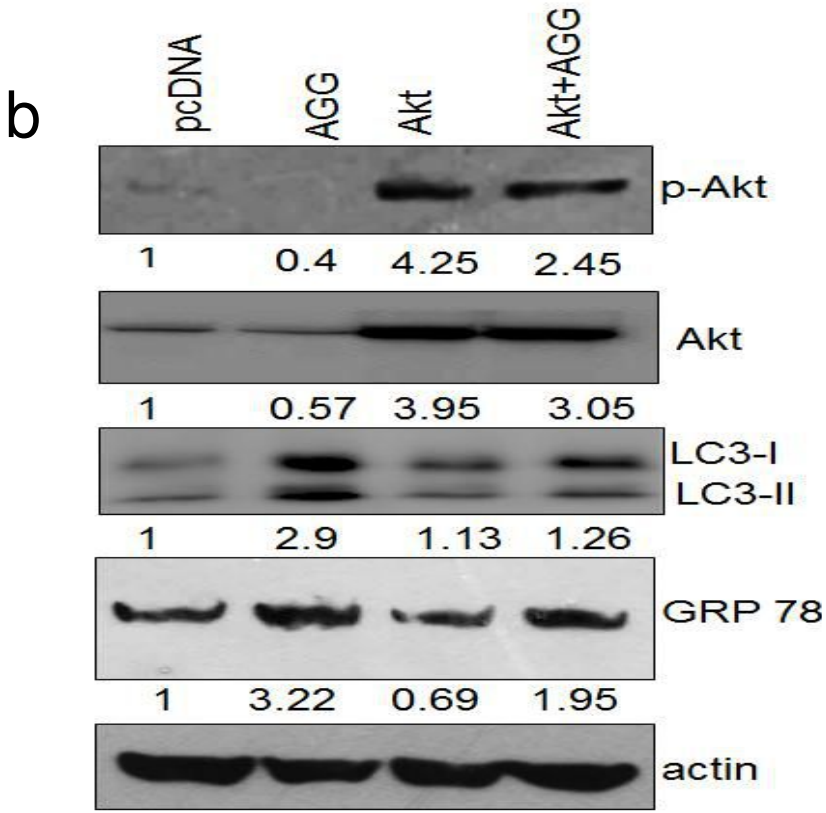
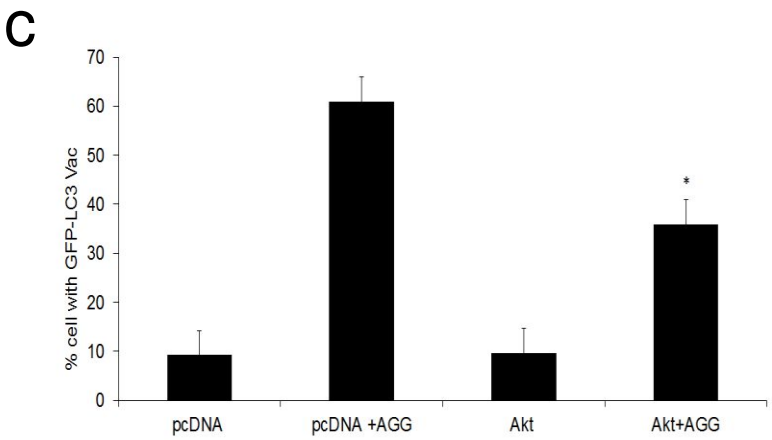


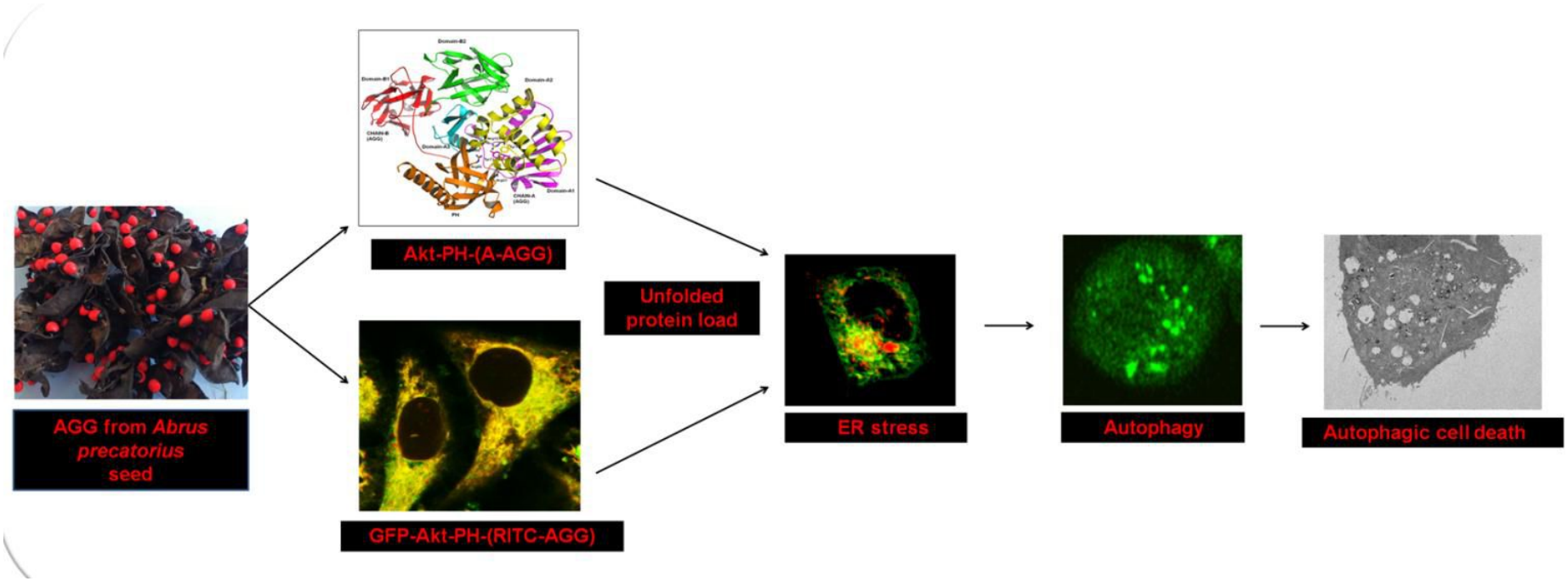
Panda et al Mol Carcinog. 2017;56(2):389-401

AGG-mediated inhibition of Akt promotes ER stress induced autophagic cell death









Panda et al Mol Carcinog. 2017;56(2):389-401

Thank You For Your Attention