

# YAP Manipulates Proliferation Via PTEN/AKT/mTOR-mediated Autophagy in Lung Adenocarcinomas

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**Primary research**

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# Abstract

Background: Autophagy is a double-edged sword during the initiation or progression of multiple tumors. Hippo pathway core-factor YAP has been proved to be involved in autophagy processes. The present study aimed to identify the mechanism underlying modulation of YAP via PTEN/AKT/mTOR-mediated autophagy in lung adenocarcinomas (LUAD).

Methods: Data of LUAD chip GSE43458 was obtained from Gene Expression Omnibus (GEO). RT-qPCR and Western blot were used to assess YAP expression in LUAD cell lines. CCK-8 assay, flow cytometry, xenograft tumor model, immunochemistry and GFP-mRFP-LC3 fusion proteins were utilized to evaluate the effect of YAP on autophagy of LUAD cells in vitro and in vivo. Autophagy inhibitor and rescue experiments were carried out to elucidate the mechanism by which YAP manipulating autophagy in LUAD cells.

Results: YAP significantly overexpressed in samples of LUAD patients and related to 5-year survival. YAP manipulates the proliferation and autophagy of A549 and H1299 LUAD cells. YAP induces activation of Akt/mTOR signaling pathway via suppressing PTEN in a Hippo-pathway-dependent manner. 3-Methyladenine impeded autophagy flux and promoted the proliferation in vitro and in vivo.

Conclusions: Hippo pathway critical transcriptional coactivators YAP manipulates the proliferation of lung adenocarcinoma, which is regulated by PTEN/AKT/mTOR autophagic signaling.

# Full Text

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# Figures

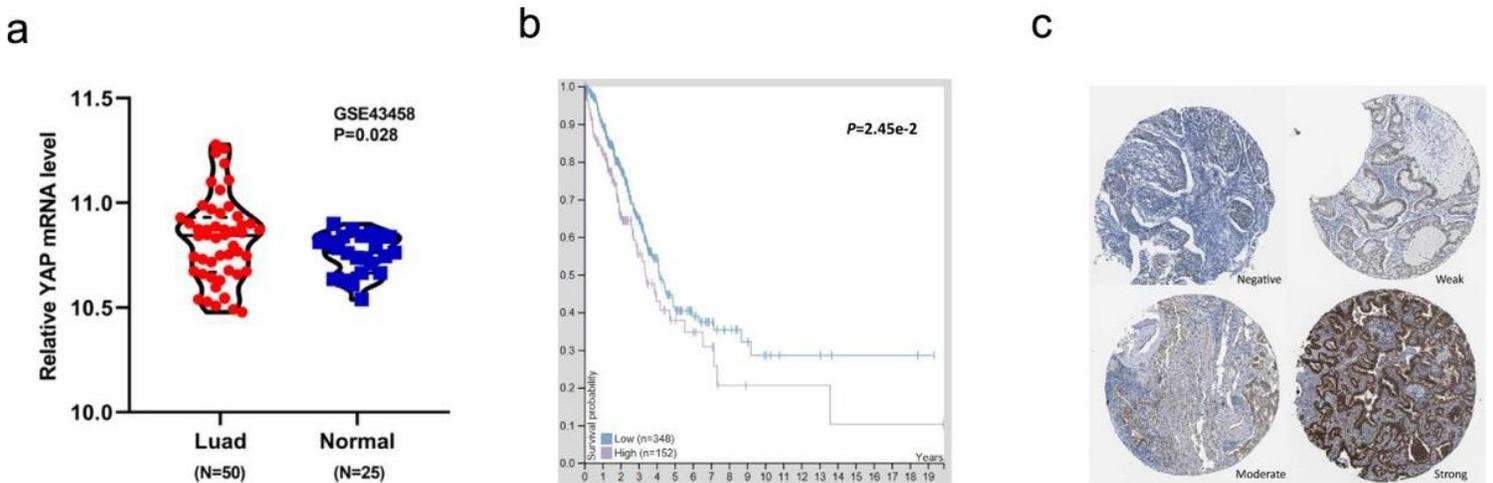
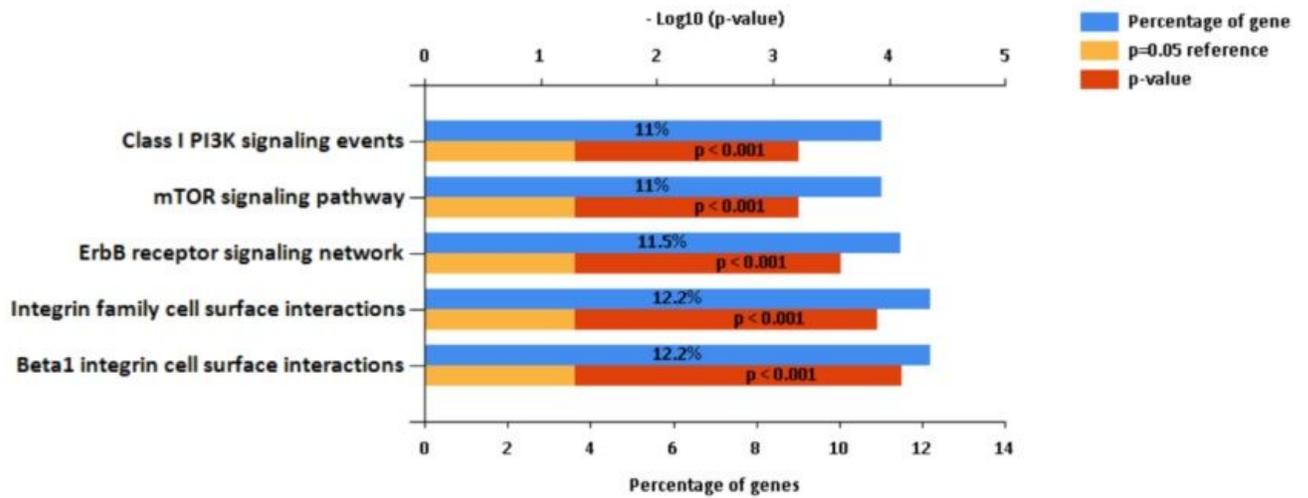


Figure 1

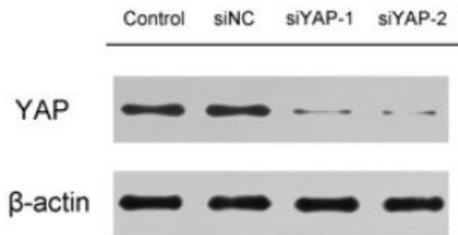
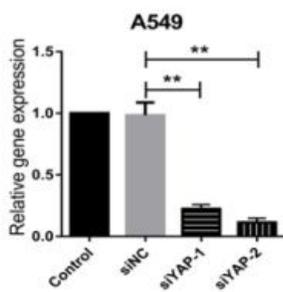
YAP is highly expressed in LUAD tissues and predicts poor prognosis. a. The expression of YAP in 50 tissues of LUAD and 25 tissues of normal lung tissues was determined of the GSE43458 at GEO database b. Kaplan-Meier survival curves and log-rank tests were used to assess the relationship between YAP levels and overall survival time of LUAD patients. The median of YAP expression levels in LUAD tissues was taken as cutoff. c. YAP levels of in four LUAD tissues The Human Protein Atlas Intensity (negative, weak, moderate, strong) [https://www.proteinatlas.org/ENSG00000137693-YAP1/pathology/lung+cancer#imid\\_19107997](https://www.proteinatlas.org/ENSG00000137693-YAP1/pathology/lung+cancer#imid_19107997).

a

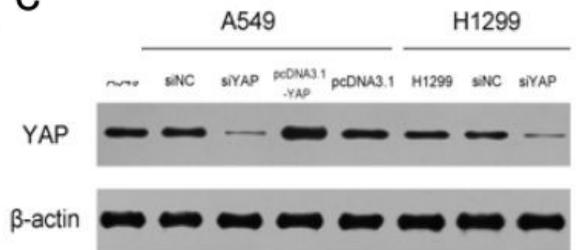
### Biological pathway enrichment analysis



b



c



d

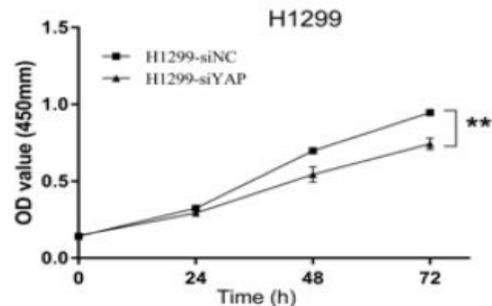
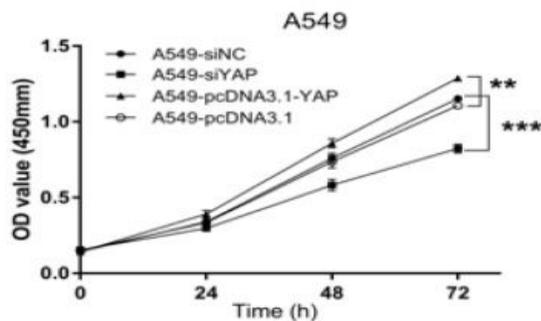
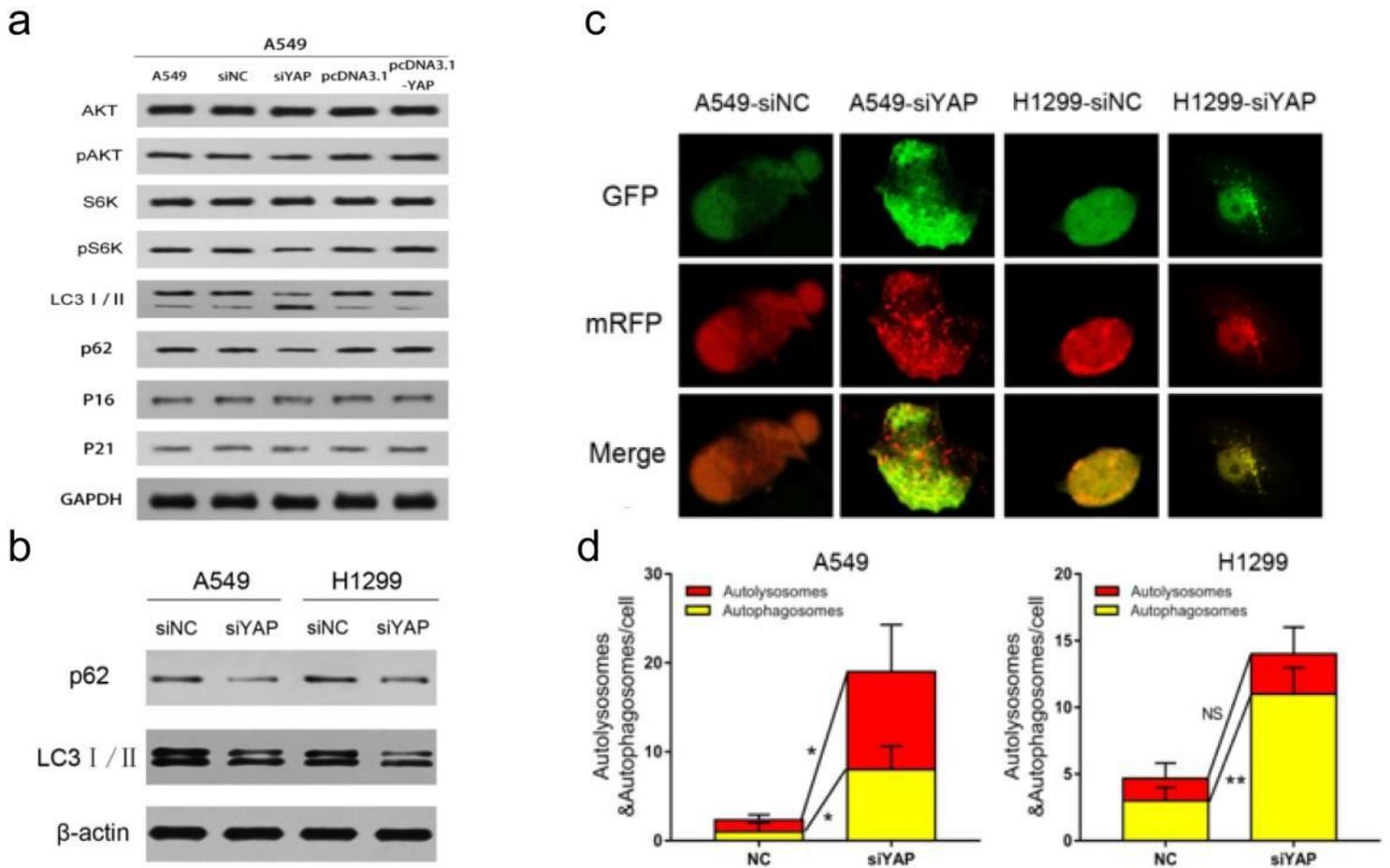


Figure 2

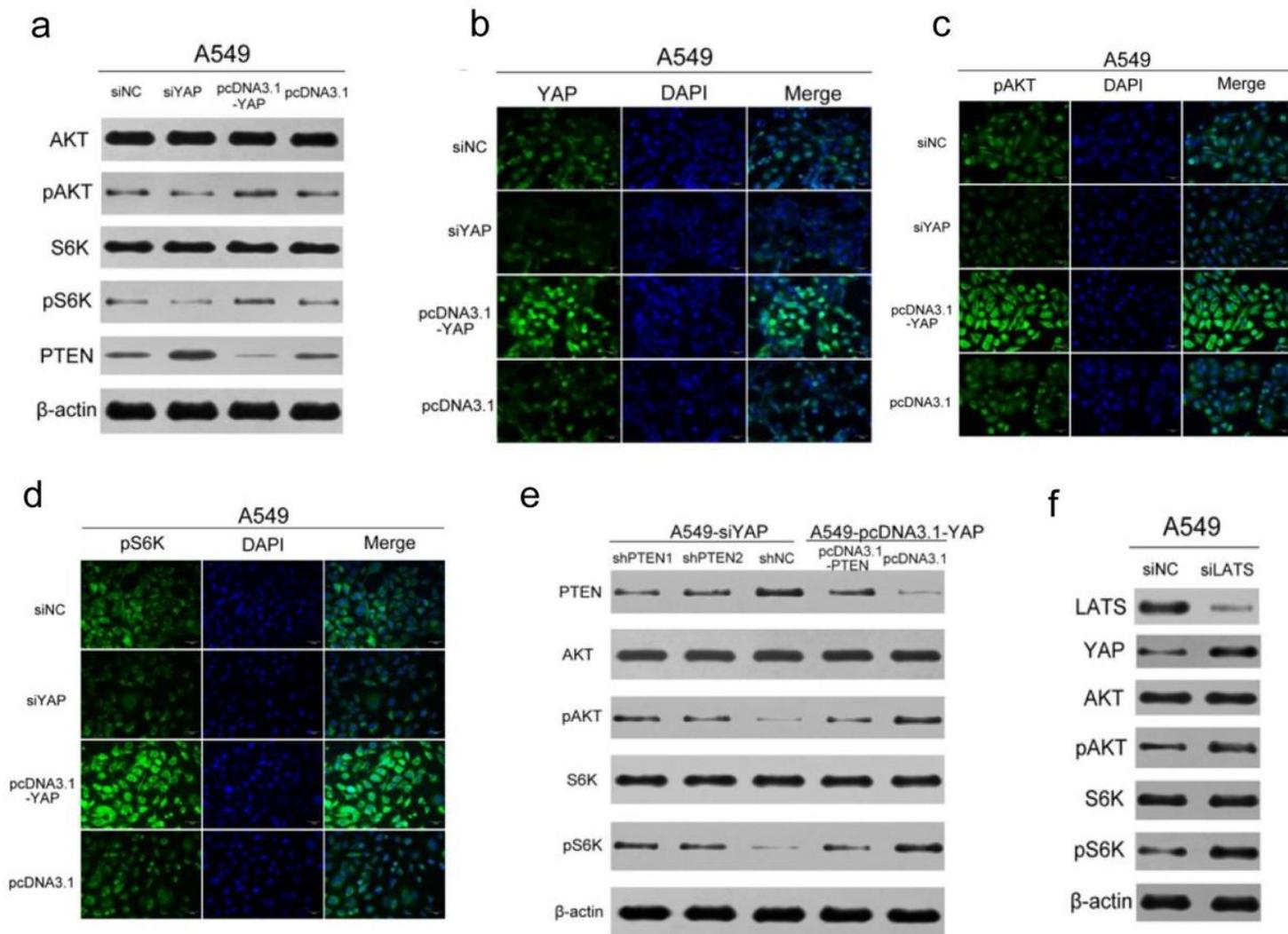
YAP targeted siRNA suppresses YAP expression in A549 and H1299 cells with reducing cell proliferation. a. The result of biological pathway enrichment analysis was shown with respect to YAP. b. siYAP-1/2 was constructed and significantly inhibited the expression of YAP in A549 cells, which detected by qPCR and

Western blot. c. The protein expression of YAP in A549 transfected with siYAP or pcDNA3.1-YAP, and H1299 with siYAP transfection were analysed by Western blot, respectively. d. CCK-8 assay was utilized to analyze cell proliferation of A549 cells with siYAP or pcDNA3.1-YAP, and H1299 with siYAP transfection. Data represent the Mean SD on three independent experiments (one-way ANOVA, \*\*P < 0.01, \*\*\*P < 0.001).



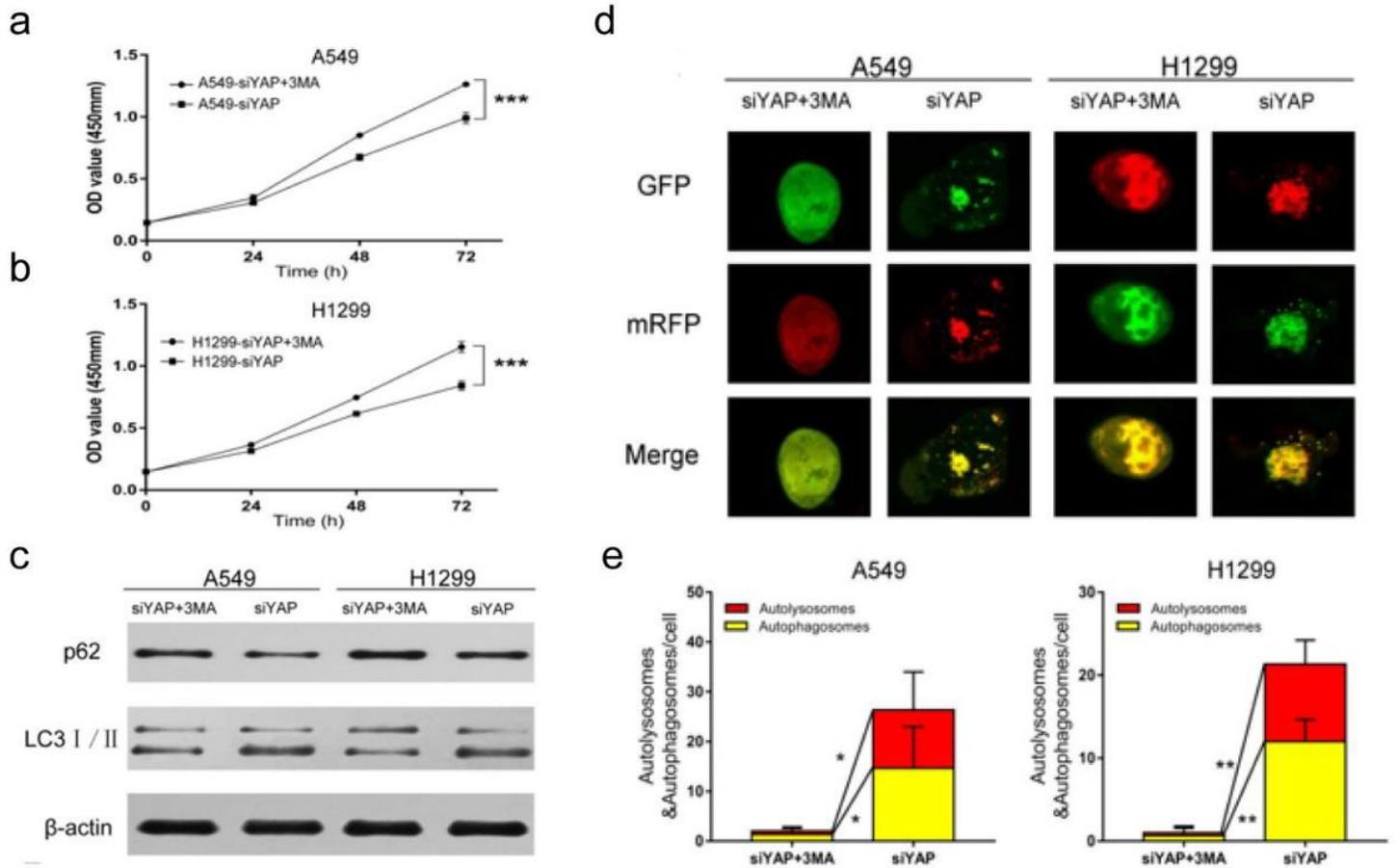
**Figure 3**

Autophagy in A549 and H1299 cell manipulated by YAP gene. a. Analysis of autophagy-related protein expression in A549 cell manipulated by YAP1 gene. Cells were analyzed by immunoblotting with indicated antibodies. b. Autophagy substrate proteins p62 and autophagy marker proteins LC3I/II were detected in A549/H1299-siYAP cellular extracts by Western blot. c. Immunofluorescence analysis of autolysosomes puncta in A549 and H1299 cells transfected with siYAP. Confocal microscopy images showing cellular localization of autophagic dots in A549/H1299-siYAP cells. The GFP stain (green puncta) was on behalf of the initial process of autophagy and the mRFP (red puncta) indicated the late process of autophagy. Magnification is  $\times 400$ . d. Quantification of autophagy activity was analyzed from 10 random visual fields for each group and shown in the graph. Data were shown as the Mean SD. Statistical analysis was calculated by one-way ANOVA. Scale bars =  $25\mu\text{m}$  (\*P < 0.05, \*\*P < 0.01).



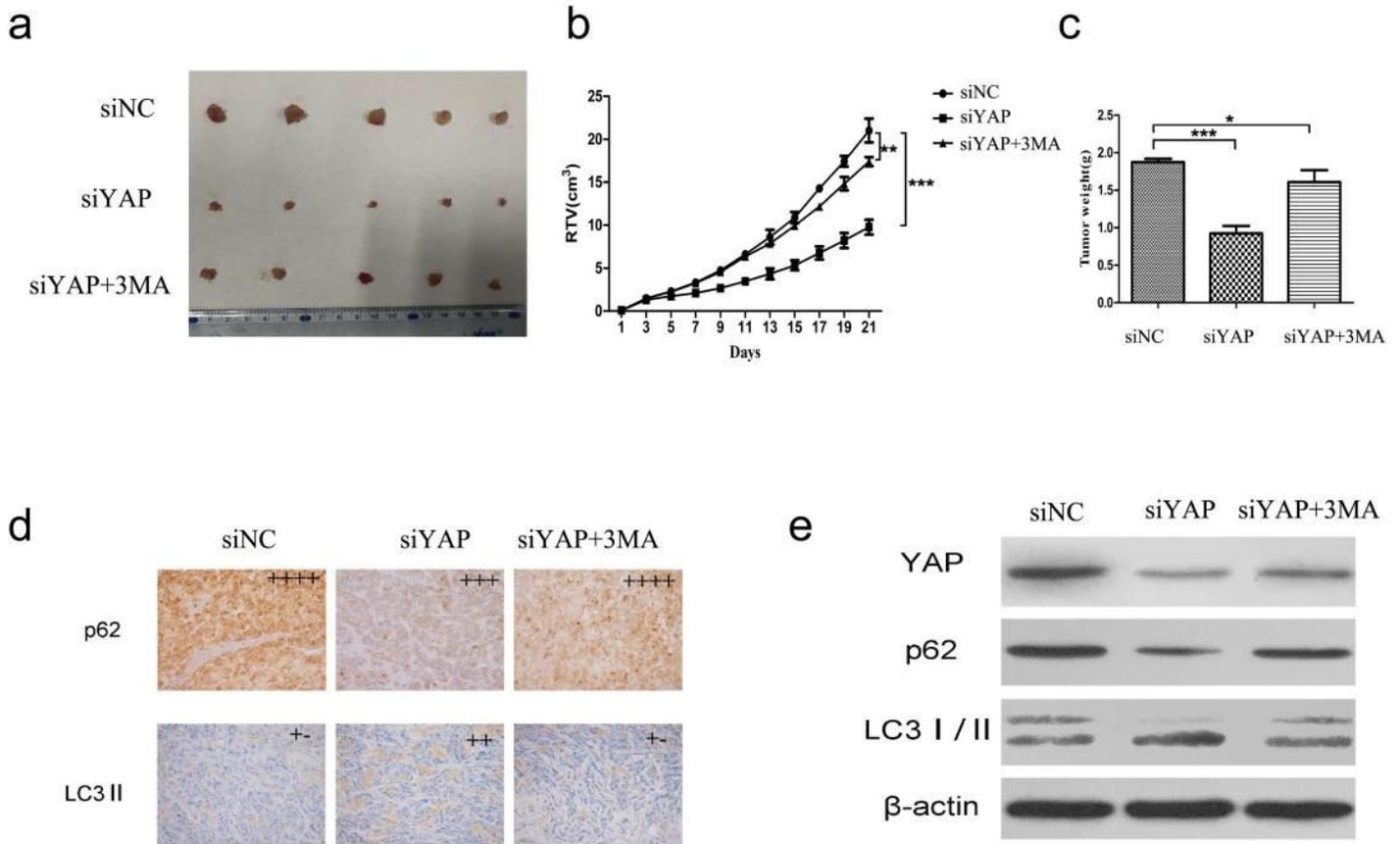
**Figure 4**

YAP induced activation of Akt/mTOR signaling pathway via suppressing PTEN in a Hippo-pathway-dependent manner. a. Following transfected with siYAP or pcDNA3.1-YAP in A549, Western blot measured the level of proteins associated with Akt/mTOR signaling pathway. b-d. Representative immunofluorescence of A549 cells transfected with siNC, siYAP, pcDNA3.1 or pcDNA3.1-YAP was observed by Inversed Fluorescent Microscope. Fluorescent staining of YAP, pAKT and pS6K was shown as green stain and fluorescence intensity indicated the relative amount in the cells. Magnification is  $\times 200$ . e. Western blot showed the expressive levels of AKT, pAKT, S6K, pS6K, and PTEN after transfection with or without shPTEN in A549-siYAP, with or without pcDNA3.1-PTEN in A549-pcDNA3.1-YAP, respectively. f. Western blot showed the expressive levels of AKT, pAKT, S6K, pS6K, and YAP after knockdown LATS, which being a upstream of YAP and a core factor in Hippo pathway.



**Figure 5**

The transformation of autophagy in A549/H1299-siYAP after treated with 3-MA. a-b. The proliferation ability of A549/H1299-siYAP and A549/H1299-siYAP+3-MA was determined by CCK-8 assay. c. A549/H1299-siYAP cells treated with 3-MA were lysated. Autophagy substrate proteins p62 and the conversion of LC3B-I to LC3B-II were detected by the Western blot. d-e. Confocal microscopy images showing cellular localization of autophagic dots in A549/H1299-siYAP or A549/H1299-siYAP+3MA cells. The GFP stain (green puncta) was on behalf of the initial process of autophagy and the mRFP (red puncta) indicated the late process of autophagy. Magnification is  $\times 400$ . Quantification of autophagy activity was analyzed from 10 random visual fields for each group and shown in the graph. Data were shown as the Mean  $\pm$  SD (one-way analysis, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).



**Figure 6**

Knockdown of YAP inhibited tumor growth through autophagy in vivo. a. Image of tumor size in A549 cells tumor xenograft treated with control , siYAP and siYAP+3MA. b, c. Represented figure indicated the tumor growth after intraperitoneal injection with or without 3MA. d. Immune staining indicated the expression of p62 and LC3 II, scale bar = 200  $\mu$ m. e. Western blot showed the expressive levels of YAP, p62, and LC3 in tumor tissues treated with or without siYAP and 3MA. Data were shown as the Mean  $\pm$  SD (one-way analysis, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001).