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Supplemental Information

Therapeutic potential of IBP as an autophagy inducer for treating lung cancer via blocking PAK1/Akt/mTOR signaling

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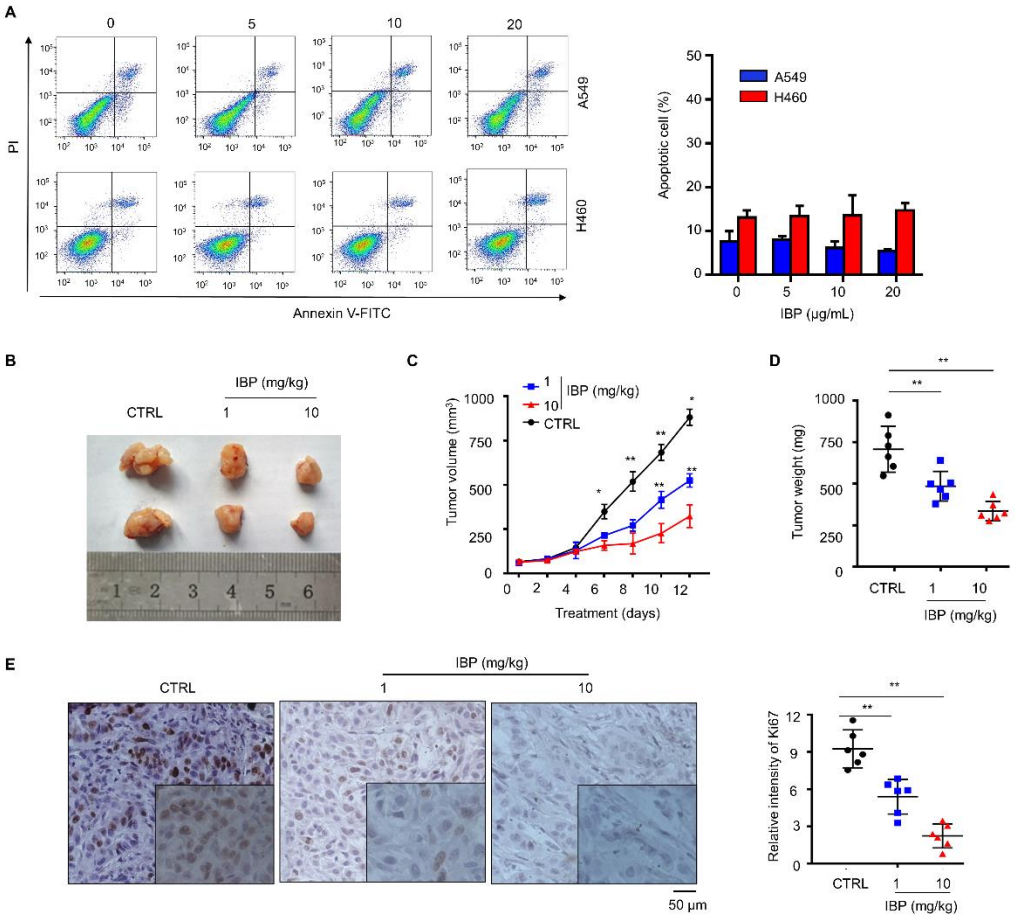

Figure S1. IBP inhibits lung cancer cells in an apoptosis-independent way. **(A)** A549 and H460 cells were incubated in the presence of IBP at different concentrations (0, 5, 10, 20 μg/mL) for 48 h. The apoptotic lung cancer cells was assessed by flow cytometry assay. **(B-D)** H460 cells were subcutaneously inoculated in the back of Balb/C mice. 1 or 10 mg/kg of IBP was administered by intraperitoneal injection every 2 days when the tumor was felt and the tumor size was measured. Mice were sacrificed on day 12, and tumors were excised, photographed and weighted. **(E)** Ki67 expression in tumor xenografts was examined by IHC. Representative images were provided as indicated; Scale bar, 50 mm. Data are mean ± SD from three independent experiments. One-way ANOVA (Tukey's post hoc); $*p < 0.05$; $**p < 0.01$.

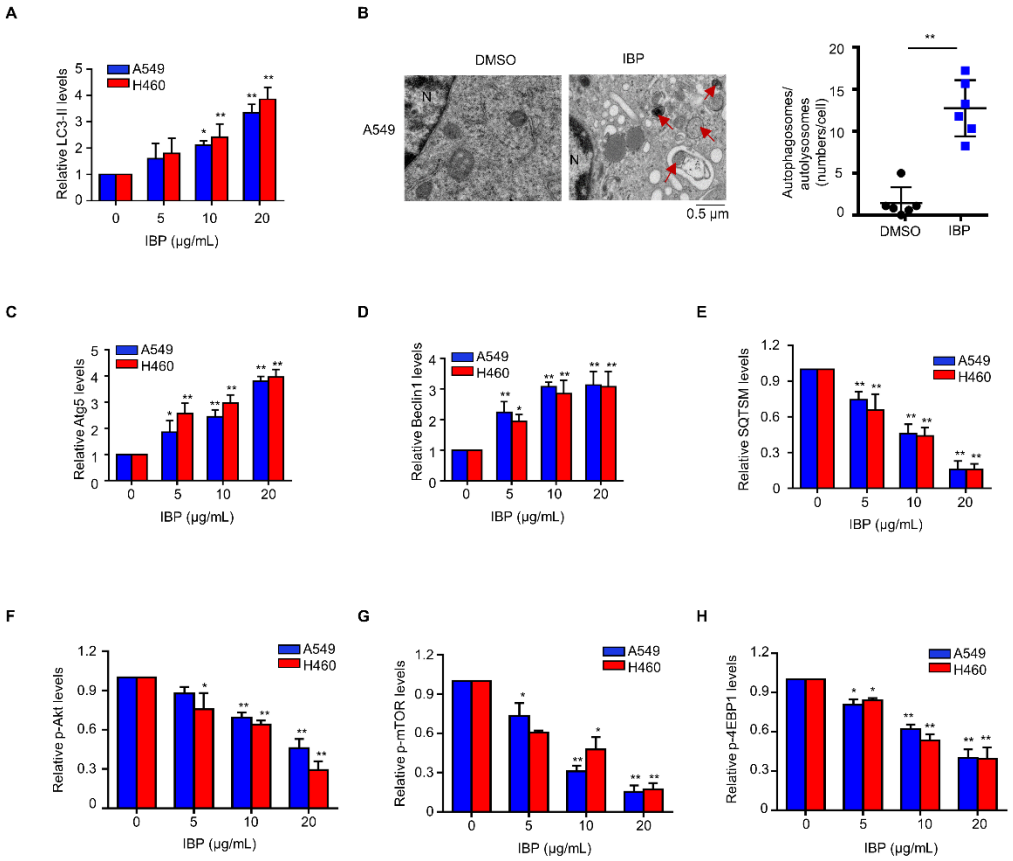

Figure S2. Densitometric quantification of the immunoblotting gel data presented in main Figures (in text) and autophagosome in A549 cells. (A) Fig. 2A, **(B)** Autophagy measured by transmission electron microscopy in A549 cells. Arrows, autophagosomes. Cells treated with DMSO or 10 μg/mL IBP for 48 hours was examined. **(C-E)** Fig. 2A. **(F-H)** Fig. 4D. Data are mean ± SD from three independent experiments. One-way ANOVA (Tukey's post hoc); *$p < 0.05$; **$p < 0.01$.

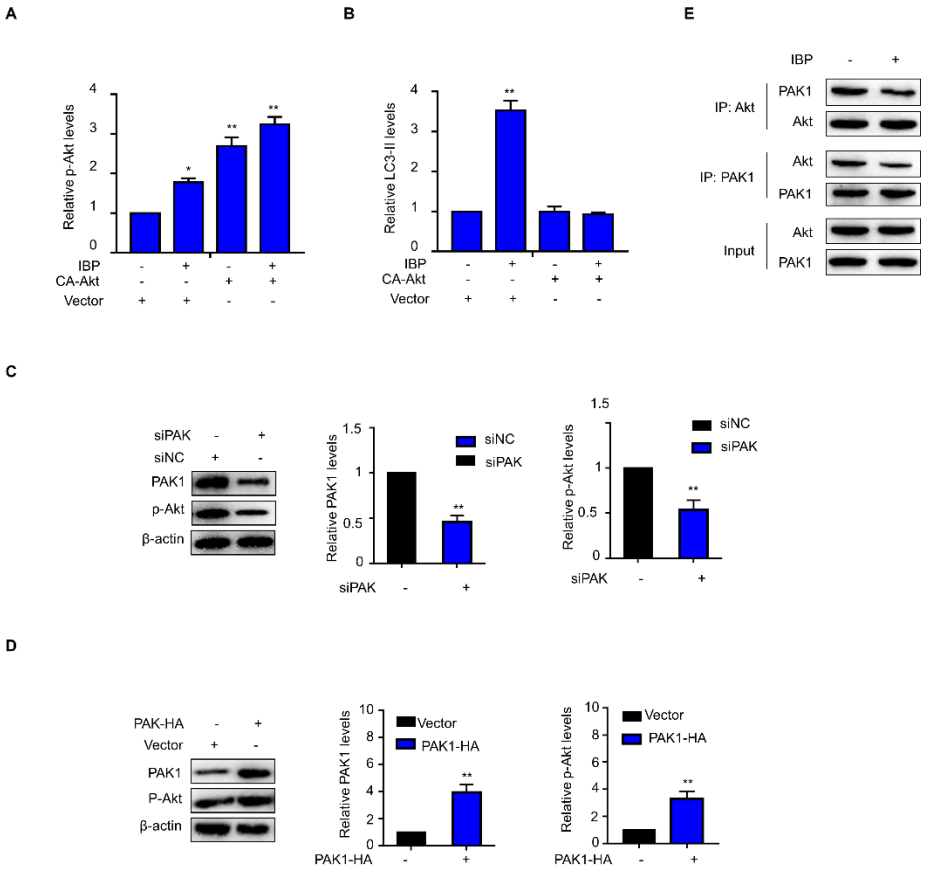

Figure S3. IBP inhibits Akt/mTOR pathway in A549 cells and Interaction of PAK1 with Akt in A549 cells. (A-B) Fig. 4E**. (C-D)** PAK1 levels and Akt phosphorylation in A549 by Western blot analysis. Cells were transfected with siPAK1 (C) or a construct encoding PAK1-HA (D) for 48 hours. **(E)** IP-western blots analysis of the interaction of PAK1 with Akt. A549 cells were treated with 10 μg/mL IBP or DMSO for 48 hours after cells were co-transfected with PAK1-HA and Akt-GFP. Data are a representation of 3 independent experiments. One-way ANOVA (Tukey's post hoc); *, p <0.05; **, p <0.01.

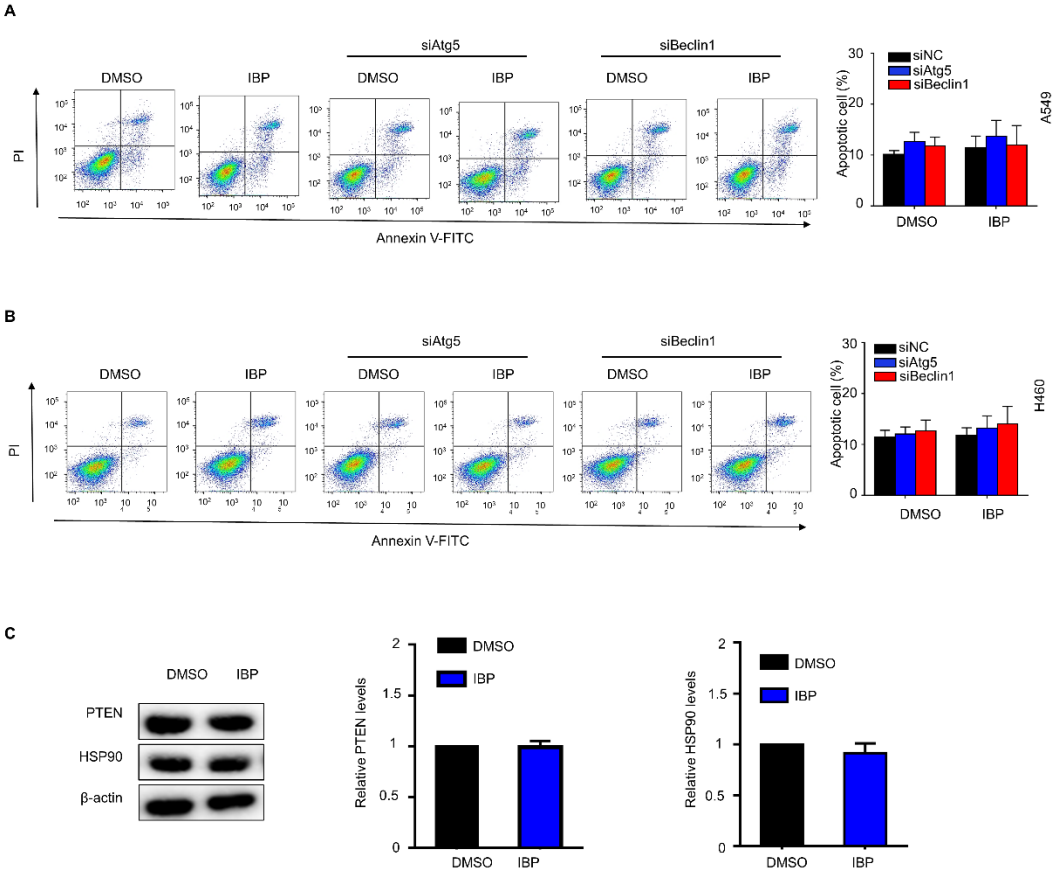

Figure S4. IBP treatment showed no obvious effect on apoptosis cells in an autophagy-independent way and PETN and HSP90 expression in A549**. (A-B))** A549 and H460 cells were transfected with siRNA against Atg5 or Beclin1 or control (50nmol/L) for 48 hours. Then cells were treated with 10 μg/mL IBP or DMSO for 48 hours. **(C)** PTEN and HSP90 in A549 by Western blot analysis. Data are shown as means ± SD for three independent experiments (cell samples, Tukey's post hoc; *$p \leq$ 0.05; **$p \leq 0.01$).