

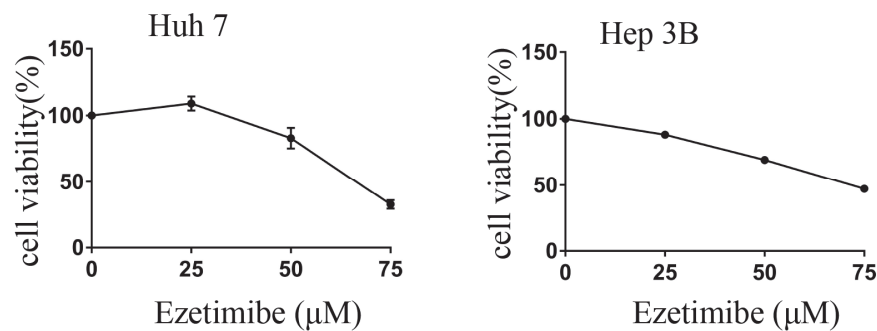
Supplementary Data

Ezetimibe induces paraptosis via targeting NPC1L1 to inhibit  
mTOR/MAPK signaling pathway in hepatoma

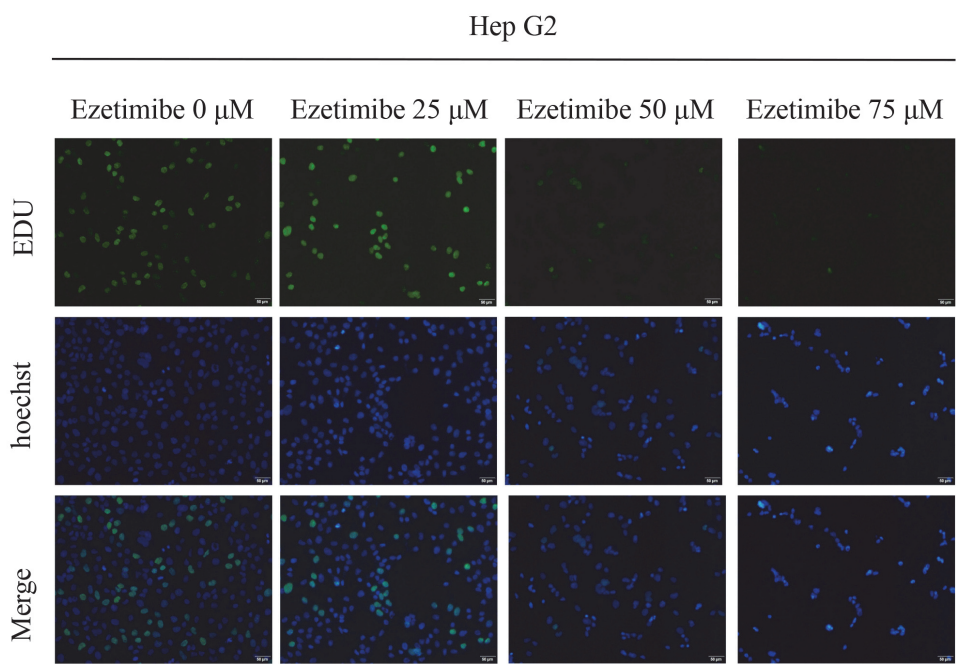
Yu-ting Yin, et al

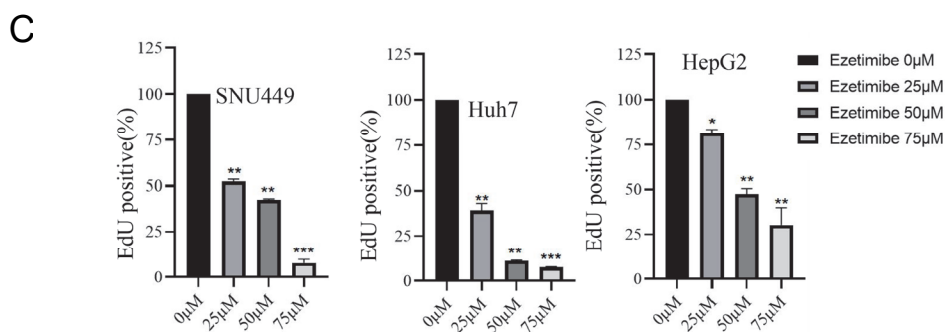
Supplementary Figures

A

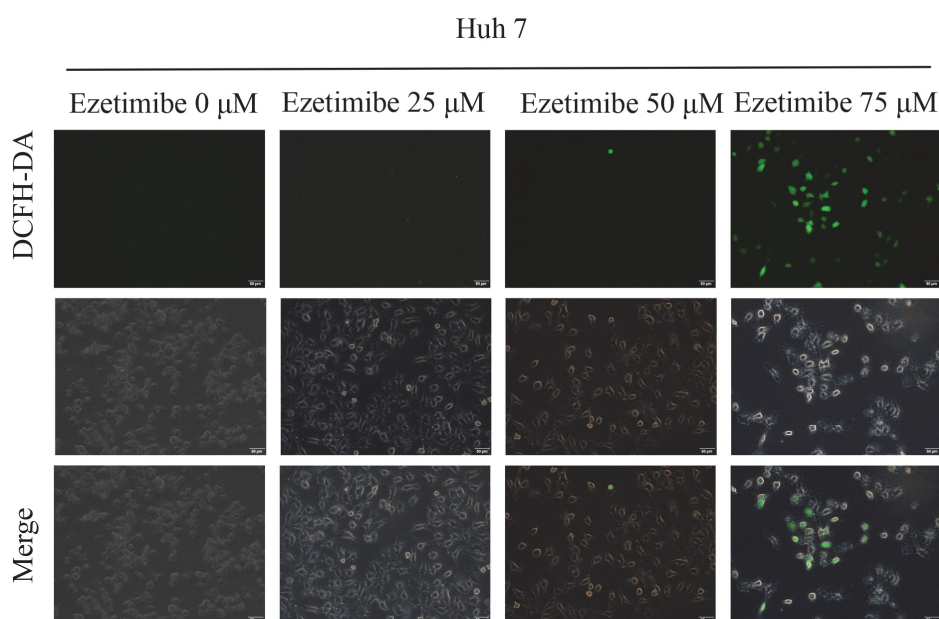


B

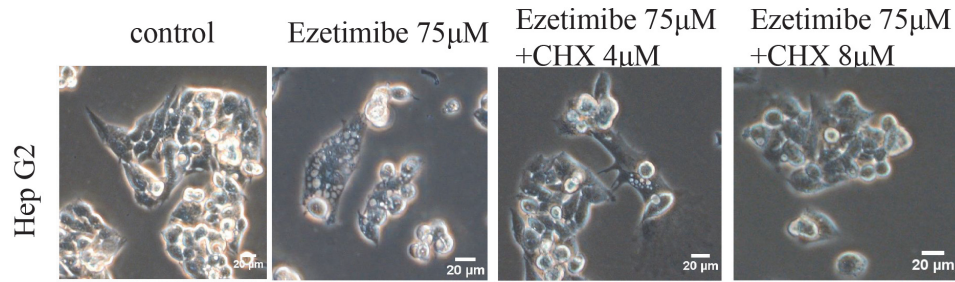




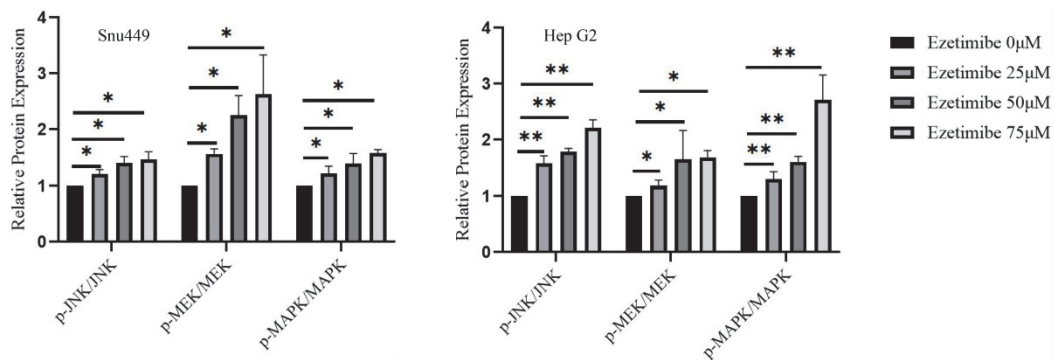
**Fig. S1. Ezetimibe inhibits proliferation of HCC cells.** (A) Cell proliferation is gradually decreased in HCC cells treated with increasing concentrations of Ezetimibe for 48h; cell viability was measured by CCK8 assay. (B) EDU staining was used to measure the cellular proliferation based on the reaction with Alexa488-azide (green). Scale bars, 50  $\mu$ m. (C) Quantification of EdU-positive cells. \*\*P < 0.01, \*\*\*P < 0.001.



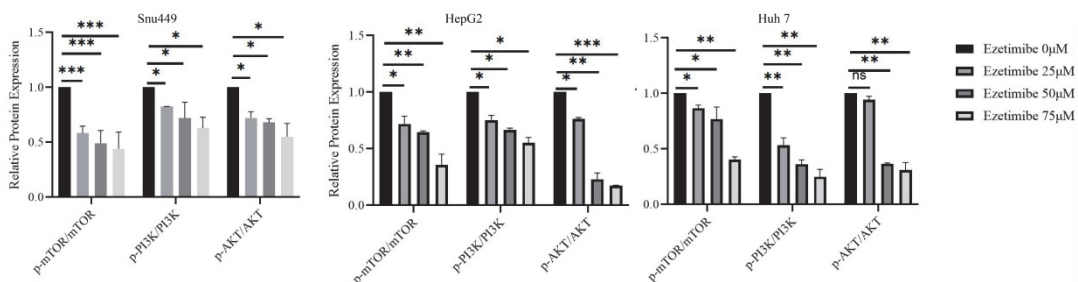
**Fig. S2. Ezetimibe induces ROS in HCC cells.** Huh-7 cells were treated with Ezetimibe ranging from 0 to 75 $\mu$ M for 24h and then the cells were stained with DCFH-DA of Cellular ROS Assay Kit (ROS exhibited green fluorescence). Scale bars, 50  $\mu$ m.



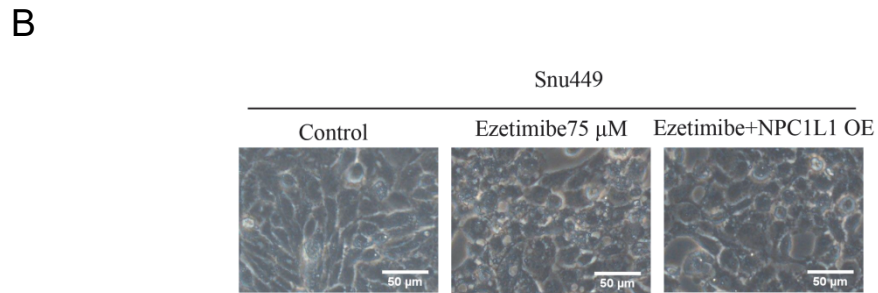
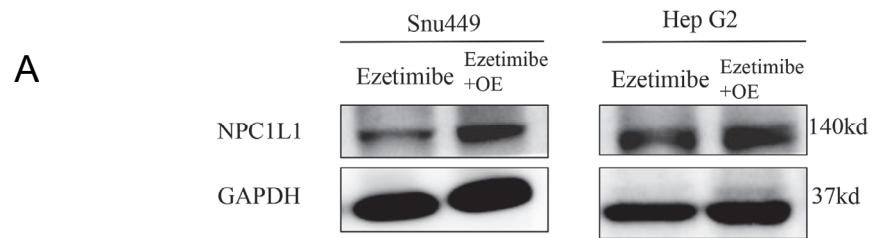
**Fig. S3. Cycloheximide reduces Ezetimibe-induced vacuolation in HCC cells.** Cycloheximide (CHX, 8μM) significantly decreased perinuclear vacuolation induced by Ezetimibe at 24h compared with cells treated with Ezetimibe alone. Scale bars, 20 μm.



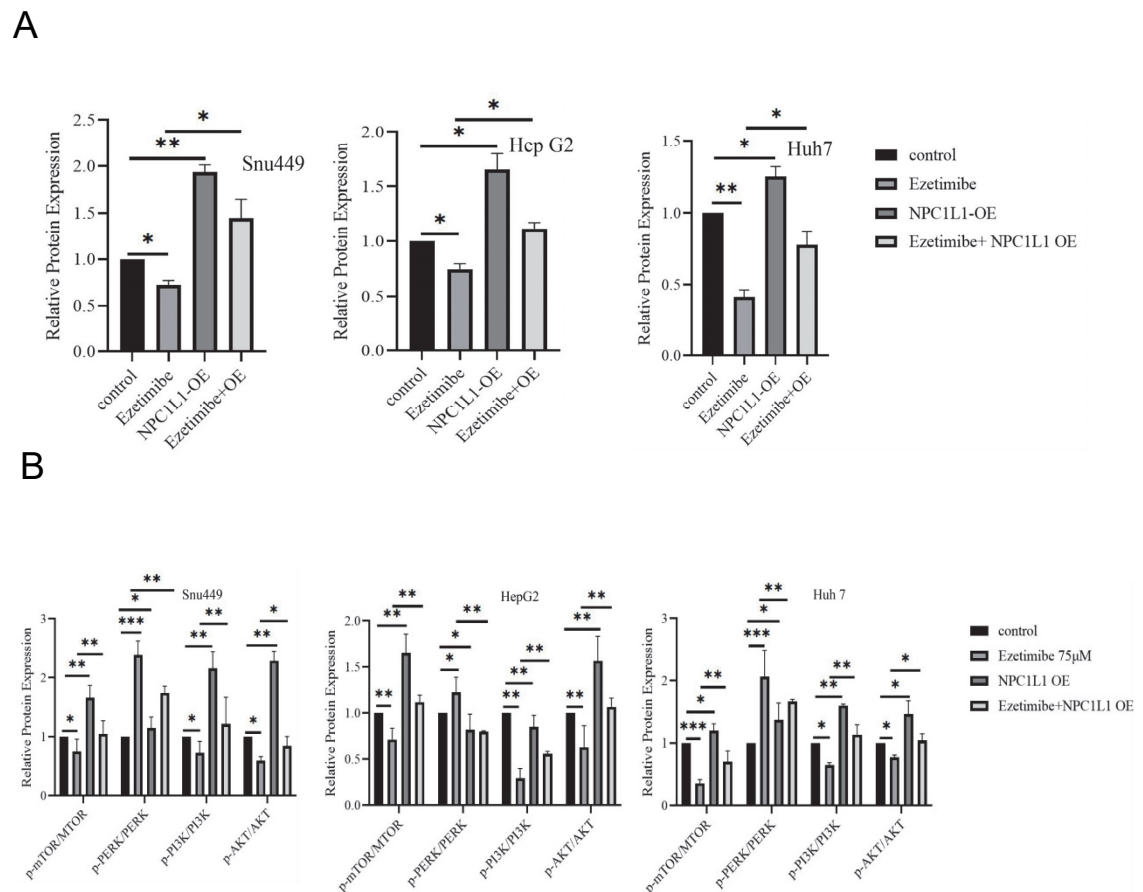
**Fig.S4. Ezetimibe upregulates the expression levels of MAPK pathway.** Relative level of MAPK signaling pathway expression was quantified by ImageJ software and bar presentation. \*P < 0.05, \*\*P < 0.01.



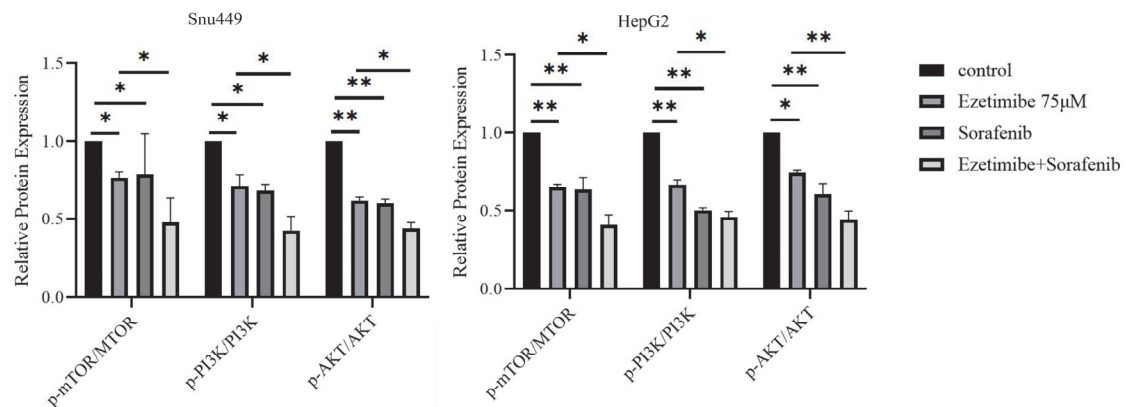
**Fig.S5. Ezetimibe decreases the expression levels of mTOR pathway.** Relative level of mTOR signaling pathway expression was quantified by ImageJ software and bar presentation. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.



**Fig. S6. Ezetimibe induces paraptosis by targeting NPC1L1.** (A) After transfecting with the over-expression plasmid, Western blot was used to determine the level of NPC1L1. (B) Overexpression of NPC1L1 could significantly eliminate vacuoles induced by Ezetimibe in SNU-449 cells. Scale bars, 50  $\mu$ m.



**Fig. S7. Overexpression of NPC1L1 inhibits the expressions of ER stress-related proteins and enhances the expression of PI3K, p-AKT, p-mTOR in SNU449, Huh-7 and HepG2 cells lines. (A) Relative level of NPC1L1 expression was quantified by ImageJ software and bar presentation. \*P < 0.05, \*\*P < 0.01. (B) Quantification the expression of PI3K/AKT/mTOR and PERK proteins. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.**



**Fig.S8. Co-treatment of Ezetimibe and Sorafenibthe downregulates the expression of mTOR signaling pathway. Relative level of mTOR signaling pathway expression was quantified by ImageJ software and bar presentation. \*P < 0.05, \*\*P < 0.01.**