Supplementary materials

Autophagy inhibition sensitizes hepatocellular carcinoma to the multikinase inhibitor linifanib

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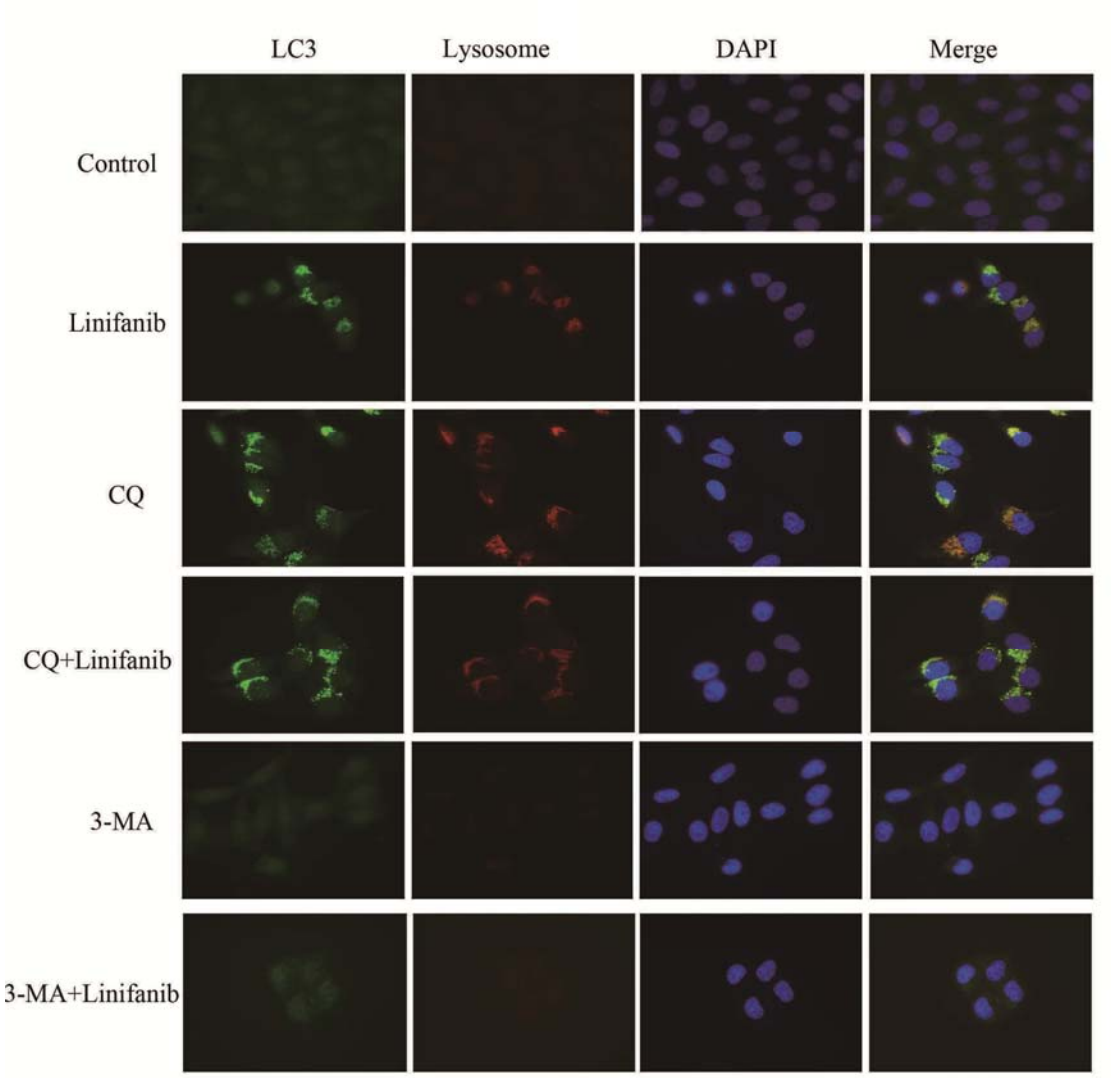

Fig. S1. Linifanib induced autophagosomes and autolysosomes formation in hepatocarcinoma cells. Bel-7404 cells were treated with 2.5 μM linifanib in the presence or absence of CQ (5 μM) or 3-MA (5 mM) for 24 h before labeled with fluorescence and imaged by fluorescence microscope. Green: FITC-labeled LC3; Red: lyso-tracker-labeled lysosome; Blue: DAPI-labeled nucleus. The yellow puncta indicated LC3 co-located with lysosome.

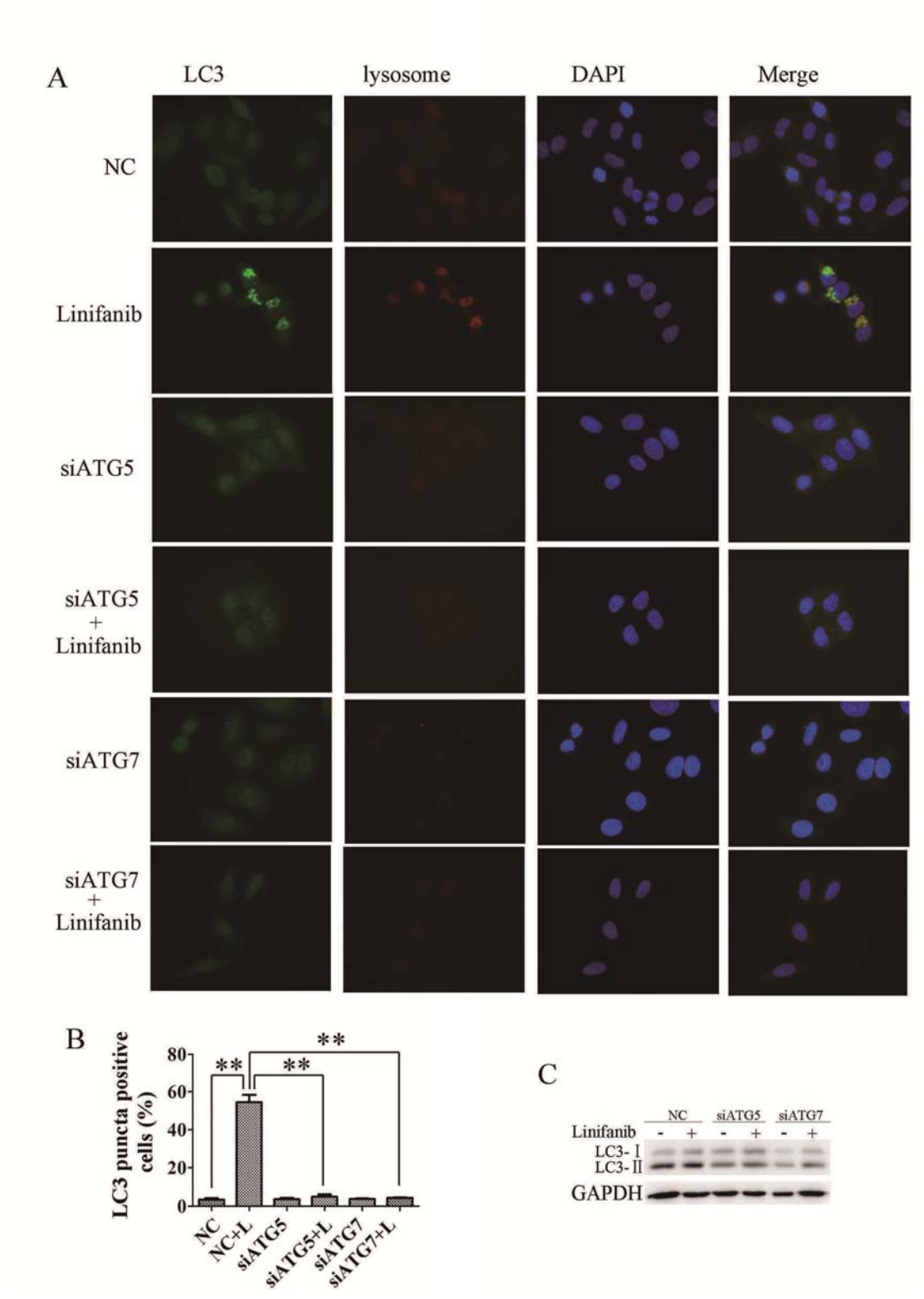

Fig. S2. Inhibition of linifanib-induced autophagy by siRNAs against ATG5 or ATG7. Bel-7404 cells were transfected with siRNA against ATG5 (siATG5) or ATG7 (siATG7) for 24 h, then treated with linifanib (L) for 24 h. A. Cells were labeled with fluorescence and imaged by fluorescence microscope. Green: FITC-labeled LC3; Red:

lyso-tracker-labeled lysosome; Blue: DAPI-labeled nucleus. B. Percentage of green or yellow puncta-positive cells was quantified and analyzed using a threshold of >5 dots/cell. The data were represented as the mean ± SD. **P < 0.01. C. Cell lysate was subjected to immunoblotting for LC3. Data were expressed as the mean±SD. ** P<0.01.

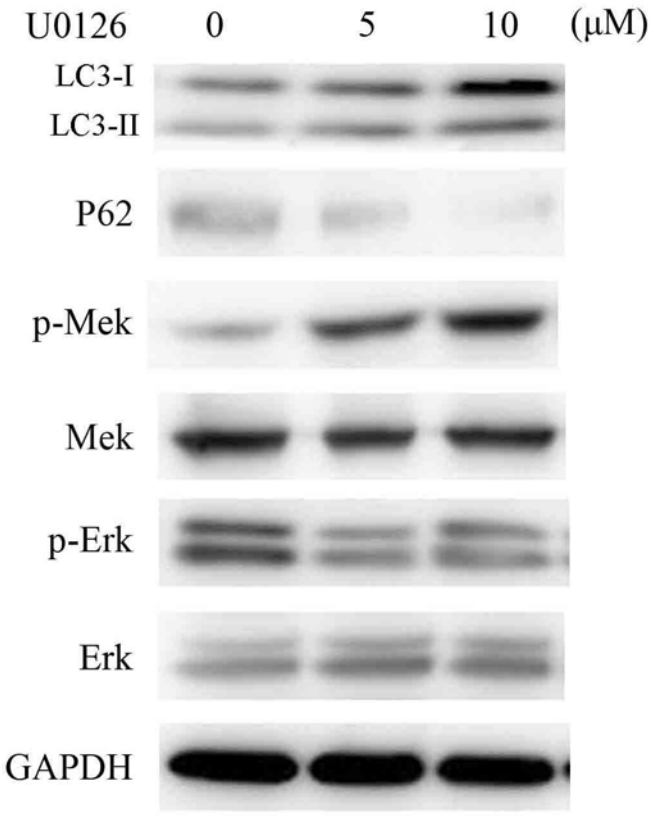

Fig. S3. The Mek/Erk-selective inhibitor U0126 induces autophagy in Bel-7404 cells. Cells were treated with U0126 for 24 hours. Cell lysates were subjected to immunoblotting analysis for LC3, p62, and phospho- or total forms of Mek, Erk.