

Figure S1. SPRED2 promotes autophagy in Hep3B and HLE cells. **(A)** TEM showing the presence of autophagosomes in control [C] and SPRED2-overexpressing (OE) [OE] cells. The area enclosed by the square is enlarged right. Arrows indicate autophagosomes. Scale bars are 1 μ m. The number of autophagosomes was counted in 135 μ m². Data are presented as the mean \pm SEM. * p < 0.05, ** p < 0.01. n = 3. **(B)** Cell lysates were prepared from control [C] and SPRED2-OE [OE] cells and the presence of each protein was evaluated by Western blotting. Band densities were digitized and semi-quantitated. Data are presented as the mean \pm SEM. * p < 0.05, ** p < 0.01. n = 3.

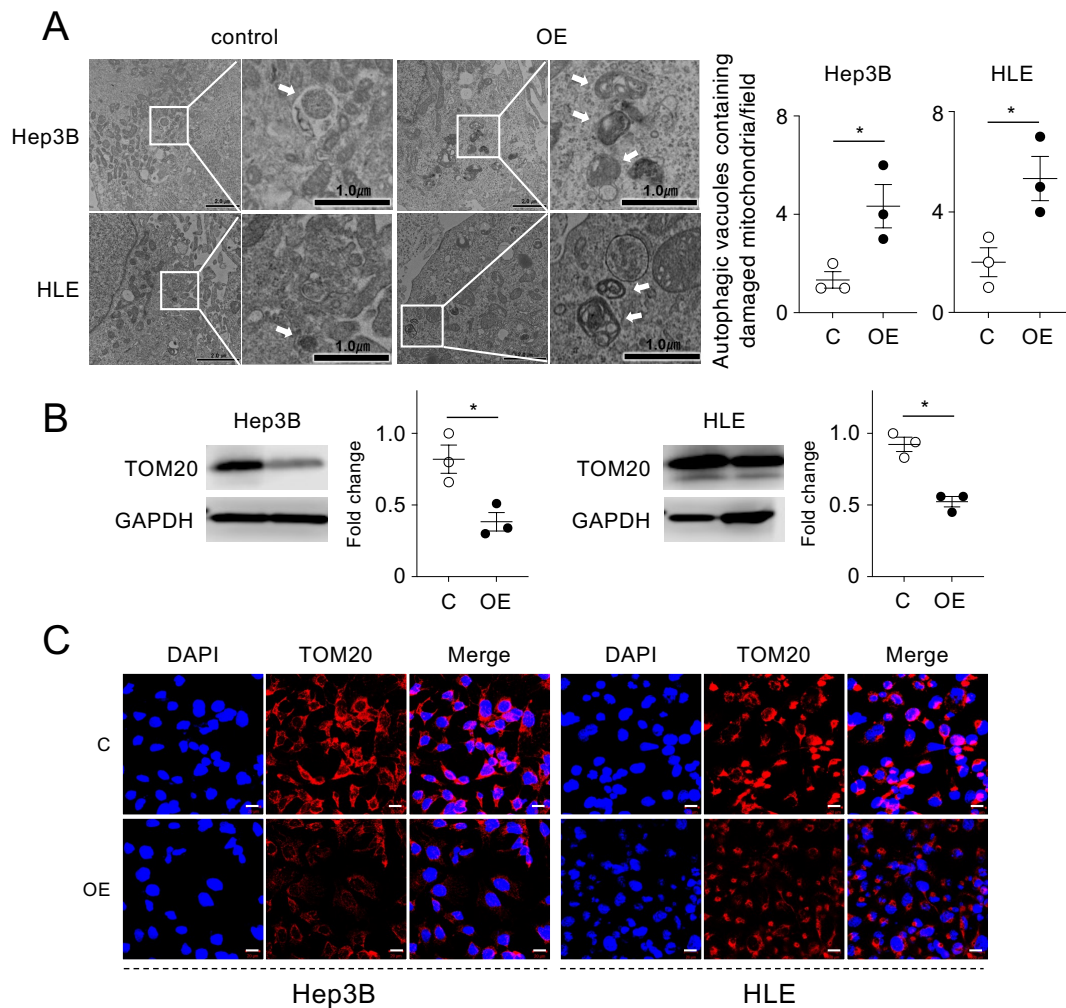


Figure S2. SPRED2 promotes mitophagy in Hep3B and HLE cells. **(A)** TEM showing the presence of autophagic vacuoles housing damaged mitochondria in control [C] and SPRED2-overexpressing [OE] cells. The area enclosed by the square is enlarged right. Arrows indicate autophagosomes. Scale bars indicate 1 μ m. The number of autophagic vacuoles containing damaged mitochondria was counted in 135 μ m². Data are presented as the mean \pm SEM. * p < 0.05. n = 3. **(B)** Cell lysates were prepared from control [C] and SPRED2-OE [OE] cells and the presence of each protein was evaluated by Western blotting. Band densities were digitized and semi-quantitated. Data are presented as the mean \pm SEM. * p < 0.05. n = 3. **(C)** Control [C] and SPRED2-OE [OE] cells were seeded on a Lab-Tek II slide, fixed in acetone and the presence of TOM20 was analyzed by immunofluorescence. Representative images showing TOM20 expression are shown. Blue: DAPI, Red: TOM20. Scale bars are 20 μ m.

Figure S3

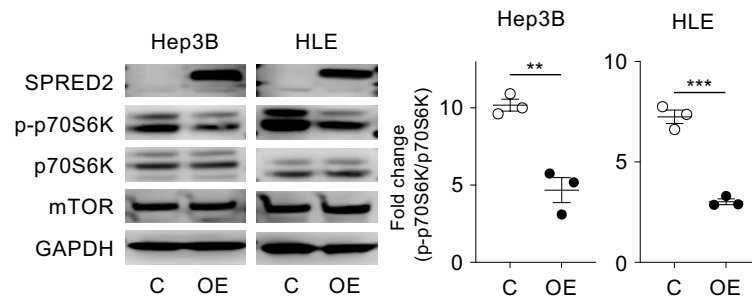


Figure S3. SPRED2 regulates autophagy through the mTORC1 signaling pathway in Hep3B and HLE cells. Cell extracts were prepared from control [C] and SPRED2-overexpressing [OE] cells, and the presence of each protein was evaluated by Western blotting. Band densities were digitized and semi-quantitated. Data are presented as the mean \pm SEM. ** p < 0.01, *** p < 0.001. n = 3

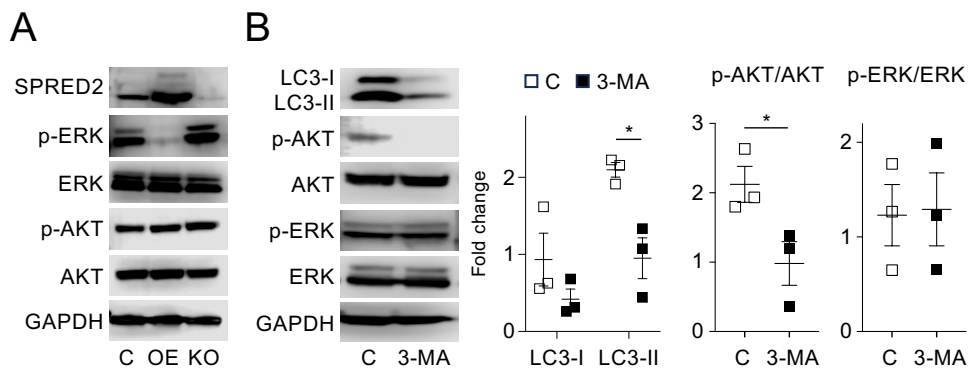


Figure S4. MAPK/ERK pathway is independent of PI3K/AKT pathway. **(A)** Cell extracts were prepared from control [C], SPRED2-overexpressing [OE] and SPRED2-knockout [KO] cells, and the presence of each protein was evaluated by Western blotting. **(B)** HepG2 cells were treated with 3 mM PI3K inhibitor 3-Methyladenine (3-MA) or vehicle control (ultra pure distilled H₂O: C) for 5 hours, after which cell lysates were prepared, and the presence of each protein was assessed by Western blotting. Band densities were digitized and semi-quantitated. Data are presented as the mean \pm SEM. * $p < 0.05$. $n = 3$