Cell Death & Disease

Supporting Information

Regulating autophagy facilitated therapeutic efficacy of sonic hedgehog pathway inhibition on lung adenocarcinoma through GLI 2 suppression and ROS production

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Figure S1 Upregulated GLI1 expression in LUAD cells after SMO activation. Cells were treated with/without SAG for 24 h, followed by extraction of the mRNA. The GLI1 mRNA level was normalized by the level of GAPDH * $P < 0.05$ v.s. Vehicle; ** $P < 0.01$ v.s. Vehicle. The experiments were repeated for 3 times.

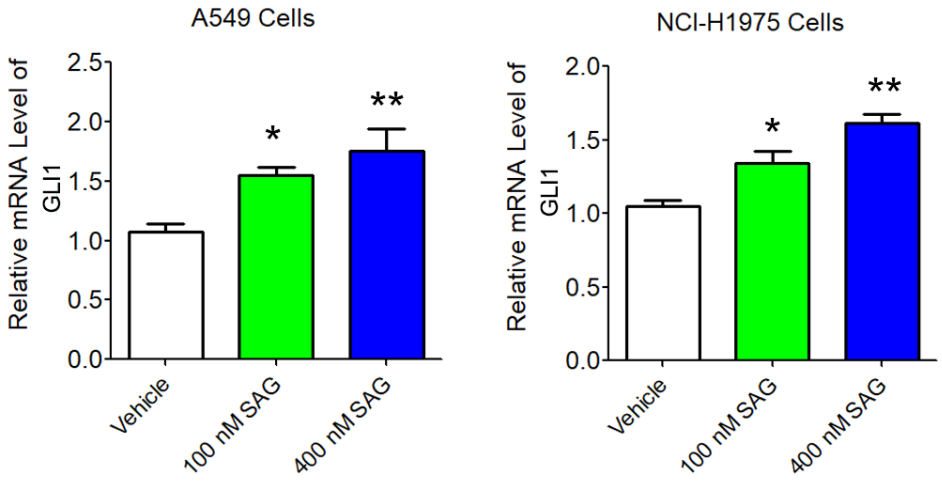

Figure S2 Statistical analysis of related protein level in Figure 2D (n = 6)

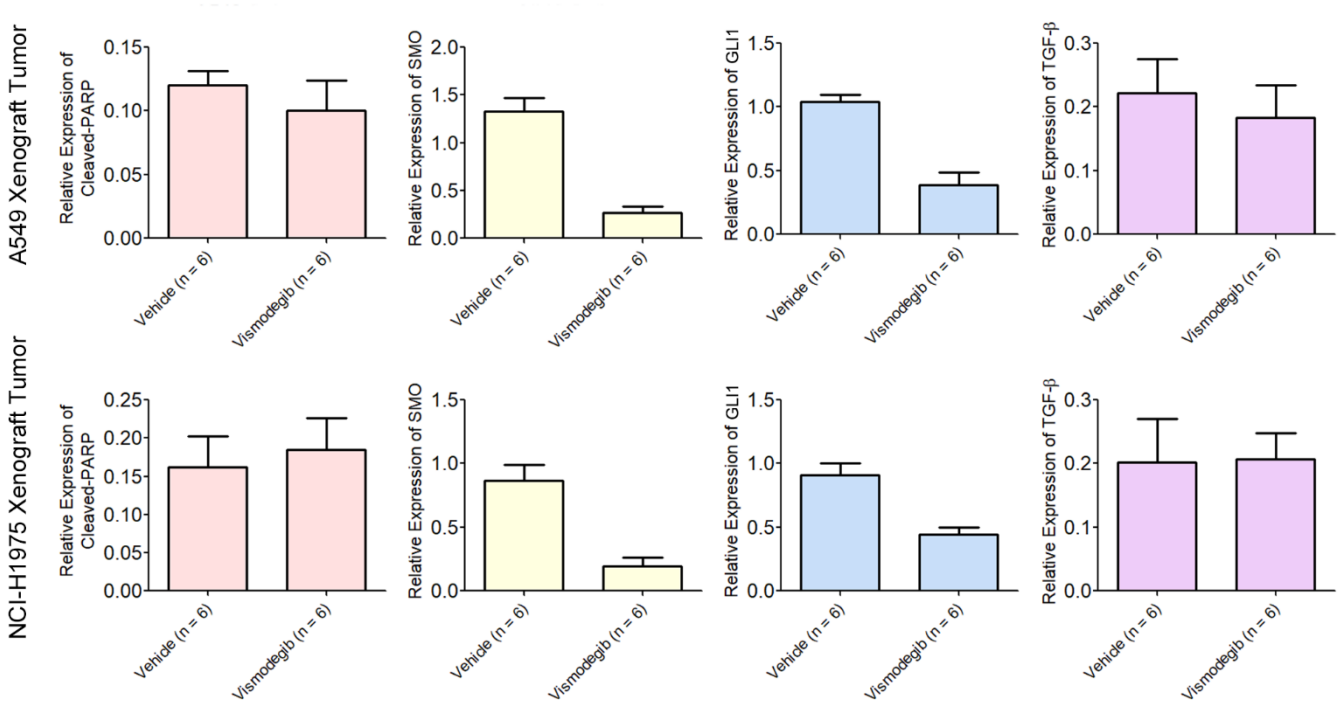

Figure S3 Enhanced transcription of ATG5 and ATG7 in A549 and NCI-H1975 cells after vismodegib treatment. A549 Cells (A) and NCI-H1975 cells (B) were lysed by Trisol and prepared for Realtime-PCR detection after vismodegib exposure (n = 3). The experiments were performed for 3 times.

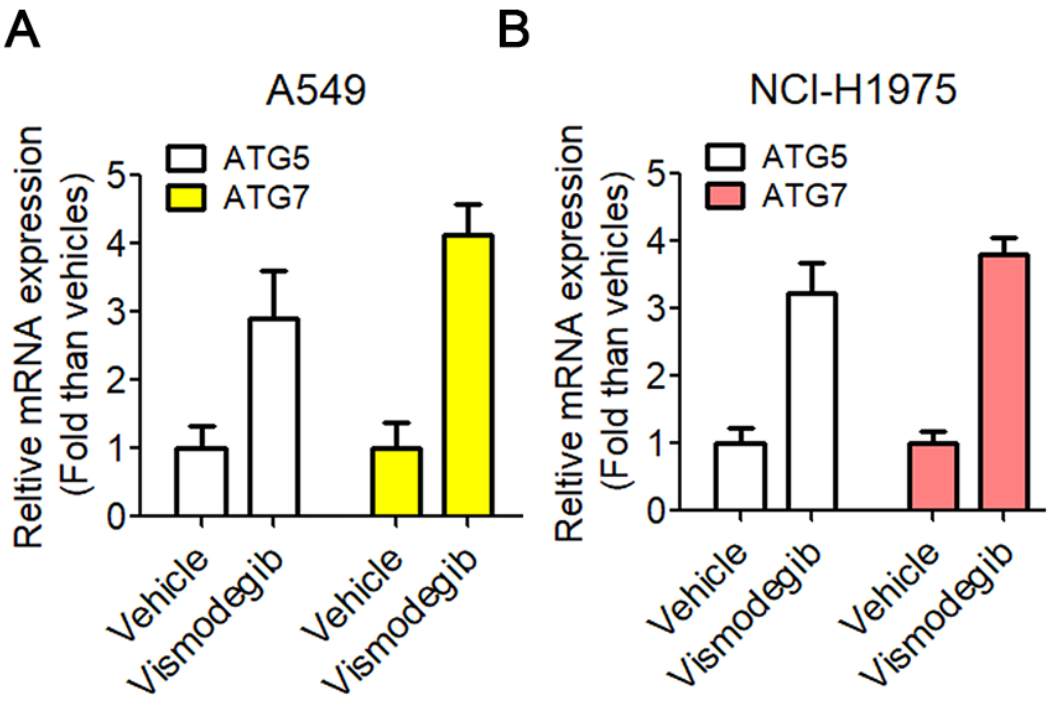

Figure S4 Autophagosomes formation *in vitro* and *in vivo* after vismodegib therapy

A, B: Suppression of GLI 1 expression and increased LC3-II level after cells exposure to vismodegib. C: Increased LC3-II expression in A549 and NCI-H1975 transplanted tumors. D: Vismodegib induced autophagic vesicles formation in A549 and NCI-H1975 cells. Cells were treated with 30 μM of vismodegib for 12h and 24 h. Then, cyto-ID green dye was applied to detect the autophagosomes formation in A549 and NCI-H1975 cells.

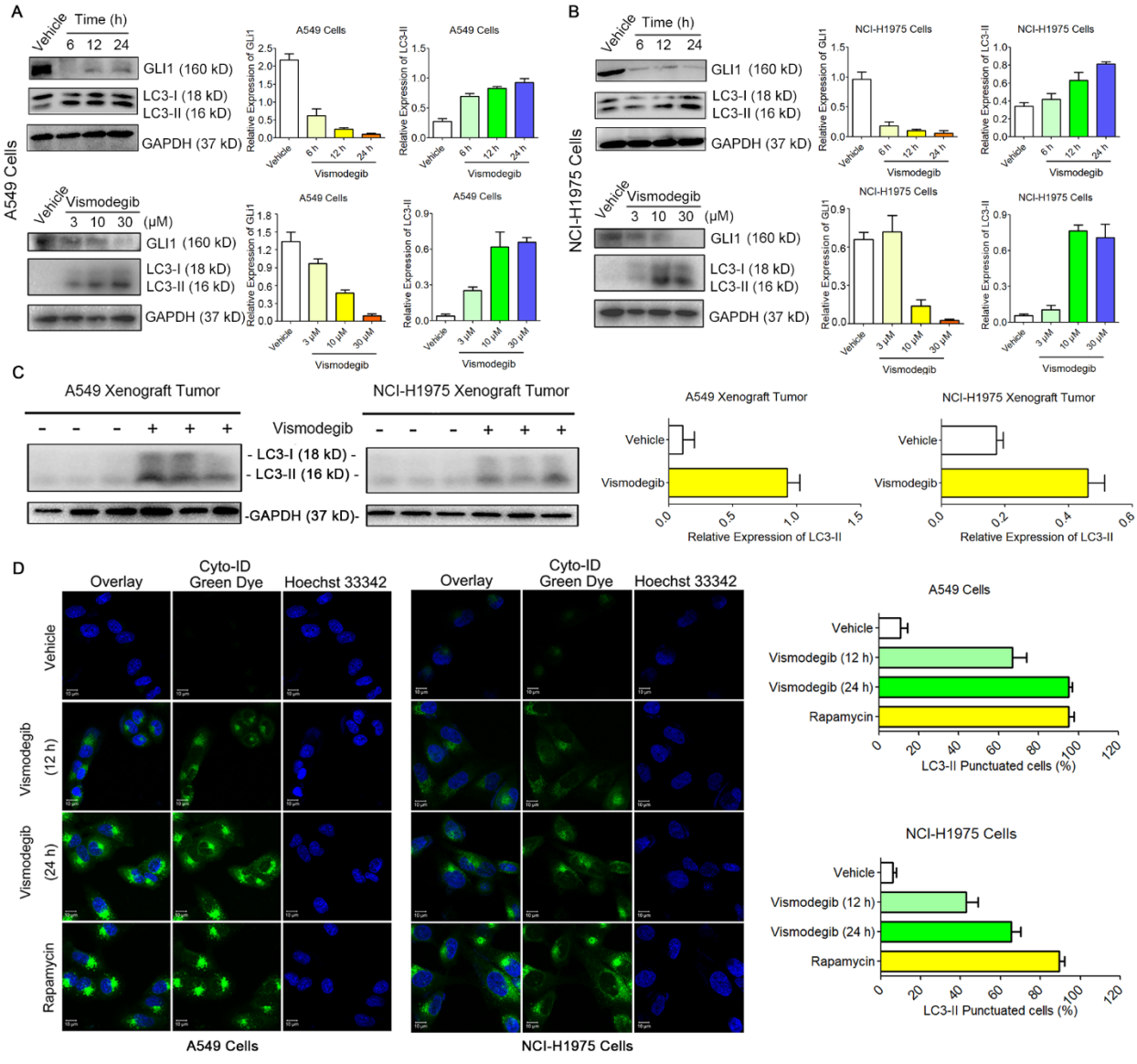

Fig. S5 Vismodegib induce neither growth inhibition (A, B) nor upregulated level of LC3-II (C) in HEK293 cells.

HEK293 cells were treated with different concentrations of vismodegib for 12-48 h. MTT assays were applied to detect the cytotoxic effect of vismodegib on HEK293 cells, and western blots were employed to monitor the change of LC3-II after vismodegib exposure. Both western blots and MTT assays have been repeated for 3 times

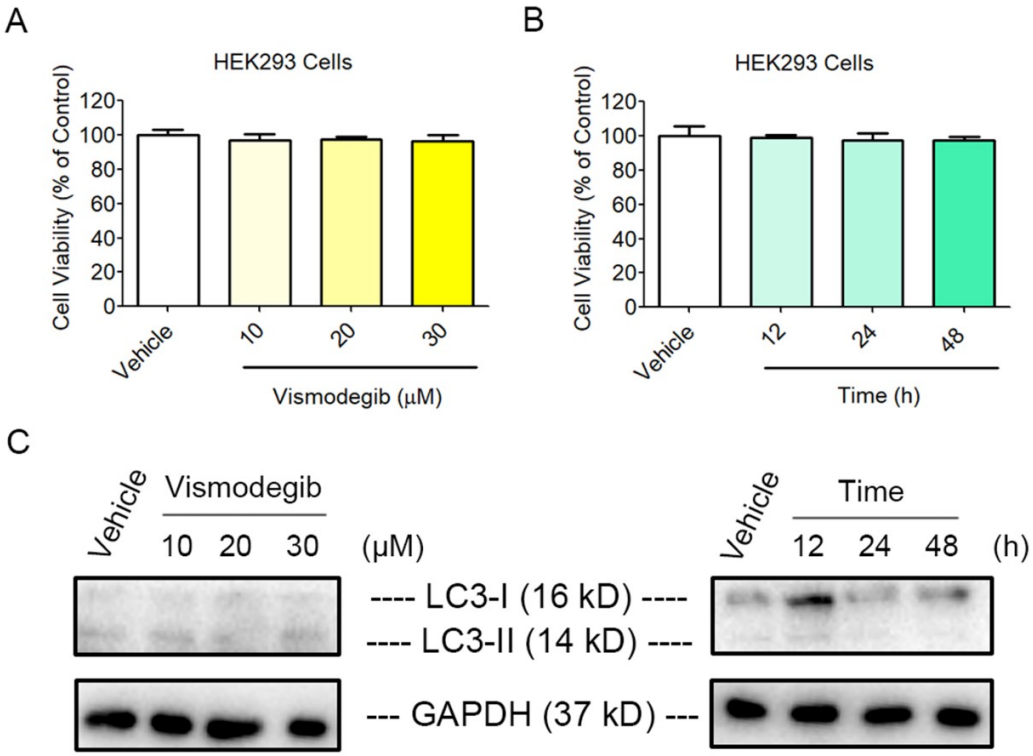

Figure S6 A549 xenograft tumors after mice were sacrificed after 28-day treatment.

A549 xenograft tumors were collected immediately when mice were sacrificed. (Cyclophosphamide: CTX)

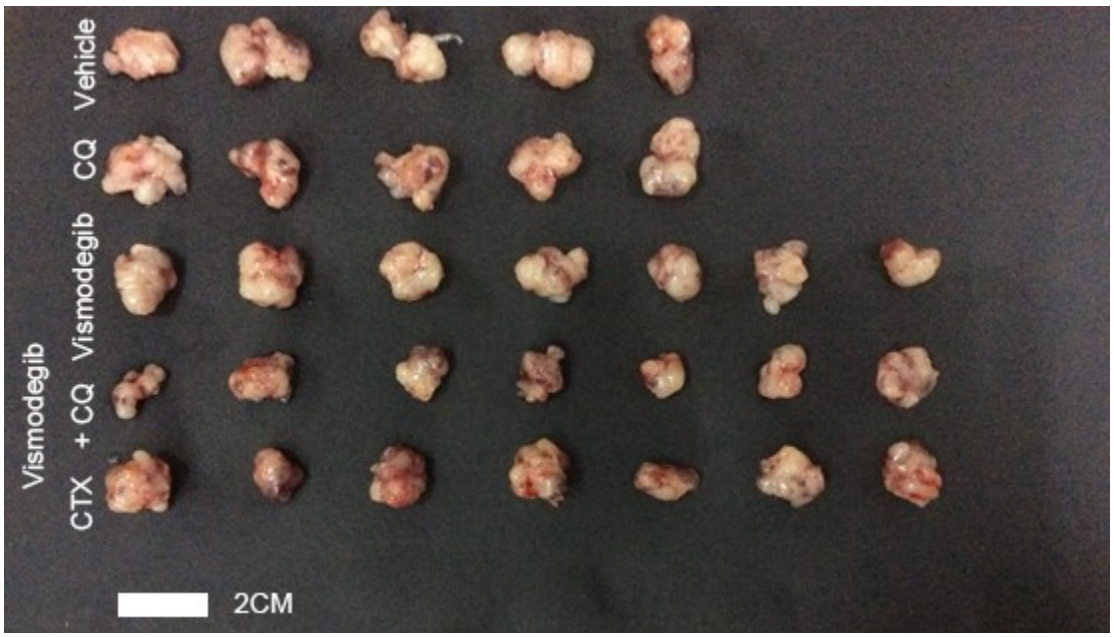

Figure S7 NCI-H1975 xenograft tumors after mice were sacrificed after 28-day treatment. NCI-H1975 xenograft tumors were collected immediately when mice were sacrificed. (Cyclophosphamide: CTX)

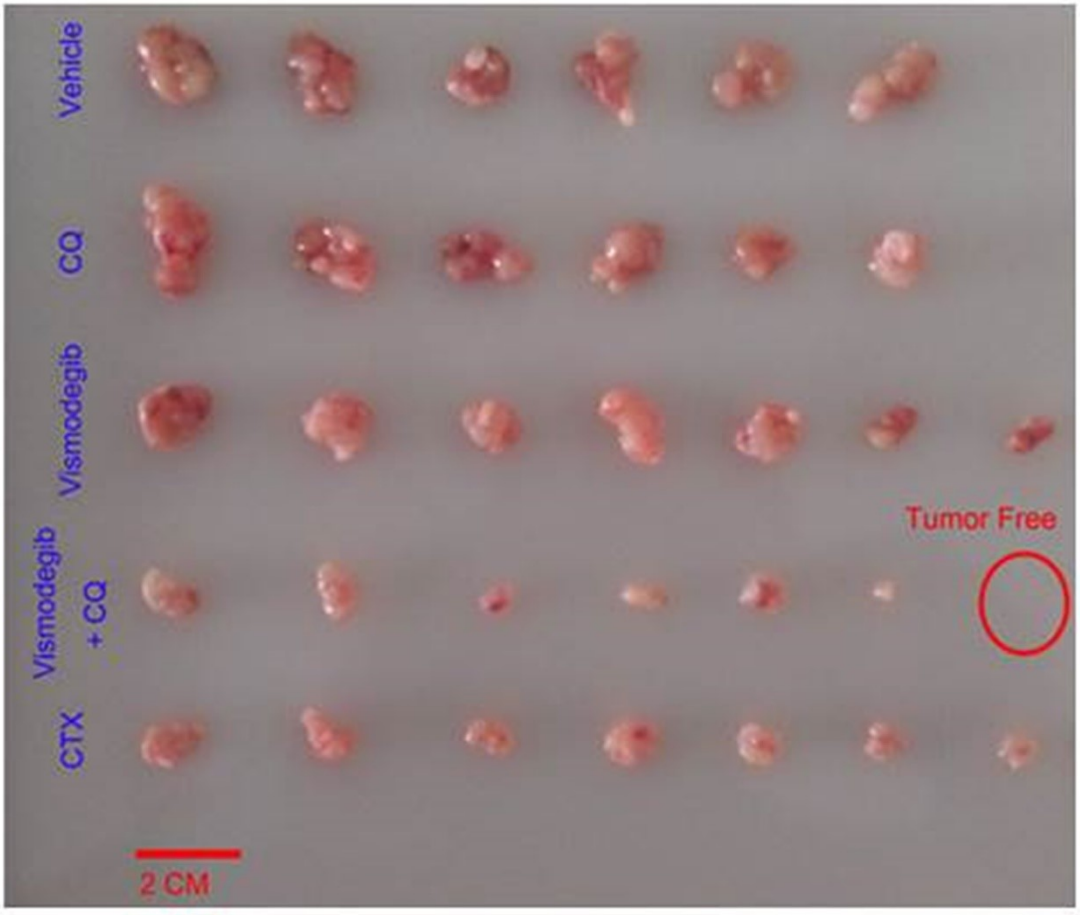

Figure S8 Statistical analysis of related protein level in Figure 6A (n = 5)

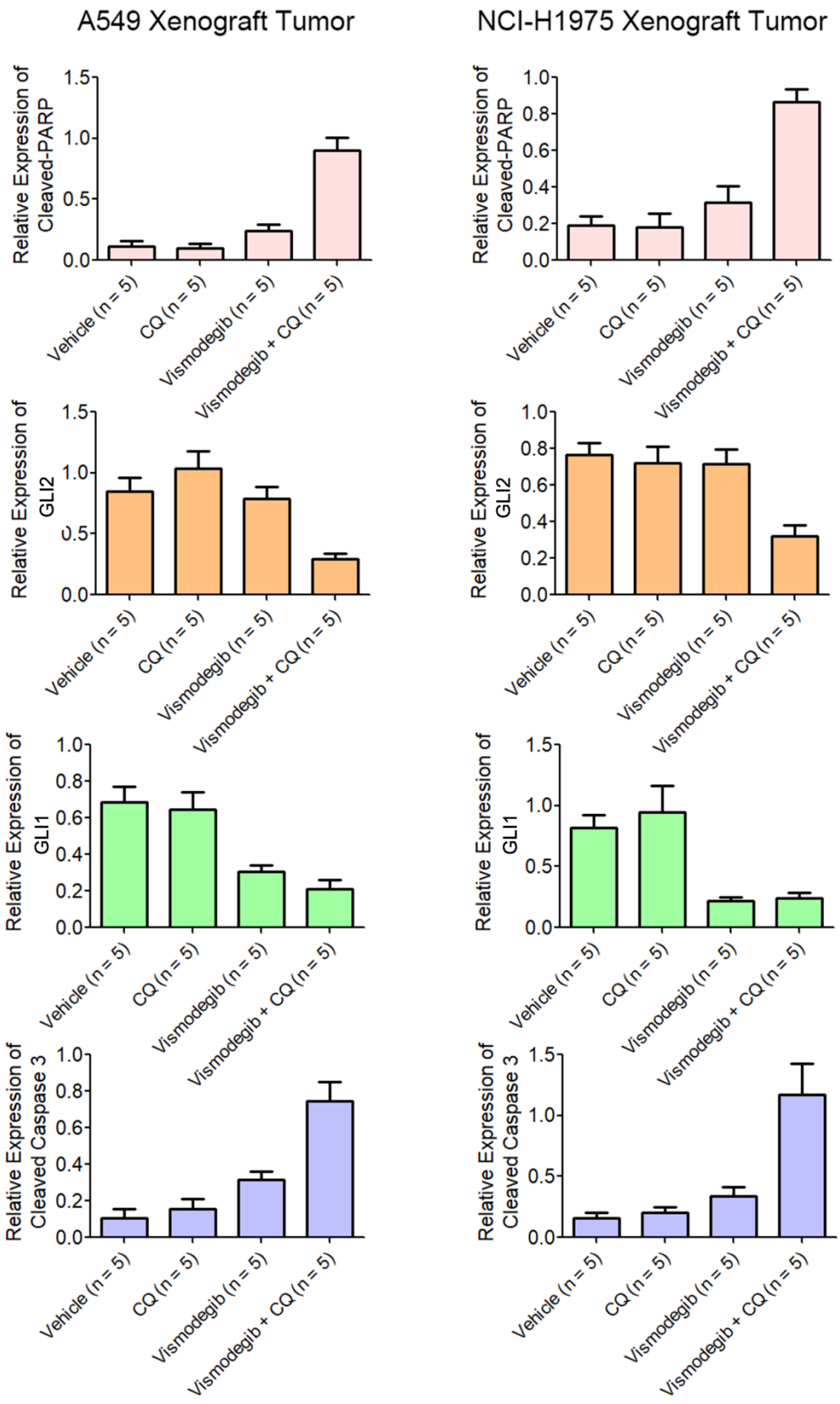

Figure S9. Caspase 3-dependent cytotoxic effect of vismodegib after autophagy blockage in A549 and NCI-H1975 cells.

Cells were treated with vismodegib, Z-VAD-fmk or chloroquine (CQ) for 72 h. The activation of Caspase 3 and the cell viability was measured by western blot and MTT assay, respectively. Both the western blot and the MTT assay have been repeated for 3 times.

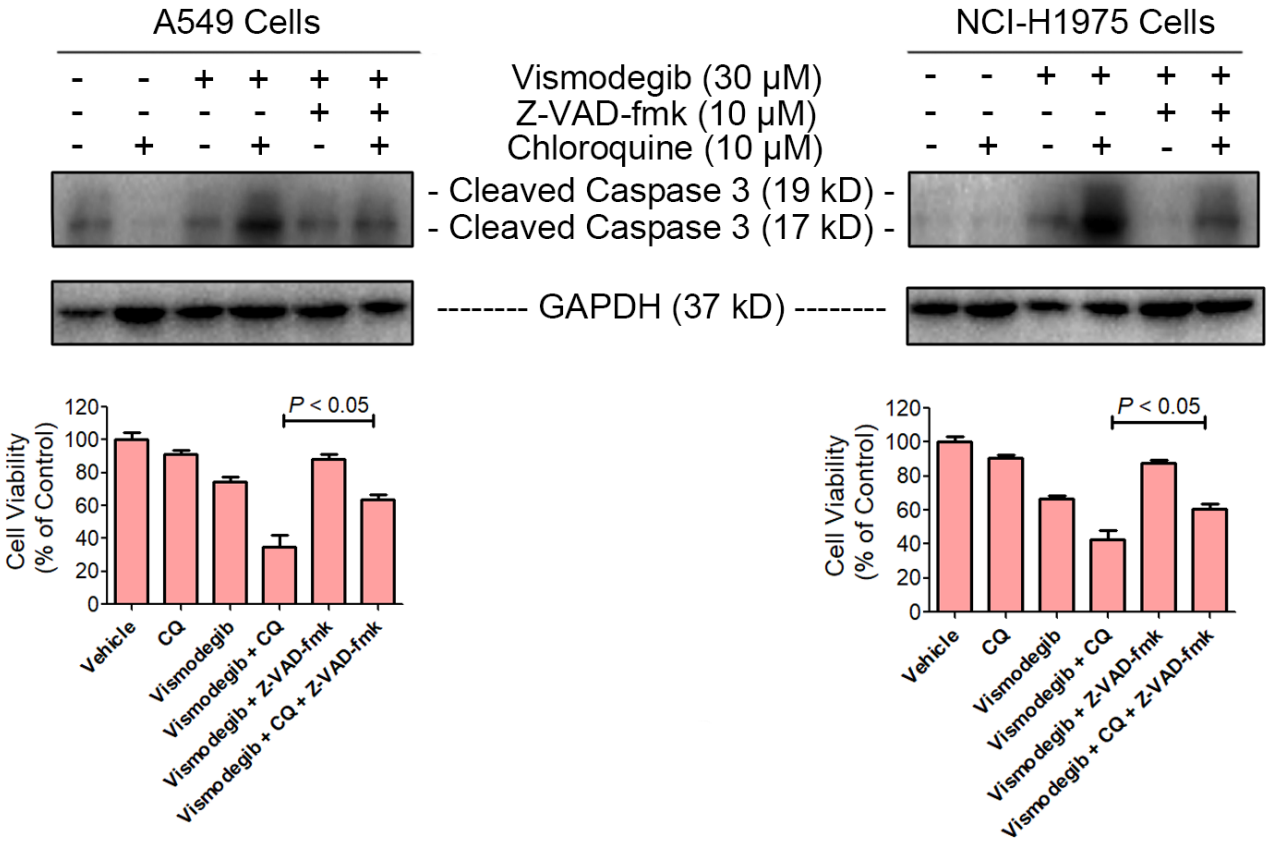

Figure S10 Knockdown of GLI2 promoted the anti-LUAD effect of vismodegib in A549 and NCI-H1975 cells

Cells were treated with anti-GLI2 siRNA for 48 h to silence the expression of GLI2, and then co-cultured with vismodegib for 72 h. The experiments were performed for 3 times.

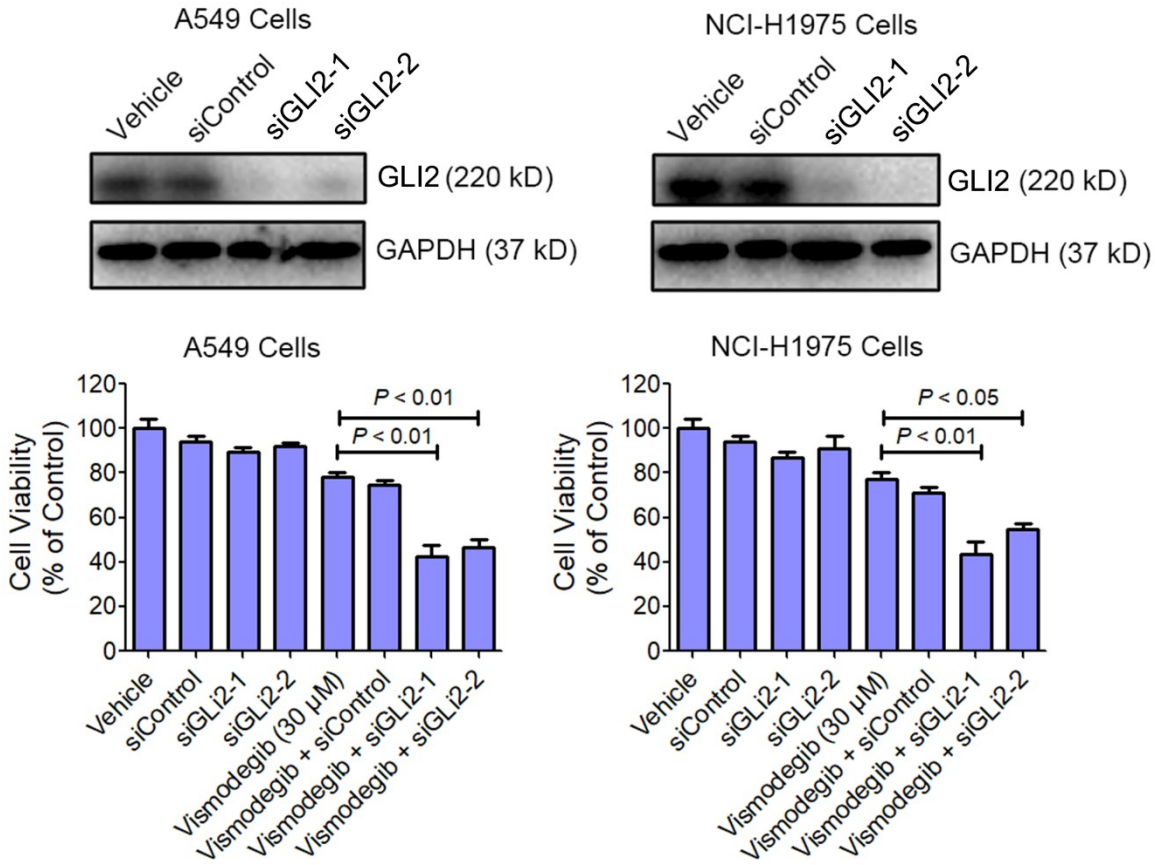

Figure S11. Repeated results of Fig. 1A, B (A), Fig. 2D (B), and Fig. 6A (C)