JCB

Wang et al., https://doi.org/10.1083/jcb.201708064

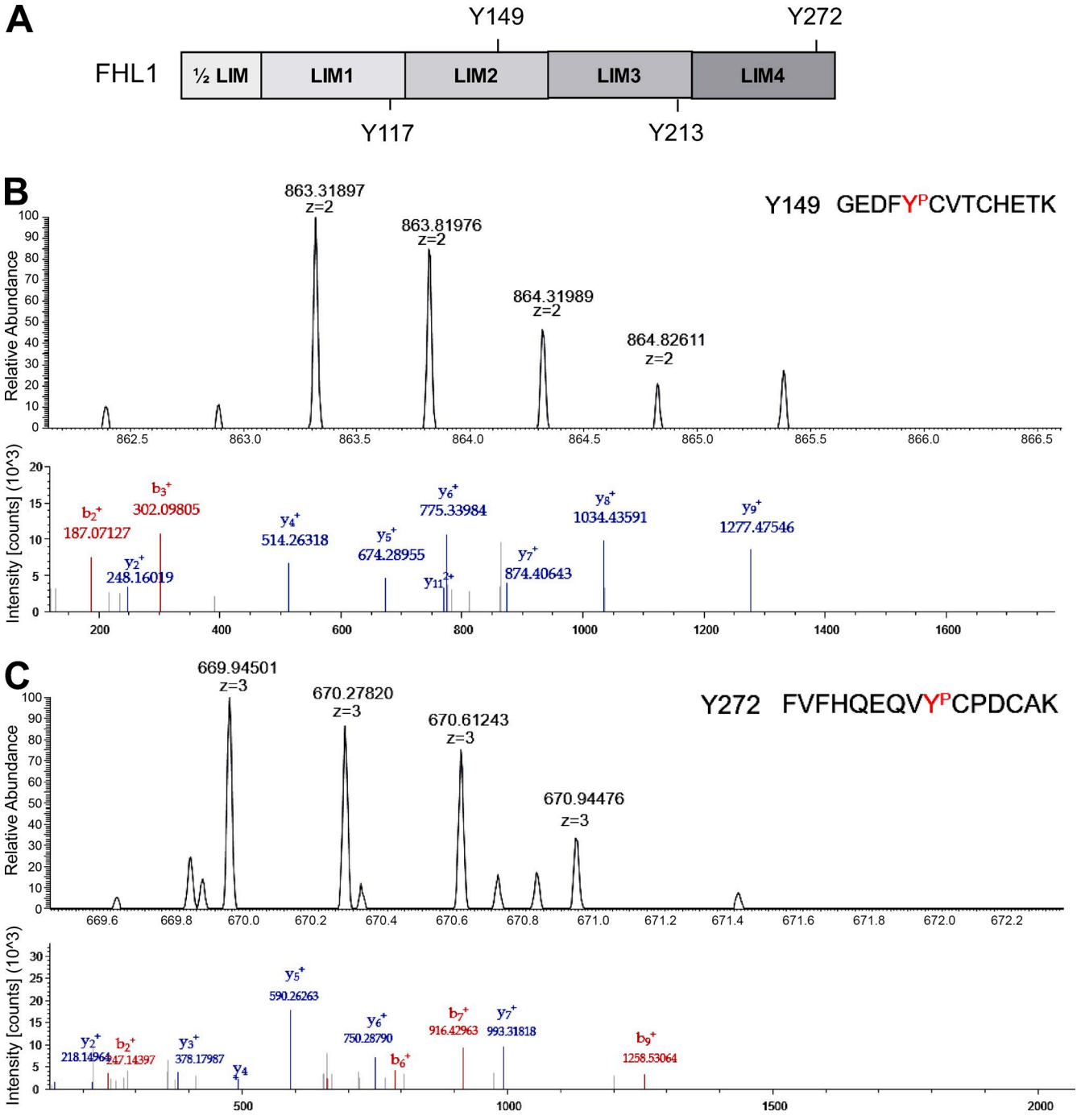

Figure S1. **c-Src mediates FHL1 phosphorylation at Tyr149 and Tyr272. (A)** Amino acid sequence of human FHL1 showing four putative tyrosine phosphorylation sites, Tyr117, Tyr149, Tyr213, and Tyr272, detected by mass spectrometry. **(B and C)** Tandem mass spectrometry spectra and sequence coverage (y and b ions) for the FHL1 phosphopeptides GEDFY^{P}CVTCHETK and FVFHQEQVY^{P}CPDCAK derived from FHL1 coexpressed with c-Src in HeLa cells.

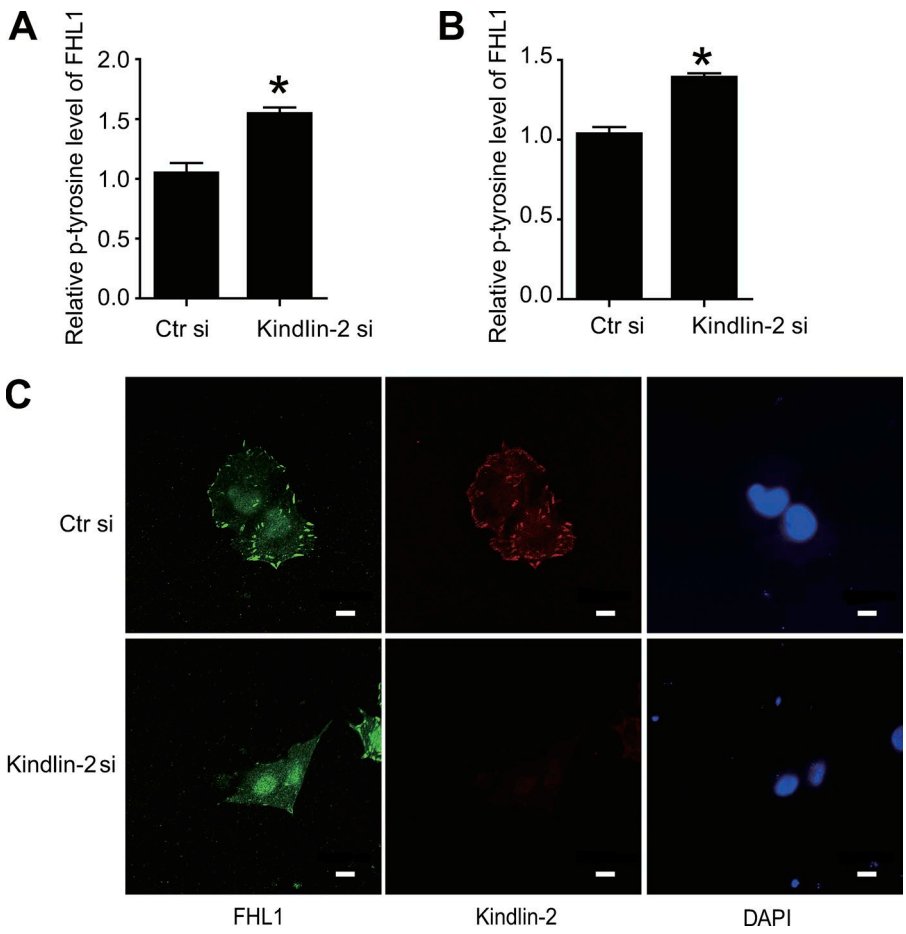

Figure S2. **Statistical analysis of Fig. 4 (D and E) and subcellular localization of FHL1 regulated by kindlin-2. (A)** Statistical analysis of Fig. 4 D. p-Tyr levels of FHL1 were detected in H1299 cells transfected with control siRNA or kindlin-2 siRNA for 48 h. **(B)** Statistical analysis of Fig. 4 E. HeLa cells were transfected with indicated plasmids, and siRNAs and p-Tyr levels of FHL1 were detected. The intensity of the p-Tyr bands from the FHL1 IP from three separate biological replicates was quantified and statistically analyzed by two-tailed Student's *t* tests. *, $P < 0.05$. **(C)** H1299 cells transfected with the control siRNA or kindlin-2 siRNA were replated on FN-coated coverslips for 4 h and stained with anti–kindlin-2 and anti-FHL1 antibodies. The nuclei were stained with DAPI. Bars, 10 µm.

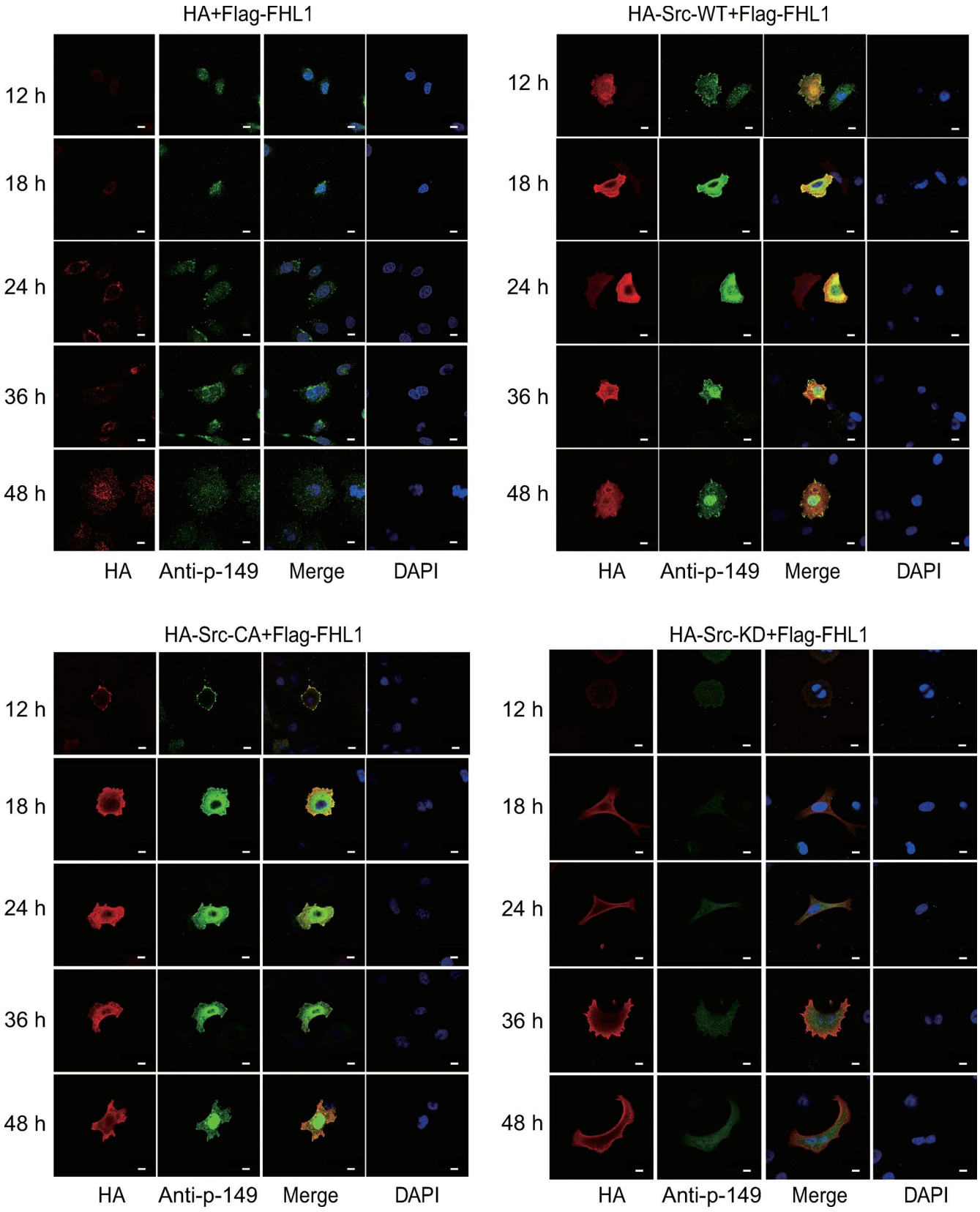

Figure S3. **Time series of FHL1 phosphorylation induced by Src.** HeLa cells transfected with different constructs of Src for different time were replated on FN-coated coverslips for 4 h and stained with anti-HA and anti–p-FHL1 antibodies. The nuclei were stained with DAPI. Bars, 10 μm.

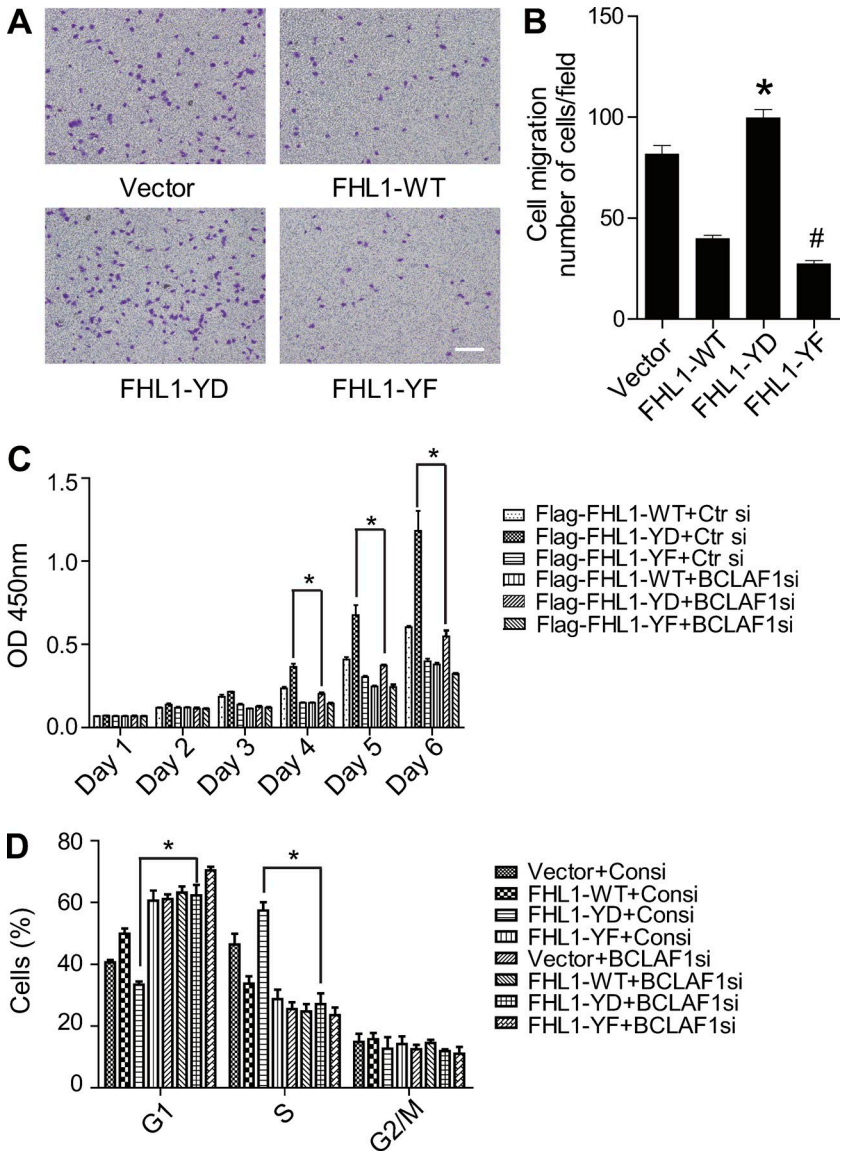

Figure S4. **FHL1 phosphorylation promotes lung cancer cell migration and statistical analysis of Fig. 7 (H and J). (A and B)** H1299 cells stably expressing FHL1-WT, FHL1-Y149-272D (FHL1-YD), and FHL1-Y149-272F (FHL1-YF) were established, and a transwell assay was performed to examine the effect of FHL1 phosphorylation on H1299 cell migration. Bar, 50 µm. *, $P < 0.05$ versus vector group; #, $P < 0.05$ versus FHL1-Y149-272D. **(C)** A WST1 assay was performed when BCLAF1 was knocked down in H1299 cells stably expressing FHL1-WT, FHL1-Y149-272D, and FHL1-Y149-272F mutants. **(D)** Effect of BCLAF1 depletion by siRNA on the distribution of the cell cycle of H1299 cells analyzed by flow cytometry. Statistical analyses were performed by one-way ANOVAs. Values shown are means ± SD of triplicate measurements. *, $P < 0.05$.

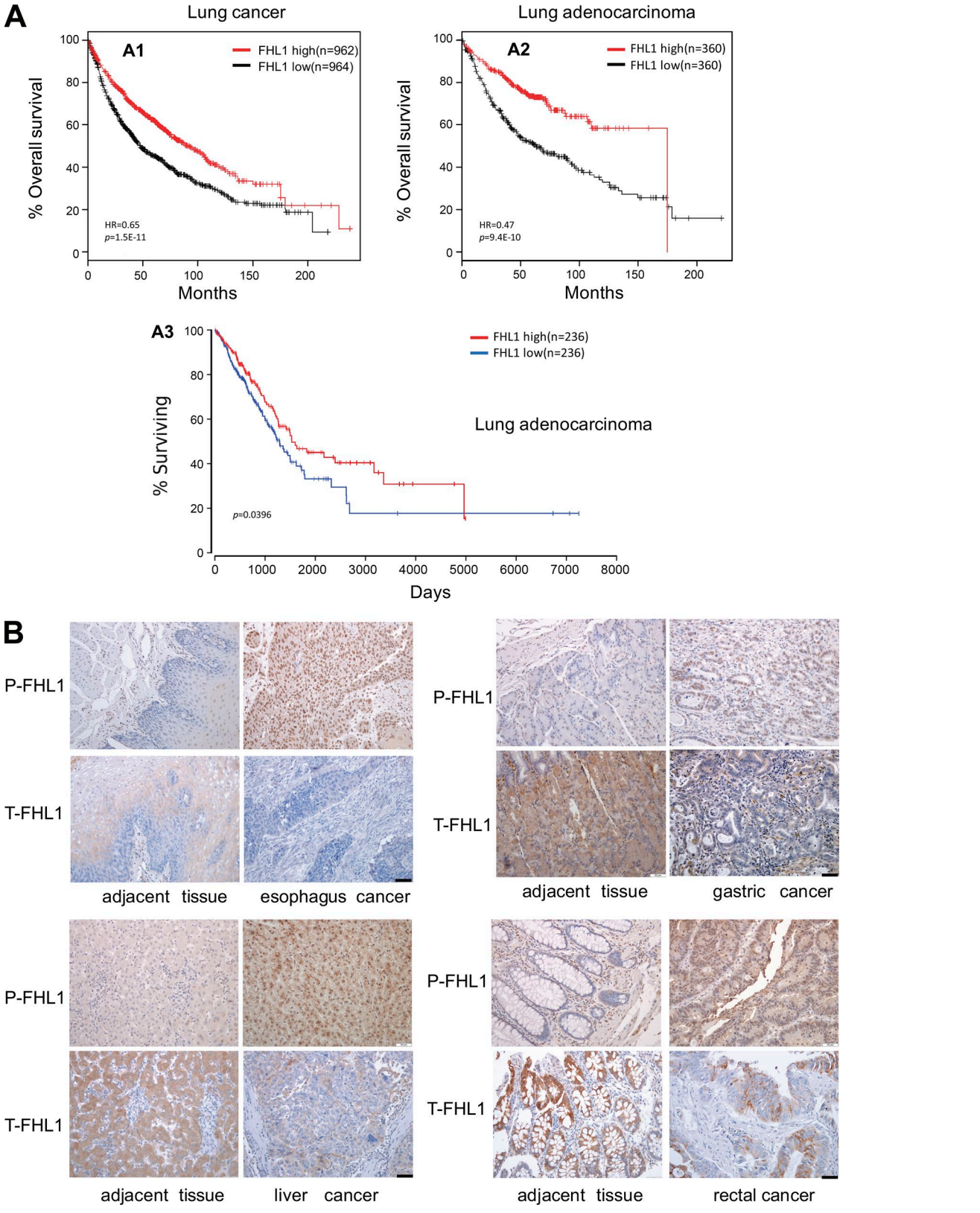

Figure S5. **Analysis of FHL1 expression by databases and tissue chips. (A)** Data of FHL1 expression were analyze by the Kaplan–Meier Plotter analysis database (A1 and A2) and the TCGA database (A3). HR, hazard ratio. **(B)** Phosphorylation of FHL1 and total FHL1 expression in tissue chips of multiple organ cancer was determined by immunohistochemistry using anti–p-FHL1 or anti-FHL1 antibodies. The images of liver cancer, gastric cancer, rectal cancer, and esophagus cancer are shown. Bars, 50 μm.

Table S1 is a separate Excel file showing mass spectrometry spectra analysis of the FHL1 interaction with BCAFL1 in the nucleus.