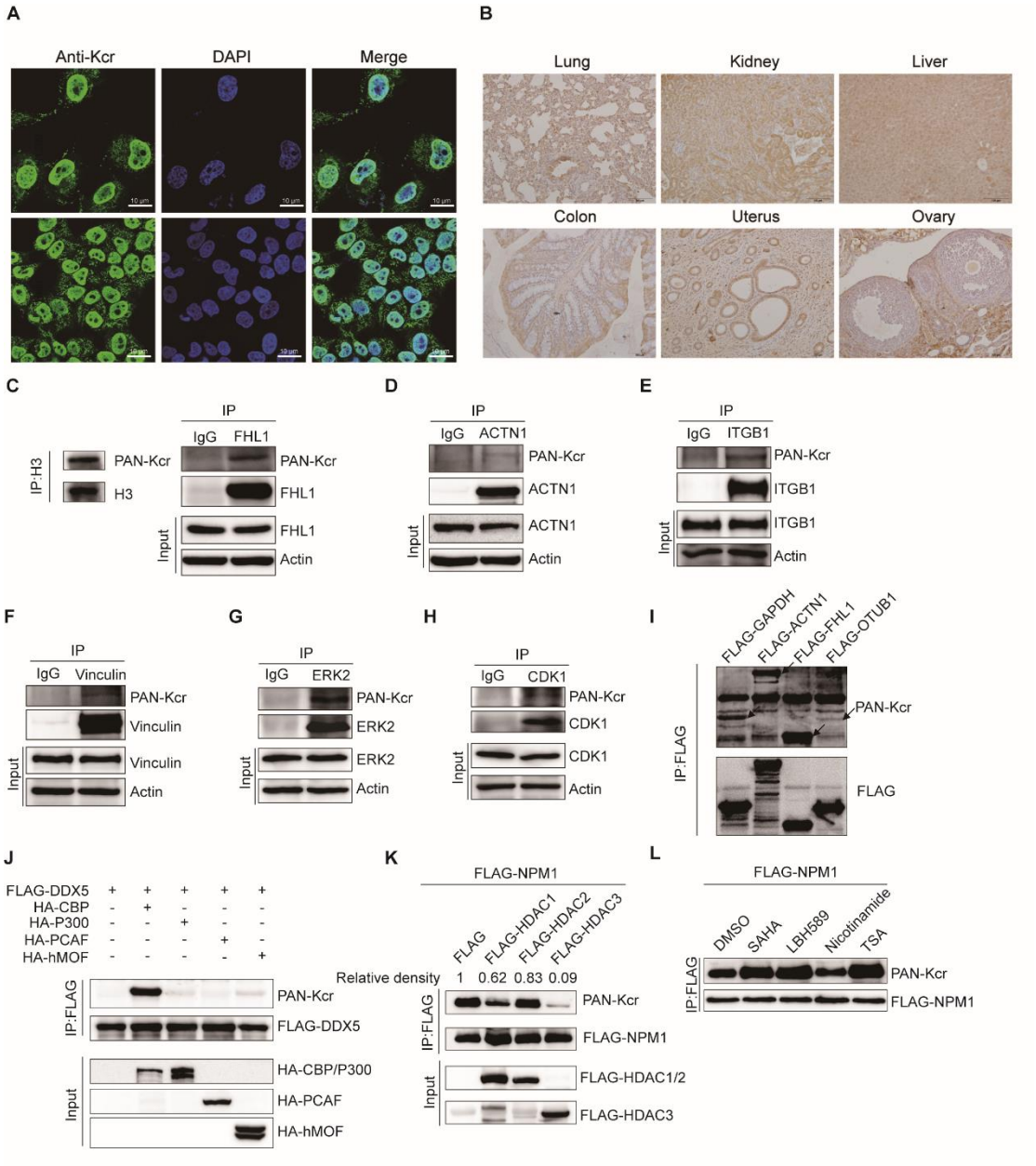

Supplementary Figure S2

(A) The crotonylation proteins were detected in H1299 and Hela cells by immunofluorescence using an anti-Kcr antibody (green), and the nuclei was stained with DAPI (blue), followed by visualization with confocal microscopy. Scale bars: 10μm.

(B) The crotonylation proteins were detected in multiple mouse tissues by immunohistochemistry using an anti-Kcr antibody.

(C-H) Endogenous validation of lysine crotonylated proteins. H1299 cells were treated

with 3μM TSA and 5mM nicotinamide for 12h, and then cell lysates were immunoprecipitated with the antibody of H3, FHL1, ACTN1, ITGB1, Vinculin, ERK2, and CDK1 respectively, followed by immunoblotting with pan-Kcr antibody.

(I) Independent validation of lysine crotonylated proteins. H1299 cells separately transfected with FLAG tagged GAPDH, ACTN1, FHL1 and OTUB1 were treated with 3μM TSA and 5mM nicotinamide for 12h, and then cell lysates were immunoprecipitated with an anti-FLAG antibody, followed by immunoblotting with pan-Kcr antibody.

(J) FLAG tagged DDX5 expression vector was co-transfected into H1299 cells separately with a variety of acetyltransferases expression vectors. Forty-eight hours post transfection, cell lysates were immunoprecipitated with an anti-FLAG antibody, followed by Western blot analysis with pan-Kcr antibody.

(K) HDAC1 and HDAC3 decrotonylated NPM1 in vivo. H1299 cells were co-transfected separately with expression vectors containing FLAG-NPM1, HA-CBP and HDAC1, HDAC2, HDAC3. Western blot analysis was performed with pan-Kcr antibody.

(L) The HDAC inhibitor SAHA and LBH589 increased the NPM1 crotonylation level. H1299 cells were transfected with FLAG-NPM1 and HA-CBP, then treated respectively with 3μM TSA, 10μM SAHA, 150nM LBH589 or 5mM nicotinamide for 12 h. Cell lysates were immunoprecipitated with an anti-FLAG antibody, followed by Western blot analysis with a pan-Kcr antibody.