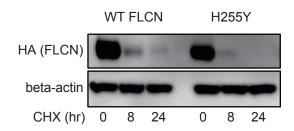


HA-tagged FLCN WT, FLCN H255Y and FLCN K508R constructs were stably transduced into FLCN-deficient UOK257 renal tumor cell line using the lentiviral doxycycline-inducible system (Clontech) and their equivalent mRNA expression after the addition of 0.3 ng/µl doxycycline for 24 hours was confirmed by real-time PCR (left panel). Western blot analysis was performed using HA antibody (6E2) and a-tubulin (Cell Signaling), and FLCN H255Y protein was expressed at a lower level relative to FLCN WT protein whereas FLCN K508R protein was expressed at a level equivalent to FLCN WT (right panel).

Supplemental Figure 2.



The cell lines used in Supplemental Figure 1 were treated with 100μ g/ml cycloheximide (CHX) for 0, 8 and 24 hours and western blot analysis was performed using HA antibody (6E2) and beta-actin (Cell Signaling). FLCN H255Y protein was expressed at a lower level relative to FLCN WT protein and degraded faster than FLCN WT.