

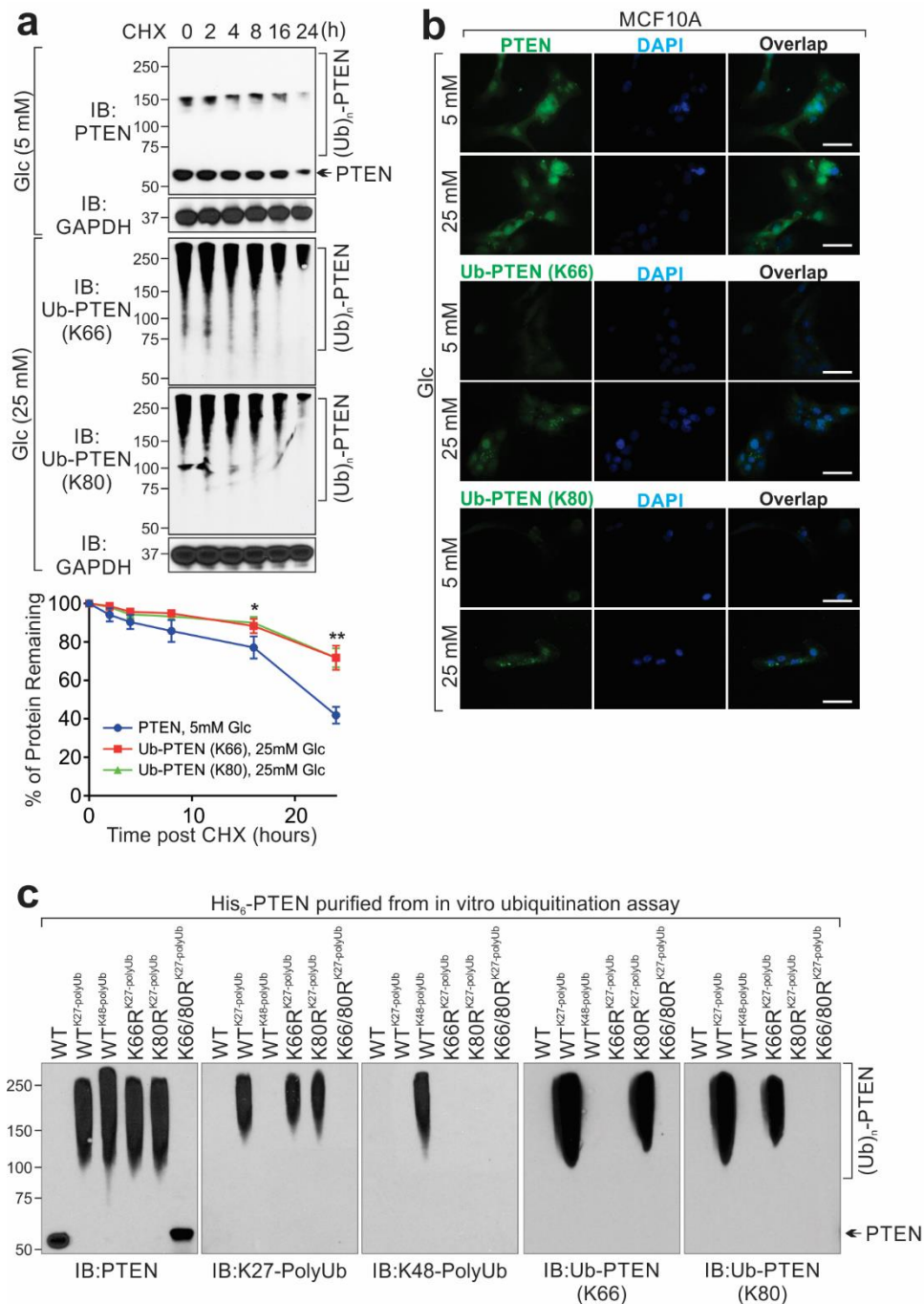


Figure S5: K27-linked polyubiquitination exhibit minimal effect on the stability and subcellular localization of PTEN.

(a) Stability of PTEN or PTEN^{K27-polyUb} recognized by Ub-PTEN (K66) or Ub-PTEN (K80) antibodies respectively after CHX treatment (100 μ g/ml) for indicated time in glucose-starved

MCF10A *PTEN*^{+/+} or MCF10A *PTEN*^{-/-} cells followed by glucose stimulation (5mM or 25mM). Top panel: Representative blots are shown. Bottom panels: Protein bands from the immunoblots were quantitated with Image J software, using the GAPDH signal for normalization. Signal intensities were then normalized to the 0 hr time point. Error bars, SD, n=3 independent experiments, one-way ANOVA. **(b)** Immunofluorescence detection of PTEN, Ub-PTEN (K66) and Ub-PTEN (K80) in MCF10A cells with glucose starvation followed by glucose stimulation (5mM or 25mM, 1 hr). Scale bars: 50 μ m. **(c)** IB detection of PTEN or indicated polyubiquitinated PTEN purified from *in vitro* ubiquitination assay (n.s. $p>0.05$ and $*p<0.05$, and $**p<0.01$).