

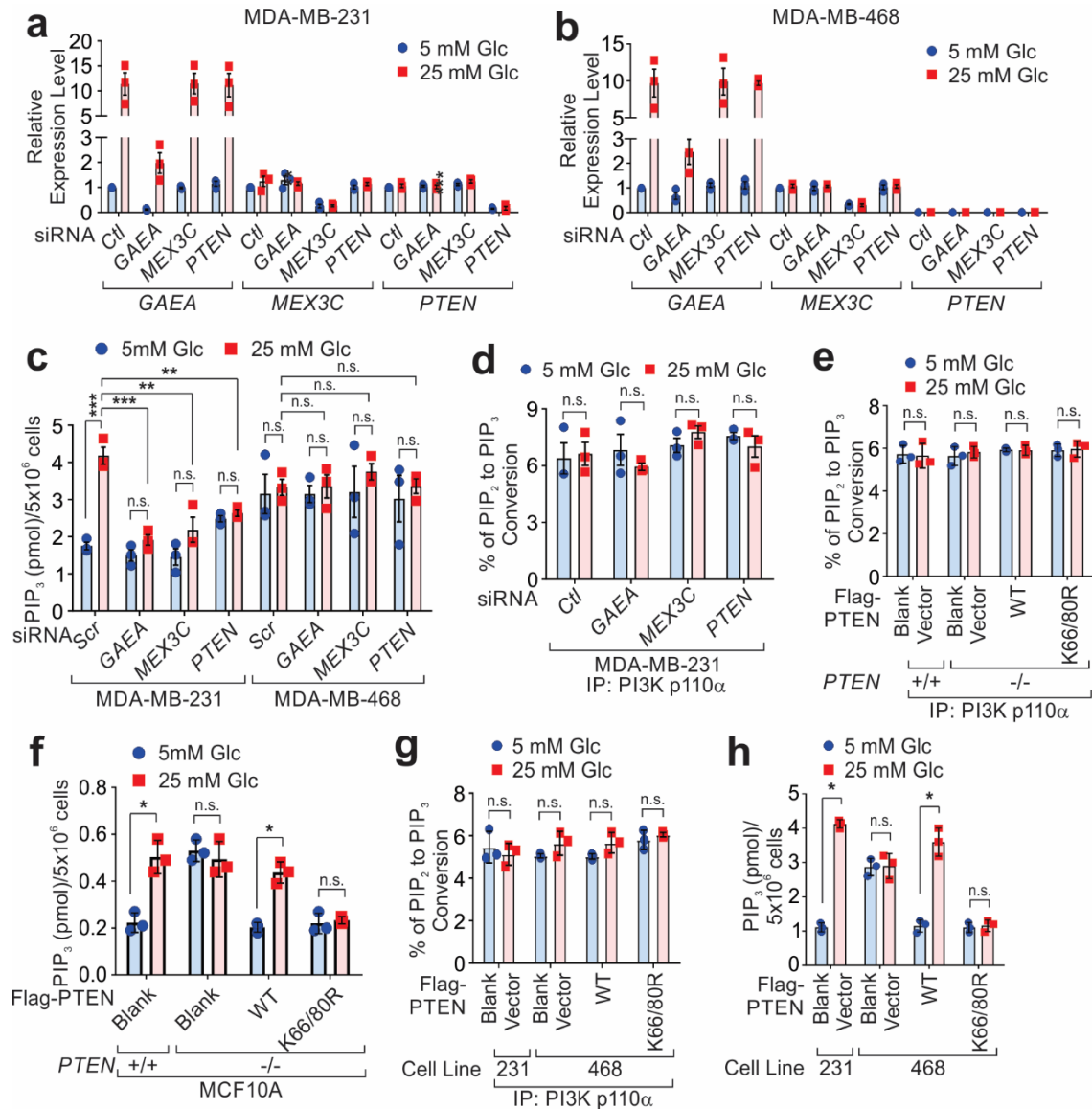


Figure S8: K27-linked polyubiquitination regulates the phosphoinositide phosphatase activity of PTEN *in vivo*.

(a, b) RT-qPCR detection of indicated gene expression in MDA-MB-231 (a) and MDA-MB-468 (b) cells transfected with indicated followed by glucose starvation and stimulation (5mM or 25mM, 1 hr). (c) Quantitative measure of cellular PIP₃ level in MDA-MB-231 and MDA-MB-468 cells transfected with indicated siRNAs. (d) Measurement of kinase activity of PI3K immunoprecipitated from MDA-MB-231 cells transfected with indicated siRNA or vectors

followed by glucose starvation and stimulation (5mM or 25mM, 1 hr). **(e, f)** Measurement of kinase activity of PI3K immunoprecipitated (e) or quantitative measure of cellular PIP₃ level (f) in MCF10A *PTEN*^{+/+} and MCF10A *PTEN*^{-/-} cells transfected with indicated vectors, followed by glucose starvation and stimulation (5mM or 25mM, 1 hr). **(g, h)** Measurement of kinase activity of PI3K immunoprecipitated (g) or quantitative measure of cellular PIP₃ level (h) in PIP₃ in MDA-MB-231 or MDA-MB-468 cells transfected with indicated vectors followed by glucose starvation and stimulation (5mM or 25mM, 1 hr). All error bars, SD, n=3 independent experiments. Student's t-test (n.s. $p>0.05$, * $p<0.05$, ** $p<0.01$, and *** $p<0.001$).