

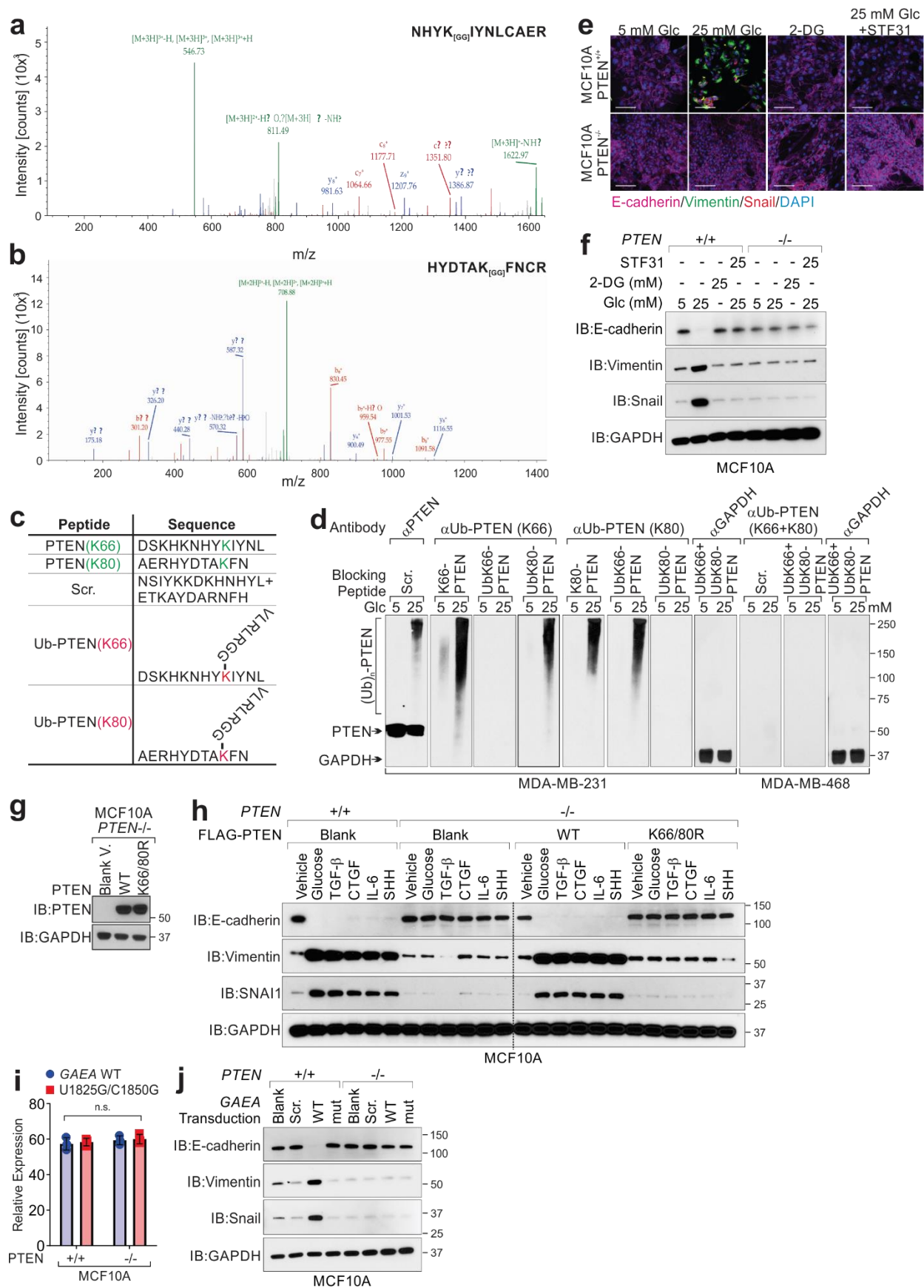


Figure S3: K27-linked polyubiquitination of PTEN promotes EMT.

(a, b) Annotated MS/MS spectrum assigned to the PTEN peptide NHY[GG]KIYNLCAER (c) and HYDTA[GG]KFNCR (d) acquired from analysis of tryptic digest by high-sensitivity LC-MS/MS on an Orbitrap Elite high-resolution mass spectrometer. (c) The peptide sequences for generating Ub-PTEN antibody. (d) Peptide blocking assay in the presence of indicated blocking peptides showing the specific IB detection of Ub-PTEN in glucose-starved MDA-MB-231 or MDA-MB-468 cells followed by glucose stimulation (5 mM or 25mM, 1 hr). IB of GAPDH serves as a negative control. (e, f) Immunofluorescence detection (e) or IB detection (f) using indicated antibodies in MCF10A *PTEN*^{+/+} or MCF10A *PTEN*^{-/-} cells followed by glucose starvation and stimulation (5 or 25 mM, 12d), or 2-DG (25 mM, 12d) or in the presences of STF31 (5 μM, 12d). Scale bars: 50 μm (e). (g, h) IB detection using indicated antibodies in MCF10A *PTEN*^{-/-} cells transfected with indicated expression constructs, followed with indicated stimuli. (i, j) RT-QPCR detection of *GAEA* (i) and IB detection using indicated antibodies (j) in MCF10A *PTEN*^{+/+} or MCF10A *PTEN*^{-/-} cells transduced with lentivirus harboring indicated expression vectors. Error bars (i), SD, n=3 independent experiments, one-way ANOVA. (n.s. $p>0.05$ and $*p<0.05$).