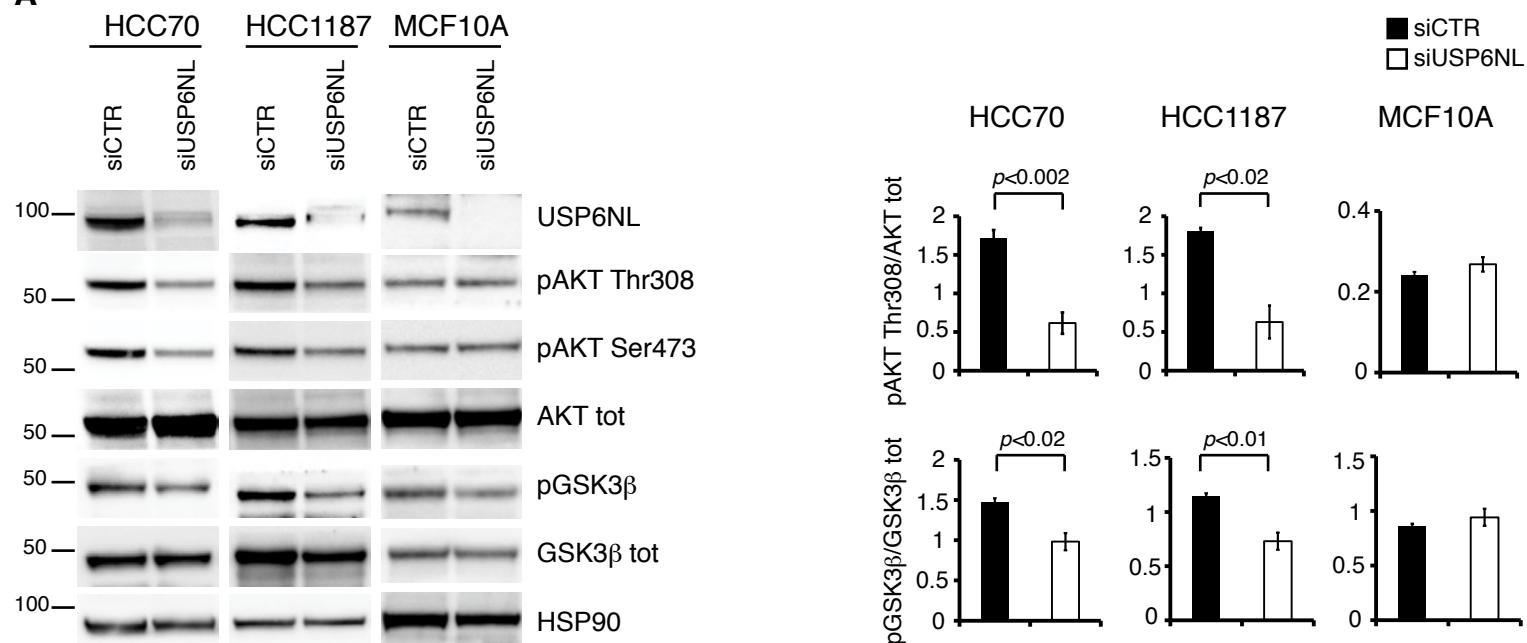
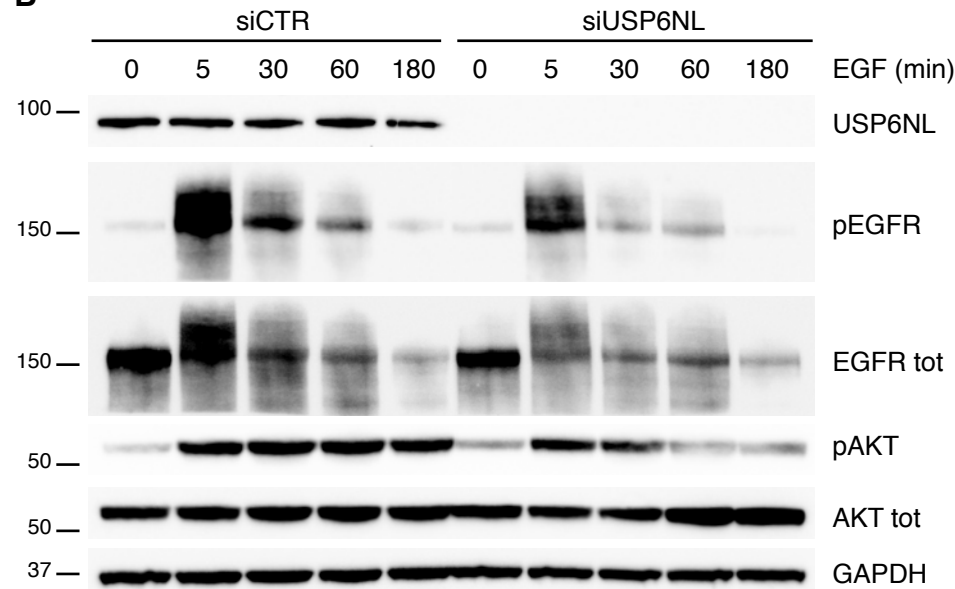


Supplementary Figure S3

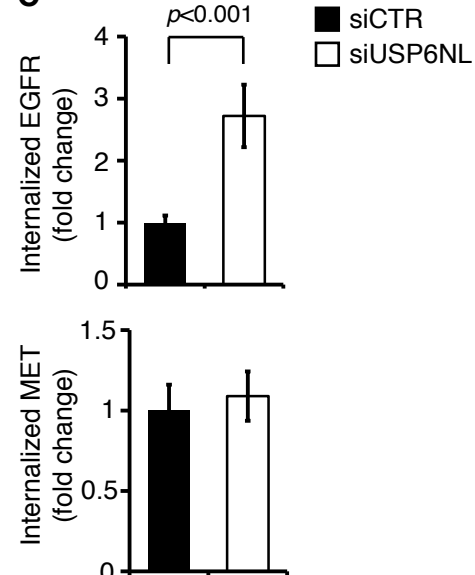
A



B



C



Supplementary Figure S3. Effects of USP6NL silencing on downregulation of AKT and EGFR. A) Total cellular lysates from the indicated cell lines, silenced with control oligos (siCTR) or USP6NL oligo (siUSP6NL) were run on distinct gels. Immunoblotting was as indicated. Detection of phospho/total proteins and of USP6NL/HSP90 was performed on the same gel. Membranes were first immunoblotted with anti-phospho antibodies, stripped and re-probed with anti-total antibodies. Depletion of USP6NL reduces phosphorylation on Thr308 of AKT and dampens the phosphorylation status of GSK3β mainly in the overexpressing BC cell lines. Densitometric analyses are shown on the right. Means ± s.e.m. of at least 3 experiments. B) HCC70 cells were silenced as indicated on top, serum starved and treated with 100 ng/ml of EGF for the indicated time. Immunoblotting was as indicated on the right. USP6NL silencing does not apparently affect the residual EGFR phosphorylation in serum starved cells (time point 0), however it severely reduces EGF-stimulated phosphorylation (at 5, 30, 60, 180 min) as well as the steady-state phosphorylation observed in growing conditions (shown in Figure 3A of main text). C) Analysis of EGFR and MET endocytosis by surface biotinylation. HCC70 cells, silenced with control oligo (siCTR) or with USP6NL oligo (siUSP6NL), were serum starved for 2 hours, surface labeled with cleavable sulfo-NHS-SS-biotin at 4°C for 30 minutes and shifted at 37 °C for 15 min in warm medium containing EGF (100 ng/ml), to stimulate EGFR endocytosis, or HGF (50 ng/ml), for MET endocytosis. Biotin was removed from proteins remaining at the cell surface by incubation with MesNa for 40 minutes at 4°C. Cells were lysed and, after correction to equivalent protein concentrations, the amount of biotinylated intracellular EGFR or MET was determined by capture-ELISA. The amount of internalized receptors was calculated relative to the total amount of cell surface-labeled receptors, after background subtraction. Data were normalized vs. the amount of internalized EGFR or MET in control cells. Mean ± s.e.m n=3, 3 technical replicates.