Figure S1

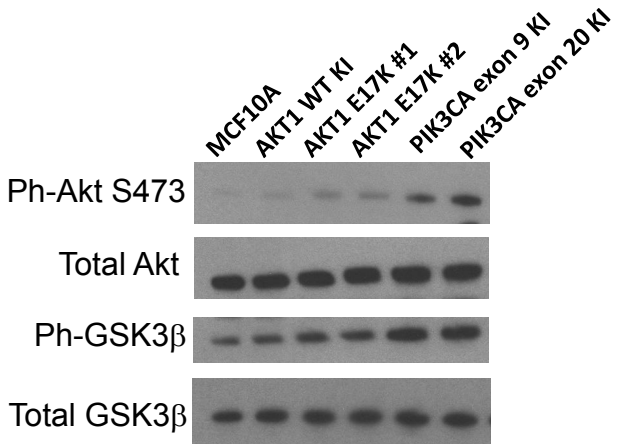

MCF10A
AKT1 WT KI
AKT1 E17K #1
AKT1 E17K #2
PIK3CA exon 9 KI
PIK3CA exon 20 KI
Ph-Akt S473
Total Akt
Ph-GSK3β
Total GSK3β

Figure S2a

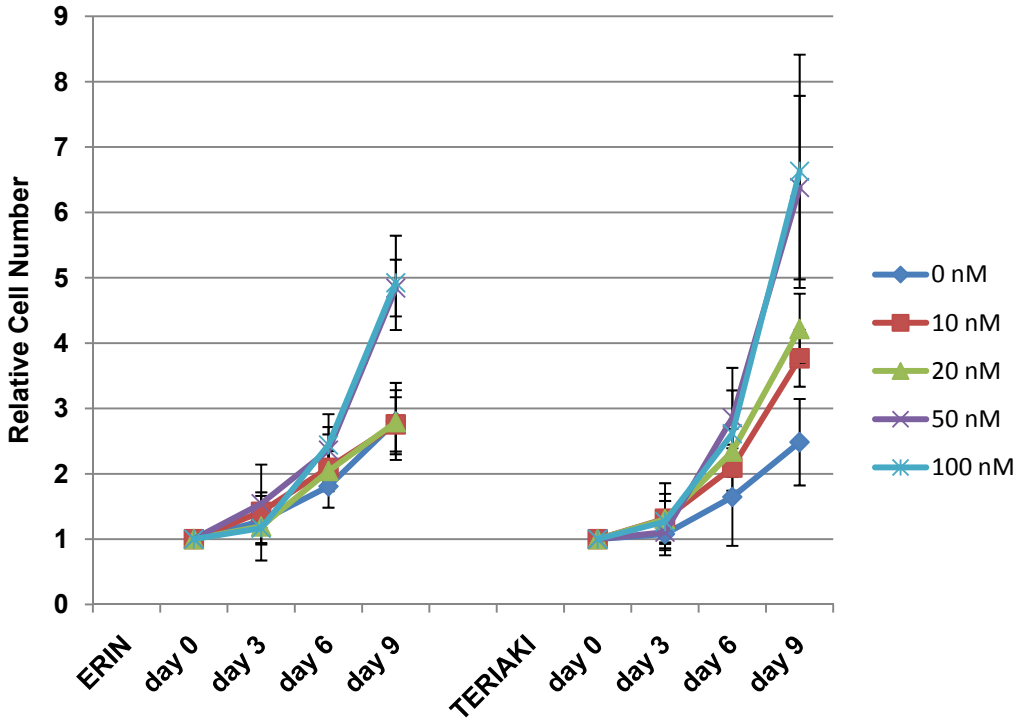
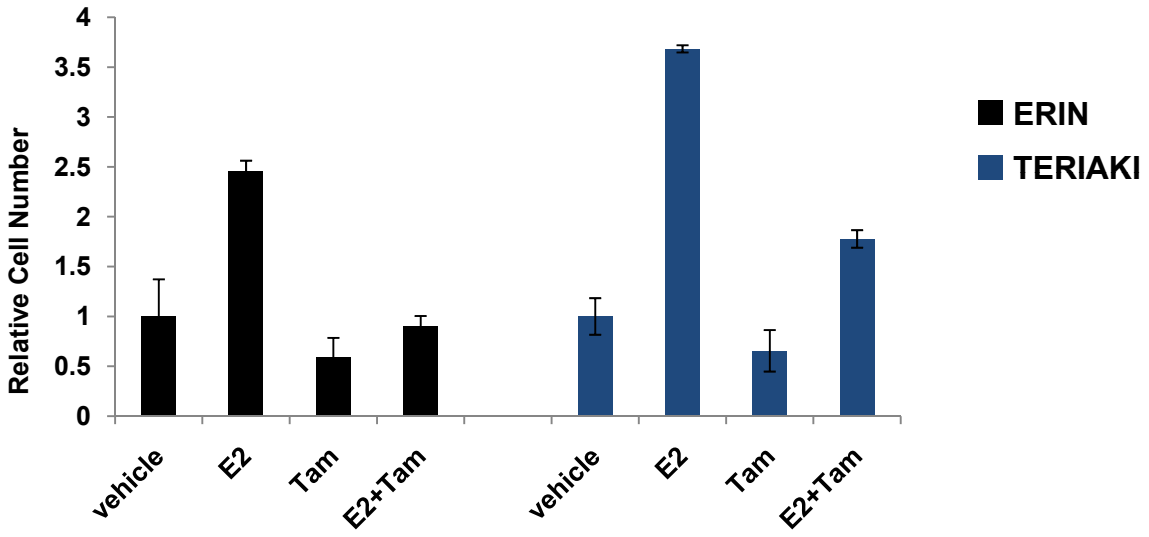

Relative Cell Number
9
8
7
6
5
4
3
2
1
0
ERIN
day 0
day 3
day 6
day 9
TERIAKI
day 0
day 3
day 6
day 9
0 nM
10 nM
20 nM
50 nM
100 nM

Figure S2b


Relative Cell Number
4
3.5
3
2.5
2
1.5
1
0.5
0
vehicle
E2
Tam
E2+Tam
vehicle
E2
Tam
E2+Tam
ERIN
TERIAKI

Supplementary Figure Legends

Figure S1. AKT1 and PIK3CA mutants differentially phosphorylate the direct Akt target GSK3β. Immunoblot analysis of parental MCF10A cells and their isogenic AKT1 and PIK3CA knock in derivatives under EGF-free conditions. Phosphorylation of Akt S473 is shown to demonstrate biochemical activation of Akt under these growth conditions.

Figure S2. AKT1 mutation does not quantitatively alter the proliferative response to estrogen and tamoxifen. (a) Dose-response of estrogen-induced proliferation. ERIN and TERIAKI cells were seeded in EGF-free, phenol red-free growth medium supplemented with vehicle or 17 β-estradiol (E2) at the indicated concentrations. Media was replaced every 3 days and cells were counted over a period of nine days. (**b)** Proliferative response to estrogen and tamoxifen. ERIN and TERIAKI cells were seeded in EGF-free, phenol red-free growth medium supplemented with vehicle, 17 β-estradiol (E2), 4-hydroxy-tamoxifen (Tam), or both. Medium was replaced every 3 days. Cells were counted after 9 days in culture. Results are averages of triplicate cell counts with standard deviations, normalized to vehicle-treated cell counts.