

# Crosstalk between ER and PI3K signaling pathways in human

## luminal type breast cancer and its therapeutic implications

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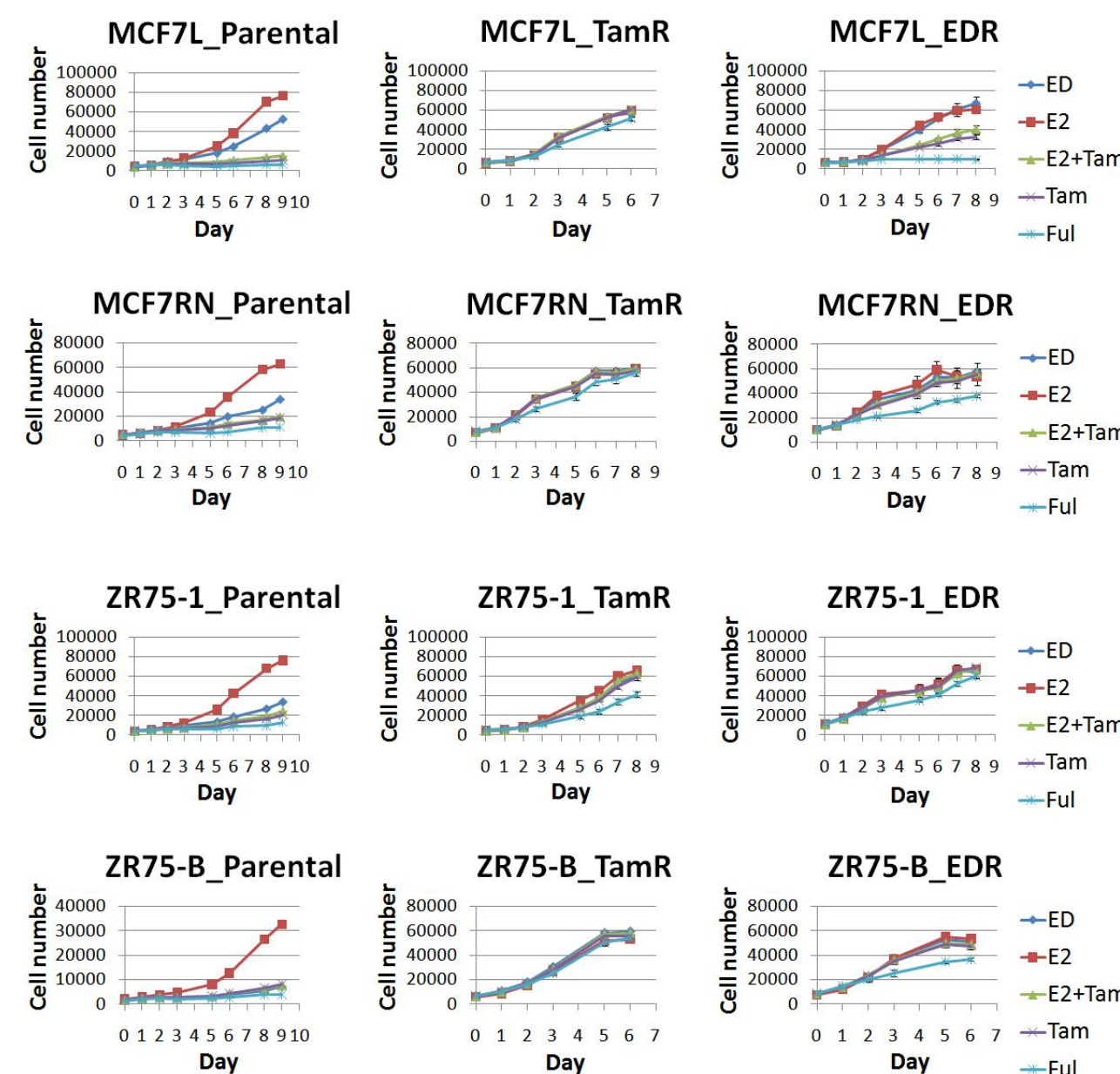
### Background

We have recently shown that the luminal B subtype breast cancer (BC) is associated with PI3K molecular signature. Breast tumors of the luminal B subtype generally have lower estrogen receptor (ER) expression levels and/or activity and are more aggressive and less sensitive to endocrine therapy when compared to luminal A tumors. To further investigate the role of PI3K signaling in BC luminal types and endocrine resistance, we have characterized an array of luminal A and B cell lines, as well as their endocrine resistant derivatives, to examine how modulation of the PI3K pathway can influence ER activity and endocrine sensitivity.

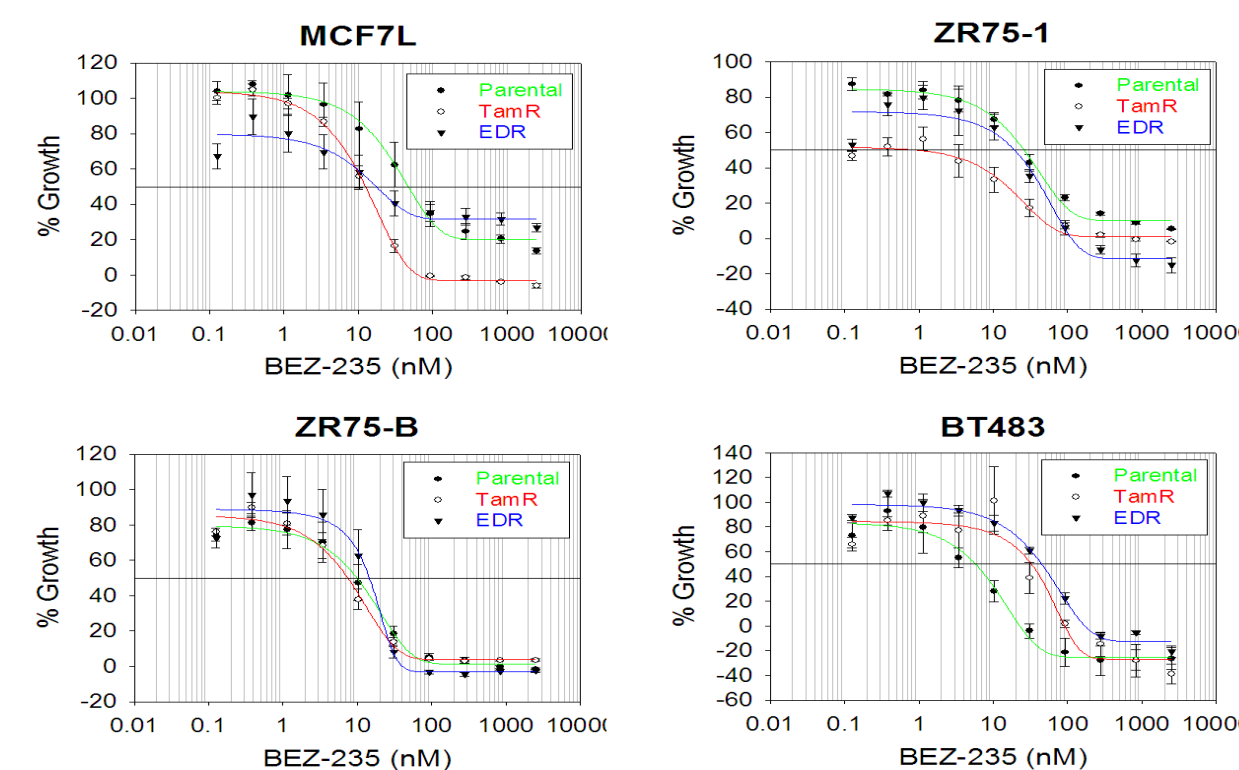
### Materials and Methods

Luminal A (ZR75-1, BT483) and luminal B BC cell lines (MCF7, ZR75-B, CAMA-1) were included in this study. Resistant clones were developed by long-term (>6 months) endocrine treatment [estrogen deprivation (ED) or ED plus Tamoxifen (Tam)]. The IC<sub>50</sub> values of the PI3K inhibitor BEZ-235 were determined in the parental and the endocrine resistant lines by Celigo *in situ* cell cytometry (Cyntellect Inc., San Diego, CA). qRT-PCR was performed to measure the effect of the inhibitor on mRNA levels of ER and its regulated genes (e.g. PR, CAV1, and IGF1R). Cells were also treated (4 days) with Tam, BEZ-235, or the combination, and growth inhibition assays were conducted to assess the drugs' efficacy. An inducible PTEN knockdown clone was established in MCF7L cell line. Endocrine sensitivity was studied *in vitro* by Celigo cytometry, and *in vivo*, using xenograft mouse model under ED conditions.

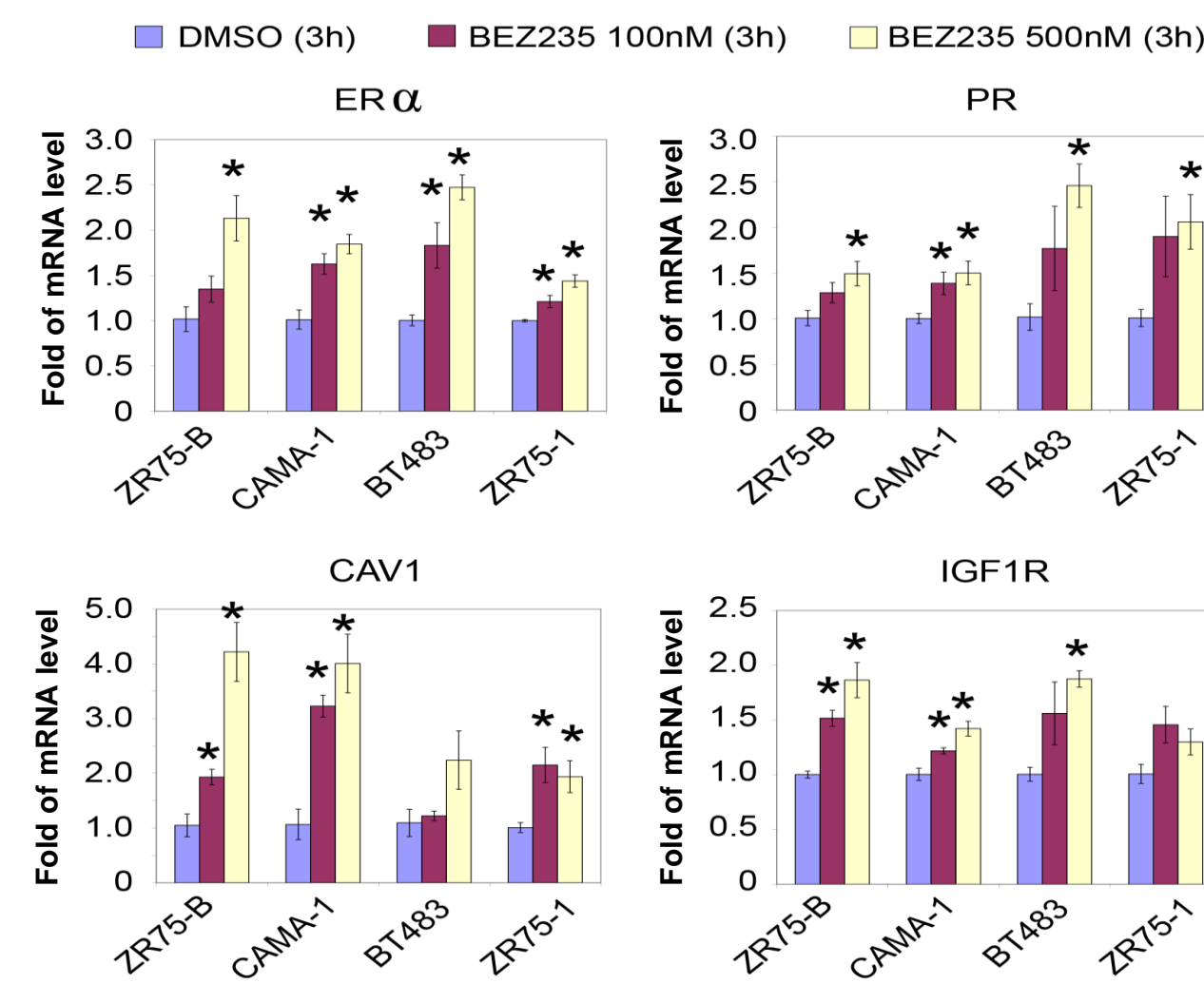
### Results



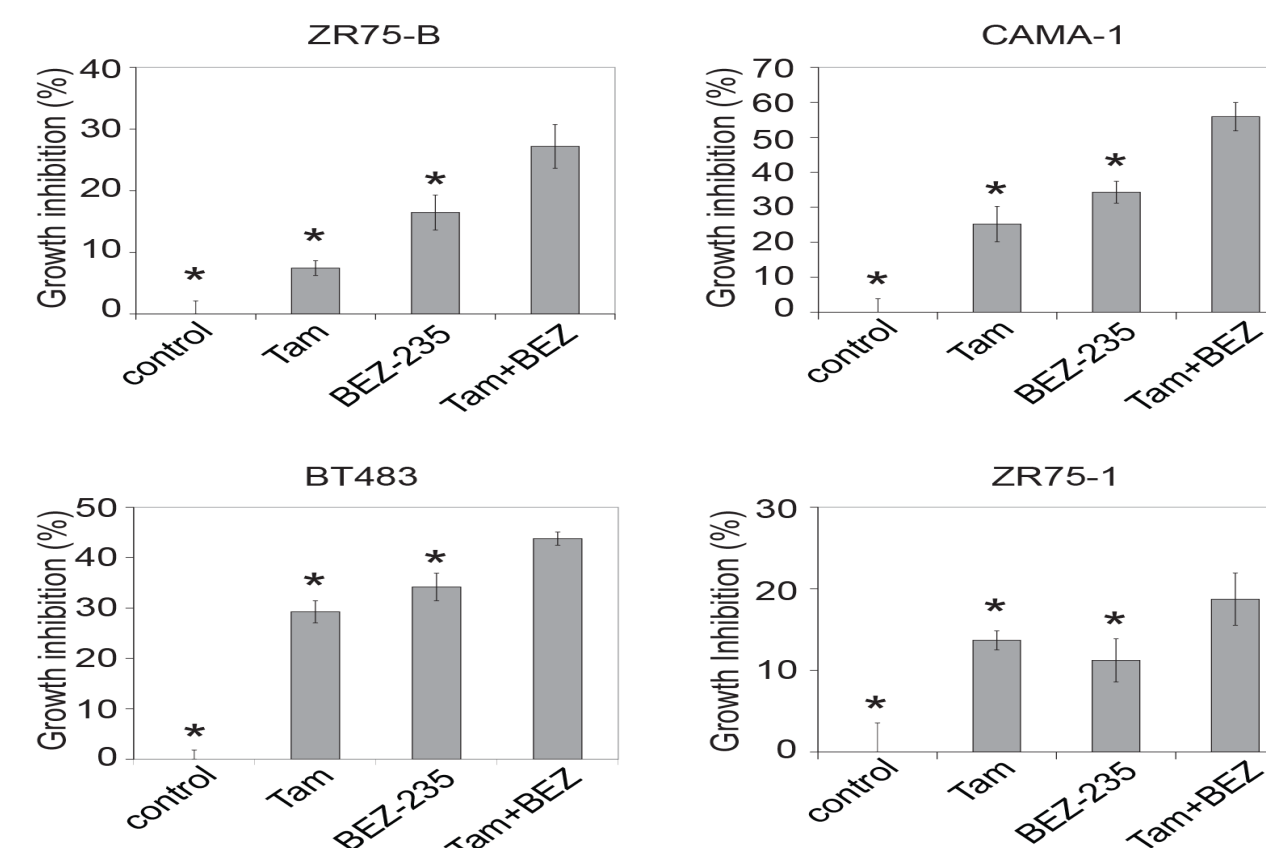
**Figure 1.** Establishment of endocrine resistant cell clones *in vitro*. Cells were cultured in phenol-red free medium with 5% charcoal-stripped fetal bovine serum (CS-FBS) w/ or w/o Tam (10<sup>-7</sup> M) for over half year. Growth curve assay was conducted by Celigo *in situ* cell cytometry for 10 days.



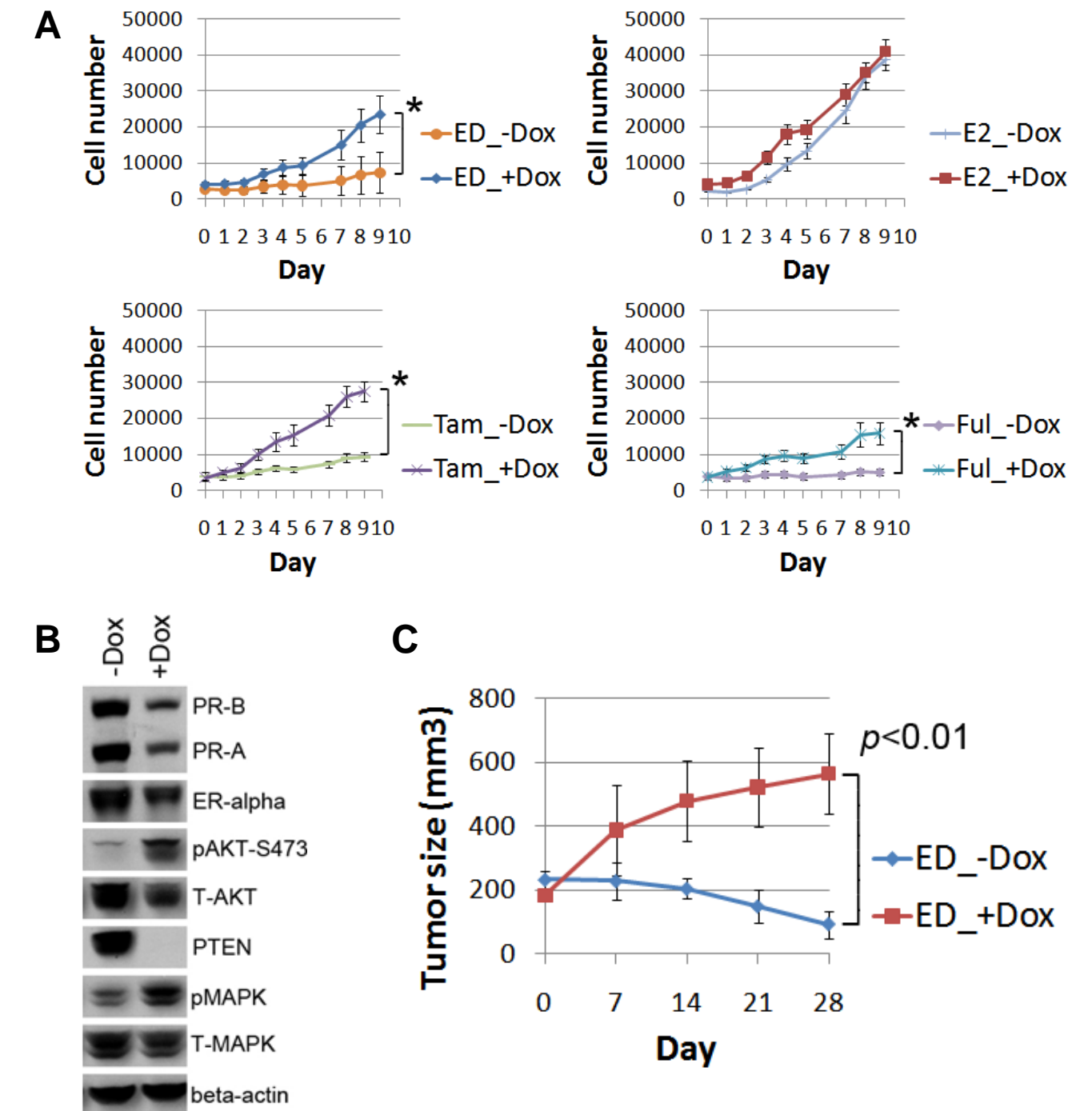
**Figure 2.** Heterogeneous response to the PI3K and mTOR dual inhibitor BEZ-235 in four endocrine-resistant cell line models. The IC<sub>50</sub> measurement of BEZ-235 was performed in parental (green) and resistant (red and blue) cell lines.



**Figure 3.** PI3K inhibition increases mRNA levels of ER and ER-regulated genes. Cells were treated by BEZ-235 (100nM and 500nM) for 3 hrs before harvesting for mRNA extraction. qRT-PCR was performed to measure the levels of ER and ER target genes (PR, CAV1, and IGF1R). Asterisks (\*) denote significant differences between BEZ-235 and control (P<0.05, t-test).



**Figure 4.** Superiority of anti-PI3K and endocrine combination therapy. Cells were treated for 4 days with Tam (100nM), BEZ-235 (10nM), or the combination of Tam+BEZ. Average growth inhibition normalized to control (regular medium) in each cell line. Asterisks (\*) denote significant differences from Tam+BEZ group (P<0.05, t-test).



**Figure 5.** PTEN knockdown renders MCF7L endocrine resistance *in vitro* and *in vivo*. A) MCF7L cells w/ or w/o doxycycline-induced shPTEN were pre-starved for 4 days in 5% CS-FBS prior to endocrine treatment. Asterisks (\*) denote significant differences (P<0.01, t-test). B) Western blot of PTEN and other biomarkers in MCF7L cells. C) Xenograft tumor growth upon PTEN knockdown under ED.

### Conclusion

- Endocrine resistant cell line models vary in response to PI3K inhibitor.
- Both luminal A and B cells acquire increased ER expression/activity and sensitivity to Tam upon PI3K inhibition.
- Upregulation of PI3K signaling by PTEN knockdown renders resistance to endo-therapy.

### Acknowledgment

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