



**Supplementary Figure S1.** Validation of LTED cell lines. **A**, Proliferation of parental and LTED MCF7 lines in E2-deprived conditions.  $1.5 \times 10^4$  cells/well of MCF7, MCF7 LTED<sup>WT</sup> and MCF7 LTED<sup>Y537C</sup> cell lines were seeded into 24-well plates tissue culture plates in RPMI 1640 media supplemented with 10% DCC-FBS with/without supplemental E2. Cell proliferation was monitored over 138 hours using the Incucyte, with images taken every 6 hours. Graph shows mean changes in confluence relative to time zero ±SEM. *n*=2 biological replicates, *n*=6 technical replicates (two-way ANOVA with Sidak's multiple comparisons test, and confidence intervals of 95%). **B**, 2000-4000 cells/well of parental HCC1428, SUM44, T47D, and ZR-75-1 cells and their LTED derivatives were seeded into 96-well tissue culture plates in RPMI 1640 media supplemented with 10% DCC-FBS with/without E2 (as indicated). After 5 days, cell viability was assessed using CellTiter-Glo. Graphs show mean percentage change in viability relative to E2-treated parental cell line. *n*=2 biological replicates, *n*=8 technical replicates, ±SEM (one-way ANOVA with Sidak's multiple comparisons test, and confidence intervals of 95%).