Supplemental information

Modeling breast cancer proliferation, drug synergies, and alternating therapies

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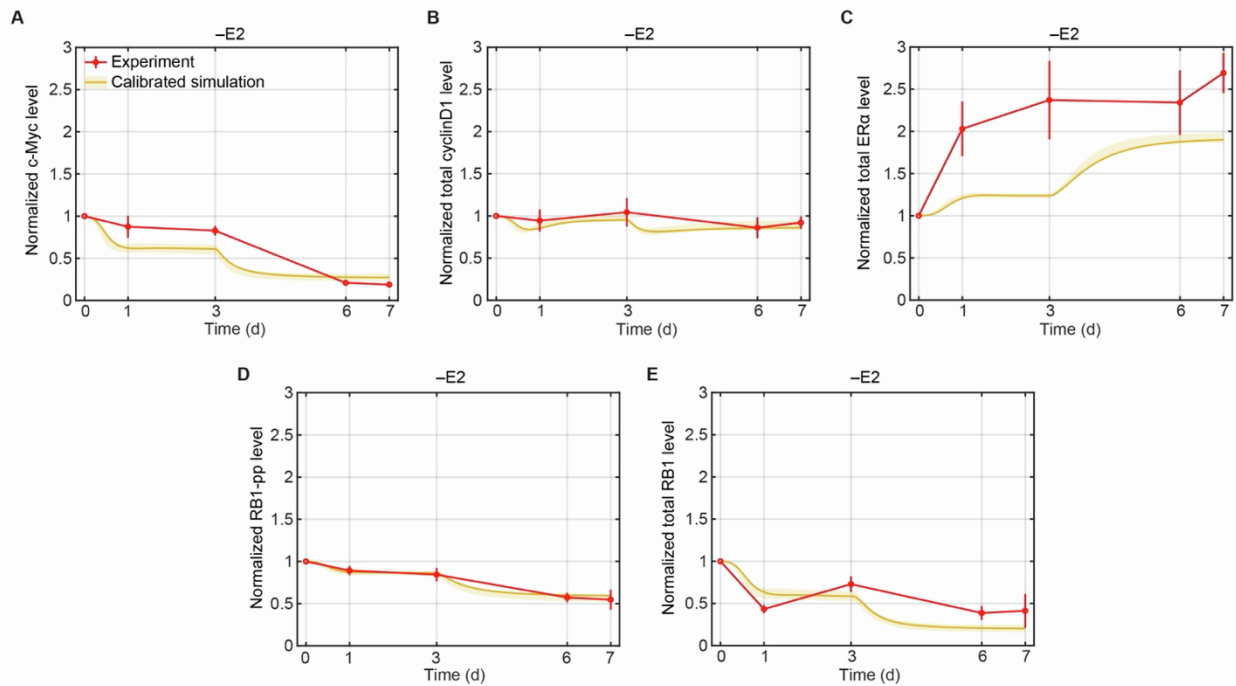
Supplemental Information

Figure S1. Model Calibration Simulations of Normalized Protein Levels for –E2 Treatment Compared to Experimental Data. Related to Figure 1.

(A) Model simulation of normalized c-Myc compared to experimental data (mean ± s.e., n=3). The experimental data are shown in red and the simulation results are shown in yellow (solid line represents the lowest cost value simulation and the shaded regions contains the central 98% of the cohort simulations).

(B) Model simulation of normalized total cyclinD1 compared to experimental data (mean ± s.e., n=3). Color, lines and the shaded regions have the same meaning as (A).

(C) Model simulation of normalized total ERα compared to experimental data (mean ± s.e., n=3).

(D) Model simulation of normalized RB1-pp compared to experimental data (mean ± s.e., n=3).

(E) Model simulation of normalized total RB1 compared to experimental data (mean ± s.e., n=3). The experimental data are re-used from previous work.[S1]
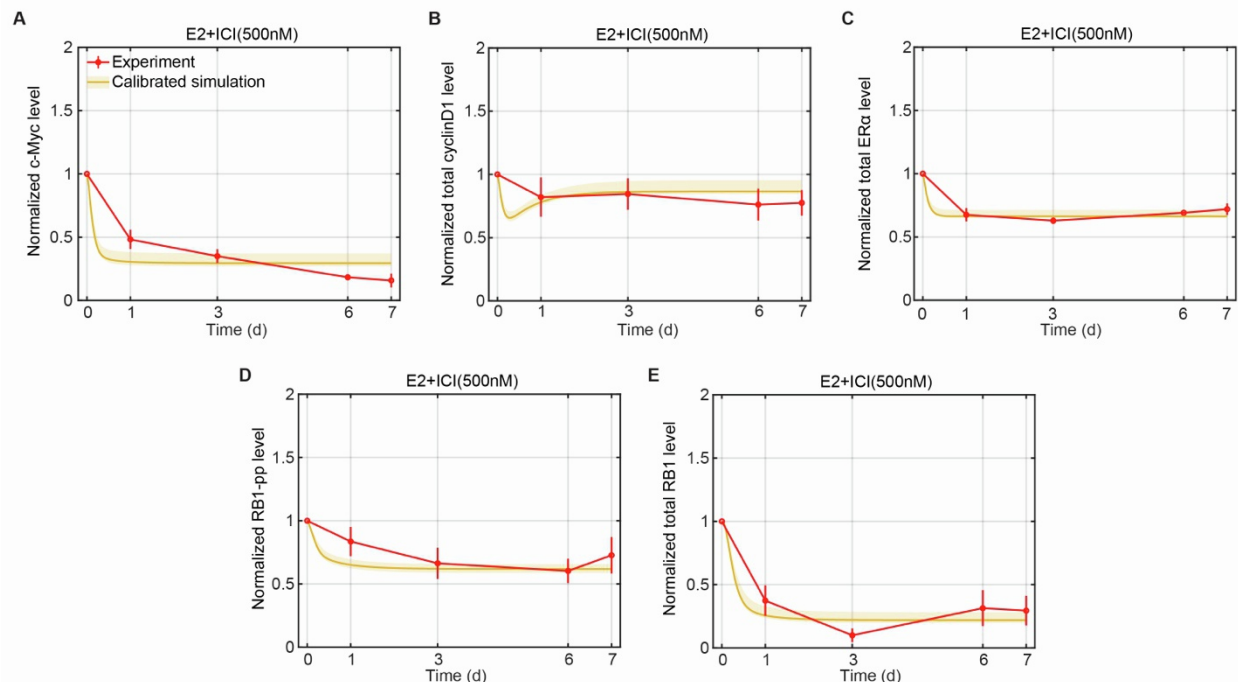
Figure S2. Model Calibration Simulations of Normalized Protein Levels for E2+ICI(500nM) Treatment Compared to Experimental Data. Related to Figure 1.

(A) Model simulation of normalized c-Myc compared to experimental data (mean ± s.e., n=3). The experimental data are shown in red and the simulation results are shown in yellow (solid line represents the lowest cost value simulation and the shaded regions contains the central 98% of the cohort simulations).

(B) Model simulation of normalized total cyclinD1 compared to experimental data (mean ± s.e., n=3). Color, lines and the shaded regions have the same meaning as (A).

(C) Model simulation of normalized total Erα compared to experimental data (mean ± s.e., n=3).

(D) Model simulation of normalized RB1-pp compared to experimental data (mean ± s.e., n=3).

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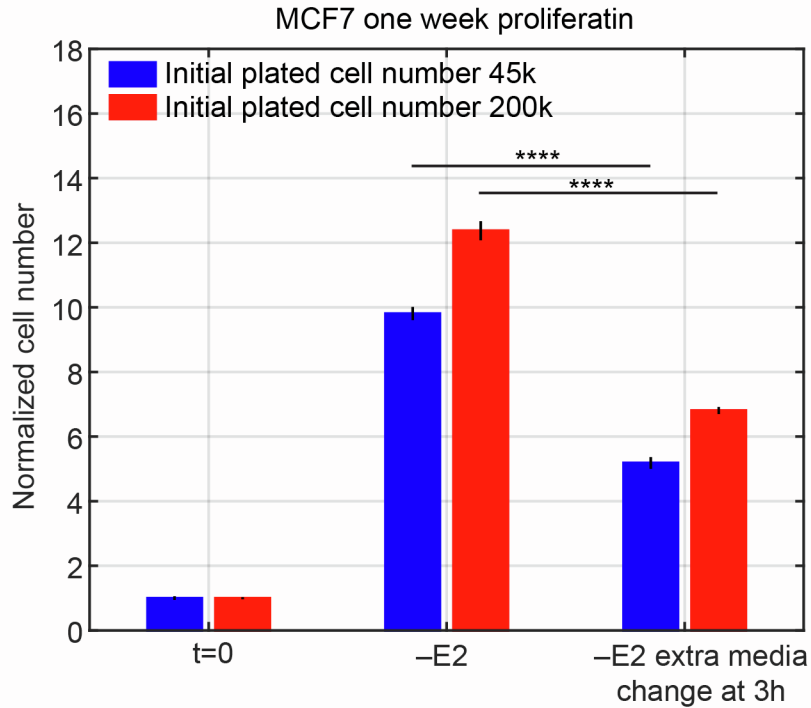
Figure S3. Extra Media Change during –E2 Treatment Decreases MCF7 Cell Proliferation. Related to STAR Methods. The bar plot shows normalized cell number relative to t=0 for the cases of 45×10^3(45k, blue bars) and 200×10^3(200k, red bars) initially plated cells (mean ± s.e., n=2 biological replicates, and 3 technical replicates for each biological replicate). The media is changed at t=0 and at day 3 to E2-deprived media in the –E2 case (middle). An extra media change to E2-deprived media at 3 hours was added in the –E2 extra media change case (right). In both cases of initially-plated cells, the extra media change, which further decreases the residual E2 level, significantly decreases the overall MCF7 proliferation at 1 week. Data were compared by two-way ANOVA, $p \le 0.0001$ (****).
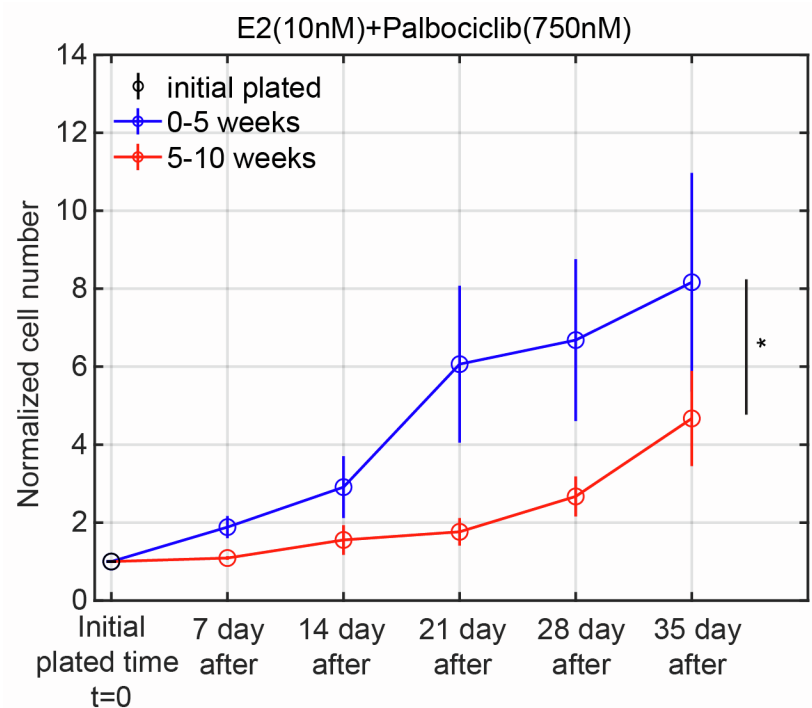
Figure S4. MCF7 Cell Proliferation Rate Decreases from 5 Weeks to 10 weeks Compared to 0 to 5 Weeks in Palbociclib Treatment. Related to STAR Methods. Plot shows normalized cell number relative to initial plated number under E2+palbo(750nM) treatment (mean ± s.e., n=3). This is a re-plot of the 10 weeks E2+palbo(750nM) treatment data in Figure 5. MCF7 cells were under E2+palbo (750nM) treatment for total 10 weeks. At 5 weeks, the cells were re-plated. The blue line is the normalized cell number from 0 to 5 weeks relative to the initial plated number at t=0. The red line is the normalized cell number from 5 to 10 weeks relative to the initial plated number at 5 weeks. The normalized proliferation from 5 to 10 weeks is significantly smaller than that from 0 to 5 weeks. Data were compared by paired t test, $p \leq 0.05$ (*).
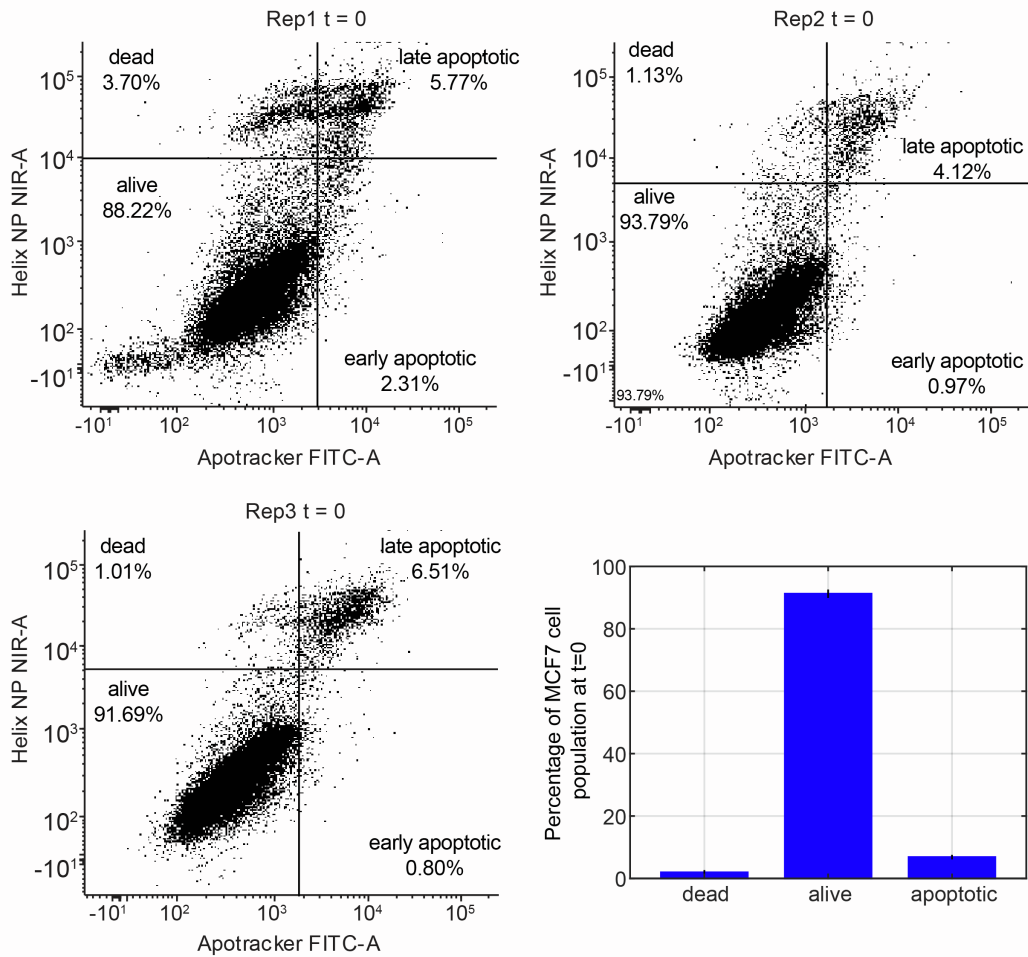
Figure S5. Apoptosis Assay of MCF7 Cells at t=0. Related to Table 1. Plots show the three

replicate measurements for apoptotic percentage of MCF7 cells at t=0 by flow cytometry. The bar

plot shows the average percentage of the three replications (mean ± s.e., n=3). The apoptotic

percentage equals the early apoptotic percentage plus the late apoptotic percentage. The alive

and apoptotic percentages were used to assign the *Nalive and Ndead* values in Table 1.
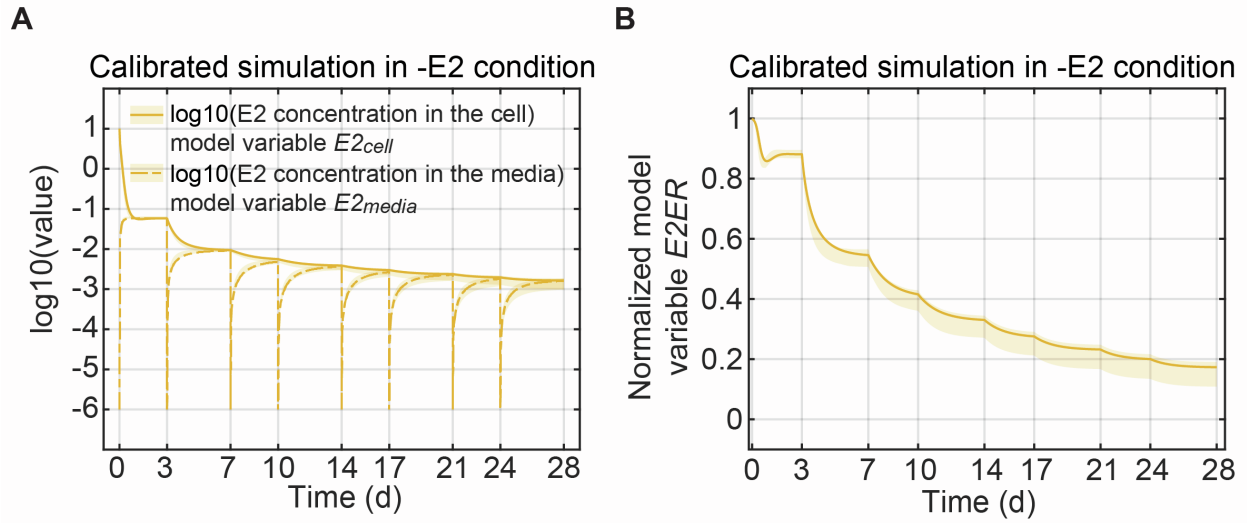
Figure S6. Simulations of Model E2 Changes during –E2 Treatment. Related to Figure 1.

(A) Simulation of E2 concentration in cells (model variable $E2_{cell}$, number 2 in Table 1) and medium (model variable $E2_{media}$, number 1 in Table 1) during –E2 treatment. The solid and dashed lines represent the lowest cost value simulation and the shaded regions contain the central 98% of the cohort simulations.

(B) Simulation of model variable $E2ER$ (number 4 in Table 1) during –E2 treatment. The lines and shaded regions have the same meaning as (A).
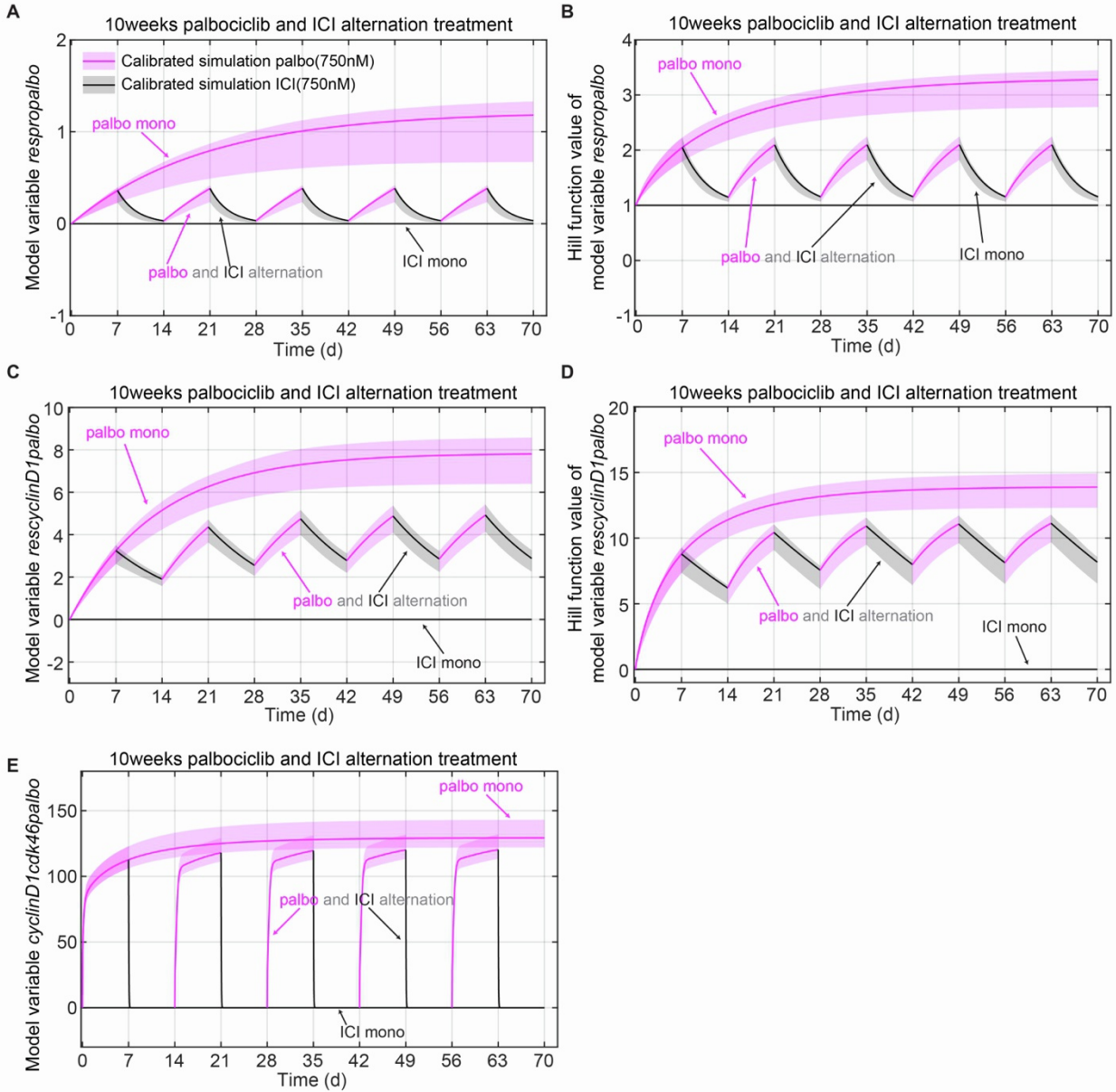
Figure S7. Simulations of Model Variable Changes during Long Time Mono and Alternating Treatments. Related to Figure 4.

(A) Simulation of model variable *respropalbo* (number 25 in Table 1) changes during the mono and alternating treatments, which are E2+palbo(750nM), E2+ICI(750nM) and E2+palbo(750nM) alternating with E2+ICI(750nM) treatments, as in Figure 4A-4D. In both the mono and alternating treatments, the E2+palbo(750nM) condition is shown in purple and the E2+ICI(750nM) condition in black. In the alternating treatment, each treatment period is 7days and starts with

E2+palbo(750nM). The solid line represents the lowest cost value simulation and the shaded regions contain the central 98% of the cohort simulations.

(B) Simulation of changes in the hill function value in the model variable *respropalbo* (denominator of equation 20) during the mono and alternating treatments shown in (A). The lines and shaded regions have the same meaning as (A).

(C) Simulation of model variable *rescyclinD1palbo* (number 7 in Table 1) changes during the mono and alternating treatments shown in (A).

(D) Simulation of changes in the hill function value in the model variable *rescyclinD1palbo* (equation 45) during the mono and alternating treatments shown in (A).

(E) Simulation of model variable *cyclinD1cdk46palbo* (number 14 in Table 1) changes during the mono and alternating treatments shown in (A).
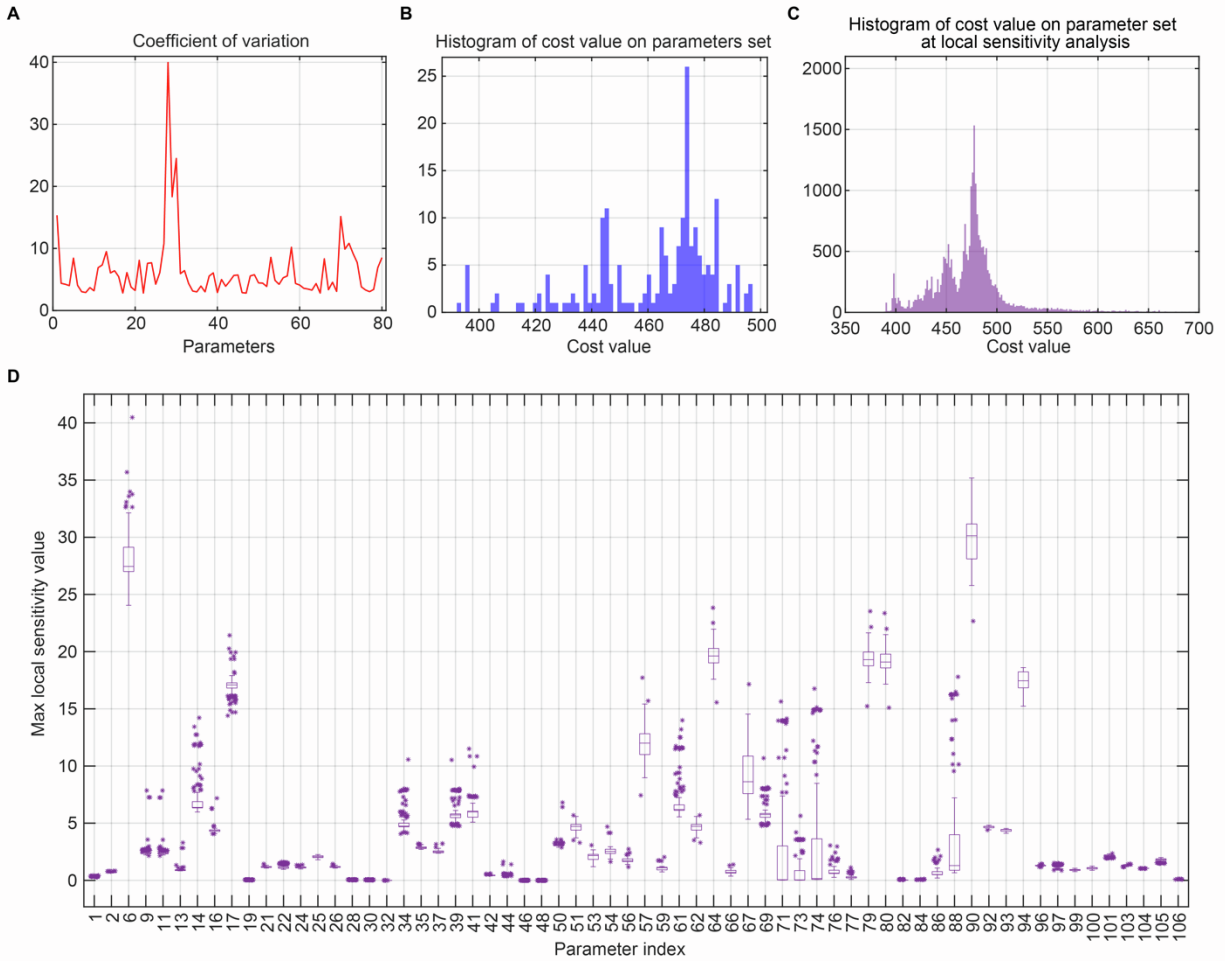
Figure S8. Profile of the Parameter Sets in the Parameter Cohort. Related to STAR Methods.

(A) Coefficients of variation of the parameters in the parameter cohort.

(B) Histogram of the cost values of each the parameter set in the parameter cohort.

(C) Boxplot of local sensitivity analysis of the model parameters. The parameter index is the index in Table 2. The analysis was performed on all 64 data-calibrated parameters in a parameter set, except hill function powers, and all parameter sets in the cohort. The maximum local sensitivity values across all proliferation data points for each parameter in the cohort are plotted in one box. The center line in each box is the median, and the bottom and top lines of each box are the 25th and 75th percentiles, respectively. The whiskers are maximum and minimum values without considering outliers. Data points are considered outliers if they lie more than 1.5×IQR (interquartile range) below the 25th percentile or above the 75th percentile.

(D) Histogram of the cost values for each perturbed parameter set used in the local sensitivity analysis.

Table S1. Times when various species were measured and used for parameter calibration or result prediction. Related to STAR Methods. E2 (control), E2+ICI (treated with ICI at a specific concentration in E2 medium), –E2 (E2 deprivation), –E2+ICI (treated with ICI at a specific concentration in –E2 medium), E2+palbo (treated with palbociclib at a specific concentration in E2 medium), E2+palbo+ICI (treated with palbociclib and ICI at a specific concentration in E2 medium),–E2+palbo (treated with palbociclib at a specific concentration in –E2 medium) and E2+abema (treated with abemaciclib at a specific concentration in E2 medium), E2+abema+ICI (treated with abemaciclib and ICI at a specific concentration in E2 medium). n: biological replications, d: days.

Figure	Condition	Species name	Measure time point	Calibration /Prediction	Model variables used for simulation
1C	E2	normalized cell number	1d, 3d, 6d, 7d, 11d. Each time point n=3	Calibration	*Nalive*
1D	–E2	normalized cell number	1d, 3d, 6d, 7d, 14d, 21d, each time point n=3	Calibration	*Nalive*
1E	E2+ICI (100nM)	normalized cell number	1d, 3d, 6d, 7d, 14d, 21d, each time point n=3	Calibration	*Nalive*
1F	E2+ICI (500nM)	normalized cell number	7d, 14d, 21d, each time point n=3	Calibration	*Nalive*
1G	E2+palbo (250nM)	normalized cell number	7d, 14d, 21d, 28d each time point n=3	Calibration	*Nalive*
1H	E2+palbo (500nM)	normalized cell number	7d, 14d, 21d, 28d each time point n=3	Calibration	*Nalive*
1I	E2+palbo (1μM)	normalized cell number	7d, 14d, 21d, each time point n=3	Calibration	*Nalive*
1J	–E2+ICI (100nM)	normalized cell number	7d, 14d, 21d, each time point n=3	Calibration	*Nalive*
1K	E2+palbo (100nM)	normalized cell number	7d, 14d, 21d, each time point n=3	Calibration	*Nalive*
2A	E2+abema (300nM)	normalized cell number	1d, 3d, 6d, 7d, 14d, 21d, each time point n=3	Calibration	*Nalive*
2B	E2+abema (500nM)	normalized cell number	1d, 3d, 6d, 7d, 14d, 21d, each time point n=3	Calibration	*Nalive*

2C	E2+abema (500nM)	c-Myc	1d, 3d, 6d, 7d, each time point n=3	Calibration	*cMyc*
2D	E2+abema (500nM)	RB1-pp	1d, 3d, 6d, 7d, each time point n=3	Calibration	*ppRb*
3A	Alternating treatment	normalized cell number	7d, E2+palbo(250nM) 14d, –E2 21d, E2+palbo(250nM) 28d, –E2 each time point n=3	Calibration	*Nalive*
3B	Alternating treatment	normalized cell number	7d, E2+palbo(500nM) 14d, E2+ICI(500nM) 21d, E2+palbo(250nM) 28d, E2+ICI(500nM) each time point n=3	Calibration	*Nalive*
3C	Alternating treatment	normalized cell number	7d, E2+palbo(750nM) 14d, E2+ICI(500nM) each time point n=3	Prediction	*Nalive*
3D	Alternating treatment	normalized cell number	7d, E2+palbo(750nM)+ICI(500nM) 14d, E2+ICI(500nM) each time point n=3	Prediction	*Nalive*
4A	Alternating treatment	normalized cell number	7d, E2+palbo(750nM) 14d, E2+ICI(750nM) 21d, E2+palbo(750nM) 28d, E2+ICI(750nM) 35d, E2+palbo(750nM) 42d, E2+ICI(750nM) 49d, E2+palbo(750nM) 56d, E2+ICI(750nM) 63d, E2+palbo(750nM) 70d, E2+ICI(750nM) each time point n=3	Calibration	*Nalive*
4A	E2+palbo (750nM)	normalized cell number	7d, 14d, 21d, 28d, 35d, 42d, 49d, 56d, 63d, 70d, each time point n=3	Calibration	*Nalive*
4A	E2+ICI (750nM)	normalized cell number	7d, 14d, 21d, 28d, 35d, each time point n=3	Calibration	*Nalive*
4E	E2+palbo (750nM)	cyclinD1	35d, 50d, each time point n=3	Calibration	*cyclinD1+* *cyclinD1cdk46+* *cyclinD1cdk46p21+* *cyclinD1Cdk46palo+* *cyclinD1cdk46abema+* *cyclinD1cdk46p21palbo+* *cyclinD1cdk46p21abema*
4E	Alternating treatment	cyclinD1	35d, E2+palbo(750nM) 70d, E2+ICI(750nM) each time point n=3	Calibration	Same as above

4F	E2+palbo (750nM)	Cdk4	35d, 50d, each time point n=3	-	*cdk46+ cdk46palbo+ cdk46abema+ cyclinD1cdk46+ cyclinD1cdk46p21+ cyclinD1cdk46abema+ cyclinD1cdk46p21palbo+ cyclinD1cdk46p21abema*
4F	Alternating treatment	Cdk4	35d, E2+palbo(750nM) 70d, E2+ICI(750nM) each time point n=3	-	Same as above
4G	E2+palbo (750nM)	Cdk6	35d, 50d, each time point n=3	-	Same as above
4G	Alternating treatment	Cdk6	35d, E2+palbo(750nM) 70d, E2+ICI(750nM)	-	Same as above
4H	E2+palbo (750nM)	cyclinE	35d, 50d, each time point n=3	-	*cyclinE+cyclinEp21*
4H	Alternating treatment	cyclinE	35d, E2+palbo(750nM) 70d, E2+ICI(750nM)	-	*cyclinE+cyclinEp21*
4I	E2+palbo (750nM)	Cdk2	35d, 50d, each time point n=3	-	-
4I	Alternating treatment	Cdk2	35d, E2+palbo(750nM) 70d, E2+ICI(750nM	-	-
4J	E2+palbo (750nM)	cyclinD1	7d, E2+palbo(750nM) 14d, E2+ICI(750nM) each time point n=3	-	*cyclinD1+ cyclinD1cdk46+ cyclinD1cdk46p21+ cyclinD1Cdk46palo+ cyclinD1cdk46abema+ cyclinD1cdk46p21palbo+ cyclinD1cdk46p21abema*
6J	E2+ICI (200nM)	normalized cell number	17d, n=3	Prediction	*Nalive*
6J	E2+palbo (50nM)+ ICI(200nM)	normalized cell number	17d, n=3	Prediction	*Nalive*
6J	E2+palbo (100nM)+ ICI(200nM)	normalized cell number	17d, n=3	Prediction	*Nalive*
6J	E2+palbo (300nM)+ ICI(200nM)	normalized cell number	17d, n=3	Prediction	*Nalive*
6K	E2+ICI (200nM)	normalized cell number	17d, n=3	Prediction	*Nalive*
6K	E2+abema (50nM)+ ICI(200nM)	normalized cell number	17d, n=3	Prediction	*Nalive*
6K	E2+abema (100nM)+ ICI(200nM)	normalized cell number	17d, n=3	Prediction	*Nalive*
S1A	–E2	c-Myc	1d, 3d, 6d, 7d, each time point n=3	Calibration	*cMyc*

S1A	–E2	cyclinD1	1d, 3d, 6d, 7d, each time point n=3	Calibration	*cyclinD1+ cyclinD1cdk46+ cyclinD1cdk46p21+ cyclinD1Cdk46palo+ cyclinD1cdk46abema+ cyclinD1cdk46p21palbo+ cyclinD1cdk46p21abema*
S1A	–E2	ERα	1d, 3d, 6d, 7d, each time point n=3	Calibration	*ER+E2ER+ ICIER*
S1A	–E2	RB1-pp	1d, 3d, 6d, 7d, each time point n=3	Calibration	*ppRb*
S1A	–E2	RB1	1d, 3d, 6d, 7d, each time point n=3	Calibration	*Rb+pRb+ppRb*
S2	E2+ICI (500nM)	c-Myc	1d, 3d, 6d, 7d, each time point n=3	Calibration	*cMyc*
S2	E2+ICI (500nM)	cyclinD1	1d, 3d, 6d, 7d, each time point n=3	Calibration	*cyclinD1+ cyclinD1cdk46+ cyclinD1cdk46p21+ cyclinD1Cdk46palo+ cyclinD1cdk46abema+ cyclinD1cdk46p21palbo+ cyclinD1cdk46p21abema*

References:

[S1] He, W., Demas, D.M., Conde, I.P., Shajahan-Haq, A.N., and Baumann, W.T. (2020). Mathematical modelling of breast cancer cells in response to endocrine therapy and Cdk4/6 inhibition. J. R. Soc. Interface 17, 20200339. https://dx.doi.org/10.1098/rsif.2020.0339.