

## Supporting information

Mathematical modeling of breast cancer cells in response to endocrine therapy and Cdk4/6 inhibition  
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## **Biological Justification for the Model**

Endocrine therapy, targeting ER signaling, is widely-used to treat ER+ breast cancer patients [1-7]. Two common endocrine therapies are E2 deprivation, via aromatase inhibitors (AIs) [5, 8], and ER down-regulators such as ICI182,780 (ICI; fulvestrant, Faslodex) [7, 9, 10]. AIs, like letrozole or anastrozole, are used in postmenopausal women for the treatment of breast cancer [8]. AIs lower the E2 level by inhibiting aromatase, which is an enzyme responsible for a key step in the synthesis of E2 [5]. The steroidal anti-estrogen ICI is also used in postmenopausal women. It competitively blocks E2 binding to the ER and causes a proteasome-dependent degradation of the receptor [11].

The central effect of steroid hormone E2 is growth maintenance and reproduction [12, 13] through the regulation of various processes, including cell proliferation and survival [14]. E2 exerts its effects by binding to nuclear receptors ER $\alpha$  and ER $\beta$  [15]. ER $\alpha$  is frequently overexpressed in breast cancer cells and is the major oncogene responsible for E2-induced enhancement of cell proliferation [14]. In the classical signaling mechanism, E2 binds to ER $\alpha$  (E2:ER) in the cytoplasm, producing conformational changes that result in homodimerization, translocation to the nucleus, and binding to estrogen response elements (ERE) in the promoter region of estrogen-responsive genes to regulate transcription [13, 14]. One important class of estrogen-responsive genes regulate progression through the G1 phase of the cell cycle [14, 16]. This progression mainly depends on transcriptional expression of regulatory proteins c-Myc and cyclinD1 [16].

27 c-Myc responds to E2 stimulation within 15 minutes, making it one of the earliest estrogen-responding  
28 genes [17-19]. The rapid induction of c-Myc by E2:ER is via the P2 promoter region of the c-Myc gene,  
29 which contains an atypical ERE region [17, 20]. c-Myc is a nuclear transcription factor with high-  
30 affinity for DNA-binding and its level is highly correlated with breast cancer cell proliferation [21, 22].  
31 The c-Myc oncogene is usually overexpressed in human breast cancer cells and is an essential positive  
32 regulator of the G1-S transition [22]. c-Myc represses transcription of the cyclin-dependent kinase  
33 inhibitor p21<sup>CIP1/WAF1</sup> (p21) by interacting with Miz-1 and several other proteins at the transcriptional  
34 start site [23] and transforming Miz-1 from an activator to a repressor [24, 25]. In addition, c-Myc also  
35 can transcriptionally repress p27<sup>KIP1</sup> (p27) expression in breast cancer cells [23] by binding to the  
36 initiator element at the start site of the TATA-less p27 promoter [26]. Moreover, the box II domain of  
37 c-Myc can interact with the N-terminal DNA-binding region of FOXO3a at the proximal Forkhead  
38 element on the p27 promoter. This interaction inhibits the FOXO3a-mediated activation of the p27  
39 promoter [27].

40

41 Besides c-Myc, E2:ER also transcriptionally up-regulates cyclinD1, whose expression is key to G1-S  
42 progression [28, 29]. ER $\alpha$  binds upstream (-96 and -29bp) of the cyclinD1 promoter region  
43 encompassing a cAMP response element (CRE-D1) [28, 30]. The up-regulation is E2-dependent and  
44 the AF-1, AF-2 and DNA binding domains of ER $\alpha$  are required [28, 30]. In addition, c-Myc has been  
45 reported to transcriptionally up-regulate [31], down-regulate [32-34], or have no effect on cyclinD1  
46 expression level [35-37]. When tested on breast cancer cells [16], induction of c-Myc failed to increase  
47 cyclinD1 expression and induction of cyclinD1 had no effect on c-Myc expression in the interval from  
48 3 to 24 hours after induction [16]. Therefore, we model the increased transcription of cyclinD1 and c-  
49 Myc as a direct effect of E2:ER.

50

51 CyclinD1 binds with cyclin-dependent kinases Cdk4 or Cdk6 (mostly Cdk4 in MCF-7 cells [38, 39]) to  
52 form holoenzymes and activate their kinase activity [40]. CyclinD1:Cdk4/6 phosphorylates

53 retinoblastoma protein (RB1) to a hypophosphorylated form (RB1-p) [16, 41-56]. The Cdk inhibitors  
54 p21 and p27, which can bind to the cyclinD1:Cdk4/6 complexes and inhibit their kinase activity [41,  
55 42, 57, 58], are downregulated by c-Myc to further activate the holoenzyme. Since p21 and p27 also  
56 inhibit the kinase activity of cyclinE:Cdk2, the increased sequestration of these inhibitors by cyclinD  
57 and their downregulation by c-Myc, serve to activate the cyclinE:Cdk2 complex [43, 44, 59]. This  
58 mechanism of cyclinE:Cdk2 activation by estrogen treatment is confirmed by several studies [45-47].  
59 Finally, active cyclinE:Cdk2 hyperphosphorylates and fully inactivates RB1 [16, 23, 41, 44, 46, 48, 49,  
60 53-56], which is a key step in the G1-S transition [16, 17, 21, 23, 43-45]. The hyperphosphorylated RB1  
61 (RB1-pp) releases E2F transcription factors that transactivate the genes required for DNA synthesis  
62 [23, 43-45]. All of these mechanisms have been well-established and recognized in ER+ breast cancer  
63 cells [16-20, 43-48, 59, 60].

64 There are numerous feedback loops involving E2F in the G1-S transition machinery [61-63]. While it  
65 is enticing to include all the well-known mechanisms around the G1-S transition, the limited data we  
66 are able to obtain does not warrant the increase in parameters, which would be practically, if not  
67 theoretically, unidentifiable.

68 With regard to proliferation, in the simple situation where total RB1 and total E2F are constant, the  
69 level of hyperphosphorylated RB1, RB1-pp, should be related to the proliferation rate. But our  
70 experimental data, as well as that of others, shows that total RB1 is not constant in response to  
71 endocrine therapy. In fact, the interactions between the RB1 and E2F families of proteins is quite  
72 complicated: there are three types of pocket proteins that bind E2F (RB1, p107, and p130) and nine  
73 types of E2F (E2F1-9), not to mention the 15 phosphorylation sites on RB1. In addition, p130 is known  
74 to increase in response to endocrine therapy. From our experimental data it appears that proliferation  
75 closely follows the level of RB1-pp (phosphorylation on site S612) and so in our simplified model we  
76 use the RB1-pp level to drive changes in proliferation.

77

78 The two common endocrine therapies used in this study, E2 deprivation and ER down-regulation,  
79 decrease the transcription factor E2:ER, down-regulate c-Myc and cyclinD1, increase p21 and p27, and  
80 inhibit cyclinD1:Cdk4/6 and cyclinE:Cdk2 kinase activity [49-53, 64]. The consequence of this  
81 decreased kinase activity is reduced phosphorylation of RB1, which limits the transcriptional activity  
82 of E2F, impeding the G1-S transition and arresting the cell in a state with characteristics of quiescence  
83 [38, 49, 50, 51, 53, 64, 65].

84

## 85 Model

86 **Table S1. Model variable description**

Variable name	Description	Initial value	Half-life
(1) <i>ER</i>	Estrogen receptor	0.002μM	4-5h [66]
(2) <i>E2ER</i>	Estrogen bound estrogen receptor	0.068μM	3-4h [66]
(3) <i>ICIER</i>	ICI 182,780 bound estrogen receptor	0μM	< 3-4h [66]
(4) <i>cyclinD1</i>	Protein cyclinD1	0.183μM	0.4h [67]
(5) <i>cyclinD1p21</i>	p21 bound cyclinD1 protein	0.219μM	-
(6) <i>cyclinD1palbo</i>	palbociclib bound cyclinD1 protein	0μM	-
(7) <i>cMyc</i>	Protein c-Myc	6.535μM	0.333h [68]
(8) <i>p21</i>	Protein p21	0.102μM	0.33-1h [69]
(9) <i>cyclinE</i>	Protein cyclinE	0.306μM	0.5h [70]
(10) <i>cyclinEp21</i>	p21 bound cyclinE protein	0.274μM	-
(11) <i>RB1</i>	Retinoblastoma protein	76.857μM	2-3h [71]
(12) <i>RB1p</i>	Hypophosphorylated RB1 (RB1-p)	0.481μM	2-3h [71]

(13) $RB1pp$	Hyperphosphorylated RB1 (RB1-pp)	7.306μM	>4h [71]
(14) $cell$	Cell number	1a.u.	-

87 a.u. arbitrary units

88

89 **Table S2. Model parameter description**

Parameter name	Description	Value	Fixed or calibrated
(1) $k_{ER}$	Translation rate of ERα	0.02μM/hour	Calibrated
(2) $kd_{ER}$	Degradation rate of ERα	0.10/hour	Fixed
(3) $kd_{E2ER}$	Degradation rate of E2ER	0.30/hour	Fixed
(4) $kb_{E2ER}$	Binding rate between E2 and ERα	4266.27/(hour×μM)	Calibrated
(5) $kub_{E2ER}$	Unbinding rate between E2 and ERα	1.0/hour	Fixed
(6) $kb_{ICIER}$	Binding rate between ICI and ERα	206.80/(hour×μM)	Calibrated
(7) $kub_{ICIER}$	Unbinding rate between ICI and ERα	1.0/hour	Fixed
(8) $kd_{ICIER}$	Degradation rate of ICIER	0.52/hour	Calibrated
(9) $k_{cyclinD1}$	Translation rate of cyclinD1	0.14μM/hour	Calibrated
(10) $kd_{cyclinD1}$	Degradation rate of cyclinD1	1.73/hour	Fixed
(11) $k_{cyclinD1E2ER}$	Increased cyclinD1 translation by E2ER	11.57	Calibrated
(12) $p_{cyclinD1E2ER_1}$	Parameter 1 of cyclinD1 increased translation by E2ER	2.61μM	Calibrated
(13) $p_{cyclinD1E2ER_2}$	Parameter 2 of cyclinD1 increased translation by E2ER	0.17	Calibrated

(14) $kb_{cyclinD1p21}$	Binding rate between cyclinD1 and p21	32.09/(hour×μM)	Calibrated
(15) $kub_{cyclinD1p21}$	Unbinding rate between cyclinD1 and p21	1/hour	Fixed
(16) $kb_{cyclinD1palbo}$	Binding rate between cyclinD1 and palbociclib	5.18/(hour×μM)	Calibrated
(17) $kub_{cyclinD1palbo}$	Unbinding rate between cyclinD1 and palbociclib	1/hour	Fixed
(18) $k_{cMyc}$	Translation rate of c-Myc	0.33μM/hour	Calibrated
(19) $kd_{cMyc}$	Degradation rate of c-Myc	2.31/hour	Fixed
(20) $k_{cMycE2ER}$	Increased translation of c-Myc by E2ER	14.18	Calibrated
(21) $p_{cMycE2ER_1}$	Parameter 1 of c-Myc increased translation by E2ER	0.03μM	Calibrated
(22) $p_{cMycE2ER_2}$	Parameter 2 of c-Myc increased translation by E2ER	6.99	Calibrated
(23) $k_{cMycRB1pp}$	Increased translation of c-Myc by RB1-pp	37.30	Calibrated
(24) $p_{cMycRB1pp_1}$	Parameter 1 of c-Myc increased translation by RB1-pp	5.50μM	Calibrated
(25) $p_{cMycRB1pp_2}$	Parameter 2 of c-Myc increased translation by RB1-pp	5.36	Calibrated
(26) $k_{p21}$	Translation rate of p21	1.29μM/hour	Calibrated
(27) $kd_{p21}$	Degradation rate of p21	1.39/hour	Fixed
(28) $p_{p21cMyc_1}$	Parameter 1 of p21 inhibited translation by c-Myc	0.24μM	Calibrated
(29) $p_{p21cMyc_2}$	Parameter 2 of p21 inhibited translation by c-Myc	2.44	Calibrated
(30) $k_{cyclinE}$	Translation rate of cyclinE	0.81μM/hour	Calibrated
(31) $kd_{cyclinE}$	Degradation rate of cyclinE	1.39/hour	Fixed
(32) $kb_{cyclinEp21}$	Binding rate between cyclinE and p21	20.94/(hour×μM)	Calibrated

(33) $k_{ub_{cyclinEp21}}$	Unbinding rate between cyclinE and p21	1.0/hour	Fixed
(34) $k_{RB1}$	Translation rate of RB1	3.27μM/hour	Calibrated
(35) $kd_{RB1}$	Degradation rate of RB1	0.35/hour	Fixed
(36) $k_{RB1RB1pp}$	Increased RB1 translation by RB1-pp	390.87μM/hour	Calibrated
(37) $p_{RB1RB1pp_1}$	Parameter 1 of RB1 increased translation by RB1-pp	10.76μM	Calibrated
(38) $p_{RB1RB1pp_2}$	Parameter 2 of RB1 increased translation by RB1-pp	7	Calibrated
(39) $k_{RB1cyclinD1}$	Phosphorylation rate of RB1 by cyclinD1	15.01/hour	Calibrated
(40) $k_{RB1pdepho}$	Dephosphorylation rate of RB1-p	23.62μM/hour	Calibrated
(41) $kd_{RB1p}$	Degradation rate of RB1-p	0.80/hour	Fixed
(42) $k_{RB1pcyclinE}$	Phosphorylation rate of RB1-p by cyclinE	5.37/hour	Calibrated
(43) $k_{RB1ppdepho}$	Dephosphorylation rate of RB1-pp	9.27μM/hour	Calibrated
(44) $kd_{RB1pp}$	Degradation rate of RB1-pp	0.05/hour	Fixed
(45) $p_{cyclinD1RB1_1}$	Parameter 1 of RB1 phosphorylation by cyclinD1	14.01μM	Calibrated
(46) $p_{cyclinD1RB1_2}$	Parameter 2 of RB1 phosphorylation by cyclinD1	3.19	Calibrated
(47) $p_{RB1pdepho_1}$	Parameter 1 of RB1-p dephosphorylation	0.68μM	Calibrated
(48) $p_{RB1pdepho_2}$	Parameter 2 of RB1-p dephosphorylation	7	Calibrated
(49) $p_{cyclinERB1p_1}$	Parameter 1 of RB1-p phosphorylation by cyclinE	6.35μM	Calibrated
(50) $p_{cyclinERB1p_2}$	Parameter 2 of RB1-p phosphorylation by cyclinE	0.35	Calibrated
(51) $p_{RB1ppdepho_1}$	Parameter 1 of RB1-pp dephosphorylation	59.85μM	Calibrated

(52) $p_{RB1ppdepho_2}$	Parameter 2 of RB1-pp dephosphorylation	2.11	Calibrated
(53) $k_{pro}$	Basal proliferation rate	$1.2 \times 10^{-4}$ /hour	Calibrated
(54) $k_{proRB1pp}$	Proliferation rate increased by RB1-pp	266.30	Calibrated
(55) $p_{proRB1pp_1}$	Parameter 1 of proliferation rate increased by RB1-pp	6.01 $\mu$ M	Calibrated
(56) $p_{proRB1pp_2}$	Parameter 2 of proliferation rate increased by RB1-pp	6	Fixed
(57) $k_{carrying}$	Carrying capacity	37.39a.u.	Calibrated
(58) $E2_{dep1}$	Estrogen concentration in E2 deprivation media 0 to 3days	$9.67 \times 10^{-5}$ $\mu$ M <sup>(2)</sup>	Calibrated
(59) $E2_{dep2}$	Estrogen concentration in E2 deprivation media 3 to 7days	$2.30 \times 10^{-5}$ $\mu$ M	Calibrated
(60) $E2$	Estrogen concentration	0.01 $\mu$ M	Fixed
(61) $ICI$	ICI 182,780 concentration	0.5 $\mu$ M	Fixed
(62) $palbo$	Palbociclib concentration	1 $\mu$ M	Fixed

90

91     The culture media, including any drugs, is changed at T = 0 and T = 3d for the experiments (E2control,  
92     E2 deprivation, +E2+ICI and E2 deprivation+ICI, +E2+palbo and E2 deprivation+palbo), so the longest  
93     period without resupplying the drugs is 4 days. In the model, the drug concentration is assumed to  
94     be constant throughout the experiment. There is no data we are aware of for the half-life of either  
95     palbociclib or fulvestrant in our in-vitro culture conditions. Most data is for the plasma half-life or  
96     terminal half-life in-vivo, which are not applicable to our case as they are determined primarily by  
97     processing in the liver and excretion through the kidneys. There is some data for the in-vitro  
98     stability of these drugs in human plasma, which is somewhat akin to our conditions. Palbociclib

99 shows less than 5% degradation in human plasma at room temperature over 3 days [72]. Fulvestrant  
100 shows no degradation in human plasma at room temperature over 7 hours [73]. Based on this data,  
101 we believe the half-life in our system is sufficiently long that assuming a constant level of drug is  
102 a reasonable approximation.

103

104 **Model Equations**

105

$$\frac{dER}{dt} = k_{ER} - kd_{ER} \times ER \quad (1)$$
$$-kb_{E2ER} \times E2 \times ER + kub_{E2ER} \times E2ER \quad (2)$$
$$-kb_{ICIER} \times ICI \times ER + kub_{ICIER} \times ICIER \quad (3)$$

106 (1) Translation and degradation of ER $\alpha$

107 (2) Binding and unbinding between ER $\alpha$  and E2

108 (3) Binding and unbinding between ER $\alpha$  and ICI 182,780

109

$$\frac{dE2ER}{dt} = -kd_{E2ER} \times E2ER \quad (4)$$
$$+kb_{E2ER} \times E2 \times ER - kub_{E2ER} \times E2ER \quad (5)$$

111 (4) Degradation of E2:ER

112 (5) Binding and unbinding between ER $\alpha$  and E2

113

$$\frac{dICIER}{dt} = kb_{ICIER} \times ICI \times ER - kub_{ICIER} \times ICIER \quad (6)$$
$$-kd_{ICIER} \times ICIER \quad (7)$$

115 (6) Binding and unbinding between ICI 182,780 and ER $\alpha$

116 (7) Degradation of ICIER

117

$$\begin{aligned} \frac{dcyclinD1}{dt} &= -kd_{cyclinD1} \times cyclinD1 \quad (8) \\ 118 \quad +k_{cyclinD1} \times \left( 1 + k_{cyclinD1E2ER} \times \frac{E2ER^{p_{cyclinD1E2ER_2}}}{p_{cyclinD1E2ER_1}^{p_{cyclinD1E2ER_2}} + E2ER^{p_{cyclinD1E2ER_2}}} \right) \quad (9) \\ &\quad -kb_{cyclinD1p21} \times cyclinD1 \times p21 + kub_{cyclinD1p21} \times cyclinD1p21 \quad (10) \\ &\quad -kb_{cyclinD1palbo} \times cyclinD1 \times palbo + kub_{cyclinD1palbo} \times cyclinD1palbo \quad (11) \end{aligned}$$

119 (8) Degradation of cyclinD1

120 (9) Basal translation of cyclinD1 and the increased translation by E2:ER

121 (10) Binding and unbinding between cyclinD1 and p21

122 (11) Binding and unbinding between cyclinD1 and palbociclib

123

$$\begin{aligned} 124 \quad \frac{dcyclinD1p21}{dt} &= -kd_{cyclinD1} \times cyclinD1p21 \quad (12) \\ &\quad +kb_{cyclinD1p21} \times cyclinD1 \times p21 - kub_{cyclinD1p21} \times cyclinD1p21 \quad (13) \end{aligned}$$

125 (12) Degradation of p21 bound cyclinD1

126 (13) Binding and unbinding between cyclinD1 and p21

127

$$\begin{aligned} 128 \quad \frac{dcyclinD1palbo}{dt} &= -kd_{cyclinD1} \times cyclinD1palbo \quad (14) \\ &\quad +kb_{cyclinD1palbo} \times cyclinD1 \times palbo - kub_{cyclinD1palbo} \times cyclinD1palbo \quad (15) \end{aligned}$$

129 (14) Degradation of palbociclib bound cyclinD1

130 (15) Binding and unbinding between cyclinD1 and palbociclib

131

$$\frac{dcMyc}{dt} = -kd_{cMyc} \times cMyc \quad (16)$$

132  $+k_{cMyc} \times (1 + k_{cMycE2ER} \times \frac{E2ER^{p_{cMycE2ER_2}}}{p_{cMycE2ER_1}^{p_{cMycE2ER_2}} + E2ER^{p_{cMycE2ER_2}}}) \quad (17)$

$$+k_{cMycRB1pp} \times \frac{RB1pp^{p_{cMycRB1pp_2}}}{p_{cMycRB1pp_1}^{p_{cMycRB1pp_2}} + RB1pp^{p_{cMycRB1pp_2}}}) \quad (18)$$

133 (16) Degradation of c-Myc

134 (17) Basal translation of c-Myc and the increased translation by E2:ER

135 (18) Increased translation of c-Myc by RB1-pp

136

$$\frac{dp21}{dt} = -kd_{p21} \times p21 \quad (19)$$

137  $+k_{p21} \times \frac{p_{p21cMyc_1}^{p_{p21cMyc_2}}}{p_{p21cMyc_1}^{p_{p21cMyc_2}} + cMyc^{p_{p21cMyc_2}}} \quad (20)$

$$-kb_{cyclinD1p21} \times cyclinD1 \times p21 + kub_{cyclinD1p21} \times cyclinD1p21 \quad (21)$$

$$-kb_{cyclinEp21} \times cyclinE \times p21 + kub_{cyclinEp21} \times cyclinEp21 \quad (22)$$

138 (19) Degradation of p21

139 (20) Basal translation and the inhibition of translation by c-Myc

140 (21) Binding and unbinding between cyclinD1 and p21

141 (22) Binding and unbinding between cyclinE and p21

142

143  $\frac{dcyclinE}{dt} = k_{cyclinE} - kd_{cyclinE} \times cyclinE \quad (23)$

$$-kb_{cyclinEp21} \times cyclinE \times p21 + kub_{cyclinEp21} \times cyclinEp21 \quad (24)$$

144 (23) Translation and degradation of cyclinE

145 (24) Binding and unbinding between cyclinE and p21

146

147 
$$\frac{dcyclinEp21}{dt} = -kd_{cyclinE} \times cyclinEp21 \quad (25)$$

$+kb_{cyclinEp21} \times cyclinE \times p21 - kub_{cyclinEp21} \times cyclinEp21 \quad (26)$

148 (25) Degradation of p21 bound cyclinE

149 (26) Binding and unbinding between cyclinE and p21

150

$$\frac{dRB1}{dt} = k_{RB1} - kd_{RB1} \times RB1 \quad (27)$$

$+k_{RB1RB1pp} \times \frac{RB1pp^{p_{RB1RB1pp_2}}}{p_{RB1RB1pp_1}^{p_{RB1RB1pp_2}} + RB1pp^{p_{RB1RB1pp_2}}} \quad (28)$

151  $-k_{RB1cyclinD1} \times cyclinD1 \times \frac{RB1^{p_{cyclinD1RB1_2}}}{p_{cyclinD1RB1_1}^{p_{cyclinD1RB1_2}} + RB1^{p_{cyclinD1RB1_2}}} \quad (29)$

$+k_{RB1pdepho} \times \frac{RB1p^{p_{RB1p_2}}}{p_{pRB1p_1}^{p_{RB1p_2}} + RB1p^{p_{RB1p_2}}} \quad (30)$

152 (27) Degradation of RB1 and basal translation

153 (28) Increased translation by E2F, modeled as proportional to RB1-pp

154 (29) Phosphorylation of RB1 by cyclinD1

155 (30) Dephosphorylation of RB1-p

156

$$\frac{dRB1p}{dt} = -kd_{RB1p} \times RB1p \quad (31)$$

$+k_{RB1cyclinD1} \times cyclinD1 \times \frac{RB1^{p_{cyclinD1RB1_2}}}{p_{cyclinD1RB1_1}^{p_{cyclinD1RB1_2}} + RB1^{p_{cyclinD1RB1_2}}} \quad (32)$

157  $-k_{RB1pdepho} \times \frac{RB1p^{p_{RB1p_2}}}{p_{pRB1p_1}^{p_{RB1p_2}} + RB1p^{p_{RB1p_2}}} \quad (33)$

$-k_{RB1pcyclinE} \times cyclinE \times \frac{RB1p^{p_{cyclinERB1p_2}}}{p_{cyclinERB1p_1}^{p_{cyclinERB1p_2}} + RB1p^{p_{cyclinERB1p_2}}} \quad (34)$

$+k_{RB1ppdepho} \times \frac{RB1pp^{p_{RB1pp_2}}}{p_{pRB1pp_1}^{p_{RB1pp_2}} + RB1pp^{p_{RB1pp_2}}} \quad (35)$

158 (31) Degradation of RB1-p

159 (32) Phosphorylation of RB1 by cyclinD1

160 (33) Dephosphorylation of RB1-p

161 (34) Phosphorylation of RB1-p by cyclinE

162 (35) Dephosphorylation of RB1-pp

163

$$\frac{dRB1pp}{dt} = -kd_{RB1pp} \times RB1pp \quad (36)$$

$$164 + k_{RB1pcyclinE} \times cyclinE \times \frac{RB1p^{p_{cyclinERB1p_2}}}{p_{cyclinERB1p_1}^{p_{cyclinERB1p_2}} + RB1p^{p_{cyclinERB1p_2}}} \quad (37)$$

$$-k_{RB1ppdepho} \times \frac{RB1pp^{p_{RB1pp_2}}}{p_{RB1pp_1}^{p_{RB1pp_2}} + RB1pp^{p_{RB1pp_2}}} \quad (38)$$

165 (36) Degradation of RB1-pp

166 (37) Phosphorylation of RB1-p by cyclinE

167 (38) Dephosphorylation of RB1-pp

168

$$169 \frac{dcell}{dt} = k_{pro} \times (1 + k_{proRB1pp} \times \frac{RB1pp^{p_{proRB1pp_2}}}{p_{proRB1pp_1}^{p_{proRB1pp_2}} + RB1pp^{p_{proRB1pp_2}}}) \times cell \times (1 - \frac{cell}{k_{carrying}}) \quad (39)$$

170 (39) Basal proliferation and the increased proliferation by RB1-pp

171 To model resistance, we added the following equation:

$$172 \frac{dres}{dt} = par1_{res} \times palbo - par2_{res} \times res \quad (40)$$

173 and added one term to the cyclinD1 equation:

$$\frac{dcyclinD1}{dt} = -kd_{cyclinD1} \times cyclinD1 \quad (8)$$

$$174 + k_{cyclinD1} \times \left( 1 + k_{cyclinD1E2ER} \times \frac{E2ER^{p_{cyclinD1E2ER_2}}}{p_{cyclinD1E2ER_1}^{p_{cyclinD1E2ER_2}} + E2ER^{p_{cyclinD1E2ER_2}}} \right) \quad (9)$$

$$-kb_{cyclinD1p21} \times cyclinD1 \times p21 + kub_{cyclinD1p21} \times cyclinD1p21 \quad (10)$$

$$-kb_{cyclinD1palbo} \times cyclinD1 \times palbo + kub_{cyclinD1palbo} \times cyclinD1palbo \quad (11)$$

$$+par3_{res} \times \frac{res^{par5_{res}}}{par4_{res}^{par5_{res}} + res^{par5_{res}}} \quad (41)$$

175  $par1_{res} = 1e^{-4}$ ,  $par2_{res} = 1e^{-3}$ ,  $par3_{res} = 0.819$ ,  $par4_{res} = 0.06$ ,  $par5_{res} = 4.87$

176

## 177 Model Summary

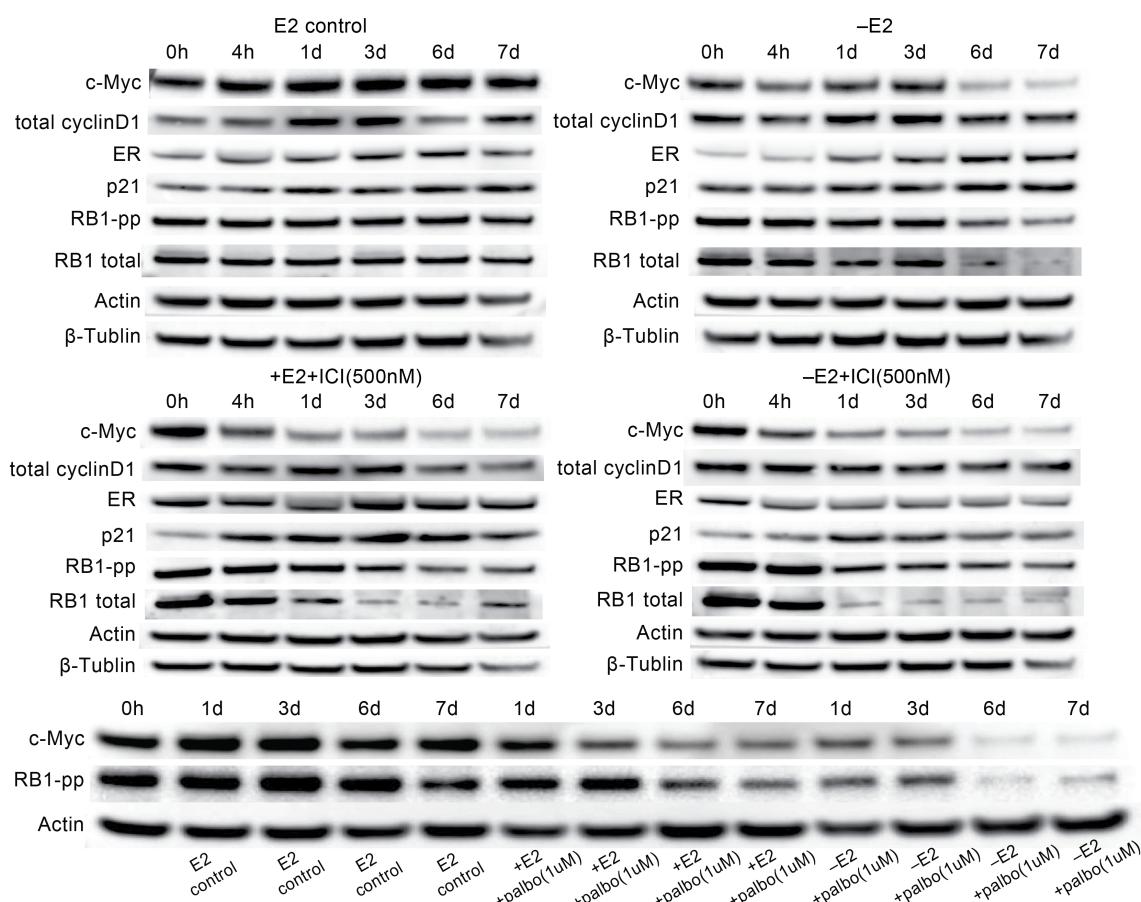
178 The mathematical model contains 14 ordinary differential equations (ODEs) and has 62 parameters.  
179 It is implemented in MATLAB (MathWorks, Inc., Massachusetts, United States). The synthesis,  
180 degradation, phosphorylation, dephosphorylation, association and dissociation reactions are modeled  
181 by mass action laws and Hill functions. ICI 182,780 and palbociclib effects are modeled by competitive  
182 binding to ER $\alpha$  [50, 60] or cyclinD1, representing cyclinD1:Cdk4/6 [74]. The ODEs are solved  
183 numerically by the *ode23tb* function. RB1 has an important role in the G1-S transition and it is  
184 inactivated by phosphorylation [75]. A total of over 15 phosphorylation sites are found on RB1 (i.e. T5,  
185 S249, S252, T356, T373, S567, S608, S612, S780, S788, S795, S807, S811, T821, T826) [76]. In late G1,  
186 cyclinE:Cdk2 kinase mediates the phosphorylation of RB1 on S612 and T373 relieving RB1's  
187 repression of E2F activity [76, 77]. As cyclinD1:Cdk4/6 kinases hypophosphorylate RB1 in G1 and  
188 cyclinE:Cdk2 kinase hyperphosphorylates RB1-p in late G1 [78-80], we chose one form of  
189 phosphorylated RB1 (S612) to represent the phosphorylation status of RB1 by cyclinE:Cdk2 kinase.

190

## 191 Data Normalization

192 Protein levels were measured by Western blotting at time points 0h, 4h, 1d, 3d, 6d and 7d for the E2  
193 deprivation, +E2+ICI and -E2+ICI conditions, and at 0h, 1d, 3d, 6d and 7d for the +E2+palbo and -  
194 E2+palbo conditions. The blots for the treatment conditions are normalized to actin and  $\beta$ -tubulin, to  
195 account for loading variation, normalized to time 0h, to account for antibody affinity variations, and  
196 normalized to the (normalized) E2 control values for each species at each time point, to account for  
197 variations in the expression levels of species in the cells used for a given experiment. Since the control  
198 cells are not subject to treatment perturbations (they continue to be grown in E2), the asynchronous  
199 population would ideally have constant expression levels of the various molecules. This is not the case

200 in our experiments as there are frequently transients during the first few time points, perhaps due to  
 201 the shock of reattachment upon plating before time 0. Furthermore, after 3 days the control cells begin  
 202 to approach confluence, while the cells under treatment conditions do not begin to approach  
 203 confluence until 7 days. At confluence, paracrine signaling and contact inhibition suppress  
 204 proliferation via cell cycle arrest [81-84]. To avoid the confounding effect of confluence on the control  
 205 cells, we assume the expression levels of the species in the control cells after 3 days are the same as  
 206 their levels at the 3d timepoint and use the 3d levels for normalization of the future timepoints of the  
 207 treated cells. This approach allows our normalization to deal with the initial transients in expression  
 208 levels while not confounding the normalization of timepoints after 3 days with the effects of confluence  
 209 on the control cells.



210

211 **Fig. S1: Representative western blot data shown here for different treatment conditions. All**  
 212 **experiments were done in triplicate.**

213

## 214 Local Sensitivity Analysis

215 Local sensitivity analysis concerns the sensitivity of a model output to changes in the input parameters  
216 [85]. The local sensitivities of the seven measured output variables are calculated with respect to the  
217 parameters at day 7 timepoint [86].

218

$$s_{ijk} = \frac{\partial \log(X_{ij})}{\partial \log(P_k)} = \frac{\partial X_{ij}}{\partial P_k} \frac{P_k}{X_{ij}} \quad (42)$$

219 where  $s_{ijk}$  is the local sensitivity value, which is the derivative of output  $X_{ij}$  with respect to parameter  
220  $P_k$  multiplied by the ratio  $P_k/X_{ij}$ . It represents the relative change in the model output induced by a  
221 small relative change in a parameter. In the equation,  $i$  indexes the outputs (protein level or cell  
222 number),  $j$  indexes the timepoints, and  $k$  indexes the parameters.

223  $s_{ijk}$  is approximated by the second order central finite difference. Each parameter is individually  
224 varied by  $\pm 5\%$  of its value. Therefore,

225

$$s_{ijk} \approx \frac{X_{ij}(P_k + 5\% \times P_k) - X_{ij}(P_k - 5\% \times P_k)}{10\% \times P_k} \frac{P_k}{X_{ij}(P_k)}$$

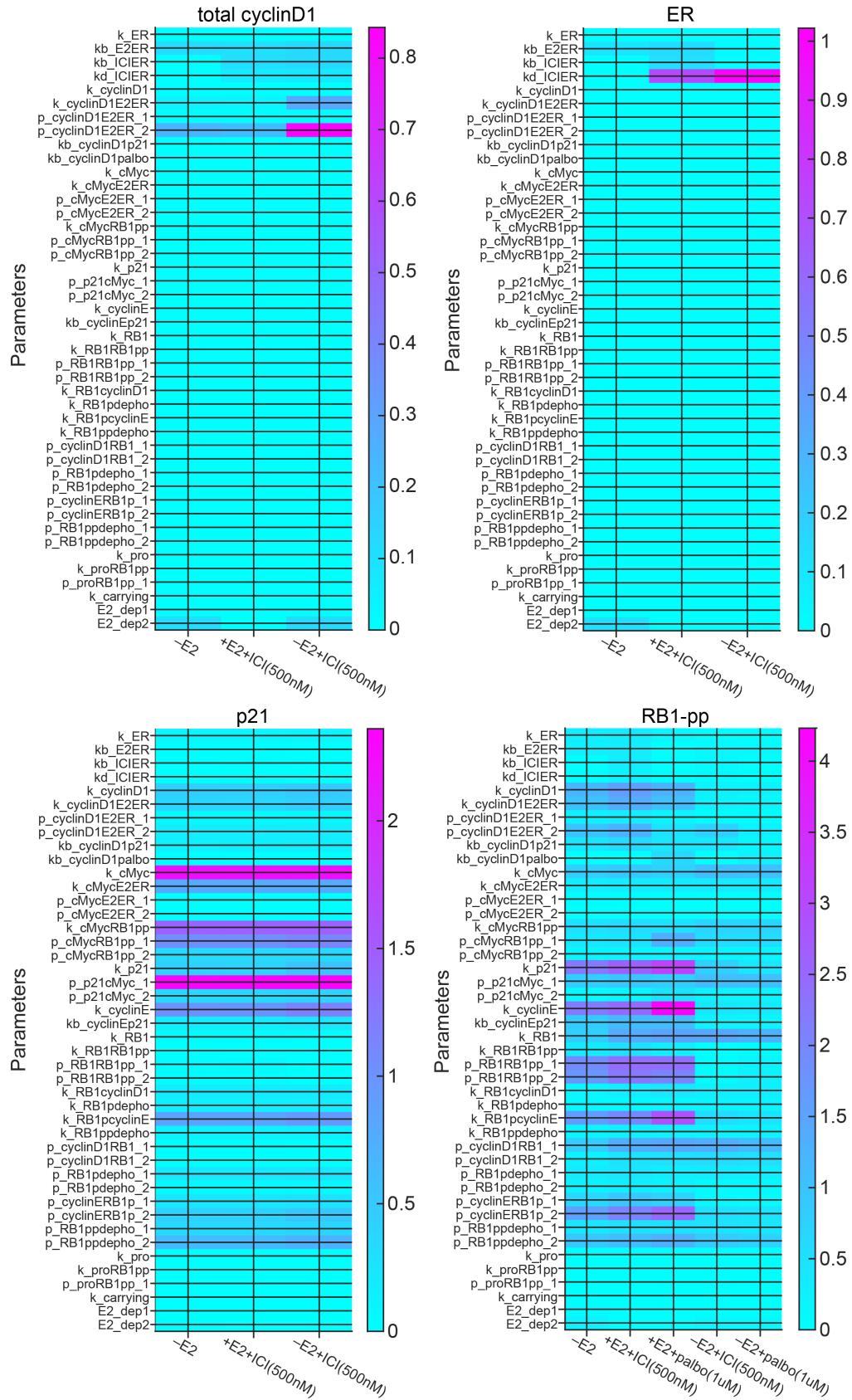
226

$$= \frac{X_{ij}(P_k + 5\% \times P_k) - X_{ij}(P_k - 5\% \times P_k)}{10\% \times X_{ij}(P_k)} \quad (43)$$

227 All parameters that were varied to fit the data were selected for local sensitivity analysis, while the  
228 fixed parameters were excluded. The 44 parameters, from top to bottom in Fig 6 and Fig. S2, are:  $k_{ER}$ ,  
229  $kb_{E2ER}$ ,  $kb_{ICIER}$ ,  $kd_{ICIER}$ ,  $k_{cyclinD1}$ ,  $k_{cyclinD1E2ER}$ ,  $p_{cyclinD1E2ER_1}$ ,  $p_{cyclinD1E2ER_2}$ ,  $kb_{cyclinD1p21}$ ,  
230  $kb_{cyclinD1palbo}$ ,  $k_{cMyc}$ ,  $k_{cMycE2ER}$ ,  $p_{cMycE2ER_1}$ ,  $p_{cMycE2ER_2}$ ,  $k_{cMycRB1pp}$ ,  $p_{cMycRB1pp_1}$ ,  $p_{cMycRB1pp_2}$ ,  $k_{p21}$ ,  
231  $p_{p21cMyc_1}$ ,  $p_{p21cMyc_2}$ ,  $k_{cyclinE}$ ,  $kb_{cyclinEp21}$ ,  $k_{RB1}$ ,  $k_{RB1RB1pp}$ ,  $p_{RB1RB1pp_1}$ ,  $p_{RB1RB1pp_2}$ ,  $k_{RB1cyclinD1}$ ,  
232  $k_{RB1pdepho}$ ,  $k_{RB1pcyclinE}$ ,  $k_{RB1ppdepho}$ ,  $p_{cyclinD1RB1_1}$ ,  $p_{cyclinD1RB1_2}$ ,  $p_{RB1pdepho_1}$ ,  $p_{RB1pdepho_2}$ ,  $p_{cyclinERB1p_1}$ ,  
233  $p_{cyclinERB1p_2}$ ,  $p_{RB1ppdepho_1}$ ,  $p_{RB1ppdepho_2}$ ,  $k_{pro}$ ,  $k_{proRB1pp}$ ,  $k_{proRB1pp_1}$ ,  $k_{carrying}$ ,  $E2_{dep1}$ ,  $E2_{dep2}$ .

234

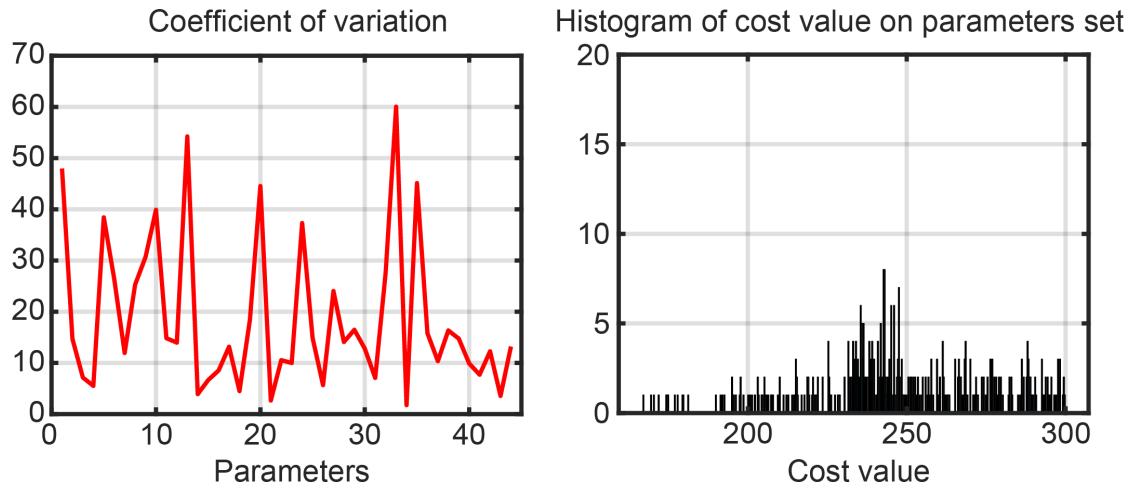
### Local sensitivity of parameters



236 **Fig. S2: Local Sensitivity of total cyclinD1, total ER, total p21 and RB1-pp.** Sensitivity of each  
237 protein at the 7d timepoint with respect to all variable parameters.

238 **Variation of the Cohort Parameters and Plots of the Individual**  
239 **Trajectories**

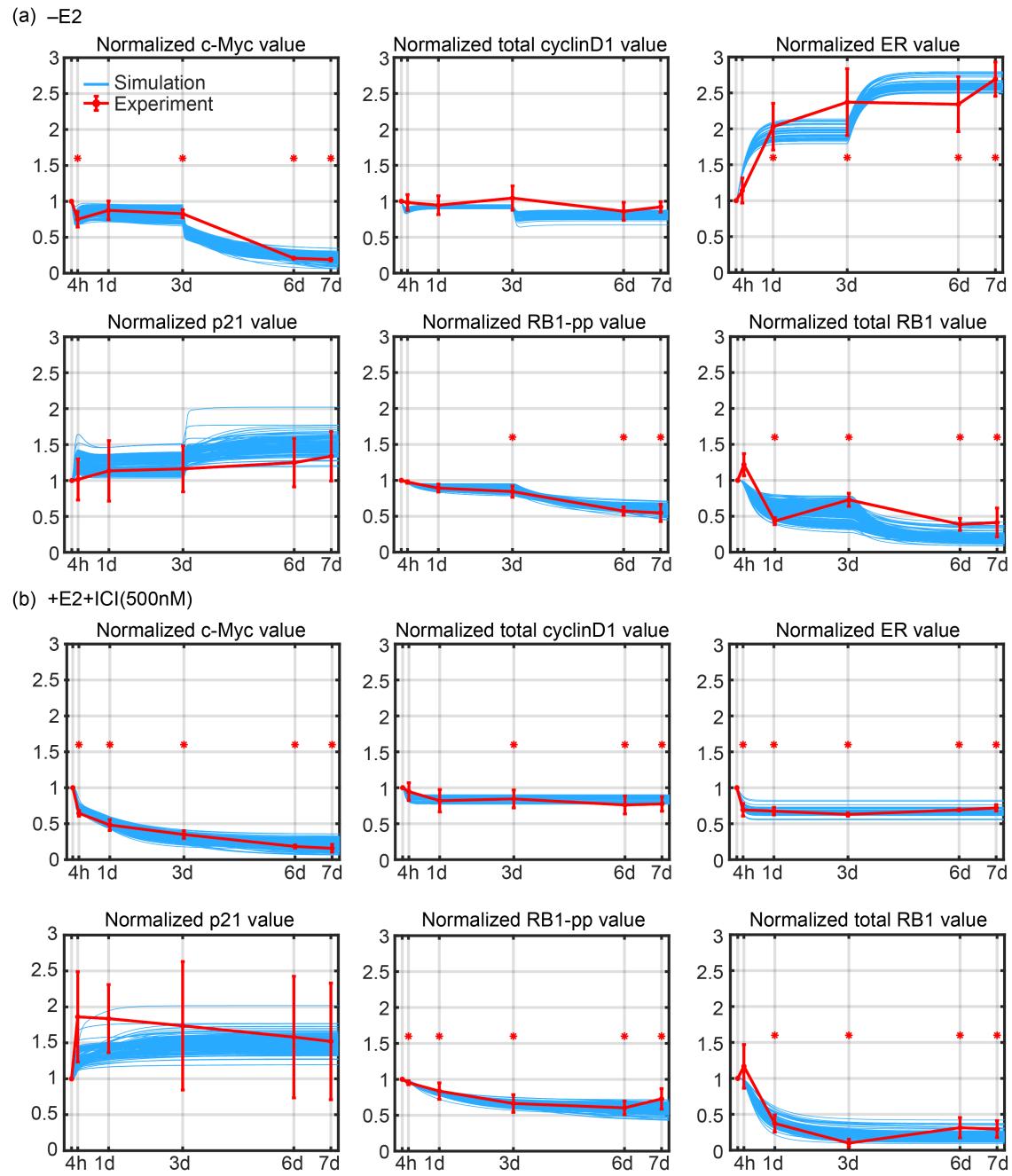
Coefficient of variation and histogram of cost value on parameters set



240

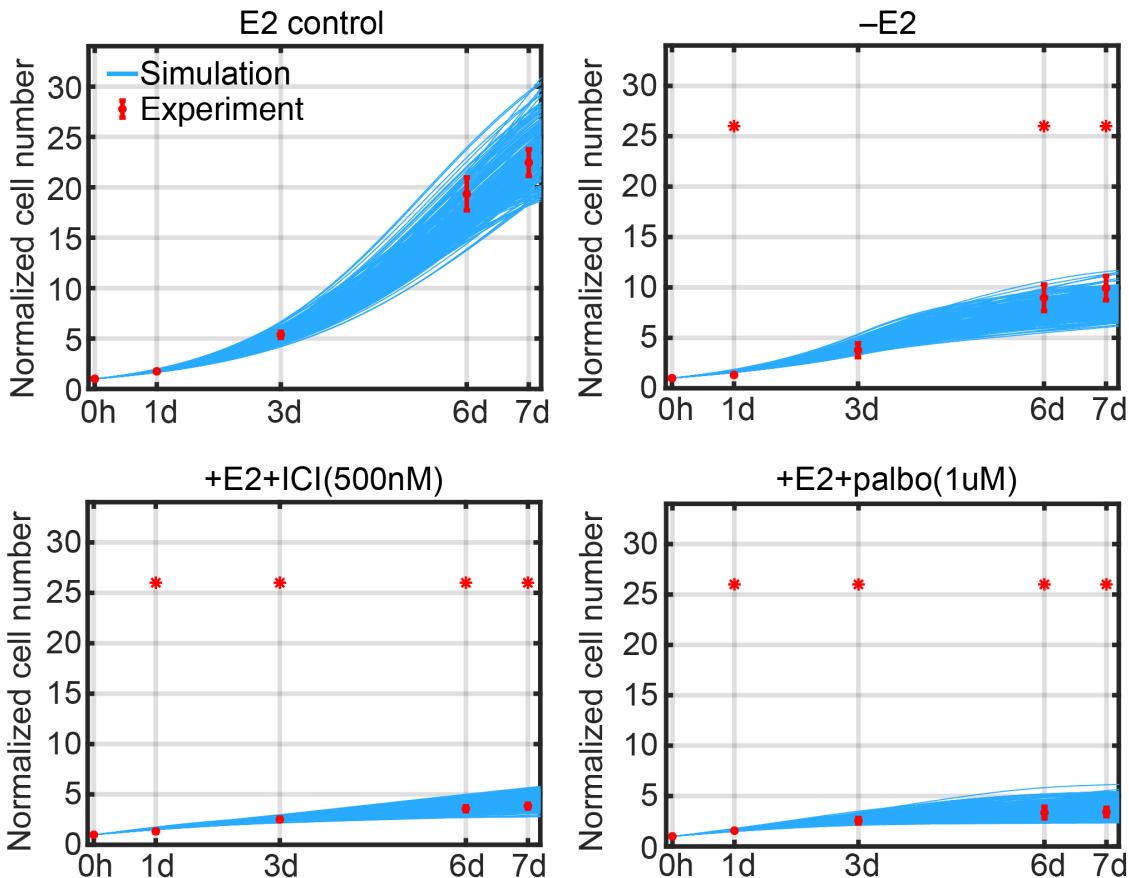
241 **Fig. S3: Coefficients of variation and histogram of cost values for parameter sets in the**  
242 **simulation cohort.** Ordering of parameters corresponds to that in the sensitivity plots.

243



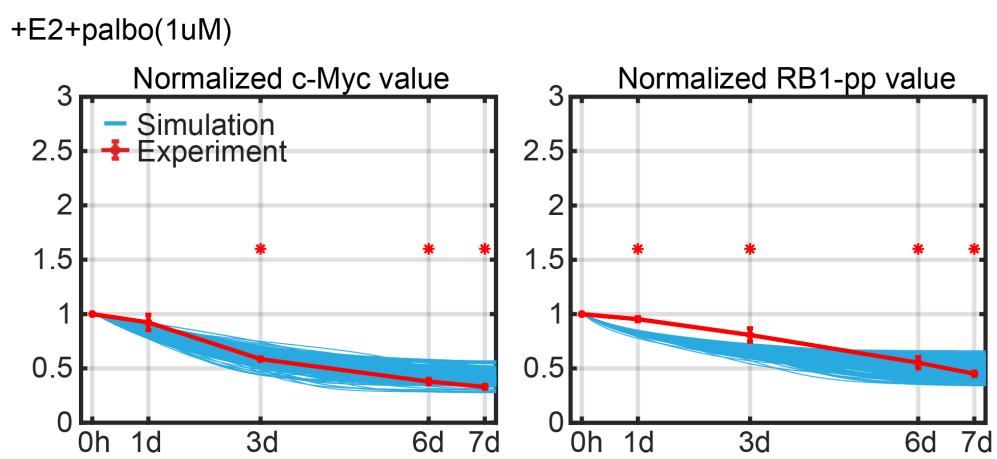
244

245 **Fig. S4: All cohort trajectories plotted with experimental protein data that was used to fit the**  
 246 **model. (a) -E2 condition. (b) +E2+ICI(500nM) condition.**



247

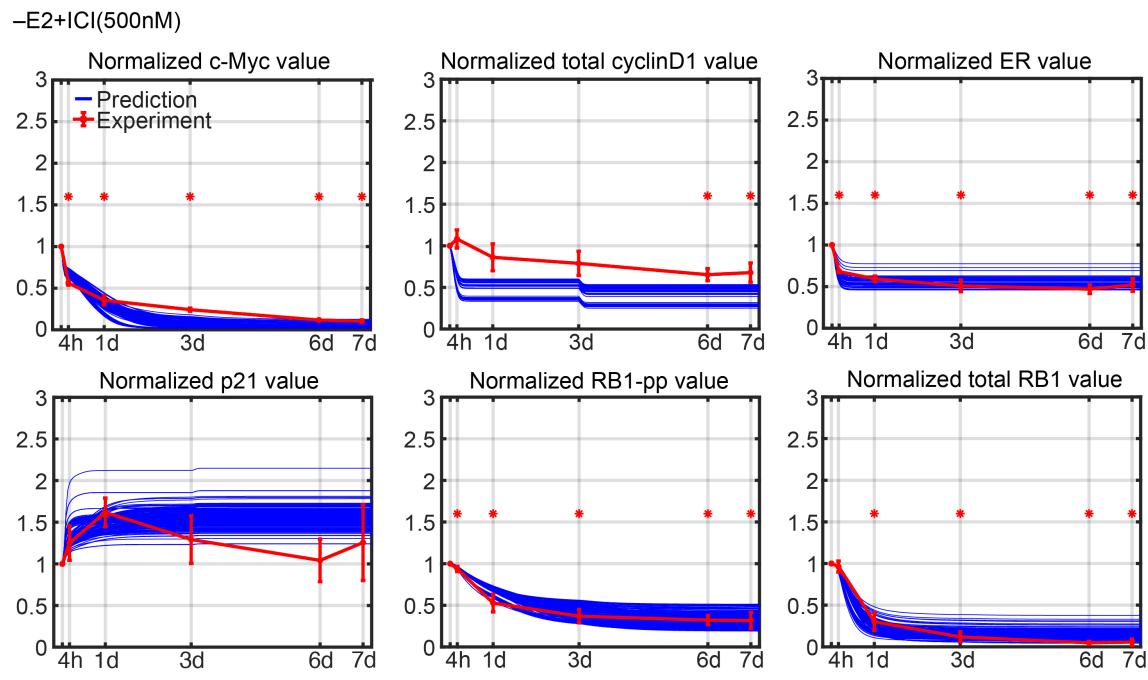
248 **Fig. S5: All cohort trajectories plotted with experimental proliferation data that was used to**  
 249 **fit the model.**



250

251 **Fig. S6: All cohort trajectories plotted with experimental protein data used to fit the**  
 252 **parameters involving palbociclib in the model.**

253

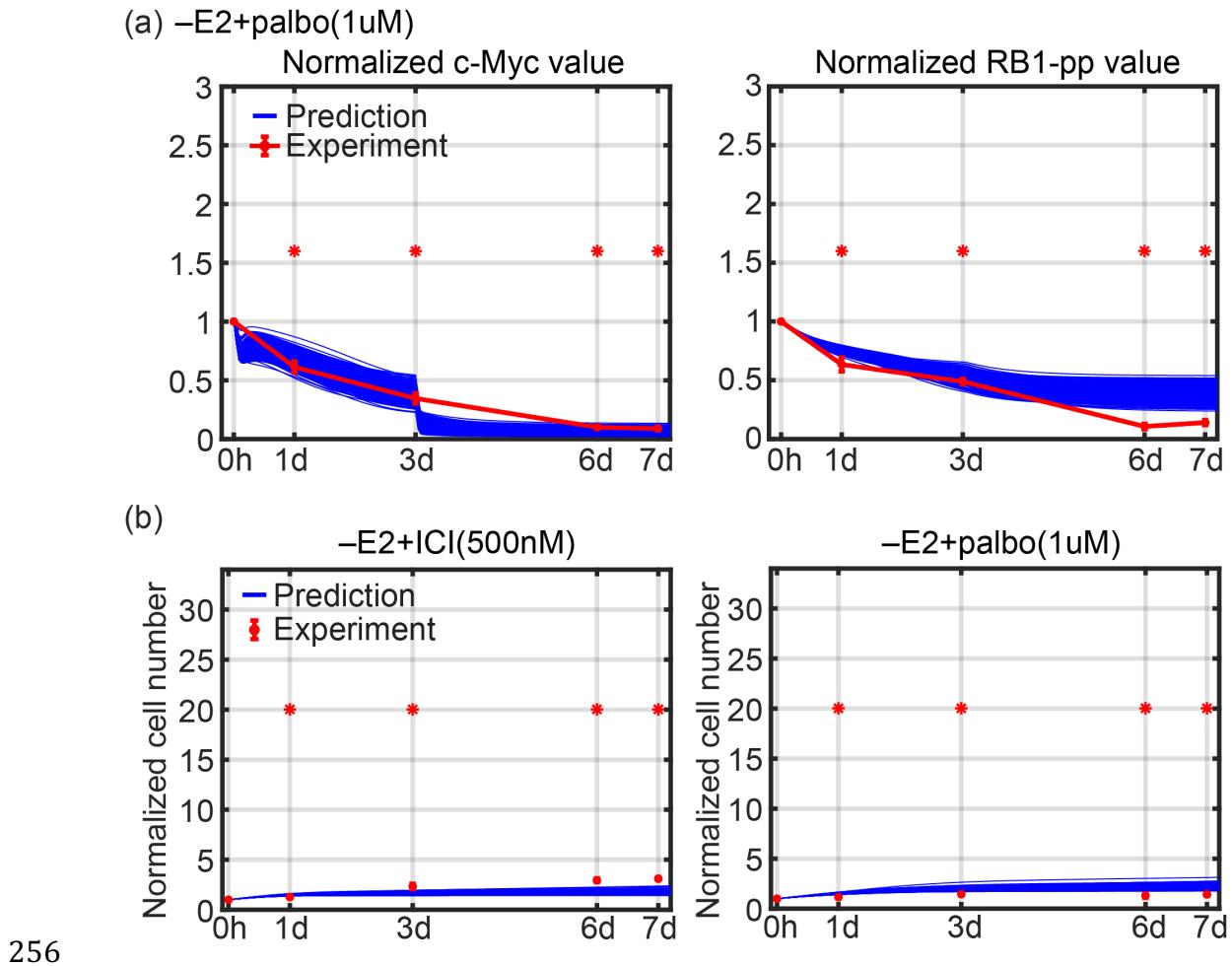


254

**Fig. S7: All cohort trajectory predictions for the -E2+ICI(500nM) case plotted with**

255

**experimental protein validation data.**



257 **Fig. S8: All cohort trajectory predictions plotted with the experimental validation data.** (a)  
258 Protein predictions for the  $-E2+palbociclib(1\mu M)$  case. (b) Proliferation predictions for the –  
259  $E2+ICI(500nM)$  and  $-E2+palbociclib(1\mu M)$  cases.

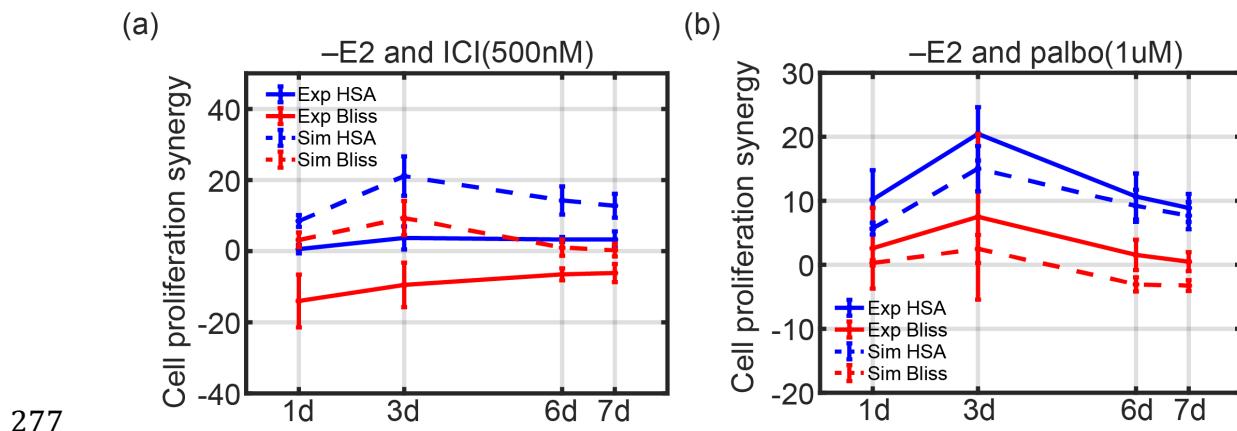
260

## 261 Synergy Analysis of the Combination Treatments

262

263 Fig. S9 shows the synergy scores of the combination treatments  $-E2+ICI(500nM)$  and  $-$   
264  $E2+palbociclib(1\mu M)$  for proliferation at different timepoints. The HSA and Bliss synergy scores are  
265 calculated using the R package SynergyFinder [87]. The score, in percent, is equal to the percent

266 inhibition of the combination therapy minus the expected inhibition. For HSA the expected inhibition  
 267 is the highest percent inhibition of the two mono therapies, and for Bliss the expected inhibition is the  
 268 sum of the percent inhibition of each mono therapy minus the product of the percent inhibitions of the  
 269 two therapies. Thus, a positive score represents a synergistic effect. There is no significant synergy,  
 270 and some antagonism indicated in the experimental results for the -E2+ICI(500nM) combination  
 271 therapy. The simulations show some synergy for this case because they slightly under-predict the true  
 272 proliferation of the combination. For the case of the -E2+palbociclib(1uM) combination, there is some  
 273 indication of synergy in the experimental results and slightly less in the simulation results due to the  
 274 slight overprediction of proliferation for the combination in the simulations. A synergistic effect  
 275 between -E2 and palbociclib is consistent with the use of palbociclib in conjunction with endocrine  
 276 therapies in the clinic [88-90].



278 **Fig. S9: HSA and Bliss cell proliferation synergy scores of -E2+ICI(500nM) and -**  
 279 **E2+palbociclib(1uM) treatments.** The scores of the experimental results are shown with solid lines  
 280 (mean value  $\pm$  s.d., n = 3). The scores of the simulation results are shown with dashed lines (mean value  
 281  $\pm$  s.d., n = 400).

282

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