

Supplementary Information for:

“Integrative modelling uncovers p21-driven drug resistance and prioritizes therapies for PIK3CA-mutant breast cancer”

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Supplementary Figures 1 to 9

Supplementary Tables 1 to 4

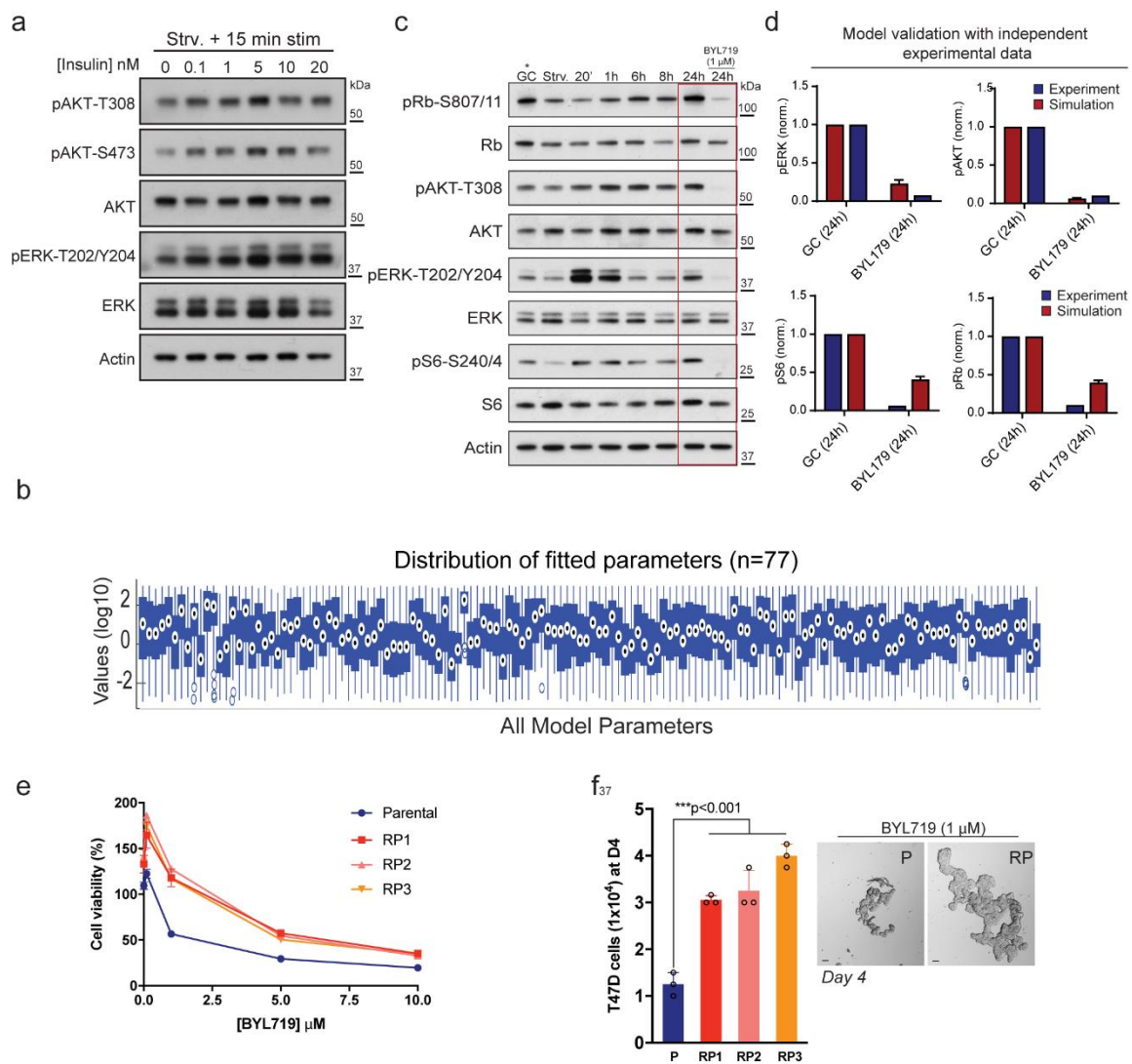
Other supplementary materials for this manuscript include the following:

Dataset 1. List of best-fitted parameter values used for ensemble model calibration.

Dataset 2. CDI scores and effect of single and combination drugs shown in Fig. 2A-F and Supplementary Fig. 3C, D.

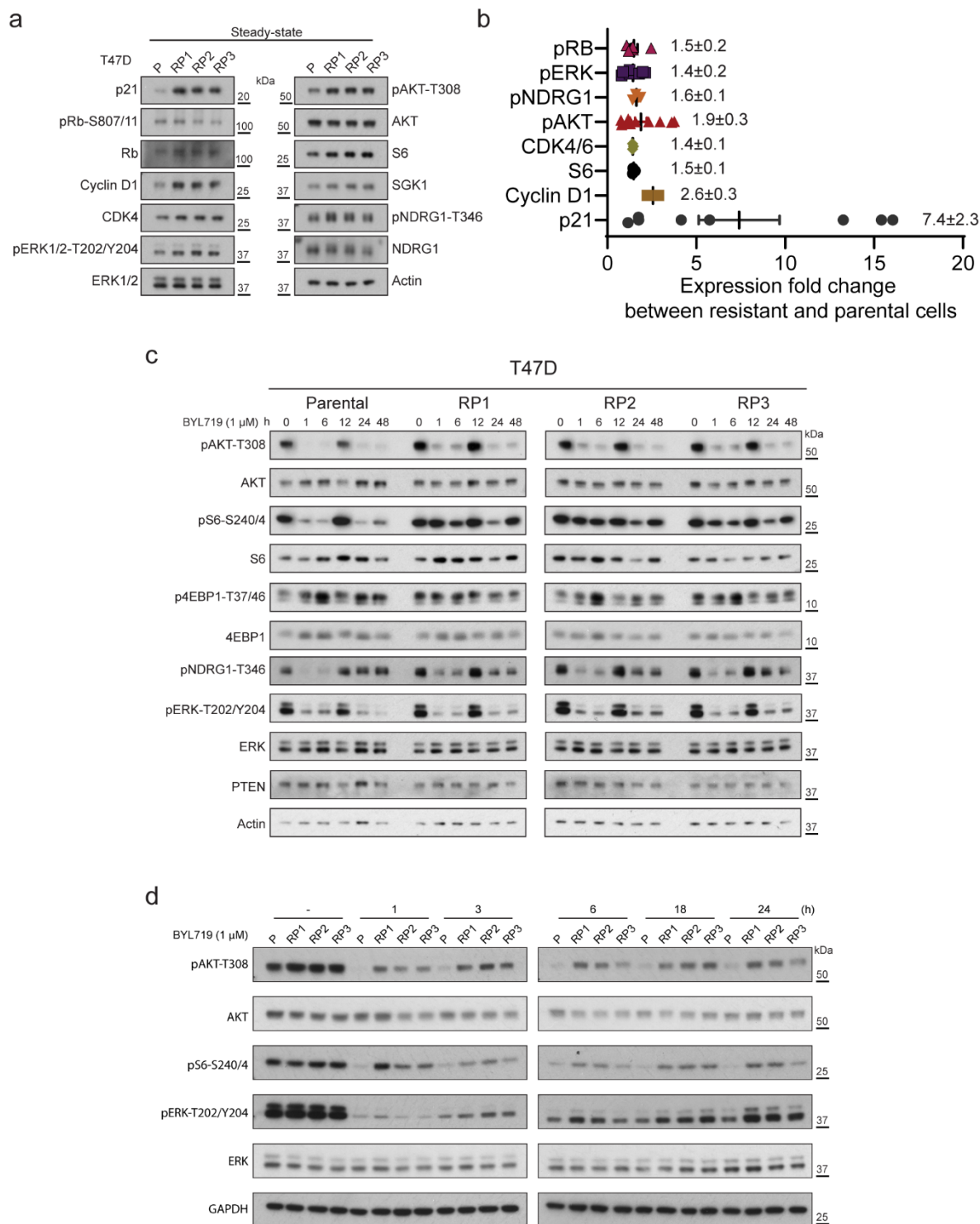
Dataset 3. Parameters and constants for the phenotypic model.

Supplementary Figures



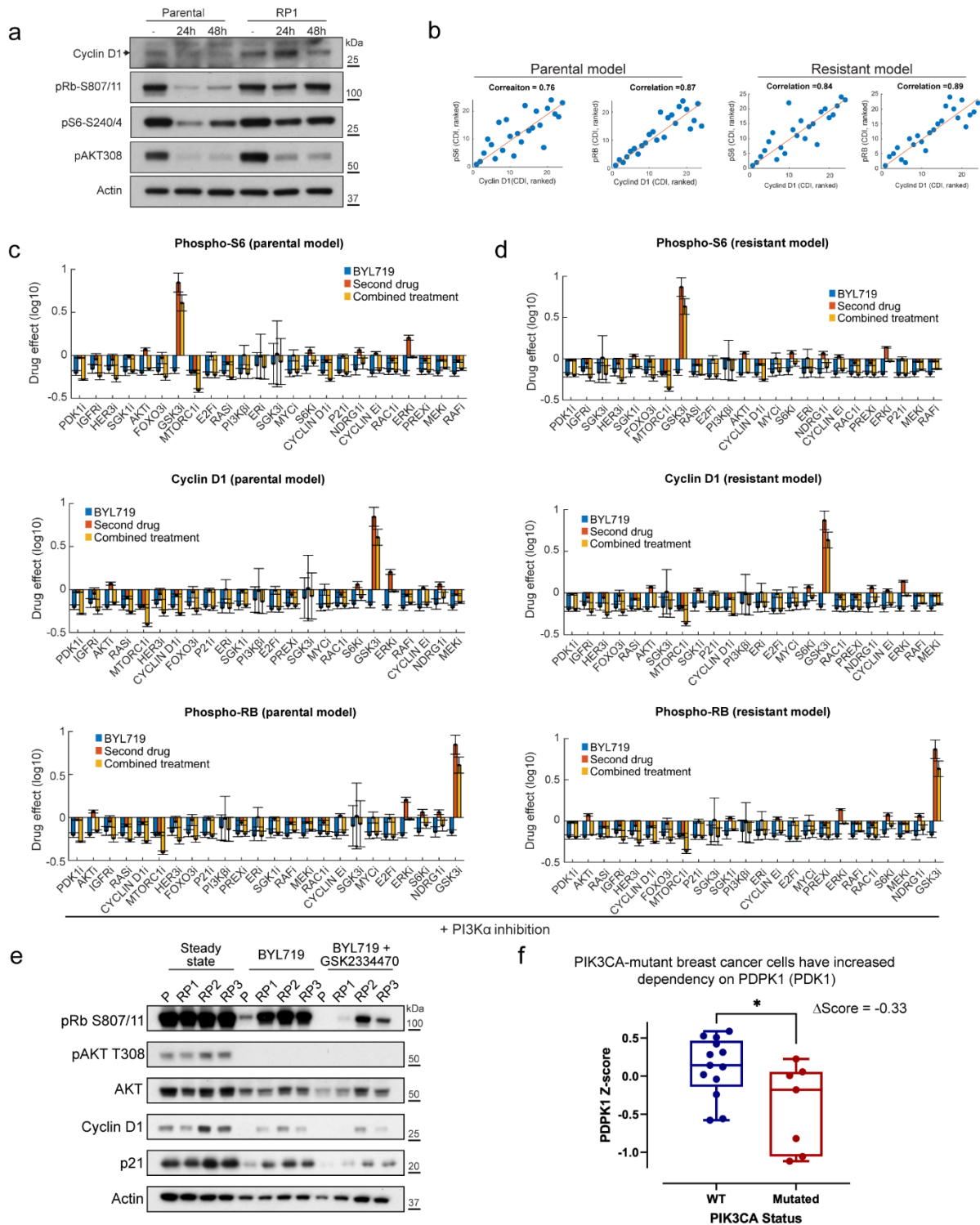
Supplementary Figure 1. (a) Parental T47D cells were starved for 24 hrs in DMEM, and stimulated for 15 min with insulin at the indicated concentrations. **(b)** Box plot of fitted parameters (n=77). **(c)** WB of parental T47D cells kept under growing conditions (GC) or starved for 24 hrs in DMEM and then stimulated with full medium for the indicated times. One well of T47D cells was stimulated with full medium plus 1 μ M BYL719 for 24h to test response to treatment. **(d)** Model validation using independent experimental data, quantified from WB images shown in (c). Red and blue bars indicate simulation and experimental data, respectively. Data represent mean values and error bars indicate standard errors (n=77). Effect of BYL719 on ERK, AKT, S6 and RB phosphorylation. **(e)** Cell viability assay of

parental and resistant T47D pools (RPs) grown under increasing concentration of BYL719, i.e. 0.1, 1, 5 and 10 μ M for 4 days, and processed with the CellTiter-Glo® 3D-Cell Viability assay. Data are represented as mean $\pm$ standard deviation (SD) of triplicate wells (n=3). **(f)** 3D growth curve of parental T47D cells and BYL719-RPs plated on p(HEMA) coated dishes and kept in full medium plus 1 μ M BYL719 for 4 days. Right, representative images of parental (P) and resistant pool 1 (RP) 4 days of treatment. Data are represented as mean $\pm$ SD, one-way ANOVA, (n=3 replicates of culture), ***p<0.001.



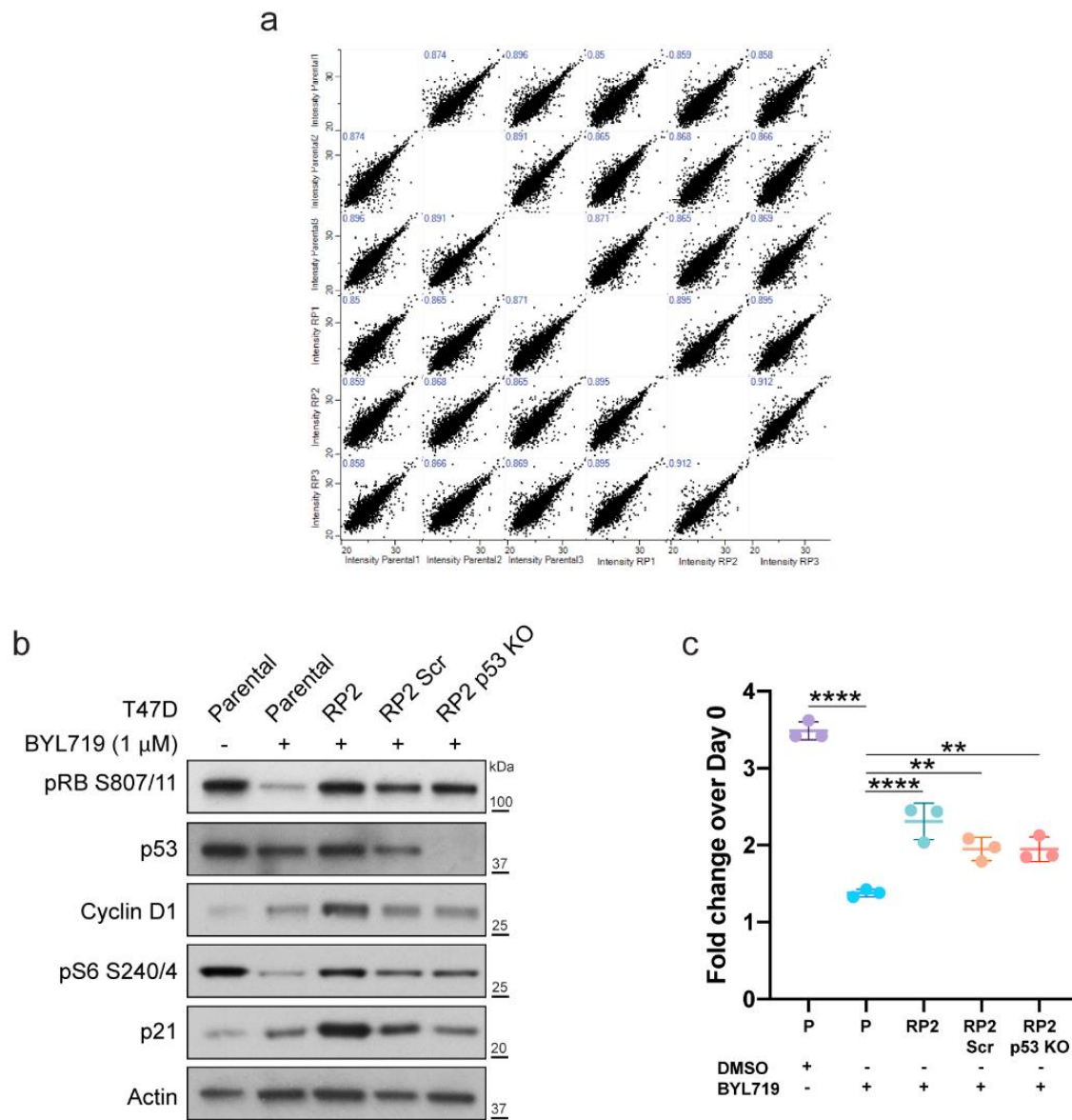
Supplementary Figure 2. (a) Representative cell lysates of parental and T47D RPs cultured in growing condition. Expression/phosphorylation of multiple network nodes was analysed by WB. **(b)** Relative fold change in expression and/or phosphorylation of signalling proteins in RPs compared to parental cells, quantified from WB data (as in panel a). Data was quantified from 3 independent experiments as mean $\pm$ standard error.

(c) Parental T47D cells and three BYL719- RPs were either left untreated or treated with BYL719 (1 μ M) for 1, 6, 12, 24 and 48h or **(d)** 1, 3, 6, 18 and 24h. Cell lysates were analysed by WB (See also Figure 1G).

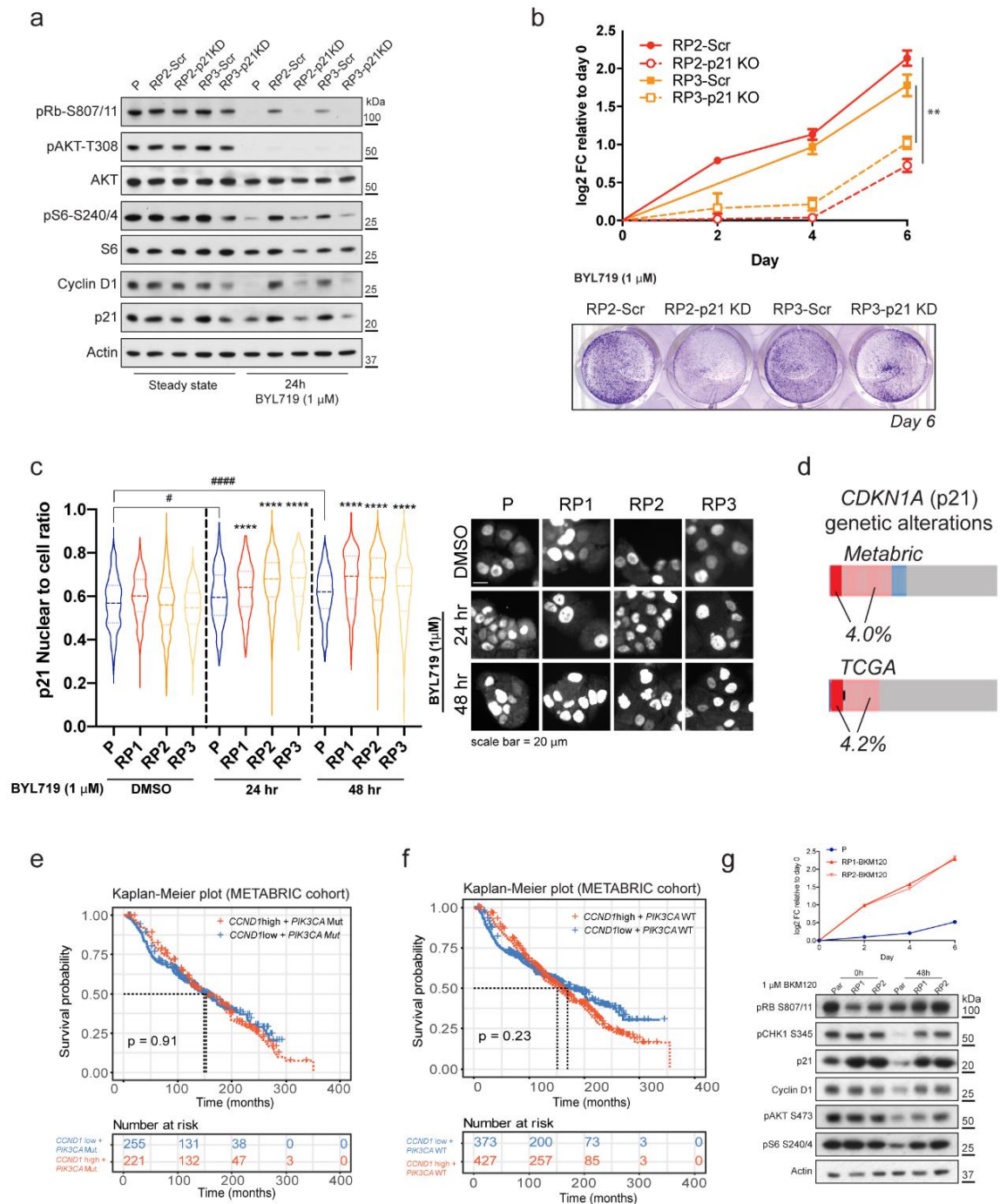


Supplementary Figure 3. (a) WB analysis of cyclin D1 and pRb in lysates of T47D parental and RP1 treated with BYL719 for the time indicated. **(b)** Correlation analysis of the synergy scores between readouts, i.e., pS6, Cyclin D1 and pRb. Effect of single and combination drugs treatments on pS6, cyclin D1 and pRb levels in parental **(c)** and resistant PI3K models **(d)**. Bars indicate the mean values and $\pm$ standard errors (see Dataset 2). **(e)** WB analysis of

p21 and cell cycle regulators in lysates of T47D cells. T47D parental cells and RP1 were treated with 1 μ M BYL719 or 0.5 μ M of the PDK1 inhibitor GSK2334470, either alone or in combination, for 24 and 48h. **(f)** Dependency scores of the *PDPK1* gene in *PIK3CA*-mutant (n=13) and -WT (n=7) breast cancer cells, DEPMAP; note that a lower negative score value indicates stronger dependency, *p<0.05.



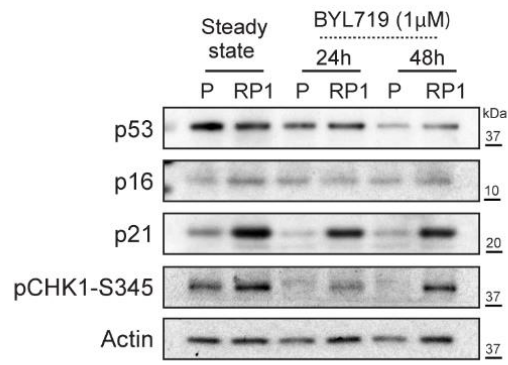
Supplementary Figure 4. (a) Pearson's correlation of phosphopeptides identified in triplicate samples of T47D parental cells and RP1, RP2 and RP3. **(b)** WB showing successful *TP53* knock-out (KO) in BYL719-RP2 cells using CRISPR. RP2 Scr samples are cells transduced with negative scramble (Scr) control. **(c)** Comparative growth curves at day 6 of the indicted cell lines upon 1 μ M BYL719 treatment. Data are presented as mean $\pm$ SD, one-way ANOVA, (n=3 replicates of culture) **p<0.01, ****p<0.0001.



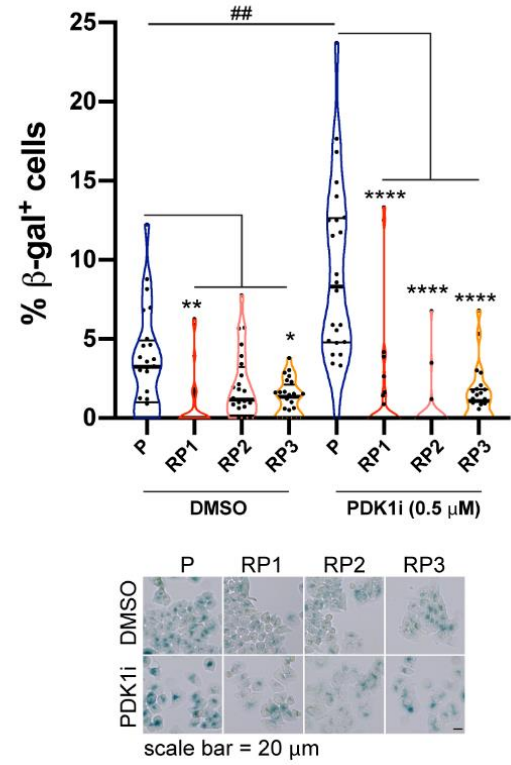
Supplementary Figure 5. (a) WB analysis of cell cycle regulators in T47D cells. CRISPR/Cas9 approach was used to transduce T47D RP1 cells with scramble (Scr) or 2 independent guide-RNAs directed at *CDKN1A* (p21KD-1, -2). Cells were either left untreated or treated with 1 μ M BYL719 for 24h. **(b)** *CDKN1A* knock-down re-sensitises T47D RP1 cells to BYL719. T47D cells were treated with 1 μ M BYL719 for 6 days and new drug added to the medium every 2 days. Representative images of cells stained with crystal violet on day

6 (bottom). Data are presented as mean $\pm$ SD, one-way ANOVA, (n=3 replicates of culture) **p<0.01. **(c)** Parental (P) and resistant T47D cells were treated with 1 μ M BYL719 for 24 and 48 hrs, and fluorescence intensities quantified using CellProfiler. The fluorescence intensity of nuclear p21 was normalised over the intensity detected in the entire cell, and results are presented as cell ratio. Data represent median and the 1st and 3rd quartile. ####p<0.0001 indicate comparisons between the same cell type upon BYL719 treatments compared to DMSO; ****p<0.0001 are comparisons between RPs and parental in the same treatment condition. **(d)** Percentage of genetic alterations affecting *CDKN1A* in human breast cancer samples (Metabric and TCGA datasets). Alterations legends: deep deletion (dark blue), amplification (red), high mRNA levels (pink), low mRNA level (blue), black (truncating mutations) and grey (unaltered). **(e, f)** Probability of overall survival (OS) of breast cancer patients (METABRIC). **(g)** *Top*: growth curves of parental and BKM-120-resistant T47D cells over 6 days of treatments in 1 μ M BKM-120. *Bottom*: WB showing markers of resistance in parental and BKM-120-resistant T47D cells.

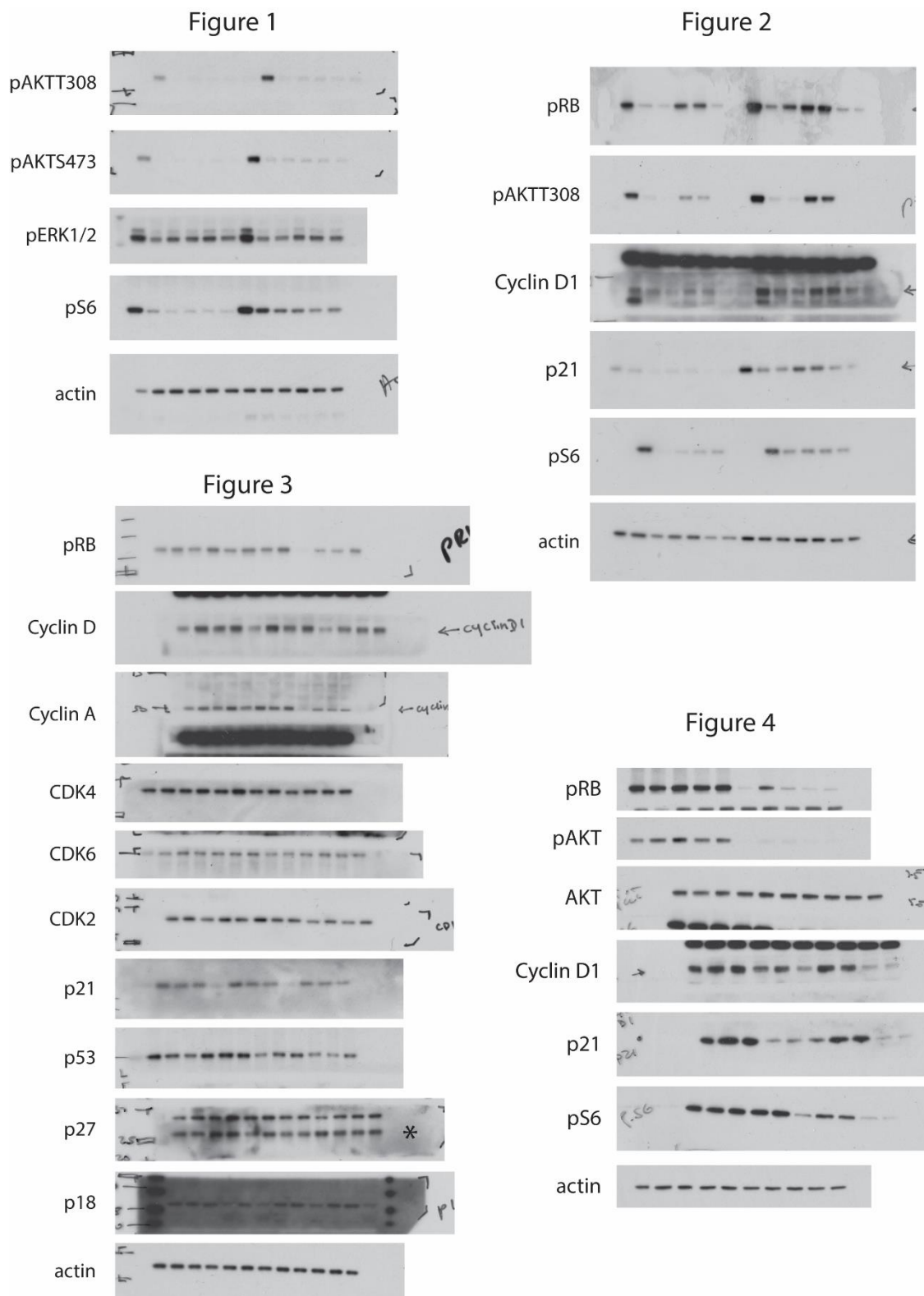
a



b



Supplementary Figure 6. (a) WB analysis of senescence regulators in T47D parental and RP1 cells treated with 1μM BYL719 for 24 and 48h. **(b)** T47D parental and RPs were treated with 0.5 μM GSK2334470 for 48hrs, then fixed and stained to detect β-gal positivity. ##p<0.01, *p<0.05, **p<0.01, ****p<0.0001.



Supplementary Figure 7. Uncropped scans of blots included in main figures 1 to 4.

Figure 5

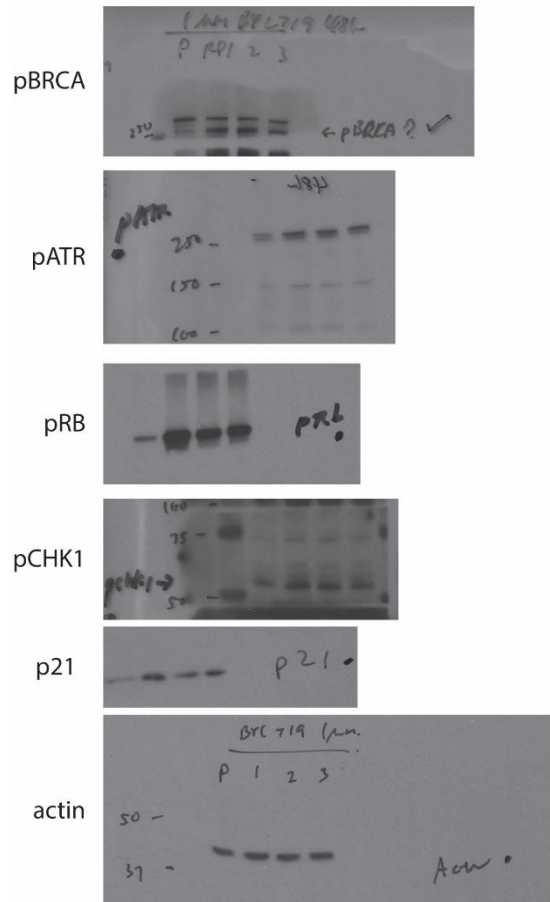


Figure 6

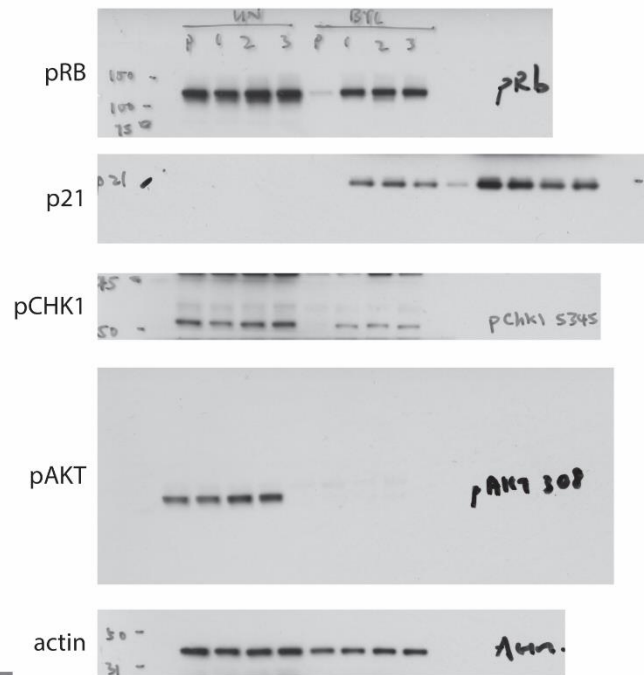
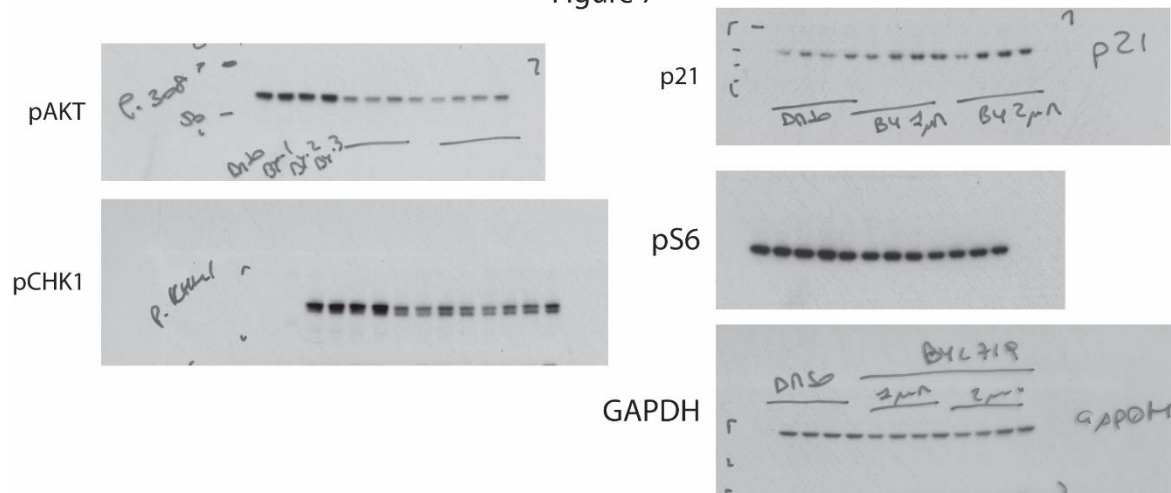
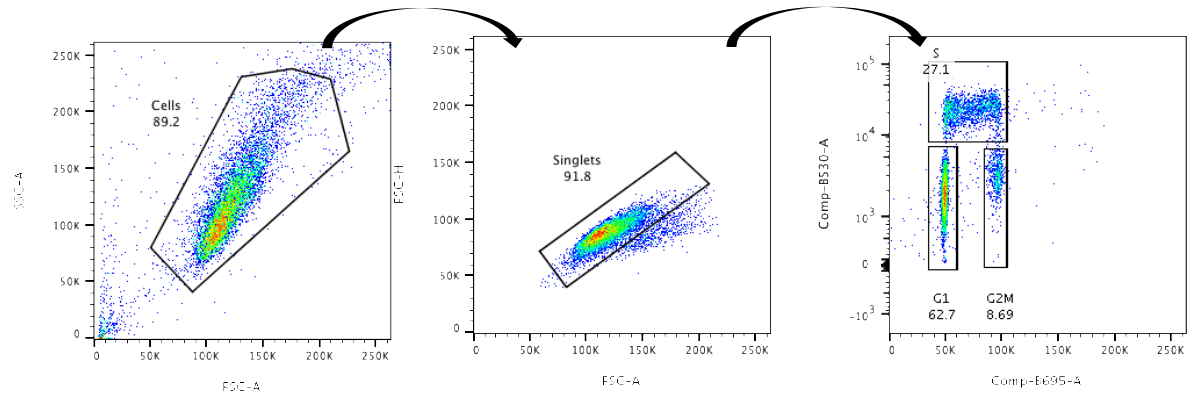


Figure 7



Supplementary Figure 8. Uncropped scans of blots included in main figures 5 to 7.



Supplementary Figure 9. Gating strategy used for cell cycle analysis in Figure 3d. Upon treatments, T47D cells were gated by the “Cells” gate in the SSC-A vs FSC-A plot. Single cells, excluding doublets, were then gated in the “Singlets” gate in the FSC-H vs FSC-A. The distribution of cell cycle phases was analysed on intact single cells by BrdU-FITC antibodies (Comp-B530-A) and propidium iodide (Comp-B695-A).

Supplementary Tables

Supplementary Table 1.

Reactions and reaction rates of the Integrated PI3K signaling network model.

	Reaction	Reaction rates
rt01	$\text{IGF} + \text{IR} \rightarrow \text{IRL}$	$\text{ka0} * \text{Ins} * \text{IR} - \text{kd0} * \text{IRa}$
rt02	$\text{IRL} \rightarrow \text{IRp}$	$\text{kc1_rt_002} * \text{IRL} - \text{vm_rt_002} * \text{IRp}$
rt03	$\text{IRp} \rightarrow \text{IRi}$	$\text{kc1_rt_003} * \text{IRp} - \text{vm_rt_003} * \text{IRi}$
rt04	$\text{PI3K}\alpha \rightarrow \text{IRp_PI3K}\alpha$	$\text{kc2_rt_004} * \text{IRp} * \text{PI3K}\alpha / (1 + \text{Ki_rt_004} * \text{pS6K}) - \text{vm_rt_004} * \text{IRp_PI3K}\alpha$
rt05	$\text{PI3K}\beta \rightarrow \text{IRp_PI3K}\beta$	$\text{kc2_rt_005} * \text{IRp} * \text{PI3K}\beta / (1 + \text{Ki_rt_004} * \text{pS6K}) - \text{vm_rt_005} * \text{IRp_PI3K}\beta$
rt06	$\text{PI3K}\alpha \xrightarrow{\text{pHER3L_PI3K}\alpha}$	$\text{kc2_rt_006} * \text{pHER3L} * \text{PI3K}\alpha - \text{vm_rt_006} * \text{pHER3L_PI3K}\alpha$
rt07	$\text{PI3K}\beta \xrightarrow{\text{pHER3L_PI3K}\beta}$	$\text{kc2_rt_007} * \text{pHER3L} * \text{PI3K}\beta - \text{vm_rt_007} * \text{pHER3L_PI3K}\beta$
rt08	$\text{PIP2} \rightarrow \text{PIP3}$	$\text{PIP2} * \text{kc2_rt_008} / (\text{Km_rt_008} + \text{PIP2}) * ((\text{mtPIK3CA} + (\text{IRp_PI3K}\alpha + \text{pHER3L_PI3K}\alpha) * (1 + \text{alpha_rt_008a} * \text{mGABp} + \text{alpha_rt_008b} * \text{tRas})) * (1 - \text{w_drg} * \text{BYL719_on} * \text{BYL719} / (\text{Ki_drg} + \text{BYL719}))) + (\text{IRp_PI3K}\beta + \text{pHER3L_PI3K}\beta) * (1 + \text{alpha_rt_008a} * \text{mGABp} + \text{alpha_rt_008c} * \text{tRac1}))$
rt09	$\text{PIP3} \rightarrow \text{PIP2}$	$\text{kc2_rt_009} * \text{PIP3} * \text{PTEN}$
rt10	$\text{GAB} \rightarrow \text{mGABp}$	$\text{kc2_rt_010} * \text{GAB} * \text{PIP3} / (1 + \text{Ki_rt_010} * \text{pErk}) - \text{vm_rt_010} * \text{mGABp}$
rt11	$\text{PDK1} \rightarrow \text{mPDK1}$	$\text{kc2_rt_011} * \text{PDK1} * \text{PIP3} - \text{vm_rt_011} * \text{mPDK1}$
rt12	$\text{PREX1} \rightarrow \text{mPREX1}$	$\text{kc2_rt_012} * \text{PREX1} * \text{PIP3} - \text{vm_rt_012} * \text{mPREX1}$
rt13	$\text{dRac1} \rightarrow \text{tRac1}$	$\text{kc2_rt_013} * \text{mPREX1} * \text{dRac1} - \text{vm_rt_013} * \text{tRac1}$
rt14	$\text{Akt} \rightarrow \text{pAkt}$	$\text{kc2_rt_014} * \text{mPDK1} * \text{Akt} / (1 + \text{ki_rt_014} * \text{ppRB}) - \text{vm_rt_014} * \text{pAkt}$
rt15	$\text{SGK1} \rightarrow \text{pSGK1}$	$\text{kc2_rt_015} * \text{SGK1} * \text{mPDK1} / (1 + \text{ki_rt_014} * \text{ppRB}) - \text{vm_rt_015} * \text{pSGK1}$
rt16	$\text{SGK3} \rightarrow \text{pSGK3}$	$\text{kc2_rt_016} * \text{mPDK1} * \text{SGK3} / (1 + \text{ki_rt_014} * \text{ppRB}) - \text{vm_rt_016} * \text{pSGK3}$
rt16a	$\text{GSK3} \rightarrow \text{pGSK3}$	$\text{kc2_rt_016a} * \text{pAkt} * \text{GSK3} - \text{vm_rt_016a} * \text{pGSK3}$

rt17	mTORC1 amTORC1	→	$mTORC1 * (kc2_rt_017a * pAkt + kc2_rt_017b * pSGK1) * (1 + \alpha_rt_017 * K4Da) - vm_rt_017 * amTORC1$
rt18	FOXO3	→ pFOXO3	$FOXO3 * (kc2_rt_018a * pAkt + kc2_rt_018b * pSGK1) - vm_rt_018 * pFOXO3$
rt19	S6K	→ pS6K	$kc2_rt_019 * S6K * amTORC1 - vm_rt_019 * pS6K$
rt20	S6	→ pS6	$S6 * (kc2_rt_020a * pS6K) - vm_rt_020 * pS6$
rt21	dRas	→ tRas	$dRas * (kc2_rt_021a * pHER3L + kc2_rt_021b * IRp / (1 + Ki_rt_004 * pS6K) + kc2_rt_021c * mGABp) / (1 + Ki_rt_021 * pErk) - vm_rt_021 * tRas$
rt22	Raf	→ aaRaf	$kc2_rt_022 * tRas * Raf / (Km_rt_022 + Raf) / (1 + Ki_rt_022 * pAkt) - kc2_rt_022 * aaRaf$
rt23	Mek	→ pMek	$kc2_rt_023 * (1 + \alpha_rt_023 * tRac1) * aaRaf * Mek / (Km_rt_023 + Mek) - vm_rt_023 * pMek$
rt24	Erk	→ pErk	$kc2_rt_024 * Erk * pMek - vm_rt_024 * pErk$
rt25	→ SGK3		$vs_rt_025 * (ER + pER) / (Km_rt_025 + (ER + pER))$
rt26	SGK3	→ ∅	$kdeg_rt_026 * SGK3$
rt27	∅	→ NDRG1	vs_rt_027
rt28	NDRG1	→ ∅	$(vs_rt_027/50) * NDRG1$
rt29	NDRG1	→ pNDRG1	$kc2_rt_029 * NDRG1 * (pSGK1 + pSGK3) - vm_rt_029 * pNDRG1$
rt30	pNDRG1	→ ∅	$kdeg_rt_030 * pNDRG1 * GSK3 / (Km_rt_030 + pNDRG1)$
rt31	∅	→ HER3	$vs_rt_031 * FOXO3 / (Km_rt_031 + FOXO3)$
rt32	HER3	→ ∅	$deg_rt_031 * HER3 * (1 + \alpha_rt_031 * (NDRG1 + pNDRG1))$
rt33	HER3 + HRG	→ HER3L	$ka_rt_033 * HER3 * HRG * HRG_on - kd_rt_033 * HER3L$
rt34	HER3L	→ pHER3L	$kc1_rt_034 * HER3L - vm_rt_034 * pHER3L$
rt34a	pHER3L	→ iHER3L	$kc1_rt_034_a * pHER3L * (1 + \alpha_rt_034_a * pErk) - vm_rt_034_a * iHER3L$
rt35	∅	→ ER	$vs_rt_035b * FOXO3 / (Km_rt_035 + FOXO3)$
rt36	ER	→ ∅	$deg_rt_035 * ER$
rt37	ER	→ pER	$kc2_rt_037 * pErk * ER - vm_rt_037 * pER$
cc01	∅	→ RB	vs_cc_001
cc02	RB	→ ∅	$(vs_cc_001/100) * RB$
cc03	RB	→ pRB	$kc1_cc_003 * RB * (K4Da + K4Da_p21) - vm_cc_003 * pRB$
cc05	pRB	→ ppRB	$kc2_cc_005 * K2Ea * pRB - vm_cc_005 * ppRB$
cc07	∅	→ E2F	$tr_cc_007a * E2F / (Km_cc_007b + E2F) + tr_cc_007b * (cMyc / (Km_cc_007a + cMyc)) * (E2F / (Km_cc_007b + E2F)) + tr_cc_007c * (cMyc / (Km_cc_007a + cMyc))$

cc08	$E2F \rightarrow \emptyset$	$E2F * (kdeg_cc_008b + kdeg_cc_008a * K2Ea) / (Km_cc_008 + E2F)$
cc09	$E2F + RB \rightarrow E2FRB$	$(ka_cc_009 * E2F * RB - kd_cc_009 * E2FRB)$
cc10	$\emptyset \rightarrow Cd$	$vs_cc_010c * amTORC1 / (Km_cc_010b + amTORC1)$
cc11	$Cd \rightarrow \emptyset$	$kdeg_cc_011 * Cd * (1 + kc2_cc_011 * GSK3) / (Km_cc_011 + GSK3)$
cc12	$Cd + CDK4 \rightarrow K4D$	$ka_cc_012 * Cd * CDK4 - kd_cc_012 * K4D$
cc13	$K4D \rightarrow K4Da$	$kc1_cc_013 * K4D - vm_cc_013b * K4Da$
cc13a	$K4Da \rightarrow CDK4$	$kc2_cc_013a * K4Da * GSK3 / (Km_cc_011 + GSK3)$
cc14	$K4Da + p21 \rightarrow K4Da_p21$	$ka_cc_014 * K4Da * p21 - kd_cc_014 * K4Da_p21$
cc14a	$K4Da_p21 \rightarrow CDK4$	$kc2_cc_014a * K4Da_p21 * GSK3 / (Km_cc_011 + GSK3)$
c15	$\emptyset \rightarrow Ce$	$vs_cc_015 * E2F / (Km_cc_015 + E2F)$
cc16	$Ce \rightarrow \emptyset$	$kdeg_cc_015 * Ce$
cc17	$Ce + CDK2 \rightarrow K2E$	$ka_cc_017 * Ce * CDK2 - kd_cc_017 * K2E$
cc18	$K2E \rightarrow K2Ea$	$kc1_cc_018a * K2E - vm_cc_018b * K2Ea$
cc19	$K2Ea + p21 \rightarrow K2Ea_p21$	$ka_cc_019 * K2Ea * p21 - kd_cc_019 * K2Ea_p21$
cc20	$\emptyset \rightarrow p21$	$vs_cc_020 * pErk / (Km_cc_020 + pErk)$
cc21	$p21 \rightarrow \emptyset$	$p21 * (deg_cc_021 + kc2_cc_021b * K2Ea / (Km_cc_021b + K2Ea))$
cc22	$p21 \rightarrow p_p21$	$kc2_cc_021a * pAkt * p21 / (Km_cc_021a + pAkt)$
cc23	$p_p21 \rightarrow \emptyset$	$p_p21 * (deg_cc_021/10 + kc2_cc_021b * K2Ea / (Km_cc_021b + K2Ea))$
cc25	$\emptyset \rightarrow cMyc$	$vs_cc_025a * amTORC1 / (Km_cc_025a + amTORC1) + vs_cc_025b * (ER + pER) / (Km_cc_025b + (ER + pER))$
cc26	$cMyc \rightarrow \emptyset$	$deg_cc_025 * cMyc * (1 + kc1_cc_026 * GSK3)$

Supplementary Table 2.

Ordinary differential equations (ODE) of the integrated PI3K signaling network model

(Reaction rates are included in Supplementary Table 1).

Left-hand Sides	Right-hand Sides
$[IGF]/dt$	- rt01
$[IR]/dt$	-rt01
$[IRL]/dt$	+rt01- rt02
$[IRp]/dt$	+rt02 - rt03
$[IRi]/dt$	+rt03
$[PI3Ka]/dt$	-rt04 - rt06
$[PI3Kb]/dt$	-rt05 - rt07
$[IRp_PI3Ka]/dt$	+rt04
$[IRp_PI3Kb]/dt$	+rt05
$[pHER3L_PI3Ka]/dt$	+rt06
$[pHER3L_PI3Kb]/dt$	+rt07
$[PIP3]/dt$	+rt08 - rt09
$[PIP2]/dt$	-rt08 + rt09
$[PDK1]/dt$	-rt11
$[mPDK1]/dt$	+rt11
$[GAB]/dt$	-rt10
$[mGABp]/dt$	+rt10
$[PREX1]/dt$	-rt12
$[mPREX1]/dt$	+rt12
$[dRac1]/dt$	-rt13
$[tRac1]/dt$	+rt13
$[Akt]/dt$	-rt14
$[pAkt]/dt$	+rt14
$[SGK1]/dt$	-rt15
$[pSGK1]/dt$	+rt15
$[SGK3]/dt$	-rt16 + rt25 - rt26
$[pSGK3]/dt$	+rt16
$[GSK3]/dt$	-rt16a
$[pGSK3]/dt$	+rt16a
$[mTORC1]/dt$	-rt17
$[amTORC1]/dt$	+rt17
$[FOXO3]/dt$	-rt18
$[pFOXO3]/dt$	+rt18
$[NDRG1]/dt$	+rt27 - rt28 - rt29

[pNDRG1]/dt	+rt29 - rt30
[S6K]/dt	-rt19
[[pS6K]/dt	+rt19
[S6]/dt	-rt20
[pS6]/dt	+rt20
[dRas]/dt	-rt21
[tRas]/dt	+rt21
[Raf]/dt	-rt22
[aaRaf]/dt	+rt22
[Mek]/dt	-rt23
[pMek]/dt	+rt23
[Erk]/dt	-rt24
[pErk]/dt	+rt24
[HER3]/dt	+rt31 - rt32 - rt33
[HRG]/dt	-rt33
[HER3L]/dt	+rt33 - rt34
[pHER3L]/dt	+rt34 - rt34a
[iHER3L]/dt	+rt34a
[ER]/dt	+rt35 - rt36 - rt37
[pER]/dt	+rt37
[RB]/dt	+cc01 - cc02 - cc03 - cc09
[pRB]/dt	+cc03 - cc05
[ppRB]/dt	+cc05
[E2F]/dt	+cc07 - cc08 - cc09
[E2FRB]/dt	+cc09
[Cd]/dt	+cc10 - cc11 - cc12
[CDK4]/dt	-cc12 + cc13a + cc14a
[K4D]/dt	+cc12 - cc13
[K4Da]/dt	+cc13 - cc13a - cc14
[Ce]/dt	+cc15 - cc16 - cc17
[CDK2]/dt	-cc17
[K2E]/dt	+cc17 - cc18
[K2Ea]/dt	+cc18 - cc19
[p21]/dt	-cc14 - cc19 + cc20 - cc21 - cc22
[K4Da_p21]/dt	+cc14 - cc14a
[K2Ea_p21]/dt	+cc19
[cMyc]/dt	+cc25 - cc26
[p_p21]/dt	+cc22 - cc23

Supplementary Table 3.

List of antibodies, small molecule inhibitors, primers and guideRNAs

MATERIALS	SOURCE	IDENTIFIER
Antibodies		
Rabbit monoclonal anti-Phospho-Akt (Ser473) (clone D9E)	Cell Signaling Technology	Cat# 4060; RRID:AB_2315049
Rabbit monoclonal anti-Phospho-Akt (Thr308) (clone D25E6)	Cell Signaling Technology	Cat# 13038; RRID:AB_2629447
Rabbit monoclonal anti- Phospho-S6 Ribosomal Protein (Ser240/244) (clone D68F8)	Cell Signaling Technology	Cat# 5364; RRID:AB_10694233
Rabbit monoclonal anti-S6 Ribosomal Protein (clone 5G10)	Cell Signaling Technology	Cat# 2217; RRID:AB_331355
Mouse monoclonal anti-Actin (clone AC-40)	Sigma-Aldrich	Cat# A3853; RRID:AB_262137
Rabbit monoclonal anti-Phospho-4E-BP1 (Thr37/46) (236B4)	Cell Signaling Technology	Cat# 2855; RRID:AB_560835
Rabbit monoclonal anti-Akt (pan) (clone 11E7)	Cell Signaling Technology	Cat# 4685; RRID:AB_2225340
Rabbit monoclonal anti-Phospho Rb (Ser807/811)	Cell Signaling Technology	Cat# 8516; RRID:AB_11178658
Rabbit monoclonal anti-Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	Cell Signaling Technology	Cat# 4370; RRID:AB_2315112
p44/42 MAPK (Erk1/2)	Cell Signaling Technology	Cat# 4696; RRID:AB_390780
Rabbit polyclonal anti-Cyclin D1	Cell Signaling Technology	Cat# 2922; RRID:AB_2228523
Mouse monoclonal anti-Cyclin A (clone AT10.2)	Santa Cruz Biotechnology	Cat# sc-53227; RRID:AB_782329
Rabbit monoclonal anti-CDK4 (clone D9G3E)	Cell Signaling Technology	Cat# 12790; RRID:AB_2631166
Mouse monoclonal anti-CDK6 (clone DCS22)	Cell Signaling Technology	Cat# 3136; RRID:AB_2229289
Rabbit monoclonal anti-CDK2 (clone 78B2)	Cell Signaling Technology	Cat# 2546; RRID:AB_2276129
Rabbit monoclonal anti-p21 Waf1/Cip1 (clone 12D1)	Cell Signaling Technology	Cat# 2947; RRID:AB_823586

Mouse monoclonal anti-p18 INK4C (clone DCS118)	Cell Signaling Technology	Cat# 2896; RRID:AB_331203
Rabbit monoclonal anti-p27 Kip1 (clone D69C12)	Cell Signaling Technology	Cat# 3686; RRID:AB_2077850
Rabbit monoclonal anti-Phospho-Chk1 (Ser345) (clone 133D3)	Cell Signaling Technology	Cat# 2348; RRID:AB_331212
Mouse monoclonal anti-PCNA (clone PC10)	Cell Signaling Technology	Cat#2586; RRID:AB_2160343
Rabbit monoclonal anti-CDKN2A/p16INK4a (clone EPR1473)	Abcam	Cat# ab108349; RRID:AB_10858268
Mouse monoclonal anti-p53 (clone 1C12)	Cell Signaling Technology	Cat# 2524; RRID:AB_331743
Rabbit polyclonal anti-53BP1	Abcam	Cat# ab21083 RRID:AB_722496
Mouse monoclonal anti-phospho-Histone H2A.X (Ser139) (clone JBW301)	Millipore	Cat# 05-636, RRID:AB_309864
Rabbit monoclonal anti-phospho-NDRG1 (Thr346) (D98G11) XP®	Cell Signaling Technology	Cat# 5482; RRID:AB_10693451
Rabbit monoclonal anti-hospho-SGK3 (Thr320) (D30E6)	Cell Signaling Technology	Cat# 5642; RRID:AB_10694357
Small Molecule inhibitors		
BYL719 (Alpelisib), PI3Kalpha inhibitor	Selleck Chemicals	Cat# S2814; CAS:1217486-61-7
MK-8776, Chk1 inhibitor	AdooQ Bioscience	Cat# SCH 900776; CAS: 891494-63-6
GSK2334470, PDK1 inhibitor	Selleck Chemicals	Cat # S7087; CAS 1227911-45-6
Quantitative PCR		
Human CDKN1A FWD:TGTCCGTCAGAACCCATGC REV: AAAGTCGAAGTTCCATCGCTC	PrimerBank – MGH-PGA	PrimerBank ID: 310832423c1
CRISPR reagents and crRNA sequences		
Hs.Cas9.TP53.1.AA /AltR1/rCrC rArUrU rGrUrU rCrArA rUrArU rCrGrU rCrCrG rGrUrU rUrUrA rGrArG rCrUrA rUrGrC rU/AltR2/	Integrated DNA Technologies (IDT)	N/A
Hs.Cas9.CDKN1A AB /AltR1/rCrUrUrUrGrUrCrArCrCrGrArGrArCrArC rCrArCrGrUrUrUrUrArGrArGrCrUrArUrGrCrU/A ltR2/	Integrated DNA Technologies (IDT)	N/A
Hs.Cas9.CDKN1A AD /AltR1/rCrCrArArGrCrUrCrUrArCrCrUrUrCrCrCr ArCrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrU/Al tR2/	IDT	N/A
Alt-R® CRISPR-Cas9 Negative Control crRNA #1, 2 nmol	IDT	Cat# 1072544

Alt-R® CRISPR-Cas9 Negative Control crRNA #2, 2 nmol	IDT	Cat#1072545
Alt-R CRISPR-cas9. tracrRNA-5'ATTO 5nmol	IDT	Cat# #1075934
Alt-R S.p. Cas9 Nuclease 3NLS	IDT	Cat#1074181
Nuclease Free Duplex Buffer 2ml	IDT	Cat# 1072570
Opti-MEM™ Reduced Serum Medium, GlutaMAX™ Supplement	Thermofisher Scientific	Cat# 51985034
Lipofectamine 3000	Thermofisher Scientific	Cat# L3000-008

Supplementary Table 4.

Ordinary differential equations (ODE) of the phenotypic model. CC: Cell Cycle signals (= cyclin D1 + cyclin E, outputs of the PI3K network model). G1: G1-phase, S: S-phase, A: Apoptosis, R: DNA repair, M: G2/M-phase. DD: DNA damage signal caused by PI3K α inhibitor BYL719. DD₀: the basal DNA damage signal that naturally occurs due to various internal and external factors but not directly caused by specific external agents such as BYL719. pCHK1: phosphorylated and activated CHK1 by DD. MK-8876: CHK1 inhibitor. The kinetic parameters and constants used for the simulation of the phenotypic model are provided in **Supplementary Dataset 3**.

Left-hand Sides	Right-hand Sides
$d[G1]/dt$	$= -kc_{g1_s} \cdot G1 \cdot CC + kr_{s_g1} \cdot S$
$d[S]/dt$	$= kc_{g1_s} \cdot G1 \cdot CC - kr_{s_g1} \cdot S - kc_{s_m} \cdot (1/(1+Ki_chk1 \cdot pCHK1)) + kc_{dd} \cdot DD / (1+Ki_chk1 \cdot pCHK1) + kc_{chk1} \cdot pCHK1 \cdot S$
$d[A]/dt$	$= kc_{dd} \cdot DD \cdot S / (1+Ki_chk1 \cdot pCHK1)$
$d[R]/dt$	$= kc_{chk1} \cdot pCHK1 \cdot S - kc_{r_m} \cdot R$
$d[M]/dt$	$= kc_{s_m} \cdot S / (1+Ki_chk1 \cdot pCHK1) + kc_{r_m} \cdot R$
	$DD = kc_{byl} \cdot (DD_0 + BYL719/Ki_{byl})$ $pCHK1 = kc_{chk_dd} \cdot DD / (1 + MK8876)$ $CC = \text{cyclin D1} + \text{cyclin E (outputs of the mechanistic model)}$