SUPPLEMENTARY MATERIAL AND METHODS

Immunohistochemistry (IHC) and image analysis

PDX and patient tumors were fixed immediately after excision in 10% buffered formalin solution for a maximum of 24h at room temperature before being dehydrated and paraffin embedded (FFPE). IHC was performed on FFPE tissue sections (3 µM). For pRb, cyclin D1 and cyclin E1, sections were dewaxed, rehydrated and antigen retrieved using a microwave at maximum power (Whirlpool JT479/WH) in EDTA 1mM pH 8 (pRb), citrate pH 6 (cyclin D1) or pH 9 (cyclin E1) for 20 minutes. After peroxide blocking, the slides were stained with the corresponded primary antibodies at dilution 1:100 (pRb and cyclin D1) or 1:300 (cyclin E1), then with Mouse (pRb and cyclin E1) or Rabbit (cyclin D1) EnVision^{TM}+System-HRP labelled polymer and finally with Liquid DAB + Substrate Chromogen System (DAKO). Harris´ hematoxylin was used to counterstain the nuclei and mounted with xylol based mounting medium. Positive and negative controls were run along the tested slides per each marker.

Staining of p16, phospho-pRb (Ser807/811), and Ki67 was undertaken following the protocol provided by Ventana Medical System, Inc. Briefly, the slides were heated in the instrument at 75ºC for 28 minutes and deparaffinized with EZ prep solution (Ventana Medical System). Then, the antigen retrieval was performed at slightly basic pH at 95ºC using the Cell Conditioning 1 buffer (CC1; Ventana Medical System) followed by staining with anti-rabbit HQ (KI67) or anti-mouse HR (p16 and phospho-pRb (Ser807/811)) and anti-HQ-HRP and DAB staining (Roche). The slides were counterstained with Hematoxylin II and bluing reagent (Ventana Medical System) and mounted with xylol based mounting medium.

A pathologist scored the different proteins expression in each sample. Total pRb and p16 were scored semiquantitatively onto life images with very strong (4+), strong (3+), moderate (2+) weak (1+) or negative staining (0). Allred scores of cyclin D1 and cyclin E1 were calculated from 0 to 8 taking into account the percentage of positive cells (0 to 5) plus the staining intensity (0 to 3). The percentage of cells with nuclear Ki67 or phospho-pRb (Ser807/811) staining was quantified in samples at baseline and after ribociclib treatment from pharmacodynamic experiments.

PDCs *ex vivo* treatment for immunoblotting

Cell suspension generated from a 500mm^3 PDX were plated in duplicated at 1x10^6 cells/well into 6 well-plates (BD Bioscience). Next day, drugs and vehicle (DMSO) were added, and cells were

incubated for 24 hours. Then, Matrigel® was melted by incubation with 1 mM EDTA in PBS on ice for 20 minutes, the spheroids were collected into a conical tube and centrifuged at 2,000 rpm for 5 minutes at 4ºC. The supernatants were removed, and the pellets were stored at -20ºC until protein lysates were prepared for Western-blot analysis (see below).

***In vitro* cell line assessment**

For half maximal inhibition concentration (IC_{50}) analysis, cells ($2x10^3$/well) were seeded into 96-well plates (BD Bioscience) and, after 24 hours, were treated for 6 days with different concentrations of ribociclib, fulvestrant or the combination of drugs. The treatments and media were refreshed every 3 days. Cell proliferation was measured at day 0 and day 6 by fixing with 4% glutaraldehyde (MERCK) in PBS, staining with 0.1% of crystal violet (Sigma-Aldrich) in methanol, solubilizing with 10% of acetic acid in PBS and measuring the absorbance of each well at 560nm. Values at day 6 were normalized with values at day 0, relativized to controls (vehicle-treated cells) and plotted as the percentage inhibition against the log concentration of ribociclib. IC_{50} was determined using a sigmoidal regression model and was defined as the concentration of drug required for a 50% reduction in growth. Each experiment was repeated three times with three technical replicates.

For biochemical analysis, cells ($1.5x10^6$/well) were seeded into p100 dishes (BD Bioscience) and, the following day, were treated for 24h or 5 days with 0.5 μM, 1 μM or 1.5 μM ribociclib, 100nM fulvestrant or the combination of drugs. Next, cells were harvested and prepared for immunoblotting analysis (see below). T47D-p16 cells were incubated with 1μg/ml doxycycline 48h prior to add the treatments for inducing p16 expression.

For enrichment assays, cells ($5.0x10^5$/well) were seeded into p100 dishes (BD Bioscience). 5% of MCF7- or T47D- cells overexpressing p16 or cyclin D1 ($2.5x10^4$/well) were mixed with 95% of MCF7- or T47D-MOCK cells ($4.75x10^5$/well), seeded together and, the following day, were treated with 1 μM ribociclib for 0, 3 or 14 days. Cells were harvested and prepared for immunoblotting analysis (see below).

PDX, PDC and cell lines protein isolation and immunoblotting

Both flash-frozen tumor pieces from pharmacodynamic assays and harvested cells were lysed in ice-cold buffer containing Tris-HCl pH7.8 20 mmol/L, NaCl 137 mmol/L, EDTA pH 8.0 2 mmol/L,

NP40 1%, glycerol 10%, supplemented with NaF 10 mmol/L, Leupeptin 10 mg/mL, Na_2VO_4 200 mmol/L, PMSF 5 mmol/L, and Aprotinin (Sigma-Aldrich). Tissue homogenization was performed on ice with a POLYTRON® system PT 1200 E (Kinematica). Lysates were centrifuged at 13,000 rpm 4ºC for 10 min and the supernatants were collected. Protein concentration was calculated using DCTM Protein Assay (Bio-Rad). A total of 30 µg of protein were separated on 12% SDS-PAGE acrylamide gels at 100V and transferred to nitrocellulose membrane for 1.5 hours at 100V. Membranes were blocked for 1 hour in 5% milk in Tris-buffered saline (TBS)-Tween and then hybridized using the corresponding primary antibodies in 5% BSA TBS-Tween. All primary antibodies were used at dilution 1:1000. Mouse and rabbit horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2000; GE Healthcare) were diluted in 5% milk in TBS-Tween and proteins were detected with Immobilon Western Chemiluminescent HRP substrate (Millipore). Images were captured with FUJIFILM LASS-4000 camera system and quantified with Image J (http://rsb.info.nih.gov).

Cell lines

MCF7 and T47D cell lines were obtained from ATCC and maintained according to the manufacturer's instructions. Cell line were authenticated (FTA Sample Collection Kit for Human Cell Authentication Service; ATCC services) and mycoplasma test was performed every 5 passages (MycoAlert™ Mycoplasma Detection Kit; LONZA). Both cell lines were grown in RPMI 1640 with GlutaMAX medium (Gibco) supplemented with 10% of heat inactivated fetal bovine serum (Gibco) and 1 nM of β-estradiol (Sigma-Aldrich). Cell lines were banked in multiple aliquots on receipt to reduce risk of phenotypic drift and were used for a maximum of 15 passages. All cells have been cultured at 37ºC with 5% CO_2 atmosphere.

Lentiviral infection

Lentiviral infection was done following the manufacturer´s indications (Sigma-Aldrich). Briefly, 293FT cells were used for the production of the virus. 293FT cells ($5x10^6$) were transfected with lentivirus and packaging (gag-pol, vsvg, rev) plasmids (Addgene) using polyethyleneimine (Sigma-Aldrich) in a DNA-PEI ratio of 1:3. Viral production was induced by adding 10mM Na Butyrate the following day. Virus were harvested 48 hours post transfection. p16-INK4A and cyclin D1 lentiviral stocks were tittered using colony forming assay (Hela cells). MCF7 and T47D cells

were infected with doxycycline-inducible pLX401-INK4A (MOI 1:2) for overexpressing p16 or pLenti-*CCND1* (MOI 1:2) and the control plasmid (pLenti-tGFP) for overexpressing cyclin D1. 8µg/ml of polybrene (Sigma-Aldrich) were added, plates were centrifuged 1 hour at 1.500 rpm at 37ºC to improve the infection and incubated overnight. For p16-INK4 cell selection, 2 µg/ml of puromycin (Invitrogene) were added to the cultured media 48 hours post-infection. After 5 days, all cells in the control plate (non-infected cells) were dead and the concentration of puromycin was reduced to 1 µg/ml (maintenance dose). For p16 expression, 1 µg/ml of doxycycline (Sigma-Aldrich) was added to the culture media 48 hours before treatments were added.

Real Time-qPCR ready custom panels

RNA was extracted from flash-frozen control and ribociclib treated PDX samples (15-30mg) by using the PerfectPure RNA Tissue kit (five Prime). The purity and integrity were assessed by the Agilent 2100 Bioanalyzer system, and cDNA was obtained using the PrimeScript RT Reagent kit (Takara). Quantitative RT-PCR was performed in the LightCycler® 480 (Roche) using LightCycler® 480 Probes Master (Roche) and ready-to-use custom 384-plates panels containing predesigned human specific primers and UPL Probes for each gene (see table below). Tbd probes were designed and tested specifically for this assay. The comparative CT method was used for data analysis, in which geoNorm algorithms were applied to select the most stably expressed housekeeping genes (GAPDH and ACTB) and geometric means were calculated to obtain normalized CT values [61].

GENE ID	ASSAY ID
ACTB	143636
BAD	142965
BAK1	100068
BAX	142318
BBC3	144371
BCL2	100083
BCL2L1	100088
BCL2L11	100096
BID	100122
BIK	145589
BIRC2	100131
BIRC3	tbd
BIRC5	101365
CCNA2	102811
CCNB1	101373

GENE ID	ASSAY ID
CCNB2	101376
CCND1	100844
CCND2	101384
CCND3	102813
CCNE1	139821
CCNE2	144468
CCNH	101394
CDC20	102870
CDC25A	102820
CDK1	101406
CDK10	tbd
CDK11A	tbd
CDK2	101416
CDK4	101418
CDK5	105690

GENE ID	ASSAY ID
CDK6	tbd
CDK7	101429
CDK8	101433
CDK9	tbd
CDKN1A	142319
CDKN1B	100855
CDKN2A	148945
CDKN2B	tbd
CDKN2C	111127
CDKN2D	110945
E2F1	102827
E2F2	102830
E2F3	102834
E2F4	102860
FASLG	145654

GENE ID	ASSAY ID
FOXO1	137191
GAPDH	141139
HRK	145616
MYC	100977
MYT1	147592
PMAIP1	100739
RB1	149106
RBL1	101543
RBL2	101547
TNFSF10	101266
TP53	101277
WEE1	102849

***tbd**: not catalog assay available.

Antibodies and reagents

Primary antibodies used for Western blot were CDK4 (12790), cyclin D2 (3741), phospho-pRb S807/811 (9308), phospho-pRb S780 (9307), phospho-CDK2 T160 (2561), phospho-AKT T308 (2965), phospho-AKT S473 (9271), AKT (9272), PARP (9542) and FGFR1 (3472) from Cell Signaling Technology; CDK6 (ab124821), cyclin D1 (ab40754) and cyclin E2 (ab40890) from Abcam; Tubulin (T- 9026) from Sigma; cyclin E1 (sc-481), CDK2 (sc162) and, human GAPDH (sc137179) from Santa Cruz Biotechnology; p16-INKA (i0883-I-AP) from ProteinTech; pRb (554136) from BD Pharmigen; ER-alpha (MS315-PO) from Neomarkers. Secondary antibodies used for Western blot were goat anti-rabbit IgG HRP linked whole antibody (NA934) and goat anti-mouse IhG HRP linked whole antibody (NA931) from Sigma-Aldrich. Antibodies used for immunohistochemistry (IHC) were cyclin D1 (RM9104) from ThermoScientific; pRb (554136) from BD Pharmigen; phospho-pRb S807/811 (8516) from Cell Signaling Technology; cyclin E1 (05-363) from Millipore; p16 (725-4713), ER (790-4324), PR (790-2223), Ki67 (790-4286) and HER2 (790-2991) from Ventana Medical Systems, Roche. Primary antibodies used for immunofluorescence (IF) were Cytokeratin 18 (ab133263) and Alexa Fluor® 568 anti-Vimentin (ab202504) both from Abcam and secondary antibody was Alexa Fluor® 488 goat anti-rabbit IgG. Ribociclib (LEE11) and alpelisib (BYL719) were provided by Novartis. Commercial trastuzumab (Herceptin) was obtained from a pharmacy. Fulvestrant and letrozole were purchased from Selleckchem. P18IN003 was purchased from Glixxlabs. Lenti ORF clone of human cyclin D1 (*CCND1*) mGFP tagged (RC204957L2) and Lenti-C-mGFP tagged empty vector (PS100071) were purchased from Origene. pLX401-INK4A vector was a gift from William Hahn (Addgene plasmid#121919; http://n2t.net/addgene:121919;RRID: Addgene 121919).

SUPPLEMENTARY FIGURES

Supplementary Figure 1

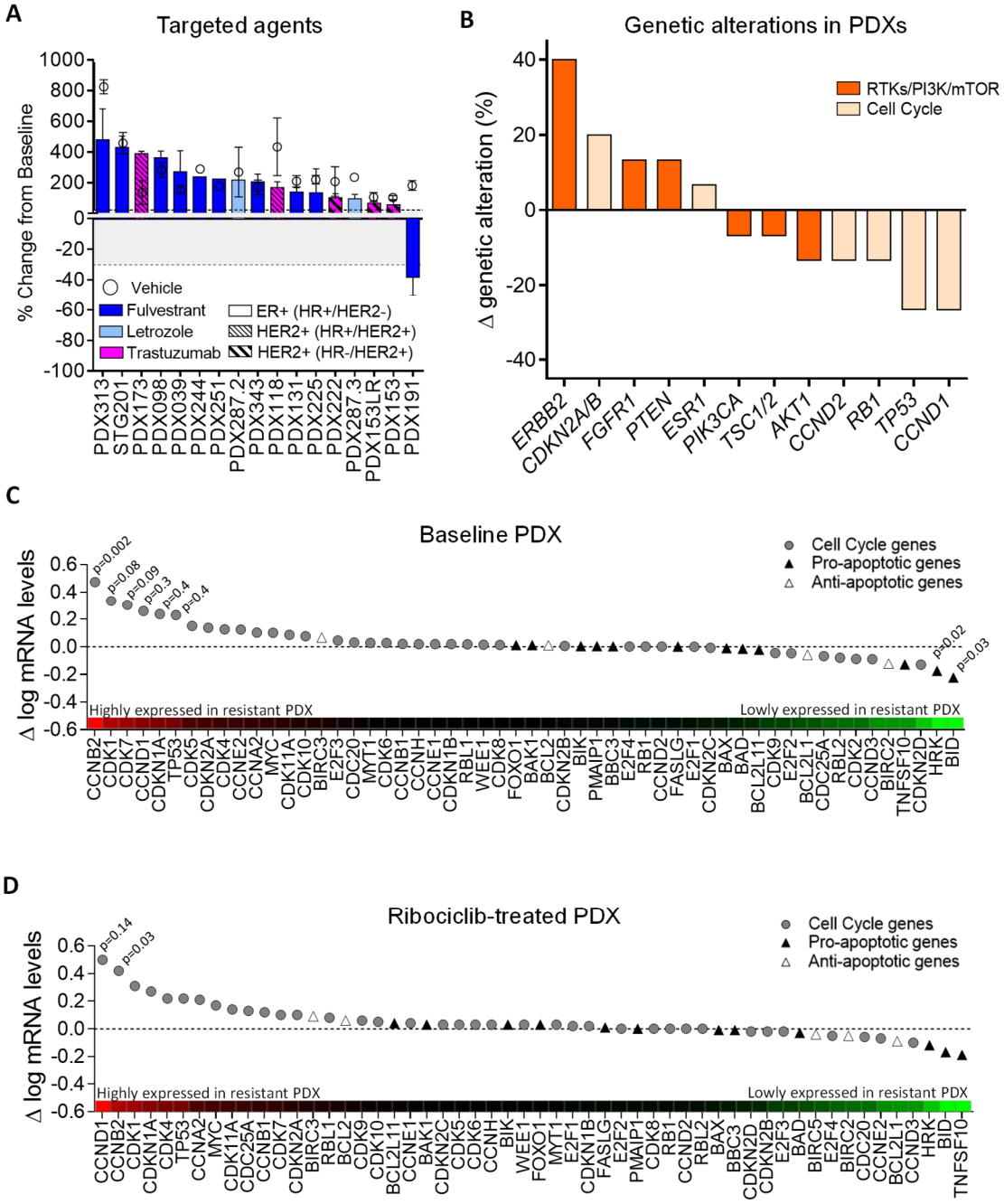

Fig. S1. Antitumor efficacy of others targeted therapies and genomic and transcriptomic analysis of PDXs. (**A**) Waterfall plot representing the growth of 12 ER$^+$ and 5 HER2$^+$ PDXs treated with endocrine therapy (10mg/mouse fulvestrant or 20mg/kg letrozole) or 10mg/kg trastuzumab (bars) and vehicle (circles). The percentage change from the initial volume is shown at day 15 of treatment. Data represent means and error bars ± SEM. Dashed lines indicate the range of PD (>20%), SD (20% to -30%) and PR/CR (<-30%). The molecular subtypes are

indicated. (**B**) Incidence of alterations in 12 genes related to PI3K and cell cycle, analyzed by IMPACT™ in untreated ribociclib-sensitive *vs*. ribociclib-resistant PDXs. For this analysis copy number amplifications (Ir≥2) and deleterious mutations (missense putative drivers, frameshift, and splice mutations) were considered. Different colors indicate the specific gene-related pathways. (**C**) and (**D**) mRNA levels of 54 cell cycle and apoptosis genes in ribociclib-resistant (n=12) *vs*. ribociclib-sensitive (n=5) PDXs measured by RT-qPCR in untreated (baseline) or treated with 75 mg/kg ribociclib for 12 days. Gene expression was normalized to housekeeping genes (*ACTB* and *GAPDH*) and, mean-centered data is provided. Symbols indicate the specific gene-related pathways. *p*-value, multiple *t*-test.

Supplementary Figure 2

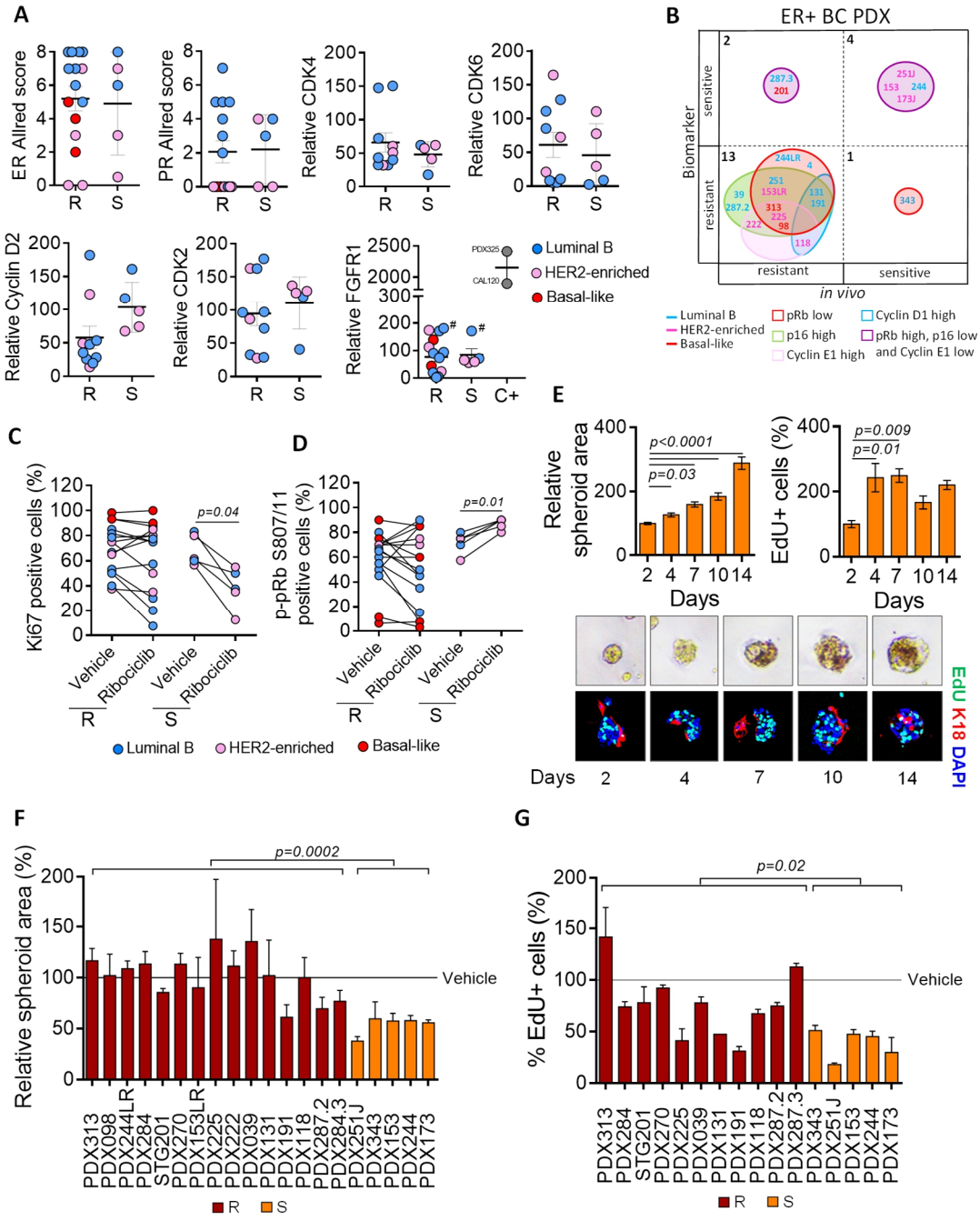

Fig. S2. Expression levels of several proteins in PDXs and spheroid area and EdU-incorporation analysis in PDCs *ex-vivo* cultures. (**A**) Quantification of the indicated proteins in untreated PDX detected by IHC and Allred score method (ER and PR) or by Western blot (CDK4, CDK6, cyclin D2, CDK2 and FGFR1) according to the PDXs ribociclib response. For FGFR1, expression levels were compared to two independent positive controls (C+; PDX325 and CAL120 cell line), which harbored high-CN *FGFR1* amplification. Different colors indicate the PDXs intrinsic subtype and hashtags indicate models harboring gene amplification. Data are

represented as means ± SEM. *p*-value, unpaired parametric *t*-test. R, resistant; S, sensitive. (**B**) Concordance analysis of the PDXs responses to ribociclib based on the analyzed biomarkers (y-axis) *vs.* the *ex vivo* response (x-axis). Singleplex or multiplex biomarkers are represented by circles of different colors and the number of PDX within each category is indicated. (**C**) and (**D**) Analysis of Ki67 (left graph) and phospho-pRb S807/811 (right graph) in vehicle and 14 days ribociclib-treated PDXs according to the PDXs ribociclib response. For illustration purposes, only the mean value of each PDX was plotted; however, for the statistical analysis all technical replicates were used. *p*-values are based on Mann-Whitney U test. Different colors represent the PDXs intrinsic subtype. R, resistant; S, sensitive. (**E**) Relative spheroid area (left graph) or percentage of Edu-positive cells (right graph) in untreated PDC287.3 for the indicated time. Data are represented as means of three independent experiments ± SEM. *p*-values are based on one-way ANOVA test with Tukey´s method correction. Underneath pictures show representative bright field or confocal microscopy images of PDC287.3 at different time points as indicated. EdU is shown in green, cytokeratin 18 (K18) in red and DAPI in blue. Magnification 40x. Quantification of the relative spheroid area (**F**) or percentage of EdU-positive cells **(G)** in the indicated PDCs after treatment with 1 µM ribociclib in *ex vivo* cultures for 7 days. Data are represented as means of three independent experiments ± SEM. *p*-values are based on the one-way ANOVA test with Tukey´s method correction. Black lines indicate the vehicle conditions. R; resistant; S; sensitive, according to the *in vivo* ribociclib anti-tumor activity.

Supplementary Figure 3

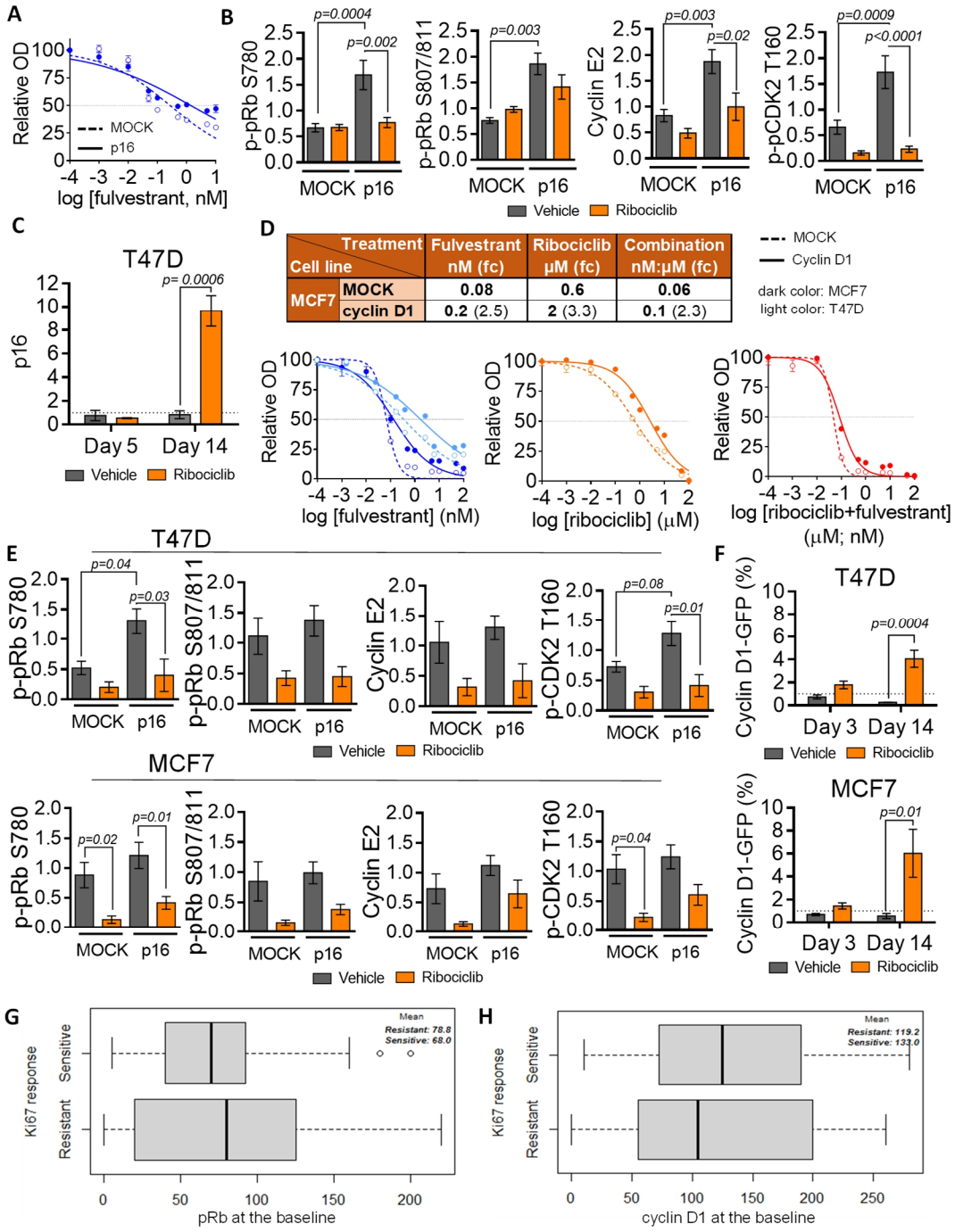

Fig. S3. Validation of p16, pRb and cyclin D1 as biomarkers of resistance to ribociclib in ER$^+$ cell lines and patients. (**A**) Representative dose-response curves of T47D cells overexpressing p16 (*CDKN2A*) compared to control cells (MOCK) after 6 days of treatment with indicated concentrations of fulvestrant. (**B**) Quantification of the expression levels of indicated proteins relative to tubulin analyzed by Western blot in T47D-p16 untreated or treated with 0.5 µM of ribociclib for 24 hours. At least three independent experiments were conducted with. Data are represented as means ± SEM. *p*-value, unpaired parametric *t*-test. (**C**) Quantification of the

expression levels of the indicated proteins relative to tubulin in three independent enrichment experiments using T47D- or MCF7-cyclin D1 cells seeded at 1:20 dilution with MOCK-transfected cells and treated with 0.5 μM ribociclib or vehicle for the indicated time. Data are represented as means ± SEM. *p*-value, unpaired parametric *t*-test. (**D**) Half-maximal inhibitory concentration (IC_{50}) values of ribociclib, fulvestrant and ribociclib plus fulvestrant in MCF7 cells overexpressing cyclin D1 (*CCND1*) and fold-change values (fc; in brackets) compared to controls (MOCK) evaluated after 6-days dose-response experiments as shown underneath. At least three independent experiments were conducted with three technical replicates per condition. (**E**) Quantification of the expression levels of the indicated proteins relative to tubulin analyzed by Western blot in T47D-cyclin D1 and MCF7-cyclinD1 untreated or treated with 0.5 μM of ribociclib for 24 hours. At least three independent experiments were conducted. Data are represented as mean ± SEM. *p*-value, unpaired parametric *t*-test. (**F**) Quantification of the expression levels of the indicated proteins relative to tubulin in an enrichment experiment using T47D- or MCF7-cyclin D1 cells seeded at 1:20 dilution with MOCK-transfected cells and treated with 1 μM ribociclib or vehicle for the indicated time. At least three independent experiments were conducted. Data are represented as means ± SEM. *p*-value, unpaired parametric *t*-test. Logistic model to evaluate the effect of pRb (**G**) and cyclin D1 (**H**) on the response to abemaciclib in in the ABC-POP trial tumor samples. The mean value of each subgroup is indicated.

Supplementary Figure 4

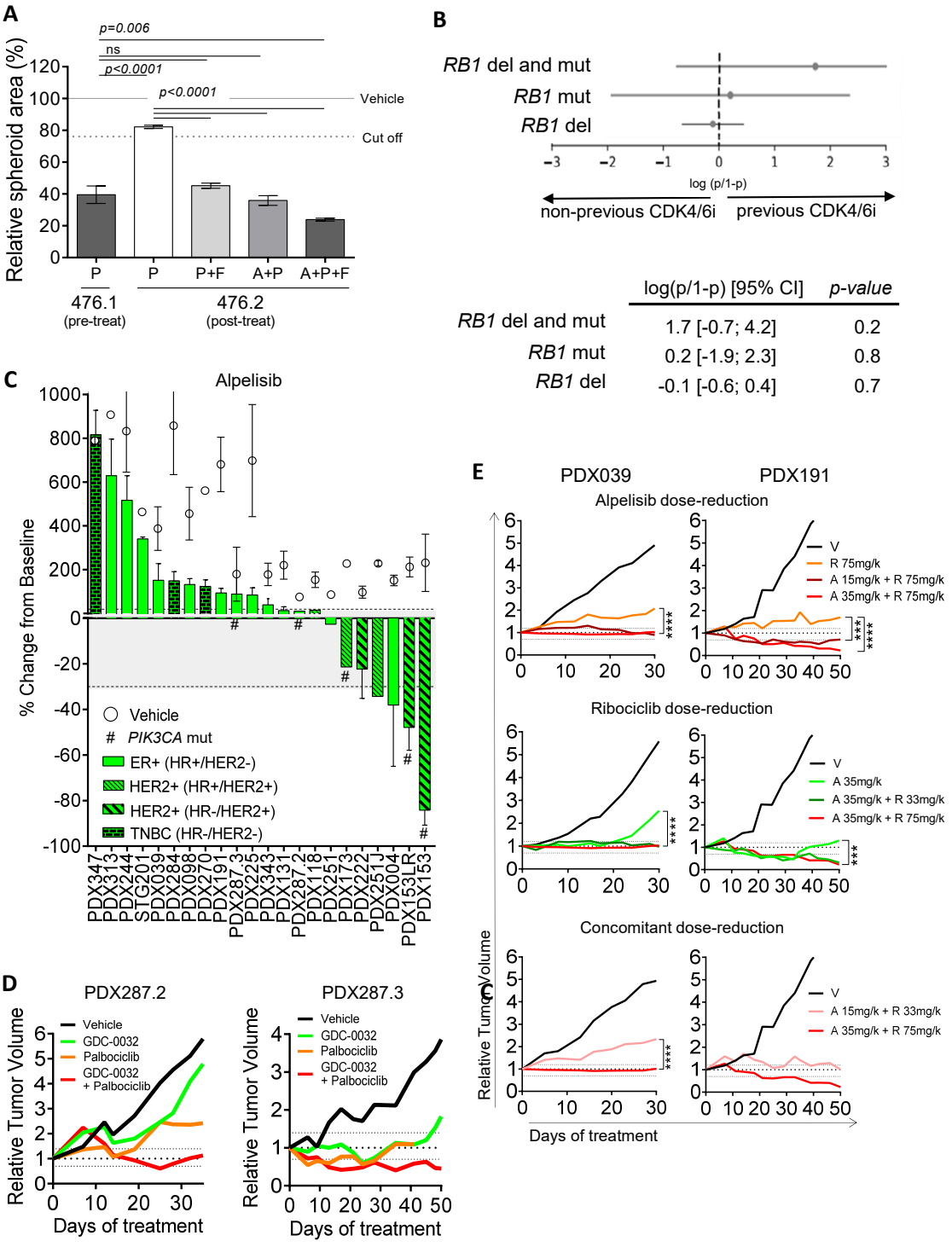

Fig. S4. Validation of RB1 homozygous loss as predictor of acquired resistance to ribociclib and antitumor efficacy of PI3K inhibitor in PDXs. (**A**) Relative spheroid area in PDX476.1 treated with 500 nm palbociclib and PDC476.2 after treatment with 500 nM palbociclib as single-agent or combined with 100 nM fulvestrant and/or 2.5 µM alpelisib in *ex vivo* cultures for 7 days. Data are presented as means of three independent experiments ± SEM. *p*-values are based on the one-way ANOVA test with Tukey´s method correction compared with the vehicle

(black line). The dashed line indicates the optimal cut-off established in Figure 3E. On the right, (**B**) Association between *RB1* alterations (only mutation, only deletion or both) and prior exposure to CDK4/6 inhibitors across metastatic breast cancer patients. Horizontal segments represent 95% confidence intervals of the logit value for each test. The size of the circle in the significant case is proportional to the negative logarithms of the logit *p*-value. (**C**) Waterfall plot representing the growth of 23 PDX treated with alpelisib 35 mg/kg (bars) or vehicle (circles). The percentage change from the initial volume is shown at day 35 of treatment. Dashed lines indicate the range of PD (>20%), SD (20% to -30%) and PR/CR (<-30%). Striped bars indicate the PDXs molecular subtypes. Hashtags indicate models harboring mutations in *PIK3CA*. Data is represented as mean and SEM. (**D**) Relative tumor growth of PDX287.2 and PDX287.3 treated with the indicated drugs and time. Dashed lines indicate the range of PD (>1.2), SD (1.2 to -0.7) and PR/CR (<-0.7). (**E**) Relative tumor growth of PDX039 and PDX191 after treatment with the indicated drugs and time. Dashed lines indicate the range of PD (>1.2), SD (1.2 to -0.7) and PR/CR (<-0.7). *p*-values are based on the two-way ANOVA test with Bonferroni´s method correction. V, vehicle; R, ribociclib; A, alpelisib; ns, non-significant.

SUPPLEMENTARY TABLES

Supplementary Table 1

		Molecular Sybtype		ER status		PR status		HER2 status		KI67 (%)	
Sample ID	**Origin**	**Patient**	**PDX**	**Patient**	**PDX**	**Patient**	**PDX**	**Patient**	**PDX**	**Patient**	**PDX**
004	Metastatic (skin)	$ER^+/HER2^-$	$ER^+/HER2^-$	pos	pos	neg	pos	neg	nd	50	25
039	Metastatic (skin)	$ER^+/HER2^-$	$ER^+/HER2^-$	pos	pos	neg	neg	neg	nd	50	80
098	Primary	$ER^+/HER2^-$	$ER^+/HER2^-$	pos	pos (low)	neg	neg	neg	neg	70	75
118	Metastatic (skin)	nd	$ER^+/HER2^+$	nd	pos	nd	neg	nd	pos	nd	50
131	Metastatic (skin)	nd	$ER^+/HER2^-$	nd	pos	nd	pos	nd	nd	nd	50
153	Primary	$HER2^+$	$HER2^+$	neg	neg	neg	neg	pos	pos	40	35
153LR	Primary		$HER2^+$		neg		neg		nd		75
173	Primary	$ER^+/HER2^+$	$ER^+/HER2^+$	pos	pos	pos	pos	pos	pos	20	60
191	Primary	$ER^+/HER2^-$	$ER^+/HER2^-$	pos	pos	pos	pos	neg	nd	30	75
STG201	Metastatic (nd)	nd	$ER^+/HER2^-$	nd	pos (low)	nd	neg	nd	nd	nd	95
222	Primary	$HER2^+$	$HER2^+$	neg	neg	neg	neg	pos	pos	80	85
225	Metastatic (skin)	nd	$ER^+/HER2^-$	nd	pos (low)	nd	neg	nd	nd	nd	50
244	Metastatic (skin)	nd	$ER^+/HER2^-$	nd	pos	nd	pos	nd	nd	nd	75
244LR	Metastatic (skin)		$ER^+/HER2^-$		pos		pos		nd		75
251J	Primary	$ER^+/HER2^+$	$ER^+/HER2^+$	pos	pos	neg	neg	pos	pos	50	45
251	Primary	$ER^+/HER2^-$	$ER^+/HER2^-$	pos	pos	pos	pos	neg	neg	30	50
270	Primary	$ER^-/HER2^-$	$ER^-/HER2^-$	neg	neg	neg	neg	neg	nd	70	85
284	Primary	$ER^-/HER2^-$	$ER^-/HER2^-$	neg	neg	pos	neg	neg	neg	45	75
287.2	Primary	$ER^+/HER2^-$	$ER^+/HER2^-$	pos	pos	pos	pos	neg	neg	80	40
313	Metastatic (skin)	nd	$ER^+/HER2^-$	nd	pos	nd	neg	nd	neg	nd	95
287.3	Primary	$ER^+/HER2^-$	$ER^+/HER2^-$	pos	pos	pos	pos	neg	neg	80	65
343	Metastatic (breast)	$ER^+/HER2^-$	$ER^+/HER2^-$	pos	pos	pos	pos	neg	nd	60	70
347	Primary	$ER^-/HER2^-$	$ER^-/HER2^-$	neg	neg	neg	neg	neg	neg	80	80

Table S1. Status ER, PR, HER2 and percentage of KI67-positive cells in matched patients and PDXs tumors. Abbreviations: pos (positive); nd (not determined).

Supplementary Table 2

Sample ID	Biopsy origen	Patient TNM	Treatments before biopsy
004	Metastasis	T2N0M1	**NeoAdj:** NA **Adj:** NA **Met:** NA
039	Metastasis	M1	**NeoAdj:** NA **Adj:** NA **Met:** L, T, Ex, F + R, Be, Ct (5 lines)
098	Primary	T1N1M0	**NeoAdj:** Ct (2 lines) **Adj:** NA
118	Metastasis	NA	**NeoAdj:** NA **Adj:** Ct, T **Met:** H + Ct (6 lines), R, La + H, HS
131	Metastasis	M0	**NeoAdj:** NA **Adj:** Ct (2 lines), T **Met:** Ct (3 lines), L, Ex, Ct+Be, Ct+F
153/153LR	Primary	T4N1M0	**NeoAdj:** Ct+H **Adj:** R+H+T+LH, Ct+H+R+LH+L
173	Primary	M1	**NeoAdj:** NA **Adj:** Ct+H+T **Met:** NA
191	Primary	NA	**NeoAdj:** NA **Adj:** NA
STG201	Metastasis	NA	**NeoAdj:** NA **Adj:** NA **Met:** NA
222	Primary	T2N2M1	**NeoAdj:** NA **Adj:** NA **Met:** Ct+H, R+H, Ct+T
225	Metastasis	M1	**NeoAdj:** NA **Adj:** NA **Met:** A
244/244LR	Metastasis	M1	**NeoAdj:** NA **Adj:** NA **Met:** Ct (3 lines), Ct+Be, Ct+R, F+Ev+Ex
251J	Primary	T2N1M0	**NeoAdj:** NA **Adj:** NA
251	Primary	T2N0M0	**NeoAdj:** NA **Adj:** L
270	Primary	T2N0M0	**NeoAdj:** NA **Adj:** NA
284	Primary	T2N0M0	**NeoAdj:** Ct **Adj:** NA
287.2	Primary	T2N1M1	**NeoAdj:** NA **Adj:** Ct, LH+T
313	Metastasis	NA	**NeoAdj:** Ct (2 lines) **Adj:** NA **Met:** Ct+R, Ct
287.3	Primary	T2N1M1	**NeoAdj:** NA **Adj:** Ct, LH+T
343	Metastasis	M0	**NeoAdj:** Ct **Adj:** R+L-A **Met:** P+Ex, Ct. Ct+GD
347	Primary	T3N2M0	**NeoAdj:** Ct **Adj:** NA

Table S2. Clinical information of patients' tumors. Abbreviations: L (letrozole); T (tamoxifen); A (anaztrazole); Ex (exemestano); F (fulvestrant); H (herceptin); La (lapatinib); Ev (everolimus); P (palbociclib); GD (GDC-0032); HS (HSP990); LH (analogs LHRH); R (radiotherapy); Be (bevacizumab); Ct (chemotherapy varius); nd (not determined); ne (not exist).

Supplementary Table 3

	Gene ID	Mut		CVN	
		aa change	allelic fc.	alteration	log ratio
PDX313	*TP53*	p.X187_splice	1.0		
	CCND2			AMP	2.1
	AKT1	p.E17K	1.0		
PDX347	*TP53*	p.E286*	1.0		
	CDK6	p.V45L	0,4		
	TSC2	p.R1706H	0.5		
PDX098	*TP53*	p.R249S	1.0		
	RB1	p.F721fs	0.9		
PDX244LR	*TP53*	p.C176R	1.0		
	ESR1	p.Y537S	0.3		
	PTEN			del	-3.0
	CDKN2A			del	-3.3
	CDKN2B			del	-3.3
	RB1	p.L694fs	0.6		
PDX284	*TP53*	p.R110P	1.0		
	CDKN2A			del	-6.1
	CDKN2B			del	-6.1
	CCND1	p.D289N	1.0		
	CCND2			AMP	2.1
STG201	*TP53*	p.M237I	1.0		
	CDKN2A			del	-4.6
	CDKN2B			del	-4.6
	AKT1			AMP	1.5
PDX270	*TP53*	p.S241A	1.0		
	AR	p.G454_G455insG	0.6		
PDX153LR	*TP53*			del	-2.1
	PIK3CA	p.K111E	0.6		
PDX225	*TP53*	p.Q167*	1.0		
	AKT1	p.E17K	0.8	AMP	1.5
	AR			AMP	1.9
PDX222	*TP53*	p.R280G	1.0		
	TSC2	p.S526T	1.0		
	AR			AMP	1.4
PDX039	*TP53*	p.V157I, 0.5	0.5		
	TSC2			del	-7,0
PDX251	*TP53*	p.Y236C	1.0	del	-2.1
	PIK3CA	p.E545K	0.6		
PDX131	*TP53*	p.Q331fs	1.0		
	ESR1	p.Y537S	0.5		
	CDK6			AMP	1.1
	CCND1			AMP	3.4
	CCNE1			AMP	1.1
	AR	p.57_60del	0.2		
PDX191	*FGFR1*			AMP	2.7
	CCND1			AMP	3.0
PDX118					
PDX287.2	*TP53*	p.T256fs	1.0		
	PIK3CA	p.H1047R	0.5		
	CCND1			AMP	3.0
PDX004					
PDX287.3	*TP53*	p.T256fs	1.0		
	PIK3CA	p.H1047R	0.5		
	RB1	p.K810N	0.25		
	CCND1			AMP	3.3
	AR	p.457_457del	0.5		
PDX251J	*TP53*	p.E287*	1.0		
	CDKN2A	p.S12*	1.0		
PDX343	*ESR1*			AMP	1.1
	FGFR1	p.W37C	0.95	AMP	3.2
	PIK3CA			AMP	1.7
PDX153	*TP53*			del	-2.1
	FGFR1			AMP	1.0
	PIK3CA	p.K111E	0,6		
PDX244	*TP53*	p.C176R	1.0		
	ESR1	p.Y537S	0.3		
	PTEN			del	-3.0
	CDKN2A			del	-4.2
	CDKN2B			del	-4.2
PDX173	*FGFR1*			AMP	1.1

Table S3. PDXs mutations and CVN. Abbreviations: AMP (amplification); del (deletion).

Supplementary Table 4

		Biomarker analysis							*Ex vivo* analysis	
PDX ID	Clinical subtype	PAM50	IMPACT data	pRb score	p16 score	Cyclin D1 score	Cyclin E1 score	Predicted response	Δ spheroid area	Predicted response
292	ER+ HER2-	HER2-enriched	11q.13 amp (lr 2.3)	2+	3+	7	5	Resistant	25	Resistant
301	ER+ HER2-	Luminal B		2+	3+	4	3	Resistant	-3	Resistant
346	ER+ HER2-	Luminal B	11q.13 amp (lr 5.1)	3+	2+	6	4	Resistant	-8	Resistant
350	ER+ HER2-	Luminal B		3+	2+	8	0	Resistant	-25	Sensitive
376	ER+ HER2-	Luminal B		3+	2+	7	5	Resistant	22	Resistant
399	ER+ HER2-	Luminal B		2+	1+	7	0	Resistant	-33	Sensitive
406	ER+ HER2-	HER2-enriched		3+	1+	5	3	Sensitive	-41	Sensitive
433	ER+ HER2-	Luminal B	11q.13 amp (lr 2.6)	4+	1+	3	4	Sensitive	-34	Sensitive
446B	ER+ HER2-	Luminal B	*CDKN2A* deepDel (lr -3.0)	4+	0	5	3	Sensitive	-30	Sensitive
450	ER+ HER2-	Luminal B		1+	1+	6	0	Resistant	-1	Resistant
BB3RC31	ER+ HER2-	Luminal B	*FAT1* p.2871fs (allel fc. 0.2)	3+	1+	7	0	Resistant	-3	Resistant
BB6RC39	ER+ HER2+	HER2-enriched		4+	1+	6	0	Sensitive	-25	Sensitive
BB6RC87	ER+ HER2+	HER2-enriched		1+	0	8	4	Resistant	12	Resistant
BB6RC160	ER+ HER2+	HER2-enriched	11q.13 amp (lr 2.9)	4+	0	6	0	Sensitive	-37	Sensitive

Table S4. Complex biomarker validation in 14 additional ER$^+$ BC PDXs. pRb and p16 score: very strong (4+), strong (3+), moderate (2+) weak (1+) or negative staining (0). Cyclin D1 and cyclin E1 Allred score takes into account the percentage of positive cells (0 to 5) plus the staining intensity (0 to 3).