SUPPLEMENTARY INFORMATION

SUPPLEMENTARY MATERIAL AND METHODS

Cell Culture

Most of the cell lines (Alexander, BT549, HCC202, Hep3B, Huh1, Huh7, JHH2, JHH7, PLC/PRF/5, skHep1, SNU387, SNU398, SNU449, GP2-293) were cultured in DMEM media (Corning). The remaining cell lines (HepG2, Li7, SNU423 and SNU475) were cultured in RPMI (Corning). All media contained 10% fetal bovine serum (FBS) (Biowest) and 100 $IUml^{-1}$ penicillin/streptomycin (Corning). All cells were grown in a humidified incubator at 37 °C with 5% CO_2, were tested regularly, and were negative for mycoplasma contamination. Individuality of each cell line was checked by STR sequencing (Genetica, LabCorp). Cell lines were passaged for fewer than 3 months after authentication. Human liver cancer cell lines were a kind gift of Drs. Scott Powers (Stony Brook) and Josep Llovet (Icahn School of Medicine at Mount Sinai), and breast cancer cell lines were a gift of Dr. Jose Silva (Icahn School of Medicine at Mount Sinai). Retroviruses were packed using GP2-293 cells (purchased from Clontech) and infections were performed as described elsewhere.[1] The infected population was selected using 1 µg/ml puromycin for 4 days.

Cell assays

For proliferation assays, cells were seeded in triplicate at 100,000 cells per well in 6-well plates. The day after plating, palbociclib was added at 1 µM. Control wells with an equivalent amount of DMSO were also included. To evaluate palbociclib in the context of resistance to sorafenib, both Huh7 parental cells and sorafenib-resistant Huh7 cells (cultured in sorafenib at 4 µM for 2 months),[2] were seeded in triplicate at a density of 80,000 cells per well in 6-well plates. The following day DMSO, palbociclib (1 µM) or

sorafenib (5 μM) were added to the medium. Cells were counted three days after using a hemocytometer. Two or three independent experiments in triplicate were performed for each cell line.

For colony formation assay, cells were seeded in duplicate at 700-1,000 cells per well in 12-well plates. The day after plating, palbociclib was added at increasing concentrations, from 0.25 μM to 10 μM. Control wells with an equivalent amount of DMSO were also included. Growth media with DMSO or the drug was replaced every 3 days. Cells were stained 2 weeks after using crystal violet (0.05%) and photographed using a digital scanner. The crystal violet was then extracted with glacial acetic acid 10% and colorimetric measurement was performed at 570 nm. Two independent experiments in duplicate or triplicate were performed for each cell line. The IC_{50} was calculated using Prism 6 software.

For the BrdU incorporation assay, control or drug-treated cells were incubated with bromodeoxyuridine (BrdU) for 4 hours and the assay was performed following the manufacturer´s instructions (Cell Proliferation ELISA, BrdU, Roche). Briefly, after removing the labeling medium, the cells were fixed, and the DNA was denatured. Then, the anti-BrdU antibody was added and bound to the BrdU incorporated into the newly synthesized cellular DNA. The immune complexes were detected by the subsequent substrate reaction. The reaction product was quantified by measuring the absorbance using a scanning multi-well spectrophotometer (ELISA reader) at 370 nm. Two independent experiments in triplicate were performed for each cell line.

For cell cycle analysis, cells were harvested and washed in PBS, fixed in 85% ethanol and stored at 4 °C for at least 30 minutes. Cells were then washed again in PBS and incubated at room temperature with 1X PBS containing DAPI (1 µg/mL) and 0.1 % Triton X-100. Data were collected using a Fortessa (BD Biosciences) and analyzed using BD FACSDiva Software (BD Biosciences). Results represent a minimum of 15,000 cells assayed for each sample. Two independent experiments were performed for each cell line.

For viability experiments, 2,500 cells were plated in 96-well plates. The day after plating, palbociclib (1 µM) or sorafenib (5 µM) was added. Control wells with an equivalent amount of DMSO were also included. The viability of the cells was measured 4 days after by performing the MTS assay following the standard protocols. Two independent experiments in triplicate were performed for each cell line.

For the study of cellular senescence, one million cells were plated in 10 cm diameter dishes and treated with DMSO or palbociclib (0.5 µM) for two weeks. Cells were replated if needed. Growth media with DMSO or the drug was replaced every 3 days. SAβGAL staining was performed at pH = 6 as previously described.[3, 4] To calculate the percentage of SAβGAL positive cells, 5 fields per condition at 200X magnification were examined manually.

For the reversibility experiments, 10^6 cells were plated in 10 cm diameter dishes and treated with DMSO or palbociclib (0.5 µM) for two weeks. Cells were replated if needed. Growth media with DMSO or the drug was replaced every 2 days. At day 10, cells were counted and 10,000 cells were replated in 6-well plates. Regular media was

used for 10 additional days. Crystal violet staining was then performed as described above. Two independent experiments in duplicate were performed for each cell line.

Vectors

For the knockdown experiments, miR30-design shRNAs targeting RB1, p107, p130, renilla luciferase, and firefly luciferase in LMP vector were used.[5] For the CRISPR experiments, px330 vector [6] was digested with BbsI and ligated with annealed oligonucleotides (Supplementary table 2). pX330-U6-Chimeric_BB-CBh-hSpCas9 was a gift from Feng Zhang (Addgene plasmid # 42230)[6]. All constructs were verified by nucleotide sequencing.

Drug Treatment

The specific CDK4/6 inhibitor palbociclib (P-7766) and sorafenib (S-8502) were purchased from LC Laboratories. Drugs for *in vitro* studies were dissolved in DMSO to yield a 10 mM stock solution and stored at -80 °C. For the *in vivo* experiments, palbociclib was dissolved in sodium lactate buffer (50 mmol/L, pH 4.0) while sorafenib was dissolved in a 4X cremophor EL/95% ethanol solution (50:50).

Western Blot Analysis

Cell or tissue samples were lysed and Western blot analysis was done as described previously.[7] Cells were washed with PBS and lysed at 4°C in 200 µl lysis buffer. Phospho-lysis buffer (50 mM Tris pH 7.5, 280 mM NaCl, 0.5% NP-40, 0.2 mM EDTA, 2 mM EGTA, 10% Glycerol) supplemented with phosphatase and protease inhibitors (cOmplete and PhosSTOP tablets, Roche) was used for cell lysis and protein

concentration was determined using the BCA Protein Assay Kit (Pierce Biochemicals). Proteins were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore). Membranes were immunoblotted with antibodies against phopho-RB1 (Ser780) (Cell Signaling Technology #8180), total RB1 (Cell Signaling Technology #9309), CDK4 (Santa Cruz Biotechnology sc-260), CDK2 (Santa Cruz Biotechnology sc-163), CCND1 (Vector Laboratories VP-C394), CCNA2 (Cell Signaling Technology #4656), p53 (Santa Cruz Biotechnology sc-6243), p21 (Santa Cruz Biotechnology sc-397), p107 (Santa Cruz Biotechnology sc-318), p130 (Santa Cruz Biotechnology sc-317), β-actin (Santa Cruz Biotechnology sc-47778), vinculin (Sigma-Aldrich V9131), phospho-ERK^{Y204} (Santa Cruz Biotechnology sc-7383) and total ERK (Santa Cruz Biotechnology sc-93). Blots were washed and incubated with a horseradish peroxidase conjugated-goat-anti-rabbit (Santa Cruz Biotechnology sc-2004) or goat-anti-mouse IgG (Santa Cruz Biotechnology sc-2005), developed using ECL Plus chemifluorescent reagent (Amersham Biosciences) and imaged using the ChemiDoc MP system (Biorad). All immunoblots were performed independently at least twice. Protein levels were quantified using ImageJ software (NIH) and normalized as indicated. The HeatMapImage module in GenePattern software (Broad Institute, MIT) was used to create the heatmaps.

Organotypic ex vivo culture of patient-derived HCC tissues

Fresh HCC tissues were obtained from liver surgeries performed as routine clinical care at Mount Sinai Medical Center through the Mount Sinai Biorepository. Anonymous use of de-identified tissues was approved by the institutional review board. Fresh HCC tissues were sliced into 300 μm-thick sections, and cultured with palbociclib (10 μM) for 48h.

Sections were then fixed in formalin and paraffin-embedded. Effects of the drugs on tissue architecture and cell proliferation were assessed by hematoxylin-eosin staining and by immunostaining of Ki67, respectively. Samples with >40% viable/morphologically-intact cells based on average of six randomly selected visual fields in H&E staining after culture with DMSO were included in the subsequent analysis.

In vivo experiments

All mouse experiments were approved by the Icahn School of Medicine at Mount Sinai (ISMMS) Animal Care and Use Committee (protocol no. IACUC-2014-0229). Mice were maintained under specific pathogen-free conditions, and food and water were provided *ad libitum*. All animals were examined prior to the initiation of studies to ensure that they were healthy and acclimated to the laboratory environment. 5-7-week-old, female athymic NCR-NU-NU or male C57BL6 (Envigo laboratories) mice were used for animal experiments with human cell lines and hydrodynamic tail vein injections, respectively.

For drug efficacy studies using a genetically-engineered mosaic mouse model of liver cancer, hydrodynamic tail vein delivery of vectors was performed in male C57BL6 mice, as described previously.[8] Mice were evaluated by bioluminescence imaging using an IVIS Spectrum system (Caliper LifeSciences, purchased with the support of NCRR S10-RR026561-01) to quantify liver tumor burden before being randomly assigned to various treatment study cohorts. Quantification was performed using Living Image software (Caliper LifeSciences). Mice were injected with fresh D-luciferin (150 mg/kg) 12 minutes before imaging. Mice were treated with vehicle (sodium lactate, n = 4) or palbociclib (100 mg/kg, n = 4), given orally once a day by gavage, based on mean group body weight. Bioluminescence imaging evaluation was repeated every week

during treatment. Investigators were not blind with respect to treatment. Tumor growth rate was calculated with the following formula: tumor growth rate = (normalized tumor signal at day 7-normalized tumor signal at day 0)/ tumor signal at day 0)*100/7, 7 being the number of days.

For Huh7 and PLC5 xenografts, cells (10 X 10^6) were harvested on the day of use and one flank was injected subcutaneously per mouse. Growth-factor-reduced Matrigel/PBS (50% final concentration) was used for Huh7. After inoculation, mice were monitored daily, weighed twice weekly, and caliper measurements begun when tumors became visible. Tumor volume was calculated using the following formula: tumor volume = (D X d^2)/2, in which D and d refer to the long and short tumor diameter, respectively. When tumors reached a size of 100-200 mm^3, mice were randomized and treated with vehicle, palbociclib (100 mg/kg), sorafenib (30 mg/kg) or a combination, given orally by gavage once a day, based on mean group body weight. No obvious toxicities were observed in the vehicle- or drug-treated animals as assessed by difference in body weight between vehicle- and drug-treated mice. The end-point of the experiment for survival studies was considered a tumor volume of 1000 mm^3 as per our approved protocol. Once the animals were sacrificed, tumors were excised, formalin-fixed and paraffin-embedded, frozen, or embedded in OCT (Tissue Tek). Tumor growth rate was calculated with the following formula: tumor growth rate = (tumor volume at day X-tumor volume at day 0)/ tumor volume at day 0)*100/X, X being the number of days.

Hydrodynamic tail vein injection

A sterile 0.9% NaCl solution/plasmid mix was prepared containing 20 μg DNA of pT3-EF1a-Myc, 20 μg of px330-p53 and 5 μg of PT2/C-Luc//PGK-SB13. C57BL/6 mice were

injected with the 0.9% NaCl solution/plasmid mix into the lateral tail vein with a total volume corresponding to 10% of body weight in 5-7 seconds. The pT3-EF1a-Myc vector was a kind gift of Dr. Xin Chen, UCSF San Francisco, PT2/C-Luc//PGK-SB13 was a gift from John Ohlfest (Addgene plasmid # 20207)[9] and pX330-U6-Chimeric_BB-CBh-hSpCas9 was a gift from Feng Zhang (Addgene plasmid # 42230)[6]. All constructs were verified by nucleotide sequencing.

Gene expression

Total RNA was isolated from frozen tumors or cell pellets using the Trizol method (Invitrogen) and was further purified with DNase treatment and RNAeasy kit (Qiagen) according to the manufacturer´s recommendations. Thermal cycling was carried out in ABI PRISM 7900 with default cycling conditions and real-time fluorescence detection. Expression fold change was calculated using the manufacturer's suggested delta-delta-Ct method. The sequence of the primers for RB1 and GAPDH is available upon request.

Histochemical staining

Tissues were fixed overnight in 4% formalin, embedded in paraffin, and cut into 5 µm thick sections. Sections were then subjected to antigen retrieval and treated with 3% H_2O_2 for 10 minutes. An enhanced streptavidin-biotin staining procedure was followed: sections were incubated with the appropriate biotinylated antibody for 30 minutes at room temperature; imaging was carried out using the ImmPRESS™ detection kit (Vector Laboratories) according to manufacturer's instructions; sections were then counterstained with Harris's hematoxylin. The following primary antibodies were used: Ki67 (Thermo Scientific RM-9106) and cleaved caspase 3 (Cell signaling, #9661). The percentage was determined by using Image J. The assessment of apoptosis in the

tissue sections was performed using the ApopTag In Situ Apoptosis Detection Kit (Millipore), according to the manufacturer's guidelines. The apoptotic index is expressed as the number of positive-nuclei per high-power field (200X magnification).

Detection of SAβGAL activity was performed as described previously [3] at pH = 6.0. Frozen sections of liver tissue or adherent cells were fixed with 0.5% gluteraldehyde in PBS for 15 min, washed with PBS supplemented with 1 mM MgCl2, and stained for three hours in PBS containing 1 mM MgCl2, 1mg/ml X-Gal, and 5 mM of each potassium ferricyanide and potassium ferrocyanide. Sections were counterstained with Eosin. The percentage of SAβGAL positive staining is expressed as the ratio of stained area and the total area at 100x magnification.

Human HCC sample analysis

Gene expression profiling of a total of 418 human samples was included. We used as training set 190 HCC samples from the TCGA database and an independent validation set (Heptromic dataset, GSE63898). The "RB1 loss-of-function" ("RB1_LOF") signature was generated from genes significantly altered (FDR < 0.05, fold change equal or greater than 2) in HCC samples with RB1 altered (either RB1 homozygous deletions (HD) or gene mutations) compared with remaining samples without RB1 alterations within the TCGA dataset. Differential mRNA expression between the 2 groups was analyzed with the GenePattern platform (www.broadinstitute.org/genepattern, Broad Institute, Boston, MA) using Comparative Marker Selection module, and false discovery rate (FDR) for multiple hypotheses testing correction. Then, "RB1_LOF" signature was validated by Nearest Template prediction (NTP, GenePattern module) in the independent dataset (Heptromic dataset).

To characterize the molecular alterations enriched between different groups of patients, we performed Gene set enrichment analysis (GSEA). Enrichment scores for each sample were calculated by ssGSEA (GenePattern module) and displayed as heatmap. DAVID software was also used for pathway analysis.[10]

SUPPLEMENTAL LEGENDS

Figure S1. Palbociclib inhibits proliferation of human liver cancer cell lines. (A) Crystal violet staining of colonies treated during 2 weeks with the corresponding doses of palbociclib (PD). Remaining cell lines not illustrated in text.

Figure S2. Palbociclib induces a reversible cell cycle arrest in human liver cancer cell lines. (A) BrdU incorporation relative to DMSO-treated cells, after 3 days of treatment with 1 µM palbociclib (PD). The mean + SD is shown. Breast cancer cell lines in pink, in blue the only HCC resistant cell line (B) Percentage of cells in G_0/G_1 after 3 days of treatment with 1 µM palbociclib (PD). The mean + SD is shown. (C) Representative cell cycle histograms. (D) Percentage of apoptotic cells (cells in sub-G_0/G_1) after 3 days of treatment with 1 µM palbociclib (PD). The mean + SD is shown. (E) Reversibility assay. Crystal violet staining after replating the cells that were pretreated as indicated. Three representative cell lines are shown.

Figure S3. RB1 loss of function correlates with resistance to palbociclib in human liver cancer cell lines. (A) Viability of the cells, measured by MTS assay, after treating the cells with DMSO, palbociclib (PD) or sorafenib (Sora) for 4 days. The mean + SD is shown. (B) Schematic of resistant cell line generation.

Figure S4. Loss of RB1 confers resistance to palbociclib in human liver cancer cell lines. (A) Schematic of *RB1*-sgRNA (single guide RNA) experiment. (B) IC_{50} values for the different cell lines were calculated by quantifying the extracted crystal violet in **figure 4C,D**. (C) Immunoblotting analysis of indicated proteins (basal levels) of cells in **figure 4C,D**.

Figure S5. Pathway analysis of human HCC samples. (A) GSEA plots of human HCC samples from TCGA for the gene sets "E2F_targets", "G2M_checkpoint" and Mitotic_spindle" in RB1 wild-type (WT) or altered samples. ES, enrichment score. NES, normalized enrichment score. (B) Five most significant Gene Ontology categories enriched in RB1 WT or altered HCC patient samples, after DAVID analysis. The p values are also shown.

Figure S6. *Ex vivo* culture and treatment with palbociclib. (A) Hematoxylin-eosin (H&E) staining (200X magnification) in a representative responder *ex vivo* human HCC sample treated with palbociclib for 2 days. (B) Percentage of cleaved-caspase 3 (CC3) positive cells in *ex vivo* human HCC samples treated with DMSO (black) or palbociclib (grey) for 2 days. The mean ± SD is shown. NA indicates not-addressed due to lack of the corresponding sample. (C) *RB1* expression, relative to *GAPDH*, in responder (R) and non-responders (NR).

Figure S7. Palbociclib, alone or in combination with sorafenib, has potent anti-tumor effects *in vivo.* (A) Schematic of the vectors used in the mosaic mouse model of liver cancer. (B) Body weight over time for different treatment groups in PLC5 xenografted mice. 5-7 mice per group are represented. The mean + SD is shown. (C) Spider plots depicting tumor growth in each group of treatment over time. The black bars represent the treatment periods (30 days).

Figure S8. Effects of mono- or combinatorial therapies. (A) Immunoblotting of designated proteins after treatment with 5 μM of sorafenib for 3 days. (B)

Immunostaining of apoptotic cells (measured by TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling) in representative tumors treated with the indicated treatment for 5 days. V, vehicle; S, sorafenib; PD, palbociclib; combo, combination of sorafenib and palbociclib. (C) Quantification of apoptotic index in the different treatment groups. The mean ± SD is shown. (D) Quantification of % of SAβGAL-positive area in the different treatment groups. The mean ± SD is shown.

Table S1. Signature of genes differentially expressed between RB1 wild-type and altered human HCC samples (TCGA) "RB1_LOSS-OF-FUNCTION" ("RB1_LOF")

Feature	Score	Fold Change	RB1_LOF_ Mean	RB1_LOF_ Std	RB1_functional _Mean	RB1_functional _ Std
WDR76	6.50	3.27	424.88	213.90	129.82	111.04
TCF19	6.03	3.03	1313.09	688.25	434.00	329.54
CHAF1B	5.90	3.13	390.71	210.40	124.98	130.88
MCM3	5.83	2.02	3934.77	1565.56	1947.32	1267.26
PAQR4	5.72	2.67	1042.94	530.50	390.89	359.07
FAM111B	5.65	3.11	586.83	331.94	188.85	171.32
EZH2	5.59	2.17	607.56	269.08	280.54	211.70
TYMS	5.42	2.38	1701.18	837.75	715.54	655.09
CCNF	5.24	2.01	451.46	195.91	224.15	189.06
MCM2	5.19	2.32	2158.51	1080.88	930.79	935.15
ZWINT	5.16	2.10	930.23	427.10	442.97	404.03
MCM6	5.15	2.22	1883.91	942.58	847.30	566.21
HELLS	5.11	2.51	192.81	103.50	76.95	89.50
C21orf58	5.03	2.33	173.86	91.63	74.69	62.69
CCNE2	4.99	4.64	481.38	361.07	103.84	97.25
CDC6	4.88	2.58	895.58	523.64	346.63	348.20
FANCI	4.79	2.14	849.76	438.85	396.16	318.33
UHRF1	4.74	3.14	527.86	354.86	167.96	224.51
NCAPG2	4.67	2.07	675.78	347.82	326.86	235.73
PRC1	4.51	2.20	1206.60	677.24	547.45	492.73
DNMT1	4.48	2.19	2267.17	1291.84	1033.43	722.27
TFDP1	4.45	2.60	2289.55	1502.99	882.00	526.43
KNTC1	4.45	2.99	798.19	566.06	267.04	240.37
CDC7	4.37	2.51	274.07	176.76	109.14	104.77
E2F8	4.34	2.59	213.28	140.42	82.37	92.88
BRIP1	4.26	2.96	166.74	122.26	56.26	59.66
KIF18B	4.26	2.60	402.26	271.00	154.81	175.70
RIBC2	4.17	4.38	86.19	75.59	19.68	32.71
CDKN2C	4.15	2.46	1140.99	765.04	464.03	446.14
FANCE	4.15	2.32	216.04	139.65	93.31	68.73
CDT1	4.14	2.01	695.33	376.99	345.63	402.19
DSCR6	4.06	5.86	63.31	61.65	10.81	19.50
FAM72D	4.02	2.07	109.93	63.39	53.08	64.89
NUSAP1	4.00	2.10	1262.03	766.02	602.09	523.34
CDH24	3.99	2.61	200.66	144.30	76.77	99.74

TROAP	3.94	2.05	473.97	277.18	231.65	269.24
E2F1	3.94	2.57	1313.86	959.74	510.37	498.51
DTL	3.94	2.57	724.03	527.70	282.08	284.15
CDK1	3.89	2.00	848.69	497.74	423.41	438.56
ZNF367	3.87	2.78	659.14	518.40	236.94	194.72
C16orf75	3.84	2.20	435.37	284.64	197.80	228.49
RBL1	3.79	2.76	177.78	141.64	64.35	60.03
POLD3	3.72	2.08	535.59	354.34	257.26	147.22
CASP8AP2	3.70	2.06	389.80	256.24	189.64	109.47
ASF1B	3.68	2.43	620.43	465.02	255.57	266.47
TP73	3.68	3.95	160.08	154.55	40.52	56.86
POLE2	3.67	2.34	207.94	151.70	88.97	89.84
KIFC1	3.67	2.23	883.93	621.04	396.62	385.50
WDHD1	3.66	2.63	246.64	197.52	93.65	95.48
CENPL	3.65	2.10	261.29	177.08	124.34	84.15
ORC1L	3.63	2.40	262.21	196.73	109.20	124.53
STIL	3.59	2.04	254.14	167.49	124.38	119.22
CKAP2L	3.55	2.04	184.97	122.94	90.52	90.36
C6orf167	3.52	2.42	243.37	191.69	100.76	87.04
FAM72A	3.50	2.19	37.43	26.88	17.09	20.01
E2F2	3.49	2.29	173.60	131.21	75.92	75.02
TUB	3.46	2.69	175.11	148.57	65.05	94.55
SKA3	3.45	2.04	228.86	156.58	112.03	115.11
TOP2A	3.39	2.36	2892.55	2315.30	1225.09	1255.04
KIF14	3.38	2.40	281.30	227.64	117.25	128.45
ATAD5	3.38	2.59	133.26	114.79	51.37	48.98
C8orf39	3.36	2.13	7.69	5.73	3.62	2.75
KIF23	3.32	2.08	377.52	266.68	181.84	249.96
SPIN4	3.31	2.01	144.54	102.41	71.86	64.51
GREB1L	3.30	2.49	196.06	167.83	78.77	77.50
FAM81A	3.29	2.70	67.60	59.83	25.02	43.79
CENPF	3.28	2.10	1410.44	1052.50	670.32	690.16
CDKN2A	3.27	2.51	916.35	788.69	364.97	490.91
IQGAP3	3.26	2.09	918.58	689.94	439.06	393.59
CLSPN	3.25	2.79	117.59	109.15	42.11	59.68
B3GNT5	3.19	2.43	571.57	498.19	234.76	248.89
ARNT2	3.17	3.00	423.17	415.40	141.25	255.97
FES	3.17	2.05	1344.69	1001.21	657.45	770.47
MESP2	3.16	3.12	94.03	95.96	30.16	34.33
CXorf57	3.14	2.45	169.11	149.37	69.03	86.77
ESCO2	3.11	2.09	127.02	97.43	60.88	80.28
DEPDC1	3.07	2.29	352.90	301.77	153.99	194.50
POLQ	3.05	2.08	152.07	120.16	73.25	82.86
CEP152	3.01	2.00	107.24	83.64	53.49	49.38
CENPI	2.99	2.37	64.42	57.96	27.13	40.12
NRM	2.93	2.61	892.64	894.84	341.95	265.71
E2F7	2.93	3.18	139.72	155.04	43.96	60.65
C1orf106	2.90	3.29	1169.06	1324.65	355.17	648.79
ARHGAP11A	2.90	2.03	469.46	385.66	231.08	222.06
C19orf57	2.87	2.26	123.40	113.33	54.64	49.90
MESP1	2.83	2.38	171.22	166.00	71.93	75.31
NUF2	2.81	2.10	360.69	313.77	172.13	190.68
MKI67	2.81	2.43	2144.85	2130.32	881.09	916.78
SBK1	2.81	2.51	113.49	112.01	45.27	86.41
BRCA2	2.80	2.12	113.39	100.55	53.57	53.99
ZMYND10	2.80	2.13	28.51	24.86	13.37	20.12
GPR137C	2.77	2.41	110.74	111.07	45.96	44.47
BARD1	2.77	2.94	62.78	71.05	21.36	28.42

SCARNA12	2.72	2.23	8.80	8.42	3.95	4.04
MGC27382	2.70	2.12	13.85	12.80	6.53	6.03
EHF	2.68	2.48	592.71	612.25	239.36	413.43
BUB1B	2.64	2.06	373.56	339.83	181.60	209.77
ASPM	2.63	2.05	1106.84	1010.25	540.29	565.00
ST6GALNAC2	2.62	3.16	285.71	354.72	90.39	122.30
RNF157	2.59	2.02	968.60	879.42	479.95	566.56
OSBPL6	2.57	2.13	197.08	191.68	92.34	99.97
BZRAP1	2.57	2.17	256.22	253.06	118.31	126.50
BMP8B	2.54	2.06	106.63	99.04	51.64	83.50
CNTFR	2.52	2.66	584.35	670.17	219.39	488.47
REEP1	2.52	2.01	104.90	93.96	52.06	97.36
ALG1L	2.52	2.49	465.78	521.37	187.41	266.87
PRR11	2.52	2.53	83.09	94.15	32.86	47.07
GNAZ	2.51	2.03	694.09	646.53	342.62	476.49
SALL2	2.51	2.17	282.87	282.75	130.21	198.41
EVC2	2.37	2.25	119.35	130.04	53.15	86.91
C17orf82	2.36	2.08	32.30	33.31	15.56	18.98
MYB	2.36	2.24	34.60	37.50	15.45	28.55
CDCA7L	2.35	2.07	611.03	636.33	294.61	278.71
ASRGL1	2.32	2.68	607.20	776.66	226.35	321.90
PLCXD1	2.32	2.07	1751.21	1807.15	847.74	1250.88
42616.00	2.32	2.29	89.71	100.52	39.15	76.96
CAND2	2.30	2.77	131.19	172.48	47.33	81.43
C16orf79	2.26	2.72	76.23	100.67	27.98	46.77
CDCA7	2.24	2.74	296.69	391.29	108.17	261.53
PRODH	2.13	3.29	3607.43	5529.71	1097.61	3305.15
PAPLN	2.02	2.02	838.58	944.71	414.89	913.40
SLC13A2	1.89	4.72	366.26	728.28	77.57	180.50
ANKRD22	1.73	10.04	725.43	1806.20	72.24	153.29
IER5	1.70	2.04	1149.05	1649.71	562.61	303.40
FAM179A	-2.56	3.70	3.80	3.21	14.04	50.98
CDHR1	-3.18	5.01	3.95	4.26	19.80	63.39
SAA2	-3.47	23.79	824.81	1160.20	19618.99	69909.68
TMEM182	-3.66	2.92	33.82	21.13	98.61	221.53
SLC16A5	-4.07	2.51	20.64	16.76	51.83	88.06
SAA1	-4.24	15.96	1703.58	2192.21	27184.16	77441.11
LDHD	-4.33	2.02	1252.85	1198.20	2526.77	2009.16
SLC19A3	-4.46	2.12	211.29	150.43	448.40	554.47
NCF1C	-4.59	2.89	10.85	10.18	31.39	50.91
SLC13A5	-4.65	2.78	2580.29	3845.74	7161.72	7384.12
SAA4	-4.66	2.46	2859.95	3182.66	7031.83	7763.02
ACSS3	-4.69	2.20	705.05	633.39	1548.59	1580.91
SHF	-4.75	2.13	275.36	259.37	587.05	481.74
NPR2	-4.76	2.00	344.01	258.40	688.72	625.72
STEAP1	-4.85	2.50	203.17	242.37	507.95	483.75
RIPPLY1	-4.85	3.74	16.22	22.37	60.71	102.05
HPR	-4.88	3.08	4434.84	7269.76	13645.12	14547.79
PHLDA1	-4.98	2.27	948.76	558.01	2156.56	2748.83
TMEM232	-4.99	2.69	2.31	2.58	6.22	7.36
CES2	-5.02	2.07	9425.82	7507.45	19534.83	16401.26
JAKMIP2	-5.02	4.49	13.28	11.60	59.56	114.85
FAM169A	-5.08	2.66	153.06	119.81	406.94	559.89
ATP6V0E2	-5.14	2.36	1552.38	1189.55	3670.64	4257.88
PATL2	-5.18	2.98	5.70	6.64	16.98	21.70
AMACR	-5.23	3.39	1446.59	1217.35	4902.39	7883.70
CES1	-5.24	2.35	37373.57	34481.79	87971.45	83189.14
SULT1B1	-5.25	4.56	16.68	19.58	76.05	136.15

AKR1C4	-5.29	2.63	2882.74	3038.02	7571.57	8000.90
GCSH	-5.33	2.35	38.11	38.05	89.49	70.68
MAP3K8	-5.40	2.41	95.39	71.40	229.49	256.57
LRRC6	-5.53	5.03	4.10	3.66	20.64	37.37
LBP	-5.55	2.73	8559.41	9312.82	23360.54	23644.56
UGT1A6	-5.56	2.42	5460.73	4654.62	13240.49	13037.51
CFHR4	-5.60	3.67	365.61	531.91	1341.51	1736.52
SH3BP5	-5.70	2.20	314.01	227.81	690.51	592.41
NEK3	-5.70	2.00	121.16	60.75	242.77	221.61
FAM176A	-5.72	2.03	684.11	473.65	1386.01	940.76
UGT1A1	-5.72	4.05	1834.64	2821.82	7427.44	10083.35
GFRA1	-5.77	3.64	306.88	436.28	1117.11	1383.47
APCS	-5.79	2.72	9576.69	9790.80	26051.18	25576.10
GNAO1	-5.89	4.15	67.32	100.87	279.35	377.70
CDK6	-5.89	3.34	339.37	505.67	1132.08	1078.93
GLUD1	-5.95	2.23	6191.05	3482.84	13829.05	13673.79
THSD1	-6.06	2.47	51.36	35.62	126.95	129.56
TRPS1	-6.21	2.88	52.43	46.11	150.79	162.68
C9	-6.23	5.96	1475.35	2397.24	8797.06	13751.58
NAT2	-6.37	5.12	42.47	65.75	217.63	308.10
TTC9	-6.38	3.14	238.66	189.84	749.90	900.02
TSC22D1	-6.42	2.39	1863.60	1010.71	4452.90	4444.10
MAP2	-7.42	3.71	195.75	160.76	727.02	818.29
CFI	-7.72	2.43	4603.81	2933.02	11209.13	7733.16

SUPPLEMENTARY REFERENCES

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Fig. S1

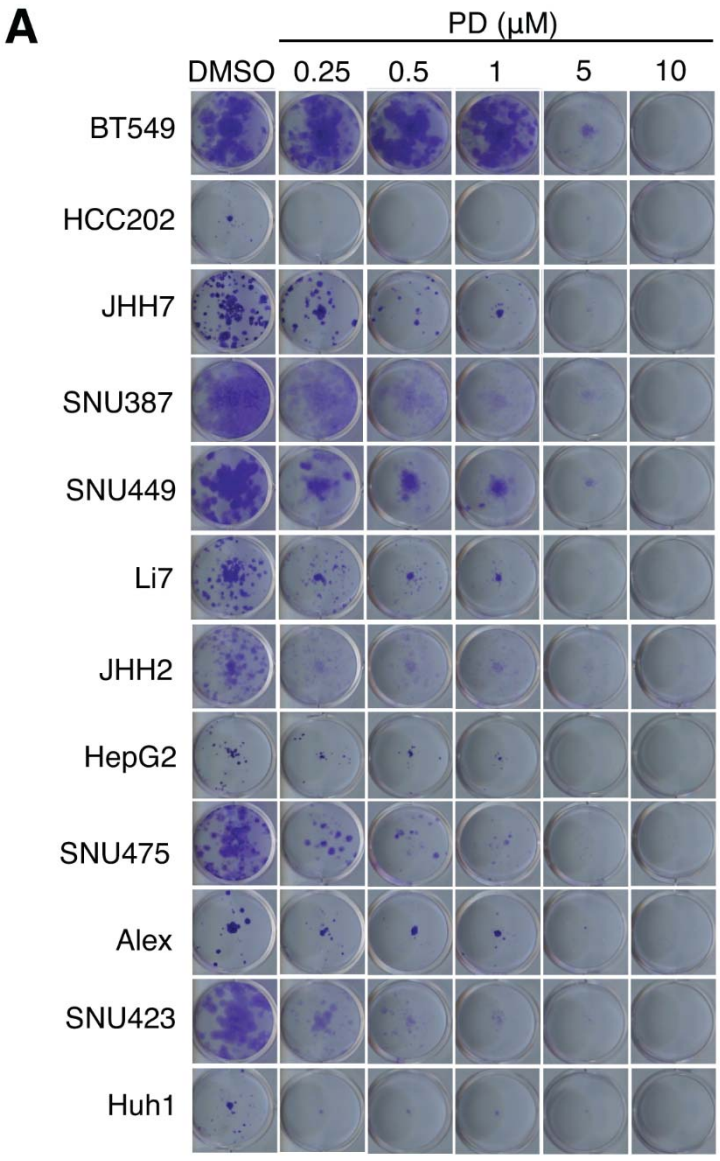

A
PD (μM)
DMSO
0.25
0.5
1
5
10
BT549
HCC202
JHH7
SNU387
SNU449
Li7
JHH2
HepG2
SNU475
Alex
SNU423
Huh1

Fig. S2

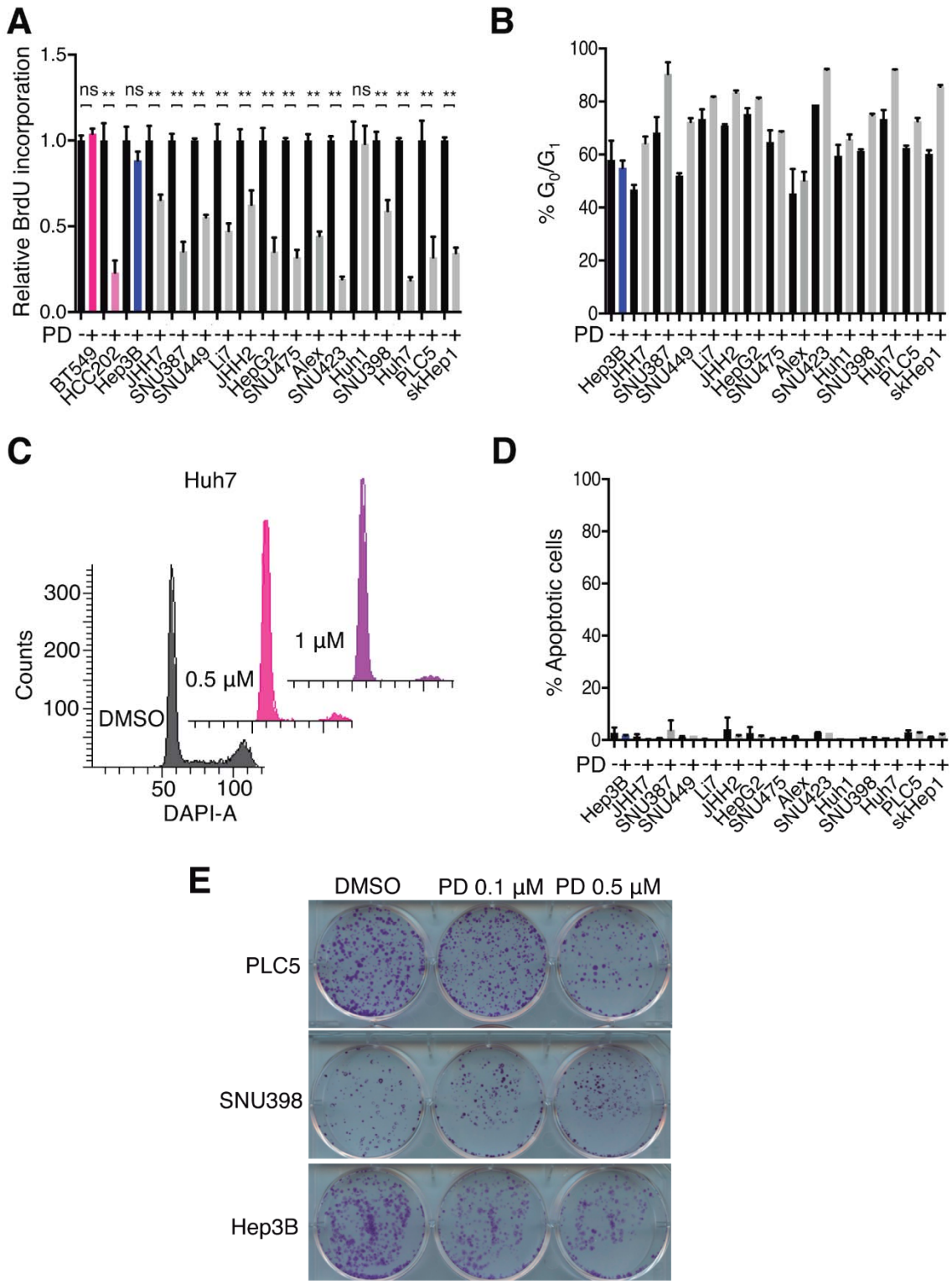

A
Relative BrdU incorporation
1.5
1.0
0.5
0.0
ns
**
PD
BT549
HCC202
Hep3B
JHH7
SNU387
SNU449
Li7
JHH2
HepG2
SNU475
Alex
SNU423
Huh1
SNU398
Huh7
PLC5
skHep1
B
% G0/G1
100
80
60
40
20
0
C
Huh7
Counts
300
200
100
DMSO
0.5 µM
1 µM
50
100
DAPI-A
D
% Apoptotic cells
E
DMSO
PD 0.1 µM
PD 0.5 µM
PLC5
SNU398
Hep3B

Fig. S3

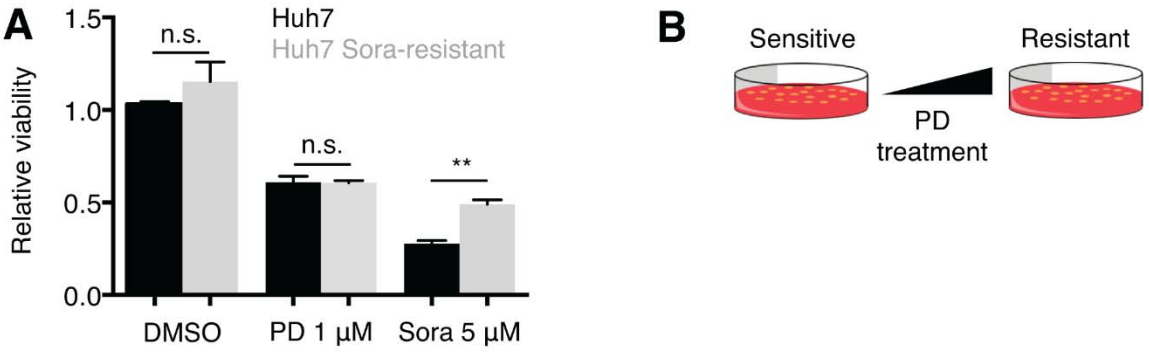
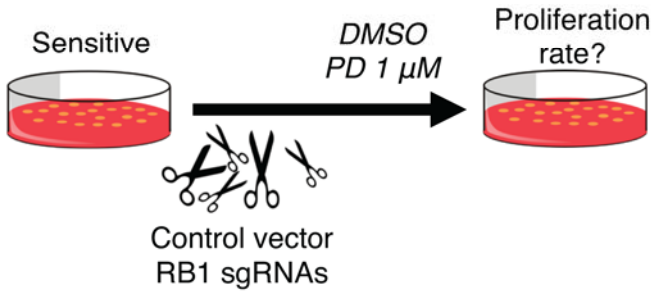

A
1.5
1.0
0.5
0.0
Relative viability
n.s.
n.s.
**
Huh7
Huh7 Sora-resistant
DMSO
PD 1 μM
Sora 5 μM
B
Sensitive
Resistant
PD
treatment

Fig. S4.

A



B

IC_{50} (uM)	skHep1	PLC5	Huh7
sh-Ren.713	0.0048	0.0051	0.0043
sh-Luc.1309	0.0054	0.0155	0.0058
sh-RB.662	0.5136	0.7931	0.0194
sh-RB.698	0.3014	1.443	0.2388
sh-p107.2285	0.0047	0.0608	0.0055
sh-p107.2483	0.0041	0.0071	0.0058
sh-p130.698	0.0039	0.0111	0.0078
sh-p130.3587	0.0053	0.0181	0.0048

C

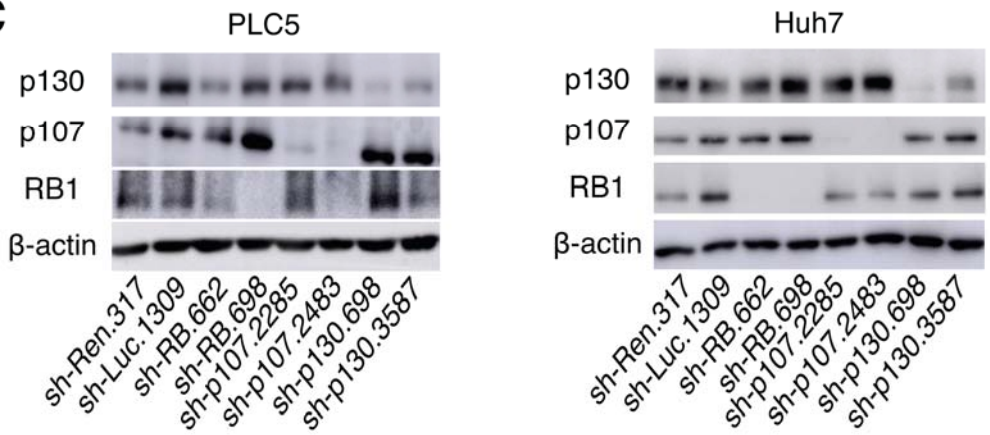
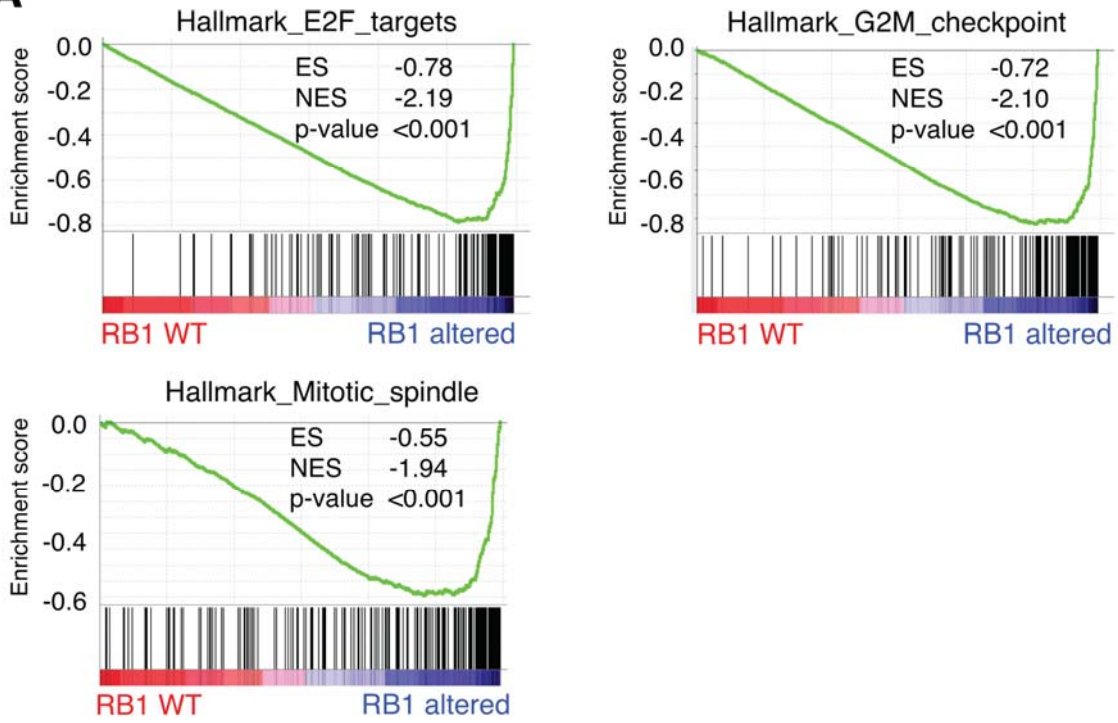


Fig. S5

A



B

RB1 altered	**p value**
GO:0007049~cell cycle	1.26E-82
cell cycle	6.33E-81
GO:0022403~cell cycle phase	9.00E-78
GO:0022402~cell cycle process	1.02E-69
GO:0000279~M phase	3.67E-69

Fig. S6

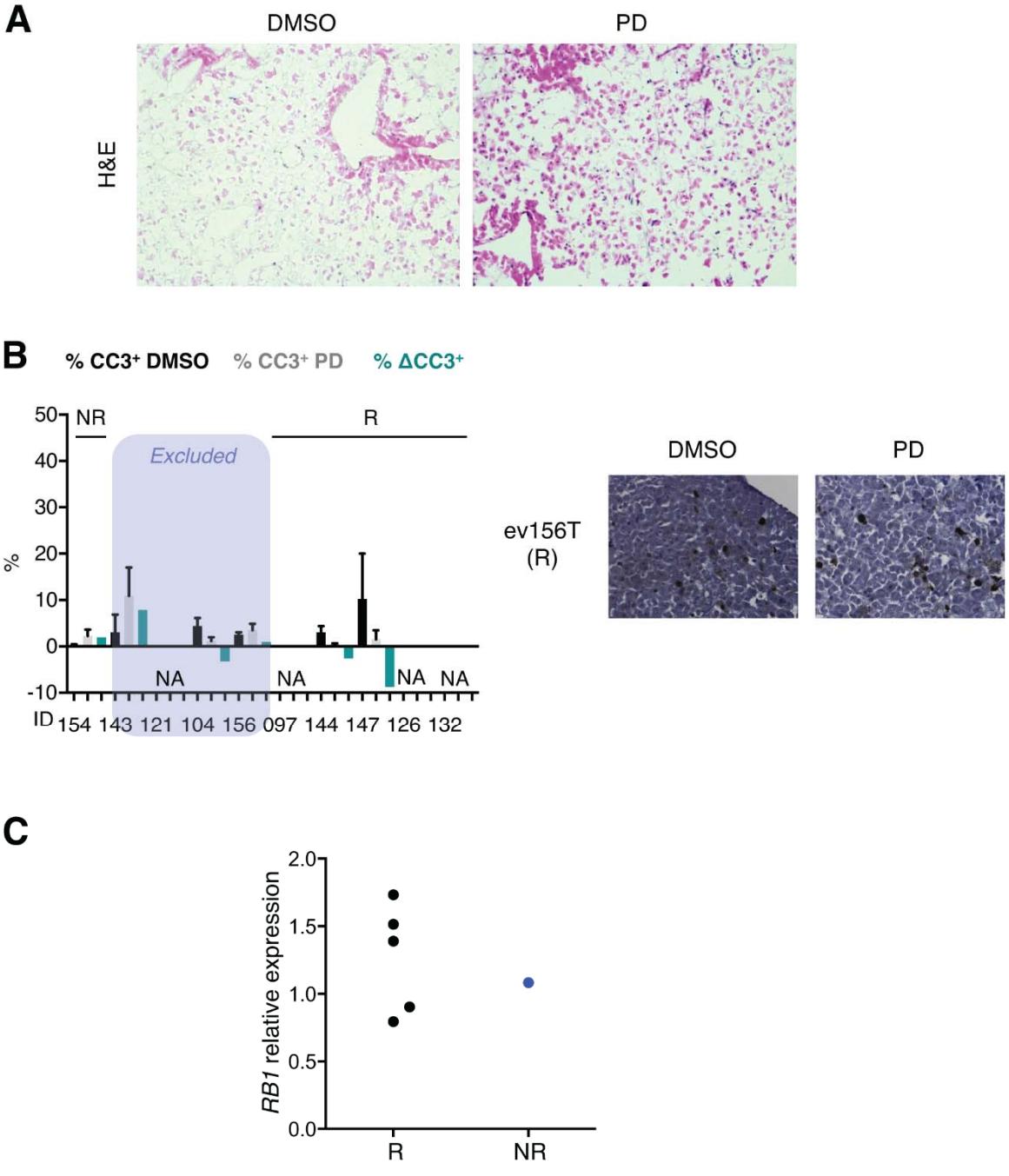

A
DMSO
PD
H&E
B
% CC3+ DMSO
% CC3+ PD
% ΔCC3+
50
40
30
20
10
0
-10
%
NR
R
Excluded
NA
NA
NA
NA
ID 154 143 121 104 156 097 144 147 126 132
DMSO
PD
ev156T
(R)
C
RB1 relative expression
2.0
1.5
1.0
0.5
0.0
R
NR

Fig. S7

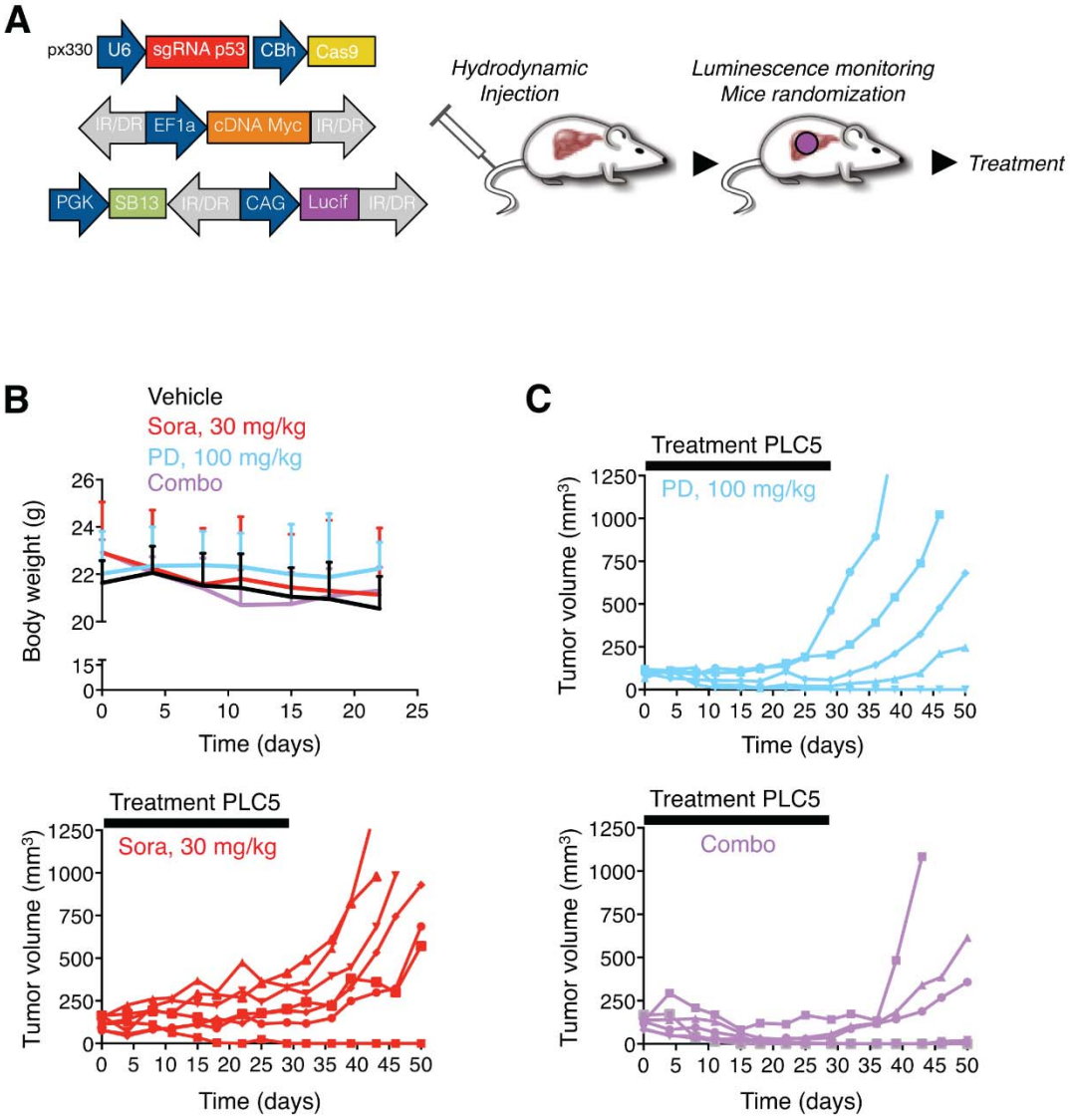
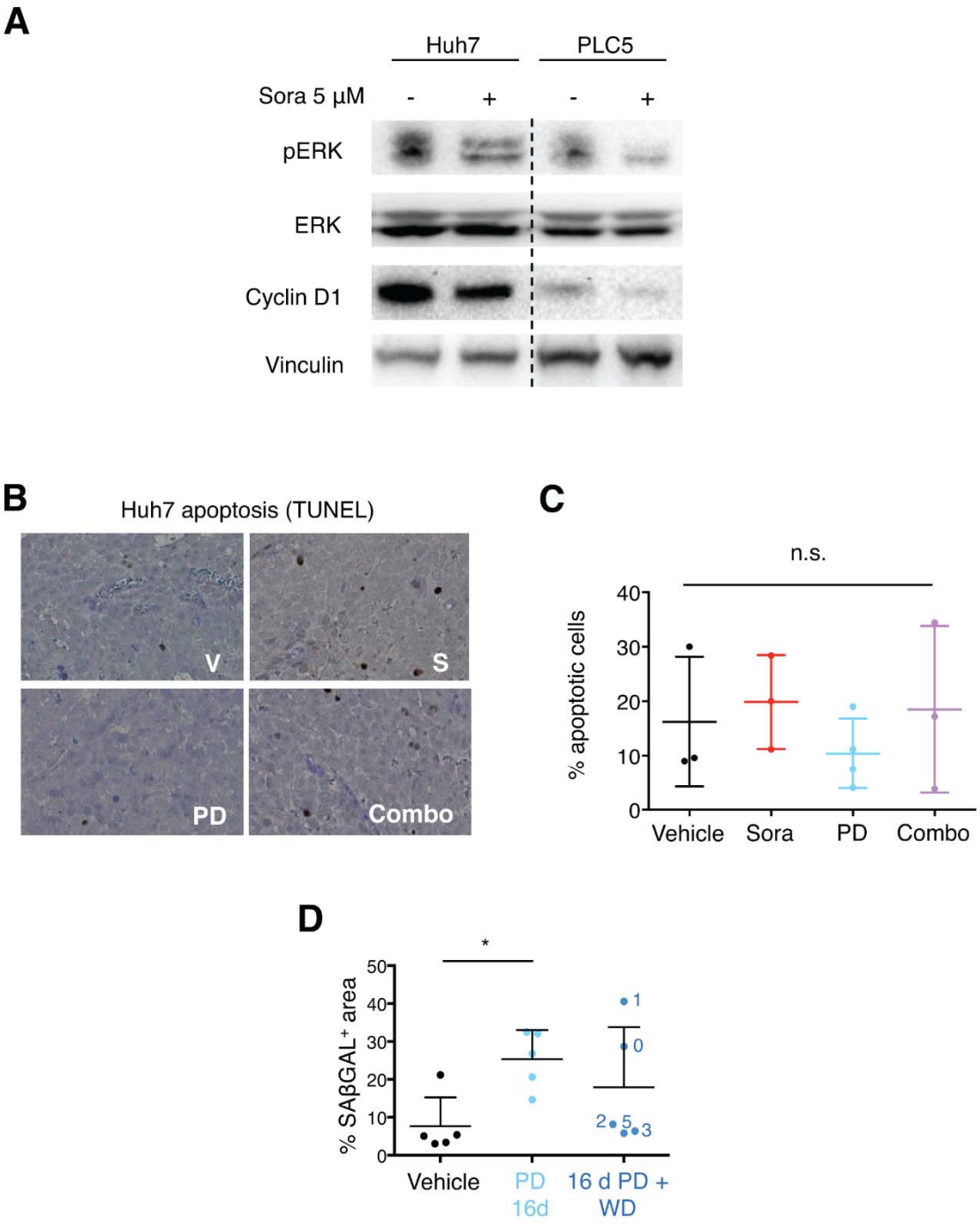

A
px330
U6
sgRNA p53
CBh
Cas9
IR/DR
EF1a
cDNA Myc
IR/DR
PGK
SB13
IR/DR
CAG
Lucif
IR/DR
Hydrodynamic Injection
Luminescence monitoring Mice randomization
Treatment
B
Vehicle
Sora, 30 mg/kg
PD, 100 mg/kg
Combo
Body weight (g)
Time (days)
Treatment PLC5
Sora, 30 mg/kg
Tumor volume (mm3)
Time (days)
C
Treatment PLC5
PD, 100 mg/kg
Tumor volume (mm3)
Time (days)
Treatment PLC5
Combo
Tumor volume (mm3)
Time (days)

Fig. S8


A
Huh7
PLC5
Sora 5 µM
-
+
-
+
pERK
ERK
Cyclin D1
Vinculin
B
Huh7 apoptosis (TUNEL)
V
S
PD
Combo
C
n.s.
% apoptotic cells
40
30
20
10
0
Vehicle
Sora
PD
Combo
D
*
% SAβGAL+ area
50
40
30
20
10
0
1
0
2
5
3
Vehicle
PD 16d
16 d PD + WD