Supplementary Figures & Tables

BET, SRC, and BCL2 family inhibitors are synergistic drug combinations with PARP inhibitors in ovarian cancer

Authors:

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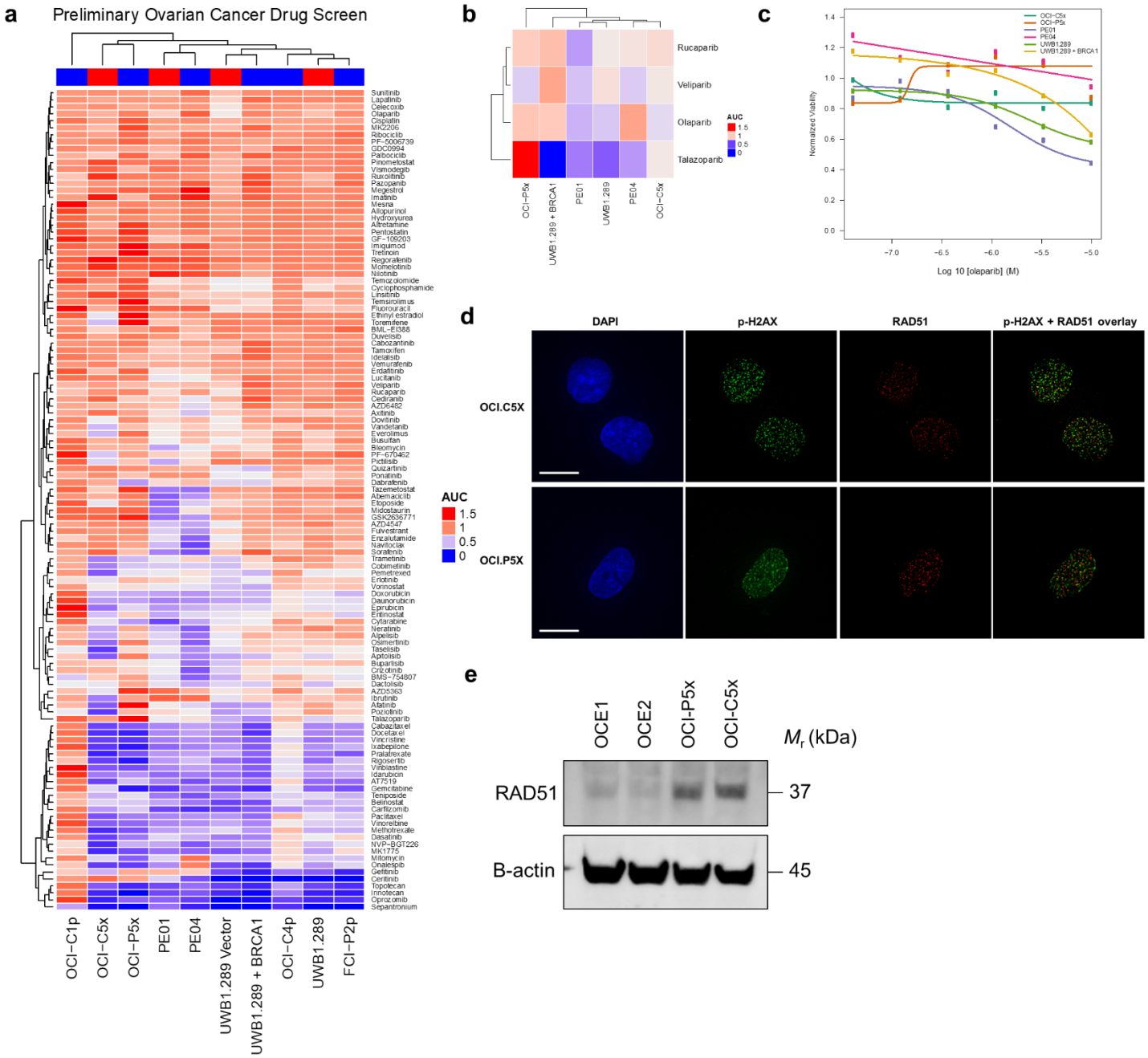
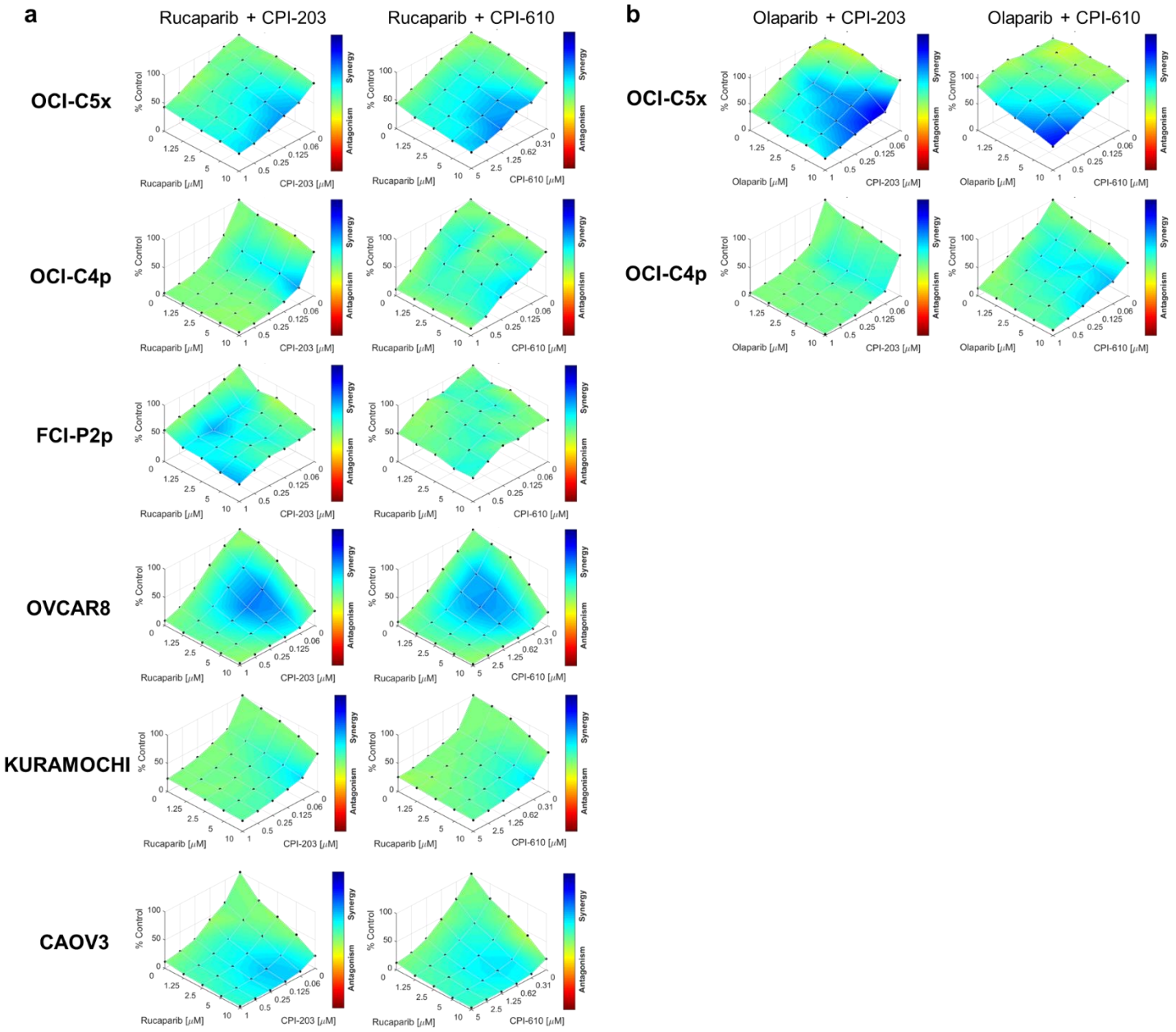
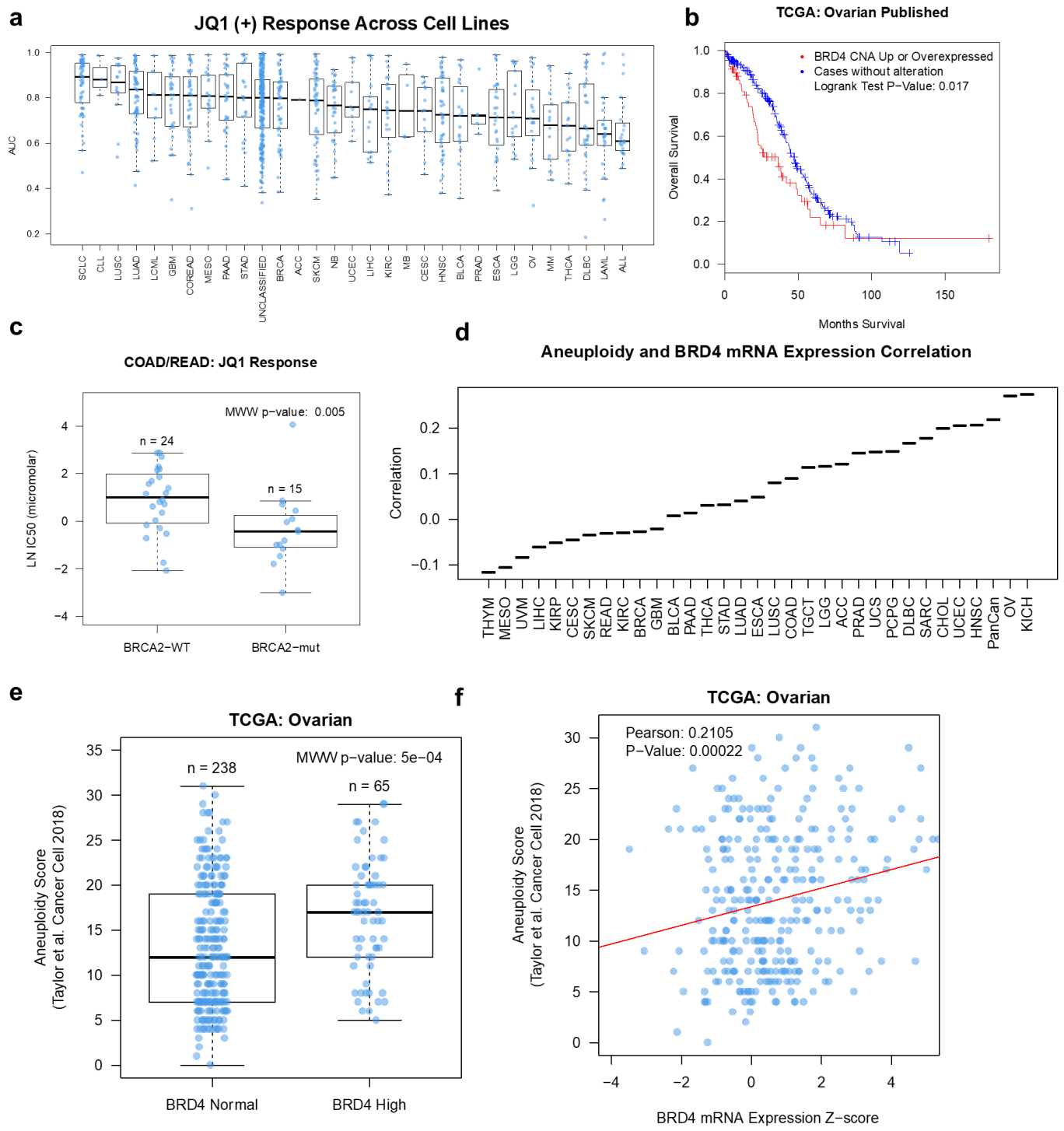


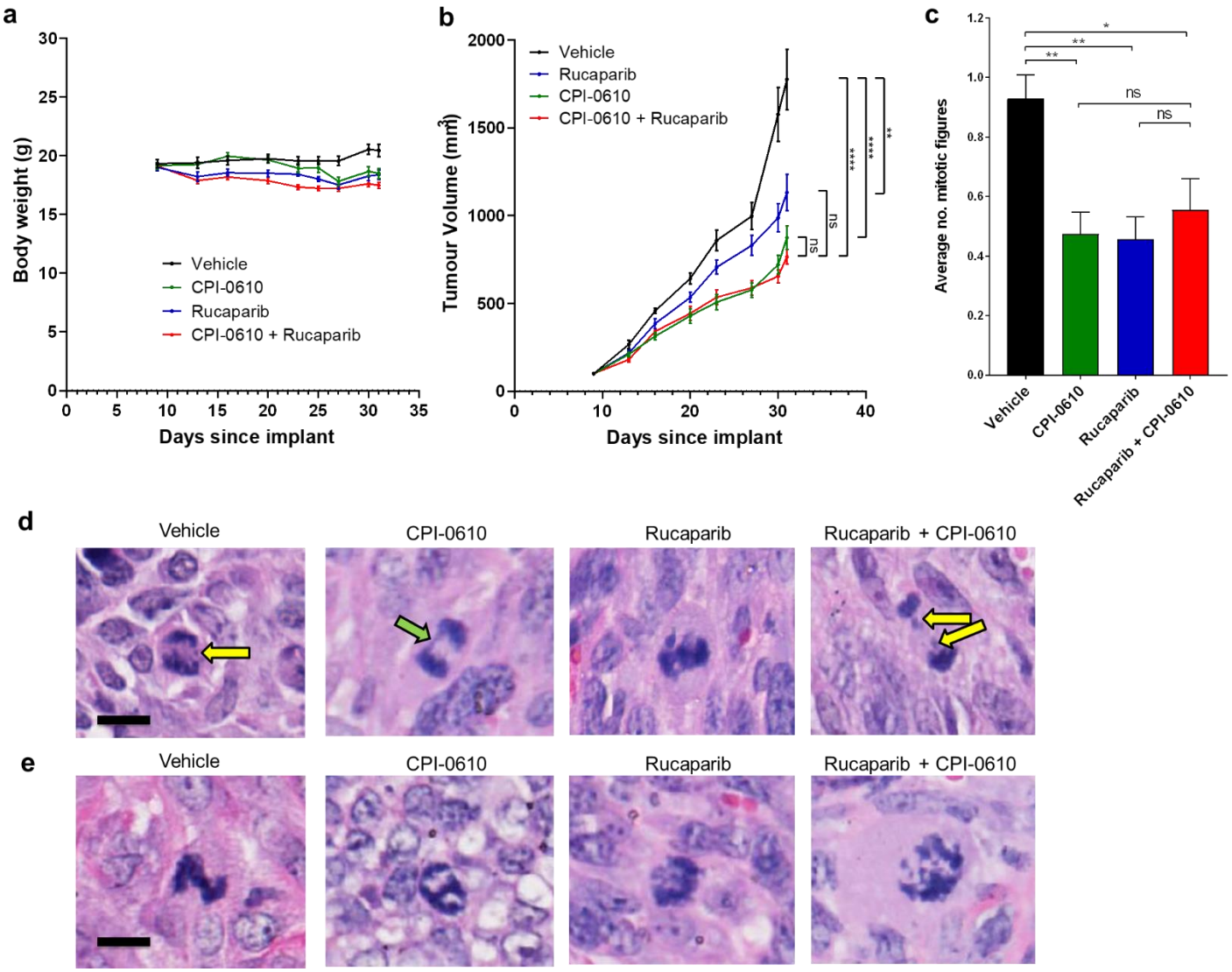
Supplementary Figure 1. (a) Preliminary drug screen of PDCs and cell lines to assess response to a panel of oncology-focused compounds. **(b)** Hierarchical clustering to PARP inhibitors. **(c)** The response to PARP inhibition for OCI-P5x and OCI-C5x is more similar to that of known BRCA1/2 reverted or restored cell lines (UWB1.289+BRCA1, PE04) than those cell lines that are PARPi sensitive with deleterious BRCA1/2 mutations (UWB1.289, PE01). **(d)** Representative images of immunofluorescence staining for RAD51 and p-HisH2AX in OCI-C5x and OCI-P5x cells following irradiation (10Gy). Cells were fixed and stained 4h post-irradiation. Scale bar = 5 μm. **(e)** Basal levels of RAD51 protein expression in OCE1, OCE2, OCI-P5x, and OCI-C5x cells.



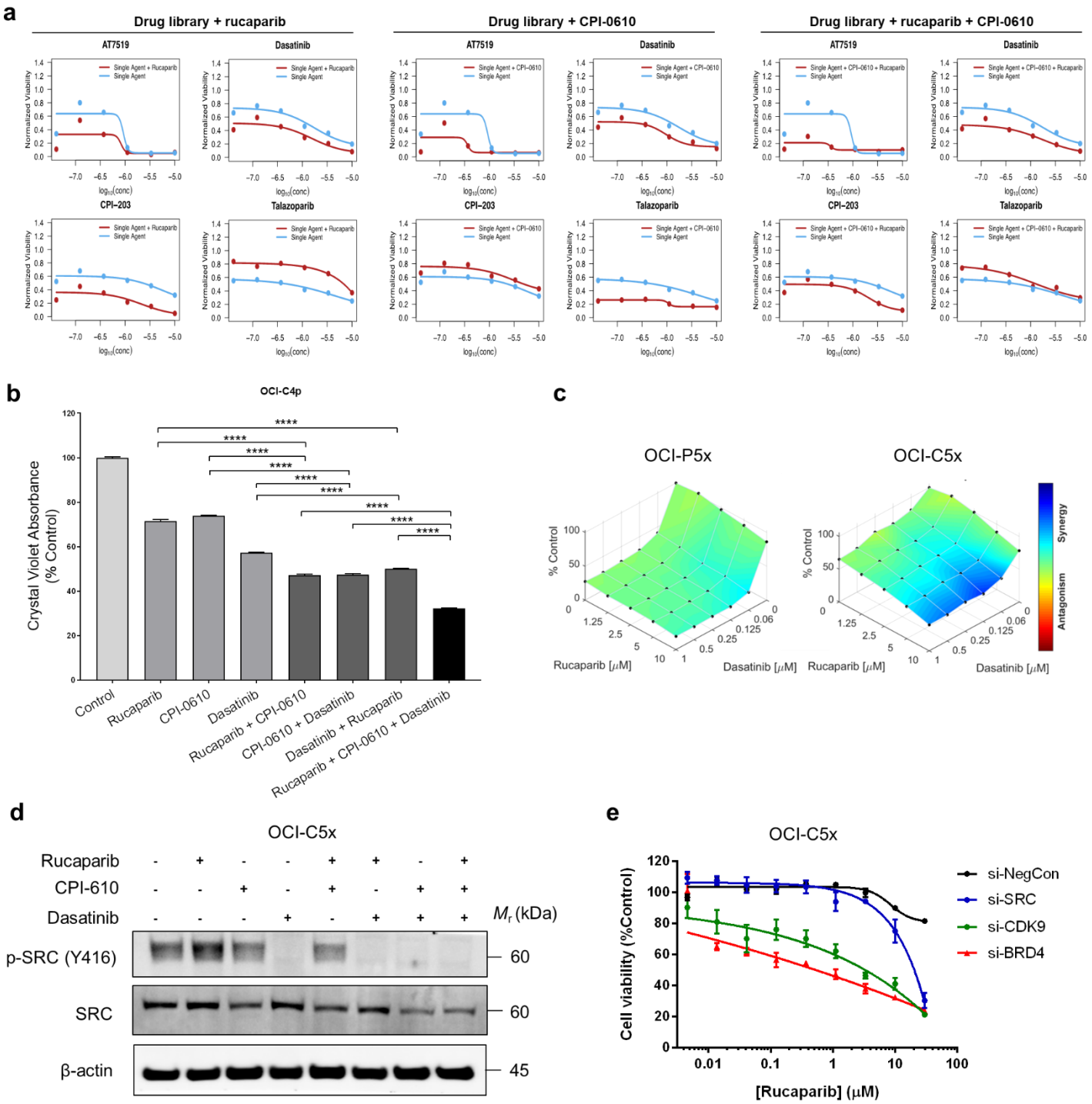
Supplementary Figure 2. PARPi (rucaparib, olaparib) and BETi (CPI-203, CPI-0610) combinations are synergistic in a panel of ovarian PDCs and cell lines. **(a)** Dose-response plots of rucaparib in combination with CPI-203 or CPI-0610 demonstrating synergistic drug combination effects in OCI-C5x, OCI-C4p, FCI-P2P, OVCAR8, KURAMOCHI, and CAOV3 cells. **(b)** Dose-response plots of olaparib in combination with CPI-203 or CPI-0610 demonstrating synergistic drug combination effects in OCI-C5x and OCI-C4p cells.



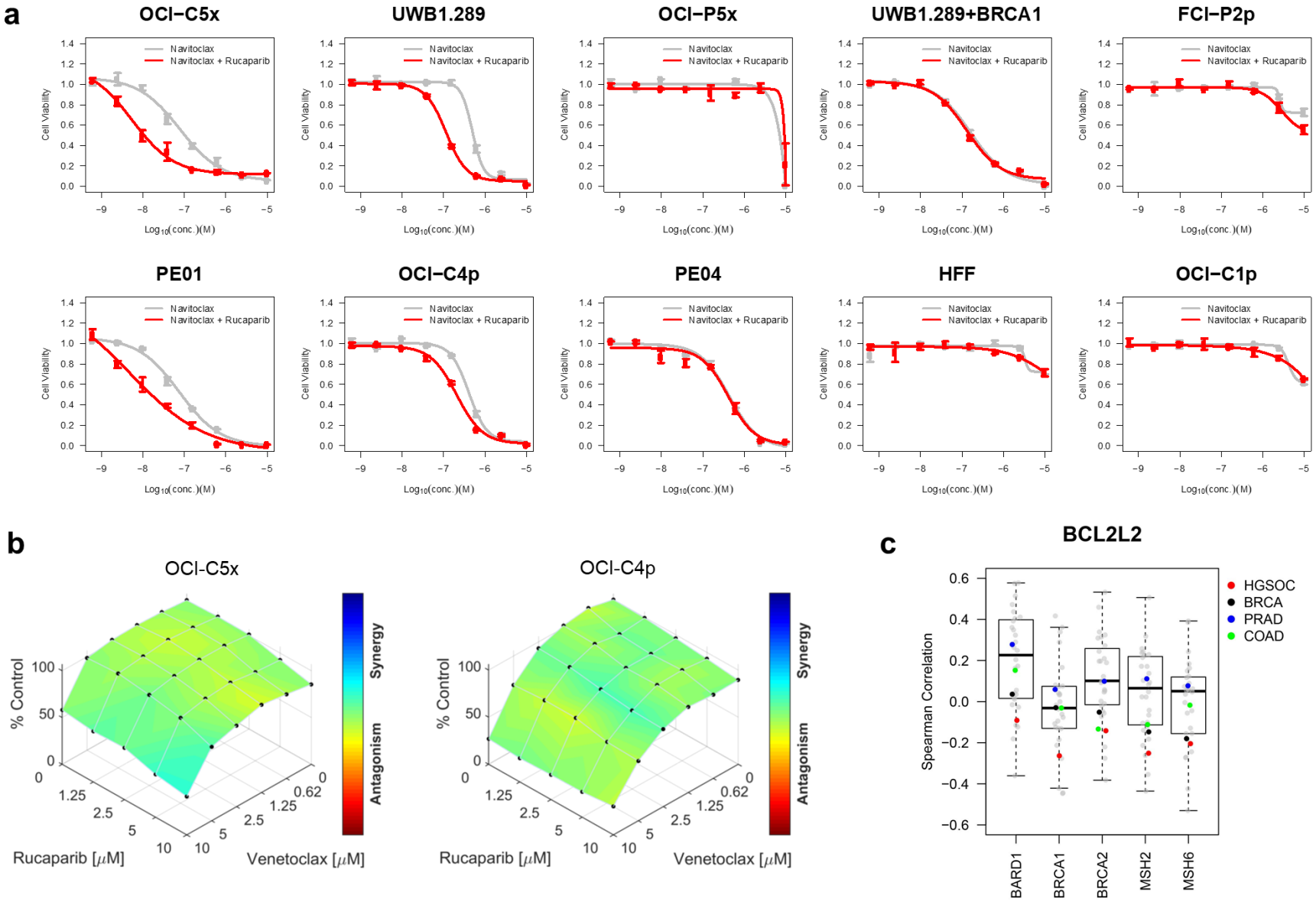
Supplementary Figure 3. (a) Ovarian cancer cell lines (OV) are the sixth most sensitive cell line type to JQ1 within the Genomics of Drug Sensitivity in Cancer (GDSC) dataset. **(b)** BRD4 overexpressed or copy number amplified HGSOC have a significantly decreased progression free survival than cases without BRD4 alteration. **(c)** BRCA1/2 mutated colorectal cancer cell lines are significantly more sensitive to JQ1 inhibition than BRCA1/2 wild type cell lines and in the GDSC project. **(d)** BRD4 mRNA expression level correlation with an aneuploidy score across individual cancer types and pan-cancer (Taylor et al. Cancer Cell 2018). **(e)** HGSOC with BRD4 overexpression or copy number amplification have a significantly higher rate of aneuploidy than those HGSOC which do not harbour such alterations. **(f)** Within the TCGA HGSOC dataset, BRD4 mRNA expression levels demonstrate a significant positive correlation with the aneuploidy score.



Supplementary Figure 4. (a) Body weight measurements from the OCI-P5x xenograft mouse study. Data is presented as mean ± SEM. **(b)** Tumour volume measurements from the OCI-P5x xenograft mouse study. Data is presented as mean ± SEM: ***p*<0·01, ****p<0·0001, ns=not significant (one-way ANOVA followed by Tukey's test). **(c)** Mitotic index of each treatment group; calculated by counting number of mitotic figures at 40X magnification (10 fields per sample), then taking the average of the fields for each sample. Data is presented as mean ± SEM: **p*<0·05, ***p*<0·01, ns=not significant (one- way ANOVA followed by Tukey's test). **(d)** Representative images of mitotic figures in each treatment group. Common irregular mitotic events were observed such as trailing chromosomes (yellow arrows), and anaphase bridges (green arrow) **(e)** Representative images from each treatment group showing a high number of cells with aberrant mitotic shapes which are the hallmarks of mitotic catastrophe. Scale bar = 10 μM.

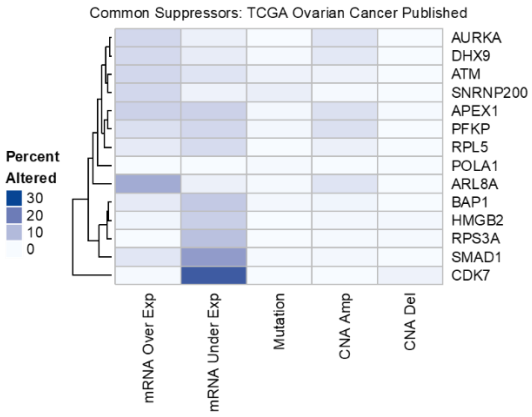


Supplementary Figure 5. Additional drug screens identify rucaparib combinations with dasatinib and/or CPI-610 as effective in ovarian PDCs. (a) Representative drug curves from the OCI-P5x combination screen utilizing a 131 clinically focused drug library in combination with rucaparib, CPI-610, or both rucaparib and CPI-610. **(b)** Clonogenic assays performed in OCI-C4p cells using rucaparib (10 μM), CPI-0610 (0.5 μM), and dasatinib (50 nM) as single agents, in double combinations, or in triple combination. Quantitation of crystal violet staining is representative of two independent experiments. Data is presented as mean ± SEM: ****p<0·0001, (one- way ANOVA followed by Tukey's test). **(c)** Dose-response plots of rucaparib in combination with dasatinib demonstrating synergistic drug combination effects in OCI-P5x and OCI-C5x cells. **(d)** Western blot analysis of phospho-SRC (Tyr 416) and SRC levels in response to treatment with rucaparib (10 μM), CPI-0610 (3 μM), and dasatinib (300 nM) for 48 h in OCI-C5x cells. **(e)** OCI-C5x cells were treated with siRNA specific to SRC, CDK9, or BRD4 for 48 h. Cells were subsequently treated with rucaparib for a further 6 days before cell viability was measured. Representative of 3 independent experiments, data is presented as mean ± SEM.



Supplementary Figure 6. The rucaparib and navitoclax drug combination demonstrates more activity in cells with known BRCA or BARD1 mutations (OCI-C5x, OCI-C4p, UWB1.289, PE01). **(a)** Dose response curves of navitoclax as a single agent (grey) and with the addition of rucaparib (red) in 10 PDCs and cell lines. **(b)** Dose-response plots of rucaparib in combination with venetoclax demonstrating little synergy in OCI-C5x and OCI-C4p cells. **(c)** Correlation of *BCL2L2* expression with BRCA1/2, MSH2/6, and BARD1 expression in The Cancer Genome Atlas (TCGA): high grade serous ovarian carcinoma (HGSOC), breast invasive carcinoma (BRCA), prostate adenocarcinoma (PRAD), and colon adenocarcinoma (COAD).

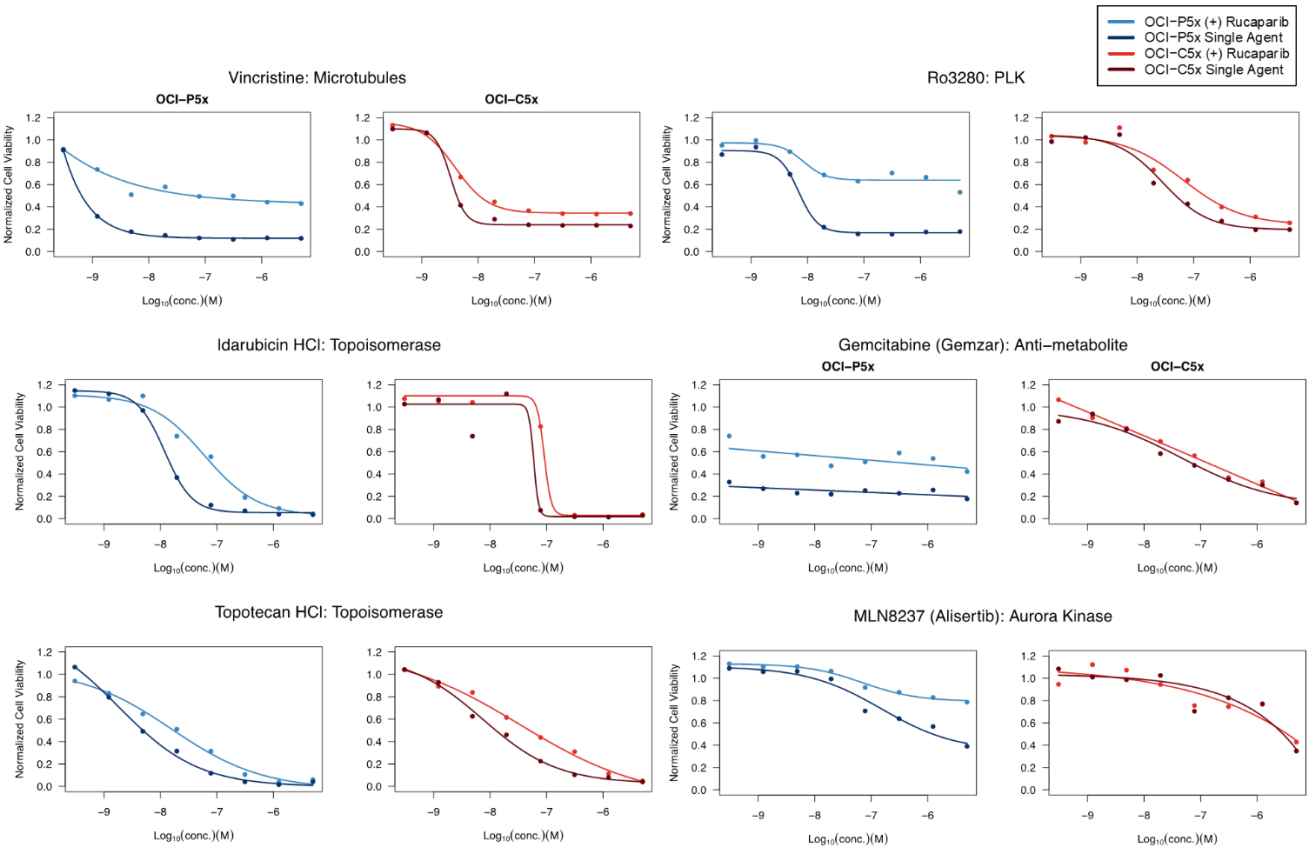
a



b

Pathway	*Genes*	*P-value*
Ribosome	RPS3A, RPL3, RPS9, RPL5, RPL11	0.001
Pyrimidine metabolism	NME4, POLD3, TYMS, NME3, POLE, RRM1, POLR2I, POLA1, UCK2, RRM2B, DPYD	0.007
Nucleotide excision repair	POLD3, RPA1, XPA, RPA2, ERCC5, RFC1, POLE, CDK7, ERCC3, ERCC1	0.015
DNA replication	POLD3, RPA1, RPA2, DNA2, RFC1, POLE, POLA1	0.016
Spliceosome	PRPF19, DHX8, EIF4A3, SNRNP200, DHX16, CDC5L	0.07
Cell cycle	MAD2L1, TP53, BUB1, PRKDC, CHEK1, CDK7, ABL1, SMC1A, ATM, CDK2, WEE1	0.076

c



Supplementary Figure 7. Rucaparib conditional drug screen identifies antagonistic drug combinations. (a) TCGA ovarian cancer analysis of the common rucaparib suppressor genes indicates frequent underexpression of CDK7 and SMAD1. **(b)** Pathway analysis of all the unique rucaparib suppressor genes from the siRNA screen indicates an enrichment for those involved in cellular processes closely involved in cell division. **(c)** Rucaparib antagonistic drugs as identified by the high-throughput combinatorial drug screen (n = 395). Drugs that commonly antagonize the effect of rucaparib target the mitotic spindle, microtubules, and DNA.

Supplementary Table 1: List of mutations in ovarian patient derived cells from BROCA sequencing

Gene	Location	AA substitution	cDNA substitution	Zygosity	OCI.C5x	OCI.P5x	OCI.P7a	OCI.P9a1	FCI.P2P	OCI.C1P	OCI.C4P
BARD1	CDS/frameshift insertion	K205fs	NM_000465:614dupA	het	1						
BRCA1	CDS/stopgain SNV	Q541X	NM_007294:C1621T	het	1						
MSH2	CDS/frameshift deletion	P472fs	NM_000251:1414delC	het	1						
MSH2	CDS/stopgain SNV	R711X	NM_000251:C2131T	het	1						
PAXIP1	CDS/frameshift deletion	F100fs	NM_007349:300delT	het	1						
POLE	CDS/frameshift deletion	1446_1446del	NM_006231:4337_4338del	het	1						
RBBP8	CDS/frameshift deletion	K355fs	NM_002894:1063delA	het	1						
RINT1	CDS/frameshift deletion	P246fs	NM_021930:736delC	het	1						
SMARCA4	CDS/nonframeshift insertion	P230delinsPGP	NM_001128844:690_691insGGCCCT	het	1						
TP53BP1	CDS/stopgain SNV	Q297X	NM_001141980:C889T	het	1						
POLQ	splicing/splicing	-	NM_199420:exon19:c.5774-1G>A	het		1					
TP53	CDS/nonsynonymous SNV	Y236N	NM_000546:T706A	hom		1					
BRCA1	CDS/stopgain SNV	L974X	NM_007294:T2921A	het			1				
FANCL	CDS/frameshift insertion/ splicing	T367fs	NM_018062:1099_1100insATTA	het			1				
BRCA1	CDS/frameshift deletion	E1000fs	NM_007294:2999delA	het				1			
BARD1	CDS/frameshift deletion	K205fs	NM_000465:614delA	het							1
MSH6	CDS/frameshift insertion/splicing	T1189fs	NM_000179:3568dupT	het							1
TP53	CDS/stopgain SNV	R342X	NM_000546:C1024T	hom							1
PIK3CA	CDS/nonsynonymous SNV	H1047L	NM_006218:A3140T	het						1	
TP53	CDS/nonsynonymous SNV	R248Q	NM_000546:G743A	hom					1		
ERCC6	CDS/stopgain SNV	Y1155X	NM_000124:C3465A	het					1		

Supplementary Table 2: Dosing regimen for OCI-P5x *in vivo* study

Group	**n**	**Tumour inoculum (s.c.) day 0**	**Treatment** (when the mean tumour size reached 100 mm^3)	**Dose (mg/kg)**	**Dosing route**	**Treatment Schedule**
1	10	5×10^6 /mouse in 0.1 ml PBS with Matrigel (1:1)	Vehicle	0	*p.o.*	QDx23days
2	10		CPI-0610	60	*p.o.*	QDx23days
3	10		Rucaparib	100	*p.o.*	BIDx22days
4	10		CPI-0610	60	*p.o.*	QDx23days
			Rucaparib	100	*p.o.*	BIDx22days

Supplementary Table 3: List of rucaparib sensitiser genes								
OCI-P5x						**OCI-C5x**		
ASXL2	HNF1A	NEK3	PRDM15	SFMBT1	TRERF1	AKT1	HAUS7	MYBL1
BPTF	HNRNPAB	NLGN2	PRDM4	SIRT2	TRIM28	ALDH18A1	HCK	MYO3B
BRCA1	ING1	NME3	PRMT1	SKIV2L	TRIM33	ALKBH3	HIST2H4B	NEK10
BRD4	ING3	PALB2	PRMT5	SLC16A2	TRIM68	ALPK1	HM13	NRK
C18orf37	JMJD2C	PARP4	PTTG1	SLC1A2	UBE2N	AOF1	HMGN2	PAK4
C1orf149	JMJD6	PARP6	PTTG2	SLC22A11	USP3	AOF2	INO80E	PHF20L1
CBX8	KDM5A	PDE1B	PWWP2B	SMARCB1	UTX	BCL2L1	JAK2	PHKG2
CLDN2	KIAA1718	PDE1C	PYGO1	SMPDL3A	UTY	BRD4	JMJD6	PIK3CA
CLDN3	LIG4	PDE2A	RABGGTA	SS18L1	WDR5	C18orf37	KMT2B	PNKP
CLOCK	LOC93349	PDE3A	RAI1	SSRP1	WNT4	CD79A	KTI12	PRKCZ
CNP	LRWD1	PDE4A	RBBP7	SUPT16H	YES1	CHKA	L3MBTL	RASSF7
DDX50	MAP2K3	PDE7A	RBL2	SUPV3L1	ZNHIT4	CLK2	LMTK3	RIPK5
DGKQ	MAP2K7	PDE8A	RCBTB1	SUV39H1	ZZZ3	CTSD	LY6E	SCYL1
DHX37	MAP3K12	PDE8B	RERE	SUZ12		DCAMKL2	MACROD1	STK17A
DQX1	MBD5	PELO	REV1	TADA1L		DUT	MAP3K12	UBE2V2
ECAT8	MBTD1	PET117	RIC8A	TADA3		EIF4A2	MAPK15	
ELAC1	MED10	PGGT1B	RLF	TAF1		ERCC3	MAPK3	
ERCC2	MED16	PHF16	RNF17	TAF12		ERCC6	MBD4	
FBXO10	MED23	PHF20L1	RPH3A	TAF1L		ERN1	MBD6	
FXR2	MED4	PHF8	RPS6KB1	TAF5L		EVI1	MCCC1	
GDPD1	MED8	PIAS2	RUVBL1	TAF7		EZH1	MED22	
GDPD2	MGC22014	PIK3CB	RUVBL2	TARBP2		FGF19	MET	
GLRA2	MIER1	PIK3CD	RYR1	TDP1		FMR1	MLL	
HDAC2	MIER3	PLCB4	S100A4	TDRD6		FRMPD4	MLL5	
HIF1A	MLLT6	PLCD1	SAFB	TGFB1		FTO	MORC1	
HIRA	MORC1	PLCD4	SATB2	TGFB2		GALK2	MORF4L1	
HIST1H4C	MSX1	PLCG2	SBDSP1	TGM3		GCKR	MPHOSPH8	
HIST2H4B	MTA1	PMAIP1	SBNO1	TLE1		GGCT	MPP4	
HMG20A	MTA3	POU5F1P4	SCML2	TLR7		GPX1	MSH2	
HMGN3	NDEL1	PRDM1	SERPINE2	TNRC6B		GRK4	MVK	

Supplementary Table 4: List of rucaparib suppressor genes							
OCI-P5x				**OCI-C5x**			
AATK	CDK7	MAP3K1	RPL11	ACTL6B	CXXC5	NUDT1	SGK
ABL1	CHAF1B	MAP3K5	RPL5	ACTR5	DAPK3	OARD1	SMAD1
ABL2	CHKB	MAP3K8	RPS3A	ADCK4	DDX31	PAF1	SMARCAL1
ACTR6	CKM	MARK2	RPS6KA3	AIFM2	DHX35	PCM1	SMPD3
ACVR1C	CLK4	MAST1	RPS6KB1	APEX1	DHX9	PDE4B	SNRNP200
ADCK2	CRY2	MAST2	SBK1	APOA2	DICER1	PFKP	TESK2
AIPL1	CSNK1A1	MET	SETMAR	ARL8A	DNA2	PHF3	TF
AK5	CSNK1D	MPP1	SFPQ	ATM	EME2	POLA1	TNK2
AKAP1	CSNK1E	MYLK2	SMAD1	ATP7A	ERCC1	POLD3	TRIM24
ALK	DHX8	NDC80	SMC1A	AURKA	FANCM	POLH	TYMS
ALPK2	DHX9	NDUFA10	SMG1	BAIAP2	FOXA2	POLQ	TYRO3
APEX1	DMAP1	NME3	SNRNP200	BAP1	GRK1	POLR2K	UBE2N
APEX2	DPH3	PFKM	SPHK2	BCL3	HMGB2	PRDM11	UTY
ARID1A	DYRK2	PFKP	STK11	BCOR	IRAK3	PRKAR1A	ZBTB7A
ARL8A	EIF4A3	PHKG2	STK39	BMI1	KCTD13	PRMT10	
ATM	EPHA4	PIM1	STK40	BRPF3	KDM5A	PSMC2	
AURKA	ERBB2	PIP5K2A	TEK	CBX5	KIAA1447	RIPK3	
AURKB	EXT1	PIP5K2C	TSSK1	CBX7	KPNA1	RIPK4	
BAP1	FGFR2	POLA1	UBR2	CDC2L2	KPNA3	RPA1	
BLM	FLJ23018	PRKCE	UCK2	CDC5L	LMTK2	RPA2	
BMX	FLT4	PRKCI	VRK2	CDIPT	MAD2L1	RPIA	
BRWD1	GCK	PRKDC	WEE1	CDK2	MAP3K14	RPL3	
BUB1	GK2	PRKX	WNK2	CDK7	MSH2	RPL5	
CAMK1G	HMGB2	PTK6	XAB2	CENPV	MUS81	RPS3A	
CD79A	HSPB8	PTK9	XRCC6	CHEK1	MYSM1	RPS9	
CDC2L5	IHPK3	RAD51L3		CHMP2A	NADK	RRP8	
CDC2L6	KIF18A	RAPGEF4		CIT	NAP1L5	S100A6	
CDC42BPA	LATS2	ROR1		CLDN6	NME4	SBDS	