Supplementary Materials and Methods

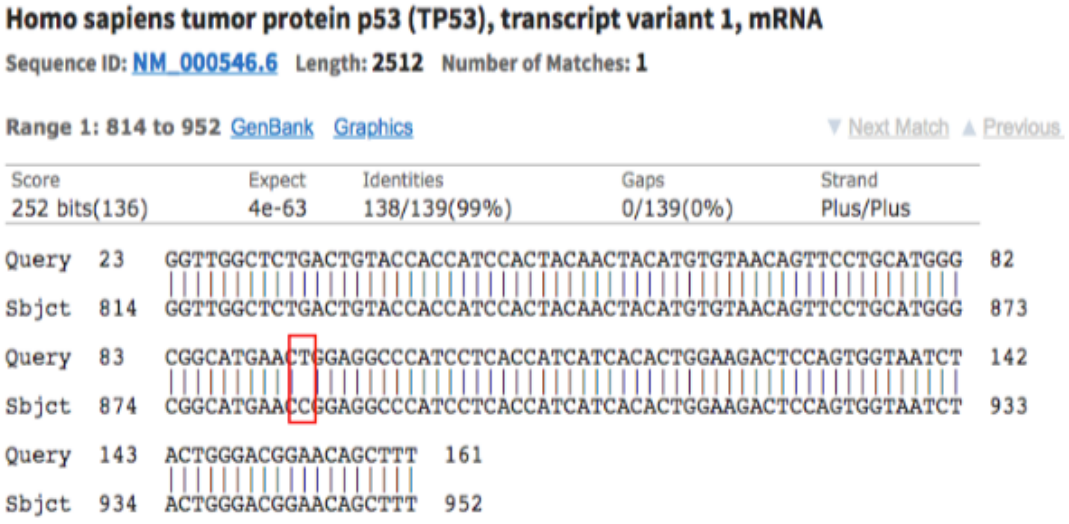
p53 genotyping of PDAC cell lines by sequencing

MIAPaCa2, BxPC3 and CFPAC-1 cells were seeded at a density of 3 x 10^5 cells/mL and incubated in 5% CO_2, 37°C overnight. RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) following manufacturers guidelines. This was followed by cDNA synthesis using Tetro CDNA synthesis kit (Bioline, London, UK). A region of ~175 bp covering mutations for all three cell lines was amplified using Phusion High-Fidelity PCR Kit (ThermoFisher Scientific, USA) (Cat. no. F553S). Sequences for the primers used to amplify the region are shown below. PCR products were resolved on a 1% agarose gel in TAE buffer followed by gel purification using QIAquick Gel Extraction Kit (Qiagen, Germany) (Cat. no. 28704). Samples were submitted for sequencing by the Genomics Facility at the University of Birmingham and analysed using SnapGene (https://www.snapgene.com/snapgene-viewer/).

	Forward Primer	Reverse Primer
Set 1	5-tcgacatagtgtggtggtgc	5-aaagctgttccgtcccagta
Set 2	5-acttttcgacatagtgtggtgg	5-tcaaagctgttccgtccca

Confirmation of p53 genotype in:

A) MIAPaCa2, (pR248W, c742 C→T)



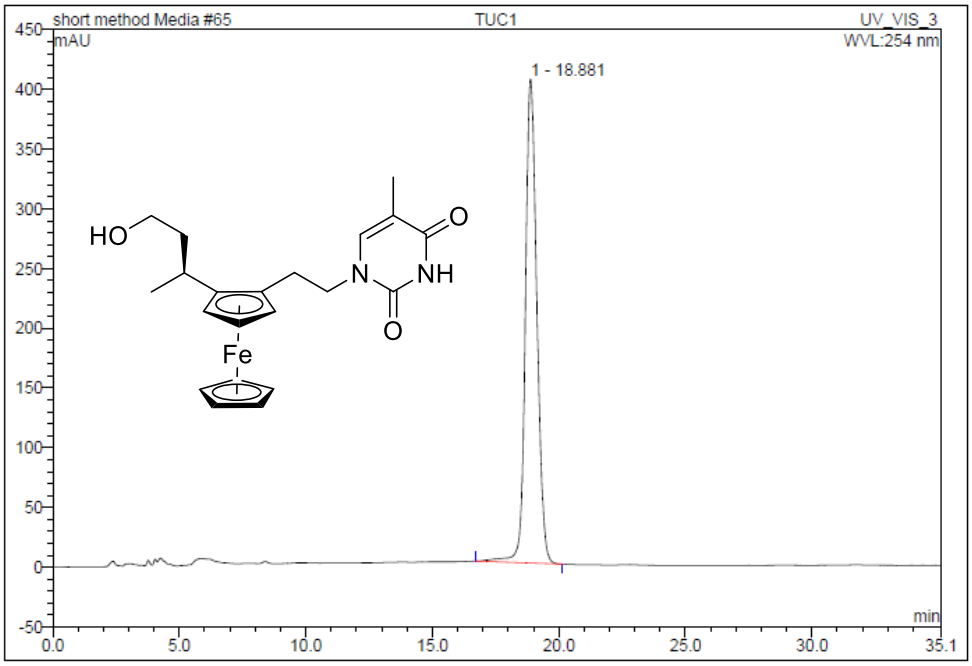
B) BxPC3, (pY220C, c659 A→G)

C) CFPAC-1, (pC242R, c724 T→C)

Sequence of primers used for qPCR

Gene	Forward Primer	Reverse Primer
BRCA2	GTTTCCACACCTGTCTCAGC	GGTGGAGGTAAAGGCAGTCT
SERTAD1	GCCGTTTCCTGATTGGTTGT	AGACCCTTGCTCAGCATCTT
HUS1	GACTTGGTGTAGTAGCCAGAA	CGGGGTGAACACACTGAAAT
GADD45A	CACTGTCGGGGTGTACGAAG	CCTGGATCAGGGTGAAGTGG
Casp3	GCCTCTTCCCCCATTCTCAT	CTTCCATGTATGATCTTTGGTTCC
MDM2	CGAGCTTGGCTGCTTCTG	GTACGCACTAATCCGGGGAG
CDKN1A	GCCGAAGTCAGTTCCTTGTG	CATGGGTTCTGACGGACATC
CDK2	CATCTTTGCTGAGATGGTGACT	ACTTGGCTTGTAATCAGGCAT
CCNE1	CCCATCATGCCGAGGGAG	CACGTTTGCCTTCCTCTTCC
CDK7	CTCGGGCAAAGCGTTATGAG	CTCTGGCCTTGTAAACGGTG
CCNT1	TGGAAAATAGCCCATCCCGT	GTGAGACGTTAAGACGCTGC
CCNG2	AGGTGAGGCTACAGTGATTCC	AGGCACAGATGCCAAACCTA
B2M	TTTGGCTCACAGTGTAAAGGG	GTCACCCCAACTATGCCATT
RBBP8	CTCAGAAAGTGCTCGCTTCC	TCTGCAGAGTTAGGGCTTCC
TBP	CCGGCTGTTTAACTTCGCTT	CACACGCCAAGAAACAGTGA

Supplementary Figures and Tables



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	18.88	n.a.	405.397	213.749	100.00	n.a.	BMB
Total:			405.397	213.749	100.00	0.000	

Figure S1: Assessment of chiral purity by HPLC of ferro-nucleobase (S,Rp)-1-[α-Methyl-(3-(hydroxy)propyl)]-2-[(thyminyl)ethyl]-ferrocene, 1-**(*S,R*$_p$)**. Samples were eluted on a cellulose 1 column with a flow rate of 1 mL min^{-1} using 40% MeCN in H_2O as the solvent.

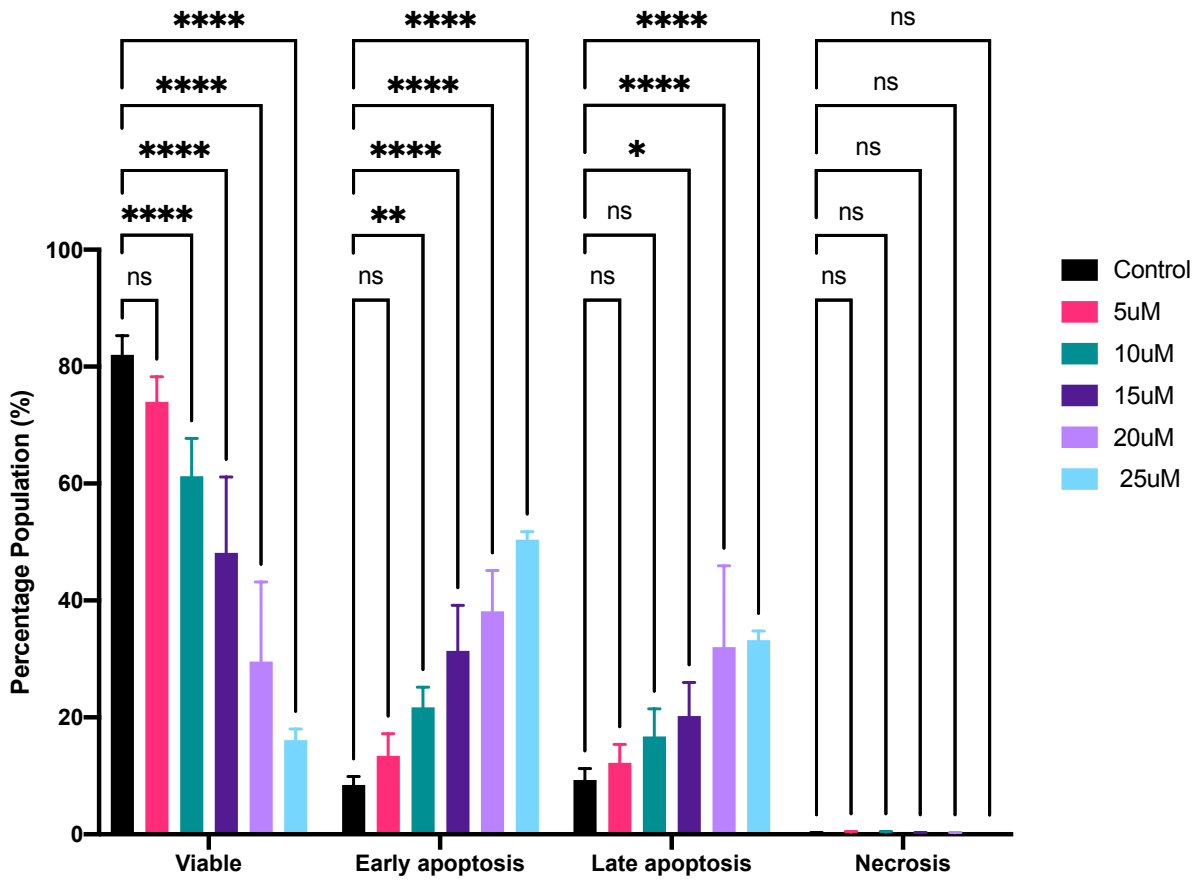


Figure S2: Induction of apoptosis as assessed by annexin and PI staining in MIAPaCa2 cells following treatment with 0-25 μM **1-(*S,R*$_p$)** for 72 hours. * and **** statistically significant P<0.05 and 0.0001 as assessed by a 2-way ANOVA followed by a *post-hoc* Dunnett's t-test. The results represent the mean of three independent experiments carried out in duplicate.

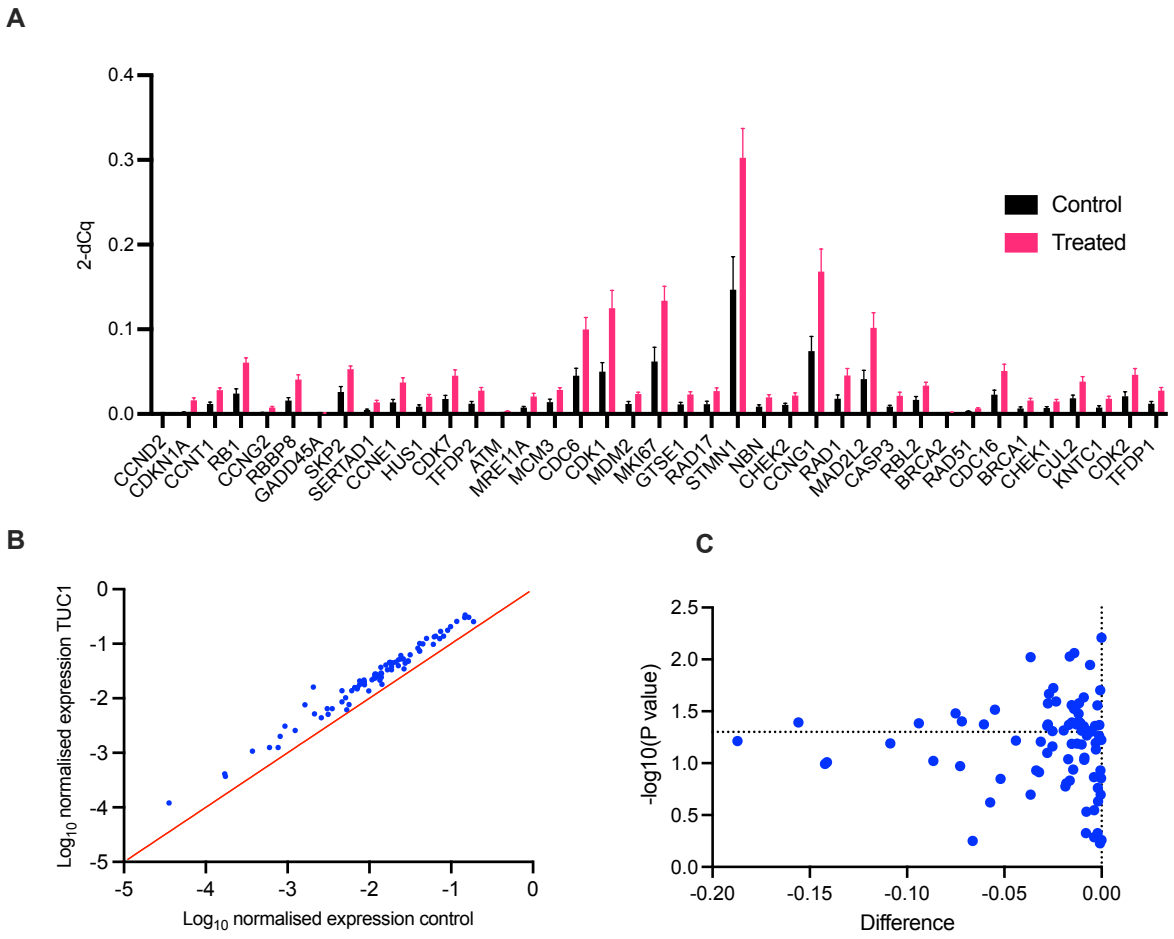


Figure S3: List of 39 genes related to DNA-repair that are statistically significantly upregulated in MIAPaCa2 cells following treatment with **1-(*S,R*$_P$)** (10 μM, 24 hours). A) Expression in control and treated cells expressed as 2^{-dCq} relative to *GAPDH*. B) Log normalised plot of data and C) volcano plot. The results represent the mean of three independent biological experiments (± SD, n=3).

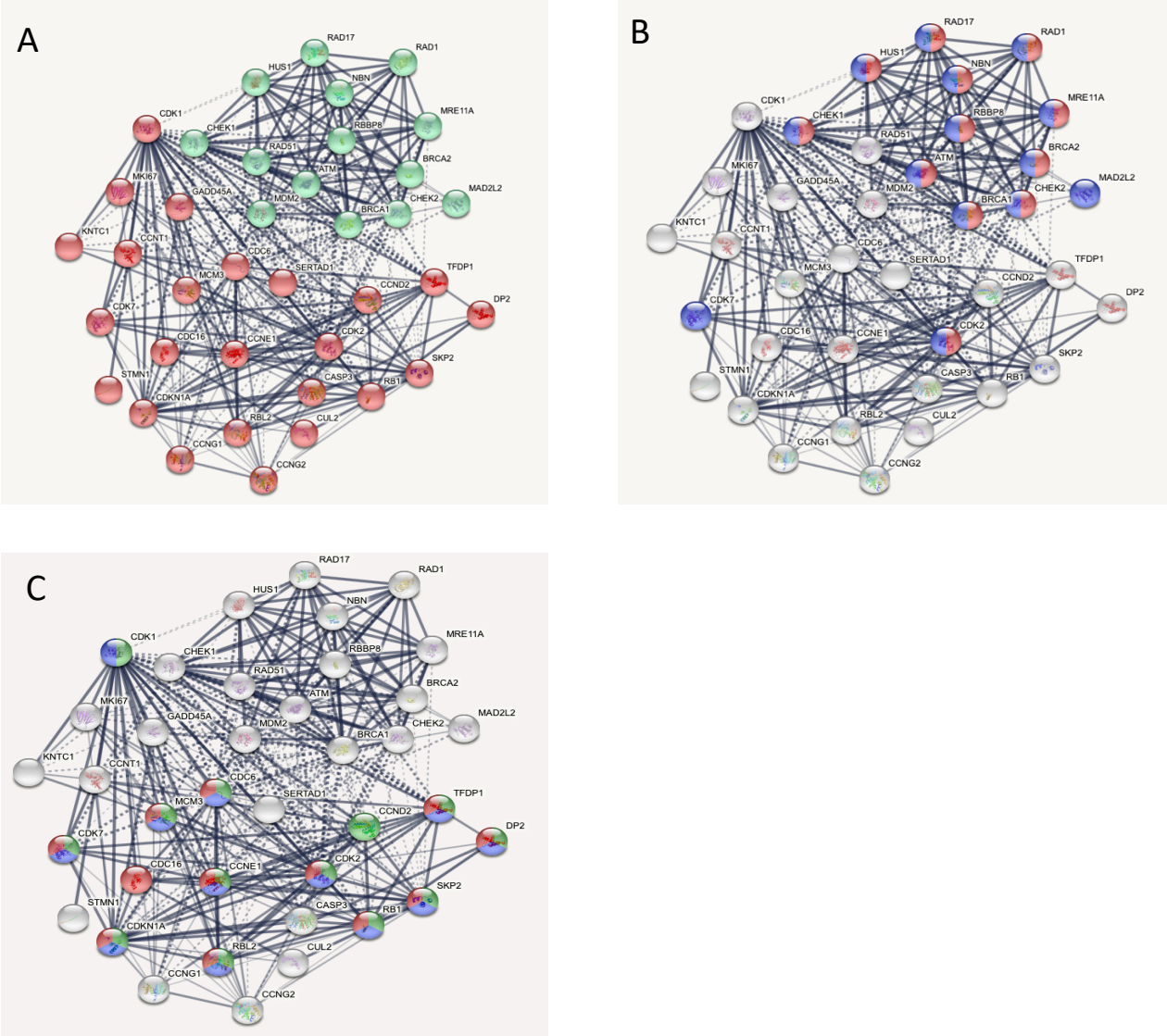
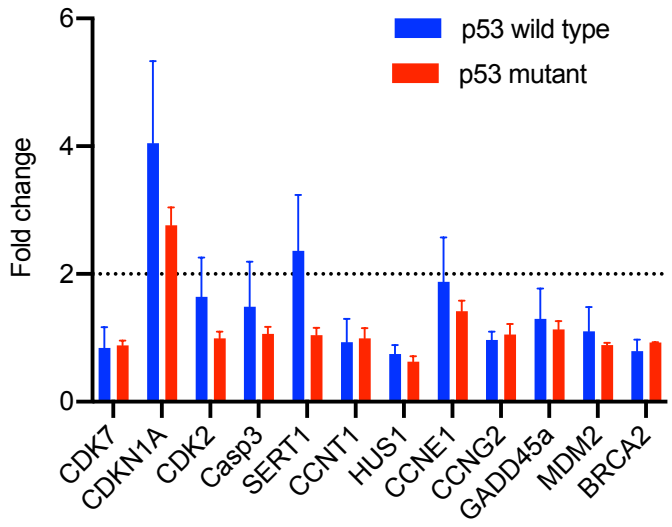


Figure S4: Kmeans clustering analysis of the 39 transcripts that were statistically significantly upregulated following treatment of MIAPaCa2 cells with **1-(*S,R*$_P$)** (10 μM, 24 hours). A) Overall Kmeans clustering analysis showing the separation of transcripts into 2 groups coloured red and green. B) Genes (*RAD17, RAD1, HUS1, NBN, MRE11A, CHEK1, CHEK2, RBBP8, ATM, BRCA1* and *MAD2L2*) related to DNA double strand break repair (Reactome pathway HSA-5693532 coloured red) and DNA repair (Reactome pathway HSA-73894 coloured blue) are linked to the first cluster. C) Genes (*CDK1, CDK7, MCM3, CDC6, CDC16, CCNE1, CCND2, TFDP1, DP2, SKP2, RB1, CDK2, RBL2* and *CDKN1A*) related to G1/S transition (Reactome pathway HSA-69206 coloured blue), S-phase (Reactome pathway HSA-69242 coloured green) and Mitotic G1-G1/S phase ((Reactome pathway HSA-453279 coloured red) are linked to cluster 2.

A



B

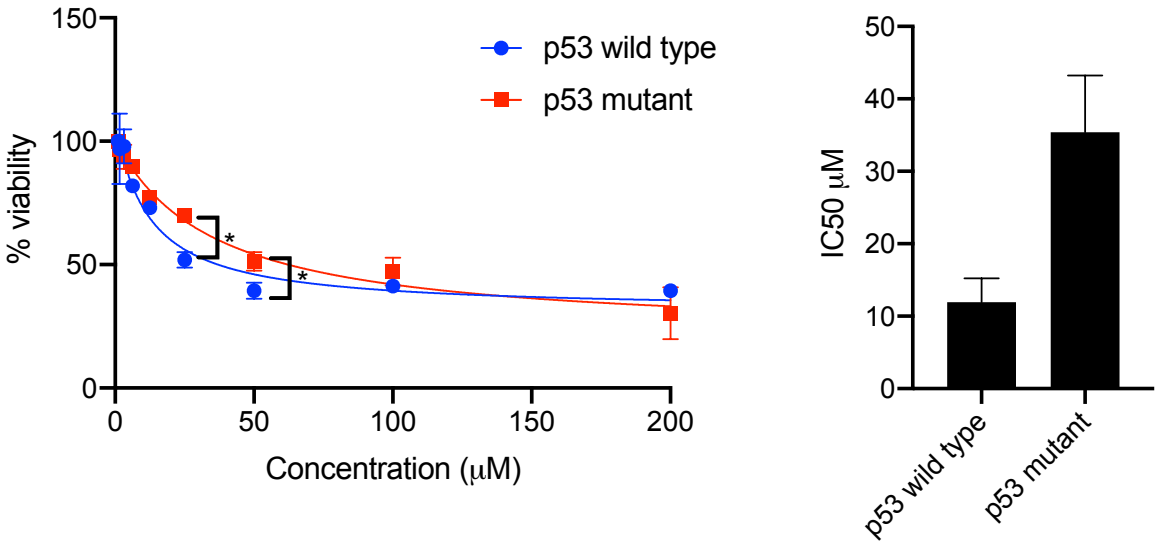


Figure S5: Transcriptional response to **1-(*S,R*$_p$)** is not affected by p53 status but p53 null cell lines are more sensitive to **1-(*S,R*$_p$)** as assessed by the MTT assay. A) Fold change in 12 genes identified in PDAC cell lines in HCT116 p53$^{+/+}$ and p53$^{-/-}$ HCT116 cells B) Cytotoxicity curves for in HCT116 p53$^{+/+}$ and p53$^{-/-}$ HCT116 cells with **1-(*S,R*$_p$)** for 72 hours as assessed by the MTT assay. The results represent the mean of three independent biological experiments (n=3). * There was a statistically significant difference ($P < 0.05$) in the concentration-response of wild type and mutant cells as assessed by a 2-way ANOVA followed by a *post-hoc* Tukey t-test.

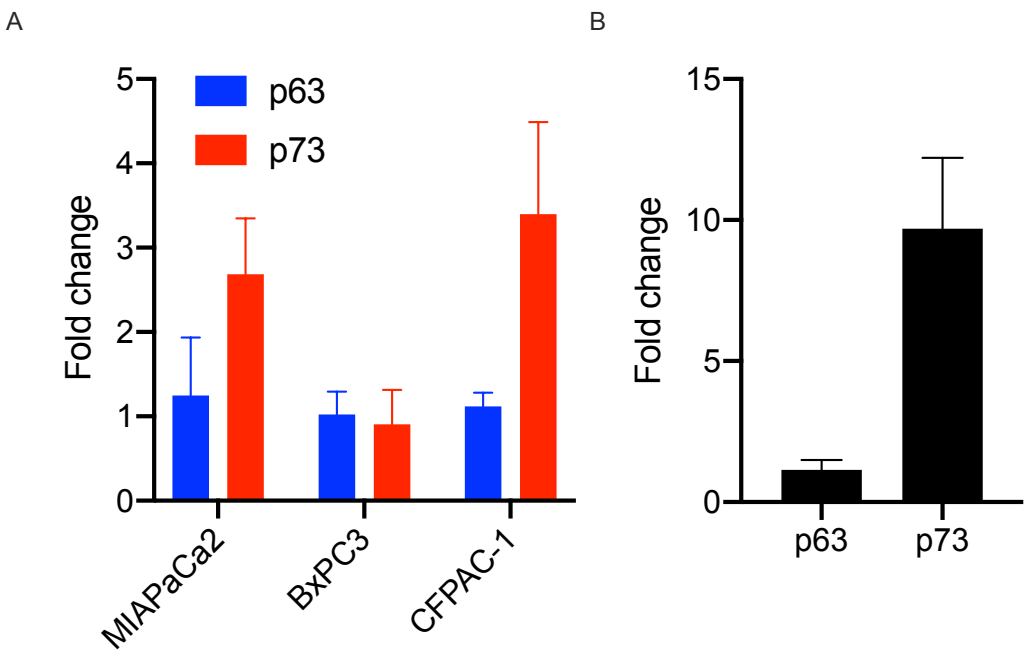


Figure S6: A) The p53 homologue p73 but not p63 is differentially regulated in PDAC cells following treatment with **1-(*S*,R_p)** as assessed by qPCR. B) p73 but not p63 is upregulated in HCT116 p53 knock cells. The results represent the mean of three independent biological experiments (n=3).

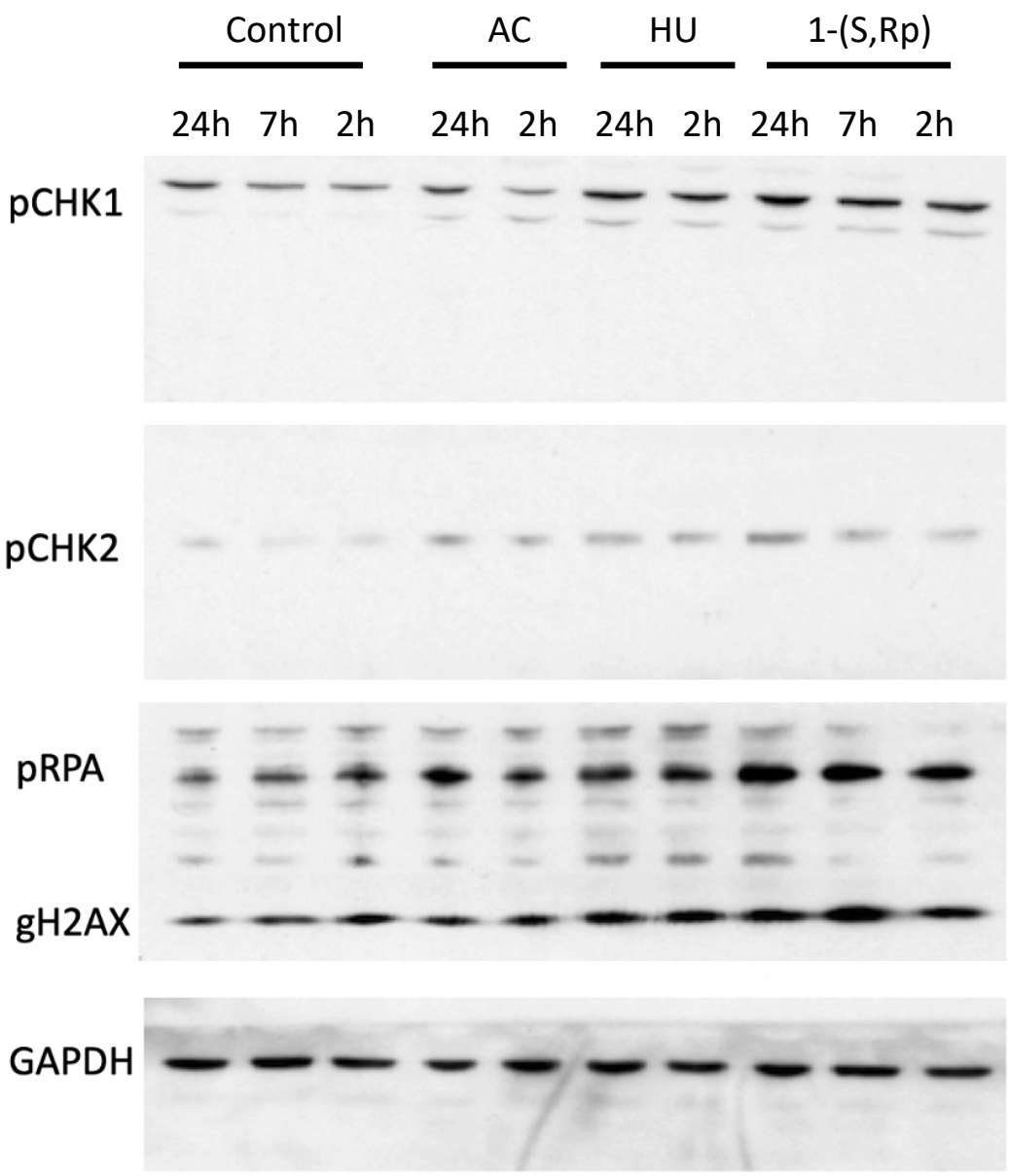


Figure S7: Western blotting analysis of cell cycle and DNA-damage response proteins in MIAPaCa2 cells. Cells were treated with 10 μM **1-(*S,R*$_p$)** for 2, 7 and 24 hours. As a positive control cells were treated with 0.5 μM hydroxyurea (HU) and 2.4 μM aphidicolin (AC) for 2 and 24 hours. Following treatment, cells were harvested and lysed in radioimmunoprecipitation assay (RIPA) buffer mixed with phosphatase inhibitor cocktail and protein kinase inhibitor. Lysates were centrifuged at 10 000 rpm for 5 min at 4°C. Protein quantification was carried out by Bradford assay (1) and mixed with Laemmli buffer. After separation on NuPAGE 4-12% Bis-Tris Gel (ThermoFisher Scientific, USA) (Cat. No. NP0322BOX), the proteins were transferred onto 0.2 μm nitrocellulose membrane that was blocked in immobilon and incubated with the primary and secondary antibodies. For detection. HRP was visualised using Luminata reagent (MerckMillipore, WBLUR0100) onto Amersham Hyperfilm ECL (GE Healthcare Life Sciences, 28906836). Antibodies used were: pChk1 (Ser 345) (1:1000) Cell Signalling Cat#2341, pChk2 (Thr 68) (1:1000) Cell Signalling Cat#2661, g-histone H2AX (Ser139)(1:250) Cell Signalling Cat# 9718, pRPA (RPA2) (Ser4/Ser8), GAPDH (1:1000) Genetex Cat#GTX627408. Secondary antibodies used were goat anti-mouse HRP conjugated (Fisher Scientific, PA1-74421) and goat anti-rabbit HRP conjugated (Fisher Scientific, PI-31460)

Table S1: List of 29 compounds identified as having a similar pattern of activity (Pearson's correlation >0.5) to **1-(S,R_p)** in the NCI-60 panel of cell lines as determined by the COMPARE algorithm (https://dtp.cancer.gov/databases_tools/compare.htm). 7 compounds (24%, highlighted in red) were identified as having inhibition of DNA-synthesis as the mode of action. COMPARE analysis was performed using the one-dose data obtained for **1-(S,R_p)** (NSC 787046) in the NCI-60 panel (https://dtp.cancer.gov/databases_tools/compare.htm). This data set was compared to the subset of all GI50 data for all the agents in the database. The mechanism of action for each target identified was obtained from CellMiner (https://discover.nci.nih.gov/cellminer/drugQuery.do) by submitting the list of NSC numbers.

Correlation	Target NSC	Target Descriptor	Mechanism of action
0.74	303812	Aphidicolin Glycinate	DNA synthesis inhibitor
0.72	95678	3HP	DNA synthesis inhibitor
0.61	182986	AZQ	Alkylating at N-7 position of guanine
0.61	51143	IMPY	DNA synthesis inhibitor | RNA synthesis inhibitor
0.59	32065	Hydroxyurea	Antimetabolite | Ribonucleotide reductase inhibitor
0.59	27640	5-FUDR	DNA synthesis inhibitor | TYMS inhibitor
0.57	26980	Mitomycin C	Alkylating at N-2 position of guanine
0.57	296934	Teroxirone	Alkylating at N-7 position of guanine
0.56	609699	Topotecan	Topoisomerase 1 inhibitor
0.56	249992	m-AMSA (amsacrine)	Topoisomerase 2 inhibitor
0.55	135758	Piperazinedione	Alkylating at N-7 position of guanine
0.55	125066	Bleomycin	DNA binder
0.55	740	Methotrexate	Antifols (impairs the function of folic acids. This inhibits production of DNA, RNA, and proteins) | DHFR inhibitor
0.55	19893	5-fluorouracil	DNA synthesis inhibitor | RNA synthesis inhibitor | TYMS inhibitor
0.54	1895	Guanazole	Alkylating at N-7 position of guanine
0.54	34462	Uracil nitrogen mustard	Alkylating at N-7 position of guanine | Alkylating agent
0.54	9706	Triethylenemelamine	Alkylating at N-7 position of guanine | Alkylating agent
0.53	6396	Thio-tepa	Alkylating at N-7 position of guanine
0.53	3088	Chlorambucil	Alkylating at N-7 position of guanine | Alkylating agent
0.52	329680	Hepsulfam	Alkylating at N-7 position of guanine
0.52	352122	Trimetrexate	Antifols (impairs the function of folic acids. This inhibits production of DNA, RNA, and proteins)
0.51	619003	MX2 HCl	Unknown
0.51	762	Nitrogen mustard	Alkylating at N-7 position of guanine | Alkylating agent
0.51	63878	Cytosine arabinoside	DNA synthesis inhibitor
0.51	153858	Maytansine	Tubulin affecting
0.5	344007	Piperazine alkylator	Alkylating at N-7 position of guanine
0.5	119875	Cisplatin	Alkylating at N-7 position of guanine | Alkylating agent
0.5	107392	5HP	DNA synthesis inhibitor
0.5	269148	Menogaril	Topoisomerase 2 inhibitor

Table S2: List of all 53 genes with more than a 2-fold increase in expression in MIAPaCa2 cells treated with **1-(*S*,R_P)** (10 μM, 24 hours) compared to untreated controls. The results represent three independent biological repeats repeated in technical triplicates (n=3). Where statistically significant (t-test on 2^{-dCq} values) *P* values are shown. Also highlighted are genes that were also more than 1.5-fold upregulated in either BxPC3 (green), CFPAC-1 (blue) or both (red) PDAC cell lines under the same experimental conditions in a single qPCR array experiment with the same set of primers (3 technical triplicates, 1 biological repeat).

GENE	Fold Change	P value
NM_001759 CCND2 Cyclin D2	3.4	0.006
NM_000389 CDKN1A Cyclin-dependent kinase inhibitor 1A	7.8	0.009
NM_001240 CCNT1 Cyclin T1	2.0	0.009
NM_000321 RB1 Retinoblastoma	2.5	0.009
NM_004354 CCNG2 Cyclin G2	4.7	0.011
NM_002894 RBBP8 Retinoblastoma binding protein 8	2.6	0.019
NM_001924 GADD45A Growth arrest and DNA-damage-inducible alpha	2.9	0.020
NM_005983 SKP2 S-phase kinase-associated protein 2 (p45)	2.0	0.022
NM_013376 SERTAD1 SERTA domain containing 1	3.0	0.023
NM_001238 CCNE1 Cyclin E1	2.7	0.025
NM_004507 HUS1 HUS1 checkpoint homolog (S. pombe)	2.3	0.026
NM_001799 CDK7 Cyclin-dependent kinase 7	2.6	0.027
NM_006286 TFDP2 Transcription factor Dp-2 (E2F dimerization partner 2)	2.3	0.028
NM_000051 ATM Ataxia telangiectasia mutated	3.5	0.028
NM_005590 MRE11A MRE11 meiotic recombination 11 homolog A (S. cerevisiae)	2.7	0.028
NM_002388 MCM3 Minichromosome maintenance complex component 3	2.0	0.030
NM_001254 CDC6 Cell division cycle 6 homolog (S. cerevisiae)	2.2	0.031
NM_001786 CDK1 Cyclin-dependent kinase 1	2.5	0.033
NM_002392 MDM2 Mdm2 p53 binding protein homolog (mouse)	2.0	0.033
NM_002417 MKI67 Antigen identified by antibody Ki-67	2.2	0.040
NM_016426 GTSE1 G-2 and S-phase expressed 1	2.0	0.040
NM_002873 RAD17 RAD17 homolog (S. pombe)	2.3	0.041
NM_005563 STMN1 Stathmin 1	2.0	0.041
NM_002485 NBN Nibrin	2.3	0.041
NM_007194 CHEK2 CHK2 checkpoint homolog (S. pombe)	2.0	0.041
NM_004060 CCNG1 Cyclin G1	2.3	0.041
NM_002853 RAD1 RAD1 homolog (S. pombe)	2.5	0.042
NM_006341 MAD2L2 MAD2 mitotic arrest deficient-like 2 (yeast)	2.0	0.042
NM_004346 CASP3 Caspase 3 apoptosis-related cysteine peptidase	2.5	0.042
NM_005611 RBL2 Retinoblastoma-like 2 (p130)	2.0	0.043
NM_000059 BRCA2 Breast cancer 2 early onset	2.5	0.043
NM_002875 RAD51 RAD51 homolog (S. cerevisiae)	2.1	0.044
NM_003903 CDC16 Cell division cycle 16 homolog (S. cerevisiae)	2.2	0.044
NM_007294 BRCA1 Breast cancer 1 early onset	2.4	0.044
NM_001274 CHEK1 CHK1 checkpoint homolog (S. pombe)	2.1	0.048
NM_003591 CUL2 Cullin 2	2.1	0.048
NM_014708 KNTC1 Kinetochore associated 1	2.3	0.049
NM_001798 CDK2 Cyclin-dependent kinase 2	2.2	0.049
NM_007111 TFDP1 Transcription factor Dp-1	2.0	0.050
NM_001168 BIRC5 Baculoviral IAP repeat containing 5	2.4	NS
NM_001826 CKS1B CDC28 protein kinase regulatory subunit 1B	2.3	NS
NM_001827 CKS2 CDC28 protein kinase regulatory subunit 2	2.2	NS
ABL1 C-abl oncogene 1 non-receptor tyrosine kinase	2.2	NS

NM_013366 ANAPC2 Anaphase promoting complex subunit 2	2.1	NS
NM_001184 ATR Ataxia telangiectasia and Rad3 related	2.1	NS
NM_004217 AURKB Aurora kinase B	2.1	NS
NM_000633 BCL2 B-cell CLL/lymphoma 2	2.4	NS
NM_005190 CCNC Cyclin C	2.3	NS
NM_001239 CCNH Cyclin H	2.1	NS
NM_001790 CDC25C Cell division cycle 25 homolog C (S. pombe)	2.2	NS
NM_003885 CDK5R1 Cyclin-dependent kinase 5, regulatory subunit 1 (p35)	2.1	NS
NM_003590 CUL3 Cullin 3	2.1	NS
NM_002358 MAD2L1 MAD2 mitotic arrest deficient-like 1 (yeast)	2.1	NS
NM_004526 MCM2 Minichromosome maintenance complex component 2	2.1	NS

References

1. M. M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254 (1976).