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SUPPLEMENTARY INFORMATION

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CRISPR–Cas9 genome editing induces a p53-mediated DNA damage response

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SUPPLEMENTARY FIGURES AND TABLES

Supplementary Table 1.

Gene set enrichment analysis (GSEA)[1] for RPE1 $p53^{-/-}$ and RPE1 $p53^{+/+}$ cells, representative example of one duplicate. In an unbiased, genome-wide screen, known essential pathways were identified in RPE1 $p53^{-/-}$, but not in RPE1 $p53^{+/+}$ cells. This is because in $p53^{+/+}$ cells, a double-strand break is a generic fitness disadvantage, which masks guide-specific fitness disadvantages.

RPE $p53^{-/-}$				
Gene Set	Description	Enrichment score	Norm. enrichment score	FDR (cutoff 0.001)
GO:0006383	transcription from RNA polymerase III promoter	0.651527	2.190364	<0.0001
GO:0016072	rRNA metabolic process	0.563517	2.119759	<0.0001
GO:0036260	RNA capping	0.609326	1.952626	0.000111
GO:0016073	snRNA metabolic process	0.557971	1.959511	0.000125
GO:0006353	DNA-templated transcription, termination	0.554843	1.966847	0.000143
GO:0034470	ncRNA processing	0.516722	1.973703	0.000167
GO:0006360	transcription from RNA polymerase I promoter	0.5945	2.006502	0.0002
GO:0006354	DNA-templated transcription, elongation	0.533292	1.915295	0.00025
GO:0022613	ribonucleoprotein complex biogenesis	0.538056	2.048674	0.00025
GO:0070972	protein localization to endoplasmic reticulum	0.534672	1.925633	0.000272
GO:0044033	multi-organism metabolic process	0.519588	1.926238	0.0003
GO:0006413	translational initiation	0.557021	2.053296	0.000333
GO:0071166	ribonucleoprotein complex localization	0.519239	1.865444	0.000599
GO:0061641	CENP-A containing chromatin organization	0.604081	1.871328	0.000615
GO:0006399	tRNA metabolic process	0.506631	1.867288	0.000642
GO:0098781	ncRNA transcription	0.520971	1.848991	0.000687
GO:0071826	ribonucleoprotein complex subunit organization	0.495206	1.839184	0.000764
RPE $p53^{+/+}$				
Gene Set	Description	Enrichment score	Normalised enrichment score	FDR (cutoff 0.001)
N/A	N/A	N/A	N/A	N/A

[1]Wang, J., Duncan, D., Shi, Z. & Zhang, B. WEB-based GEne SeT AnaLysis Toolkit (WebGestalt): update 2013. *Nucleic acids research* **41**, W77-83 (2013).

Supplementary Table 2.

Tested compounds inhibiting Type I interferon (IFN) and innate immune receptor signaling. None of them significantly increased the rate of homologous recombination in the GFP repair – based assay.

Compound	Target molecule	Target pathway	Conc. range	Manufacturer	Cat. no
Ruxolitinib	JAK 1/2 inhibitor	IFN-α	0.1-10nM	Selleckchem	S1378
Tofacitinib	JAK 3 inhibitor	IFN-α	0.1-10nM	Selleckchem	S5001
Pepinh-MYD	Small molecule MyD88 inhibitor	TLR	1-50uM	Invivogen	tlrl-pimyd
Pepinh-TRIF	Small molecule TRIF inhibitor	TLR	1-50uM	Invivogen	tlrl-pitrif
Irak 1/4 inhibitor I	IRAK 1, 4	TLR	100nM-1uM	Sigma	I5409-5MG
IL1 receptor antagonist	IL1 protein inhibitor	IL1-β	2-30ng/µl	Sigma	SRP3327
Anti-hTNF-α-hIgG4	TNF neutralizing antibody	TNF	0.1-25ng/µl	Invivogen	htnfa-mab4
Anti-hIL-6-IgG	IL6 neutralizing antibody	IL6	0.1-25ng/µl	Invivogen	mabg-hil6-3
Anti-hIFN-β-IgG	IFN-β neutralizing antibody	IFN-β	0.1-25ng/µl	Invivogen	mabg2-hifnb-3
Anti-hIFN-α-IgA	IFN-α neutralizing antibody	IFN-α	0.1-25ng/µl	Invivogen	maba-hifna-3
BX795[2]	IKKε/TBK1 inhibitor	TLR signaling	5nM-1µM	Invivogen	tlrl-bx7
CYT387	JAK 1/2 inhibitor	IFN-α signaling	5-500ng/µl	Invivogen	inh-cy87
VX-765	Caspase 1/IL1 converting enzyme inhibitor	Inflammasome	0.5-50ng/µl	Invivogen	inh-vx765i-1
Amlexanox	IKKε/TBK1 inhibitor	TLR	0.1µM-10µM	Sigma	SML0517
C16[2]	PKR inhibitor	TLR signaling	1µM	Sigma	I9785

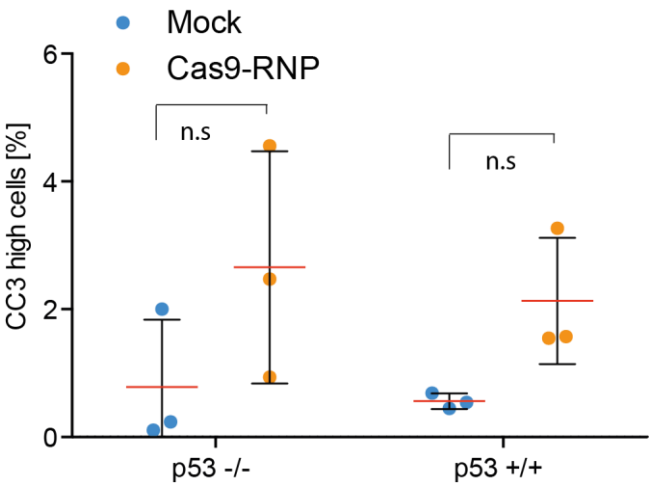
MyD88, Myeloid differentiation primary response gene 88 ; TLR, Toll-Like Receptor; TRIF, TIR-domain-containing adapter-inducing interferon-β; IL, interleukin; IKKε, Inhibitor-κB kinase ε; TBK1, TANK Binding Kinase 1

[2]Muerdter, F. et al. Resolving systematic errors in widely used enhancer activity assays in human cells. *Nature methods* 15, 141-149 (2018).

Supplementary Table 3. Statistics for all figures.

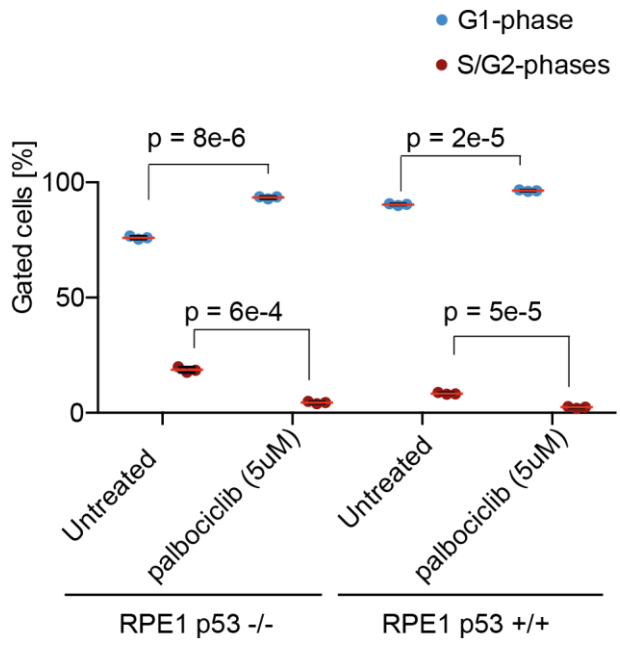
Figure	Cell line	Comparison	p value	t value	DF
Fig. 2a	p53 -/-	G1 mock vs Cas9	0.7751	-0.30949	3.3659
	p53 +/+	G1 mock vs Cas9	0.00252	-6.8323	3.9465
	p53 -/-	G2/S mock vs Cas9	0.6852	-0.44518	3.1226
	p53 +/+	G2/S mock vs Cas9	0.001721	7.9592	3.7711
Fig. 2c		p53 -/- vs p53 +/+	1.61E-07	12.908	9.9099
		p53 -/- vs primary RPE	1.01E-08	17.134	9.9641
		p53 -/- vs repair oligo only	3.16E-10	38.975	7.8064
Fig. 2d	p53 -/-	Control vs Palbociclib	0.01317	4.7104	3.4483
	p53 +/+	Control vs Palbociclib	0.01639	5.8894	2.4751
Fig. S1	p53 -/-	CC3 expression mock vs Cas9	0.2144	-1.5439	3.2119
	p53 +/+	CC3 expression mock vs Cas9	0.1083	-2.7337	2.0603
Fig. S2	p53 -/-	G1 cell cycle stage Palbociclib vs untreated	7.76E-06	-34.029	3.7745
	p53 +/+	G1 cell cycle stage Palbociclib vs untreated	2.43E-05	-22.775	3.9526
	p53 -/-	G2 cell cycle stage Palbociclib vs untreated	5.8E-04	18.384	2.7489
	p53 +/+	G2 cell cycle stage Palbociclib vs untreated	4.86E-05	18.79	3.9845

Supplementary Figure 1.



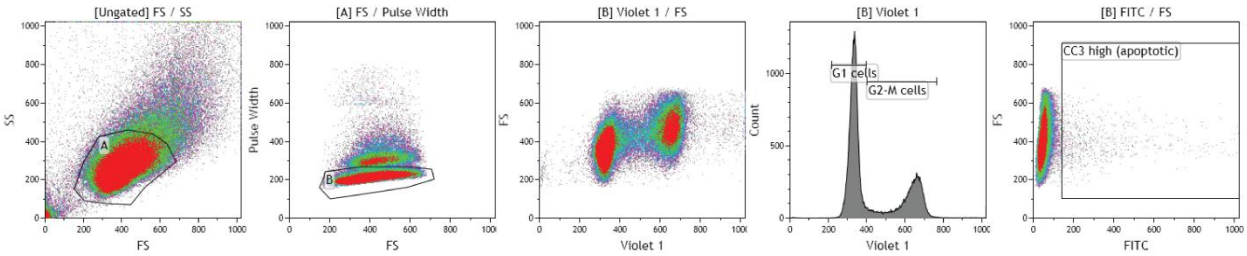
Cleaved Caspase 3 in RPE1 cells 2 days after CRISPR-Cas RNP transfection (n=3 biologically independent samples, representative replicate of two independent experiments). The statistical significance was calculated using Welch's t-test (n.s., not significant; $p>0.21$ $p53^{+/+}$ and $p>0.10$ for $p53^{-/-}$ cells). The mean is indicated with red line and standard error as bars.

Supplementary Figure 2.



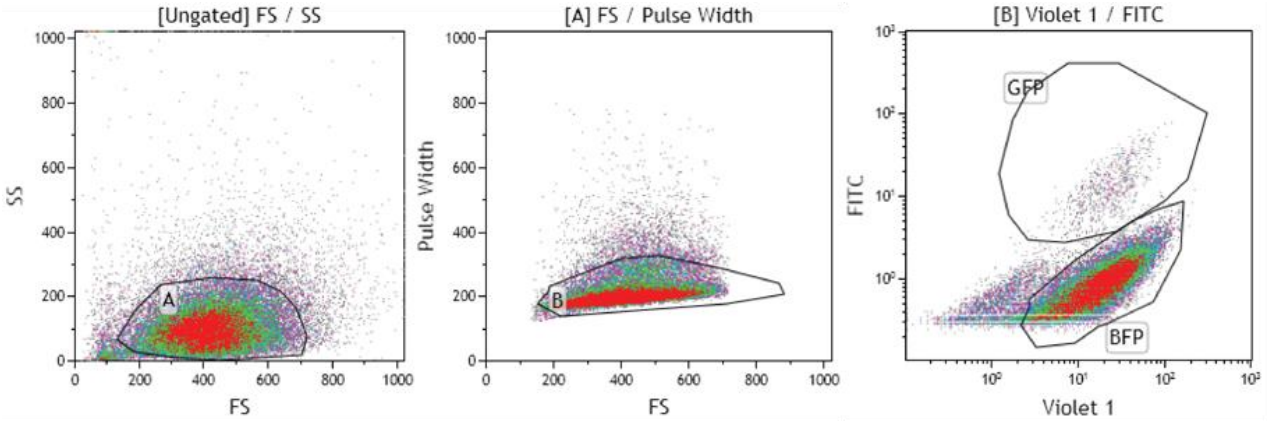
Cell cycle stage in cells treated with 5µM Palbociclib (n=3 biologically independent samples, representative replicate of two independent experiments). The statistical significance was calculated using Welch's t-test. The mean is indicated with red line and standard error as bars

Supplementary Figure 3.



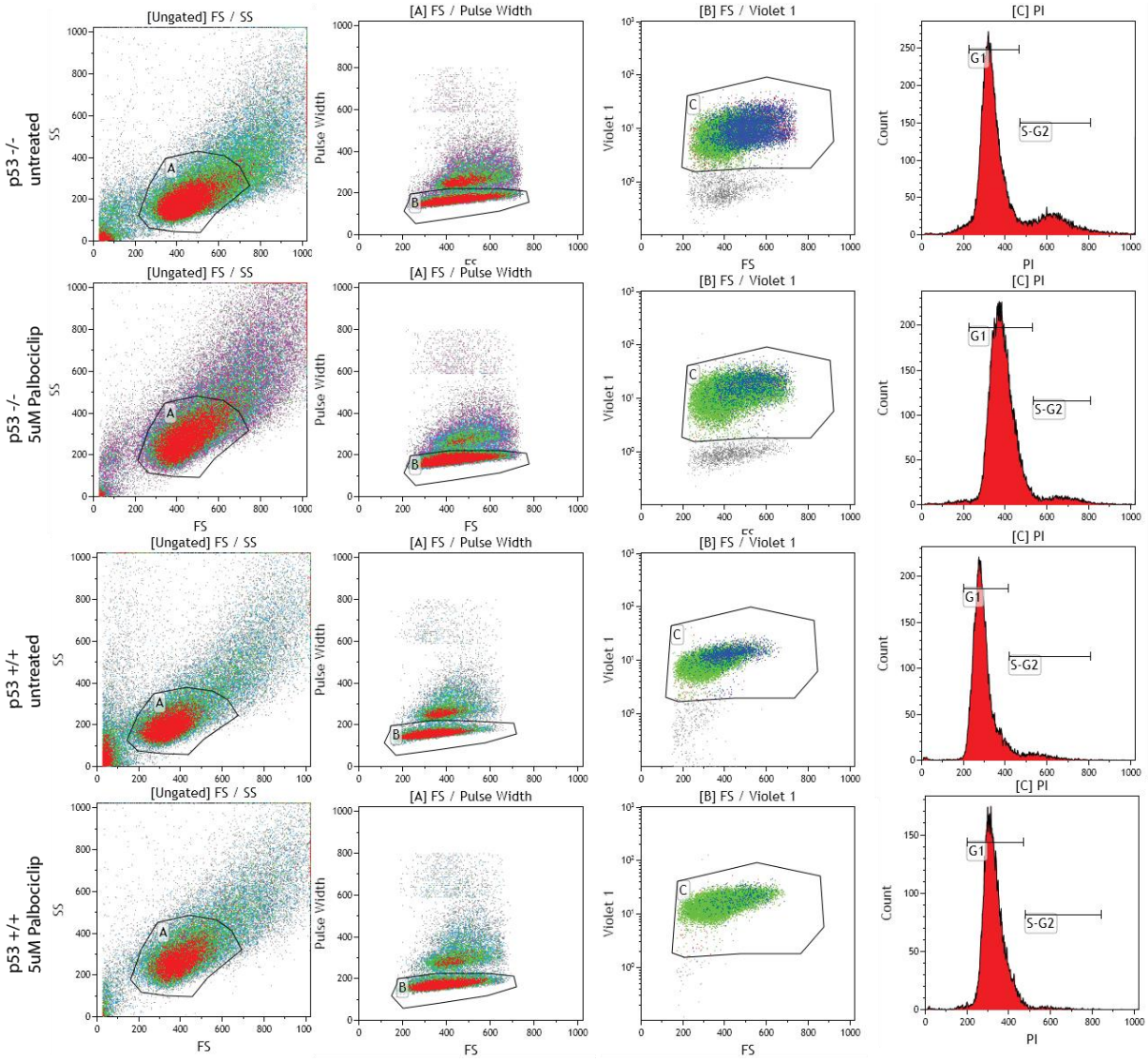
Flow cytometry gating that was used in determining the cell cycle phase (violet channel, Fig. 2a) and the expression of Cleaved Caspase 3 (CC3) (FITC channel, **Supplementary Fig. 2**). We measured 3 independent samples, in two independent experiments (n=2x3). Below is a representative sample of three, from one of the independent experiments.

Supplementary Figure 4.



Flow cytometry gating used in determining the GFP+ population (Figs. 2c, 2d, 2e, 2f).

Supplementary Figure 5.



Flow cytometry gating used in determining the cell cycle stage in Palbociclib-treated RPE1 cells expressing the BFP-cassette. (**Supplementary Fig. 4**). We performed three independent samples, in two independent experiments (n=2x3). Below is one representative sample out of three, from one of the two independent experiments.

Supplementary References

1. Wang, J., Duncan, D., Shi, Z. & Zhang, B. WEB-based GEne SeT AnaLysis Toolkit (WebGestalt): update 2013. *Nucleic acids research* **41**, W77-83 (2013).
2. Muerdter, F. et al. Resolving systematic errors in widely used enhancer activity assays in human cells. *Nature methods* **15**, 141-149 (2018).