Supplementary Information

This file contains Supplementary Methods, Supplementary Tables 1-3, and Supplementary Figures 1-4.

Supplementary Methods contain descriptions and catalogue numbers of antibodies, kits, chemicals, plasmids, cell lines, and sequences for siRNAs, guide RNAs, primers and probes.

Supplementary Tables 1-3 contain statistical values for Figure 5.

Supplementary Figure 1 contains sequences of $p53^{-/-}$ clones.
Supplementary Figure 2 contains sequences of $Rb^{-/-}$ clones
Supplementary Figure 3 shows transfection efficiency of CRISPR RNP.
Supplementary Figure 4 shows gating strategy for NHEJ assay.

Supplementary Methods

Reagents

Reagent	Source	Identifier or Sequence
Antibodies & Enzymes		
Rabbit polyclonal anti-DNA PKcs	Abcam	ab70250
Rabbit polyclonal anti-Ku80/XRCC5	Novus Biologicals	NB100-503
Ku70 (D10A7) Rabbit mAb	Cell Signaling Technology	4588S
Rabbit polyclonal anti-Mre11	Novus Biologicals	NB100-142
Rabbit polyclonal anti-Rad50	Novus Biologicals	NBP2-20054
Rabbit polyclonal anti-Nbs1	Novus Biologicals	NB100-143
Rabbit polyclonal anti-PARP1	Novus Biologicals	NBP2-13732
Rabbit polyclonal anti-PLK3	Novus Biologicals	NBP2-32530
SirT6 (D8D12) Rabbit mAb	Cell Signaling Technology	12486S
RPA34 (RPA2) Mouse Monoclonal Antibody [Clone ID: OTI1H10]	OriGene	TA500765
RPA34 (RPA2) Mouse Monoclonal Antibody [Clone ID: OTI7C12]	OriGene	TA500786
Rabbit monoclonal [EPR2877Y] to RPA32/RPA2	Abcam	ab76420
Rabbit polyclonal anti-CIRBP	Proteintech	10209-2-AP
Rabbit monoclonal [EPR18783] anti-CIRP	Abcam	ab191885
Goat polyclonal anti-CIRP	Abcam	ab106230
Mouse monoclonal [PAb 240] anti-p53	Abcam	ab26
Rabbit polyclonal anti-p53	Abcam	ab131442

Rabbit polyclonal anti-Rb	Abcam	ab226979
PTEN (D4.3) XP Rabbit mAb	Cell Signaling Technology	9188S
Ras (G12V Mutant Specific) (D2H12) Rabbit mAb	Cell Signaling Technology	14412S
Sv40 Large T Antigen (D1E9E) Rabbit mAb	Cell Signaling Technology	15729S
Rabbit polyclonal anti-Histone H3	Abcam	ab1791
Rabbit polyclonal anti-beta actin	Abcam	ab8227
Poly/Mono-ADP Ribose (E6F6A) Rabbit mAb	Cell Signaling Technology	83732
Goat anti-mouse IgG H&L (HRP)	Abcam	ab6789
Goat anti-rabbit IgG H&L (HRP)	Abcam	ab6721
Rabbit Anti-Goat IgG H&L (HRP)	Abcam	ab6741
Mouse Anti-Cyclobutane Pyrimidine Dimers (CPDs) mAb (Clone TDM-2)	Cosmo Bio	CAC-NM-DND-001
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Biotin	ThermoFisher Scientific	62-654-0
Starbright™ Blue 520 Goat Anti-Mouse IgG	Bio-Rad	12005866
Starbright™ Blue 520 Goat Anti-Rabbit IgG	Bio-Rad	12005869
Streptavidin - HRP	ThermoFisher Scientific	434323
MS-grade trypsin	ThermoFisher Scientific	90058
rhRPA	Enzymax	61
Alt-R™ S.p. Cas9 Nuclease V3	IDT	1081058

Kits		
EpiQuik Nuclear Extraction Kit	Epigentek	OP-0002-1
PARP Universal Colorimetric Assay Kit	Trevigen	4677-096-K
Annexin V FLUOS Staining Kit	Roche	1988549
QIAamp DNA Blood Mini Kit	Qiagen	51104
Q5 Site-Directed Mutagenesis Kit	New England Biolabs	E0554
One Shot TOP10 Chemically Competent Cells	ThermoFisher Scientific	C404006
Zero Blunt TOPO PCR Cloning Kit for Sequencing	ThermoFisher Scientific	45-003-1
TOPO TA Cloning Kit for Subcloning	ThermoFisher Scientific	450641
Phusion Hot Start Flex DNA Polymerase	New England Biolabs	M0535
QIAprep Spin Miniprep Kit	Qiagen	27106
EndoFree Plasmid Maxi Kit	Qiagen	12362
NHDF Nucleofector® Kit	Lonza	VPD-1001
Quick-RNA™ MiniPrep	Zymo Research	R1054
Dual-Luciferase Reporter 1000 Assay System	Promega	E1980
Protamine Sulfate Coated ELISA plate	Cosmo Bio	CSR-NM-MA-P002
KAPA 2G Robust HotStart ReadyMix	Roche	07961375001
S-Trap Micro Column	Protifi	C02-micro-80
Wizard Genomic DNA Purification Kit	Promega	A1120

QIAquick Gel Extraction Kit	Qiagen	28706
NEBNext Q5 Hot Start HiFi PCR Master Mix	New England Biolabs	M0543L
Chemicals		
Cytochalasin B from Drechslera dematioidea	Sigma-Aldrich	C2743
Methylene Blue	Sigma-Aldrich	M9140
Agarose, low gelling temperature	Sigma-Aldrich	A9414
50% Hydrogen Peroxide	Thermo-Fisher	H341
Antifade mounting medium with DAPI	Vector laboratories	H-1200
Acridine Orange	ThermoFisher Scientific	A1301
Geneticin™ Selective Antibiotic (G418 Sulfate)	ThermoFisher Scientific	10131035
Puromycin	ThermoFisher Scientific	A1113803
Etoposide	Sigma-Aldrich	E1383
N-Nitroso-N-ethylurea,ISOPAC ®	Sigma-Aldrich	N3385
6-thioguanine	Chem-Impex	01491
Nitro blue tetrazolium chloride	Thermo Fisher	N6495
TDRL-505	Sigma-Aldrich	5305350001
Alt-R® Cas9 Electroporation Enhancer	IDT	1075915
SYBR Gold	ThermoFisher Scientific	S11494
siRNA		
Non-target	Dharmacon	UGGUUUACAUGUCGACUAA
anti-CIRBP	Dharmacon	GACAGATCTCAGAAGTGGTGGTCGT
Primers & Probes		
Pem-F	IDT	GCTAAGTGCTTAGTAAAGCAATAGACTGCAT
Pem-R	IDT	GGACTAGTAATTGTTTAACATGTGGGAAGTT
hGAPDH-F	IDT	GAGTCCACTGGCGTCTTCAC

hGAPDH-R	IDT	GGTTCACACCCATGACGAACA
bwGAPDH-F	IDT	GAGTCCACTGGCGTCTTCAC
bwGAPDH-R	IDT	ATGGTTCACGCCCATGACAAA
hCIRBP-F	IDT	CTGGAGCAGGTCTTCTCAAAGT
hCIRBP-R	IDT	TCCTTAGCGTCGTCAATGTTCT
bwCIRBP-F	IDT	CAGTCACTGGAGCAGGTCTT
bwCIRBP-R	IDT	GCATCATCGATGTTCTCAAAGGT
PTEN ddPCR probe	IDT	/56-FAM/ACCGCCAAG/ZEN/TCCAGAGCCAT/3IABkFQ/
UCE.359 ddPCR probe	IDT	/5HEX/ATGAGACGG/ZEN/GAGACAAGTGATGCT/3IABkFQ/
UCE.359-F	IDT	ATCTGAGACTTGTGACAT
UCE.359-R	IDT	GTGTTAATTGGTAATGACTATT
RB1 KO up-F	IDT	TCGGGAAGAGACAGGTTCAG
RB1 KO up-R	IDT	TCGCTCCCGAACCCAAA
RB1 KO down 1-F	IDT	ACTAAAAGGTCTGAGTGAGTGC
RB1 KO down 1-R	IDT	CTCATATGAGAAGCCCAGAAGG
RB1 KO down 2-F	IDT	ACTAAAAGGTCTGAGTGAGTGC
RB1 KO down 2-R	IDT	CTTTCGAAGCAGAACTCCAAAC
TP53 KO up-F	IDT	GCAGAAATAGGTAGACACTGAGGAA
TP53 KO up-R	IDT	ACGGGAGAAACGAGAGTCAGAA
TP53 KO down 1-F	IDT	CTTCCTGTGTTGGAAAGCCATG
TP53 KO down 1-R	IDT	CACCAAGCAGAGGTTTAAGCAC
TP53 KO down 2-F	IDT	CTTCCTGTGTTGGAAAGCCATG
TP53 KO down 2-R	IDT	CTGCCCCTTCTTAGACTTCAGG
PTEN KO up-F	IDT	CAGTCGCTGCAACCATCCA
PTEN KO up-R	IDT	ATCCGACCAGGGTTAAATGTCATAG
PTEN KO down 1-F	IDT	AAGAGGGTCATTTAAAAGGCCC
PTEN KO down 1-R	IDT	TCTGACACAATGTCCTATTGCC
PTEN KO down 2-F	IDT	TAGGGCGAATGGAATCCTAAAC
PTEN KO down 2-R	IDT	TCTGACACAATGTCCTATTGCC
PTEN fidelity-F	IDT	TGGCTGCTGAGGAGAAGC
PTEN fidelity-R	IDT	GCATCCGTCTACTCCCACG
PTEN fidelity-R (cow SNP)	IDT	GCATCCGTCTATTCCCACG
PTEN ddPCR-F	IDT	TTCTGCCATCTCTCTCC

PTEN ddPCR-R	IDT	CAGGTCAAGTCTAAGTCG
Lego-iC2-bwCIRBP-F	IDT	CCGCGAATTCGCCACCATGGCATCAGATGAGGGC
Lego-iC2-bwCIRBP-R	IDT	CCGCGCGGCCGCTTACTCGTTGTGTGTAGCGTAACTGTCGTAGC
CRISPR Guide RNAs		
Alt-R® CRISPR-Cas9 tracrRNA	IDT	1072533
Alt-R® CRISPR-Cas9 tracrRNA, ATTO™ 550	IDT	1075928
RB1 KO crRNA 1	IDT	GCTGCTCTACGGGGGGTTTT
RB1 KO crRNA 2	IDT	GGCTGCTCTACGGGGGGTTT
RB1 KO crRNA 3	IDT	AGACACCAGCTGATACTACG
TP53 KO crRNA 1	IDT	GTCACAGGCAGAACTCGGCG
TP53 KO crRNA 2	IDT	CGTGGAGCCCCCTCTGAGTC
TP53 KO crRNA 3	IDT	AAATAGGGGAGTGATAACCG
PTEN KO crRNA 1	IDT	GCTAACGATCTCTTTGATGA
PTEN KO crRNA 2	IDT	AGATCGTTAGCAGAAACAAA
PTEN KO crRNA 3	IDT	ATTCTCTGGATCAGAGTCAG
PTEN fidelity crRNA	IDT	GCTAACGATCTCTTTGATGA
Plasmids		
pPB-SV40 large T (LT)	Ref. [1]	
pPB-LT^{K1}	Ref. [1]	
pPB-LT$^{\Delta 434-444}$	Ref. [1]	
pPB- SV40 small T (ST) + LT	Ref. [1]	
pPB-H-Ras V12	Ref. [1]	
pPB-*hTERT*	Ref. [1]	
Super PiggyBac Transposase Expression Vector	System Biosciences	PB210PA-1
pRP[Exp]-Neo-CMV-hCIRBP	VectorBuilder	VB210330-1269gmt
pRP[Exp]-Neo-CMV-bwCIRBP	VectorBuilder	VB210330-1268bpc
pRP[Exp]-Neo-CMV-bwCIRBP-RGG 9R/A	VectorBuilder	VB220320-1032yqx
pRP[Exp]-Neo-CMV-Puro	VectorBuilder	VB220318-1212uym
pCMV-Luc	Ref. [2]	
pCMV-RL	Promega	E2261

NHEJ reporter construct	Ref. [3]	
pCMV-ISce1	Ref. [3]	
pDsRed	Ref. [3]	
LeGO-iC2-Luciferase	This manuscript	
LeGO-iC2-bwCIRBP	This manuscript	
pp53-TA-Luc	Clontech (Takara)	631914
pE2F-TA-Luc	Clontech (Takara)	631914
pRb-TA-Luc	Clontech (Takara)	631914
Lego-iC2	Addgene	27345
pVSV-G	Addgene	138479
psPAX2	Addgene	12260
pmaxGFP	Lonza	VPD-1001
Cell Lines		
14B10SF	Ref. [4]	Primary dermal fibroblasts isolated by the authors from adult bowhead whale
14B11SF	Ref. [4]	Primary dermal fibroblasts isolated by the authors from adult bowhead whale
18B9SF	Ref. [5]	Primary dermal fibroblasts isolated by the authors from adult bowhead whale
NHDF	ATCC	PCS-201-012, Lot 64540954
HDF637	ATCC	PCS-201-012, Lot 63792061
YAHSF	ATCC	CRL-2691
MJ		Neonatal foreskin fibroblasts provided by collaborator
BT1SF		Primary dermal fibroblasts isolated by the authors from adult *Bos taurus*
BT2SF		Primary dermal fibroblasts isolated by the authors from adult *Bos taurus*
WTMSF9		Primary dermal fibroblasts isolated by the authors from adult wild-caught *Mus musculus*
WTMSF10		Primary dermal fibroblasts isolated by the authors from adult wild-caught *Mus musculus*
BL6AMSF2		Primary dermal fibroblasts isolated by the authors from adult, normal C57BL/6 *Mus musculus*
Lenti-X 293T	Takara	632180
$Ku80^{-/-}$ MEFs	ATCC	CRL-3261

References

1. Tian, X. *et al.* Evolution of telomere maintenance and tumour suppressor mechanisms across mammals. *Philosophical Transactions of the Royal Society B: Biological Sciences* **373**, (2018).
2. Tian, X. *et al.* SIRT6 Is Responsible for More Efficient DNA Double-Strand Break Repair in Long-Lived Species. *Cell* **177**, 622-638.e22 (2019).
3. Mao, Z. *et al.* SIRT6 promotes DNA repair under stress by activating PARP1. *Science (1979)* **332**, 1443–1446 (2011).
4. Suydam, R. *et al.* Subsistence harvest of bowhead whales (Balaena mysticetus) by Alaskan Eskimos during 2014.
5. Suydam, R. *et al.* Subsistence harvest of bowhead whales (Balaena mysticetus) by Alaskan Natives during 2018.

Supplementary Tables

Supplementary Table 1. ANOVA for Figure 5a

Uncorrected Fisher's LSD	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Individual P Value
Unmodified					
CRISPR vs. CRISPR +TDRL-505	0.05767	-0.03855 to 0.1539	No	ns	0.1232
CRISPR vs. CRISPR + RPA	-0.1992	-0.3692 to -0.02927	Yes	*	0.0371
Substitutions					
CRISPR vs. CRISPR +TDRL-505	0.03891	0.03454 to 0.04328	Yes	***	0.0007
CRISPR vs. CRISPR + RPA	0.01569	-0.001850 to 0.03323	No	ns	0.0614
Insertions >1 bp					
CRISPR vs. CRISPR +TDRL-505	0.01742	-0.01002 to 0.04486	No	ns	0.1119
CRISPR vs. CRISPR + RPA	0.001321	-0.02206 to 0.02470	No	ns	0.8306
Insertions 1 bp					
CRISPR vs. CRISPR +TDRL-505	0.005787	-0.01362 to 0.02519	No	ns	0.328
CRISPR vs. CRISPR + RPA	0.01687	0.01075 to 0.02299	Yes	**	0.007
Complex Deletions					
CRISPR vs. CRISPR +TDRL-505	0.0279	0.02401 to 0.03180	Yes	**	0.0011
CRISPR vs. CRISPR + RPA	0.04349	0.009846 to 0.07713	Yes	*	0.0308
Deletions ≤20bp					
CRISPR vs. CRISPR +TDRL-505	-0.1519	-0.2873 to -0.01646	Yes	*	0.0404
CRISPR vs. CRISPR + RPA	0.1241	0.03022 to 0.2180	Yes	*	0.0296
Deletions >20 bp					
CRISPR vs. CRISPR +TDRL-505	0.004173	-0.001390 to 0.009736	No	ns	0.0841
CRISPR vs. CRISPR + RPA	-0.002245	-0.03359 to 0.02910	No	ns	0.7871

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
Unmodified								
CRISPR vs. CRISPR +TDRL-505	0.3298	0.2722	0.05767	0.0224	3	3	2.579	2
CRISPR vs. CRISPR + RPA	0.3298	0.5291	-0.1992	0.0395	3	3	5.044	2
Substitutions								
CRISPR vs. CRISPR +TDRL-505	0.07402	0.0351	0.03891	0.001	3	3	38.32	2
CRISPR vs. CRISPR + RPA	0.07402	0.05833	0.01569	0.0041	3	3	3.849	2
Insertions >1 bp								
CRISPR vs. CRISPR +TDRL-505	0.03177	0.01434	0.01742	0.0064	3	3	2.732	2
CRISPR vs. CRISPR + RPA	0.03177	0.03044	0.001321	0.0054	3	3	0.2431	2
Insertions 1 bp								
CRISPR vs. CRISPR +TDRL-505	0.05978	0.05399	0.005787	0.0045	3	3	1.283	2
CRISPR vs. CRISPR + RPA	0.05978	0.04291	0.01687	0.0014	3	3	11.86	2
Complex Deletions								
CRISPR vs. CRISPR +TDRL-505	0.0917	0.0638	0.0279	0.0009	3	3	30.83	2
CRISPR vs. CRISPR + RPA	0.0917	0.04821	0.04349	0.0078	3	3	5.562	2
Deletions ≤20bp								
CRISPR vs. CRISPR +TDRL-505	0.3699	0.5218	-0.1519	0.0315	3	3	4.826	2
CRISPR vs. CRISPR + RPA	0.3699	0.2458	0.1241	0.0218	3	3	5.687	2
Deletions >20 bp								
CRISPR vs. CRISPR +TDRL-505	0.04299	0.03881	0.004173	0.0013	3	3	3.228	2
CRISPR vs. CRISPR + RPA	0.04299	0.04523	-0.002245	0.0073	3	3	0.3082	2

Supplementary Table 2. ANOVA for Figure 5b

Uncorrected Fisher's LSD	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Individual P Value
CRISPR - CRISPR +TDRL-505					
Deletions >3 bp	-0.03388	-0.1015 to 0.03374	No	ns	0.3007
Insertions >1 bp	0.02337	-0.04425 to 0.09100	No	ns	0.4708
Unmodified	0.2158	0.1482 to 0.2834	Yes	****	<0.0001
Deletions ≤3 bp	-0.06979	-0.1374 to -0.002167	Yes	*	0.044
Insertions 1 bp	-0.2277	-0.2954 to -0.1601	Yes	****	<0.0001
Substitutions	0.08478	0.01715 to 0.1524	Yes	*	0.0176
Complex Deletions	0.007456	-0.06017 to 0.07508	No	ns	0.8165

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
CRISPR - CRISPR +TDRL-505								
Deletions >3 bp	0.021	0.05489	-0.03388	0.03153	3	3	1.075	14
Insertions >1 bp	0.05066	0.02728	0.02337	0.03153	3	3	0.7413	14
Unmodified	0.4719	0.2561	0.2158	0.03153	3	3	6.844	14
Deletions ≤3 bp	0.08296	0.1528	-0.06979	0.03153	3	3	2.214	14
Insertions 1 bp	0.2444	0.4722	-0.2277	0.03153	3	3	7.223	14
Substitutions	0.1069	0.02209	0.08478	0.03153	3	3	2.689	14
Complex Deletions	0.02218	0.01473	0.007456	0.03153	3	3	0.2365	14

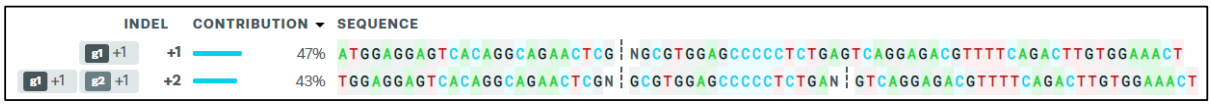
Supplementary Table 3. ANOVA for Figure 5c

Uncorrected Fisher's LSD	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Individual P Value
CRISPR - CRISPR + bwCIRBP					
Deletions >20 bp	-0.001176	-0.04744 to 0.04509	No	ns	0.9573
Insertions >1 bp	0.0102	-0.03607 to 0.05647	No	ns	0.6437
Deletions ≤20bp	0.007581	-0.03869 to 0.05385	No	ns	0.7305
Insertions 1 bp	0.02677	-0.01950 to 0.07303	No	ns	0.2351
Substitutions	-0.003783	-0.05005 to 0.04249	No	ns	0.8633
Complex Deletions	0.007353	-0.03892 to 0.05362	No	ns	0.7383
Unmodified	-0.04694	-0.09321 to -0.0006692	Yes	*	0.0472

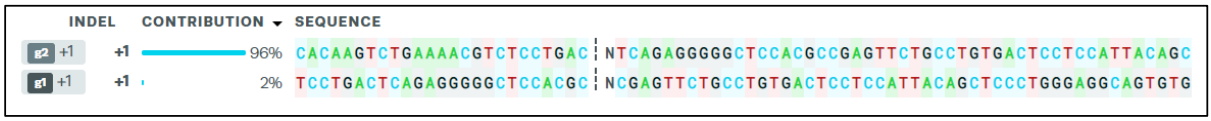
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
CRISPR - CRISPR + bwCIRBP								
Deletions >20 bp	0.0616	0.06277	-0.001176	0.02157	3	3	0.0545	14
Insertions >1 bp	0.01988	0.009684	0.0102	0.02157	3	3	0.4726	14
Deletions ≤20bp	0.4482	0.4406	0.007581	0.02157	3	3	0.3514	14
Insertions 1 bp	0.05208	0.02531	0.02677	0.02157	3	3	1.241	14
Substitutions	0.04352	0.0473	-0.003783	0.02157	3	3	0.1753	14
Complex Deletions	0.06763	0.06028	0.007353	0.02157	3	3	0.3408	14
Unmodified	0.3071	0.354	-0.04694	0.02157	3	3	2.176	14

a ***TP53* upstream**

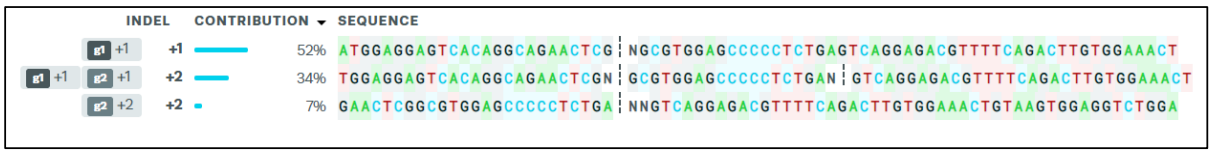
TP53$^{-/-}$ clone 13



TP53$^{-/-}$ clone 17



TP53$^{-/-}$ *RB1*$^{-/-}$ clone 1



TP53$^{-/-}$ *RB1*$^{-/-}$ clone 26

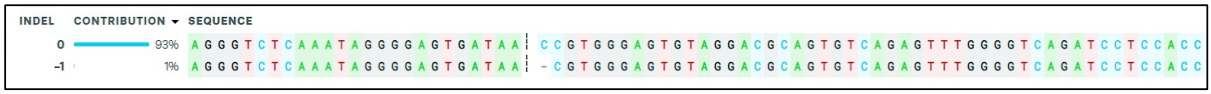
b ***TP53* downstream**

TP53$^{-/-}$ clone 13

TP53$^{-/-}$ clone 17

TP53$^{-/-}$ *RB1*$^{-/-}$ clone 1

TP53$^{-/-}$ *RB1*$^{-/-}$ clone 26

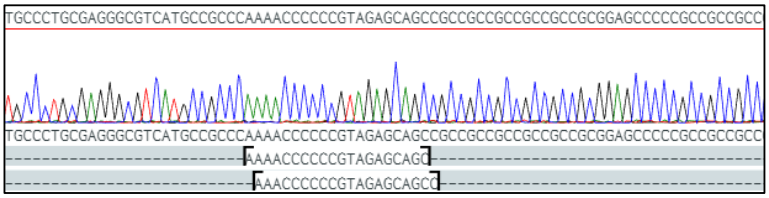


Supplementary Figure 1. Sequences of TP53 CRISPR knockout clones. a, Results of Synthego ICE deconvolution of Sanger sequencing traces from PCR products amplified from the upstream *TP53* target site for the indicated cell lines. Estimated allele frequencies close to 100% are considered to indicate homozygous alleles, while the presence of 2 alleles with estimated frequencies close to 50% indicate heterozygosity at this site. Predicted alleles with single-digit frequencies are considered to result from background noise in Sanger traces. **b**, Results of Synthego ICE deconvolution of Sanger sequencing traces from PCR products amplified from the downstream *TP53* target site for the indicated cell lines.

a

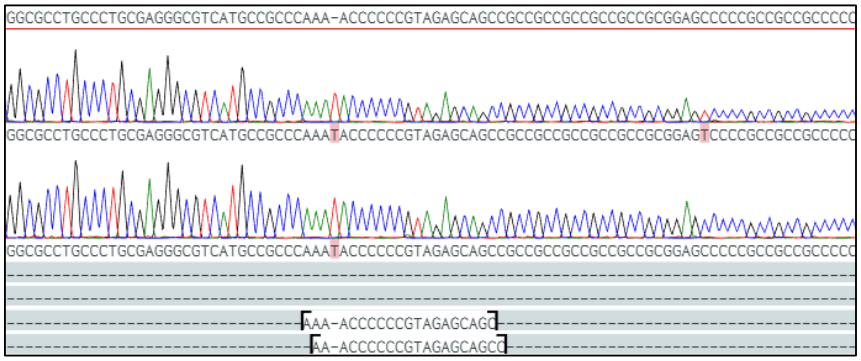
RB1 upstream

TP53$^{-/-}$ clone 13



TP53$^{-/-}$ clone 17

TP53$^{-/-}$ *RB1*$^{-/-}$ clone 26



b

RB1 downstream

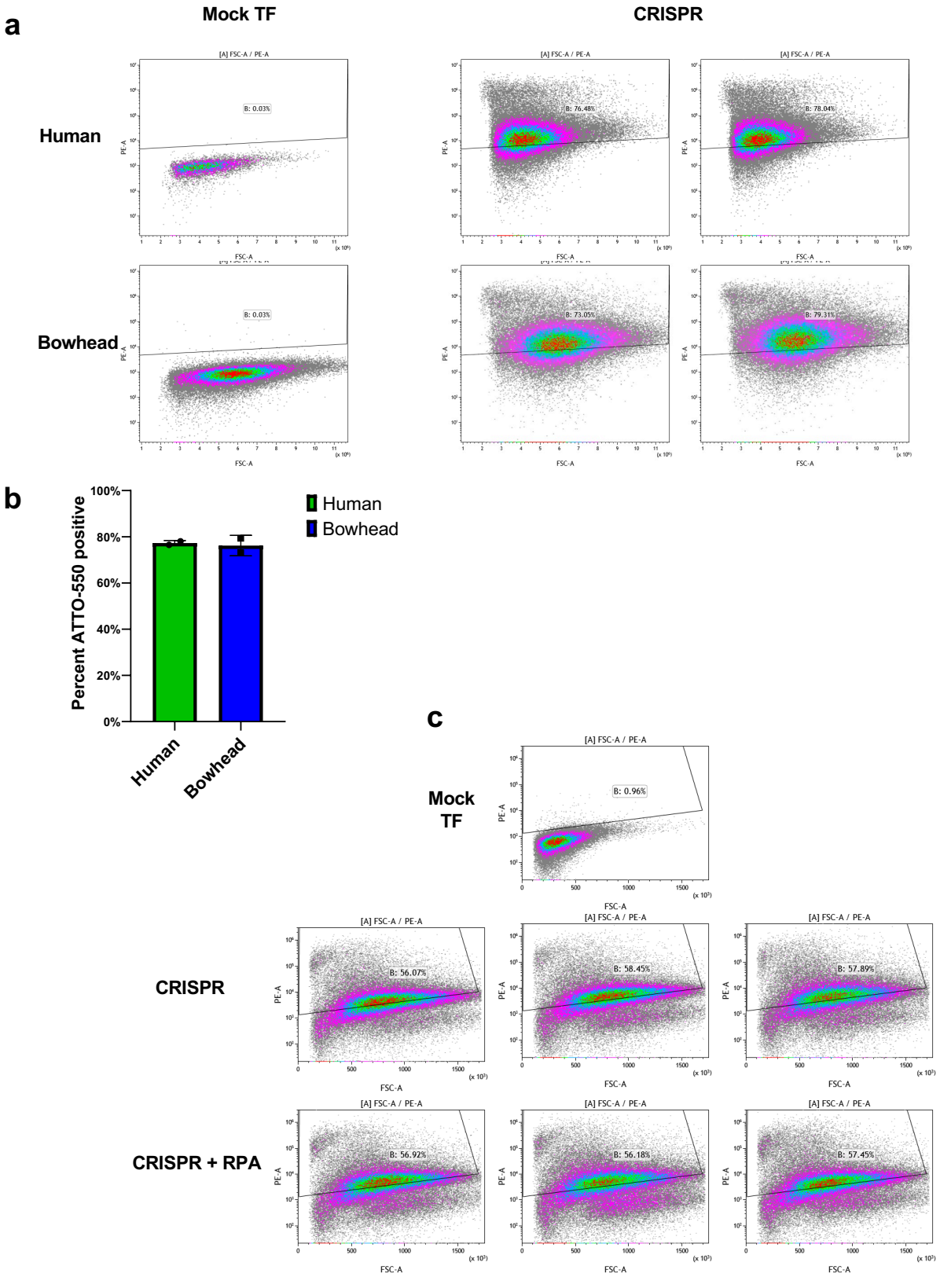
TP53$^{-/-}$ clone 13

TP53$^{-/-}$ clone 17

TP53$^{-/-}$ *RB1*$^{-/-}$ clone 1

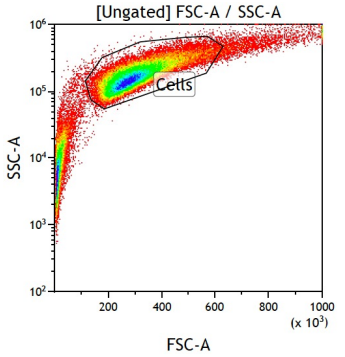
TP53$^{-/-}$ *RB1*$^{-/-}$ clone 26

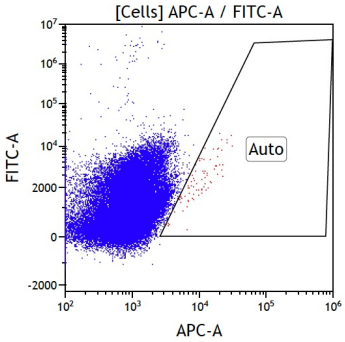
Supplementary Figure 2. Sequences of RB1 CRISPR knockout clones. a, Results of Synthego ICE deconvolution of Sanger sequencing traces from PCR products amplified from the upstream *RB1* target site for the indicated cell lines. Estimated allele frequencies close to 100% are considered to indicate homozygous alleles, while the presence of 2 alleles with estimated frequencies close to 50% indicate heterozygosity at this site. Predicted alleles with single-digit frequencies are considered to result from background noise in Sanger traces. For PCR products that failed Synthego ICE analysis, individual alleles were sequenced through TOPO cloning followed by Sanger sequencing of bacterial colonies, and sequenced alleles are shown as alignments to the reference sequence, with target site guide RNA sequences aligned below (Benchling). **b**, Results of Synthego ICE deconvolution of Sanger sequencing traces from PCR products amplified from the downstream *RB1* target site for the indicated cell lines.

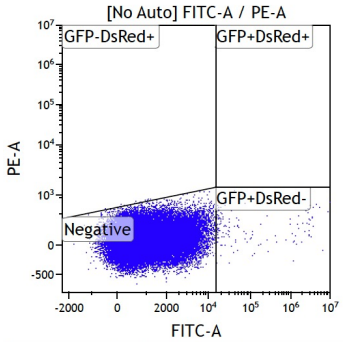

a
Mock TF
CRISPR
Human
Bowhead
[A] FSC-A / PE-A
B: 0.03%
B: 76.48%
B: 78.04%
B: 0.03%
B: 73.05%
B: 79.31%
PE-A
FSC-A
b
Percent ATTO-550 positive
100%
80%
60%
40%
20%
0%
Human
Bowhead
c
Mock TF
CRISPR
CRISPR + RPA
B: 0.96%
B: 56.07%
B: 58.45%
B: 57.89%
B: 56.92%
B: 56.18%
B: 57.45%

Supplementary Figure 3. Transfection efficiency of CRISPR RNP with ATTO 550-labeled tracRNA. a, Flow cytometry measurement of percentage ATTO-550 positive cells immediately (0h) post transfection in human and bowhead primary fibroblasts. ATTO-550 positive gate was set based on mock-transfected control. Plots in each row show results by species for mock transfection and replicate ATTO-550 labeled RNP transfections. **b**, Quantification of average percentage ATTO-550 positive cells by species. **c**, Flow cytometry measurement of percentage ATTO-550 positive cells immediately (0h) post transfection in human primary fibroblasts with or without co-transfection of rhRPA. ATTO-550 positive gate was set based on mock-transfected control. Plots in each row show results of triplicate transfections.

Mock transfection

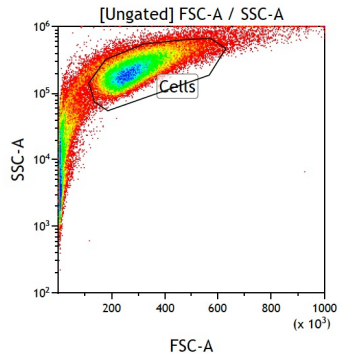


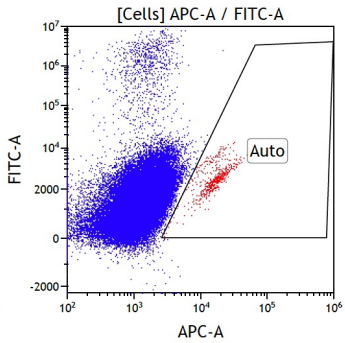


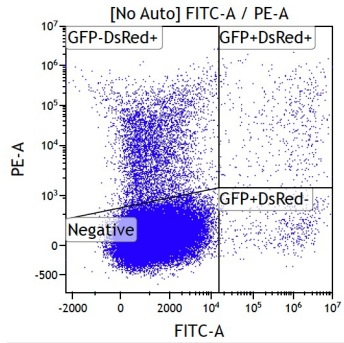


Gate	Number	%Gated
All	51,052	100.0
GFP-DsRed+	14	0.0
GFP+DsRed-	140	0.3
GFP+DsRed+	0	0.0
Negative	50,898	99.7

I-Sce1/DsRed transfection







Gate	Number	%Gated
All	52,429	100.0
GFP-DsRed+	4,840	9.2
GFP+DsRed-	443	0.8
GFP+DsRed+	485	0.9
Negative	46,661	89.0

Supplementary Figure 4. Gating strategy for NHEJ and HR assays. Mock transfected cells were used to set positive/negative gates that were applied to all samples within a given experiment. The live, single cell population was gated based on FSS-SSC and cellular debris were excluded. Autofluorescent cells were gated out based on fluorescence along the APC channel. For calculating NHEJ or HR efficiency, the total numbers of GFP+ and DsRed+ cells were calculated from the FITC-PE plot. Fluorescence compensation was performed in Kaluza.