SPARCLE, a p53-induced IncRNA, controls apoptosis after genotoxic stress by promoting PARP-1 cleavage

Highlights
- SPARCLE is a p53-induced, nuclear IncRNA that mediates DNA-damage-induced apoptosis
- SPARCLE inhibits repair of DNA single-stranded and double-stranded breaks
- SPARCLE binds to PARP-1 and promotes caspase-3 cleavage of PARP-1
- The N-terminal fragment of PARP-1 restores apoptosis in SPARCLE-deficient cells

In brief
Meza-Sosa et al. characterized a p53-regulated long non-coding RNA named SPARCLE. SPARCLE is needed to induce apoptosis by enhancing caspase-3-mediated PARP-1 cleavage in response to DNA damage.
**SPARCLE, a p53-induced IncRNA, controls apoptosis after genotoxic stress by promoting PARP-1 cleavage**

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https://doi.org/10.1016/j.molcel.2022.01.001

**SUMMARY**

p53, master transcriptional regulator of the genotoxic stress response, controls cell-cycle arrest and apoptosis following DNA damage. Here, we identify a p53-induced IncRNA suicidal PARP-1 cleavage enhancer (**SPARCLE**) adjacent to miR-34b/c required for p53-mediated apoptosis. **SPARCLE** is a ∼770-nt, nuclear IncRNA induced 1 day after DNA damage. Despite low expression (<16 copies/cell), **SPARCLE** deletion increases DNA repair and reduces DNA-damage-induced apoptosis as much as p53 deficiency, while its overexpression restores apoptosis in p53-deficient cells. **SPARCLE** does not alter gene expression. **SPARCLE** binds to PARP-1 with nanomolar affinity and causes apoptosis by acting as a caspase-3 cofactor for PARP-1 cleavage, which separates PARP-1’s N-terminal (NT) DNA-binding domain from its catalytic domains. NT-PARP-1 inhibits DNA repair. Expressing NT-PARP-1 in **SPARCLE**-deficient cells increases unrepaired DNA damage and restores apoptosis after DNA damage. Thus, **SPARCLE** enhances p53-induced apoptosis by promoting PARP-1 cleavage, which interferes with DNA-damage repair.

**INTRODUCTION**

p53 is the master transcriptional regulator of the cellular genotoxic stress response. Inactivating mutations of **TP53**, the gene encoding p53, occur in almost every type of cancer and are linked to poor prognosis. **TP53** is the most frequently mutated gene in cancer, indicating its potency as a tumor suppressor. Mice carrying inactivating **TP53** mutations develop tumors spontaneously within six months of birth. Depending on the severity of the stress and the target cell, p53 can promote cell repair and survival or cell-cycle arrest, senescence, or apoptosis by controlling the expression of both coding and non-coding RNAs (ncRNAs). p53 activates the transcription of over a hundred genes, including the CDK4/6 inhibitor **CDKN1A/p21**, to block cell-cycle progression and the bcl-2 family genes to promote apoptosis (Kastenhuber and Lowe, 2017; Lane, 1992; Vogelstein et al., 2000).

No single protein-coding p53 transcriptional target gene explains the strong effect of p53 on tumorigenesis and malignancy, prompting researchers to investigate p53-induced ncRNAs (Bieging-Rolett et al., 2020; Chaudhary and Lal, 2017; Dangelmaier et al., 2019; Grossi et al., 2016; Léveillé et al., 2015; Sánchez et al., 2014). The miR-34 microRNA (miRNA) family is the most studied p53-regulated ncRNA (He et al., 2007; Hermeking, 2007; Raver-Shapira et al., 2007). In humans, the miR-34 family has three members: miR-34a on chromosome 1 and miR-34b and miR-34c, located in the same transcriptional unit on chromosome 11 (Figure 1A). miR-34a is considered a tumor suppressor—its overexpression (OE) enhances p53-mediated cell-cycle arrest or apoptosis (Rokavec et al., 2014) and its expression is reduced in some cancers. The miR-34 family directly or indirectly suppresses many known p53-regulated genes. However, miR-34a deletion has minor effects on apoptosis and cell-cycle progression after genotoxic stress (Navarro and Lieberman, 2015).
Figure 1. miR-34b/c knockout disrupts p53-mediated apoptosis after DNA damage
(A) miR-34b/c and SPARCLE genomic locus on human chromosome 11. The promoter contains two potential binding sites for p53 (p53RE1 and p53RE2). BTG4 is on the opposite strand.
(B) miR-34a, miR-34b, and miR-34c TALEN-guided deletions in HCT116 used in this study.
(C–E) miR-34a (C) and miR-34b/c (D and E) expression analyzed by qRT-PCR in untreated and DOX-treated WT, miR-34a knockout (KO), and miR-34b/c KO HCT116.
(F and G) Untreated or DOX-treated WT, miR-34a KO, miR-34b/c KO, and p53 KO HCT116 analyzed by flow cytometry for annexin V staining (F) and cell-cycle profile (G) 48 h after treatment.

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and does not cause a strong in vivo phenotype (Concepcion et al., 2012). This puzzling result might be explained if other miR-34 family members, miR-34b and miR-34c, substitute for miR-34a. However, genetic deletion of all 3 miR-34 miRNAs (Concepcion et al., 2012) in mice did not impair the p53 response to genotoxic damage or lead to an increase in spontaneous tumors within the first year of life, unlike haploinsufficiency or deletion of p53. Deletion of the miR-34 family and the 3 genes of the miR-449 family, which share a 7-nt seed sequence, profoundly impaired ciliogenesis, leading to respiratory dysfunction, infertility, and early mortality (Song et al., 2014). However, these problems were considered unrelated to p53.

To understand the lack of a strong miR-34a deletion phenotype and the functional redundancy of the miR-34 family, we generated miR-34b/c null HCT116. Unexpectedly, miR-34b/c deletion (unlike miR-34a deletion) reduced DNA-damage-induced apoptosis as much as p53 deficiency. However, expressing the deleted miRNAs did not rescue the phenotype, suggesting that something other than miR-34b/c deficiency was responsible. Here, we show that an IncRNA adjacent to the miR-34b/c cluster that we named suicidial PARP-1 cleavage enhancer (SPARCLE), which is not expressed in miR-34b/c-deleted cells and is induced late after DNA damage by p53 binding to a p53 response element (p53RE) that also induces miR-34b/c, regulates apoptosis after DNA damage. Cells genetically deficient in miR-34b/c, SPARCLE, or the p53RE that controls miR-34b/c and SPARCLE expression phenocopy the lack of DNA-damage-induced cell death as strongly as p53-deficient cells. Even though SPARCLE expression is delayed and low after DNA damage, it powerfully promotes apoptosis in cells with unrepaired DNA damage by inhibiting late DNA-damage repair.

RESULTS

miR-34b/c knockout cells are resistant to DNA-damage-induced apoptosis

To investigate whether the lack of a DNA-damage phenotype in miR-34a knockout (KO) cells and mice could be due to redundancy of other family members (miR-34b and miR-34c), HCT116 cells deficient in miR-34a or miR-34b and miR-34c were generated using transcription-activator-like effector nucleases (TALENs) (Figure 1B). As expected, each of these miRNAs was induced by doxorubicin (DOX) in wild-type (WT) HCT116 cells, but not in cells in which the corresponding mRNA gene was deleted (figures 1C–1E) or in p53 hypomorphic cells (Figure S1A). As previously reported, miR-34a deletion did not affect basal or DNA-damage-induced apoptosis or cell-cycle profile (Navarro and Lieberman, 2015), in contrast to p53 hypomorphic cells (Bunz et al., 1999) (referred to hereafter as p53 KO cells) that showed greatly reduced sub G1 cells, a sign of apoptosis, after DOX (Figures 1F and 1G). Deletion of miR-34b and miR-34c reduced DOX-induced apoptosis almost as much as p53 KO (Figure 1F), resulting in fewer sub G1 cells, and an increase in arrested cells. No cell-cycle profile change was observed under basal conditions, but DOX-treated miR-34b/c KO cells had increased G1 cells (Figure 1G). To determine whether the apoptotic defect in miR-34b/c KO cells was due to miR-34b/c, miR-34b and miR-34c mimics were transfected into miR-34b/c KO HCT116. Surprisingly, mature miR-34b and miR-34c mimics did not rescue apoptosis of miR-34b/c KO cells, suggesting that lack of these two miRNAs was not responsible for protection from apoptosis (Figure 1H).

SPARCLE is a ~770-nt nuclear IncRNA

Reduced apoptosis in damaged miR-34b/c KO cells might be caused by off-target TALEN disruption of another gene or effects of miR-34b/c disruption on expression of a nearby gene on human chromosome 11. Multiple miR-34b/c KO clones had the same phenotype (data not shown), suggesting that an off-target deletion was not responsible for the loss of DNA-damage-induced apoptosis. 3-kb upstream of the miR-34b/c pre-miRNA sequence, a putative non-conserved IncRNA named LOC728196 was annotated (Figure 1A). LOC728196 was intermittently removed from public databases, raising questions about whether it is a bona fide IncRNA. However, a previous report confirmed LOC728196 expression by qRT-PCR in some glioma and astrocytoma cell lines and linked high expression in glioma to poor prognosis (Wang et al., 2018). For reasons that will be explained later, we renamed this putative IncRNA SPARCLE. SPARCLE’s expression was not detected above background by qRT-PCR under basal conditions in WT and p53 KO HCT116 but was induced after DOX treatment only in p53 WT cells (Figure 2A). Expression was first detected 24 h after DOX at a low level (mean ~1 copy/cell) and further increased to a mean of ~8 copies/cell by 48 h. SPARCLE was not detected in untreated, p53 KO or miR-34b/c KO cells (Figure 2A).

Because the size and properties of the SPARCLE transcript are not known, we characterized it further. 5’ rapid amplification of cDNA ends (RACE) was used to define the SPARCLE transcription start site (TSS) (Figure 2B). Two TSS’s located 29-nt (TSS1) and 14-nt (TSS2) downstream of the annotated LOC728196 TSS were cloned at equal frequencies (Figure S1B). In this paper TSS1 coordinates will be arbitrarily used for annotation. To define the length of SPARCLE, we tried different approaches. SPARCLE was not detected by northern blot even after DNA damage or by 3’ RACE (data not shown). Since 3’ RACE relies on poly(A) tails to amplify 3’-ends, SPARCLE may not be polyadenylated. Circular RACE, which does not depend on polyadenylation for amplification, amplified a reproducible and strong signal, which was cloned and sequenced (Figure 2C). None of the sequences had a poly(A) tail. All the clones ended 771 nt downstream of TSS1. Thus, SPARCLE is a ~770 nt IncRNA that likely is not polyadenylated (Figure S1C).

Next, we assessed whether SPARCLE’s sequence is conserved upstream of miR-34b/c in other species (Figure S1D).
SPARCLE is well conserved among primates and some other mammals, including cow, sheep, and pig, with identities of 80%–95% in ~40% of the 771 nt SPARCLE sequence. A similar large region is not well conserved in rodents; however, a ~150-nt region immediately downstream of TSS1 is highly conserved (>70%) in all mammals analyzed, suggesting that this region may be functional. A conserved upstream sequence of the miR-34b/c cluster was not found in fish, reptiles, or any invertebrates. A sequence analysis using the coding potential calculator (CPC) versions 1 and 2 (Kang et al., 2017) indicated that SPARCLE is unlikely to be translated (CPC1 coding potential, −1.319; CPC2 coding probability, 0.0093). Thus, SPARCLE is a bona fide mammalian lncRNA.

To determine SPARCLE's cellular localization, WT HCT116 cells were separated into nuclear and cytoplasmic fractions (CFs) 48 h after DOX treatment, and RNA was extracted from each fraction and analyzed by qRT-PCR for SPARCLE. Two nuclear ncRNAs, MALAT1 and U6, and the cytoplasmic miRNA miR-7 were amplified as controls (Figure 2D). SPARCLE was only detected in the nuclear fraction (NF), while the other transcripts were localized as expected. To confirm SPARCLE’s nuclear localization, single-molecule RNA fluorescence in situ hybridization (smFISH) (Orjalo et al., 2011; Raj et al., 2008) was performed using SPARCLE probes in WT HCT116 48 h after adding medium or the double-strand DNA break (DSB)-inducing agent necrostatin (NCS). SPARCLE was not detected in un-stressed cells, but nuclear puncta were observed in NCS-treated cells (Figure 2E). No signal was detected in NCS-treated SPARCLE KO cells (see below), indicating that the smFISH assay was specific (Figure S1E). Thus, SPARCLE is a low abundance, DNA-damage-induced, nuclear lncRNA.

**SPARCLE's promoter contains one functional p53 response element**

Just 5’ to SPARCLE’s TSS1 are two predicted p53REs, p53RE1 (beginning at −1,301 nt) and p53RE2 (at −32 nt). To determine whether SPARCLE is induced by p53, its expression was analyzed in p53 KO and WT HCT116 cells treated with the DNA-damaging agents DOX and NCS or nutlin-3, an Mdm2 inhibitor that activates p53 without causing genotoxic stress. As expected, SPARCLE was detected after all these p53-activating stimuli, but only in p53-sufficient cells (Figure 2F). Thus, p53 activation is all that is needed to induce SPARCLE. To determine whether the two putative p53REs upstream of SPARCLE’s TSS are functional, the complete region that contains one or both p53REs or the complete region in reverse orientation (1ER35p + 2ER35p) were cloned upstream of a luciferase reporter gene. HCT116 were transfected with these reporter plasmids, treated or not with DOX, and assessed for luciferase activity 48 h later (Figure 2G). Luciferase activity was detected only after DOX in cells transfected with the p53RE1 + p53RE2 or p53RE2 reporters, indicating that p53RE2 is the only functional p53RE. To corroborate this finding, chromatin immunoprecipitation (ChIP) assays were performed using a p53 antibody or control IgG in WT and p53 KO HCT116 (Figure 2H), p53 bound to p53RE2 to a limited extent in untreated cells, but binding increased dramatically after DOX. Similarly, NCS treatment of WT HCT116 showed time-dependent binding of p53 to the p53RE2 beginning 24 h and increasing by 48 h after adding NCS (Figure 2I). p53 did not bind to p53RE1 after DNA damage. As positive control, p53 binding to the promoter of the p53 target gene CDKN1A/p21 was verified. To examine whether p53-dependent upregulation of SPARCLE after DNA damage occurs in other cells, p53 was knocked down or not in lung adenocarcinoma (A549), hepatocellular carcinoma (HepG2), and poorly differentiated colon carcinoma (RKO), and the knocked down or control cells were treated or not with DOX. SPARCLE, TP53, p53, and CDKN1A/p21 expression levels were measured by qPCR 24 h after (Figure S2). SPARCLE and CDKN1A were significantly upregulated in all three DOX-treated cell lines knocked down with a control siRNA, but their induction was strongly suppressed when p53 was knocked down (Figure S2). Thus, SPARCLE is a p53-induced lncRNA induced at low levels after genotoxic stress.

**Cells lacking p53RE2 resist DNA-damage-induced apoptosis that is rescued by SPARCLE overexpression**

Since SPARCLE’s induction depends on p53 binding to p53RE2, p53RE2 KO HCT116 clones were generated using TALENs to further investigate the role of SPARCLE in DNA-damage induced
apoptosis (Figure 3A). Two clones were generated that behaved similarly (data not shown). As expected, deleting p53RE2 abrogated p53 binding to SPARCLE’s promoter (data not shown) and SPARCLE expression after genotoxic stress (Figure 3B, left). Deleting p53RE2 also blocked DOX-induced miR-34b/c (Figure 3B, middle and right), suggesting that SPARCLE and
Molecular Cell

**A**

**SPARCLE KO**

5' GCCAA**[(ATC...CAA)]**TCCAG 3'

WT SPARCLE KO C1

SPARCLE KO C2

**B**

SPARCLE relative to GAPDH

![Graph showing SPARCLE levels with DOX](legend on next page)

miR-34b

relative to U6

miR-34c

relative to U6

![Graph showing miRNA levels with DOX](legend on next page)

**C**

HCT116

Annexin V+ cells,%

- DOX

+ DOX

![Graph showing Annexin V+ cell percentages](legend on next page)

**D**

Cells in cell cycle phase, %

G2/M

S

G1

Sub G1

![Graph showing cell cycle distribution](legend on next page)

**E**

HCT116

Annexin V+ cells, %

- NCs

+ NCs

![Graph showing Annexin V+ cell percentages](legend on next page)

**F**

HCT116

Annexin V+ cells, %

- DOX

+ DOX

![Graph showing Annexin V+ cell percentages](legend on next page)

**G**

A549

Annexin V+ cells, %

0 24 48

![Graph showing Annexin V+ cell percentages](legend on next page)

**H**

HCT116

Annexin V+ cells, %

0 24 48

![Graph showing Annexin V+ cell percentages](legend on next page)

**I**

DOX

NCS

X-rays

CPT

Arsenite

Nutlin-3

![Graph showing treatment effects](legend on next page)

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miR-34b/c are co-regulated or may form part of the same transcriptional unit. p53RE2 deficient cells did not have a significantly altered cell-cycle profile under basal conditions (Figure 3C), DOX treatment of p53RE2-deficient HCT116 cells, like miR34b/c KO cells (Figures 1F and 1G), reduced annexin V staining and apoptosis and increased G1 arrest compared with WT HCT116 (Figures 3C–3E). Moreover, p53RE2 deletion and p53 KO reduced DOX-induced apoptosis in HCT116 similarly.

Because p53RE2 deletion abrogates both SPARCLE and miR-34b/c expression, to determine which of these transcripts is responsible for promoting apoptosis, we performed rescue experiments. As before, transfection of miR-34b and miR-34c mimics did not increase apoptosis in p53RE2-deficient cells (Figure 3F). However, transfection of plasmids encoding different length SPARCLE constructs into p53RE2 KO HCT116 restored NCS-induced apoptosis to the level in WT HCT116 (Figure 3G). SPARCLE constructs encoding the first 275 bases, 1.2, 2.2, and 3.2 kb after SPARCLE’s TSS all restored apoptosis comparably. Thus, SPARCLE is needed for apoptosis and the first 275 bases of SPARCLE is active. Since SPARCLE KO-associated resistance to apoptosis was rescued by an exogenous plasmid, SPARCLE is likely a trans-acting IncRNA rather than a local chromatin modifier.

**SPARCLE deficiency reduces apoptosis in response to DNA damage**

To confirm the critical role of the 5′-end of SPARCLE in DNA-damage-induced apoptosis, HCT116 that lack the first 253 nt of SPARCLE (called SPARCLE KO) were generated using CRISPR-Cas9n (Figure 4A). Two KO clones gave similar results (data not shown). SPARCLE was not detected in SPARCLE KO cells even after DOX (Figure 4B, left), but both miR-34b and miR-34c were expressed without significant change compared with WT HCT116 (Figure 4B, middle and right). After DOX, apoptosis—assessed by annexin V staining 48 h later—was reduced compared with WT HCT116 to the same extent in SPARCLE KO, p53RE2 KO, and p53 KO HCT116 (Figure 4C).

As previously observed for miR-34b/c KO and p53RE2 KO cells, SPARCLE KO had no significant effect on cell-cycle profile under basal conditions but showed fewer subG1 and increased G1 phase arrested cells after DOX (Figure 4D). Moreover, OE of either 275 nt or 3 kb SPARCLE comparably restored NCS-induced apoptosis in both SPARCLE KO and p53 KO cells (Figure 4E). The SPARCLE 3-kb construct contains the miR-34b/c precursor sequence, but the shorter 275-nr SPARCLE (SPARCLE 275) does not (Figure 3G).

Nonetheless, expression of both constructs caused similar amounts of DNA-damage-induced apoptosis (Figure 4E). Moreover, transfection of miR-34b and miR-34c mimics did not rescue DNA-damage-induced apoptosis in SPARCLE KO or p53 KO cells (Figure 4F).

To examine whether SPARCLE regulated DNA-damage-induced apoptosis in other cells, SPARCLE KO clones were generated in two additional p53-sufficient cell lines—A549 and HepG2 (Figures S3A and S3B, left). As for HCT116, SPARCLE KO eliminated DOX-induced SPARCLE expression, but both miR-34b and miR-34c were induced by DOX in SPARCLE KO cells (Figures S3A and S3B, middle and right). SPARCLE KO dramatically reduced apoptosis in DOX-treated A549 and HepG2 (Figure 4G). Ectopic expression of SPARCLE 275, but not a 275-nt control RNA, transcribed from the opposite strand of SPARCLE (named ELCRAPS), in SPARCLE KO cells (Figure S3C), completely rescued DOX-induced apoptosis in all three SPARCLE KO lines (Figure 4H). In these experiments, ectopic SPARCLE and ELCRAPS were similarly overexpressed to ~40 copies/cell, which was modestly more than SPARCLE levels induced after DOX in WT cells (~8–16 copies/cell) (Figure S3C). Transfection of SPARCLE or ELCRAPS had no effect on miR-34b or miR-34c expression at baseline or after DOX (Figures S3D and S3E). Thus, SPARCLE, but not miR-34b/c, activates DNA-damage- and p53-induced apoptosis in multiple cell types.

**SPARCLE promotes apoptosis in response to single- and double-stranded DNA damage**

Until now, DNA damage was induced using two DSB-inducing agents, DOX or NCS, with similar results. To determine whether SPARCLE plays a role in other types of DNA damage, WT, SPARCLE KO, and p53 KO HCT116 were treated with a variety of agents that induce genotoxic stress or with nutlin-3, which activates a p53 response and caspase-3 independently of DNA damage. Apoptosis was measured by annexin V staining 24 and 48 h later (Figure 4I). Apoptosis in response to nutlin-3 or agents that cause DSB (DOX, NCS, and X-rays) or single-strand breaks (SSB) (camptothenic [CPT]) was strongly inhibited to a similar extent in SPARCLE KO and p53 KO HCT116 compared with WT HCT116. However, apoptosis in response to arsenite, which causes oxidative damage to DNA and abasic sites, was comparable in WT, p53 KO, and SPARCLE KO HCT116. Thus, SPARCLE plays a major role in p53-mediated apoptosis in response to both SSB and DSB but may not be important in responding to oxidative DNA damage.
Figure 5. SPARCLE does not affect gene expression but interacts with DNA repair proteins
(A) Volcano plots showing few significant differentially expressed genes comparing duplicate samples of WT, SPARCLE KO (KO), and SPARCLE overexpressing (OE) HCT116 before and after DOX. Blue genes are significantly downregulated; red genes, upregulated. Dotted lines indicate 1 log2 fold change and FDR < 1e−5. See also Figure S4.
(B) SPARCLE RNA antisense purification-mass spectrometry (RAP-MS) experimental scheme (B) and 10 SPARCLE-interacting proteins with greatest peptide coverage isolated from nuclei of DOX-treated WT HCT116 (C).

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**SPARCLE does not regulate gene expression**

Most nuclear IncRNAs whose function has been defined act as chromatin or transcription regulators (Huarte, 2015; Ulitsky and Bartel, 2013). To determine if SPARCLE regulates gene expression, RNA-seq of WT, SPARCLE KO, and SPARCLE KO overexpressing SPARCLE 275 (SPARCLE OE) HCT116 that were untreated or treated with DOX for 48 h was compared in duplicate samples. As expected, DOX altered the expression of about a thousand genes, including well-known p53-regulated genes (Figures S4A–S4C; Table S1). However, surprisingly, when basal and DOX-induced gene expression were compared in WT, SPARCLE KO and SPARCLE OE cells, there were few consistent changes that could be linked to SPARCLE (Figures 5A and S4B). Similar results were found when the arbitrary threshold chosen to identify differentially expressed genes was changed. In fact, the expression profile of SPARCLE KO and OE cells were virtually identical. Moreover, SPARCLE KO did not significantly alter the basal or NCS-induced expression of TP53, BTG4 (the nearby coding gene in the same locus), PARP1 or key p53-regulated genes, including CDKN1A, BAX, and NOXA, as measured by qRT-PCR (Figure S4D). Thus, SPARCLE is not a transcriptional regulator.

**SPARCLE binds to PARP-1**

To get a clue to how SPARCLE functions, RNA antisense purification-mass spectrometry (RAP-MS) (Engreitz et al., 2013; McHugh et al., 2015) was performed to pull down SPARCLE-interacting proteins from nuclei of DOX-treated WT and SPARCLE KO HCT116 (Figure 5B). Three 5’ biotinylated probes, antisense to the functional first 275 nt of SPARCLE, were used for pull-down (Table S3). 557 proteins with a minimum of 3 unique peptides were pulled down and identified in WT cells, and only 5 were found in SPARCLE KO cells, indicating assay specificity for identifying candidate SPARCLE-interacting proteins (Table S2). After filtering the 557 proteins based on their percent coverage (≥17%), 206 proteins were chosen as candidate SPARCLE interactors. Among these, multiple proteins involved in the DNA-damage response (DDR) (PARP-1, Ku80/XRCC5, PRKDC/XRCC7/DNA-PKcs) and splicing (SF3B3, PRPF8, SNRNP200) were found. Others included IFL3/NF10, the larger subunit of the NFAT transcription factor, which interacts with DNA-PKcs (Ting et al., 1998); the heat shock protein HSPA8; Supt16H, a histone chaperone in the FACT chromatin remodeling complex; and the fatty acid synthase (FASN) (Figure 5C).

Because cells lacking SPARCLE are defective in DNA-damage-induced apoptosis, we focused on PARP-1, Ku80, and DNA-PKcs, which sense DNA breaks and assemble on and repair damaged DNA (Ceccaldi et al., 2016; Spagnolo et al., 2012). In particular, PARP-1 senses and binds to both SSB and DSB and rapidly catalyzes PARylation, the addition of poly(ADP) ribose (PAR) to recruit DNA repair factors to sites of DNA damage (Beck et al., 2014; Wei and Yu, 2016). When DNA damage is low, PARP-1 enhances survival (D’Amours et al., 2001; Halappanavar et al., 1999; Oliver et al., 1998). However, when damage is extensive, PARP-1 promotes apoptosis (D’Amours et al., 2001; Halappanavar et al., 1999; Oliver et al., 1998). Ku80 and DNA-PKcs are key factors in DSB repair by non-homologous end joining (NHEJ) (Ceccaldi et al., 2016; Chang et al., 2017; Li and Xu, 2016).

RNA antisense pull-down followed by western blot (RAP-WB) was used to assess the candidate SPARCLE-interacting DDR proteins identified by RAP-MS. RAP-WB confirmed an interaction of SPARCLE with both full-length (FL) and the caspase-3-cleaved N-terminal (NT) fragment of PARP-1 in WT cells. No signal was detected in SPARCLE KO HCT116 harvested 48 h after DOX (Figure 5D). DNA-PKcs was not detected in the input cell lysates (data not shown), and Ku80 was not detected in the RAP-WB (Figure 5D). qRT-PCR amplified SPARCLE in RNA immunoprecipitated (RIP) using PARP-1 antibody, but not using anti-Ku80 (Figure 5E). SPARCLE was not amplified in SPARCLE KO HCT116 or in untreated WT HCT116, confirming the specificity of the RIP assay. These data suggest that SPARCLE binds to PARP-1.

Microscale thermophoresis (MST) confirmed the interaction between SPARCLE and PARP-1. Alexa647-labeled human recombinant PARP-1 (R-PARP-1) or Ku80 were incubated with in vitro transcribed SPARCLE 275 or ELCRAPS RNA (Figure 5F). SPARCLE 275 directly interacted with PARP-1 (but not with Ku80) with nanomolar affinity (Kd, 13.7 ± 6.4 nM), but PARP-1 did not interact with ELCRAPS RNA. Thus, SPARCLE and PARP-1 bind tightly. Ku80 and DNA-PKcs may have been pulled down with SPARCLE in the RAP-MS proteome because they associate with PARP-1 in DDR complexes, but they are unlikely to directly interact strongly with SPARCLE.

**SPARCLE deficiency enhances DNA-damage repair**

Because SPARCLE interacts with PARP-1 and increases apoptosis after DNA damage, we hypothesized that SPARCLE interferes with DDR. To test this hypothesis, the extent of NCS-induced unrepaired DNA damage was compared in WT and SPARCLE KO HCT116 by counting γH2A.X foci that assemble at DSB using confocal microscopy (Figures 6A and 6B), and the percentage of cells with unrepaired DNA breaks marked with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) by flow cytometry (Figure 6C). WT and SPARCLE KO cells both similarly increased γH2A.X foci for the first 36 h after adding NCS (Figure 6A). However, at 48 h, when SPARCLE is well expressed, SPARCLE KO cells had ~3-fold...
Figure 6. SPARCLE inhibits DNA repair

(A) γH2A.X foci (green) by immunofluorescence microscopy after NCS treatment of WT and SPARCLE KO HCT116. DAPI staining in blue. Shown are representative images (left) and the number of foci/cell in 3 independent experiments (right).

(B) γH2A.X foci (green) by immunofluorescence microscopy 48 h after adding NCS or medium to WT and SPARCLE KO HCT116 transfected with empty vector (EV) or an expression plasmid encoding the 5’ 275 nt of SPARCLE (SPARCLE 275). Shown are representative images (left) and image quantification of 3 experiments.

(C) TUNEL staining 48 h after adding medium or NCS to WT and SPARCLE KO HCT116 transfected 24 h earlier with EV or SPARCLE 275.

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fewer γH2A.X foci. Similarly, 48 h after NCS treatment, virtually no SPARCLE KO cells were TUNEL+, while about a third of WT HCT116 had unrepaired TUNEL-stained DNA breaks. Ectopic expression of SPARCLE 275 significantly increased the number of γH2A.X foci in NCS-treated WT HCT116 and significantly increased γH2A.X foci and TUNEL+ cells in SPARCLE KO cells, confirming that SPARCLE inhibits DNA repair, leaving more unrepaired DNA damage, and that the 5’ 275-nt fragment is the active region (Figures 6B and 6C). To confirm this finding, DNA damage was compared over 48 h in DOX-treated WT and SPARCLE KO HCT116 cells by COMET assay (single-cell gel electrophoresis) (Figure 6D). The comets in WT HCT116 cells contained more DNA tail fragments that migrated more rapidly to produce longer tails than in SPARCLE KO HCT116, leading to significantly larger COMET tail moments beginning at 24 h when SPARCLE is first detected.

To confirm that SPARCLE 275 interferes with DSB repair and evaluate which DSB repair pathway it inhibits, a SPARCLE 275 expression plasmid or empty vector (EV) was co-transfected in WT HCT116 with homologous recombination (HR) or NHEJ GFP reporter constructs that restore GFP fluorescence if they are repaired (Seluanov et al., 2010). Expression of SPARCLE 275 significantly reduced HR by ~50% and NHEJ by ~35% (Figure 6E). Thus, SPARCLE reduced both HR- and NHEJ-mediated DNA repair.

**SPARCLE enhances caspase-3-mediated cleavage of PARP-1**

To understand how SPARCLE interaction with PARP-1 inhibits DDR, we first examined whether SPARCLE KO affects expression or cleavage of PARP-1 under basal conditions 24 and 48 h after inducing DSB by adding NCS to HCT116 or DOX to A549 and HepG2. PARP-1 cleavage is a hallmark of apoptosis (Figure 7A). Activated caspase-3 and caspase-7 cleave 116-kDa FL PARP-1 into 24-kDa NT and 89-kDa CT domains, inactivating PARP-1 by separating the NT DNA-binding domain from the auto-activation and catalytic domains (Chaitanya et al., 2010; D’Amours et al., 2001; Smulson et al., 1998). Without DNA damage, PARP-1 was expressed comparably in SPARCLE KO and WT cells, and cleaved PARP-1 was not detected (Figures 7B and S5A). After DNA damage, a PARP-1 immunoreactive band was detected of the size expected for NT-PARP1 after caspase cleavage. NT-PARP1 was detected at 24 h but increased after 48 h, paralleling changes in SPARCLE expression. SPARCLE KO cells had no apparent change in FL PARP-1 compared with WT cells but significantly less cleaved NT-PARP1, suggesting that SPARCLE promotes PARP-1 cleavage (Figures 7B and S5A). To confirm that SPARCLE increases PARP-1 cleavage, we ectopically expressed SPARCLE 275 or EV in WT and SPARCLE KO HCT116 cells (Figure 7C). As in untransfected cells, NT-PARP1 was significantly reduced in SPARCLE KO compared with WT cells after EV transfection. However, ectopic SPARCLE 275 greatly increased NT-PARP1 in both WT and SPARCLE KO HCT116. Similar results were obtained in WT and SPARCLE KO A549 and HepG2 cells transfected with SPARCLE 275 but no increase in NT-PARP1 was observed in cells that expressed ELCRAPS (Figure S5B). Thus, SPARCLE enhances PARP-1 cleavage. Reduced PARP-1 cleavage and apoptosis after DNA damage in SPARCLE KO cells could be caused by reduced caspase-3 activity. However, although p53 KO reduced caspase-3 activity 48 h after adding NCS, SPARCLE KO had no effect on activated caspase-3 (Figure S5C), indicating that SPARCLE’s effect on PARP-1 cleavage was not due to a change in caspase-3 activation.

To investigate whether enhanced PARP-1 cleavage was a direct or indirect effect, R-PARP1 and active caspase-3 (R-caspase-3, recombinant caspase-3) (Figure S5D) were incubated for 10 min with in vitro transcribed SPARCLE 275 or ELCRAPS (Figure S5E). Because cells express few copies of SPARCLE, to mimic cellular conditions SPARCLE was added at very low concentrations ranging from 1/100th to 1/1,000th the molar concentration of PARP1. Even at the lowest concentration, SPARCLE dramatically enhanced PARP-1 cleavage, while ELCRAPS was inactive (Figure 7D). To determine whether the first 275 nt of SPARCLE were essential to enhance PARP-1 cleavage, we generated truncated in vitro transcribed RNA versions of SPARCLE encoding its first 75 nt (SPARCLE 75) or first 178 nt (SPARCLE 178) (Figure S5E). Although NT-PARP1 was readily detected after a brief incubation of FL PARP1 with SPARCLE 275 and caspase-3, no cleaved PARP1 was detected when SPARCLE 75, SPARCLE 178, or ELCRAPS substituted for SPARCLE 275 or when caspase-3 was omitted (Figure 7E). Thus, most of the 5’-end of SPARCLE 275 is needed to enhance caspase-3-mediated PARP-1 cleavage.

To investigate if PARP1 catalytic activity is important for SPARCLE function, apoptosis was analyzed in cells treated with the PARP inhibitor Olaparib, added at the same time as NCS. PARP-1 inhibition did not restore apoptosis in SPARCLE KO or p53 KO HCT116 to WT levels (Figure S5F). These data suggest that the main mechanism by which SPARCLE increases apoptosis is by enhancing PARP1 cleavage by caspase-3, rather than by directly altering its catalytic activity.

**NT-PARP-1 interferes with DNA repair and restores DNA-damage-induced apoptosis in SPARCLE KO cells**

Because caspase-3 separates the DNA-binding and catalytic domains of PARP1, we hypothesized that NT-PARP1 would bind to DNA breaks without recruiting DNA repair factors and thus block DNA repair. In support of this hypothesis, two studies showed that NT-PARP1 binds to DNA breaks and decreases DSB repair in vitro (Smulson et al., 1998; Yung and Satoh, 2001). SPARCLE’s enhancement of PARP1 cleavage to...
Figure 7. SPARCLE enhances caspase-3 cleavage of PARP-1 to inhibit DNA repair and increase apoptosis after DNA damage
(A) PARP-1 domains. Fl-Fill are zinc-finger domains. Arrow shows the caspase-3/7 cleavage site.
(B) PARP-1 immunoblot of lysates from untreated and NCS-treated WT and SPARCLE KO HCT116. α-tubulin probed as loading control. Representative blot (top); densitometry quantification of 3 blots (bottom). FL, full-length; NT, N-terminal
(C) PARP-1 immunoblot of lysates from WT and SPARCLE KO HCT116 cells transfected with empty vector or SPARCLE 275. α-tubulin probed as loading control. Representative blot (top); densitometry quantification of 3 blots (bottom).

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generate more NT-PARP-1 would then lead to more unrepaired DNA damage and thereby increase apoptosis. To test this hypothesis, the effect of NT-PARP-1 ectopic expression on DNA repair and apoptosis in WT and SPARCLE KO cells was analyzed (Figures 7F–7H). Modest PARP-1–NT OE, which did not alter FL PARP-1 levels (Figure 7F), increased TUNEL (Figure 7G) and annexin V (Figure 7H) staining similarly in WT and SPARCLE KO cells under basal conditions suggesting that NT-PARP-1 interfered with repair of endogenous DNA damage, such as occurs during DNA replication. As expected, NCS treatment of EV-transfected cells led to significantly less TUNEL staining and apoptosis in SPARCLE KO than WT cells. However, these differences became insignificant in cells transfected to express NT-PARP-1. Because NT-PARP-1 expression rescues SPARCLE deficiency, the main mechanism by which SPARCLE inhibits DNA repair and increases apoptosis is likely by enhancing caspase-3 cleavage of PARP-1.

**SPARCLE KO tumors are relatively resistant to chemotherapy**

Our model suggests that SPARCLE KO cells will be relatively resistant to DNA damage because they more efficiently repair damage and are less likely to undergo apoptosis. To test SPARCLE’s *in vivo* importance, WT or SPARCLE KO HCT116 (3 × 10^6 cells/mouse) were implanted subcutaneously into nude mice (10 mice/group) and 2 weeks later, when tumors were clearly palpable, mice were treated weekly intraperitoneally with DOX (Figure 7I). SPARCLE KO had no significant effect on tumor size before chemotherapy or for the first 3 weeks of chemotherapy, suggesting that SPARCLE KO cells did not affect tumor cell proliferation or survival in the absence of therapy. However, after 3 weeks of treatment, SPARCLE KO tumors became significantly larger. Because of tumor size, mice had to be sacrificed 40 days after implantation and 4 days after the fourth DOX dose. At sacrifice, SPARCLE KO HCT116 xenografts had reduced TUNEL staining compared with WT xenografts (Figure 7J). Thus, SPARCLE inhibits DNA repair and promotes apoptosis after DNA damage *in vivo* and is expressed at such low levels and so late after DNA damage that it has not been clearly annotated in the transcriptome. It also was not identified as a p53-induced IncRNA in studies that screened for p53-induced ncRNAs and uncovered p53-regulated lncRNAs including *lincRNA-p21* (Huarte et al., 2010), *TP53TG1* (Diaz-Lagaras et al., 2016), *MEG3* (Zhu et al., 2015), *Neat1* (Adriaens et al., 2016), *DDSR1* (Sharma et al., 2015), *DINO* (Schmitt et al., 2016), *LINP1* (Zhang et al., 2016), *PURPL* (Li et al., 2017), *PINC* (Chaudhary et al., 2017), and *GUARDIN* (Hu et al., 2018).

It is surprising that a poorly expressed IncRNA so potently affects cell death. However, the mechanism of action for SPARCLE acting as a cofactor for caspase-3 cleavage of PARP-1 is strongly supported by the *in vitro* cleavage experiment with purified active caspase-3 and PARP-1 and *in vitro* transcribed SPARCLE. Addition of SPARCEL to WT HCT116 lysates probed for FL (endogenous) and NT- (exogenous) PARP-1 cleavage and increases in WT HCT116 lysates probed for FL (endogenous) and NT- (exogenous) PARP-1 cleavage and increases 1 day after DNA damage is triggered, we speculate that SPARCLE’s role is to promote DNA damage repair, as has been previously suggested (D’Amours et al., 2001; Smulson et al., 1998). Unrepaired DNA breaks then trigger apoptosis. SPARCLE reduces p53-mediated apoptosis and increases G1 arrest. Because SPARCLE only begins to be expressed 1 day after DNA damage is triggered, we speculate that SPARCLE’s main mode of action. PARP-1 cleavage helps maintain ATP levels, which would otherwise become depleted secondary to NAD+ depletion during PARylation. Cells need ATP to undergo apoptosis rather than die by necrosis (Gibson and Kraus, 2012; Li and Yu, 2015; Schreiber et al., 2006). However, the potent pro-apoptotic effect of NT-PARP-1 in the setting of DNA damage, which we demonstrate here, has not been fully appreciated (Wang et al., 2018). Our model is that cleaved NT-PARP-1 binds to DNA, but because it lacks the other PARP-1 domains, fails to recruit DNA repair factors to DNA breaks and consequently blocks DNA damage repair, as has been previously suggested (D’Amours et al., 2001; Smulson et al., 1998). Unrepaired DNA breaks then trigger apoptosis. SPARCLE reduces p53-mediated apoptosis and increases G1 arrest. 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to bring PARP-1 and caspase-3 together and promote its cleavage. Cells are estimated to contain \(-2 \times 10^9\) PARP-1 molecules/cell \((\text{Liu et al., 2017})\). Our data suggest that SPARCLE is about ten thousand times less abundant after DNA damage \(\sim 8-16\) copies/cell than PARP-1. Thus, the 1:1,000 SPARCLE:PARP-1 molar ratio used in our \textit{in vitro} cleavage experiment may come close to the physiological ratio. The strong nonmolar affinity we measured for the PARP-1:SPARCLE interaction may be important for its potent biological effect despite its low expression. Although SPARCLE binds to both FL and NT-PARP-1, our model is that once cleaved, NT-PARP-1 detaches allowing another FL molecule to bind. We are unaware of any previous example of an lncRNA acting as a protease cofactor.

lncRNAs have previously been shown to act as protein scaffolds \((\text{Chu et al., 2011})\). However, most of the examples of lncRNA scaffolds involve assembling protein complexes on chromatin \((\text{examples are the telomerase RNA TERC and HO-TAIR and ANRIL that interact with polycomb repressor complexes})\) or on RNA that take advantage of sequence complementarity to enhance binding. However, lncRNAs can also bind to proteins independently of interactions with other nucleic acids. One lncRNA example is \textit{NKILA} the cytoplasmic lncRNA that binds to the NF-\textit{k}B/I\textit{k}B complex and stabilizes it by masking I\textit{k}B phosphorylation sites \((\text{Huang et al., 2018})\).

After DSB induction, \textit{SPARCLE}-deficient miR-34b/c KO, p53RE2 KO, and \textit{SPARCLE} KO HCT116 arrested in G1, while p53 KO HCT116 arrested at G2/M \((\text{Figures 1G, 3C, and 4D})\). p53 activation can cause both G1 and G2/M arrest, which it does by regulating many genes. One of the most important p53 induced genes is \textit{CDKN1A} which encodes for p21, which inhibits CDK4/6 that plays an important role in progression from G1. \textit{SPARCLE} mostly affects cell death by interfering with DNA repair. Unrepaired DSBs in \textit{SPARCLE} sufficient cells are expected to trigger the ATR response and activate CHK1 to cause G2/M arrest. However, in the absence of \textit{SPARCLE}, this checkpoint would not be as strongly induced, but other p53-induced checkpoints could become more prominent. p53-induced genes other than \textit{SPARCLE}, such as \textit{CDKN1A}, are likely responsible for the difference in cell-cycle arrest between p53 KO cells and miR-34b/c KO, p53RE2 KO, and \textit{SPARCLE} KO cells.

The \textit{PARP-1} cleavage promoting activity of the \(\sim 770\) nt lncRNA was contained within its 5’ 275 nt sequence and required more than the first 178 5’ sequence. Even though we found that the first 275 nt are critical for \textit{PARP-1} cleavage, it is possible that the rest of the lncRNA may also have a functional role. Future work will need to identify the critical residues and two-dimensional and three-dimensional structures important for \textit{SPARCLE} binding and function. Although a few \textit{SPARCLE} SNPs have been reported, it is unknown whether they have functional consequences or might be linked to diseases such as cancer. Based on the strong pro-apoptotic effect of \textit{SPARCLE} post-DNA damage, one might expect that \textit{SPARCLE} expression might be suppressed by mutation or promoter methylation in p53-sufficient cancers or that \textit{SPARCLE} suppression might occur in chemotherapy or irradiated tumors as a mechanism of resistance. It will be worthwhile to look for a link between \textit{SPARCLE} and prognosis and drug resistance of p53-sufficient tumors. It is also possible that \textit{SPARCLE} has other activities, such as in splicing, based on the candidate RAP-MS-interacting proteins, which we did not investigate. Future studies could investigate some of the other potential \textit{SPARCLE}-interacting proteins that were detected in the \textit{SPARCLE} pull-down, which we did not pursue in this study.

The expression of the miR-34b/c cluster and \textit{SPARCLE} are closely linked. They both use the same p53RE in their promoter and may even be expressed in the same primary transcript. The primary transcript of the miR-34b/c cluster has not been defined. Since deletion of miR-34b and miR-34c also reduced \textit{SPARCLE} expression \((\text{Figure 2A})\), but the converse was not true \((\text{deletion of 253 nt of \textit{SPARCLE} did not delete mature miR-34b or miR-34c})\) \((\text{Figures 4B, S3A, and S3B})\), one intriguing possibility is that \textit{SPARCLE} and these miRNAs are transcribed on the same primary transcript transcript that is processed by DROSHA into \textit{SPARCLE} (or a longer precursor) and a pre-miRNA encoding miR-34b and miR-34c. Such a model would explain its low copy number and the fact that \textit{SPARCLE} is 5’-capped but likely does not have a poly(A) tail. Processing of a shared primary transcript or the stability of each of its products might be regulated after genotoxic damage since miR-34b/c are both similarly expressed at 24 and 48 h, while \textit{SPARCLE} expression greatly increases between 24 and 48 h \((\text{Figures 2A and S1A})\). However, additional work is needed to define the biogenesis of \textit{SPARCLE} and its potential link to miR-34b/c.

Limitations of the study

\textit{SPARCLE}’s low copy number makes it challenging to study. For example, attempts to use smFISH to colocalize \textit{SPARCLE} with other factors \((\text{for example with \textit{PARP-1} or DNA repair foci})\) were unsuccessful. Therefore, we were unable to verify that \textit{SPARCLE} reduced the recruitment of DNA repair factors to DNA-damage sites. Although \textit{SPARCLE} localized mostly to the nucleus, we do not know if \textit{SPARCLE} catalyzes \textit{PARP-1} cleavage by caspase-3 \((\text{which has been found in the nucleus})\) \((\text{Kamada et al., 2005; Luo et al., 2010})\) when \textit{PARP-1} is bound to DNA breaks or in solution. Structural studies of \textit{SPARCLE} interacting with \textit{PARP-1} and caspase-3 could lead to a better understanding of \textit{SPARCLE}’s role in promoting caspase-3 cleavage of \textit{PARP-1}. The formation of a stable complex might require using a catalytically dead mutant of caspase-3 or RNase-resistant \textit{SPARCLE}. The \textit{SPARCLE} primary transcript remains to be defined. One attractive hypothesis that could be explored using \textit{Drosha} and/or \textit{Dicer} KO cell lines is that \textit{SPARCLE} and miR-34b/c are transcribed as one transcript that is processed by the RNAi machinery.

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Supplemental information can be found online at https://doi.org/10.1016/j.molcel.2022.01.001.

ACKNOWLEDGMENTS
We thank Dipanjan Chowdhury (Dana Farber Cancer Institute) for NHEJ antibodies, Vera Gorbonuova (University of Rochester) for DSB repair reporter plasmids, David Valle-Garcia (National University of Mexico) for bioinformatic advice, and Jonathan Salazar-León (National University of Mexico) for assistance with the graphical abstract using BioRender. This work was supported by NIH R01DA039566 (J.L.), Pew Latin American Fellowship in the Biomedical Sciences and Mexican National Council of Science and Technology (CONACyT) Postdoctoral Fellowship (CVU: S31537) (K.F.M.-S.), Glaxo Smith Kline-Immune Disease Institute Alliance Fellowship (F.N.), CR1 Irvington Postdoc-
toral Fellowship (R.M.), US Department of Defense Breast Cancer Breakthrough Fellowship (Y.Z.), DQAPA-PAEPIT In213119 (L.P.-M.) and In211719 (G.P.-A.) Intramural Research Program of the National Cancer Institute (NCI), Center for Cancer Research (CCR), NIH (AL), and CONACyT grant IFC 2016-2282 (L.P.-M.). K.F.M.-S. is member of the Mexican National System of Researchers (SNI).

AUTHOR CONTRIBUTIONS

DECLARATION OF INTERESTS
The authors declare no competing interests.

Received: February 5, 2020
Revised: January 4, 2022
Accepted: January 4, 2022
Published: January 31, 2022

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## REAGENT OR RESOURCE

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### Critical commercial assays

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### Reagent or Resource Source Identifier

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### Deposited data

- Original images for Western blots and microscopy (Mendeley dataset)
  - This study
  - [https://doi.org/10.17632/wx7t4dyjm9.1](https://doi.org/10.17632/wx7t4dyjm9.1)
- RNA-seq
  - This study
  - GEO: GSE144510

### Experimental models: Cell lines

| Human cell line: A549                                         | ATCC                          | Cat# CCL-185; RRID: CVCL_0023 |
| Human cell line: HCT116                                        | ATCC                          | Cat# CCL-247; RRID: CVCL_0291 |
| Human cell line: HCT116 p53-/- (Bunz et al., 1999)             | ATCC                          | N/A                          |
| Human cell line: HEK-293T                                       | ATCC                          | Cat# CRL-3216; RRID: CVCL_0063|
| Human cell line: HepG2                                         | ATCC                          | Cat# HB-8065; RRID: CVCL_0027 |
| Human cell line: RKO                                          | ATCC                          | Cat# CRL-2577; RRID: CVCL_0504|

### Experimental models: Organisms/Strains

- **nu/nu mice**
  - The Jackson Laboratory
  - Cat# 007850

### Oligonucleotides

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**RESOURCE AVAILABILITY**

**Lead contact**
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Judy Lieberman, judy.lieberman@childrens.harvard.edu.

**Materials availability**
All unique/stable reagents generated in this study will be made available on request to the lead contact but may require a completed Materials Transfer Agreement.

**Data and code availability**
- The accession number for RNA-seq data reported in this paper is GEO: GSE144510. The Mendeley dataset associated to this study containing original Western blot and microscopy images can be found at DOI: https://doi.org/10.17632/wx7t4dyjm9.1
- No original code was generated in this study.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Cell lines and culture conditions**
The human colorectal cancer HCT116, human lung adenocarcinoma A549, human hepatocellular carcinoma HepG2, human colon carcinoma RKO and HEK-293T cell lines were obtained from ATCC. p53 hypomorphic HCT116 (Bunz et al., 1999), called p53 KO in...
this manuscript, were a kind gift of Dr. Bert Vogelstein, Johns Hopkins University, Baltimore, MD. Cells were cultured at 37°C in 5% CO₂ in Dulbecco’s modified Eagle’s medium (DMEM) (ThermoFisher Scientific) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin G, 100 μg/mL streptomycin sulfate, 6 mM HEPES, 1.6 mM L-glutamine and 50 μM 2-mercaptoethanol (2-ME).

**Animal experiments**

Mouse experiments were conducted in the Harvard Medical School Animal Facility using protocols approved by the Harvard Medical School Institutional Animal Care and Use Committee (IACUC). 6-weeks-old male nuf/nuf mice (Jackson Laboratories) were subcutaneously inoculated in the flank with 3x10⁶ WT or SPARCLE KO HCT116 cells. Beginning fourteen days later, animals received 8 mg/kg doxorubicin (DOX) intraperitoneally weekly for four weeks. Tumor size was monitored every other day. Mice were sacrificed when any tumor in the experiment reached the maximal allowable size (100 mm³). Excised tumors were fixed in 4% formaldehyde for 24 hr at room temperature and embedded in paraffin.

**METHOD DETAILS**

**Cell treatments**

Cells were seeded (2.5x10⁵ cells/well) in 6-well plates overnight before each treatment. Treatment was with medium or 200 ng/mL neocarzinostatin (NCS), 1 μM doxorubicin (DOX), 50 μM nutlin-3, 4 Gy ionizing radiation (IR) administered with an RS 2000 irradiator (RadSource), 100 nM CPT or 15 μM sodium meta-arsenite. Unless otherwise indicated, treated cells were analyzed 48 hr later.

**RNA extraction**

RNA, isolated using TRIRizol reagent (15596026, Thermo Fisher Scientific), was treated with the TURBO DNA-free kit (AM1907, Thermo Fisher Scientific) following the manufacturer’s directions. RNA concentration was determined by Nanodrop (Thermo Fisher Scientific).

**qRT-PCR**

For small RNAs, reverse transcription (RT) reactions were performed using the TaqMan® MicroRNA Reverse Transcription Kit (4366597, ThermoFisher Scientific) in combination with the TaqMan® MicroRNA Assay (ThermoFisher Scientific) specific for each small RNA (hsa-miR-34a-5p, hsa-miR-34b-3p, hsa-miR-34b-5p, hsa-miR-34c-5p, U6). Total RNA (100 ng) was used for each RT reaction, performed according to the manufacturer’s protocol. RT negative controls without enzyme or RNA were analyzed in parallel. qPCR reactions were performed using 1 μL cDNA, the specific forward primer included in the TaqMan MicroRNA Assay and the TaqMan® Universal PCR Master Mix no AmpErase® UNG (4364341, ThermoFisher Scientific) following the manufacturer’s protocol using a Bio-Rad CFX96 qPCR instrument.

For larger RNAs DNase-treated total RNA (500 ng) was used to generate cDNA using the SuperScript IV VILO Master Mix (11756050, Thermo Fisher Scientific), which contains both oligo dT and random hexamers. The PCR protocol suggested for the SsoFast EvaGreen Supermix reagent (1725204, Bio-Rad) was followed using a final volume of 20 μL, 1 μL of cDNA and 500 nM primers. All primers were designed to work using a Tm of 60°C. Primers used are listed in Table S3.

**Cell cycle analysis**

Treated and untreated cells were trypsinized 48 hr after genotoxic stress, washed once with 1X PBS and then permeabilized using 70% ethanol at -20°C for 1 hr. Cells were centrifuged at 5,000 rpm at room temperature for 2 min and pellets were washed twice with 1X PBS and resuspended in 100 μL 1X PBS. 1 μL of RNase A (EN0531, ThermoFisher Scientific) was added and cells were incubated at room temperature for 30 min before adding propidium iodide (PI) and incubating at room temperature for 1 hr. After adding 300 μL of 1X PBS to each sample, stained cells were analyzed on a FACSCanto II flow cytometer using FlowJo software (BD Biosciences). Differences between cell cycle profiles were analyzed by one-way ANOVA using the Holm-Sidak method and Prism software (Graphpad).

**Annexin V staining**

Treated and untreated cells were trypsinized 48 hr after treatment, washed once with 1X PBS and resuspended in 100 μL 1X Annexin V binding buffer (BD Biosciences), 4 μL Annexin V AlexaFluor647-conjugated antibody (A23204, ThermoFisher Scientific) and 1.8 μL LIVE/DEAD fixable violet dead cell dye (L34963, ThermoFisher Scientific). After incubation at room temperature for 30 min, 300 μL 1X Annexin V binding buffer was added to each sample. Stained cells were analyzed on a FACSCanto II flow cytometer using FlowJo software.

**miRNA mimics and plasmid transfection**

24 hr before any treatment, cells were transfected with 50 nM of the corresponding miRNA mimics (Dharmacon) or with 1 μg of the indicated plasmid using Lipofectamine 2000 (11668019, ThermoFisher Scientific) in Opti-MEM I reduced serum medium (11058021, ThermoFisher Scientific) following the manufacturer’s suggested protocol.
Gene knockdown
24 hr before DOX treatment, cells were reverse-transfected with 20 nM of either TP53 SMARTpool siRNAs (L-003329-00-0005, Dharmacon) or the AllStars Negative Control siRNA (1027281, Qiagen) as a negative control using Lipofectamine RNAiMAX (13778075, ThermoFisher Scientific) in Opti-MEM I reduced serum medium (11058021, ThermoFisher Scientific) following the manufacturer’s suggested protocol.

5' and 3' rapid amplification of cDNA ends (RACE)
5' and 3' RACE were performed using 1 μg of DNase-treated nuclear RNA from doxorubicin-treated HCT116 WT cells following the suggested protocols using the FirstChoice® RLM-RACE kit (AM1700, ThermoFisher Scientific). RACE products were purified with the QIAquick PCR purification kit (28104, Qiagen) and cloned using the CloneJET PCR cloning kit (K1231, ThermoFisher Scientific). Multiple colonies were picked and sequenced. Specific primers used for these experiments are listed in Table S3.

Circular RACE (cRACE)
20 μg of nuclear RNA from DOX-treated HCT116 WT cells was treated with TURBO DNase (AM2239, ThermoFisher Scientific) following the manufacturer’s directions. Half of the DNA-free RNA was treated with 2 U of tobacco acid pyrophosphatase (TAP) for 1 hr at 37°C to remove the 5’ cap, and the other half was treated the same except that TAP was not added. Reactions were then treated with 10 U of T4 RNA ligase (New England Biolabs) and 1 mM ATP in a final volume of 100 μl overnight at room temperature. RNA was ethanol precipitated, washed and resuspended in 20 μl of nuclease-free water. 3 μl of RNA were mixed with 1 μl of 10 mM deoxyribonucleotide triphosphates (dNTPs), 2 μl of the SPARCLE cRACE specific primer and 7 μl of nuclease-free water and then heated at 65°C for 5 min and cooled to 25°C. Then, reverse transcription was performed using SuperScript III Reverse Transcriptase (18080093, ThermoFisher Scientific) and 1 μl RNaseOUT (10777019, ThermoFisher Scientific) following the manufacturer’s protocol. 2 μl of this reaction was mixed with 1 μl of each PCR primer (10 mM), 6 μl of nuclease-free water and 10 μl of the 2X Phusion polymerase mix (New England Biolabs) and amplified as follows: one cycle at 98°C for 30 s, 35 cycles at 98°C for 10 s, 60°C for 10 s and 72°C for 10 s and one cycle at 72°C for 3 min. PCR products were purified with the QIAquick PCR purification kit (28104, Qiagen) and cloned using the CloneJET PCR cloning kit (K1231, ThermoFisher Scientific). Then, multiple colonies were picked and sequenced. Gene-specific primers used for cRACE are listed in Table S3.

Luciferase assays
Cells were transfected with 1 μg of the corresponding plasmid 24 hr before treatment using Lipofectamine 2000 (11668019, ThermoFisher Scientific) following the manufacturer’s suggested protocol. Treated and untreated cells were trypsinized 48 hr after genotoxic stress, washed once with 1X PBS and then lysed in 50 μl of 1X passive lysis buffer from the Dual luciferase reporter assay kit (E1910, Promega), incubated on ice for 15 min and centrifuged at 13,000 rpm at 4°C for 10 min. Supernatants were stored at -70°C before assaying luminescence of 2.5 μl of each sample using a Synergy 2 Microplate Reader (Biotek) following the manufacturer’s directions. For these assays, SPARCLE’s promoter was cloned into the pGL3-Basic plasmid (E1751, Promega) in forward and reverse orientation, while the deletion of individual p53REs was done by PCR and subcloning. Multiple clones were picked and verified by sequencing. Sequences of cloning primers are listed in Table S3.

Chromatin immunoprecipitation (ChIP)
Treated cells were fixed with 1% formaldehyde in fresh medium for 10 min at room temperature before adding 2.5 M glycerine to a final concentration of 0.125 M to quench the reaction with gentle shaking at room temperature for 5 min. Medium was removed and cells were washed once with ice cold 1X PBS. Then 1 ml of ice cold 1X PBS was added to each 10 cm plate (around 8-9 plates per condition to get 40x10^6 cells) and cells were scraped, collected and spun at 1,500 rpm at 4°C for 5 min. Cell pellets were resuspended in 1 ml of Solution I (10 mM Hepes pH 7.5, 10 mM EDTA, 0.5 mM EGTA and 0.75% Triton X-100) supplemented with protease inhibitors and 1 mM phenylmethylsulfonyl fluoride (PMSF) and the cOmplete protease inhibitor cocktail (4693159001, MilliporeSigma) and incubated at 4°C for 10 min with frequent turning. Nuclei were spun at 2,000 rpm at 4°C for 5 min, supernatants were discarded and pellets were gently resuspended in 1 ml of Solution II (10 mM Hepes pH 7.5, 200 mM NaCl, 1 mM EDTA and 0.5 mM EGTA) supplemented with PMSF and the protease inhibitor cocktail. Samples were then spun at 2,000 rpm at 4°C for 5 min, supernatants were discarded and pellets were resuspended in 1 ml of lysis buffer (25 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.1% SDS and 0.5% sodium deoxycholate) supplemented with PMSF and the protease inhibitor cocktail and incubated on ice for 10 min. DNA shearing was performed using a sonicator with an Amplitude of 30 and 26 pulses of 30 sec with 1 min of rest intervals. Lysates were transferred to 1.5 ml tubes and spun at 13,000 rpm for 4°C for 10 min to remove cellular debris. Supernatants (chromatin in solution) were transferred to new tubes and 40 μg of chromatin were diluted in lysis buffer supplemented with PMSF and protease inhibitors in a final volume of 400 μl. 4 μl (1%) was allocated as the Input and stored at -70°C until the reverse crosslinking step to free and purify DNA. For p53 ChIP, 4 μg of p53 antibody (DO-1, sc-126, Santa Cruz Biotechnology) was added to each tube of chromatin and incubated overnight at 4°C with rotation. Then, 40 μl of protein G Dynabeads (10003D, ThermoFisher Scientific) were added and incubated for 4 hr at 4°C with rotation. Beads were pelleted using a magnet and washed for 5 min with 1 ml of each of the following buffers at 4°C with rotation: 2 washes with ChIP Wash Buffer 1 (low salt buffer: 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8 and 150 mM NaCl), 1 wash with ChIP Wash Buffer 2 (high salt buffer: 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8
and 500 mM NaCl), 1 wash with ChIP Wash Buffer 3 (LiCl buffer: 0.25 M LiCl, 1% NP40, 1% sodium deoxycholate, 1mM EDTA and 10 mM Tris–HCl pH 8) and 1 wash with TE buffer (1 mM EDTA and 10 mM Tris–HCl pH 7.5). After discarding the TE supernatant, 100 µL of ChIP Elution Buffer (1% SDS and 0.1 M NaHCO₃) was added and samples were mixed well. Input samples were also mixed with 100 µL of ChIP Elution Buffer. ChIP and Input samples were incubated for 1 hr at 65°C with shaking (1,200 rpm). To reverse the cross-link and elute chromatin from the beads, 4 µL of 5 M NaCl (0.2 M final concentration) was added and incubated overnight at 65°C with shaking. Beads were magnetically pelleted and supernatants were transferred to new tubes before adding 1 µL of RNase A (T3018L, New England Biolabs) and incubating at 37°C for 30 min. Then, 5 µL of proteinase K was added and the mixture was incubated at 56°C for 1 hr. DNA was purified using the MinElute PCR purification kit (28004, Qiagen) using 40 µL of the kit’s elution buffer. DNA was stored at -20°C until 1 µL was used for qPCR. Primers used for this assay are listed in Table S3.

**SPARCLE Taqman qPCR**

DNase-treated total RNA (1 µg) was used to generate cDNA using the SuperScript IV VILO Master Mix (11756050, ThermoFisher Scientific), which contains both oligo dT and random hexamers, in a final volume of 20 µL following manufacturer’s protocol. qPCR was performed using the AmpliTaq DNA polymerase with 5’ 3’exonuclease activity (N8080166, Applied Biosystems) in a final volume of 20 µL of cDNA, 2 µM of each forward and reverse primer, 0.4 µM of SPARCLE Taqman probe, 0.4 µM of dNTPs, 50 units of AmpliTaq DNA polymerase, 1x PCR buffer with pre-added MgCl₂ and DNase/RNase-free distilled water up to 20 µL. The cycling parameters were 95°C for 3 min and 40 cycles of: 95°C for 15 s, 60°C for 1 min, using a Bio-Rad CFX96 PCR machine. To calculate absolute SPARCLE copy numbers, the Ct values, from equal amounts of total RNA, were used to calculate copies per µg of RNA by extrapolating the copy number from standard curves performed with known amounts of SPARCLE plasmid (1 to 625 copies). SPARCLE copies per µg were converted to copies per cell assuming 20 pg of total RNA per cell. The Taqman probe and the forward and reverse primers used for this assay are listed in Table S3.

**Cell fractionation**

Treated and untreated cells were trypsinized 48 hr after genotoxic stress, washed once with 1X PBS and then resuspended in 300 µL of 1X PBS plus 300 µL of 0.1% Igepal and immediately spun at 6,000 rpm for 15 s at room temperature. Supernatants were saved as cytoplasmic fractions and stored at -70°C until protein quantification or processed for RNA extraction using TRizol. Nuclear pellets were then gently resuspended in cold 0.05% Igepal and spun at 6,000 rpm for 15 s at room temperature. Supernatants were discarded to eliminate cytoplasmic contamination. For RNA extraction, TRizol (15596026, ThermoFisher Scientific) was directly added and samples were processed following manufacturer’s directions. To extract nuclear proteins, pellets were resuspended in 50 µL of lysis buffer supplemented with 1 mM PMSF and the cOmplete protease inhibitor cocktail (493159001, MilliporeSigma), incubated on ice for 15 min and spun at 13,000 rpm at 4°C for 10 min. Supernatants were saved as nuclear fractions and stored at -70°C until protein quantification.

**Immunoblot**

Cells were lysed in lysis buffer (50 mM Tris-Cl pH 7.4, 150 mM NaCl, 1% Triton X-100) supplemented with 1 mM PMSF and the cOmplete protease inhibitor cocktail (493159001, MilliporeSigma). Protein concentration was determined by using the Pierce BCA protein assay (ThermoFisher Scientific). 7 µg of protein in cell lysates was added to SDS loading buffer (50 mM Tris–HCl pH 6.8, 10% glycerol, 2% SDS, 1% 2-mercaptoethanol and 0.1% bromophenol blue), boiled for 5 min, analyzed by SDS-PAGE and transfer to Immobilon-P PVDF membranes (IPVH00010, MilliporeSigma), which were then blocked with 5% milk in TBS-T buffer (150 mM NaCl, 20 mM Tris–HCl pH 7.6, 0.05% Tween-20) for 1 hr before overnight incubation with primary antibody at 4°C. After three washes of 5 min with TBS-T, membranes were incubated with secondary antibody for 1 hr at room temperature and then washed three times with TBS-T before adding the SuperSignal West Femto maximum sensitivity substrate (34094, ThermoFisher Scientific) for chemiluminescent detection. Antibodies used were PARP-1 (B-10, sc-74470, Santa Cruz Biotechnology), Ku80/Ku86 (B-1, sc-5280, Santa Cruz Biotechnology), lamin A (E-1, sc-376248, Santa Cruz Biotechnology), caspase-3 ([ABM1C12], ab208161, Abcam), β-actin (JLA20-c, Developmental Studies Hybridoma Bank) and α-tubulin (T5168, Sigma).

**Single-molecule FISH (smFISH)**

Single-molecule RNA fluorescence in situ hybridization (FISH) for SPARCLE was performed using a pool of 23 Quasar® 570 single-labeled probes designed and purchased from Stellaris Biosearch Technologies following the manufacturer’s directions, except for substituting a 72 hr incubation time for the hybridization step. Cells were imaged using an Axiovert 200M microscope (Pan Apochromat, 1.4 NA: Carl Zeiss) at 63X. Images were analyzed with SlideBook 4.2 software (Intelligent Imaging Innovations). All images shown are representative of at least three independent experiments.

**SPARCLE truncations**

Full-length SPARCLE, SPARCLE fragments and the reverse sequence of the first 275 nt of SPARCLE (ELCRAPS) were amplified by PCR using the Phusion High-Fidelity DNA polymerase (M0530S, New England Biolabs) following the manufacturer’s protocol and subcloned into the pcDNA3.1 plasmid (V80020, ThermoFisher Scientific). Multiple clones were picked and verified by sequencing. Primer sequences are listed in Table S3.
PARP-1 N-terminal domain (PARP-1-NT) cloning
The PARP-1 N-terminal domain expression vector was generated by PCR amplification using the Phusion High-Fidelity DNA polymerase (M0530S, New England Biolabs) following the manufacturer’s protocol and subcloned into the NotI and XhoI sites of the pCDNA3.1(+)/myc-His A plasmid (V80020, ThermoFisher Scientific). Multiple clones were picked and correct cloning was verified by sequencing. Primer sequences are listed in Table S3.

Generation of miR-34b/c KO and p53RE2 KO HCT116 cells using TALENs
To remove miR-34b/c or p53RE2, TALENs were designed to disrupt the sequence of each of these loci in HCT116 cells. TALENs were generated using the TALE Toolbox kit (100000019, Addgene). Cells were transfected with 2 μg of each TALEN using Lipofectamine 2000 (11668019, ThermoFisher Scientific) and plated by limiting dilution in 100 mm dishes 48 hr post-transfection. Single clones were tested for miR-34b/c or p53RE2 sequence by qPCR and edited clones were verified by sequencing.

Generation of SPARCLE KO HCT116, A549 and HepG2 cells using CRISPR/Cas9
To generate SPARCLE KO HCT116 cells lacking the first 275 nt of SPARCLE, two sgRNAs (sgRNA 1 and sgRNA 2) flanking this genomic region were independently cloned into the pSpCas9n(BB)-2A-Puro (PX462) V2.0 plasmid (62987, Addgene).

For generating SPARCLE KO A549 and HepG2 cells, the lentiCRISPR v2 plasmid (52961, Addgene) was used to generate a single cut that leads to random and different-sized deletions within the SPARCLE’s region complementary to each designed sgRNA. Cells were infected (A549, HepG2) or co-infected (HCT116) with the corresponding lentivirus derived from the corresponding constructs in 35 mm dishes and selected with 2 μg/mL puromycin (P7255, Sigma) for two weeks and cloned by seeding one cell per well in 96-well plates. Clones were analyzed by sequencing and qPCR, and 2 or 3 independent clones were selected. Although two versions of the CRISPR/Cas9 system were used to generate SPARCLE KO cell lines in this study, 250-300 clones were screened for each CRISPR experiment and cell line, only 2-3 complete KO clones were found for each cell line. This suggests that the genomic locus of SPARCLE may have inherent unknown characteristics that make gene editing challenging within this region. sgRNA sequences are listed in Table S3.

RNA-seq libraries and sequencing
RNA from untreated or 48 hr DOX-treated WT, SPARCLE KO (KO), or SPARCLE KO over-expressing (OE) HCT116 cells was isolated following the TRIzol protocol (15596026, ThermoFisher Scientific). Illumina RNA-seq libraries were obtained from 500 ng of total RNA using the NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina (E7760S, New England Biolabs) following the manufacturer’s instructions. Libraries were quality controlled, pooled and sequenced in a NextSeq 550 using the high-output mode.

RNA antisense purification-mass spectrometry (RAP-MS)
Treated and untreated WT and SPARCLE KO HCT116 cells seeded in 150 mm plates were UV-crosslinked and processed for RAP-MS experiments 48 hr post-treatment as described (McHugh et al., 2015). Briefly, UV-crosslinked cells (40×10^6 per condition) were collected to prepare nuclear lysates. Lysates were pre-cleared and then, incubated with 5 μg of each of the three SPARCLE spanning biotinylated probes (Table S3) for 2 hr at 67°C with intermittent shaking at 1,100 rpm (30 s shaking, 30 s off). Lysates were incubated with pre-cleared, streptavidin-coated Dynabeads M-280 (11205D, ThermoFisher Scientific) for 30 min at 67°C with intermittent shaking (30 s shaking, 30 s off). Beads were washed and proteins were eluted using 125 U of benzamidine (71206, Millipore). Finally, proteins were precipitated using 10% trichloroacetic acid (TCA) overnight with gentle rotation. Next day, 40 μL of protein A or G Dynabeads (10001D/10009D, ThermoFisher Scientific) were added and incubated at 4°C for 1 hour. To wash unbound material, beads were pelleted at 2,500 rpm for 30 seconds and supernatant was removed. Beads were resuspended in 500 μL of RIP buffer and a total of three RIP washes followed by one 1X PBS wash, were performed. To isolate
coprecipitated RNAs, beads and inputs were mixed with TRIzol and total RNA was extracted following the manufacturer's protocol. SPARCLE KO cells were used as negative control for these experiments.

**TUNEL assay**
Untreated and 48 hr DOX-treated WT and SPARCLE KO HCT116 cells were processed following the protocol of the APO-BrdU TUNNEL Assay kit (A23210, ThermoFisher Scientific) and fluorescence of cells was analyzed on a FACS Canto II flow cytometer using FlowJo software.

**COMET assay**
WT and SPARCELKO HCT116 cells treated with DOX for indicated times were processed to perform alkaline COMET assay following the protocol of the CometAssay kit (4250-050-K, Trevigen). Fluorescence images were acquired using a Zeiss 800 laser scanning confocal microscope at 20x magnification and analyzed using Zeiss Zen software. At least 50 cells were analyzed for each condition. DNA damage in COMET assay images was analyzed using the OpenCомet plugin for ImageJ and reported as tail moment, which combines measurements of the amount of DNA in the tail with the distance it has migrated.

**HR and NHEJ reporter assays**
DNA double strand break (DSB) repair was assessed using HR and NHEJ reporter plasmids as described (Seluanov et al., 2010). Briefly, reporter plasmids, digested with HindIII (R0104, New England Biolabs), were co-transfected with 1 μg of the first 275 bp of SPARCLE (SPARCLE 275) expression plasmid into HCT116 WT cells as linear DNA using 0.5 μg of the linearized NHEJ reporter plasmid or 2 μg of the linearized HR reporter plasmid. Cells were analyzed on a FACSCanto II flow cytometer using FlowJo software 3 days later.

**Immunofluorescence**
WT or SPARCLE KO HCT116 (7x10⁵), seeded onto 18 mm circular glass coverslips (18CIR-1, ThermoFisher Scientific) were treated with indicated DNA damaging agents for 48 h and then washed once with 1X PBS and fixed with 3.7% formaldehyde at room temperature for 10 min. Cells were washed twice with 1X PBS and permeabilized with 0.5% Triton at room temperature for 10 min. Permeabilized cells were washed once with 1X PBS and then blocked using IF buffer (5% BSA, 0.1% Triton X-100 in PBS) at room temperature for 30 min. Primary anti-phosphohistone H2A.X (Ser139) (γH2A.X, 9718S, Cell Signaling) at 1:500 was incubated overnight at 4°C with gentle shaking. After 3 washes with 0.05% TBS-T, the goat anti-rabbit IgG (H+L) highly cross-absorbed Alexa Fluor 488 secondary antibody (A-11034, ThermoFisher Scientific) added at 1:1000 in DAPI-containing IF buffer was incubated at room temperature in the dark for 1 hr. Washed coverslips were mounted onto glass slides using VECTASHIELD antifade mounting medium (H-1000, Maravai LifeSciences). Fluorescence images were acquired using an Axiovert 200M microscope (Pan Apochromat, 1.4 NA; Carl Zeiss) and analyzed using SlideBook 4.2 software (Intelligent Imaging Innovations).

**In vitro transcription**
2 μg of linearized plasmid encoding full-length SPARCLE (SPARCLE 3K), the first 75 nt (SPARCEL 75), 178 nt (SPARCLE 178) or 275 nt (SPARCEL 275) of SPARCLE sequence or the reverse sequence of SPARCLE 275 or ELCRAPS were in vitro transcribed using the MEGAScript T7 transcription kit (AM1334, ThermoFisher Scientific) following the manufacturer’s directions, but with increased incubation time (6 hr).

**PARP-1 in vitro cleavage**
50 ng of human recombinant full-length PARP-1 protein (11040-H08B, Sino Biological) were incubated at 37°C for 10 min with 0.5 units of human recombinant cleaved Caspase 3 protein (ab52101, Abcam) and with the indicated amount of in vitro transcribed SPARCEL 75, SPARCEL 178, SPARCEL 275 or ELCRAPS. Cleavage products were detected by immunoblot using an antibody that recognizes full-length and N-terminal PARP-1 (B-10, sc-74470, Santa Cruz Biotechnology).

**Microscale thermophoresis (MST)**
Human recombinant PARP-1 (11040-H08B, Sino Biological) was incubated at 37°C for 30 min to achieve binding equilibrium. Reaction mixtures were taken up into MST capillaries and measurements were acquired using a Monolith NT.115 (NanoTemper Technologies). Data were fit using the Hill equation and K_D values were determined using the MO.Affinity analysis software (NanoTemper Technologies, Munich, Germany).

**PARP-1 inhibition**
Seeded HCT116 cells were incubated with medium or NCS and DMSO or 10 μM Olaparib (PARP-1 inhibitor) and analyzed 48 hr later for annexin V staining by flow cytometry.
Caspase-3 activity assay
To measure caspase-3 activity, cells were trypsinized 48 hr after indicated treatment, washed once with 1X PBS and resuspended in 500 μL of 1X PBS containing 0.5 μL of the CellEvent Caspase-3/7 green detection reagent (C10423, Thermo Fisher Scientific) and 1.8 μL LIVE/DEAD fixable violet dead cell dye (L34963, ThermoFisher Scientific). After 35 min incubation at 37°C, stained cells were analyzed on a FACSCanto II flow cytometer using FlowJo software.

TUNEL immunohistochemistry
Slides of paraffin-embedded sections of WT- and SPARCLE KO-HCT116 cells-derived tumors were assessed for TUNEL using the TUNEL assay kit-HRP-DAB (ab206386, Abcam) following the manufacturer’s protocol, except for substitution of xylene with Safe-Clear Xylene Substitutes (23-314629, Fisher Scientific) for sample rehydration and counterstaining. TUNEL signal was analyzed using an Eclipse TE300 inverted microscope (40X, Nikon).

QUANTIFICATION AND STATISTICAL ANALYSIS

Quantification
ImageJ software was used to quantify signal intensities of bands in western blots, the number of foci in immunofluorescence microscopy slides and TUNEL+ tumor cells in immunohistochemistry slides and the tail moment of COMET assays.

Statistics
Data are presented as mean ± standard error. Kolmogorov-Smirnov normality tests were applied to the data. For multiple paired comparisons, student’s t-tests were used to determine p-values, except for cell cycle analysis, survival curves and tumor size comparisons where differences were analyzed by one-way ANOVA using the Holm-Sidak method. QuickCalcs and Prism softwares (Graphpad) were used to perform all the statistical tests. p-values <0.05 were considered significant.

RNA-seq analysis
RNA-seq reads were aligned against the human transcriptome (Ensembl v85 annotation) with HISAT 2.02 using the following parameters: --no-unal --rna-strandness R. Alignments with QS <10 or falling within Encode blacklisted regions were eliminated. Coverage bigwig files were generated with the bamCoverage program from the Deeptools suite v3.3.0. Read count tables were obtained with featureCounts from the Rsmbred package v1.22.3 in a R 3.3.3 environment with the following parameters: allowMultiOverlap=T, largestOverlap=T, strandSpecific=2. The read count table was analyzed with edgeR v3.12.1. After deleting poorly expressed genes (cpm<1), calculating normalization factors, and estimating dispersion, differentially expressed genes between treated/untreated WT, KO and OE cells were calculated using glm modeling. A gene was considered to be differentially expressed if it had a log2 fold change > 1 and a FDR <1e-5.