

# Regulation of poly(A)-specific ribonuclease activity by reversible lysine acetylation

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## SUPPORTING TABLES

**Table S1:** Primers used for site-directed mutagenesis and sequencing

Primer name	Primer sequence with underlined mutation site 3'→5'
K220R PARN Fwd	CAGACTTTGAGCTGG <u>AGG</u> TATCCG
K220R PARN Rvs	AATGCCTTTCGGATA <u>CCT</u> CCAGCT
K243R PARN Fwd	GAGCGATATATAGTTATCAGC <u>AGA</u> GTAGATGAAGAAGAACGC
K243R PARN Rvs	GCGTTCTTCTTCATCTACT <u>TCT</u> GCTGATAACTATATATCGCTC
K250R PARN Fwd	GAAGAAGAACGC <u>AGA</u> AGAAGA
K250R PARN Rvs	CTGCTGCTCTCTTCT <u>TCT</u> GCGTTC
K499R PARN Fwd	GCTGTCAATACCAGC <u>AGA</u> TATGCA
K499R PARN Rvs	ATAGCTTTCTGCATA <u>TCT</u> GCTGGT
K566R PARN Fwd	GCTCCCAGCACAGTAGGA <u>AGG</u> AGAAATTTGAGTCCTAGTCAAGAGG
K566R PARN Rvs	CCTCTTGACTAGGACTCAAATTTCT <u>CCT</u> TCCTACTGTGCTGGGAGC
CMV sequencing Fwd	CGCAAATGGGCGGTAGGCGTG
PARN (bp1364- 1383) K499 & K566 sequencing Fwd	TCATGTGTCTGCCAGATCAA

**Table S2:** Primers used to generate and sequence CRISPR-induced KO cells

<b>Primer name</b>	<b>Explanation</b>	<b>Primer sequence 3'→5'</b>
PARN gRNA Fwd	Exon3 gRNA + BbsI	CACCGTTTGTTAATGCAGAGACTGA
PARN gRNA Rvs	Exon3 gRNA + BbsI	AAACTCAGTCTCTGCATTAACAAA
SIRT1 gRNA Fwd	Exon1 gRNA +BsmBI	CACCGCTCCCCGGCGGGGGACGACG
SIRT1 gRNA Rvs	Exon1 gRNA + BsmBI	AAACCGTCGTCCCCCGCCGGGGAGC
CRISPR U6 Fwd	Sequencing primer for gRNA insertion	ACTATCATATGCTTACCGTAAC
Primer 1 Fwd	PCR primer	CGACATTATAGAGGCACTGAATGA
Primer 1 Rvs	PCR primer	ACCTCTCTTCTGGAGTGTCAA
Primer 2 Fwd	PCR primer	TTTAGACGCTGGAAGTGCCT
Primer 2 Rvs	PCR primer	ACTGAAAATGCTAAACTTTCCCCAA

**Table S3:** Antibodies used for western blotting and immunoprecipitation

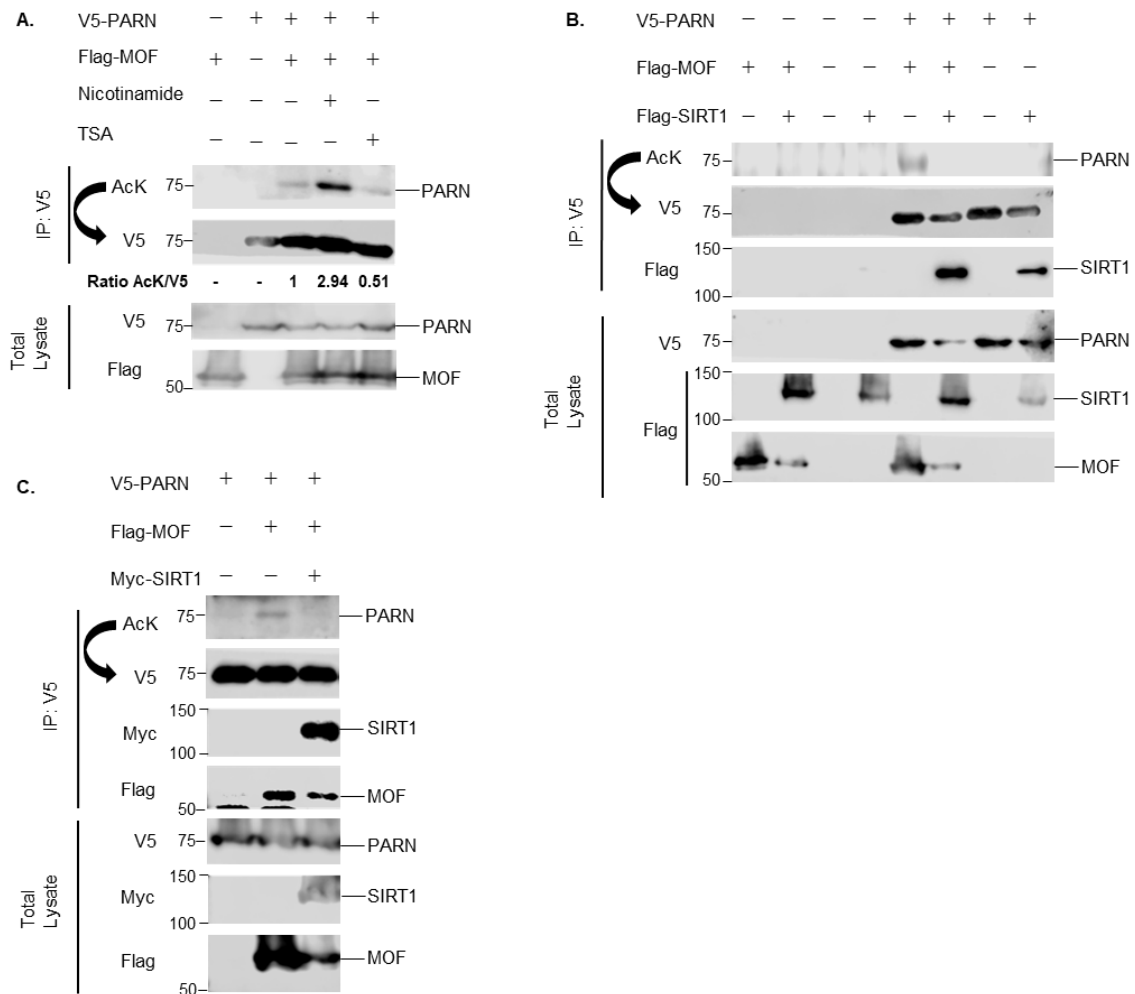
<b>Antibody name</b>	<b>Vendor, catalog number</b>	<b>Western blot dilution</b>
Acetyl-lysine (AcK)	Cell Signaling Technology, 9441s	1:500
V5	Thermo Fisher Scientific, MA5-15253	1:1000
Flag	Sigma-Aldrich, F1804	1:1000
HA	Sigma-Aldrich, H3663	1:1000
Myc	Cell Signaling Technology, 5605T	1:1000
PARN	Abcam, ab188333	1:1000
Vinculin	Santa Cruz Biotechnology, sc-73614	1:1000
B-Actin	Sigma-Aldrich, a1978	1:3000
SIRT1	Santa Cruz Biotechnology, sc-74504	1:1000
Secondary Mouse IgG HRP linked	GE Healthcare, NA931	1:5000
Secondary Rabbit IgG HRP linked	GE Healthcare, NA934	1:3000

**Table S4:** Primers used for RT-qPCR

<b>Primer name</b>	<b>Primer sequence 3'→5'</b>
PARN Fwd	CAGCAGAAACATGCCAAAGA
PARN Rvs	CCAAGAGTCTGGGGAAAACA
hTR Fwd	TTTGTCTAACCTAACTGAGAAGG
hTR Rvs	CTCTAGAATGAACGGTGGAAGG
snoRNA10 Fwd	CTCAGCTCCGCTTAACCACA
snoRNA10 Rvs	TGTCGTGCATTAGGAGAGCC
scaRNA8 Fwd	GGAGGCTGATACACAAATTG
scaRNA8 Rvs	GTATCTGTCCGTTACGATTTTC
scaRNA13 Fwd	ATACTCCAGAGGAAGCTGAG
scaRNA13 Rvs	TCTCGACTGCAAAGCTTCTC
GAPDH Fwd	CATGTTTCGTCATGGGTGTGAACCA
GAPDH Rvs	AGTGATGGCATGGACTGTGGTCAT

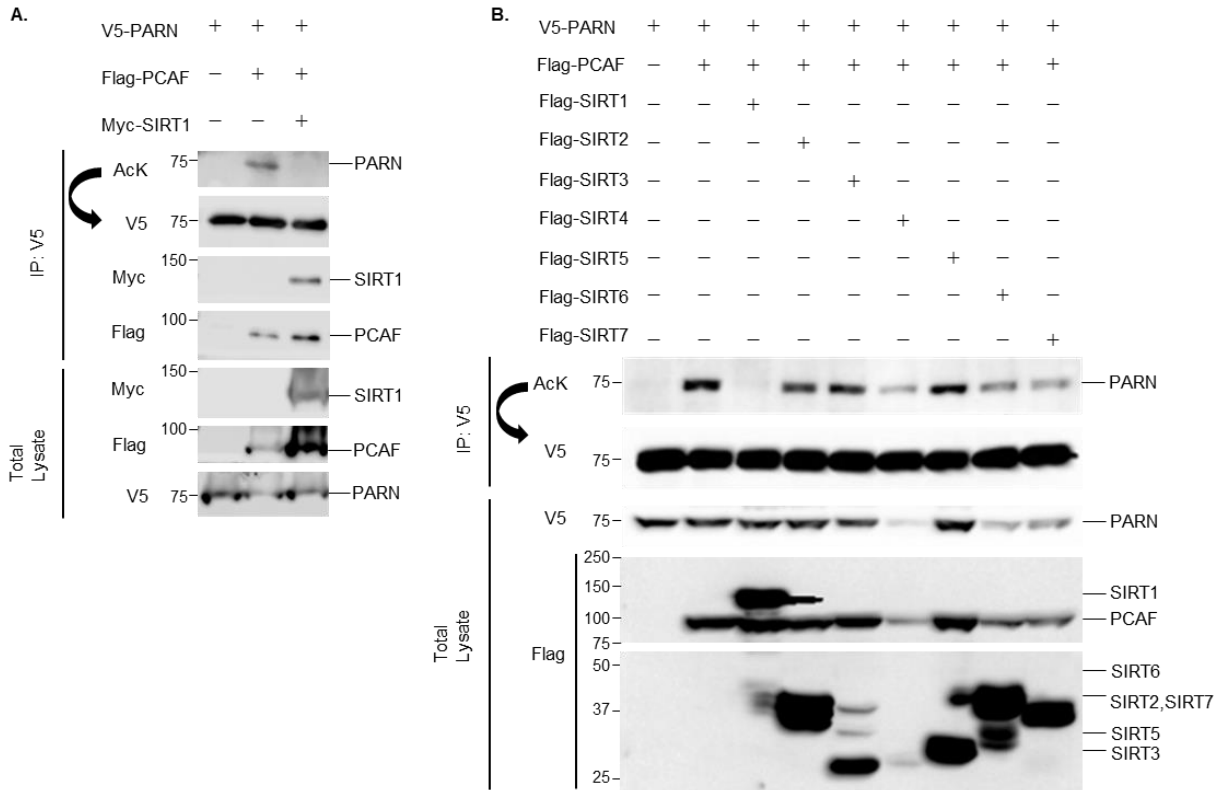
SUPPORTING FIGURES

Figure S1



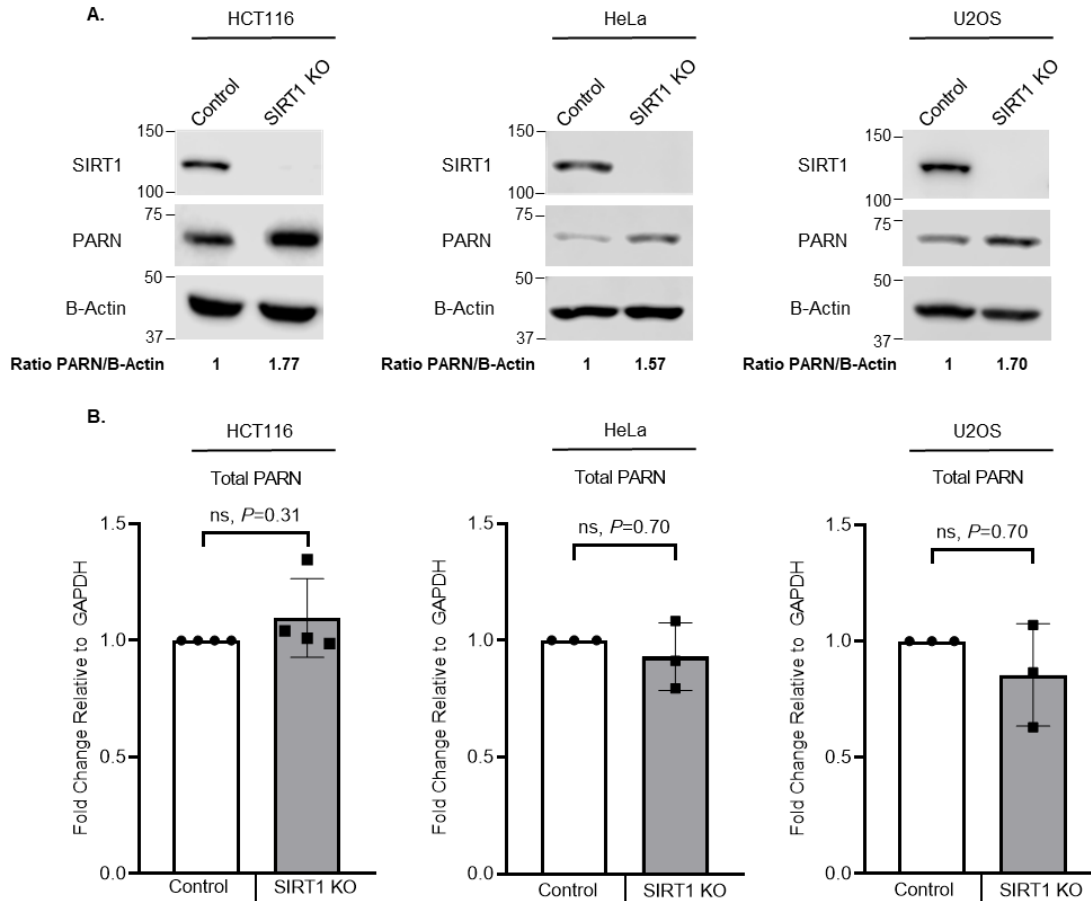
**Figure S1. SIRT1 deacetylates MOF-acetylated PARN.** (A-C) The indicated plasmids were transfected in 293T cells for 48 h. Protein extracts were immunoprecipitated with anti-V5 antibody and immunoblotted with a non-specific anti-acetyl lysine (AcK) antibody. Membranes were stripped under low stringency conditions and re-blotted using anti-V5 antibody. (A) Nicotinamide treatment enhances MOF-mediated PARN acetylation. V5-tagged PARN was co-transfected with Flag-tagged MOF in 293T cells for 48 h, and subsequently, cells were treated with different lysine deacetylase inhibitors for 18 h. Trichostatin A (TSA) is an inhibitor of class I and II lysine deacetylases, whereas nicotinamide inhibits the class III deacetylases, called sirtuins. The ratio of AcK to V5 was quantified and normalized to WT-PARN. (B) SIRT1 deacetylates MOF-acetylated PARN. V5-tagged PARN was co-transfected with Flag-tagged MOF and Flag-tagged SIRT1. (C) SIRT1 deacetylates MOF-acetylated PARN. V5-tagged PARN was co-transfected with Flag-tagged MOF and Myc-tagged SIRT1.

**Figure S2**



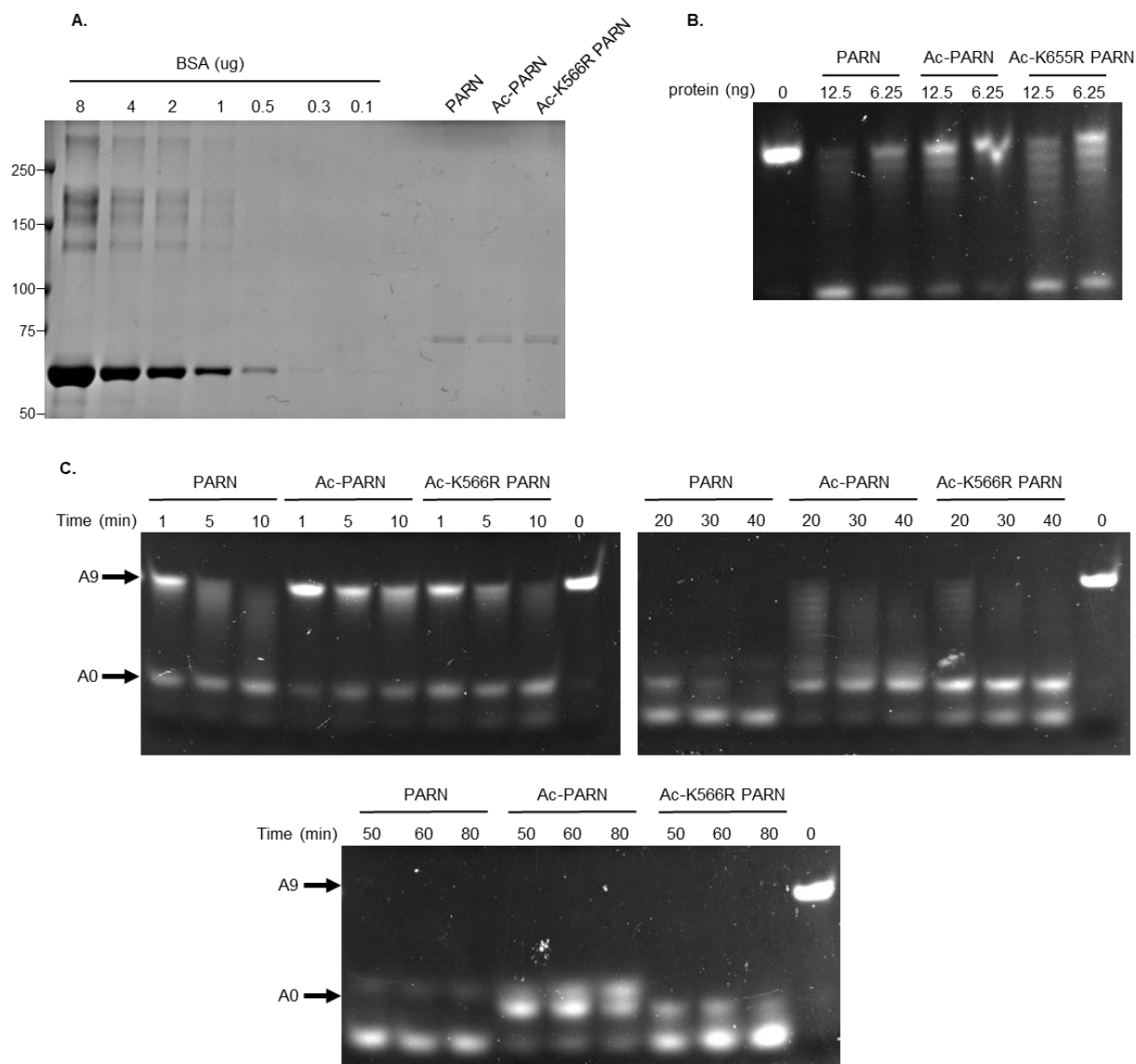
**Figure S2. SIRT1 deacetylates PCAF-acetylated PARN.** (A-B) The indicated plasmids were transfected in 293T cells for 48 h. Protein extracts were immunoprecipitated with anti-V5 antibody and immunoblotted with a non-specific anti-acetyl lysine (AcK) antibody. Membranes were stripped under low stringency conditions and re-blotted using anti-V5 antibody. (A) SIRT1 deacetylates PCAF-acetylated PARN. V5-tagged PARN was co-transfected with Flag-tagged PCAF and Myc-tagged SIRT1. (B) SIRT1, SIRT4, SIRT6 and SIRT7 deacetylate MOF-acetylated PARN. V5-tagged PARN was co-transfected with Flag-tagged PCAF and Flag-tagged SIRT1-7.

Figure S3



**Figure S3. SIRT1 loss increases PARN protein levels.** (A) Western blots of SIRT1 WT and SIRT1 KO HCT116, HeLa and U2OS cells, reveal that loss of SIRT1 increases PARN protein levels. The ratio of PARN to B-Actin was quantified and normalized to SIRT1 WT (control) cells. (B) RT-qPCR of HCT116 (n=4), HeLa (n=3) and U2OS (n=3) cells demonstrates that SIRT1 KO has no effect on PARN transcript levels. Data are presented as the mean  $\pm$  standard deviation. For statistical analyses, unpaired, two-sided Wilcoxon-Mann-Whitney tests were performed.  $P > 0.05$  was considered non-significant (ns).

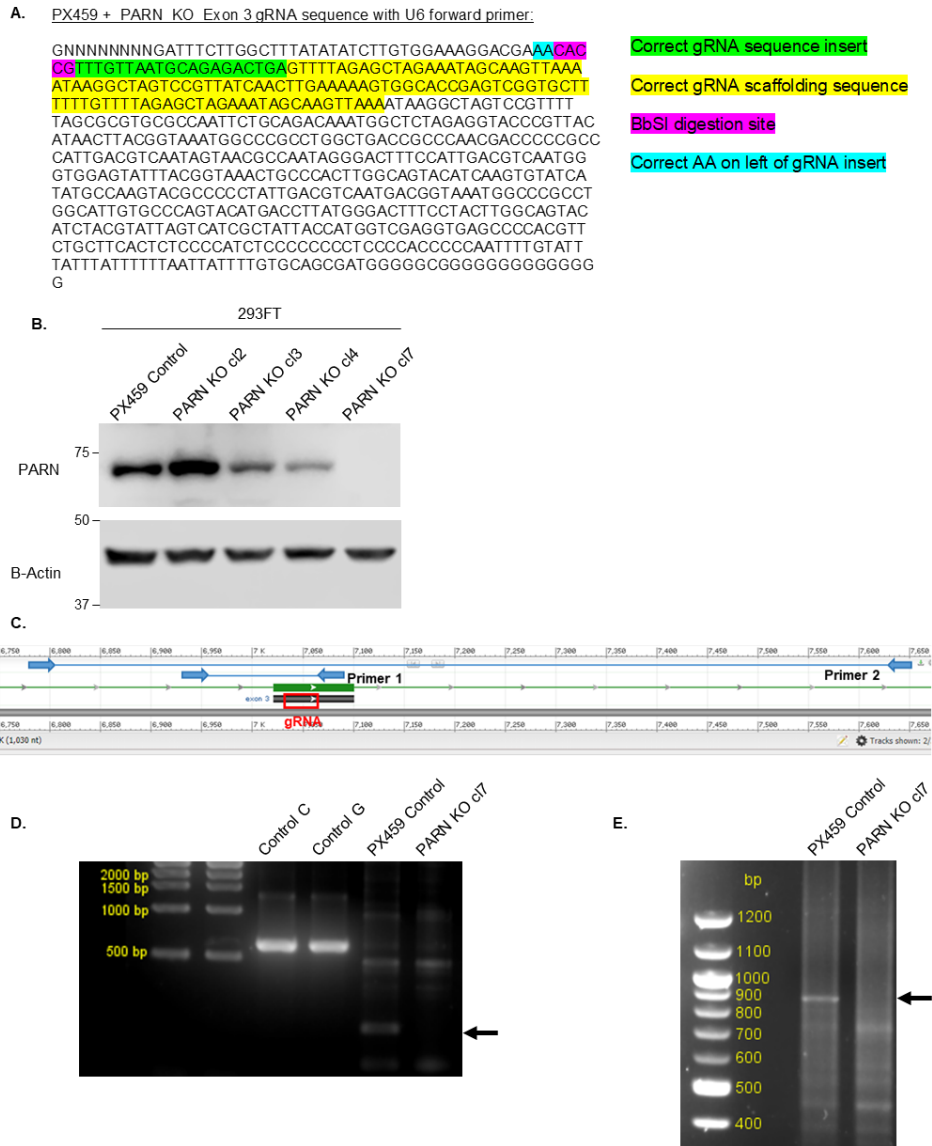
Figure S4



**Figure S4. Reducing PARN's acetylation rescues its enzymatic activity.** (A) Coomassie blue staining was used to determine concentrations of purified WT PARN (PARN), p300-acetylated WT PARN (Ac-PARN) and p300-acetylated K566R PARN (Ac-K566R PARN). (B) *In vitro* 3' exoribonuclease activity assay comparing the enzymatic activity of purified PARN, Ac-PARN and Ac-K566R PARN serial dilutions. Purified PARN, Ac-PARN and Ac-K566R PARN were incubated with 5' - fluorescein RNA probe at 30°C for 1 h. After the reaction was stopped, samples were loaded onto a 20% polyacrylamide/50% Urea/TBE gel. The same reaction mix without any protein was used as a control (0 ng). (C) *In vitro* activity time course assay comparing the enzymatic activity of PARN, Ac-PARN and Ac-K566R PARN. 5' - fluorescein RNA probe was incubated with 100 ng of purified PARN, Ac-PARN and Ac-K566R PARN at 30°C over the indicated time. After the reaction was stopped, samples were processed as described in (B). The same reaction mix without any protein was used as a control (0 min).

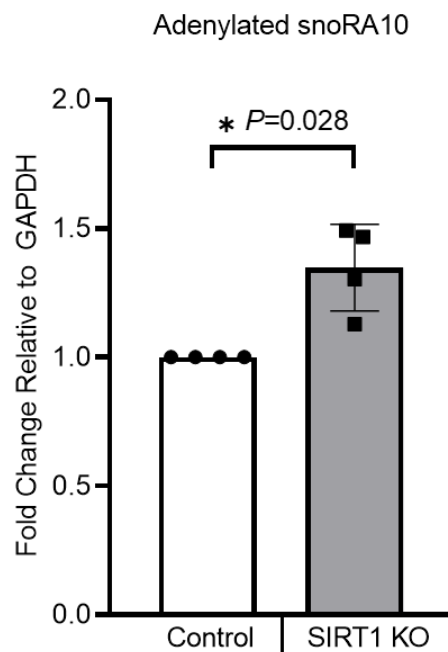


Figure S5



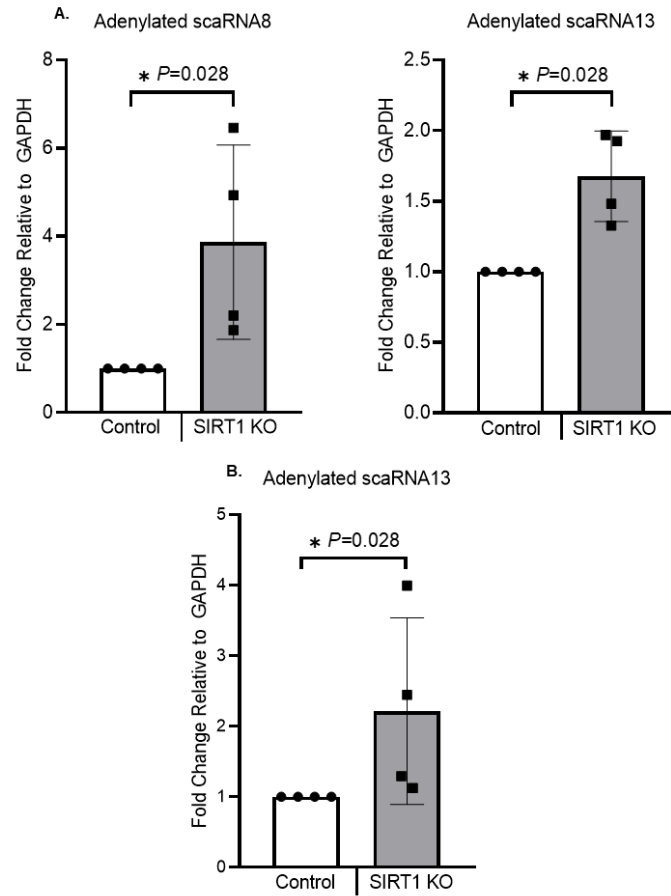
**Figure S5. Generating 293FT PARN KO cells with CRISPR-Cas9.** (A) Sequencing with the U6 forward primer confirmed PARN guide RNA (gRNA) targeting exon 3 (green) was incorporated into the PX459 plasmid. Sequencing results also confirmed the correct scaffolding sequence (yellow), BbSI digestion site (purple) and amino acid sequence (blue) were adjacent to the gRNA insert. (B) Western blot analysis of PARN KO clones (cl) reveals PARN KO c17 has no detectible PARN protein. 293FT cells were transfected with PX459 control and PX459 containing PARN gRNA as shown in (A), selected with puromycin and expanded into monoclonal cell lines. Each clone was tested for PARN protein expression. (C) Primers used to amplify regions around the gRNA target site (red box). (D-E) Agarose gels of PX459 control and PARN KO c17 PCR products after DNA amplification. (D) PCR was performed using primer 1, and the black arrow indicates the expected band size (151 bp) for the PCR product. Control C and control G from the Surveyor assay kit were used as controls. (E) PCR was performed using primer 2, and the black arrow indicates the expected band size (868 bp) for the PCR product. This data confirmed PARN KO was successful as there was a deletion upstream and downstream of exon 3.

Figure S6



**Figure S6. SIRT1 loss increases levels of adenylated snoRNA10.** RT-qPCR of adenylated small nucleolar RNA 10 (snoRNA10/snoRA10) was performed in HCT116 control and SIRT1 KO cells (n=4). Data is presented as the mean  $\pm$  standard deviation. Statistical analysis was performed using an unpaired, two-sided Wilcoxon-Mann-Whitney test (\*,  $P<0.05$ ).

Figure S7



**Figure S7. SIRT1 loss increases levels of adenylated scaRNA8 and scaRNA13.** (A) RT-qPCR of adenylated small cajal body RNA 8 (scaRNA8) and small cajal body RNA 13 (scaRNA13) in HCT116 control and SIRT1 KO cells (n=4). (B) RT-qPCR of adenylated small cajal body RNA 13 (scaRNA13) in HeLa control and SIRT1 KO cells (n=4). Data are presented as the mean  $\pm$  standard deviation. Statistical analyses were performed using unpaired, two-sided Wilcoxon-Mann-Whitney tests (\*,  $P < 0.05$ ).