A cautionary tale of sense-antisense gene pairs:
Independent regulation despite inverse correlation of expression

SUPPLEMENTARY INFORMATION

Table S1: Sequence of sgRNAs, primers, siRNAs and LNA gapmeRs used in this study:

sgRNA sequences

Name	Target	Sequence
Control sgRNA	EGFP	5'-GGGCGAGGAGCTGTTCACCG-3'
NOP14-AS1 sgRNA #1	NOP14-AS1	5'-GACGGACGGTCGCACAGACG-3'
NOP14-AS1 sgRNA #2	NOP14-AS1	5'-GGTCGCACAGACGCGGAACA-3'
NOP14-AS1 sgRNA #3	NOP14-AS1	5'-ACAGCCAAGCAGCGACCCGC-3'
NOP14 sgRNA #1	NOP14	5'-GCGGCCCGGCACGTGTCTTA-3'
NOP14 sgRNA #2	NOP14	5'-CGTTCGAGGTGAAAGTTAAC-3'
TP53 sgRNA	TP53	5'-GCTACCTGCTCCCTGGACGG-3'
LIPE-AS1 sgRNA #1	LIPE-AS1	5'-GCCTATCGCTGCCCGAGTT-3'
LIPE-AS1 sgRNA #2	LIPE-AS1	5'-GGGCCTCTGCGGAGTGGACC-3'
CEACAM1 sgRNA #1	CEACAM1	5'-GCCGTGCTCGAAGCGTTCC-3'
CEACAM1 sgRNA #2	CEACAM1	5'-GCTGGGGCGGGGTTTGTCC-3'

RT-qPCR primers

Name	Target	Sequence
NOP14-AS1 F	NOP14-AS1	5'-CCAGAGGTGCATTTCAGGAT-3'
NOP14-AS1 R	NOP14-AS1	5'-AAGGCAGGAAGATTGCTTCA-3'
NOP14 F	NOP14	5'-GAGGTTTGCTCTGGAACAGC-3'
NOP14 R	NOP14	5'-TCGATGTCTGCCAAAGACTG-3'
MFSD10 F	MFSD10	5'-GTCTACTTCCTCTACCTCTTCCT-3'
MFSD10 R	MFSD10	5'-CTGCTGTAGGCTACTGAACTG-3'
TP53 F	TP53	5'-GTGACACGCTTCCCTGGATT-3'
TP53 R	TP53	5'-TGTTTCCTGACTCAGAGGGG-3'
CDKN1A F	CDKN1A	5'-CGAAGTCAGTTCCTTGTGGAG-3'
CDKN1A R	CDKN1A	5'-CATGGGTTCTGACGGACAT-3'
CyclophilinA F	Cyclophilin A	5'-GTCAACCCCACCGTGTTCTT-3'
CyclophilinA R	Cyclophilin A	5'-CTGCTGTCTTTGGGACCTTGT-3'
45S pre-rRNA F	45S pre-rRNA	5'-CGGTCGTGTGTGGGTTGACT-3'
45S pre-rRNA R	45S pre-rRNA	5'-CTCCTTCCTTCCGAGGCAGA-3'
pri-miR21 F	pri-miR21	5'-TTTTGTTTTGCTTGGGAGGA-3'
pri-miR21 R	pri-miR21	5'-AGCAGACAGTCAGGCAGGAT-3'
tRNA Lys F	tRNA Lys	5'-CCCGAACAGGGACTTGAAC-3'
tRNA Lys R	tRNA Lys	5'-GCCCGGATAGCTCAGTCG-3'
CEACAM1 F	CEACAM1	5'-CTTCACAGAGTGCGTGTACC-3'
CEACAM1 R	CEACM1	5'-TGGATTCAGTAGTGAGCTGGG-3'
LIPE-AS1 F	LIPE-AS1	5'-ACCGGGAGGCTCTGATTGTT-3'
LIPE-AS1 F	LIPE-AS1	5'-ACCAGAGTTGGTTCAGTTCCTTT-3'

PCR primers

Name	Target	Sequence
XbaI VP160 F	VP160	5'-ATGCTCTAGAGACGCGCTGGACGATTTCG-3'
BamHI VP160 R	VP160	5'-ATATGGATCCCAACATATCCAAATCGAAGTCATCGAGC-3'
5'RACE GSP	NOP14-AS1	5'-GGGTGCTGGGGTTCTCCATTCAGGACA-3'
3'RACE GSP	NOP14-AS1	5'-GTCTCCTCCCACCTCCGAGAGTGCAGTG-3'
AgeI EGFP	EGFP	5'-ATACCGGTCCACCATGGTGAGCAAGGGCGAGGAGC-3'
BamHI EGFP	EGFP	5'-ATAGGATCCCTTGTACAGCTCGTCCATG-3'
NOP14 cDNA F	NOP14	5'-CCATGGCGAAGGCGAAGAAGGTCGGGG-3'
NOP14 cDNA R	NOP14	5'-TTATTTTTTGAACTTTTTCCTCTTCAGAGCCTTCC-3'
AgeI NOP14 F	NOP14	5'-CAGGACCGGTCCACCATGGCGAAGGCGAAGAAG-3'
BamHI NOP14 R	NOP14	5'-GCCGGATCCTTTTTTGAACTTTTTCCTCTTCAGAG-3'

LNA GapmeRs

Name	Target	Sequence
NOP14-AS1-LNA Gapmer#1	NOP14-AS1	5’-GGAAAGATGGTGTGAATTGG-3’
NOP14-AS1-LNA Gapmer#2	NOP14-AS1	5’-AAAATGCTTGCGGATCTCAC-3’

siRNAs

Name	Target	Sequence
siNOP14(1)	NOP14	5’-CCAATCCGTTCGAGGTGAAAGTTAA-3’
siNOP14(2)	NOP14	5’-GGAAAGAGCTGATTGAAGA-3’

Table S1 contains all oligonucleotide sequences used in this study. All sequences are given in 5'-3' direction.

Table S2: Microarray data

Table S2 contains the primary data obtained from the microarray analysis. Microarray data are given as log2 signal intensities plus the microarray annotation as provided by the manufacturer.

SUPPLEMENTARY MATERIALS AND METHODS

Microarray

A549 and HepG2 cells were treated with 50 µM Etoposide, 50 µM Cisplatin, 20 µM Bleomycin or vehicle control DMSO for eight hours. Total RNA was isolated using Trizol and 7 µg RNA were labeled with Cy3 mono-reactive dye (Amersham Biosciences) using the SuperScript Indirect cDNA labeling system (Invitrogen) with anchored oligo-dT primers. To investigate the expression of lncRNAs after induction of DNA damage, NCode Human Non-Coding RNA Microarrays (Invitrogen) were hybridized in duplicate with 232 ng labeled cDNA each using the Agilent gene expression hybridization kit according to the manufacturer's protocol. The arrays were scanned on an Agilent scanner and the data was extracted using Agilent Feature Extraction Software (FE).

Data analysis was performed in the GeneSpring GX 10.0 software (Agilent). Data normalization was performed using the Quantile method (Bolstad et al., 2003) and the data was filtered for a minimum expression of 7. The significance of the differential expression was determined by means of an unpaired t-test with adjusted p values corrected by the Benjamini-Hochberg method (Hochberg and Benjamini, 1990) for multiple tests. Only lncRNA candidates with at least a 3-fold difference in expression between control and experimental groups with an adjusted p-value of less than 0.05 were considered as hits.

5'- and 3'-RACE

RNA was extracted from HepG2 cells treated with 50 µM Etoposide for 16h and reverse transcribed using the SMARTer RACE cDNA Amplification Kit (Clontech) according to manufacturer's instructions. 5'- and 3'- RACE PCR products were generated using Advantage 2 PCR Enzyme System (Clontech) according to the manufacturer's instructions. The gene-specific primers used for 5'-and 3'-RACE can be found in Suppl. Table S1. The PCR products obtained were gel eluted and cloned into the pCR2.1-TOPO vector using the TOPO TA cloning kit (ThermoFisher Scientific) and sequenced using the M13 reverse primer.

Virus production and transduction

Virus production was performed as described earlier (1). Briefly, one day prior to transfection, HEK293T cells ($4x10^5$ cells / well, in a total of 2 ml media) were seeded in Poly-L-Lysine coated 6-well plates (Greiner Bio-One). Cells were co-transfected with 1.2 µg LentidCas9-KRAB-PURO iv sgRNA / LentidCas9-VP160-PURO iv sgRNA (containing the indicated sgRNA) / Lenti EGFP Blast / Lenti NOP14 Blast, 0.9 µg psPAX2 (packaging plasmid) and 0.3 µg pMD2.G (envelope plasmid) using 6 µl Lipofectamine 2000 (ThermoFisher Scientific) in 200 µl Opti-MEM (ThermoFisher Scientific). 48 hours post transfection, the lentivirus-containing medium was filtered using a low protein binding 0.45 µm syringe filter (Millipore). 0.5 ml of the filtered virus along with Polybrene (Final concentration: 8 µg / ml) was then added to NCI-H460 or A549 cells which were seeded in a 6 well plate one day prior to transduction ($1x10^5$ cells / well, in a total of 2 ml medium). 24 hours post-transduction, the medium was replaced with 2 µg/ml Puromycin (ThermoFisher Scientific) and / or 6 µg/ml Blasticidin (InvivoGen) containing media. For Puromycin selection, cells were incubated in Puromycin-containing medium for three days. For Blasticidin selection, cells were incubated in Blasticidin-containing medium for six days. Untransduced cells were used to monitor for a complete selection. The stable cell lines obtained were used for further experiments.

siRNA / Antisense LNA GapmeR knockdown

RNA interference (RNAi) was performed as described earlier with some minor modifications (2). For *TP53* knockdown using siPOOLs (3), NCI-H460 cells ($2x10^5$ cells per well) were reverse transfected with 10 nM (final concentration) of siPOOL control or siPOOL *TP53* (siTOOLs Biotech) using 6 µl RNAiMAX reagent in 6-well plates. 48 hours post transfection, cells were treated with 1 µM Doxorubicin (final concentration) or vehicle control DMSO. 24 hours post treatment, cells were lysed in TRI reagent for RNA extraction or RIPA buffer for protein extraction.

For NOP14 knockdown using siRNAs, NCI-H460 cells ($2x10^5$ cells per well) were reverse transfected with 40 nM (final concentration) of the control siRNA or two independent siRNAs against *NOP14* (Eurofins Genomics) using 2 µl RNAiMax reagent in 12-well plates. 24 hours post transfection, cells were treated with DMSO or 1 µM Doxorubicin (final concentration). 24 hours post treatment, cells were lysed in TRI reagent for RNA extraction or RIPA buffer for protein extraction.

For *NOP14-AS1* knockdown using Antisense LNA GapmeRs, NCI-H460 or A549 cells ($4x10^5$ cells per well) were reverse transfected with 25 nM (final concentration) of the control or two independent LNA GapmeRs (Exiqon) against *NOP14-AS1* using 6 µl RNAiMAX reagent (ThermoFisher Scientific) in 6-well plates. 24 hours post transfection, cells were treated with 1 µM Doxorubicin (final concentration) or vehicle control DMSO. 24 hours post treatment, cells were lysed in TRI reagent for RNA extraction or RIPA buffer for protein extraction.

RNA extraction and Reverse Transcription - quantitative Polymerase Chain Reaction (RT-qPCR)

RNA was isolated using Trizol (ThermoFisher Scientific) as described earlier (4) or TRI reagent (Sigma-Aldrich) according to the manufacturer's recommendations with some minor modifications. Cells were lysed using 1 ml TRIzol or TRI reagent per well of a 6 well plate. 200 µl Chloroform was added per ml of lysate, vortexed briefly and centrifuged for 15 minutes at 4^oC and 13000g. Following phase separation, the RNA was isopropanol precipitated from the aqueous layer. The precipitate was incubated with DNase I (Roche) for 30 min at 37°C for removal of genomic DNA. Thereafter, the RNA was subjected to Phenol:Chloroform extraction to remove any protein contaminations and precipitated overnight at -80°C using ethanol. A total of 1-2 µg RNA was reverse transcribed to generate cDNA using random hexamer primers and RevertAid H-Minus Reverse Transcriptase (ThermoFisher Scientific) as per the manufacturer's recommendations. To further control for any residual genomic DNA contamination, a minus-RT reaction was also performed where reverse transcriptase was replaced by water. The cDNA generated was diluted by a factor of 20 or 40 and RT-qPCR was performed using PowerSYBRGreen PCR Master Mix (ThermoFisher Scientific) in a StepOnePlus qPCR instrument (Life Technologies). Quantification was performed using the ΔΔCt method and the housekeeping gene *PPIA* (*Cyclophilin A*) was used for normalization.

Western Blot

Protein lysates were prepared as described earlier with some minor modifications (5). Briefly, cells were rinsed with ice-cold phosphate-buffered saline (PBS, Sigma-Aldrich) and incubated for 30 min on ice in 200 µl RIPA buffer per well of a 6-well plate. Following lysis, the crude lysate was subjected to centrifugation for 30 min at 4^oC and 17000g to precipitate cellular debris. The protein concentration was determined using the BCA assay and equal amounts (10 µg / 20 µg) of lysates were separated on self-cast 10% SDS-PAGE gels and transferred to Nitrocellulose membranes. The membranes were blocked using 5% milk in TBST (50 mM Tris, 150 mM NaCl, 0.05% Tween 20) and then incubated with primary antibodies according to the manufacturer's recommendations. The following primary antibodies were used:

NOP14 (#HPA039596, Sigma-Aldrich), TP53 (#554293, BD Pharmingen) and GAPDH (#MAB374, EMD Millipore). Following incubation with the primary antibody, the membranes were washed with TBST and incubated with secondary antibodies which were either HRP-coupled (Dianova) or NIR-fluorescent reagent (LI-COR IRDye® 800CW) conjugated. Chemiluminescence detection was performed using Super Signal West Pico chemiluminescent substrate (ThermoFisher Scientific) on a ChemoCam Imager (Intas). Fluorescence detection was performed using LI-COR Biosciences Odyssey®. Image Studio™ Lite was used for image analysis and band quantification.

Cellular fractionation

Fractionation was performed as described in (6). Briefly, cells from 70-90% confluent 10 cm dishes were harvested in 1 ml PBS by scraping and resuspended in 1 ml resuspension buffer (10 mM Tris pH7.4; 10 mM NaCl; 3 mM $MgCl_2$). The suspension was resuspended in 300 µl RSBG40 (10 mM Tris pH7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.5 mM DTT, 10% glycerol, 0.5% NP40, 40 U/ml RiboLock, 10 mM Vanadylribonucleoside) and incubated 3 min on ice. Following centrifugation at 4,500 g, 4 °C for 3 min, the supernatant was used to extract cytoplasmic RNA using 1.6 ml Trizol. The pellet was gently resuspended in 100 µl RSBG40 + sodium deoxycholate (final conc. = 0.33%) and Tween-20 (final conc. = 0.66%). Following 5 min incubation on ice, the nuclei were centrifuged at 4,500 g, 4 °C for 3 min and washed with 400 µl RSBG40 (without detergents). The nuclei were centrifuged at 9,300 g, 4 °C for 5 min and the pellet was resuspended in 1 ml Trizol. RNA isolation and RT-qPCR for the different fractions was performed as described above.

Copy number determination

The per cell copy number for *NOP14-AS1* was determined as described in (7). Briefly, *NOP14-AS1* was cloned into pCRII-TOPO vector (ThermoFisher Scientific) and was used to generate a standard curve using serial dilutions of this plasmid. Total RNA from one million NCI-H460 cells was used to determine *NOP14-AS1* expression. This was compared to the standard curve to estimate the per cell copy number of *NOP14-AS1* in these cells.

BrdU cell proliferation assay

5000 cells from each stable cell line were seeded per well of a black walled, clear bottomed 96 well plate (ThermoFisher Scientific). 24 hours post seeding, cells were treated with either 1 µM Doxorubicin or vehicle control DMSO for 18 hours following which 10 µl BrdU labelling agent was added to each well and the cells were incubated at 37 °C for another 6 hours. Cell proliferation rates were determined as described in (7) using the Cell Proliferation ELISA, BrdU Kit (Roche) as per the manufacturer's instructions.

Clonogenic assay

Clonogenic assay was performed as described in (8). Briefly, 1000 cells from each stable cell line were seeded per well of a 6 well plate and incubated at 37°C and 5% CO2 in a humidified chamber. 4 days post seeding, cells were washed with PBS and incubated in 2 ml of staining solution (6.0% glutaraldehyde and 0.5% crystal violet in PBS) for 30 min. Cells were then rinsed with water to remove excess staining.

Figure S1

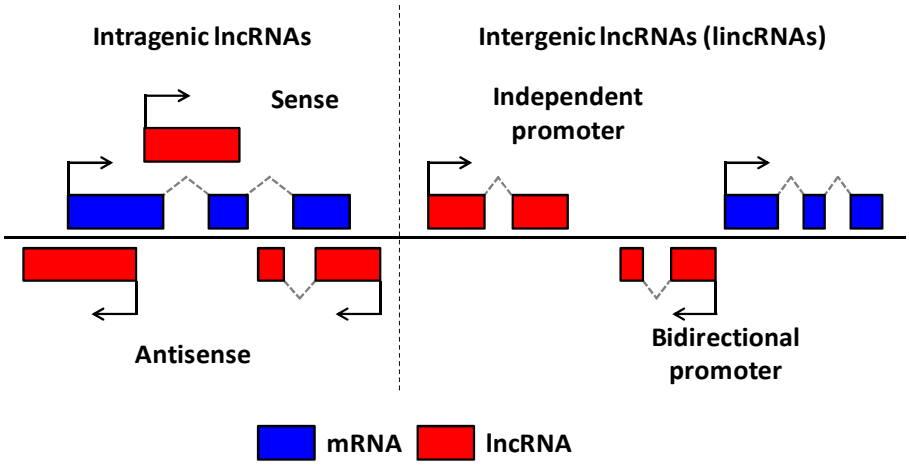

Figure S1: LncRNA genomic distribution

A schematic representation of lncRNA loci distribution across the genome. LncRNAs can be either intragenic (left) or intergenic (right). Intragenic lncRNAs can further be classified as sense or antisense depending on their orientation relative to the second gene in the locus. Intergenic lncRNAs can be transcribed either from independent promoters or bidirectional promoters of other genes.

Figure S2

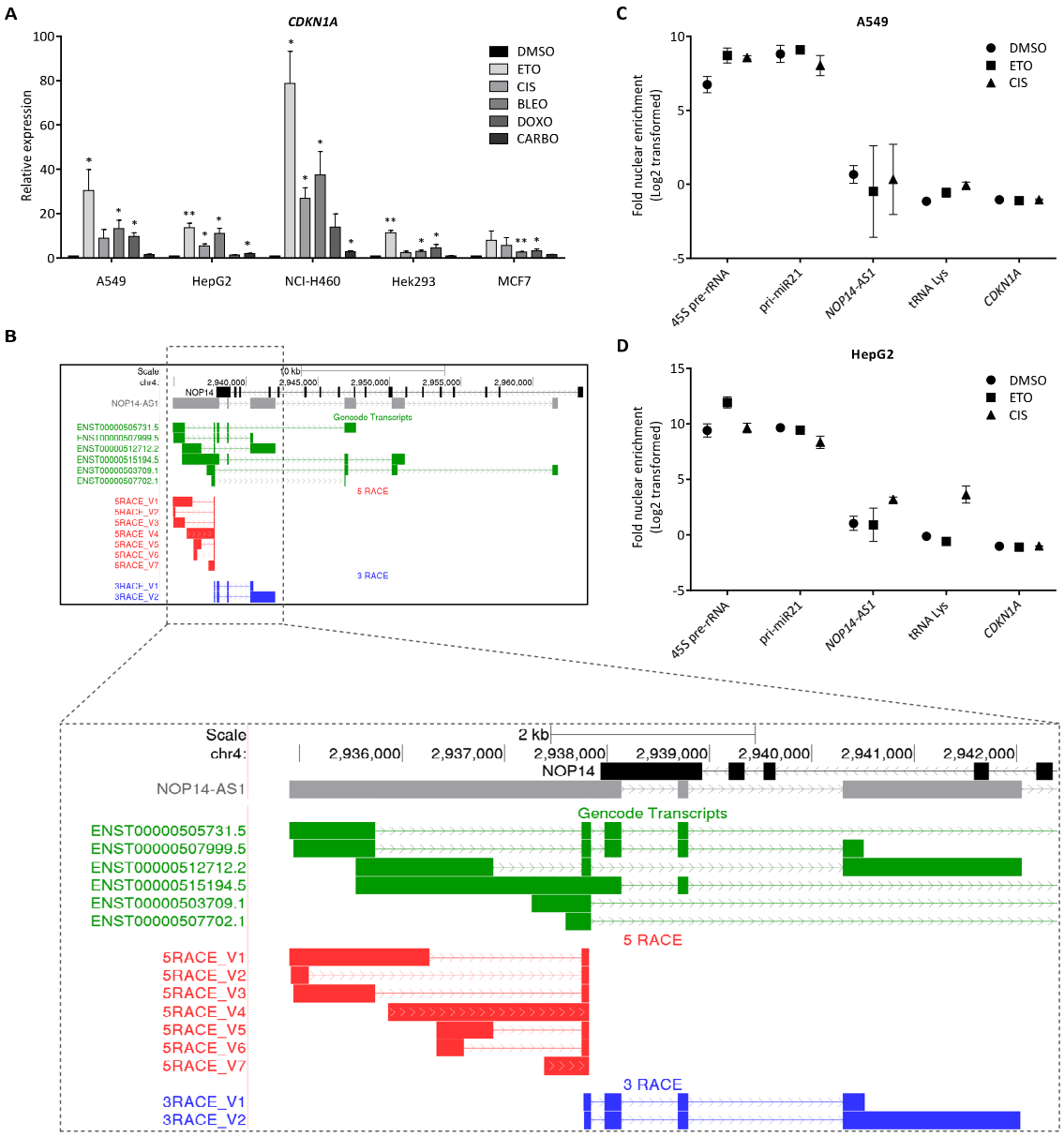

Figure S2: *NOP14-AS1*: An antisense transcript induced upon DNA damage

A: A549 / HepG2 / NCI-H460 / Hek293 / MCF7 cells were treated with 50 µM Etoposide (ETO) / 50 µM Cisplatin (CIS) / 20 µM Bleomycin (BLEO) / 1 µM Doxorubicin (DOXO) / 50 µM Carboplatin (CARBO) or vehicle control DMSO for 8 hours. RT-qPCR results for *CDKN1A* normalized to *Cyclophilin A* and DMSO control. Error bars represent SEM (n≥3). * p<0.05; ** p<0.01; *** p<0.001 compared to DMSO control, unpaired two-sided t-test.

B: Sanger sequencing results of the clones obtained from 5'- (red) and 3'-RACE (blue) for *NOP14-AS1* performed on the cDNA obtained from HepG2 cells compared to annotated isoforms (green).

C,D: (C) A549 / (D) HepG2 cells were treated with 50 µM Etoposide (ETO) / 50 µM Cisplatin (CIS) or vehicle control DMSO for 8 hours. RT-qPCR was performed on RNA from the nuclear as well as the cytoplasmic fractions and their ratio is depicted as fold nuclear enrichment. Error bars represent SEM (n=2).

Figure S3

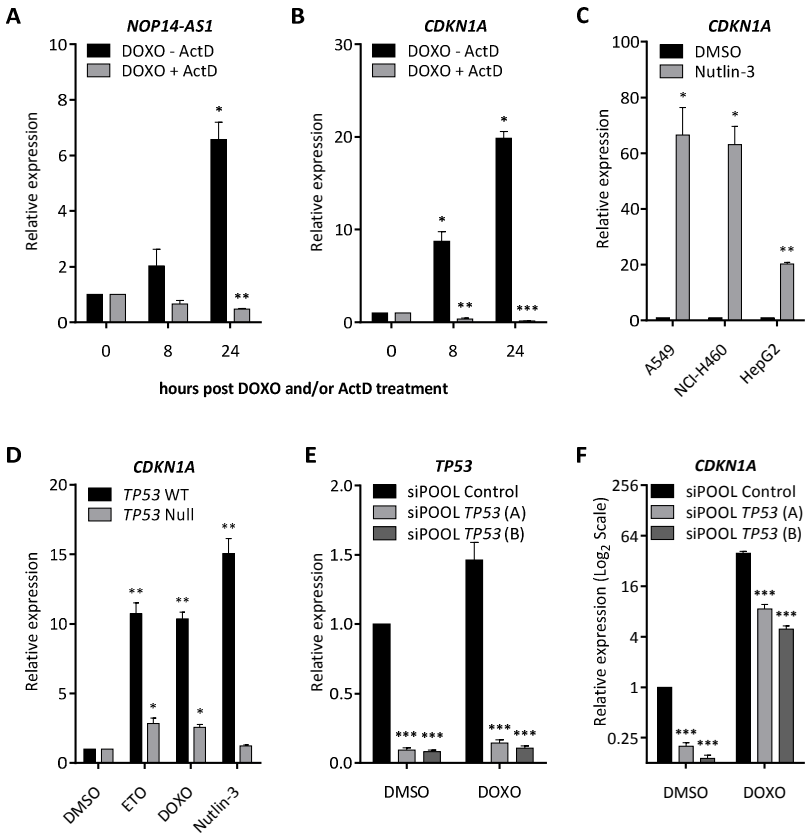

Figure S3: *NOP14-AS1* is induced in a *TP53*-dependent manner

A,B: A549 cells were treated with 1 µM Doxorubicin (DOXO) and either 10 µg/ml Actinomycin D (ActD) or vehicle control DMSO. RT-qPCR results for (A) *NOP14-AS1* and (B) *CDKN1A* normalized to *Cyclophilin A* and untreated controls. Error bars represent SEM (n=3). * p<0.05; ** p<0.01; *** p<0.001 compared to untreated controls, unpaired two-sided t-test.

C: A549 / NCI-H460 / HepG2 cells were treated with either 10 µM Nutlin-3 or vehicle control DMSO for 24 hours. RT-qPCR results for *CDKN1A* normalized to *Cyclophilin A* and DMSO controls. Error bars represent SEM (n=3). * p<0.05; ** p<0.01; *** p<0.001 compared to DMSO controls, unpaired two-sided t-test.

D: HCT116 TP53 WT / TP53 Null cells were treated with 50 µM Etoposide (ETO) / 1 µM Doxorubicin (DOXO) / 10 µM Nutlin-3 or vehicle control DMSO for 12 hours. RT-qPCR results for *CDKN1A* normalized to *Cyclophilin A* and DMSO controls. Error bars represent SEM (n=3). * p<0.05; ** p<0.01; *** p<0.001 compared to DMSO controls, unpaired two-sided t-test.

E,F: NCI-H460 cells were transfected with either one of the two indicated siPOOLs against *TP53* or siPOOL Control. These were then treated with either 1 µM Doxorubicin (DOXO) or vehicle control DMSO for 24 hours. RT-qPCR results for (E) *TP53* and (F) *CDKN1A* normalized to Cyclophilin A and siPOOL Control + DMSO control. Error bars represent SEM (n=5). * p<0.05; ** p<0.01; *** p<0.001 compared to siPOOL Control, unpaired two-sided t-test.

Figure S4

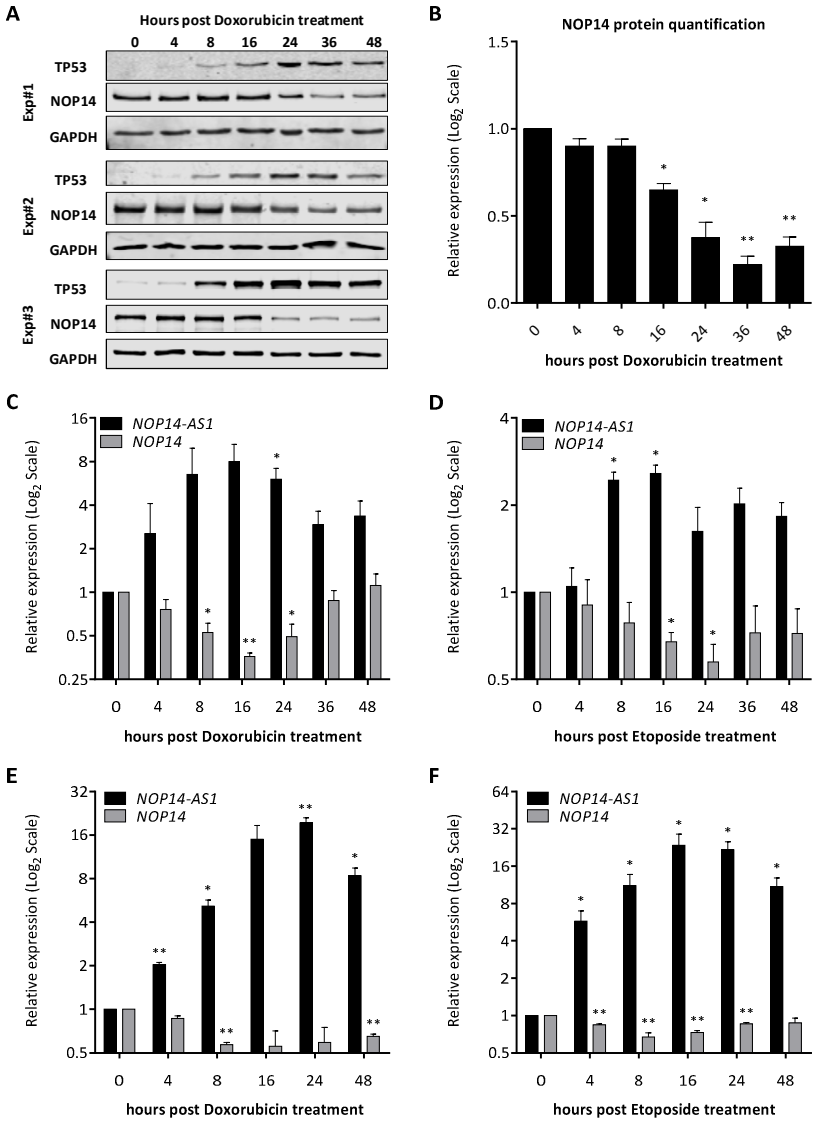

Figure S4: Inverse co-regulation between *NOP14-AS1* and *NOP14*

A,B: NCI-H460 cells were treated with 1 µM Doxorubicin (DOXO) for the indicated time points. (A) Western blot results for TP53 and NOP14. GAPDH was used as a loading control. Images from Exp#3 were also used in Fig. 3B in the main text. (B) Quantification of the NOP14 protein expression normalized to GAPDH and untreated control. Error bars represent SEM (n=3). * p<0.05; ** p<0.01; *** p<0.001 compared to untreated control, unpaired two-sided t-test.

C,D: A549 cells were treated with (C) 1 µM Doxorubicin (DOXO) or (D) 50 µM Etoposide (ETO) for the indicated times. RT-qPCR results for *NOP14-AS1* and *NOP14* normalized to *Cyclophilin A* and untreated control. Error bars represent SEM (n=3). * p<0.05; ** p<0.01; *** p<0.001 compared to untreated control, unpaired two-sided t-test.

E,F: HepG2 cells were treated with (E) 1 µM Doxorubicin (DOXO) or (F) 50 µM Etoposide (ETO) for the indicated times. RT-qPCR results for *NOP14-AS1* and *NOP14* normalized to *Cyclophilin A* and untreated control. Error bars represent SEM (n=3). * p<0.05; ** p<0.01; *** p<0.001 compared to untreated control, unpaired two-sided t-test.

Figure S5

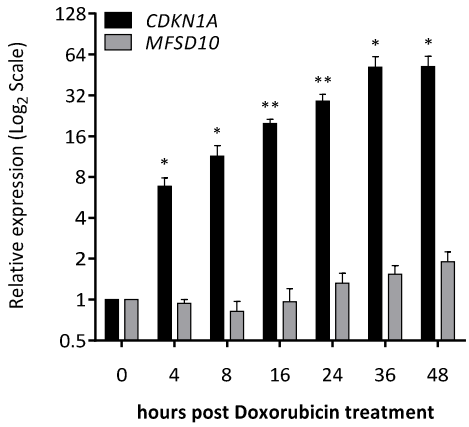

Figure S5: *MFSD10* is not regulated upon DNA damage

NCI-H460 cells were treated with 1 µM Doxorubicin (DOXO) for the indicated times. RT-qPCR results for *CDKN1A* and *MFSD10* normalized to *Cyclophilin A* and untreated control. Error bars represent SEM (n=3). * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared to untreated control, unpaired two-sided t-test.

Figure S6

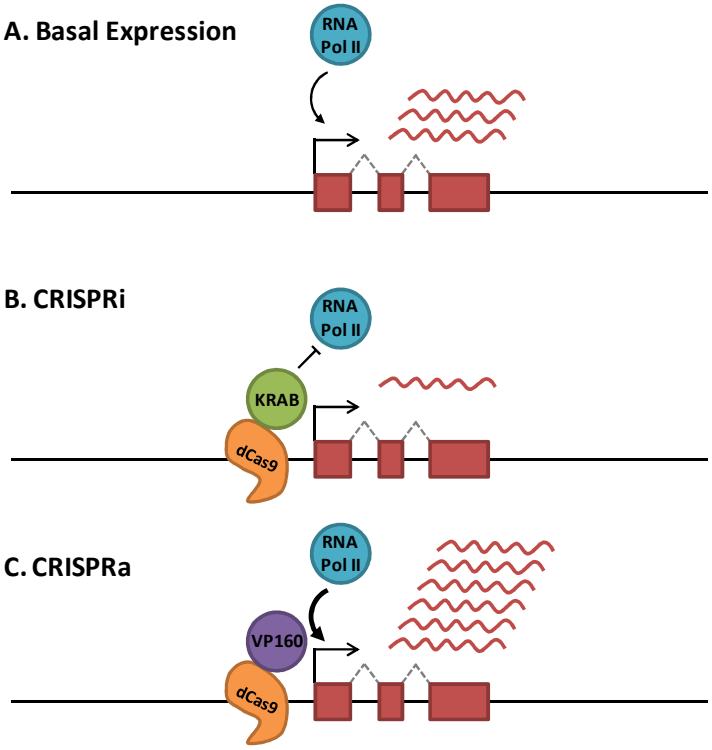

Figure S6. CRISPRi/a mediated modulation of transcription

Schematic representation of dCas9-KRAB- / dCas9-VP160-mediated modulation of transcription of a target gene.

A: Basal gene expression from the endogenous promoter in absence of any dCas9 effectors.

B: Reduced gene expression from the endogenous promoter in presence of dCas9-KRAB in the vicinity of the promoter.

C: Increased gene expression from the endogenous promoter in presence of dCas9-VP160 in the vicinity of the promoter.

Figure S7

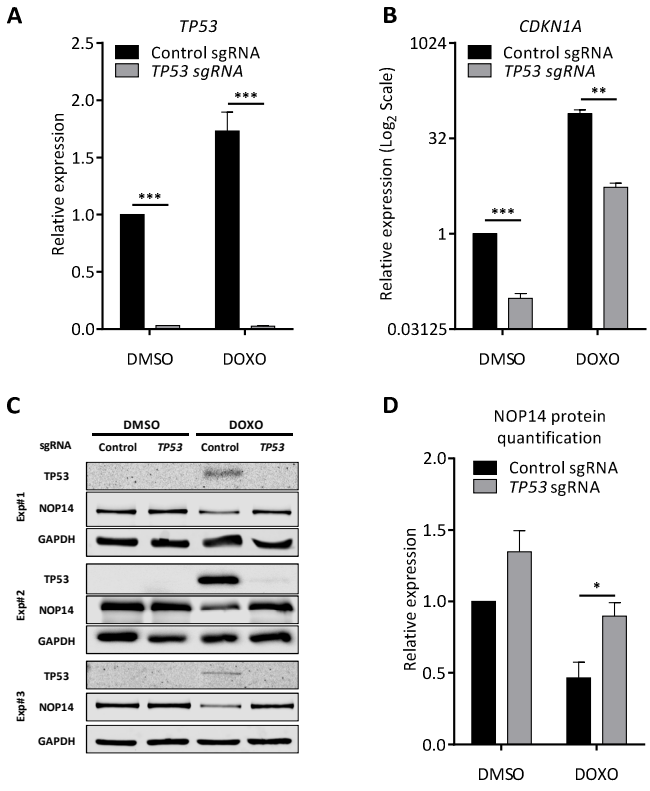

Figure S7. p53 knockdown partially rescues DNA damage induced NOP14 repression.

A-D: NCI-H460 cells were transduced with Lenti dCas9-KRAB-PURO iv sgRNA containing either a control sgRNA or an sgRNA targeting the *TP53* promoter. These were then treated with either 1 µM Doxorubicin (DOXO) or vehicle control DMSO for 24 hours. RT-qPCR results for (A) *TP53* and (B) *CDKN1A* normalized to *Cyclophilin A* and control sgRNA + DMSO controls. Error bars represent SEM (n=5). * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared to control sgRNA, unpaired two-sided t-test. (C) Western blot results for TP53 and NOP14. GAPDH was used as a loading control. Images from Exp#3 were also used in Fig. 3B in the main text. (D) Quantification of the NOP14 protein expression normalized to GAPDH and Control sgRNA + DMSO control. Error bars represent SEM (n=3). * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared to Control sgRNA, unpaired two-sided t-test.

Figure S8

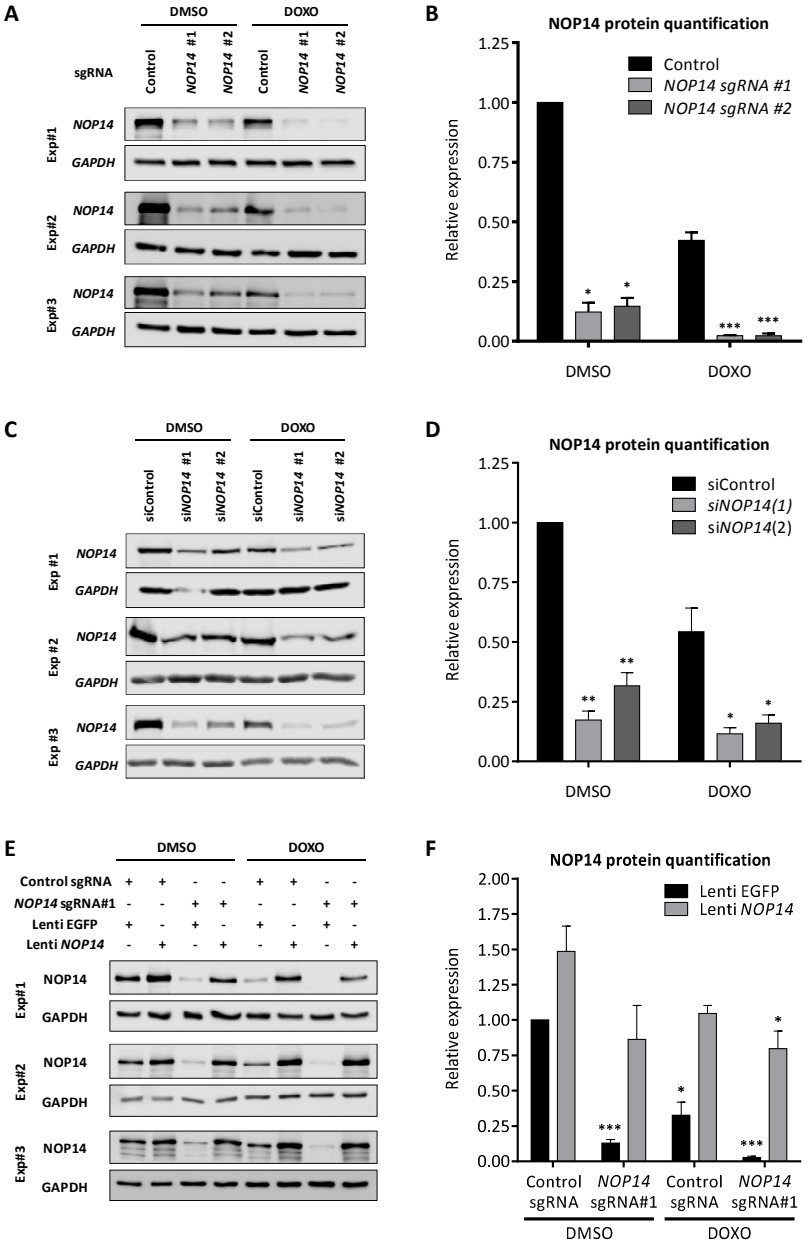

Figure S8: *NOP14* does not regulate *NOP14-AS1* expression upon DNA damage

A,B: NCI-H460 cells were transduced with Lenti dCas9-KRAB-PURO iv sgRNA containing either a control sgRNA or two independent sgRNAs targeting the *NOP14* promoter. These were then treated with either 1 µM Doxorubicin (DOXO) or vehicle control DMSO for 24 hours. (A) Western blot results for NOP14. GAPDH was used as a loading control. Images from Exp#1 were also used in Fig. 4A in the main text. (B) Quantification of the NOP14 protein expression normalized to GAPDH and Control sgRNA + DMSO control. Error bars represent SEM (n=3). * p<0.05; ** p<0.01; *** p<0.001 compared to Control sgRNA, unpaired two-sided t-test.

C,D: NCI-H460 cells were transfected with either a control siRNA (siControl) or two independent siRNAs (si*NOP14*(1) and si*NOP14*(2)) targeting the *NOP14* mRNA. These were then treated with either 1 µM Doxorubicin (DOXO) or vehicle

control DMSO for 24 hours. RT-qPCR results for (D) Western blot results for NOP14. GAPDH was used as a loading control. Images from Exp#3 were also used in Fig. 4C in the main text. (E) Quantification of the NOP14 protein expression normalized to GAPDH and siControl + DMSO control. Error bars represent SEM (n=3). * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared to control siRNA, unpaired two-sided t-test.

E-F: NCI-H460 cells were transduced with Lenti dCas9-KRAB-PURO iv sgRNA containing either a control sgRNA or an sgRNA targeting the *NOP14* promoter in combination with either Lenti EGFP Blast or Lenti NOP14 Blast. These were then treated with either 1 μM Doxorubicin (DOXO) or vehicle control DMSO for 24 hours. (G) Western blot results for NOP14. GAPDH was used as a loading control. Images from Exp#1 were also used in Fig. 4E in the main text. (H) Quantification of the NOP14 protein expression normalized to GAPDH and control sgRNA + Lenti EGFP + DMSO control. Error bars represent SEM (n=3). * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared to control sgRNA + DMSO controls, unpaired two-sided t-test. Error bars represent SEM (n=3). * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared to untreated control, unpaired two-sided t-test.

Figure S9

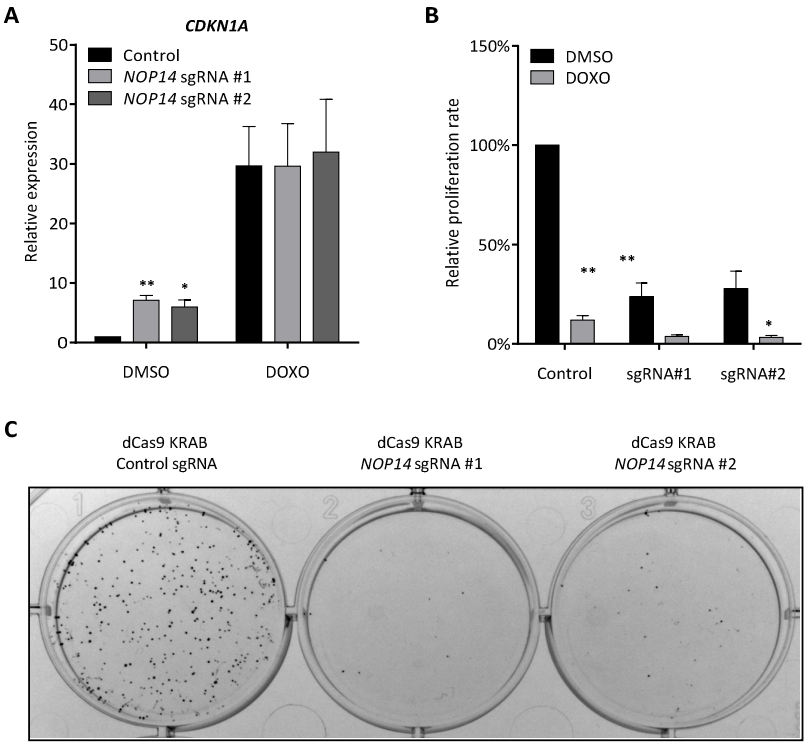

Figure S9: *NOP14* knockdown results in reduced cell proliferation

A,B: NCI-H460 cells were transduced with Lenti dCas9-KRAB-PURO iv sgRNA containing either a control sgRNA or two independent sgRNAs targeting the *NOP14* promoter. These were then treated with either 1 µM Doxorubicin (DOXO) or vehicle control DMSO for 24 hours. (A) RT-qPCR results for *CDKN1A* normalized to *Cyclophilin A* and DMSO controls. (B) Proliferation rates normalized to dCas9-KRAB control sgRNA + DMSO control. Error bars represent SEM (n=4). * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared to dCas9-KRAB control sgRNA + DMSO / dCas9-KRAB control sgRNA + DOXO, unpaired two-sided t-test.

C: NCI-H460 cells were transduced with Lenti dCas9-KRAB-PURO iv sgRNA containing either a control sgRNA or two independent sgRNAs targeting the *NOP14* promoter. Equal numbers of cells for each stable cell line were seeded and a clonogenic assay was performed.

Figure S10

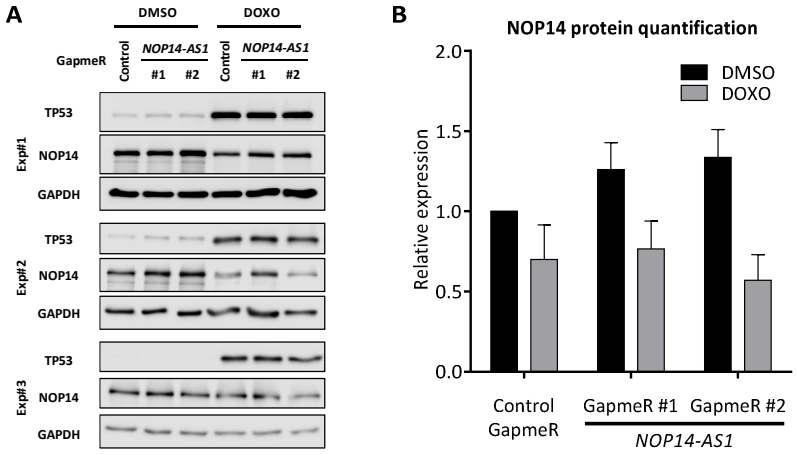

Figure S10: *NOP14-AS1* does not regulate NOP14 protein expression upon DNA damage

A-C: NCI-H460 cells were transfected with either a control GapmeR or two independent GapmeRs (GapmeR#1 and GapmeR#2) targeting the *NOP14-AS1* lncRNA. These were then treated with either 1 µM Doxorubicin (DOXO) or vehicle control DMSO for 24 hours. (A) Western blot results for NOP14. GAPDH was used as a loading control. Images from Exp#1 were also used in Fig. 5C in the main text. (B) Quantification of the NOP14 protein expression normalized to *GAPDH* and control GapmeR + DMSO control. Error bars represent SEM (n=3). * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared to control GapmeR, unpaired two-sided t-test.

Figure S11

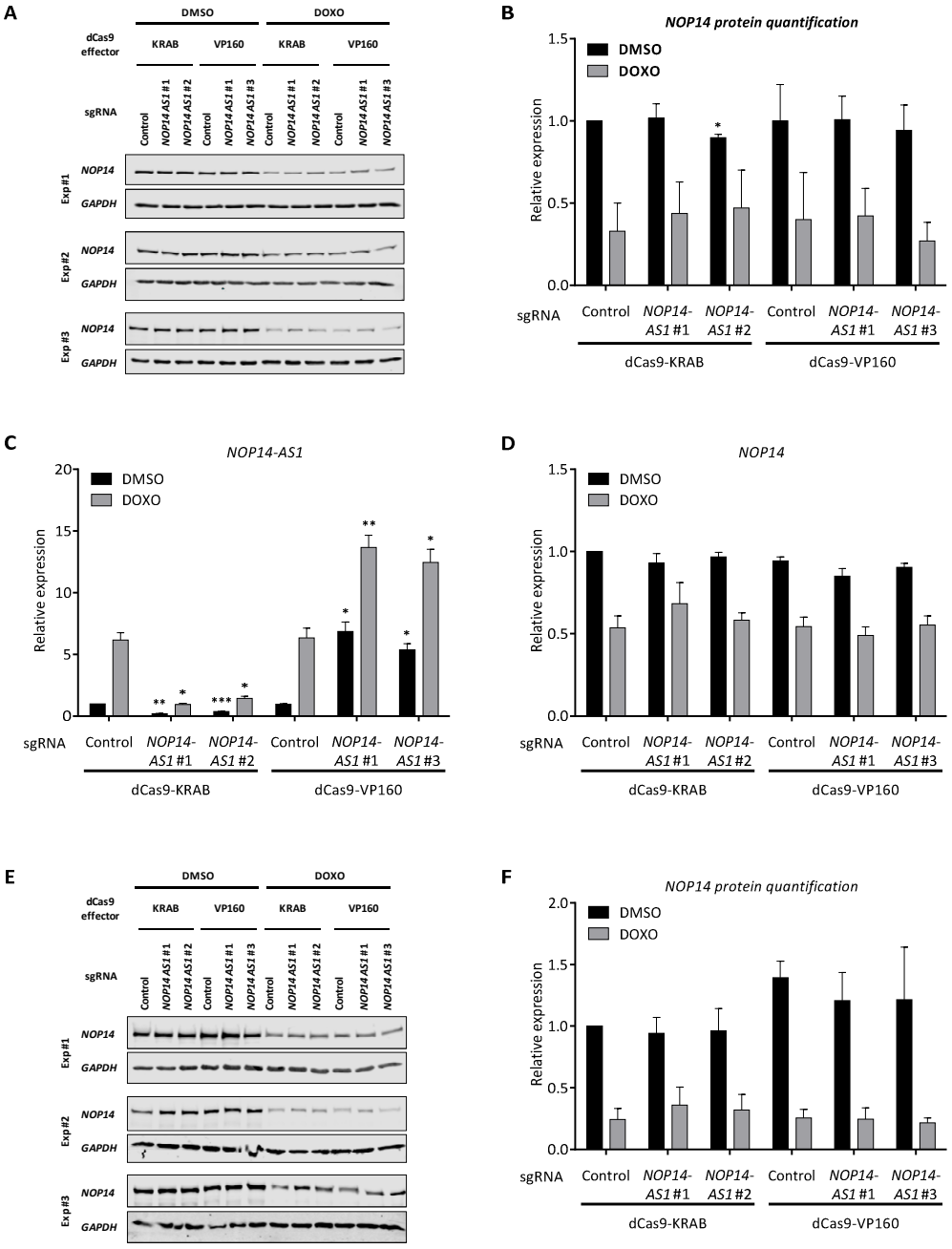

Figure S11: *NOP14-AS1* does not regulate NOP14 expression upon DNA damage

A,B: NCI-H460 cells were transduced with Lenti dCas9-KRAB-PURO iv sgRNA / Lenti dCas9-VP160-PURO iv sgRNA containing either a control sgRNA or two independent sgRNAs targeting the *NOP14-AS1* promoter. These were then treated with either 1 µM Doxorubicin (DOXO) or vehicle control DMSO for 24 hours. (A) Western blot results for NOP14. GAPDH was used as a loading control. (B) Quantification of the NOP14 protein expression normalized to GAPDH and dCas9-KRAB control sgRNA + DMSO control. Error bars represent SEM (n=3). * p<0.05; ** p<0.01; *** p<0.001 compared to dCas9-KRAB control sgRNA + DMSO / dCas9-KRAB control sgRNA + DOXO, unpaired two-sided t-test.

C-F: A549 cells were transduced with Lenti dCas9-KRAB-PURO iv sgRNA / Lenti dCas9-VP160-PURO iv sgRNA containing either a control sgRNA or two independent sgRNAs targeting the *NOP14-AS1* promoter. These were then treated with either 1 µM Doxorubicin (DOXO) or vehicle control DMSO for 24 hours. RT-qPCR results for (C) *NOP14-AS1* and (D) *NOP14* normalized to *Cyclophilin A* and control sgRNA + DMSO controls. Error bars represent SEM (n=3). * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared to control sgRNA, unpaired two-sided t-test. (E) Western blot results for NOP14. GAPDH was used as a loading control. (F) Quantification of the NOP14 protein expression normalized to GAPDH and dCas9 KRAB control sgRNA + DMSO control. Error bars represent SEM (n=3). * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared to dCas9-KRAB control sgRNA + DMSO / dCas9-KRAB control sgRNA + DOXO, unpaired two-sided t-test.

Figure S12

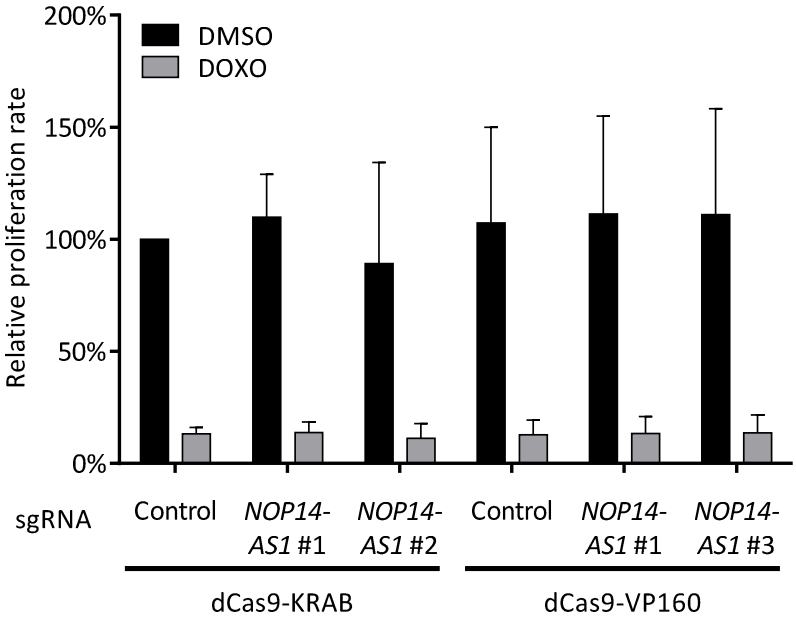

Figure S12: *NOP14-AS1* does not regulate cell proliferation upon DNA damage

NCI-H460 cells were transduced with Lenti dCas9-KRAB-PURO iv sgRNA / Lenti dCas9-VP160-PURO iv sgRNA containing either a control sgRNA or two independent sgRNAs targeting the *NOP14-AS1* promoter. These were then treated with either 1 µM Doxorubicin (DOXO) or vehicle control DMSO for 24 hours. Proliferation rates normalized to dCas9-KRAB control sgRNA + DMSO control. Error bars represent SEM (n=6). * $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared to dCas9-KRAB control sgRNA + DMSO / dCas9-KRAB control sgRNA + DOXO, unpaired two-sided t-test.

SUPPLEMENTARY SEQUENCES

All sequences are given in 5'-3' direction.

>5RaceV1

CTCTTCCGGCCGGAGTCAGTGGCGGGGTTCTCGCCGGTCCTGCGGGTCGCTGCTTGGCTGTGGGTTAGCAGCACCCTGAGCGGAGCGGAAGAGCTGCCCGCCGCCGC
GTAACCCCGAGGCCGAGACTCCTGGCCTGGCCTCCTTGGGCCCACGACGCCTGCCCCGCGGAGCGGACCCATGGGCCTGAGGTTTGTGTGCGGGGCCTCAGGCGAGC
GCTGGCGCAGCCTGTCCCCCATCGTCTGCGCCCTGCGGGCCTGGCCCTCCCCGTGGAAGGACCGCGGGCTTCGGGGCCCCACGCTGGGGGGCTGACTCCTGCCACAC
ACACAGCGGTGGCCGCGGGCCTGGGGCGGCAGCACAGCCATTCTGCTCGGGCTGCGTGAGCTGACCCCGCTCTCTGTGCACCTACTTTTGCTGCGTTTGGTGCTGAGC
CTGAAGTCCCAGGACAGGCGGCTGAGGGGGAAAGATGGTGTGAATTGGGTGGGCAGAGACAACTGAAAGCCACAGGACCCGGTTGGCACCTTTTTGGCCGCCTGCG
CCTTTGCCTGCAGCCCCGCTCCCTGGGTCACCCGAAGGAGGAGCTGGCGCTCTTTCCGGGCGCAGAAGCACCCGACCCACGCTGGAGGAAACGGTCCCTCTGGGCAG
GAGAAGCTTTGGGCCTGGCTGGAGCACACTCACGGAACCACGCCAACGCCTGGCCTGTAGCCAGCTCCCACCTCCCCTCCCACCTTCTAAAATGCTTGCGGATCTCACG
TGGCCGACCCTAACCAGATACACATGGGGAAAGGAATCAGGTGGCACACATACCGCCTTTACTCCATAGAGAACTTCCAATTGTGGGTAAGAACAAAGCCCCACCTCT
TCCTAATAGATGCAGTTTTCCACCTGCAGCCAAAAAGACAACCTGTTATTAGCTTTTTTCCCAGAGGACAGGCATCTCTATGAAACTGCCATTGCAAAATTTGCCGACAG
TGAGATAACCAGCTCCATTTTGCTTCTAACCTCCATGCCCTCCTTGTTTACTGCTGCACGCAGGTTGAACTGTGGGAGGAACTTAGTTTAAAACAAAGATGATAACAGCC
CTTTCCCAAAGGAAACCTTTCCTGGGGATTAGCCTGCTTTTGTGGGACCAACAGATTAGCCACAAGATTAGAAACTACGGTTGAAGAGTCACGCAGCGGGAGGCTACA
GGATTCTGACCCTCCCCAAACTGCTCCTGGCGATAACATCACCATCGTAAAGCCTAAGATCAGCACTTGGTGGGTCACCTGGCACCACCCAGGCTCATCTGATGTTGTG
GCACCCAGGAACTGACTCAGTGCGAAAACAGCTTCGACTCCCTGTGATTTCATCTCTGACCTGACCAATGGGTCGGCACAGACGCAACCCAGTCTCCTCCCACCTCCGA
GAGTGCAGTGTCCTGAATGGAGAACCCCAGCACCCA

>5RaceV2

AGTGGCGGGGTTCTCGCCGGTCCTGCGGGTCGCTGCTCGGCTGTGGGTTAGCAGCACCCTGAGCGGAGCGGAAGAGCTGCCCGCCGCCGCGTAACCCCGAGGCCGA
GACTCCTGGCCTGGCCTCCTTGGGCCCACGACGCCTGCCCCGCGGAGCGGACCCATGGGCCTGAGGTCGGCACAGACGCAACCCAGTCTCCTCCCACCTCCGAGAGTG
CAGTGTCCTGAATGGAGAACCCCAGCACCCA

>5RaceV3

CCTGCGGGTCGCTGCTCGGCTGTGGGTTGGCAGCACCCTGAGCGGAGCGGAAGAGCTGCCCGCCGCCGCGTAACCCCGAGGCCGAGACTCCTGGCCTGGCCTCCTTG
GGCCCACGACGCCTGCCCCGCGGAGCGGACCCATGGGCCTGAGGTTTGTGTGCGGGGCCTCAGGCGAGCGCTGGCGCAGCCTGTCCCCCATCGTCTGCGCCCTGCGG
GCCTGGCCCTCCCCGTGGAAGGACCGCGGGCTTCGGGGCCCCACGCTGGGGGGCTGACTCCTGTCACACACACAGCGGTGGCCGCGGGCCTGGGGCGGTAGCACAG
CCATTCTGCTCGGGCTGCGTGAGCTGACCCCGCTCTCTGTGCACCTACTTTTGCTGCGTTTGGTGCTGAGCCTGAAGTCCCAGGACAGGCGGCTGAGGGGGAAAGATG
GTGTGAATTGGGTGGGCAGAGACAACTGAAAGCCACAGGACCCGGTTGGCACCTTTTTGGCCGCCTGCGCCTTTGCCTGCAGCCCCGCTCCCTGGGTCACCCGAAGGA
GGAGCCGGCGCTCTTTCCGGGCGCAGAAGCACCCGACCCACGCTGGAGGAAACGGTCCCTCTGGGCAGGAGAAGCTTTGGGCCTGGCTGGAGCACACTCACGGAAC
CACGCCAACGCCTGGCCTGTAGCCAGCTCCCACCTCCCCTCCCACCTTCTAAAATGCTTGCGGATCTCACGTGGCCGACCCTAACCAGATACACATGGGGAAAGGAATC
AGGTGGCACACATACCGCCTTTACTCCATAGAGAACTTCCAATTGTGGGTCGGCACAGACGCAACCCAGTCTCCTCCCACCTCCGAGAGTGCAGTGTCCTGAATGGAGA
ACCCCAGCACCCA

>5RaceV4

ACAGTGAGATAACCAGCTCCATTTTGCTTCTAACCTCCATGCCCTCCTTGTTTACTGCTGCACGCAGGTTGAACTGTGGGAGGAACTTAGTTTAAAACAAAGATGATAAC
AGCCCTTTCCCAAAGGAAACCTTTCCTGGGGATTAGCCTGCTTTTGTGGGACCAACAGATTAGCCACAAGATTAGAAACTACGGTTGAAGAGTCACGCAGCGGGAGGC
TACAGGATTCTGACCCTCCCCAAACTGCTCCTGGCGATAACATCACCATCGTAAAGCCTAAGATCAGCACTTGGTGGGTCACCTGGCACCACCCAGGCTCATCTGATGTT
GTGGCACCCAGGAACTGACTCAGTGCGAAAACAGCTTCGACTCCCTGTGATTTCATCTCTGACCTGACCAATGGGTACTTCTGACTCACTGGCTGCCCCCCACCCACCAG
CTTATCTTTAAAAACTCCAAACCCCAGATGCTCAGGGAGACCGAGTAATAATAAAACTCCAGTCTCCCACACAGCTAGCTCTGCGTGAATTACCTTTTCTCTGTTGCAAAT
GCCCTTTCTTGATAAATTGGCTGTCTAGGTAACAGGGAAGGTGAACCCACTGGGCAGTTACACTTTTATCCACCCTTCCAGCTGGATCACGCGCCCTCTTCAATGACATG
ACACGCAGCTAGAGTGTATGAGCTTGCTGCCGGCACCTTCCGTCCTGGATCGCATAGCAAGGCCTCAATGGACAGCCAGGTGCCTGGTCCGGTCCCACTTTTTTTTCTG
TCCTGTTCTCGTTTCCTCTTTGCTTGGCCAAAGAGGGTTGGGCCAGGGTCCTGAGTGCAAAGGCTTGGCTTGGTGAATTAAAGTGGCAGTAGCCGTGTGGCAGAGAAA
ATTTGTAGATGGGTGAAATCCTGGGCCTACACAGTGACAAAGTGTATATAGACAGCCAGCCTAGTTGGTGGATGTGACAACCCCAGACTCTTCTAGATTTGTGGATCC
GTGCACCTGCCAGACCTGTCTCTGAGCCATCTGGGGAGGCTGGGTAAGATTTCCCCCGTCAGGGCCTGAGCCTCATCTCCAAGCTGCTGGCTTCAGCCCAGCTGCCACC
AGCGCTGTCTCTGTCTTGCGCTTTCCAGAGAGAGACTGACTCATTTTGAGCTCCAGGCCTACCAGGCAGCCCAACCGCGGCAGTAACTGCCTCTCACACTGAGGATGGC
TCATGCTCTGGTCCAGCCATGGCTTGAACACCTCCCAACAAGAACAAAGCCAGACTCATTCTCCCTAGCATCTTCTTCCTTGCTCAAAGATCCCACACCCCGTTAGAAGCT
GGAACTGGGTCCTTGTCACCTTCCTCACCTCCCCAGTCGTAGTCTGGTTAATCCTTACATCTCACCACGGTCACCGTGGCTAGATGTGACCTCCGCGATGCTTCAGGCAC
GTACCAGGGCAGCCTGGGCAGGGCTGGTCACTGTGATCTCATGAGGTGTGAACTTGACCCCGGCCTTCTTTCCTGGGATGCCGGCTAAGGACACAAGTTCAGTGTTTG
CCCTGGCTTGTAGCCCAGCGGGGGCTCCGTCATCCCCTGGGGAATGCCTCGGCCTGCAAGACCGCATACCGCATTGGAACAGGGAAACCACCTACTTCAGCACCTCCC
TCAGCAAGGTGGCAGGTCCCAACAAGCCTTGCACCACGAGGAAGTGGCAGTGGCATTCGGGATATGGCTCCCTGGCCAGCTTGTGAAGTTCAAGGGGCTCGTTACTTC
TTACAGCTCACAGTCCCTCCCGGGTCCAGCACACCTGAGGGCTGACCTCCCTCTTGATTAGTGCAGGGGAAAAGCACTTTAAAAAGCTGTGTCATGCGTAACTTGCACA
TCTTCTGATTCTGACCTGAAACTGCATCCCAGGTCGGCACAGACGCAACCCAGTCTCCTCCCACCTCCGAGAGTGCAGTGTCCTGAATGGAGAACCCCAGCACCCA

>5RaceV5

AGGGAGACCGAGTAATAATAAAACTCCAGTCTCCCACACAGCTAGCTCTGCGTGAATTACCTTTTCTCTGTTGCAAATGCCCTTTCTTGATAAATTGGCTGTCTAGGTAA
CAGGGAAGGTGAACCCACTGGGCAGTTACACTTTTATCCACCCTTCCAGCTGGATCACGCGCCCTCTTCAATGACATGACACGCAGCTAGAGTGTATGAGCTTGCTGCC
GGCACCTTCCGTCCTGGATCGCATAGCAAGGCCTCAATGGACAGCCAGGTGCCTGGTCCGGTCCCACTTTTTTTTCTGTCCTGTTCTCGTTTCCTCTTTGCTTGGCCAAAG
AGGGTTGGGCCAGGGTCCTGAGTGCAAGGGCTTGGCTTGGTGAATTAAAGTGGCAGTAGCCGTGTGGCAGAGAAAATTTGTAGATGGGTGAAATCCTGGGCCTACA

CAGTGACAAAGTGTATATAGACAGCCAGCCTAGTTGGTGGATGTGACAACCCCAGACTCTTCTAGATTTGTGGATCCGTGCACCTGCCAGACCTGTCTCTGAGCCATCT
GGGGAGGCTGGGTCGGCACAGACGCAACCCAGTCTCCTCCCACCTCCGAGAGTGCAGTGTCCTGAATGGAGAACCCCAGCACCCA

>5RaceV6

GGGagaCCGagtAATAATAAAACCCCagtCTCCCACACAGCTAGCTctGCGTGAATTACCTTTTCTCTGTTGCAAATGCCCTTTCTCGATAAATTGGCTGTCTAGGTAACAG
GGAAGGTGAACCCACTGGGCAGTTACACTTTTATCCACCCTTCCAGCTGGATCACGCGCCCTCTTCAATGACATGACACGCAGCTAGAGTGTATGAGCTTGCTGCCGGC
ACCTTCCGTCCTGGATCGCATAGCAAGGCCTCAATGGACAGCCAGGTCGGCACAGACGCAACCCAGTCTCCTCCCACCTCCGaGAGTGCAGTGTCCTGAATGGAGAAC
CCCAGCACCCA

>5RaceV7

agtgTTTGCCctgGCttGtAGCCCAGCGGGGGCTCcgtCATCCCctGGGGAATGCCTCGGCCTGCAAGACCGCATACCGCATTGGAACAGGGAAACCACCTACTTCAGCACC
TCCCTCAGCAAGGTGGCAGGTCCCAACAAGCCTTGCACCACGAGGAAGTGGCAGTGGCATTCGGGATATGGCTCCCTGGCCAGCTTGTGAAGTTCAAGGGGCTCGTT
ACTTCTTACAGCTCACAGTCCCTCCCGGGTCCAGCACACCTGAGGGCTGACCTCCCTCTTGATTAGTGCAGGGGAAAAGCACCTTAAAAAGCTGTGTCATGCGTAACTT
GCACATCTTCTGATTCTGACCTGAAACTGCATCCCAGGTCGGCACAGACGCAACCCAGTCTCCTCCCACCTCTGAGAGTGCAGTGTCCTGAATGGAGAACCCCAGCACC
CA

>3RACEV1

GTCTCCTCCCACCTCCGAGAGTGCAGTGTCCTGAATGGAGAACCCCAGCACCCAGAAGCATCCACTGAAGGACTAAATTCTACACATGAAGGCAAACGTCCGTGGTTG
ACAGAAATTACACTGGCCAGAATCCCCAGTCCCCATGAGGCTTGTCCAGACGCAGTGAACCAGTCGCAGCTGATAACACACAGACCATTCCCGATCCCAGAGGTGCAT
TTCAGGATTCATTCTATTTCATCAGAGACGGGGTCTTCCTGTGTTGCCCAGGCTGGTCTCGAACTCCTGGGCTGAAGCAATCTTCCTGCCTTGGCCTCCCAGAGGGTTGG
AATTGCAGATGTGAGGTGTGTCCTGGAACCCTGGCTGCACCCACAGATACAGCCACTCCTGGGCCCATGTGGATGTAACCTTCGAGTGCCGGAATGCCTCGTCTCCATG
GAGACCAAACTCAAGGGCCTGACTGGCCCAGTCTACCGAGACCATGAGACCTGCTGTGCCCCTCGGCCAGGCCAACCCAAATTCTTTTAAATAAAAGGCACAAGCCAC
AGCCACACCAGC

>3RACEV2

CTCCTCCCACCTCCGAGAGTGCAGTGTCCTGAATGGAGAACCCCAGCACCCAGAAGCATCCACTGAAGGACTAAATTCTACACATGAAGGCAAACGTCCGTGGTTGAC
AGAAATTACACTGGCCAGAATCCCCAGTCCCCATGAGGCTTGTCCAGACGCAGTGAACCAGTCGCAGCTGATAACACACAGACCATTCCCGATCCCAGAGGTGCATTTC
AGGATTCATTCTATTTCATCAGAGACGGGGTCTTCCTGTGTTGCCCAGGCTGGTCTCGAACTCCTGGGCTGAAGCAATCTTCCTGCCTTGGCCTCCCAGAGGGTTGGAA
TTGCAGATGTGAGGTGTGTCCTGGAACCCTGGCTGCACCCACAGATACAGCCACTCCTGGGCCCATGTGGATGTAACCTTCGAGTGCCGGAATGCCTCGTCTCCATGG
AGACCAAACTCAAGGGCCTGACTGGCCCAGTCTACCGAGACCATGAGACCTGCTGTGCCCCTCGGCCAGGCCAACCCAAATTCTTTTAAATAAAAGGCACAAGCCACA
GCCACACCAGCAGGAAGTGGATACAGGATAAGGGGCAGGGCTCACATCTCATCCTCATCAGCCTTTGACCAGGCGACGGAGTCTGTGATAGCTAGCTTATCTGCTCTA
TGCTCCAGGGACCTGGCCGGAACGAGCCGAGTTGCTGTCCTCATGGAACTCTCATCCTAGTGGGGCAGAGACAAGGACGGTGTAACGTGCCCGGGGGTCAGCACCAC
AGACAAAACGCAGGGAGCACGGCACAGTGTGGGCCAGGGAAGGCCTGGCTTCCCAAGGGTGTGGGCGCGAGCAGCATGGCTGTGGGGAGTTCTAGGTGCAGAGAA
CTACTCCAGAGACGAAGCCAGGCCTGCGAGGAGGCCGGAGCGCATGAGGGGAAGGCACGAGGCAAGGCTGCCAGGGCTGTGCTGGTGGTGCGGGACGGTCCTGTG
GGGGTAGCTGCGGAGATGGCACTGATGCCTTCGGCTCCAGCAGCATCCTGGGCTGGTGCGGGCCAATAAGGGGCAGGGTTGGGGCAGGATGAAAGGCACAAGCCA
CAGCCATGTCAAGCAGGAAGTGGATGCAGGGTACAGCCTGTCAGGGTCAGGGTGAAAGGCAGGGAGGCGGGTAGGCCATAGGTGAATTTGGAGCAGCCTGTAGAG
GACAGGATGTGCCATCAAAGACTTGTTCTTGGGTCCAGCTGAGACTGTCCCTTGGGGCTTCCTGTCACCTTGTGCAGGATAGTGAGACCATCCTTGGAAAACCCATCGG
GACTGCAGAGCGCGGCTCAGAGCTGACTGTCCAGGCCTGGCAGGGGCTGACCCCTGGGCGCTGGTGCACCCTCCGTGCGGCCTCTTGGAGGAAGCACACAAGGCGA
CAGTTCCCAGCTGCCGCCTGAGATGCAGCATCTGCTTTACTCCAGCCCAGCACTGCTCACCGTCACTGCCCACTCTTATGATTTTACTTCCCTTCATATTTGTGATTTTGCA
AAGATGGTGTTGACTTACAGCTGCAGCATCAAATGACTGACTGCCACCAGCTTGGCCCCAGCTCAGGGACATGTCTGAGCCCAGGCAGAGCACCCTAGTGGCCGGGA
AGCCTCACCTGGAGCTCCTGCGGGTGGCTGCAGTCCGCCAGGTGATCCGTGAGGAGGGCTTGGAGAGGCCCCATGATGGCGTGGAAGGATGGCAGGGACCCGTACA
TGAGCACGCAGCGCTTCAGCAGGGCCAGGCCCACAGCCAGGCAGGACAGTCTGTGAGGGCAGGAGGCAAGAGGAGGTCCAACTTGTTCTGTTTGTCACTGACAAAAC
CAATAACGTGGCAAAAATGAACTTCCCCACTTCCACTAGGCTGAAAACTCCAGTGTGGCCACCTTGGCCGGCCAGGGTTGGGCCCATGCAACCACGGGCAAGGCAGGC
TCAGGGTCAGGCCTAACGCAGGAGGGGAGGCTGTGGGAACCGCAGCGCCAGCTGGGATGGGGCGGCTCCATTTTTGGTTCCAAATTATGATTTGTGAAATCAAGCAA
ATGTATTTTGAAAATGAAAAGAAAAAGGGACAGCAA

SUPPLEMENTARY REFERENCES

1. Goyal, A., Myacheva, K., Gross, M., Klingenberg, M., Duran Arque, B. and Diederichs, S. (2016) Challenges of CRISPR/Cas9 applications for long non-coding RNA genes. *Nucleic acids research*.
2. Hammerle, M., Gutschner, T., Uckelmann, H., Ozgur, S., Fiskin, E., Gross, M., Skawran, B., Geffers, R., Longerich, T., Breuhahn, K. *et al.* (2013) Posttranscriptional destabilization of the liver-specific long noncoding RNA HULC by the IGF2 mRNA-binding protein 1 (IGF2BP1). *Hepatology*, **58**, 1703-1712.
3. Hannus, M., Beitzinger, M., Engelmann, J.C., Weickert, M.T., Spang, R., Hannus, S. and Meister, G. (2014) siPools: highly complex but accurately defined siRNA pools eliminate off-target effects. *Nucleic acids research*, **42**, 8049-8061.
4. Gutschner, T., Baas, M. and Diederichs, S. (2011) Noncoding RNA gene silencing through genomic integration of RNA destabilizing elements using zinc finger nucleases. *Genome research*, **21**, 1944-1954.
5. Gutschner, T., Hammerle, M., Pazaitis, N., Bley, N., Fiskin, E., Uckelmann, H., Heim, A., Grobeta, M., Hofmann, N., Geffers, R. *et al.* (2014) Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) is an important protumorigenic factor in hepatocellular carcinoma. *Hepatology*, **59**, 1900-1911.
6. Wang, Y., Zhu, W. and Levy, D.E. (2006) Nuclear and cytoplasmic mRNA quantification by SYBR green based real-time RT-PCR. *Methods*, **39**, 356-362.
7. Seiler, J., Breinig, M., Caudron-Herger, M., Polycarpou-Schwarz, M., Boutros, M. and Diederichs, S. (2017) The lncRNA VELUCT strongly regulates viability of lung cancer cells despite its extremely low abundance. *Nucleic acids research*, **45**, 5458-5469.
8. Franken, N.A., Rodermond, H.M., Stap, J., Haveman, J. and van Bree, C. (2006) Clonogenic assay of cells in vitro. *Nature protocols*, **1**, 2315-2319.