SUPPLEMENTARY INFORMATION

SUPPLEMENTAL METHODS

Cell synchronization

Cells were synchronized by double thymidine block. Briefly, 50% confluent cells were treated with 2mM Thymidine for 16h, washed twice with 1x PBS and reincubated with regular media for 9h. Then, cells were treated with a second round of 2mM thymidine treatment for 16h. At this point cells were considered as synchronized and were washed twice with 1x PBS (time 0), incubated with regular media to release the block and collected at different time points (1, 2, 3, 6 hours) to obtain populations at different phases of the cell cycle. Synchronization was evaluated by FACS analysis of DNA content with PI staining.

Fluorescence-Activated Cell Sorting analysis of cell cycle and apoptosis

To measure cell cycle distribution $1x10^6$ cells were harvested, washed twice with cold 1x PBS and taken to a final volume of 1 ml of 0.9% NaCl. Then, cells were fixed by adding 1 ml of ice-cold 95% ethanol dropwise while vortexing. Samples were allowed to stand at room temperature for 30 min before staining or stored at 4 °C for up to a week. Immediately before analysis, cells were spun out of fixing solution and resuspended in 1 ml of a 5 µg/ml PI solution (Propidium Iodide, Sigma, P4170). Thirty minutes before analysis, 20 µl of a 10 mg/ml of RNase A (ThermoScientific, EN0531) were added to each sample and incubated at 37°C in the dark. Unstained cells were used to set up cytometer settings.
To measure apoptosis $1.0x10^5$ cells were trypsinized, washed with cold 1x PBS and resuspended in 100 µl of Binding Buffer 1x (BD Pharmingen, 556454). Then, cells were added 5 µl of a 1:4 dilution of Annexin V-APC (BD Pharmingen, 556420) and/or 5 µl of a 50 µg/ml solution of PI (Propidium Iodide, Sigma, P4170) and were vortexed briefly. Unstained cells as well as single-stained cells were used to set up cytometer settings. After 15 min of incubation at room temperature in the dark, extra 400 µl of Binding Buffer 1x were added to the cells.
All samples were analyzed by FACS in a BD FACSCanto II flow cytometer. Data were recorded and analyzed using BD FACSDiva software.

RNA extraction and qRT-PCR

Total RNA extraction from cells or human samples was performed using the Maxwell® 16 LEV simply RNA Purification Kit (Promega) or using the TRIzol reagent, following the manufacturer's procedures. The RNA concentration was measured using a NanoDrop 1000 Spectrophotometer. For reverse transcription, 1 µg of RNA was incubated with 20 units Moloney Murine Leukemia Virus Retro-transcriptase (MMLV-RT, Invitrogen, 28025013) in MMLV-RT buffer, 5 µM DTT, 0.5 mM dNTPs (Sigma, GE28-4065-64) and 10 ng/ml random primers (Invitrogen, 48190-011) in a final volume of 40 µl. The reaction was set at 37 °C for 60 min and 95 °C for 1 min in a C1000 Thermal Cycler (Bio-Rad) and immediately placed at 4 °C. The

qRT-PCR was performed with 5 µl SYBR® Green Supermix (Bio-Rad, 1708884), 0.27 µM of each primer and 2 µl of the cDNA mix in a final volume of 10 µl in the CFX96 Real-Time system (Bio-Rad). The mixture was incubated at 95 °C for 3 min, then at 95 °C for 15 s, 60 °C for 15 s and 72 °C for 25 s for 34 cycles, and finally, 1 min at 95 °C and 1 min at 65 °C. The results were analyzed with Bio-Rad CFX manager software. Relative gene expression was normalized to the RPLP0 a housekeeping gene, a known invariant control for HCC, and expressed as $2^{-\Delta CT}$. The primers used were designed using the Primer3 online tool (http://primer3.ut.ee/)(21) and are listed in Supplementary Table S1C.

Cloning

The sequence of NIHCOLE was cloned by GenScript Cloning Services using a SmaI cloning site into a pUC57 backbone. The inserts were excised from pUC57 by SacI/XhoI digestion for insertion into pCAGGS for overexpression in cell lines, and by KpnI/XhoI digestion for sense insertion and NotI/HindII for antisense insertion into pcDNA3.1 for *in vitro* transcription. After overnight ligation at 16 °C, 1 µl of the ligation product was electroporated into XL1-Blue electroporation-competent cells. Electroporated cells were then seeded onto LB agar plates with 100 µg/mL ampicillin. Resistant clones were re-plated into a new ampicillin LB plate. Clones were tested by colony PCR using the KAPA TAQ PCR Kit (KapaBiosystems, KK1015). Minipreps of positive clones (Macherey-Nagel, 740588250) were purified and corroborated by restriction enzyme digestion pattern. Checked clones were verified by sequencing and maxipreps were purified (Macherey-Nagel, 740414.50) and aliquoted in 500 ng stocks.

RNA *in vitro* transcription

For *in vitro* transcription, full-length NIHCOLE and NICHOLEas templates were prepared either by plasmid linearization or by PCR amplification using forward primers with a T7 promoter adapter. Templates for SM2 and SM3 were prepared by PCR amplification using full-length NIHCOLE as template and forward primers with a T7 promoter adapter (Supplementary Table S1C). Template for LUC was provided in the *in vitro* transcription kit. Purified templates were transcribed using the HiScribe™ T7 Quick High Yield RNA Synthesis Kit (New England Biolabs, E2040S) either unlabeled or labeled with biotin, using a biotin RNA labeling mix (Sigma, 11685597910) following the manufacturer's instructions. After transcription RNA was treated with RNase-free DNase I (ThermoScientific, EN0521) to remove the DNA template and the RNA was cleaned up using RNA Clean and Concentrator-100 columns (ZymoResearch, R1019). After that, RNAs were refolded by temperature treatment ramping down from 65°C (10 min) to 4°C (1°C decrease every 40 s). RNA concentration was determined using the Qubit RNA HS kit (Invitrogen, Q32855) in a Qubit 4 Fluorometer (Invitrogen). Size and integrity of RNAs were assayed by automated RNA electrophoresis in a 4200 TapeStation System (Agilent).

DNA damage induction

Cells were either mock-treated or treated with DNA-PKcs inhibitor NU7441 (Tocris, 3712) or with 2 or 10 Gray (Gy) of gamma ionizing radiation (IR). Radiation was delivered using a Siemens Oncor Impression Plus linear accelerator equipped with a 6 MeV X-ray source. For the immunofluorescence and comet assay experiments, cells were irradiated using a ^{137}Cs-source GammaCell tissue irradiator at 2.9 Gy/min).

Immunoblotting

For the analysis of proteins by immunoblotting, whole-cell extracts were obtained by lysis with RIPA Buffer (150 mM NaCl, 25 mM Tris-HCl pH 7.5, 2 mM EDTA. 0.1 % deoxycholate, 0.1 % SDS, 1 % Triton X-100) supplemented with cOmplete Protease Inhibitor Cocktail) following the standard protocol. Alternatively, for the separate analysis of the whole-cell extract and chromatin-bound proteins, cell extraction buffer (CEB, Invitrogen, FNN0011) was used following the manufacturer's instructions. In summary, cells were harvested by scraping and transferred to conical 15 ml tubes, collected by centrifugation, and washed twice with ice-cold 1x PBS. Lysis was performed with CEB freshly supplemented with 1 mM PMSF (Sigma, 10837091001) and cOmplete Protease Inhibitor Cocktail using an approximate ratio of 1 ml per 10^8 cells. Then, cells were vortexed and incubated on ice for 30 min with occasional vortexing. Cell lysates were clarified by centrifugation at 14000 rpm at 4°C for 10 minutes. The supernatant (whole-cell extract) was transferred into a new tube and the pellet, containing the chromatin-bound proteins, was resuspended in freshly prepared 1 % SDS solution using the same volume as for CEB. The resuspended pellet was boiled for 5 min and sonicated 5 s on/5 s off twice.
Protein quantitation was performed directly with nanodrop by measuring total absorbance at 280 nm or, in the extracts obtained with CEB, with a detergent compatible protein quantitation kit (BioRad DC Protein Assay, 500112) following the recommended protocol. After quantitation, 25 µg of low molecular weight proteins or 50 µg of high molecular weight proteins were diluted to a final volume of 20 µl in 1x LDS sample buffer (Invitrogen, NP0007) with 1 % β-mercaptoethanol as reducing agent. Proteins were separated by SDS-PAGE on 12 % gels (24). After electrophoresis, proteins were transferred to nitrocellulose membranes following standard procedures. Then, membranes were blocked with 5 % non-fat milk for 1 h and probed overnight at 4°C with primary antibodies anti-PARP1 (Cell Signaling, 9542), anti-GAPDH (Cell Signaling, 5174), diluted 1:1000 in 5 % non-fat milk. Then, HRP-conjugated anti-mouse (Millipore, SAB3701132, 1:7000) or anti-rabbit (Millipore, SAB3700956, 1:10000) secondary antibodies were used to probe the membranes for 1 h at RT. Finally, membranes were developed with ECL-plus Western blotting substrate (Perkin-Elmer, NEL103001EA) for the chemiluminescent detection of proteins in a Chemidoc MP.

Negative stain electron microscopy

To visualize NIHCOLE, 3 µl of the purified lncRNA at 0.94 µM were incubated with freshly glow-discharged carbon-coated 400 mesh copper electron microscopy grids (Electron Microscopy Sciences) and stained using 2% (w/v) uranyl-formate stain solution pH-buffered with 25 mM NaOH. For evaluating

the Ku-NIHCOLE pulldown experiments, 3 µl of the eluted samples from the pulldown were used to prepare negative stain electron microscopy grids using 2% (w/v) uranyl-acetate as stain solution. Grids were visualized using a Tecnai 12 transmission electron microscope (Thermo Fisher Scientific Inc.) equipped with a lanthanum hexaboride cathode operated at 120 keV. Micrographs were recorded at a nominal magnification of 61320x that corresponds to 2.5 Å/pix at the specimen level on a 4kx4k TemCam-F416 CMOS camera (TVIPS). Images were collected in low-dose conditions using Serial EM (28,29), and those images corresponding to the Ku-NIHCOLE pulldown experiments were CTF-corrected in Relion (30). Particles corresponding to clusters of Ku-bound to NIHCOLE were first manually selected using e2boxer.py routine in EMAN2 (31). Each of those images was then analyzed in order to estimate the approximate number of Ku molecules in each Ku-NIHCOLE cluster using e2boxer.py routine in EMAN2.

Fluorescence Polarization Anisotropy

Protein-probe complexes were prepared as for EMSA but using 10x less concentration of labeled probe in a dark 384-well microplate. After incubation samples were submitted to FPA detection in a PHERAstar FSX (BMG Labtech). Non-linear regression was performed to estimate *Kd*, and R^2.

Expression and purification of XRCC4/LIG4

Full-length XRCC4 and DNA-Ligase IV were co-expressed in baculovirus harboring a C-terminal 8xHis-tag in XRCC4 and a N-terminal twin-strep-tag in DNA-Ligase IV. pFBDM-twin-strep-tag-DNA-LigaseIV/XRCC4-8xHis-tag was transfected into sf9 cells for viral production. For protein expression, insect High Five cells were infected with DNA-LigaseIV/XRCC4 baculovirus at a MOI of 2 and incubated for 70 hr at 26°C. Cells were lysed by homogenization in lysis buffer (50 mM HEPES, pH 8.0, 250 mM NaCl, 10 % (w/v) glycerol, 0.5 mM TCEP) supplemented with EDTA-free protease inhibitors (Roche) and TURBO DNase (Life Technologies). After a 20-minute incubation in ice, the slurry was clarified by centrifugation at 50,000 × g for 60 min, passed through a 0.5 µm filter and over Talon resin (Clontech) equilibrated with lysis buffer. The resin was then washed with lysis buffer supplemented with 5 mM imidazole and eluted in buffer supplemented with 300 mM imidazole. Fractions containing the DNA Ligase 4/XRCC4 complexes were identified by SDS-PAGE (Fig. S7A), pooled, loaded onto a Hitrap-Strep column (GE Healthcare), and subsequently eluted with buffer supplemented with 2.5 mM d-desthiobiotin. Finally, DNA-Ligase IV/XRCC4 rich fractions were pooled, concentrated, and loaded into a 26/60 Superdex 200 column (GE Healthcare) (Fig. S7B). Peak fractions from the size exclusion column were aliquoted, flash-frozen, and stored at −80°C.

Bioinformatic functional analyses

Expression analyses from TCGA and INSERM RNA-seq data were performed as previously described (33,34). To predict lncRNA function, we performed a guilt-by-association analysis (GBA) using the

samples of the TCGA from HCC (33). The Pearson correlation value was calculated between NIHCOLE and the remaining set of protein-coding genes. The correlation matrix obtained was used as input for giTools (35) where an enrichment analysis of GO categories was performed for each lncRNA using a method based on the Z-score to calculate the enrichment p-values (36).

Analysis of RNA secondary structure

To analyze the secondary structure of NIHCOLE and NIHCOLE structural motifs, the full sequence of NIHCOLE was submitted to the two most frequently used web-tools for the prediction of RNA secondary structure: The RNAfold Web Server from The Vienna RNA Web Suite (37) of the University of Vienna (http://rna.tbi.univie.ac.at/#webservices) and the UNAfold Web Server (38), previously Mfold, from the RNA Institute of the University at Albany (http://unafold.rna.albany.edu/). In both cases, minimum free energy (MFE) predictions at 37 °C were obtained using a model based on base-pair probability.