Supplementary Information for

Pericentromeric noncoding RNA changes DNA binding of CTCF and inflammatory gene expression in senescence and cancer

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Supplementary Information Methods

Cell Culture
TIG-3 cells (1-3) and IMR-90 cells were obtained from the Japanese Cancer Research Resources Bank and American Type Culture Collection, respectively. TIG-3 cells, IMR-90 cells and IMR-90/ER:H-RasV12 cells (1) were cultured in Dulbecco’s Modified Eagle’s (DME) medium (Nacalai Tesque) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin (Sigma-Aldrich) at physiological oxygen conditions (92% N₂, 5% CO₂, and 3% O₂) at 37°C. RPE-1/hTERT cells (4) and HEK-293T cells (1) were cultured in DME medium (Nacalai Tesque) supplemented with 10% FBS and penicillin/streptomycin (Sigma-Aldrich) at a 5% CO₂ incubator at 37°C. SVts8 cells (5) were cultured in DME medium (Nacalai Tesque) supplemented with 10% FBS and penicillin/streptomycin (Sigma-Aldrich) in a 5% CO₂ incubator at 34°C. Mouse embryonic fibroblasts (MEFs) were generated from CD-1 mice as previously described (6) and then cultured in DME medium (Nacalai Tesque) supplemented with 10% FBS and penicillin/streptomycin (Sigma-Aldrich) at physiological oxygen conditions (92% N₂, 5% CO₂, and 3% O₂) at 37°C. All cell lines used were negative for mycoplasma.

To induce doxorubicin (DXR)-induced senescence, TIG-3 and RPE-1/hTERT cells were cultured in medium containing DXR at concentrations of 250 and 150 ng/mL, respectively. One day before DXR treatment, TIG-3 and RPE-1/hTERT cells were plated at a density of 5,000 and 3,637 cells·cm⁻², respectively. These cells were not passaged after DXR treatment. To cause X-ray (XRA)-induced senescence, IMR-90, SVts8, and RPE-1/hTERT cells were exposed to 10-, 12-, and 40-gray (Gy) irradiation, respectively, with a CP-160 X-ray machine (Faxitron X-ray Corporation). After XRA irradiation, IMR-90, SVts8, and RPE-1/hTERT cells were plated at a density of 2,500, 9,090 and 4,000 cells·cm⁻², respectively. These cells were not passaged for 10 days after XRA irradiation. The induction of oncogene-induced senescence in IMR-90/ER:H-RasV12 cells were performed as previously described (1).

Plasmid Construction
Human centromeric satellite α (hSATα) and pericentromeric satellite II (hSATII) RNAs were cloned from the cDNA derived from DXR-treated RPE-1/hTERT cells and inserted into a pGEM-T Easy Vector (Promega). These cDNAs were tandemly connected in triplet and then subcloned into either a pcDNA3 vector (Invitrogen) or MaRX-puro retrovirus vector (7). Mouse centromeric minor satellite (MinSAT) and pericentromeric major satellite (MajSAT) RNAs were cloned from cDNAs derived from MEFs and inserted into a pGEM-T Easy Vector (Promega). These cDNAs were subsequently subcloned into a pcDNA3 vector (Invitrogen) or MaRX-puro retrovirus vector (7).

The 3xFLAG-tagged CTCF cDNA was cloned into a pcDNA3 vector (Invitrogen), MaRX-puro retrovirus vector (7), or pLenti CMV GFP Puro (658-5) (#17448, Addgene) using an In-Fusion HD Cloning Kit (Clontech) according to the manufacturer’s instructions. For the construction to delete all 11 zinc finger (ZF) domains (CTCFΔZF1-11), the following primers were used: 5’-GAAAGGTGTA AAGAAGACAT TCGGCCAGAG TGCCGTAGAG GGGGAATG GAGGAG-3’ (Forward) and 5’-CTCTCTTATT TTCCCCCTCT AGCCCATCTG GGGCAATGT CTTCTTTACA CCTTTTC-3’ (Reverse). For the construction of CTCFΔZF1 and CTCFΔZF10, indicating
the deletion of ZF domains 1 and 10, respectively, the primers were used as previously reported (8). For the construction of CTCFΔZF3-6, which spans the deletion of ZF domains 3–6, the following primers were used: 5'-CAGGTACTCG TCCTACAGAA AATGTGGCC-3' (Forward) and 5'-GGCCACATTT TCTGAGGAC GAGTACCTG-3' (Reverse).

All cDNAs were sequenced on a Genetic Analyzer 3130 (Applied Biosystems) using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

**RNA Interference (RNAi) and Overexpression**

The knockdown of CTCF and hSATII RNA were performed by the transfection of small interfering RNAs (siRNAs) using Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher Scientific), according to the manufacturer’s instructions. siRNAs targeting CTCF (Thermo Fisher Scientific, #HSS116455 and #HSS116456) and Negative Control (Duplex High GC Duplex) siRNA (Thermo Fisher Scientific, #46-2000) were used at a concentration of 50 nM for 2 days. Two siRNAs targeting hSATII RNA (#1: 5'-UUUCCAUUCC AUUCCAUUC-3' and #2: 5'-AAUCAUCGA UGGUCUCGA-3') and one for Negative Control (5'-AUGAACGUGA AUUGCUCAA-3') were used at the concentration of 20 nM for 2 days. As shown in Fig. 1H, siRNA #2 targeting hSATII RNA was treated to proliferating or XRA-induced senescent SVts8 cells. Knockdown efficiency was evaluated using real-time quantitative polymerase chain reaction (RT-qPCR).

Retroviral gene transfer into SVts8 cells ([SI Appendix](#), Fig. S5) was conducted by transient transfection of LinXE ecotropic packaging cells with a MaRX-puro vector and the vector containing hSATII DNA, as previously described (7). Infected cell populations were selected for 3 days in the presence of 1 μg/mL puromycin. Retroviral gene transfer into early-passage primary MEF cells (P1–P3, [SI Appendix](#), Fig. S6) was conducted by transient transfection of LinXE ecotropic packaging cells with a MaRX-puro vector and the vector containing MajSAT DNA, as previously described (6, 7). Infected cell populations were selected for 5 days in the presence of 1 μg/mL puromycin.

SVts8 ([Fig. 2E](#)) or HEK-293T ([Fig. 2C and D](#)) cells were transfected with a vector designed to express hSATII RNA and/or CTCF using X-tremeGENE HP DNA Transfection Reagent (Roche), according to the manufacturer’s instructions. After 24 hours of transfection, the cells were scraped and analyzed. We monitored the transfection efficiency of CTCF using EGFP expression (the same backbone plasmid with CTCF) and confirmed almost 100% efficiency in each experiment. Although CTCF overexpression generally causes cell cycle arrest, in SVts8 cells in which pRb and p53 are inactivated by large T antigen, we could avoid cell cycle arrest and thereby examined the effect of CTCF overexpression within 24 h. SVts8 cells ([Fig. 1E and F](#) and [3; SI Appendix](#), Fig. S2A–C) were transfected with a vector designed to express hSATII RNA using X-tremeGENE HP DNA Transfection Reagent (Roche) a total of four times every other day, according to the manufacturer’s instructions. Cells were scraped and analyzed 24 hours after the final transfection.

Lentiviruses encoding 3xFLAG-tagged CTCF or CTCFΔZF were generated by using Lentiviral Packaging Mix (Sigma-Aldrich, #SHP001) according to the manufacturer’s instructions.

**Reverse Transcription PCR (RT-PCR)**
Total RNA was extracted with TRIzol Reagent (Thermo Fisher Scientific). After removing genomic DNA contamination using a TURBO DNA-free Kit (Applied Biosystems), the extracted RNA underwent reverse transcription using a PrimeScript RT Master Mix (TaKaRa). RT-PCR was performed on a Veriti Thermal Cycler (Applied Biosystems) using KOD-Plus Neo DNA polymerase (Toyobo). The primers used for RT-PCR are as follows: human GAPDH: 5′-GCCACATCGCTCAGCACAC-3′ (Forward) and 5′-CATCACGCCCA CAGTTTCC-3′ (Reverse); mouse GAPDH: 5′-CAACTACATGTCTGTACATGTTC-3′ (Forward) and 5′-CGCCAGTACATCTCCACGAC-3′ (Reverse); EXOtic-hSATα: 5′-GTTTAAACTTAAAGCTCAACGAAAGGCCACAA-3′ (Forward) and 5′-CTATTTTGCA TCTAGAAGGT CAATGGCAGA-3′ (Reverse); EXOtic-hSATII: 5′-GCAGTACATCAAATGGGCGTGGAAGGCCACAA-3′ (Forward) and 5′-CGCCATTTCGATGATTGGATCC-3′ (Reverse); EXOtic Control (Backbone): 5′-GACTTTAGAGGGTACCGTGA TCCG-3′ (Forward) and 5′-GCTGGCAACT AGAAGGCACA G-3′ (Reverse). The amplification products were separated using 1%–3% agarose gel electrophoresis and detected by ethidium bromide staining.

**RT-qPCR**
Total RNA was extracted with TRIzol Reagent (Thermo Fisher Scientific). After removing genomic DNA contamination using a TURBO DNA-free Kit (Applied Biosystems), the extracted RNA underwent reverse transcription using a PrimeScript RT Master Mix (TaKaRa). RT-qPCR was performed on a StepOne Plus PCR system (Thermo Fisher Scientific) using SYBR Premix Ex Taq II (Tli RNaseH Plus, TaKaRa, #RR820A). The primers used for RT-qPCR are as follows: hSATII: 5′-AATCATCGAATGGTCTCGATTTCTG-3′ (Forward) and 5′-ATAATTCCAT TCGATTTCCAC-3′ (Reverse); hSATα: 5′-AAGGTCAATGGCAGAAAAGAA-3′ (Forward) and 5′-CAACGAAGGCACACAAGAT-3′ (Reverse); human ACTB: 5′-AGAGCTACGACTGCCTGAC-3′ (Forward) and 5′-AGCAGATGTGGCGTACAG-3′ (Reverse); MajSAT: 5′-ATATCCATTCGATTTCCAC-3′ (Forward) and 5′-CTTGCCATAT TCCACGTCCT-3′ (Reverse); MinSAT: 5′-TTGGAAACGGAATTTG-3′ (Forward) and 5′-CGGTTTCCAAATATGTGTTTT-3′ (Reverse); murine ACTB: 5′-CGCACCAGTGCGCCATGGA-3′ (Forward) and 5′-TACAGCAGGGAGCATCGT-3′ (Reverse). LMNB1 (9) and CTCF (10) were detected as previously described. CDKN2A, IL6, CXCL8, IL1A, IL1B, CXCL10, IFNA1 and IFNB1 were detected as previously described (2). The quantity of all samples was obtained using the standard curve method according to the manufacturer’s protocol and was normalized to the housekeeping gene ACTB.

**Northern Blot**
Total RNA was extracted from proliferating or senescent TIG-3 and IMR-90/ER:H-RasV12 cells by TRIzol Reagent (Invitrogen) according to manufacturer’s protocols. After genomic DNA digestion using a TURBO DNA-free Kit (Applied Biosystems), total RNA samples (3–9 μg) were denatured in a 0.5 × MOPS (Nacalai Tesque), 2.2 M formaldehyde (Wako), and 50% formamide (Sigma-Aldrich) solution at 65°C for 15 minutes and then electrophoresed on 1% agarose gels containing 1× MOPS and 2.2 M formaldehyde solution (Wako). After transfer onto a Hybond-N+ membrane (Amersham/GE Healthcare) and followed by cross-linking with ultraviolet light, the membrane was prehybridized in Church buffer (11). A 32P-labeled DNA probe targeting hSATII RNA was generated from
the pcDNA3-hSATII RNA plasmid (described in the “Plasmid Construction” section) with the primer 5′-TATAATTCCA TTCGATTCC-3′ and the Megaprime DNA Labeling System (Amersham/GE Healthcare) according to manufacturer’s protocols. The membrane was hybridized overnight with the purified probe using Sephadex G-50 DNA Grade NICK Columns (GE Healthcare) in Church buffer at 55°C. The membrane was then washed twice with 1× SSC buffer containing 0.1% SDS at 55°C for 5 minutes, followed by 0.2× SSC buffer containing 0.1% SDS at 55°C for 15 minutes. The hybridized 32P signal was visualized on high-performance chemiluminescence film (Amersham/GE Healthcare).

RNA Pull-down Assay
RNA pull-down assays were performed using a RiboTrap Kit (MBL, #RN1011/RN1012) according to the manufacturer’s instructions. Briefly, 5-bromo-UTP was randomly incorporated into hSATα, hSATII, MinSAT, and MajSAT RNAs upon transcription using vectors containing the full-length RNAs as templates (in vitro transcription). Next an anti-BrdU antibody conjugated with Dynabeads Protein G (Thermo Fisher Scientific, #10004D) were bound to the in vitro-synthesized RNA before incubating at 4°C for overnight with SVts8 cell lysates for hSATα and hSATII RNA or MEF lysates for MinSAT and MajSAT RNA. Finally, the samples were washed, eluted, and subjected to western blot or mass spectrometric analysis.

Western Blotting
Cell pellets were lysed in lysis buffer (0.1 M Tris-HCl pH 7.5, 10% glycerol, and 1% SDS), boiled for 5 minutes, and then centrifuged for 10 minutes at 15,000 rpm. All protein concentrations were determined by BCA Protein Assay Reagent (Pierce). Each cell lysate was electrophoresed by SDS-PAGE and transferred onto PVDF membranes (Millipore). After blocking with 5% skim milk (Megumilk) or 5% bovine serum albumin (Sigma-Aldrich) in Tris-buffered saline with 0.1% Tween 20 (TBST), the membrane was treated with primary antibodies to p16 (IBL, #11104, 1:250 dilution), lamin-B1 (Abcam, #ab16048, 1:1,000 dilution), GAPDH (Proteintech, #60004-1-lg, 1:10,000 dilution), vinculin (Sigma-Aldrich, #V9131, 1:1,000), CTCF (Cell Signaling Technology, #3418, 1:1,000 dilution), DDDK-tag (MBL, #M185-3L, 1:5,000), and ras (Oncogene, #OP41, 1:1,000 dilution) overnight at 4°C in blocking buffer. Membranes were then washed three times in TBST and incubated with an enhanced chemiluminescence (ECL) anti-mouse IgG, horseradish peroxidase-linked whole antibody (GE Healthcare, NA931V) or ECL anti-rabbit IgG, horseradish peroxidase-linked whole antibody (GE Healthcare, NA934V) for 1 hour at room temperature. After washing the membrane three times in TBST, the signal was resolved with SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific) and imaged on a FUSION imaging system (Vilber-Lourmat).

Mass Spectrometric Analysis
The hSATII RNA-binding proteins were purified using a RiboTrap Kit (MBL) as described in the “RNA Pull-down Assay” section. The eluate was then concentrated approximately 10-fold by Amicon Ultra 0.5 mL Centrifugal Filters (30K, Merck Millipore). Samples were reduced in 1× Laemmli sample buffer with 10 mM TCEP at 100°C for 10 minutes, alkylated with 50 mM iodoacetamide at ambient temperature for 45 minutes, and subjected to SDS-PAGE. Electrophoresis was stopped at a migration distance of 2 mm from the top
edge of the separation gel. After Coomassie Brilliant Blue staining, protein bands were excised, destained, and finely cut prior to in-gel digestion with Trypsin/Lys-C Mix (Promega) at 37°C for 12 hours. The resulting peptides were extracted from gel fragments and analyzed with an Orbitrap Fusion Lumos Mass Spectrometer (Thermo Scientific) combined with an UltiMate 3000 RSLC nano-flow HPLC (Thermo Scientific). Tandem mass spectrometry spectra were searched against a Homo sapiens protein sequence database in SwissProt using Proteome Discoverer 2.2 (Thermo Scientific), in which peptide identification filters were set at “false discovery rate (FDR) < 1%.” Gene ontology analysis was performed by Metascape (12).

Chromatin Immunoprecipitation (ChIP) Followed by ChIP-Seqencing (ChIP-seq)
ChIP was essentially performed as described (2) with minor modifications. Briefly, cells at 70%–80% confluency were cross-linked with 1% formaldehyde for 10 minutes at room temperature and quenched with 125 mM glycine for 5 minutes at room temperature. The cross-linked cells were scraped into a microcentrifuge tube and washed twice with ice-cold phosphate-buffered saline (PBS). The washed cells were lysed in ChIP lysis buffer (1% SDS, 50 mM Tris-HCl pH 8.0, 10 mM EDTA, and 1× protease inhibitor cocktail) and then sonicated using a Bioruptor (Cosmo Bio Corporation) set to pulse on high (30 seconds of sonication, followed by 30 seconds of rest) at 4°C for 15 minutes. Lysates were cleared by centrifugation at maximum speed for 10 minutes at 4°C, and the chromatin-containing supernatants were transferred into new centrifuge tube. After sixfold dilution with ice-cold ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH 8.0, 150 mM NaCl, and 1× protease inhibitor cocktail), the CTCF (Cell Signaling Technology, #3418) or Rabbit (DA1E) mAb IgG XP Isotype Control (Cell Signaling Technology, #3900) antibody was added, followed by overnight rotation at 4°C. The next day, samples were incubated with Dynabeads Protein G (Thermo Fisher Scientific, #10004D) at 4°C for 30 minutes before a wash process, according to the manufacturer’s instruction. Dynabeads Protein G were collected on a magnet and washed three times with wash buffer I (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.0, and 150 mM NaCl), wash buffer II (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.0, and 500 mM NaCl), and wash buffer III (0.25% LiCl, 1% NP-40, 1% Na-DOC, 1 mM EDTA, and 10 mM Tris-HCl pH 8.0), followed by two washes with ice-cold TE (10 mM Tris-HCl pH 8.0 and 1 mM EDTA) and elution by ChIP elution buffer (1% SDS and 0.1 M Na₂CO₃). The eluted DNA was incubated overnight at 65°C for to reverse cross-links, followed by incubation in the presence of Proteinase K Solution (Wako) at 50°C for 2 hours. The samples were subsequently cleaned by phenol–chloroform extraction, precipitated with ethanol, and resuspended in either TE buffer or low TE buffer (10 mM Tris-HCl pH 8.0, and 0.1 mM EDTA) for ChIP-seq. ChIP-qPCR analyses were performed on a StepOne Plus PCR system (Thermo Fisher Scientific) using SYBR Premix Ex Taq II (Tli RNaseH Plus, TaKaRa, #RR820A). The primers used for qPCR are as follows: human imprinting control region (ICR): 5’-CCCATCTTGC TGACCTCAC-3’ (Forward) and 5’-AGACCTGGGA CGTTTCTGTG-3’ (Reverse) (13); hSATα: 5’-AAGGTCAATG GCAGAAAAGA A-3’ (Forward) and 5’-CAACGAAGGC CACAAGATGT C-3’ (Reverse); mouse ICR: 5’-GTCACTCAGG CATAGCATTC-3’ (Forward) and 5’-GTCTGCCCGAG CAATATGTAG-3’ (Reverse) (14); MinSAT: 5’-
TTGGAAACGG GATTTGTAGA-3’ (Forward) and 5’-CGGTTTCCAA CATATGTGTT TT-3’ (Reverse).

Libraries for ChIP-seq were prepared with SMARTer ThruPLEX DNA-Seq Kit (Takara Bio USA) according to the manufacturer’s protocol. The amplicon libraries for sequencing were quantified using a LabChip GX Touch (PerkinElmer) and sequenced using 2× 75-bp MiSeq Reagent Kits v3 (Illumina) on an Illumina MiSeq, according to the manufacturer’s recommendations.

**Chromosome Conformation Capture (3C)-qPCR**

3C-qPCR was performed as previously described (15). In brief, to make a single-cell suspension hSATII RNA-overexpressed SVts8 cells were filtered through a 40-μm cell strainer and cross-linked with 1% formaldehyde for 10 minutes. After quenching with 125 mM glycine, the cross-linked cells were resuspended in lysis buffer (10 mM Tris-HCl pH 8.0, 10 mM NaCl, 0.2% NP-40, and 1× protease inhibitor cocktail) and subjected to enzymatic digestion using BgII (700 units, New England Biolabs) at 37°C for overnight, followed by ligation with T4 DNA ligase (400 units, New England Biolabs) at 16°C for 4 hours in a large volume. Next, cross-link reversal was performed by incubating samples overnight at 65°C in the presence of Proteinase K Solution (480 μg, Wako). The digested and ligated chromatin samples were then cleaned by phenol–chloroform extraction and precipitated with ethanol. The precipitated samples were subsequently cleaned using a QIAquick PCR Purification Kit (Qiagen) and resuspended in nuclease-free water. 3C-qPCR was performed using a StepOne Plus PCR system (Thermo Fisher Scientific) and SYBR Premix Ex Taq II (Tli RNaseH Plus, TaKaRa, #RR820A), and data were normalized to a GADPH control. The primers used for 3C-qPCR are as follows: 3C-Constant: 5'-CGGATAAGAG AAAGGAGGTG TTGG-3'; 3C-T1: 5'-AGGTGATGAAA TCCTACCAGC AGTG-3'; 3C-T2, 5'-TGAGATTACAGGCATGAGCC AC-3'; 3C-T4: 5'-ATGTAGGGAA GTGATGGGAG AG-3'; 3C-T6: 5'-TACTGTCTTCA AAGGCAGGCA CC-3'; 3C-T7: 5'-ACAACGTCTT TTCCCAACTA CC-3'; 3C-T15: 5'-GGGAGGAGAT TGACTACAAA GGAC-3'; 3C-T22: 5'-AGGTTGACGT GAGCTGAGAT TG-3'; 3C-T26: 5'-AGACCACCTGACAGAGATAA CC-3' and gGapdh-Fwd: 5'-GGGAGGTAGA GGGGTGATGT TTGG-3'; gGapdh-Rev: 5'-ATGGCATGGA CTGTGGTCTG-3'. The relative cross-linking frequency was calculated by setting the cross-linking frequency of a bacterial artificial chromosome-containing amplified locus at 100%.

**Assay for Transposase-Accessible Chromatin (ATAC)-seq**

ATAC-seq was performed as previously described (16). The amplicon libraries for sequencing were quantified using a LabChip GX Touch (PerkinElmer) and KAPA Library Quantification Kit (KAPA Biosystems, #KK4824) and sequenced using 2 × 75-bp MiSeq Reagent Kits v3 (Illumina) on an Illumina MiSeq, according to the manufacturer’s recommendations.

**RNA Immunoprecipitation (RIP)**

To evaluate the binding of CTCF and hSATII RNA, HEK-293T cells were transfected with vector constructs designed to express either CTCF (WT) or CTCFΔZF and hSATII RNA using X-tremeGENE HP DNA Transfection Reagent (Roche), according to the
manufacturer’s instructions. After 24 hours, the cells were scraped and lysed, followed by RIP assay. XRA-induced senescent IMR-90 cells were infected by lentiviruses with 10 μg/mL polybrene on Day 8 after XRA irradiation. The infected cells were then scraped on Day 10 and lysed, followed by RIP assay. RIP assay was performed using a Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, #17-700) according to the manufacturer’s instructions. After removing genomic DNA contamination using a TURBO DNA-free Kit (Applied Biosystems), immunoprecipitated RNA underwent subjected to reverse transcription using a PrimeScript RT Master Mix (TaKaRa). Next, qPCR was performed to quantify the immunoprecipitated RNA using a StepOne Plus PCR system (Thermo Fisher Scientific) and SYBR Premix Ex Taq II (Tli RNaseH Plus, TaKaRa, #RR820A). The primers used for qPCR are listed in the “RT-qPCR” and “Chromatin Immunoprecipitation (ChIP) Followed by ChIP Sequencing (ChIP-seq)” sections. The percent input (% Input) was calculated.

Electrophoretic Mobility Shift Assay (EMSA)

Human full-length CTCF cDNA was cloned into a pGEM-6P-1 plasmid (GE Healthcare) to produce recombinant CTCF with glutathione S-transferase (GST)-tagged N-terminal (GST-CTCF). The plasmid was transformed into Escherichia coli BL21 host strains (TaKaRa), and GST-CTCF expression was induced under 0.2 M isopropyl β-D-thiogalactopyranoside and 100 μM ZnSO₄ at 20°C for 2 hours. After collecting E. coli cells by centrifugation, the pellet was resuspended in PBS containing 1x protease inhibitor cocktail, followed by sonication using a Bioruptor (Cosmo Bio Corporation) set to pulse on high (20 seconds of sonication, followed by 30 seconds of rest) at 4°C for 3 minutes. GST-CTCF was purified by GST Sepharose 4B (GE Healthcare), followed by GST-tag removal using a PreScission Protease (GE Healthcare) according to the manufacturer’s protocol. hSATII and hSATα RNAs were transcribed in vitro by a MEGAscript Kit (Invitrogen/ Thermo Fisher Scientific). EMSA was performed using a LightShift Chemiluminescent EMSA Kit (Thermo Fisher Scientific) according to the manufacturer’s protocol. Briefly, recombinant CTCF was purified as described, and 10 fmol/μL biotin-labeled ICR probe (17) and/or hSATII or hSATα RNA at a concentration of 10, 5, or 2.5 pmol/μL were incubated for 20 minutes at room temperature. After incubation, the mixture was loaded on a 5% polyacrylamide gel and underwent electrophoresis (100 V) at 4°C for 110 minutes in 0.5x TBE. After transfer onto a Hybond-N+ membrane (Amersham/GE Healthcare), samples were cross-linked with ultraviolet light. The signals of biotin-labeled probes were detected using a Chemiluminescent Nucleic Acid Detection Module Kit (Thermo Fisher Scientific) according to the manufacturer’s protocol. Chemiluminescence signals were detected using a FUSION imaging system (Vilber-Lourmat).

Immunofluorescence Imaging

To detect multipolar or chromosomal bridge formation, hSATII RNA-expressing SVts8 or MajSAT RNA-expressing MEFs were seeded on PLL-coated glass cover slips (Matsunami Glass, #C1210). After 24 hours, the cells were fixed with ice-cold methanol at -20°C for 10 minutes, and permeabilized with 0.1% Triton X-100. After blocking with 15% goat serum (Sigma-Aldrich, #G9023), cells were incubated with α-tubulin (Sigma-Aldrich, #T9026, 1:5,000), γ-tubulin (Sigma-Aldrich, #T3559, 1:5,000), or pericentrin (Abcam, #ab4448, 1:1,000) antibodies overnight at 4°C. The next day, the blots were washed twice
with PBS and then incubated with a Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (Thermo Fisher Scientific, #A11008, 1:1,000) and Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 (Thermo Fisher Scientific, #A11032, 1:1,000) at room temperature for an hour. The slides were mounted using ProLong Diamond Antifade Mountant with DAPI (Thermo Fisher Scientific) and examined using a BZ-X710 fluorescence microscope (Keyence).

**Karyotype Analysis**
Cells were treated with nocodazole (100 ng/mL) for 12 (MEFs) or 4 (SVts8) hours. Karyotype analysis of mitotic cells was performed as previously described (18).

**Focus Formation Assay**
MajSAT RNA-expressing MEFs (1–5× 10^4) previously described in the “RNA Interference (RNAi) and Overexpression” section were seeded into 6-cm-diameter dishes. The cells were maintained at physiological oxygen conditions (92% N_2, 5% CO_2, and 3% O_2) for approximately a month, and the medium was changed weekly until the cells were photographed and counted. After the approximately 1-month culture, cells were stained with 0.05% crystal violet in 20% methanol. Dishes were scanned, and piled-up foci were counted.

**Anchorage-Independent Soft Agar Colony Formation Assay**
A total of 1 × 10^3 cells were suspended in DME medium containing 0.4% SeaPlaque Agarose (Lonza, #50101) and 10% FBS and layered on DME medium containing 0.6% SeaPlaque Agarose and 10% FBS in 6-well plates in triplicate. After 2–3 weeks of incubation, the number of colonies was counted.

**RNA Sequencing (RNA-Seq)**
Total RNA was extracted from satellite RNA-overexpressed or XRA-irradiated (12 Gy) SVts8 cells using TRIzol Reagent (Invitrogen), details of which are found in the “Cell Culture” or “RNA Interference (RNAi) and Overexpression” sections. After genomic DNA digestion using a TURBO DNA-free Kit (Applied Biosystems), sequencing libraries were prepared using a NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs) according to the manufacturer’s instruction, followed by 150-bp paired-end sequencing performed by Annoroad Corporation using a HiSeq X.

**Extraction and Application of Exosome-Like Extracellular Vesicles (EVs)**
To cause DXR-induced senescence, RPE-1/hTERT cells were cultured in medium containing DXR at concentrations of 150 ng/mL. One day before DXR treatment, RPE-1/hTERT cells were plated at a density of 3,637 cells•cm^-2. These cells were not passaged after DXR treatment. To induce XRA-induced senescence, RPE-1/hTERT cells were exposed to 40-Gy irradiation with a CP-160 X-ray machine (Faxitron X-ray Corporation). After XRA irradiation, RPE-1/hTERT cells were plated at a density of 4,000 cells•cm^-2. These cells were not passaged for 10 days after XRA irradiation. EVs were collected from senescent RPE-1/hTERT cells cultured for 3 days in DME medium containing 5% FBS by EV depletion using ultracentrifugation at 100,000 × g for 16 hours. The number of EVs was counted using a LM10 Nanoparticle Characterization System (NanoSight) as
previously described (3, 4). The total RNA of EVs was extracted using TRIzol LS Reagent (Thermo Fisher Scientific) according to the manufacturer’s protocols. Regarding the addition of EVs to cells, after the collected EVs were mixed in FBS-depleted medium at a density of $2 \times 10^9$ particles/mL, the medium of host cells was changed with the EV-containing medium daily for one week. Subsequently, karyotyping or anchorage-independent growth assay of EV-treated cells were performed. Designer exosome production using EXOtic devices was performed as previously reported (19), and EV extraction and application to cells were similarly performed as described.

**RNA In Situ Hybridization (RNA-ISH)**

Tissue samples were obtained from a patient who underwent surgical resection at Cancer Institute, Japanese Foundation for Cancer Research (JFCR). Tissue samples were collected after obtaining the appropriate institutional review board approval (approval number: 2013–1090) and written informed consent of the patient. hSATII RNA was detected on formalin-fixed paraffin-embedded (FFPE) sections in primary colon cancer specimens using an Advanced Cell Diagnostics (ACD) RNAscope® 2.5 HD Reagent Kit-BROWN (ACD, #322300) and the RNAscope® Target Probe - Hs-HSATII (ACD, #504071) according to the manufacturer’s instructions. For each sample ($n = 10$), two images ($\times 100$) of normal mucosa, submucosa and tumor were randomly selected. The area of hSATII RNA positivity and total cells were analyzed using NIH ImageJ software. The hSATII RNA-positive area per field (%) of each type of cell was calculated as the proportion of the total positive area to the total area of cells.

**Organoid Culture Experiments**

Organoids prepared from small intestinal tumors ($Apc^{\Delta716}$ or $Apc^{\Delta716} Trp53^{R270H/R270H}$) were cultured as previously described (20). Total RNA was collected using an RNeasy Plus Micro Extraction Kit (Qiagen) after organoid culture for 3 days.

**In Vivo Allograft Assays**

MEF/Vector or MEF/MajSAT RNA ($5 \times 10^6$ cell) in Hank’s Balanced Salt Solution (Gibco/Thermo Fisher Scientific) were subcutaneously injected with an equal volume of Matrigel (BD Pharmingen) into 4- or 5-week-old female BALB/c-nu/nu mice (Charles River Laboratories). After 20 or 30 days of cell injection, the mice were euthanized, and tumor weight was measured. All animal procedures were performed using protocols approved by the JFCR Animal Care and Use Committee in accordance with the relevant guidelines and regulations (approval number: 1804-05).

**Bioinformatical Analysis**

The sequence and processing data have been deposited in the DNA Data Bank of Japan with the accession numbers DRA009771 (https://ddbj.nig.ac.jp/DRASearch/submission?acc=DRA009771) for RNA-seq, DRA010750 (https://ddbj.nig.ac.jp/DRASearch/submission?acc=DRA010750) for ChIP-seq, and DRA010749 (https://ddbj.nig.ac.jp/DRASearch/submission?acc=DRA010749) for ATAC-seq. All other data supporting the findings of this study are available within the article.
Screening for unique transcripts showing increased chromatin accessibility and active transcription

To screen for loci showing increased chromatin accessibility and being actively transcribed during cellular senescence (Fig. 1A), we first performed a comparative analysis of ATAC-seq data between proliferating and X-ray-induced senescent IMR-90 cells. The resulting paired-end FastQ reads of ATAC-seq underwent quality control with FastQC (version 0.11.8) and trimmed with TrimGalore (version 0.6.4; https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). The trimmed reads were mapped against Homo sapiens UCSC hg19 using Bowtie 2 (version 2.3.5) alignment software (21). SAMtools (version 1.9) was used to sort and convert SAM to BAM files (22, 23). Uniquely mapped reads were used for peak calling using MACS 2 (version 2.1.4) with the command “$ macs2 callpeak --nomodel --nolambda --keep-dup all --call-summits -f BAMPE -g hs,” and peaks were filtered by an enrichment score (q < 0.01) (24). Using the read-depth normalized matrix of ATAC-seq signal for all consensus peaks, differential loci of chromatin accessibility between proliferating and X-ray-induced senescent IMR-90 cells were determined using DiffBind (version 2.14.0), resulting in 16,325 peaks displaying significantly altered chromatin accessibility, FDR < 0.05 and region width < 10 k based on the consensus peaks identified in at least two replicates. Based on 14,356 and 1969 of the 16,325 peaks showing “Up”- and “Down”-regulated in X-ray-induced senescent IMR-90 cells compared to proliferating IMR-90 cells, respectively. We defined the total 16,325 peaks as “Differential Peaks” (Fig. 1A and B). Peak distributions showing significantly altered chromatin accessibility in the senescent cells (SI Appendix, Fig. S1A) were analyzed by the cis-regulatory element annotation system (25).

Next, to identify transcripts containing the 16,325 regions displaying significantly altered chromatin accessibility in X-ray-induced senescent IMR-90 cells, we referred to GRCh37/hg19 and RepeatMasker databases using BEDTools (26), resulting in the identification of 652 transcripts in these regions. The referral GRCh37/hg19 database was from the UCSC genome annotation database for the February 2009 assembly of the human genome (hg19, GRCh37 Genome Reference Consortium Human Reference 37), whereas the RepeatMasker database [DNA, LINE, LTR, RC, RNA (7SK), rRNA, Satellite, SINE and snRNA families] was referred from hg19 - February 2009 - RepeatMasker open-4.0.5 - Repeat Library 20140131.

We then calculated the gene expression level of these 652 transcripts in proliferating and X-ray-induced senescent IMR-90 cells. Publicly available data of proliferating and senescent IMR-90 cells (GEO: GSE130727) (27) were reanalyzed using four samples: GSM3752532 (IMR-90 PDL15 1) and GSM3752533 (IMR-90 PDL15 2) for proliferating IMR-90 cells and GSM3752534 (IMR-90 IR+ 1) and GSM3752535 (IMR-90 IR+ 2) for X-ray-induced senescent IMR-90 cells. For comparative analysis of RNA-seq, we used original datasets from published data as internal controls. In the transcriptome analysis of coding genes and some noncoding RNAs, the trimmed paired-end FastQ reads by TrimGalore (version 0.6.4) were aligned to a comprehensive gene annotation file (GENCODE, GRCh37, release 34) using HISAT2 (version 2.1.0) (28) using default settings. SAMtools (version 1.9) was used to sort and convert SAM to BAM files (22, 23). The counts mapped to the transcripts were computed using featureCounts (29). In the transcriptome analysis of repetitive elements, the trimmed paired-end FastQ reads by TrimGalore (version 0.6.4) were mapped against H. sapiens UCSC hg19 using the Bowtie
2 (version 2.3.5) alignment software (21). The counts of repetitive elements were calculated by RepEnrich2 (30) using default settings. These counts (coding genes, some noncoding RNAs and repetitive elements) were then normalized using the trimmed mean of M-values method in EdgeR (version 3.28.1) (31). Among the 652 transcripts, 47 differentially expressed transcripts were identified (significance determined with FDR < 10^{-10}) and are shown as red (upregulated; 32 transcripts) or blue (downregulated; 15 transcripts) dots in the volcano plot (Fig. 1C).

The volcano plots were visualized using the “ggplot2” package (Fig. 1B and C). The peaks of uniquely mapped reads by ATAC-seq and RNA-seq in hSATII loci are shown using the Integrative Genomics Viewer for visualization (Fig. 1D) (32, 33).

**RNA-seq analysis**

For RNA-seq analysis in hSATα- or hSATII-overexpressed and X-ray-induced senescent SVts8 cells (Fig. 1E–G; SI Appendix, Fig. S2B–D), the resulting FastQ reads containing more than 50% of below Q20 and more than 5% of N were removed, as performed by Annoroad Corporation. The trimmed reads were aligned to a comprehensive gene annotation file (GENCODE, GRCh37, release 34) using HISAT2 (version 2.1.0) (28) using default settings, and the number of transcripts per kilobase million (TPM) was estimated by StringTie (version 2.0) (34). To summarize and visualize RNA-seq data in hSATα- or hSATII-overexpressed and X-ray-induced senescent SVts8 cells, principal component analysis was performed using singular value decomposition approach implemented in R function (prcomp) on TPM values (SI Appendix, Fig. S2C). For comparative analysis of RNA-seq, we used original datasets from our RNA-seq data as internal controls. Each heatmap column regarding SASP-related gene expression in hSATα- or hSATII-overexpressed and X-ray-induced senescent SVts8 cells shows the value normalized to a z-score using each TPM value (Fig. 1E). Gene Set Enrichment Analysis (GSEA; Fig. 1G; SI Appendix, Fig. S2D) was carried out using the GSEA tool from the Broad Institute (35). The scatter plots (Fig. 1F; SI Appendix, Fig. S2B) were visualized using the “ggplot2” package.

For RNA-seq analysis in cells, multivesicular endosomes (MVEs) or exosomes derived from DKO1 (human colon cancer cell line; SI Appendix, Fig. S7A), publicly available data (GEO: GSE130727) (36) were reanalyzed using 12 samples; GSM3584509 (DKO1_Exo_Cell_RNA_1), GSM3584510 (DKO1_Exo_Cell_RNA_2), GSM3584511 (DKO1_Exo_Cell_RNA_3), and GSM3584512 (DKO1_Exo_Cell_RNA_4) for cells; GSM3584513 (DKO1_Exo_MV_RNA_1), GSM3584514 (DKO1_Exo_MV_RNA_2), GSM3584515 (DKO1_Exo_MV_RNA_3), and GSM3584516 (DKO1_Exo_MV_RNA_4) for MVEs; and GSM3584517 (DKO1_Exo_Low_RNA_1), GSM3584518 (DKO1_Exo_Low_RNA_2), GSM3584519 (DKO1_Exo_Low_RNA_3), and GSM3584520 (DKO1_Exo_Low_RNA_4) for exosomes. For comparative analysis of RNA-seq, we used original datasets from published data as internal controls. The trimmed paired-end FastQ reads by TrimGalore (version 0.6.4) were mapped against *H. sapiens* UCSC hg19 using the Bowtie 2 (version 2.3.5) alignment software (21). The counts of repetitive elements were calculated by RepEnrich2 (30) using default settings. Counts per million are shown (SI Appendix, Fig. S7A).

**ATAC-seq analysis**


For ATAC-seq analysis in empty vector- or hSATII-overexpressed SVts8 cells (Fig. 3E), the resulting FastQ reads underwent quality control with FastQC (version 0.11.8) and trimmed with Trimgalore (version 0.6.4). The trimmed sequences were mapped against *H. sapiens* UCSC hg19 using Bowtie 2 (version 2.3.5) alignment software (21). Uniquely mapped reads were used for peak calling using MACS 2 (version 2.1.4) with the command “$ macs2 callpeak --nomodel --nolambda --keep-dup all --call-summits -f BAMPE -g hs,” and peaks were filtered by an enrichment score (q < 0.01) (24). Each specific peak was identified using BEDTools using intersect.

ChIP-seq analysis

For ChIP-seq analysis (Fig. 3A–C, 3E), FastQ reads underwent quality control with FastQC (version 0.11.8) and trimmed with Trimgalore (version 0.6.4) using default settings. The trimmed sequences were mapped against *H. sapiens* UCSC hg19 using Bowtie 2 (version 2.3.5) alignment software (21). Uniquely mapped reads were used for peak calling using MACS 2 (version 2.1.4), and the peaks were filtered by an enrichment score (p < 0.001) (24). The 36,084 specific peaks detected in empty vector-expressed SVts8 cells or the 33,941 specific peaks detected in hSATII RNA-overexpressed SVts8 cells were identified using BEDTools using intersect. Normalized bigwigs were generated by deepTools (version 3.5.1) (37) “computeMatrix” command and the tracks in chr4: 76,930,000 to 77,030,000 were visualized with RNA-seq and ATAC-seq data using Integrative Genomics Viewer (Fig. 3E) (32, 33). For the enrichment of peaks from ChIP-seq data, we used deepTools to generate read abundance from all datasets around peak center ± 2-kb region, using “computeMatrix.” These matrices were then used to create profiles (Fig. 3B, left) and heatmaps (Fig. 3B, right) split into two clusters using the k-means algorithm using deepTools commands “plotProfile” or “plotHeatmap,” respectively.

Statistical Analysis

Parametric statistical analyses were performed using the unpaired two-tailed Student’s *t*-test (Fig. 3F; SI Appendix, Figs. S1C and S3 G, I, J, and L, and S5 B–E, S6 B–E, and S7 E–G), or one-way analysis of variance (ANOVA), followed by the Dunnett’s (Fig. 2F and 4A; SI Appendix, Fig. S2A and S7C) or Tukey’s (Fig. 1H and 2D and E and 3C; SI Appendix, Fig. S2 E and F and S3C and D and S5G) multiple comparisons post hoc test using the R software for statistical computing (64-bit version 3.6.1). Non-parametric statistical analyses were performed using the Wilcoxon rank-sum test (Fig. 3B and 4D–E), or the Kruskal-Wallis *H* test (one-way ANOVA on ranks) followed by the Steel’s multiple comparisons post hoc test (SI Appendix, Fig. S6F) using the R software for statistical computing. A *P*-value < 0.05 was considered statistically significant. All experiments, except for mass spectrometric analysis, were repeated at least twice.

Statistical Analysis

Parametric statistical analyses were performed using the unpaired two-tailed Student’s *t* test (Fig. 3F and SI Appendix, Figs. S1C, S3 G, I, J, and L, S5 B–E, S6 B–E, and S7 E–G) or one-way ANOVA, followed by the Dunnett’s (Figs. 2F and 4A and SI Appendix, Figs. S2A and S7C) or Tukey’s (Figs. 1H, 2 D and E, and 3C and SI Appendix, Figs. S2 E and F, S3 C and D, and S5G) multiple comparisons post hoc test using the R software for statistical computing (64-bit version 3.6.1). Nonparametric statistical analyses were
performed using the Wilcoxon rank-sum test (Figs. 3B and 4D and E) or the Kruskal–Wallis $H$ test (one-way ANOVA on ranks) followed by the Steel’s multiple comparisons post hoc test (SI Appendix, Fig. S6F) using the R software for statistical computing. $P < 0.05$ was considered statistically significant. All experiments, except for mass spectrometric analysis, were repeated at least twice.
Fig. S1. Pericentromeric satellite RNA is upregulated during cellular senescence.

(A) Peak distribution of the 16,325 regions dramatically altered (FDR < 0.05) during cellular senescence in IMR-90 cells by ATAC-seq (Fig. 1A and B). (B–F) Pre-senescent cells were rendered senescent by treatment with 4-OHT to activate oncogenic H-RasV12 (IMR-90/ER;H-RasV12, B–D) or serial passage (TIG-3, E and F). These cells were subjected to Western blotting (B), RT-qPCR (C and E), and Northern blot (D and F) to detect hSATII RNA and senescence markers. Replicative senescent cells rendered senescent by serial passage were collected at 1 week (W), 2 months (M), and 3 months after the cessation of proliferation (E and F). PDL, population doubling level. The relative expression indicates the value normalized to that of proliferating (control) cells (C). Each bar represents mean ± SD of three technical replicates repeated in two independent experiments (C). Each column shows the values normalized to z-score after calculated as a fold change from proliferating (PDL 41) TIG-3 cells (E). ***P < 0.001 by the unpaired two-sided t-test.
Fig. S2. Pericentromeric satellite RNA promotes SASP-like inflammatory gene expression.

(A) RT-qPCR analysis of SASP-like inflammatory genes in hSATα- and hSATII RNA-overexpressing SVts8 cells. The relative expression indicates the value normalized to that of empty vector-expressed cells. (B) Scatterplot showing the biological replicate in Fig. 1F. (C) PCA of RNA-seq performed in Fig. 1E. (D) GSEA of signatures associated with
IL6-JAK-STAT3 and SASP in hSATII RNA-overexpressed SVts8 cells. NES, normalized enrichment score. (E and F) The effect of the knockdown of hSATII RNA on the expression level of hSATII RNA and SASP-like inflammatory genes in proliferating (control) and X-ray-induced (E) or replicative senescent (F) IMR-90 cells. The relative expression shows a value normalized to that of control siRNA-treated proliferating cells. Each bar represents mean ± SD of three technical replicates, repeated in two independent experiments (A, E, F). *P < 0.05, **P < 0.01 or ***P < 0.001 by one-way ANOVA followed by the Dunnett’s multiple comparisons post hoc test (A) or Tukey’s multiple comparisons post hoc test (E, F).
Fig. S3. Pericentromeric satellite RNA is bound to CTCF and regulated by CTCF.

(A) Silver staining for hSATII RNA-binding proteins. (B and C) Western blot analysis of FLAG-tagged CTCF (WT: wild type), CTCFΔZF1-11 (deletion of ZF domains 1-11), CTCFΔZF1, or CTCFΔZF10 (B) and RIP assay (C) of HEK-293T cells. (D) RIP assay of
X-ray-induced senescent IMR-90 cells overexpressing FLAG-tagged CTCF or CTCFΔZF1-11. (E) RT-PCR analysis of hSATII RNA, and Western blot analysis using antibodies; anti-FLAG (CTCF), anti-CTCF and anti-GAPDH. (F) Western blot analysis of CTCF knockdown by siRNA. (G and H) RT-qPCR (G) or Western blot (H) analysis of hSATII RNA, CTCF, and SASP genes in serial passage-induced senescent TIG-3 cells. The relative expression shows the value normalized to that of early-passage cells. (I and J) RT-qPCR analysis of MajSAT RNA and SASP-like inflammatory genes in DXR-induced senescent MEFs (I) or MajSAT RNA-expressing MEFs (J). The relative expression indicates the value normalized to that of control treatment (I) or empty vector-expressed (J) cells. (K) Immunoprecipitated proteins bound to MinSAT or MajSAT RNA. (L) ChIP-qPCR analysis for CTCF binding to ICR or MinSAT locus. (M) Scheme of hSATII RNA regulation by CTCF. Although CTCF normally suppresses the expression of hSATII RNA and SASP-like inflammatory genes at a low level, the reduction of CTCF provokes the expression of hSATII RNA during cellular senescence. Subsequently, the upregulated hSATII RNA disturbs CTCF binding to DNA, which induces SASP-like inflammatory gene expression. Each bar represents mean ± SD of three technical replicates repeated in two independent experiments (C, D, G, I, J, L). *P < 0.05, **P < 0.01, ***P < 0.001 or N.S. (not significant) by the one-way ANOVA followed by the Tukey’s multiple comparisons post hoc test (C, D) or the unpaired two-sided t-test (G, I, J, L).
Fig. S4. Pericentromeric satellite RNA provokes a change of chromatin interaction at CXCL10/11 loci.

(A–F) Hi-C data in IMR90 (A), HMEC (B), HUVEC (C), HNEK (D), GM12878 (E), and K562 (F) cells (assembly: hg19, location: chr4 76,800,000-77,200,000) were obtained from 3D Genome browser (38, 39). The region considered in 3C assay (Fig. 3F) is surrounded by a broken line. (G) A predictive model of hSATII RNA-induced SASP-like
inflammatory gene expression by the disruption of CTCF-sustained chromatin organization.
Fig. S5. Pericentromeric human satellite RNA provokes chromosomal instability.

(A) The retrovirus transduction of hSATII RNA in SVts8 cells was validated by RT-PCR. (B and C) Immunostaining of SVts8 cells for microtubules (α-tubulin), centrosomes (pericentrin), and DNA (DAPI). Percentage of multipolar cells (B) or chromosome-bridged cells (C). Scale bar, 5 μm. Each bar represents mean ± SEM of three biological replicates (total of 90 cells per condition). (D and E) Karyotype analysis (n = 20) (D) and anchorage-independent growth analysis (E) of hSATII RNA-overexpressed SVts8 cells. Scale bar, 500 μm. Data are mean ± SEM (n = 3, total of 15 independent fields per condition). (F and G) Immunostaining (F) and percentage of multipolar cells (G) in SVts8 cells. Scale bar, 5 μm. Each bar represents mean ± SEM of three biological replicates (total of 90 cells per condition). *P < 0.05, **P < 0.01, or ***P < 0.001 by the unpaired two-sided t-test (B, C, D, E) or one-way ANOVA followed by the Tukey’s multiple comparisons post hoc test (G).
Figure. S6

(A–C) RT-PCR of MajSAT RNA overexpression in MEFs (A). Immunostaining for microtubules, centrosomes and DNA. Percentage of multipolar cells (B) or chromosome-bridged cells (C). Each bar represents mean ± SEM of three biological replicates (total of 90 cells per condition). Scale bar, 5 μm. (D) Focus formation assay. Representative photo (left) and the number of piled-up colonies (right). Each bar represents mean ± SEM of three biological replicates (total of 90 cells per condition). (E) Karyotype analysis of MEFs. Data are mean ± SEM (n = 3, total of 60 cells per condition). (F) MEFs were subcutaneously injected into nude mice (n = 6). The weight (left) and photo (right) of tumors after 20 (MEF/MajSAT RNA #2) or 30 (MEF/Vector, MajSAT #1 or MajSAT #3) days. Scale bar, 10 mm. *P < 0.05, **P < 0.01, or ***P < 0.001 by the unpaired two-sided t-test (B, C, D, E) or the Kruskal-Wallis H test (one-way ANOVA on ranks) and Steel’s multiple comparisons post hoc test (F).

Fig. S6. Pericentromeric mouse satellite RNA provokes chromosomal instability.

(A–C) RT-PCR of MajSAT RNA overexpression in MEFs (A). Immunostaining for microtubules, centrosomes and DNA. Percentage of multipolar cells (B) or chromosome-bridged cells (C). Each bar represents mean ± SEM of three biological replicates (total of 90 cells per condition). Scale bar, 5 μm. (D) Focus formation assay. Representative photo (left) and the number of piled-up colonies (right). Each bar represents mean ± SEM of three biological replicates (total of 90 cells per condition). (E) Karyotype analysis of MEFs. Data are mean ± SEM (n = 3, total of 60 cells per condition). (F) MEFs were subcutaneously injected into nude mice (n = 6). The weight (left) and photo (right) of tumors after 20 (MEF/MajSAT RNA #2) or 30 (MEF/Vector, MajSAT #1 or MajSAT #3) days. Scale bar, 10 mm. *P < 0.05, **P < 0.01, or ***P < 0.001 by the unpaired two-sided t-test (B, C, D, E) or the Kruskal-Wallis H test (one-way ANOVA on ranks) and Steel’s multiple comparisons post hoc test (F).
Figure. S7

(A) RNA-seq (DKO1) (GSE125905)

(B) Soft agar assay (SVts8)

(C) Karyotype analysis (SVts8)

(D) RT-PCR (SVts8)

(E) Soft agar assay (SVts8)

(F) Karyotype analysis (SVts8)

(G) RT-qPCR (Small intestinal organoids)

Fig. S7. Pericentromeric satellite RNA in small EVs provokes chromosomal instability.

(A) Count per million mapped reads of hSATII RNA detected in cells, multi-vesicular endosome (MVE) or exosomes secreted from cells of human colon cancer cell line, DKO1, (GSE125905) (36). (B) Comparison of anchorage-independent growth of SVts8 cells treated with small EVs derived from proliferating or X-ray-induced senescent RPE-1/hTERT cells. The number of colonies was counted (total of 5 independent fields per condition). Scale bar, 20 μm. (C) Number of chromosomes in the colonies from SI Appendix, Fig. S7B, or parent SVts8 cells (n = 8 per condition). (D) The incorporation of Nluc, hSATA, or hSATII RNA in designer exosomes produced by the EXOTic devices into SVts8 cells was confirmed by RT-PCR. (E) Comparison of anchorage-independent growth of SVts8 cells treated with Nluc (Control) or hSATII RNA-incorporated small EVs by EXOTic devices. The number of colonies was counted (n = 20 per condition). (F) The number of chromosomes in the colonies from SI Appendix, Fig. S7E (n = 20 per condition). (G) RT-qPCR analysis of MajSAT, CTCF, and SASP factor genes in a malignant organoid derived from colon cancer (Apc<sup>37T</sup> Trp53<sup>R270H/R270H</sup>) compared with its nonmalignant
organoid ($Apc^{\Delta 716}$). The relative expression shows the value normalized to that of nonmalignant organoids ($Apc^{\Delta 716}$). Each bar represents mean ± SD of three technical replicates repeated in two independent experiments (G). In the boxplot, the bottom and top hinges indicate the first and third quartile, respectively. The horizontal lines into the boxes indicate the median. The upper and lower whiskers define the highest and lowest value within 1.5 times of the interquartile range, respectively. *$P < 0.05$, **$P < 0.01$, or ***$P < 0.001$ by the unpaired two-sided $t$-test (E, F, G) or one-way ANOVA followed by the Dunnett’s multiple comparisons post hoc test (C).
Table S1. A list of hSATII RNA binding proteins.

| Accession | Description | # Unique Peptides |
|------------|-------------|------------------|
| Q9NZB2     | Constitutive coactivator of PPAR-gamma-like protein 1 OS=Homo sapiens GN=FAM120A PE=1 SV=2 | 43 |
| Q08211     | ATP-dependent RNA helicase A OS=Homo sapiens GN=DHX9 PE=1 SV=4 | 42 |
| Q9NR30     | Nucleolar RNA helicase 2 OS=Homo sapiens GN=DDX21 PE=1 SV=5 | 41 |
| Q1KMD3     | Heterogeneous nuclear ribonucleoprotein U-like protein 2 OS=Homo sapiens GN=HNRNPUL2 PE=1 SV=1 | 41 |
| Q12906     | Interleukin enhancer-binding factor 3 OS=Homo sapiens GN=ILF3 PE=1 SV=3 | 37 |
| Q00839     | Heterogeneous nuclear ribonucleoprotein U OS=Homo sapiens GN=HNRNPU PE=1 SV=6 | 35 |
| Q96KR1     | Zinc finger RNA-binding protein OS=Homo sapiens GN=ZFR PE=1 SV=2 | 34 |
| Q9Y6M1     | Insulin-like growth factor 2 mRNA-binding protein 2 OS=Homo sapiens GN=IGF2BP2 PE=1 SV=2 | 31 |
| Q9NZI8     | Insulin-like growth factor 2 mRNA-binding protein 1 OS=Homo sapiens GN=IGF2BP1 PE=1 SV=2 | 28 |
| O43390     | Heterogeneous nuclear ribonucleoprotein R OS=Homo sapiens GN=HNRNPR PE=1 SV=1 | 25 |
| P19338     | Nucleolin OS=Homo sapiens GN=NCL PE=1 SV=3 | 25 |
| O60596     | Heterogeneous nuclear ribonucleoprotein Q OS=Homo sapiens GN=SYNCRIP PE=1 SV=2 | 24 |
| P22626     | Heterogeneous nuclear ribonucleoprotein 2 B2/B1 OS=Homo sapiens GN=HNRNPA2B1 PE=1 SV=2 | 24 |
| P61978     | Heterogeneous nuclear ribonucleoprotein K OS=Homo sapiens GN=HNRNPK PE=1 SV=1 | 23 |
| P49711     | Transcriptional repressor CTCF OS=Homo sapiens GN=CTCF PE=1 SV=1 | 22 |
| P04264     | Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6 | 19 |
| P51991     | Heterogeneous nuclear ribonucleoprotein A3 OS=Homo sapiens GN=HNRNPA3 PE=1 SV=2 | 19 |
| P14868     | Heterogeneous nuclear ribonucleoprotein L OS=Homo sapiens GN=HNRNPL PE=1 SV=2 | 18 |
| Q58KZ1     | DBIRD complex subunit ZNF326 OS=Homo sapiens GN=ZNF326 PE=1 SV=2 | 18 |
| P49750     | YLP motif-containing protein 1 OS=Homo sapiens GN=YLPM1 PE=1 SV=3 | 18 |
| P09651     | Heterogeneous nuclear ribonucleoprotein A1 OS=Homo sapiens GN=HNRNPA1 PE=1 SV=5 | 16 |
| Q16630     | Heterogeneous nuclear ribonucleoprotein A1 OS=Homo sapiens GN=HNRNPA1 PE=1 SV=5 | 16 |
| P67809     | Nuclease-sensitive element-binding protein 1 OS=Homo sapiens GN=YBX1 PE=1 SV=3 | 15 |
| P35527     | Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=5 | 15 |
| Q12905     | Interleukin enhancer-binding factor 2 OS=Homo sapiens GN=ILF2 PE=1 SV=2 | 14 |
| O00571     | ATP-dependent RNA helicase DDX3X OS=Homo sapiens GN=DDX3X PE=1 SV=3 | 14 |
| Q41403     | Heterogeneous nuclear ribonucleoprotein D0 OS=Homo sapiens GN=HNRNPD PE=1 SV=1 | 13 |
| Q722W4     | Zinc finger CCCH-type antiviral protein 1 OS=Homo sapiens GN=Z3HAV1 PE=1 SV=3 | 13 |
| Q13242     | Serine/arginine-rich splicing factor 9 OS=Homo sapiens GN=SRSF9 PE=1 SV=1 | 13 |
| Q14966     | Zinc finger protein 638 OS=Homo sapiens GN=ZNF638 PE=1 SV=2 | 13 |
| P38159     | RNA-binding motif protein, X chromosome OS=Homo sapiens GN=RBMX PE=1 SV=3 | 12 |
| P31942     | Heterogeneous nuclear ribonucleoprotein H3 OS=Homo sapiens GN=HNRNPH3 PE=1 SV=2 | 12 |
| Q99729     | Heterogeneous nuclear ribonucleoprotein A/B OS=Homo sapiens GN=HNRNPA1 PE=1 SV=2 | 12 |
| Q96QR8     | Transcriptional activator protein Pur-beta OS=Homo sapiens GN=PURB PE=1 SV=3 | 12 |
| O00425     | Insulin-like growth factor 2 mRNA-binding protein 3 OS=Homo sapiens GN=IGF2BP3 PE=1 SV=2 | 12 |
| P35908     | Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2 | 12 |
| O76021     | Ribosomal L1 domain-containing protein 1 OS=Homo sapiens GN=RS1L1D1 PE=1 SV=3 | 12 |
| P13645     | Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6 | 12 |
| P55265     | Double-stranded RNA-specific adenosine deaminase OS=Homo sapiens GN=ADV ADAR PE=1 SV=4 | 12 |
| Q96SI9     | Spermatid perinuclear RNA-binding protein OS=Homo sapiens GN=STRBPE PE=1 SV=1 | 11 |
| P17844     | Probable ATP-dependent RNA helicase DDX5 OS=Homo sapiens GN=DDX5 PE=1 SV=1 | 11 |
| P16989     | Y-box-binding protein 3 OS=Homo sapiens GN=YBX3 PE=1 SV=4 | 10 |
| Q07955     | Serine/arginine-rich splicing factor 1 OS=Homo sapiens GN=SRSF1 PE=1 SV=2 | 10 |
| Q91CD5     | Nuclear receptor coactivator 5 OS=Homo sapiens GN=NCOA5 PE=1 SV=2 | 10 |
| Q15424     | Scaffold attachment factor B1 OS=Homo sapiens GN=SAFB PE=1 SV=4 | 10 |
| Q9BUU2     | Heterogeneous nuclear ribonucleoprotein U-like protein 1 OS=Homo sapiens GN=HNRNPUL1 PE=1 SV=2 | 10 |
| Accession  | Long name                                                                                   | Cell Line | OS       | GN       | PE | SV | Score |
|------------|---------------------------------------------------------------------------------------------|-----------|----------|---------|----|----|-------|
| P46777     | 60S ribosomal protein L5 OS=Homo sapiens GN=RPL5 PE=1 SV=3                                 | 9         |          |         |    |    |       |
| P52272     | Heterogeneous nuclear ribonucleoprotein M OS=Homo sapiens GN=HNRNPM PE=1 SV=3              | 9         |          |         |    |    |       |
| P18124     | 60S ribosomal protein L7 OS=Homo sapiens GN=RPL7 PE=1 SV=1                                 | 9         |          |         |    |    |       |
| P61247     | 40S ribosomal protein S3a OS=Homo sapiens GN=RPS3A PE=1 SV=2                               | 9         |          |         |    |    |       |
| P07910     | Heterogeneous nuclear ribonucleoproteins C1/C2 OS=Homo sapiens GN=HNRNPC PE=1 SV=4         | 9         |          |         |    |    |       |
| P31943     | Heterogeneous nuclear ribonucleoprotein H OS=Homo sapiens GN=HNRPH1 PE=1 SV=4              | 9         |          |         |    |    |       |
| P35637     | RNA-binding protein FUS OS=Homo sapiens GN=FUS PE=1 SV=1                                   | 9         |          |         |    |    |       |
| Q00577     | Transcriptional activator protein Pur-alpha OS=Homo sapiens GN=PURA PE=1 SV=2              | 8         |          |         |    |    |       |
| O14979     | Heterogeneous nuclear ribonucleoprotein D-like OS=Homo sapiens GN=HNRNPDL PE=1 SV=3        | 8         |          |         |    |    |       |
| P11940     | Polyadenylate-binding protein 1 OS=Homo sapiens GN=PABPC1 PE=1 SV=2                        | 8         |          |         |    |    |       |
| Q9UBU9     | Nuclear RNA export factor 1 OS=Homo sapiens GN=NXF1 PE=1 SV=1                              | 8         |          |         |    |    |       |
| P6578      | 60S ribosomal protein L4 OS=Homo sapiens GN=RPL4 PE=1 SV=5                                 | 8         |          |         |    |    |       |
| O00308     | NEDD4-like E3 ubiquitin-protein ligase WWP2 OS=Homo sapiens GN=WWP2 PE=1 SV=2              | 8         |          |         |    |    |       |
| Q99575     | Ribonucleases P/MRP protein subunit POP1 OS=Homo sapiens GN=POP1 PE=1 SV=2                 | 8         |          |         |    |    |       |
| Q96MU7     | YTH domain-containing protein 1 OS=Homo sapiens GN=YTHDC1 PE=1 SV=3                       | 8         |          |         |    |    |       |
| P62701     | 40S ribosomal protein L4 OS=Homo sapiens GN=RPS4X PE=1 SV=2                               | 8         |          |         |    |    |       |
| P39023     | 60S ribosomal protein L3 OS=Homo sapiens GN=RPL3 PE=1 SV=2                                | 7         |          |         |    |    |       |
| Q32P28     | Prolyl 3-hydroxylase 1 OS=Homo sapiens GN=P3H1 PE=1 SV=2                                  | 7         |          |         |    |    |       |
| Q9UHX1     | Poly(U)-binding-splicing factor PUF60 OS=Homo sapiens GN=PUF60 PE=1 SV=1                  | 7         |          |         |    |    |       |
| Q92804     | TATA-binding protein-associated factor 2N OS=Homo sapiens GN=TAFA5 PE=1 SV=1              | 7         |          |         |    |    |       |
| Q14151     | Scaffold attachment factor B2 OS=Homo sapiens GN=SABF2 PE=1 SV=1                          | 7         |          |         |    |    |       |
| Q11142     | Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1                       | 7         |          |         |    |    |       |
| Q12849     | G-rich sequence factor 1 OS=Homo sapiens GN=GRSF1 PE=1 SV=3                              | 7         |          |         |    |    |       |
| P78406     | mRNA export factor OS=Homo sapiens GN=RAE1 PE=1 SV=1                                      | 7         |          |         |    |    |       |
| Q92841     | Probable ATP-dependent RNA helicase DDX17 OS=Homo sapiens GN=DDX17 PE=1 SV=2              | 7         |          |         |    |    |       |
| Q02878     | 60S ribosomal protein L6 OS=Homo sapiens GN=RPL6 PE=1 SV=3                                | 7         |          |         |    |    |       |
| Q13151     | Heterogeneous nuclear ribonucleoprotein A0 OS=Homo sapiens GN=HNRNPA0 PE=1 SV=1           | 6         |          |         |    |    |       |
| O43809     | Cleavage and polyadenylation specificity factor subunit 5 OS=Homo sapiens GN=NUDT21 PE=1  | 6         |          |         |    |    |       |
| Q9H2U1     | ATP-dependent RNA helicase DHX36 OS=Homo sapiens GN=DHX36 PE=1 SV=2                       | 6         |          |         |    |    |       |
| P62424     | 60S ribosomal protein L7a OS=Homo sapiens GN=RPL7A PE=1 SV=2                             | 6         |          |         |    |    |       |
| P08621     | U1 small nuclear ribonucleoprotein 70 kDa OS=Homo sapiens GN=SNRNP70 PE=1 SV=2            | 6         |          |         |    |    |       |
| Q96TS8     | Msx2-interacting protein OS=Homo sapiens GN=SPEN PE=1 SV=1                               | 6         |          |         |    |    |       |
| Q8N684     | Cleavage and polyadenylation specificity factor subunit 7 OS=Homo sapiens GN=CPSF7 PE=1   | 6         |          |         |    |    |       |
| P05338     | 60S acidic ribosomal protein P0 OS=Homo sapiens GN=RPLP0 PE=1 SV=1                        | 5         |          |         |    |    |       |
| P60709     | Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1                                   | 5         |          |         |    |    |       |
| P84098     | 60S ribosomal protein L19 OS=Homo sapiens GN=RPL19 PE=1 SV=1                             | 5         |          |         |    |    |       |
| P43243     | Matrix-3 OS=Homo sapiens GN=MATR3 PE=1 SV=2                                             | 5         |          |         |    |    |       |
| P26373     | 60S ribosomal protein L13 OS=Homo sapiens GN=RPL13 PE=1 SV=4                             | 5         |          |         |    |    |       |
| Q9H0D6     | 5'-3' exoribonuclease 2 OS=Homo sapiens GN=XRN2 PE=1 SV=1                                | 5         |          |         |    |    |       |
| O95793     | Double-stranded RNA-binding protein Staufen homolog 1 OS=Homo sapiens GN=STAU1 PE=1 SV=2 | 5         |          |         |    |    |       |
| P02768     | Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2                                            | 5         |          |         |    |    |       |
| P61254     | 60S ribosomal protein L26 OS=Homo sapiens GN=RPL26 PE=1 SV=1                             | 5         |          |         |    |    |       |
| Q15007     | Pre-mRNA-splicing regulator WTAP OS=Homo sapiens GN=WTAP PE=1 SV=2                       | 5         |          |         |    |    |       |
| P40429     | 60S ribosomal protein L13a OS=Homo sapiens GN=RPL13A PE=1 SV=2                           | 5         |          |         |    |    |       |
| P26368     | Splicing factor U2AF 65 kDa subunit OS=Homo sapiens GN=U2AF2 PE=1 SV=4                    | 5         |          |         |    |    |       |
| P42285     | Superkiller viralicidal activity 2-like 2 OS=Homo sapiens GN=SIV2L2 PE=1 SV=3             | 5         |          |         |    |    |       |
| Q99590     | Protein SCAF11 OS=Homo sapiens GN=SCAF11 PE=1 SV=2                                      | 5         |          |         |    |    |       |
| P62280     | 40S ribosomal protein S11 OS=Homo sapiens GN=RPS11 PE=1 SV=3                             | 5         |          |         |    |    |       |
| Q9UKM9     | RNA-binding protein Raly OS=Homo sapiens GN=RALY PE=1 SV=1                                | 5         |          |         |    |    |       |
| Accession | Description |
|-----------|-------------|
| Q9UH17    | DNA dC->dU-editing enzyme APOBEC-3B OS=Homo sapiens GN=APOBEC3B PE=1 SV=1 |
| P09012    | U1 small nuclear ribonucleoprotein A OS=Homo sapiens GN=SNRPA PE=1 SV=3 |
| Q9UL40    | Zinc finger protein 346 OS=Homo sapiens GN=ZNF346 PE=1 SV=1 |
| O60832    | HACA ribonucleoprotein complex subunit 4 OS=Homo sapiens GN=DKC1 PE=1 SV=3 |
| Q13310    | Polyadenylate-binding protein 4 OS=Homo sapiens GN=PABPC4 PE=1 SV=1 |
| P50914    | 60S ribosomal protein L14 OS=Homo sapiens GN=RPL14 PE=1 SV=4 |
| P62913    | 60S ribosomal protein L11 OS=Homo sapiens GN=RPL11 PE=1 SV=2 |
| P02538    | Keratin, type II cytoskeletal 6A OS=Homo sapiens GN=KRT6A PE=1 SV=3 |
| O95232    | Lac7-like protein 3 OS=Homo sapiens GN=LUC7L3 PE=1 SV=2 |
| Q86YZ3    | Hornerin OS=Homo sapiens GN=HRNR PE=1 SV=2 |
| O15504    | H/ACA ribonucleoprotein complex subunit 4 OS=Homo sapiens GN=DKC1 PE=1 SV=3 |
| Q13310    | Polyadenylate-binding protein 4 OS=Homo sapiens GN=PABPC4 PE=1 SV=1 |
| P50914    | 60S ribosomal protein L14 OS=Homo sapiens GN=RPL14 PE=1 SV=4 |
| P62913    | 60S ribosomal protein L11 OS=Homo sapiens GN=RPL11 PE=1 SV=2 |
| P02538    | Keratin, type II cytoskeletal 6A OS=Homo sapiens GN=KRT6A PE=1 SV=3 |
| O95232    | Lac7-like protein 3 OS=Homo sapiens GN=LUC7L3 PE=1 SV=2 |
| Q86YZ3    | Hornerin OS=Homo sapiens GN=HRNR PE=1 SV=2 |
| O15504    | H/ACA ribonucleoprotein complex subunit 4 OS=Homo sapiens GN=DKC1 PE=1 SV=3 |
| Q13310    | Polyadenylate-binding protein 4 OS=Homo sapiens GN=PABPC4 PE=1 SV=1 |
| P50914    | 60S ribosomal protein L14 OS=Homo sapiens GN=RPL14 PE=1 SV=4 |
| P62913    | 60S ribosomal protein L11 OS=Homo sapiens GN=RPL11 PE=1 SV=2 |
| P02538    | Keratin, type II cytoskeletal 6A OS=Homo sapiens GN=KRT6A PE=1 SV=3 |
| O95232    | Lac7-like protein 3 OS=Homo sapiens GN=LUC7L3 PE=1 SV=2 |
| Q86YZ3    | Hornerin OS=Homo sapiens GN=HRNR PE=1 SV=2 |
| O15504    | H/ACA ribonucleoprotein complex subunit 4 OS=Homo sapiens GN=DKC1 PE=1 SV=3 |
| Q13310    | Polyadenylate-binding protein 4 OS=Homo sapiens GN=PABPC4 PE=1 SV=1 |
| P50914    | 60S ribosomal protein L14 OS=Homo sapiens GN=RPL14 PE=1 SV=4 |
| P62913    | 60S ribosomal protein L11 OS=Homo sapiens GN=RPL11 PE=1 SV=2 |
| P02538    | Keratin, type II cytoskeletal 6A OS=Homo sapiens GN=KRT6A PE=1 SV=3 |
| O95232    | Lac7-like protein 3 OS=Homo sapiens GN=LUC7L3 PE=1 SV=2 |
Q86U42  Polyadenylate-binding protein 2  OS=Homo sapiens  GN=PABPN1  PE=1  SV=3  
P02545  Prelamin-A/C  OS=Homo sapiens  GN=LMNA  PE=1  SV=1  
Q13610  Periodic tryptophan 1 homolog  OS=Homo sapiens  GN=PWP1  PE=1  SV=1  
P62316  Small nuclear ribonucleoprotein Sm D2  OS=Homo sapiens  GN=SNRPD2  PE=1  SV=1  
Q16629  Serine/arginine-rich splicing factor 7  OS=Homo sapiens  GN=SRSF7  PE=1  SV=1  
P11022  SRSF protein kinase 1  OS=Homo sapiens  GN=SRPK1  PE=1  SV=2  
Q9Y520  Protein PRRC2C  OS=Homo sapiens  GN=PRRC2C  PE=1  SV=4  
P06748  Nucleophosmin  OS=Homo sapiens  GN=NPM1  PE=1  SV=2  
Q5639  Elongation factor 1-alpha 2  OS=Homo sapiens  GN=EEF1A2  PE=1  SV=1  
Q86VM9  Zinc finger CCCH domain-containing protein 18  OS=Homo sapiens  GN=ZC3H18  PE=1  SV=2  
P08865  40S ribosomal protein SA  OS=Homo sapiens  GN=RPSA  PE=1  SV=4  
P50412  Transcription factor AP-1  OS=Homo sapiens  GN=JUN  PE=1  SV=2  
P13243  Serine/arginine-rich splicing factor 5  OS=Homo sapiens  GN=SRSF5  PE=1  SV=1  
P46781  40S ribosomal protein S9  OS=Homo sapiens  GN=RPS9  PE=1  SV=3  
P08708  40S ribosomal protein S17  OS=Homo sapiens  GN=RPS17  PE=1  SV=2  
P99019  40S ribosomal protein S19  OS=Homo sapiens  GN=RPS19  PE=1  SV=2  
Q7L2E3  Putative ATP-dependent RNA helicase DHX30  OS=Homo sapiens  GN=DHX30  PE=1  SV=1  
Q00059  Transcription factor A, mitochondrial  OS=Homo sapiens  GN=TFAM  PE=1  SV=1  
P51114  Fragile X mental retardation syndrome-related protein 1  OS=Homo sapiens  GN=FXR1  PE=1  SV=3  
P18621  60S ribosomal protein L17  OS=Homo sapiens  GN=RPL17  PE=1  SV=3  
P15015  Zinc finger protein 646  OS=Homo sapiens  GN=ZNF646  PE=1  SV=1  
P15393  Splicing factor 3B subunit 3  OS=Homo sapiens  GN=SF3B3  PE=1  SV=4  
P16590  116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens  GN=EFTUD2  PE=1  SV=1  
P12874  Splicing factor 3A subunit 3  OS=Homo sapiens  GN=SF3A3  PE=1  SV=1  
P15233  Non-POU domain-containing octamer-binding protein OS=Homo sapiens  GN=NONO  PE=1  SV=4  
P19391  Zinc finger protein 678  OS=Homo sapiens  GN=ZNF678  PE=1  SV=2  
P15149  Plecin OS=Homo sapiens  GN=PLEC  PE=1  SV=3  
P30876  DNA-directed RNA polymerase II subunit RB2  OS=Homo sapiens  GN=POLR2B  PE=1  SV=1  
P20700  Lamin-B1  OS=Homo sapiens  GN=LMNB1  PE=1  SV=2  
P62081  40S ribosomal protein S7  OS=Homo sapiens  GN=RPS7  PE=1  SV=1  
P12956  X-ray repair cross-complementing protein 6  OS=Homo sapiens  GN=XRCC6  PE=1  SV=2  
Q96Q89  Kinesin-like protein KIF20B  OS=Homo sapiens  GN=KIF20B  PE=1  SV=3  
P62910  60S ribosomal protein L32  OS=Homo sapiens  GN=RPL32  PE=1  SV=2  
PQ9NY46  Sodium channel protein type 3 subunit alpha OS=Homo sapiens  GN=SCN3A  PE=1  SV=2  
P46782  40S ribosomal protein S5  OS=Homo sapiens  GN=RPS5  PE=1  SV=4  
P18077  Dermcidin OS=Homo sapiens  GN=DCD  PE=1  SV=2  
P05164  Myeloperoxidase OS=Homo sapiens  GN=MPO  PE=1  SV=1  
P18077  60S ribosomal protein L35a  OS=Homo sapiens  GN=RPL35A  PE=1  SV=2  
Q5SZL2  Centrosomal protein of 85 kDa-like OS=Homo sapiens  GN=CEP85L  PE=1  SV=1  
P49790  Nuclear pore complex protein Nap153 OS=Homo sapiens  GN=NUP153  PE=1  SV=2  
P62891  60S ribosomal protein L39  OS=Homo sapiens  GN=RPL39  PE=1  SV=2  
Q9Y4K1  Beta/gamma crystallin domain-containing protein 1 OS=Homo sapiens  GN=CRYBG1  PE=1  SV=3  
O75808  Calpain-15 OS=Homo sapiens  GN=CAPN15  PE=1  SV=1  
P17RY0  Cytoplasmic polyadenylation element-binding protein 4 OS=Homo sapiens  GN=CPEB4  PE=1  SV=1  
Q92800  Histone-lysine N-methyltransferase EZH1 OS=Homo sapiens  GN=EZH1  PE=1  SV=2  
Q92673  Sortilin-related receptor OS=Homo sapiens  GN=SORL1  PE=1  SV=2
| Gene ID  | Gene Name                                      | Organism   | Gene ID  | Gene Name                                      | Organism   |
|---------|------------------------------------------------|------------|---------|------------------------------------------------|------------|
| P08670  | Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4     |            | O00746  | Nucleoside diphosphate kinase, mitochondrial OS=Homo sapiens GN=NME4 PE=1 SV=1 |            |
| P02751  | Fibronection OS=Homo sapiens GN=FN1 PE=1 SV=4 |            | Q9UMS4  | Pre-mRNA-processing factor 19 OS=Homo sapiens GN=PRPF19 PE=1 SV=1 |            |
| Q15459  | Splicing factor 3A subunit 1 OS=Homo sapiens GN=SF3A1 PE=1 SV=1 |            | Q5VWQ0  | Round spermatid basic protein 1 OS=Homo sapiens GN=RSBN1 PE=1 SV=2 |            |
| Q9BWU0  | Kanadaptin OS=Homo sapiens GN=SLC4A1AP PE=1 SV=1 |            | O95639  | Cleavage and polyadenylation specificity factor subunit 4 OS=Homo sapiens GN=CPSF4 PE=1 SV=1 |            |
| Q6NZY4  | Zinc finger CCHC domain-containing protein 8 OS=Homo sapiens GN=ZCCHC8 PE=1 SV=2 |            | P04406  | Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3 |            |
| Q6NSZ9  | Zinc finger and SCAN domain-containing protein 25 OS=Homo sapiens GN=ZSCAN25 PE=1 SV=3 |            | Q14011  | Cold-inducible RNA-binding protein OS=Homo sapiens GN=CIORBP PE=1 SV=1 |            |
| Q6XE24  | RNA-binding motif, single-stranded-interacting protein 3 OS=Homo sapiens GN=RBMS3 PE=1 SV=1 |            | P62304  | Small nuclear ribonucleoprotein E OS=Homo sapiens GN=SNRPE PE=1 SV=1 |            |
| P42766  | 60S ribosomal protein L35 OS=Homo sapiens GN=RPL35 PE=1 SV=2 |            | Q9NPE3  | H/ACA ribonucleoprotein complex subunit 3 OS=Homo sapiens GN=NOP10 PE=1 SV=1 |            |
| Q92794  | Histone acetyltransferase KAT6A OS=Homo sapiens GN=KAT6A PE=1 SV=2 |            | Q14692  | Ribosome biogenesis protein BMS1 homolog OS=Homo sapiens GN=BMS1 PE=1 SV=1 |            |
| Q14683  | Structural maintenance of chromosomes protein 1A OS=Homo sapiens GN=SMC1A PE=1 SV=2 |            | Q9NQ29  | Putative RNA-binding protein Luc7-like 1 OS=Homo sapiens GN=Luc7L PE=1 SV=1 |            |
| P17020  | Zinc finger protein 16 OS=Homo sapiens GN=ZNF16 PE=1 SV=3 |            | P56537  | Eukaryotic translation initiation factor 6 OS=Homo sapiens GN=EIF6 PE=1 SV=1 |            |
| Q13595  | Transformer-2 protein homolog alpha OS=Homo sapiens GN=TRA2A PE=1 SV=1 |            | A6NKH3  | Putative 60S ribosomal protein L37a-like protein OS=Homo sapiens GN=RPL37AP8 PE=5 SV=2 |            |
| P62266  | 40S ribosomal protein S23 OS=Homo sapiens GN=RPS23 PE=1 SV=3 |            | Q9N656  | Dolichyl-diphosphooligosaccharide–protein glycosyltransferase 48 kDa subunit OS=Homo sapiens GN=DDOST PE=1 SV=4 |            |
| P39656  | mRNA cap guanine-N7 methyltransferase OS=Homo sapiens GN=RNMT PE=1 SV=1 |            | O3148  | Oral-facial-digital syndrome 1 protein OS=Homo sapiens GN=OFD1 PE=1 SV=1 |            |
| P78332  | RNA-binding protein 6 OS=Homo sapiens GN=RBM6 PE=1 SV=5 |            | P06124  | Tyrosine-protein phosphatase non-receptor type 11 OS=Homo sapiens GN=PTPN11 PE=1 SV=2 |            |
| Q10570  | Cleavage and polyadenylation specificity factor subunit 1 OS=Homo sapiens GN=CPSF1 PE=1 SV=2 |            | Q13435  | Splicing factor 3B subunit 2 OS=Homo sapiens GN=SF3B2 PE=1 SV=2 |            |
| Q9NUL3  | Double-stranded RNA-binding protein Staufen homolog 2 OS=Homo sapiens GN=STAUF2 PE=1 SV=1 |            | P01782  | Immunoglobulin heavy variable 3-9 OS=Homo sapiens GN=IGHV3-9 PE=1 SV=2 |            |
| P61353  | 60S ribosomal protein L27 OS=Homo sapiens GN=RPL27 PE=1 SV=2 |            | Q8IXT5  | RNA-binding protein 12B OS=Homo sapiens GN=RBMS12B PE=1 SV=2 |            |
| P05165  | Propionyl-CoA carboxylase alpha chain, mitochondrial OS=Homo sapiens GN=PCCA PE=1 SV=4 |            | P62888  | 60S ribosomal protein L30 OS=Homo sapiens GN=RPL30 PE=1 SV=2 |            |
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