Supplemental Methods

Cell Culture

AS iPSC (AS del 1-0) and PWS iPSC (PWS del 1-7) lines were generated and maintained as previously described(1). Briefly, iPSCs were cultured in hESC medium with the following components. Dulbecco's modified Eagle's medium/F-12, 20% Knock-Out serum replacer, 0.1 mM non-essential amino acids, 1 mM L-glutamine, 0.1 mM b-mercaptoethanol, and 4 ng/mL bFGF. Colonies were grown on mouse embryonic fibroblasts (MEFs) and split mechanically approximately every 7 days.

ChIP qPCR

ChIP qPCR was performed using Millipore EZ-Magna ChIP G – Chromatin Immunoprecipitation Kit (17-409) following manufacturer's instructions. Briefly, 6-10 million cells were used for fixation. Sonication took place in 0.5 mL of nuclear lysis buffer. 4 µl of ChIPAb+ CTCF (17-10044) antibody was added to 50 µl of sonicated DNA aliquot with 450 µl ChIP dilution buffer for immunoprecipitation overnight. The washed immune-DNA complexes were reverse-crosslinked in 100 µl ChIP Elution buffer followed by removal of magnetic beads. Instead of using provided column for DNA purification, the process was carried out using phenol-chloroform. 200 µl of nuclease-free water and 200 µl of phenol-chloroform were added to the 100 µl reverse-crosslinked ChIP Elution buffer. After centrifugation, aqueous phase was transferred to a new tube. 200 µl of

chloroform was added for extra step of purification. The aqueous phase was transferred to a new tube and the precipitation took place at -20 °C for 30 minutes after adding 1 mL of 100% ethanol, 4 µg of glycogen, and 1/10 of total volume of 5M NaCl. Centrifuged DNA pellet was washed with 75% ethanol. The dry DNA pellet was reconstituted with 50 µl of nuclease-free water. The DNA was further diluted between 1:2 to 1:5 ratio for qPCR. 5 µl of diluted DNA was added to a total 20 µl reaction using SYBR green PCR master mix from Thermo Fisher Scientific (4309155). qPCR primers are listed in SI Appendix Table S1.

ChIP-seq

ChIP material was obtained following the same process in ChIP qPCR. Library preparation and sequencing runs were performed at the Genomics Core of the Yale Stem Cell Center. FASTQ files were mapped to the human genome (hg19) as described previously (2, 3). Normalized signals and CTCF peaks were identified in individual replicates using the ENCODE3 ChIP-Seq pipeline (https://www.encodeproject.org/pipelines/ENCPL138KID/). Differential CTCF binding between AS and PWS iPSCs was determined using DiffBind (Ross-Innes et al Nature 2012) (SI Appendix, Table S4).

CRISPR and single-stranded oligonucleotide (ssODN) design

CRISPR gRNA sequences were designed using CRISPR Genome Engineering Resource (http://crispr.mit.edu)(4). The gRNA sequence was cloned in px459 V2

vector following the published protocol(5, 6). ssODNs were designed following the previously described protocol(7). The sequence of CRISPRs and ssODNs used in this paper are listed in SI Appendix, Table S2.

Generation of genome-manipulated AS iPSC clones

6-10 million AS iPSCs were treated with 10 μM ROCKi (Calbiochem; Y-27632)(8) 24 hours prior to electroporation. iPSCs were dissociated into single cells by treatment with Accutase for 30 minutes. The cells were resuspended in 800 μl of cold PBS. 10 μg of each of two CRISPRs flanking the deletion/inversion region were added to the mixture. Electroporation took place using BioRad Gene Pulser Xcell with the following conditions: 250 V, 500 μF, 0.4 cm cuvettes. In the case of LoxP insertion, 2 ssODNs were also added along with 2 CRISPRs. Electroporated cells were transferred to a 15 conical tubes and centrifuged at 1000 rpm for 3 minutes to remove debris. Cells were then resuspended in hESC media and seeded on DR4 MEFs. For the first 24 hours, ROCKi was added to the hESC medium to increase cell survival. For the second 24 hours, ROCKi along with 1 μg/ml puromycin were added for positive selection. For the third 24 hours, only puromycin was added to the medium. Regular hESC medium was changed everyday until visible colonies were formed. The total process took about 2 weeks after electroporation. Individual colonies were picked and screened using primer pairs to detect for deletion or inversion. Primers were designed as described[9] and listed in SI Appendix, Table S3 . To generate the

24kb deletion iPSCs, LoxP-positive iPSCs were electroporated with 5ug of Cre-IRES-PuroR (Addgene #30205). Cells were selected with puromycin at 24 hours for a total of 48 hours. After the 48 hours of selection regular media was changed daily until colonies were isolated. Recombination of LoxP sites and deletion of the 24kb region was confirmed in the resulting clones by PCR.

4C-seq

For 4C-seq, nuclei were harvested as described previously(2). Briefly, approximately 2 million of iPSCs and iPSC-derived neurons were fixed in formaldehyde (1% final) and quenched in glycine (150 mM final) at room temperature on a shaker for 10 and 5 minutes, respectively. Cells were harvested and centrifuged for 5 minutes at 2000 g at 4 °C. Cell pellets were lysed in 1 mL of cell lysis buffer containing proteinase inhibitor cocktail 3 (Calbiochem) on ice for 20 minutes prior to dounce homogenization with pestle A for 10 strokes and pestle B for 30 strokes. The nuclei were collected at 2000 g, 4 °C for 5 minutes, washed and resuspended in 500 µl of 1x restriction enzyme buffer (NEB Cutsmart). The following circularized chromatin conformation capture (4C) material preparation procedure was adapted from previously described protocols(9). The nuclei in NEB Cutsmart buffer was incubated at 65 °C for 10 minutes on a thermal mixer with 800 rpm after adding 15 µl of 10% SDS. 150 µl of 10% Triton X-100 was added and incubated at 37 °C for 10 minutes on a nutator. 200 units of NlaIII (NEB) and 16.5 uL 10x NEB Cutsmart

were added for the first digestion at 37 °C overnight on a nutator. An additional 50 units of NlaIII was used for an additional 2 hours to ensure complete digestion. NlaIII was heat inactivated prior to splitting into 3 ligation reactions. Each of the ligation solutions were prepared on ice with the following components: 220 µl heat inactivated digested material, 745 µl of 10X T4 Ligase buffer, 745 µl 10% Triton X-100, 800 µl BSA, 5.5 mL nuclease-free water, and 2000 units of NEB T4 DNA ligase. Prepared ligation solutions were incubated at 15 °C overnight. 15 µl of 20 mg/ml proteinase K (NEB) was added to the ligation solution, and incubated at 65 °C overnight to reverse the crosslinking. Reverse-crosslinked 3C material was purified using phenol-chloroform followed by chloroform prior to ethanol precipitation at -80 °C overnight. The 4C library was pelleted, air-dried, and resuspended in 10 mM Tris-HCl (pH 8.0). The second digestion was performed at 37 °C overnight with components listed in the followings: 445 µl of 3C library, 50 µl of 10X DpnII buffer, 150 units of DpnII. After digestion, DpnII was inactivated at 65 °C for 25 minutes. The following components were added to the heat-inactivated material for second ligation at 15 °C for 4 hours: 1.4 mL 10X T4 ligase buffer, 12.6 mL cold nuclease-free water, 6000 units of NEB T4 ligase. The 4C material was ethanol precipitated and column purified using Zymo Research DNA concentrator kit. 150 µl 10 mM Tris-HCL (pH 8.0) was used to elute 4C material.

4C viewpoint primers were selected from human NlaIII - DpnII primer pair database provided by Tayan's group

(http://compgenomics.weizmann.ac.il/tanay/?page_id=367) with minor modifications. Primer pair sequences and illumina adapters are listed in SI Appendix, Table S5 and S6. Sequential PCR was performed to construct 4C library using Expand Long Template Polymerase (Roche). Genomic primer pairs of 4C viewpoints were used for the first PCR, and the products were purified by Zymo Research DNA concentrator kit. The purified PCR products were eluted in 10 mM Tris (pH 8.0) as the template for the second PCR. Illumina barcoded adapters were used as primers for the second PCR, and the barcoded 4C library were purified by Zymo Research DNA concentrator kit. Both first and second PCR reactions were done in 8 replicates. 2 nM 4C library from each sample was pooled together before loading on the Illumina NextSeq 500. All samples were done in replicates.

Reads were first de-multiplexed to separate samples and trimmed to remove Illumina adapters using cutadapt. Reads within each sample were then separated according to the genomic sequences and trimmed using cutadapt. Each viewpoint from each sample was mapped to hg19 reference genome using Bowtie 2. The generated bam files were plugged into R package (r3Cseq) to analyze chromatin interactions of each viewpoint(10).

RNA isolation and qRT-PCR

RNA was isolated using RNABee (AMSBIO, LLC) following the manufacturer's instructions. 1 μg of RNA was used for reverse transcription using BioRad iScript

Advanced cDNA Synthesis Kit. 30 ng of cDNA was loaded in a 20 µl qPCR reaction. qPCR was performed using BioRad CFX Touch Real-Time PCR Detection System. Relative expression was calculated using the delta delta Ct method.

Neural differentiation

iPSC-derived 10 wk neurons were differentiated using a monolayer protocol as described(11). Briefly, 2-day post-split iPSCs were switched to N2B27 medium supplemented with 500 ng/mL Noggin (R&D Systems) for 14 days with a complete change of medium every other day. Colonies were split on to poly-ornithine and laminin-coated plates with 1:2 ratio using StemPro EZ Passage tool (Life Technologies) for another week with a complete change of medium every other day. These cells were trypsinized and replated to poly-ornithine and laminin-coated plates and supplemented with ROCKi overnight. Cells were kept in N2B27 for a few more days before switching to neural differentiation medium for a week. For terminal differentiation, neurons were plated at a density of 200,000 cells per well of a 6 well plate. Terminally differentiated neurons were kept in neuron differentiation medium for another 5 weeks to obtain mature neurons.

PRO-seq

PRO-seq was carried out as described(12-14) with the following modifications. iPSCs grown on 10cm dishes were washed twice with cold PBS, scraped, and

pelleted. Pellets were resuspended in ice-cold permeabilization buffer, pulsed with a dounce homogenizer, and incubated on ice for 5 minutes. Permeabilization was confirmed by trypan blue staining. Permeabilized cells were counted and flash frozen in aliquots of 1 x 10^6 each.

PRO-seq library production

PRO-seq libraries from ~1 million permeabilized cells were constructed as in Mahat et. al. (15), with the following modifications. After the run-on reaction, RNAs were extracted with RNA purification columns (Norgen, product #37500). After the 3'-ligation, the cap-removal, end repair, 5'-ligation, and reverse transcription reactions were all performed on the beads by doubling of reaction volumes. cDNA was eluted from the beads by brief heating to 95^{o}C. A second elution was performed with water, and samples were pre-amplified 5 cycles before storing. Cycling parameters as as in Mahat et. al. Test amplifications of serial dilutions from each pre-amplification were used to determine the number of cycles for full amplification, with a maximum of 15 cycles. Fully amplified libraries were PAGE purified on a 8% PAGE gel, quantified and sized by Qbit and Agilent tapestation, respectively and sequenced on an Illumina NextSeq 500 (75 bp – high throughput kit) at the Center for Genome Innovation (Storrs, CT).

Mapping of PRO-seq data

Adapter sequences were trimmed with FASTX-Toolkit and filtered for a minimum of 15 bases. All reads were then trimmed to a maximum of 36 bases and then reverse complimented. Reads were first mapped with Bowtie (16) to a copy of the mouse rDNA repeat (GenBank: BK000964.1) with the -K1 option, and unaligned reads were then mapped to the mouse genome (mm10) filtering for unique matches. Only the final 3'-base representing position of the polymerase was reported to output files used in all analyses.

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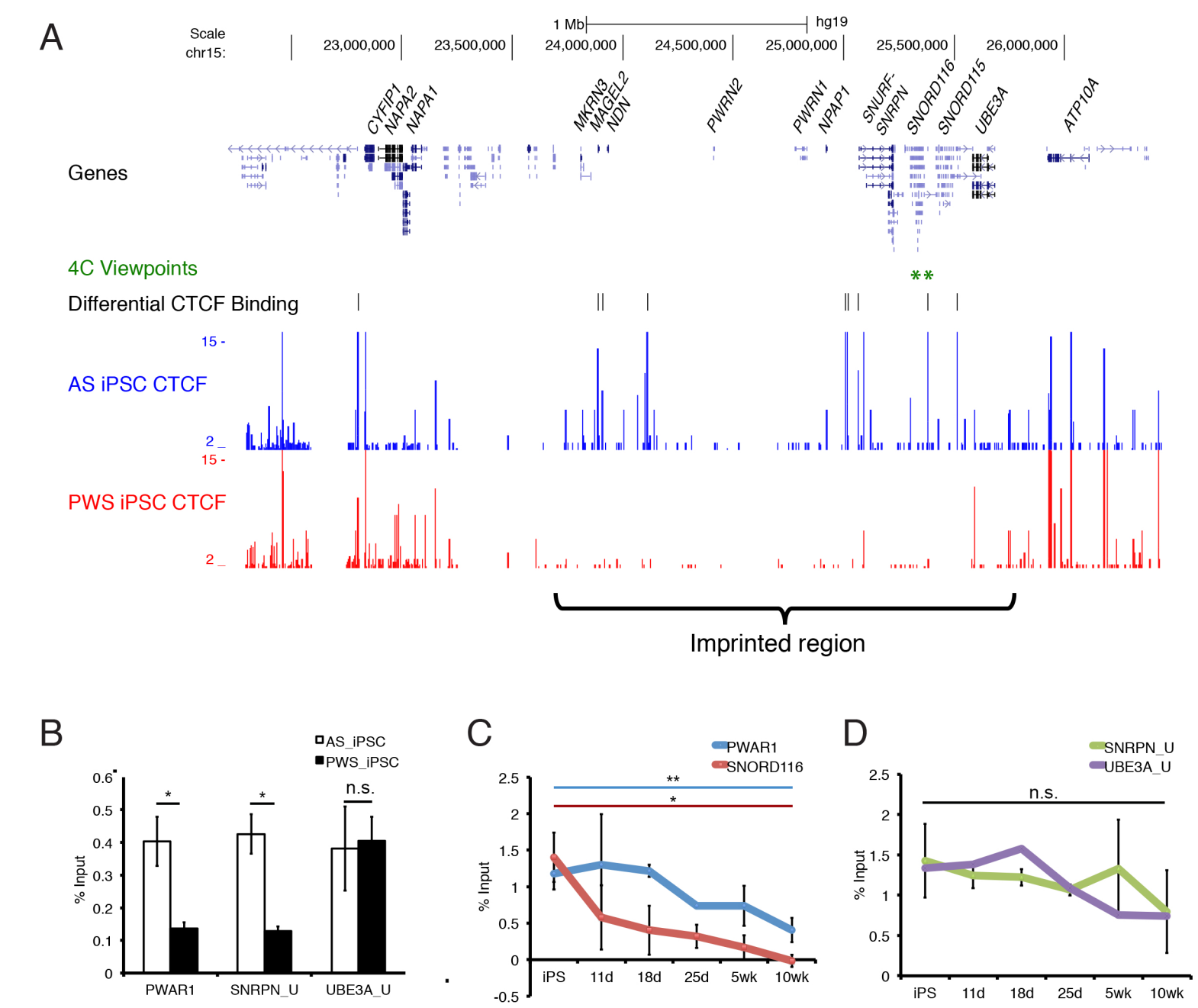
Supplemental Figure Legends

Suppl. Fig. 1. CTCF is enriched on the paternal allele of *PWAR1.* ChIP-seq for CTCF was performed in AS and PWS iPSCs harboring deletion of the 15q11-q13 region on the maternal and paternal alleles, respectively. CTCF binding on the paternal allele (blue) and maternal allele (red) are shown in A. The imprinted region is designated. Asterisks mark the CTCF sites used as anchors for 4C-seq. Black bars indicate significant differentially bound CTCF sites based on ChIP-Seq data. ChIP-qPCR was used to quantify CTCF enrichment on the paternal and maternal alleles in AS and PWS iPSCs, respectively, in B. ChIP-qPCR was used to quantify CTCF enrichment at *PWAR1* and *SNORD116* sites (C) and at sites upstream of SNRPN and upstream of *UBE3A* (D) during a time course of neural differentiation of AS iPSCs.

Suppl. Fig. 2. RNAPII is stalled at *PWAR1* and at the first exon of *SNORD115* in AS iPSCs. PRO-seq was used to map RNAPII density in AS, ΔI, ΔI-P, and ΔS-115 iPSCs. Plus strand RNAPII density is shown in red. Minus strand RNAPII density is shown in blue. Absent signals in ΔI, ΔI-P, and ΔS-115 iPSC lanes reflect engineered deletions.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NAPA1
MKRN3
MAGEL2
NDN
PWRN2
PWRN1
NPAP1
SNURF-
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SNORD116
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UBE3A
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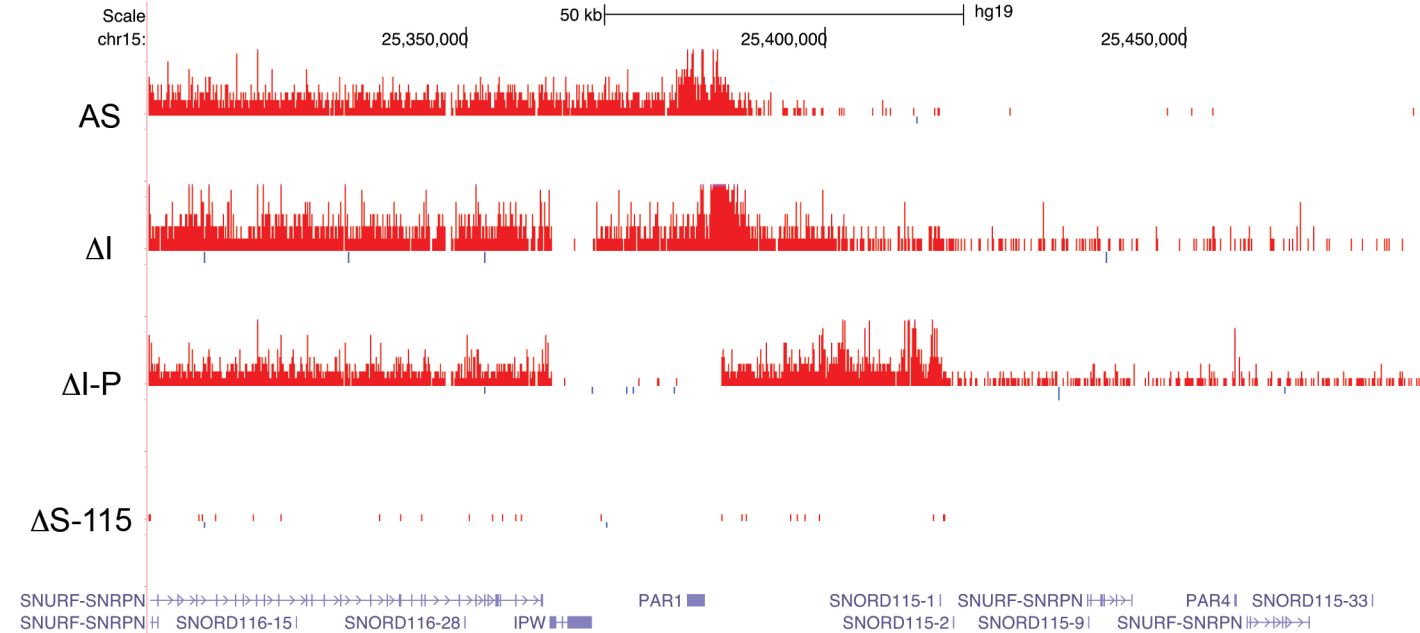
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SNURF-SNRPN
SNURF-SNRPN
SNORD116-15
SNORD116-28
IPW
PAR1
SNORD115-1
SNURF-SNRPN
SNORD115-2
SNORD115-9
PAR4
SNORD115-33
SNURF-SNRPN

Table S1. ChIP-qPCR primers

Primer name	sequence
SNRPN_U_F	GGTCTCTCAGTTGGCTCCTG
SNRPN_U_R	ATGGTGGATACTTGGCTTGG
PAR1_F	CAGGGAACGCTCTTCAACAT
PAR1_R	AACCAGTTCCAAACCTGACG
UBE3A_U_F	TGCTTCTGAACCCTGAATCC
UBE3A_U_R	CATGGACAAGTGTGTGTTGCT
SNRPN_exon_F	ATCTGTCTGAGGAGCGGTCAGT
SNRPN_exon_R	TCCCCAGGCTGTCTCTTGAG
SNORD116_F	GTTGGTGTTGCCTAGCATCC
SNORD116_R	CCTTGCAGGTCTTGGAAATC
PAR1_mi_F	CAGGGAAAGGGAGTTTGTTG
PAR1_mi_R	CGTGGGATTGTTTGATAGTGTG

Table S2. CRISPR guide RNA sequences for genome engineering

CRISPR name	sequence
IPW 5' #1	TATACAGAGCAATACGATCATGG
IPW 5' #2	GGAAAGGTTGGATTAAACTAAGG
IPW 5' #4	TCTAAGAATTCCACTGGTGAAGG
IPW 3'	AACTAGCACATACAAGGAACTGG
PAR1 5'	GTTCTGAAGCAAGGTATACCTGG
PAR1 3'	ATATAACCAAATTGTCCGTTAGG
SNRPN_intron 1	CAAGAACCTGGCATATACGAGGG
SNORD115-45	GACTCCATAGATTAACCCCCTGG

Table S3. PCR primers for clone-screening

Primer name	sequence
IPW_T1	TTGCACATAAATATTGCCTTTCA
IPW_T2	ACTGCCCTCCCTTTACCCTA
IPW_T3	CTAGCCTTCCCCTTCCATCT
IPW_T4	TGGGAGAATAAGAAGCGTTAAGA
PAR1_T1	CCTCCCTCAAATTGCTCTTTT
PAR1_T2	TTGTGCAAATGCAATATGTGA
PAR1_T3	TCATATACGAGTTGAGTCCCAAT
PAR1_T4	TGGTCTTTGGAAGGAGATGG
PAR1_T4’	TGTTTTAATCTGCGTCCTTTTG
SNRPN_intron_T1	GCTGAAAGACATTCGTTTGGA
SNORD115-45_T4	CGCCACAATGGTGTCTTTTT
IPW_F	TCTTCTGCCTCCTGTCTCGT
IPW_R	TCCCATCACCACAGTGAAAA
PAR5_F	AGGTGCTTTTGCTTTGCCTA
PAR5_R	TCTCTGAACCCCAACAGCTT

Table S5. 4C viewpoint primers for 4C-seq library preparation (1st PCR)

Genomic primer	sequence
SNRPN_U_NlaIII	**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**TCATAAGAAGTTGAAGCATG
IPW_NlaIII	**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**GCTTTCTGGGAGAAGCCATG
PWAR1_NlaIII	**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**ATATGCCCACATTCCACATG
UBE3A_U_NlaIII	**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**TTTGGATACCTTTCATCATG
SNRPN_DpnII	**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT**CAGTTGGCTCCTGTATCATT
IPW_DpnII	**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT**CCATAACCATCTAGTCCACAA
PWAR1_DpnII	**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT**GCCCCAGGGAAAATAGTACC
UBE3A_U_DpnII	**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT**TACTGTGGGTTTTTGGTTTT

Table S6. Illumina HT adapter primers (2nd PCR)

Illumina HT adapters	
Truseq_HT_D504	AATGATACGGCGACCACCGAGATCTACACGGCTCTGAACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq_HT_D505	AATGATACGGCGACCACCGAGATCTACACAGGCGAAGACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq_HT_D506	AATGATACGGCGACCACCGAGATCTACACTAATCTTAACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq_HT_D507	AATGATACGGCGACCACCGAGATCTACACCAGGACGTACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq_HT_D705_revcom	CAAGCAGAAGACGGCATACGAGATTTCTGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq_HT_D706_revcom	CAAGCAGAAGACGGCATACGAGATACGAATTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq_HT_D707_revcom	CAAGCAGAAGACGGCATACGAGATCTGAAGCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT