CENP-A associated lncRNAs influence chromosome segregation in human cells

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ABSTRACT

Transcription occurs ubiquitously throughout non-coding parts of the genome, including at repetitive α-satellite DNA elements which comprise the majority of human centromeres. The function of temporally regulated centromeric transcription, and transcripts, is consequently a topic of intense investigation. In this study, we use high throughput approaches to identify and describe lncRNAs associated with the centromere specific histone variant CENP-A that arise from the transcription of specific centromeres at early G1, which we then show are physically associated with centromeres, and which are functionally necessary for accurate chromosome segregation. Targeted depletion of one such centromeric RNA, which originates from a single centromere, is sufficient to increase the frequency of chromosome segregation defects. These data support the emerging paradigm of the necessity of centromere-specific lncRNAs in the integrity of faithful chromosome segregation.

INTRODUCTION

Centromeres are specialized chromosomal domains essential for kinetochore formation and attachment of the microtubule spindle, and consequently required for faithful chromosome segregation during cell division (1). The integrity of this chromatin region is maintained by a unique epigenetic landscape that is defined by the centromeric histone variant CENP-A, histone post-translational modifications, DNA methylation, chromatin-binding kinetochore proteins, and non-coding RNAs (2,3). Indeed, despite the lack of known genes in most centromeres, centromeric transcription is ubiquitous across eukaryotic species (4-19). In many of the species studied, the repetitive sequence of centromeres has contributed to the challenge in characterizing centromere-derived long non coding RNAs (lncRNAs). In humans, centromeric DNA consists of 171bp monomer α-satellite repeats (20). Arranged in a tandem fashion, these AT-rich repeats lack genes and share 40-100% sequence identity across different chromosome

centromeres (21). The organization of monomeric or dimeric units of this base sequence into higher order repeats (HOR) can serve to distinguish individual centromeres (22). Recent work has demonstrated that lncRNAs are transcribed during late mitosis - early G1 by RNA polymerase II (RNAPII) from centromeric human α-satellite DNA, where they interact with the essential inner kinetochore proteins CENP-A and CENP-C (8,12,19). Excitingly, this phenomenon appears to be evolutionarily conserved across humans, Drosophila and Xenopus (17,18). In mammals, over-expression or down-regulation of centromeric α-satellite RNAs is associated with errors of chromosome segregation arising from centromeric defects during mitosis (10-12,19,23). Finally, a recent study in Drosophila confirms the importance of centromere transcription-assisted loading of CENP-A via factors such as FACT and Mis18α (24). The accumulation of evidence from multiple organisms synthesized into a working hypothesis suggests that centromeric transcripts and transcription are both essential for the accurate spatial and temporal targeting of centromeric proteins at the centromere, and consequently, contribute to accurate segregation of chromosomes during mitosis. Several outstanding questions remain to be answered: whether all centromeres in a given set of chromosomes are uniformly transcribed; the identity of these transcripts; whether centromeric transcripts have a discrete length reflecting a defined transcriptional unit; whether they bind *in cis* or *in trans* across different centromeres in a genome; the consequences of their loss or over-abundance; and finally, the mechanism by which cenRNAs might bind kinetochore proteins directly. In this study, we report our progress in investigating five out of these six important questions.

In our previous study (19), we reported that in early G1, RNAPII transcribes human centromeres, generating ~1.3kb long non-coding centromeric RNAs (cenRNAs) which are associated with soluble pre-assembly CENP-A/H4/HJURP- and CENP-A- chromatin-complexes. Depletion of these transcripts using α-satellite consensus sequence shRNAs leads to defective CENP-A loading in early G1 at a subset of centromeric fibers, resulting in chromosome mis-segregation and other cellular defects. These data, along with work of others cited above, strongly suggested a role for cenRNAs in maintaining the integrity of centromeric chromatin and cell division. In this study, to better characterize centromeric RNAs and their function, we performed soluble and chromatin bound CENP-A associated RIP-seq (RNA-immunoprecipitation coupled to high throughput sequencing) from HeLa cells at early G1. Using this strategy, we identify several hundred cenRNAs which map to individual chromosomal centromeres, with strong homology to known centromeric α-satellite repeats, and which range from 300-2500 bases in length. Focusing our efforts on a subset of these cenRNAs, we show that one cenRNA, approximately ~1kb long, transcribes uniquely from chromosome 17, co-localizes with centromeric markers, and appears to be poly-adenylated. Functional characterization of this cenRNA by down-regulation of the transcript, demonstrates that its loss increases the frequency of chromosome segregation defects. These data support the emerging paradigm that centromeric RNAs are crucial players in the maintenance of centromeric integrity in higher eukaryotes.

MATERIAL AND METHODS

Tissue culture

HeLa cells were grown at 37°C, 5% CO_2, in Dulbecco's modified Eagle's medium high in glucose and

L-glutamine (#11965; Thermo Fisher Scientific/Gibco, Grand Island, NY) supplemented with 10% Fetal Bovine Serum (#26140-079; Thermo Fisher Scientific/Gibco) and 1X Pen/Strep solution (#10378-016; Thermo Fisher Scientific/Gibco).

Cells were synchronized by double thymidine block (0.5mM, #T9250; Sigma-Aldrich, Saint Louis, MO). After a first block of 19 hours, cells were released for 9 hours, followed by a second thymidine block of 16 hours. Then, cells were released for the appropriate time (10 hours for mitosis and 11 hours for early G1, as described previously in (19)).

Antibodies

Antibodies are commercially available. Supplemental Table 1 lists all antibodies used for each experiment.

shRNA

shRNAs incorporated into pGFP-V-RS plasmid vectors were purchased from Origene (Rockville, MD). Supplemental Table 2 lists sequences of these shRNA.

Primers

Primers were purchased from Integrated DNA Technologies (Coralville, IA). Supplemental Table 3 lists primer sequence used for each experiment.

BLAST on cenRNA#1

BLAST was performed on untranslated nucleotide sequence (blastn). Sensitive settings were: word size = 7 and expect value threshold = 10, with no low complexity filtering or repeat masking. Multiple blastn runs with variations of these parameters did not yield additional hits. To make our searching results more intuitive to interpret (as opposed to E-values and bit scores), a negative control sequence consisting of random sequence of the same length as cenRNA#1 (19) and with the same GC percent (47.4%, which is close to the human genome average of 46%; (25)) was generated. Random GC matched sequence for control was generated from the random DNA server at UC Riverside (http://www.faculty.ucr.edu/~mmaduro/random.htm; Morris Maduro, accessed on February 2, 2015). The reference sequence of the 17bp CENP-B box (5'-CTTCGTTGGAAACGGGA-3') is from (26). Adapter sequences identified in cenRNA#1 are described in (27).

RNA Immuno-Precipitation – sequencing (RIP-seq)

RNAs associated to CENP-A or agarose bead (mock-IP) were purified and identified following the protocol described in (28). Briefly, for each condition, five F175 flasks of HeLa cells at a final confluency of 80% were used per RIP-seq. Trypsinized cells were washed two times with cold 1X PBS; 0.1% Tween 20 (#P2287, Sigma-Aldrich), before fixation with 0.1% formaldehyde (#15680, Electron Microscopy Sciences, Hatfield, PA) in 1X PBS. The reaction was stopped by addition of 125mM glycine (#50046, Sigma-Aldrich). Then, nuclei were isolated in TM2 buffer (20mM Tris–HCl, pH 8.0 (#15568-025, Thermo Fisher Scientific); 2mM $MgCl_2$ (#AM9530G, Thermo Fisher Scientific); 0.5mM PMSF (#78830, Sigma-Aldrich); 1X complete protease inhibitor cocktail (#05892953001, Roche, Indianapolis, IN) complemented with 0.5% Nonidet™ P40 Substitute (NP40, #74385, Sigma-Aldrich) and 10mM Ribonucleoside Vanadyl Complex (RVC, #1402, New England Biolabs—NEB, Ipswich, MA), and washed in TM2 buffer complemented with 10mM RVC. Chromatin was MNase-digested (MNase, #N3755, Sigma-Aldrich) in 0.1M TE buffer (0.1M NaCl (#24740-011, Thermo Fisher Scientific); 10mM Tris–HCl, pH 8.0; 0.2mM EGTA (#03777, Sigma-Aldrich) in presence of 2mM $CaCl_2$ (#746495, Sigma-Aldrich). After addition of 10mM EGTA to stop the MNase action, nuclear pellet was resuspended in 1mL low-salt buffer (0.5X PBS, 5mM EGTA, 0.5mM PMSF, 1X complete protease inhibitor cocktail) complemented with 50 units of murine RNase inhibitor (#M0314S, NEB), and chromatin was extracted overnight at 4°C in an end-over-end rotator. After centrifugation, the supernatant was precleared with 30µL of protein A/G Plus agarose beads (#sc-2003,Santa Cruz Biotechnology, Dallas, TX) for 30 min at 4°C in an end-over-end rotator, and then, incubated with the anti-CENP-A primary antibody or no antibody for a minimum of 4 hours and a maximum of 12 hours at 4°C in an end-over-end rotator. CENP-A/primary antibody complex and mock-IP sample were immunoprecipitated with 50µL of protein A/G plus agarose beads for 2 hours at 4°C on the end-over-end rotator. Beads were washed three times with 1mL low-salt buffer containing murine RNase inhibitor and complete protease inhibitor cocktail. To isolate immuno-precipitated RNA, RNA–protein complex was eluted (elution buffer: 1% SDS; 0.1M sodium bicarbonate (#S6014, Sigma-Aldrich)), denaturated, treated sequentially with proteinase K (#AM2548, Thermo Fisher Scientific/Ambion) and DNaseI (#AM2222, Thermo Fisher Scientific/Ambion), and purified by phenol:chloroform:isoamylalcohol method. RNA concentration was determined by measurement on a UV-spectrophotometer and the quality of RNA was verified using a Bioanalyzer (#5067-1511, Agilent Technologies, Santa Clara, CA).

RIP-seq library construction and sequencing

To prepare RIP samples for sequencing, libraries were constructed with the Illumina TruSeq ChIP Library Preparation Kit (IP-202-1012 & IP-202-1024, Illumina, San Diego, CA). RIP libraries were barcoded and run in multiplex on a HiSeq 2500 instrument (Illumina) by the NCI-Sequencing Core Facility. Cluster generation was performed using an Illumina HiSeq PE Cluster Kit v4 cBot (#PE-401-4001, Illumina). The sequencer was run for 125 cycles in paired-end mode. Base calling accuracy was high for each sample, with > 90% of bases with a quality score of at least Q30 (99.9% accuracy in Phred scale). RIP-Seq experiments were performed in duplicate, and included both, input and mock-IP samples as negative controls. Raw reads total for each sample ranged from 49 to 112 million reads.

Computational analysis

To identify putative centromeric transcripts from the RIP-seq results, we followed a computational pipeline as described in our recently published methods book chapter on this topic (28). Briefly, raw reads in fastq format were cropped to remove low quality base calls from read ends with Trimmomatic (29). These pre-processed reads were then used in a first pass alignment to the repeating rDNA subunit (U13369.1, retrieved from GenBank) with Bowtie2 (30) in sensitive mode, to deplete ribosomal transcripts *in silico*. The unaligned reads from this process were used for further analysis. These reads were mapped to the Build 38 reference genome (hg38) using Tophat v2.1.0 (31). For Tophat alignment, up to three read mismatches (and an edit distance of three) were allowed for alignment, and only a single best alignment was reported for each read (the '-g 1' option). The centromeric sequence included in this version of the reference allowed us to map reads deriving from centromeric transcripts. Transcripts were defined from these alignments by reference-guided transcript assembly using Cufflinks (31). These assemblies were merged and transcript abundances were estimated with Cufflinks. Quantitative tracks for browser visualization were generated using deepTools (32). For visualization, Bowtie2 alignments were run in local mode (which allows soft-clipping), and enrichment relative to input was plotted as the subtraction of the depth normalized coverage in the input sample from the depth normalized coverage in the IP sample.

Open reading frame (ORF) finding was performed using the NCBI Orf Finder tool (https://www.ncbi.nlm.nih.gov/orffinder/), selecting "ATG only" to define ORF start sites. To search for TATA-boxes, we used the "fuzznuc" Emboss package that can map the consensus motif with amibiguity codes ("TATAWAAR"; (33)). For evidence of other gene elements, we used Promoter 2.0 and Genscan on the region +/- 2kb from the cenRNAs (34,35).

Motif enrichment was performed with MEME (36). Since this tool limits the amount of sequence that may be input at one time, we separated out transcript sequences into four subsets and ran them individually. The maximum motif size was set to 171bp, the length of the α-satellite monomer. We analyzed the ten highest reported scoring motifs. Sequence logos for the top-scoring motif in each subset is presented in Supplemental Figure 1A. Each of these motifs were highly similar to α-satellite monomer (88-98% identical over 98-100% of the query).

To generate distance trees for the complete set of cenRNAs, we aligned them using MUSCLE (Multiple Sequence Comparison by Log-Expectation; (37)), and calculated a tree based on average distance using percent identity (Supplemental Figure 1B). These calculations were implemented with Jalview (38). The results were then visualized in Dendroscope 3 to make the tree more interpretable (39). Since branching patterns can differ by algorithm, we generated multiple trees for comparison. Representative of this analysis, trees using alternative alignments Clustal and clustering performed with Unweighted Pair Group Method with Arithmetic Mean (UPGMA), including bootstrap results (using Geneious v.10.0.6; (40)), are shown in Supplemental Figure 1C-F. We note that a fraction of transcripts do not cluster at the bootstrap level of 70% support, but despite this, separation of transcripts into clusters was observed consistently.

Poly-adenylation of candidate cenRNAs

Total RNAs from HeLa cells were purified with Trizol reagent (#15596026, Thermo Fisher Scientific/Ambion) following manufacturer instructions. To run out genomic DNA contamination, these total RNAs were incubated with 4 units of DNase I (#M0303S, NEB) for 30 min at 37°C. The reaction was stopped by addition of 50mM EDTA (#351-027-721, Quality Biological, Gaithersburg, MD) and incubated at 75°C for 10 min. Then, total RNAs were purified using the Zymo Clean & Concentratator kit (#R1015, Zymo Research, Irvine, CA) before being retro-transcribed using SuperScript IV kit (#18091050, Thermo Fisher Scientific) with either polydT primers or random hexamers following manufacturer instructions. PCR reaction was run using Dream Taq PCR master mix kit (#K1071, Thermo Fisher Scientific) with α-satellite or cenRNA#4 primers (Supplemental Table 3). Cycling conditions for PCR were: 30s at 98°C; 30 cycles: 5s at 98°C, 30s at 57°C, 20s at 72°C; 300s at 72°C. Finally, PCR products were analyzed on 2% agarose gel.

RNA fluorescence *in situ* hybridization (RNA FISH)

Custom Stellaris RNA FISH probes labeled with Quasar dyes (*i.e.*, Quasar®570 or Quasar®670) were designed against specific cenRNAs and purchased from Biosearch Technologies (Petulama, CA). 75% confluent HeLa cells on poly-L lysine coverslip in 6-well plate were washed three times with HANKS buffer (#14170112, Thermo Fisher Scientific) and fixed with 4% paraformaldehyde (#15714, Electron Microscopy Sciences) in 1X PBS for 10 min at room temperature (RT). After washing cells with 1X PBS two times for 10 min, cells were made permeable in 70% ethanol (#61509-0010, Acros Organics, Pittsburgh, PA) overnight at 4°C. In order to confirm that the observed RNA FISH signal results from the hybridization of the probe with an RNA molecule and not genomic DNA, for every RNA FISH experiment, cells were rinsed once with 1X PBS in presence or absence of 1mg/mL of RNase A (#12091-021, Thermo Fisher Scientific). Cells were pre-incubated with 2X SSC (#AM9763, Thermo Fisher Scientific/Ambion); 10% deionized formamide (#15745, Electron Microscopy Sciences) for 5 min, and incubated with hybridization mix (0.1µM RNA FISH probe set diluted in 10% dextran sulfate (#S4030, EMD Millipore, Billerica, MA), 2X SSC, 10% deionized formamide), for 4 hours to overnight at 37°C in the dark. Finally, cells were washed twice with 10% deionized formamide in 2X SSC for 30 min at 37°C and once with 2X SSC for 5 min at RT. Coverslips were mounted on cells with anti-fade mounting medium Prolong Gold with DAPI (#P36935, Thermo Fisher Scientific).

Immuno-fluorescence / RNA FISH (IF/RNA FISH)

IF/RNA FISH experiments were performed as described previously (28). Custom Stellaris RNA FISH probes were purchased at Biosearch Technologies. Briefly, 75% confluent cells on poly-L lysine coverslip in 6-well plate were washed three times with HANKS buffer and fixed with 4% paraformaldehyde in 1X PBS for 10 min at RT. After permeabilization in 0.1% Triton X-100 (#T8787,

Sigma-Aldrich) in 1X PBS for 5 min at RT, cells were incubated with anti-CENP-B primary antibody in IF buffer (1X PBS; 1% normal goat serum (#005-000-121, Jackson ImmunoResearch, West Grove, PA); 50 units of murine RNase inhibitor) overnight at 4°C. After three washes in 1X PBS, cells were incubated with secondary antibody (goat anti-rabbit IgG (H+L) secondary antibodies, Alexa Fluor®488 or Alexa Fluor®568 conjugate (Thermo Fisher Scientific) in IF buffer for 1 hour at RT in the dark. To validate the RNA signal, half of slides were treated with 1X PBS complemented with 1mg/mL of RNase A. Cells were fixed with 4% paraformaldehyde in 1X PBS for 10 min, and washed twice with 1X PBS for 10 min. Then, cells were treated for RNA FISH as described earlier in "RNA FISH" section.

Electroporation of HeLa cells - shRNA depletion of centromeric RNAs

$1.2x10^6$ cells were mixed with 100µL of RT nucleofection reagent (#VACA-1001, Lonza, Allendale, NJ) and 7µL of plasmid containing shRNA, or a GFP control to quantify transfection efficiency (500ng/µL of Scrambled, cenRNA#4A, cenRNA#4B, α-satellite A, α-satellite B, H3-GFP). After electroporation with Nucleofector® Device (Lonza, Walkersville, MD) using program O-005 (for high viability), HeLa cells were resuspended in 500µl of warm media. 200µL of cells were transferred to a 6-well plate. Two days later, cells were stained for RNA FISH to validate the depletion in cenRNA. Otherwise, they were selected with 0.5µg/mL puromycin until day 6, at which they were fixed and treated for IF.

Immuno-fluorescence

HeLa cells were grown on poly-D-Lysine-treated coverslips in 6-well plate. After two washes with cold 1X PBS, cells were prefixed for 30s with cold 4% paraformaldehyde in PEM (80mM K-PIPES pH6.8; 5mM EGTA pH7; 0,2mM $MgCl_2$). After washing three times with cold PEM, soluble proteins were extracted for 5 min on ice with 0.5% Triton X-100 in CSK (10mM K-PIPES pH6.8, 100mM NaCl, 300mM sucrose, 1mM EGTA, 3mM $MgCl_2$). Few drops of 4% paraformaldehyde in PEM were added for 5 min. Coverslips were then incubated with fresh 4% paraformaldehyde in PEM for 40 min on ice. After three washes with PEM, cells were permeabilized with 0.5% Triton X-100 in PEM for 30 min at RT, washed again three times, and blocked in 1X TBS, 3% Bovine Serum Albumin, 5% normal goat serum for 1 hour at RT. Finally, cells were incubated with the primary antibody diluted in 1X TBS, 1% Bovine Serum Albumin, 5% normal goat serum overnight at 4°C in a humidified chamber. Cells were washed three times for 5 min at RT with 1X TBS, 0.1% Tween 20, and incubated with secondary antibody for 1 hour at RT. After washing, coverslips were mounted on slides with anti-fade mounting medium Prolong Gold with DAPI.

Microscopy observation and analysis

RNA FISH, IF and IF/RNA FISH slides were observed with a DeltaVision or DeltaVision Elite RT microscopy imaging system (GE Healthcare, Pittsburgh, PA) controlling an interline charge-coupled device camera (Coolsnap, Tucson, AZ) mounted on an inverted microscope (IX-70; Olympus, Center Valley, PA). Images were captured by using a 60X objective at 0.2 μm z-sections and analyzed with Image J (1.50e; Java 1.6.0_20, NIH, Bethesda, MD).

Statistical analysis

On each figure is indicated the number of repetition for each experiment (generally N=3 biological replicates; typically, a minimum of 100 cells - n=100 - scored per phenotype measured). Standard error was determined for all quantification measures. To test the significance of chromosome segregation defect measurements, a two-tailed Fisher's exact test was performed. For all tests, α was assumed to be 0.05. Significant p-values are indicated on the figures or tables with an asterisk each time it was evaluated.

RESULTS

Identification of centromeric RNAs by RIP-seq originating from discrete centromeric satellite sequences

To design an unbiased approach to identify potentially repetitive centromeric RNA sequences, we constructed libraries from RIP experiments followed by high-throughput sequencing (RIP-seq), and identified several hundred RNA species associated with CENP-A at centromeres (Figure 1A; (28)). We refined a computational pipeline to assemble putative centromere-derived transcripts from RIP-seq reads (Figure 1B; (28)). Briefly, reads deriving from ribosomal transcripts were depleted *in silico,* mapped to Build 38 reference sequence including centromere sequence models, and assembled into putative transcripts using the Build 38 reference as a guide. This analysis yielded 432 putative transcripts that were at least 300bp in length (the approximate library fragment length), with abundance $\geq$ 1 fragment per kilobase per million mapped reads (FPKM) in the chromatin fraction of each of two replicates, and which mapped to centromeres (Figure 1C-F and Supplemental Table 4). The complete set of candidate cenRNAs has a median size of 443bp, and a maximum size of 2450bp (Figure 1G). They were detected on every chromosome except for chromosomes 6, 13, and 14, although low abundance transcript predictions were defined on these chromosomes as well (Figure 1H). Most displayed reduced enrichment in shRNA α-satellite samples (60-66% of cenRNAs). These results demonstrate the pervasive nature of centromeric transcription in the HeLa transcriptome, and suggest that centromeric transcripts span a size range from 300-2500 bases, arising from transcripton of a large fraction of centromeres in the human nucleus.

We next searched for recurring motifs in these centromeric RNA sequences using the algorithm MEME. Reassuringly, the top scoring motifs were highly similar to the canonical α-satellite monomer (Supplemental Figure 1A), confirming the predominance of α-satellite sequence in these transcripts.

To analyze the sequence heterogeneity in our putative cenRNAs, we generated trees computed on percent identity distance (Supplemental Figure 1B-F). Although α-satellite sequence is predominant in these transcripts, sufficient differences exist to separate them into four distinct groups, or 'clades.' Although transcripts from the same centromere tended to group together, the branching was not strictly by chromosomal origin. These results demonstrate the diversity of centromeric transcribed sequences, and show that they are separable and identifiable in sequencing experiments.

From the RIP-seq centromere-mapping putative transcripts identified above, we selected three candidate cenRNAs for follow-up experiments, based on relative abundance and enrichment relative to controls (cenRNA#2, cenRNA#3, cenRNA#4; Figure 1C-E). Interestingly, each selected cenRNA was also detected in soluble pre-assembly CENP-A RIP-seq, but at less than half the abundance, supporting a relatively stable chromatin association for these targets. A reduction of enrichment was also observed in RIP samples transfected with α-satellite shRNA, suggesting these cenRNAs are depleted in our knockdown experiments (8.4-79% for cenRNA#2, 24.9-31.2% for cenRNA#3, and 9.2-31.7% for cenRNA#4; Supplemental Table 4). Each of these transcripts was defined by alignment to centromeric reference assembly, containing sequence in previously described α-satellite monomers (20). Using this approach, we observed that cenRNA#2 maps to chromosome 3 with high sequence similarity to the HOR DSZ1 (41), although RepeatMasker classifies the majority of the transcript region as scaffold attachment region (SAR) class satellite rather than ALR/alpha (42,43). In contrast, cenRNA#3 and cenRNA#4 mapped with very high homology to centromeric sequences on chromosome 17, specifically to the D17Z1 α-satellite array (44,45). Thus, these results show the existence of multiple centromeric long non-coding RNAs originating from several chromosomes. In addition, multiple RNAs can be transcribed from one chromosome. We interpret these data to indicate two points. First, that individual centromeres from different chromosomes are competent for transcription, and second, that transcription likely does not have a distinctive single start site per centromere.

We analyzed the sequence characteristics of the cenRNAs for evidence of coding potential. None of them included an open-reading frame (ORF) with 100 or more codons (a commonly used cut-off for defining a lncRNA; (46)). We also searched for protein alignments to six-frame translated cenRNA sequence. Neither cenRNA had evident similarity to human protein, with best hits all from predicted microbial protein sequence (Supplemental Datafile 1). This analysis supports the commonly accepted notion that centromeric transcripts are non-coding.

Centromeric RNAs are present at discrete centromeric loci in the nucleus during late mitosis-early G1

We, and others, have previously shown that human centromeres are preferentially transcribed by RNAPII at late mitosis – early G1 (8,19). To assess the abundance of expression of these newly-identified cenRNAs in human cells at early G1, we synchronized HeLa cells to that specific time of the cell cycle and performed RNA FISH. Then, we measured the frequency of cells expressing these

cenRNAs by counting cells with a bright RNA FISH focus consistent with known appearance of transcriptionally active foci.

For RNA FISH localization controls, we chose MALAT1 and NEAT1, two well described nuclear lncRNAs, which are ubiquitously expressed in human cells (47). MALAT1 was highly expressed with punctated spots all over the nucleus, whereas NEAT1 signal was more discrete with an average of 7 spots per cell (Figure 2A). This distribution is consistent with the localization of MALAT1 and NEAT1 described in the literature (47), implicating our approach conserve the spatial localization of lncRNAs. Both MALAT1 and NEAT1 were expressed in more than 80% of late mitosis – early G1 synchronized cells (Figure 2B). To exclude an unspecific hybridization of the RNA probes to genomic DNA, cells were treated with RNase A. As expected, the RNA FISH signal was diminished substantially after RNase A treatment, confirming the the ribonucleic nature of the observed signal and validating the method (Supplemental Figure 2).

Next, we studied the localization of centromeric α-satellite RNAs (Figure 2). Consensus centromeric transcripts were revealed by probes designed against the consensus sequence of α-satellite repeats derived from (20). On average, seven discrete nuclear spots were observed in 34% of late mitosis – early G1 synchronized cells, but none in cells treated with RNase A (Figure 2B and Supplemental Figure 2). This result suggests that several centromeric RNAs are transcribed from multiple centromeres and are localized in multiple nuclear localizations at the end of mitosis.

Next, we analyzed the localization of the three selected cenRNAs, namely cenRNA#2, cenRNA#3 and cenRNA#4 (Figure 1) using the same approach. Using probes designed against cenRNA#2, we observed RNA FISH signals were diffuse and similar to background and the RNase A-treated control, suggesting these probes were prone to non-specific recognition of other RNAs in the genome (Figure 2A and Supplemental Figure 2). In contrast, RNA FISH experiments performed with RNA probes designed against cenRNA#3 and cenRNA#4 expressed well defined and unique foci in synchronized HeLa cells (Figure 2A). cenRNA#3 and cenRNA#4 were observed in ~28% and ~37% of late mitosis - early G1 synchronized cells, respectively (Figure 2B). These signals were lost upon RNase A treatment, confirming the ribonucleic nature of the analyzed foci (Supplemental Figure 2).

Next, we characterized these transcripts by determining the chromatin domain to which they are localized. For this purpose, we performed a co-IF/RNA FISH to observe their relative localization to centromeres, using CENP-B, which marks centromeric DNA (Figure 3A). Since these RNAs were originally identified by CENP-A-chromatin RIP-seq (Figure 1), we expected to observe a co-localization or partial co-localization of cenRNAs with CENP-B domains. Reassuringly, in cells expressing centromeric α-satellite transcripts, ~74% of them displayed overlap between CENP-B and α-satellite RNA signals (Figure 3B). The absence of RNA FISH signal after RNase A treatment confirm that the observed α-satellite foci were indeed centromeric transcripts and not genomic sequence (Figure 3B). Next, we assessed the relative localization of cenRNA#4 to centromeres by repeating this co-IF/RNA FISH experiment (Figure 3C). As observed for α-satellite transcripts, cenRNA#4 was also found overlapping with CENP-B signal in ~70% of cells expressing cenRNA#4 (Figure 3C). Upon RNase A treatment, the RNA signal was lost (Figure 3C). Thus, our results indicate that in late mitosis - early G1 cells, cenRNA#4 and α-satellite transcripts localize adjacent to CENP-

B domains.

Centromeric transcripts are poly-adenylated

Since centromeric RNAs have been reported to be polyadenylated in Drosophila (18), we were curious whether human centromeric transcripts might also contain a poly(A) tail. To test this hypothesis, we retro-transcribed DNase I-treated total human RNAs with either poly(dT) or random hexamer primers, amplified cDNAs and compared profiles of PCR products on agarose gel (Supplemental Figure 3). Using primers designed against centromeric consensus sequence (α-satellite), we observed discrete bands every ~170 bases (from 170 to 680 bases) after retro-transcription with both poly(dT) or random hexamer primers (Supplemental Figure 3A). These data suggest that these centromeric transcripts are likely polyadenylated. To confirm the ribonucleic nature of the analyzed molecules, several controls were performed. To prove the absence of contaminant in our reaction mix, the experiment was performed without total human RNAs (-RT in Supplemental Figure 3A). We did not observe PCR products in this condition. We also tested the property of primers to self-anneal or dimerize by running a PCR reaction without cDNA (-cDNA in Supplemental Figure 3A). No PCR products were amplified. Altogether, these data suggest that centromeric RNAs are polyadenylated. We decided to further test this hypothesis that cenRNAs present a poly(A) tail by repeating the experiment with primer sets designed against cenRNA#4 (Supplemental Figure 3B).Similarly to centromeric α-satellite transcripts, several discrete bands were observed every ~170 bases after retro-transcription with either poly(dT) or random hexamer primers (Supplemental Figure 3B). However, when total RNAs were retro-transcribed, an additional band with an approximate size of 50 bases was amplified. The same band is observed in control conditions (-RT, and –cDNA; Supplemental Figure 3B). The difference between profiles between the assay (poly(dT) and random hexamers) and controls (-RT, and –cDNA) indicated that cenRNA#4 is poly-adenylated as well (Supplemental Figure 3B).

It has recently been reported in Xenopus (17), that centromeric RNAs appear to be spliced before they function to assist in CENP-A/CENP-C loading. We attempted to investigate whether human cenRNAs are processed using a 3'-5' RACE experiment; however, these results were inconclusive. Thus, while we were unable to definitively conclude whether human centromeric RNAs undergo splicing, these data suggest that polyadenylation is a maturation step that cenRNAs undergo before they bind CENP-A/HJURP complexes.

Down-regulation of centromeric RNAs leads to chromosome segregation defects.

In our earlier work, the down-regulation of consensus α-satellite RNAs by shRNA treatment led to an accumulation of mitotic defects (19). Indeed, parallel and previous work in Drosophila, Xenopus, and mammals demonstrate that loss of centromeric RNAs leads to mitotic defects underpine by the loss of CENP-A and CENP-C (15,17-19,48). Here, in order to investigate the function of cenRNA#4, we generated an shRNA construct designed against cenRNA#4 and analyzed the outcomes of the down-regulation of this transcript (Figure 4).

HeLa cells were transfected with the H3-GFP or shRNA constructs and treated with puromycin to select for transfected cells (Figure 4A). At Day 2, the efficiency of transfection was assessed by quantifying the percentage of cells expressing H3-GFP and by an RNA FISH approach (Supplemental Figure 4). The percentage of cells expressing α-satellite RNAs or cenRNA#4 (58% and 43%, respectively) after transfection with a scrambled sequence was similar to non-transfected cells (Supplemental Figure 4C-D). Compared to cells transfected with scrambled shRNA, we observed a decrease in the number of cells expressing centromeric RNAs (58% versus 34%) and cenRNA#4 (43% versus 22%), validating our approach of centromeric RNAs down-regulation (Supplemental Figure 4C-D). At Day 6, HeLa cells were synchronized at late mitosis - early G1 and stained for α-tubulin and CENP-B to visualize the mitotic spindle and centromeres, respectively (Figure 4A). We next scored for phenotypes related to chromosome segregation defects (Figure 4B and Supplemental Figure 5A-B). Cells transfected with scrambled shRNA displayed 30% of phenotypes related to chromosome segregation defects (Figure 4B). This percentage is explained by the high level of chromosome instability of HeLa cells (49,50). However, the down-regulation of α-satellite transcripts (α-sat) increased this percentage up to 50%, indicating the major role of these RNAs in the correct segregation of chromosomes (Figure 4B and Supplemental Figure 5B). Similarly, the proportion of cells with chromosome defects increase with cenRNA#4 down-regulation (up to 45%; Figure 4B and Supplemental Figure 5B). Overall, these data suggest that centromeric transcripts identified in these experiments are involved in faithful cell division.

DISCUSSION

Previously, we and others have shown that transcription is required for proper centromere function in human cells and is limited to late mitosis - early G1 (19). Here, we characterize the origin, localization, and putative function of these centromere-derived long non-coding RNAs. High throughput CENP-A RIP-seq experiments revealed several novel transcripts (Figure 1), which were polyadenylated. Curiously, only 30-40% of HeLa cells had signal for cenRNAs (Figure 2). In addition, the number of foci per nucleus was also less than the number of centromeres per nucleus (Figure 2 and Figure 3). Furthermore, the α-satellite RNAs and CENP-B foci were juxta-positioned (Figure 4), results that were recapitulated for chromosome-specific cenRNAs (cenRNA#4). Finally, knocking-down a chromosome-specific cenRNA resulted in mitotic defects (Figure 4). Altogether, these data reveal cenRNAs from different chromosomes are important for genome stability.

One specific question emerges from our observations. Why are cenRNA FISH signals only found in 30-40% of the cells at early G1? We consider several possible explanations. The most plausible one is that the temporal window when cenRNAs are actively transcribed is very short, thus reducing the odds of capturing the event by bright RNA FISH, as this requires fixing the cells. Consequently, this could mean that not all centromeres produce cenRNAs at precisely the same time. Another possibility is cenRNA molecules are long lived, and not required in high abundance, allowing the deposition of CENP-A over several cell cycles. Thus, only a fraction of the centromeres may need to undergo transcription per cell cycle, potentially sporadically. A third possibility is the very low abundance and unstable nature of cenRNAs might complicate the detection of all cenRNAs, except during active

transcription, both by RIP-seq or RNA FISH. Finally, a fourth possible explanation is that cenRNAs could function both *in cis* and *in trans*. In other words, cenRNAs transcribed from one centromere are also involved in function at other centromeres. This interpretation would be consistent with the observation that most human centromeres consist of very similar α-satellite sequences, thus mildly diverged sequences may not be sufficient to inhibit trans-localization or activity of cenRNAs. In this scenario, cenRNAs are either transported to other centromeres by chaperones or by diffusion of the RNA molecules. In the former case, the responsible chaperone, likely HJURP, remains to be identified. In the latter case, proximity would be a limiting factor. The closer another centromere is to the centromere producing the cenRNAs, the greater the likelihood that diffusion results in trans use of the cenRNAs. Indeed, a recent study used a novel method to analyze Hi-C data and showed that in human lymphoblastoid cells several centromeres are in close proximity, creating regulatory communities (51). Thus, our observation that there are less α-satellite RNA FISH signals than centromere signals is consistent with the possibility of trans-acting cenRNA (Figure 5).

Past reports describe the importance of centromeric transcripts in chromosome stability (10-12,19,23). We show that down-regulating centromeric transcripts using α-satellite consensus sequence shRNA decreases the level of expression of several specific cenRNAs, leading to CENP-A depletion and chromosome defects. One may argue that in a *trans* model, the down-regulation of one specific transcript should not lead to mitotic defects, as other cenRNAs will prevent the absence of the lost cenRNAs. However, the fine-tune regulation of transcription at centromeres can impede this rescue, by not re-adjusting the level of centromeric transcripts, leading to a decrease of the cenRNAs pool. Indeed, work on human artificial chromosomes suggest that the level of transcription at the centromere is balanced by post-translational modifications present at this chromatin domain, and not by cenRNAs themselves (48,52). Indeed, too much transcription is just as deleterious for CENP-A assembly as is too little. One speculative interpretation is that over-production of HAC cenRNAs might result in trans-migration of those HAC-cenRNAs to other centromeres, effectively diluting CENP-A at the HAC.

Thus, in this "Goldilocks" model, overexpression of cenRNAs might result in the concentration of cenRNA exceeding its titer, followed by mislocalization of surplus cenRNA. This mislocalization might be facilitated by histone chaperones other than HJURP (53,54), resulting in ectopic deposition of CENP-A (53,55). Ectopic CENP-A nucleosomes have the intrinsic capacity to be sites of ectopic kinetochore formation, which in turn can result in dicentric chromosomes. Ectopic CENP-A has also been found in colorectal cancers and misexpression of other kinetochore components has recently been shown to correlate with poor prognosis in various cancers (55,56). It will therefore be extremely interesting to study the relationship and co-dependence between over-expressed cenRNAs and other over-expressed centromere and kinetochore proteins.

Taken together, in this follow up report, we find that multiple cenRNAs are produced from various chromosomes, ranging in size from 300-2000 bases, and these transcripts were found to be poly-adenylated and localized adjacent to functional centromeres. Furthermore, despite only 30% of cells showing cenRNA signal, knockdown of chromosome-specific cenRNAs resulted in accumulated mitotic defects. Future work will be necessary to pinpoint the mechanism by which these cenRNAs assist in CENP-A loading at centromeres. We can envision 4 models by which cenRNAs can assist in CENP-A localization. First, cenRNAs might serve as true honing sequences, using an RNA-DNA

hybrid mechanism to help tether HJURP/CENP-A/H4 complexes to centromeric sequences; second, cenRNAs may be structurally involved in a pre-assembly complex that stabilizes or co-folds CENP-A/H4, thus acting as co-chaperone; third, cenRNAs may sequester or protect pre-assembly CENP-A/H4, preventing their fortuitious association with H3 complexes, or H3-containing chromatin before they are temporally loaded onto centromeres. Finally, based on several reports of cenRNAs binding CENP-C, it is tempting to speculate that cenRNAs in a pre-assembly complex with CENP-A/H4 and HJURP only permit association of these complexes to "active" centromeres because cenRNAs have a preference for pre-existing CENP-C-chromatin bound molecules. In this final mechanism, which is speculative and will require rigorous testing, we suggest that cenRNAs may act as a genetic-epigenetic toggle, reinforcing CENP-A localization only to active centromeres, rather than just to centromeres which contain alpha satellite sequences. This model would also address a long standing mystery in the field, wherein non-homologous CENP-As from distant species, when expressed in human cells, can localize to and rescue the loss of human CENP-A (57). In the absence of a true genetic centromere in human cells, it is has been puzzling to consider how, for example, yeast CENP-A can associate with centromeres of a species removed by several million years of evolution. In this model, it can be imagined that because the chaperone HJURP may bind non-cognate CENP-As, and can presumably also bind cenRNAs arising from active centromeres, it provides a mechanism for exogenous CENP-As to localize, and rescue human CENP-A loss from centromeres. Indeed, if intra-chromosomal use of cenRNAs is the mechanism, this might have also have implications for the evolution of centromere DNAs. The centromeres of most species consist of one type of satellite DNA. One outstanding question remains how it is possible that the centromeres of all chromosomes consist of the same type of satellite DNA. It is tempting to speculate that some sort of transposition had to have occurred. For instance, there are centromere-specific transposable elements (TEs) and the centromeres of *Arabidopsis thaliana*, which are devoid of TEs, can be invaded by an LTR retrotransposon from *A. lyrata* (58). A recent study comparing centromeres from different *Zea mays* inbred lines showed that CENP-A repositioned, followed by invasion of a transposable element (59). Altogether, centromere-specific transposition events have been shown to occur in plants and furthermore the centromere-associated protein CENP-B has been domesticated from a pogo-like transposase twice (60,61). The question that remains is, beyond the transposition events on evolutionary timescales, has transposition behavior been domesticated by centromeres via trans-acting cenRNAs. These, and other fascinating evolutionary and mechanistic questions discussed above, await future investigation.

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FIGURE LEGENDS

Figure 1: New RNAs associated to CENP-A chromatin identified by RIP-seq are originating from centromeres. (A) Schematic of RIP-seq protocol. (B) Summary of downstream data analysis pipeline (described in detail in (28)). (C-E) Screenshots of read coverage from CENP-A RIP-seq experiments for each of three identified transcripts. Input and depth-normalized read depths are presented, arbitrary scale. (F) Characteristics of novel cenRNAs identified by RIP-seq. Higher-order repeat (HOR) classification for each transcript. Data include locus coordinates, size of the sequenced RNA, higher-order repeat (HOR) or closest matching, GenBank accession, current assembly array, and repeat supra-chromosomal family (SF). (G) Size distribution of 432 detected centromeric transcripts. (H) Chromosome distribution of 432 detected centromeric transcripts.

Figure 2: cenRNAs display discrete nuclear localization. (A) Long non-coding RNAs NEAT1 and MALAT1 and centromeric transcripts are observed by RNA FISH in early G1 synchronized HeLa cells. (B) The frequency of cells expressing NEAT1 and MALAT1 and centromeric transcripts observed in A was quantified and tabulated (N=3).

Figure 3: cenRNAs and CENP-B domains partially co-localized. (A) Schematic of the co-IF/RNA FISH experiment. (B & C) G1-synchronized HeLa cells are stained for CENP-B, as marker of centromeres and centromeric transcripts (α-satellite in B and cenRNA#4 in C). Cells displaying an overlap of the IF and RNA signals were counted amongst cells expressing α-satellite transcripts and cenRNA#4. No signal was observed after RNase treatment (+RNase) (N=3).

Figure 4: The down-regulation of cenRNAs is accompanied with chromosome defects. (A) Schematic of the down-regulation of cenRNAs followed by co-IF experiment. (B) Transfected HeLa cells with either scrambled or α-satellite or cenRNA#4 shRNA are synchronized at late mitosis – early G1 and stained for CENP-B (marker of centromeres) and α-tubulin (marker of mitotic spindle). Cells were counted and categorized for their chromosome defects (N=3).

Figure 5: Working model. In a *cis* model, specific cenRNAs are transcribed from one centromere and stay associated to this specific centromere, leading to chromosome defects (*e.g.,* lagging chromosomes, chromatin bridge) of this exact chromosome and the loss of CENP-A at this centromere. On the other hand, in a *trans* model, a specific cenRNAs transcribed from one centromere will act on different chromosomes. Its loss will cause genome instability on different chromosomes.

Supplemental Figure 1: Description of centromeric transcripts identified by RIP-seq. (A) Sequence logos for the most abundant motif, in each of four subsets (~100 each) of putative centromeric transcript sequences. Motif length and significance as reported by MEME are indicated. (B-F) Pairwise percent identity matrix of 432 centromeric transcripts detected via CENP-A Rip-seq. Percent identity is displayed in shades of grey, with lighter shades indicating more similar. Hierarchical clustering was performed, which separates the transcripts into four groups (indicated at left), based on the four top most dendrogram branch points. A color code (right) indicates the chromosome of origin for each transcript. The three transcripts described in detail in this work are indicated at right.

Supplemental Figure 2: cenRNAs are not observed after RNase treatment. As in figure 2, RNAs were stained by RNA FISH in early G1 synchronized HeLa cells after treatment with RNase A treatment. A long exposure staining is shown to highlight the absence of RNA FISH signal (N=3).

Supplemental Figure 3: RNAs transcribed from centromeres are poly-adenylated. (A) Using primers designed against centromeric α-satellite, total RNAs were amplified after retro-transcription with either poly-(dT) or random hexamer primers. (B) As in A, total RNAs were retro-transcribed and amplified with primers designed against cenRNA#4. bp: base pairs; MW: molecular weight (50bp ladder); -RT: no RT; -DNA: no cDNA (N=3).

Supplemental figure 4: cenRNAs are down-regulated by shRNA approach. (A) Schematic of the RNA FISH and IF experiments. (B) HeLa cells are fixed and observed under microcope. H3-GFP positive cells are tabulated. (C & D) HeLa cells are stained for α-satellite transcripts (B) or cenRNA#4 (C) two days post-transfection with an shRNA constructs. Cells displaying an RNA signal were counted and tabulated (graphs on the right) (N=4 minimum).

Supplemental figure 5: cenRNAs depletion leads to chromosome defects. (A) Schematic of the down-regulation of cenRNAs followed by co-IF experiment. (B) Transfected HeLa cells with either scrambled or α-satellite or cenRNA#4 shRNA are synchronized at late mitosis – early G1 and stained for CENP-B (marker of centromeres) and α-tubulin (marker of mitotic spindle). Cells were counted and categorized for their chromosome defects (N=3).

Supplemental Table 1: List of antibodies.

Target Protein	Reference	Manufacturer	Method
CENP-A “3-19”	ab13939	Abcam	RNA Immuno-Precipitation – sequencing, Immuno-Fluorescence
CENP-B “H65”	sc-22788	Santa Cruz	Immuno-Fluorescence
α-Tubulin “DM1A”	62204	Thermo Fisher Scientific	Immuno-Fluorescence

Supplemental Table 2: List of shRNA sequence.

Target Sequence	Sequence (5’ to 3’)
Scrambled	GCACTACCAGAGCTAACTCAGATAGTACT
α-satellite A	TGTGTGCATTCAACTCACAGAGTTG
α-satellite B	CAACTCACAGAGTTGAACCTTCCTT
cenRNA#4 A	GATGACTGCATTCAACTCACGGAGTTGAA
cenRNA#4 B	CACCTCACAGAGTTGACCATTCCTATTGA

Supplemental Table 3: List of primer sequence.

Target Sequence		Sequence (5’ to 3’)	Method
α-satellite SetA	Forward	TGCATTCAACTCACAGAGTTGAAC	PCR
	Reverse	CATCACAAAGAAGTTTCTGAGAATGC	PCR
α-satellite SetB	Forward	CAACTCACAGAGTTGAACCTTCCTT	PCR
	Reverse	AGTTGAATGCACACATCACAAAGA	PCR
cenRNA#4 SetA	Forward	ACTCCTTTTGAGAGCGCAGT	PCR
	Reverse	GGTGCAGTTATCTCGTTTCCA	PCR
cenRNA#4 SetB	Forward	CTCTCTTTCTGTGGCATCTG	PCR
	Reverse	GAATGGTCAACTCTGTGAGG	PCR
GAPDH	Forward	AGTCAACGGGTTTGGTCGTAT	PCR
	Reverse	CTGCAAATGAGCCCCAGCC	PCR

Supplemental Table 4

tracking_id	locus	length	FPKM_rep1 IPCENPA chromatin	FPKM_rep1 IPCENPA supernatant	FPKM_rep1 IP control chromatin	FPKM_rep1 IP control supernatant	FPKM_rep2 cenpa	FPKM_rep2 input	FPKM_rep2 mock	IPCENPA max
CenRNA2	chr3:93470360-93470800	440	1442.66	682.546	1178.22	1338.85	4108.4	818.165	4680.33	4108.4
CenRNA3	chr17:23016255-23016556	301	91.1096	35.5475	82.5076	106.99	167.024	33.7938	66.7278	167.024
cenRNA:chr17:26594525-26594960	chr17:26594525-26594960	435	32.144	22.0417	19.2611	23.4381	112.202	16.9335	98.4654	112.202
CenRNA4	chr17:26603730-26604659	929	51.2469	24.2789	48.3182	57.4155	100.647	15.0331	64.5019	100.647
cenRNA:chr17:24150840-24151314	chr17:24150840-24151314	474	8.51129	2.35105	10.4807	8.64865	49.6636	7.00909	24.2729	49.6636
cenRNA:chr19:25422901-25423243	chr19:25422901-25423243	342	3.72908	0	2.34643	0	20.8466	3.61158	11.0357	20.8466
cenRNA:chr12:35011195-35011560	chr12:35011195-35011560	365	4.79721	0	6.03832	15.5297	17.5938	2.91578	8.94051	17.5938
cenRNA:chr5:47491561-47491892	chr5:47491561-47491892	331	2.02234	0	1.01788	1.15493	14.2213	1.61278	5.3192	14.2213
cenRNA:chr19:25600817-25601375	chr19:25600817-25601375	558	1.43702	0.893292	1.44807	2.46457	13.3581	1.22914	5.20249	13.3581
cenRNA:chr17:24740075-24740585	chr17:24740075-24740585	510	3.33136	0	1.88812	2.38038	11.8747	1.61453	3.83711	11.8747
cenRNA:chr19:26750190-26750573	chr19:26750190-26750573	383	1.43677	1.78523	2.53221	2.4627	11.1449	3.43898	6.14374	11.1449
cenRNA:chr20:27194762-27195193	chr20:27194762-27195193	431	2.25664	5.60928	4.54646	0.644826	10.5868	0.643181	5.56844	10.5868
cenRNA:chr5:48001328-48001892	chr5:48001328-48001892	564	2.11917	0.878231	1.42366	1.21151	10.5828	2.17515	4.41731	10.5828
cenRNA:chr17:26098145-26098474	chr17:26098145-26098474	329	2.05366	0	3.10088	0	10.3681	0.935842	6.75183	10.3681
cenRNA:chr5:48535895-48536223	chr5:48535895-48536223	328	4.13931	7.71102	0.520831	3.54574	9.32917	1.41468	2.72173	9.32917
cenRNA:chr10:40924813-40925129	chr10:40924813-40925129	316	2.28051	2.83175	1.1476	3.90636	8.6335	0.519518	3.74818	8.6335
cenRNA:chr12:36782697-36783180	chr12:36782697-36783180	483	2.74413	1.13704	4.37759	5.75126	8.25386	1.77312	4.81603	8.25386
cenRNA:chr4:50562740-50563124	chr4:50562740-50563124	384	2.85736	1.77519	0.359709	2.44885	7.98949	2.4426	11.2785	7.98949
cenRNA:chr20:28313055-28313356	chr20:28313055-28313356	301	2.60313	0	0	0	7.97587	0	0.855484	7.97587
cenRNA:chr11:51814030-51814362	chr11:51814030-51814362	332	6.02098	2.49264	7.07122	5.73093	7.59961	1.60057	3.29932	7.59961
cenRNA:chr18:16740369-16740720	chr18:16740369-16740720	351	1.75145	0	2.64517	2.00088	7.58089	0.199578	1.15192	7.58089
cenRNA:chr17:25033865-25034240	chr17:25033865-25034240	375	1.50482	0	2.65199	3.43892	7.32898	1.02904	8.41417	7.32898
cenRNA:chr12:36513965-36514305	chr12:36513965-36514305	340	1.89152	0	0.476069	1.08033	7.163	0.215516	1.24391	7.163
cenRNA:chr1:123518947-123519369	chr1:123518947-123519369	422	1.1758	0	1.7766	3.35967	6.78881	0.67022	2.70785	6.78881
cenRNA:chr17:24187861-24188440	chr17:24187861-24188440	579	5.42243	0	0.17076	1.93751	6.6067	1.54605	2.45395	6.6067
cenRNA:chr12:35427176-35427564	chr12:35427176-35427564	388	4.19155	0	4.22145	4.78984	6.5533	0.637015	5.51506	6.5533
cenRNA:chr19:27146703-27147147	chr19:27146703-27147147	444	1.06609	0	2.41646	0.609292	6.54066	1.21547	4.20927	6.54066
cenRNA:chr15:18780386-18781210	chr15:18780386-18781210	824	1.22422	1.52241	0.411316	0.700045	6.48343	1.07066	1.61208	6.48343
cenRNA:chr10:41013772-41014155	chr10:41013772-41014155	383	1.43677	0	1.80872	1.6418	6.47958	1.47385	8.03412	6.47958
cenRNA:chr10:41068409-41068823	chr10:41068409-41068823	414	1.22144	0	1.23033	0	6.17058	1.25318	4.82205	6.17058
cenRNA:chr2:92598948-92599257	chr2:92598948-92599257	309	2.42185	0	0	0	6.11177	0	0.796015	6.11177
cenRNA:chr4:51107215-51107566	chr4:51107215-51107566	351	3.5029	0	1.76345	3.00132	6.00154	0.997889	15.5509	6.00154
cenRNA:chr11:53631485-53631880	chr11:53631485-53631880	395	4.03557	0	0.338711	3.07453	5.82434	1.22667	1.32752	5.82434
cenRNA:chr18:15885398-15885724	chr18:15885398-15885724	326	4.20465	0	0	1.20054	5.68572	0.239496	1.38231	5.68572
cenRNA:chr22:14878202-14878530	chr22:14878202-14878530	328	2.06966	0	0.520831	2.36383	5.5975	0	2.72173	5.5975
cenRNA:chr2:93866822-93867203	chr2:93866822-93867203	381	5.81288	0	0	3.32115	5.50509	0.165634	0.478	5.81288
cenRNA:chr10:39979064-39979448	chr10:39979064-39979448	384	2.85736	0	0.359709	0	5.41224	0.32568	1.40981	5.41224
cenRNA:chr19:26880103-26880489	chr19:26880103-26880489	386	2.82552	1.75543	2.13423	1.61439	5.352	0.483082	3.25295	5.352
cenRNA:chr11:54102184-54102514	chr11:54102184-54102514	330	2.03789	0	1.0257	2.3276	5.14427	0	0.670006	5.14427
cenRNA:chr2:92655343-92655754	chr2:92655343-92655754	411	1.23947	1.54033	0.936356	0.708286	5.14344	0.282591	2.44658	5.14344
cenRNA:chr1:123128673-123129060	chr1:123128673-123129060	387	1.40493	0	0.353736	3.21091	5.06892	0.320272	2.31067	5.06892
cenRNA:chr1:123070673-123071106	chr1:123070673-123071106	433	1.11828	2.77971	0.844884	0	5.04454	1.6574	3.31136	5.04454
cenRNA:chr12:35774447-35774979	chr12:35774447-35774979	532	2.32869	0	3.32419	2.21869	5.04365	0.97373	3.321	5.04365
cenRNA:chr4:51124734-51125721	chr4:51124734-51125721	987	2.25856	0	0.650473	0.553541	5.01007	0.846597	2.44318	5.01007
cenRNA:chr5:47310450-47310774	chr5:47310450-47310774	324	2.13594	0	0.537484	0	5.00627	0.243318	1.40438	5.00627
cenRNA:chr17:25211131-25211437	chr17:25211131-25211437	306	9.9488	0	2.50285	1.41992	4.93143	0	0	9.9488
cenRNA:chr1:123729941-123730366	chr1:123729941-123730366	425	2.31908	0	1.46004	1.98795	4.81204	0.264384	0.381491	4.81204
cenRNA:chr17:26572643-26573713	chr17:26572643-26573713	1070	2.041	1.08787	0.881747	0.833722	4.58023	0.69854	3.55184	4.58023
cenRNA:chr17:24751971-24752310	chr17:24751971-24752310	339	1.90528	0	4.31575	5.44092	4.46645	1.73665	12.5294	4.46645
cenRNA:chr1:123550662-123550969	chr1:123550662-123550969	307	2.46508	0	1.86047	0	4.44332	0.280745	1.62039	4.44332
cenRNA:chr2:92398223-92398597	chr2:92398223-92398597	374	1.51376	0	0	0.864835	4.36888	0	2.48945	4.36888
cenRNA:chr12:35620221-35620564	chr12:35620221-35620564	343	1.85131	0	0	1.0574	4.34011	0.843763	1.82625	4.34011
cenRNA:chr3:92699663-92700018	chr3:92699663-92700018	355	1.7052	2.11831	0	0	4.30557	1.16589	1.12154	4.30557
cenRNA:chr1:124757430-124757981	chr1:124757430-124757981	551	2.19957	0.91153	1.10823	0.838294	4.23481	0.668924	3.61957	4.23481
cenRNA:chr8:45463531-45463856	chr8:45463531-45463856	325	4.23802	0	0	0	4.20257	0	0	4.23802
cenRNA:chr11:52722842-52723364	chr11:52722842-52723364	522	5.60684	0	1.61416	2.74725	4.19239	0.639389	1.5816	5.60684
cenRNA:chr11:54270091-54270498	chr11:54270091-54270498	407	1.26434	0	3.50213	1.44497	4.10599	0.864768	5.82312	4.10599
cenRNA:chr10:41177487-41177861	chr10:41177487-41177861	374	1.51376	0	0.381105	0	4.09583	0.172526	2.98734	4.09583
cenRNA:chr11:52882791-52883243	chr11:52882791-52883243	452	1.03108	0	0.779062	0.589304	4.09335	0.35268	3.05339	4.09335
cenRNA:chr1:124083165-124083543	chr1:124083165-124083543	378	1.47858	1.83713	0.372259	0.844762	4.00076	0.505564	2.918	4.00076
cenRNA:chr11:53682092-53682929	chr11:53682092-53682929	837	2.79745	1.98791	2.31618	2.28525	3.96838	0.410295	1.57875	3.96838
cenRNA:chr15:18910721-18911789	chr15:18910721-18911789	1068	1.46125	0	0.589198	0.167132	3.95766	0.400094	2.50169	3.95766
cenRNA:chr1:123411054-123411469	chr1:123411054-123411469	415	1.21555	0	0.612199	0	3.94767	1.10857	1.5996	3.94767
cenRNA:chr17:25973919-25974288	chr17:25973919-25974288	369	1.56005	0	0.392744	1.78249	3.93952	0.355589	1.02619	3.93952
cenRNA:chr20:26755647-26756136	chr20:26755647-26756136	489	2.68547	0	0.676432	1.02334	3.87722	0.204147	1.76743	3.87722
cenRNA:chr11:53245157-53245505	chr11:53245157-53245505	348	5.3631	0	0.449974	1.02112	3.86879	0.407405	0.587863	5.3631
cenRNA:chr5:48914932-48915256	chr5:48914932-48915256	324	2.13594	0	0.537484	0	3.85098	0.486636	1.40438	3.85098
cenRNA:chr15:18499643-18500068	chr15:18499643-18500068	425	1.15954	0	0	0	3.76595	0.396576	0.381491	3.76595
cenRNA:chr1:124474661-124475040	chr1:124474661-124475040	379	1.47004	3.65303	3.33098	4.19942	3.71248	1.34038	2.41762	3.71248
cenRNA:chr1:122733695-122734050	chr1:122733695-122734050	355	1.7052	0	0.429237	2.92218	3.69049	0.38863	1.12154	3.69049
cenRNA:chr11:53244246-53244701	chr11:53244246-53244701	455	1.01854	0	1.28266	0.582143	3.67601	0.232263	0.670285	3.67601
cenRNA:chr7:58832241-58832635	chr7:58832241-58832635	394	2.70477	0	0	0.772739	3.65966	1.23323	0.444869	3.65966
cenRNA:chr7:60699852-60700209	chr7:60699852-60700209	357	1.68293	0	0.423637	0	3.64235	0.19178	0	3.64235
cenRNA:chr12:36058889-36059260	chr12:36058889-36059260	371	1.54121	1.91484	0.776015	0.880499	3.61401	0.175651	0.506908	3.61401
cenRNA:chr15:18586782-18587204	chr15:18586782-18587204	422	1.1758	0	0	0	3.60656	0.402132	0.773671	3.60656
cenRNA:chr1:123527773-123528211	chr1:123527773-123528211	438	2.18788	0	0.551007	1.87559	3.55308	0.623601	2.15957	3.55308
cenRNA:chr11:53081559-53081908	chr11:53081559-53081908	349	1.77546	0	0.446898	2.02828	3.52214	0	1.75153	3.52214

Supplemental Table 4

tracking_id	locus	length	FPKM_rep1 IPCENPA chromatin	FPKM_rep1 IPCENPA supernatant	FPKM_rep1 IP control chromatin	FPKM_rep1 IP control supernatant	FPKM_rep2 cenpa	FPKM_rep2 input	FPKM_rep2 mock	IPCENPA max
cenRNA:chr15:18710948-18711350	chr15:18710948-18711350	402	1.29684	0	0	0	3.50952	0.739145	2.98632	3.50952
cenRNA:chr11:52858028-52858472	chr11:52858028-52858472	444	1.06609	2.65009	0.805486	1.21858	3.4627	0.48619	2.80618	3.4627
cenRNA:chr19:27043834-27044255	chr19:27043834-27044255	421	1.18132	0	0.892465	0	3.41033	0.269345	1.94325	3.41033
cenRNA:chr19:26488684-26489092	chr19:26488684-26489092	408	2.51607	0	1.26715	0	3.4046	0.14341	1.24159	3.4046
cenRNA:chr20:27673257-27673868	chr20:27673257-27673868	611	1.87181	0	0.471588	0.713445	3.37885	0.355812	1.43757	3.37885
cenRNA:chr11:51854616-51855242	chr11:51854616-51855242	626	1.20306	0.747926	1.06087	0.343917	3.36614	0.68608	1.58396	3.36614
cenRNA:chr17:23416004-23416360	chr17:23416004-23416360	356	1.694	0	0	0	3.36075	0.19304	0	3.36075
cenRNA:chr1:124741036-124741705	chr1:124741036-124741705	669	1.09076	0	0.687065	0.623658	3.34744	0.55986	2.15426	3.34744
cenRNA:chr3:91915094-91915451	chr3:91915094-91915451	357	1.68293	0	0.423637	0	3.33882	0.19178	0.553456	3.33882
cenRNA:chr8:45518922-45519636	chr8:45518922-45519636	714	1.49054	0	0.500757	0.28409	3.31875	0.170019	0.981311	3.31875
cenRNA:chr8:45487270-45487711	chr8:45487270-45487711	441	1.07983	1.34211	0	0	3.31246	0.123113	0.355291	3.31246
cenRNA:chr10:41359869-41360228	chr10:41359869-41360228	359	1.66119	0	0	0	3.29576	0.378613	1.09264	3.29576
cenRNA:chr3:91128662-91129022	chr3:91128662-91129022	360	1.65053	0	0	0	3.27462	0	0	3.27462
cenRNA:chr9:43402462-43402880	chr9:43402462-43402880	418	1.19819	0	0.603467	0	3.2428	0.273189	0	3.2428
cenRNA:chr1:123692055-123692735	chr1:123692055-123692735	680	1.06532	0.662334	0.402629	0.609119	3.17324	0.546809	1.40269	3.17324
cenRNA:chr11:52976427-52976850	chr11:52976427-52976850	423	1.17033	0	0	0	3.16747	0.800526	1.54015	3.16747
cenRNA:chr10:40398653-40399110	chr10:40398653-40399110	457	1.01035	0	0	1.15493	3.0995	0.345595	1.66225	3.0995
cenRNA:chr1:124061800-124062124	chr1:124061800-124062124	324	2.13594	0	0	0	3.08078	0.243318	1.40438	3.08078
cenRNA:chr11:53808898-53809328	chr11:53808898-53809328	430	1.13341	4.22591	0.570868	2.59092	3.06763	0.258431	0.745803	4.22591
cenRNA:chr5:49090924-49091415	chr5:49090924-49091415	491	1.77764	1.10488	0.671649	1.01611	3.04776	0.405406	1.46245	3.04776
cenRNA:chr11:53301922-53302385	chr11:53301922-53302385	463	1.9731	1.22626	0.248479	2.81935	3.02653	0.449945	0	3.02653
cenRNA:chr10:40633507-40634570	chr10:40633507-40634570	1063	1.76376	1.46237	0.666725	0.840549	3.02542	0.670724	2.32276	3.02542
cenRNA:chrX:59985721-59986095	chrX:59985721-59986095	374	1.51376	0	0	0	3.00361	0	0.497889	3.00361
cenRNA:chr19:25265630-25265941	chr19:25265630-25265941	311	2.37993	0	1.19755	1.35879	3.00308	0	3.12904	3.00308
cenRNA:chr19:26445762-26446185	chr19:26445762-26446185	423	1.17033	0	0.294723	0	2.9563	0.533684	0.770075	2.9563
cenRNA:chr10:40483376-40483706	chr10:40483376-40483706	330	2.03789	0	1.0257	1.1638	2.93959	0	0	2.93959
cenRNA:chr11:51727101-51727510	chr11:51727101-51727510	409	1.25179	0	0	0	2.93601	0.142698	0.823621	2.93601
cenRNA:chr11:53752632-53753170	chr11:53752632-53753170	538	2.28631	2.84236	1.34389	3.04966	2.88862	0.260732	0.752444	2.88862
cenRNA:chr16:37232571-37232936	chr16:37232571-37232936	365	1.59907	0	1.20766	0.913511	2.88424	0.91118	1.05182	2.88424
cenRNA:chr19:25739948-25740281	chr19:25739948-25740281	333	3.98373	0	1.50385	0	2.8733	0.226931	1.30979	3.98373
cenRNA:chr5:48250476-48250874	chr5:48250476-48250874	398	2.64809	0	0	0	2.86644	0.301853	2.17778	2.86644
cenRNA:chr10:41069147-41069832	chr10:41069147-41069832	685	1.58122	0	0.53121	0.301366	2.85452	0.300597	1.90848	2.85452
cenRNA:chr3:91561159-91561718	chr3:91561159-91561718	559	1.43292	0	0.54148	0.40959	2.84505	0.490254	0.707409	2.84505
cenRNA:chr15:19695956-19696388	chr15:19695956-19696388	432	1.12328	0	0.282885	0	2.83756	0.128062	0.739144	2.83756
cenRNA:chr18:18392536-18392952	chr18:18392536-18392952	416	8.46794	7.51686	0.30463	0	2.83741	0	0	8.46794
cenRNA:chr11:54115109-54115410	chr11:54115109-54115410	301	2.60313	0	0	0	2.81501	0.889312	0	2.81501
cenRNA:chr17:23411806-23412307	chr17:23411806-23412307	501	1.7169	0	0.432477	0	2.78876	0.195782	3.67253	2.78876
cenRNA:chr11:52617632-52618167	chr11:52617632-52618167	535	3.0764	0.956149	0.581238	0.439664	2.77631	0.175417	0.253117	3.0764
cenRNA:chr5:47956316-47956753	chr5:47956316-47956753	437	2.19744	0	0.276707	0	2.77558	0.375795	0.723001	2.77558
cenRNA:chr10:41295831-41296219	chr10:41295831-41296219	388	1.39718	1.7361	0.351788	0	2.77255	0	0.919177	2.77255
cenRNA:chr10:40127909-40129567	chr10:40127909-40129567	1658	1.73307	0.43112	0.305754	0.396482	2.75399	0.711846	3.13853	2.75399
cenRNA:chr8:45530197-45530952	chr8:45530197-45530952	755	1.37875	0.571498	0.463214	0.262791	2.73805	0.524241	1.66419	2.73805
cenRNA:chr11:54059889-54060246	chr11:54059889-54060246	357	10.0976	2.09068	1.27091	1.92271	2.73176	1.15068	2.76728	10.0976
cenRNA:chr19:26225541-26226032	chr19:26225541-26226032	491	1.77764	0	0	0	2.72694	0.506758	1.16996	2.72694
cenRNA:chr1:122579786-122580228	chr1:122579786-122580228	442	1.07521	1.33637	0.812374	0	2.71624	0.122587	1.41509	2.71624
cenRNA:chr11:53266324-53266800	chr11:53266324-53266800	476	7.50906	0	0.70926	2.14602	2.71026	0.321081	1.54434	7.50906
cenRNA:chr17:26431782-26432191	chr17:26431782-26432191	409	2.50357	0	1.26087	2.86126	2.71017	0.428094	0	2.71017
cenRNA:chr12:35274079-35274472	chr12:35274079-35274472	393	2.71931	0	1.3694	0	2.69817	0.464944	3.57807	2.71931
cenRNA:chr1:124052682-124053024	chr1:124052682-124053024	342	3.72908	2.31596	0	1.06495	2.68989	0.424891	2.45238	3.72908
cenRNA:chr17:25941782-25942210	chr17:25941782-25942210	428	1.14372	0	0	1.96085	2.68278	0.39117	1.12887	2.68278
cenRNA:chr5:47588517-47588877	chr5:47588517-47588877	360	1.65053	0	0	0.942868	2.67923	0	1.08563	2.67923
cenRNA:chr5:47558432-47558878	chr5:47558432-47558878	446	1.05712	0	0.798713	0	2.67057	0.361577	0	2.67057
cenRNA:chr11:51092592-51093123	chr11:51092592-51093123	531	3.11454	0.967995	0.784585	1.33533	2.67017	0.443976	1.02501	3.11454
cenRNA:chr19:25224641-25225090	chr19:25224641-25225090	449	1.04394	0	0.788765	0.596644	2.63731	0.238049	2.06094	2.63731
cenRNA:chr11:53375117-53375704	chr11:53375117-53375704	587	1.32697	0	0	1.13796	2.63478	0.378352	1.52863	2.63478
cenRNA:chr5:49367931-49369179	chr5:49367931-49369179	1248	1.20826	0	0.304505	0.138202	2.61808	0.248129	1.27301	2.61808
cenRNA:chr17:26548702-26549136	chr17:26548702-26549136	434	2.22665	0	0.560763	0.636265	2.61155	0	0.366301	2.61155
cenRNA:chr5:48893293-48893658	chr5:48893293-48893658	365	1.59907	0	0	0.913511	2.59581	0	0.525912	2.59581
cenRNA:chr4:51080855-51081274	chr4:51080855-51081274	419	1.19251	0	0	0	2.58196	0.271896	0.78466	2.58196
cenRNA:chr11:53916809-53917157	chr11:53916809-53917157	348	1.7877	0	0.449974	0	2.57919	0.407405	1.17573	2.57919
cenRNA:chr12:37081816-37082218	chr12:37081816-37082218	402	1.29684	0	0.653101	0.741036	2.57365	0.295658	2.55971	2.57365
cenRNA:chr4:51434565-51435109	chr4:51434565-51435109	544	1.49696	0	0.565662	0	2.56682	0.170716	0.246334	2.56682
cenRNA:chr10:40806906-40807345	chr10:40806906-40807345	439	2.17839	0	0.27431	0	2.555	0.49672	3.22532	2.555
cenRNA:chr17:23055469-23055837	chr17:23055469-23055837	368	3.13927	0	0	0	2.54809	0.178886	2.06497	3.13927
cenRNA:chr17:24364189-24364629	chr17:24364189-24364629	440	2.16899	0	0	0	2.54398	0.123644	0	2.54398
cenRNA:chr5:48540120-48540472	chr5:48540120-48540472	352	1.73967	0	0	0.993722	2.50999	0.198237	0.57209	2.50999
cenRNA:chr19:24934236-24934645	chr19:24934236-24934645	409	1.25179	1.55561	0	0	2.48432	0.998886	1.23543	2.48432
cenRNA:chr5:47797583-47797937	chr5:47797583-47797937	354	1.71655	0	2.59253	1.96106	2.47667	0.195606	2.25799	2.47667
cenRNA:chr19:27162721-27163465	chr19:27162721-27163465	744	1.87608	0	0.709083	0	2.45556	0.535002	1.08077	2.45556
cenRNA:chr5:46985117-46985548	chr5:46985117-46985548	431	1.12832	0	1.42077	1.28965	2.4431	0.514545	0	2.4431
cenRNA:chr10:41176757-41177282	chr10:41176757-41177282	525	2.38017	0	0.599581	0.90708	2.43434	0.633335	3.65547	2.43434
cenRNA:chr11:53980600-53981014	chr11:53980600-53981014	414	1.22144	0	2.46066	1.39599	2.42416	0.556969	1.20551	2.42416
cenRNA:chr7:60544383-60544872	chr7:60544383-60544872	489	1.79031	0	0	0	2.42326	0.102073	1.17829	2.42326
cenRNA:chr5:48547165-48547503	chr5:48547165-48547503	338	3.83846	0	0	1.09614	2.42259	0.874673	1.2621	3.83846
cenRNA:chr10:40105175-40105966	chr10:40105175-40105966	791	2.58712	0	0	0.49312	2.41324	0.0983723	0.567783	2.58712
cenRNA:chr11:54040087-54040654	chr11:54040087-54040654	567	1.40096	0	0.352939	1.60184	2.40231	0.239663	0.922186	2.40231
cenRNA:chr11:53564416-53564870	chr11:53564416-53564870	454	1.02269	0	0.772725	0	2.39912	0.233208	3.36505	2.39912

Supplemental Table 4

tracking_id	locus	length	FPKM_rep1 IPCENPA chromatin	FPKM_rep1 IPCENPA supernatant	FPKM_rep1 IP control chromatin	FPKM_rep1 IP control supernatant	FPKM_rep2 cenpa	FPKM_rep2 input	FPKM_rep2 mock	IPCENPA max
cenRNA:chr12:35619403-35619762	chr12:35619403-35619762	359	1.66119	2.06372	0.418174	0.948955	2.39692	0.56792	2.18527	2.39692
cenRNA:chr21:12739650-12740106	chr21:12739650-12740106	456	1.01443	0	0.510993	0	2.37977	0.231326	1.33516	2.37977
cenRNA:chr9:44621024-44621538	chr9:44621024-44621538	514	2.46582	0	0.207048	0	2.37354	0.0937303	0.811485	2.46582
cenRNA:chr11:51982309-51982862	chr11:51982309-51982862	553	1.45787	0	0.5509	1.25015	2.36826	0.0831306	0.959622	2.36826
cenRNA:chr5:50036126-50036487	chr5:50036126-50036487	361	3.27997	0	0.412842	0.936855	2.36635	0.373786	2.15741	3.27997
cenRNA:chr5:49543637-49544426	chr5:49543637-49544426	789	1.29802	0.538046	0.8722	0.742226	2.34344	0.246778	1.28191	2.34344
cenRNA:chr1:122599682-122600046	chr1:122599682-122600046	364	1.60911	0	1.21524	0.919243	2.32187	0.366759	2.11685	2.32187
cenRNA:chr9:43800776-43801222	chr9:43800776-43801222	446	1.05712	0	0.266238	0.604169	2.28906	0.361577	0	2.28906
cenRNA:chrX:61425086-61425572	chrX:61425086-61425572	486	2.71448	0	1.13956	1.55159	2.28613	0.103175	0.595505	2.71448
cenRNA:chr3:92951512-92951939	chr3:92951512-92951939	427	2.29789	0	0.578686	0.656602	2.2804	0.523941	3.02407	2.29789
cenRNA:chr16:38019280-38019628	chr16:38019280-38019628	348	1.7877	2.22065	0	3.06336	2.25679	0.814811	1.17573	2.25679
cenRNA:chr1:122510753-122511060	chr1:122510753-122511060	307	2.46508	0	0.620158	0	2.22166	0.842234	3.24079	2.46508
cenRNA:chr12:36547531-36547967	chr12:36547531-36547967	436	1.10355	0	0.277921	0	2.19038	0.629073	0	2.19038
cenRNA:chr11:52759524-52759898	chr11:52759524-52759898	374	1.51376	0	0.762209	0.864835	2.18444	0.172526	0	2.18444
cenRNA:chr10:41130545-41130961	chr10:41130545-41130961	416	2.41941	0	1.21852	0	2.18263	0.275812	2.38788	2.41941
cenRNA:chr5:48333691-48334149	chr5:48333691-48334149	458	2.01261	0	0.253452	0.575154	2.17912	0.229474	0.662237	2.17912
cenRNA:chr12:35770494-35770890	chr12:35770494-35770890	396	2.67614	3.32545	0.67384	1.52914	2.17258	0.762617	0.88033	3.32545
cenRNA:chr9:44895369-44895808	chr9:44895369-44895808	439	1.0892	0	0.27431	0	2.16193	0.12418	1.07511	2.16193
cenRNA:chr3:92358403-92358821	chr3:92358403-92358821	418	1.19819	0	0	0.684719	2.16187	0.136594	0.394196	2.16187
cenRNA:chr10:41345572-41345991	chr10:41345572-41345991	419	2.38503	0	0	0	2.15164	0.135948	0.78466	2.38503
cenRNA:chr12:35965549-35967067	chr12:35965549-35967067	1518	1.34283	0.238599	0.580172	0.438858	2.1477	0.28453	2.02122	2.1477
cenRNA:chr7:60397425-60397866	chr7:60397425-60397866	441	1.07983	1.34211	0	0	2.14336	0.123113	1.06587	2.14336
cenRNA:chr1:122462444-122462864	chr1:122462444-122462864	420	1.18689	0	1.19556	0.678267	2.1415	0.676536	0.780963	2.1415
cenRNA:chr16:37516257-37516613	chr16:37516257-37516613	356	1.694	2.10441	0	0	2.13866	0.19304	2.22836	2.13866
cenRNA:chr11:53838565-53838943	chr11:53838565-53838943	378	1.47858	0	0.744519	0.844762	2.13374	0	0.486333	2.13374
cenRNA:chr17:23009293-23009927	chr17:23009293-23009927	634	1.18045	0	0	0	2.13091	0.0673191	0.194275	2.13091
cenRNA:chr11:53600894-53601337	chr11:53600894-53601337	443	2.14126	0	0.539277	0.611886	2.12511	0.122065	0.352266	2.14126
cenRNA:chr20:26582901-26583258	chr20:26582901-26583258	357	1.68293	0	1.69455	0	2.1247	0.38356	1.66037	2.1247
cenRNA:chr20:28548431-28549646	chr20:28548431-28549646	1215	1.74701	1.55201	0.817662	0.428194	2.11804	0.484048	2.5473	2.11804
cenRNA:chr11:52679616-52680919	chr11:52679616-52680919	1303	1.37706	0.570906	0.694101	1.31259	2.11357	0.261849	1.20907	2.11357
cenRNA:chr1:124253782-124254140	chr1:124253782-124254140	358	1.67199	0	0	0	2.11092	0.571607	0.549865	2.11092
cenRNA:chr4:49889166-49889613	chr4:49889166-49889613	447	1.05269	0	0.265123	0	2.08952	0.240042	1.0391	2.08952
cenRNA:chr3:93133399-93133935	chr3:93133399-93133935	536	2.30026	0	0.38631	1.31497	2.07588	0.437205	0.50469	2.30026
cenRNA:chr2:93224579-93225006	chr2:93224579-93225006	427	1.14894	0	0	0.656602	2.07309	0.261971	3.02407	2.07309
cenRNA:chr11:53153976-53154291	chr11:53153976-53154291	315	4.5996	0	0	1.31312	2.07296	0	0	4.5996
cenRNA:chr11:52697347-52697708	chr11:52697347-52697708	361	1.63998	2.0374	0.412842	0	2.07056	0.373786	1.61805	2.07056
cenRNA:chr11:52795266-52796417	chr11:52795266-52796417	1151	2.13214	1.32588	0.604498	1.21936	2.06932	0.577717	1.31623	2.13214
cenRNA:chr5:48205766-48206194	chr5:48205766-48206194	428	1.14372	0	0.288028	0.653618	2.06367	0	2.25775	2.06367
cenRNA:chr1:124505506-124505912	chr1:124505506-124505912	406	2.54143	0	0.959934	0.726121	2.06333	0.434561	2.09016	2.54143
cenRNA:chr11:52470580-52470919	chr11:52470580-52470919	339	1.90528	0	0.479527	0	2.06144	0.217081	1.25294	2.06144
cenRNA:chr20:27603311-27603762	chr20:27603311-27603762	451	1.03533	0	0	0	2.0551	0	1.02199	2.0551
cenRNA:chr5:48183312-48183764	chr5:48183312-48183764	452	1.03108	0	0.259687	0.589304	2.04668	0.35268	0	2.04668
cenRNA:chr11:52049738-52050078	chr11:52049738-52050078	340	1.89152	0	0.952137	1.08033	2.04657	0	3.10977	2.04657
cenRNA:chr5:48245126-48245649	chr5:48245126-48245649	523	1.59686	0	0	1.82568	2.01748	0.364205	1.83934	2.01748
cenRNA:chr21:12552940-12553283	chr21:12552940-12553283	343	1.85131	6.89867	2.79578	1.0574	2.00313	0.421881	1.82625	6.89867
cenRNA:chr17:24598540-24599205	chr17:24598540-24599205	665	1.10032	0	0	1.88735	1.98632	0	0	1.98632
cenRNA:chr11:52447518-52447886	chr11:52447518-52447886	368	1.56963	0	0.790307	1.79343	1.98185	0	0	1.98185
cenRNA:chr11:53765538-53765906	chr11:53765538-53765906	368	3.13927	0	0.395154	1.79343	1.98185	0.536657	0.516243	3.13927
cenRNA:chr11:53554142-53554649	chr11:53554142-53554649	507	2.52362	0	0.423795	0	1.97367	0.0959257	0	2.52362
cenRNA:chr20:26781218-26781610	chr20:26781218-26781610	392	1.367	0	1.03259	0	1.97289	0	0	1.97289
cenRNA:chr3:91824394-91824786	chr3:91824394-91824786	392	1.367	0	1.03259	0	1.97289	0.467454	0.449673	1.97289
cenRNA:chr4:49858740-49859432	chr4:49858740-49859432	692	1.03889	0.645908	0.261762	0	1.96926	0.0592497	0.512964	1.96926
cenRNA:chr17:25442364-25442921	chr17:25442364-25442921	557	1.44114	0	0	0.411939	1.95093	0	1.66008	1.95093
cenRNA:chr3:92618206-92618577	chr3:92618206-92618577	371	1.54121	0	0	0	1.94601	0.175651	0.506908	1.94601
cenRNA:chr11:54019720-54021679	chr11:54019720-54021679	1959	1.43509	0.178502	0.361701	1.14912	1.94364	0.261986	0.614301	1.94364
cenRNA:chr17:22815930-22816652	chr17:22815930-22816652	722	3.91286	0.608199	0.492961	0.279667	1.94259	0.278953	1.28804	3.91286
cenRNA:chr18:15695333-15695780	chr18:15695333-15695780	447	1.05269	0	0.79537	0	1.89956	0	0.346367	1.89956
cenRNA:chr11:52872607-52872958	chr11:52872607-52872958	351	1.75145	0	0.440862	0	1.89522	0.399156	0.575959	1.89522
cenRNA:chr15:19488615-19488915	chr15:19488615-19488915	300	2.62744	0	0	0	1.89417	0.2992	1.72691	2.62744
cenRNA:chr12:36465198-36465550	chr12:36465198-36465550	352	3.47934	2.16107	0.437901	0.993722	1.88249	0.198237	1.71627	3.47934
cenRNA:chr11:54251259-54252721	chr11:54251259-54252721	1462	1.20241	0	0.555577	0.802302	1.88174	0.320102	0.725826	1.88174
cenRNA:chr11:51683118-51683569	chr11:51683118-51683569	451	1.03533	0	0.78227	0.591731	1.86828	0.354133	1.02199	1.86828
cenRNA:chr11:52682213-52682789	chr11:52682213-52682789	576	2.05	1.69917	1.37722	1.56265	1.85016	0.467598	0.224906	2.05
cenRNA:chr10:41198004-41198850	chr10:41198004-41198850	846	1.18199	0	0.893543	0.450601	1.84949	0.26967	2.20501	1.84949
cenRNA:chr11:51966780-51967479	chr11:51966780-51967479	699	1.02407	0	0.258029	0	1.84874	0.11681	1.3484	1.84874
cenRNA:chr5:48036665-48036995	chr5:48036665-48036995	330	2.03789	0	0.51285	0	1.83724	0	0.670006	2.03789
cenRNA:chr20:27814202-27814858	chr20:27814202-27814858	656	2.24487	0.697826	0.141402	0.641761	1.82361	0.192037	1.66259	2.24487
cenRNA:chr5:49133860-49134317	chr5:49133860-49134317	457	1.01035	0	0.25447	0	1.82323	0	0.664898	1.82323
cenRNA:chr20:26898110-26898493	chr20:26898110-26898493	383	1.43677	0	1.08523	0	1.81428	0.327522	0.472595	1.81428
cenRNA:chr1:124338393-124338725	chr1:124338393-124338725	332	2.00699	0	0.505087	0	1.80943	0.457304	0	2.00699
cenRNA:chr9:43563175-43563885	chr9:43563175-43563885	710	1.50242	0	0.252374	0.286354	1.80822	0.399873	0.329711	1.80822
cenRNA:chr1:124531006-124531641	chr1:124531006-124531641	635	1.17768	0.732156	0.890147	0.673332	1.80703	0.268646	0.775281	1.80703
cenRNA:chr3:92585513-92586450	chr3:92585513-92586450	937	1.37916	0.428793	0.260661	0.591514	1.80534	0.196668	0.908099	1.80534
cenRNA:chr11:52708236-52709536	chr11:52708236-52709536	1300	1.1507	0	0.464005	0.13162	1.78693	0.446366	1.66703	1.78693
cenRNA:chr1:123057503-123057810	chr1:123057503-123057810	307	2.46508	0	0.620158	0	1.77733	0.561489	3.24079	2.46508
cenRNA:chr11:53849046-53849433	chr11:53849046-53849433	387	2.80986	0	0.353736	0.802728	1.77412	0.160136	0	2.80986
cenRNA:chr10:41217318-41217731	chr10:41217318-41217731	413	3.68218	0	2.16356	0	1.77161	2.65848	6.8645	3.68218

Supplemental Table 4

tracking_id	locus	length	FPKM_rep1 IPCENPA chromatin	FPKM_rep1 IPCENPA supernatant	FPKM_rep1 IP control chromatin	FPKM_rep1 IP control supernatant	FPKM_rep2 cenpa	FPKM_rep2 input	FPKM_rep2 mock	IPCENPA max
cenRNA:chr11:51289660-51290100	chr11:51289660-51290100	440	1.08449	0	0	0	1.76122	0.123644	0.356824	1.76122
cenRNA:chr5:47735127-47735516	chr5:47735127-47735516	389	1.38952	0	0.69972	0	1.75468	0.475143	3.65656	1.75468
cenRNA:chr17:26006654-26007276	chr17:26006654-26007276	622	1.82204	0	0	0.694483	1.75416	0.277084	0.999542	1.82204
cenRNA:chr1:123459117-123459558	chr1:123459117-123459558	441	1.07983	1.34211	0.271954	0	1.75366	0.246227	1.42116	1.75366
cenRNA:chr12:36488356-36488693	chr12:36488356-36488693	337	1.93337	2.40133	0	0	1.74315	0.220276	1.27139	2.40133
cenRNA:chr11:52011311-52011885	chr11:52011311-52011885	574	2.06122	0	0	0.392798	1.73626	0.235077	1.58295	2.06122
cenRNA:chr17:23973175-23973856	chr17:23973175-23973856	681	1.06307	0	0	0.911747	1.7272	0.181884	0.699862	1.7272
cenRNA:chr17:22941094-22941749	chr17:22941094-22941749	655	2.2499	0	0.70859	0.321598	1.72615	0.256622	0.370291	2.2499
cenRNA:chr20:27854938-27855543	chr20:27854938-27855543	605	1.26675	0	0.159573	0	1.71497	0.0722383	1.66777	1.71497
cenRNA:chr1:122725297-122725665	chr1:122725297-122725665	368	1.56963	0	0	0.896716	1.69872	0.357771	3.09746	1.69872
cenRNA:chr19:26972753-26973390	chr19:26972753-26973390	637	1.17219	0	0.295332	0.335096	1.6928	0.267393	2.50791	1.6928
cenRNA:chr11:54335435-54336609	chr11:54335435-54336609	1174	2.08136	1.29431	0.524536	1.4879	1.6912	0.207775	0.513955	2.08136
cenRNA:chr11:52588727-52589284	chr11:52588727-52589284	557	2.16172	0	0.181528	0.823877	1.6908	0.493065	1.18577	2.16172
cenRNA:chr12:36228334-36230026	chr12:36228334-36230026	1692	1.01601	0.210621	0.341427	0.193699	1.68181	0.328447	1.17089	1.68181
cenRNA:chr9:45412381-45412834	chr9:45412381-45412834	453	1.02687	1.27634	0.517254	0	1.66772	0	0.33788	1.66772
cenRNA:chr7:58956541-58956967	chr7:58956541-58956967	426	1.15422	0	0.29067	0	1.66608	0.131586	8.73407	1.66608
cenRNA:chr3:91886337-91886846	chr3:91886337-91886846	509	1.67122	0	0	0.477659	1.65893	0.190576	0.824971	1.67122
cenRNA:chr3:91811133-91811533	chr3:91811133-91811533	400	1.31031	0	0.329937	0	1.65476	0	0	1.65476
cenRNA:chr4:50842454-50842965	chr4:50842454-50842965	511	1.66018	0	0.836395	0.474505	1.64797	0.189318	1.36587	1.66018
cenRNA:chr17:24526204-24526549	chr17:24526204-24526549	345	1.82537	0	0.459443	0	1.64591	0	0	1.82537
cenRNA:chr19:26602103-26602448	chr19:26602103-26602448	345	1.82537	0	0.918885	1.04261	1.64591	0.207989	1.8007	1.82537
cenRNA:chr11:54272528-54273095	chr11:54272528-54273095	567	2.10144	0	1.23529	0.800919	1.64369	0.31955	2.07492	2.10144
cenRNA:chr11:53658264-53658722	chr11:53658264-53658722	458	1.00631	0	0.253452	0.575154	1.63434	0.344212	0.662237	1.63434
cenRNA:chr11:52306198-52306934	chr11:52306198-52306934	736	2.38065	0.592068	0.71983	0.544499	1.6332	0.217244	1.56735	2.38065
cenRNA:chr9:43423820-43424501	chr9:43423820-43424501	681	1.06307	0	0.267852	0	1.63124	0.121256	0.699862	1.63124
cenRNA:chr3:93328436-93328978	chr3:93328436-93328978	542	1.50593	0	0.189684	0.860892	1.63086	0.257609	0.49562	1.63086
cenRNA:chr11:52465481-52466218	chr11:52465481-52466218	737	1.90092	0.590949	0.359234	0	1.63011	0.108417	0.469317	1.90092
cenRNA:chr19:25119081-25120272	chr19:25119081-25120272	1191	1.02268	0	0.257732	0	1.61578	0.204181	0.757598	1.61578
cenRNA:chr12:36814656-36815679	chr12:36814656-36815679	1023	1.54196	0	0.233151	0.88181	1.6148	0.246277	1.21839	1.6148
cenRNA:chr5:47926212-47926617	chr5:47926212-47926617	405	1.27715	0	0.96479	0	1.61293	0.436759	1.26044	1.61293
cenRNA:chr1:123555932-123556621	chr1:123555932-123556621	689	1.04538	0	0	0	1.60411	0.238478	0.516164	1.60411
cenRNA:chr19:25072669-25073018	chr19:25072669-25073018	349	3.55092	0	0.446898	0	1.60097	0.20231	4.67075	3.55092
cenRNA:chr3:92396509-92396916	chr3:92396509-92396916	407	2.52869	0	0.955125	1.44497	1.59677	0.144128	1.24781	2.52869
cenRNA:chr3:92666889-92667297	chr3:92666889-92667297	408	1.25803	0	0	0	1.58881	0.286819	0.413864	1.58881
cenRNA:chr4:50188823-50189231	chr4:50188823-50189231	408	1.25803	1.56337	0	0.718882	1.58881	0	0.413864	1.58881
cenRNA:chr4:51132967-51133861	chr4:51132967-51133861	894	1.09924	0	0.277003	0.419065	1.58774	0.167198	0.723774	1.58774
cenRNA:chr17:26326121-26326787	chr17:26326121-26326787	666	1.09791	0	0.138313	0	1.58559	0	0.361395	1.58559
cenRNA:chr11:51560455-51561295	chr11:51560455-51561295	840	1.19321	0	0.100225	0.22744	1.57981	0.317603	0.916566	1.57981
cenRNA:chr19:25779809-25780276	chr19:25779809-25780276	467	1.94258	0	0.24464	0	1.57752	0	0	1.94258
cenRNA:chr4:50707213-50707594	chr4:50707213-50707594	381	1.45322	0	0	0	1.57288	0.165634	0.956	1.57288
cenRNA:chr5:48377230-48377727	chr5:48377230-48377727	497	1.74069	0	0.219233	0	1.57077	0.198493	1.14566	1.74069
cenRNA:chr9:44499269-44499824	chr9:44499269-44499824	555	1.44946	0	0.365149	0	1.56974	0.0826513	0.238522	1.56974
cenRNA:chr7:59542160-59542571	chr7:59542160-59542571	411	2.47894	0	1.24847	0.708286	1.56539	0.141296	2.03882	2.47894
cenRNA:chr19:26935495-26935993	chr19:26935495-26935993	498	1.73469	0	0.218477	0	1.56535	0.0989041	0.856278	1.73469
cenRNA:chr1:122138610-122138932	chr1:122138610-122138932	322	2.17052	0	2.18469	3.71826	1.56529	1.23626	0	2.17052
cenRNA:chr11:52750006-52750447	chr11:52750006-52750447	441	1.07983	0	0.271954	0	1.55881	0.246227	0	1.55881
cenRNA:chr1:123979957-123980370	chr1:123979957-123980370	413	1.22739	0	0	0	1.55016	0.699601	0	1.55016
cenRNA:chr11:53794359-53794772	chr11:53794359-53794772	413	3.68218	0	0.927241	2.10417	1.55016	0.13992	0.807588	3.68218
cenRNA:chr12:36854676-36855089	chr12:36854676-36855089	413	1.22739	0	1.23632	1.40278	1.55016	0.13992	1.21138	1.55016
cenRNA:chr18:19555252-19555606	chr18:19555252-19555606	354	3.43309	0	1.29627	4.90266	1.54792	0	0	3.43309
cenRNA:chr11:51641632-51642075	chr11:51641632-51642075	443	1.07063	0	1.07855	0	1.54553	0.48826	0	1.54553
cenRNA:chr5:48622293-48622971	chr5:48622293-48622971	678	1.06986	0	0	0.305856	1.54509	0.12203	1.0565	1.54509
cenRNA:chr19:25546424-25547308	chr19:25546424-25547308	884	1.11551	0	0.0937005	0	1.5441	0.0424181	0.489655	1.5441
cenRNA:chr3:92827630-92827985	chr3:92827630-92827985	355	1.7052	0	0	0	1.5377	0	1.12154	1.7052
cenRNA:chr11:52285364-52286016	chr11:52285364-52286016	652	2.83139	0	0.285352	0.971316	1.53337	0.452124	0.745588	2.83139
cenRNA:chr11:53116440-53117183	chr11:53116440-53117183	743	1.87959	0	0.355205	1.07475	1.52699	0.268001	0.773421	1.87959
cenRNA:chr4:50747682-50748128	chr4:50747682-50748128	446	1.05712	1.3139	0.266238	1.20834	1.52604	0.241051	3.13041	1.52604
cenRNA:chr9:44251165-44251701	chr9:44251165-44251701	536	1.53351	0	0.193155	0	1.52231	0.087441	0.252345	1.53351
cenRNA:chr1:122761559-122761976	chr1:122761559-122761976	417	1.20392	0	0.909525	0.68799	1.52054	0.411741	0	1.52054
cenRNA:chr4:51719445-51720071	chr4:51719445-51720071	626	1.20306	0	0.303106	0	1.52019	0.137216	0.791979	1.52019
cenRNA:chr11:52468696-52469145	chr11:52468696-52469145	449	1.04394	0	0.525844	0.596644	1.50703	0.238049	0	1.50703
cenRNA:chr20:27794182-27795173	chr20:27794182-27795173	991	1.92601	0	0.728044	0.183571	1.50693	0.329584	0.951143	1.92601
cenRNA:chr19:26247238-26247657	chr19:26247238-26247657	419	2.38503	0	0	0	1.50615	0.271896	1.17699	2.38503
cenRNA:chr11:53603618-53604218	chr11:53603618-53604218	600	1.28292	0	0	0.366736	1.50527	0.07316	0.211132	1.50527
cenRNA:chr11:53931742-53932704	chr11:53931742-53932704	962	1.00006	0.414573	0.336022	0.190633	1.50471	0.152117	0.329244	1.50471
cenRNA:chr1:122726785-122727112	chr1:122726785-122727112	327	2.08588	0	0	0	1.50435	0	0.685758	2.08588
cenRNA:chr1:124555142-124555592	chr1:124555142-124555592	450	1.03962	0	0.261835	0	1.5008	0.237064	0.342071	1.5008
cenRNA:chr12:35681712-35682253	chr12:35681712-35682253	541	3.77615	0	1.14152	0.431739	1.49945	0	1.24277	3.77615
cenRNA:chr11:51496033-51496453	chr11:51496033-51496453	420	2.37378	0	0.896671	0	1.49905	0.135307	0	2.37378
cenRNA:chr5:48449166-48449862	chr5:48449166-48449862	696	1.03037	0	0.64904	0.883714	1.48808	0.411348	1.01752	1.48808
cenRNA:chr11:52748519-52749461	chr11:52748519-52749461	942	1.02732	0.425872	0.51777	0.587483	1.48389	0.117197	0.338217	1.48389
cenRNA:chr17:23153815-23155037	chr17:23153815-23155037	1222	1.23925	0	0.124926	0.283492	1.47687	0.0565537	0.408019	1.47687
cenRNA:chr11:53525770-53526749	chr11:53525770-53526749	979	1.63	0.405431	0.410765	0.186428	1.47153	0.334715	0.321983	1.63
cenRNA:chr11:52391351-52392022	chr11:52391351-52392022	671	1.08605	0	0.273638	0.931445	1.47043	0	0.178746	1.47043
cenRNA:chr5:48647733-48648189	chr5:48647733-48648189	456	1.01443	0	0	0	1.46447	0.115663	1.33516	1.46447
cenRNA:chr15:17537697-17538370	chr15:17537697-17538370	673	1.08137	0	0.136231	0	1.4641	0.0616714	0.53393	1.4641
cenRNA:chr11:52347105-52348212	chr11:52347105-52348212	1107	1.11827	0	0.281817	0	1.4639	0.127578	0.552265	1.4639

Supplemental Table 4

tracking_id	locus	length	FPKM_rep1 IPCENPA chromatin	FPKM_rep1 IPCENPA supernatant	FPKM_rep1 IP control chromatin	FPKM_rep1 IP control supernatant	FPKM_rep2 cenpa	FPKM_rep2 input	FPKM_rep2 mock	IPCENPA max
cenRNA:chr12:35690131-35690774	chr12:35690131-35690774	643	1.15601	0	0.582515	0.991418	1.46077	0.263704	1.14153	1.46077
cenRNA:chr5:49747416-49747904	chr5:49747416-49747904	488	2.69507	0	0.452566	0.5135	1.45915	0.102438	0.295625	2.69507
cenRNA:chr9:44278748-44279361	chr9:44278748-44279361	613	1.24171	0	0.312839	0	1.45693	0	0.817409	1.45693
cenRNA:chr1:123557258-123557685	chr1:123557258-123557685	427	1.14894	0	0.289343	0	1.45117	0.261971	1.13403	1.45117
cenRNA:chr17:24953969-24954553	chr17:24953969-24954553	584	1.33756	0	0.505464	0	1.44863	0	0.440238	1.44863
cenRNA:chr11:51441676-51442072	chr11:51441676-51442072	396	1.33807	0	0.67384	0	1.44839	0.152523	1.3205	1.44839
cenRNA:chr1:123610460-123611045	chr1:123610460-123611045	585	1.33401	0	0	0.381333	1.44478	0.0760719	0.658605	1.44478
cenRNA:chr15:19697940-19698368	chr15:19697940-19698368	428	1.14372	0	0	0.653618	1.44457	0.26078	1.50516	1.44457
cenRNA:chr1:124064764-124065161	chr1:124064764-124065161	397	1.33102	0	0.335147	0	1.44076	1.06205	0.875698	1.44076
cenRNA:chr11:52345163-52345592	chr11:52345163-52345592	429	2.27708	0	0.286725	1.30132	1.43804	0.2596	0	2.27708
cenRNA:chr20:28299579-28300135	chr20:28299579-28300135	556	5.78116	0	0.18205	0	1.43479	0.164827	1.90269	5.78116
cenRNA:chr1:122899028-122899394	chr1:122899028-122899394	366	3.17828	0	0.400058	0.907846	1.43318	0.362212	1.0453	3.17828
cenRNA:chr3:93474763-93475288	chr3:93474763-93475288	525	2.38017	0	0.19986	0.45354	1.43196	0.0904765	0.52221	2.38017
cenRNA:chr17:25842790-25843442	chr17:25842790-25843442	652	1.13256	0.704115	0.142676	0.323772	1.43115	0.193767	0.186397	1.43115
cenRNA:chrX:61670116-61670515	chrX:61670116-61670515	399	1.31714	3.27349	0.994968	0.752622	1.42576	0.300281	1.29986	3.27349
cenRNA:chr20:27438283-27438650	chr20:27438283-27438650	367	1.57933	0	0.397592	0	1.42434	0	1.55829	1.57933
cenRNA:chr19:25581321-25581621	chr19:25581321-25581621	300	2.62744	0	0	0	1.42062	0.5984	1.72691	2.62744
cenRNA:chr4:49756355-49757298	chr4:49756355-49757298	943	1.02593	0.850584	0.430888	0	1.42013	0.0780249	1.01327	1.42013
cenRNA:chr11:51143036-51143500	chr11:51143036-51143500	464	1.96538	0	0.247508	1.12333	1.41868	0.112047	0.646708	1.96538
cenRNA:chr11:52627854-52628287	chr11:52627854-52628287	433	2.23656	0	0.281628	0	1.41247	0	1.10379	2.23656
cenRNA:chr11:53861602-53861971	chr11:53861602-53861971	369	3.1201	0	0.785488	0	1.40697	0.177795	0.513095	3.1201
cenRNA:chr3:92366738-92367270	chr3:92366738-92367270	532	1.55246	1.93001	0.586622	0	1.40101	0.177042	0.510923	1.93001
cenRNA:chr19:24908795-24909492	chr19:24908795-24909492	697	1.54239	0	0.129543	0.881906	1.39223	0.46915	0.169239	1.54239
cenRNA:chr1:123346957-123347459	chr1:123346957-123347459	502	2.56659	0	0.646508	1.46711	1.38964	0.487789	2.53387	2.56659
cenRNA:chr20:28317146-28317550	chr20:28317146-28317550	404	1.28365	0	0.646463	0.733504	1.38954	0	2.11141	1.38954
cenRNA:chr11:51141091-51141561	chr11:51141091-51141561	470	1.92031	0	0.725511	0.548797	1.38618	0.109479	0.315945	1.92031
cenRNA:chr17:23103073-23103511	chr17:23103073-23103511	438	1.09394	1.35963	3.03054	2.50078	1.38176	0.24944	1.07978	1.38176
cenRNA:chr4:49718514-49718886	chr4:49718514-49718886	372	1.53196	0	0	1.75044	1.38167	0.349194	3.52707	1.53196
cenRNA:chr11:54098805-54099144	chr11:54098805-54099144	339	1.90528	0	0.959055	1.08818	1.37429	0	1.25294	1.90528
cenRNA:chr5:49511240-49512044	chr5:49511240-49512044	804	1.26533	0	0.106281	0.964723	1.37067	0.240565	0.694244	1.37067
cenRNA:chr11:53251143-53251818	chr11:53251143-53251818	675	1.07674	0	0.678235	0	1.36064	0.122814	1.59493	1.36064
cenRNA:chr11:54301082-54302262	chr11:54301082-54302262	1180	1.03425	0	0.0651622	0.295743	1.35394	0.0884966	0.766173	1.35394
cenRNA:chr3:91658284-91658895	chr3:91658284-91658895	611	1.87181	0	0.314392	0	1.35154	0.142325	1.8483	1.87181
cenRNA:chr17:24692478-24692854	chr17:24692478-24692854	376	1.49597	0	0	0	1.34925	0.170501	0	1.49597
cenRNA:chr11:52737387-52737898	chr11:52737387-52737898	511	1.66018	0	0.209099	0	1.34834	0	1.0927	1.66018
cenRNA:chr11:53825053-53825395	chr11:53825053-53825395	342	1.86454	0	0.469287	2.12989	1.34494	0.212446	0.613094	1.86454
cenRNA:chr3:93230726-93231138	chr3:93230726-93231138	412	1.2334	0	0.621184	1.40964	1.3352	0.281209	2.84038	1.3352
cenRNA:chr11:52990656-52991239	chr11:52990656-52991239	583	1.34113	0.833718	0.506813	0.383367	1.33145	0.305911	0.882825	1.34113
cenRNA:chr19:26458002-26458449	chr19:26458002-26458449	447	3.15806	0	0.265123	0	1.32969	0.840147	1.73183	3.15806
cenRNA:chr11:54237176-54238210	chr11:54237176-54238210	1034	1.21714	0	0.306727	0.174013	1.31859	0.242997	1.10198	1.31859
cenRNA:chr1:124500811-124501365	chr1:124500811-124501365	554	1.45365	0	0	0	1.3119	0.165781	0.478424	1.45365
cenRNA:chr11:52082783-52083338	chr11:52082783-52083338	555	1.44946	0	0.912873	0.414314	1.30812	0.330605	0.715567	1.44946
cenRNA:chr7:59085472-59085993	chr7:59085472-59085993	521	1.60708	0	0.404831	0	1.30525	0.0916332	0.528886	1.60708
cenRNA:chr11:54123668-54124051	chr11:54123668-54124051	383	1.43677	0	1.08523	0.820899	1.29592	0.163761	0.472595	1.43677
cenRNA:chr17:23177999-23178489	chr17:23177999-23178489	490	1.78396	0	0.89871	0.509857	1.28782	0.406845	1.76116	1.78396
cenRNA:chr17:23133938-23134784	chr17:23133938-23134784	846	1.18199	0	0.496413	0.2253	1.28042	0.0898901	0.389119	1.28042
cenRNA:chr11:52830623-52831542	chr11:52830623-52831542	919	1.06057	0	0.089087	0.202164	1.27659	0.322637	0.814706	1.27659
cenRNA:chr11:53295221-53295856	chr11:53295221-53295856	635	3.53305	0	0.445074	1.01	1.27555	0.201484	0.19382	3.53305
cenRNA:chr10:41447011-41447647	chr10:41447011-41447647	636	1.17493	0	0.444033	0.671759	1.27257	0.134009	2.32041	1.27257
cenRNA:chr11:52384360-52384961	chr11:52384360-52384961	601	1.27966	0.79552	0.161197	0.365802	1.27045	0.0729738	0.631782	1.27966
cenRNA:chr11:53191148-53191571	chr11:53191148-53191571	423	2.34066	1.45448	0.589447	2.00643	1.26699	0.133421	0	2.34066
cenRNA:chr16:38016735-38017158	chr16:38016735-38017158	423	1.17033	0	0.294723	0	1.26699	0.133421	0	1.26699
cenRNA:chr11:54189530-54190313	chr11:54189530-54190313	783	1.31157	0	0.550815	0.499982	1.26288	0.598448	0.287842	1.31157
cenRNA:chr7:59898072-59898749	chr7:59898072-59898749	677	1.07214	0	0.135068	0.306508	1.25806	0.183436	0.529375	1.25806
cenRNA:chr3:92888929-92889860	chr3:92888929-92889860	931	1.04296	0.864704	0.525649	0.198808	1.25539	0.15864	0.801182	1.25539
cenRNA:chr12:35992710-35993895	chr12:35992710-35993895	1185	1.02896	0.639868	0.388972	0.882688	1.25411	0.293479	1.01634	1.25411
cenRNA:chr11:53304574-53305365	chr11:53304574-53305365	791	1.29356	0	0.325953	0.98624	1.24554	0.147558	1.41946	1.29356
cenRNA:chr10:41493821-41494758	chr10:41493821-41494758	937	1.37916	0.428793	0.086887	0.197171	1.24506	0.0786672	1.02161	1.37916
cenRNA:chr11:52614908-52615590	chr11:52614908-52615590	682	2.12165	0	0.400929	0.303274	1.24479	0.3025	0.698386	2.12165
cenRNA:chr11:53637381-53637808	chr11:53637381-53637808	427	3.44683	0	0.86803	0	1.24386	0.261971	1.13403	3.44683
cenRNA:chr11:52778775-52780298	chr11:52778775-52780298	1523	2.86655	0	0.722457	1.31157	1.24231	0.305252	0.943845	2.86655
cenRNA:chr12:36522876-36524738	chr12:36522876-36524738	1862	1.36734	0.377942	0.344623	0.260683	1.23458	0.277352	1.10056	1.36734
cenRNA:chr8:44594360-44595046	chr8:44594360-44595046	686	1.05194	0	0.132524	0	1.23437	0.179981	1.38508	1.23437
cenRNA:chr12:34998907-34999446	chr12:34998907-34999446	539	1.51959	0	0.191404	0	1.23424	0.259944	0.500113	1.51959
cenRNA:chr17:26270660-26271274	chr17:26270660-26271274	614	1.23865	0	0.156034	0.354086	1.22975	0.141273	0.611546	1.23865
cenRNA:chr11:52806198-52806850	chr11:52806198-52806850	652	3.39767	0	0.570703	1.61886	1.2267	0.258357	1.49118	3.39767
cenRNA:chr18:18528554-18528947	chr18:18528554-18528947	393	2.71931	0	0	0.776889	1.22644	0	0.447259	2.71931
cenRNA:chr7:59669642-59670334	chr7:59669642-59670334	692	2.07778	0	0	0.297007	1.21906	0.0592497	0.341976	2.07778
cenRNA:chr7:58378749-58379667	chr7:58378749-58379667	918	1.06207	0	0.35685	0.202449	1.21447	0.282705	0.932405	1.21447
cenRNA:chr12:36531171-36533621	chr12:36531171-36533621	2450	1.00868	0.418222	0.33898	0.256414	1.21437	0.153456	0.774998	1.21437
cenRNA:chr18:15917121-15917555	chr18:15917121-15917555	434	2.22665	0	0	0	1.20533	0.253857	0.732602	2.22665
cenRNA:chr11:54289548-54290286	chr11:54289548-54290286	738	1.423	0	0.597594	0.271222	1.19886	0.054106	0.624575	1.423
cenRNA:chr2:94046568-94046928	chr2:94046568-94046928	360	1.65053	6.15144	0	0	1.19077	0.564277	0	6.15144
cenRNA:chr11:51678914-51679313	chr11:51678914-51679313	399	1.31714	0	2.98491	0	1.18813	0	0.433288	1.31714
cenRNA:chr17:24809917-24810585	chr17:24809917-24810585	668	1.09313	0	0.275423	1.56254	1.18402	0.187026	0.359823	1.18402
cenRNA:chr3:91009604-91009965	chr3:91009604-91009965	361	1.63998	0	0	0	1.18318	0	1.61805	1.63998
cenRNA:chr17:26266579-26266979	chr17:26266579-26266979	400	1.31031	0	0.329937	0	1.18197	0.149362	0.431042	1.31031

Supplemental Table 4

tracking_id	locus	length	FPKM_rep1 IPCENPA chromatin	FPKM_rep1 IPCENPA supernatant	FPKM_rep1 IP control chromatin	FPKM_rep1 IP control supernatant	FPKM_rep2 cenpa	FPKM_rep2 input	FPKM_rep2 mock	IPCENPA max
cenRNA:chr3:93282114-93283208	chr3:93282114-93283208	1094	1.13468	0	0.357441	0.162227	1.17806	0.0970878	0.373579	1.17806
cenRNA:chr11:51528828-51529930	chr11:51528828-51529930	1102	1.12452	0	0	0.160775	1.16752	0.0641459	0.462795	1.16752
cenRNA:chr16:36345826-36346773	chr16:36345826-36346773	947	1.02037	0	0.085711	0.389005	1.1668	0.155205	1.00778	1.1668
cenRNA:chr20:28336851-28337254	chr20:28336851-28337254	403	3.87064	0	0	0	1.16386	0	0	3.87064
cenRNA:chr5:49407661-49408105	chr5:49407661-49408105	444	2.13217	0	0.268495	0.609292	1.15423	0.48619	1.05232	2.13217
cenRNA:chr20:27075758-27076203	chr20:27075758-27076203	445	1.06158	0	0	0	1.14936	0.484137	0.698583	1.14936
cenRNA:chr11:53296905-53297311	chr11:53296905-53297311	406	3.81214	0	0.959934	0	1.14629	0.144854	0	3.81214
cenRNA:chr20:28041136-28042216	chr20:28041136-28042216	1080	1.44113	0.358464	0.217908	0.164832	1.14494	0.197294	0.569367	1.44113
cenRNA:chr12:36366027-36366473	chr12:36366027-36366473	446	1.05712	1.3139	0.266238	0	1.14453	0.241051	0.695646	1.3139
cenRNA:chr15:19095211-19095856	chr15:19095211-19095856	645	1.15071	0	0.144962	0	1.14249	0.0656241	0.757535	1.15071
cenRNA:chr22:14910304-14910711	chr22:14910304-14910711	407	1.26434	0	0.318375	0	1.14055	0	0	1.26434
cenRNA:chr17:25597444-25598092	chr17:25597444-25598092	648	1.71429	0	0.287947	0	1.1347	0.130353	0.752368	1.71429
cenRNA:chr4:51235620-51235988	chr4:51235620-51235988	368	1.56963	0	0	0	1.13248	0	1.03249	1.56963
cenRNA:chr11:51276659-51277471	chr11:51276659-51277471	812	1.24856	0	0.104873	0.475971	1.12709	0.0949513	1.37009	1.24856
cenRNA:chr7:59793816-59794226	chr7:59793816-59794226	410	1.2456	0	0.62732	0	1.12366	0.283987	0.819554	1.2456
cenRNA:chr11:51176830-51177362	chr11:51176830-51177362	532	1.55246	0	0.586622	0.443737	1.12081	0.265563	0	1.55246
cenRNA:chr11:51798688-51799307	chr11:51798688-51799307	619	1.22357	0	0.308271	0.349777	1.10435	0.139554	0.805472	1.22357
cenRNA:chr17:24882900-24883521	chr17:24882900-24883521	621	1.21764	0	0.460166	0	1.099	0.0694388	0	1.21764
cenRNA:chr4:50608432-50609053	chr4:50608432-50609053	621	1.21764	0	0.306777	0	1.099	0.138878	0.80157	1.21764
cenRNA:chr11:54094659-54095032	chr11:54094659-54095032	373	1.52281	1.892	0.766759	0	1.09874	0	1.50258	1.892
cenRNA:chr1:122933713-122934128	chr1:122933713-122934128	415	1.21555	0	0.3061	0	1.09658	0.277142	0.7998	1.21555
cenRNA:chr11:52670738-52671692	chr11:52670738-52671692	954	1.01079	0	0.339626	0	1.09501	0.0768742	1.22018	1.09501
cenRNA:chr1:124754461-124754918	chr1:124754461-124754918	457	1.01035	0	0	1.15493	1.09394	0.115198	1.99469	1.09394
cenRNA:chr11:52868853-52869562	chr11:52868853-52869562	709	1.00362	0	0.126439	0	1.0871	0	0.495553	1.0871
cenRNA:chr11:53922752-53923127	chr11:53922752-53923127	375	4.51445	1.86968	1.89428	1.71946	1.08577	0.857537	0.989902	4.51445
cenRNA:chr5:48471029-48471404	chr5:48471029-48471404	375	1.50482	0	0.378856	0	1.08577	0	1.9798	1.50482
cenRNA:chr5:47565226-47565644	chr5:47565226-47565644	418	1.19819	0	0	1.36944	1.08093	0	0	1.19819
cenRNA:chr3:93151408-93152289	chr3:93151408-93152289	881	1.12049	0	0.282355	0.213581	1.07895	0.0852144	0.491838	1.12049
cenRNA:chrX:61063675-61064395	chrX:61063675-61064395	720	1.47306	0	0.371165	1.4038	1.06373	0.224035	0.969808	1.47306
cenRNA:chr17:23206055-23206478	chr17:23206055-23206478	423	1.17033	0	0.589447	0	1.05582	0	0.385038	1.17033
cenRNA:chr15:19059864-19060545	chr15:19059864-19060545	681	1.06307	0.660933	0.133926	0.303916	1.05551	0.121256	0.349931	1.06307
cenRNA:chr15:19433431-19433767	chr15:19433431-19433767	336	1.9477	0	0	1.11237	1.05363	0	1.28079	1.9477
cenRNA:chr11:52647375-52647973	chr11:52647375-52647973	598	1.28951	0	0	0.368617	1.04745	0	0.636644	1.28951
cenRNA:chr9:44436592-44437062	chr9:44436592-44437062	470	1.92031	0	0.241837	0	1.03963	0.109479	0.947835	1.92031
cenRNA:chr4:50542479-50543389	chr4:50542479-50543389	910	1.07418	0.445288	0.0902294	0.409512	1.03437	0.163387	0.825153	1.07418
cenRNA:chr11:53268022-53268714	chr11:53268022-53268714	692	1.55834	0	0.261762	0	1.03152	0.236999	0.512964	1.55834
cenRNA:chr4:50948077-50949168	chr4:50948077-50949168	1091	1.13854	0	0.143462	0	1.02788	0.12989	1.49939	1.13854
cenRNA:chr1:123371606-123371991	chr1:123371606-123371991	385	1.42068	0	0	0	1.02513	0.161929	1.40192	1.42068
cenRNA:chr11:54173629-54174014	chr11:54173629-54174014	385	4.26203	0	0	1.62343	1.02513	0	0	4.26203
cenRNA:chr11:51502875-51503351	chr11:51502875-51503351	476	1.87726	0	0.47284	0	1.01635	0	0.308868	1.87726
cenRNA:chr2:92557211-92557732	chr2:92557211-92557732	521	2.41062	0	0	0	1.01519	0.2749	1.05777	2.41062
cenRNA:chr11:51823244-51823811	chr11:51823244-51823811	567	1.40096	0	0.352939	0.800919	1.0115	0.479325	1.61383	1.40096
cenRNA:chr1:123606381-123606950	chr1:123606381-123606950	569	1.39319	0	0.350983	0.79648	1.00589	0.0794448	0.687806	1.39319
cenRNA:chr11:54334500-54334934	chr11:54334500-54334934	434	2.22665	1.3837	0	0	1.00444	0.634642	1.0989	2.22665
cenRNA:chr3:92604590-92604979	chr3:92604590-92604979	389	2.77903	0	0	0	1.00267	0	0.91414	2.77903
cenRNA:chr11:53428821-53429980	chr11:53428821-53429980	1159	2.37847	0	0.466209	0.302275	1.00209	0.090451	1.39217	2.37847
cenRNA:chr11:52326532-52327239	chr11:52326532-52327239	707	2.5191	0	0.888621	0.288076	1.0005	0.114936	0.49754	2.5191