

SUPPLEMENTARY INFORMATION

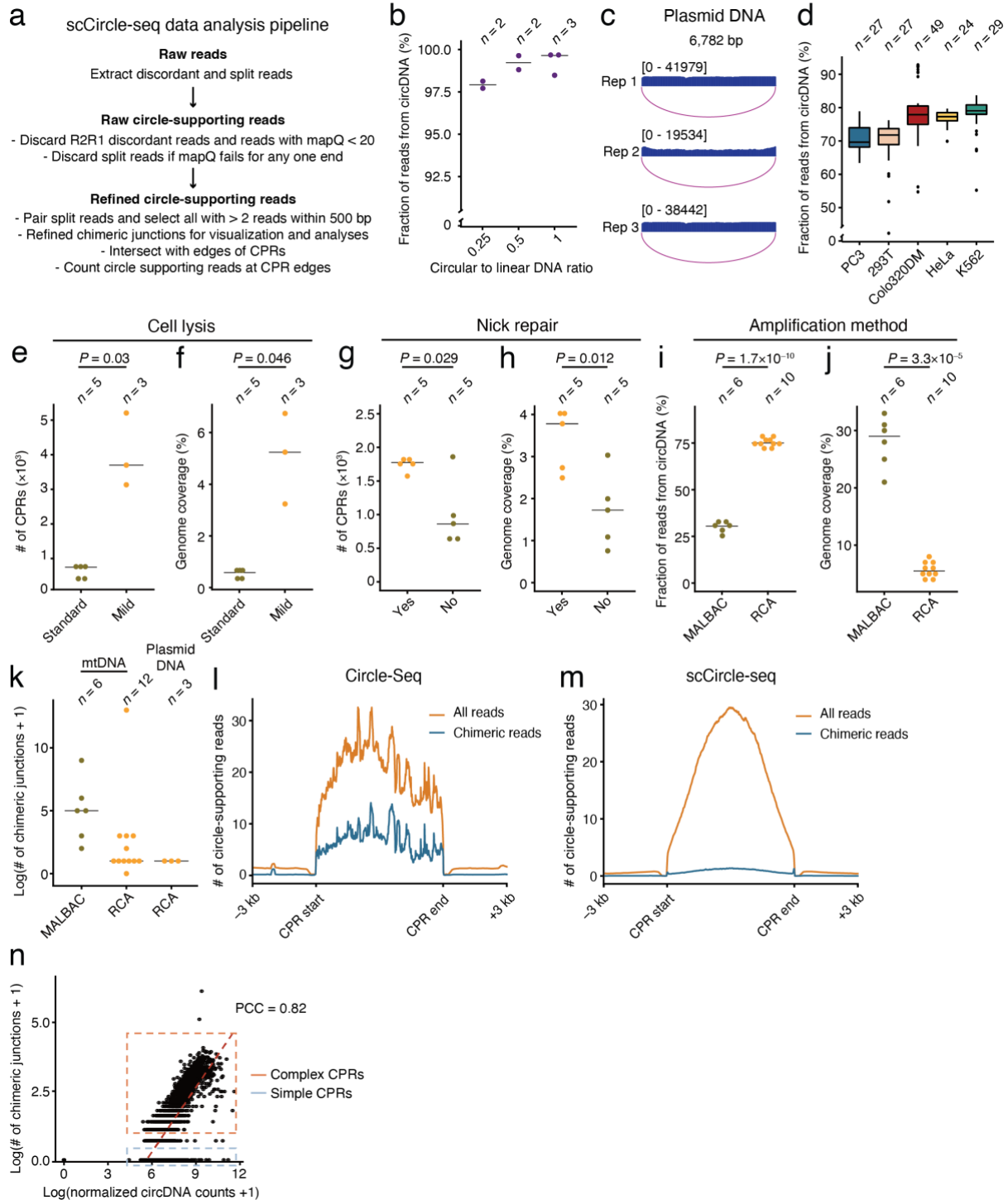
scCircle-seq unveils the diversity and complexity of circular DNAs in single cells

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| | |
|--------------------------|--------|
| 1. Supplementary Figures | pg. 2 |
| 2. Supplementary Methods | pg. 12 |
| 3. Supplementary Tables | pg. 19 |

1. Supplementary Figures

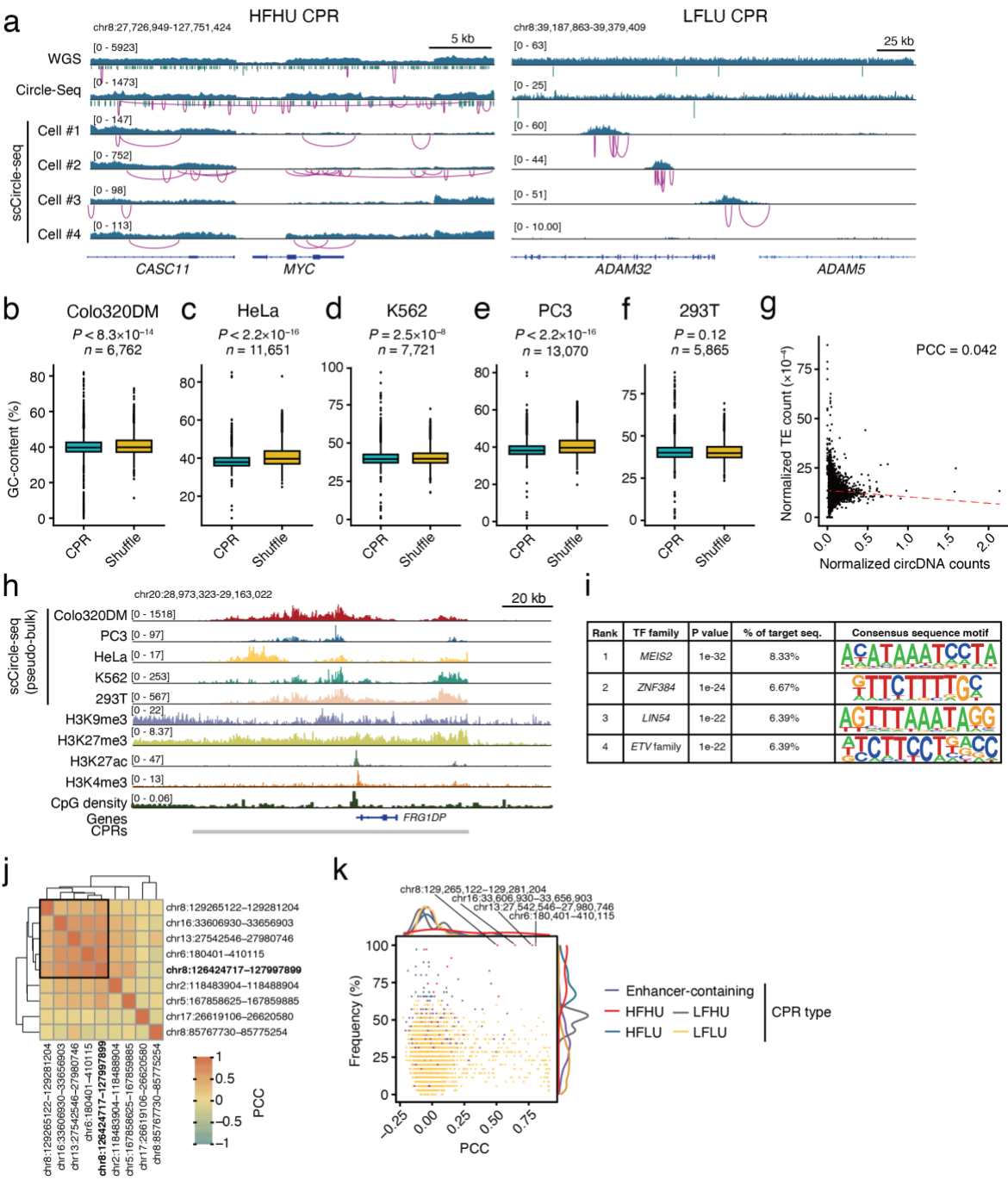
Supplementary Figure 1



Supplementary Fig. 1. scCircle-seq implementation and optimization. **(a)** Schematic description of the computational pipeline used to process scCircle-seq data and call circle-producing regions (CPRs). bp, base-pair. **(b)** Efficiency of linear genomic DNA (gDNA) removal during the scCircle-seq procedure, assessed in an experiment in which different ratios

of circular and linear DNA were manually mixed and used as input for scCircle-seq. *n*, number of samples. Each dot represents one sample. **(b)** Coverage (blue) and chimeric junctions identified (magenta arches) in three replicate (Rep) experiments in which scCircle-seq was applied to circular plasmid DNA. The numbers in squared brackets represent the intensity range of the corresponding track. **(d)** Percentage of all reads classified as circle-supporting reads for each of the five cell lines subjected to scCircle-seq. *n*, number of single cells analyzed. Boxplots extend from the 25th to the 75th percentile, horizontal bars represent the median, and whiskers extend from $-1.5 \times \text{IQR}$ to $+1.5 \times \text{IQR}$ from the closest quartile, where IQR is the inter-quartile range. Black dots, outliers. In each boxplot, the minimum and maximum are defined, respectively, by the uppermost and lowermost outlier dot or extremity of the corresponding whisker. **(e)** Number of CPRs identified by scCircle-seq in PC3 cells lysed as in Circle-Seq (standard) or using milder lysis conditions as in scCircle-seq. *n*, number of single cells (colored dots). *P*, t-test, two-tailed. **(f)** Same as in (e) but showing the genome coverage of CPRs. **(g, h)** Same as in (e) and (f), respectively, but comparing the use of a nick repair step in scCircle-seq (Yes) or not as in Circle-Seq (No). **(i)** Fraction of all sequencing reads assigned to circDNA in a standard scCircle-seq experiment using rolling circle amplification (RCA) or using a different DNA amplification method (multiple annealing and looping based amplification cycles or MALBAC¹³). *n*, number of single cells (colored dots). *P*, t-test, two-tailed. **(j)** Genome coverage of CPRs identified by scCircle-seq using RCA or MALBAC. *n*, number of single cells (colored dots). *P*, t-test, two-tailed. **(k)** Number of chimeric junctions detected by scCircle-seq for circular mitochondrial DNA (mtDNA) and circular plasmid DNA using two different amplification methods. *n*, number of single cells (colored dots). **(l, m)** Coverage of all reads and circle-supporting reads along the CPRs identified by Circle-Seq (l) and scCircle-seq (m) in PC3 cells. kb, kilobase. **(n)** Correlation between the number of circDNAs and chimeric junctions in the CPRs detected by scCircle-seq in Colo320DM cells. PCC, Pearson's correlation coefficient. Each black dot in the scatterplot represents a CPR. Dashed red line, linear regression. Complex and simple CPRs are marked by the dashed rectangles.

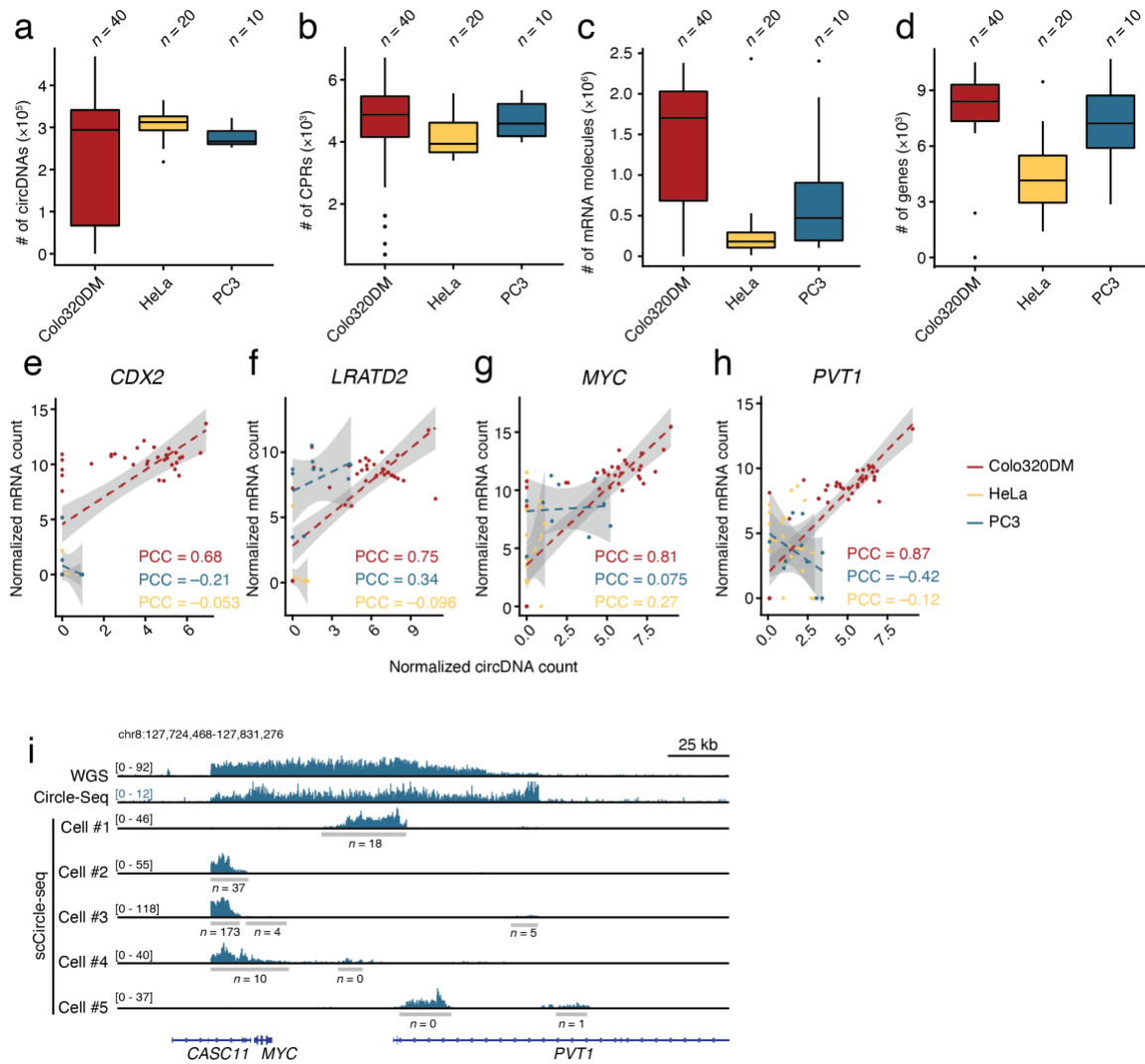
Supplementary Figure 2



Supplementary Fig. 2. Genomic distribution of circDNAs identified by scCircle-seq. **(a)** Integrative Genomics Viewer (IGV) tracks showing the coverage (dark blue) and chimeric reads (magenta arches) detected by whole genome sequencing (WGS), Circle-Seq, and scCircle-seq in one high-frequency high-uniformity (HFHU) circle-producing region (CPR) on chromosome (chr) 8 and in one low-frequency low-uniformity (LFLU) CPR on chromosome (chr) 8, in Colo320DM cells. The numbers in squared brackets represent the intensity range of

the corresponding track. Protein-coding genes overlapping with the regions are shown at the bottom. kb, kilobase. **(b-g)** Distributions of the GC-content of CPRs versus the same number of regions after random permutation of their genomic coordinates (shuffle) for each cell line profiled by scCircle-seq. *n*, number of CPRs. *P*, t-test, two-tailed. Boxplots extend from the 25th to the 75th percentile, horizontal bars represent the median, and whiskers extend from $-1.5 \times \text{IQR}$ to $+1.5 \times \text{IQR}$ from the closest quartile, where IQR is the inter-quartile range. Black dots, outliers. In each boxplot, the minimum and maximum are defined, respectively, by the uppermost and lowermost outlier dot or extremity of the corresponding whisker. **(g)** Correlation between normalized transposon element (TE) counts and normalized circDNA counts inside the CPRs identified by scCircle-seq in HeLa cells. Each dot represents a CPR. PCC, Pearson's correlation coefficient. Dashed red line, linear regression. **(h)** IGV tracks showing the coverage of circDNAs detected in the five cell lines profiled by scCircle-seq and various histone marks profiled by chromatin immunoprecipitation and sequencing (ChIP-seq) in HeLa cells, in the indicated region on chromosome (chr) 20. The numbers in squared brackets represent the intensity range of the corresponding track. **(i)** Transcription factor motif enrichment inside enhancers overlapping with CPRs in Colo320DM cells. Only significantly enriched motifs are shown. **(j)** Heatmap representation of the co-occurrence of five HFHU CPRs (black square) in Colo320DM cells. The region in bold corresponds to an ecDNA encompassing the *MYC* oncogene previously detected in the same cell line²⁵. PCC, Pearson's correlation coefficient. **(k)** Frequency versus correlation (PCC) between the corresponding CPR and the *MYC*-containing ecDNA described in (j), for each CPR detected by scCircle-seq in Colo320DM cells. Each dot represents a CPR. The genomic coordinates of the four CPRs frequently co-occurring with the *MYC* ecDNA shown in (j) are displayed. Marginal distributions are shown on the top and right side of the scatterplot.

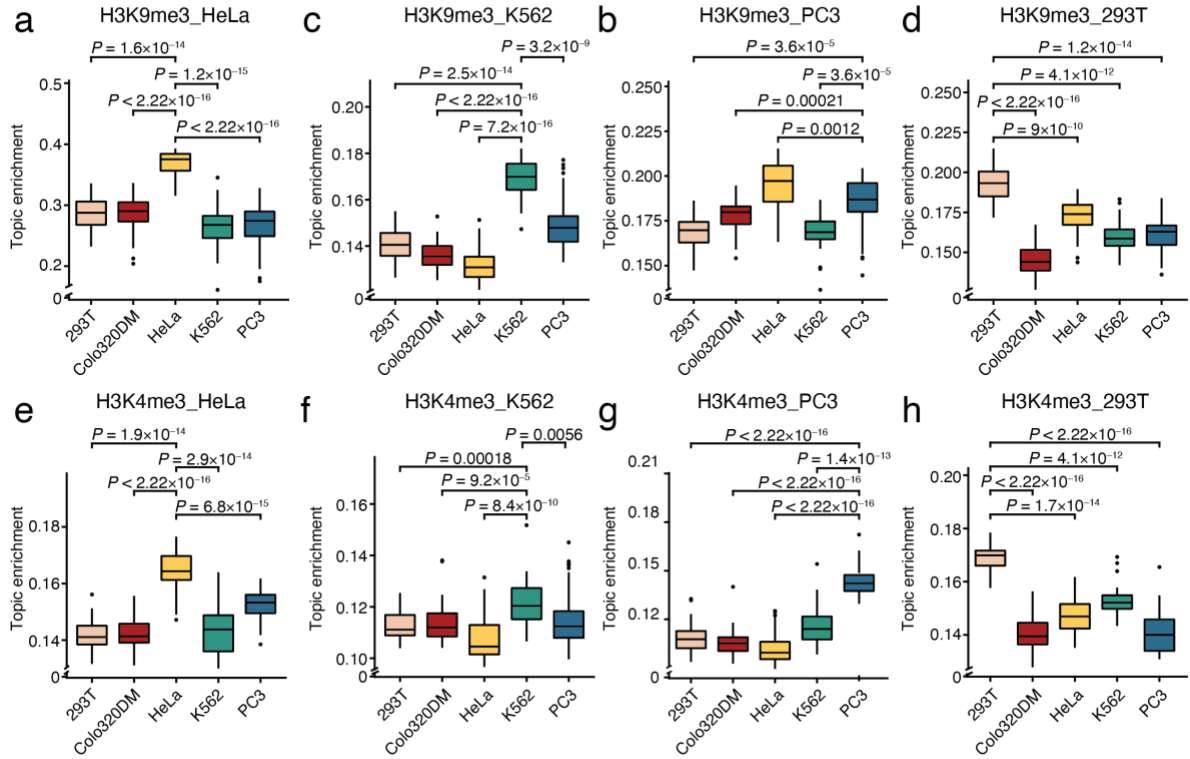
Supplementary Figure 3



Supplementary Fig. 3. Simultaneous profiling of circDNAs and RNA in the same cell. **(a, b)** Distributions of the number of circDNAs (a) and circle-producing regions (CPRs) (b) detected by scCircle-seq in three cell lines profiled by simultaneous scCircle-seq and scRNA-seq by Smart-seq2²⁶. n , number of single cells analyzed. **(c, d)** Distributions of the number of RNAs (c) and genes (d) detected by Smart-seq2 in the same cells shown in (a, b). In (a-d), the boxplots extend from the 25th to the 75th percentile, horizontal bars represent the median, and whiskers extend from $-1.5 \times \text{IQR}$ to $+1.5 \times \text{IQR}$ from the closest quartile, where IQR is the inter-quartile range. Black dots, outliers. In each boxplot, the minimum and maximum are defined, respectively, by the uppermost and lowermost outlier dot or extremity of the corresponding whisker. **(e-h)** Correlations between the normalized circDNA counts and the mRNA counts of genes from the corresponding CPRs in each of the three cell lines profiled by simultaneous scCircle-seq and Smart-seq2. Each dot represents one cell. Only four genes for which the

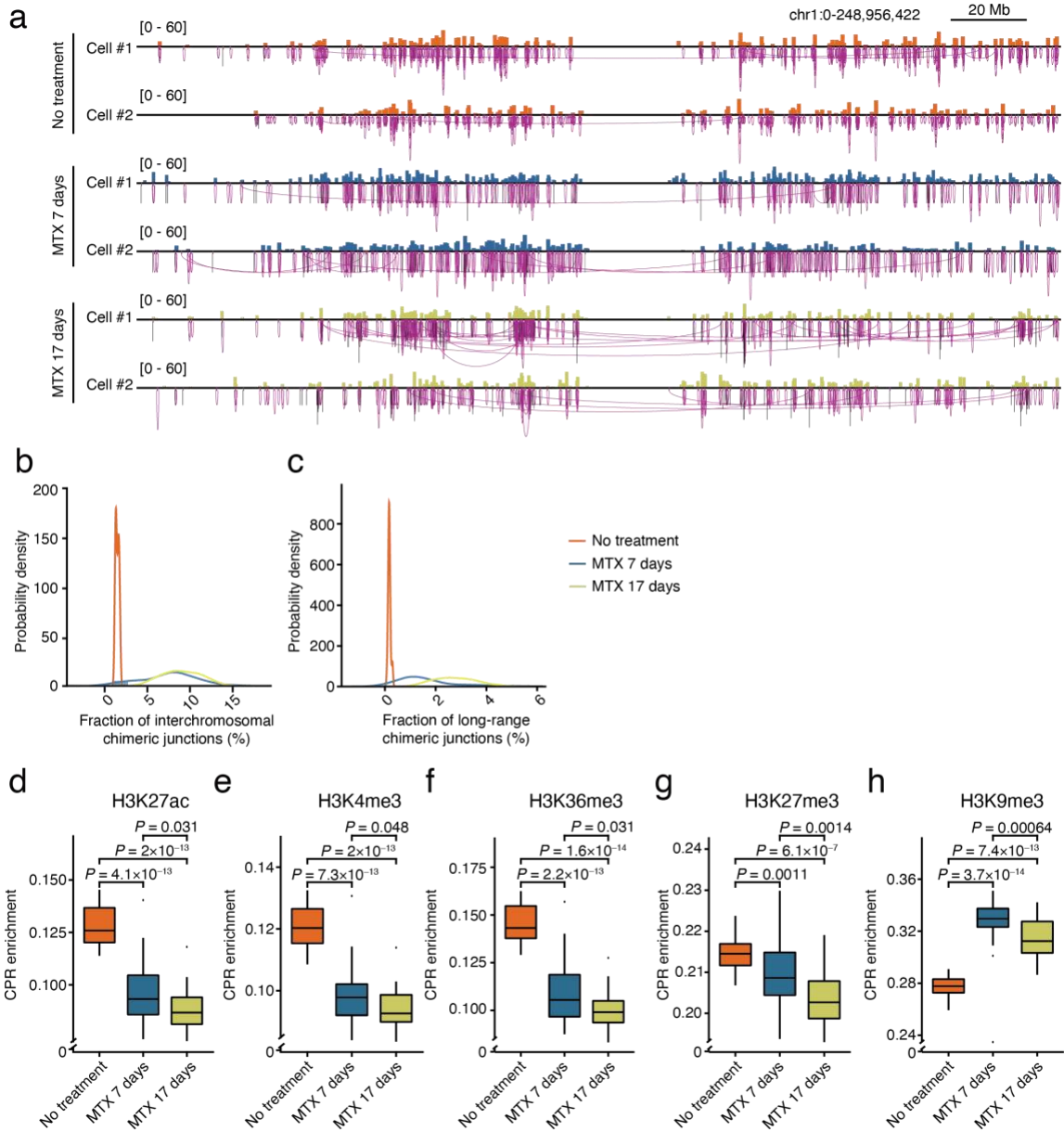
correlation was significant in at least one cell line are shown. PCC, Pearson's correlation coefficient. Dashed lines, linear regression. Grey shades, 95% confidence bands. (i) Integrative Genomics Viewer (IGV) tracks showing the coverage (dark blue) by whole genome sequencing (WGS), Circle-Seq, and scCircle-seq of the indicated region on chromosome (chr) 8 encompassing the *MYC* oncogene, in Colo320DM cells profiled by simultaneous scCircle-seq and Smart-seq2. Gray bars, CPRs. *n*, number of chimeric junction-supporting reads for the corresponding CPR. The numbers in squared brackets represent the intensity range of the corresponding track.

Supplementary Figure 4



Supplementary Fig. 4. Enrichment of topics identified by cisTopic²⁷ related to circDNAs in different chromatin types. (a-d) Topic enrichment in histone H3K9me3 chromatin immunoprecipitation and sequencing (ChIP-seq) peaks available in the Encyclopedia of DNA Elements (ENCODE) for four of the five cell lines profiled by scCircle-seq. P , t-test, two-tailed. (e-h) Same as in (a-d) but for histone H3K4me3 peaks. All boxplots in the figure extend from the 25th to the 75th percentile, horizontal bars represent the median, and whiskers extend from $-1.5 \times \text{IQR}$ to $+1.5 \times \text{IQR}$ from the closest quartile, where IQR is the inter-quartile range. Black dots, outliers. In each boxplot, the minimum and maximum are defined, respectively, by the uppermost and lowermost outlier dot or extremity of the corresponding whisker.

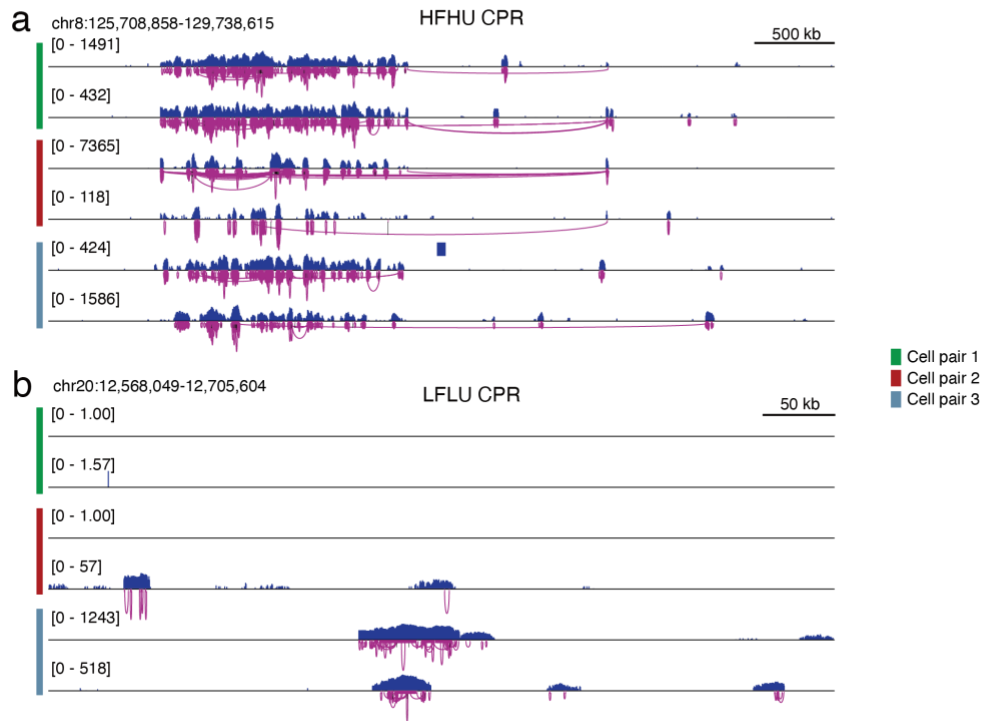
Supplementary Figure 5



Supplementary Fig. 5. Rewiring of the circDNA landscape upon replication stress. **(a)** Integrative Genomics Viewer (IGV) tracks showing the coverage (orange, blue, and green) and chimeric reads (magenta arches) detected by scCircle-seq in HeLa cells treated or not with methotrexate (MTX), in the indicated region on chromosome (chr) 1. The numbers in squared brackets represent the intensity range of the corresponding track. For simplicity, only two cells per condition are shown. **(b, c)** Probability density distribution of the frequency of circDNA chimeric junctions connecting reads that align to sequences on different chromosomes or far apart (> 100 kilobases) along the same chromosome, for HeLa cells treated or not with MTX. n , total number of CPRs. **(d-h)** Enrichment of circle-producing regions (CPRs) inside chromatin

immunoprecipitation and sequencing (ChIP-seq) peaks for the indicated histone marks available in the Encyclopedia of DNA Elements (ENCODE), for HeLa cells treated or not with MTX. *P*, t-test, two-tailed.

Supplementary Figure 6



Supplementary Fig. 6. Divergence of circDNAs between daughter cells after mitosis. **(a)** Integrative Genomics Viewer (IGV) tracks showing the coverage (blue) and chimeric reads (magenta arches) detected by scCircle-seq in three pairs of HeLa daughter cells picked immediately after mitosis, in a high-frequency high-uniformity (HFHU) circle-producing region (CPR) on chromosome (chr) 8. The numbers in squared brackets represent the intensity range of the corresponding track. **(b)** Same as in (a) but for a low-frequency low-uniformity (LFLU) CPR on chr 20.

2. Supplementary Methods

Step-by-step scCircle-seq protocol

REAGENTS

- DPBS, with MgCl₂ and CaCl₂ (Sigma, Cat. No. D8662)
- TrypLE Express Enzyme (1X), phenol red (Thermo Fisher Scientific, Cat. No. 12605036)
- PBS (10X), pH 7.4, (Thermo Fisher Scientific, Cat. No. AM9625)
- EDTA (0.5 M), pH 8.0 (Thermo Fisher Scientific, Cat. No. AM9260G)
- TAPS (GOLDBIO, Cat. No. T-780)
- Poly (ethylene glycol) (Sigma, Cat. No. 89510)
- Nuclease-free water (Thermo Fisher Scientific, Cat. No. AM9932)
- UltraPure 1M Tris-HCl, pH 8.0 (Thermo Fisher Scientific, Cat. No. 15568025)
- MgCl₂ (1 M) (Thermo Fisher Scientific, Cat. No. AM9530G)
- Sodium chloride solution (Sigma, Cat. No. S6546)
- Pierce DTT (Dithiothreitol) (Thermo Fisher Scientific, Cat. No. 20290)
- IGEPAL CA-630 (Sigma, Cat. No. I8896)
- Triton X-100 (Sigma, Cat. No. 93418)
- Hoechst 33342 Solution (20 mM, 12.3 mg/ml) (Thermo Fisher Scientific, Cat. No. 62249)
- UltraPure BSA (50 mg/mL) (Thermo Fisher Scientific, Cat. No. AM2616)
- Dynabeads MyOne Silane (Thermo Fisher Scientific, Cat. No. 37002D)
- NEBNext FFPE DNA Repair Mix (NEB, Cat. No. M6630L)
- 1 pg/μL linear lambda DNA as linear spike-in DNA
- 1 pg/μL plasmid as circular spike-in DNA
- QIAGEN Protease (QIAGEN, Cat. No. 19157)
- Protease Inhibitor Cocktail Set VII (Sigma, Cat. No. 539138-1SET)
- Plasmid-Safe ATP-Dependent DNase (Lucigen, Cat. No. E3101K)
- ATP Solution (100 mM) (Thermo Fisher Scientific, Cat. No. R0441)
- phi29 DNA Polymerase (NEB, Cat. No. M0269L)
- Deoxynucleotide (dNTP) Solution Mix (NEB, Cat. No. N0447L)
- Exo-Resistant Random Primer (Thermo Fisher Scientific, Cat. No. SO181)
- Pyrophosphatase, inorganic (0.1 U/μL) (Thermo Fisher Scientific, Cat. No. EF0221)
- DNA Clean & Concentrator-5 (Zymo, Cat. No. D4014)
- Tn5 transposase assembled with Nextera adaptors
- Nextera dual index PCR primer (custom-made by IDT based on the sequences in the Nextera XT DNA Library Preparation Kit, Illumina)
- NEBNext Ultrall Q5 PCR Mastermix (NEB, cat. no. M0544L)
- Agencourt RNAClean XP (Beckman Coulter, Cat. No. A63987)
- Agencourt AMPure XP (Beckman Coulter, Cat. No. A63881)

- Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Cat. No. Q32851)
- Bioanalyzer High Sensitivity DNA Kit (Agilent, Cat. No. 5067-4627)

CONSUMABLES

- Sterile Disposable Scalpel (No. 10) (VWR, Cat. No. 233-5363)
- Eppendorf DNA LoBind tubes 5 mL (Sigma, Cat. No. EP0030108310)
- Eppendorf DNA LoBind tubes 0.5 mL (Sigma, Cat. No. EP0030108035)
- Eppendorf DNA LoBind tubes 1.5 mL (Sigma, Cat. No. EP0030108051)
- 15 mL conical tubes (Fisher Scientific, Cat. No. 430790)
- Falcon 5 mL Round Bottom Polystyrene Tube, Sterile (Fisher Scientific, Cat. No. 10100151)
- Sapphire Filter tips, low retention (Greiner Bio-One, Cat. No. 771265, 773265, 738265, 750265)
- 50 µm CellTrics cell strainer (Sysmex, Cat. No. 04-004-2327)
- 20 µm CellTrics cell strainer (Sysmex, Cat. No. 04-004-2325)
- 10 µm CellTrics cell strainer (Sysmex, Cat. No. 04-004-2324)
- Qubit Assay Tubes (Thermo Fisher Scientific, Cat. No. Q32856)
- Bioanalyzer High-sensitivity DNA Kit (Chips) (Agilent, Cat. No. 5067-4626)

EQUIPMENT

- Cell counter (Invitrogen Countess II FL Automated Cell Counter or equivalent)
- Centrifuge (Eppendorf Microcentrifuge 5424 and 5810 or equivalent)
- Sample mixer (Thermo Scientific Tube Revolver Rotator or equivalent)
- Non-contact liquid handler (Dissipix GmbH I.DOT)
- Incubator (Binder GmbH KB 53 or Boekel Scientific 241000 or equivalent)
- Thermoshaker (Eppendorf Thermomixer F or equivalent)
- PCR cyclers for tubes or strips (Analytik Jena Biometra TRIO or equivalent)
- Magnetic stand (Invitrogen DynaMag-2/-5 Magnet or equivalent)
- Fluorometers (Qubit 2.0 Fluorometer or equivalent)
- Fragment analyzer (Agilent 2100 Bioanalyzer or equivalent)

PROCEDURE

Cell preparation

1. Wash the cells with 1×DPBS with MgCl₂ and CaCl₂ pre-warmed to 37 °C
2. Aspirate the DPBS from the flask
3. Add TrypLE Express Enzyme and incubate at 37 °C to dissociate cells
4. Add equal volume of culture medium to neutralize the enzyme and resuspend the cells
5. Count the cells and transfer 1×10⁶ cells to a new Eppendorf tube

NOTE: For suspension cells, steps 1-3 can be skipped

6. Pellet the cells at 100-400×g for 5 min

NOTE: The centrifugation time needs to be optimized for every cell type cells

7. Wash the cells once with 1xPBS/5 mM EDTA
8. Pellet the cells at 100-400 g for 5 min
9. Resuspend the cells with proper amount of 1xDPBS and dilute them into a proper concentration for mouth-pipetting

NOTE: Unless otherwise specified, perform all the subsequent steps by keeping the samples on ice and holding them in hand for as short as possible

Nuclei extraction and DNA nick repair

10. Prepare the following working solutions:

- Nucleus Isolation Buffer (1 mL)

| | |
|-------------------------|--------|
| Tris-HCl, pH 8.0 (1 M) | 10 µL |
| NaCl (5 M) | 2 µL |
| MgCl ₂ (1 M) | 3 µL |
| IGEPAL CA-630 (10%) | 30 µL |
| BSA (10%) | 100 µL |
| DTT (1 M) | 2 µL |
| Tween-20 (10%) | 10 µL |
| Nuclease-free water | 845 µL |

NOTE: Prepared fresh buffer before nucleus isolation every time

- Nick Repair Mix (40 µL for 100 samples)

| | |
|---------------------------------------|--------|
| NEBNext FFPE DNA Repair Mix | 4.9 µL |
| NEBNext FFPE DNA Repair Buffer (10 x) | 15 µL |
| Linear spike-in DNA (1 pg/µL) | 10 µL |
| Circular spike-in DNA (1 pg/µL) | 10 µL |

NOTE: Prepared fresh nick repair mix right before reaction every time

- 2x Triton Lysis (1 mL)

| | |
|------------------------|--------|
| Tris-HCl, pH 8.0 (1 M) | 40 µL |
| NaCl (5 M) | 8 µL |
| TritonX-100 (10%) | 20 µL |
| DTT (1 M) | 30 µL |
| EDTA (0.5 M) | 4 µL |
| Nuclease-free water | 898 µL |

NOTE: Store at -20 °C for up to 1 year

- 2x Trans8k (1 mL)

| | |
|---------------------------|--------|
| TPAS buffer (1 M, pH 8.5) | 20 µL |
| MgCl ₂ (1 M) | 10 µL |
| PEG 8k (50%) | 320 µL |
| Nuclease-free water | 650 µL |

NOTE: Store at -20 °C for up to 1 year

11. Mouth-pipette the desired number of single cell into separate PCR tubes (one cell per tube) pre-filled with 6.75 μ L of Nucleus Isolation Buffer and 0.25 μ L of Dynabeads MyOne Silane beads
12. Place the PCR tubes on ice for 30 min
13. Vortex the PCR tubes for 1 min and then centrifuge at 500 \times g for 5 min at 4 °C
14. Transfer 5.6 μ L of supernatant from each tube into new PCR tube without disturbing the bead pellet
NOTE: The supernatant can be subjected to the Smart-seq2 protocol or stored at –20 °C for up to a week
15. Dispense 0.4 μ L of Nick Repair Mix into of each tube without touching the liquid surface
16. Spin down the liquid and mix by gentle vortexing
NOTE: Make sure to vortex very gently to avoid excessive genomic DNA fragmentation
17. Incubate the samples for 1 hour at 20 °C while slowly rotating to keep the beads in suspension

Cell lysis

18. Prepare the following mix:

Lysis buffer (for 100 samples)

| | |
|----------------------------|-------------|
| QIAGEN Protease (60 mg/ml) | 4 μ L |
| 2 x Triton lysis | 150 μ L |

NOTE: Prepare fresh lysis buffer for every new experiment

19. Dispense 1.54 μ L of Lysis Buffer into of each tube without touching the liquid surface
20. Spin down the liquid and mix by gentle vortexing
NOTE: Make sure to vortex very gently to avoid excessive genomic DNA fragmentation
21. Incubate the samples in a PCR thermocycler for 1 hour at 50 °C with lid temperature set to 90 °C
22. Prepare the following solution:

Inhibition mix (for 100 samples)

| | |
|-------------------------------------|------------|
| Protease Inhibitor Cocktail Set VII | 5 μ L |
| Nuclease-free water | 45 μ L |

23. Dispense 0.5 μ L of Inhibition Mix into of each tube without touching the liquid surface
24. Spin down the liquid and mix by gently vortexing them
NOTE: Make sure to vortex very gently to avoid excessive genomic DNA fragmentation
25. Incubate the samples at room temperature for 1 hour

Linear DNA digestion

26. Prepare the following mix:

● Digestion mix (for 100 samples)

| | |
|--|------------|
| ATP Solution (100 mM) | 10 μ L |
| Plasmid-Safe Reaction Buffer (10 x) | 50 μ L |
| Plasmid-Safe ATP-Dependent DNase (10 U/ μ L) | 10 μ L |
| DTT (25 mM) | 10 μ L |
| Nuclease-free water | 40 μ L |

NOTE: The quantity of Plasmid-Safe ATP-Dependent DNase can range between 1 and 5 U for each sample depending on the ploidy of the cell

27. Dispense 1.2 µL of Digestion Mix into of each tube without touching the liquid surface

28. Spin down the liquid and mix by gentle vortexing

NOTE: Make sure to vortex very gently to avoid excessive genomic DNA fragmentation

29. Place the samples in a PCR thermocycler with lid set at 90 °C and run the following program:

i. 37 °C 20 hours

ii. 70 °C 10 min

iii. 4 °C Hold on

Amplification of circular DNA

30. Prepare the following mix:

Amplification mix (for 100 samples)

| | |
|---------------------------------------|--------|
| phi29 DNA Polymerase (10 U/µL) | 80 µL |
| phi29 buffer (10x) | 100 µL |
| dNTP Solution Mix (10 mM) | 100 µL |
| Exo-Resistant Random Primer (500 mM) | 100 µL |
| Pyrophosphatase, inorganic (0.1 U/µL) | 10 µL |
| Nuclease-free water | 110 µL |

31. Dispense 5 µL of Amplification Mix into of each tube without touching the liquid surface

32. Spin down the liquid and mix by gentle vortexing

NOTE: Make sure to vortex very gently to avoid excessive genomic DNA fragmentation

33. Place the samples in a PCR thermocycler with lid set at 90 °C and run the following program:

i. 30 °C 3 hours

ii. 65 °C 10 min

iii. 4 °C Hold on

34. Clean up the samples using the DNA Clean & Concentrator-5 according to the manufacturer's instructions and elute each sample with 30 µL of elution buffer

35. Measure the concentration of each sample using Qubit

NOTE: The amount of DNA retrieved typically varies between 25 and 90 ng per sample

Library preparation

36. Prepare the following mix:

Tagmentation Mix (for 50 samples):

| | |
|-----------------------------|--------|
| Trans8k (2 x) | 250 µL |
| Tn5 transposase* (0.125 µM) | 10 µL |

*Pre-assembled with Nextera adapters

NOTE: For 4 ng input DNA (size ranging between 2 and 6 kb), 0.2 µL of 0.125 µM Tn5 is sufficient to generate a high-quality library

Tnp Removal Mix (for 50 reactions):

| | |
|----------------------------|------|
| NaCl (5 M) | 6 µL |
| EDTA (0.5 M) | 9 µL |
| QIAGEN Protease (60 mg/mL) | 2 µL |

37. Dispense 4 ng of amplified DNA from each sample into a new PCR tube
38. Add nuclease-free water to make the total volume up to 4.8 µL
39. Dispense 5.2 µL of Tagmentation Mix into each sample and mix by vortexing
- NOTE:** The samples should be thoroughly vortexed as the Tagmentation Mix is viscous
40. Place the samples in a PCR thermocycler with lid set at 90 °C and run the following program:
 - i. 55 °C 10 min
 - ii. 4 °C Hold on

NOTE: Transfer the tubes into a PCR thermocycler pre-cooled at 4 °C to repress early activation of the tagmentation reaction, which otherwise might result in excessive fragmentation. Then start the program. After step i. is finished, immediately take the tubes out of the thermocycler, and place them on ice.

41. Dispense 2 µL of Tnp Removal Mix into each tube as quickly as possible and mix by vortexing
42. Place the samples in a PCR thermocycler with lid set at 90 °C and run the following program:
 - i. 50 °C 30 min
 - ii. 70 °C 15 min
 - iii. 4 °C Hold on

NOTE: Skip the step after all the samples are placed in the PCR cyclor

43. Prepare the following mix:

Library Preparation Mix (for 50 samples):

| | |
|-------------------------|--------|
| Q5 Master Mix (2x) | 750 µL |
| MgCl ₂ (1 M) | 25 µL |
| Nuclease-free water | 70 µL |

44. Dispense 17 µL of Library Preparation Mix and 3 µL of Nextera dual index PCR primer into each tube and mix by vortexing
45. Place the samples in a PCR thermocycler pre-chilled at 4 °C with lid set at 105 °C and run the following program:
 - i. 72 °C 5 min
 - ii. 98 °C 30 sec
 - iii. 98 °C 10 sec
 - iv. 62 °C 1 min
 - v. 72 °C 2 min
 - Go to step iii. 6 times
 - vi. 72 °C 5 min
 - vii. 4 °C Hold on

Library clean-up

46. Pool 8 samples with different indexes into 1 tube

47. Perform a double-size selection of the mixed library using by first adding 0.3x Agencourt AMPure XP and then 0.8x Agencourt AMPure XP
48. Elute each sample with 30 μ L elution buffer
49. Check the size distribution of the final libraries on a Bioanalyzer

NOTE: The final library size should range from 400 to 900 bp

3. Supplementary Tables

Supplementary Table 1. Summary of the sequencing data described in this study. Because of its large size, this table is provided as a separate Excel file.

Supplementary Table 2. Summary of publically available downloaded data.

| Cell line | Method | Database | Accession Number |
|-----------|-------------------------|----------|------------------|
| Colo320DM | Whole genome sequencing | SRA | SRX5055021 |
| PC3 | Whole genome sequencing | SRA | SRX5055020 |
| PC3 | ChIP-seq for H3K4me3 | SRA | GSM3768252 |
| PC3 | ChIP-seq for H3K9me3 | ENCODE | ENCSR339ZMJ |
| PC3 | ChIP-seq for H3K27ac | SRA | GSM1383871 |
| PC3 | ChIP-seq for H3K27me3 | ENCODE | ENCSR881TWJ |
| HeLa | ChIP-seq for H3K4me3 | SRA | GSM3398461 |
| HeLa | ChIP-seq for H3K9me3 | SRA | GSM4710592 |
| HeLa | ChIP-seq for H3K27ac | SRA | GSM2990412 |
| HeLa | ChIP-seq for H3K27me3 | SRA | GSM2990413 |
| K562 | ChIP-seq for H3K4me3 | ENCODE | ENCSR000EW |
| K562 | ChIP-seq for H3K9me3 | SRA | GSM5175742 |
| K562 | ChIP-seq for H3K27ac | SRA | GSM5593404 |
| K562 | ChIP-seq for H3K27me3 | ENCODE | ENCSR000AKQ |
| 293T | ChIP-seq for H3K4me3 | SRA | GSM5954235 |
| 293T | ChIP-seq for H3K9me3 | ENCODE | ENCSR000FCJ |
| 293T | ChIP-seq for H3K27ac | SRA | GSM5954237 |
| 293T | ChIP-seq for H3K27me3 | SRA | GSM5269337 |