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Movie S5 - Single particle tracking Halo-CTCF in "enriched" prometaphase cells

Supplemental Fig. S1
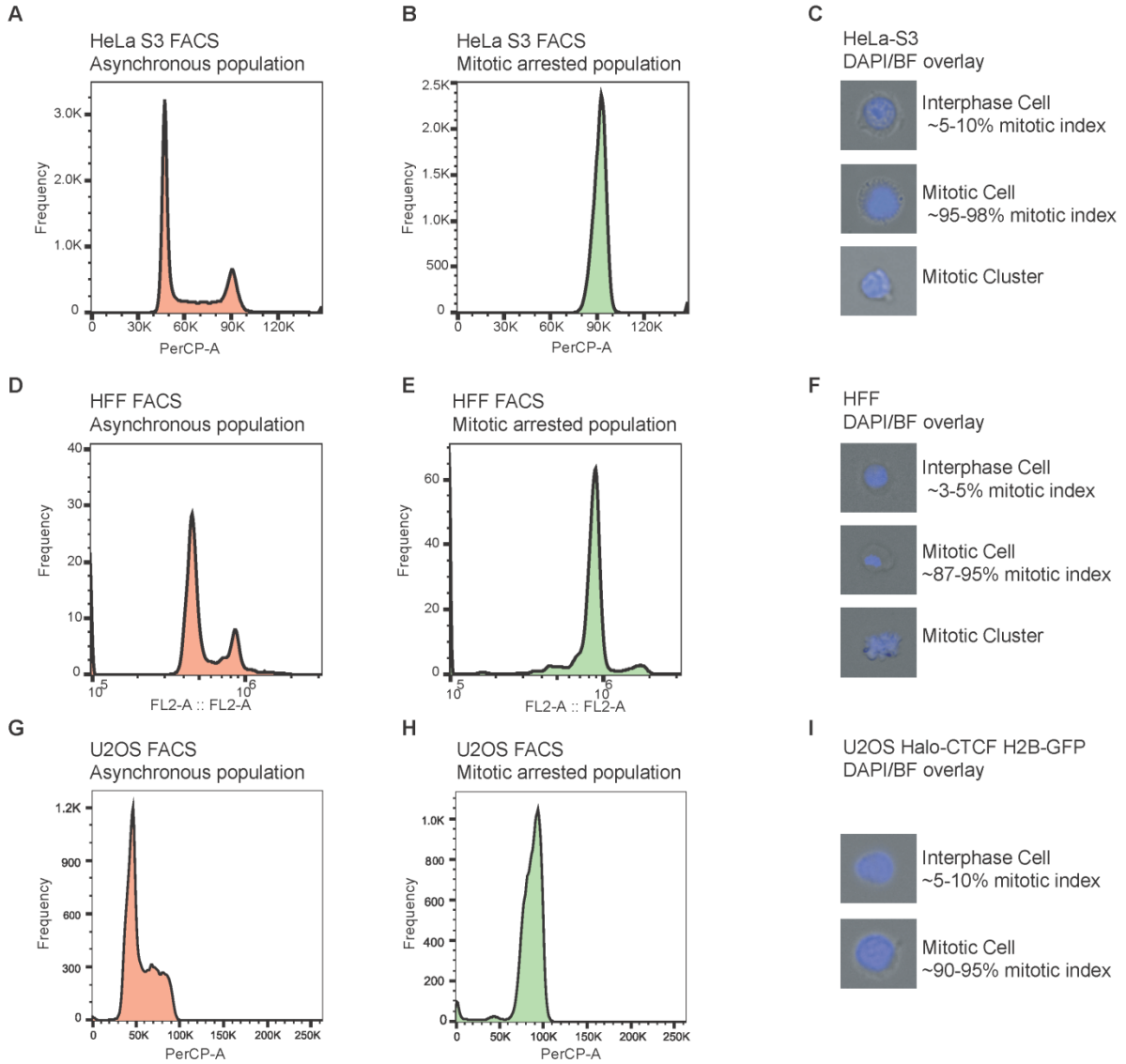
Supplemental Fig. S1 – Flow cytometry using propidium iodide staining and microscopy using DAPI staining show synchronization in M-phase by Nocodazole. (**A-B**) Cell cycle flow cytometry for non-synchronized (**A**) and nocodazole arrested (**B**) HeLa-S3 cell populations. (**C**) DAPI staining of an interphase cell, nocodazole arrested prometaphase cell and purified mitotic chromatin of HeLa-S3 cells and their average scored mitotic index in percentage of total population. (**D-E**) Cell cycle flow cytometry for non-synchronized (D) and nocodazole arrested (E) HFF cell populations. (**F**) DAPI staining of an interphase cell, nocodazole arrested

prometaphase cell and purified mitotic chromatin of HFF and their average scored mitotic index in percentage of total population. (**G-H**) Cell cycle flow cytometry for non-synchronized (**G**) and nocodazole arrested (**H**) U2OS Halo-CTCF H2B –GFP cell populations. (**I**) DAPI staining of an interphase cell and nocodazole arrested prometaphase cell of U2OS Halo-CTCF H2B-GFP and their average scored mitotic index in percentage of total population. Note that the scales of x-axes of the different plots are different dependent on the setting of the FACS equipment.

Supplemental Fig. S2 – 5C interaction interaction maps obtained with non-synchronized HeLa-S3 cells (**A**), mitotic HeLa-S3 cells (**B**), and mitotic chromatin purified from mitotic HeLa-S3 cells (**C**). Heatmap shows two 2 MB regions located on chromosome 1 (hg19 chr1: 46740122-48740121 (upper left)) and chromosome 11 (hg19 chr11: 33003550-35003549 (lower right)). Data was binned in 20 kb bins.
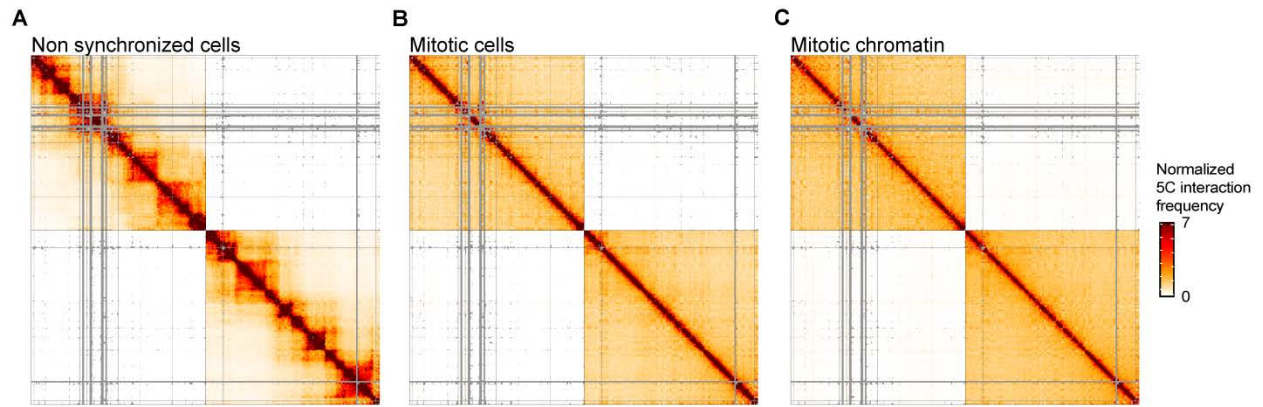
Supplemental Fig. S3

A HeLa-S3 V-plot Non Synchro-
nous on CTCF motif with
ENCODE CTCF ChIP peak

fragment length

distance from CTCF motif (bp)

B HeLa-S3 V-plot Mitotic Cell
on CTCF motif with ENCODE
CTCF ChIP peak

distance from CTCF motif (bp)

C HeLa-S3 V-plot Mitotic Chromatin
on CTCF motif with ENCODE
CTCF ChIP peak

distance from CTCF motif (bp)

Normalized
ATAC-seq
signal
3
0

D HeLa-S3 V-plot Non Synchro-
nous on CTCF motif with
ATAC peak proximal to TSS

fragment length

distance from CTCF motif (bp)

E HeLa-S3 V-plot Mitotic Cell
on CTCF motif with ATAC
peak proximal to TSS

distance from CTCF motif (bp)
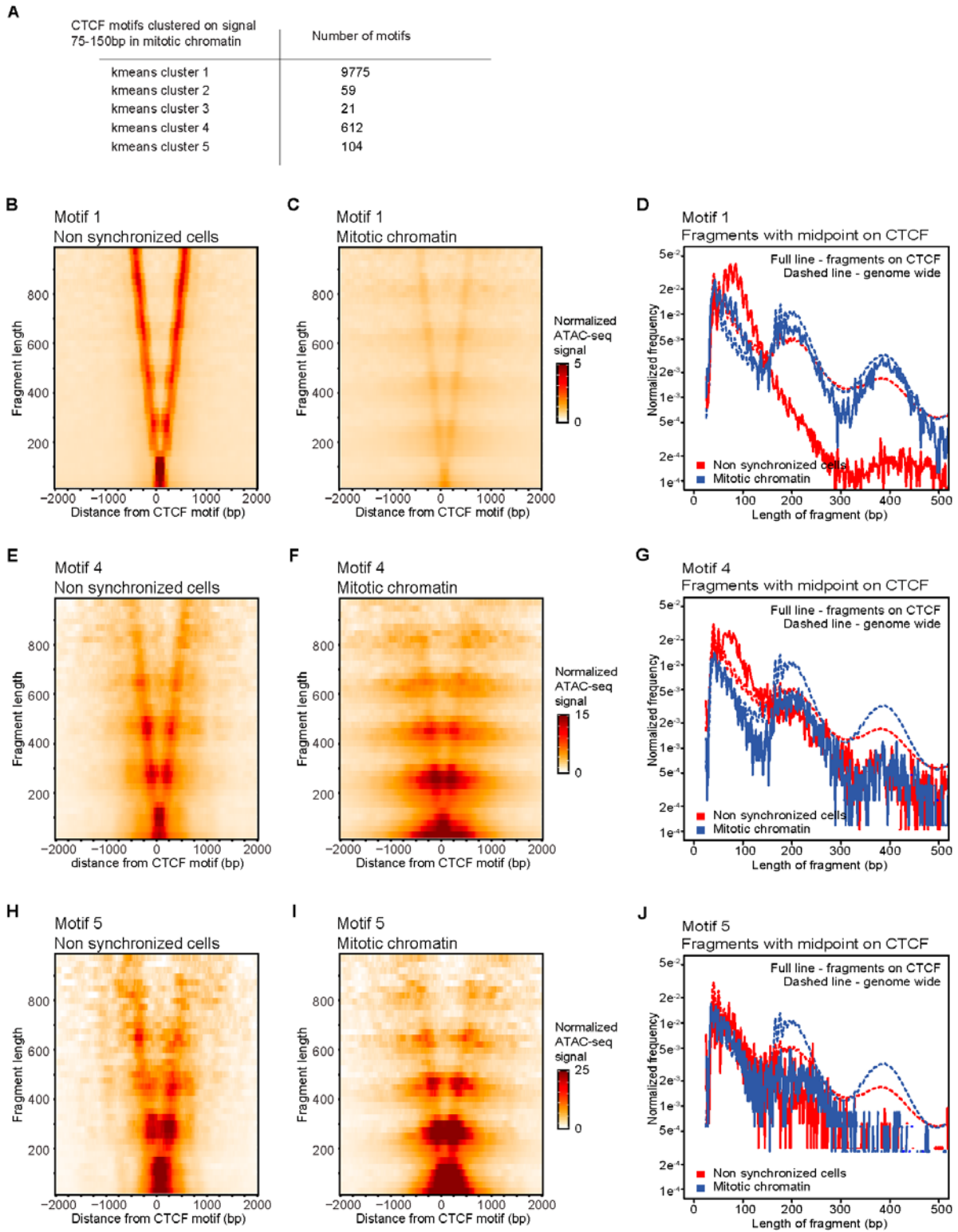
F HeLa-S3 V-plot Mitotic Chromatin
on CTCF motif with ATAC peak
proximal to TSS

distance from CTCF motif (bp)

Normalized
ATAC-seq
signal
12
0

G HeLa-S3 V-plot Non Synchro-
nous on CTCF motif with
ATAC peak distal to TSS

fragment length

distance from CTCF motif (bp)

H HeLa-S3 V-plot Mitotic Cell
on CTCF motif with ATAC
peak distal to TSS

distance from CTCF motif (bp)

I HeLa-S3 V-plot Mitotic Chromatin
on CTCF motif with ATAC
peak distal to TSS

distance from CTCF motif (bp)

Normalized
ATAC-seq
signal
5
0

J HeLa-S3 V-plot Non Synchro-
nous on CTCF motif with
ATAC peak in Mitotic Cell

fragment length

distance from CTCF motif (bp)

K HeLa-S3 V-plot Mitotic Cell
on CTCF motif with ATAC
peak in Mitotic Cell

distance from CTCF motif (bp)

L HeLa-S3 V-plot Mitotic Chromatin
on CTCF motif with ATAC
peak in Mitotic Cell

distance from CTCF motif (bp)

Normalized
ATAC-seq
signal
10
0

Supplemental Fig. S3 – (**A-C**) Normalized V-plots at CTCF motifs that display ENCODE CTCF ChIP peaks. V-plots are made based on ATAC-seq data from non-synchronized cells (**A**), mitotic cells (**B**) and mitotic chromatin (**C**). (**D-F**) Normalized V-plots at CTCF motifs within 2kb of TSS in non-synchronized cells (**D**), mitotic cells (**E**) and mitotic chromatin (**F**). (**G-I**) Normalized V-plots at CTCF motifs 2 kb or more away from any TSS in non-synchronized cells (**G**), mitotic cells (**H**) and mitotic chromatin (**I**). (**J-L**) Normalized V-plots at CTCF motifs with mitotic ATAC-seq peak in non-synchronized cells (**J**), mitotic cells (**K**) and mitotic chromatin (**L**).
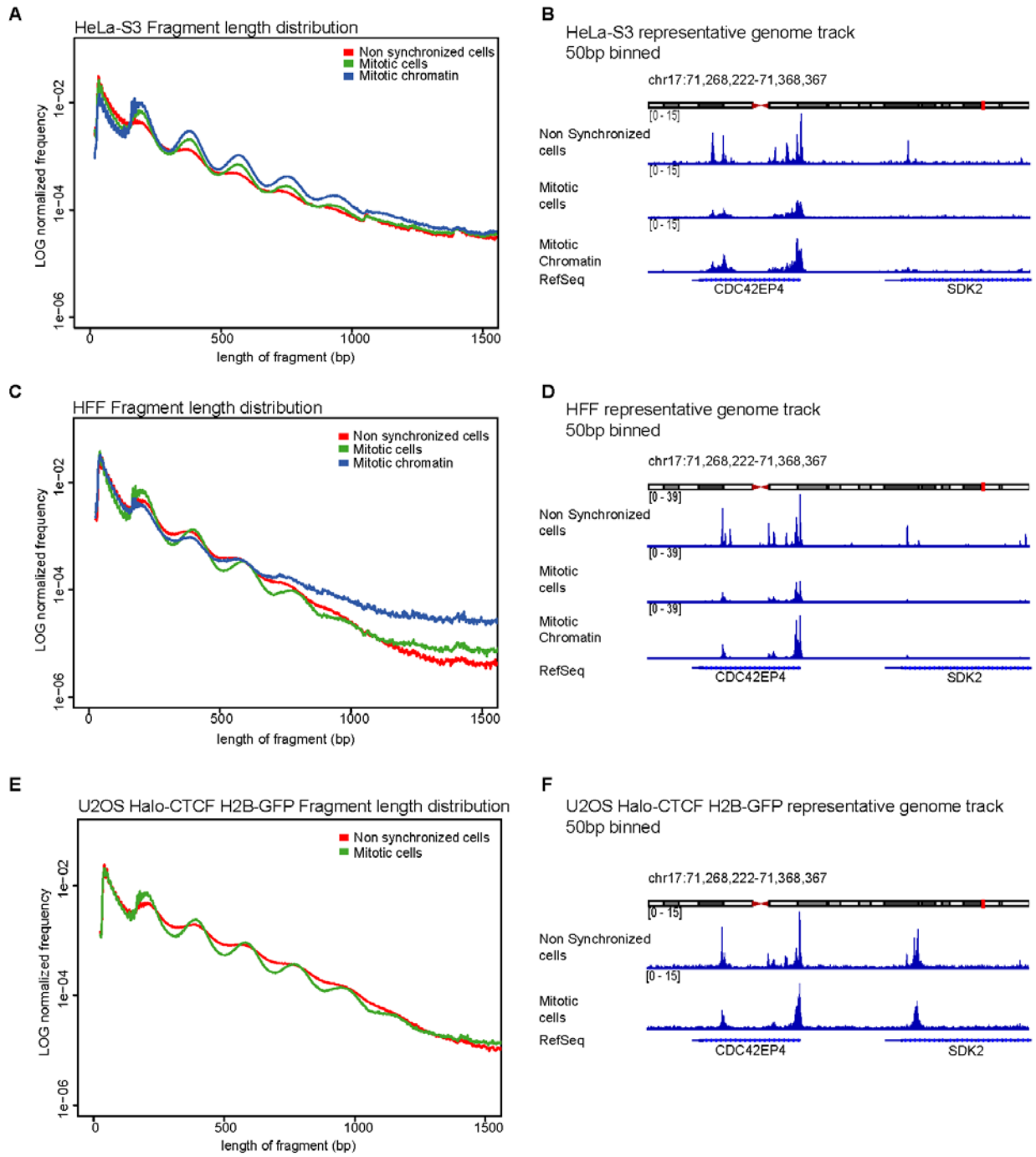
Supplemental Fig. S4

A

CTCF motifs clustered on signal 75-150bp in mitotic chromatin	Number of motifs
kmeans cluster 1	9775
kmeans cluster 2	59
kmeans cluster 3	21
kmeans cluster 4	612
kmeans cluster 5	104

B Motif 1
Non synchronized cells

C Motif 1
Mitotic chromatin

D Motif 1
Fragments with midpoint on CTCF

E Motif 4
Non synchronized cells

F Motif 4
Mitotic chromatin

G Motif 4
Fragments with midpoint on CTCF

H Motif 5
Non synchronized cells

I Motif 5
Mitotic chromatin
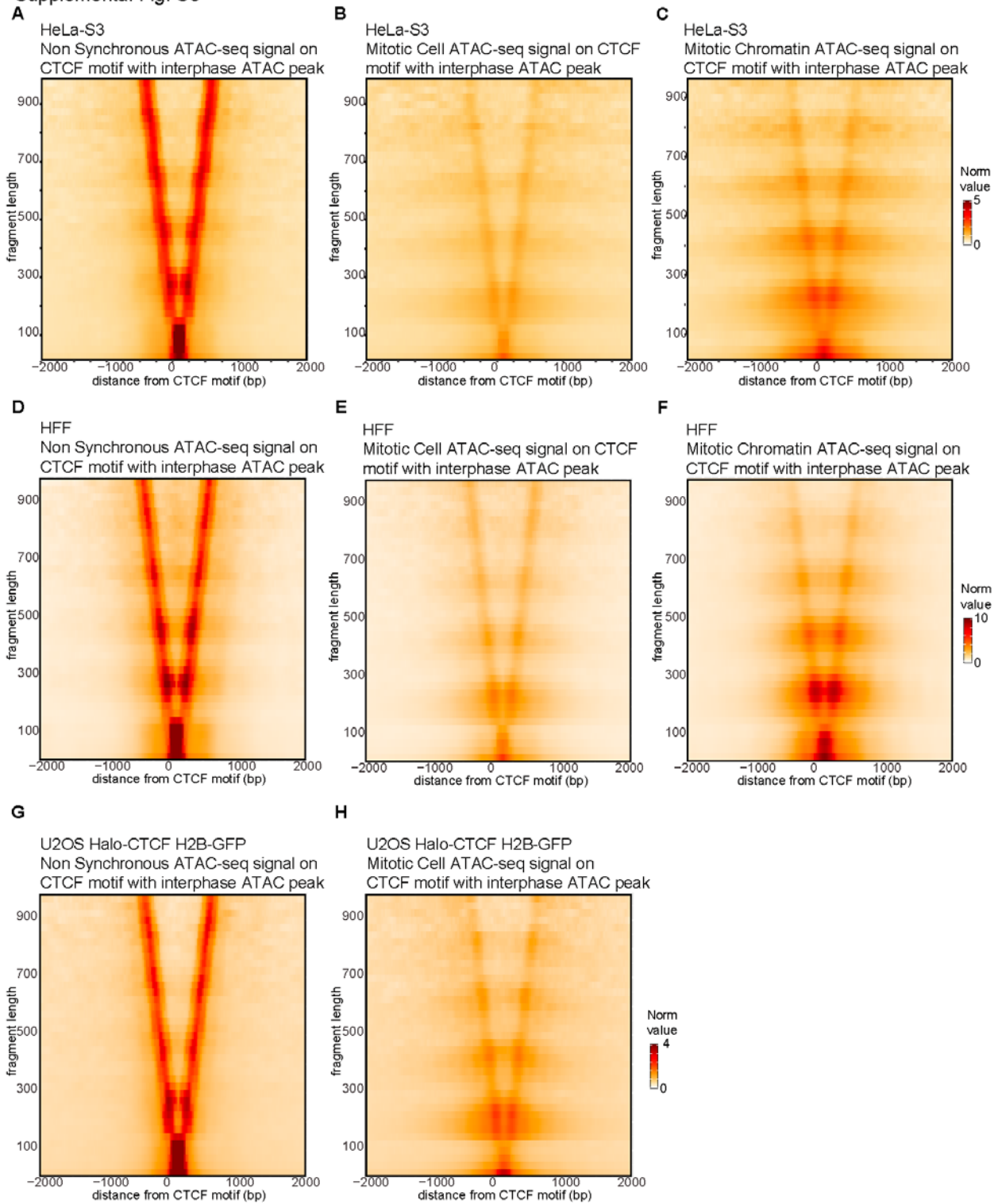
J Motif 5
Fragments with midpoint on CTCF

Supplemental Fig. S4 – (**A**) K-means clustering in 5 clusters of ATAC-seq signal of 75-150 bp length reads in mitotic chromatin at 2kb window from interphase accessible CTCF motifs. (**B-C**) V-plots of ATAC-seq data obtained with non-synchronized cells (**B**) and mitotic chromatin (**C**) at CTCF motifs in cluster 1 representing 9,975 CTCF motifs. (**D**) Fragment length distribution of reads with their midpoint on a CTCF motif from cluster 1. (**E-F**) V-plots of ATAC-seq data obtained with non-synchronized cells (**E**) and mitotic chromatin (**F**) on CTCF motifs in cluster 4 of kmeans clustering representing 612 CTCF motifs. (**G**) Fragment length distribution of reads with their midpoint on a CTCF motif from cluster 4. (**H-I**) V-plots of ATAC-seq data obtained with non-synchronized cells (**H**) and mitotic chromatin (**I**) on CTCF motifs in cluster 5 of kmeans clustering representing 104 CTCF motifs. (**G**) Fragment length distribution of reads with their midpoint on a CTCF motif from cluster 5.
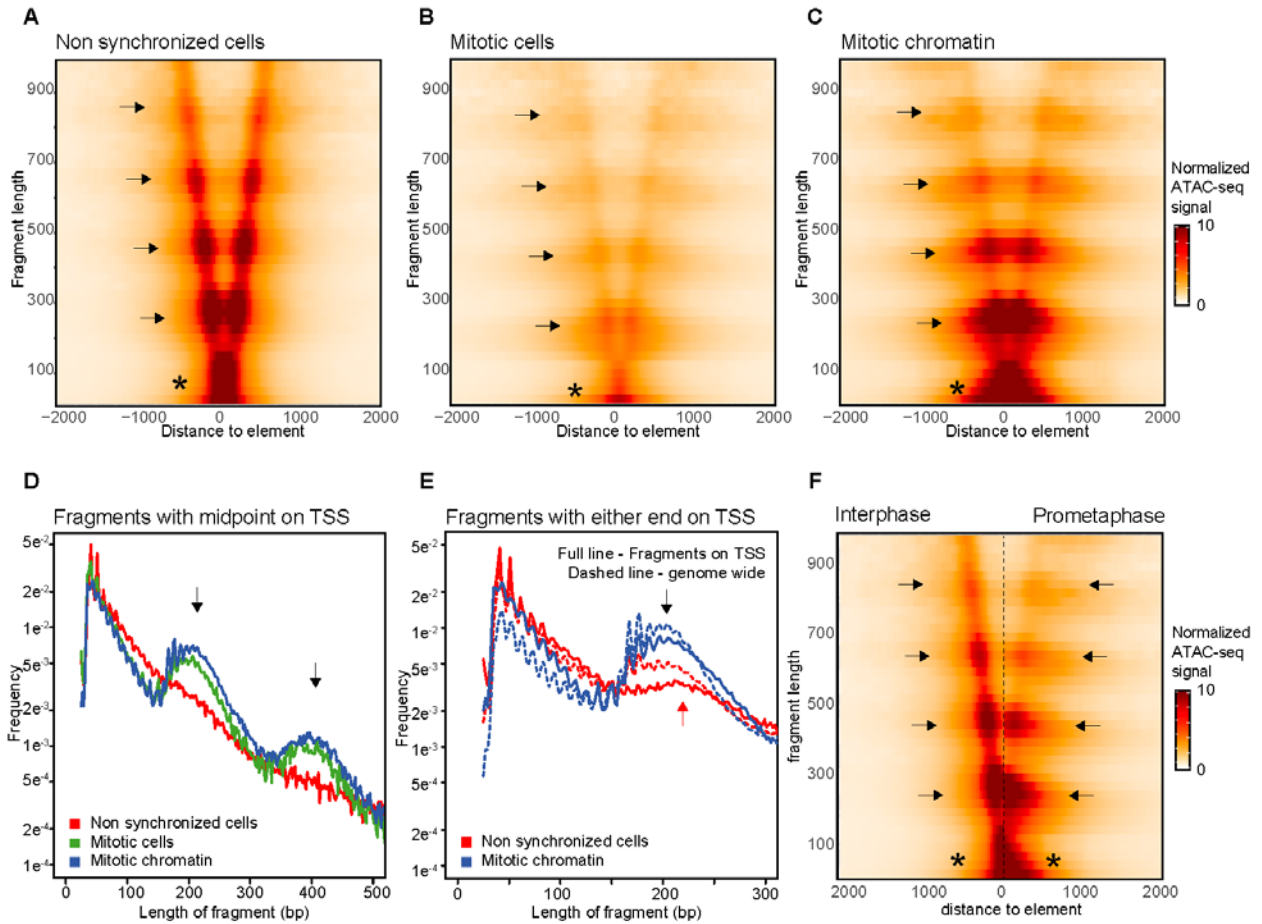
Supplemental Fig. S5

Supplemental Fig. S5 – ATAC-seq fragment length distributions and representative genome tracks for data obtained with HeLa-S3 (**A-B**), HFF (**C-D**) and U2OS Halo-CTCF H2B-GFP (**E-F**).

Supplemental Fig. S6

A HeLa-S3
Non Synchronous ATAC-seq signal on
CTCF motif with interphase ATAC peak

B HeLa-S3
Mitotic Cell ATAC-seq signal on CTCF
motif with interphase ATAC peak

C HeLa-S3
Mitotic Chromatin ATAC-seq signal on
CTCF motif with interphase ATAC peak

D HFF
Non Synchronous ATAC-seq signal on
CTCF motif with interphase ATAC peak

E HFF
Mitotic Cell ATAC-seq signal on CTCF
motif with interphase ATAC peak

F HFF
Mitotic Chromatin ATAC-seq signal on
CTCF motif with interphase ATAC peak

G U2OS Halo-CTCF H2B-GFP
Non Synchronous ATAC-seq signal on
CTCF motif with interphase ATAC peak

H U2OS Halo-CTCF H2B-GFP
Mitotic Cell ATAC-seq signal on
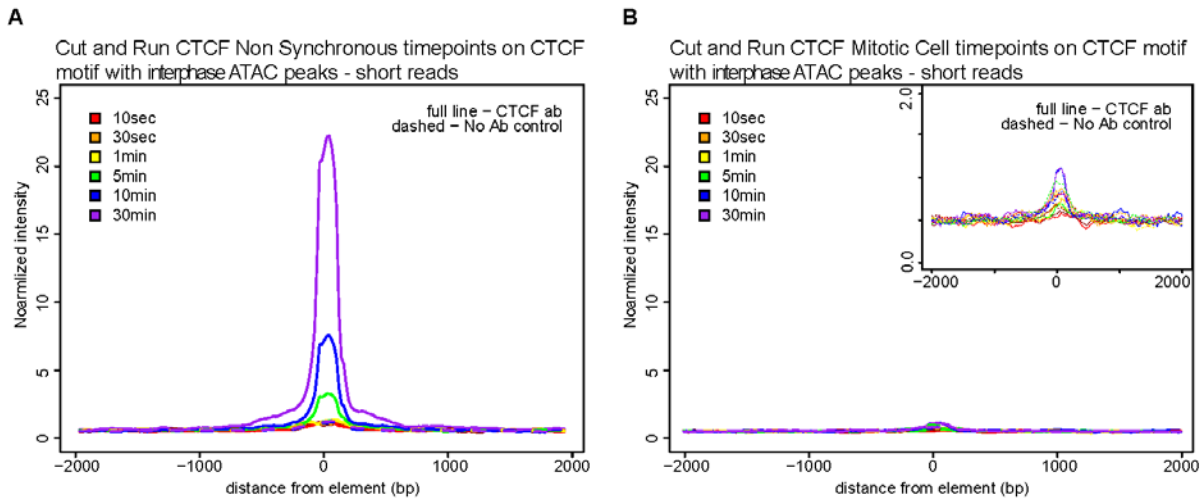CTCF motif with interphase ATAC peak

Supplemental Fig. S6 – Normalized V-plots of ATAC-seq data obtained with HeLa-S3 cells at CTCF motifs that display ATAC-seq peaks in interphase for non-synchronized cells (**A**), mitotic cells (**B**) and purified mitotic chromatin (**C**). Normalized V-plots of ATAC-seq data obtained with HFF cells at CTCF sites that display ATAC-seq peaks in interphase for non-synchronized cells (**D**), mitotic cells (**E**) and purified mitotic chromatin (**F**). Normalized V-plots of ATAC-seq data obtained with U2OS Halo-CTCF H2B-GFP cells at CTCF sites that display ATAC-seq peaks in interphase for non-synchronized cells (**A**), mitotic cells (**B**) and purified mitotic chromatin (**C**)
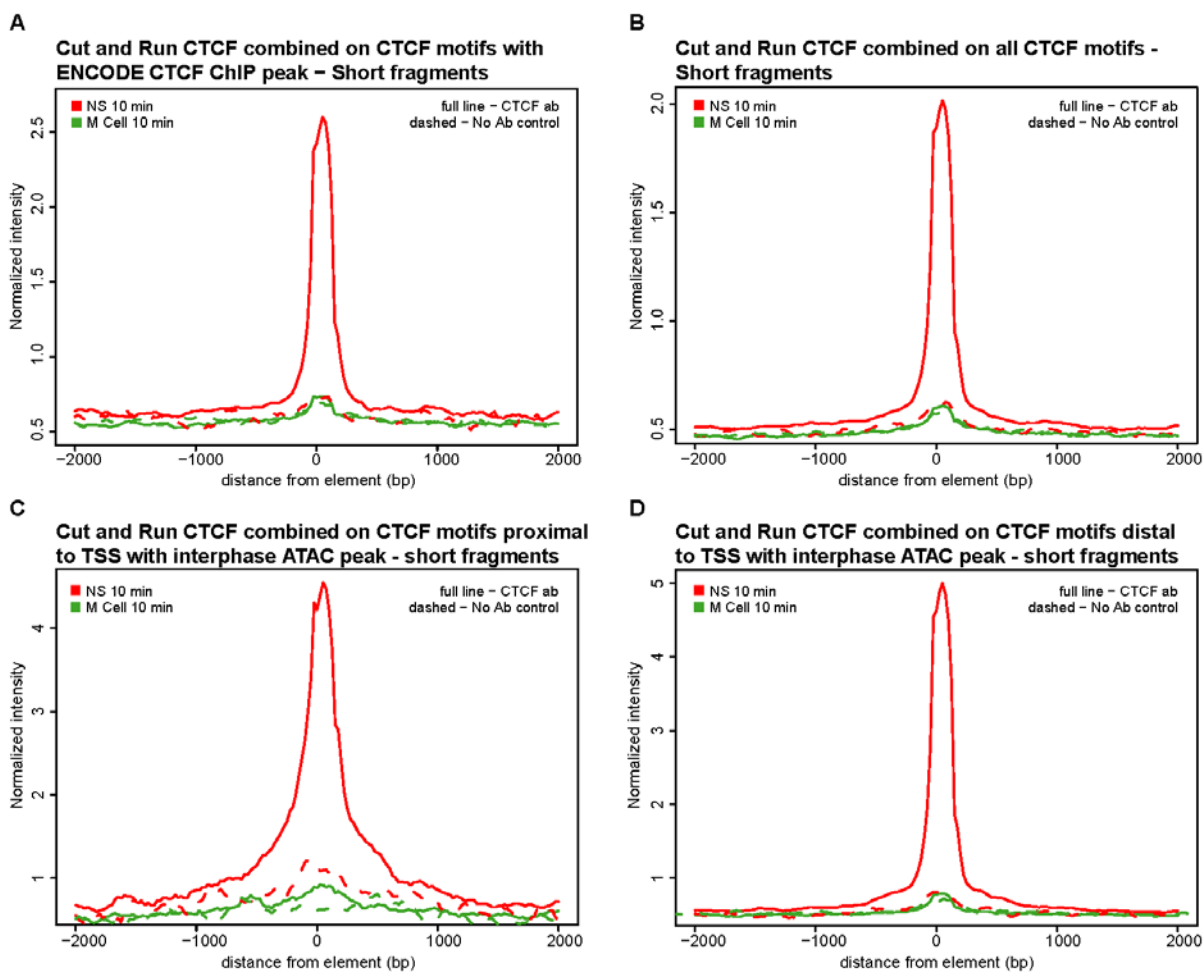
Supplemental Fig. S7

Supplemental Fig. S7 – ATAC-seq data obtained with HeLa-S3 in non-synchronozed cells (**A**), mitotic cells (**B**), and purified mitotic chromatin (**C**) represented as V-plots aggregated on all TSS that display an ATAC-seq peak in interphase. (**D**) Fragment length distribution of non-synchronized cells, mitotic cells and mitotic chromatin for all reads with their midpoint on any TSS. (**E**) Fragment length distribution of non-synchronized cells and mitotic chromatin for all reads with either end near any TSS compared to the genome-wide distribution (dashed line). (**F**) Side-to-side comparison of V-plots for non-synchronized cells and mitotic chromatin up to 2 kb upstream of TSS.
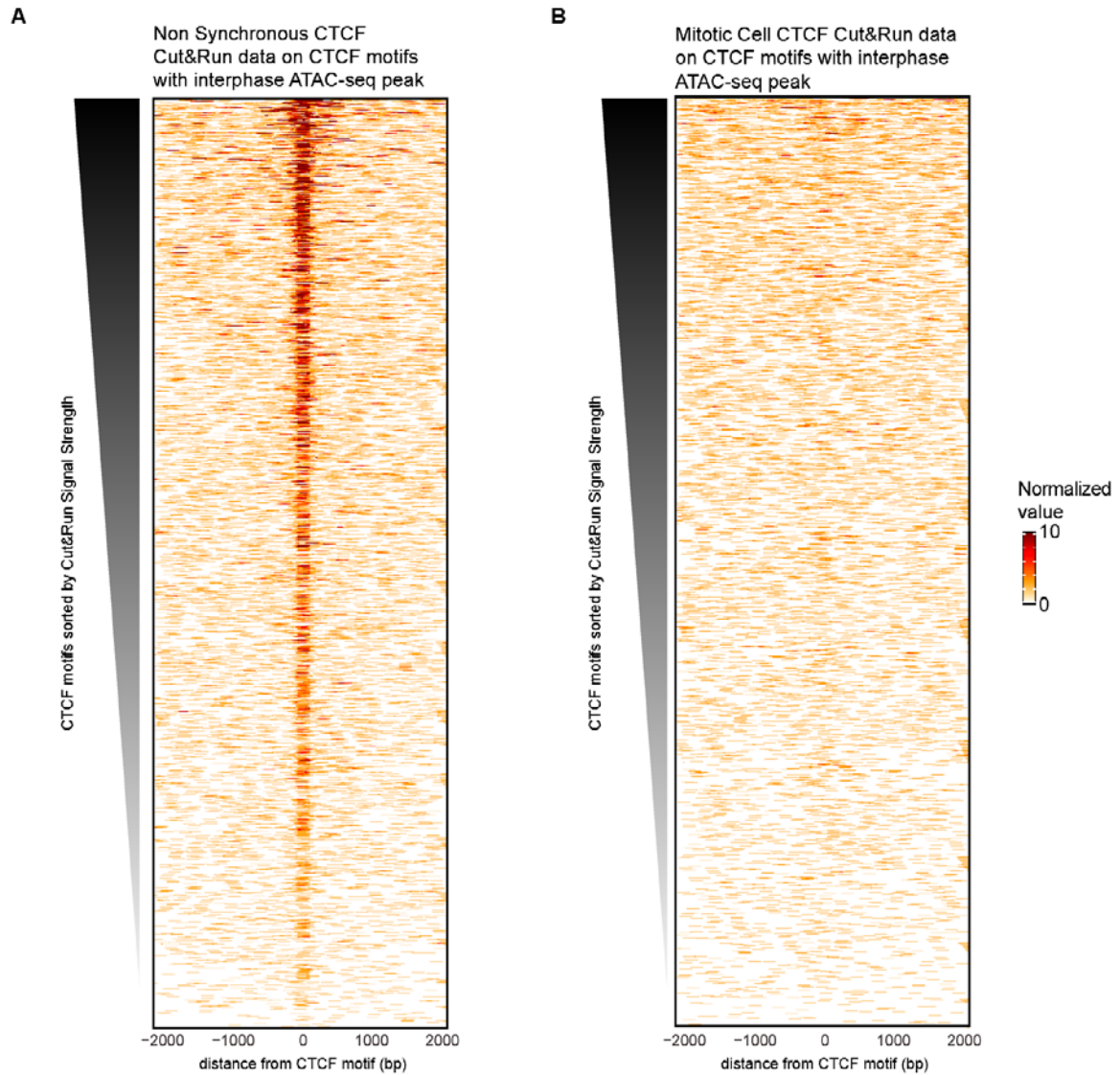
Supplemental Fig. S8 – Aggregation plots of CTCF CUT&RUN signal for reads shorter than 120bp obtained with different digestion times with pA-MNase (10 seconds to 30 min). Data are aggegated at CTCF motifs that display ATAC-seq peaks in interphase for HeLa-S3 non-synchronized cells (**A**) and mitotic cells (**B**). Insert in **B** shows CUT&RUN signal of mitotic cells at a y-axis from (0-2).

Supplemental Fig. S9 – (**A**) CTCF CUT&RUN signal obtained with non-synchronized and mitotic HeLa-S3 cells for reads shorter than 120bp aggregated at all CTCF motifs that display ENCODE CTCF ChIP peaks (6,893 sites). (**B**) CTCF CUT&RUN signal obtained with non-synchronized and mitotic HeLa-S3 cells for reads shorter than 120bp on all CTCF motifs (42,066 sites). (**C**) CTCF CUT&RUN signal obtained with non-synchronized and mitotic HeLa-S3 cells for reads shorter than 120bp on all CTCF motifs that display an ATAC-seq peak within 2 kb to a TSS in interphase (1,181 peaks). (**D**) CTCF CUT&RUN signal obtained non-synchronized and mitotic HeLa-S3 cells for reads shorter than 120bp on all CTCF motifs that display an interphase ATAC-seq peak further than 2 kb away from a TSS in interphase (9,459 peaks).
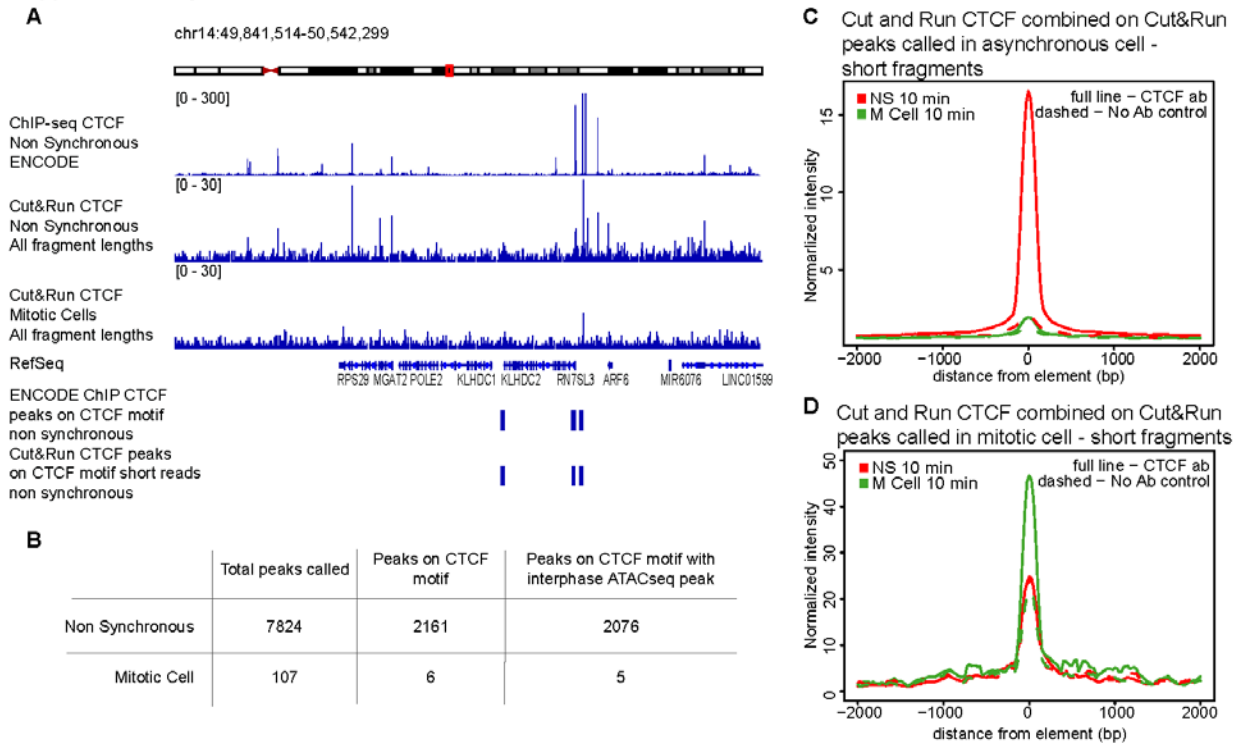
Supplemental Fig. S10 – Aggregation of CUT&RUN data heatmap obtained with HeLa-S3 cells for all reads shorter than 120bp on CTCF motifs that display an ATAC-seq peak in interphase sorted on CUT&RUN signal strength in interphase for non-synchronized cell (**A**) and mitotic cells (**B**).
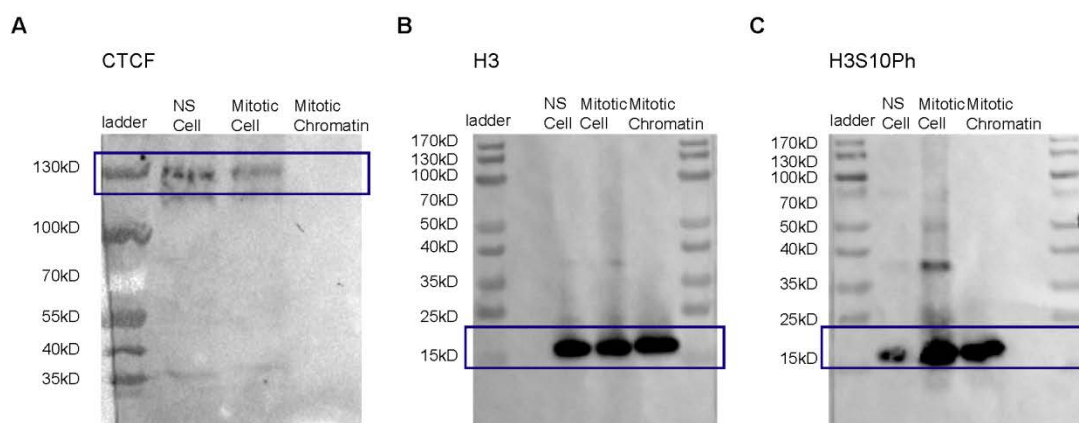
Supplemental Fig. S11 – De novo peak calling on CUT&RUN CTCF data obtained with non-synchronized and mitotic HeLa-S3 cells. (**A**) Visualization of ENCODE non synchronized CTCF ChIP-seq data and CUT&RUN data obtained with non-synchronized and mitotic HeLa-S3 cells for a representative region. The lower two tracks show the locations of CTCF motifs that display peaks called on ChIP-seq and CUT&RUN data respectively (CTCF motifs were stretched to 2kb to enhance visualization). (**B**) Number of peaks called on CTCF CUT&RUN data obtained with non-synchronized and mitotic HeLa-S3 cells and their overlap with CTCF motifs and ATAC-seq peaks. (**C**) CTCF CUT&RUN signal in non-synchronized and mitotic cells for reads shorter than 120bp on all peaks called in non-synchronized cells (7,824 peaks). (**D**) CTCF CUT&RUN signal in non-synchronized and mitotic cells for reads shorter than 120bp on all peaks called in mitotic cells (107 peaks).

Supplemental Fig. S12 – Western blots of lysates from non-synchronized HeLa-S3 cells, mitotic HeLa-S3 cells and mitotic chromatin purified from mitotic HeLa-S3 cells probed with antibodies detecting CTCF (**A**), H3 (**B**) and H3S10ph (**C**).
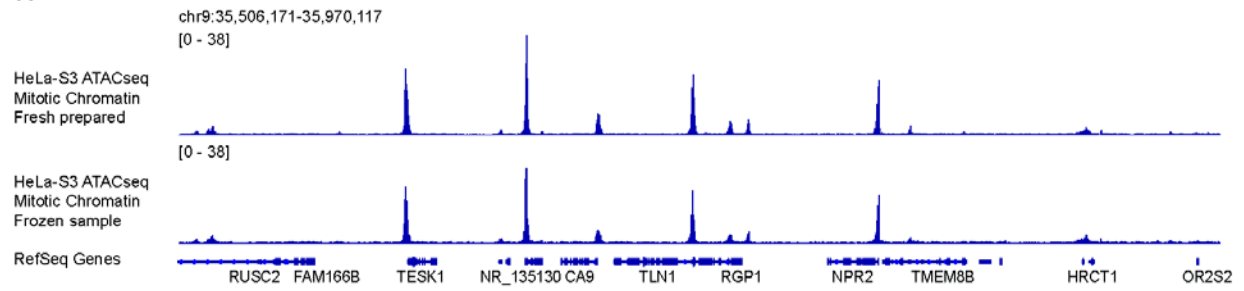
Supplemental Fig. S13
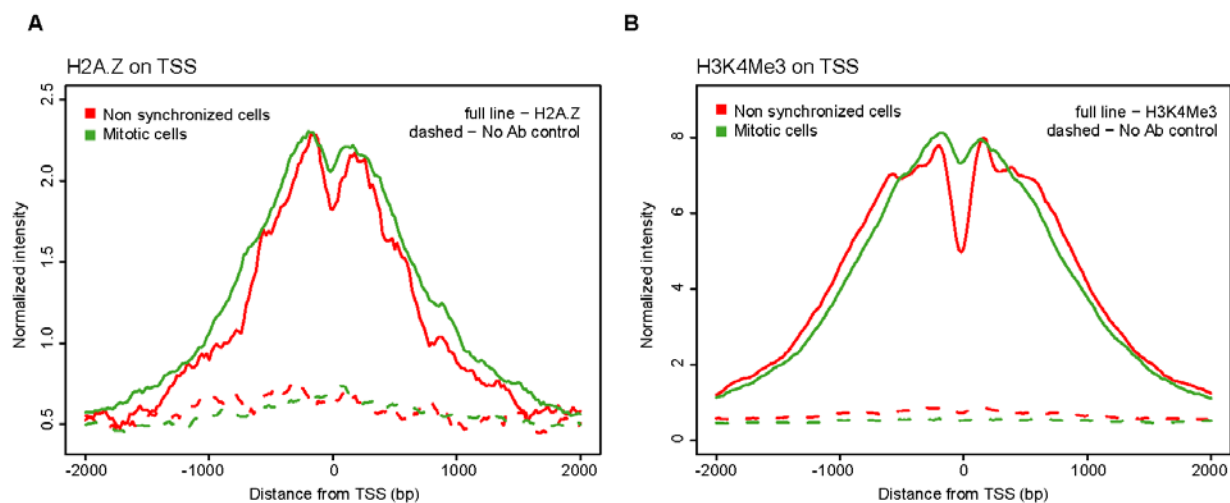
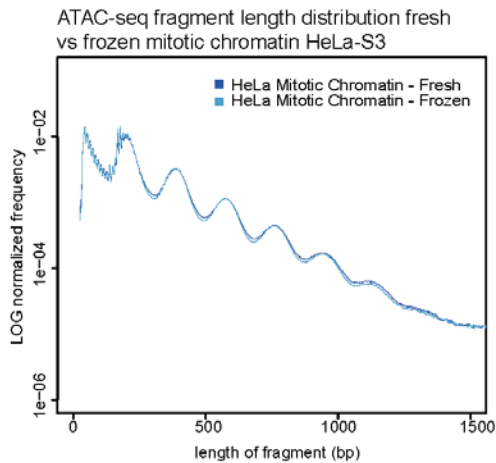**Supplemental Fig. S13** – (**A**) H2A.Z CUT&RUN signal of fragments larger than 120bp on TSSs that display ATAC-seq peaks in interphase for non-synchronized HeLa-S3 cells (red) and mitotic HeLa-S3 cells (green). (**B**) H3K4Me3 CUT&RUN signal of fragments larger than 120bp on TSSs that display ATAC-seq peaks in interphase for non-synchronized HeLa-S3 cells (red) and mitotic HeLa-S3 cells (green).
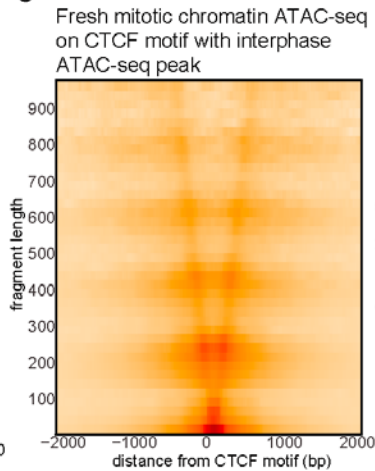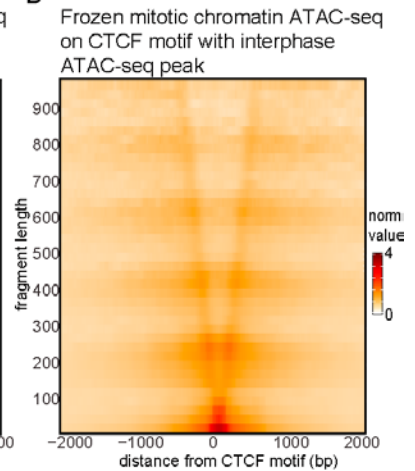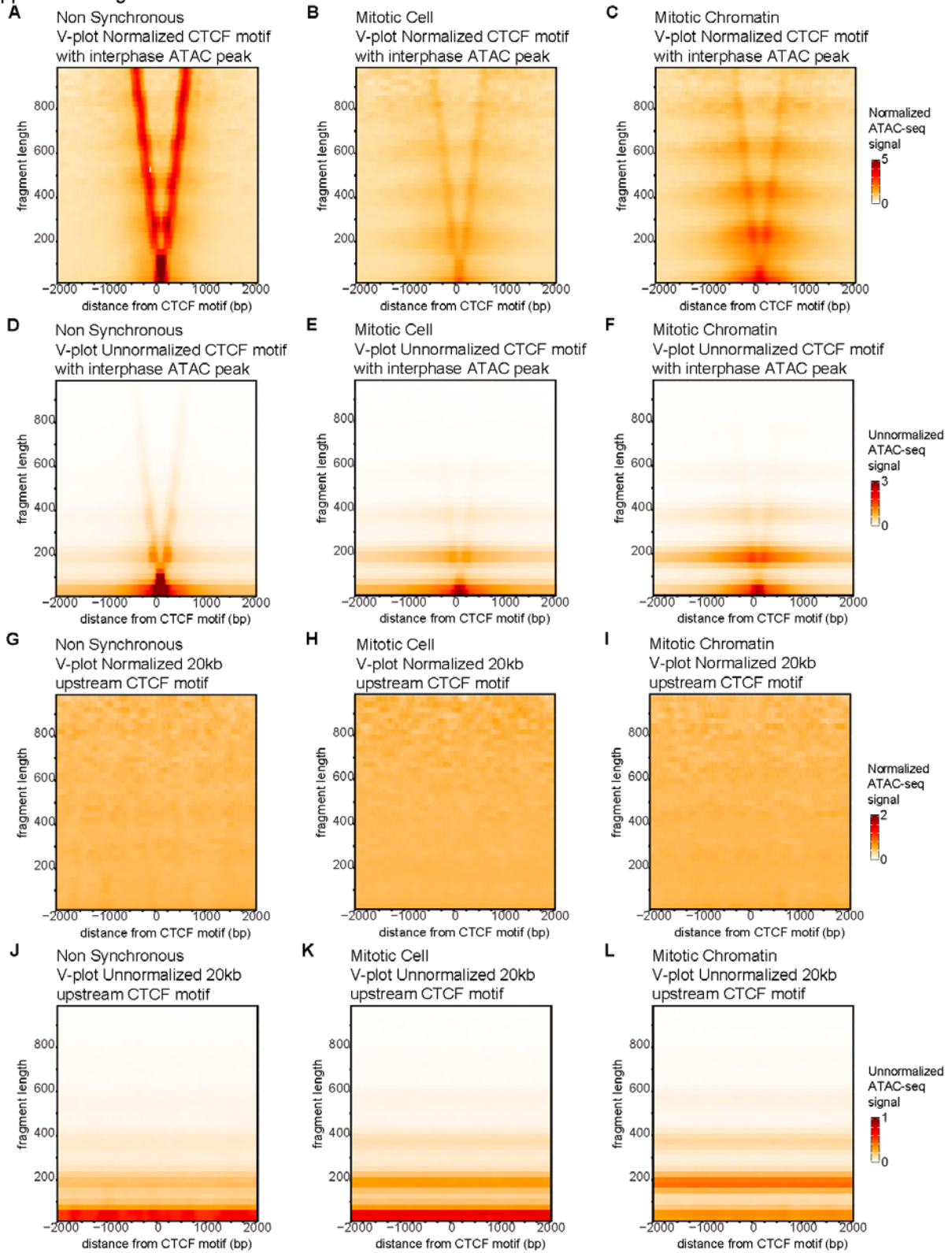
Supplemental Fig. S14 – Comparison of ATAC-seq data with freshly prepared and frozen stored mitotic chromatin from mitotically synchronized HeLa-S3. (**A**) representative genomic tracks, (**B**) fragment length distribution, (**C-D**) V-plots at CTCF motifs that display ATAC-seq peaks in interphase (**C-D**).

Supplemental Fig. S15

A
Non Synchronous
V-plot Normalized CTCF motif
with interphase ATAC peak

B
Mitotic Cell
V-plot Normalized CTCF motif
with interphase ATAC peak

C
Mitotic Chromatin
V-plot Normalized CTCF motif
with interphase ATAC peak

Normalized
ATAC-seq
signal
5
0

D
Non Synchronous
V-plot Unnormalized CTCF motif
with interphase ATAC peak

E
Mitotic Cell
V-plot Unnormalized CTCF motif
with interphase ATAC peak

F
Mitotic Chromatin
V-plot Unnormalized CTCF motif
with interphase ATAC peak

Unnormalized
ATAC-seq
signal
3
0

G
Non Synchronous
V-plot Normalized 20kb
upstream CTCF motif

H
Mitotic Cell
V-plot Normalized 20kb
upstream CTCF motif

I
Mitotic Chromatin
V-plot Normalized 20kb
upstream CTCF motif

Normalized
ATAC-seq
signal
2
0

J
Non Synchronous
V-plot Unnormalized 20kb
upstream CTCF motif

K
Mitotic Cell
V-plot Unnormalized 20kb
upstream CTCF motif

L
Mitotic Chromatin
V-plot Unnormalized 20kb
upstream CTCF motif

Unnormalized
ATAC-seq
signal
1
0

Supplemental Fig. S15 - V-plots normalized (**A-C**) and unnormalized (**D-F**) by the genome-wide fragment length distribution for HeLa-S3 ATAC-seq signal on all CTCF motifs that display an ATAC-seq peak in interphase for non-synchronized cells (**A/D**), mitotic cells (**B/E**) and purified mitotic chromatin (**C/F**). V-plots normalized (**G-I**) and unnormalized (**J-L**) by the genome-wide fragment length distribution for HeLa-S3 ATAC-seq signal on a region 20kb upstream of every CTCF motifs representing a random region in non-synchronized cells (**G/J**), mitotic cells (**H/K**) and purified mitotic chromatin (**I/L**).

Supplemental tables

Supplemental Table T1 – 5C mapping statistics

		Reads Sequenced	Valid reads mapped
R1	Asynchronous	100,986,155	99,609,624
	Mitotic Cell	67,042,262	65,620,645
	Mitotic Cluster	38,212,480	36,918,691
R2	Asynchronous	181,209,251	175,402,702
	Mitotic Cell	181,966,242	175,402,702
	Mitotic Cluster	173,399,970	166,197,088
Combined	Asynchronous	282,195,406	275,012,326
	Mitotic Cell	249,008,504	240,312,381
	Mitotic Cluster	211,612,450	203,115,779

Supplemental Table T2 – Antibodies used for western blot and Cut&Run

	Company + catalogue number	Western blot concentration	Cut&Run concentration
CTCF	CST #2899	1:1000	1:100
H2A.Z	Abcam #ab4174	x	1:100
H3K4Me1	Abcam #ab8895	x	1:50
H3K4Me3	Abcam #ab8580	x	1:100
H3	Abcam #ab1791	1:1000	x
H3S10Ph	Abcam #ab5176	1:1000	x

Supplemental Movies

Supplemental Movie S1 – Live cell imaging halo-CTCF overlaid with brightfield

Supplemental Movie S2 – Live cell imaging H2B-GFP on left and halo-CTCF on right

Supplemental Movie S3 – Single particle tracking Halo-CTCF in interphase cells

Supplemental Movie S4 - Single particle tracking Halo-CTCF in nocodazole arrested mitotic

cells

Supplemental Movie S5 - Single particle tracking Halo-CTCF in "enriched" prometaphase cells