Supplementary information

Standardizing chromatin research: a simple and universal method for ChIP-seq

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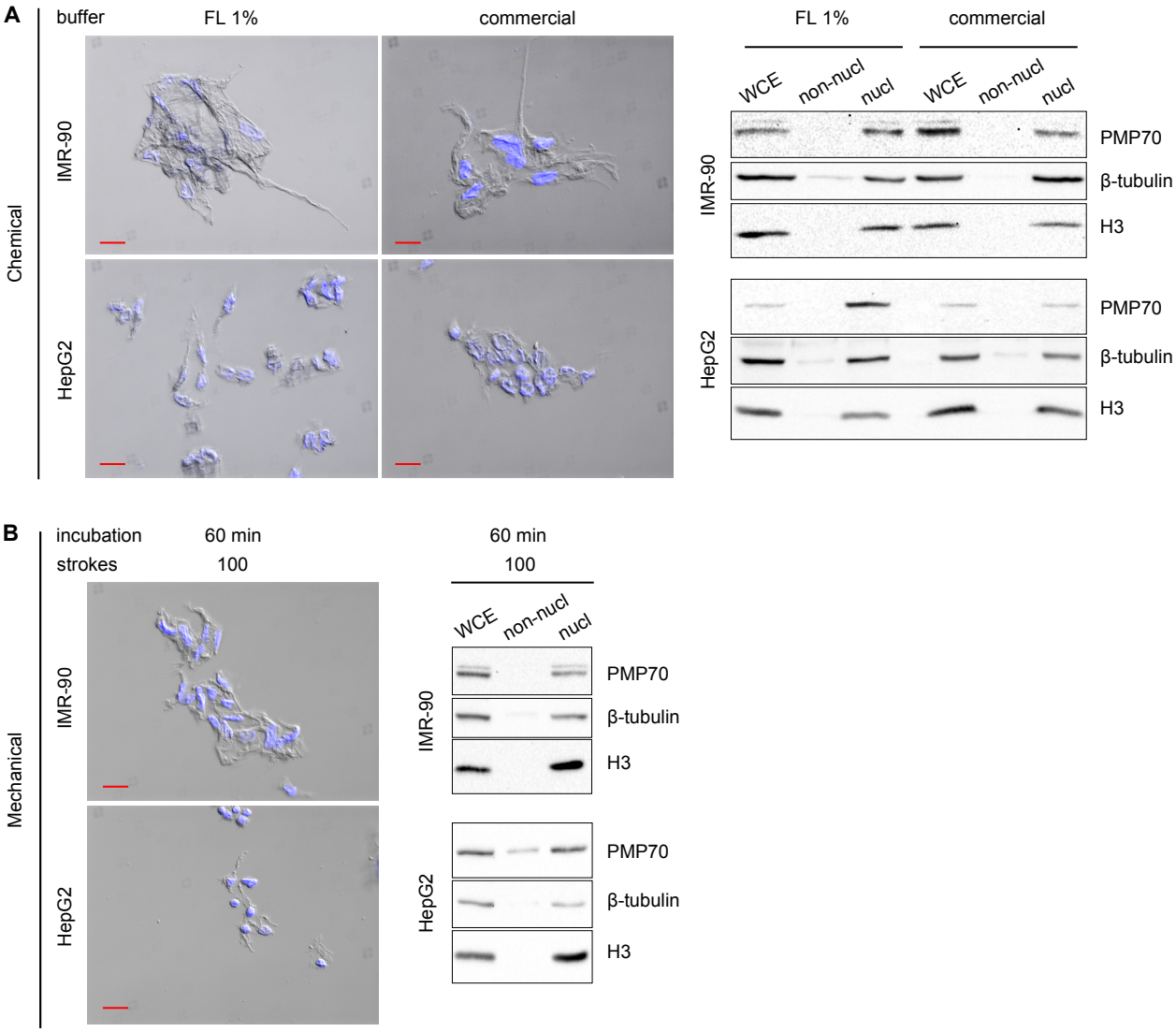
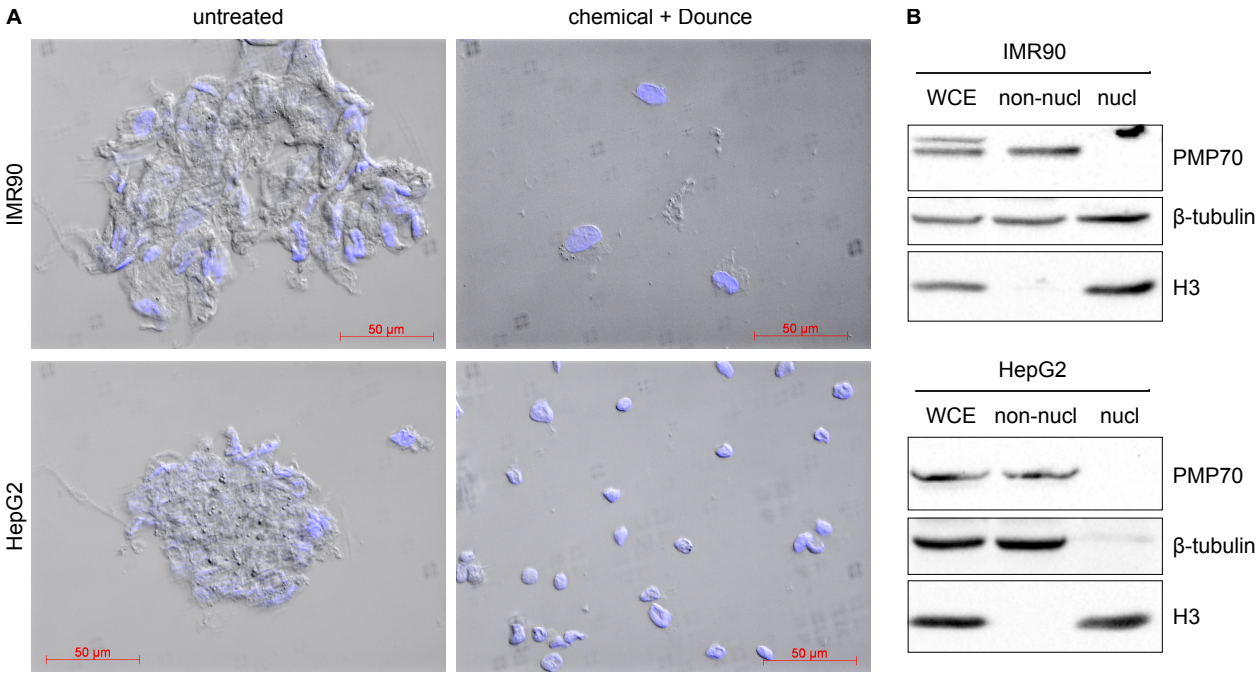
SUPPLEMENTARY FIGURES

Supplementary Figure S1: Stringent chemical and mechanical state-of-the-art treatments are not sufficient to isolate nuclei from fixed cells.



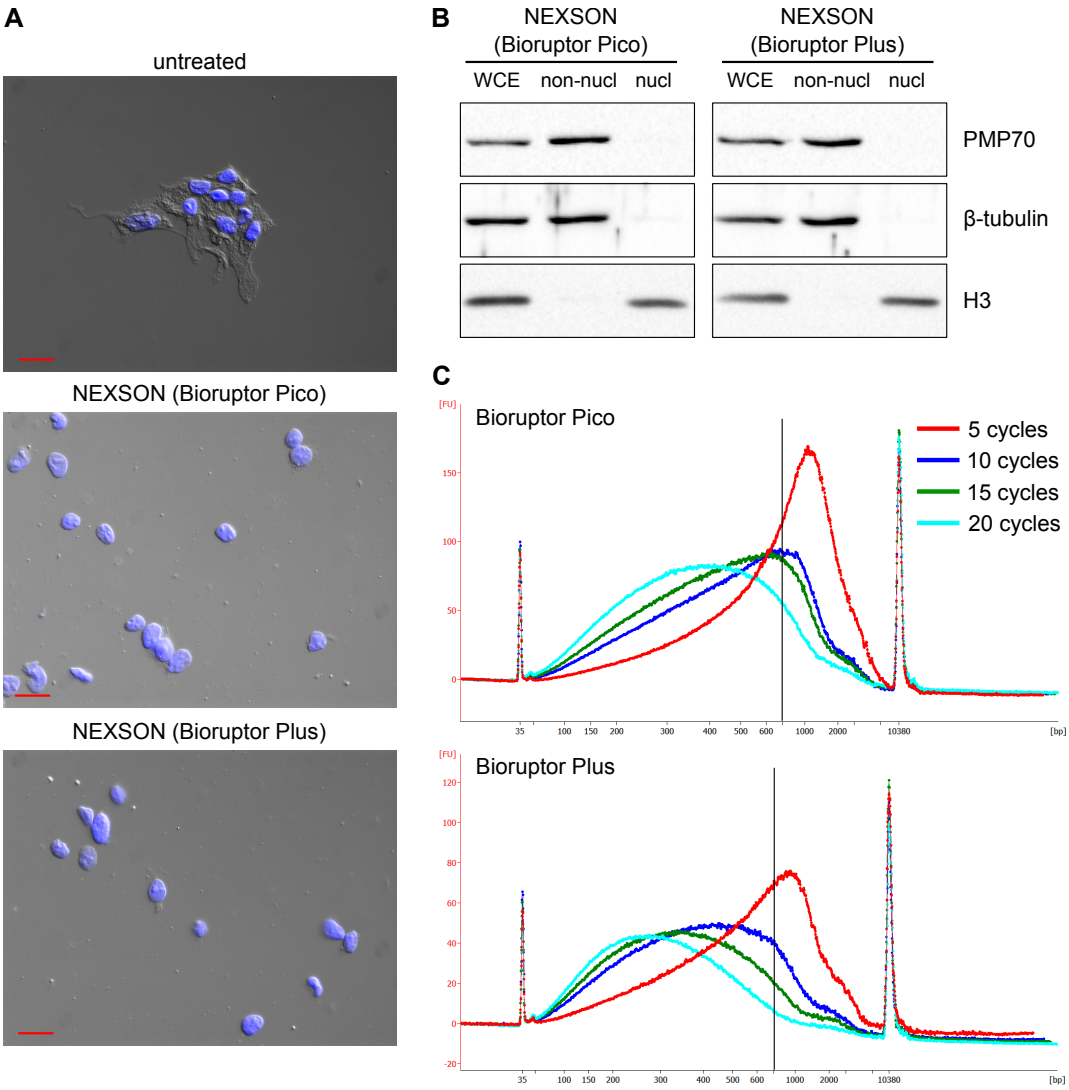
Microscope pictures and Western blot analysis of five minute formaldehyde-fixed IMR-90 and HepG2 cells undergoing enhanced chemical (**A**) or mechanical (**B**) treatments for typical nuclei isolation. (**A**) The following nuclei isolation buffers were used: FL 1%, containing 1% of Igepal; Commercial**:** cell lysis buffer commercially available from the company Covaris. (**B**) Cell pellets were incubated up to 60 minutes in FL buffer containing 0.5% Igepal and treated with a maximum of one hundred Dounce homogenizer strokes. (**A,B**) Left: Microscope pictures show the merge between DAPI (blue, nuclei) and DIC channels. Red scale bar: 20 µm. Right: Western blot analysis of the non-nuclear (non-nucl) and nuclear (nucl) fractions was carried out after the indicated treatment. Cytoplasmic (PMP70 and β-tubulin) and nuclear (histone H3) markers were used to inspect the nucleus-cytoplasm fractionation. Whole cell extract (WCE), collected prior the treatment, served as control.

Supplementary Figure S2: Effective nuclei extraction on unfixed cells with state-of-the-art treatments



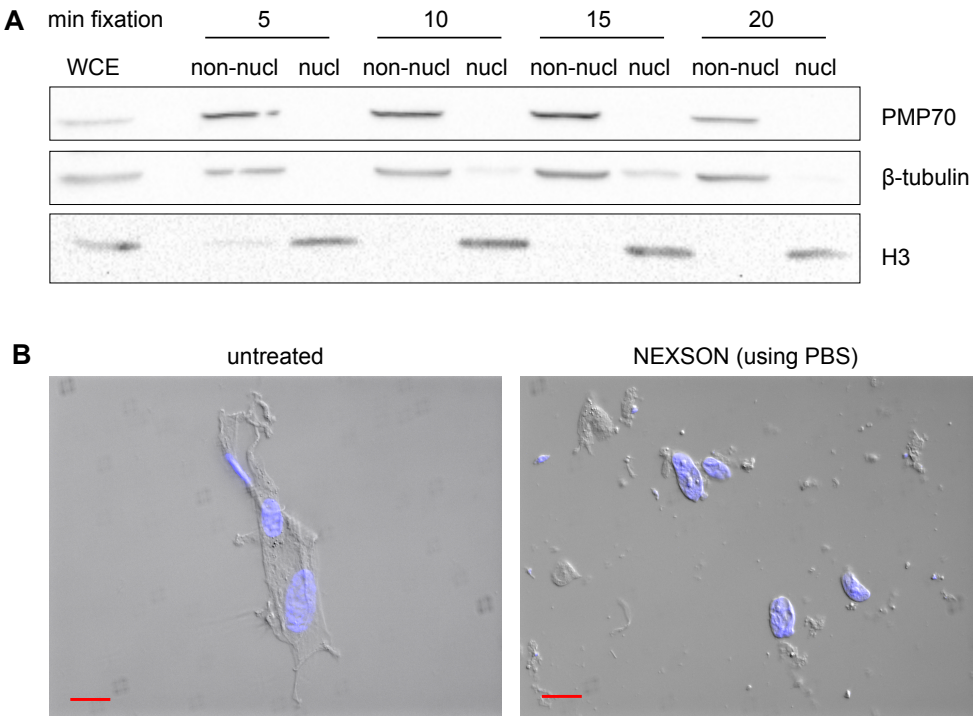
Nuclei were extracted from IMR90 and HepG2 cells using state-of-the-art nuclei extraction procedures. To this end, unfixed cell pellets were resuspended in FL buffer with 0.5% Igepal and treated with 30 Dounce homogenizer strokes. Non-nuclear and nuclear fractions were separated after the respective treatment. (**A**) Microscope pictures were taken prior (untreated, cell pellets resuspended in PBS) and after nuclei extraction treatment (Chemical + Dounce). Pictures show the merge between DAPI (nuclei) and DIC channels. Red scale bar: 50 µm. (**B**) Western blot analysis of the non-nuclear (non-nucl) and nuclear (nucl) fractions obtained after nuclei extraction. Whole cell extract (WCE) was collected prior fractionation and served as control. Cytoplasmic (PMP70 and β-tubulin) and nuclear (histone H3) markers were used to inspect effective nucleus-cytoplasm fractionation.

Supplementary Figure S3: NEXSON also works with bath-type shearing devices



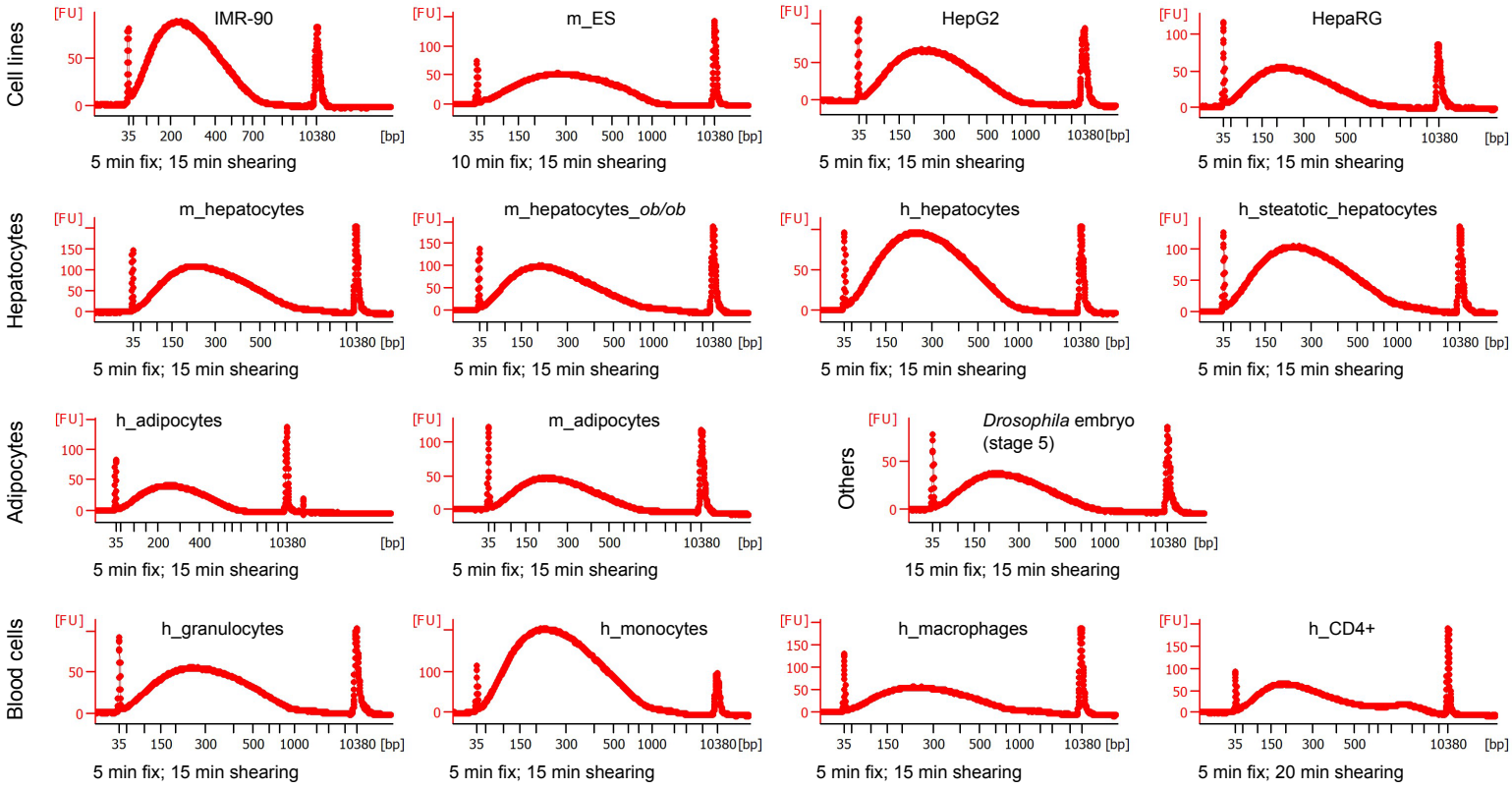
NEXSON procedure and chromatin shearing was carried out using two different types of Bioruptor sonicators (Diagenode). Five minutes formaldehyde-fixed HepG2 cells were treated as described in the Materials and Methods section. (**A**) Microscope pictures showing cells prior treatment (untreated) and after completed nuclei extraction using either Bioruptor Pico or Bioruptor Plus devices. Pictures show the merge between DAPI and DIC channels. Red scale bar: 20 µm. (**B**) Western blot analysis of fractions collected prior nuclei extraction (WCE: Whole Cell Extract) and after NEXSON treatment (non-nuclear and nuclear). Separation of nuclear (histone H3) and non-nuclear (PMP70 and β-tubulin) markers into the respective fractions indicates successful fractionation. (**C**) Chromatin shearing time curves of nuclear extract using Bioruptor Pico (upper panel) and Bioruptor Plus (lower panel) devices. Nuclei were resuspended in shearing buffer and treated with increasing number of cycles (from 5 to 20) as indicated by colored lines; each cycle consists of 30 seconds ON and 30 second OFF for both instruments. For chromatin shearing, Bioruptor Plus was set to power “High”. An aliquot of the sample was collected at each time point and analyzed by capillary electrophoresis (Bioanalyzer). X axis: bp, Y axis: Fluorescence units (FU). Vertical line indicates 800 bp size.

Supplementary Figure S4: NEXSON is tolerant to fixation time and buffer compositions.



(**A**) Western blot analysis of non-nuclear and nuclear fractions obtained after NEXSON treatment of HepG2 cells fixed up to twenty minutes in 1% formaldehyde. Cytoplasmic (PMP70 and β-tubulin) and nuclear (histone H3) markers were detected in the indicated fractions (WCE: Whole Cell Extract; non-nucl: non-nuclear fraction; nucl: nuclear fraction). Independent of the fixation time, NEXSON treatment results in effective nucleus-cytoplasm fractionation. (**B**) Microscopy analysis of five minute formaldehyde-fixed IMR-90 cells prior and after NEXSON treatment. NEXSON was conducted in PBS (phosphate-buffered saline), a buffer not used for nuclei extraction. The successful nuclei extraction shows that ultrasound-assisted nuclei extraction works independently of detergents, making NEXSON an interesting tool for multiple assays that rely on nuclei extraction.

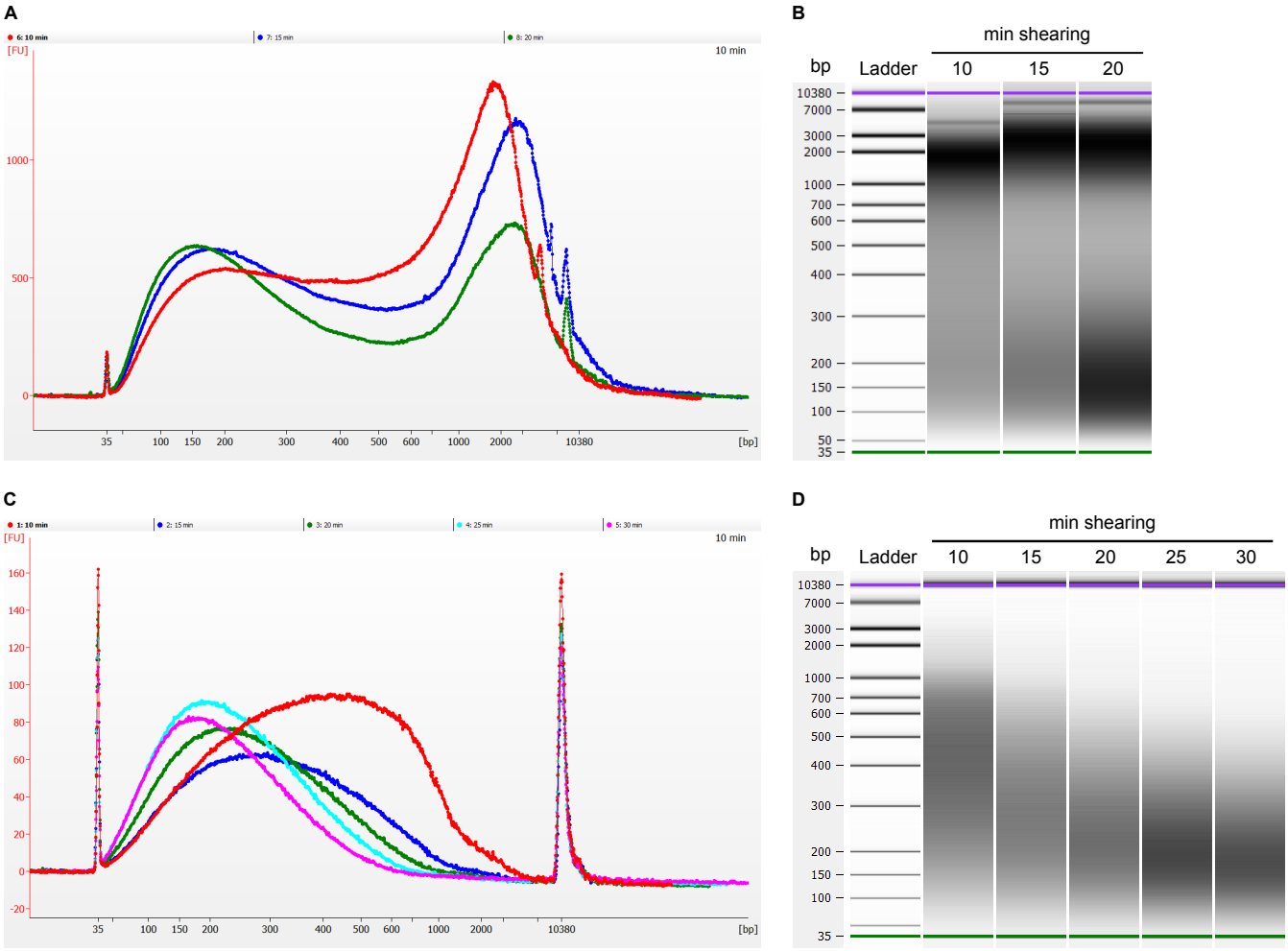
Supplementary Figure S5: Reproducibility of chromatin shearing across cell types.



Size distribution of sheared chromatin in multiple cell types. Nuclei were extracted using NEXSON for all shown cell types (fixed in 1% formaldehyde for the indicated time). Chromatin was fragmented without varying shearing buffer composition, detergent content or sonication power. Efficiency of chromatin shearing was inspected by visualization of the DNA size distribution using capillary electrophoresis (Agilent Bioanalyzer). To this an aliquot of each chromatin was de-crosslinked and proteinase K-treated prior to DNA extraction and analysis. Optimal shearing is achieved when most of the DNA fragments have a size distribution from 100-800 bp. For most samples, 15 minutes sonication resulted in an optimal chromatin size distribution. Only the CD4+ cells required 20 instead of 15 minutes shearing to concentrate most of the DNA fragments between 100-800 bp. The x-axis indicates the DNA size in base pairs (bp), the y-axis indicates fluorescence units (FU).

The tested cell types include cell lines as IMR-90, mouse embryonic stem cells (m_ES), HepG2 and HepaRG; hepatocytes (steatotic or normal; *ob/ob*: derived from leptin-deficient mice), adipocytes isolated from adipose tissue, blood cells purified from blood (granulocytes, monocytes, *in vitro* derived macrophages, CD4+) and whole *Drosophila* embryos (stage 5: cellularization). Abbreviations: h: human, m: mouse. Any variation in fixation or sonication time is indicated below the respective plot.

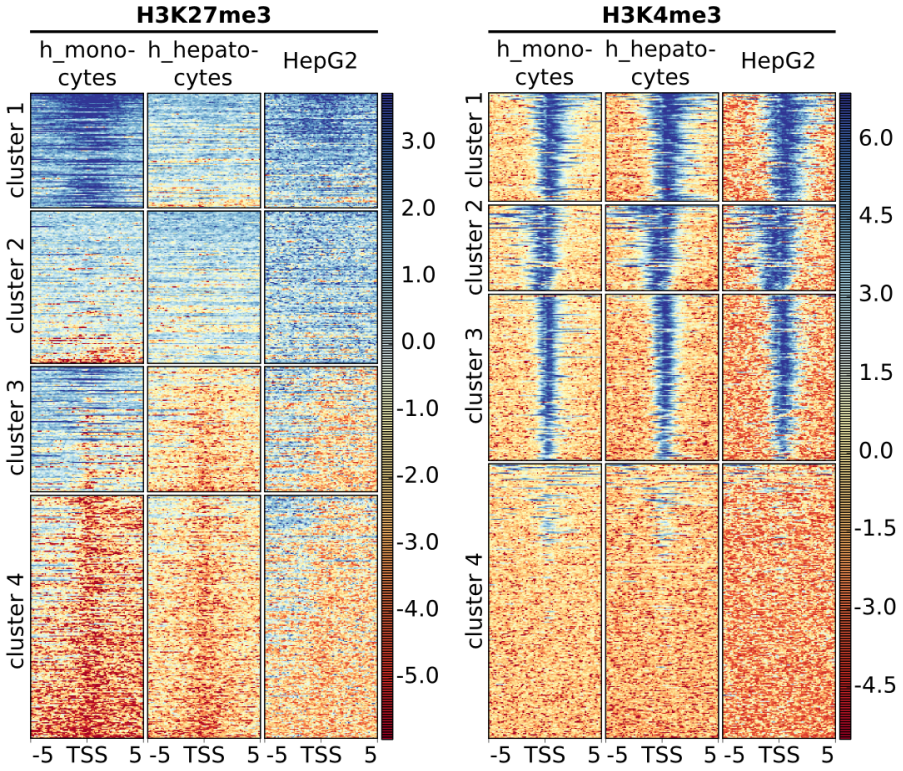
Supplementary Figure S6: Effect of nuclei extraction on chromatin quality.



(**A,B**) Shearing time course of mouse hepatocytes without nuclei extraction. (**C,D**) Shearing time course of HepG2 cells after nuclei extraction using NEXSON. Cells were fixed for 5 minutes in 1% formaldehyde, chromatin was sheared 10-30 minutes and size distribution was analyzed using capillary electrophoresis (Agilent Bioanalyzer). The size distribution at each time point is presented as electropherogram (left) and gel-like image (right). Different colours correspond to different sonication times: red (10 minutes), blue (15 minutes), green (20 minutes), light blue (25 minutes), pink (30 minutes).

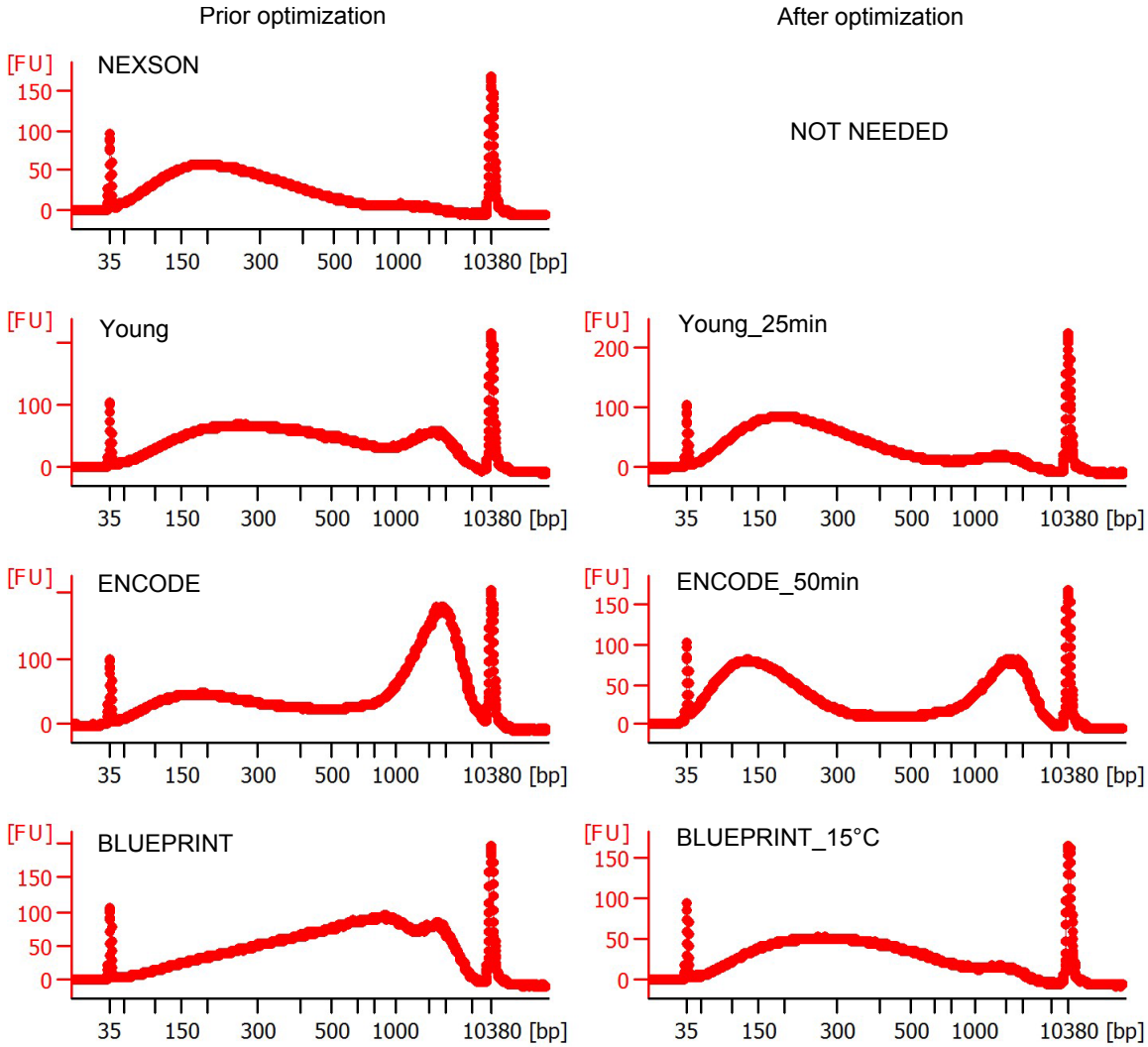
The results show that nuclei isolation is a key determinant to reach a gradual reduction in chromatin size upon sonication. Conversely, if nuclei are not isolated, prolongation of the shearing time results in chromatin that consists of two fractions: < 300 bp and > 1 kb.

Supplementary Figure S7: ChIP-seq of NEXSON-treated cells yields high-quality and cell-type-specific signals.



Histone modification ChIP-seq enrichment over all TSS annotated in RefSeq genes. Promoters are marked by H3K27me3 (left heatmap) and H3K4me3 (right heatmap) in a cell-type-specific manner. The heatmap shows regions of 5 kb up- and downstream of the TSS with each row representing a distinct TSS. The signal intensity is measured as log2 ratio of ChIP over input signal. Regions were clustered by the *k*-means algorithm. This figure shows the same samples as Figure 5.

Supplementary Figure S8: Chromatin shearing after applying several protocols for chromatin preparation.



Chromatin fragment size distribution obtained after application of four different protocols for chromatin preparation on the same 10 minutes, formaldehyde-fixed HepG2 cell batch. From the top to the bottom: NEXSON (this study), Young, ENCODE, BLUEPRINT. Chromatin was sonicated, decrosslinked and DNA was purified to inspect the size distribution by capillary electrophoresis. Left panel shows chromatin size distribution obtained after 20 minutes of Covaris sonication. At this point, only NEXSON gives an optimal size distribution. To concentrate the sample into the desired size range (100-800 bp), further optimizations were needed for the remaining protocols (right panel). In particular, Young and ENCODE protocols required extended sonication to shorten chromatin (25 min and 50 min, respectively). The BLUEPRINT protocol uses high SDS concentration (1% SDS) in the shearing buffer. To prevent SDS precipitation, water bath temperature of the Covaris sonicator was increased from 4 °C to 15 °C to achieve optimal shearing after 20 min of sonication.

SUPPLEMENTARY TABLES

Supplementary Table S1. IMR-90 ChIP-seq quality control measures after read mapping.

NEXSON-treated IMR-90 samples used either 200,000, 100,000, 10,000, 1,000 or 100 cells per ChIP reaction as indicated, whereas Roadmap samples used approximately 10 million cells per ChIP. FRiP measures the global ChIP enrichment as fraction of mapped reads that fall into peak regions. FRiP scores were computed on the genomic regions obtained by merging the peak regions called for each of the two Roadmap biological replicates.

Sample	**# Reads**	**% Mapped reads**	**% Duplicated reads**	**FRiP** (using Roadmap peak regions)
H3K27me3 NEXSON 200k	27,680,734	99.23	3.03	0.22
H3K27me3 NEXSON 100k	48,638,306	97.59	9.60	0.22
H3K27me3 NEXSON 10k	48,416,637	94.63	19.77	0.16
H3K27me3 NEXSON 1k	48,181,213	76.43	50.09	0.12
H3K27me3 NEXSON 100	37,024,835	46.41	69.03	0.09
H3K27me3 Roadmap replicate 1	53,266,810	48.73	4.37	0.35
H3K27me3 Roadmap replicate 2	43,738,370	75.41	8.74	0.25
H3K4me3 NEXSON 100k	23,234,613	85.30	30.63	0.61
H3K4me3 NEXSON 10k	20,225,592	63.27	37.90	0.32
H3K4me3 NEXSON 1k	14,760,128	36.10	50.28	0.12
H3K4me3 NEXSON 100	14,525,157	16.43	48.71	0.04
H3K4me3 Roadmap replicate 1	16,845,165	58.76	15.97	0.85
H3K4me3 Roadmap replicate 2	40,385,339	78.44	35.23	0.73