Cryo-ET reveals nucleosome reorganisation in condensed mitotic chromosomes *in vivo*

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SUMMARY

Chromosomes condense during mitosis in most eukaryotes. This transformation involves rearrangements at the nucleosome level and has consequences for transcription, but the details remain unclear. Here, we use cryo-electron tomography to determine the 3-D arrangement of nucleosomes and other large nuclear features in frozen-hydrated fission-yeast cells. Nucleosomes can form irregular clusters in both interphase and mitotic cells, but they are smaller than expected for Hi-C domains. The nucleosomes are co-mingled with two features: nucleosome-free pockets and megadalton-sized “megacomplexes”. Compared to interphase, the nucleosomes in mitotic chromosomes pack into slightly larger clusters. However, nearest-neighbor distance analysis reveals that mitotic nucleosome clusters have the same internal packing density as in interphase. Furthermore, mitotic chromosomes contain fewer megacomplexes. This uneven chromosome condensation helps explain a longstanding enigma of mitosis: most genes are repressed but a subset is upregulated.

INTRODUCTION

Chromatin structure influences key nuclear activities such as transcription, DNA repair and replication (Dixon et al., 2016). The fundamental unit of chromatin is the nucleosome, which consists of ~147 bp of DNA wrapped around a histone octamer Luger, 1997]. In mammalian cells, 2 - 35 nucleosomes pack into irregular “clutches” (Ricci et al., 2015) and more than 500 nucleosomes (calculated from nucleosome spacing) are thought to associate as topologically associating domains (Dixon et al., 2012). Likewise, in the fission yeast *Schizosaccharomyces pombe*, some 300 - 7,000 nucleosomes are thought to associate as compact globular chromatin bodies called domains (Mizuguchi et al., 2014; Kakui et al., 2017; Tanizawa et al., 2017). These studies suggest that chromatin higher-order structure arises from physical interactions of large groups of nucleosomes.

In mitotic cells, chromosomes condense into discrete structures that can be resolved in a light microscope. The factors involved in condensation have been well characterized (Hirano, 2016) and a number of models have been proposed for the large-scale organisation of chromatin domains (Maeshima and Eltsov, 2008). However, the molecular details of chromatin reorganisation are still unknown. Knowledge of how chromatin condenses in 3-D at the single-nucleosome level is needed to explain the nearly global transcriptional repression that happens to the mitotic cells of most eukaryotes (Struhl, 1998). Likewise, a 3-D model of chromatin could also explain how a subset of genes escape this mitotic repression and get upregulated (Rustici et al.; Oliva et al.; Peng et al.).

59 Some insights on *in vivo* chromatin organisation were made possible by new
60 methods, including chromatin-conformation capture (Hi-C), super-resolution
61 microscopy, and traditional EM of cells stained with DNA-proximal osmium (Beliveau et
62 al.; Pombo and Dillon, 2015; Ou et al.). However, the resultant models are limited
63 because these methods rely on population-averaged data, or have low resolution, or
64 perturb the sample due to the fixation, dehydration and staining. Cryo-EM is a label-free
65 method to visualize cells in a life-like frozen-hydrated state (McDowall et al., 1986;
66 Eltsov et al., 2008). Cryo-electron tomography (cryo-ET) goes further and can reveal the
67 3-D positions of these complexes at ~ 4-nm resolution (Gan and Jensen, 2012). Using
68 cryo-ET, we previously showed that nucleosomes in picoplankton and budding yeast
69 pack irregularly, but we did not find evidence for mitotic chromosome compaction (Gan
70 et al., 2013; Chen et al., 2016).
71 Fission-yeast mitotic-chromosome condensation resembles human chromosome
72 condensation in both morphological and Hi-C phenotypes (Hiraoka et al., 1984;
73 Naumova et al., 2013; Yam et al., 2013; Kakui et al., 2017; Tanizawa et al., 2017). We
74 have now used cryo-ET to directly visualize chromatin both before and after mitotic
75 condensation *in vivo* in the fission yeasts *S. pombe* and *S. japonicus*. To obtain
76 cryotomograms with sufficient contrast to resolve nucleosomes *in vivo*, we imaged cells
77 that were thinned by cryomicrotomy. To better understand the heterogeneous
78 complexes in the crowded nucleoplasm, we also took advantage of recent advances in
79 phase-contrast hardware and image-classification software (Bharat and Scheres, 2016;
80 Khoshouei et al., 2017). We found that in interphase, nucleosomes frequently associate
81 as small clusters or short chains. Nucleosome-free pockets and megadalton-sized

82 “megacomplexes” are interspersed among these nucleosome clusters. In mitosis,
83 chromosomes condense unevenly and have slightly larger nucleosome clusters. Mitotic
84 chromosomes also have nucleosome-free pockets, but fewer megacomplexes. These
85 phenotypes are conserved in both types of fission yeasts and lead to a model that
86 explains how some genes can be transcriptionally upregulated in mitosis.

87 **RESULTS**

88

89 **S. pombe subcellular structures are revealed at molecular resolution by cryo-ET**

90 To understand how native chromatin organisation differs between interphase and
91 mitosis, we imaged *S. pombe* cells by cryo-ET of frozen-hydrated sections
92 (cryosections, ~ 90 - 130 nm nominal thickness). To ensure that the imaged cells had a
93 known cell-cycle state, we arrested temperature-sensitive *cdc25-22* cells in G2 phase
94 (Fantes, 1979) and cold-sensitive *nda3-KM311* cells in prometaphase (Hiraoka et al.,
95 1984). Fluorescence microscopy confirmed that the latter cells have the well-known
96 mitotic chromosome-condensation phenotype (Fig. 1A, S1). In a typical cryotomogram
97 of an *S. pombe* cell, we could recognize organelles such as the endoplasmic reticulum,
98 vesicles, and the nucleus due to their membrane morphologies (Fig. 1B and C). To
99 further assess the quality of the cryosections and data, we performed subtomogram
100 averaging of cytoplasmic ribosomes (Fig. S2A, B). The resulting average was similar to
101 a low-pass-filtered budding-yeast ribosome crystal structure (Fig. S1C) (Ben-Shem et
102 al., 2011). Therefore, the conformation of large complexes are preserved at the
103 molecular level.

104

105 **Interphase chromatin is arranged as loosely packed nucleosomes and irregular**
106 **nucleosome clusters**

107 G2-phase cell nuclei are filled with many nucleosome-like granular densities. For the
108 sake of brevity, herein we call these densities nucleosomes due to their size, shape,
109 abundance, location, and condensation phenotype all being consistent with

110 nucleosomes (see below). Even if a minority of these densities are not nucleosomes,
111 the conclusions we make below are still valid. Nucleosomes frequently associate as
112 small irregular clusters less than 50-nm wide (Fig. 2B). Chain-like nucleosome
113 configurations are also abundant (Fig. 2C). *S. pombe* linker DNA is only 7-bp,
114 corresponding to ~ 2.5 nm (Lantermann et al., 2010), meaning that most nucleosomes
115 should pack closely with at least two other nucleosomes. These two nucleosomes
116 correspond to the -1 and +1 positions in the sequence. Because linker DNA is
117 unresolved in our cryotomograms, we do not know if a cluster contains nucleosomes
118 from a single or multiple sequences of nucleosomes. The remaining nucleosomes are
119 not clustered together (Fig. 2D). Efforts to automatically identify nucleosome clusters
120 with distinct motifs failed due to the irregular nucleosome packing. Small nucleosome-
121 free "pockets" (< 50 nm) are also abundant (Fig. 2E). Densities much larger than
122 nucleosomes (> 20 nm) are spread throughout the nucleus (Fig. 2F). These densities
123 are consistent with being multi-megadalton nuclear assemblies such as pre-ribosomes,
124 spliceosomes, and transcription preinitiation complexes (Gleizes et al., 2001; Allen and
125 Taatjes, 2015; Oesterreich et al., 2016); we call these "megacomplexes" for brevity.
126 Therefore, in interphase, nucleosomes associate as small irregular clusters that co-
127 mingle with megacomplexes and nucleosome-free pockets.
128

129 **Condensed mitotic chromosomes contain fewer megacomplexes**

130 Unlike in G2-phase cells, in each cryotomogram of a prometaphase cell we found a
131 single position that has many features consistent with its being a section through a
132 condensed chromosome. For example, nucleosome-like densities are abundant within

133 these positions, but much rarer outside (Fig. 3A-C). Compared to G2-phase cells, in
134 which abundant megacomplexes are interspersed with the chromatin (Fig. 2A),
135 megacomplexes are mostly absent from these large contiguous regions in
136 prometaphase cells (Fig. 3A). These positions are hundreds of nanometers wide,
137 matching the size of condensed chromosomes seen by fluorescence microscopy (Fig.
138 1A, S1). If these positions are indeed chromosomes, there should be a large contiguous
139 volume in the cell with few megacomplexes. To test this hypothesis, we sampled a
140 larger nuclear volume by cryo-ET of 5 nearly sequential cryosections (summing to ~ 700
141 nm thick) of the same cell (Fig. S3A). We saw a single megacomplex-poor region in
142 each of these cryosections, which is consistent with these positions being sections
143 through one or two of the three fission-yeast chromosomes (Fig. S3B-F). Taken
144 together, we located mitotic chromosomes and found they have fewer megacomplexes
145 within.
146
147 **Mitotic chromosomes condense unevenly and support transcription**
148 In conventional (defocus) cryotomograms, we noticed that most nucleosomes appear
149 more densely packed in prometaphase cells (Fig. S4). To better understand how
150 chromatin is organised, we imaged cells by Volta phase contrast cryo-ET (Fig. S5).
151 Volta cryo-EM data have much more contrast, making it possible to locate and
152 determine the orientations of smaller protein complexes and to resolve protein
153 complexes packed to near-crystalline density inside of cells (Engel et al., 2015;
154 Khoshouei et al., 2017). The Volta cryotomograms confirmed what we learned from
155 defocus cryo-ET: prometaphase nucleosomes more frequently packed into larger

156 clusters (Figs. 4A and B, S5). Importantly, we confirmed the presence of some loosely
157 packed nucleosomes and pockets in prometaphase cells (Figs. 4C and S5B, D),
158 meaning that condensation is uneven. In summary, mitotic chromosomes consist of
159 both densely and loosely packed nucleosomes.

160 The existence of loosely packed nucleosomes in prometaphase cells suggests
161 that mitotic chromatin is permissive to transcriptional machinery, which is supported by
162 the observation that some genes are up-regulated during mitosis (Rustici et al., 2004;
163 Oliva et al., 2005; Peng et al., 2005). However, these earlier studies could not
164 determine if *S. pombe* undergoes active mitotic RNA polymerase II transcription or if
165 some stable mRNAs are left over from G2 phase. Phosphorylation of RNA polymerase
166 II at Serine 2 of the carboxy-terminal domain (CTD) heptamer repeat is a conserved
167 marker of transcription elongation (Komarnitsky et al., 2000; Harlen and Churchman,
168 2017). Using immunofluorescence, we confirmed the existence of RNA polymerase II
169 with phospho-Ser2 CTD, and therefore active transcription, in prometaphase
170 chromosomes (Fig. S6). To rule out the possibility of abnormal transcriptional activity in
171 the mutants, we imaged asynchronous wild-type cultures, in which a small minority of
172 cells was undergoing mitosis. We also detected phospho-Ser2 CTD signal in both early
173 and late mitotic cells. Therefore, transcription is not shut-down globally in mitotic *S.*
174 *pombe* cells.

175 To analyse the nucleosome rearrangement more objectively, we performed
176 template matching to find their 3-D positions in Volta cryotomograms. This analysis
177 showed that nucleosomes formed clusters in both G2-phase and prometaphase cells,
178 but appear more crowded in the latter (Fig. 5A, B). If chromosomes condense via a

179 uniform accretion of all nucleosomes, the average nucleosome nearest-neighbor
180 distance (NND) distribution should shorten. However, the NND distributions of
181 nucleosome hits in G2-phase and prometaphase cells were indistinguishable (two-tailed
182 t-test, $p > 0.05$) (Fig. 5C). While mitotic chromatin might not condense by compaction of
183 nearest-neighbor nucleosomes, they might instead condense from changes in
184 interactions between groups of nucleosomes. We tested this hypothesis by creating
185 histograms of 10th NNDs and found that the population shifted to shorter distances in
186 prometaphase cells (two-tailed t-test, $p < 0.001$) (Fig. 5D), which explains the more-
187 crowded appearance of prometaphase nucleosomes. Taken together, our analyses
188 reveal that chromosomes condense by the merging of small nucleosome clusters,
189 resulting in a closer association between distant nucleosomes (Fig. 5E, F).
190 As an alternative approach to studying nucleosomes *in vivo*, we performed cryo-
191 ET on the undigested chromatin from lysates of G2-phase and prometaphase *S. pombe*
192 cells. Cryotomograms of cell lysis products can reveal more clearly the positions of
193 nucleosomes (Cai et al., 2017). Subtomogram averages of nucleosome template-
194 matching hits revealed unambiguous features of mononucleosomes including the
195 groove between two DNA gyres (Fig. S7), demonstrating that our template-matching
196 approach can locate most nucleosomes. In the lysates, there are many large
197 nucleosome-free pockets wider than 50 nm in G2-phase chromatin. In contrast,
198 prometaphase chromatin remained as more-compact masses with only a few, smaller
199 pockets (Fig. S8). Therefore, prometaphase chromatin has denser nucleosome packing
200 both *in vivo* and *in vitro*.
201

202 ***S. japonicus* and *S. pombe* chromosomes condense by similar means**

203 To test whether the formation of larger nucleosome clusters during condensation is
204 conserved, we imaged *S. japonicus*, a bigger fission yeast that also undergoes mitotic
205 condensation (Robinow and Hyams, 1989). *S. japonicus* has the same genome size (~
206 12Mb) as *S. pombe*, but has a nucleus at least 8-fold more voluminous. Because *S.*
207 *japonicus* is a less-developed model organism, we used asynchronous wild-type cells.
208 These cells were frozen, cryosectioned, and subjected to Volta cryo-ET just like for *S.*
209 *pombe*. To determine whether a cell was in mitosis or interphase at the time of freezing,
210 we looked for cells with cytoplasmic microtubules, which exist only in interphase, or
211 cells with nuclear microtubules, which exist only in mitosis (Yam et al., 2013). We found
212 densely packed nucleosomes in *S. japonicus* mitotic chromosomes (Fig. S9A-D).
213 Furthermore, NND and 10th-NND analyses showed that the nucleosome NND
214 distributions were similar in interphase and mitosis (two-tailed t-test, $p > 0.05$), but that
215 the 10th-NND distribution was shorter in mitosis (two-tailed t-test, $p < 0.001$) (Fig. S9E,
216 F). Therefore, the formation of larger nucleosome clusters in mitosis is a conserved
217 phenotype in fission yeasts.

218

219 **Dinucleosomes are irregular *in vivo***

220 The crowded nature of the nucleus makes it unfeasible to manually search for
221 reproducible nucleosome-packing motifs. We therefore performed reference-free 2-D
222 class-averaging analysis on di-nucleosomes extracted from our cryotomograms. Our
223 previous 2-D classification of natural *S. cerevisiae* chromatin *in vitro* showed that
224 abundant motifs can be found automatically this way (Cai et al., 2017). For example,

225 pairs of nucleosomes sometimes packed face-to-face *in vitro*, which if present *in vivo*
226 would limit access to regulatory positions like the acidic patch (Luger et al., 1997).
227 However, the same analysis of di-nucleosomes in *S. pombe* did not reveal any classes
228 containing face-to-face-packed nucleosomes (Fig. S10), meaning that such inter-
229 nucleosome interactions must be extremely rare. Instead, di-nucleosomes were packed
230 irregularly, including edge-to-edge packing, with ~ 3-nm gaps in between. G2-phase
231 nucleosomes (Fig. S10A and C) were more irregularly packed in terms of the separation
232 between candidate nucleosomes than in prometaphase cells (Fig. S10B and D). Taken
233 together, our 2-D classification analyses did not find evidence of ordered nucleosome
234 packing *in vivo*.
235
236 **A subset of nucleosomes may be partially unwrapped**
237 In a rare instances, we were able to resolve densities that resemble linker DNA
238 spanning two nucleosome-like densities in *S. pombe* G2-phase chromatin (Fig. S11A,
239 B). Many of these linker-DNA-like densities were much longer than the average ~ 2 nm
240 expected from MNase-digestion experiments (Fig. S11C-D) and nucleosome-mapping
241 studies (Lantermann et al., 2010). Furthermore, in the NND analysis, there are a large
242 number of nucleosome pairs that have NND values greater than 12 nm, i.e., the gaps
243 between adjacent nucleosomes are wider than 2 nm (Fig. 5C). This inter-nucleosome
244 gap was absent from tetranucleosomes reconstituted with the same linker DNA length
245 as *S. pombe* (Ekundayo et al., 2017). One explanation is that the larger inter-
246 nucleosome separation *in vivo* reflects the high end of the distribution of linker DNA
247 lengths. Alternatively, some nucleosomes might be partially unwrapped – such as may

248 be expected of fragile or pre-nucleosomes (Fei et al., 2015; Kubik et al., 2015), resulting
249 in apparently longer linker DNA (Fig. S11E, F). The possibility of partially unwrapped
250 nucleosomes led us to test whether the nucleosomes *in vivo* resembled
251 mononucleosomes. To do this, we performed 2-D and 3-D classification of nucleosome
252 template-matching hits (Bharat and Scheres, 2016). The 3-D classes taken from the
253 Volta cryotomograms had the correct size and shape of nucleosomes, but they lacked
254 the characteristic DNA gyres seen in the side view of the low-pass-filtered crystal
255 structure of the mononucleosome (Fig. S12) (Luger et al., 1997). This difference might
256 be due to either the insufficient signal-to-noise ratio of cryo-ET data or the
257 conformational heterogeneity caused by biological factors such as partial unwrapping or
258 both.

DISCUSSION

We have directly visualized chromatin in both interphase and mitotic fission-yeast cells by cryo-ET. The condensation phenotypes are subtle. In both interphase and mitotic cells, most of the nucleosomes are packed into small irregular clusters. While the nucleosome clusters are slightly larger in mitosis, the nearest-neighbor distance does not shorten. In other words, there is no wholesale nucleosome accretion as commonly depicted in textbooks. The most notable -- yet still subtle -- changes are that mitotic chromatin contains fewer megacomplexes *in vivo* and that this chromatin remains more compact than interphase chromatin after being released from lysed cells. We propose a model of mitotic condensation that incorporates both structural and dynamic principles that takes into account recently published counter-intuitive Hi-C results (Fig. 6).

Cryo-ET reveals new insights into higher-order chromatin organisation

One of the long-standing goals of structural cell biology is to determine how a chain of sequential nucleosomes folds within minimally perturbed cells. Such a model requires the visualization of all the linker-DNA segments, but cryo-ET at present can resolve only a tiny minority of this DNA *in vivo*. Despite this limitation, the shortness of fission-yeast linker DNA (Fig. S10) tightly constrains our interpretation of our cryotomograms. Any given nucleosome density should have at least two adjacent nucleosome densities, which correspond to the -1 and +1 sequential positions. Each of these adjacent nucleosomes would be connected via short linker DNAs to another nucleosome, corresponding to -2 and +2 positions relative to the middle nucleosome. This line of

282 reasoning fails for nucleosome-depleted positions, but these are in the minority. This
283 linker-DNA imposed close packing supports our model that nucleosomes form small
284 clusters and possibly small chains.

285 Our 2-D and 3-D classification analyses suggest that chromatin structure is
286 irregular from the level of individual nucleosomes to mitotic chromosomes. Unlike the
287 crystal structure (Luger et al., 1997), nucleosomes *in vivo* are largely conformationally
288 heterogeneous. This heterogeneity may arise from interactions with small proteins,
289 assembly/disassembly intermediates, partial unwrapping, or a combination of all these
290 factors. Out of these possibilities, the partial unwrapping of *S. pombe* nucleosomes *in*
291 *vivo* was predicted nearly a decade ago (Zlatanova et al., 2009). This variability in
292 nucleosome structure and linker DNA length can then give rise to irregular
293 oligonucleosome folding (Wong et al., 2007; Collepardo-Guevara and Schlick, 2014).
294 Furthermore, the rarity of face-to-face nucleosome packing precludes chromatin-fiber
295 interdigitation by nucleosome stacking as a major folding motif. Irregular chromatin is
296 therefore a conserved feature in single-celled eukaryotes (Gan et al., 2013; Chen et al.,
297 2016). A recent study using a new DNA negative-staining method concluded that
298 chromatin structure is also irregular in mammalian cells (Ou et al., 2017), which is
299 consistent with earlier EM studies of other mammalian cells (McDowall et al., 1986;
300 Eltsov et al., 2008; Fussner et al., 2012; Nishino et al., 2012).
301

302 **Cryo-ET, Hi-C, and live-cell imaging paint a complex picture of chromatin**

303 Hi-C is a “proximity-based” method wherein two DNA segments can be detected if they
304 are close enough to chemically cross-link (Lieberman-Aiden et al., 2009). In this

305 interpretation, Hi-C indirectly probes chromatin structure, and is revealing new principles
306 of chromatin organization in *S. pombe* (Mizuguchi et al., 2014; Kakui et al., 2017;
307 Tanizawa et al., 2017). A common Hi-C structural feature is the domain (also called
308 TADs), a contiguous sequence in which nucleosomes are more likely to interact with
309 other nucleosomes within this sequence than outside. *S. pombe* domains range from
310 40kb to 1Mb long, corresponding to 300 - 7,000 sequential nucleosomes. If domains are
311 monolithic globular bodies as universally depicted in the literature, they should be visible
312 in our cryotomograms as large, densely packed nucleosomes clusters. Such a cluster
313 would be 7 - 20 nucleosomes wide, assuming a simple cuboid shape; other globular
314 shapes would be equally large. The nucleosome clusters we saw were generally much
315 smaller. We propose that small nucleosome clusters, which we see at the single-cell
316 level by cryo-ET, correspond to different loci in each cell. If these clusters belong to Hi-
317 C domains, they would sum to the much-larger structure due the effects of population
318 averaging. As the simplest example, we illustrate how four sequential nucleosomes
319 could give rise to a Hi-C structure that is larger than what can be found in individual
320 cells (Fig. S13). Cell-to-cell variation may also explain differences between single-cell
321 and population-based Hi-C studies (Nagano et al., 2013; Flyamer et al., 2017; Nagano
322 et al., 2017; Stevens et al., 2017).
323 Two recent studies unexpectedly reported that short-range Hi-C contacts are less
324 probable in mitotic *S. pombe* cells than in interphase cells (Kakui et al., 2017; Tanizawa
325 et al., 2017), echoing a previous study of mitotic human cells (Naumova et al., 2013).
326 Interestingly, quiescent B cells, which have condensed chromosomes, have lower short-
327 range Hi-C contact probabilities than active B cells, which have decondensed

328 chromosomes (Kieffer-Kwon et al., 2017). If Hi-C detections arise solely from
329 nucleosome-nucleosome proximity, then the nucleosomes should move further apart in
330 mitosis; we observed the opposite. What else, then, could explain the lower short-range
331 Hi-C contact frequency in mitotic cells? A recent study found that within compact (160
332 nm) mammalian chromatin domains, all nucleosome motion is correlated (Nozaki et al.,
333 2017). We propose that short-range Hi-C contacts in *S. pombe* and some mammalian
334 cells is also dependent on chromatin dynamics (Hihara et al., 2012; Kakui et al., 2017).
335 These dynamics slow down in mitotic cells due to the increase in nucleosome cluster
336 size, and lowers the frequency that crosslinkable groups can interact with each other.
337

338 **The role of uneven condensation in transcriptional regulation**

339 The strong correlation between mitotic chromosome condensation and transcriptional
340 repression is well established (Taylor, 1960; Prescott and Bender, 1962). Mechanistic
341 evidence of mitotic repression came from the observation that mitotic condensation is
342 coincident with transcription-factor displacement (Martinez-Balbas et al., 1995). Yet,
343 even though transcription is largely downregulated in mitotic *S. pombe* cells, (Oliva et
344 al.), many cell-cycle-regulated genes actually get expressed more (Rustici et al.; Oliva
345 et al.; Peng et al.). In agreement, our immunofluorescence experiments detected
346 elongating RNA polymerase II in mitotic *S. pombe* chromosomes. We propose that
347 chromatin structure at multiple size scales permits access to regulatory sequences,
348 somewhat independently of dynamics. At the mononucleosome level, many chromatin-
349 regulatory complexes recognize and bind to the nucleosome's face (McGinty and Tan,
350 2015). Because face-to-face nucleosome stacking is exceptionally rare in mitotic cells,

351 this critical surface remains available for interactions. At the level of the chromosome,
352 fewer megacomplexes are interspersed within the densely packed nucleosomes.
353 However, uneven condensation leaves nucleosome-free positions that could remain
354 permissive to the occasional passage or assembly of megacomplexes such as
355 spliceosomes and transcription preinitiation complexes. If uneven chromosome
356 condensation is highly conserved, the mechanisms proposed here could explain the
357 recent observations of chromatin accessibility and transcription in mitotic mammalian
358 cells (Hsiung et al., 2015; Teves et al., 2016; Palozola et al., 2017).

359 **MATERIALS AND METHODS**

360

361 **Cell culture**

362 *S. pombe* cell culture (Forsburg, 2003), G2-phase arrest (Ducommun et al.) and
363 prometaphase arrest (Hiraoka et al.) were performed as previously reported. *nda3-*
364 *KM311* cells were grown overnight in yeast-extract supplemented (YES) medium (30
365 g/L glucose, 5 g/L yeast extract, 225 mg/L adenine, 225 mg/L histidine, 225 mg/L
366 leucine, 225 mg/L uracil) at 30°C with shaking. When the optical density at 600 nm
367 (OD_{600}) reached ~ 0.2, the cultures were cooled by incubation in a 20°C shaker. After a
368 10-hr incubation at this lower temperature, more than 90% of cells were arrested in
369 prometaphase. *cdc25-22* cells were grown in YES medium at 25°C with shaking
370 overnight. When the OD_{600} reached ~ 0.2, the cultures were warmed to 36°C in a water
371 bath and then transferred to a 36°C shaker. After 4 hr, the majority of cells were
372 arrested at G2 phase. *S. japonicus* cells were grown in YES medium at 30°C with
373 shaking overnight until mid-log phase (OD_{600} ~ 0.6) (Yam et al.).

374

375 **Fluorescence microscopy**

376 Cells were stained with 4',6-diamidino-2-phenylindole (DAPI) following a published
377 protocol (Toda et al.). Cells (1 ml) were fixed with 2.5% formaldehyde for 60 min at the
378 restrictive temperature for mutants. The cells were washed twice by centrifugation at
379 1500 *x g* for 1 min and resuspension with distilled water. They were pelleted at 1500 *x g*
380 for 1 min and stained by resuspension in 50 µl PBS, pH 7.4, containing 1 µg/ml DAPI.
381 Ten µl of the sample was then added to a glass slide and imaged using a PerkinElmer

382 Ultraview Vox Spinning Disc confocal microscope (PerkinElmer, Waltham, MA). Images
383 were recorded using a 100x oil-immersion objective.
384
385 **Immunofluorescence**
386 Log-phase cells (10 ml) were fixed with 3.7% formaldehyde for 90 min at 30°C (or at the
387 restrictive temperature for arrested mutants). Cells were then pelleted by centrifugation
388 at 1500 *x g* for 5 min. Cells were then resuspended in 1 ml PEM buffer (0.1 M PIPES
389 pH 6.95, 2 mM EGTA, 1 mM $MgSO_4$). Cells were washed once with 1 ml PEM this way
390 and then resuspended in 1 ml PEMS (1.2 M sorbitol in PEM). Next, cells were
391 spheroplasted for 15 minutes by incubation with lysis-enzyme cocktail (Abcam, cat#
392 ab206997), diluted 1:1000 in PEMS at 30°C. Cells were washed with PEMS. The pellet
393 was then resuspended in PEMS with 1% Triton X-100 and incubated at 22°C for 5 min.
394 Cells were then washed twice with PEM and incubated in PEMBAL (PEM, 1% BSA, 100
395 mM L-Lysine hydrochloride) at 22°C for 1 hour. Cells were then incubated with rabbit
396 anti-RNA polymerase II CTD-repeat YSPTSPS (phospho S2) antibody in PEMBAL
397 (1:1000 dilution) at 22°C for 1 hour. Cells were washed three times with 1 ml PEMBAL
398 and then incubated with Alexa Fluor 488-coupled donkey anti-rabbit antibodies at 22°C
399 for 1 hour. Cells were then washed three times with PEM and resuspended in 50 µl of 1
400 µg/ml DAPI.
401
402 **Self-pressurized freezing**
403 Self-pressurized freezing was performed based on a previous method, with
404 modifications (Yakovlev and Downing, 2011). *S. pombe* cells were grown in YES

405 medium until the OD_{600} reached 0.2 - 0.6. Cells were pelleted by centrifuging at 1500 *x*
406 *g* for 5 min. Concentrated dextran stock (40 kDa, 60% w/v, in YES) was added to the
407 cell pellet as an extracellular cryoprotectant to a final concentration of 30%. The cells
408 were then quick-spun to remove air bubbles and then loaded into a copper tube (0.45 /
409 0.3 mm outer / inner diameters) with a syringe-type filler device (Part 733-1,
410 Engineering Office M. Wohlwend GmbH). The tube was sealed by crimping both ends
411 with flat-jaw pliers. The sealed tube was held horizontally, ~ 3 cm above the liquid-
412 ethane cryogen surface, and then dropped in. The flattened ends of the tube were then
413 removed with a tube-cutting device under liquid nitrogen.
414
415 **Vitreous sectioning**
416 Vitreous sectioning was performed as previously described with modifications (Chen et
417 al., 2016). A perforated- or continuous-carbon grid was coated with 10-nm gold colloids
418 as fiducials for cryotomographic alignment. Gold solution (5 µl at 5.7 x 10^{12} particles/ml)
419 in 0.1 mg/ml BSA was applied to the grid and then air-dried. Frozen-hydrated cells were
420 cut into a 70-nm-thick frozen-hydrated ribbon using a 35° diamond knife (Cryo35,
421 Diatome, Nidau, Switzerland) in a Leica UC7/FC7 cryo-ultramicrotome (Leica
422 Microsystems, Vienna, Austria) at -150°C. Once the ribbon was ~ 3 mm long, the
423 colloidal-gold-coated EM grid was placed underneath the ribbon. To minimize occlusion
424 by grid bars at high tilt during cryotomographic imaging, the grid as aligned so that the
425 ribbon was in between and parallel with the grid bars. The ribbon was then attached to
426 the grid by operating the Crion in “charge” mode for ~ 30 seconds. The grid was stored
427 in liquid nitrogen until imaging.

428

429 **Cell lysis**

430 Log-phase *S. pombe* cells were pelleted by centrifuging at 1500 *x g* for 5 min and then
431 spheroplasted for 15 minutes by incubation with lysis-enzyme cocktail at either 30°C or
432 at the restrictive temperature for mutants. The spheroplasts were pelleted at 1500 *x g*
433 for 2 min at 22°C and then lysed in 20 µl lysis buffer (50 mM EDTA and a 1:1000
434 dilution of protease inhibitor cocktail, Abcam, cat# ab206997) on ice for 15 min.

435

436 **Plunge freezing**

437 Plunge freezing was done using a Vitrobot Mk IV (Thermo, Waltham, MA) operated at
438 4°C with 80% humidity. *S. pombe* cell lysates (4 µl) were applied to a freshly glow-
439 discharged perforated-carbon grid. The grid was blotted once (blot force: 1, blot time: 3
440 seconds) with filter paper (Whatman #1001055) and then plunged into liquid ethane.

441

442 **Cryo-electron tomography**

443 Tilt series were collected using FEI TOMO4 on a Titan Krios cryo-TEM (Thermo,
444 Waltham, MA) operated at 300 KeV and equipped with a field-emission gun, a Volta
445 phase-plate device, and a Falcon II direct-detection camera. Details of the imaging
446 parameters are shown in Table S1. Image alignment, CTF compensation, low-pass
447 filtering, and 3-D reconstruction were all done using the IMOD software package
448 (Kremer et al., 1996; Mastronarde, 1997; Xiong et al., 2009). Note that Volta
449 cryotomograms were not CTF compensated. Cryotomograms were visualized as

450 tomographic slices with 3dmod and as isosurfaces with UCSF Chimera (Pettersen et
451 al., 2004).
452
453 **Template matching**
454 Template matching was done using PEET (Heumann, 2016). A manually-selected
455 subtomogram containing a nucleosome-like particle served as a template. The influence
456 of the surrounding nucleoplasmic densities were suppressed by the application of a
457 spherical mask. A search was performed on a grid with a 10-nm spacing within the
458 nucleus. Template-matching hits within 6 nm were considered as duplicates and the
459 extra hit was automatically removed. The cross-correlation coefficient cutoff criteria
460 were the same as in our previous report (Cai et al., 2017). Briefly, the average cross-
461 correlation coefficients of all template-matching hits was used as an initial cutoff. This
462 cutoff was then manually adjusted (if needed) to minimize the number of detectable
463 false positives and false negatives. To further remove false positives from
464 megacomplexes, another round of template matching was performed using a ribosome-
465 like density selected within the tomogram as a template. The nucleosome hits that were
466 within 12.5 nm of these megacomplexes were then removed with a Matlab script
467 (available on request; Mathworks, Natick, MA).
468 For dinucleosome template matching, a manually-selected subtomogram of a di-
469 nucleosome served as a template. A cylindrical mask (15-nm diameter, 22-nm height),
470 rotated to align with the long axis of the dinucleosome template, was used to suppress
471 the background surrounding densities. The search grid with a 25-nm spacing was
472 generated within the nucleus. Owing to the missing-wedge artifact, nucleosomes have

473 higher resolution in the X-Y plane. Therefore, the angular search was limited only to the
474 Z axis. Hits within 15 nm were considered as duplicates. The cross-correlation
475 coefficient cutoff criteria were the same as described above for mononucleosomes.
476 False positives from megacomplexes fell into their own classes in the subsequent 2-D
477 classification run (see below) and were removed.
478
479 **Reference-free 2-D and 3-D classification**
480 Subtomogram classification and 3-D averaging of nucleosomes were done in RELION
481 1.4 and RELION 2.0 (Scheres, 2012a, b; Bharat et al., 2015; Kimanius et al., 2016),
482 following the workflow of our previous study (Cai et al., 2017). The template-matching
483 hit coordinates were imported into RELION and then particles were extracted with a
484 15.6-nm box and masked with a 13.5-nm-diameter sphere. For 2-D classification, the
485 number of classes was set to 50 and the resolution of was limited to 3 nm. All
486 nucleosome-like classes were selected. For 3-D classification, the number of classes
487 was set to between 10 and 20, and the resolution was limited to 2-3 nm. Two to three
488 rounds of 3-D classification were performed. False-positive were manually removed in
489 between rounds.
490 For di-nucleosome 2-D classification, the box size and the mask diameter were
491 set to 30 nm and 26 nm, respectively. As a default setting, RELION produces
492 projections of all densities within the boxes, including the nucleoplasmic densities above
493 and below dinucleosomes. To include only the dinucleosome densities, a python script
494 (available on request) was used to generate projections from a 17.5-nm thick
495 tomographic slice. The resolution of the data was limited to 2 nm to suppress the effects

496 of high-resolution noise. The number of classes was set to 100, but many of the classes
497 were very similar and therefore merged.
498
499 **Nearest-neighbor distance analysis**
500 Nearest-neighbor distance analysis of template-matching hits (Figs. 5 and S9) was
501 performed as previously described (Cai et al., 2017). The coordinates of the
502 nucleosome hits were imported into Matlab. NND and 10th NND were calculated using
503 the Matlab function nearestneighbour.m (custom script available on request).
504
505 **Statistical analysis**
506 Two-tailed t-tests for NND values were performed in Excel using the TTEST function.
507 The number of NND values analysed for G2-phase and prometaphase *S. pombe*,
508 interphase and mitotic *S. japonicus* were 10292, 11835, 9397, 4474, respectively.
509
510 **Data sharing**
511 A cryotomogram, corresponding to Fig. 4, was deposited in the EMDataBank as EMD-
512 6846. The tilt series for all cryotomograms presented in this manuscript were deposited
513 in the Electron Microscopy Public Image Archive as EMPIAR-10125.
514
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Contributions

S.C - experiments, project design, writing, CC - training, YH - training, ZYT - experiments, JS - training, LG - project design, writing.

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734

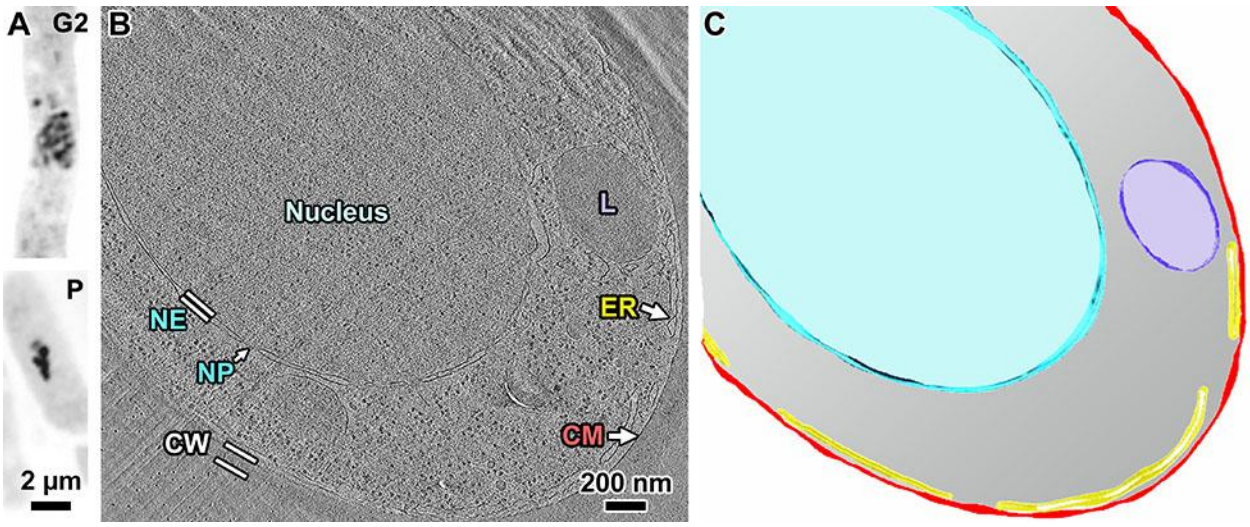
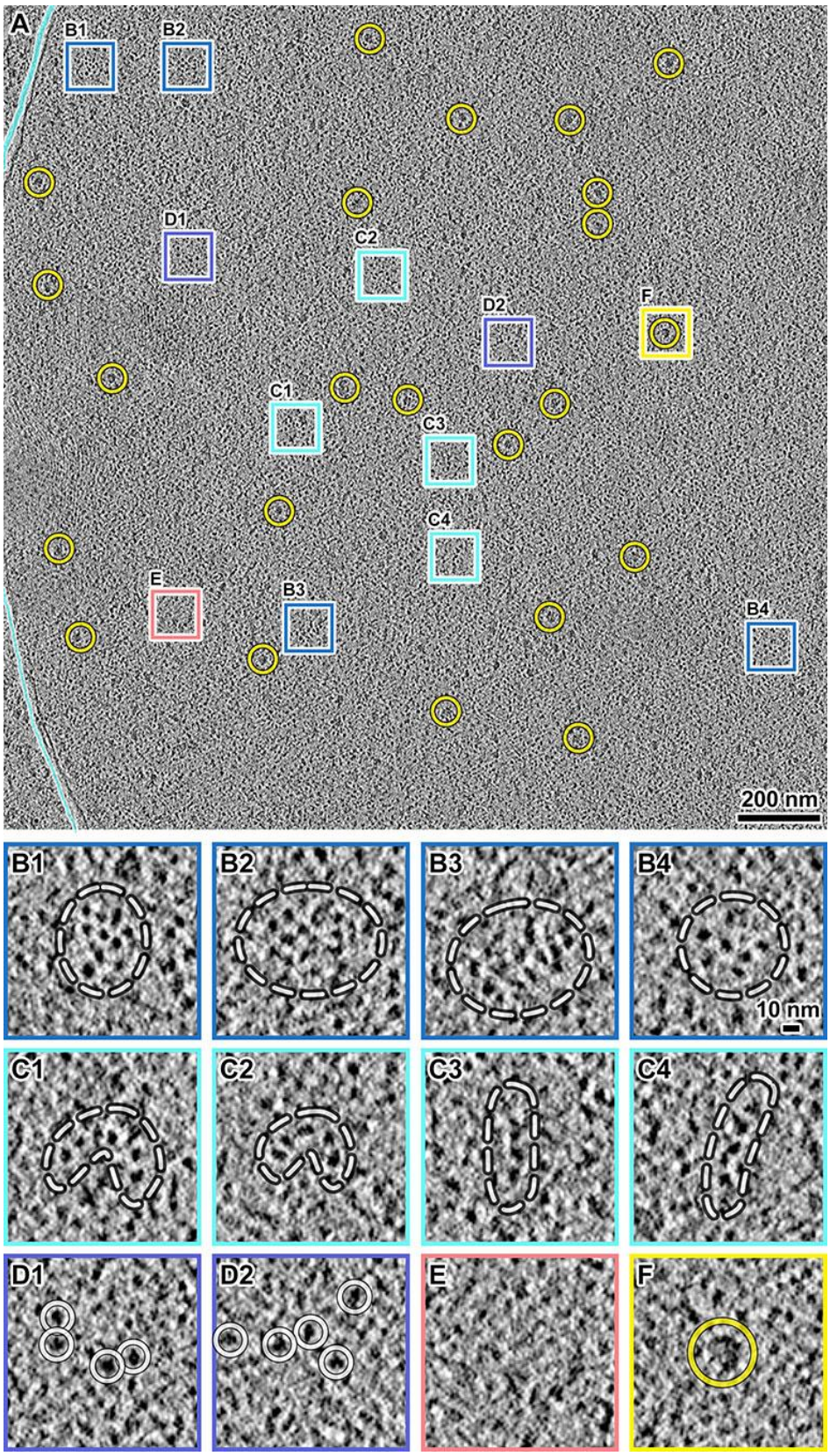


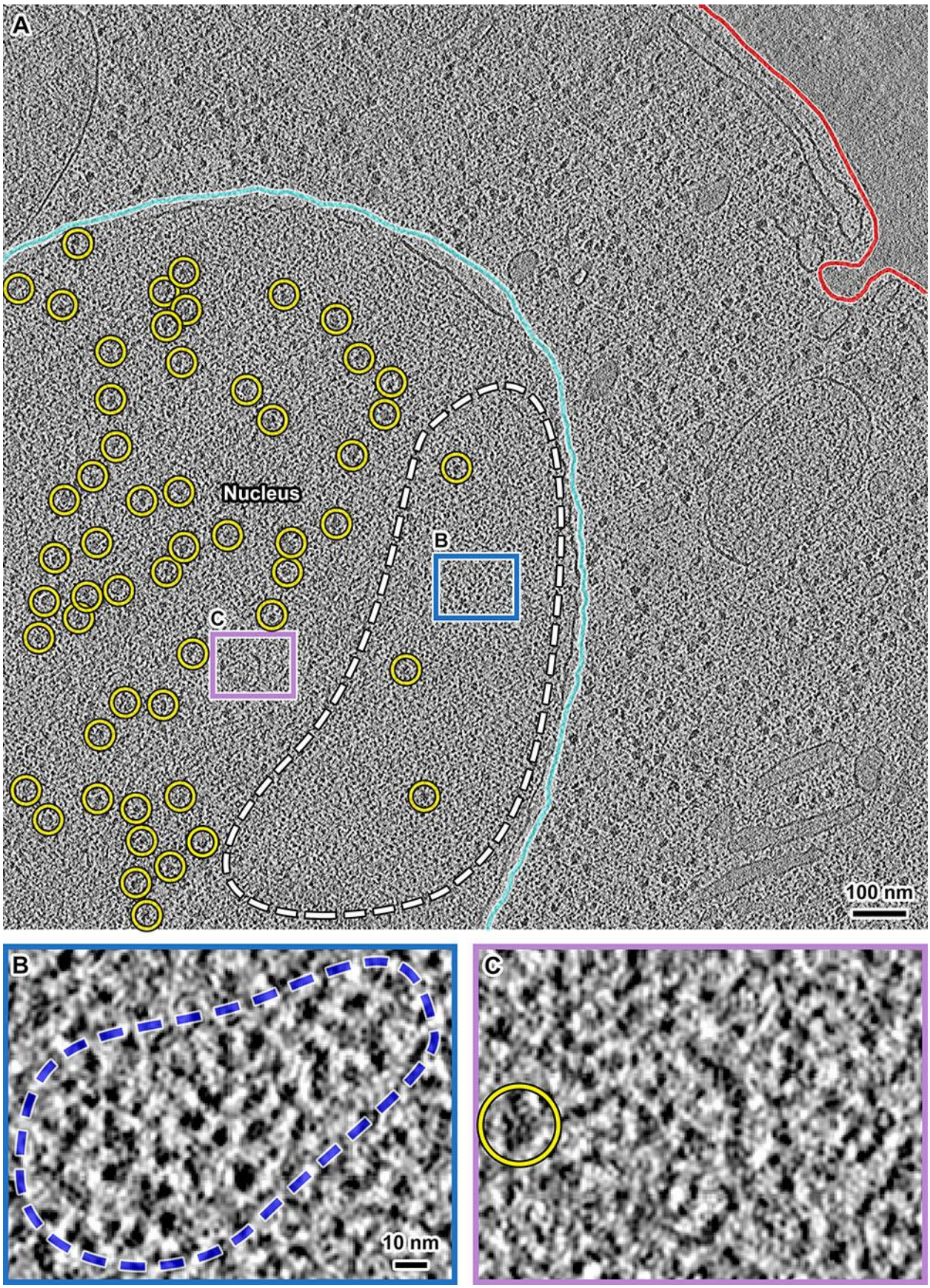
Figure 1. Strategy to study chromosome condensation in *S. pombe*.

(A) Fluorescence images of DAPI-stained G2-phase and prometaphase (P) cells, with contrast inverted for clarity. (B) Cryotomographic slice (25 nm) of a G2-phase cell. NE: nuclear envelope; NP: nuclear pore; L: lipid body; ER: endoplasmic reticulum; CM: cell membrane; CW: cell wall. The wavy features in the upper portion of the cryotomogram are crevasses from sectioning; the thin parallel lines running from 2 to 8 O'clock are knife marks. (C) Segmentation of the cryotomographic slice in panel B, showing the cytological features with the same color scheme as the text labels from panel A.


A
B1
B2
D1
C2
D2
F
C1
C3
C4
E
B3
B4
200 nm
B1
B2
B3
B4
10 nm
C1
C2
C3
C4
D1
D2
E
F

744

745 **Figure 2. *S. pombe* G2-phase chromatin consists of irregularly packed**
746 **nucleosomes and dispersed megacomplexes.**
747 (A) Cryotomographic slice (10 nm) of an interphase nucleus. Yellow circles: a subset of
748 megacomplexes. The cyan lines denote the nuclear envelope. (B1 - B4) Four examples
749 of nucleosome clusters. (C1 - C4) Four examples of nucleosome chains. In panels B
750 and C, the white dashed lines delineate the approximate boundaries of the nucleosome
751 clusters. Owing to the close packing and particulate nature of nucleosomes, it is not
752 possible to annotate the exact “outline” of a nucleosome cluster. It is also not possible to
753 determine how the nucleosomes are connected by linker DNAs, which generally cannot
754 be seen. (D1 - D2) Two examples of loosely packed nucleosomes, which are circled.
755 (E) An example position that has few nucleosomes. (F) An example megacomplex
756 (circled).



757

758 **Figure 3. Condensed *S. pombe* mitotic chromosomes contain few**

759 **megacomplexes.**

760 (A) Cryotomographic slice (11 nm) of a prometaphase cell. Cyan line: nuclear envelope,

761 red line: plasma membrane, yellow circles: megacomplexes. The position circled with

762 the dashed white line is a condensed chromosome. Rectangular boxes are enlarged 6-

763 fold in panels B and C. (B) An example position with many nucleosome-like densities

764 forming a cluster, circled with a dashed blue line. (C) An example position without

765 nucleosome-like densities. Yellow circle: a megacomplex.

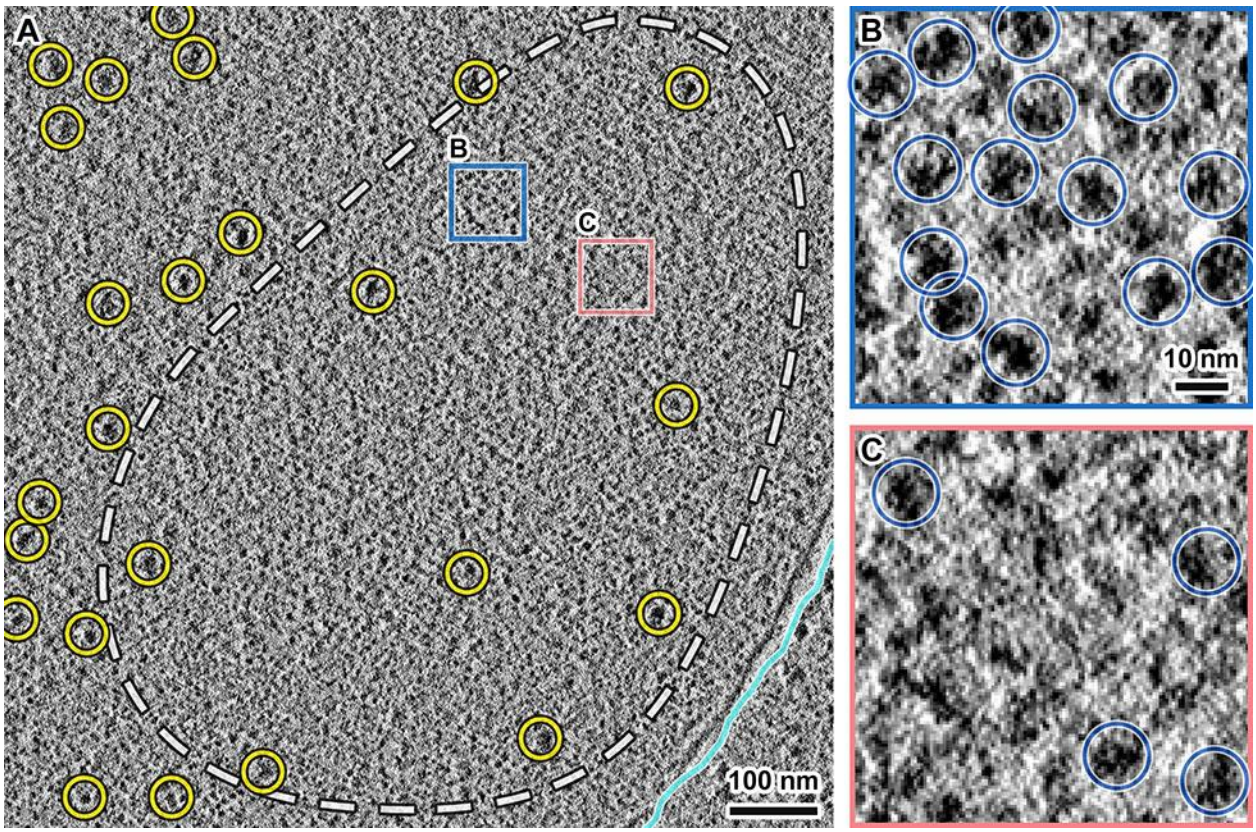


Figure 4: Larger nucleosome clusters and loosely packed nucleosomes coexist within *S. pombe* mitotic condensed chromosomes.

(A) Cryotomographic slice (11 nm) of a nucleus in a prometaphase cell, imaged with Volta phase contrast. White dashed line: approximate chromosome boundary. Yellow circles: megacomplexes. The positions in the blue and salmon boxes are enlarged six-fold in panels B and C, respectively. (B) A position within the mitotic chromosome that contains many closely packed nucleosomes. (C) A position within the mitotic chromosome that contains fewer, loosely packed nucleosomes. Blue circles: nucleosomes.

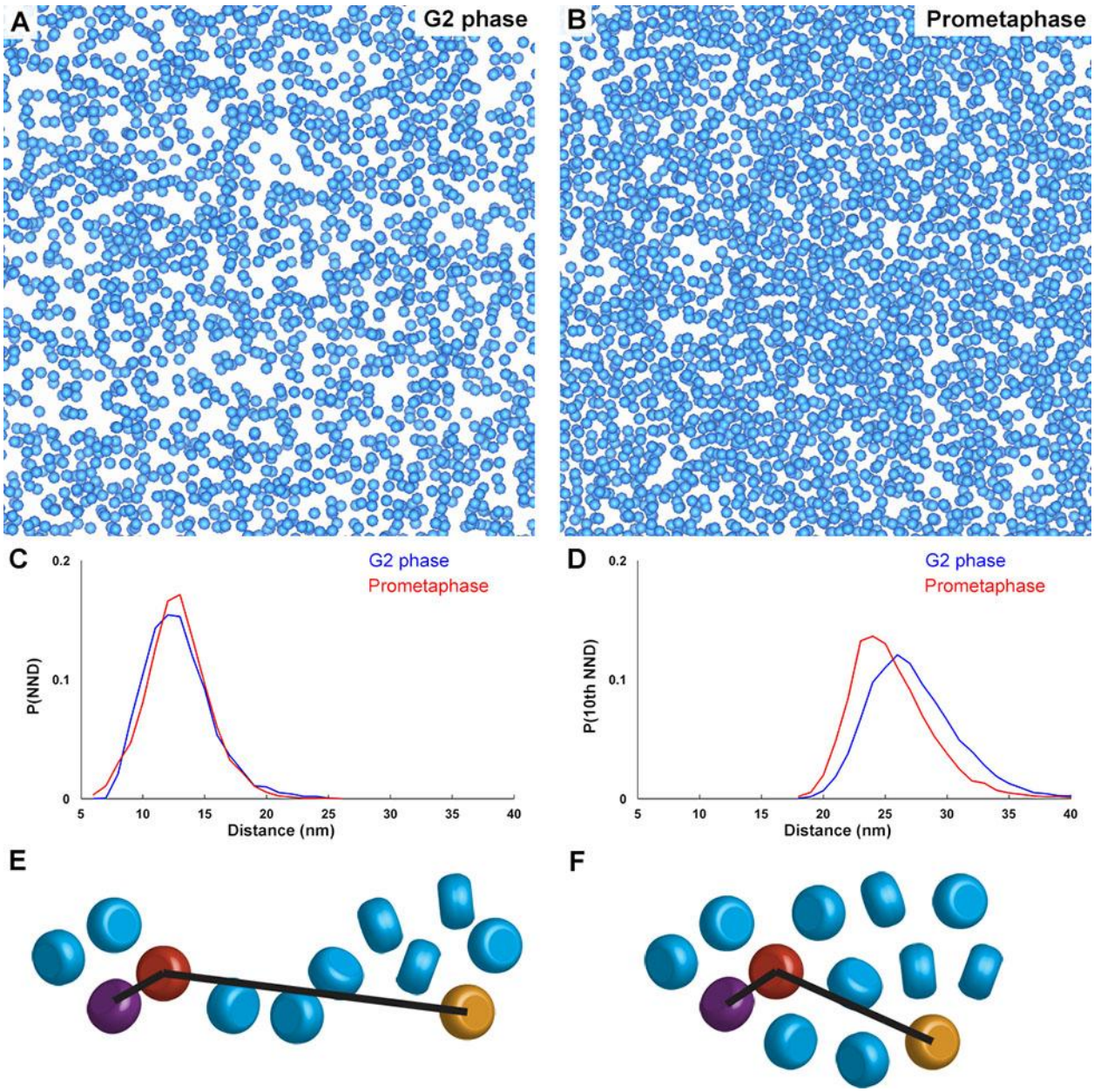


Figure 5: *S. pombe* nucleosome clusters are larger in prometaphase but have the same local packing density.

(A and B) Template-matching hits of nucleosome positions (blue spheres) in Volta cryotomograms of a G2-phase (A) and a prometaphase (B) cell. (C) Nearest-neighbor distance (NND) analysis of template-matching hits. X-axis: NND, 1-nm bins, Y-axis:

783 normalized probability. (D) Tenth NND analysis of template-matching hits. The X- and
784 Y- axes are the same as in panel C. (E and F) Cartoons showing how a small (E) and a
785 large cluster (F) of nucleosomes (rounded cylinders) could have the same NND, but
786 different 10th NND. In each panel, the purple and orange nucleosomes are the
787 respective nearest and 10th-nearest neighbors of the red nucleosome. These cartoons
788 do not show linker DNA, most of which cannot be seen in the Volta cryotomograms.

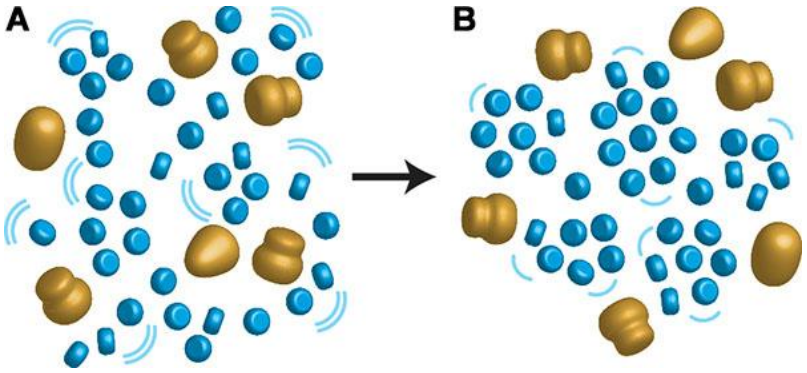


Figure 6: Mitotic chromatin is slightly more compact and less dynamic. (A) Interphase chromatin consists of both small nucleosome clusters and loosely packed nucleosomes. Blue pills: nucleosome; large gold bodies: megacomplexes. Megacomplexes (pre-ribosomes, spliceosomes, transcription preinitiation complexes) are interspersed between the nucleosomes and nucleosome-free pockets. (B) In condensed mitotic chromosomes, most nucleosomes form large clusters, thereby excluding or inhibiting the assembly of megacomplexes. Furthermore, chromatin dynamics (magnitude proportional to the size and number of the curved lines) also decrease. In both interphase and mitotic chromatin, ordered motifs such as face-to-face nucleosome-nucleosome interactions are largely absent.

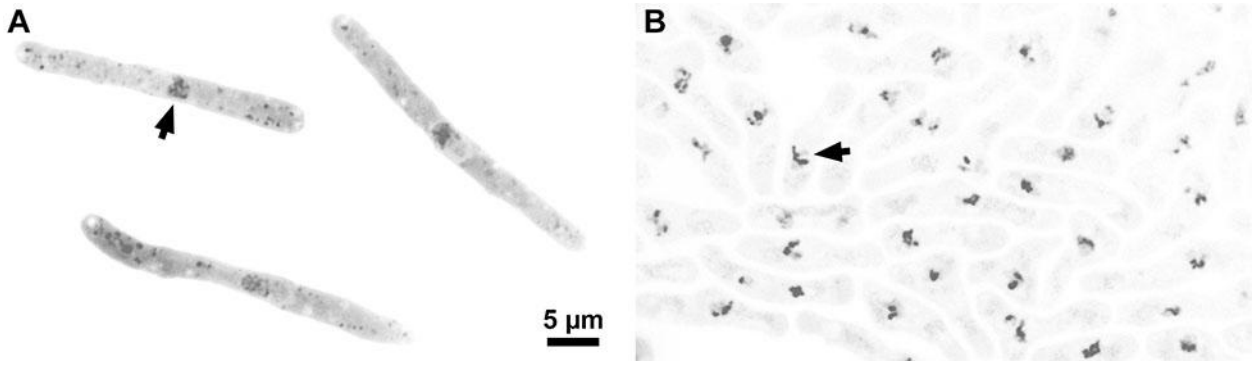


Figure S1: Synchronisation of *S. pombe*.

(A and B) Fluorescence micrographs of DAPI-stained G2-phase (A) and prometaphase (B) cells. The contrast is inverted for clarity. Arrows point to an example nucleus in each panel.

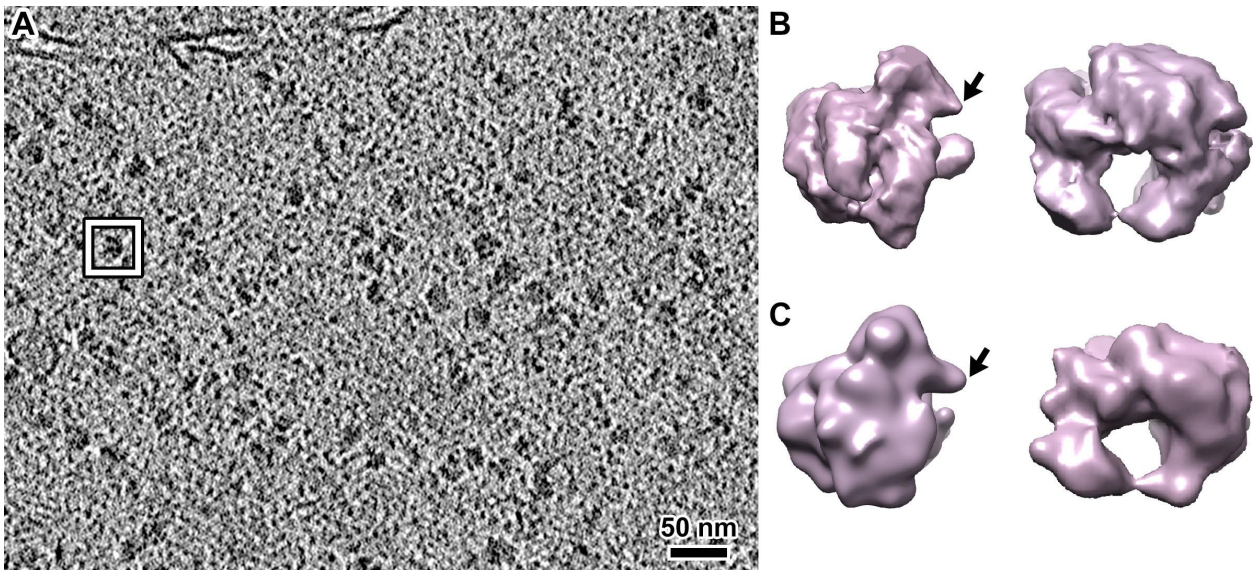
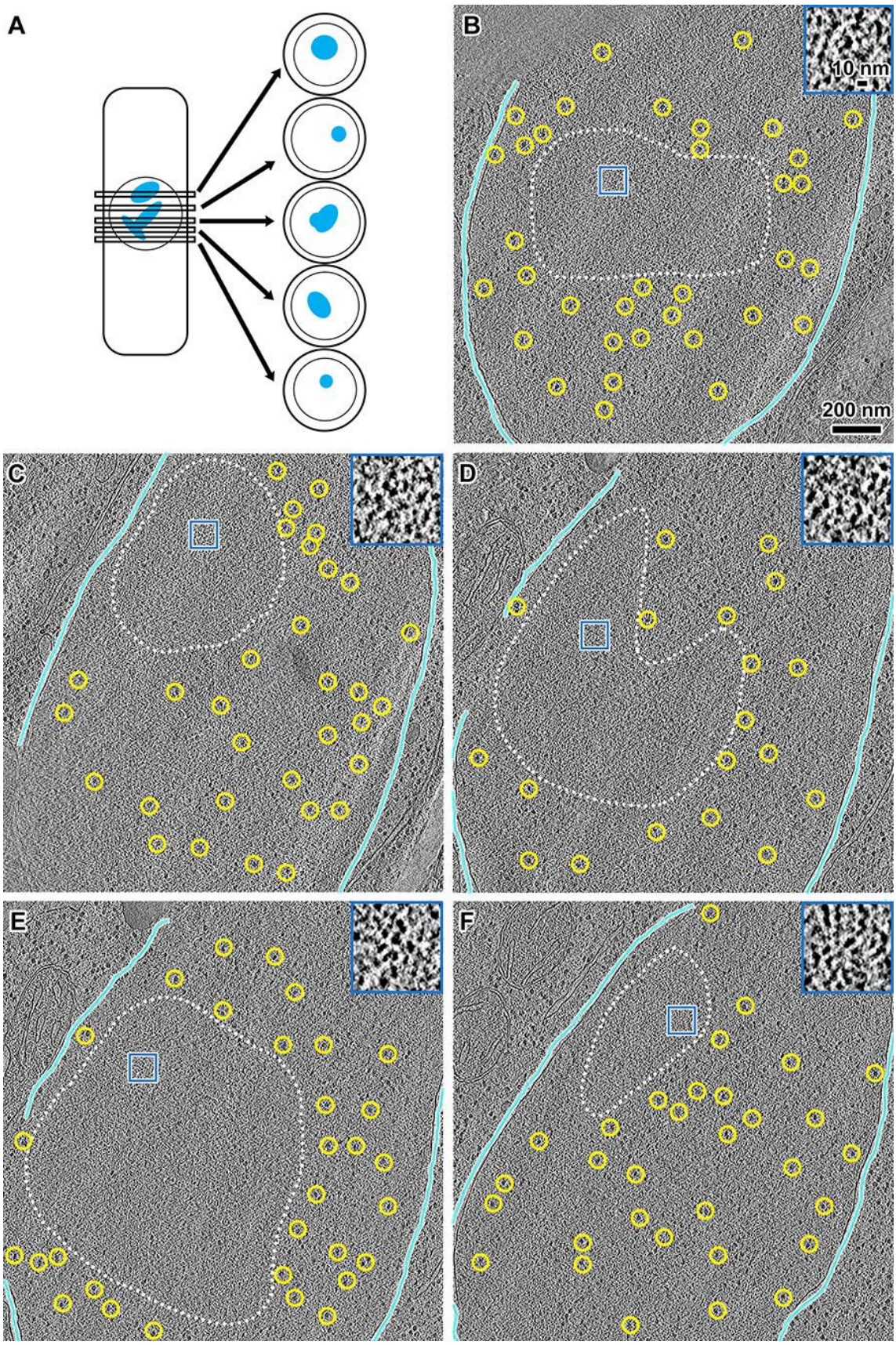


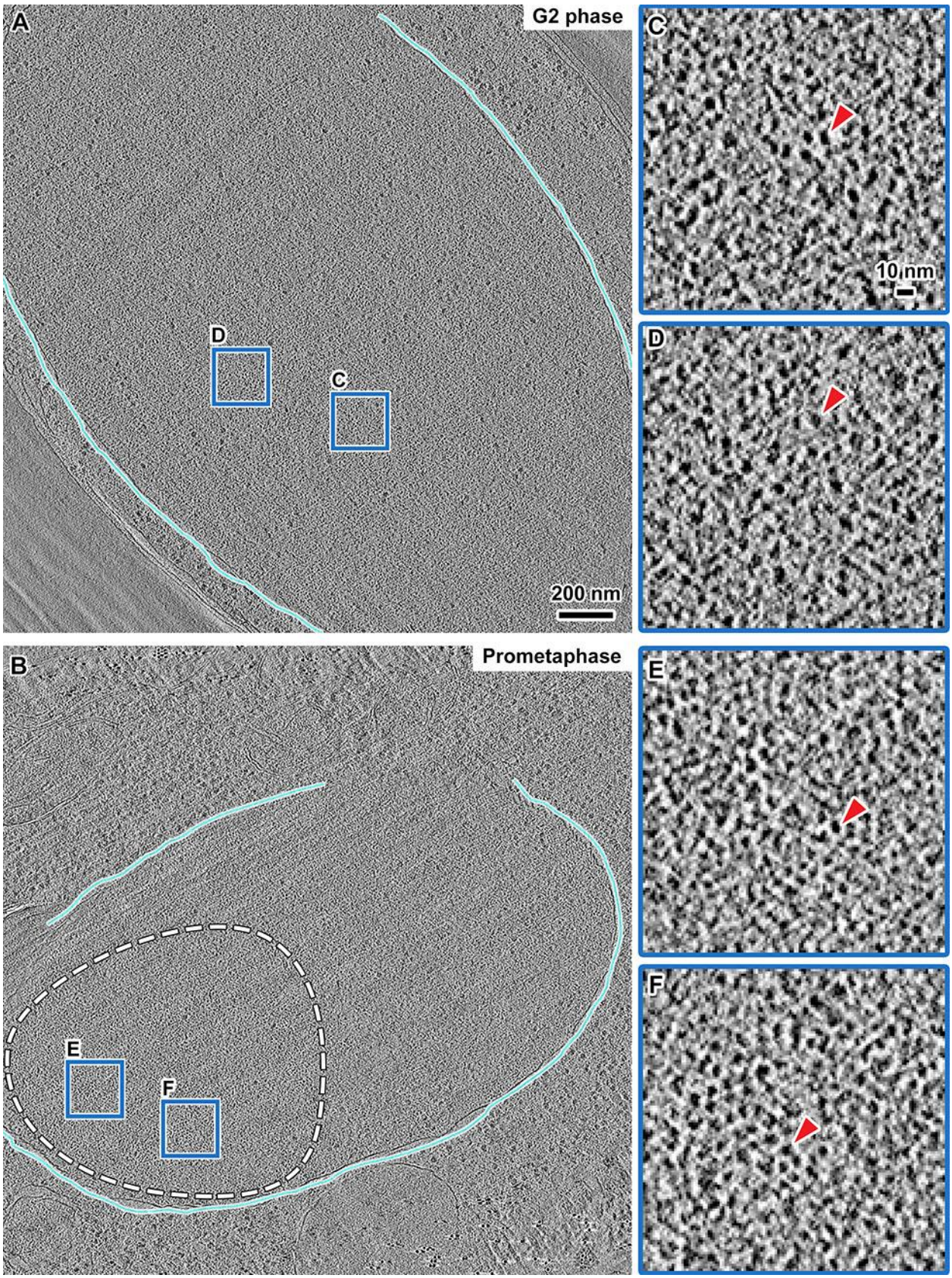
Figure S2. Cryotomograms reveal molecular detail *in vivo*.

(A) Cryotomographic slice (25 nm) of a *S. pombe* cytoplasm position that contains many ribosomes, from the cell shown in Fig. 1B and C. (B) Subtomogram average of cytoplasmic ribosomes. (C) Crystal structure of *S. cerevisiae* 80S ribosome (PDB 4V88; (Ben-Shem et al., 2011)), low-pass filtered to 50 Ångstroms resolution. In panels B and C, the left image is related to the right image by a 90° rotation around the vertical axis, followed by a 180º rotation around the horizontal axis. Notice that in the orientation presented on the left, the “beak” motif (arrow) points to the right in both the simulated density map and the subtomogram average, indicating that the cryotomogram has the correct handedness. The two maps are not expected to be identical because the cytoplasmic ribosome map is an average of multiple conformational states.



818

819 **Figure S3. Serial cryotomography reveals the location of mitotic chromosomes.**
820 (A) A cartoon showing nearly serial cryosections of a cell. Some cryosections were not
821 imaged due to ice contamination, damage, grid-bar blockage, etc. (B - F)
822 Cryotomographic slices (10 nm) of nearly serial cryosections from a single
823 prometaphase cell. Cyan lines delineate the nuclear envelope; there are gaps where the
824 nuclear envelope densities were ambiguous. Dotted lines: approximate boundaries of
825 condensed chromosomes. Yellow circles: examples of megacomplexes. For clarity, the
826 few megacomplexes inside the dotted lines lines are not annotated. Inset: four-fold
827 enlargements of blue boxes.



828

829 **Figure S4. An example of nucleosome reorganisation in prometaphase cells.**

830 (A and B) Cryotomographic slices (11 nm) of nuclei in a G2-phase (A) and a

831 prometaphase (B) cell. The cyan lines denote the nuclear envelope; there are gaps

832 where the nuclear-envelope densities were ambiguous. Dashed circle in panel B:

833 approximate outline of a condensed chromosome. (C - F) Six-fold enlargements of

834 intranuclear positions that are enriched in nucleosomes, from the corresponding boxes

835 in panels A and B. Red arrowheads point to nucleosomes.

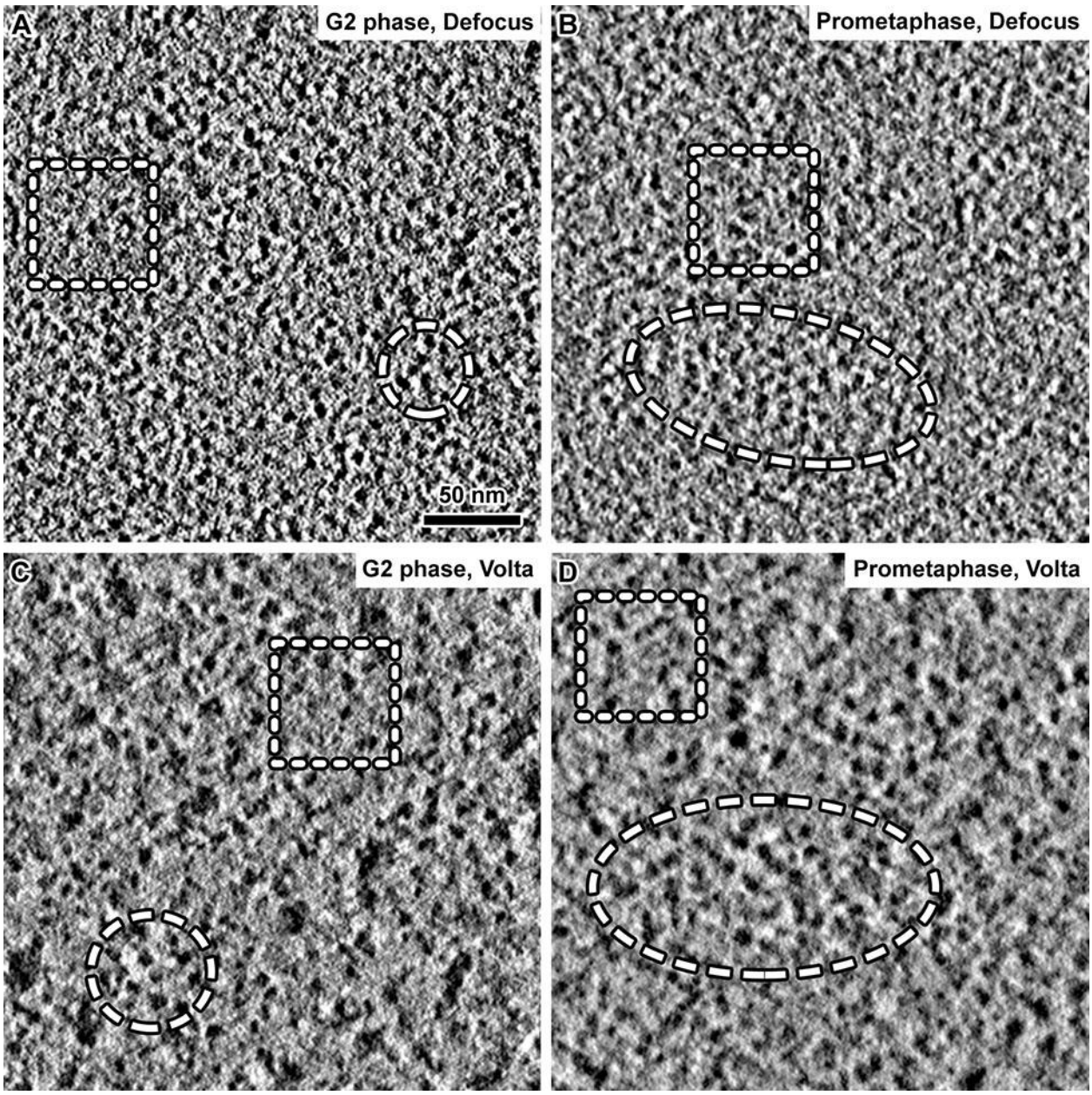


Figure S5. Nucleosome reorganisation is distinguishable in both defocus and Volta cryotomograms.

(A and C) Cryotomographic slices (10 nm) of positions in G2-phase chromatin imaged with defocus (A) and Volta (C) phase contrast. (B and D) Cryotomographic slices (10 nm) of positions in prometaphase chromatin imaged with defocus (B) or Volta (D) phase

842 contrast. Dashed circles: densely packed nucleosomes. Dashed squares: loosely-

843 packed nucleosomes.

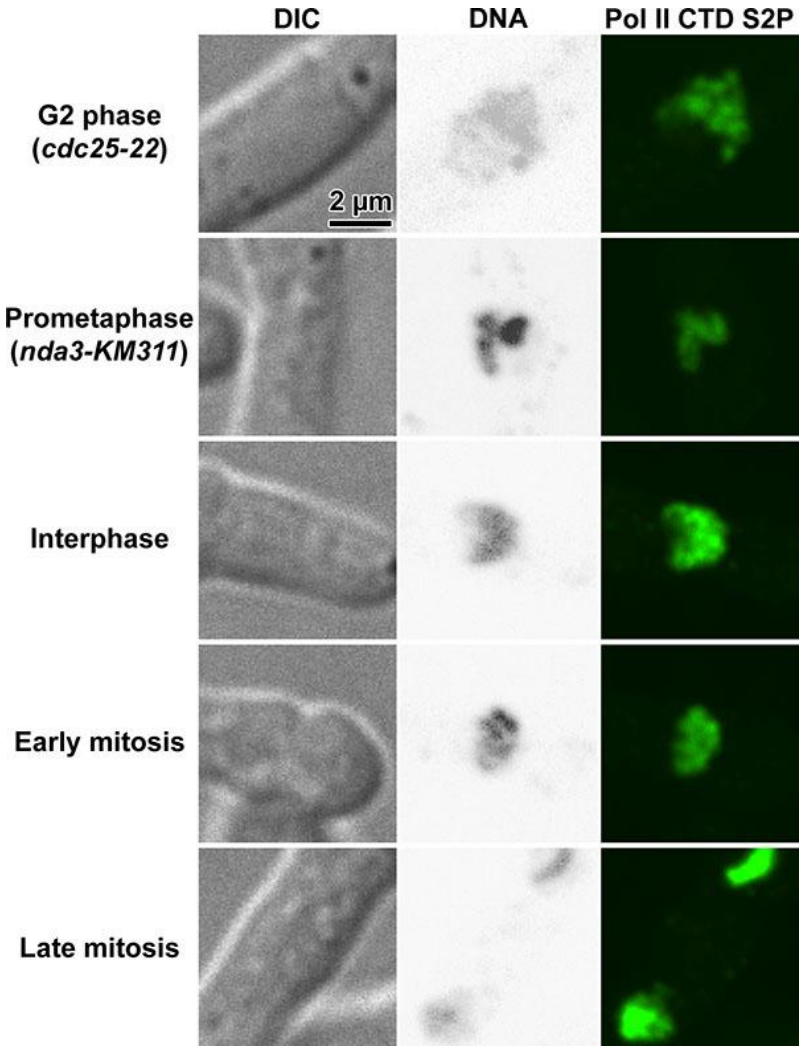


Figure S6. Transcription is not completely repressed during mitosis.

DIC and fluorescence images of G2-phase, prometaphase and unsynchronized wild type cells. Left: DIC; Middle: DAPI-stained DNA with inverted contrast; Right: immunofluorescence of RNA polymerase II C-terminal domain repeat, phosphorylated at Serine 2 (Pol II CTD S2P). Top to bottom: *cdc25-22* cell arrested in G2 phase, *nda3-KM311* cell arrested in prometaphase, unsynchronized cell in interphase, unsynchronized cell in early mitosis, unsynchronized cell in late mitosis. In the unsynchronized cultures, the cell-cycle states were determined based on the chromosome morphology.

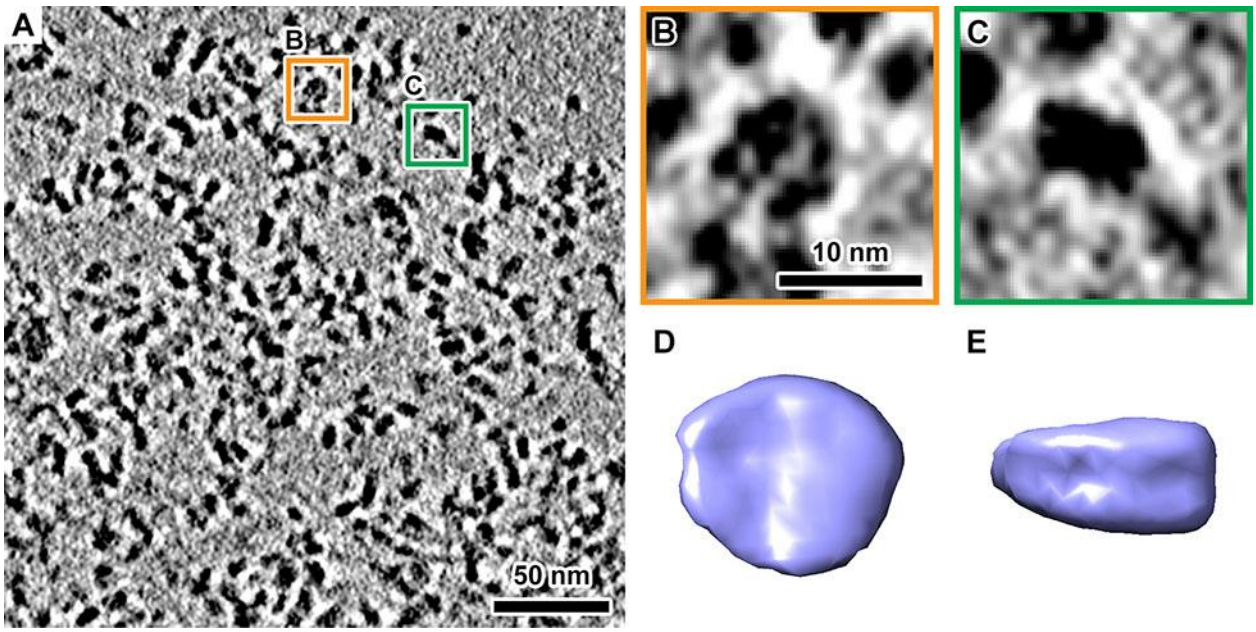


Figure S7. Nucleosomes remain clustered in cell lysates.

(A) Cryotomographic slice (11 nm) showing the chromatin released from unsynchronised *S. pombe* cells. Nucleosomes oriented face-on (orange box) and side-on (green box) are enlarged 5-fold in panels B and C, respectively. (D and E): Face- and side-on views of a nucleosome subtomogram average of particles template-matched from the cryotomogram shown in panel A. Notice that DNA gyres are visible in the side view.

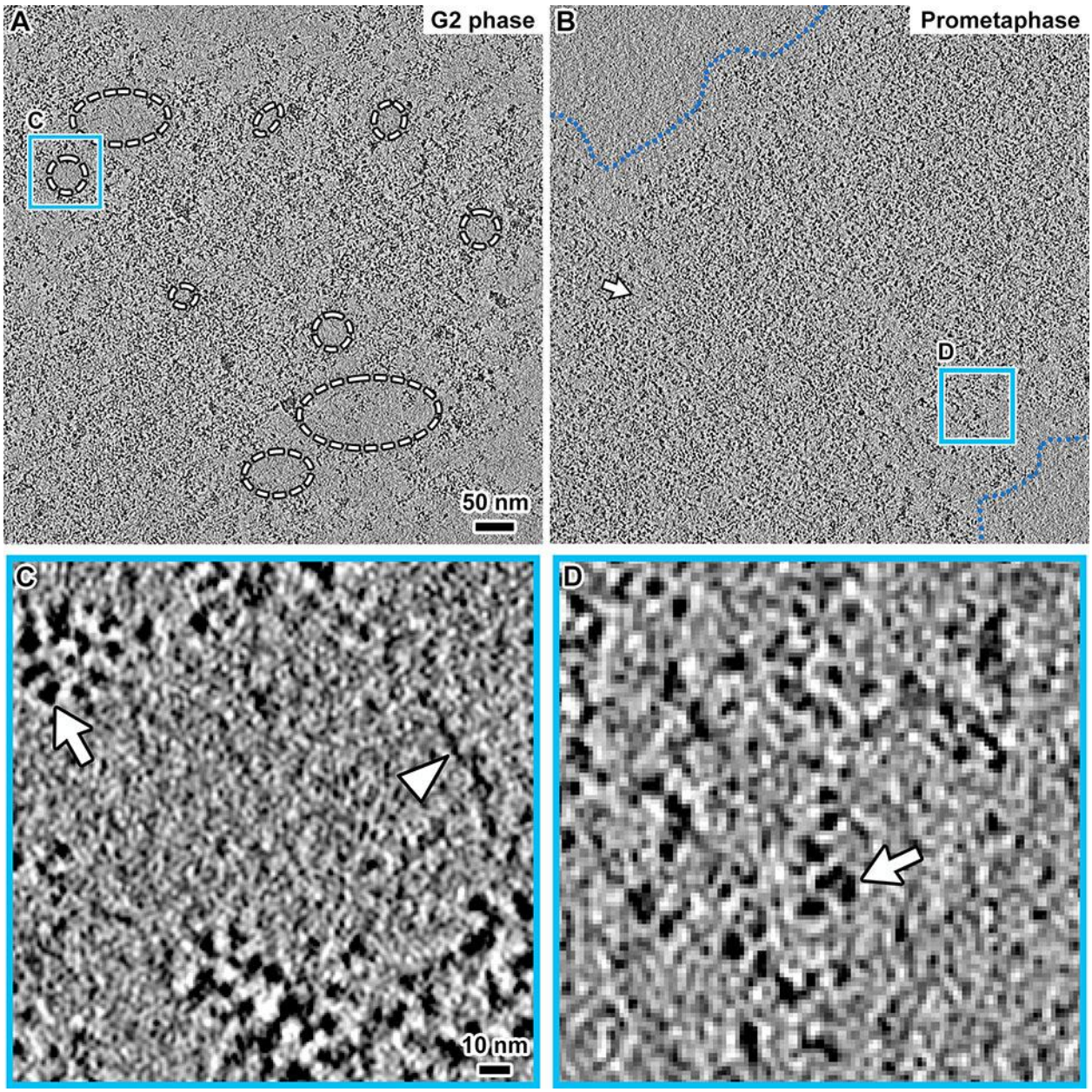
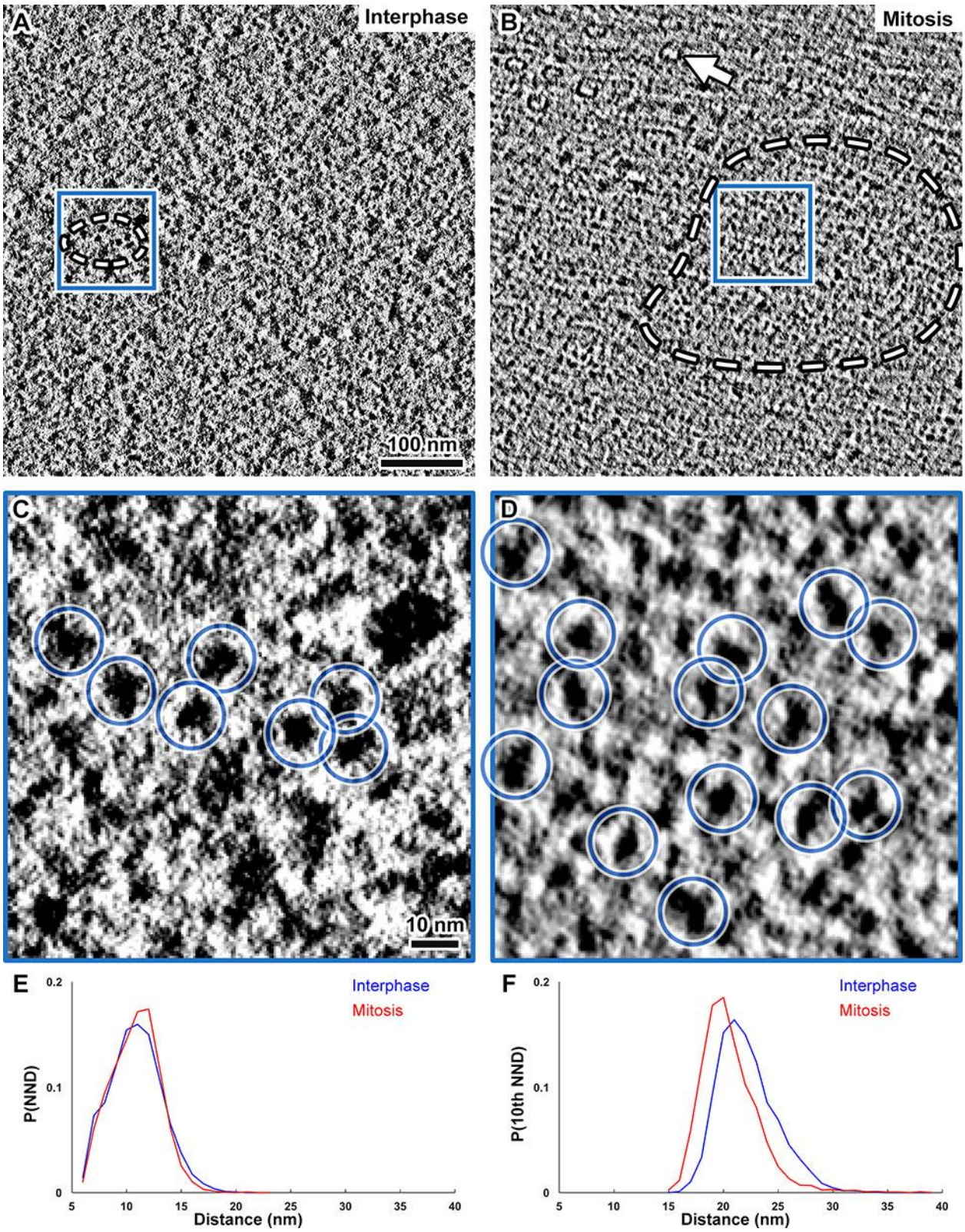


Figure S8. *S. pombe* prometaphase chromatin is also more compact than G2-phase chromatin *in vitro*.

(A and B) Cryotomographic slices (30 nm) of chromatin from G2 phase (A) and prometaphase (B) cell lysates. Dashed circles in panel A: examples of large nucleosome-free gaps. Blue dotted lines in panel B: boundaries of chromatin mass. Arrow in panel B: examples of small nucleosome-free gap. (C and D) Four-fold

869 enlargement of blue boxes in panels A and B, respectively. Arrows: nucleosomes.

870 Arrowhead: DNA.



871

872 **Figure S9. *S. japonicus* nucleosomes also pack into larger clusters in mitosis.**

873 (A and B) Volta cryotomographic slices (11 nm) of *S. japonicus* intranuclear positions in
874 an interphase (A) and a mitotic (B) cell. Dashed circles: nucleosome clusters. Arrow in
875 panel B: a spindle microtubule. (C and D) Five-fold enlargements of nuclear positions
876 that have nucleosomes in panels A and B. Nucleosomes are circled in blue. (E)
877 Nearest-neighbor and (F) tenth nearest-neighbor distance analyses of nucleosome
878 template-matching hits.

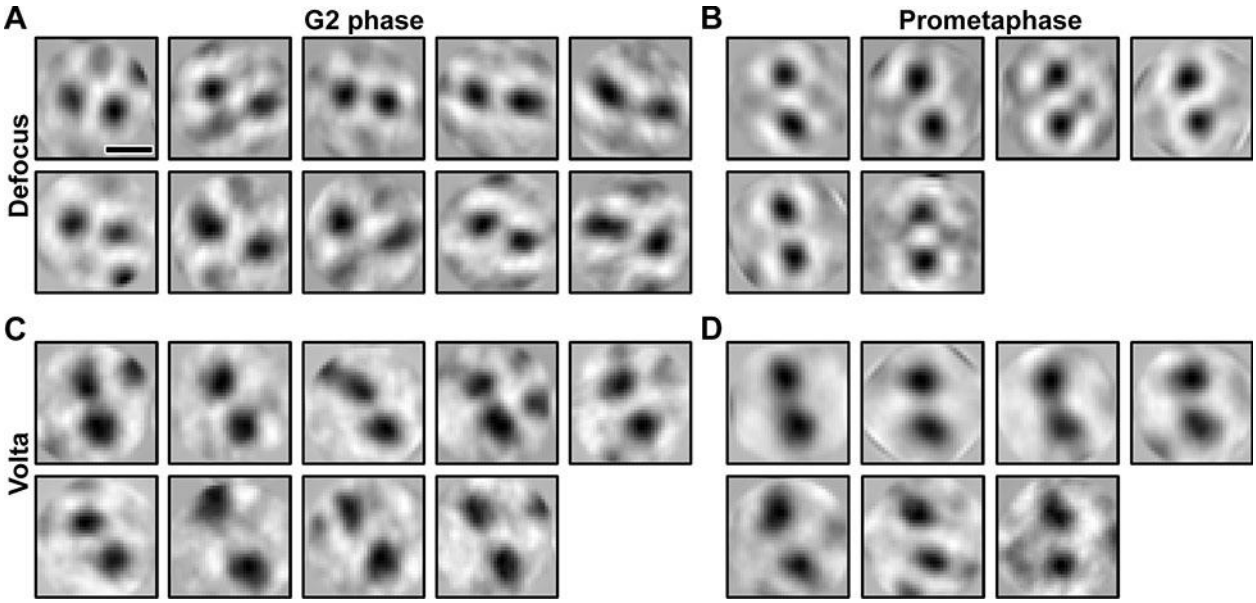


Figure S10. *S. pombe* nucleosomes do not stack face-to-face in either G2-phase or mitotic chromosomes.

(A and C) Two-dimensional classification of dinucleosome template-matching hits in defocus (A) and Volta (C) cryotomograms of G2-phase cells. (B and D) Two-dimensional classification of dinucleosome template-matching hits in defocus (B) and Volta (D) cryotomograms of prometaphase cells. Note that the 2-D classes showing megacomplexes (false positives) or tri-nucleosomes are not shown. Also note that some class averages, e.g., upper-left one in panel A, have a weak third density. This additional density may be from a lower-occupancy nucleosome position or a nucleosome that was truncated by the tomographic slice because it was not in the same exact plane as the other two. Scale bar in panel A: 10 nm.

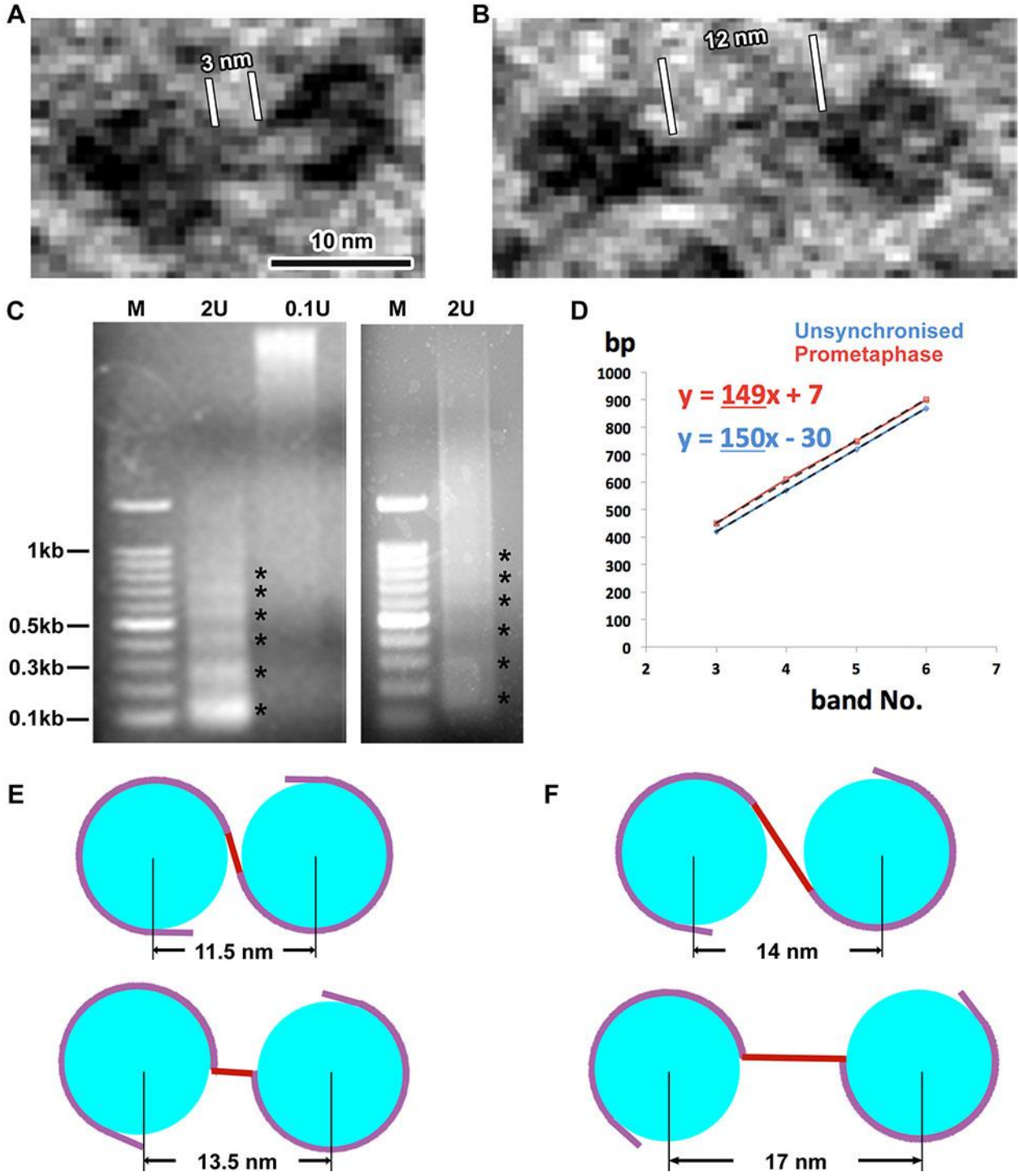
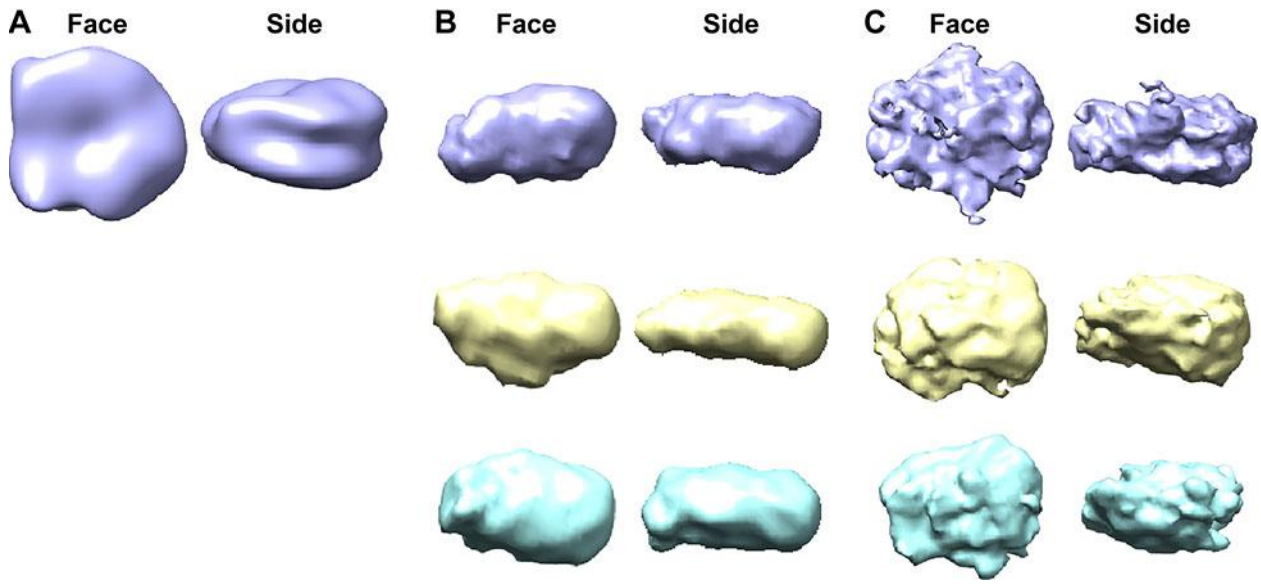


Figure S11. A subset of nucleosomes might be partially unwrapped *in vivo*.

(A and B) Volta cryotomographic slices (10 nm) of a di-nucleosome-like density in a *S. pombe* G2-phase cell, showing densities consistent with short (A) and long (B) linker

895 DNA. (C) The chromatin in unsynchronised (left; most cells are in G2-phase) and
896 prometaphase (right) cells were fixed and then digested with 2 units or 0.1 units of
897 MNase. DNA bands corresponding to mono- and oligonucleosomes are indicated by
898 asterisks. (D) Nucleosome-repeat length plot of oligomer band number vs. base pairs.
899 The monomer and dimer were excluded from the linear regression because the
900 'nibbling' of linker DNA at the ends would cause an overestimate of the slope and
901 therefore, the nucleosome repeat length. (E and F) Cartoons showing fully (E) and
902 partially (F) wrapped di-nucleosomes, drawn to scale for the nucleosome diameter and
903 DNA length (but not DNA thickness). Red: *S. pombe* linker DNA. For the fully wrapped
904 nucleosomes, the linker DNA is 2.5 nm (7 bp) long. For the partially wrapped example
905 (10 bp unwrapped from the end of each nucleosome, the linker DNA becomes 6 nm
906 long). The upper and lower panels show the extreme examples that minimize (upper) or
907 maximize (lower) the inter-nucleosome separation for a given linker length. Upper
908 panels: straight linker DNA. Lower panels: linker DNA bent at an energetically
909 unfavorable ring angles at the entry/exit site.



910

911 **Figure S12. 3-D class averages of nucleosome-like densities do not reveal high-**

912 **resolution mononucleosome-like features.**

913 (A) A simulated density map of a mononucleosome crystal structure (PDB 1ID3; (White

914 et al., 2001)), low-pass filtered to 40 Ångstroms resolution. (B and C) Isosurface

915 renderings of representative 3-D class averages of template-matching hits in the nuclei

916 of a defocus (B) and a Volta (C) cryotomogram.

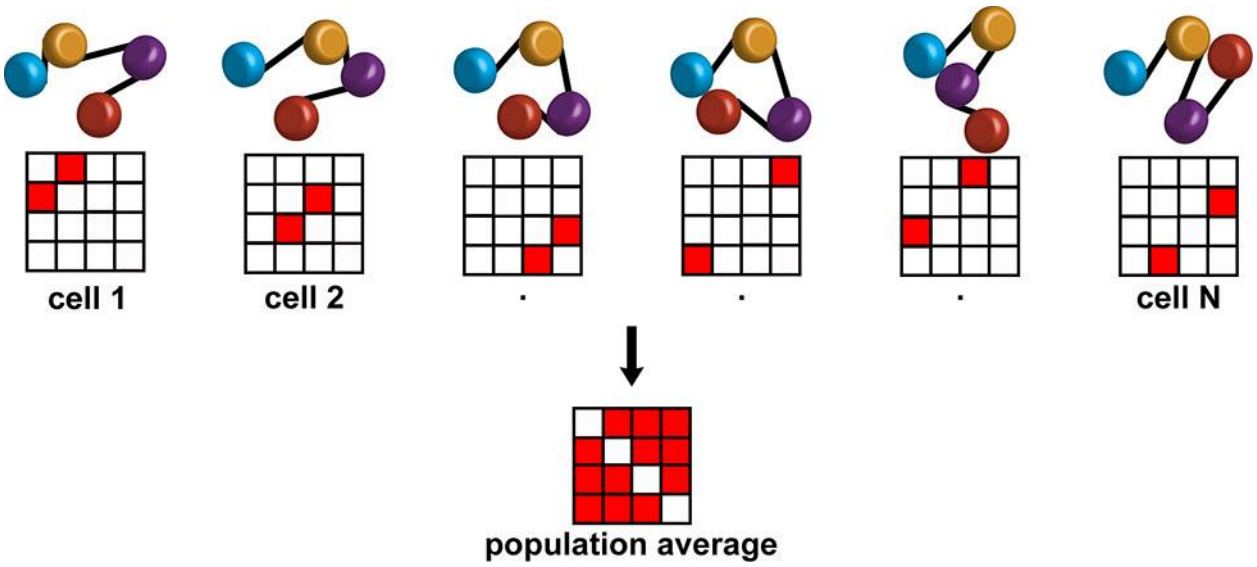


Figure S13. Reconciliation of *S. pombe* cryo-ET and Hi-C chromatin models. The blue, orange, purple and red rounded cylinders represent four sequential nucleosomes. Black lines indicate the connectivity between nucleosomes. Each column represents the nucleosomal arrangement of a different cell, at the time of fixation. The nucleosome pairs that are in physical contact have the potential to form crosslinks that can result in their detection, which is plotted in the contact matrices in the second row. Cell-to-cell variation at the time of fixation can result in the populational average shown in the Hi-C-based contact map (lower half). For clarity, only four nucleosomes are illustrated, but the concept here scales to clusters containing many more nucleosomes.

927 **Table S1. Cryo-EM and image-analysis specifics.**

EM grid	C-flat 4-2-2, EMS continuous carbon
Microscope	FEI Titan Krios
Energy	300 keV
Gun type	FEG
Camera	Falcon II Direct Detector
Tomography software	Leginon & FEI Tomo
Nominal magnification	8,700, 11000, 18000
Calibrated pixel size	9 Å, 7.3 Å, 4.6 Å
Defocus (nominal)	-8-12 µm
Defocus (fitted)	-9-13 µm
Defocus (nominal, Volta contrast)	-0.5 µm
Cumulative dose	80 - 150 e^- / $Å^2$
Dose fractionation	1 / cosine
Tilt range	±60°
Tilt increment	1° or 2°
Tomogram processing & visualization	IMOD 4.9
Template matching	PEET 1.10 - 1.11
File format conversion	Bsoft 1.8.8
Subtomogram classification & averaging	RELION 1.4, 2.0
Density map visualization	UCSF Chimera 1.11
Figure creation and compositing	Adobe Photoshop and Illustrator CS6, Google sheets

928

929 **Table S2**. Cryotomogram details

Tomogram ID	Cells	Cell-cycle state, sample type	Figure	Dose	Pixel size (Å)	Defocus † (µm)		DPC/ VPC
						nom	ref	
15Jun11_03	*pombe*	G2, cell	1B, C, S2	103	7.3	12	13-17	DPC
17May10_01	*pombe*	G2, cell	2, S5A, S10A	80	7.3	12	13	DPC
17May09_18	*pombe*	PM, cell	3	80	7.3	12	12	DPC
15Nov16_10	*pombe*	PM, cell	4, 5B, D	100	4.6	0.5		VPC
16Mar19_123	*pombe*	G2, cell	5A, C, S12	100	4.6	0.5		VPC
16Dec21_20	*pombe*	PM, cell	S3B	150	7.3	12	13	DPC
16Dec21_18	*pombe*	PM, cell	S3C	150	7.3	12	13	DPC
16Dec21_13	*pombe*	PM, cell	S3D	150	7.3	12	13	DPC
16Dec21_12	*pombe*	PM, cell	S3E	150	7.3	12	13	DPC
16Dec21_06	*pombe*	PM, cell	S3F	150	7.3	12	13	DPC
15Jun11_07	*pombe*	G2, cell	S4A, C, D	103	7.3	12	13-17	DPC
15May25_02	*pombe*	PM, cell	S4B, E, F	100	7.3	12	10-17	DPC
17May09_13	*pombe*	PM, cell	S5B, S10B	80	7.3	12	13	DPC
17May10_02	*pombe*	G2, cell	S5C, S10C	80	7.3	0.5		VPC

16Dec06_10	*pombe*	PM, cell	S5D	150	7.3	0.5		VPC
16Nov14_05	*pombe*	Unsynchronised, lysate	S7	150	7.3	12	12	DPC
17Jul08_04	*pombe*	G2, lysate	S8A, C	108	7.3	12	14	DPC
17Jul08_01	*pombe*	PM, lysate	S8B, D	100	7.3	12	14	DPC
16Aug12_03	*japo*	Interphase, cell	S9A, C	100	5.8	0.5		VPC
16Jul25_14	*japo*	Mitosis, cell	S9B, D	100	7.3	0.5		VPC
16Dec06_7	*pombe*	PM, cell	S10D	150	7.3	0.5		VPC
15Nov16_09	*pombe*	G2, cell	S11A, B	100	4.6	0.5		VPC

Cell type: *pombe* = *S. pombe*; *japo* = *S. Japonicus*, G2 = G2 phase, PM = prometaphase, Dose, in electrons / $Å^2$; † Nominal (nom) and refined (ref) defocus values; DPC = defocus phase contrast; VPC = Volta phase contrast