Nuclear blebs are associated with destabilized chromatin packing domains

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SUMMARY STATEMENT

36 Nuclear blebs are linked to various pathologies, including cancer and premature aging
37 disorders. We investigate alterations in higher-order chromatin structure within blebs, revealing
38 fragmentation of nanoscopic heterochromatin domains.

39

40 ABSTRACT

41 Disrupted nuclear shape is associated with multiple pathological processes including premature
42 aging disorders, cancer-relevant chromosomal rearrangements, and DNA damage. Nuclear
43 blebs (i.e., herniations of the nuclear envelope) have been induced by (1) nuclear compression,
44 (2) nuclear migration (e.g., cancer metastasis), (3) actin contraction, (4) lamin mutation or
45 depletion, and (5) heterochromatin enzyme inhibition. Recent work has shown that chromatin
46 transformation is a hallmark of bleb formation, but the transformation of higher-order structures
47 in blebs is not well understood. As higher-order chromatin has been shown to assemble into
48 nanoscopic packing domains, we investigated if (1) packing domain organization is altered
49 within nuclear blebs and (2) if alteration in packing domain structure contributed to bleb
50 formation. Using Dual-Partial Wave Spectroscopic microscopy, we show that chromatin packing
51 domains within blebs are transformed both by B-type lamin depletion and the inhibition of
52 heterochromatin enzymes compared to the nuclear body. Pairing these results with single-
53 molecule localization microscopy of constitutive heterochromatin, we show fragmentation of
54 nanoscopic heterochromatin domains within bleb domains. Overall, these findings indicate that
55 translocation into blebs results in a fragmented higher-order chromatin structure.

56

57 INTRODUCTION

58 The mammalian cell nucleus is a membrane-enclosed organelle that provides an enclosure for
59 chromatin, the assembly of DNA and associated proteins that regulates critical processes such
60 as gene transcription, replication, and DNA repair. Chromatin, chromatin proteins, and
61 chromatin-related processes directly influence nuclear mechanics and shape.[1-5] Nuclear
62 stability is further maintained by multiple processes, including by the nuclear lamina, a
63 meshwork of type V intermediate filament proteins called lamins.[6] Besides its role in
64 maintaining nuclear stiffness and stability, the lamina plays critical roles in regulating gene
65 expression and DNA replication through chromatin interactions.[2] Located immediately
66 underneath the inner nuclear membrane, the lamina consists of four major types of lamin
67 proteins: lamin A, lamin C, lamin B1, and lamin B2. A-type lamins, which consist of lamins A and
68 C, are primarily associated with developmental roles, contribute to nuclear stiffness, mainly
69 expressed in differentiated cells, and are spatially located near the nucleoplasm.[1, 7] B-type

70 lamins, in contrast, are expressed in all cell types throughout development and differentiation,
71 provide global integrity of chromatin structure through chromatin-tethering, and are tightly
72 associated with the inner nuclear membrane.[7-9] In mammalian cells, lamins interact with
73 heterochromatin to form lamina-associated domains (LADs), identified through the DamID
74 technique which maps protein-DNA interactions in a genome-wide manner, and are typically
75 transcriptionally repressive environments.[10-12] Disruption of these LADs has been linked to
76 epigenetic changes in cancer and pre-malignant processes such as the onset and evasion of
77 senescence.[13]

78

79 Abnormal nuclear morphology and disruption of genome organization are associated with
80 pathologies such as laminopathies (e.g., Hutchinson-Gilford progeria syndrome), cancer, and
81 cardiac disorders.[2, 14, 15] Among the most radical transformations in nuclear shape is the
82 protrusion of chromatin from the nuclear surface, known as a nuclear bleb, that are associated
83 with pathological transformation.[2, 6, 15, 16] While these blebs are highly associated with
84 gene-rich euchromatin and are believed to only contain lamin A/C,[7] recent evidence has
85 indicated that non-canonical blebs also contain B-type lamins.[2, 17, 18] A- and B-type lamins
86 both contribute to nuclear mechanics and morphology, and depletion of either has been widely
87 shown to induce both abnormal nuclear shape and a higher propensity for nuclear rupture,
88 increased presence of micronuclei, and more nuclear blebbing events.[14, 16, 19] Nucleus
89 micromanipulation force measurements reveal that the nucleus is softer upon inhibition of either
90 histone deacetylation (HDAC) or histone methyltransferase (HMT) which leads to nuclear bleb
91 formation independent of lamins.[2, 15] Thus, chromatin and lamins resist external antagonistic
92 forces from actin contraction[7, 20-22] and compression[23, 24], as well as internal transcription
93 forces[4], to maintain nuclear shape. These studies indicate that nuclear mechanics are
94 influenced by the balance of euchromatin and heterochromatin, and that perturbation of this
95 balance can result in abnormal nuclear morphology and DNA damage, both hallmarks of human
96 disease.[15, 16, 25] At the nuclear periphery, the dynamics of cytoskeleton reorganization and
97 chromatin structural changes contribute to mechano-transduction and transcription, independent
98 of lamins. For example, mechanosensitive ion channels embedded in the plasma membrane
99 activate Ca^{2+} signaling upon cellular stress, which can contribute to heterochromatin
100 reorganization and chromatin mobility.[15, 26-28]

101

102 Recent work demonstrates that chromatin assembles into higher-order polymeric domain
103 structures (nanodomains, packing domains, cores), which range between 50-200 nm in size

104 and contain ~200 Kbp to 2Mbp of genomic content across multiple cell types.[29-31] A crucial
105 feature of these domains is the formation of high-density centers with surrounding regions of
106 decreased density until a transition into low-density space with RNA-polymerase activity forming
107 primarily at the boundary. In the context of these findings, the structure of the genome
108 assembles from disordered nucleosomes (5 to 25nm) transitioning into domains (50-150nm)
109 and then into territorial polymers (>200nm). As has been previously shown, within the regime of
110 chromatin assembling into domains, chromatin acts as a power-law polymer with dimension, D
111 relating how the mass is distributed within the occupied volume. Notably, within supra-
112 nucleosomal length scales, chromatin is not assembled purely as a space-fulling globule (D=3)
113 nor is it as a poorly structured polymer with monomers primarily favors solvent interactions
114 (D=5/3), instead it is typically within these ranges and varies from cell to cell. A key feature
115 identified in this higher-order assembly is the coupling between heterochromatin centers (dense
116 cores) with euchromatic periphery and a corrugated periphery [30]. As power-law polymeric
117 assemblies, the structure of these chromatin packing domains is quantifiable by the relationship
118 $M \propto r^D$, relating how genome content fills an occupied volume as a function of its radial distance,
119 r. Notably, this organization can be directly measured by live-cell dual-Partial Wave
120 Spectroscopic (dual-PWS) Microscopy and quantified by the fractal dimension, D **(Materials**
121 **and Methods)**. In addition to quantifying packing domain structure in live cells, PWS
122 microscopy allows measurement of the effective diffusion coefficient, D_e, and the fractional
123 moving mass (FMM) which quantifies the fraction of chromatin demonstrating coherent motion
124 within a diffraction limited volume. Utilizing this technique, we have previously demonstrated
125 that B-type lamin depletion is associated with increased levels of chromatin fractional moving
126 mass and repositioning of heterochromatin cores [32, 33].

127

128 A major challenge in studying alteration in chromatin due to blebbing is that these represent
129 infrequent, but critical events in nuclear structure. As such, they are difficult to assess using
130 sequencing-based methods that measure ensemble chromatin organization such as Hi-C or
131 ChIP-Seq and require the utilization of microscopic methods that can directly quantify changes
132 in high-order genome structure. Therefore, in this study, we utilize live-cell PWS microscopy to
133 investigate the interplay between the disruption of the nuclear lamina and heterochromatin
134 enzymes in the structure of higher-order chromatin within blebs. Our results indicate distinct
135 roles for the nuclear lamina and heterochromatin remodeling processes in regulating higher-
136 order chromatin domains both of which are associated with bleb formation. Finally, pairing our

137 findings with super-resolution microscopy, we show that a key transformation of higher-order

138 chromatin within blebs is that of nanoscopic cores.

139

140 **RESULTS**

141 **B-type lamin depletion or heterochromatin loss promote aberrant nuclear morphology in**

142 **HCT-116 cells**

143 Bleb formation has been identified in numerous cell types, but the frequencies of bleb formation

144 have been shown to depend on multiple factors. Therefore, we first investigated the role of

145 processes well established to induce bleb formation: inhibition of B-type lamins and disruption in

146 heterochromatin enzymes.[2, 34] To assess the impact of lamin degradation on nuclear

147 morphology, we applied the AID system to HCT116 colorectal carcinoma epithelial cells to

148 induce simultaneous degradation of lamin B1 and lamin B2 as previously described.[32, 33, 35]

149 Using immunofluorescence imaging, we quantified the percentages of nuclear blebbing in

150 HCT116$^{LMN(B1\&B2)-AID}$ cells before and after depletion of B-type lamins by auxin treatment for 24

151 hours. We found that in comparison to untreated controls, auxin treatment promoted a

152 significant increase in the percentage of cells containing nuclear blebs (2.07% vs 6.23%; 4.163

153 $\pm$ 1.033 (Mean difference $\pm$ SEM), p-value = 0.016, Student's t-test) **(Fig. 1A; Fig. S1A)**, in

154 agreement with past studies. [2, 36, 37] Previous work from this group has demonstrated that

155 inhibition of HDACs to increase euchromatin content in mammalian cells or inhibition of histone

156 methyltransferases to decrease heterochromatin content results in a softer nucleus and

157 promotes nuclear blebbing, without perturbing lamins.[2, 15] We therefore hypothesized that in

158 addition B-type lamin loss increasing nuclear blebbing, heterochromatin loss would also result in

159 a substantial increase in nuclear deformations in HCT-116 cells. To test this, we treated

160 HCT116$^{LMN(B1\&B2)-AID}$ cells with either GSK343, an inhibitor of the histone methyltransferase

161 Enhancer of Zeste Homolog 2 (EZH2), or Trichostatin A (TSA), an inhibitor of class I and II

162 HDACs for 24 hours.

163

164 Our results confirmed that GSK343 and TSA treatment significantly increased the percentage of

165 blebbed nuclei within HCT-116 cells; with TSA treatment also inducing micronuclei formation

166 and associated with severely deformed nuclear periphery **(Fig. 1B; Fig. S1A)**. The effect of TSA

167 treatment on nuclear blebbing frequency was drastically higher than that of auxin or GSK343 in

168 HCT116$^{LMN(B1\&B2)-AID}$ cells (Auxin 6.23%, GSK343 6.99%, TSA 15.32%). Next, we explored how

169 combined treatment of either auxin and GSK343 or auxin and TSA would impact the frequency

170 of nuclear deformations. In contrast to prior work, combination of inhibition of b-type lamins with

171   disruption of heterochromatin resulted in only slightly increased rates of nuclear deformations in
172   comparison to lamin depletion, GSK343, or TSA treatment alone (Auxin + GSK343 7.97%,
173   Auxin + TSA 23.56%) **(Fig. 1C; Fig. S1A)**. Using immunofluorescence microscopy, we
174   visualized these blebs and compared relative levels of H3K27me3 and H3K27ac between the
175   untreated control and auxin, GSK343, and TSA-treated conditions within the cell nucleus **(Fig.**
176   **1D)**. Consistent with prior reports, we observe a reduction in H3K27me3 levels upon auxin-
177   induced degradation of B-type lamins, GSK343, and TSA treatment, however, only a
178   concomitant increase in H3K27ac levels was observed in the auxin-treatment group **(Fig. 1E,**
179   **F)**. Taken together, these results indicated that TSA-mediated heterochromatin disruption
180   promotes nuclear deformations to similar levels or greater levels than B-type lamin loss in HCT-
181   116 cells.

182

183   **Nanoscale chromatin packing domains are disrupted within nuclear blebs**
184   We recently demonstrated that the decreased DNA density is conserved across multiple bleb
185   mechanisms and is a consistently preserved feature of blebs. We investigate here in greater
186   detail the influence in the change of higher-order chromatin organization upon bleb formation.
187   We have previously shown that live-cell PWS microscopy, which does not resolve each
188   individual domain but measures the local ensemble in individual nuclei (**Materials and**
189   **Methods**), is sensitive to detecting the assembly into supra-nucleosome structures in individual
190   cells with measurements comparable to those observed on electron microscopy by measuring
191   the variations in the visible-light interference spectrum from within the nucleus. [29, 30, 38-40]
192   Likewise, we have shown that by analyzing the temporal interference spectrum at a single-
193   wavelength dual-PWS microscopy can measure the temporal evolution of chromatin density and
194   the fractional moving mass (FMM), which measures the volume fraction of- and mass of-
195   chromatin moving coherently with a sensitivity to mass density fluctuations as low as $\sim 5*10^{-21}$
196   grams, and the effective diffusion coefficient (D_e) within the nucleus (ranging between
197   $\sim 0.065 \mu M^2/s$ to $3.5*10^{-5} \mu M^2/s$). In the context that the mass of an individual nucleosome $\sim 10^{-19}$
198   grams, the typical values of FMM measured represent the movement of nucleosome clutches
199   moving coherently (as an ensemble). With respect to the D_e, the observed values are typically
200   between the observed rate of diffusion for genomic loci ($\sim 10^{-4} \mu M^2/s$) and the rate of mRNA
201   through the nucleus ($\sim 5*10^{-2} \mu M^2/s$).[30] Given these considerations, we utilized dual-live cell
202   PWS microscopy to probe the higher-order structure of chromatin in blebs, nuclei with blebs,
203   and stable nuclei.

204

205 Applying dual-PWS to the three well known processes that contribute to bleb formation, we
206 investigated the structure of higher order chromatin and mobility in B-type lamin depletion,
207 HDAC inhibition, and in EZH2 inhibition. As each perturbation have a distinct means to promote
208 bleb formation, we first evaluated the structure of chromatin packing domains observed within
209 blebs in all conditions (controls, EZH2i, HDACi, and lamin depletion) to see if any commonalities
210 were present **(Table 1; Fig. 2A-F)**. Overall, this indicated that independent of the mechanism of
211 bleb formation, relative to the nuclear body higher-order chromatin organization within blebs
212 was associated with a lower-likelihood of well-formed packed domains (low D) and fragmented
213 clutches (decreased FMM) with increased mobility (D_e). Comparing the observed behavior of
214 chromatin domains across the nuclei in these conditions, we observed that treatment with EZH2
215 or TSA resulted in decreased D with an associated increase in FMM in comparison to untreated
216 controls whereas D and FMM increased upon B-type lamin depletion, indicating that disruption
217 of heterochromatin enzymatic processes result in fragmentation of chromatin domains and the
218 loss of coherent chromatin motion. Next, we compared the behavior of chromatin domains
219 within blebs across all conditions and unexpectedly observed that domains associated due to
220 lamin B depletion had a comparably higher D and FMM compared to those that occur due to
221 inhibition of heterochromatin remodeling enzymes **(Fig. 2A, B, D, E; Fig. S1B, C)**. As such,
222 these results suggest that the domains translocating into blebs are larger in size and more
223 stable with the disruption of the lamina compared to the other conditions. As removal of B-type
224 lamins led to a significant increase in nuclear blebbing, these findings point to a bleb-associated
225 chromatin phenotype, in which nuclear blebs contain fewer packing domains, and B-type lamin
226 degradation could reduce the barriers for nuclear bleb formation that results from the
227 restructuring or redistribution of packing domains.

228

229 To further characterize the temporal dynamics of nuclear blebbing induced by either B-type
230 lamin loss or heterochromatin disruption, we used dual-PWS to measure chromatin mobility
231 between nuclear bleb and nuclear body in live cells. As TSA treatment resulted in the most
232 substantial increase in the frequency of nuclear deformations above, we treated
233 HCT116$^{LMN(B1\&B2)-AID}$ cells with either DMSO or TSA. By measuring the spectral interference at a
234 single wavelength as a function of time from within nuclei and within blebs, we could directly
235 image and measure how mass was transitioning between these phases.[39] As discussed
236 previously, variations in the temporal interference quantifies the FMM while the spatially-
237 average signal is inversely proportional the chromatin volume concentration (chromatin
238 density).[39, 41] Utilizing this approach, we can visualize without labels the temporal evolution

239 of chromatin density in both the nucleus and the bleb with a temporal resolution of 50ms per

240 frame (acquired over 15s total in this instance). On imaging chromatin motion in the DMSO

241 control nuclei, its visually apparent that density moves randomly over these timescales. In

242 contrast, within a TSA treated cell with a visually apparent bleb, chromatin decreases rapidly

243 adjacent to the nuclear bleb **(Fig. 3A, B; Movie S1-3)**. Between the nuclear bleb and the main

244 nuclear body in TSA treatment, we observed the active transit of small amounts of mass as

245 evidenced by increased local chromatin density and the visual changing of density in the bleb.

246 Comparing the density of chromatin within DMSO associated and TSA induced blebs, TSA

247 blebs unexpectedly had higher chromatin concentration compared to the DMSO control group

248 **(Fig. 3C)**. Our results therefore further suggest that the mobilization of chromatin packing

249 domains is an active process during nuclear blebbing induced by B-type lamin and

250 heterochromatin loss.

251

252 **Super-resolution imaging of chromatin heterochromatin domains in nuclear blebs**

253 Nuclear lamins and heterochromatin have been shown to act in parallel to maintain the

254 mechanical properties of the nucleus but the consequence of these on chromatin nanodomains

255 in bleb formation have not been investigated.[28, 34, 42] Additionally, chromatin structure and

256 dynamics are often closely related, which may support mechanisms of either granting or limiting

257 access to regions with high local chromatin concentration[43]. In the context of prior work

258 suggesting that chromatin domains are composed of high-density, presumably heterochromatic

259 centers[29-31, 44], we investigated the transformation in constitutive heterochromatin domains

260 between the nuclear bleb and the nuclear body in spontaneously forming blebs (controls), in

261 lamin B1/B2 depletion-associated blebs, and in heterochromatin enzyme-inhibited blebs (TSA)

262 using super-resolution microscopy. Due to the limitation of bleb formation being a low-frequency

263 process (< 15% of the time), we were only able to identify blebs in a few nuclei in total in HCT-

264 116 cells **(Fig. 4A-C)**. Given this limitation, we utilized a second cell-line model, U2OS cells, that

265 were associated with higher rates of bleb formation upon HDAC inhibition with TSA **(Fig. 4D-E)**.

266

267 Visually, we observed distinct differences in H3K9me3 chromatin nanodomains in these three

268 conditions. In blebs formed spontaneously **(Fig. 4A)**, blebs formed in B-type lamin depletion

269 **(Fig. 4B)**, and blebs formed due to inhibition of histone deacetylases **(Fig. 4C, E)**, it is visually

270 apparent that nanoscopic heterochromatin domains are observed. Within the nuclear body, as

271 previously demonstrated[32, 33], auxin-induced depletion of B-type lamins resulted in reduced

272 peripheral heterochromatic cores at the nuclear periphery, however, domains formed within the

273   nuclear interior were typically larger in size **(Fig. S2)**. With respect to heterochromatin
274   nanodomains in GSK343 treated HCT116 cells[32, 33] and TSA treated U2OS cells, these were
275   smaller than those in control cells and in lamin B-depletion as expected due to the inhibition of
276   heterochromatin enzymes within the nuclear body **(Fig. 4D-F; Fig. S2)**. In contrast to prior work,
277   we found that the nuclear blebs arising from either B-type lamin degradation or TSA treatment
278   contained heterochromatin around the periphery of blebs and within the center of the bleb **(Fig.**
279   **4C, E, F; Fig. S2)**. This finding highly contradicts the plethora of research stating that all nuclear
280   blebs are devoid of heterochromatin.[45-47] Instead, these results show that independent B-
281   type lamin loss, HDAC inhibition gives rise to non-canonical nuclear blebs enriched in
282   heterochromatin around near their boundaries and the transfer of nanoscopic heterochromatin
283   domains into the bleb. This also challenges the notion that euchromatin enrichment is the most
284   reliable marker of nuclear blebs[17], and further suggests that other cellular mechanisms could
285   play a role in the morphological properties of these herniations.

286

287   **DISCUSSION**
288   In this work, we found that nuclear packing domains are transformed within blebs induced by
289   the loss of either B-type lamins or inhibition of heterochromatin enzymes. Specifically, the
290   domains observed within blebs were typically poorly formed, with increased fragmentation and a
291   higher effective diffusion coefficient compared to the domains observed in the nuclear body
292   independent of the conditions. Despite the conserved differences across groups, we notably
293   saw that in domains associated with lamin B depletion, these were larger than those produced
294   by inhibition of heterochromatin remodeling enzymes (GSK343 inhibition of EZH2 and TSA
295   inhibition of HDACs) suggesting that the barrier to movement of domains or nucleosome
296   clutches is larger in the loss of b-type lamins whereas the inhibition of heterochromatin enzymes
297   fragments domains to facilitate deformations in the nuclear border **(Fig. 2A, D)**. Given these
298   findings, one possible and interesting explanation is that domains or clutches of nucleosomes
299   move in concert into blebs through transiently evolving defects in the nuclear lamina. As such,
300   depletion of b-type lamins may increase the frequency of barrier disruption events or potentially
301   result in larger transient defects that allow passage of larger domains into the bleb body. Further
302   supporting these findings were the observation that the structure of heterochromatin domains
303   upon B-type lamin depletion are larger in size compared to those in nuclei treated with
304   heterochromatin enzyme inhibitors on super-resolution microscopy **(Fig. 4A, B).** Likewise,
305   although limited the low frequency of bleb events, domains observed within all blebs were

306   smaller in size and more disperse than those observed in the adjacent nuclear body **(Fig. 4A,**
307   **B)**.

308

309   The changes observed in chromatin domains within blebs could be related to functional
310   consequences in signaling, possibly arising from applied mechanical stress when the nuclear
311   lamina or heterochromatin are disrupted. For example, using single-nucleus isolation and
312   micromanipulation assays, we previously demonstrated that nuclei with reduced
313   heterochromatin levels are softer and succumb to nuclear blebbing, while nuclei with more
314   heterochromatin levels are stiffer and resist blebbing.[2] This chromatin histone-modification-
315   based nuclear rigidity could be related to the differential transcriptional responsiveness (i.e.,
316   transcriptional plasticity) previously observed in low- versus high-chromatin packing areas upon
317   exposure to external stressors.[48] In many cases, nuclear blebbing is a marker of cell death
318   (i.e., apoptosis) and is often observed during normal developmental processes or in response to
319   various extracellular stressors. The transformation of domains within blebs upon either B-type
320   lamin or heterochromatin enzyme disruption could potentially accelerate these processes by
321   increasing DNA damage or cytoskeletal reorganization. Alternatively, bleb formation could be a
322   necessary event to maintain the stability of the remaining chromatin domains to ensure their
323   continued optimal function by maintaining the optimal conditions for remaining domains to
324   function.

325

326   Although loss of B-type lamins and inhibition of heterochromatin enzymes both induced nuclear
327   morphological changes and increased FMM within nuclear blebs, it is important to consider that
328   these perturbations may not always reflect the same changes in cell phenotype. While B-type
329   lamins are required for proper spatial positioning of heterochromatin and gene-specific loci[8,
330   32, 33], B-type lamin loss and heterochromatin disruption may impact different cellular
331   mechanisms that give rise to these morphological changes. For example, in previous work, we
332   found that decreasing heterochromatin promoted decreased nuclear rigidity and increased
333   nuclear blebbing without necessarily altering lamins.[2] Conversely, removal of B-type lamins
334   resulted in both a reduction of heterochromatin and increased nuclear blebbing.[8, 32, 33]
335   Therefore, while lamins and heterochromatin interact, depletion of either could have differential
336   effects on chromatin organization. In this work, we also found that HDAC or EZH2 inhibition
337   promoted more nuclear deformations in comparison to auxin treatment to remove B-type lamins.
338   As combined treatment of either auxin and TSA or auxin and GSK343 did not result in a more
339   significant increase in these deformations in comparison to TSA or GSK343 alone, our results

340 support previous findings that conclude disruption of chromatin alone is sufficient to cause
341 nuclear blebbing[2]. However, the distortions in the nucleus caused in part by the breakdown of
342 connections between chromatin and the nuclear lamina may be intensified by pressure
343 gradients resulting from external influences.[49]

344

345 These external factors, such as confinement imposed by the actin cytoskeleton or the
346 surrounding environment, could further contribute to the increased deformation of the nucleus.
347 Additionally, processes such as HDACi or HMTi could act by expanding the volume of
348 heterochromatin centers or destabilizing packing domains altogether. In theory, as weak,
349 unstable packing domain (i.e., nascent domain) cores expand in size, one possible
350 consequence could be increased variations in temporally active processes, such as gene
351 transcription, resulting in amplified chromatin motion. Consequently, modifications to higher-
352 order chromatin assemblies could promote bleb formation by degrading packing domains and/
353 or altering chromatin-based nuclear mechanics. However, further assessment is needed to
354 confirm this theoretical interplay between packing domain formation, nuclear mechanics, and
355 transcription.

356

357 The complexity of interactions within the genome results in varying chromatin dynamics at
358 different length scales. Intrinsic characteristics of chromatin, which involve the dynamic
359 rearrangement of histones, interactions among chromosome segments, chromatin remodelers,
360 replication proteins, and transcriptional regulators are required for proper spatiotemporal
361 genome organization. Other than Dual-PWS, several techniques have been utilized to
362 investigate the contributions of chromatin dynamics to this organization. For example, a
363 combination of photoactivated localization microscopy (PALM) and tracking of single
364 nucleosomes was recently applied to assess nucleosome-nucleosome interactions and cohesin-
365 RAD21 in domain formation and dynamics.[50] In line with our results, TSA treatment increased
366 chromatin dynamics. Recently, proximity ligation-based chromatin assembly assays have been
367 applied to investigate the kinetics of nuclear lamina binding to newly replicated DNA in mouse
368 embryonic fibroblasts.[51] Finally, computational models have been applied to probe the time
369 evolution of the chromatin over the G1 phase of the interphase in *Drosophilla* that successfully
370 predict dynamic positioning of all LADs at the nuclear envelope.[52] While Chromatin Scanning
371 Transmission Electron Microscopy (ChromSTEM) does not have live-cell imaging capabilities to
372 resolve chromatin mobility[29], future work may involve this high-resolution imaging technique to

373   investigate how the shift in chromatin dynamics seen here could be related to shifts in chromatin

374   density, volume, and shape.

375

376   **CONCLUSION**

377   The formation of nuclear blebs is believed to be associated with aberrant gene expression in

378   pathological conditions; nevertheless, our understanding of chromatin structure within blebs and

379   the mechanisms resulting in their formation remain poorly understood. The biophysical

380   characteristics of cell nuclei, including their mechanical properties and architecture, play a

381   crucial role in shaping cell phenotype, shape, and function. Our research demonstrates that the

382   transformation of chromatin nanoscopic packing domains may contribute to bleb formation given

383   the structures seen within nuclear blebs on live-cell nanoscopic imaging and super resolution

384   microscopy. This indicates that histone modifications converge in altering chromatin packing

385   domains with the resulting change in structure influencing nuclear mechanics and morphology.

386   As blebs are associated with increased DNA damage, it highlights the need for further

387   investigation into how the change in chromatin structure (both nucleosome modifications and

388   higher-order domains) contribute to transformation of chromatin function within bleb

389   compartments. Future work further investigating blebs can help us understand what happens to

390   gene-transcription within these deformations and how the reintegration of these components of

391   the genome happen during mitosis. Finally, future work to decouple how additional bleb-

392   promoting mechanisms (compression, contraction, translocation) simultaneously at different

393   length scales to organize the genome remains open for further investigation.

394

395

396   **MATERIALS AND METHODS**

397   **HCT116 Cell Culture**

398   HCT116$^{LMN(B1\&B2)-AID}$ cells and U2OS cells were grown in McCoy's 5A Modified Medium (#16600-

399   082, Thermo Fisher Scientific, Waltham, MA) supplemented with 10% FBS (#16000-044,

400   Thermo Fisher Scientific, Waltham, MA) and penicillin-streptomycin (100 µg/ml; #15140-122,

401   Thermo Fisher Scientific, Waltham, MA). To create these cells, HCT116 cells (ATCC, #CCL-

402   247) were tagged with the AID system as previously described.[32, 33] All cells were cultured

403   under recommended conditions at 37°C and 5% CO_2. All cells in this study were maintained

404   between passage 5 and 20. Cells were allowed at least 24 h to re-adhere and recover from

405   trypsin-induced detachment. All imaging was performed when the surface confluence of the dish

406 was between 40–70%. All cells were tested for mycoplasma contamination (ATCC, #30-1012K)

407 before starting perturbation experiments, and they have given negative results.

408

409 **Auxin Treatment**

410 HCT116$^{LMN(B1\&B2)-AID}$ cells were plated at 50,000 cells per well of a 6-well plate (Cellvis, P12-

411 1.5H-N). To induce expression of OsTIR1, 2 µg/ml of doxycycline (Fisher Scientific, #10592-13-

412 9) was added to cells 24 hours prior to auxin treatment. 1000 µM Indole-3-acetic acid sodium

413 salt (IAA, Sigma Aldrich, #6505-45-9) was solubilized in RNase-free water (Fisher Scientific,

414 #10-977-015) before each treatment as a fresh solution and added to HCT116$^{LMN(B1\&B2)-AID}$ cells.

415

416 **GSK343 Treatment**

417 HCT116$^{LMN(B1\&B2)-AID}$ cells were plated at 50,000 cells per well of a 6-well plate (Cellvis, P12-

418 1.5H-N). Cells were given at least 24 hours to re-adhere before treatment. GSK343 (Millipore

419 Sigma, #SML0766) was dissolved in DMSO to create a 10 mM stock solution. This was further

420 diluted in complete cell media to a final treatment concentration of 10 µM.

421

422 **Trichostatin A (TSA) Treatment**

423 HCT116$^{LMN(B1\&B2)-AID}$ cells were plated at 50,000 cells per well of a 6-well plate (Cellvis, P12-

424 1.5H-N). Cells were given at least 24 hours to re-adhere before treatment. TSA (Millipore

425 Sigma, #T1952) was diluted in complete cell medium and added to cells at a final treatment

426 concentration of 300 nM.

427

428 **Immunofluorescence Sample Preparation**

429 HCT116$^{LMN(B1\&B2)-AID}$ cells at a low passage (<P10) were plated at 100,000 cells per well of a 6-

430 well glass-bottom plate (Cellvis, #P06-1.5H-N). Following auxin treatment, cells were washed

431 twice with 1x Phosphate Buffered Saline (PBS) (Gibco, #10010031). Cells were fixed with 4%

432 paraformaldehyde (PFA) (Electron Microscopy Sciences, #15710) for 10 minutes at room

433 temperature, followed by washing with PBS 3 times for 5 minutes each. Cells were

434 permeabilized using 0.2% TritonX-100 (10%) (Sigma-Aldrich, #93443) in 1x PBS, followed by

435 another wash with 1x PBS for 3 times for 5 minutes each. Cells were blocked using 3% BSA

436 (Sigma-Aldrich, #A7906) in PBST (Tween-20 in 1x PBS) (Sigma-Aldrich, #P9416) at room

437 temperature. The following primary antibodies were added overnight at 4°C: anti-H3K27ac

438 (Abcam, #ab177178, dilution 1:7000) and anti-H3K27me3 (Abcam, #ab6002, dilution 1:200).

439 Cells were washed with 1x PBS 3 times for 5 minutes each. The following secondary antibodies

440 were added for 1 hour at room temperature: Goat anti-Rabbit IgG (H+L) Alexa Fluor 568

441 (Abcam, #ab175471, dilution 1:1000) and Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed

442 Secondary Antibody, Alexa Fluor Plus 647 (Thermo Fisher Scientific, #A32728, dilution 1:200).

443 Cells were washed with 1x PBS 3 times for 5 minutes each. Finally, cells were stained with

444 DAPI (Thermo Fisher Scientific, #62248, diluted to 0.5 µg/mL in 1x PBS) for 10 minutes at room

445 temperature. Prior to imaging, cells were washed with 1x PBS twice for 5 minutes each.

446

447 **Immunofluorescence Imaging**

448 Live and fixed cells were imaged using the Nikon SoRa Spinning Disk confocal microscope

449 equipped with a Hamamatsu ORCA-Fusion Digital CMOS camera. Live cells were imaged

450 under physiological conditions (37°C and 5% CO_2) using a stage top incubator (Tokai Hit).

451 Images were collected using a 60x/ 1.42 NA oil-immersion objective mounted with a 2.8x

452 magnifier. mClover was excited with a 488 nm laser, Alexa Fluor 647 was excited with a 640 nm

453 laser, and DAPI was excited with a 405 nm laser. Imaging data were acquired by Nikon

454 acquisition software.

455

456 **Dual-PWS Imaging**

457 For live-cell measurements, cells were imaged and maintained under physiological conditions

458 (5% CO^2 and 37°C) using a stage-top incubator (In Vivo Scientific, Salem, SC; Stage Top

459 Systems). Live-cell PWS measurements were obtained using a commercial inverted microscope

460 (Leica, DMIRB) using a Hamamatsu Image-EM charge-coupled device (CCD) camera (C9100-

461 13) coupled to a liquid crystal tunable filter (LCTF, CRi VariSpec) to acquire monochromatic,

462 spectrally resolved images ranging from 500-700 nm at 2-nm intervals as previously

463 described.[38-40] Broadband illumination was provided by a broad-spectrum white light LED

464 source (Xcite-120 LED, Excelitas). The system is equipped with a long pass filter (Semrock

465 BLP01-405R-25) and a 63x oil immersion objective (Leica HCX PL APO). All cells were given at

466 least 24 hours to re-adhere before treatment (for treated cells) and imaging. Briefly, PWS

467 measures the spectral interference signal resulting from internal light scattering originating from

468 nuclear chromatin. This is related to variations in the refractive index (RI) distribution and is

469 captured by the microscope by calculating the standard deviation of the spectral interference at

470 each pixel (Σ). Chromatin packing scaling D was then calculated using maps of Σ. Although it is

471 a diffraction-limited imaging technique, PWS can measure chromatin packing behaviors

472 because the RI of chromatin is proportional to the local density of macromolecules (e.g., DNA,

473 RNA, proteins). PWS senses the complex inhomogeneous RI distribution of chromatins with

474   length scale sensitivity around $20 - 200$ nm, and associated it with fractal coefficient, as

475   previously described.[30, 39, 41, 48] PWS measurements were normalized by the reflectance of

476   the glass medium interface (i.e., to an independent reference measurement acquired in a region

477   lacking cells on the dish). This allows acquisition of the interference signal that is directly related

478   to RI fluctuations within the cell. Changes in *D* resulting from each condition are quantified by

479   averaging cells, taken across 3 technical replicates. Average *D* was calculated by first

480   averaging *D* values from PWS measurements within each cell nucleus and then averaging

481   these measurements over the entire cell population for each treatment condition.

482

483   **Dynamic PWS Measurements**

484   Temporal PWS data was acquired as previously described.[32, 39] Briefly, dynamics

485   measurements (Σ_t^2, fractional moving mass (m_f), and diffusion) are collected by acquiring

486   multiple backscattered wide-field images at a single wavelength (550 nm) over time

487   (acquisition time), to produce a three-dimensional image cube, where Σ_t^2 is temporal

488   interference and t is time. Diffusion is extracted by calculating the decay rate of the

489   autocorrelation of the temporal interference as previously described.[39] The fractional moving

490   mass is calculated by normalizing the variance of Σ_t^2 at each pixel. Using the equations and

491   parameters supplied and explained in detail in the supplementary information of our recent

492   publication [39], the fractional moving mass is obtained by using the following equation to

493   normalize Σ_t^2 by ρ_0, the density of a typical macromolecular cluster:

494

495    $$\Sigma_t^2 \left(\frac{\pi \rho_0}{2\Gamma^2 k^3 n_i}\right)\left(\frac{NA_i}{NA_c}\right)^2 \left(\frac{n_1}{n_m - n_1}\right)^2 = \rho_0 V_{cm}\varphi = m_c\varphi = m_f \textbf{ (1)}.$$

496

497   With this normalization, Σ_t^2 is equivalent to m_f, which measures the mass moving within the

498   sample. This value is calculated from the product of the mass of the typical moving cluster (m_c)

499   and the volume fraction of mobile mass (φ). m_c is obtained by $m_c = V_{cm}\rho_0$, where V_{cm} is the

500   volume of the typical moving macromolecular cluster. To calculate this normalization, we

501   approximate $n_m = 1.43$ as the refractive index (RI) of a nucleosome, $n_1 = 1.37$ as the RI of a

502   nucleus, $n_i = 1.518$ as the refractive index of the immersion oil, and $\rho_0 = 0.55$ g cm^{-3} as the dry

503   density of a nucleosome. Additionally, $k = 1.57E5$ cm^{-1} is the scalar wavenumber of the

504   illumination light, and Γ is a Fresnel intensity coefficient for normal incidence. $NA_c = 1.49$ is the

505   numerical aperture (NA) of collection and $NA_i = 0.52$ is the NA of illumination. As stated

506   previously[39], Σ_t^2 is sensitive to instrument parameters such as the depth of field, substrate

507   refractive index, etc. These dependencies are removed through normalization with the proper

508   pre-factor calculated above for obtaining biological measurements. It should also be noted that

509   backscattered intensity is prone to errors along the transverse direction[39]. Due to these

510   variations, these parameters are more accurate when calculating the expected value over each

511   pixel.

512

513   Chromatin volume concentration is calculated by Fresnel reflection coefficient. Recall that the

514   reflectance at a RI mismatch interface:

515
$$R_s = \left| \frac{n_1 \cos \theta_i - n_2 \cos \theta_t}{n_1 \cos \theta_i + n_2 \cos \theta_t} \right|^2 = \left| \frac{n_1 \cos \theta_i - n_2 \sqrt{1 - \left(\frac{n_1}{n_2} \sin \theta_i\right)^2}}{n_1 \cos \theta_i + n_2 \sqrt{1 - \left(\frac{n_1}{n_2} \sin \theta_i\right)^2}} \right|^2 \ \textbf{(2)}.$$

516
$$R_p = \left| \frac{n_1 \cos \theta_t - n_2 \cos \theta_i}{n_1 \cos \theta_t + n_2 \cos \theta_i} \right|^2 = \left| \frac{n_1 \sqrt{1 - \left(\frac{n_1}{n_2} \sin \theta_i\right)^2} - n_2 \cos \theta_i}{n_1 \sqrt{1 - \left(\frac{n_1}{n_2} \sin \theta_i\right)^2} + n_2 \cos \theta_i} \right|^2 \ \textbf{(3)}.$$

517

518   Although the chromatin is inhomogeneous and not infinitely large, the correlation between

519   chromatin average RI and reflection coefficients still holds as confirmed by Finite Difference

520   Time Domain (FDTD) simulations **(Fig. S3)**. Briefly, we used home-built FDTD software to

521   simulate the entire PWS imaging system, from incident to light-matter interaction and then

522   collection.[53] Light beams representing the characteristics of experimental NA_i are first

523   introduced to the simulation space. The simulation space contains a layer of glass and random

524   media that represents chromatin average RI and packing behavior. The EM wave after light-

525   matter interaction is then collected with the same NA_c and far field PWS image is analyzed the

526   same way in experiments. We did a series of simulations with different average RI and

527   measured the mean reflection coefficients. We have fitted:

528
$$\frac{R}{R_0} = 0.36\phi^2 - 1.1\phi + 1.0 \ \textbf{(4)}$$

529

530   where ϕ is chromatin volume concentration, related to RI through Gladstone-Dale equation [48]:

531

532
$$n_{nucleus} = 1.3446 + 0.0882\phi \ \textbf{(5)}.$$

533

534   **SMLM Sample Preparation and Imaging**

535   Primary antibody rabbit anti-H3K9me3 (Abcam, #ab176916, dilution 1:2000) was aliquoted and

536   stored at –80°C. The secondary antibody goat anti-rabbit AF647 (Thermo Fisher Scientific, #A-

537 21245, dilution 1:1000) was stored at 4°C. The cells were plated on No. 1 borosilicate bottom

538 eight-well Lab-Tek Chambered cover glass with at a seeding density of 1.25 x 10^4. After 48

539 hours, the cells were fixed in 3% paraformaldehyde in PBS for 10 minutes, and then

540 subsequently washed with PBS once for five minutes. Thereafter the samples were quenched

541 with freshly prepared 0.1% sodium borohydride in PBS for 7 minutes and rinsed with PBS three

542 times at room temperature. The fixed samples were permeabilized with a blocking buffer (3%

543 bovine serum albumin (BSA), 0.5% Triton X-100 in PBS) for 20 minutes and then incubated with

544 rabbit anti-H3K9me3 in blocking buffer for 1-2 hours at room temperature and rinsed with a

545 washing buffer (0.2% BSA, 0.1% Triton X-100 in PBS) three times. The fixed samples were

546 further incubated with the corresponding goat secondary antibody–dye conjugates, anti-rabbit

547 AF647, for 40 minutes, washed thoroughly with PBS three times at room temperature and

548 stored at 4°C. Imaging of samples was performed on a STORM optical setup built on the

549 commercially available Nikon Ti2 equipped with a Photometric 95B sCMOS camera and a 1.49

550 NA 100X oil immersion objective lens. Samples were illuminated with the MPB Communications

551 2RU-VFL-P-2000-647-B1R 647 nm 200 mW laser. Image acquisition was performed at 20-30

552 ms exposure for 10-15k frames.

553

554 **Data and Image Analysis**

555 We used GraphPad Prism 10.1.1 for making all plots. For immunofluorescence imaging,

556 maximum intensity projection of Z-series images was performed using FIJI.[54] To quantify

557 nuclear deformation frequency, we considered blebs to be herniations that were still connected

558 to the nuclear body. We considered ruptures to be cells that were no longer intact, and we

559 considered micronuclei to be herniations that were no longer connected to the nuclear body and

560 of similar sizes to nuclear blebs. For each field of view, the number of nuclei and the number of

561 each nuclear deformation type was manually counted using FIJI. We then determined the

562 percentages of total cells within each tested condition that displayed each nuclear deformation

563 type.

564

565 **Super-resolution Data Analysis:**

566 We used the Thunder-STORM FIJI Plug-in [55]to apply Maximum Likelihood Estimation fitting of

567 a gaussian point spread function to our image stack. Localization datasets were then put into

568 our Python script that utilized DBSCAN (epsilon=50, min_pts=3) to cluster our localized

569 heterochromatic events. Heterochromatin domain size was estimated by fitting a polygon to the

570 peripheral cluster points using the *scipy* Convex Hull method. Outlier clusters smaller than twice

571 the mean uncertainty of our localization (~ 25 nm) or larger than 800 nm were removed from the

572 analysis. Results displayed are concatenations of identified heterochromatic domains across all

573 cells in that condition.

574

575 **Statistical Analysis and Quantification**

576 Statistical analysis was performed using GraphPad Prism 10.1.1 and Microsoft Excel. Pairwise

577 comparisons were calculated on datasets consisting of, at a minimum, biologically independent

578 duplicate samples using a two-tailed unpaired t test or Mann-Whitney test. The type of statistical

579 test is specified in each case. Experimental data are presented either the mean ± SEM or mean

580 ± SD, as stated in figure legends. A P value of < 0.05 was considered significant. Statistical

581 significance levels are denoted as follows: n.s. = not significant; *P<0.05; **P<0.01; ***P<0.001;

582 ****P<0.0001. Sample numbers (# of nuclei, n), the number of replicates (N), and the type of

583 statistical test used is indicated in figure legends.

584

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591

592 **COMPETING INTERESTS**

593 The authors declare no competing interests.

594

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603

604 **DATA AVAILABILITY**

605 All relevant data can be found within the article and its supplementary information. The cell lines

606 have been authenticated and are available upon request. Further information and requests for

607 resources and reagents should be directed to and will be fulfilled by the lead contact, Vadim

608 Backman (v-backman@northwestern.edu).

609

610 **AUTHORS' CONTRIBUTIONS**

611 E.M.P. and L.A. wrote the paper and performed immunofluorescence and Dual-PWS imaging

612 and analysis. N.A. and L.A. conducted SMLM imaging and analysis. N.A. assisted with

613 representative Dual-PWS images. Y.S. conducted density estimations from Dual-PWS data.

614 R.G. set up the optical system for all super resolution image acquisition. E.M.P, A.S., L.A., and

615 V.B. conceptualized the project and edited the manuscript.

616

617 **DIVERSITY AND INCLUSION STATMENT**

618 One or more of the authors of this paper self-identifies as an underrepresented minority.

619

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MAIN FIGURE TITLES AND LEGENDS

Figure 1. Aberrant nuclear morphology is induced by the loss of B-type lamins or heterochromatin.

(A) Percentages of nuclear deformations compiled over each field of view for untreated (control) and 24-hour auxin conditions in HCT116[LMN(B1&B2)-AID] cells. Each dot represents a technical replicate (N = 3; Control n = 1102, Auxin n = 1081). **(B)** Percentages of nuclear deformations compiled over each field of view for DMSO (vehicle control), 24-hour GSK343, and 24-hour TSA treatment conditions. Each dot represents a technical replicate (N = 3; DMSO n = 295, GSK343 n = 1096, TSA n = 456). **(C)** Percentages of nuclear deformations compiled over each field of view for DMSO (vehicle control), 24-hour GSK343 with auxin, and 24-hour TSA with auxin

793   treatment conditions in HCT116[LMN(B1&B2)-AID] cells. Each dot represents a technical replicate (N =

794   3; DMSO n = 295, GSK343 + Auxin n = 933, TSA + Auxin n = 456). For **(A-C)**, bar plots are

795   represented as mean ± SEM. Unpaired two-tailed t-test with Holm-Šídák test for multiple

796   comparisons applied in **(B-C)**. **(D)** Representative images of H3K27me3 (magenta), H3K27ac

797   (yellow), Lamin B1/B2-AID (green), DAPI (blue), and merged fluorescence for control, 24-hour

798   auxin, 24-hour GSK343, 24-hour GSK343 + Auxin, 24-hour TSA, and 24-hour TSA + Auxin

799   treatment conditions in HCT116[LMN(B1&B2)-AID] cells. Scale bar = 10 µm. **(E)** Corrected total cell

800   fluorescence measurements of H3K27me3 and **(F)** H3K27ac for control, 24-hour auxin, 24-hour

801   GSK343, 24-hour GSK343 + Auxin, 24-hour TSA, and 24-hour TSA + Auxin treatment

802   conditions in HCT116[LMN(B1&B2)-AID] cells. Each dot represents a cell nucleus. Violin plots show the

803   median and quartiles. Error bars represent mean ± SD. One-way ANOVA with Dunnett's test for

804   multiple comparisons. For **(D-F)**, data are representative of two technical replicates (N = 2, total

805   n > 150 for each condition). *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001

806

807   **Table 1. Dual-PWS measurements of nuclear blebs.**

Condition	Average D (Mean ± SEM)	Average FMM (g) (Mean ± SEM)	Average D_e $(\mu M^2/s)$ (Mean ± SEM)
Control (Untreated)	2.30	$1.76*10^{-19}$	$5.09*10^{-3}$
Control (DMSO)	2.22	$1.89*10^{-19}$	$5.12*10^{-3}$
(-) Lamin B1/B2	2.37 (± 0.03)	$2.26*10^{-19}$ (±	$4.35*10^{-3}$ (± 0.0003)
(-) EZH2	2.27 (± 0.03)	$2.16*10^{-19}$ (± $1.53*10^{-20}$)	$4.71*10^{-3}$ (± 0.0001)
(-) HDACs	2.20 (± 0.03)	$1.99*10^{-19}$ (± $1.53*10^{-20}$)	$4.34*10^{-3}$ (± 0.0001)

808

809

810   **Figure 2. Loss of B-type lamins, EZH2i, and HDACi induce a bleb-associated chromatin**

811   **phenotype.**

812   **(A)** Representative PWS D maps and D values for the nuclear bodies and nuclear blebs for

813   control and 24-hour auxin-treatment conditions in HCT116[LMN(B1&B2)-AID] cells. **(B)** Fractional

814   moving mass values for nuclear bodies and nuclear blebs for control and 24-hour auxin-

815   treatment conditions in HCT116[LMN(B1&B2)-AID] cells. **(C)** Effective diffusion coefficient values for the

816   nuclear bodies and nuclear blebs for control and 24-hour auxin-treatment conditions in

817   HCT116[LMN(B1&B2)-AID] cells. **(D)** Representative PWS D maps and D values for the nuclear bodies

818   and nuclear blebs for DMSO (vehicle control), 24-hour GSK343, and 24-hour TSA-treatment

819   conditions in HCT116^LMN(B1&B2)-AID cells. **(E)** Fractional moving mass values for the nuclear

820   bodies and nuclear blebs for DMSO (vehicle control, 24-hour GSK343, and 24-hour TSA-

821   treatment conditions in HCT116^LMN(B1&B2)-AID cells. **(F)** Effective diffusion coefficient values for the

822   nuclear bodies and nuclear blebs for DMSO (vehicle control), 24-hour GSK343, and 24-hour

823   TSA-treatment conditions in HCT116^LMN(B1&B2)-AID cells. For **(A-F)**, each dot represents a cell

824   nucleus. (Control n = 2451, Auxin n = 2140, Control Blebs n = 200, Auxin Blebs n = 129, DMSO

825   n = 741, GSK343 n = 790, TSA n = 498, DMSO Blebs n = 564, GSK343 Blebs n = 467, TSA

826   Blebs n = 77). Error bars represent mean ± SD. Data are compiled from three technical

827   replicates (N = 3). Violin plots show the median and quartiles for the unpaired two-tailed t-test

828   between selected groups. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001. Scale bars = 5

829   μm.

830

831   **Figure 3. Chromatin density decreases rapidly in the boundary adjacent to the nuclear**

832   **bleb.**

833   **(A)** Representative mClover signal, fractional moving mass "hot" heat map, and individual

834   frames of the temporal interference signal, inversely proportional to chromatin density over the

835   imaging acquisition time of live HCT116^LMN(B1&B2)-AID cells for DMSO (control) and 24-hour TSA

836   treatment from Dual-PWS. Arrows indicate nuclear blebs. Data are representative of three

837   technical replicates (N = 3; DMSO n = 741, TSA n = 498). Fluorescent, fractional moving mass,

838   and individual frame images have the same scaling between treatment conditions. Scale bars =

839   5 μm. **(B)** Representative chromatin volume concentration (CVC) of a bleb over the acquisition

840   time of dynamic PWS signal. The zig-zag behavior of the temporal signal originated from the

841   moving nature of chromatin and is used to measure FMM. The general trend indicates mass

842   moving out and then moving into the bleb. **(C)** Bleb average CVC for DMSO (control) and 24-

843   hour TSA treatment measured from dynamics PWS signal. Data are the same as of (A). ***P ≤

844   0.001

845

846   **Figure 4. Heterochromatic nanodomains are reorganized during nuclear bleb formation.**

847   **(A)** Representative SMLM images of HCT116^LMN(B1&B2)-AID cells with zoomed-in views before and

848   after 24-hour auxin treatment or 24-hour TSA treatment. Yellow: H3K9me3. Data are

849   representative of three technical replicates (N = 3; Control n = 1, Auxin (- Lamin B1/B2) n = 1,

850   TSA (- HDAC) n = 1). Scale bars = 5 μm for whole nucleus, 1 μm for inset of whole nucleus

851   (red) and nuclear bleb (blue). **(B)** Representative SMLM images of U2OS cells with zoomed-in

852   views before and 24-hour TSA treatment. Yellow: H3K9me3. Data are representative of three

853   technical replicates (N = 3; Control n = 3, TSA (- HDAC) n = 3). Scale bars = 5 µm for whole

854   nucleus, 1 µm for inset of nuclear bleb (blue). **(C)** Quantification of the number and size of

855   heterochromatin nanodomains in control and TSA treatment conditions for U2OS cells.

856

857   **SUPPLEMENTARY FIGURE TITLES AND LEGENDS**

858   **Figure S1. Nuclear blebbing induces the redistribution and reorganization of packing**

859   **domains.**

860   **(A)** Averages of all three replicates for percentages of nuclear deformations compiled over each

861   field of view for control, 24-hour auxin, DMSO (vehicle control), 24-hour GSK343, 24-hour TSA,

862   24-hour GSK343 with auxin, and 24-hour TSA with auxin treatment conditions. Each dot

863   represents a technical replicate (N = 3; Control n = 1102, Auxin n = 1081, DMSO n = 295,

864   GSK343 n = 1096, TSA n = 456, GSK343 + Auxin n = 933, TSA + Auxin n = 456). **(B)** Averages

865   of all three replicates for D values, fractional moving mass, and effective diffusion coefficient for

866   the nuclear bodies and nuclear blebs for control and 24-hour auxin-treatment conditions in

867   HCT116$^{LMN(B1\&B2)\text{-}AID}$ cells. **(C)** Averages of all three replicates for D values, fractional moving

868   mass, and effective diffusion coefficient for DMSO (vehicle control) 24-hour GSK343, and 24-

869   hour TSA-treatment conditions in HCT116$^{LMN(B1\&B2)\text{-}AID}$ cells. For **(A-C)** means of all replicates

870   are presented above each bar in the plots. Error bars represent mean ± SEM. For **(B-C)**, Data

871   are compiled from three technical replicates (N = 3; Control n = 2451, Auxin n = 2140, Control

872   Blebs n = 200, Auxin Blebs n = 129, DMSO n = 741, GSK343 n = 790, TSA n = 498, DMSO

873   Blebs n = 564, GSK343 Blebs n = 467, TSA Blebs n = 77).

874

875   **Figure S2. Characterization of heterochromatin nanodomains from SMLM images.**

876   Quantification of the number and size of heterochromatin nanodomains in control, loss of lamin

877   B1/B2, and TSA treatment conditions for HCT116$^{LMN(B1\&B2)\text{-}AID}$ cells.

878

879   **Figure S3. FDTD simulation confirms the correlation between PWS Intensity with nuclear**

880   **average RI and CVC.**

881   **(A)** Schematic of FDTD simulation setup. Light is illuminated from objective and focused on the

882   cell glass interface. Random media with autocorrelation coefficients representing chromatins are

883   placed into the simulation space. By solving Maxwell equations numerically, the back scattering

884   light intensity field is resolved and used to synthesize simulation PWS images. **(B)** Negative

885   correlation between PWS normalized reflectance and media average RI and phi. n = 10 for
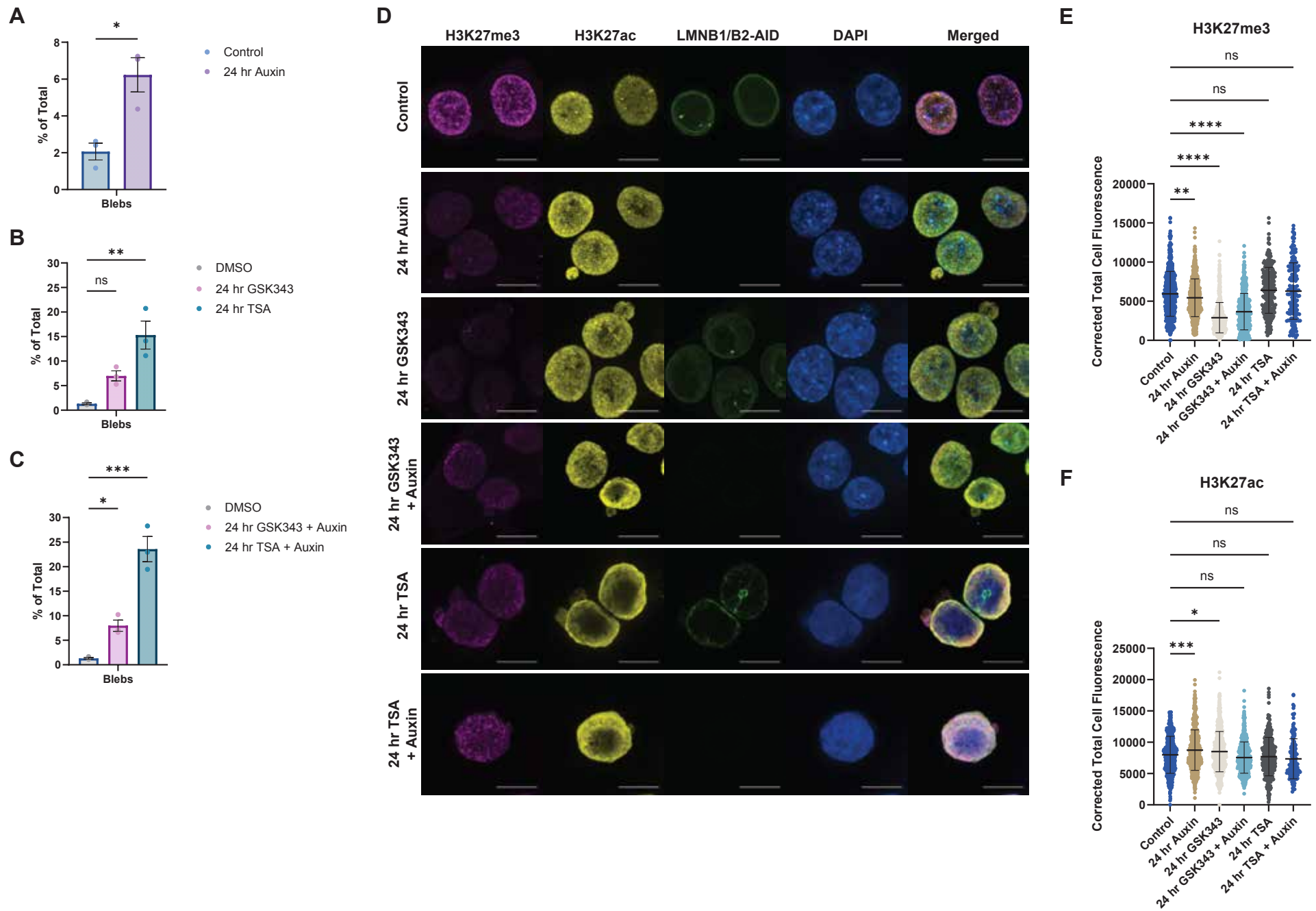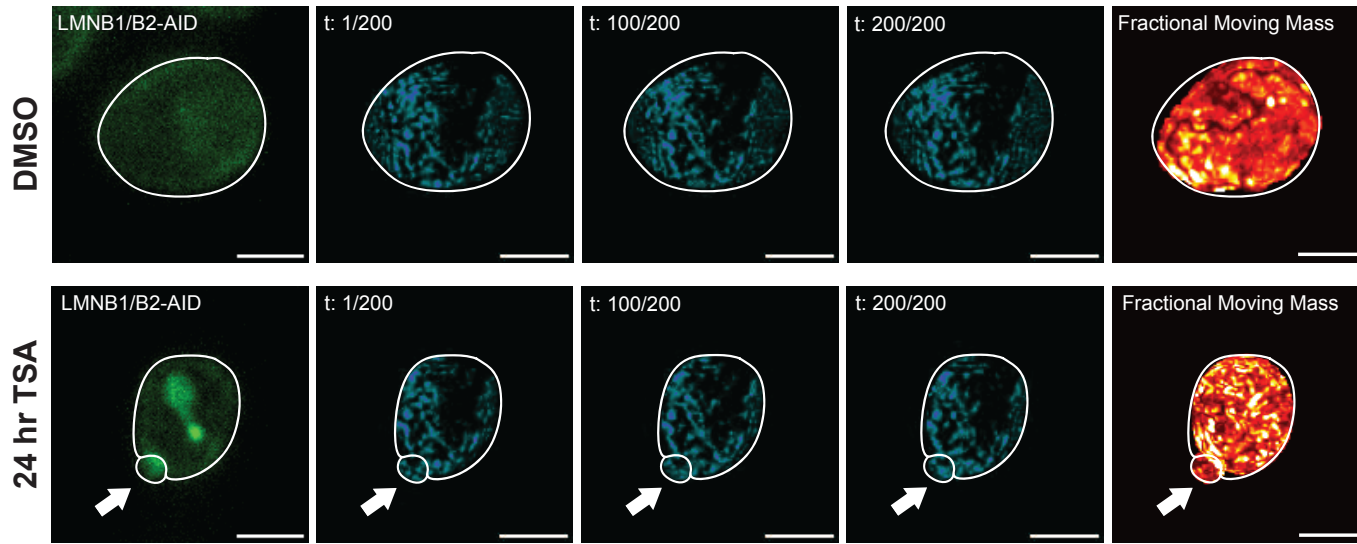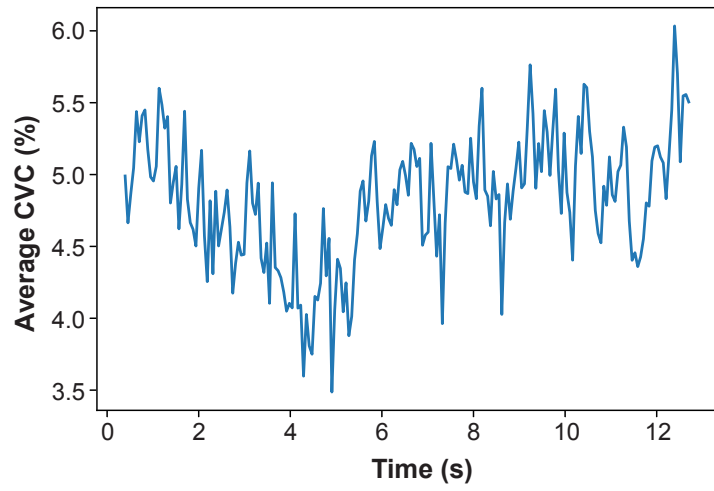
886   each condition.

887

Figure 1

Figure 2

Figure 3

Figure 4

A HCT116

DMSO

B (-) Lamin B1/B2

C (-) HDACs

H3K9me3

D U2OS

Control

E (-) HDACs

H3K9me3

F U2OS

Control
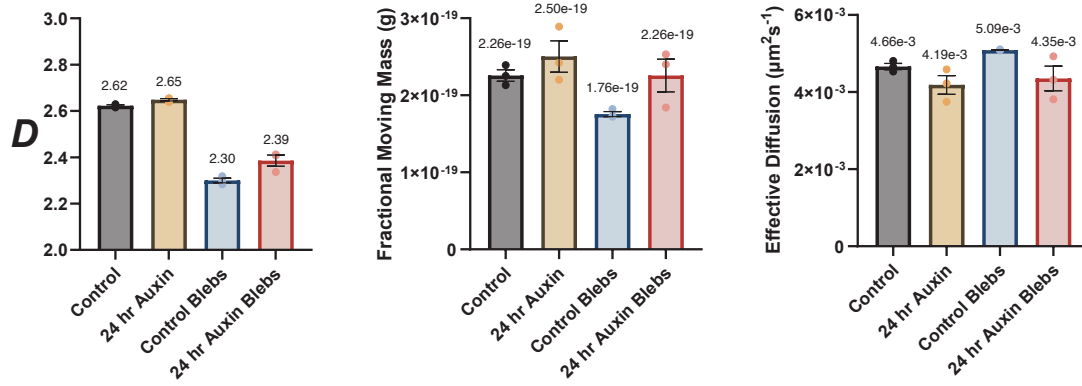
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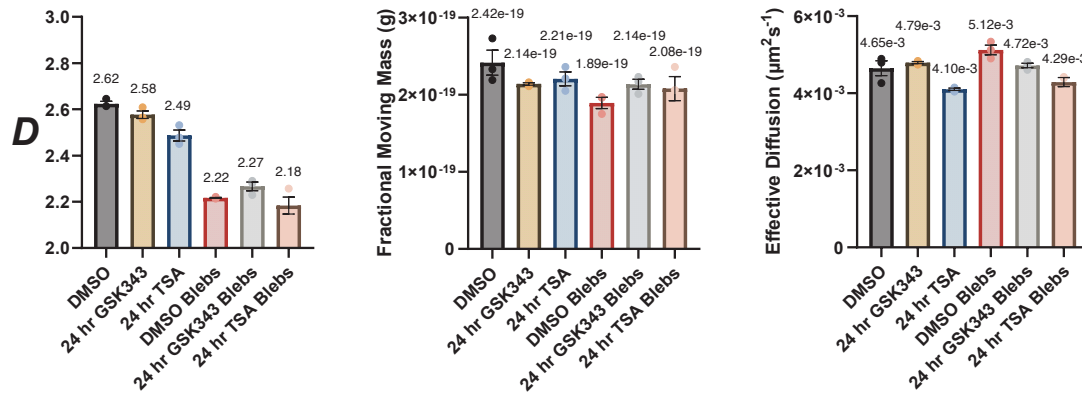(-) HDAC - Nuclear Bleb

Count / Normalized Cluster Area ($A/\pi * r_0^2$)
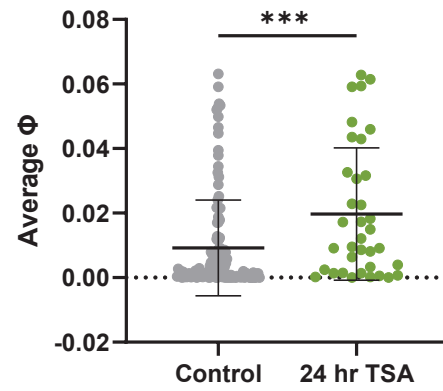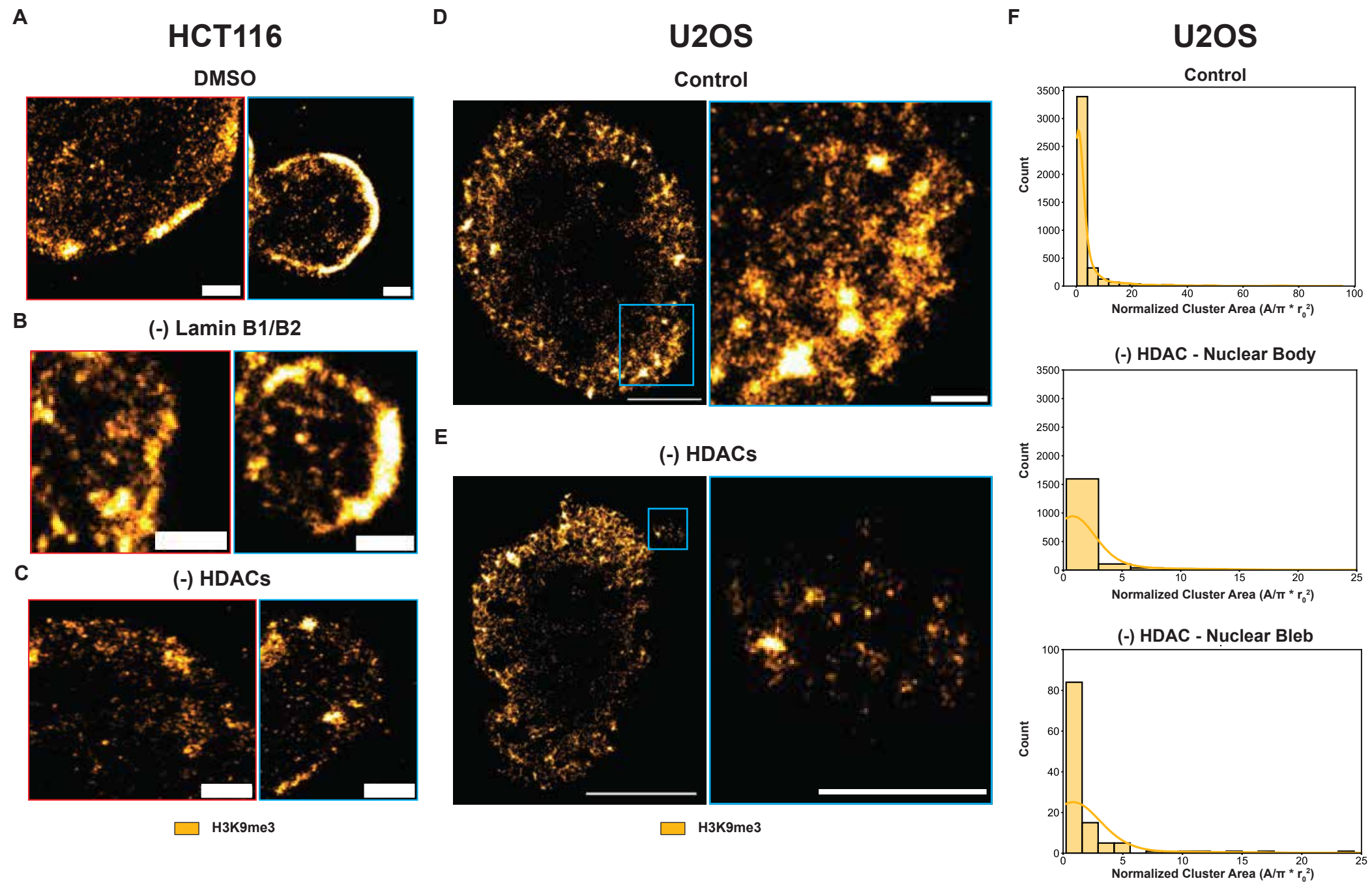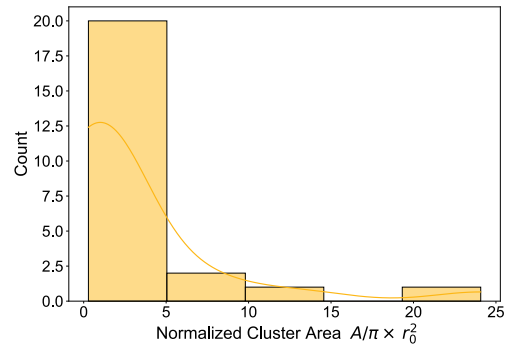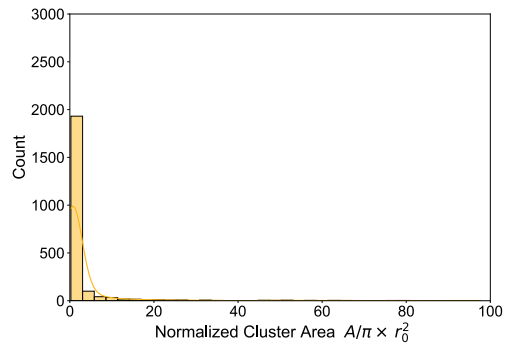
Supplementary Figure 1
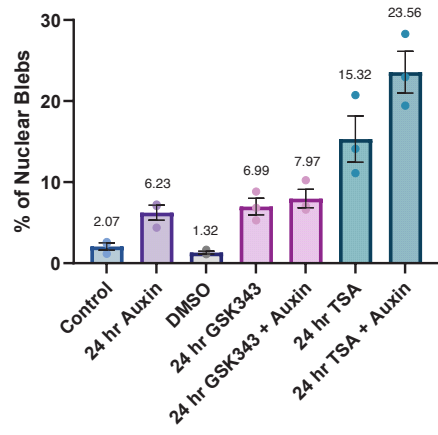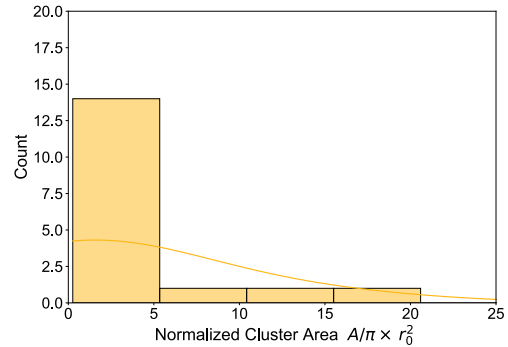
HCT116

DMSO - Nuclear Body
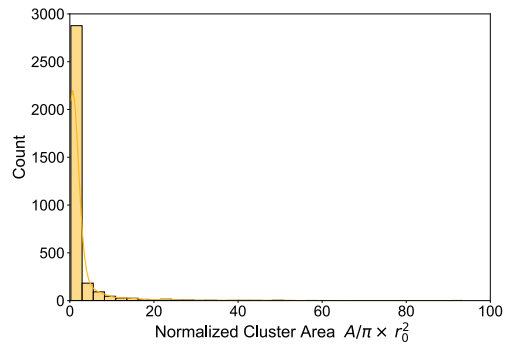
DMSO - Nuclear Bleb
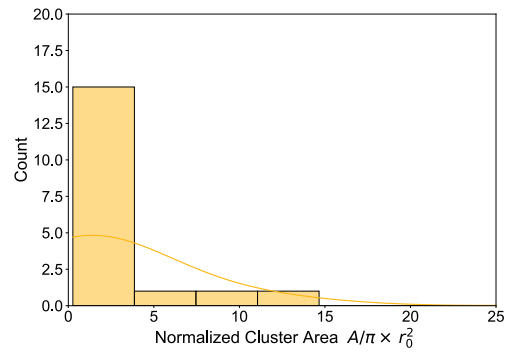
(-) Lamin B1/B2 - Nuclear Body

(-) Lamin B1/B2 - Nuclear Bleb

(-) HDAC - Nuclear Body

(-) HDAC - Nuclear Bleb

A

B