1   **TITLE**

2   **Depletion of lamins B1 and B2 alters chromatin mobility and induces differential gene**

3   **expression by a mesoscale-motion dependent mechanism**

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51 **ABSTRACT**

BACKGROUND

B-type lamins are critical nuclear envelope proteins that interact with the 3D genomic architecture. However, identifying the direct roles of B-lamins on dynamic genome organization has been challenging as their joint depletion severely impacts cell viability. To overcome this, we engineered mammalian cells to rapidly and completely degrade endogenous B-type lamins using Auxin-inducible degron (AID) technology.

RESULTS

Paired with a suite of novel technologies, live-cell Dual Partial Wave Spectroscopic (Dual-PWS) microscopy, *in situ* Hi-C, and CRISPR-Sirius, we demonstrate that lamin B1 and lamin B2 depletion transforms chromatin mobility, heterochromatin positioning, gene expression, and loci-positioning with minimal disruption to mesoscale chromatin folding. Using the AID system, we show that the disruption of B-lamins alters gene expression both within and outside lamin associated domains, with distinct mechanistic patterns depending on their localization. Critically, we demonstrate that chromatin dynamics, positioning of constitutive and facultative heterochromatic markers, and chromosome positioning near the nuclear periphery are significantly altered, indicating that the mechanism of action of B-type lamins is derived from their role in maintaining chromatin dynamics and spatial positioning.

CONCLUSIONS

Our findings suggest that the mechanistic role of B-type lamins is stabilization of heterochromatin and chromosomal positioning along the nuclear periphery. We conclude that degrading lamin B1 and lamin B2 has several functional consequences related to both structural disease and cancer.

KEY WORDS

78 Nuclear lamina; Auxin-inducible Degron System; 3D Chromatin Organization; Lamin Associated

79 Domains; Topologically Associated Domains; Partial Wave Spectroscopic Microscopy; CRISPR-

80 Sirius; *in situ* Hi-C

81

82 **BACKGROUND**

83 Chromatin is the complex macromolecular polymer-assembly formed by folding the genome and

84 its associated proteins within the confines of the cell nucleus. Numerous studies indicate that

85 the interplay of physical, chemical, and molecular principles leads to the emergence of the

86 major organizational features of the genome, including polymer-polymer interactions, chromatin

87 loop extrusion[1,2], phase separation[3,4], the physical interaction of chromatin with stable

88 architectural elements of the nucleus (i.e., nuclear envelope)[5-7], and chromatin dynamics[8,9]. Our

89 understanding is limited, however, about the mechanisms governing chromatin folding due to

90 the tethering that occurs at the nuclear periphery.

91

92 It is widely believed that the nuclear lamina is the structural limit on the spatial distribution of 3D

93 genome organization in the nucleus[10]. Lamin proteins are structural components of the nuclear

94 lamina that interact with both the cytoskeleton and the genome[11]. Lamins interact with chromatin

95 either indirectly or directly through chromatin binding proteins[12]. Mechanistically, these lamin-

96 proteins are also involved in several critical processes, including transcription, DNA repair, and

97 replication. Lamin dysregulation is associated with more than 15 diseases, termed

98 laminopathies[11,13,14]. The four main lamin isoforms found in mammals are lamin A/C, lamin B1,

99 and lamin B2, respectively[11]. Although lamin B1, lamin B2, and lamin A/C form overlapping

100 networks, B-type lamins localize primarily to the nuclear periphery adjacent to the inner nuclear

101 membrane, while A-type lamins can extend into the nucleoplasm[6,15]. In contrast to A-type

102 lamins, B-type lamins remain tightly associated with the nuclear membrane and have been

103 mapped using DamID to reveal the existence of dynamic and functional euchromatin lamin B1

104 domains.[16] Prior work identified that inhibition of lamin A/C and lamin-B receptor (LBR) alters

105 A/B compartment segregation, specifically chromatin region localization[17,18]. Differential

106 inhibition of lamin B1 or B2, in contrast, has been shown to have variable effects on

107 compartmentalization. These varied effects are hypothesized to be due to the persistence of the

108 conjugate B-protein.[6,14,19] As B-type lamins have similar domain structures, lamin B1 and lamin

109 B2 were conjectured to have redundant functions[20]. It has also been postulated that silencing B-

110 type lamin expression leads to a dramatic increase of lamin A mobility in the nucleoplasm to

111 maintain chromatin organization and transcription.[21] These varied hypotheses illustrate a need

112 to uncover how lamins B1 and B2 contribute to normal cellular physiology.

113

114 Mechanistically, B-type lamins are thought to regulate chromatin structure and gene

115 transcription by physical attachment of chromatin to constrain chromatin dynamics and

116 structure[6]. This mechanism has been evidenced by the recruitment of genes to the nuclear

117 lamina within regions termed lamin-associated domains (LADs), associated with transcriptional

118 repression, B-compartments, and heterochromatin nucleosome markers (e.g., H3K9me2/3)[16,22-

119 24]. Paradoxically, transcriptionally rich territories exist adjacent, and interspersed within, these

120 sites of transcriptional repression, suggesting that the nuclear lamina forms a complex

121 environment for transcriptional regulation[25]. Physically, LADs are variably sized, with domains

122 spanning from 100 kb to 10 Mb and a median size of 0.5 to 1 Mb from ChIP-seq analysis of

123 lamin A/C and lamin B1.[24] However, the reported relative sizes of LADs can also vary between

124 molecular and imaging techniques or cell type. For example, while imaging studies in mouse

125 and human cells report LADs to be 10 kb to 10 Mb in size, ChIP-seq studies in *Drosophila* cells

126 report LADs to vary between 7 and 700 kb[23,26,27].

127

128 Despite the broad role of B-lamins in physiology, the essentiality and redundancy of B-lamin

129 proteins poses a formidable challenge to understanding their distinct role in regulating cell

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130   function. To overcome this limitation, we generated an auxin-inducible degron system targeting

131   both B-type lamins simultaneously (see **Materials and Methods).** Utilizing this engineered cell

132   line and based on prior studies of lamin A/C compared to lamin-B receptor in nuclear

133   inversion[17,18], we tested the hypothesis that the mechanism of action of B-type lamins is due to

134   their role in tethering the genome to the nuclear periphery. In the predominant model of lamin

135   B1/B2 at present, these lamins act by the differential constraint of inner nuclear matrix to

136   regulate chromatin higher-order structure and dynamics[5,6,16,23]. Based on these prior studies, we

137   hypothesized that simultaneous LMNB1 and LMNB2 inhibition would result in (1) increased

138   chromatin chain fluctuations, (2) internal translocation of heterochromatin domains, (3)

139   dissociation of genes from the nuclear periphery, and (4) that the genes normally located on the

140   nuclear periphery would be differentially expressed due to the transformation in mesoscale (~10

141   – 1000 nm)[28-30] chromatin folding.

142

143   Surprisingly, although we observed an increase in chromatin density fluctuations,

144   heterochromatic translocation, and gene loci internalization, mesoscale chromatin folding was

145   minimally altered. Indeed, contact scaling, topologically associated domains, chromatin loops,

146   and A/B compartments were minimally perturbed with the joint inhibition of lamin B1 and lamin

147   B2. Further, this effect was similar both within and outside of LAD segments. Likewise, we

148   observed substantial upregulation and downregulation of genes both within and outside of

149   LADs. Phenotypically, changes in expression for genes within LADs were associated with

150   structural disorders and laminopathies (e.g., scoliosis) whereas differentially expressed genes

151   outside LADs were associated with malignancies (e.g., ovarian cancer). Based on these

152   findings, we propose several possible indications: (1) an independent role of B-type lamins in

153   regulation of cellular function independent of mesoscale chromatin regulation and (2) the

154   possibility of a hysteresis in mesoscale chromatin folding induced by association with B-type

155 lamins that is not lost upon joint inhibition. This hysteresis could be due to maintenance of

156 interaction with lamin A/C or direct chromatin interactions with the nuclear envelope.

157

158 **RESULTS**

159 **Addition of the mAID tag to B-type lamins does not impact proper localization of lamin**

160 **A/C**

161 Prior attempts at simultaneous elimination of lamin B1 and lamin B2 have not been successful

162 due to their critical roles in cellular viability and other important cellular processes[13]. To

163 overcome this problem, we utilized the Auxin Inducible Degron system, which allowed

164 simultaneous inhibition of B-type lamins at short timescales[31,32]. In brief, the auxin-dependent

165 degradation pathway found in plants can be introduced into non-plant eukaryotic species to

166 induce rapid depletion of a protein of interest upon exposure to the phytohormone, auxin. This is

167 achieved by fusing a destabilizing domain (degron) to the protein of interest **(Figure 1A-B).** In

168 cells expressing the F-box protein from *Oryza Sativa* (OsTIR1), the addition of auxin results in

169 OsTIR1 forming a functional SCF (Skp1-Cullin-F-box) ubiquitin ligase. Proteins fused with the

170 7 kDa degron termed mini-AID (mAID) derived from the IAA17 protein of *Arabidopsis thaliana*

171 are rapidly degraded. Importantly, this degradation system is reversible and tunable, allowing for

172 greater control of target protein degradation **(Figure 1D, S1B)**. To achieve this in B-type lamins,

173 we CRISPR-engineered HCT116 colorectal carcinoma epithelial cells and knocked in the mini

174 auxin-inducible degron and mClover at the end of endogenous lamin B1 (LMNB1) and lamin B2

175 (LMNB2) gene loci **(Figure 1B)** [33]. For simplicity, we will hereon refer to these engineered cell

176 lines as HCT116^LMNB1-AID, HCT116^LMNB2-AID and HCT116^LMN(B1&B2)-AID.

177

178 To confirm that addition of the mAID tag degron does not alter the functionality of the tagged

179 lamin proteins, we used immunofluorescence to ensure the continued association of lamin

180 B1/B2 with other proteins that localize to the same compartment as the untagged proteins (i.e.,

181   lamin A/C). Genomic localization has previously demonstrated utility as an indicator of functional

182   retention in transcription factor binding [34,35]. As expected, our results indicate that LMNB1-

183   mAID-mClover, LMNB2-mAID-mClover, and LMNB1/B2-mAID-mClover proteins localize

184   properly to the nuclear lamina in cells **(Figure 1C, S1A)**. We observed a strong overlap between

185   the mClover-signal (hence LMNB1 and/ or LMNB2) and Lamin A/C immunostaining signal. This

186   result suggests that addition of the mAID-mClover tag does not alter proper localization and

187   allows for retention of LMNB1 and LMNB2 protein function.

188

189   **Auxin treatment results in rapid and reversible depletion of targeted B-type lamins**

190   We next investigated the kinetics and reversibility of mAID-tagged lamin degradation to confirm

191   the suitability of this approach for reversible inhibition of B-type lamins. A major advantage of

192   the AID system is that conditional depletion of target proteins has less off-target effects than

193   traditional perturbation methods such as RNA interference[31]. This allows examination of

194   resulting changes in chromatin structure and transcription at the most relevant time scales, in

195   which these changes are directly associated with target protein degradation. Further, reversal of

196   target degradation can be achieved by simply removing auxin from cell culture media. We first

197   evaluated the impact of auxin treatment and the reversal process on B-type lamin degradation.

198   To induce the expression of OsTIR1, we added 2 mg/mL doxycycline to cell media 24 hours

199   prior to auxin treatment. Western blot of global protein levels and fixed-cell flow cytometric

200   analysis indicated that 1000 μM auxin treatment resulted in rapid degradation of AID-tagged

201   lamins in HCT116$^{LMNB1-AID}$, HCT116$^{LMNB2-AID}$, and HCT116$^{LMN(B1\&B2)-AID}$ cells **(Figures 1E-F, S1C-**

202   **D)**. Nearly 80% of LMNB1-mAID was degraded within the first 6 hours of auxin treatment.

203   Notably, lamin levels returned to basal levels 3 days after auxin removal. From these results, we

204   confirmed the utility of this mAID system as a means for rapid and reversible degradation of

205   endogenous LMNB1 and LMNB2 proteins to subsequently investigate their role in chromatin

206   folding and cellular function.

207

Acute depletion of B-type lamins alters nuclear morphology

As the nuclear envelope is thought to function in the confinement of the genome, we first began by analyzing the effect of B-type lamin inhibition on nuclear morphology. As expected, we observed morphological alterations when either of the lamins is depleted. To quantify these effects, we used ImageStreamX, a flow cytometry based high throughput microscopy imaging platform. At the 8-hour auxin treatment time point for all three cell lines, the population of cells included both mClover-positive (intact lamins) and mClover-negative (degraded lamins) cells. Using this flow cytometry-based analysis of DAPI-stained nuclear area, we found that lamin degradation resulted in enlarged nuclear volume in all three models **(Figure 1G, S2A-B)**. In addition to the change in nuclear volume, confocal sections in HCT116$^{LMN(B1\&B2)-AID}$ cells revealed lamin-specific spherical and discrete puncta, which has been previously demonstrated in the nucleus of cultured cells [36,37] **(S2C)**. Additionally, we noticed a notable increase in the presence of nuclear deformations, such as nuclear blebbing **(Figure 1H, S1E)**. To evaluate if acute depletion of B-type lamins transformed nuclear morphology due to secondary effects on cell viability, we used Fluorescent-activated cell sorting (FACS) to analyze cell death in HCT116$^{LMNB1-AID}$, HCT116$^{LMNB2-AID}$ and HCT116$^{LMN(B1\&B2)-AID}$ cells. Using fluorescently labeled Annexin V (Annexin V^{APC}) and DAPI staining to measure overall apoptotic and necrotic cell death,[38] we confirmed that the acute depletion of both B-type lamins did not induce notable apoptosis or necrosis at 12, 24, or 48 hours of auxin treatment in comparison to the untreated conditions **(S2D)** As such, this indicated that the alterations in nuclear morphology were a direct consequence of B-type lamin removal and that subsequent effects explored were not secondarily due to processes associated with cellular death.

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Acute depletion of both B-type lamins induces minimal changes in mesoscale chromatin structure

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233   Owing to the macroscopic alterations in nuclear morphology observed above as well as prior

234   work showing the role of lamin A/C and LBR in mesoscale chromatin organization, we

235   hypothesized that the depletion of both B-type lamins would produce global alterations in

236   chromatin folding, topologically associated domains, loops, and contact scaling. To test this, we

237   performed *in situ* Hi-C[25], which can capture structural changes in the kilobase-level DNA-DNA

238   contacts[2], in the AID cell lines (HCT116[LMNB1-AID], HCT116[LMNB2-AID], and HCT116[LMN(B1&B2)-AID]

239   cells). For each cell type, we generated more than a 1 billion contacts from cells before (~

240   1,354,831,021) and after 24 hours of auxin treatment (~ 1,210,053,449).

241

242   Multiple methods have been proposed for quantifying the mesoscale organization of the

243   genome based on Hi-C data, including (1) analysis of A/B compartment switching[39], (2)

244   eigenvector decomposition[25], (3) measurement of contact scaling $(|s|)$[40], and (4) evaluation of

245   topologically associated domain stability[41-43]. Using Hi-C data, the relationship between

246   chromatin packing behavior and genome connectivity can be conceptualized by the contact

247   probability scaling exponent (*s*). The probability (*P*) of contact between two monomers

248   separated by length (*N*) along a linear chromatin chain follows a power-law scaling relationship:

249    $P \propto N^s$. [40] Notably, upon depletion of both lamin B1 and lamin B2, we observed minimal

250   changes in mesoscale chromatin structure **(Figure 2A, S3A-C)**. Depletion of both B-type lamins

251   at 24 hours did not result in major weakening or switching of chromosomal A/B compartments

252   **(Figure 2B)**, markedly alter the frequency or size of topologically associated domains **(Figure**

253   **2D, S3D)**, nor change the frequency of contacts as measured by *|s|* **(Figure 2C)**. For example,

254   using TopDom[44] analysis, a domain-calling algorithm, we found that the mean TAD size slightly

255   decreased (control ~ 348 kb versus 24 hr. Auxin ~ 326 kb) while the number of TADs only

256   modestly increased upon 24 hours of auxin treatment (control 7979; 24 hr. Auxin 8491) **(Figure**

257   **2D)**. This is in major contrast to the findings observed in LBR depletion where compartment

258   switching, and TADs were markedly transformed in thymocytes lacking LBR compared to WT

259   controls[17].

260

261   Next, we hypothesized that one potential explanation of the limited changes in mesoscale

262   chromatin structure upon joint lamin B1 and lamin B2 inhibition could be due to confinement of

263   effects being limited to local alterations around LADs. To perform this segmental analysis, we

264   utilized publicly available DNA adenine methyltransferase identification (DamID) data for Lamin

265   B1 in HCT-116 cells[45,46]. We computationally segmented the genome into contacts that spanned

266   both LADs and non-LADs (across each), across only LADs, or across only non-LADs **(Figure**

267   **3A, S3E)**. As prior work has shown that LADs are associated with B-compartments[23,24], we

268   hypothesized that contact scaling would decay slower within B1 LADs. As expected, we

269   observed a lower $|s|$ (calculated between 10^5-10^6 bp) within LADs compared to within non-LADs

270   in untreated cells **(Figure 3E)**.

271

272   When we compared $|s|$ for chromosomes associated with the periphery (e.g., chromosome 3) to

273   those associated with the nuclear center (e.g., chromosome 19), we observed a greater

274   difference between $|s|$ in chromosome 3 **(Figures 3B-C)**. Further, analysis of each chromosome

275   demonstrated an inverse relationship between $|s|$ within LADs and the percent coverage of the

276   chromosome confined within LADs **(Figure 3D)**. However, despite the large difference in $|s|$ at

277   baseline and inverse relationship between $|s|$ and LAD coverage, only minor changes in $|s|$ were

278   observed upon B-lamin depletion with a larger magnitude of effect observed within the non-LAD

279   portions **(Figure 3E)**. Likewise, as expected, there was no relationship between $|s|$ within non-

280   LADs and the chromosomal coverage of lamin B1 **(S3F).** This finding was similarly extended

281   into analysis of TADs. Analysis of TAD size and number within and outside of LADs confirmed

282   that the median size of TADs within LAD regions is higher than TADs outside of LAD regions.

283   However, B-lamin depletion did not differentially impact the size or number of TADs within or

284   outside of LADs **(Figure 3F)**. Overall, these findings indicated that although genomic segments

285   that associate with B-type lamins have a distinct mesoscale structure, the disruption of Lamin

286   B1/B2 surprisingly may not produce phenotypic effects through alterations in higher-order

287   chromatin folding structure.

288

289   **Disruption of B-type lamins alters chromatin dynamics but has a minimal effect on**

290   **higher-order structure in live cells**

291   Although we did not observe major alterations to chromatin folding by *in situ* Hi-C, we

292   hypothesized that disruption of B-type lamins could impart a differential effect on cellular

293   function by changing chromatin dynamics in live cells. To test this hypothesis, we used dual-

294   mode live-cell Partial Wave Spectroscopic (PWS) microscopy to detect both chromatin

295   structural changes and chromatin mobility variations. PWS microscopy provides label-free

296   measurements of nanoscale structural changes with a sensitivity to structures between ~ 20

297   and 200 nm in live cells without the use of cytotoxic labels.[47-49] Briefly, in dual-mode PWS

298   microscopy, variations in nanoscopic-macromolecular structure are measured by analyzing the

299   spectral-dependence in light scattering from the packing of chromatin into higher-order

300   structures, such as chromatin packing domains, while temporal variations in scattering allow

301   measurement of chromatin mobility.[40,50-52] These domains are characterized by polymeric

302   fractal-like behavior, high chromatin-packing density, and a radial decrease in mass-density

303   from the center to the periphery.[29,53] As the primary macromolecular assembly within the

304   nucleus is chromatin, this method has been shown to detect changes in chromatin folding

305   comparable to Hi-C and electron microscopy.[40] Given that higher-order chromatin structure is

306   determined by the polymeric folding across length-scales between 10 – 200 nm and is

307   approximately a power-law (quantified by $|s|$ and is observed in multiple polymer models), this

308   distribution in packing corresponds to the distribution of the refractive index quantified by mass

309   scaling (chromatin packing scaling, *D*).[53] This scaling parameter is calculated for each pixel

310 within the region of interest (i.e., nucleus) within a given coherence volume, which is determined

311 by the depth of field longitudinally and the axial plane for each pixel. Therefore, spatial

312 variations of macromolecular density and motion that can occur upon perturbation, such as

313 degradation of B-type lamins, can be measured using PWS.

314

315 First, we confirmed with PWS that OsTIR1 expression (upon doxycycline treatment) does not

316 alter D **(S4E)**. As $|s|$ and D are inversely related based on prior experimental studies and

317 polymer modeling[40,52], we hypothesized that that nuclear average D would increase slightly after

318 inhibition of lamin B1 and lamin B2 due to the small decrease in $|s|$ observed within non-LAD

319 portions of the genome.[40] Upon the addition of auxin to HCT116$^{LMN(B1\&B2)-AID}$ cells, the average D

320 increased by a minimal amount of 0.8% but statistically significant amount across the nucleus

321 (0.021 ± 0.002 (SEM), $n = 1822$, *p-value* < 0.0001) **(Figure 4A-B)** with larger but still modest

322 changes observed within HCT116$^{LMNB1-AID}$ and HCT116$^{LMNB2-AID}$ **(S4A-B,** $n = 482$ & 401,

323 respectively)**. We verified a strong correlation between the change in D and mClover signal with

324 a 24-hour auxin treatment time course **(S4E)**. Further, the magnitude of this effect was

325 substantially smaller than that observed in other conditions, such as in transcription inhibition by

326 actinomycin D in BJ fibroblast cells (7% decrease).[40] Overall, the small increase in D and

327 decrease in $|s|$ suggested that the regulatory role of nuclear B-type lamins was independent of

328 mesoscale chromatin folding in both live and fixed cells.

329

330

331 Utilizing the temporal capabilities of dual-PWS, we then evaluated the influence of B-type lamin

332 degradation of chromatin dynamics[50,51]. Although dual-PWS lacks molecular specificity,

333 variations in temporal interference from macromolecular movement allow quantification of the

334 fractional moving mass (the magnitude of chromatin evolving in time) and the diffusion

335 coefficient without labels. Fractional moving mass, calculated from the product of the mass of

336   the moving macromolecular cluster and the volume fraction of moving mass, quantifies the

337   physical and dynamic properties chromatin, as not all motion within the cell is specifically

338   diffusive.[50] Unexpectedly, although minor changes in higher-order chromatin structure were

339   observed upon lamin B1/B2 depletion, there were much larger changes in the fractional moving

340   mass on treatment with auxin in HCT116$^{LMN(B1\&B2)-AID}$ cells (18.4%) (2.780 ± 1.275e-21 (SEM), n

341   = 1822, *p-value* < 0.0001) **(Figure 4C).** Similar changes were observed in HCT116$^{LMNB1-AID}$ and

342   HCT116$^{LMNB2-AID}$ **(S4C).** These results suggest that upon lamin B1 and lamin B2 degradation,

343   there is an increase in molecular motion occurring universally within the cell across a large

344   range of dynamic processes.[50] Indeed, we further observed that inhibition of lamin B1 and lamin

345   B2 resulted in a decrease in the ensemble diffusion coefficient of 11.4% compared to the

346   untreated control (0.000 ± 2.664e-5 (SEM), n = 1822, *p-value* < 0.0001) **(Figure 4D)**, with

347   similar findings in HCT116$^{LMNB1-AID}$ and HCT116$^{LMNB2-AID}$ **(S4D).** Taken together, our temporal

348   analysis indicates that B-type lamin degradation results in overall greater magnitude of

349   chromatin turnover with a slower rate of motion.

350

351   **Lamins B1 and B2 mechanistically determine chromatin mobility and heterochromatin**

352   **localization throughout the nucleus**

353   Based on our observations above on the slight differential effect in *|s|* in LAD vs non-LADs and

354   the alterations in chromatin mobility, we investigated if the mechanistic role of B-type lamins is

355   in the stabilization of chromatin cores to the nuclear periphery. To distinguish between whole-

356   nuclei and regional chromatin structure, we segmented the nucleus into regions of equal size

357   spanning the nuclear edge and the center of the nuclear interior **(Figure 4E)**. This spatial

358   analysis involved segmenting the nucleus into six non-overlapping ribbons to evaluate variations

359   in the location of chromatin packing domains. Structurally, *D* near the nuclear periphery was

360   lower than that of the nuclear interior, with minimal differences observed between the periphery

14

361 and the interior during lamin B1 lamin B2 inhibition **(Figure 4F)**, with similar trends observed in

362 Lamin B1 and B2 inhibition separately **(S4F)**. Further, D was strongly correlated with the

363 distance from the periphery (Pearson correlation coefficient = 0.95). This differential spatial

364 response of the change in D could potentially be explained by the movement of chromatin

365 between the periphery and nuclear interior. Unexpectedly, although fractional moving mass was

366 increased and diffusion was decreased throughout the nucleus, the differences between the

367 nuclear periphery and the center in HCT116$^{LMN(B1\&B2)-AID}$ cells was minimal **(Figures 4G-H)**, with

368 comparable results observed in HCT116$^{LMNB1-AID}$ and HCT116$^{LMNB2-AID}$ **(S4G-H)**.

369

370 Overall, these findings suggested that the mechanistic role of lamin B1 and lamin B2 was in

371 confinement of chromatin mobility throughout the cell nucleus and suggested that when lamin

372 B1 and lamin B2 are removed, chromatin moves away from the nuclear periphery with rippling

373 effects into the nuclear interior regions. We reasoned that if LMNB1 and LMNB2 depletion leads

374 to the detachment of chromatin from the nuclear periphery, as indicated by our dynamic PWS

375 results, this shift would be accompanied by heterochromatin redistribution and peripheral

376 decompaction. Using a combination of immunofluorescence and western blot analysis, we

377 confirmed an internal shift and global decrease in heterochromatin (H3K9me2/3 and

378 H3K27me3) and an increase in euchromatin (H3K27ac) upon 24 hours of Auxin treatment

379 **(Figure 5A-B, S5A-C)**. To estimate changes in chromatin compaction upon the removal of B-

380 type lamins, we used spinning disk confocal microscopy to measure the coefficient of variation

381 in DAPI-stained HCT116$^{LMN(B1\&B2)-AID}$ cells. Previously used to assess the degree of

382 heterogeneity of DNA signal across the nucleus[54], the coefficient of variation is calculated as the

383 standard deviation of the DAPI intensity values divided by the mean value of nuclear pixel

384 intensity for each nucleus. Our results demonstrated that upon auxin treatment, chromatin

385 compaction slightly decreased in HCT116$^{LMNB1-AID}$ (1.11%), HCT116$^{LMNB2-AID}$ (2.31%), and

386 HCT116$^{LMN(B1\&B2)-AID}$ (2.86%) cells **(Figure 5C, S5E-F)**. Therefore, the removal of B-type lamins

387   results in destabilization of heterochromatin localization. Taken together, these results indicate

388   that although mesoscale organization is maintained, the shift in heterochromatin at the nuclear

389   periphery induces global nuclear changes due to alterations in chromatin mobility.

390

391   **Auxin treatment results in lamina-dependent changes in 3D spatial localization and**

392   **dynamic chromatin reorganization**

393   Owing to the differential effect of LB1/B2 depletion on chromatin mobility and spatial

394   distributions observed in HCT116$^{LMN(B1\&B2)-AID}$, we next tested if re-localization of

395   heterochromatin was paired with gene-re-localization from the nuclear periphery. Specifically,

396   although Hi-C demonstrated muted changes in |s|, compartments, and TADs, it remained

397   possible that heterochromatin cores were moving independent of the relocation of genes in 3D

398   space. To test this, we utilized CRISPR-Sirius, a DNA imaging system for imaging of

399   chromosome-specific loci with a high signal-to-noise ratio in live cells [55] to measure the spatial

400   distribution of genes in live cells upon lamin B1 and lamin B2 depletion. Since depletion of both

401   lamins B1 and B2 resulted in nuclear wide changes in mobility and heterochromatin localization

402   while mesoscale structures were maintained, we hypothesized that gene loci would similarly

403   relocate in response in HCT116$^{LMN(B1\&B2)-AID}$ upon auxin treatment.

404

405   To assess the effect of B-type lamin degradation on chromosome-specific loci in live

406   HCT116$^{LMN(B1\&B2)-AID}$ cells, we labelled genomic regions on chromosomes 3 and 19 using

407   CRISPR-Sirius. Chromosomes 3 and 19 were chosen to represent chromosomes that are

408   localized relatively near the nuclear periphery and the nuclear interior, respectively.[55-60]

409   Specifically, we chose one intronic gene region on Chromosome 3 (*XXYLT1*) containing ~ 333

410   tandem repeats, and one intronic gene region on Chromosome 19 (*TCF3*) containing ~ 36

411   tandem repeats as a reference to the nuclear interior. To measure the spatial distance and

412   dynamics of the *XXYLT1* gene loci, we labeled them with CRISPR-Sirius-8XMS2 and the MS2

16

413   capsid protein MCP-HaloTag, which contains a nuclear localization sequence.[55] We visualized

414   2-3 pairs of loci in individual cells **(Figure 5D-E)** and quantified the average distance between

415   the nuclear periphery and either the *XXYLT1* or *TCF3* gene loci, normalized to the nuclear

416   radius **(Figure 5F)**. Our results demonstrated that the average distance between the nuclear

417   periphery and *XXYLT1* significantly increased upon lamin degradation, indicating that the

418   targeted loci moved towards the nuclear interior, as expected. In conjunction with relocation of

419   heterochromatin and nuclear wide changes in chromatin mobility, the average distance between

420   the nuclear periphery and TCF3 also increased, although to a less significant degree. These

421   results indicate that upon B-type lamin degradation, chromatin reorganizes throughout the

422   nuclear area, especially at the nuclear periphery.

423

424   **Acute B-type lamin depletion alters gene expression within both LADs and Non-LADs**

425   Although the changes in mesoscale chromatin structure were muted, we hypothesized that

426   alterations in chromatin localization and dynamics could still result in profound changes in gene

427   expression. To test this, we utilized RNA-Sequencing to measure gene expression changes

428   between untreated cells to 12-hour auxin-treated cells, 48-hour auxin-treated cells, and cells

429   that were auxin-treated for 48 hours followed by a 6-day wash off **(S6A)**. Given the global

430   changes in nuclear organization observed, we hypothesized that depletion of B-type lamins

431   would result in differential gene expression within both LAD and non-LAD territories. We

432   anticipated that genes within LADs would experience upregulation once freed from the

433   heterochromatic nuclear periphery. Likewise, as mesoscale structure was preserved, we

434   anticipated that there would be limited association between gene expression changes and TAD

435   structure**.** To identify broad transcriptomic phenotypes following LMNB1/B2 degradation, we

436   analyzed differentially expressed genes (DEGs) at each timepoint **(S6B)**. B-type lamin depletion

437   led to widespread differential expression: 887 genes were upregulated, and 830 genes were

438   downregulated after 48 hours of auxin treatment, when the effect of lamin depletion was most

17

439   penetrant (adjusted P value < 0.01 and absolute log fold change > 1). Further, restoration of B-

440   type lamins after growing HCT116[LMN(B1&B2)-AID] cells in auxin-free media for 6 days after 48 hours

441   of auxin treatment returned cells to basal levels of gene expression. To summarize, these

442   findings indicate that B-type lamins have a distinct mechanistic role in transcriptional regulation

443   that is independent of mechanisms responsible for maintaining higher-order chromatin folding.

444

445   Based on prior work, we anticipated that many of the differentially expressed genes after 48

446   hours of B-lamin depletion would be found within LADs. Using the same publicly available

447   Lamin B1 DamID data from HCT116 cells, we again segmented the genome into regions within

448   LADs and regions outside LADs[45]. After 48 hours of lamin depletion, we identified nearly 22% of

449   enriched genes within LADs (adjusted P value < 0.01 and absolute log fold change > 1) **(Figure**

450   **6A)**. Anomalously, lamin B1 and lamin B2 depletion predominantly generated the highest

451   number DEGs outside of LAD boundaries for all three timepoints, with the greatest number at

452   48 hours of auxin treatment (374 genes within LADs, 1331 genes outside of LADs at 48 hours)

453   **(S6C-D)**. Unexpectedly, in both LAD and Non-LAD regions, there was no clear trend towards

454   upregulation or downregulation in gene expression. Further, the log fold change of DEGs

455   outside of LADs was larger in magnitude than within LADs, indicating that they were

456   disproportionally influenced by loss of B-type lamins **(S6E)**.

457

458   With respect to the properties of DEGs within LADs and Non-LADs, we performed a gene

459   ontology analysis using Metascape and the Disease Gene Network Database. Notably, DEGs

460   within LADs were enriched for a handful of cancers and laminopathies such as scoliosis

461   **(Figures 6B-C)**. Together, these data suggest that LADs maintain transcriptionally quiet states

462   for many genes and upon depletion of B-type lamins, unevenly upregulate genes leading to

463   altered transcriptional states that contribute to laminopathies and cancers. We also analyzed the

464   transcriptional divergence of genes within and outside of LADs. Transcriptional divergence is a

18

465   measure of the change in expression between minimally expressed transcripts and highly

466   expressed transcripts. Using the Gini equation, a common metric borrowed from mainstream

467   economics for measuring equality across a population, we calculated the Gini coefficients for

468   each timepoint to quantify transcriptional divergence[61]. Upon 48 hours of auxin treatment, the

469   Gini coefficient for all genes decreased modestly from ~ 0.87 to 0.85. We also found that

470   transcriptional divergence was generally higher within LADs versus non-LADs **(S6F)** and that

471   genes within LADs were more heterogeneously expressed upon B-type lamin depletion **(S6E)**.[62]

472   Given that DEGs within LADs were associated with laminopathies and structural disorders while

473   DEGs in non-LADs were primarily associated with malignancy, these results indicate distinct

474   mechanistic consequences of gene positioning.

475

476   **DISCUSSION**

477   In this work, we generated clonal cell lines where endogenous copies of LMNB1 and LMNB2

478   were fused with mAID to achieve rapid and reversible degradation of B-type lamins in the cell

479   through auxin exposure. Using this approach, we investigated the role of B-type lamins on

480   chromatin organization, dynamics, cell function, and gene expression. Our results demonstrate

481   that auxin treatment results in an enlarged nuclear area and can effectively deplete B-type

482   lamins within 24 hours without resulting in apoptosis or necrosis. The consequence of lamin B1

483   and lamin B2 depletion had surprising effects on nuclear structure and gene expression.

484   Critically, our findings indicate that the functional consequences of B-type lamin degradation do

485   not depend on changes to the mesoscale chromatin structure (i.e., TADs, contact scaling, or

486   compartments), but rather on alterations in chromatin dynamics and the spatial distribution of

487   the genome. However, lamins regulate spatial chromatin organization as we observe that lamin

488   depletion leads to the movement of gene-specific loci towards the nuclear interior and increased

489   density fluctuations in the chromatin polymer.

490

19

491   In recent Hi-C studies comparing conventional and inverted nuclei that form from LBR depletion

492   or Lamin A/C depletion, relatively few changes in compartment and chromatin connectivity are

493   observed[17]. While B-type lamin depletion leads to shifts in gene expression within and outside of

494   LADs, our results demonstrate that these changes are not from compartment switching, TAD

495   depletion, or changes in chromatin packing efficiency. Instead, lamin B1 and lamin B2 seem to

496   exert their effect on chromatin structure independent of the regulatory mechanisms confining the

497   genome at the mesoscale. Although B-type lamin depletion led to increased nuclear volume, the

498   overall maintenance of loops, TADs, and compartments indicates that genome topology is

499   robust to perturbation of chromatin elasticity.[43] These modest effects on higher order chromatin

500   structure could be due to multiple mechanisms, including (1) redundancy of LMNA proteins after

501   depleting LMNB1 and LMNB2, (2) a distinct role for LBR in chromatin organization, or (3) the

502   evolution of hysteresis in chromatin marks due to association with B-type lamin domains that is

503   not lost on their disruption.

504

505   Our findings highlight the need for development of methods for the investigation of chromatin

506   dynamics as well as evolution in chromatin modeling to better understand how the temporal

507   evolution of chromatin can alter gene transcription without alterations in mesoscale structure.

508   While Hi-C methods enable the reconstruction of 3D chromosome and genome structures, one

509   of the key limitations of this technique is that that long-range frequencies can be noisy and

510   unreliable, often depending on experimental factors such as the length of restriction fragments,

511   GC content, and read depth.[63] Further, this is a fixed-cell technique and thus does not allow for

512   millisecond resolution of chromatin structure and dynamics. Consequently, Hi-C and derivative

513   techniques are incapable of detecting the complexities of dynamic chromatin reorganization

514   alone. Thus, incorporating multi-modal imaging techniques in addition to Hi-C can provide a

515   better method for characterizing nanoscale structural alterations in chromatin packing in real

516   time.

20

517

518   Future work pairing super-resolution microscopy in combination with PWS microscopy and high-

519   resolution electron microscopy, involving labeling for markers specific to heterochromatin, could

520   help elucidate the nanoscopic changes in chromatin structure during lamin B disruption.[30,40]

521   Such an approach can also aid in providing further understanding of the mechanisms that

522   maintain the phenotypes involving degraded lamins or inverted nuclei. Despite these limitations,

523   our findings provide a deep and surprising role of B-type lamins in cell type-specific 4D genome

524   organization and transcription. In this work, increased fluctuations in density appear to be

525   associated with the translocation of genes spatially and translocation of heterochromatin

526   markers. Theoretical work regarding fluctuations in density has been previously shown to

527   independently regulate gene expression.[64] Upon lamin degradation, heterochromatic marks

528   slightly shifted from the nuclear periphery towards the interior, indicating greater variability in

529   accessible chromatin loci.

530

531   The functional consequence of the transformation in chromatin organization on gene expression

532   patterns occurs both for genes within and outside of LADs upon depletion of LMNB1 and

533   LMNB2. As such, our work provides further evidence that LADs function not solely through the

534   effect on transcriptional repression but are involved in the maintenance of transcriptionally

535   active genomic sites. Contradictory findings from previous studies may be due to several

536   factors. First, some LADs are cell-type specific, whereas others are conserved (i.e., constitutive

537   LADs).[23,24,62] This could impact the resulting changes seen in genome-nuclear lamina

538   interactions due to changes in gene expression (lamin perturbation). Further investigation in

539   other cell types could provide a method to better understand how lamins maintain cell-type

540   specific structural organization in normal and disease development. For example, A-type lamins

541   and perinuclear lamin B2 have been previously shown to be involved in age-related

542   neurodegeneration.[65] Our results therefore warrant additional studies using neurons or iPSCs

543   utilizing similar inhibitory mechanisms presented here.

544

545   **CONCLUSION**

546   Our investigation focuses on understanding the mechanisms by which the rapid inhibition of

547   both B-type lamins simultaneously would alter genome organization, gene expression, and cell

548   function. Our results in mammalian cells indicate a direct link between transcription changes

549   mediated by B-type lamins near LADs that is not due to higher-order chromatin structure

550   transformation. Transcriptionally, expression in both LAD and non-LAD territories are

551   transformed, with distinct disease associations from the expression patterns depending on the

552   genomic territory (LADs associated with structural disorders and non-LADs with malignancies).

553   Further, our results demonstrate that upon B-type lamin degradation, chromatin conformation

554   and chromatin connectivity show subtle changes. However, the greatest alterations in chromatin

555   conformation and connectivity occur closest to the nuclear periphery in comparison to the

556   nuclear interior. Consequently, these findings demonstrate a distinct mechanistic role of B-type

557   lamins in chromatin organization and cellular function. Our results therefore warrant further

558   investigation of the dynamic relationship between the nuclear lamina and chromatin

559   organization.

560
561   **MATERIALS AND METHODS**

562   For more details, see Key Resources Table.

563

564   **HEK293T Cell Culture**

565   HEK293T cells (ATCC, #CRL-1573) were grown in Dulbecco's Modified Eagle's Medium

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

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

568 All cells were cultured under recommended conditions at 37°C and 5% CO_2. All cells in this

569 study were maintained between passage 5 and 20. Cells were allowed at least 24□h to re-

570 adhere and recover from trypsin-induced detachment. All cells were tested for mycoplasma

571 contamination (ATCC, #30-1012K) before starting experiments, and they have given negative

572 results.

573

574 **HCT116 Cell Culture**

575 HCT116 cells (ATCC, #CCL-247) were grown in McCoy's 5A Modified Medium (#16600-082,

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

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

578 Fisher Scientific, Waltham, MA). All cells were cultured under recommended conditions at 37°C

579 and 5% CO_2. All cells in this study were maintained between passage 5 and 20. Cells were

580 allowed at least 24□h to re-adhere and recover from trypsin-induced detachment. All imaging

581 was performed when the surface confluence of the dish was between 40–70%. All cells were

582 tested for mycoplasma contamination (ATCC, #30-1012K) before starting perturbation

583 experiments, and they have given negative results.

584

585 **METHOD DETAILS**

586 **Transfection and Colony Isolation for Creating AID cell lines**

587 Stable transfection was achieved as previously described [33]. Briefly, we generated conditional

588 human HCT116 mutants by homology-directed repair (HDR)-mediated gene tagging using

589 CRISPR-Cas9. Around 60-70% confluent cells expressing OsTIR1 were plated at $3x10^5$ cells in

590 a 6-well plate and cultured for 24 hours at 37°C. On the next day of transfection, 4 mL of 200

591 ng/mL CRISPR plasmid, 3 mL of 200 ng/mL donor plasmid, 90 mL of Opti-MEM I Reduced

592 Serum Medium (Gibco, #31985070), and 8 mL of FuGENE 6 Transfection Reagent (Promega,

593 #E2691) were mixed and incubated at room temperature for 15 minutes before being applied to

594   the cells. For antibiotic selection and colony formation, Hygromycin B Gold (Gibco, #10687010),

595   100 mg/mL was used. For colony isolation, single colonies were picked under a stereo

596   microscope and transferred to a 96-well plate containing 10 mL of trypsin/ EDTA and

597   neutralized with 200 mL of media. After 2 weeks of culture, the cells were transferred to a 24-

598   well plate. After a few days of culture, genomic DNA was isolated. Briefly, the cells were

599   harvested, lysed with SDS buffer (100 mM NaCl, 50 mM Tris-Cl pH 8.1, 5mM EDTA, and 1%

600   wt/vol SDS) and treated with proteinase k (New England Biolabs, #P8102) (0.6 mg/mL) at

601   55□°C for 2 hours. Then, the lysis solutions were treated with PCI (phenol/ chloroform/ isoamyl

602   alcohol) and used in EtOH precipitation. Genomic DNA pellets were washed with 70% EtOH

603   and resuspended in RNase-containing water. The PCR reaction was set up using 0.5 U of Taq

604   DNA Polymerase (G Biosciences, #786-447) (1x PCR Buffer), 0.5 mM primers, and 1 mL of

605   genomic DNA from the HCT116 CMV-OsTIR1 parental cells to a 20 mL total volume reaction

606   mixture. PCR was performed using the following conditions: 30 cycles of 98□°C for 2 min,

607   55□°C for 30□s and 68□°C for 0.5□min/kb. PCR products were examined for biallelic insertion

608   using agarose gel electrophoresis. Initially, progenitor cells were produced by integrating

609   OsTIR1 (CMV-OsTIR1) into the safe-harbor AAVS1 locus. We then co-transfected the donor

610   template plasmid with Cas9 and sgRNAs that targeted the STOP codon of the LMNB1 or

611   LMNB2 genes in HCT116 cells expressing OsTIR1. To generate cells with mAID in both genes,

612   we simultaneously transfected the two donor templates with two sgRNA that target both genes.

613   Cells expressing mClover for each protein target (LMNB1, LMNB2, or LMNB1 & B2) were

614   sorted and grown as single cell colonies. We PCR-screened 179 colonies in total to identify

615   clones with homozygous LAMINB1-mAID (5+/40 colonies), LAMIN B2-mAID (5+/55 colonies) as

616   well as clones that had homozygous mAID in both genes (3+/84 colonies).

617

618   **Primers and sgRNA**

24

619   Appropriate primers were designed to check the insertion by PCR. For creating stable cell lines,

620   primer sets detected both the wild-type (WT) (1-1.5 kb) and inserted alleles (1-1.5 kb plus the

621   size of the insertion), and another primer set to detect only the inserted allele. The first primer

622   set was designed outside of the homology arms. Primers and sgRNA sequences used to create

623   all AID cell lines are listed in **Supplementary Tables 1 – 3**.

624

625   **Plasmids for AID Cell lines**

626   The donor construct contained the AID domain fused to mClover and an intervening T2A site

627   with a hygromycin resistance marker. The construct was flanked by 50-base pair homology

628   arms corresponding to the last exon region of the sgRNA recognition sequence and Cas9

629   cleavage site. To identify a CRISPR–Cas9 targeting site, we chose an appropriate sequence

630   within 50 bp upstream or downstream from the stop codon. The following target finder sites

631   were used to construct the CRISPR–Cas9 plasmid: IDT custom Alt-R guide design and

632   WEG CRISPR finder. Construction of the CRISPR–Cas9 plasmid and donor plasmids have

633   been previously described [33]. The AAVS1 T2 CRISPR in pX330 plasmid (Addgene plasmid #

634   72833; http://n2t.net/addgene:72833; RRID: Addgene_72833) is based on pX330-U6-

635   Chimeric_BB-CBh-hSpCas9 from Dr. Feng Zhang (Addgene #42230) (Cong et al, Science,

636   2013) and was a gift from Masato Kanemaki. The AAVS1 target sequence is described in Mali

637   et al (Mali et al, Science, 2013). pMK232 (CMV-OsTIR1-PURO) was a gift from Masato

638   Kanemaki (Addgene plasmid # 72834; http://n2t.net/addgene:72834; RRID: Addgene 72834).

639   pMK364 (CMV-OsTIR1-loxP-PURO-loxP) was a gift from Masato Kanemaki (Addgene plasmid

640   # 121184; http://n2t.net/addgene:121184; RRID: Addgene_121184). pMK290 (mAID-mClover-

641   Hygro)   was   a   gift   from   Masato   Kanemaki   (Addgene   plasmid   #   72828;

642   http://n2t.net/addgene:72828; RRID: Addgene_72828).

643

644   **Auxin Treatment**

25

645   For auxin treatment, HCT116$^{LMN(B1\&B2)-AID}$ cells were plated at 50,000 cells per well of a 6-well

646   plate (Cellvis, P12-1.5H-N). To induce expression of OsTIR1, 2 µg/ml of doxycycline (Fisher

647   Scientific, #10592-13-9) was added to cells 24 hours prior to auxin treatment. For live-cell flow

648   cytometry, western blots, RT-qPCR, RNA-seq, and *in situ* Hi-C experiments, 1000 µM 3-

649   Indoleacetic acid (IAA, Sigma Aldrich, #12886) was solubilized in 100% EtOH before each

650   treatment as a fresh solution. For live-cell confocal microscopy, fixed-cell flow cytometry, fixed-

651   cell immunofluorescence, CRISPR-Sirius fluorescent imaging, and Dual PWS experiments,

652   1000 µM Indole-3-acetic acid sodium salt (IAA, Sigma Aldrich, #6505-45-9) was solubilized in

653   RNase-free water (Fisher Scientific, #10-977-015) before each treatment as a fresh solution and

654   added to HCT116$^{LMN(B1\&B2)-AID}$ cells. Optimal auxin treatment time was determined based on the

655   results from western blot, immunofluorescence, and flow cytometry experiments

656   **(Supplementary Fig. 5)**.

657

658   **Fixed-Cell Flow Cytometry (FACS) Analysis**

659   Flow cytometry analysis for HCT116$^{LMNB1-AID}$, HCT116$^{LMNB2-AID}$ and HCT116$^{LMN(B1\&B2)-AID}$ cells for

660   AID system verification and nuclear morphology experiments was performed on the Amnis

661   ImageStreamXTM, located at the University of Virginia Flow Cytometry Core Facility in

662   Charlottesville, VA. To assess the degree of apoptosis induced by auxin treatment, we used the

663   Annexin V APC Kit (Cayman Chemical, #601410) and followed the manufacturer's protocol.

664   Flow cytometry analysis for HCT116$^{LMN(B1\&B2)-AID}$ cells to determine proper auxin treatment

665   concentration was performed on a BD LSRFortessa Cell Analyzer FACSymphony S6 SORP

666   system, located at the Robert H. Lurie Comprehensive Cancer Center Flow Cytometry Core

667   Facility at Northwestern University in Evanston, IL. For all FACS analysis the same protocol was

668   used. After 24 hours of doxycycline treatment followed by auxin treatment, cells were harvested

669   and fixed. Briefly, cells were washed with DPBS (Gibco, #14190-144), trypsinized (Gibco,

670   #25200-056), neutralized with media, and then centrifuged at 500 x g for 5 minutes. Cells were

671 then resuspended in 500 µL of 4% PFA and DPBS and fixed for 10 minutes at room

672 temperature, followed by centrifugation and resuspension in cold FACS buffer (DPBS with 1%

673 of BSA and 2mM EDTA added) at 4°C until analysis could be performed the following day. Data

674 were analyzed using FlowJo software.

675

676 **Protein Detection & Antibodies**

677 HCT116$^{LMNB1-AID}$, HCT116$^{LMNB2-AID}$ and HCT116$^{LMN(B1\&B2)-AID}$ cells were lysed using Radio Immuno

678 Precipitation Assay (RIPA) buffer (Sigma-Aldrich, #R0278) with protease inhibitor added

679 (Sigma-Aldrich, #P8340). Cell lysates were quantified with a standard Bradford assay using the

680 Protein Assay Dye Concentrate (BioRad, #500-0006) and BSA as a control. Heat denatured

681 protein samples were resolved on a 4-12% bis-tris gradient gel, transferred to a PVDF

682 membrane using the Life Technologies Invitrogen iBlot Dry Transfer System (Thermo Fisher

683 Scientific, IB1001) (20V for 7 minutes), and blocked in 5% nonfat dried milk (BioRad, #120-

684 6404) in 1x TBST. Whole-cell lysates were blotted against the following primary antibodies:

685 Lamin B1 (Cell Signaling, #13435, dilution 1:1000), Lamin B2 (Cell Signaling, #12255, dilution

686 1:1000), and alpha-tubulin (Thermo Fischer Scientific, #62204, dilution 1:2000). The following

687 secondary antibodies were used: anti-rabbit IgG HRP (Promega, #W4018). Blots were

688 incubated with the primary antibody overnight at 4□°C, followed by incubation with the

689 secondary antibodies for 1 hour at room temperature. To develop blots for protein detection,

690 chemiluminescent substrates were used (Thermo Fischer Scientific, #32106).

691

692 **Quantitative Real-Time PCR & RNA Isolation**

693 Total RNA from transfected cells was harvested using the RNeasy Plus Mini Kit (Qiagen,

694 #74134) following the manufacturer's protocol. 1 mg of RNA was converted to cDNA using the

695 Applied Biosystems High-Capacity RNA-to-cDNA Kit (Thermo Fisher Scientific, #4387406). The

696 GAPDH or HPRT-1 gene was used as an internal control for analysis. RT-qPCR was performed

697   on a StepOnePlus Applied Biosystems instrument with SYBR Green. RNA quantity was

698   measured using the Nanodrop 2000 Spectrophotometer at 260nm.

699

700   **Fixed-Cell Immunofluorescence**

701   HCT116[LMN(B1)-AID] cells, HCT116[LMN(B2)-AID] cells, or HCT116[LMN(B1&B2)-AID] cells at a low passage

702   (<P10) were plated at 100,000 cells per well of a 6-well glass-bottom plate (Cellvis, #P06-1.5H-

703   N). Following auxin treatment, cells were washed twice with 1x Phosphate Buffered Saline

704   (PBS) (Gibco, #10010031). Cells were fixed with 4% paraformaldehyde (PFA) (Electron

705   Microscopy Sciences, #15710) for 10 minutes at room temperature, followed by washing with

706   PBS 3 times for 5 minutes each. Cells were permeabilized using 0.2% TritonX-100 (10%)

707   (Sigma-Aldrich, #93443) in 1x PBS, followed by another wash with 1x PBS for 3 times for 5

708   minutes each. Cells were blocked using 3% BSA (Sigma-Aldrich, #A7906) in PBST (Tween-20

709   in 1x PBS) (Sigma-Aldrich, #P9416) at room temperature. The following primary antibodies

710   were added overnight at 4°C: lamin B1 (Abcam, #ab16048, dilution 1:1000), lamin B2 (Abcam,

711   #ab155319, dilution 1:1000. Cells were washed with 1x PBS 3 times for 5 minutes each. The

712   following secondary antibody was added for 1 hour at room temperature: Invitrogen Goat anti-

713   Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 646 (Thermo Fisher

714   Scientific, #A-21245). Cells were washed with 1x PBS 3 times for 5 minutes each. Finally, cells

715   were stained with DAPI (Thermo Fisher Scientific, #62248, diluted to 0.5 µg/mL in 1x PBS) for

716   10 minutes at room temperature. Prior to imaging, cells were washed with 1x PBS twice for 5

717   minutes each.

718

719   **Preparation of Hi-C Libraries for *in situ* Hi-C**

720   *In-situ* Hi-C was performed as previously described [2]. Briefly, 2-5 million cells at 80% confluence

721   were detached and pelleted by centrifugation at 300xG for 5 minutes. Cells were resuspended

722   in fresh medium at a concentration of 1E6 cells per 1 mL media. In a fume hood, 1%

28

723 formaldehyde was used to crosslink cells, with 10 minutes of incubation at room temperature

724 with mixing. 2.5M glycine solution was added to a final concentration of 0.2M to quench the

725 reaction, and the reaction was incubated at room temperature for 5 minutes with gentle rocking.

726 Samples were centrifuged at 300xG for 5 minutes at 4°C. Cells were resuspended in 1mL of

727 ice-cold PBS and spun at 300xG for 5 minutes at 4°C. Cell pellets were flash-frozen in liquid

728 nitrogen and either stored at -80°C or used immediately for lysis and restriction digest. Nuclei

729 were permeabilized. 250 µL of Hi-C lysis buffer (10mM Tris-HCl pH 8.0, 10mM NaCl, 0.2%

730 Igepal CA930 (Sigma-Aldrich, #I3021)) with 50 µL of protease inhibitors (Sigma Aldrich,

731 #P8340) was added to each crosslinked pellet of cells. After incubation on ice for >15 minutes,

732 samples were centrifuged at 2500xG for 5 minutes and washed with 500 µL of ice-cold Hi-C

733 lysis buffer. Pelleted nuclei were resuspended in 50 µL of 0.5% sodium dodecyl sulfate (SDS)

734 (Sigma-Aldrich, #436143) and incubated at 62°C for 10 minutes. Next, 145 µL of water (Fischer

735 Scientific, #10-977-015) and 25 µL of 10% Triton X-100 (Sigma Aldrich, #93443) were added to

736 quench the SDS. Samples were mixed and incubated at 37°C for 15 minutes. DNA was

737 digested with 100U of MboI restriction enzyme (New England Biolabs, #R0147) and 25 µL of

738 10X NEBuffer 2 (New England Biolabs, #B7002S). Chromatin was digested overnight at 37°C

739 with rotation. Samples were incubated at 62°C for 20 minutes to inactivate MboI, and then

740 cooled to room temperature. The ends of restriction fragments were labeled using biotinylated

741 nucleotides (Thermo Fisher Scientific, #19524016) and ligated in a small volume (~ 900 µL)

742 using 10X NEB T4 DNA ligase buffer (New England Biolabs, #B0202) and DNA Polymerase I,

743 Large (Klenow) Fragment (New England Biolabs, #M0202) after 1 hour of incubation at 37°C.

744 Samples were mixed and incubated at room temperature for 4 hours prior to reversal of

745 crosslinks. We added 50 µL of 20 mg/mL proteinase K (New England Biolabs, #P8102) and 120

746 µL of 10% SDS and incubated samples at 55°C for 30 minutes. Next, 130 µL of 5M sodium

747 chloride was added and samples were incubated at 68°C overnight. Ligated DNA was purified

748 and sheared to a length of ~400 bp, as previously described [2] using a LE220-plus Focused-

749   ultrasonicator (Covaris, #500569) and AMPure XP beads (Beckman Coulter, #A63881). DNA

750   was quantified using the Qubit dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific,

751   #Q33230) and undiluted DNA was run on a 2% agarose gel to verify successful size selection.

752   Point ligation junctions were pulled down with 10 m/mL Dynabeads MyOne Steptavidin T1

753   beads (Thermo Fisher Scientific, #65601) and prepared for Illumina sequencing using Illumina

754   primers and protocol (Illumina, 2007) as previously described [2]. Paired-end sequencing was

755   performed using the Illumina HiSeq 2000 OR 2500 platform. A no-ligation control was also

756   used.

757

758   **Hi-C Data Processing and Analysis**

759   Juicebox was used to visualize Hi-C contact maps[66]. All Hi-C data reported were produced

760   using Illumina paired-end sequencing. We followed the Hi-C data processing pipeline that has

761   previously been described [2]. This pipeline uses the Burrows-Wheeler single end aligner (BWA)

762   [67] to map each read end separately to the hg19 reference genome, removes reads that map to

763   the same fragment, removes duplicate or near-duplicate reads, and filters the remaining reads

764   based on the mapping quality score. All analysis (i.e., aggregate peak analysis) and annotations

765   (i.e., annotation of domains, assigning loci to subcompartments, and peaks) were performed as

766   previously described [2,68]. All contact matrices were KR-normalized with Juicer. Domains were

767   annotated using TopDom.

768

769   **Dual PWS Imaging**

770   Briefly, PWS measures the spectral interference signal resulting from internal light scattering

771   originating from nuclear chromatin. This is related to variations in the refractive index distribution

772   (Σ) (extracted by calculating the standard deviation of the spectral interference at each pixel),

773   characterized by the chromatin packing scaling (D). D was calculated using maps of Σ, as

774   previously described [40,50,52,53]. Measurements were normalized by the reflectance of the glass

30

775 medium interface (i.e., to an independent reference measurement acquired in a region lacking

776 cells on the dish). This allows us to obtain the interference signal directly related to refractive

777 index (RI) fluctuations within the cell. Although it is a diffraction-limited imaging modality, PWS

778 can measure chromatin density variations because the RI is proportional to the local density of

779 macromolecules (e.g., DNA, RNA, proteins). Therefore, the standard deviation of the RI (Σ) is

780 proportional to nanoscale density variations and can be used to characterize packing scaling

781 behavior of chromatin domains with length scale sensitivity around $20 - 200$ nm, depending on

782 sample thickness and height. Changes in D resulting from each condition are quantified by

783 averaging over nearly 2000 cells, taken across 3 technical replicates. Live-cell PWS

784 measurements obtained using a commercial inverted microscope (Leica, DMIRB) using a

785 Hamamatsu Image-EM charge-coupled device (CCD) camera (C9100-13) coupled to a liquid

786 crystal tunable filter (LCTF, CRi VariSpec) to acquire monochromatic, spectrally resolved

787 images ranging from 500-700 nm at 2-nm intervals as previously described [47,50,51]. Broadband

788 illumination is provided by a broad-spectrum white light LED source (Xcite-120 LED, Excelitas).

789 The system is equipped with a long pass filter (Semrock BLP01-405R-25) and a 63x oil

790 immersion objective (Leica HCX PL APO). Cells were imaged under physiological conditions

791 (37°C and 5% CO_2) using a stage top incubator (In vivo Scientific; Stage Top Systems). All cells

792 were given at least 24 hours to re-adhere before treatment (for treated cells) and imaging.

793

794 **Dynamic PWS Measurements**

795 Dynamic PWS measurements were obtained as previously described.[50] Briefly, dynamics

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

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

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

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

800 autocorrelation of the temporal interference as previously described.[50] The fractional moving

31

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

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

803   publication [50], the fractional moving mass is obtained by using the following equation to

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

805

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

806
807   With this normalization, Σ_t^2 is equivalent to m_f, which measures the mass moving within the

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

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

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

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

812   nucleus, n_i = 1.518 as the refractive index of the immersion oil, and ρ_0 = 0.55 g cm^{-3} as the dry

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

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

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

816   previously[50], Σ_t^2 is sensitive to instrument parameters such as the depth of field, substrate

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

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

819   backscattered intensity is prone to errors along the transverse direction[50]. Due to these

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

821   pixel.

822

823   **Regional PWS Analysis**

824   We used PWS to calculate *D* values via measuring the variations in spectral light interference

825   resulting from light scattering due to heterogeneities in chromatin density as previously

826 described [52]. The same cells used to analyze average chromatin packing scaling in whole

827 nuclear regions were used for regional chromatin packing scaling analysis. For the periphery

828 characterization, individual nuclei were segmented into 6 ribbons of 260 nm width each using

829 MATLAB. The remaining region at the center of the nucleus was classified as the center. We

830 calculated the average D for each pixel, followed by averaging all these values to estimate the

831 average D in each region.

832

833 **Confocal Imaging**

834 For CRISPR-Sirius, when MCP-Halo was added to cells for fluorescent imaging, HaloTag-

835 JF646 was added to the cells at 10 μM 24 h before imaging and incubated overnight at 37°C

836 and 5% CO_2. On the day of imaging, live cells were washed three times with DPBS (Gibco,

837 #14190-144) and further incubated with phenol-red free media (Cytiva, #SH30270.01). The

838 optical instrument was built on a commercial inverted microscope (Eclipse Ti-U with the perfect

839 focus system, Nikon). Images of live cells were collected using a 100x objective and sent to an

840 electron-multiplying CCD (iXon Ultra 888, Andor). A 637 nm laser (Obis, Coherent) was co-

841 illuminated through a 100x/ 1.49 NA (numerical aperture) oil objective lens (SR APO TIRF,

842 Nikon) with an average power at the sample of 3 to 10 kW/cm^3. The microscope stage

843 incubation chamber was maintained at 37 °C and supplemented with 5% CO_2. For single image

844 acquisition, at least 50 frames were taken at 30 ms exposure time and a gain of 150. Z-stack

845 images were acquired at 0.024 μm per step for a total of 401-701 frames depending on the size

846 of the nuclei being imaged. [47,50,51] Images of fixed-cells previously transfected with CRISPR-

847 Sirius plasmids were imaged using the Nikon SoRa Spinning Disk confocal microscope

848 equipped with a Hamamatsu ORCA-Fusion Digital CMOS camera. Images were collected using

849 a 60x/ 1.42 NA oil-immersion objective mounted with a 2.8x magnifier. mClover was excited with

850 a 488 nm laser, HaloTag-JF646 was excited with a 640 nm laser, and DAPI was excited with a

851 405 nm laser. Imaging data were acquired by Nikon acquisition software.

852

Lentivirus Packaging

853

854   HEK293T cells were used to produce lentiviral particles using FuGENE HD Transfection

855   Reagent (Promega, #E2311) following the manufacturer's protocol. Briefly, one day before

856   transfection, HEK293T cells at low passage (<P10) were plated at 100,000 cells per well of a

857   12-well plate (Cellvis, P12-1.5H-N). At the time of transfection, cells reached a confluency of 70-

858   80%. For lentivirus packaging, a master mix of DNA was prepared in reduced serum media

859   (OptiMEM, Gibco, #31985-070). This master mix contained the lentiviral packaging plasmid

860   pCMV-VSV-G (a gift from Bob Weinberg, Addgene plasmid # 8454) and pCMV-dR8.2 (a gift

861   from Bob Weinberg, Addgene plasmid # 8455). For packaging each virus, the following amounts

862   of each plasmid were mixed: 0.5 μg transfer vector + 0.45 μg pCMV-dR8.2 + 0.05 μg pCMV-

863   VSV-G. Media was changed 24 hours post-transfection to fresh DMEM. Lentiviral particles were

864   harvested 60 hours after transfection. The viral supernatants were filtered using a 33 mm

865   diameter sterile syringe filter with a 0.45 μm pore size hydrophilic PVDF membrane (Millipore

866   Sigma, SLHVR33RS) and added to HEK293T cells. The virus was immediately used or stored

867   at –80 °C. Polybrene (8 μg/mL; Sigma-Aldrich) was supplemented to enhance transduction

868   efficiency.

869

CRISPR-Sirius Labelling

870

871   Plasmids were obtained from Addgene as bacterial stabs and streaked onto LB-ampicillin

872   plates. Upon overnight growth and single colony selection, a single colony was inoculated into

873   LB-ampicillin liquid culture overnight. Plasmid DNA isolation was performed using QIAprep Spin

874   Miniprep kit (Qiagen, # 27104) following the manufacturer's protocol. pHAGE-TO-dCas9-P2A-

875   HSA (Addgene plasmid # 121936; http://n2t.net/addgene:121936; RRID: Addgene_121936),

876   pHAGE-EFS-MCP-HALOnls (Addgene plasmid # 121937; http://n2t.net/addgene:121937; RRID:

34

877   Addgene_121937), and pPUR-hU6-sgRNA-Sirius-8XMS2 (Addgene plasmid # 121942;

878   http://n2t.net/addgene:121942; RRID: Addgene_121942) were gifts from Thoru Pederson.

879

880   **CRISPR-Sirius Transduction**

881   For live-cell CRISPR-Sirius, HCT116$^{LMN(B1\&B2)-AID}$ cells were transfected with CRISPR-dCas9 and

882   donor plasmids using FuGENE HD Transfection Reagent (Promega, #E2311) following the

883   manufacturer's protocol. Briefly, cells at low passage (<P10) were plated at 100,000 cells per

884   well of a 6-well glass-bottom plate (Cellvis, P06-1.5H-N). 24 hours after plating, 50 μL dCas9,

885   50 μL MCP-HALOnls, and 100 μL sgRNA lentiviral particles were added to each well. 24 hours

886   after transduction, lentiviral particles were removed by replacing media.

887

888   **CRISPR-Sirius Transfection**

889   For additional quantification of foci and distances of foci to the nuclear periphery,

890   HCT116$^{LMN(B1\&B2)-AID}$ cells were co-transfected with 200 ng MCP-HaloTag, 400 ng of dCas9

891   plasmid DNA, and 2 μg of plasmid DNA for the desired guide RNAs using Lipofectamine LTX

892   and Plus Reagent. Cells were incubated for 24 hours prior to overnight staining with HaloTag-

893   JF646 before fixation and imaging.

894

895   **Data and Image Analysis**

896   We used GraphPad Prism 9.3.1 or Excel for statistical analysis and for making all boxplots.

897   Flow cytometric analysis (FACS) data were analyzed using FlowJo software version 10.6.1. To

898   localize the fluorescent puncta, we used ImageJ software to first generate max projections of

899   the Z-stack images/50 frame single layer acquisition. Max projected images were then

900   background subtracted using the standard rolling ball algorithm with a radius of 12.0. Processed

901   images were then input into the Thunder-STORM ImageJ plugin with a peak intensity threshold

902   coefficient of 5.0 to locate the fluorescent puncta associated with the CRISPR Sirius tagged loci.

903   Puncta coordinates were saved in Microsoft Excel as a .csv file to be input to the Python

904   algorithm. The processed image was then fed into a Python computer vision algorithm that

905   segmented out the nucleus and determined the coordinates for the nuclear periphery as well as

906   the centroid of the nucleus. To quantify the spatial distance to the nuclear periphery, only pairs

907   of loci lying in the same foci plane were analyzed. Distances were measured from each

908   fluorescent puncta to the nearest nuclear periphery coordinate as well as the centroid of the

909   segmented nucleus. To account for differences in cell area, the distance from each puncta to

910   the centroid was divided by the radius, assuming a circular area. This was further verified by

911   dividing the distance by the nuclear area. To detect loci numbers, maximum intensity projection

912   of Z-series images was performed.

913

914   **Coefficient of Variation Analysis**

915   To assess chromatin compaction through the Coefficient of Variation (CV) analysis, DAPI-

916   stained cells (see section Fixed-cell immunofluorescence) treated with Auxin (see section Auxin

917   treatment) were imaged on a Nikon SoRa Spinning Disk confocal microscope (see section

918   Confocal imaging). Following a published workflow[54], we used ImageJ to create masks of each

919   nucleus. The coefficient of variation of individual nuclei was calculated in MATLAB, with CV =

920   σ/μ, where σ represents the standard deviation of the intensity values and μ representing the

921   mean value of intensity of the nucleus.

922

923   **RNA-Seq Library Preparation**

924   Total RNA extraction was performed on samples from colon carcinoma epithelial HCT116 cells

925   and HCT116$^{LMN(B1\&B2)-AID}$ cells using the RNeasy Plus Mini Kit (Qiagen, #74134) following the

926   manufacturer's protocol. The conditions for these samples were control, 12-hour auxin, 48-hour

927   auxin, and 48-hour auxin with 6 days of removal by changing cell culture media. These samples

928   were collected with three biological replicates per condition. RNA-Seq libraries were prepared

929   using the NEBNext Ultra Directional RNA Library Prep Kit for Illumina (New England BioLabs,

930   #E7760), according to the company's instruction. Library quality was measured using the Qubit

931   2.0 and Bioanalyzer.

932

933   **RNA-Seq Data Analysis**

934   Bulk mRNA sequencing was conducted in The Genome Analysis and Technology Core in

935   Charlottesville, VA at the University of Virginia. Paired-end reads were acquired using NextSeq

936   2000 (75bp) system on high throughput mode. Reads were aligned to the hg19 genome using

937   HISAT2 and quantified using StringTie. Read counts were normalized and compared for

938   differential gene expression using the DESeq2 package in R. Heatmaps were generated using

939   the pheatmap package. Other plots were generated using the ggplot2 package. We used

940   Metascape (https://metascape.org/gp/index.html#/main/step1) to perform pathway enrichment

941   analysis. The publicly available hg19 DamID track was downloaded from the UCSC genome

942   browser database. We used bedtools to compare gene coordinates with the DamID LAD

943   coordinates. We specifically used the "reldist", "closest", and "coverage" options of bedtools.

944

945   **QUANTIFICATION AND STATISTICAL ANALYSIS**

946   Experimental data are presented as the mean ± SD of three independent experiments unless

947   otherwise stated. Statistical analysis was performed using two-sided Student's t-test for

948   comparing two sets of data with normal distribution assumed, unless stated otherwise. For

949   datasets with a Gaussian distribution, parametric tests were applied. For datasets with no

950   Gaussian distribution, non-parametric tests were applied. In all cases two-tailed test were run

951   and multiple comparison corrections were applied for datasets with more than two groups and

952   multiple comparisons. Statistical analysis for PWS, coefficient of variation, and CRISPR-Sirius

953   plots has been performed in GraphPad Prism. A P value of < 0.05 was considered significant.

954   Statistical significance levels are denoted as follows: ns; *P<0.05; **P<0.01; ***P<0.001;

37

955 ****P<0.0001. Sample numbers (# of nuclei, n) and the number of replicates (N) is indicated in

956 figure legends.

957

958 **DECLARATIONS**

959

960 **ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

961 Not applicable.

962

963 **CONSENT FOR PUBLICATION**

964 Not applicable.

965

966 **AVAILABILITY OF DATA AND MATERIALS**

967 The original source code during the current study is available in the Backman Lab/ Lamin-

968 Project GitHub repository (https://github.com/BackmanLab/Lamin-Project). Datasets used

969 and/or analyzed during the current study are available from the corresponding author on

970 reasonable request. Raw data is available from the corresponding authors upon reasonable

971 request. The cell lines have been authenticated and are available upon request. Further

972 information and requests for resources and reagents should be directed to and will be fulfilled by

973 the lead contact, Vadim Backman (v-backman@northwestern.edu).

974

975 **COMPETING INTERESTS**

976 The authors declare no competing interests.

977

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1003

1004   **AUTHORS' CONTRIBUTIONS**

1005   E.P., X.W, J.Y., and N.A., and F.S. conducted the experiments; E.P. wrote the paper; L.C and

1006   J.Y. conducted RNA-seq analysis; E.P and N.A. assisted with PWS imaging and performed

1007   analysis; S.R., L.C., and L.A. conducted *in* situ Hi-C analysis; A.D. wrote the code for analyzing

1008   regional chromatin packing scaling and dynamics; V.A., L.C., and L.A. edited the manuscript.

1009

1014

1015   **INCLUSION AND DIVERSITY**

1016   One or more of the authors of this paper self-identifies as an underrepresented minority.

1017

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1228   **MAIN FIGURE TITLES AND LEGENDS**

1229   **Figure 1: Auxin treatment results in rapid B-type lamin degradation and altered nuclear**

1230   **morphology.**

1231   A.   Auxin treatment promotes the interaction between TIR1 and the degron tag (mAID),

1232   which is fused to the target protein. In the presence of auxin, the target protein is rapidly

1233   degraded upon proteasomal mediated poly-ubiquitination.

1234   B.   Each gene of interest was targeted for degradation by co-transfecting progenitor cells

1235   with the donor template plasmid with Cas9 and sgRNAs targeting the STOP codon of

1236   the sequence.

1237   C.   The nuclear localization of LMNB1-AID (green) and LMNA (red) in HCT116$^{LMN(B1\&B2)-AID}$

1238   cells was examined by immunofluorescence. Cells were stained with DAPI to indicate

1239   the nuclear region. Scale bar = 10 µm. N = 3, data was obtained from three independent

1240   biological replicates.

1241   D.   The AID system provides a rapid and reversible method for targeted protein degradation.

1242   HCT116$^{LMN(B1\&B2)-AID}$ cells express OsTIR1 upon doxycycline addition (left). Auxin

1243   treatment results degraded LMNB1 and LMNB2, indicated by loss of mClover (middle).

1244   Removal of auxin allows for the re-expression of LMNB1 and LMNB2, indicated by the

1245   presence of mClover (right).

1246   E.   Western blots reveal that AID-tagged LMNB1 and LMNB2 are degraded within 24 hours

1247   of auxin treatment, as the target protein expression is no longer detectable in

1248   HCT116$^{LMN(B1\&B2)-AID}$ cells.

1249   F.   Single cell-level degradation kinetics demonstrate a loss of B-type lamin proteins. Within

1250   24 hours of auxin treatment, the majority of mClover signal is lost in HCT116$^{LMN(B1\&B2)-AID}$

1251   cells.

1252   G.   Fixed-cell flow cytometry results demonstrate an increase in nuclear area (relative DAPI-

1253   stained area) following target protein degradation (green curve), indicated by a low

1254   mClover signal, in comparison to untreated cells with high mClover signal (black curve).

1255   H. Immunofluorescence shows the presence of nuclear blebbing in B-type lamin-deficient

1256   cells. Scale bar = 5 μm. N = 3, data was obtained from three independent biological

1257   replicates.

1258

1259   **Figure 2: In-situ Hi-C reveals that mesoscale chromatin structure is overall preserved**

1260   **upon B-type lamin degradation.**

1261   A. Representative normalized Hi-C trans-interaction matrices for all chromosomes in the

1262   control and 24-hour auxin treatment conditions are shown for HCT116$^{LMN(B1\&B2)-AID}$ cells.

1263   B. The eigenvectors for chromosomes 2 and 19 located at the nuclear periphery and

1264   interior, respectively, are shown. The A compartment (pink) and B compartment (purple)

1265   for each chromosome are indicated. Eigenvectors computed by Juicer are the first

1266   eigenvector of the correlation matrix of the binned Hi-C contacts.

1267   C. Contact probability scaling for HCT116$^{LMN(B1\&B2)-AID}$ cells are shown for the control and 24-

1268   hour auxin treatment conditions. Absolute values of *s* are indicated.

1269   D. The raindrop plot demonstrates TAD sizes for the control and 24-hour treatment

1270   conditions revealed by TopDom. The number of TADs for each condition are indicated

1271   below the boxplot.

1272

1273   **Figure 3: Loss of B-type lamins has a negligible impact on the connectivity of the**

1274   **genome.**

1275   A. The schematic demonstrates contacts across LAD segments and non-LAD segments for

1276   a representative chromosome.

1277   B. Contact scaling for chromosome 3 located at the nuclear periphery is shown. The violin

1278   plot indicates contact scaling for each chromosome, either across/ within LADs or

1279   across/ within non-LADs. Absolute values of *s* are indicated.

50

1280   C. Contact scaling for chromosome 19 located at the nuclear interior is shown. The violin

1281   plot indicates contact scaling for each chromosome, either across/ within LADs or

1282   across/ within non-LADs. Absolute values of *s* are indicated.

1283   D. The scatter plot for the inverse relationship between lamin B1 coverage and |*s*| in LAD

1284   segments is shown. The exponential distribution (gray curve) is fitted to the merged

1285   control (black) and 24-hour auxin treatment condition (purple) samples.

1286   E. The violin plot indicates contact scaling within LADs and non-LADs for both the control

1287   (untreated) and 24-hour auxin treatment conditions.

1288   F. The rain drop plot demonstrates that TAD sizes do not change in LAD versus non-LAD

1289   segments, or in the control versus 24-hour auxin treatment conditions.

1290

1291   **Figure 4: Dual-PWS microscopy reveals differential higher-order chromatin structure and**

1292   **dynamics upon loss of B-type lamins.**

1293   A. Representative images of HCT116$^{LMN(B1\&B2)-AID}$ cells obtained from PWS are shown for

1294   both the control and 24-hour auxin treatment conditions. Scale bar = 5 μm. N = 3, data

1295   was obtained from three independent biological replicates.

1296   B. Violin plots demonstrate that chromatin packing scaling (D) is significantly increased in

1297   HCT116$^{LMN(B1\&B2)-AID}$ cells.

1298   C. Violin plots demonstrate that fractional moving mass is significantly increased in

1299   HCT116$^{LMN(B1\&B2)-AID}$ cells upon 24 hours of auxin treatment.

1300   D. Violin plots demonstrate that the diffusion coefficient is significantly decreased in

1301   HCT116$^{LMN(B1\&B2)-AID}$ cells upon 24 hours of auxin treatment. (B-D). The truncated violin

1302   plots extend from the minimum to the maximum value. The line in the middle of each plot

1303   is the median value of the distribution, and the lines above and below are the third and

1304   first quartiles, respectively. Data was obtained from three technical replicates for each

1305   condition. ****P<0.0001. Welch's correction was applied.

1306   E. The schematic indicates that region 1 is the nuclear periphery while region 7 is the

1307   nuclear interior. Scale bar = 5 µm.

1308   F. Regional PWS measurements of D HCT116$^{LMN(B1\&B2)-AID}$ cells are shown, along with the

1309   change in D within each region.

1310   G. Regional PWS measurements of fractional moving mass for HCT116$^{LMN(B1\&B2)-AID}$ cells

1311   are shown, along with the change in fractional moving mass within each region.

1312   H. Regional PWS measurements of the diffusion coefficient for HCT116$^{LMN(B1\&B2)-AID}$ cells

1313   are shown, along with the change in the diffusion coefficient within each region.

1314

1315   **Figure 5: Positioning of heterochromatic markers, chromatin compaction, and**

1316   **chromosome positioning are significantly altered upon auxin treatment.**

1317   A. Line plots for the distribution of heterochromatic and euchromatic marks are shown (red)

1318   in comparison to DAPI signals (blue) for the representative nuclei from spinning disk

1319   confocal microscopy (Lamin B1/B2 in green, histone marks in magenta, DAPI in blue).

1320   B. Western blots reveal that H3K27me3 expression decreases by ~25% within 24 hours of

1321   auxin treatment. Within this time frame, the target protein expression is no longer

1322   detectable in HCT116$^{LMN(B1\&B2)-AID}$ cells.

1323   C. The coefficient of variation plot for HCT116$^{LMN(B1\&B2)-AID}$ cells demonstrates chromatin

1324   decompaction upon 24 hours of auxin treatment. The truncated violin plots extend from

1325   the minimum to the maximum value. The line in the middle of each plot is the median

1326   value of the distribution, and the lines above and below are the third and first quartiles,

1327   respectively. Data was obtained from three technical replicates for each condition (N=3,

1328   Control (n = 798), 24-hour auxin (n = 845)). ****P<0.0001. Welch's correction was

1329   applied.

1330   D. Box plot showing the number of foci per cell for XXYLT1 and TCF3 loci; $n = 42$ cells

1331   (XXYLT1) and 25 cells (TCF3). The line within each box represents the mean; the outer

1332   edges of the box are the 25th and 75th percentiles and the whiskers extend to the

1333   minimum and maximum values.

1334   E. Representative images of gene loci XXYLT1 and TCF3 in live cells. B-type lamin

1335   depletion alters the subnuclear localization preference of genomic loci, mostly at the

1336   nuclear periphery. Scale bar = 5 μm.

1337   F. Box plot showing distances of XXYLT1 and TCF3 loci to the center $n□=□53$ cells

1338   (XXYLT1) and $n=$ 28 cells (TCF3). The line within each box represents the mean; the

1339   outer edges of the box are the 25th and 75th percentiles and the whiskers extend to the

1340   minimum and maximum values. ****$P<0.0001$.

1341

1342   **Figure 6: Loss of both B-type lamins perturbs the expression of genes positioned inside**

1343   **and outside LADs.**

1344   A. Heatmaps showing the top 100 DEGs within versus outside of LAD boundaries upon 48

1345   hours of auxin treatment defined by publicly available DamID data (adjusted P value <

1346   0.01 and absolute log fold change > 1).

1347   B. DisGeNET results indicate terms upon 12 hours of auxin treatment within LADs related

1348   to structural changes and cancer.

1349   C. DisGeNET results indicate terms upon 12 hours of auxin treatment outside of LADs

1350   related to cancer and structural changes.

1351

1352   **Figure 8: Proposed model of the effect of Lamin B1 and B2 degradation on chromatin**

1353   **organization.**

1354   Schematic depicting how inhibition of B-type lamins leads to a shift in higher-order chromatin

1355   structure and dynamics. Loss of lamins B1 and B2 promotes chromatin blending, chromatin

1356   chain fluctuations, and displacement of both heterochromatin-associated domains and

1357   chromosome-specific loci, especially at the nuclear periphery. Gene expression is dramatically

53

1358   altered as a function of heterochromatin reorganization upon the loss of structural constraints at

1359   the nuclear periphery.

Figure 1
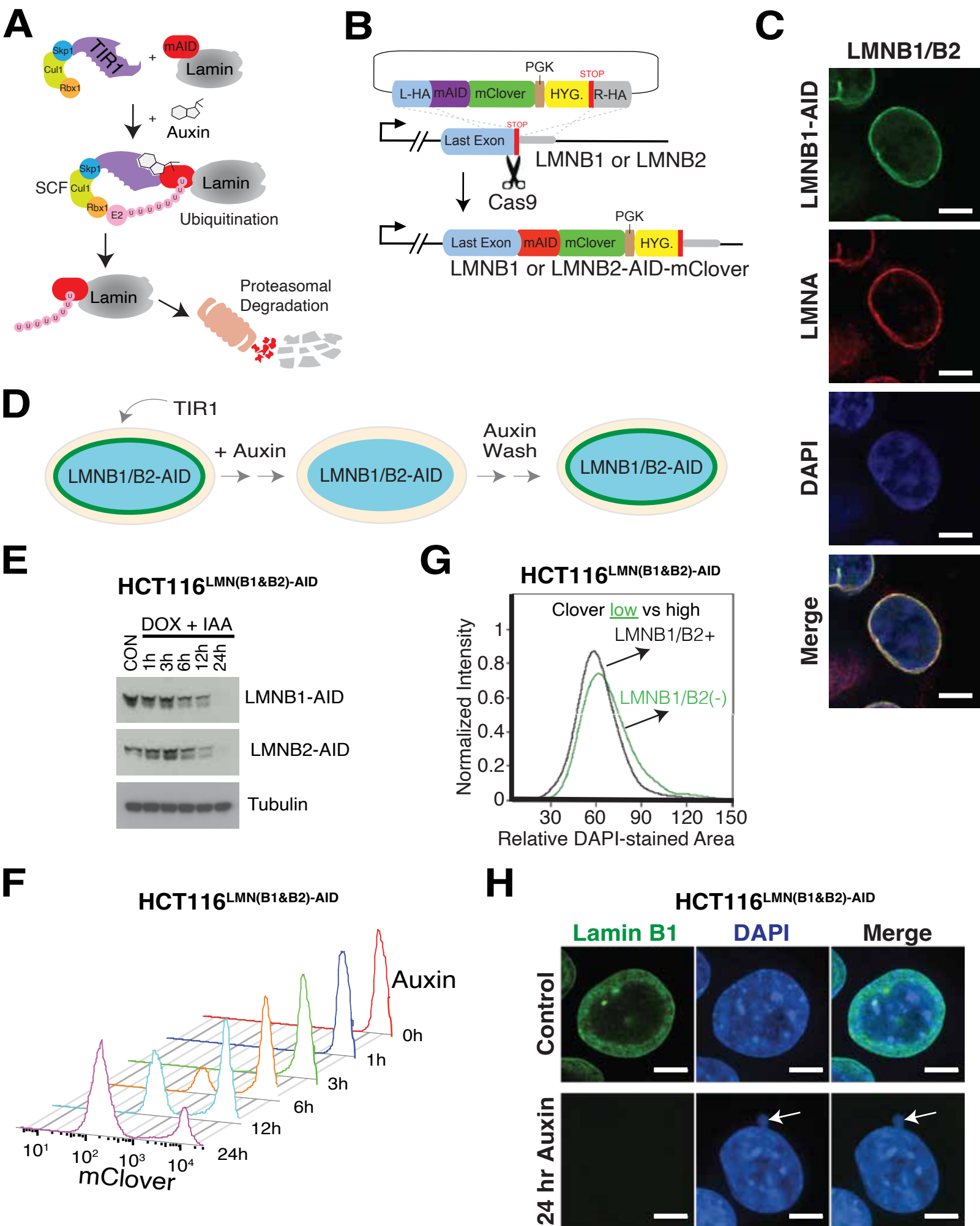
Figure 2

A — HCT116$^{LMN(B1\&B2)-AID}$; Control and 24 hr Auxin contact maps (All Chromosomes)
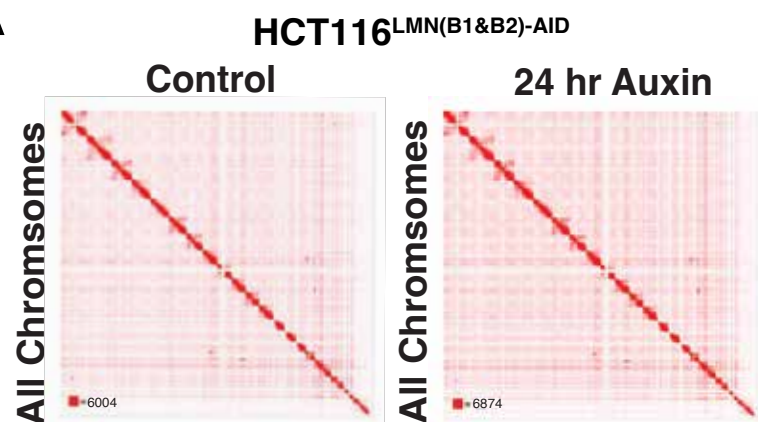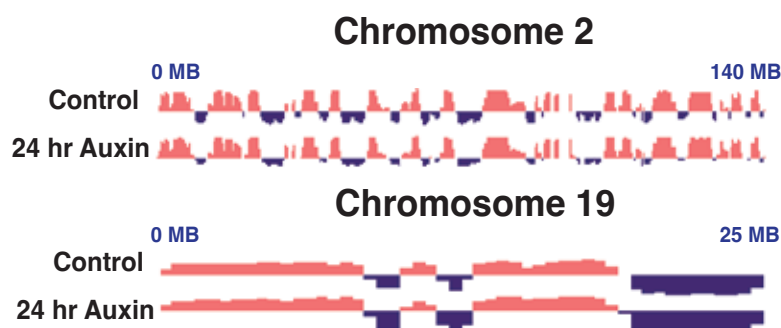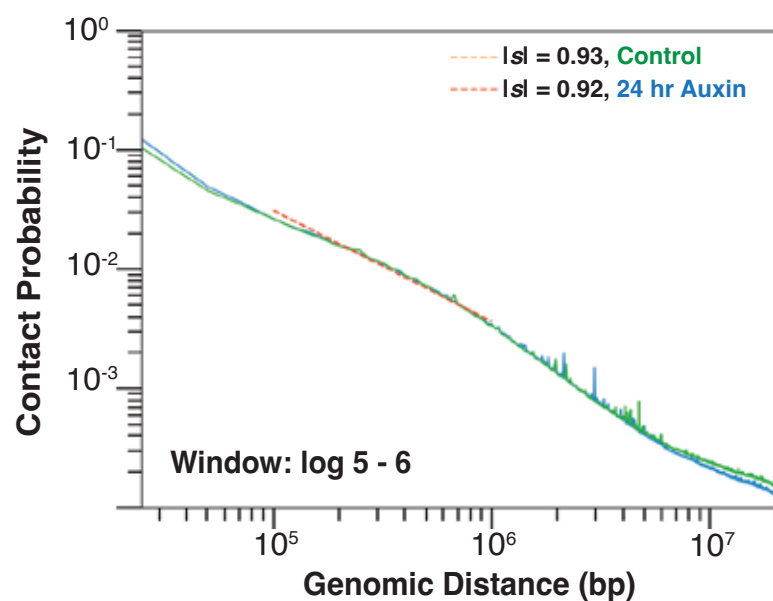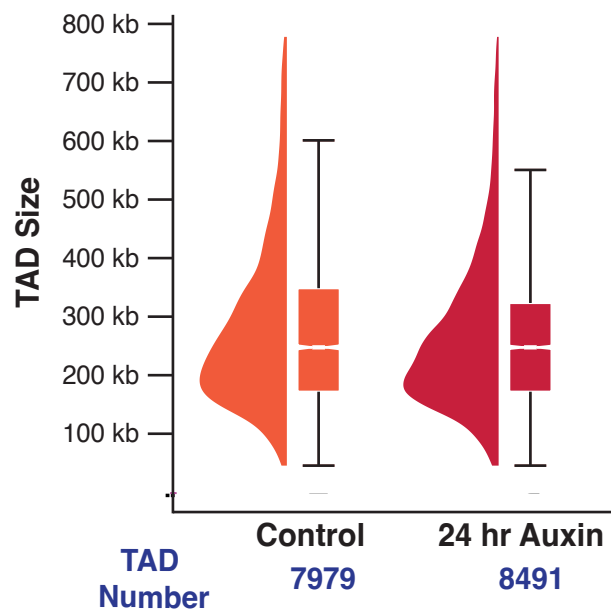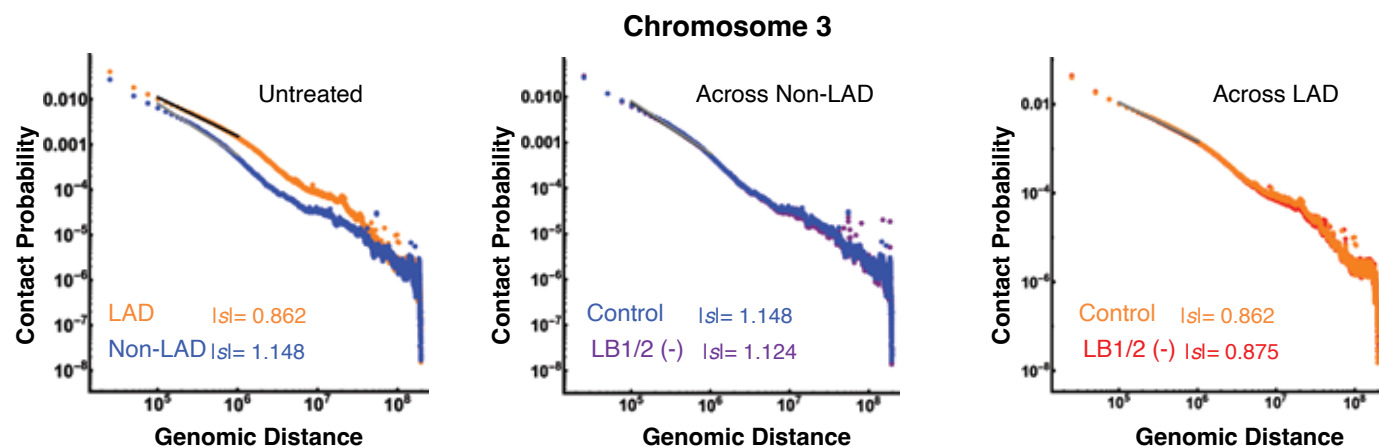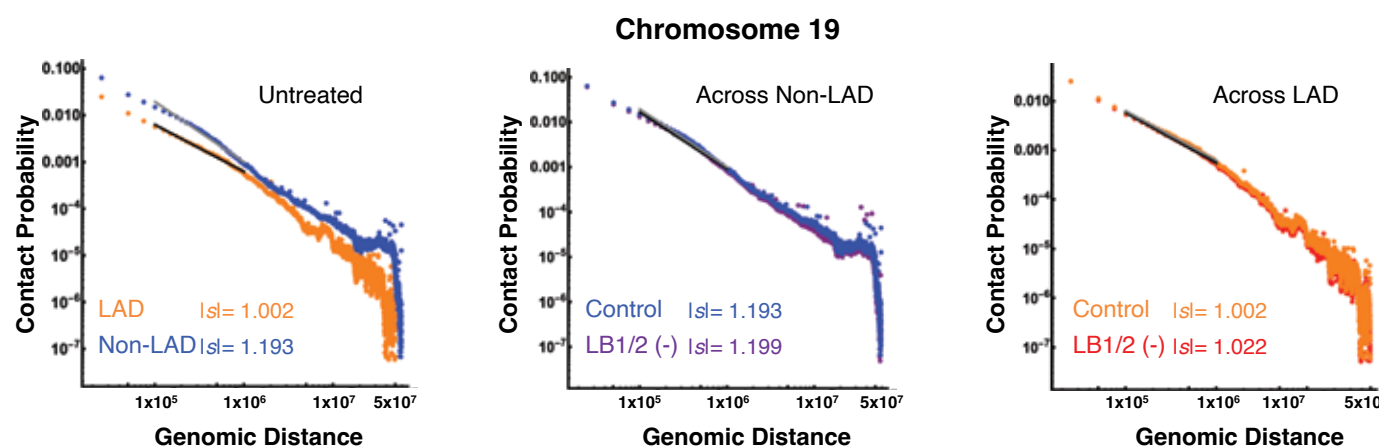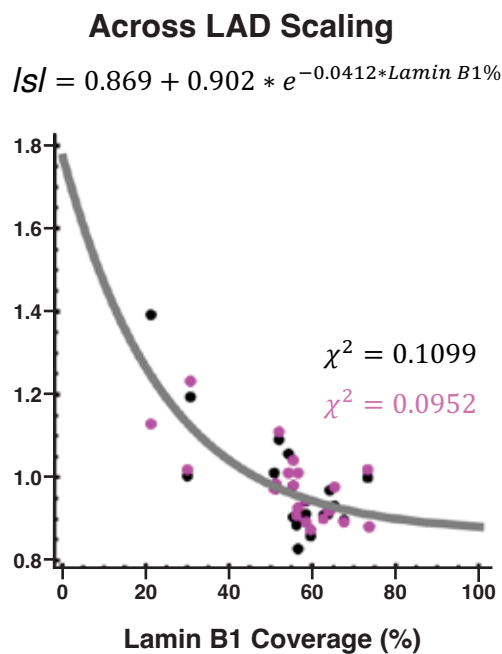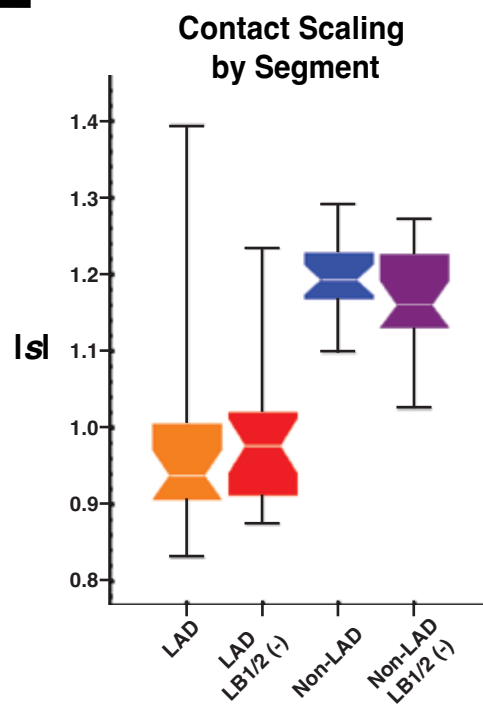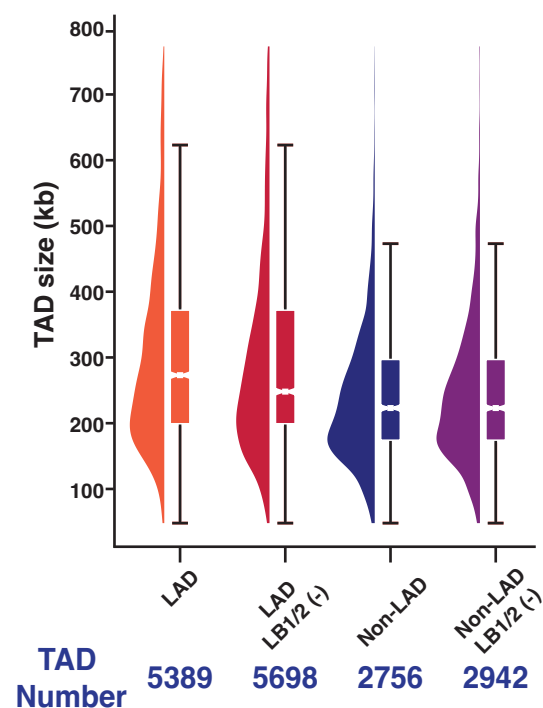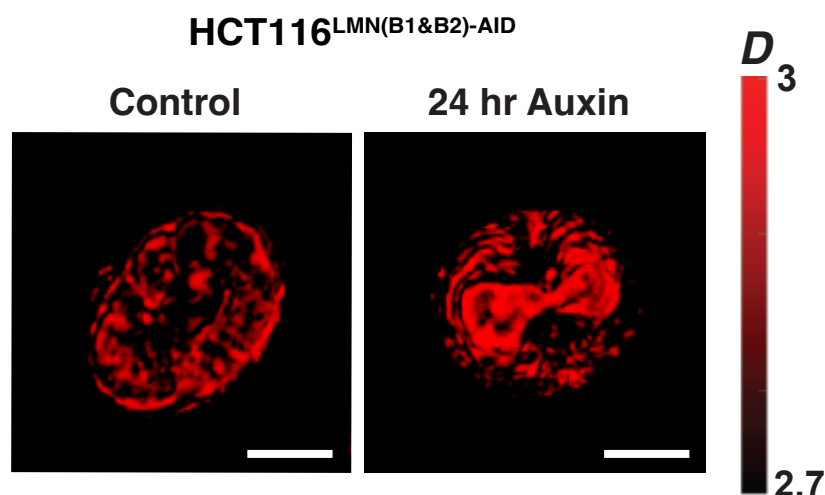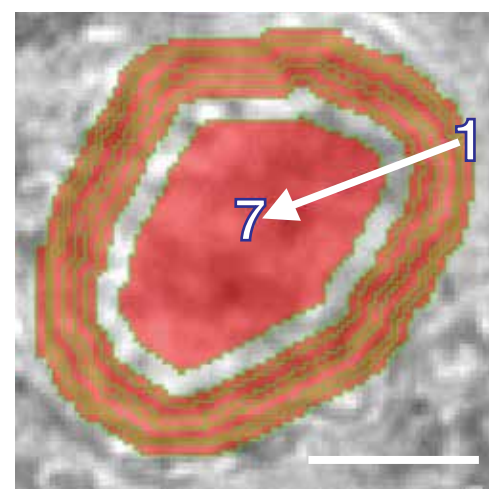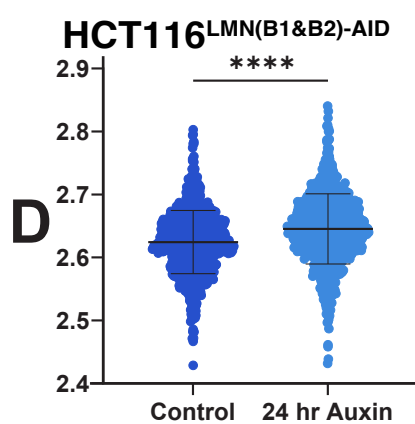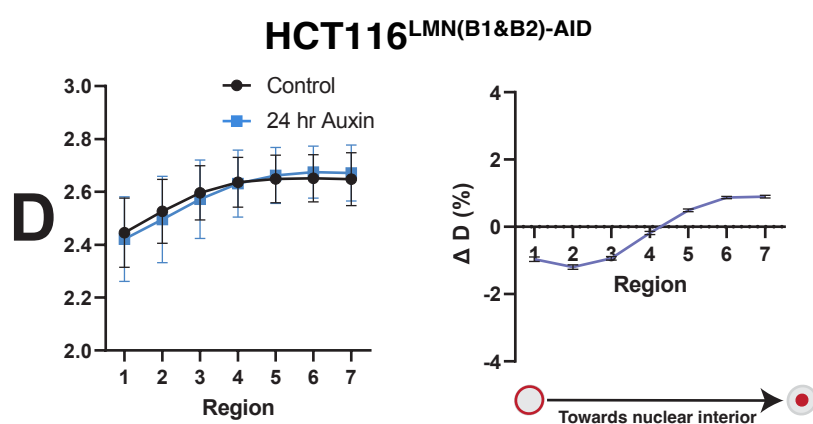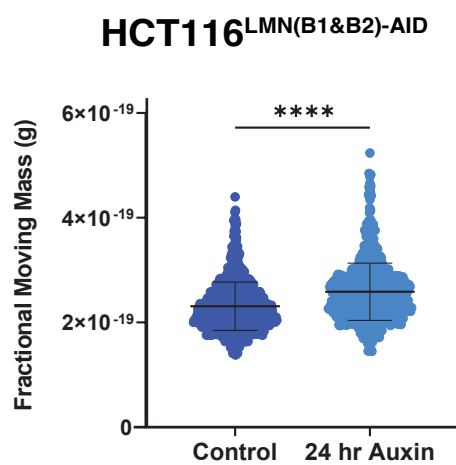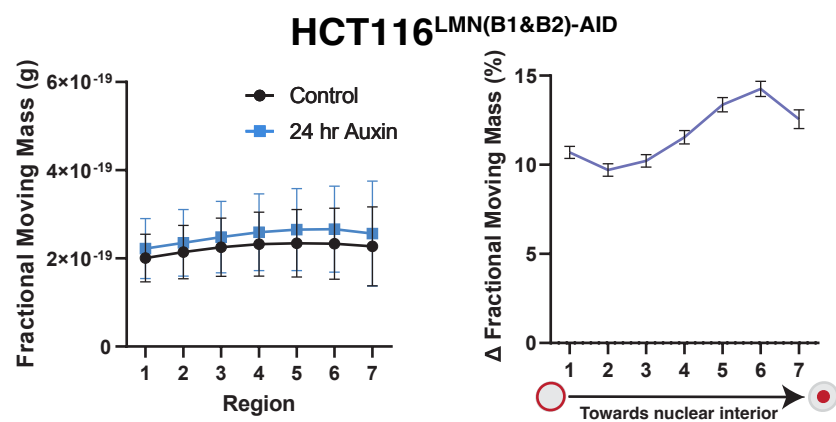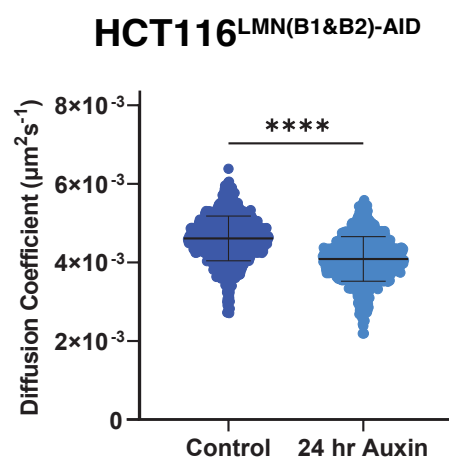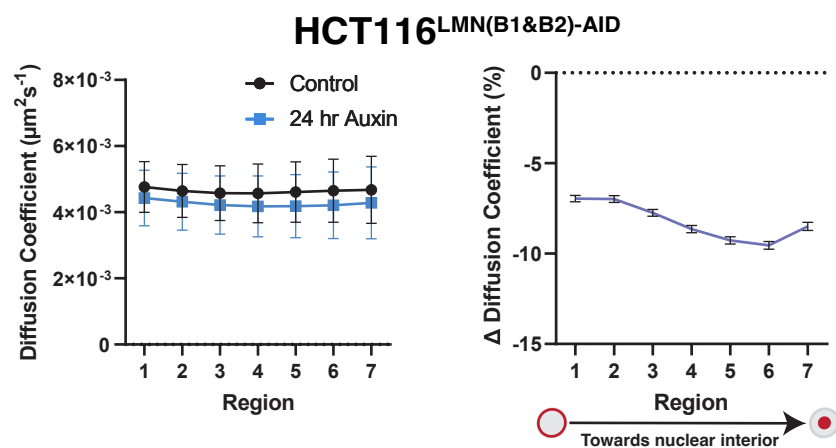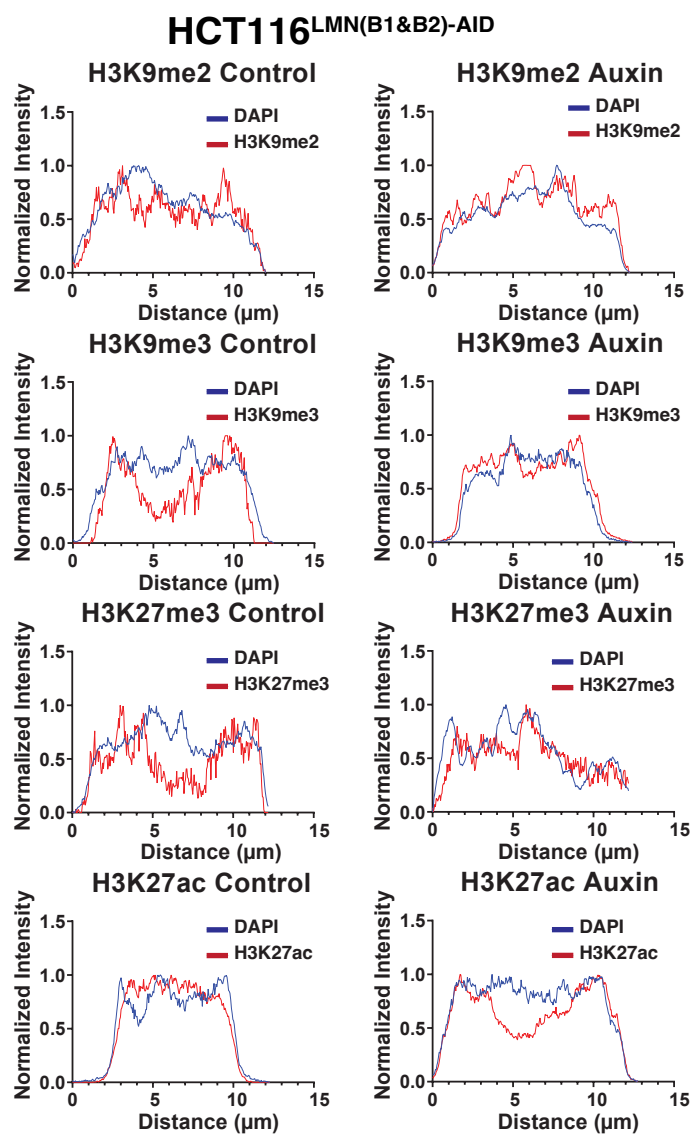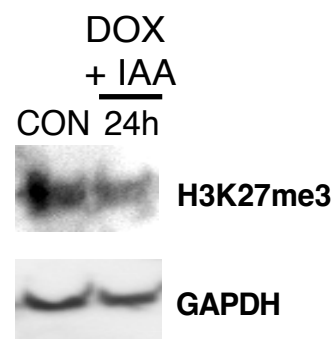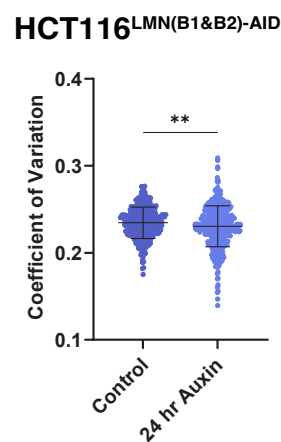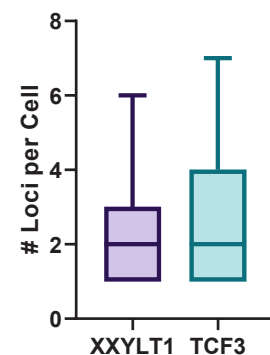
B — Chromosome 2 (0 MB – 140 MB) and Chromosome 19 (0 MB – 25 MB), Control and 24 hr Auxin

C — Contact Probability vs Genomic Distance (bp); |s| = 0.93, Control; |s| = 0.92, 24 hr Auxin; Window: log 5 - 6

D — TAD Size; TAD Number: Control 7979, 24 hr Auxin 8491

Figure 3

Figure 4

Figure 5

Figure 6

A
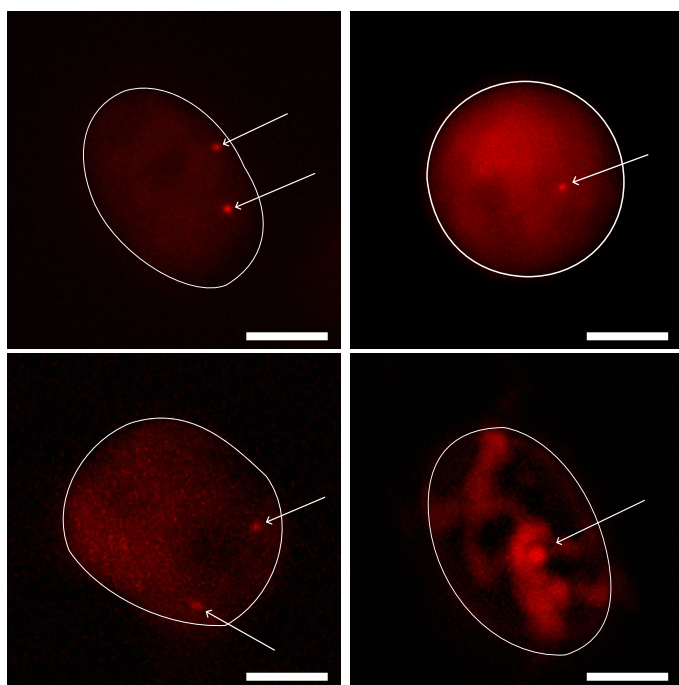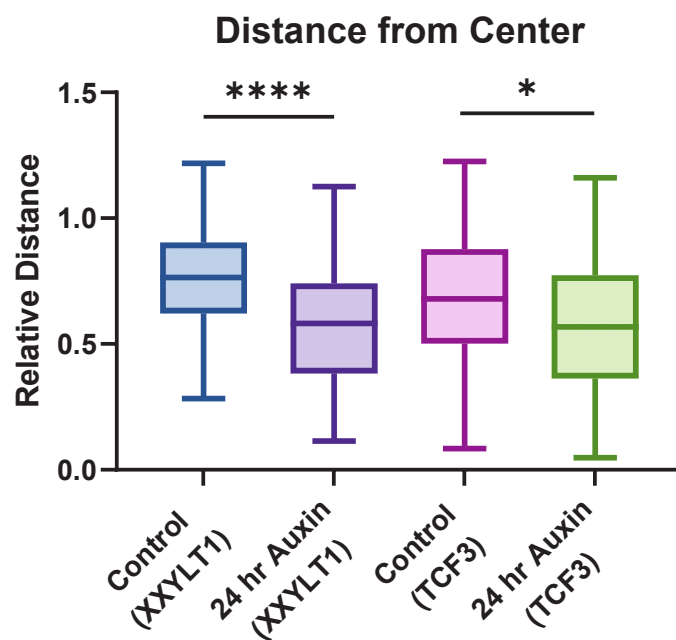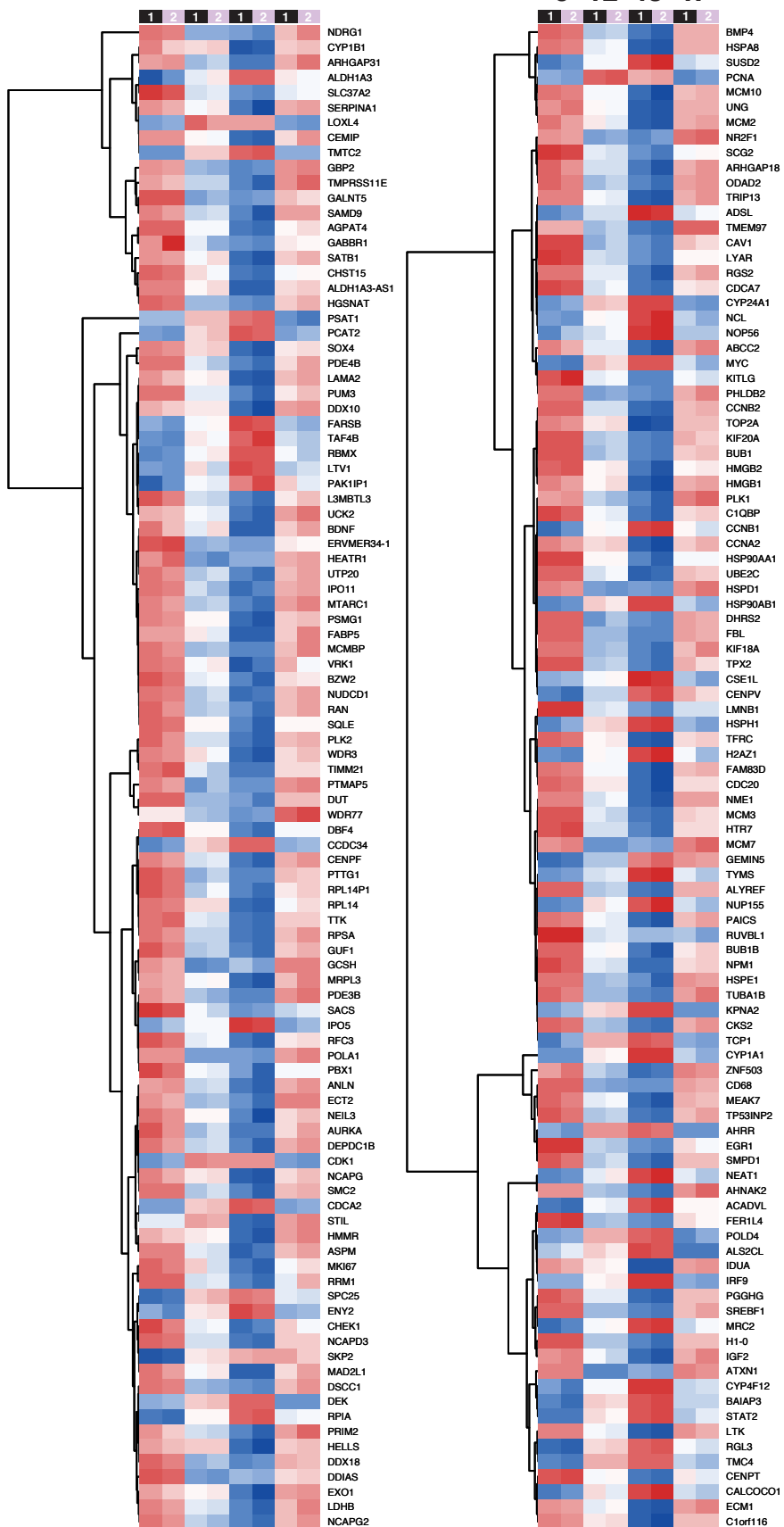
B

DisGeNET, 12 hours within LADs: Structural
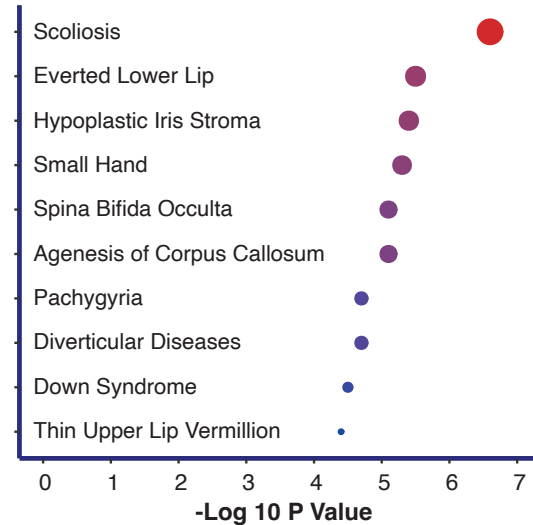
DisGeNET, 12 hours within LADs: Cancer
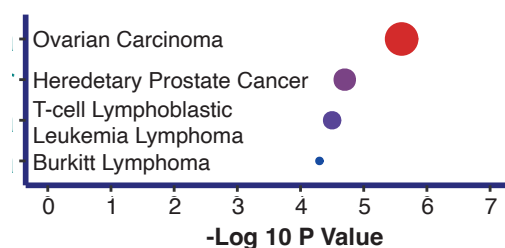
C

DisGeNET, 12 hours outside LADs: Cancer
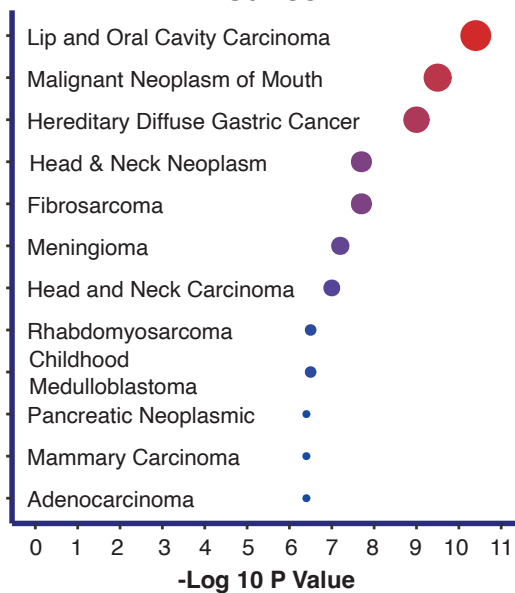
DisGeNET, 12 hours outside LADs: Structural

(+) Lamin B1/B2

Compartments

Chromatin Territories

A B

TAD

Cohesin

Chromatin Chain

Histone

DNA

Nucleosomes

(-) Lamin B1/B2

Compartments

Chromatin Territories

A B

TAD

Cohesin

Chromatin Chain

Histone

DNA

Nucleosomes

Differential Gene Expression ↑
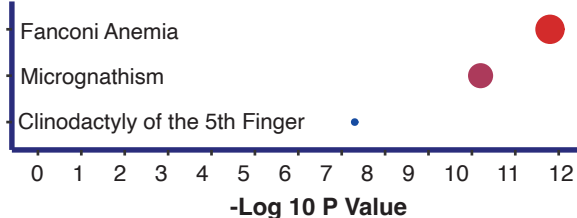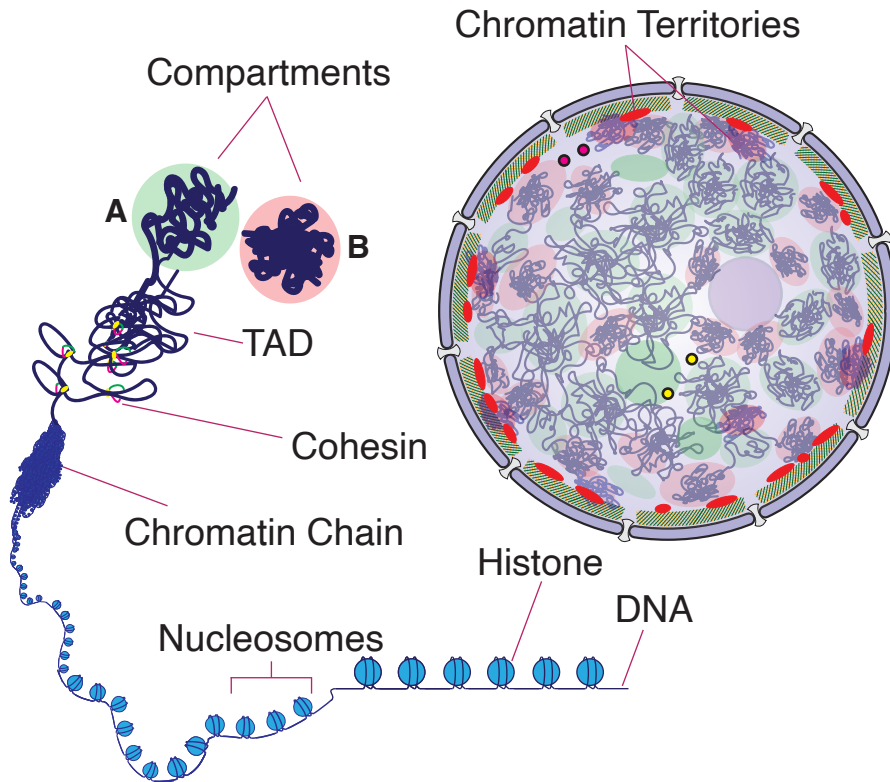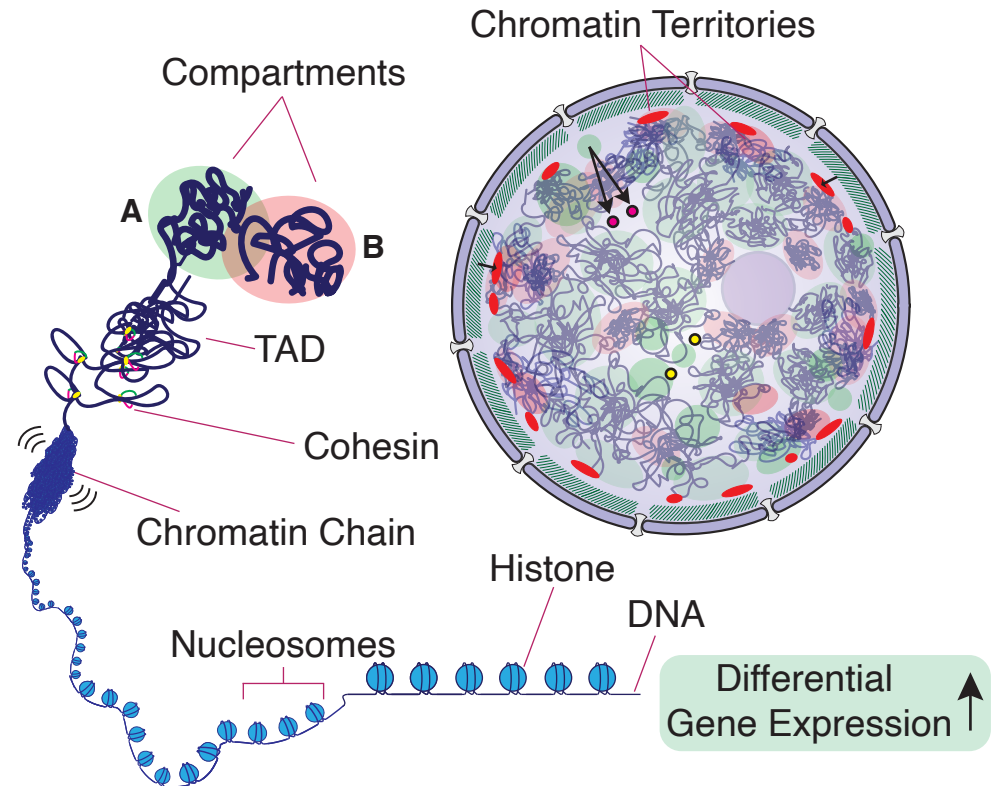
Nuclear Pore Complex   Inactive TAD   Active TAD   Nucleosomes

XXYLT1 gene loci   TCF3 gene loci   Cohesin   Chromatin   LAD

Nucleolus   Nuclear lamina   Lamin A/C   Lamin B1/B2

Supplementary Figure 1

A
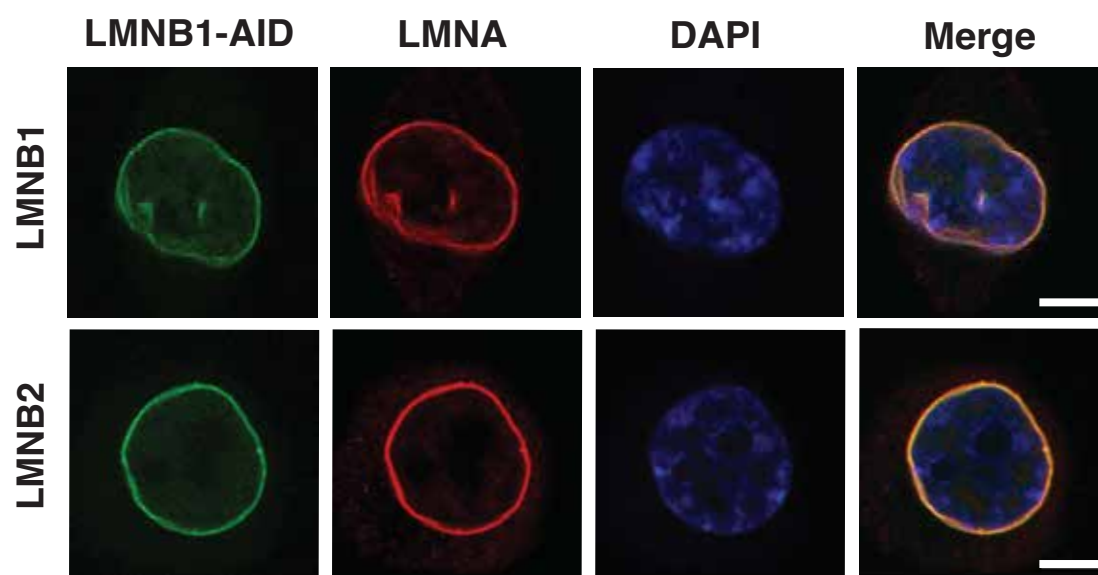
B
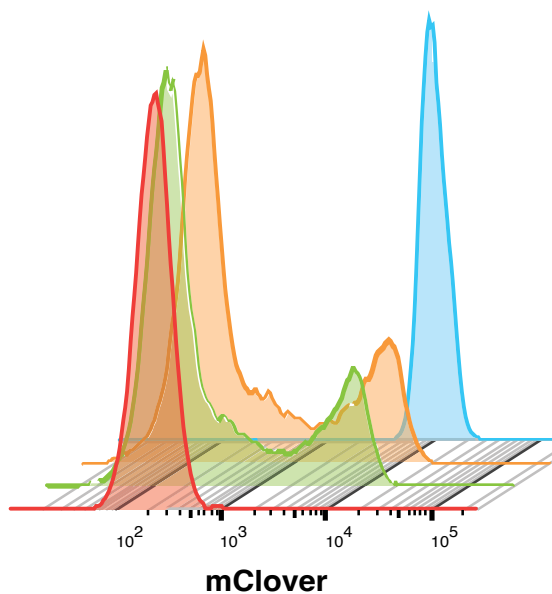
	Sample Name, [IAA]	Mean: FITC-A
🟥	WT HCT116	245
🟩	LMNB1/B2 24 hr, 1000 μm	1682
🟧	LMNB1/B2 24 hr, 500 μm	1639
🟦	LMNB1/B2 0 hr, 0 μm	9938

	Sample Name, [IAA]	Median: FITC-A
🟥	WT HCT116	228
🟩	LMNB1/B2 24 hr, 1000 μm	185
🟧	LMNB1/B2 24 hr, 500 μm	177
🟦	LMNB1/B2 0 hr, 0 μm	9328

C

D

E

Supplementary Figure 2

Supplementary Figure 3

A

HCT116^LMN(B1)-AID

B

HCT116^LMN(B2)-AID

C

HCT116^LMN(B1&B2)-AID
Chromsome 14

D

E

F

Supplementary Figure 4

Supplementary Figure 5

Supplementary Figure 6