Supplementary Figures for

Depletion of lamins B1 and B2 promotes chromatin mobility and induces differential gene expression by a mesoscale-motion dependent mechanism

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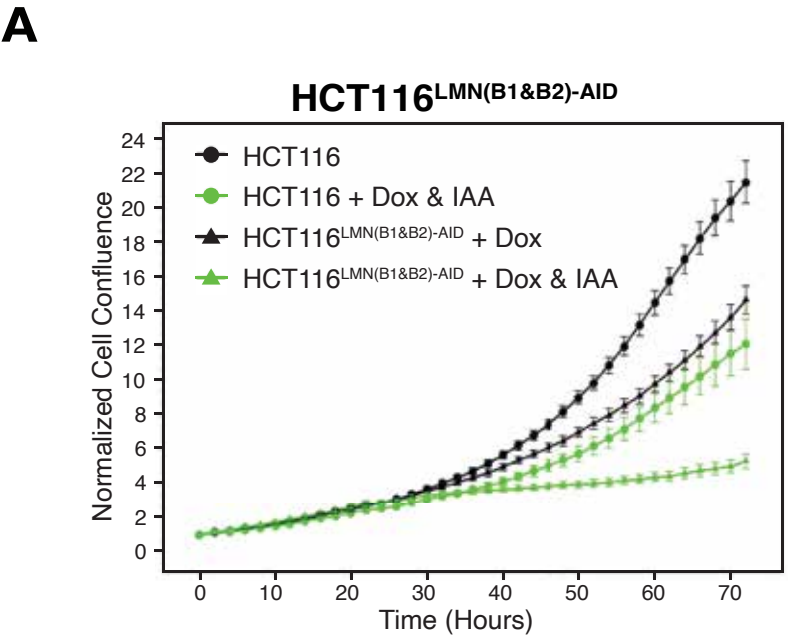
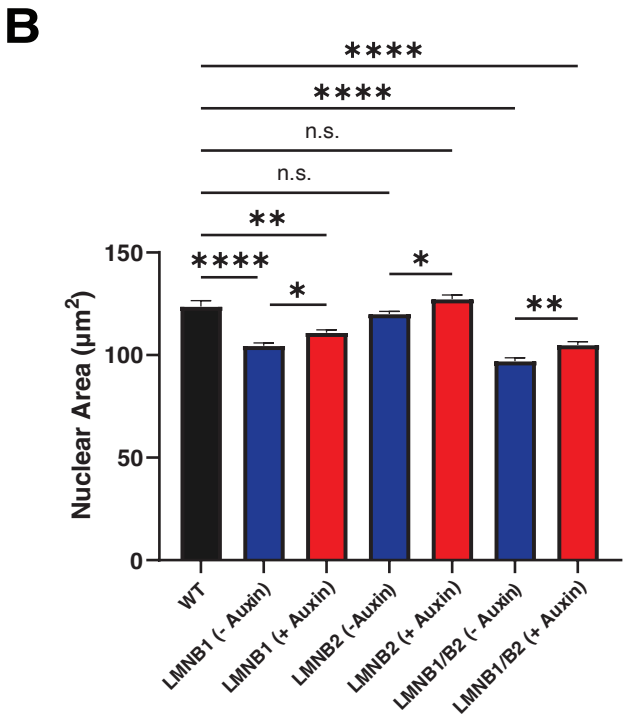
Fig. S1


A
HCT116LMN(B1&B2)-AID
HCT116
HCT116 + Dox & IAA
HCT116LMN(B1&B2)-AID + Dox
HCT116LMN(B1&B2)-AID + Dox & IAA
Normalized Cell Confluence
Time (Hours)


B
Nuclear Area (μm2)
****
****
n.s.
n.s.
**
****
*
*
**
WT
LMNB1 (- Auxin)
LMNB1 (+ Auxin)
LMNB2 (-Auxin)
LMNB2 (+ Auxin)
LMNB1/B2 (- Auxin)
LMNB1/B2 (+ Auxin)

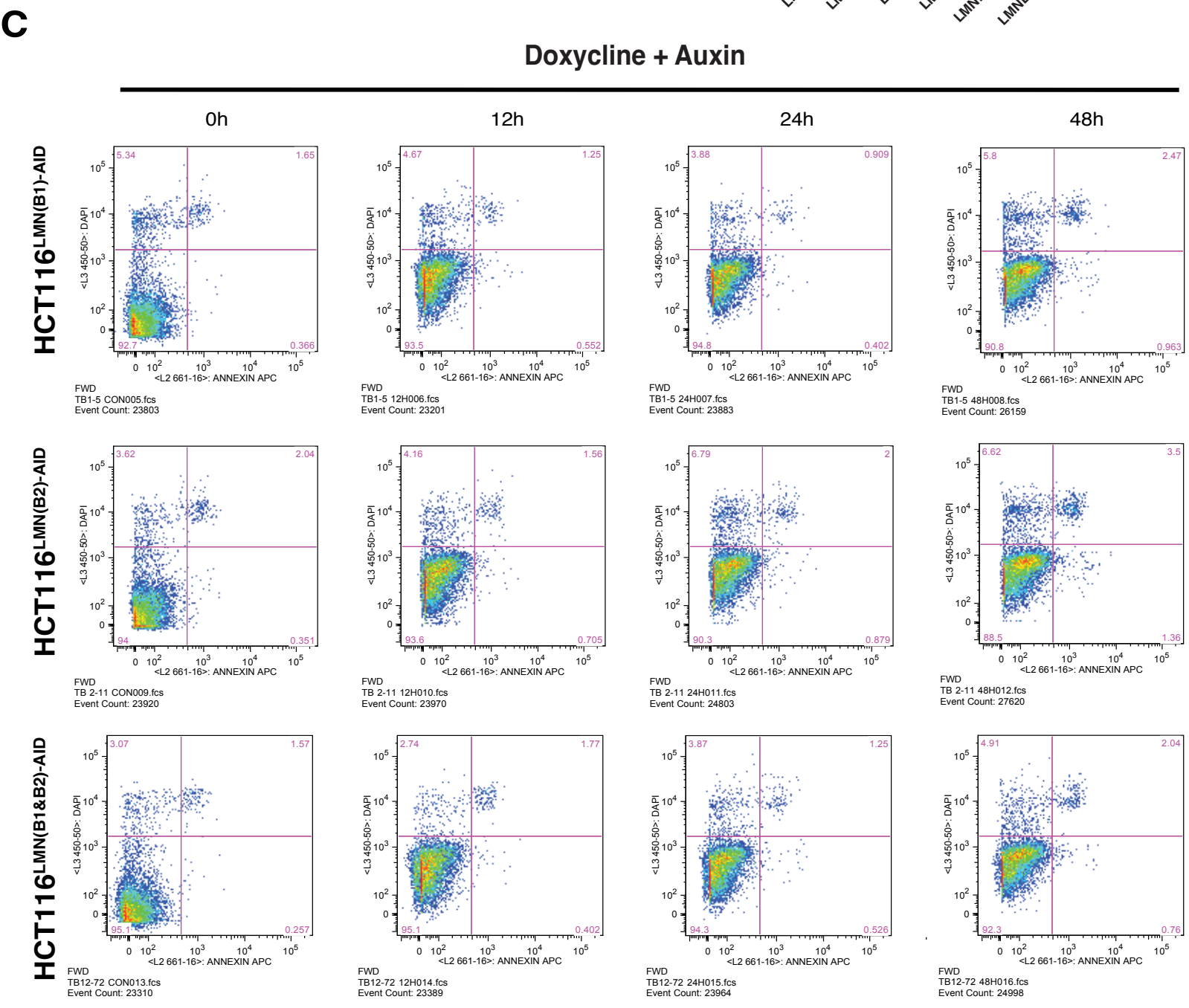

C
Doxycline + Auxin
0h
12h
24h
48h
HCT116LMN(B1)-AID
HCT116LMN(B2)-AID
HCT116LMN(B1&B2)-AID
<L3 450-50>: DAPI
<L2 661-16>: ANNEXIN APC
FWD
TB1-5 CON005.fcs
Event Count: 23803
TB1-5 12H006.fcs
Event Count: 23201
TB1-5 24H007.fcs
Event Count: 23883
TB1-5 48H008.fcs
Event Count: 26159
TB 2-11 CON009.fcs
Event Count: 23920
TB 2-11 12H010.fcs
Event Count: 23970
TB 2-11 24H011.fcs
Event Count: 24803
TB 2-11 48H012.fcs
Event Count: 27620
TB12-72 CON013.fcs
Event Count: 23310
TB12-72 12H014.fcs
Event Count: 23389
TB12-72 24H015.fcs
Event Count: 23964
TB12-72 48H016.fcs
Event Count: 24998

Fig. S1

Degradation of B-type lamins increases nuclear area but does not induce apoptosis.

A. Time-course imaging in a 96-well plate using the Incucyte Live-Cell Analysis System to assess the growth rate of HCT116 and HCT116$^{LMN(B1\&B2)\text{-}AID}$ cells. Growth rate data every 2 hours for a total of 72 hours (normalized to the 0-hour condition). We measured growth rates with and without the addition of doxycycline to induce TIR1, as well as with and without the addition of both doxycycline and auxin to degrade B-type lamins. Error bars: SEM. Data was compiled from three biological replicates (N = 3).
B. Quantification of DAPI-stained area from fixed-cell immunofluorescence (Mean + SEM). Data was compiled from two independent biological replicates (N = 2). Significance was calculated by one-way ANOVA with Šidák's test for multiple comparisons (n.s. = not significant; *<0.05; **<0.01). WT HCT116 (Control n = 66), LMNB1 (Control n = 257; Auxin n = 289), LMNB2 (Control n = 272; Auxin n = 196), LMNB1/B2 (Control n = 209; Auxin n = 257).
C. Auxin treatment to induce acute depletion of B-type lamins in HCT116$^{LMN(B1)\text{-}AID}$ cells, HCT116$^{LMN(B2)\text{-}AID}$ cells, and HCT116$^{LMN(B1\&B2)\text{-}AID}$ cells does not substantially induce cell death. Viable, in-tact cells are present in the bottom left quadrant, dead cells are shown in the top right quadrant, and apoptotic cells are shown in the bottom right quadrant in the plots. At least 20,000 events were recorded during the experiment.

Fig. S2

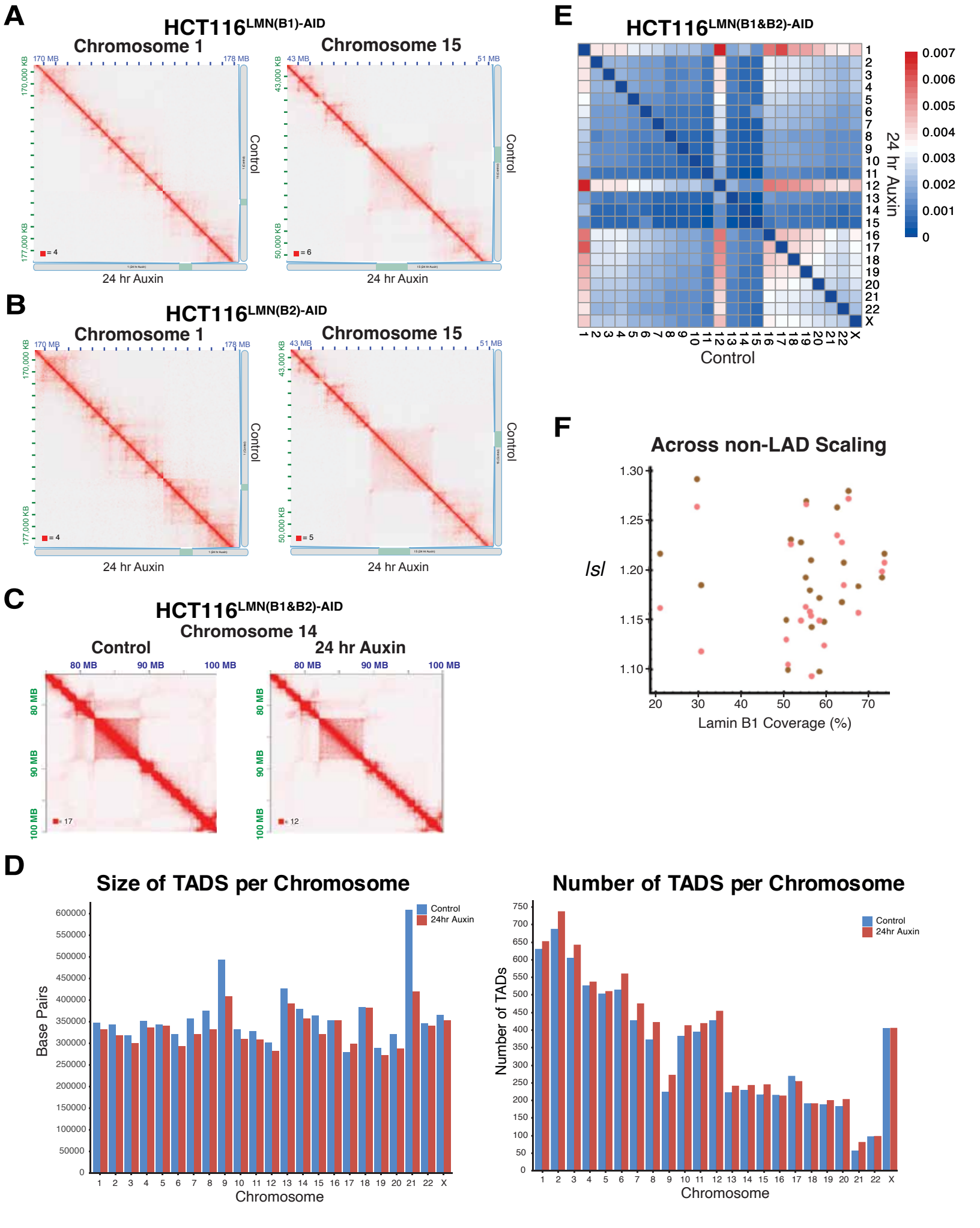

A
HCT116LMN(B1)-AID
Chromosome 1
Chromosome 15
170 MB
178 MB
43 MB
51 MB
170,000 KB
177,000 KB
43,000 KB
50,000 KB
Control
24 hr Auxin
B
HCT116LMN(B2)-AID
C
HCT116LMN(B1&B2)-AID
Chromosome 14
Control
24 hr Auxin
80 MB
90 MB
100 MB
D
Size of TADS per Chromosome
Number of TADs per Chromosome
Base Pairs
Number of TADs
Chromosome
Control
24hr Auxin
E
HCT116LMN(B1&B2)-AID
F
Across non-LAD Scaling
Lamin B1 Coverage (%)

Fig. S2

The mesoscale structure of chromatin is overall preserved upon B-type lamin degradation.

A. The normalized Hi-C trans-interaction matrices for chromosomes 1 and 15 in the control and 24-hour auxin treatment are shown for HCT116$^{LMN(B1)\text{-}AID}$ cells. 15 kb resolution.
B. Normalized Hi-C trans-interaction matrices for chromosomes 1 and 15 in the control and 24-hour auxin treatment conditions for HCT116$^{LMN(B2)\text{-}AID}$ cells. 15 kb resolution.
C. Normalized Hi-C interaction matrices for HCT116$^{LMN(B12\&B2)\text{-}AID}$ cells for chromosome 14 at 50 kb resolution from 75 Mb to 100 Mb.
D. TAD sizes for each chromosome for HCT116$^{LMN(B1\&B2)\text{-}AID}$ cells in the control and 24-hour auxin treatment conditions.
E. Normalized Hi-C trans-interaction matrix for the mean contact frequency, which is the ratio of observed/ expected interchromosomal contact frequency.
F. Scatter plot for the relationship between lamin B1 coverage and I*s*I in non-LAD segments.

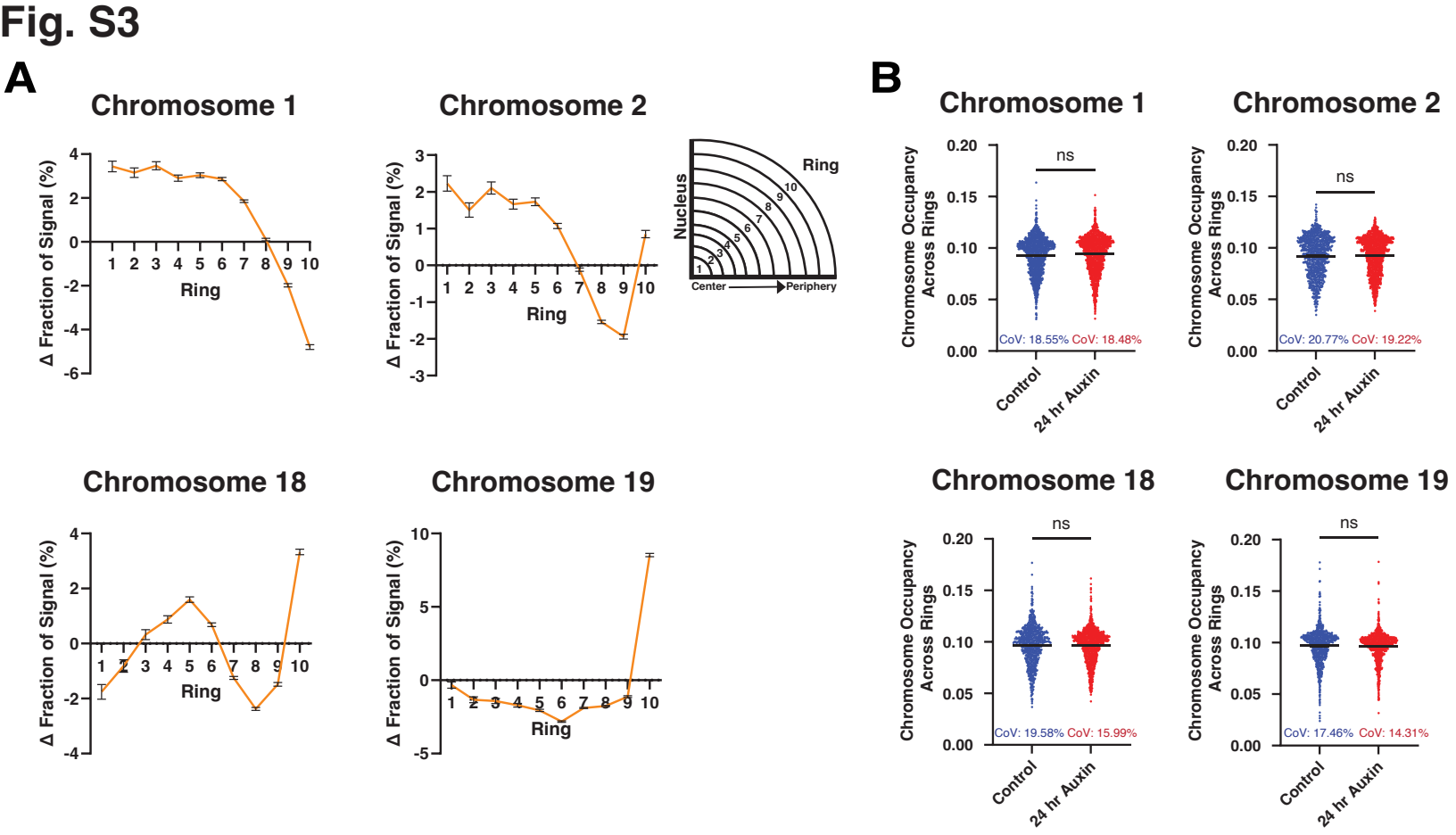


Fig. S3

Chromosome occupancy is shifted upon the addition of auxin.

A. Percent change between concentric rings for each chromosome and condition. Error bars: SD.

B. Chromosome occupancy of nuclei in compiled rings before and after 24-hour auxin treatment. The coefficient of variation (CoV) for each condition and chromosome is obtained by dividing the standard by the mean and is expressed as a percentage. Data was compiled from three independent biological replicates (N = 3). Significance was calculated by unpaired t test (n.s. = not significant). Chr. 1 (Control n = 1660; Auxin n = 1960), Chr. 2 (Control n = 1010; Auxin n = 1740), Chr. 18 (Control n = 890; Auxin n = 1480), Chr. 19 (Control n = 830; Auxin n = 1200). The violin plots extend from the minimum to the maximum value. The line in the middle of each plot is the median value of the distribution, and the lines above and below are the third and first quartiles, respectively. Each dot represents one cell.

Fig. S4

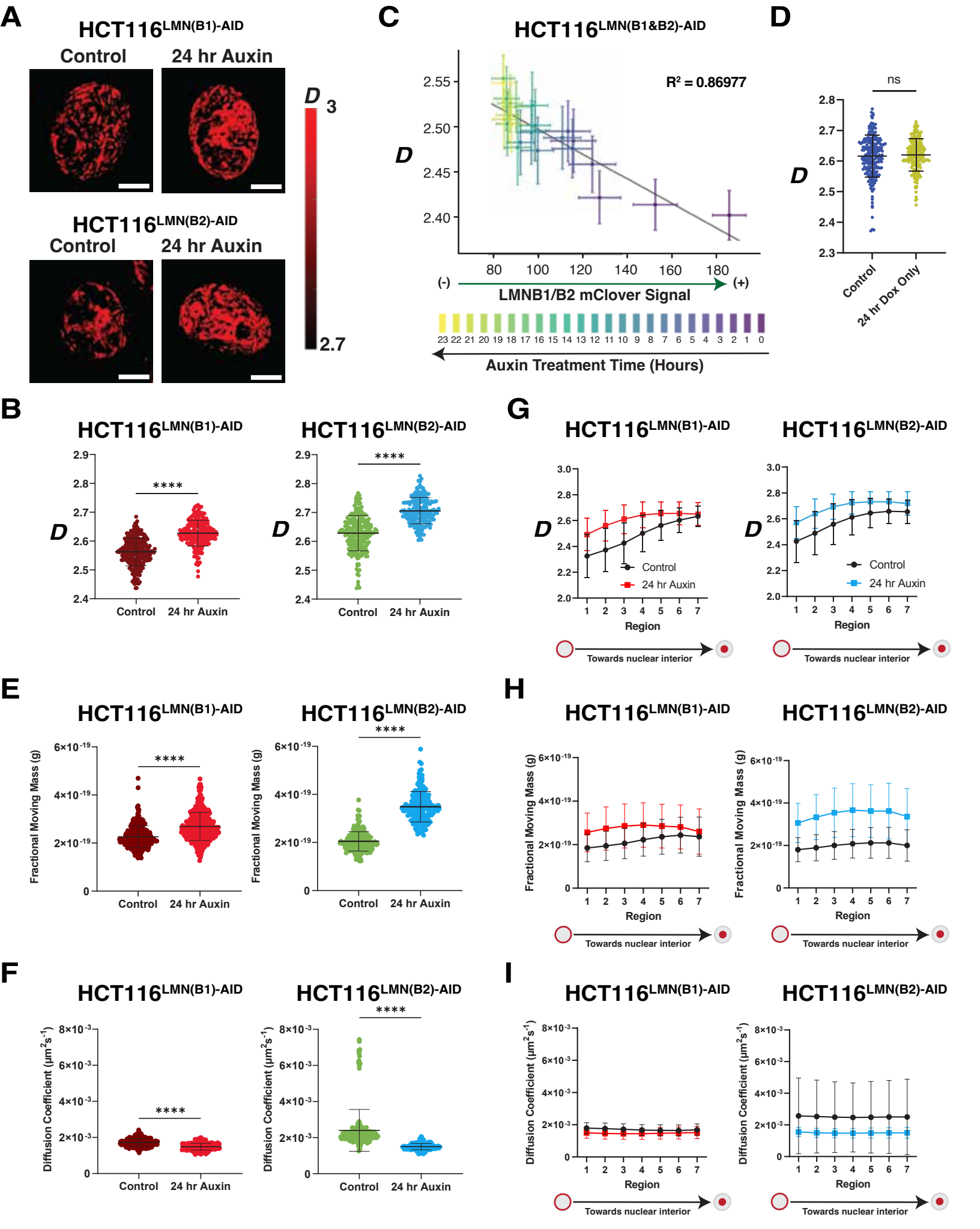

A
HCT116LMN(B1)-AID
Control
24 hr Auxin
HCT116LMN(B2)-AID
Control
24 hr Auxin
D
3
2.7
C
HCT116LMN(B1&B2)-AID
R² = 0.86977
D
2.55
2.50
2.45
2.40
80 100 120 140 160 180
(-)
(+)
LMNB1/B2 mClover Signal
23 22 21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
Auxin Treatment Time (Hours)
D
ns
D
2.8
2.7
2.6
2.5
2.4
2.3
Control
24 hr Dox Only
B
HCT116LMN(B1)-AID
HCT116LMN(B2)-AID
D
****
Control
24 hr Auxin
G
HCT116LMN(B1)-AID
HCT116LMN(B2)-AID
D
Control
24 hr Auxin
Region
Towards nuclear interior
E
HCT116LMN(B1)-AID
HCT116LMN(B2)-AID
Fractional Moving Mass (g)
6×10-19
4×10-19
2×10-19
0
****
Control
24 hr Auxin
H
HCT116LMN(B1)-AID
HCT116LMN(B2)-AID
Fractional Moving Mass (g)
Region
Towards nuclear interior
F
HCT116LMN(B1)-AID
HCT116LMN(B2)-AID
Diffusion Coefficient (μm2s-1)
8×10-3
6×10-3
4×10-3
2×10-3
0
****
Control
24 hr Auxin
I
HCT116LMN(B1)-AID
HCT116LMN(B2)-AID
Diffusion Coefficient (μm2s-1)
Region
Towards nuclear interior

Fig. S4

Dual-PWS reveals differential higher-order chromatin structure and dynamics upon B-type lamin loss.

A. Representative PWS images of HCT116$^{LMN(B1)\text{-}AID}$ cells and HCT116$^{LMN(B2)\text{-}AID}$ cells before and after 24-hour auxin treatment. Scale bar = 5 μm. Data are representative of three independent biological replicates (N = 3).
B. Violin plots for D in HCT116$^{LMN(B1)\text{-}AID}$ cells and HCT116$^{LMN(B2)\text{-}AID}$ cells before and after 24-hour auxin treatment.
C. Live-cell PWS 24-hour auxin treatment time course in HCT116$^{LMN(B1\&B2)\text{-}AID}$ cells. Error bars: SD. Data are representative of three independent biological replicates (N = 3; n = 634).
D. Doxycycline treatment does not alter D in HCT116$^{LMN(B1\&B2)\text{-}AID}$ cells. Data was compiled from three independent biological replicates (N = 3). Significance was calculated by unpaired t test with Welch's correction applied (n.s. = not significant). (Control n = 507; Auxin n = 519).
E. Violin plots for fractional moving mass in HCT116$^{LMN(B1)\text{-}AID}$ cells and HCT116$^{LMN(B2)\text{-}AID}$ cells before and after 24-hour auxin treatment.
F. Violin plots for diffusion coefficient in HCT116$^{LMN(B1)\text{-}AID}$ cells and HCT116$^{LMN(B2)\text{-}AID}$ cells before and after 24-hour auxin treatment.
G. Regional PWS measurements of D in HCT116$^{LMN(B1)\text{-}AID}$ cells and HCT116$^{LMN(B2)\text{-}AID}$ cells before and after 24-hour auxin treatment.
H. Regional PWS measurements of fractional moving mass in HCT116$^{LMN(B1)\text{-}AID}$ cells and HCT116$^{LMN(B2)\text{-}AID}$ cells before and after 24-hour auxin treatment.
I. Regional PWS measurements of diffusion coefficient for HCT116$^{LMN(B1)\text{-}AID}$ cells and HCT116$^{LMN(B2)\text{-}AID}$ cells before and after 24-hour auxin treatment.

For A-B and E-I: Data was compiled from three independent biological replicates (N = 3). Significance was calculated by unpaired t test with Welch's correction applied (****< 0.0001). Lamin B1 (Control n = 694; Auxin n = 585), Lamin B2 (Control n = 568; Auxin n = 525). Violin plots extend from the minimum to the maximum value. The line in the middle of each plot is the median value of the distribution, and the lines above and below are the third and first quartiles, respectively. Each dot represents one cell.

Fig. S5

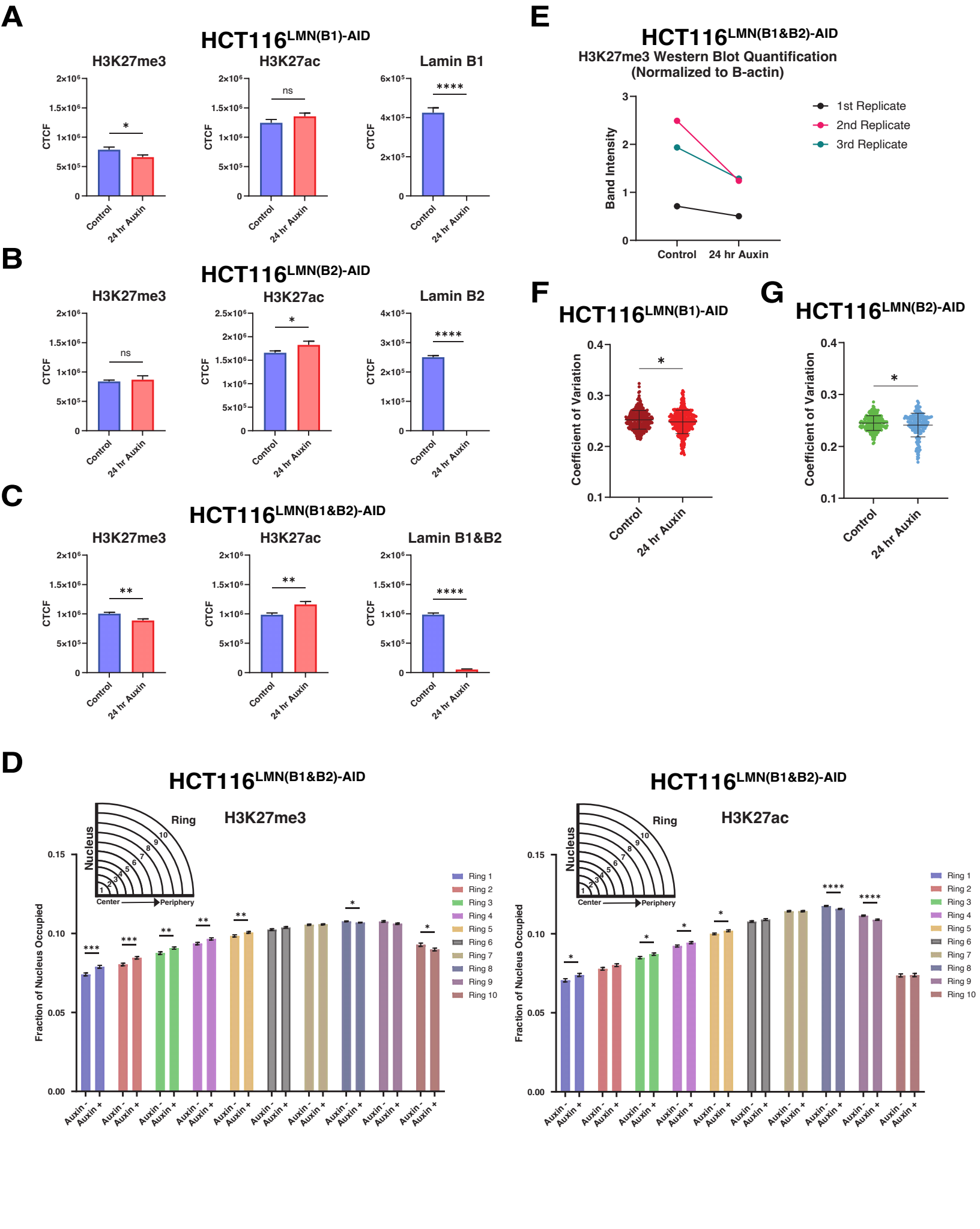

A
HCT116LMN(B1)-AID
H3K27me3
H3K27ac
Lamin B1
CTCF
Control
24 hr Auxin
ns
*
****
B
HCT116LMN(B2)-AID
H3K27me3
H3K27ac
Lamin B2
C
HCT116LMN(B1&B2)-AID
H3K27me3
H3K27ac
Lamin B1&B2
**
D
HCT116LMN(B1&B2)-AID
H3K27me3
H3K27ac
Ring
Nucleus
Center
Periphery
Fraction of Nucleus Occupied
Auxin -
Auxin +
Ring 1
Ring 2
Ring 3
Ring 4
Ring 5
Ring 6
Ring 7
Ring 8
Ring 9
Ring 10
E
HCT116LMN(B1&B2)-AID
H3K27me3 Western Blot Quantification
(Normalized to B-actin)
Band Intensity
1st Replicate
2nd Replicate
3rd Replicate
F
HCT116LMN(B1)-AID
Coefficient of Variation
G
HCT116LMN(B2)-AID

Fig. S5

Chromatin decompaction promotes redistribution of heterochromatic marks.

A. Bar plots of H3K27me3, H3K27ac, and Lamin B1 corrected total cell fluorescence in HCT116$^{LMN(B1)\text{-}AID}$ cells before and after 24-hour auxin treatment (Mean + SEM). (Control n = 281; 24-hr Auxin n = 193).
B. Bar plots of H3K27me3, H3K27ac, and Lamin B2 corrected total cell fluorescence in HCT116$^{LMN(B2)\text{-}AID}$ cells before and after 24-hour auxin treatment (Mean + SEM). (Control n = 249; 24-hr Auxin n = 152).
C. Bar plots of H3K27me3, H3K27ac, and Lamin B1&B2 corrected total cell fluorescence in HCT116$^{LMN(B1\&B2)\text{-}AID}$ cells before and after 24-hour auxin treatment (Mean + SEM). (Control n = 383; 24-hr Auxin n = 367).
D. Graphing of the average fraction and SEM of H3K27me3 and H3K27ac fluorescent signals present in each one of ten concentric nuclear rings. Data was compiled from two independent biological replicates (N = 2). Significance was calculated by unpaired t test (*< 0.05; **< 0.01; ***< 0.001; ****< 0.0001). (H3K27me3 (Control n = 455; Auxin n = 510), H3K27ac (Control n = 448; Auxin n = 447)).
E. Western blot analysis for H3K27me3 in HCT116$^{LMN(B1\&B2)\text{-}AID}$ cells. GAPDH was used as a loading control. Three independent biological replicates (N = 3) are shown.
F. Coefficient of variation plot for HCT116$^{LMN(B1)\text{-}AID}$ cells showing chromatin decompaction upon 24 hours of auxin treatment. (Control n = 770; Auxin n = 492).
G. Coefficient of variation plot for HCT116$^{LMN(B2)\text{-}AID}$ cells. (Control n = 650; Auxin n = 393).

For A-C and F-G: Data was compiled from three independent biological replicates (N = 3). Significance was calculated by unpaired t test (n.s. = not significant; *<0.05; **<0.01; ***<0.001; ****<0.0001). For F-G: Violin plots extend from the minimum to the maximum value. The line in the middle of each plot is the median value of the distribution, and the lines above and below are the third and first quartiles, respectively.

Fig. S6

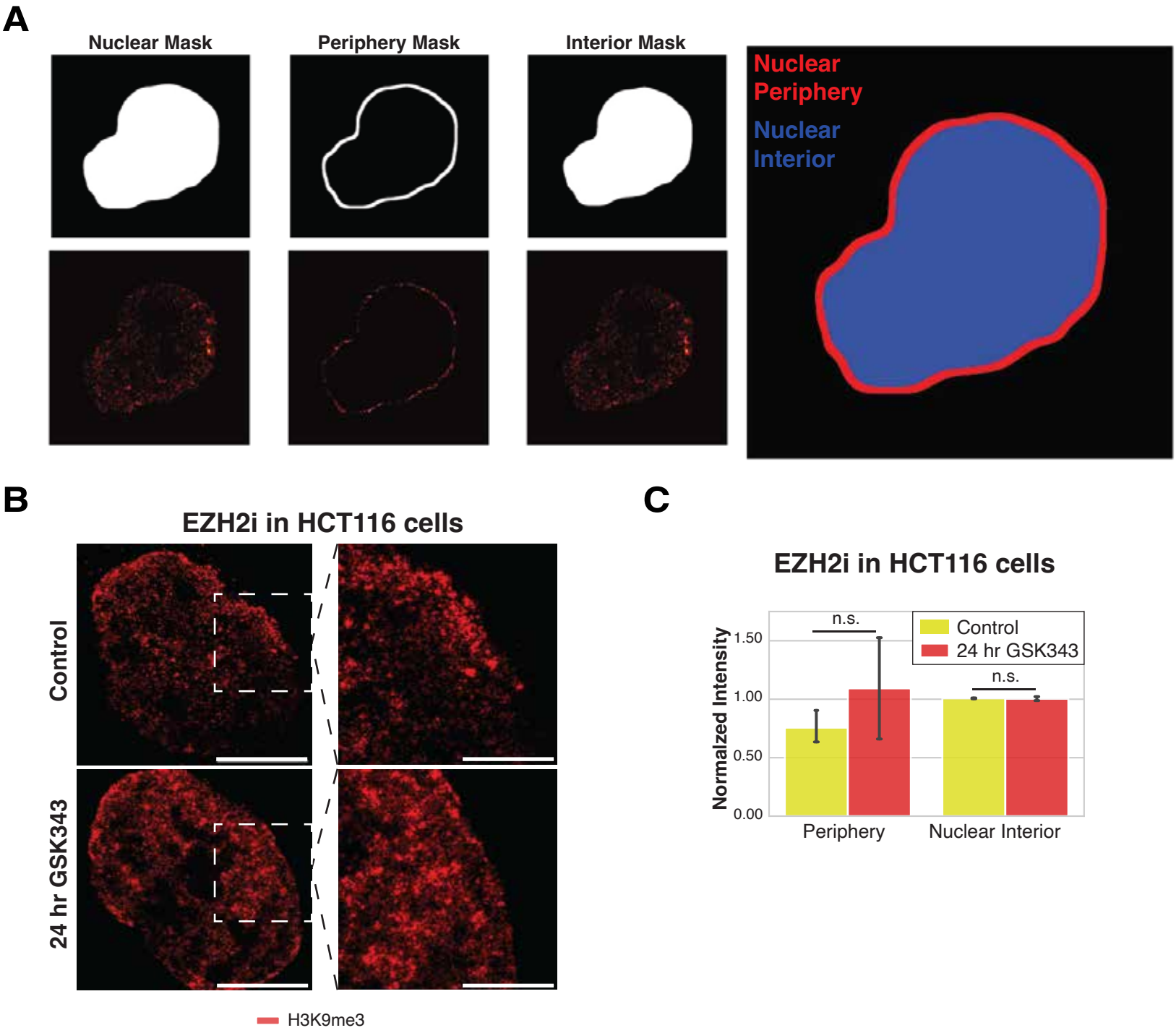

A
Nuclear Mask
Periphery Mask
Interior Mask
Nuclear Periphery
Nuclear Interior
B
EZH2i in HCT116 cells
Control
24 hr GSK343
H3K9me3
C
EZH2i in HCT116 cells
Normalzed Intensity
1.50
1.00
0.50
0.00
n.s.
n.s.
Control
24 hr GSK343
Periphery
Nuclear Interior
D
EZH2i in HCT116 cells
D
*
Fractional Moving Mass (g)
6x10-19
4x10-19
2x10-19
0
****
Diffusion Coefficient (μm2s-1)
8x10-3
6x10-3
4x10-3
2x10-3
0
****
Control
24 hr GSK343

Fig. S6

SMLM allows sub-diffraction resolution to assess heterochromatin redistribution without perturbing B-type lamins.

A. Example of Normalized STORM Intensity (NSI) analysis pipeline. Top: Representative images of binary masks used to specify the regions of interest for NSI analysis. Bottom: Representative masked HCT116$^{LMN(B1\&B2)\text{-}AID}$ nucleus corresponding to the binary masks above. Right: Overlay of the nuclear interior and periphery masks in blue and red, respectively.
B. STORM images of HCT116 cells after 24-hour GSK343 treatment (10 μM) compared to the vehicle control (DMSO). Scale bars = 5 μm, Scale bars (zoom) = 2 μm.
C. Quantification of NSI after EZH2 inhibition. Error bars: SD. Significance was calculated by unpaired t test (n.s. = not significant). (Control n = 4; GSK343 n = 5).
D. Violin plots for D, fractional moving mass, and the diffusion coefficient in HCT116 cells after 24-hour GSK343 treatment (10mM) compared to the vehicle control (DMSO). Data was compiled from three independent biological replicates (N = 3). Significance was calculated by unpaired t test (*<0.05; ****< 0.0001). (Control n = 707; Auxin n = 807). The truncated violin plots extend from the minimum to the maximum value. The line in the middle of each plot is the median value of the distribution, and the lines above and below are the third and first quartiles, respectively. Each dot represents one cell.

Fig. S7

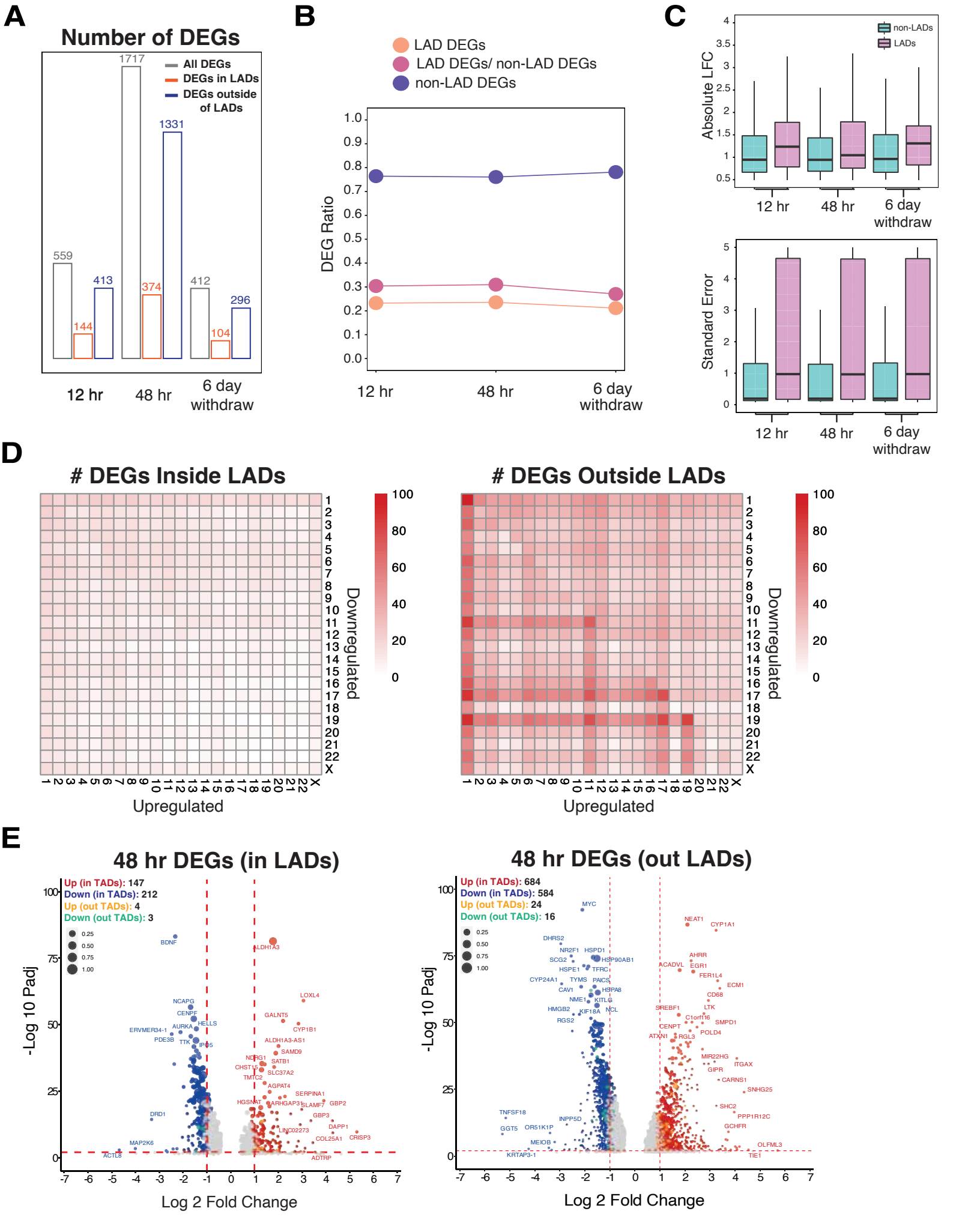

A
Number of DEGs
All DEGs
DEGs in LADs
DEGs outside of LADs
559
144
413
1717
374
1331
412
104
296
12 hr
48 hr
6 day withdraw
B
LAD DEGs
LAD DEGs/ non-LAD DEGs
non-LAD DEGs
DEG Ratio
12 hr
48 hr
6 day withdraw
C
non-LADs
LADs
Absolute LFC
Standard Error
12 hr
48 hr
6 day withdraw
D
DEGs Inside LADs
DEGs Outside LADs
Downregulated
Upregulated
E
48 hr DEGs (in LADs)
Up (in TADs): 147
Down (in TADs): 212
Up (out TADs): 4
Down (out TADs): 3
48 hr DEGs (out LADs)
Up (in TADs): 684
Down (in TADs): 584
Up (out TADs): 24
Down (out TADs): 16
-Log 10 Padj
Log 2 Fold Change

Fig. S7

Degradation of B-type lamins promotes differential gene expression near LAD boundaries.

A. Bar plot showing the number of all DEGs compared to the number of DEGs within versus outside of LAD boundaries defined by publicly available DamID data for all three auxin treatment conditions (adjusted P value < 0.01 and absolute log fold change > 1).
B. Dot plot showing the ratios of DEGs within LADs, outside of LADs, and all DEGs.
C. Box plot showing the absolute log fold change and standard error for DEGs within LADs and outside of LADs for all three auxin treatment conditions.
D. Heatmaps showing the number of DEGs within and outside of LADs in relation to their upregulation or downregulation.
E. Volcano plots showing DEGs within and outside of LADs in relation to TADs after 48 hours of auxin treatment (adjusted P value < 0.01 and absolute log fold change > 1). “Up” refers to upregulated genes and “Down” refers to downregulated genes.

Fig. S8

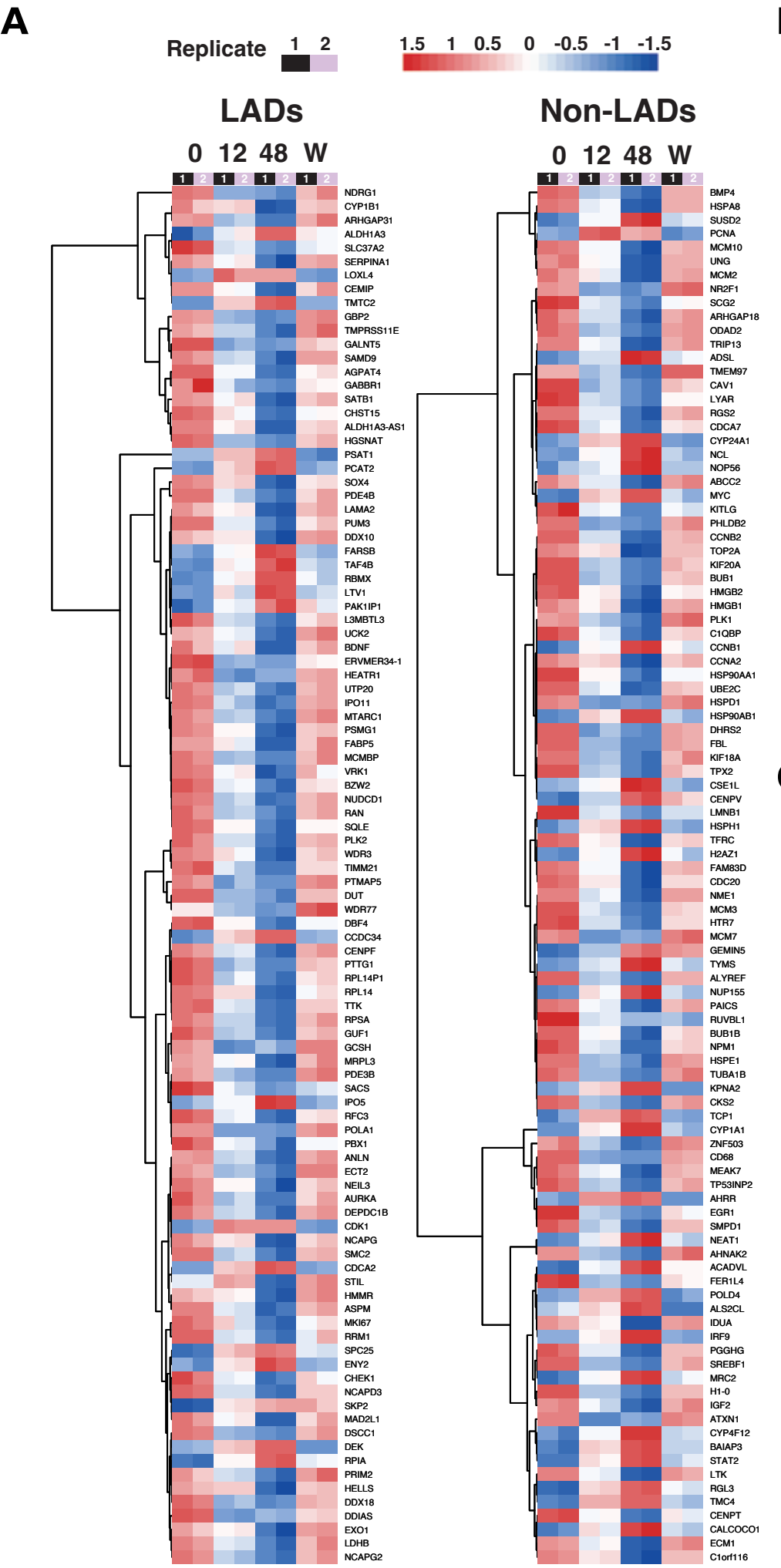

A
Replicate 1 2
1.5 1 0.5 0 -0.5 -1 -1.5
LADs
Non-LADs
0 12 48 W
NDRG1 CYP1B1 ARHGAP31 ALDH1A3 SLC37A2 SERPINA1 LOXL4 CEMIP TMTC2 GBP2 TMPRSS11E GALNT5 SAMD9 AGPAT4 GABBR1 SATB1 CHST15 ALDH1A3-AS1 HGSNAT PSAT1 PCAT2 SOX4 PDE4B LAMA2 PUM3 DDX10 FARSB TAF4B RBMX LTV1 PAK1IP1 L3MBTL3 UCK2 BDNF ERVMER34-1 HEATR1 UTP20 IPO11 MTARC1 PSMG1 FABP5 MCMBP VRK1 BZW2 NUDCD1 RAN SQLE PLK2 WDR3 TIMM21 PTMAP5 DUT WDR77 DBF4 CCDC34 CENPF PTTG1 RPL14P1 RPL14 TTK RPSA GUF1 GCSH MRPL3 PDE3B SACS IPO5 RFC3 POLA1 PBX1 ANLN ECT2 NEIL3 AURKA DEPDC1B CDK1 NCAPG SMC2 CDCA2 STIL HMMR ASPM MKI67 RRM1 SPC25 ENY2 CHEK1 NCAPD3 SKP2 MAD2L1 DSCC1 DEK RPIA PRIM2 HELLS DDX18 DDIAS EXO1 LDHB NCAPG2
BMP4 HSPA8 SUSD2 PCNA MCM10 UNG MCM2 NR2F1 SCG2 ARHGAP18 ODAD2 TRIP13 ADSL TMEM97 CAV1 LYAR RGS2 CDCA7 CYP24A1 NCL NOP56 ABCC2 MYC KITLG PHLDB2 CCNB2 TOP2A KIF20A BUB1 HMGB2 HMGB1 PLK1 C1QBP CCNB1 CCNA2 HSP90AA1 UBE2C HSPD1 HSP90AB1 DHRS2 FBL KIF18A TPX2 CSE1L CENPV LMNB1 HSPH1 TFRC H2AZ1 FAM83D CDC20 NME1 MCM3 HTR7 MCM7 GEMIN5 TYMS ALYREF NUP155 PAICS RUVBL1 BUB1B NPM1 HSPE1 TUBA1B KPNA2 CKS2 TCP1 CYP1A1 ZNF503 CD68 MEAK7 TP53INP2 AHRR EGR1 SMPD1 NEAT1 AHNAK2 ACADVL FER1L4 POLD4 ALS2CL IDUA IRF9 PGGHG SREBF1 MRC2 H1-0 IGF2 ATXN1 CYP4F12 BAIAP3 STAT2 LTK RGL3 TMC4 CENPT CALCOCO1 ECM1 C1orf116
B
DisGeNET, 12 hours within LADs: Structural
Scoliosis
Everted Lower Lip
Hypoplastic Iris Stroma
Small Hand
Spina Bifida Occulta
Agenesis of Corpus Callosum
Pachygyria
Diverticular Diseases
Down Syndrome
Thin Upper Lip Vermillion
0 1 2 3 4 5 6 7
-Log 10 P Value
DisGeNET, 12 hours within LADs: Cancer
Ovarian Carcinoma
Hereditary Prostate Cancer
T-cell Lymphoblastic Leukemia/ Lymphoma
Burkitt Lymphoma
0 1 2 3 4 5 6 7
-Log 10 P Value
C
DisGeNET, 12 hours outside LADs: Cancer
Lip and Oral Cavity Carcinoma
Malignant Neoplasm of Mouth
Hereditary Diffuse Gastric Cancer
Head & Neck Neoplasm
Fibrosarcoma
Meningioma
Head and Neck Carcinoma
Rhabdomyosarcoma
Childhood Medulloblastoma
Pancreatic Neoplasmic
Mammary Carcinoma
Adenocarcinoma
0 1 2 3 4 5 6 7 8 9 10 11
-Log 10 P Value
DisGeNET, 12 hours outside LADs: Structural
Fanconi Anemia
Micrognathism
Clinodactyly of the 5th Finger
0 1 2 3 4 5 6 7 8 9 10 11 12
-Log 10 P Value

Fig. S8

Degradation of B-type lamins promotes differential gene expression near LAD boundaries.

A. Heatmaps showing the top 100 DEGs within versus outside of LAD boundaries upon 48 hours of auxin treatment defined by publicly available DamID data (adjusted P value < 0.01 and absolute log fold change > 1).
B. DisGeNET results indicate terms upon 12 hours of auxin treatment within LADs related to structural changes and cancer.
C. DisGeNET results indicate terms upon 12 hours of auxin treatment outside of LADs related to cancer and structural changes.

Fig. S9

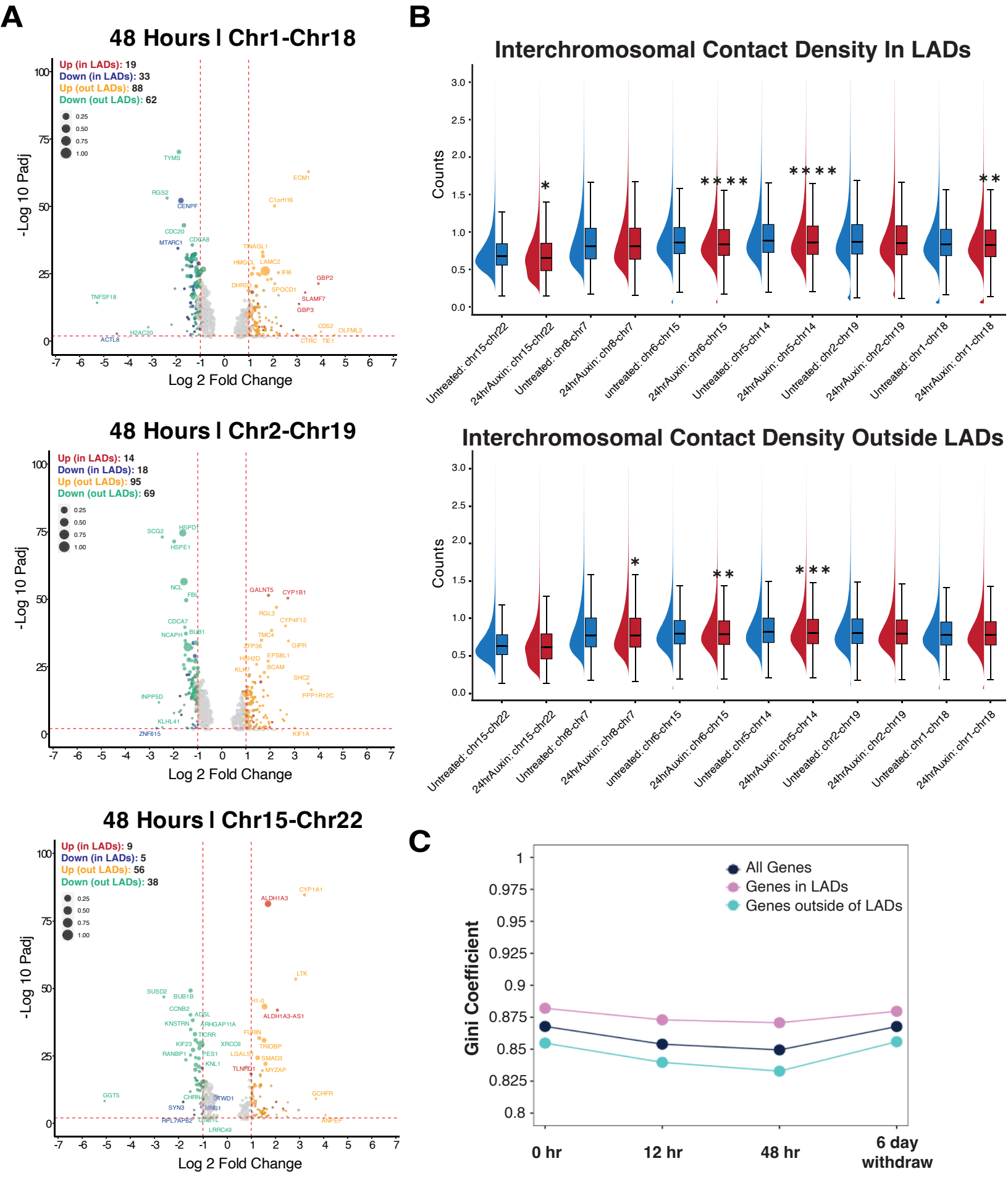

A
48 Hours | Chr1-Chr18
Up (in LADs): 19
Down (in LADs): 33
Up (out LADs): 88
Down (out LADs): 62
-Log 10 Padj
Log 2 Fold Change
48 Hours | Chr2-Chr19
Up (in LADs): 14
Down (in LADs): 18
Up (out LADs): 95
Down (out LADs): 69
-Log 10 Padj
Log 2 Fold Change
48 Hours | Chr15-Chr22
Up (in LADs): 9
Down (in LADs): 5
Up (out LADs): 56
Down (out LADs): 38
-Log 10 Padj
Log 2 Fold Change
B
Interchromosomal Contact Density In LADs
Counts
Untreated: chr15-chr22
24hrAuxin: chr15-chr22
Untreated: chr8-chr7
24hrAuxin: chr8-chr7
untreated: chr6-chr15
24hrAuxin: chr6-chr15
Untreated: chr5-chr14
24hrAuxin: chr5-chr14
Untreated: chr2-chr19
24hrAuxin: chr2-chr19
Untreated: chr1-chr18
24hrAuxin: chr1-chr18
Interchromosomal Contact Density Outside LADs
Counts
C
Gini Coefficient
All Genes
Genes in LADs
Genes outside of LADs
0 hr
12 hr
48 hr
6 day withdraw

Fig. S9

Chromosomes with decreased interaction frequencies had more DEGs outside LADs.

A. Volcano plots showing DEGs specific to chromosome pairs with decreased interaction frequency within and outside of LADs 48 hours of auxin treatment (adjusted P value < 0.01 and absolute log fold change > 1). “Up” refers to upregulated genes and “Down” refers to downregulated genes.
B. Raindrop plot comparing interchromosomal contact frequencies for the control and 24-hour treatment conditions. Error bars: SEM. Data was pooled from two independent biological replicates (N = 2). Significance was calculated by unpaired t test (**< 0.01).
C. Dot plot showing the Gini coefficient as a measure of transcriptional divergence for all three conditions.