Supplemental Materials

Molecular Biology of the Cell

Wang et al.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1: Cell geometry altered nuclear morphology and chromatin compaction. (A) Low magnification images of fibronectin patterns (yellow). Scale bar: 20 μm. (B) Representative confocal images of NIH 3T3 cells labeled with phalloidin (red) and Hoechst (blue) cultured on these patterns. Scale bar: 20 μm. Images below are Imaris generated surface plot of nucleus in AP and IP substrates. (C) Dot plot quantifying the nuclear maximum projected area, nuclear height, and nuclear volume normalized to the AP nuclear volume. Data is presented as mean ± SD with $20 < n < 30$. *** $P < 0.001$. Two sample student's t test. (D) Representative color map showing the compaction of chromatin (stained with Hoechst) in nucleus of cells cultured on AP and IP substrates. Dot plot showing the normalized Hoechst mean intensity in the nucleus on AP and IP substrates. Data is presented as mean ± SD with $16 < n < 20$. * $P < 0.05$. Two sample student's t test. (E) Representative confocal (up) and thresholded (below) images showing Chr2 (red), and Chr6 (green) in nuclei (blue) of cells on AP and IP substrates. Scale bar: 5 μm. Experiments were performed in triplicates.

Figure S2: Effects of cell geometry on the radial distance distribution and its correlation with CT length and gene density. Radial distance difference matrix shows the difference of the radial distance between two heterologous chromosomes in AP and IP substrates. Row and column labels are the chromosome numbers painted in this study. Experiments were performed in triplicates.

Figure S3: Thresholded and raw Pol II S5P images. (A) Representative images of Chr1, 2, 3, 11, and 5 (green) with Pol II S5P (purple) in AP and IP substrates. (B) Representative raw immunofluorescence images of DNA (green) and Pol II S5P (red). Scale bar: 5 μm.

Figure S4: Activated form of RNA Pol II was revealed as pocket like structures at the surface of CTs. (A) Chromosome paint combined with immunofluorescent images of Chr2 (green), and 5S RNA pol2 (pink) with the nuclear outline (white) in xy plane. Scale bar: 5 μm. The right images are the orthogonal views of the region cropped by the orange box. (B) Illustration of the 3D erosion quantification for the distribution of 5S RNA pol2 pockets (pink) on CTs (green). (C) Bar graphs showing the distribution of 5S RNA pol2 pockets on all the painted CTs in shell 1, 2, and 3. Data is given as mean ± SE with $60<n<100$. *$P < 0.05$; **$P<0.01$; ***$P < 0.001$ Two sample student's t test. Experiments were performed in triplicates.

Figure S5: Thresholded images of all the CT pairs painted in this work, and the intermingling degree quantification of all the CT pairs. (A) Thresholded images of all the CT pairs painted in this study in AP and IP substrates. (B) Bar graph quantifying the intermingling degree of all the CT pairs in AP and IP substrates. Data is presented as mean ± SE with $20 < n < 30$. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. Mann-Whitney U test. (C) Intermingling difference matrix shows the difference of the intermingling degree between two CT pairs in AP and IP substrates. Row and column labels are the CT pairs painted in this study. Experiments were performed in triplicates.

Figure S6: Cell geometry regulates intermingling degrees. (A) Representative images of Chr2-6, Chr5-9, Chr11-15, and Chr2-10 in AP and IP substrates. (B) Representative images showing the intermingling between Chr5 and Chr9. (C) Bar graph quantifying the intermingling degree between Chr5 and Chr9. Data is presented as mean ± SE with $40<n<50$. N.S indicates not significant. Mann-Whitney U test. Experiments were performed in triplicates.

Figure S7: Transcription is required for the increase in intermingling degrees, but not for the interior localization and reorientation of chromosomes. (A) Representative images of nucleus stained by Hoechst, Chr11 (purple), Chr15 (green) Right: zoomed in image of the cropped regions by light blue and orange boxes. (B) Bar graph showing the intermingling degree of Chr11 and Chr15. Data is presented as mean ± SE with $20<n<30$. *** $P<0.001$. N.S denotes not significant. Mann-Whitney U test. (C,F) Bar graph showing the normalized radial distance of Chr11 and Chr15. Data is presented as mean ± SE with $20<n<30$. * $P<0.05$. n.s denotes not significant. Mann-Whitney U test. (D, E, G, H) Bar graph showing the orientation of Chr11 and Chr15. Data is presented as mean ± SE with $50<n<100$. *** $P<0.001$; ** $P<0.01$; * $P<0.05$. n.s denotes not significant. Two sample student's t test. Experiments were performed in triplicates.

Figure S8: Compartmentalization of transcription factors affects chromosome intermingling. (A) (B) Representative images of nucleus stained by Hoechst and MRTF-A (red). Scale bar: 5 μm. (C) (D) Representative images of nucleus stained by Hoechst and p65 (red). Scale bar: 5 μm. (E) Bar graph showing the nuclear to cytoplasmic (N/C) ratio of MRTF-A and p65. Data is presented as mean ± SE with 40<n<50. *** P<0.001. Two sample student's t test. Experiments were performed in triplicates.

Figure S9: Mapping the CT orientations from top and side views.
(A) Orientation map for all the painted chromosomes in AP and IP nucleus in XY plane. Each double-arrow line represents the major axis of one chromosome. γ_{xy} was measured as the angle between the major axis of chromosome and the major axis of nucleus. (B) The distribution of γ_{xy} for all the painted chromosomes in AP and IP nucleus in XY plane. Inset: bar graph quantifying the γ_{xy} in AP and IP substrates. Data is presented as mean ± SD with 1000 < n < 1500. *** P < 0.001. Mann-Whitney U test. (C) Orientation map for all the painted chromosomes in AP and IP nucleus in XZ plane. Each double-arrow line represents the major axis of one chromosome. γ_{xz} was measured as the angle between the major axis of chromosome and the major axis of nucleus. (D) The distribution of γ_{xz} for all the painted chromosomes in AP and IP nucleus in XZ plane. Inset: bar graph quantifying the γ_{xz} in AP and IP substrates. Data is presented as mean ± SD with 1000 < n < 1500. *** P < 0.001. Mann-Whitney U test. (E) Side view orientation mapping for Chr5-9, Chr2-10, and Chr15-11 in AP and IP substrates. Experiments were performed in triplicates.

Figure S10: Illustration of the z score calculation for chromosome activity. Scatter plot of the transcription activity of 19 chromosomes in AP substrates. The black solid line indicates the average activity of the 19 chromosomes, and the distance between the solid line and the dashed lines indicates one standard deviation (σ). The z score is defined by the difference between the activity of each chromosome and the average activity, divided by one standard deviation (σ), as seen from the equation.

Figure S11: Coupling between chromosome reorganization and transcriptome change. (A) Heatmap of chromosome activity in anisotropic (AP) and isotropic (IP) substrates. (B) Scatter plot of radial distance change and chromosome activity change between AP and IP substrates. (C)Randomized heatmap of chromosome activity. (D) Scatter plot of radial distance change randomized chromosome activity change. (E) Heatmap of interchromosome activity distance in anisotropic (AP) and isotropic (IP) substrates. (F) Scatter plot between intermingling change and interchromosome activity distance change between AP and IP substrates. (G)Randomized heatmap of interchromosome activity distance. (H) Scatter plot between intermingling change and randomized interchromosome activity distance change. Experiments were performed in triplicates.

Figure S12: NF-κB target genes and SRF/ MRTF-A target genes. (A) (C) NF-κB target genes and SRF/ MRTF-A target genes across the genome above the foldchange of 1.2 between AP and IP substrates. Values are log2 foldchange of expression of genes in IP substrates divided by that in AP substrates. Positive values (light color) indicate upregulation in IP substrates and negative values (dark color) indicate downregulation, compared to AP substrates. (B) (D) NF-κB target genes and SRF/ MRTF-A target genes on the painted chromosome pairs. Experiments were performed in triplicates.

Figure S13: Correlations between chromosome position and compaction. (A) Scatter plot between the fold change of chromosome volume and the fold change of normalized radial distance (NRD) in IP substrates vs AP substrates. (B) The scatter plot between chromosome intermingling and chromosome decompaction factor. Inset: bar graph showing the Pearson's correlation coefficient of this correlation in AP and IP substrates. Values on top of the bar graphs are p values of the correlations. (C) (D) Scatter plots between normalized radial distance and chromosome decompaction factor in AP (C) and IP (D) substrates.

Figure S14: Metaphase Spread of NIH3T3 cells. (A) Metaphase Spread showing the chromosomes of NIH3T3 cells. Average chromosome copy number was 64 with a standard deviation of +/- 5 chromosomes within the population.

Figure S15: DNA intensity distribution. Widefield images of nuclei in AP cells (A) and CI cells (B). (C) A histogram showing the frequency distribution of the Nuclear (DNA) Intensity for AP and CI cells.
N= 101 (AP) and 97 (CI)

Figure S16: Effect of Fixation on 3D chromatin structure. (A & B) Images of nuclei before and after fixation. The merged image represents the overlap. In order to quantify this, we have calculated the correlation coefficient (P) of the entire nucleus in 3D before and after fixation. The correlation coefficients for 5 nuclei are represented in the boxplot (C).

Supplementary Movie. Demonstrates the algorithm in 2D for packing 10 ellipses into an enclosing ellipse that deforms stepwise into an enclosing circle; the packing of the inscribed ellipses is updated at each step so as to minimize the pairwise overlap.

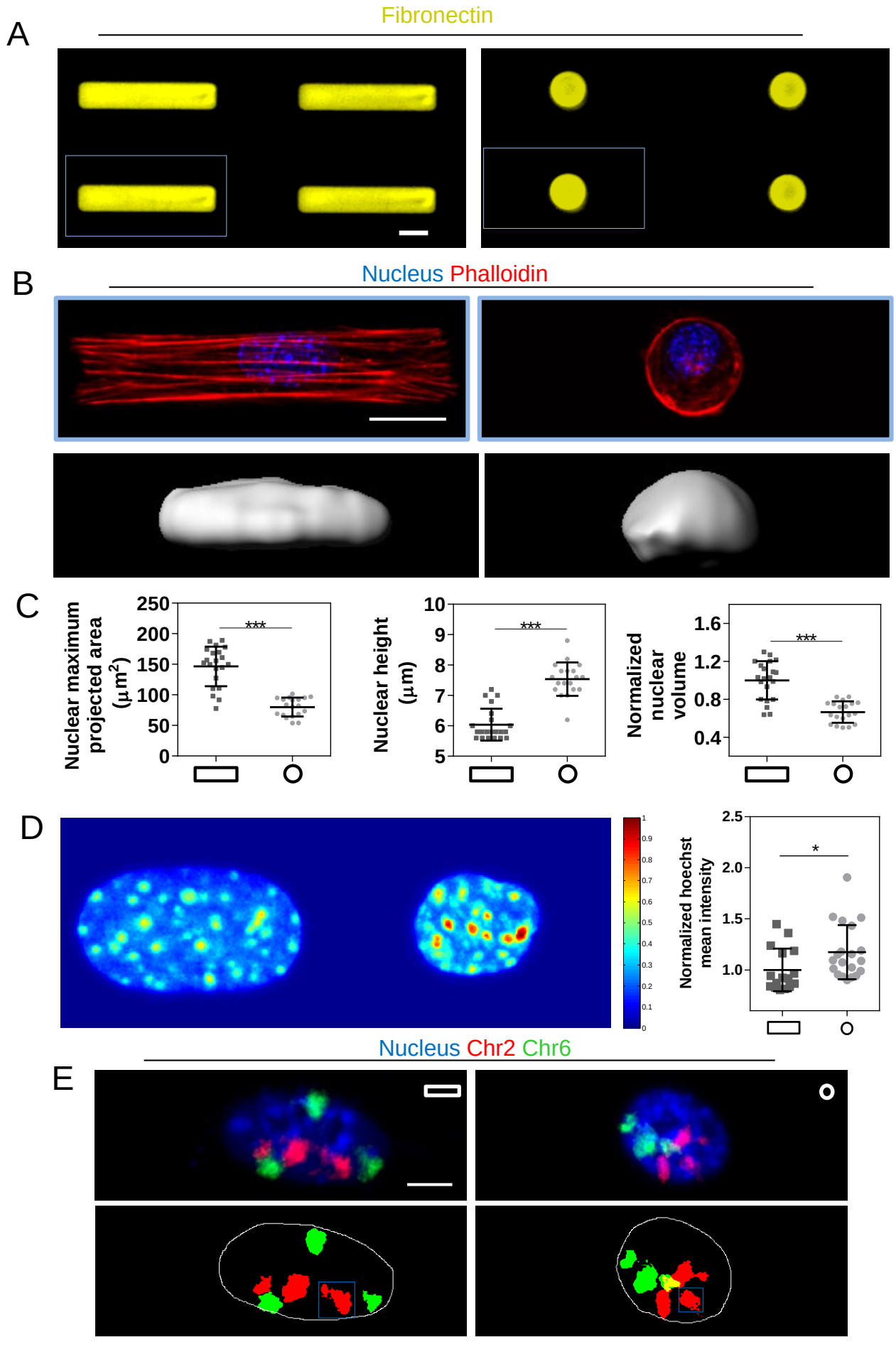

A
Fibronectin
B
Nucleus Phalloidin
C
Nuclear maximum projected area (μm²)
250
200
150
100
50
0
***
Nuclear height (μm)
10
9
8
7
6
5
***
Normalized nuclear volume
1.6
1.2
0.8
0.4
***
D
Normalized hoechst mean intensity
2.5
2.0
1.5
1.0
*
Nucleus Chr2 Chr6
E

Figure S1

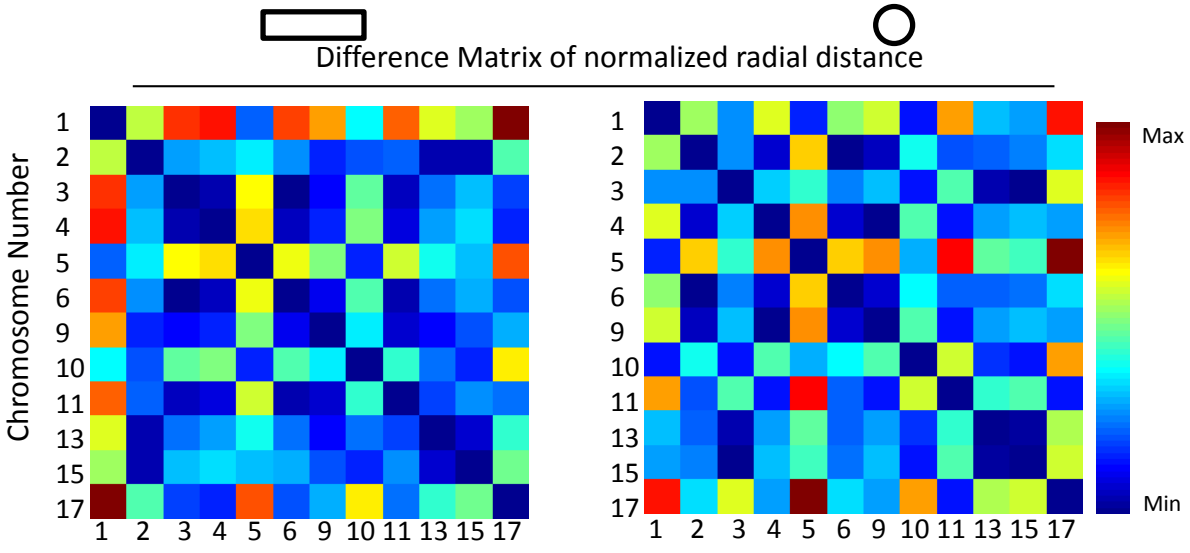
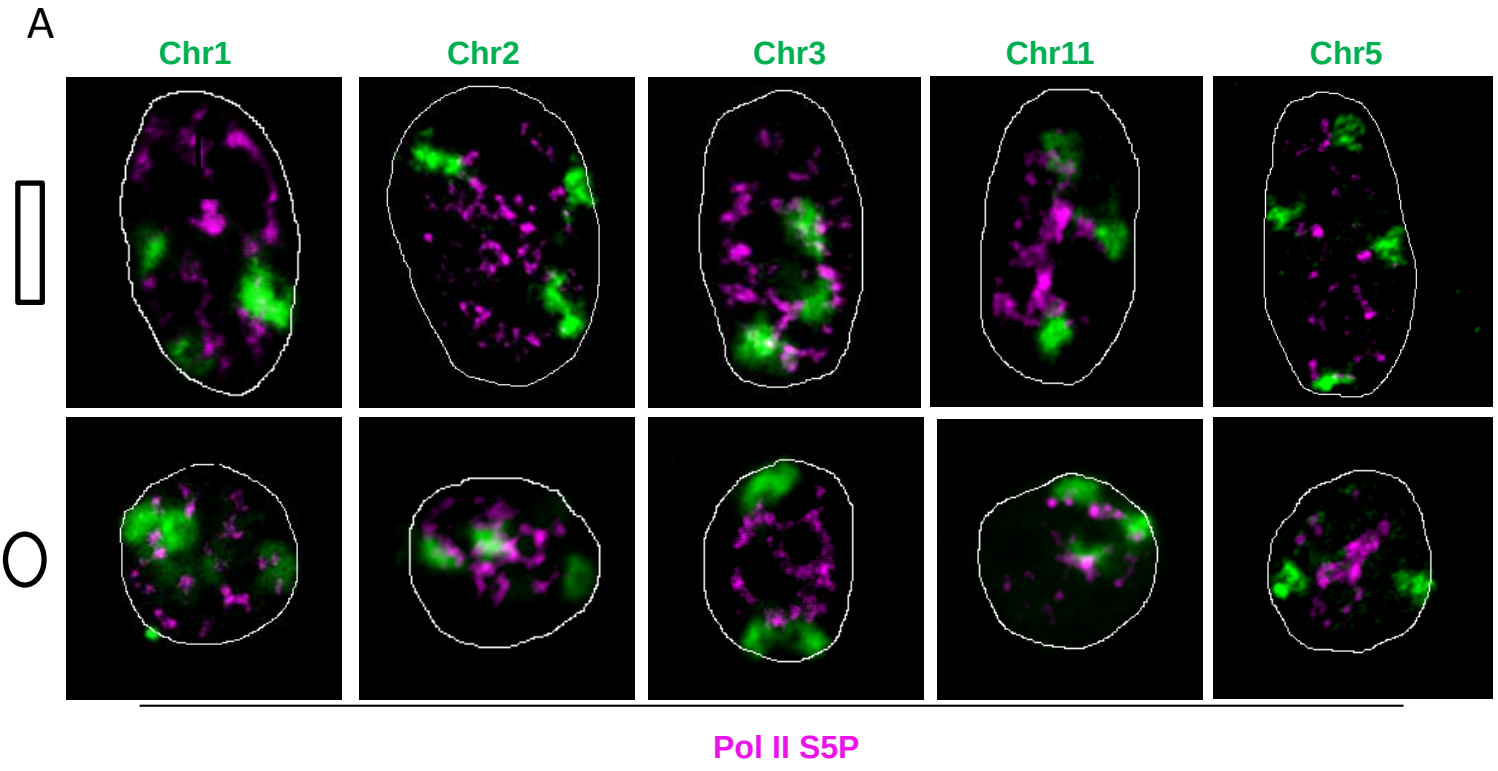

Difference Matrix of normalized radial distance
Chromosome Number
1
2
3
4
5
6
9
10
11
13
15
17
Max
Min

Figure S2



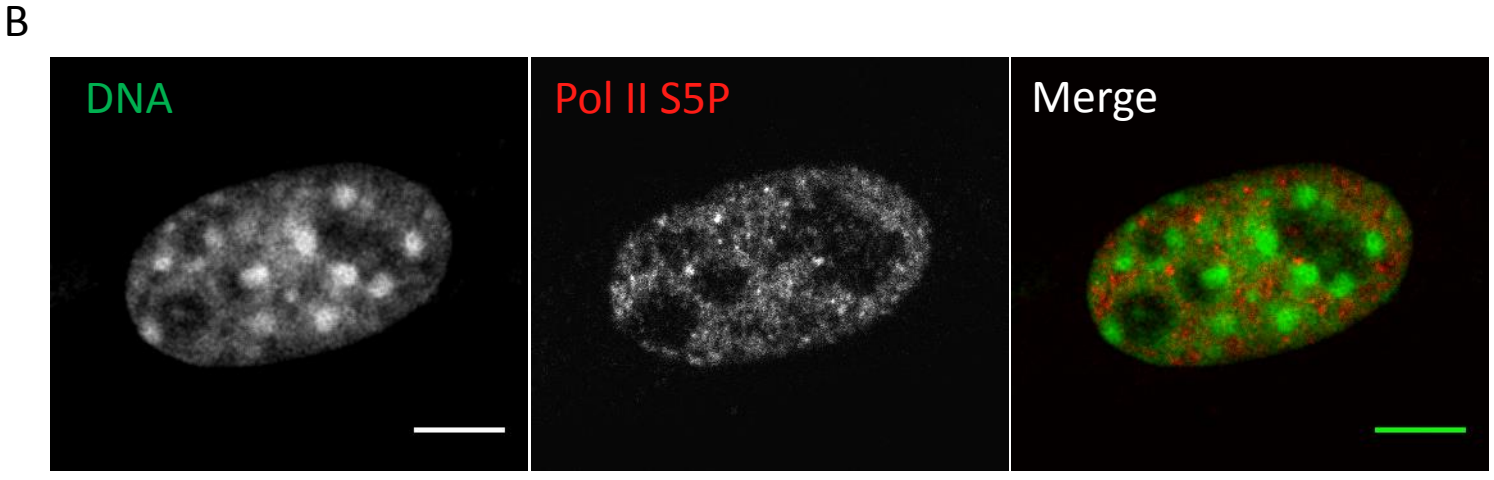


Figure S3

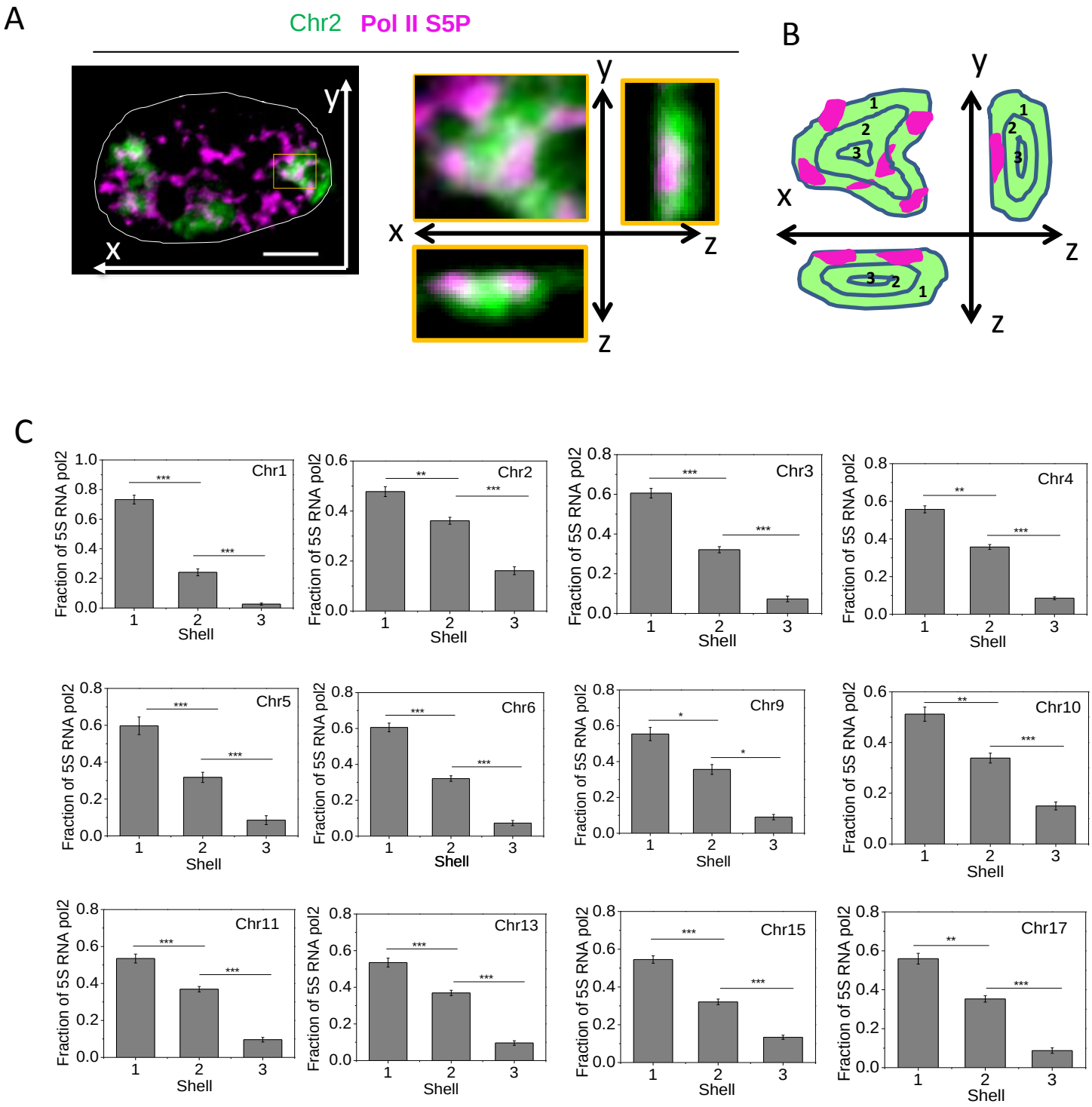

A
Chr2
Pol II S5P
y
x
z
B
C
Fraction of 5S RNA pol2
Shell
Chr1
Chr2
Chr3
Chr4
Chr5
Chr6
Chr9
Chr10
Chr11
Chr13
Chr15
Chr17

Figure S4

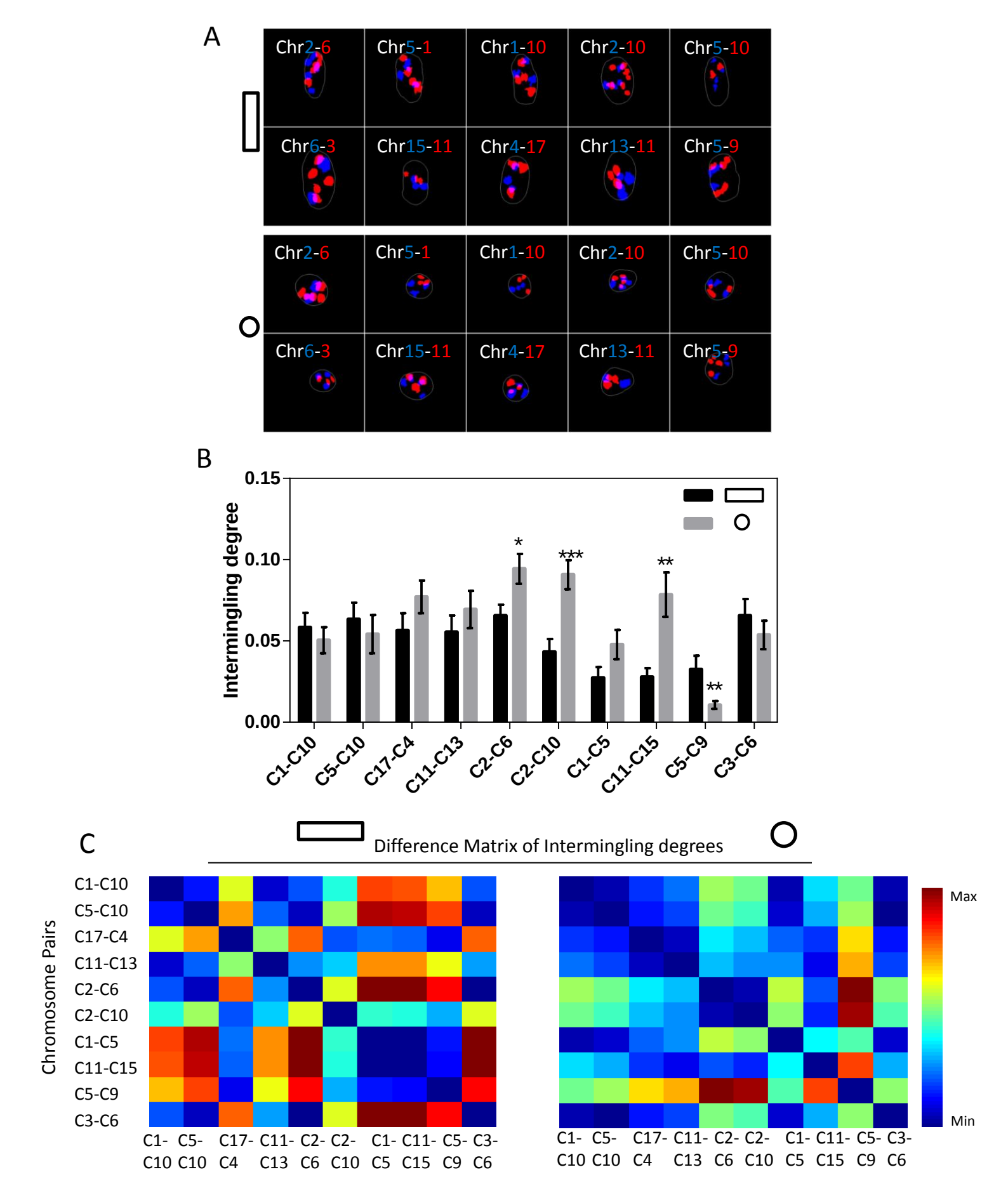

A
Chr2-6
Chr5-1
Chr1-10
Chr2-10
Chr5-10
Chr6-3
Chr15-11
Chr4-17
Chr13-11
Chr5-9
B
Intermingling degree
0.15
0.10
0.05
0.00
*
***
**
**
C1-C10
C5-C10
C17-C4
C11-C13
C2-C6
C2-C10
C1-C5
C11-C15
C5-C9
C3-C6
C
Difference Matrix of Intermingling degrees
Chromosome Pairs
Max
Min

Figure S5

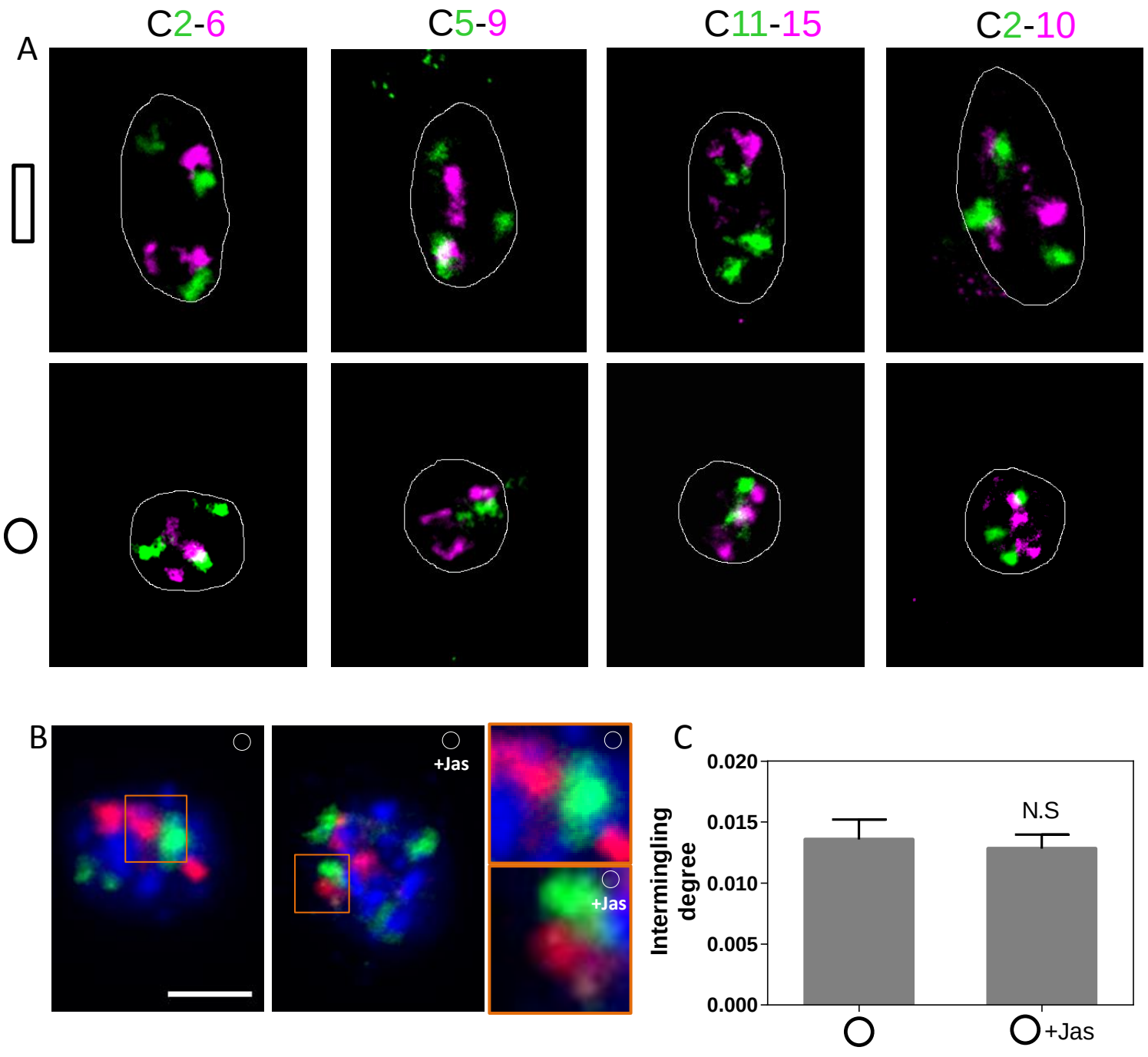

C2-6
C5-9
C11-15
C2-10
A
B
+Jas
+Jas
C
Intermingling degree
0.020
0.015
0.010
0.005
0.000
N.S
+Jas

Figure S6

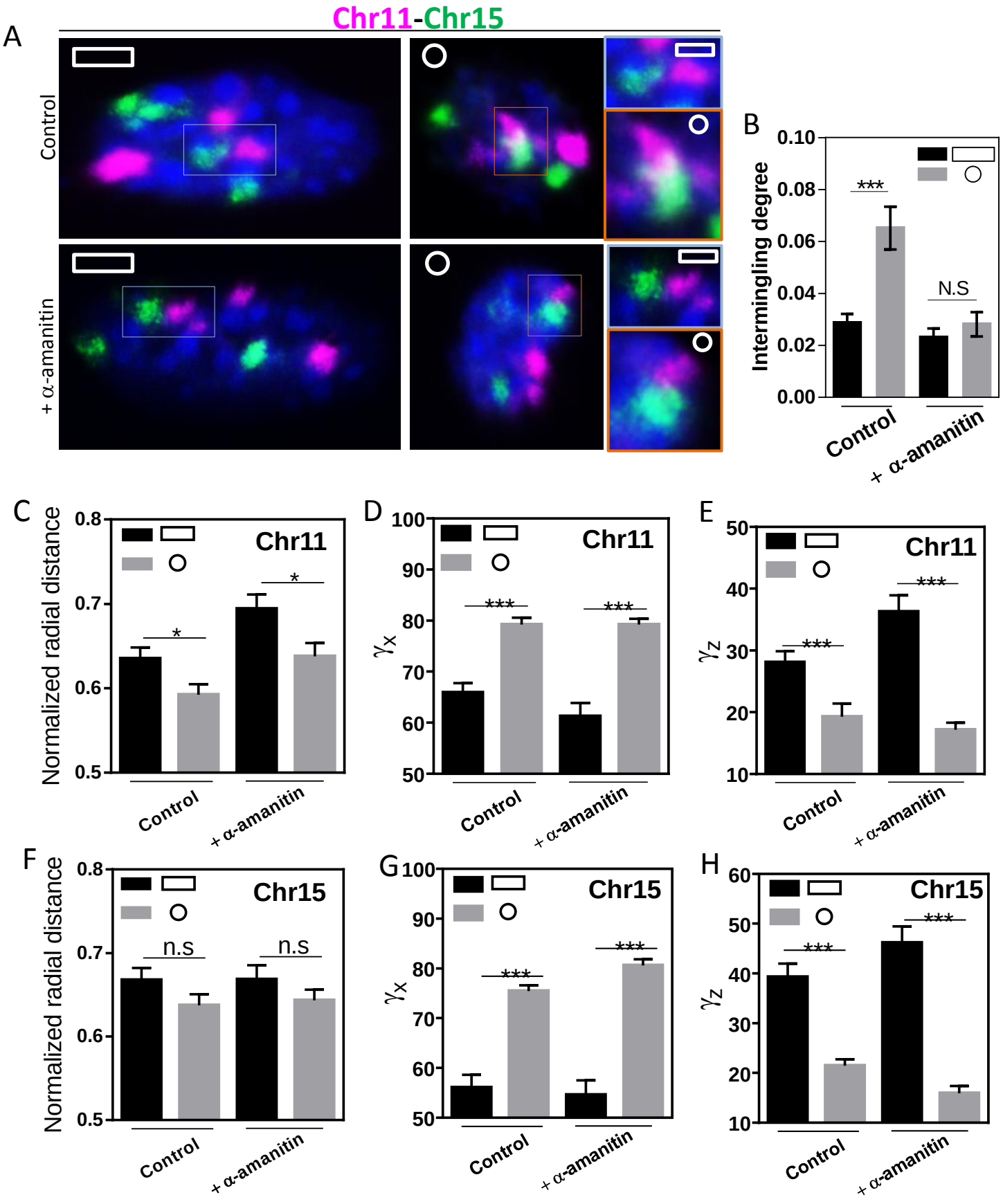

A
Chr11-Chr15
Control
+ α-amanitin
B
Intermingling degree
0.10
0.08
0.06
0.04
0.02
0.00
***
N.S
Control
+ α-amanitin
C
Normalized radial distance
0.8
0.7
0.6
0.5
Chr11
*
*
Control
+ α-amanitin
D
100
90
80
70
60
50
γx
Chr11
***
***
Control
+ α-amanitin
E
50
40
30
20
10
γz
Chr11
***
***
Control
+ α-amanitin
F
Normalized radial distance
0.8
0.7
0.6
0.5
Chr15
n.s
n.s
Control
+ α-amanitin
G
100
90
80
70
60
50
γx
Chr15
***
***
Control
+ α-amanitin
H
60
50
40
30
20
10
γz
Chr15
***
***
Control
+ α-amanitin

Figure S7

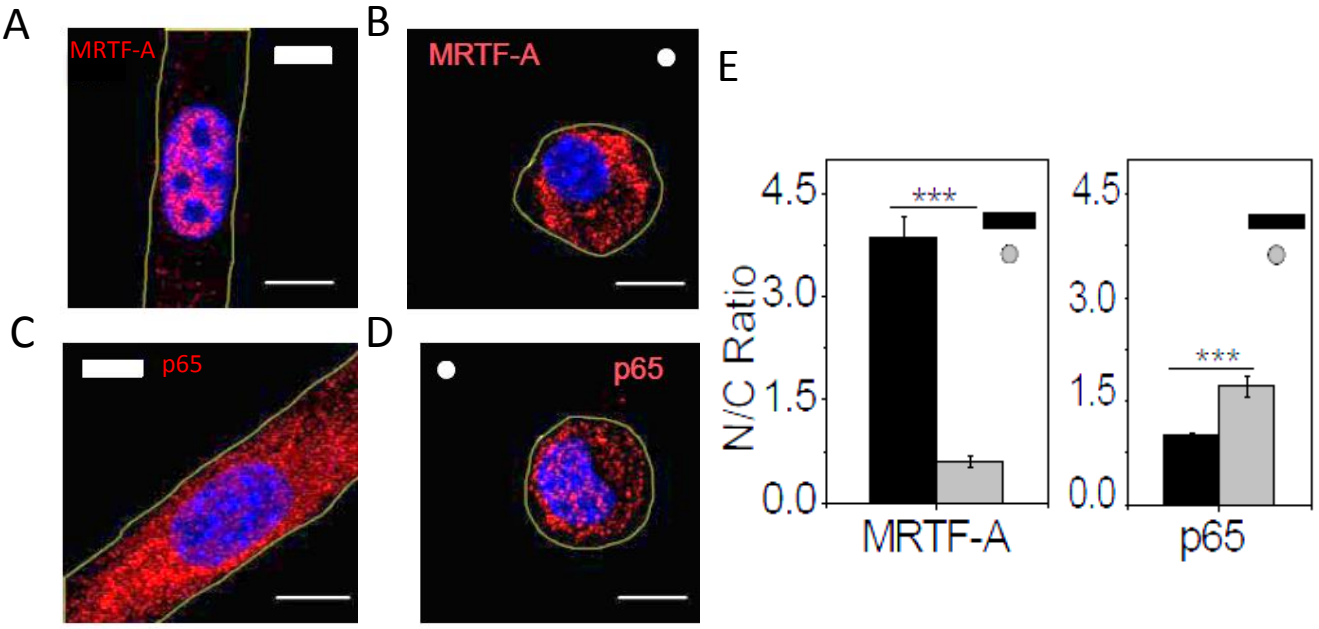
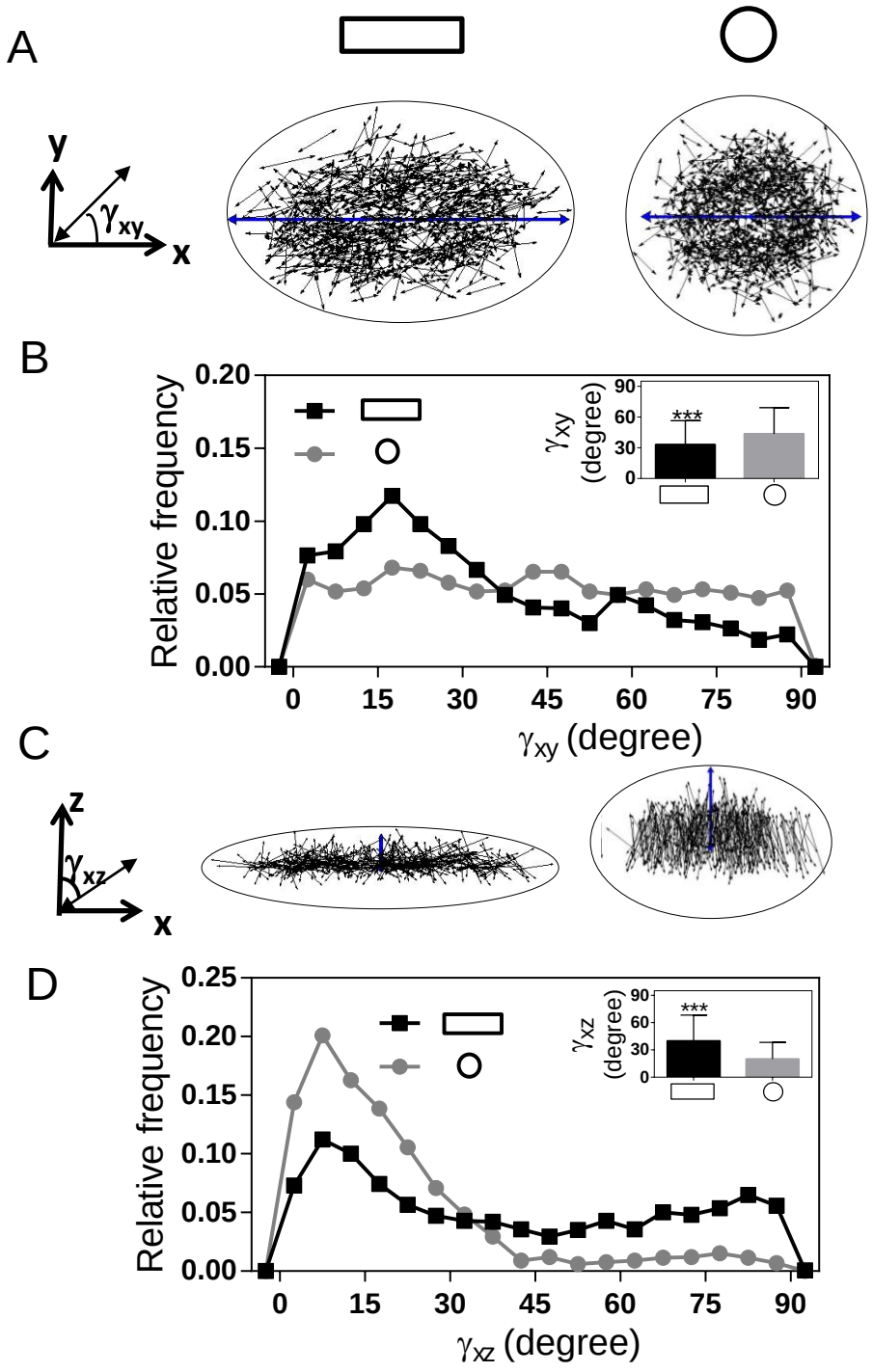

A
MRTF-A
B
MRTF-A
C
p65
D
p65
E
N/C Ratio
4.5
3.0
1.5
0.0
***
MRTF-A
4.5
3.0
1.5
0.0
***
p65

Figure S8


A
y
x
γ_{xy}
B
Relative frequency
0.20
0.15
0.10
0.05
0.00
0
15
30
45
60
75
90
γ_{xy} (degree)
***
γ_{xy} (degree)
C
z
x
γ_{xz}
D
Relative frequency
0.25
0.20
0.15
0.10
0.05
0.00
γ_{xz} (degree)
***
γ_{xz} (degree)

E

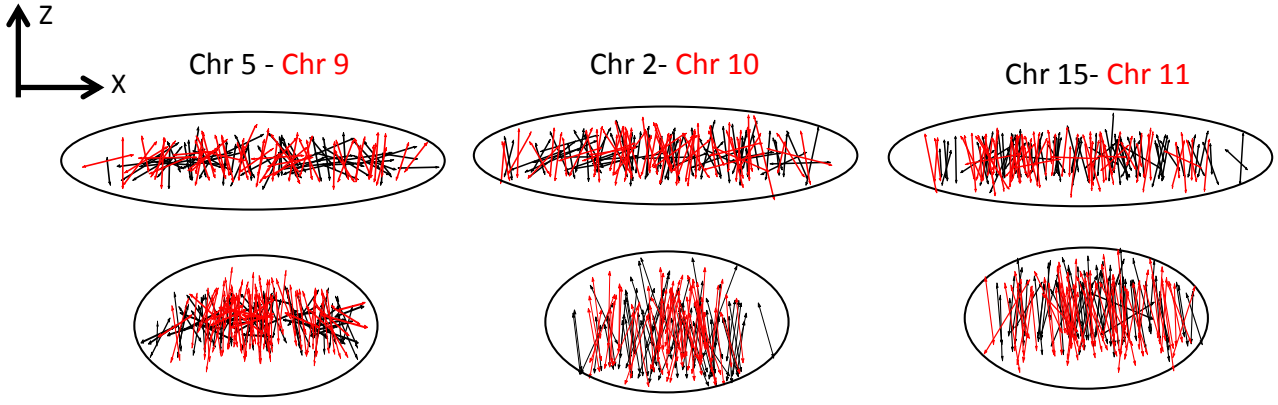
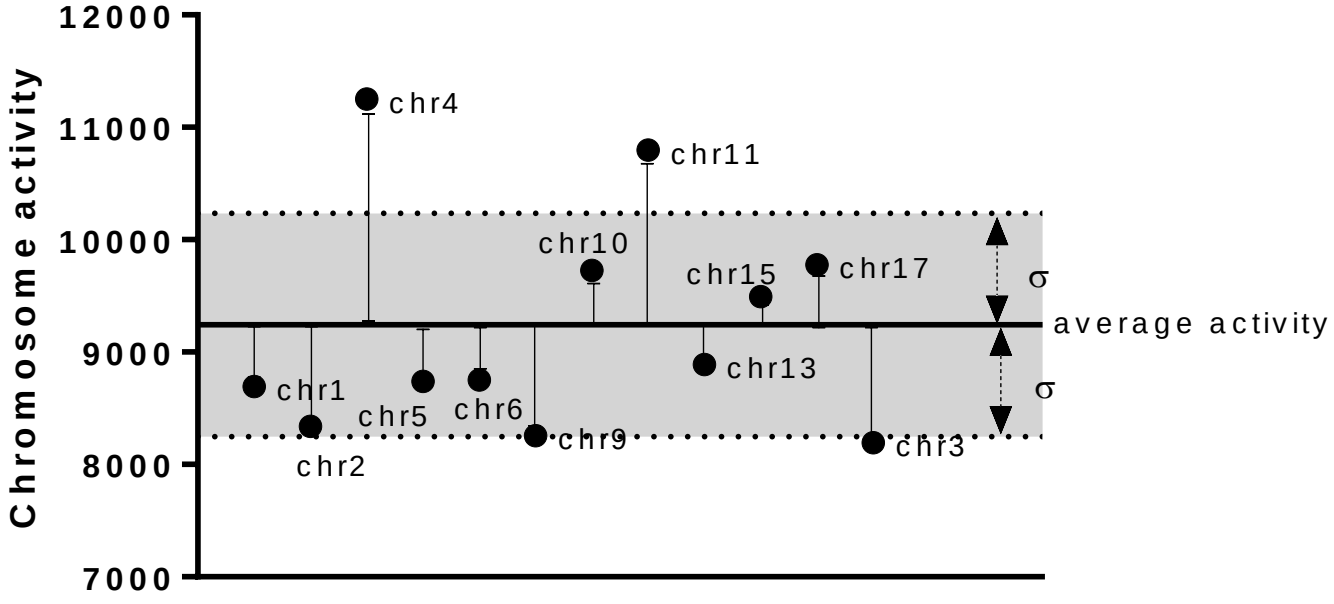

z
x
Chr 5 - Chr 9
Chr 2- Chr 10
Chr 15- Chr 11

Figure S9



$$z = \frac{chromosome\ activity\ - average\ activity}{standard\ deviation\ (\sigma)}$$

Figure S10

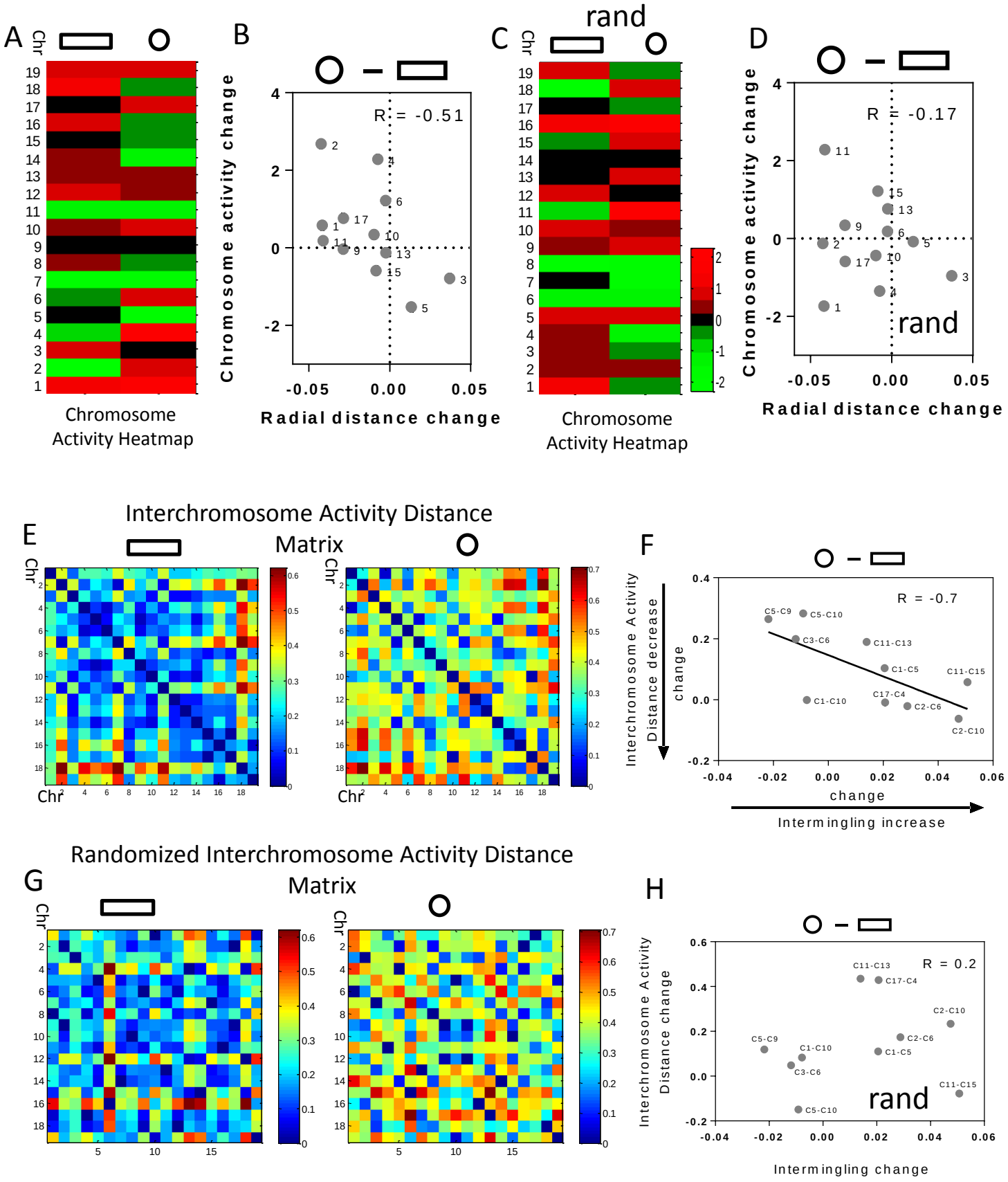

A
Chr
Chromosome
Activity Heatmap
B
Chromosome activity change
R = -0.51
Radial distance change
C
rand
Chr
Chromosome
Activity Heatmap
D
Chromosome activity change
R = -0.17
rand
Radial distance change
Interchromosome Activity Distance
Matrix
E
Chr
F
Interchromosome Activity
Distance decrease
change
R = -0.7
C5-C9
C5-C10
C3-C6
C11-C13
C1-C5
C11-C15
C1-C10
C17-C4
C2-C6
C2-C10
change
Intermingling increase
Randomized Interchromosome Activity Distance
Matrix
G
Chr
H
Interchromosome Activity
Distance change
R = 0.2
C11-C13
C17-C4
C2-C10
C5-C9
C1-C10
C2-C6
C1-C5
C3-C6
C11-C15
C5-C10
rand
Intermingling change

Figure S11

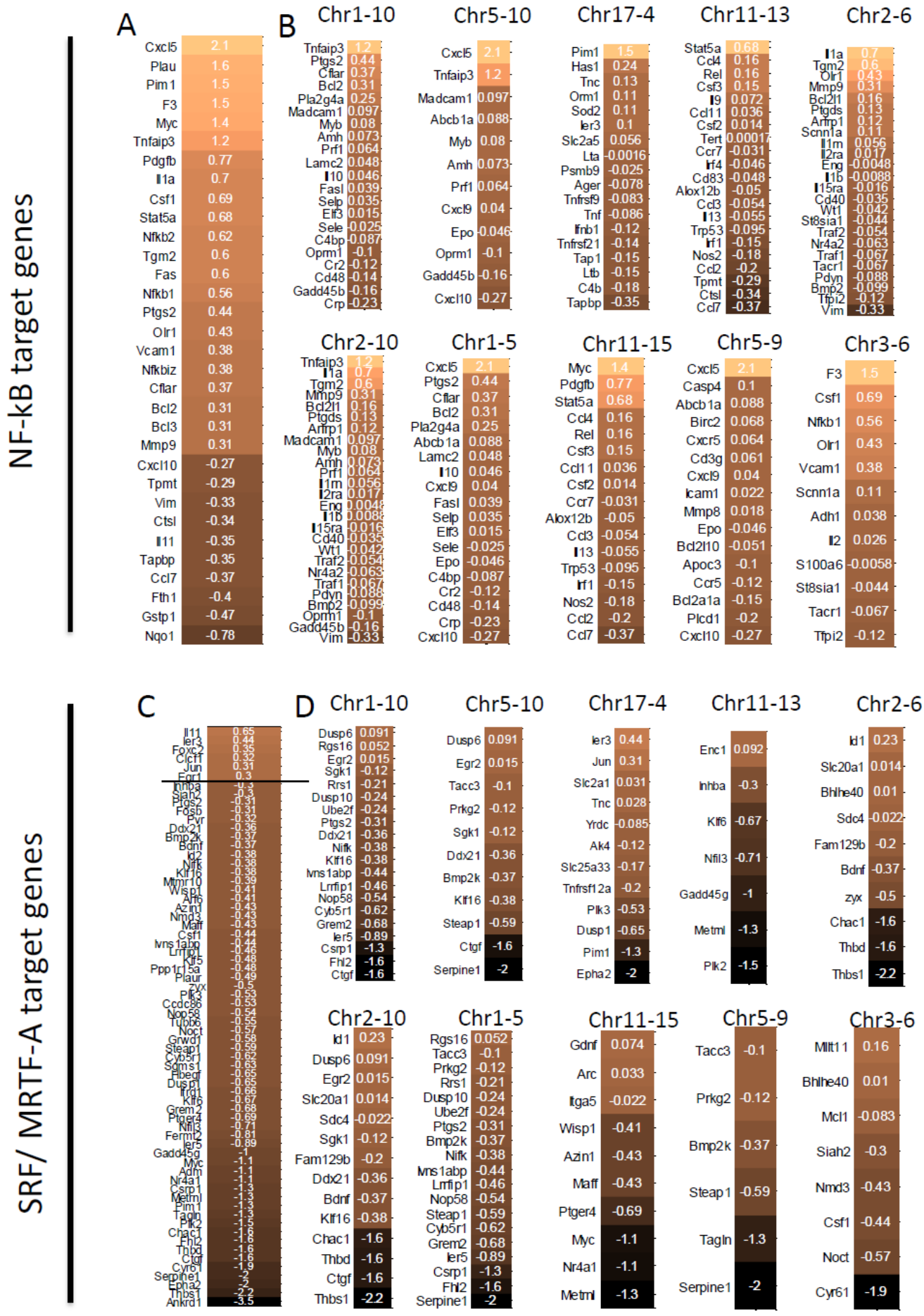

NF-kB target genes
A
Cxcl5 2.1
Plau 1.6
Pim1 1.5
F3 1.5
Myc 1.4
Tnfaip3 1.2
Pdgfb 0.77
Il1a 0.7
Csf1 0.69
Stat5a 0.68
Nfkb2 0.62
Tgm2 0.6
Fas 0.6
Nfkb1 0.56
Ptgs2 0.44
Olr1 0.43
Vcam1 0.38
Nfkbiz 0.38
Cflar 0.37
Bcl2 0.31
Bcl3 0.31
Mmp9 0.31
Cxcl10 -0.27
Tpmt -0.29
Vim -0.33
Ctsl -0.34
Il11 -0.35
Tapbp -0.35
Ccl7 -0.37
Fth1 -0.4
Gstp1 -0.47
Nqo1 -0.78
B
Chr1-10
Tnfaip3 1.2
Ptgs2 0.44
Cflar 0.37
Bcl2 0.31
Pla2g4a 0.25
Madcam1 0.097
Myb 0.08
Amh 0.073
Prf1 0.064
Lamc2 0.048
Il10 0.046
Fasl 0.039
Selp 0.035
Elf3 0.015
Sele -0.025
C4bp -0.087
Oprm1 -0.1
Cr2 -0.12
Cd48 -0.14
Gadd45b -0.16
Crp -0.23
Chr5-10
Cxcl5 2.1
Tnfaip3 1.2
Madcam1 0.097
Abcb1a 0.088
Myb 0.08
Amh 0.073
Prf1 0.064
Cxcl9 0.04
Epo -0.046
Oprm1 -0.1
Gadd45b -0.16
Cxcl10 -0.27
Chr17-4
Pim1 1.5
Has1 0.24
Tnc 0.13
Orm1 0.11
Sod2 0.11
Ier3 0.1
Slc2a5 0.056
Lta -0.0016
Psmb9 -0.025
Ager -0.078
Tnfrsf9 -0.083
Tnf -0.086
Ifnb1 -0.12
Tnfrsf21 -0.14
Tap1 -0.15
Ltb -0.15
C4b -0.18
Tapbp -0.35
Chr11-13
Stat5a 0.68
Ccl4 0.16
Rel 0.16
Csf3 0.15
Il9 0.072
Ccl11 0.036
Csf2 0.014
Tert 0.00017
Ccr7 -0.031
Irf4 -0.046
Cd83 -0.048
Alox12b -0.05
Ccl3 -0.054
Il13 -0.055
Trp53 -0.095
Irf1 -0.15
Nos2 -0.18
Ccl2 -0.2
Tpmt -0.29
Ctsl -0.34
Ccl7 -0.37
Chr2-6
Il1a 0.7
Tgm2 0.6
Olr1 0.43
Mmp9 0.31
Bcl2l1 0.16
Ptgds 0.13
Arfrp1 0.12
Scnn1a 0.11
Il1rn 0.056
Il2ra 0.017
Eng -0.0048
Il1b -0.0088
Il15ra -0.016
Cd40 -0.035
Wt1 -0.042
St8sia1 -0.044
Traf2 -0.054
Nr4a2 -0.063
Traf1 -0.067
Tacr1 -0.067
Pdyn -0.088
Bmp2 -0.099
Tfpi2 -0.12
Vim -0.33
Chr2-10
Tnfaip3 1.2
Il1a 0.7
Tgm2 0.6
Mmp9 0.31
Bcl2l1 0.16
Ptgds 0.13
Arfrp1 0.12
Madcam1 0.097
Myb 0.08
Amh 0.073
Prf1 0.064
Il1rn 0.056
Il2ra 0.017
Eng 0.0048
Il1b 0.0088
Il15ra -0.016
Cd40 -0.035
Wt1 -0.042
Traf2 -0.054
Nr4a2 -0.063
Traf1 -0.067
Pdyn -0.088
Bmp2 -0.099
Oprm1 -0.1
Gadd45b -0.16
Vim -0.33
Chr1-5
Cxcl5 2.1
Ptgs2 0.44
Cflar 0.37
Bcl2 0.31
Pla2g4a 0.25
Abcb1a 0.088
Lamc2 0.048
Il10 0.046
Cxcl9 0.04
Fasl 0.039
Selp 0.035
Elf3 0.015
Sele -0.025
Epo -0.046
C4bp -0.087
Cr2 -0.12
Cd48 -0.14
Crp -0.23
Cxcl10 -0.27
Chr11-15
Myc 1.4
Pdgfb 0.77
Stat5a 0.68
Ccl4 0.16
Rel 0.16
Csf3 0.15
Ccl11 0.036
Csf2 0.014
Ccr7 -0.031
Alox12b -0.05
Ccl3 -0.054
Il13 -0.055
Trp53 -0.095
Irf1 -0.15
Nos2 -0.18
Ccl2 -0.2
Ccl7 -0.37
Chr5-9
Cxcl5 2.1
Casp4 0.1
Abcb1a 0.088
Birc2 0.068
Cxcr5 0.064
Cd3g 0.061
Cxcl9 0.04
Icam1 0.022
Mmp8 0.018
Epo -0.046
Bcl2l10 -0.051
Apoc3 -0.1
Ccr5 -0.12
Bcl2a1a -0.15
Plcd1 -0.2
Cxcl10 -0.27
Chr3-6
F3 1.5
Csf1 0.69
Nfkb1 0.56
Olr1 0.43
Vcam1 0.38
Scnn1a 0.11
Adh1 0.038
Il2 0.026
S100a6 -0.0058
St8sia1 -0.044
Tacr1 -0.067
Tfpi2 -0.12
SRF/ MRTF-A target genes
C
Il11 0.65
Ier3 0.44
Foxc2 0.35
Cfl1 0.32
Jun 0.31
Egr1 0.3
Inhba -0.3
Siah2 -0.3
Ptgs2 -0.31
Fos b -0.31
Pvr -0.32
Ddx21 -0.36
Bmp2k -0.37
Bdnf -0.37
Id2 -0.38
Nfk -0.38
Klf16 -0.38
Mtmr10 -0.39
Wisp1 -0.41
Arf6 -0.41
Azin1 -0.43
Nmd3 -0.43
Maff -0.43
Csf1 -0.44
Ivns1abp -0.44
Lrrfip1 -0.46
Klf5 -0.48
Ppp1r15a -0.48
Plaur -0.49
Zyx -0.5
Plk3 -0.53
Ccdc86 -0.53
Nop58 -0.54
Tubb6 -0.55
Noct -0.57
Grwd1 -0.58
Steap1 -0.59
Cyb5r1 -0.62
Sgms1 -0.65
Fibegf -0.65
Dusp1 -0.65
Irf1 -0.66
Klf6 -0.67
Grem2 -0.68
Ptger4 -0.69
Nfil3 -0.71
Fermt2 -0.81
Ier5 -0.89
Gadd45g -1
Myc -1.1
Adm -1.1
Nr4a1 -1.1
Csrp1 -1.3
Metrnl -1.3
Pim1 -1.3
Tagln -1.5
Plk2 -1.5
Chac1 -1.6
Fhl2 -1.6
Thbd -1.6
Ctgf -1.6
Cyr61 -1.9
Serpine1 -2
Epha2 -2
Thbs1 -2.2
Ankrd1 -3.5
D
Chr1-10
Dusp6 0.091
Rgs16 0.052
Egr2 0.015
Sgk1 -0.12
Rrs1 -0.21
Dusp10 -0.24
Ube2f -0.24
Ptgs2 -0.31
Ddx21 -0.36
Nifk -0.38
Klf16 -0.38
Ivns1abp -0.44
Lrrfip1 -0.46
Nop58 -0.54
Cyb5r1 -0.62
Grem2 -0.68
Ier5 -0.89
Csrp1 -1.3
Fhl2 -1.6
Ctgf -1.6
Chr5-10
Dusp6 0.091
Egr2 0.015
Tacc3 -0.1
Prkg2 -0.12
Sgk1 -0.12
Ddx21 -0.36
Bmp2k -0.37
Klf16 -0.38
Steap1 -0.59
Ctgf -1.6
Serpine1 -2
Chr17-4
Ier3 0.44
Jun 0.31
Slc2a1 0.031
Tnc 0.028
Yrdc -0.085
Ak4 -0.12
Slc25a33 -0.17
Tnfrsf12a -0.2
Plk3 -0.53
Dusp1 -0.65
Pim1 -1.3
Epha2 -2
Chr11-13
Enc1 0.092
Inhba -0.3
Klf6 -0.67
Nfil3 -0.71
Gadd45g -1
Metrnl -1.3
Plk2 -1.5
Chr2-6
Id1 0.23
Slc20a1 0.014
Bhlhe40 0.01
Sdc4 -0.022
Fam129b -0.2
Bdnf -0.37
zyx -0.5
Chac1 -1.6
Thbd -1.6
Thbs1 -2.2
Chr2-10
Id1 0.23
Dusp6 0.091
Egr2 0.015
Slc20a1 0.014
Sdc4 -0.022
Sgk1 -0.12
Fam129b -0.2
Ddx21 -0.36
Bdnf -0.37
Klf16 -0.38
Chac1 -1.6
Thbd -1.6
Ctgf -1.6
Thbs1 -2.2
Chr1-5
Rgs16 0.052
Tacc3 -0.1
Prkg2 -0.12
Rrs1 -0.21
Dusp10 -0.24
Ube2f -0.24
Ptgs2 -0.31
Bmp2k -0.37
Nifk -0.38
Ivns1abp -0.44
Lrrfip1 -0.46
Nop58 -0.54
Steap1 -0.59
Cyb5r1 -0.62
Grem2 -0.68
Ier5 -0.89
Csrp1 -1.3
Fhl2 -1.6
Serpine1 -2
Chr11-15
Gdnf 0.074
Arc 0.033
Itga5 -0.022
Wisp1 -0.41
Azin1 -0.43
Maff -0.43
Ptger4 -0.69
Myc -1.1
Nr4a1 -1.1
Metrnl -1.3
Chr5-9
Tacc3 -0.1
Prkg2 -0.12
Bmp2k -0.37
Steap1 -0.59
Tagln -1.3
Serpine1 -2
Chr3-6
Mllt11 0.16
Bhlhe40 0.01
Mcl1 -0.083
Siah2 -0.3
Nmd3 -0.43
Csf1 -0.44
Noct -0.57
Cyr61 -1.9

Figure S12

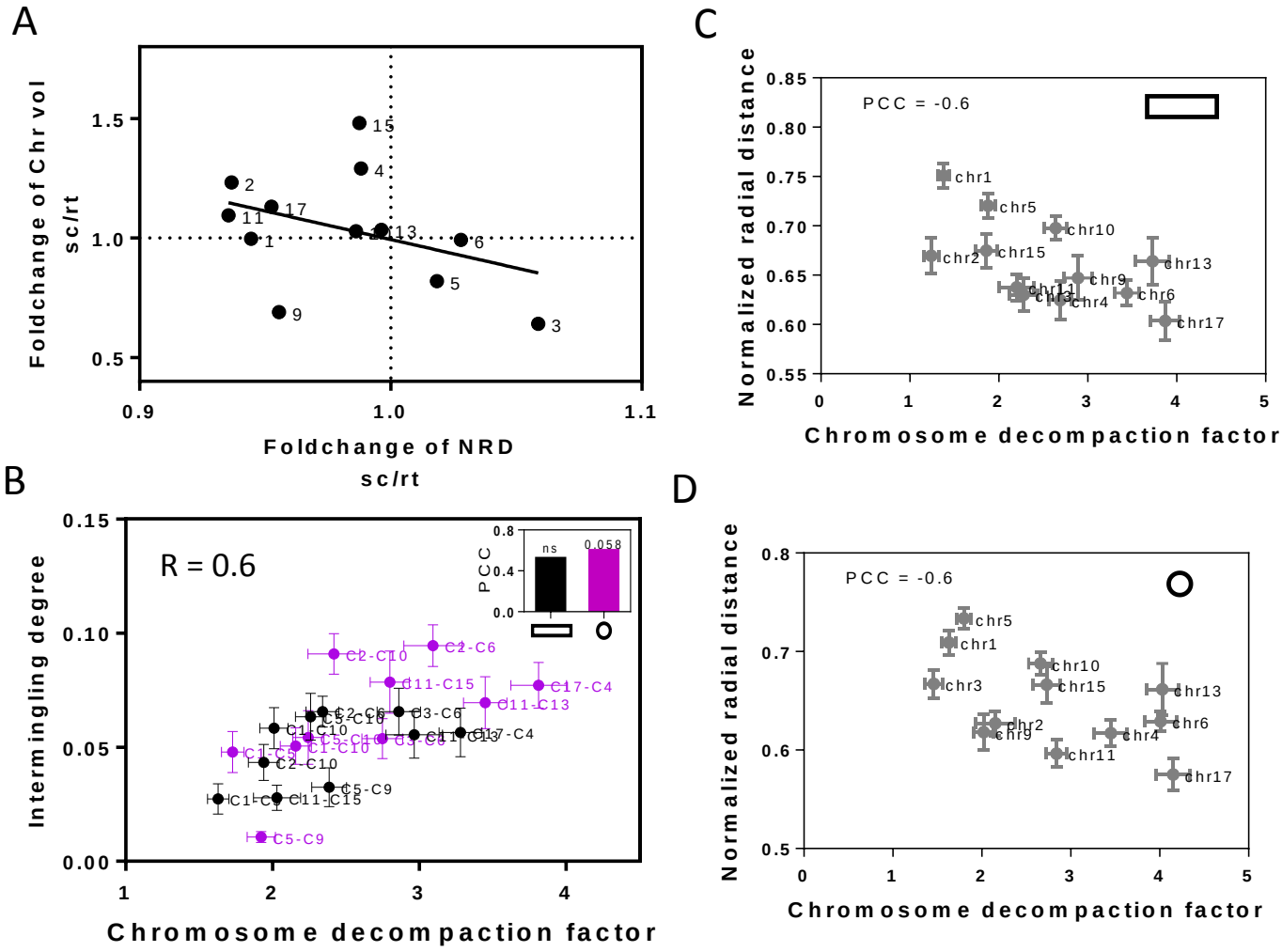

A
Foldchange of Chr vol sc/rt
Foldchange of NRD sc/rt
B
R = 0.6
Intermingling degree
Chromosome decompaction factor
PCC
ns
0.058
C
PCC = -0.6
Normalized radial distance
Chromosome decompaction factor
D
PCC = -0.6
Normalized radial distance
Chromosome decompaction factor

Figure S13

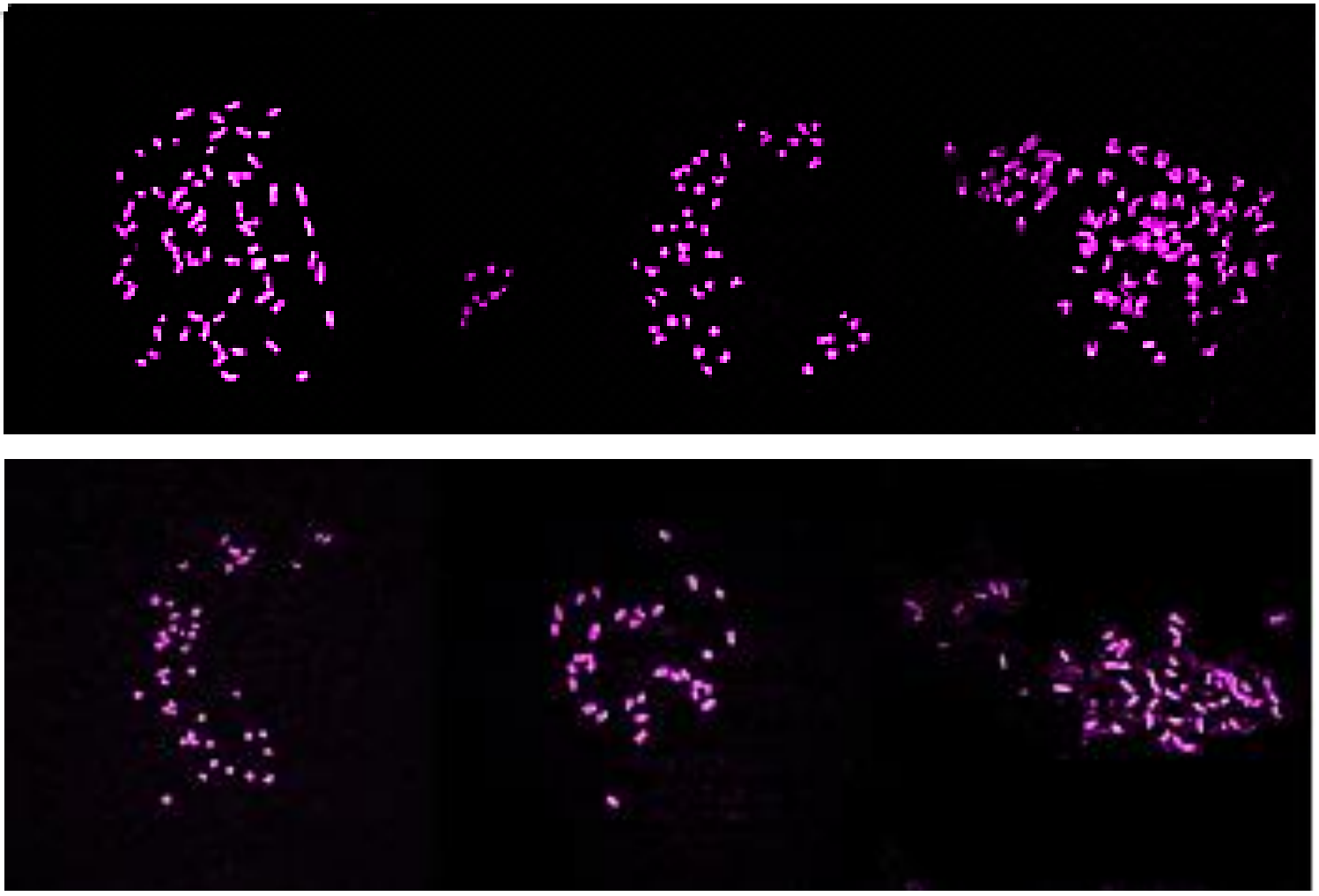
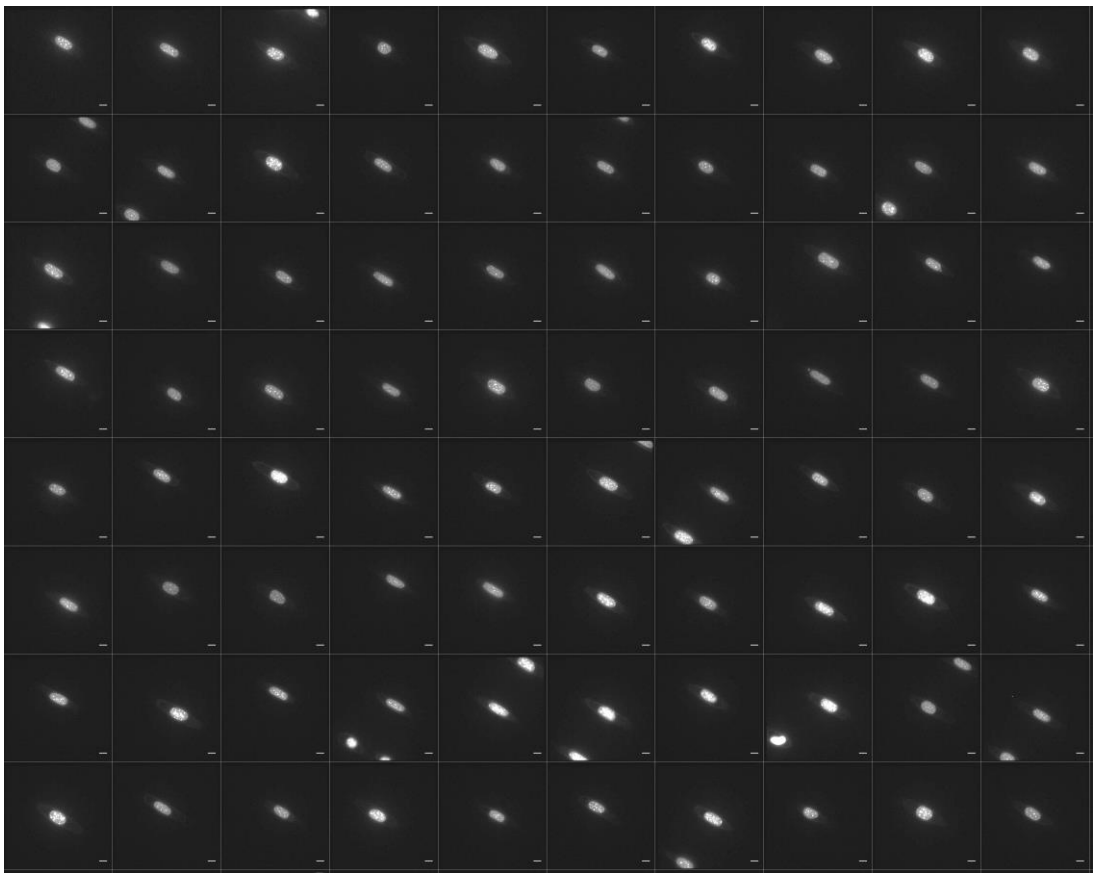
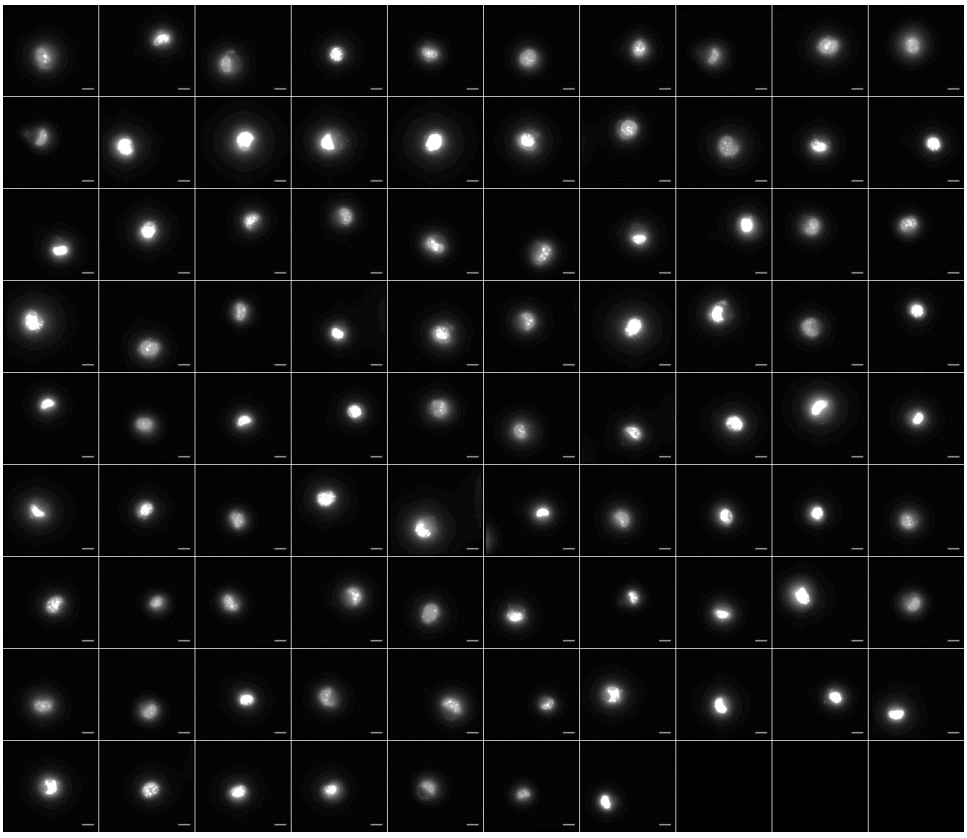


Figure S14

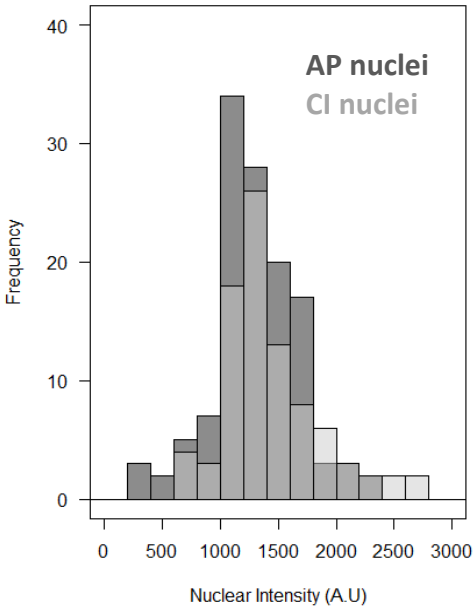
A



B



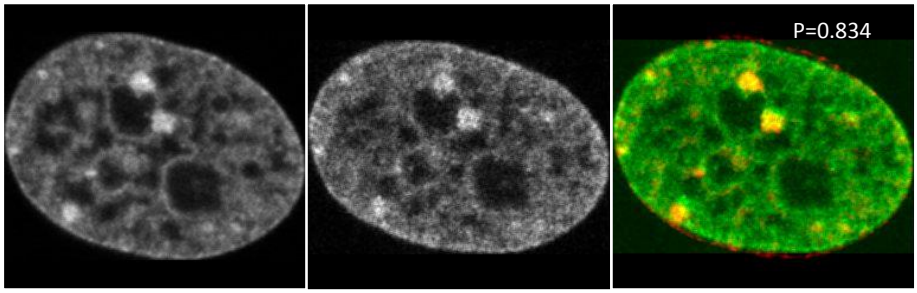
C



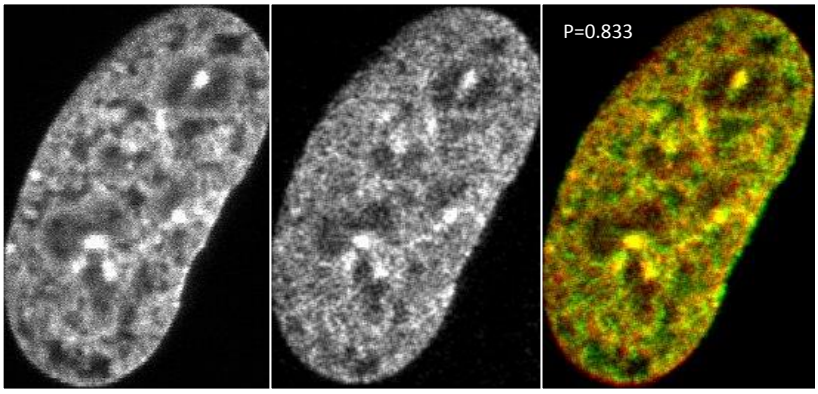
SD : 415.136 (AP)
SD: 414.2639 (CI)

Figure S15

A



B



C

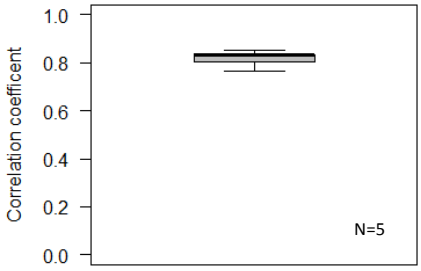


Figure S16