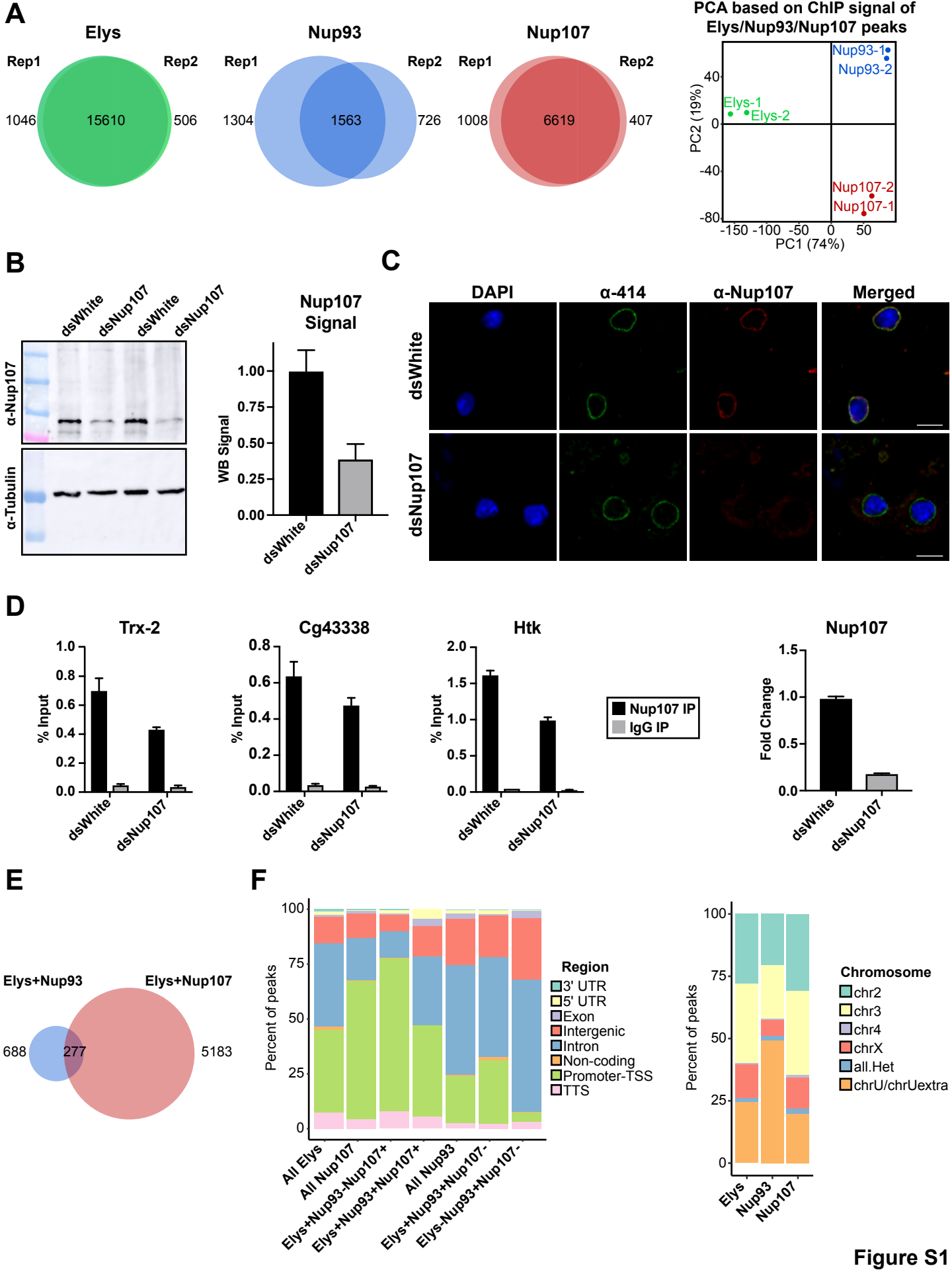

A
Elys
Rep1
Rep2
1046
15610
506
Nup93
Rep1
Rep2
1304
1563
726
Nup107
Rep1
Rep2
1008
6619
407
PCA based on ChIP signal of Elys/Nup93/Nup107 peaks
Nup93-1
Nup93-2
Elys-1
Elys-2
Nup107-2
Nup107-1
PC2 (19%)
PC1 (74%)
B
dsWhite
dsNup107
dsWhite
dsNup107
α-Nup107
α-Tubulin
Nup107 Signal
WB Signal
dsWhite
dsNup107
C
DAPI
α-414
α-Nup107
Merged
dsWhite
dsNup107
D
Trx-2
Cg43338
Htk
Nup107
% Input
Fold Change
Nup107 IP
IgG IP
dsWhite
dsNup107
E
Elys+Nup93
Elys+Nup107
688
277
5183
F
Percent of peaks
Region
3' UTR
5' UTR
Exon
Intergenic
Intron
Non-coding
Promoter-TSS
TTS
All Elys
All Nup107
Elys+Nup93-Nup107+
Elys+Nup93+Nup107+
All Nup93
Elys+Nup93+Nup107-
Elys-Nup93+Nup107-
Chromosome
chr2
chr3
chr4
chrX
all.Het
chrU/chrUextra
Elys
Nup93
Nup107

Figure S1

Supplemental Figure S1, related to Figure 1.

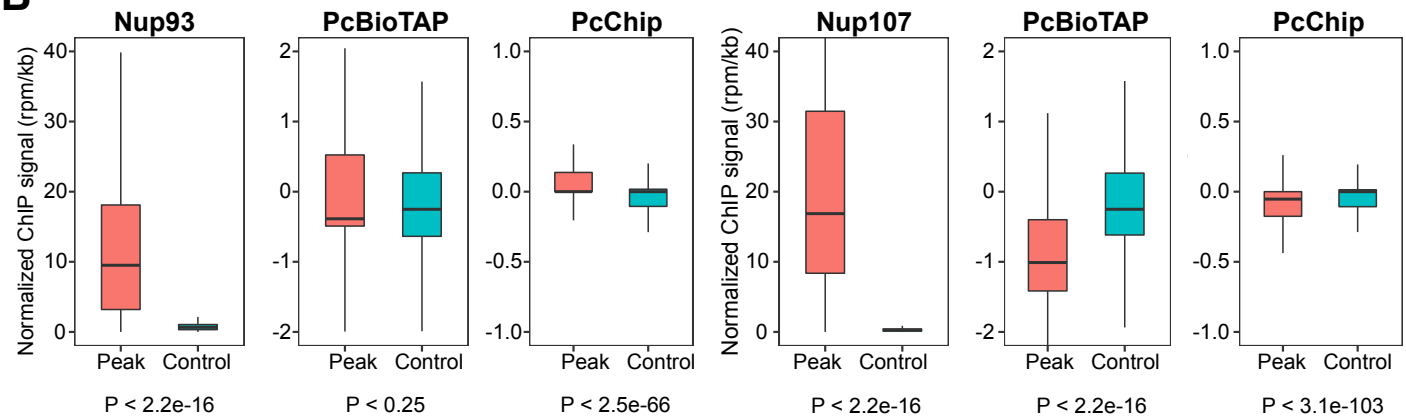
(A) Venn diagrams showing overlap between called peaks of two independent ChIP-seq replicates (Rep1 and Rep2) for each Nup, as labeled, in S2 cells (left) and respective principal component analysis (PCA) for ChIP-seq Nup peak datasets (right). **(B)** Western Blot of S2 cells treated with dsWhite and dsNup107 RNAi for 3 rounds (total of 6 days), and blotted against Nup107 antibody (top). Graph depicts quantification of Nup107 western signal normalized to tubulin signal (bottom). Molecular weight markers are 250, 150, 100, 75, 55 and 37 kDa, top-bottom. Bars represent mean +/- SD from 3 biological replicates. **(C)** Representative IF images of cells treated with RNAi against control (dsW), or Nup107 (dsNup107). Cells are stained for 414 (green), Nup107 (red) and DNA (DAPI, blue) **(D)** ChIP-qPCR of S2 cells treated with RNAi against control (dsWhite) or Nup107 transcripts (dsNup107) using generated Nup107 antibody or rabbit IgG, at genes identified as targets of Nup107 (left). RT-qPCR of Nup107 transcript levels in RNAi treatments is shown at right. Bars represent mean ChIP signal normalized to input (left) and mean expression levels normalized to dsWhite (right) + SEM (3 biological replicates). For (B)-(D) RNAi treatments were performed for 3 rounds, 144 hours total. **(E)** Venn diagram comparing overlap between Elys/Nup93 ChIP-seq peaks with Elys/Nup107 ChIP-seq peaks. **(F)** Distribution of Nup ChIP-seq peaks relative to genomic elements as labeled (left), and relative to the 4 chromosomes and heterochromatic/repetitive elements of the fly genome (right).

A

Chromatin Color P - Values					
	Yellow	Red	Green	Blue	Black
Elys	P < 0.01	P < 0.01	P = 1.0	P = 1.0	P = 1.0
Nup107	P < 0.01	P < 0.01	P = 1.0	P = 1.0	P = 1.0
Elys/Nup107	P < 0.01	P < 0.01	P = 0.54	P = 1.0	P = 1.0
Nup93	P = 1.0	P < 0.01	P = 1.0	P < 0.01	P = 1.0
Elys/Nup93	P = 1.0	P < 0.01	P = 1.0	P < 0.01	P = 1.0

Chromatin State P - Values									
	State 1	State 2	State 3	State 4	State 5	State 6	State 7	State 8	State 9
Elys	P < 0.01	P = 1.0	P < 0.01	P = 1.0	P = 1.0	P = 1.0	P = 1.0	P = 1.0	P = 1.0
Nup107	P < 0.01	P = 1.0	P = 1.0	P = 1.0	P = 1.0	P = 1.0	P = 1.0	P = 1.0	P = 1.0
Elys/Nup107	P < 0.01	P = 1.0	P = 0.54	P = 1.0	P = 1.0	P = 1.0	P = 1.0	P = 1.0	P = 1.0
Nup93	P < 0.01	P = 1.0	P < 0.01	P = 1.0	P = 1.0	P < 0.01	P = 1.0	P = 1.0	P = 1.0
Elys/Nup93	P = 1.0	P = 1.0	P < 0.01	P = 1.0	P = 1.0	P < 0.01	P = 1.0	P = 1.0	P = 1.0

B



C

Top 20 most enriched GO terms for Nup93-Pc targets
GO:0010092~specification of organ identity
GO:0048098~antennal joint development
GO:0061332~Malpighian tubule bud morphogenesis
GO:0007383~specification of segmental identity, antennal segment
GO:0042693~muscle cell fate commitment
GO:1902339~positive regulation of apoptotic process involved in morphogenesis
GO:0035223~leg disc pattern formation
GO:0042684~cardioblast cell fate commitment
GO:0042682~regulation of compound eye cone cell fate specification
GO:0048735~haltere morphogenesis
GO:0042659~regulation of cell fate specification
GO:0035288~anterior head segmentation
GO:0035284~brain segmentation
GO:0007487~analia development
GO:0007483~genital disc morphogenesis
GO:0030539~male genitalia development
GO:0009997~negative regulation of cardioblast cell fate specification
GO:0007380~specification of segmental identity, head
GO:0035051~cardiocyte differentiation
GO:0007384~specification of segmental identity, thorax

D

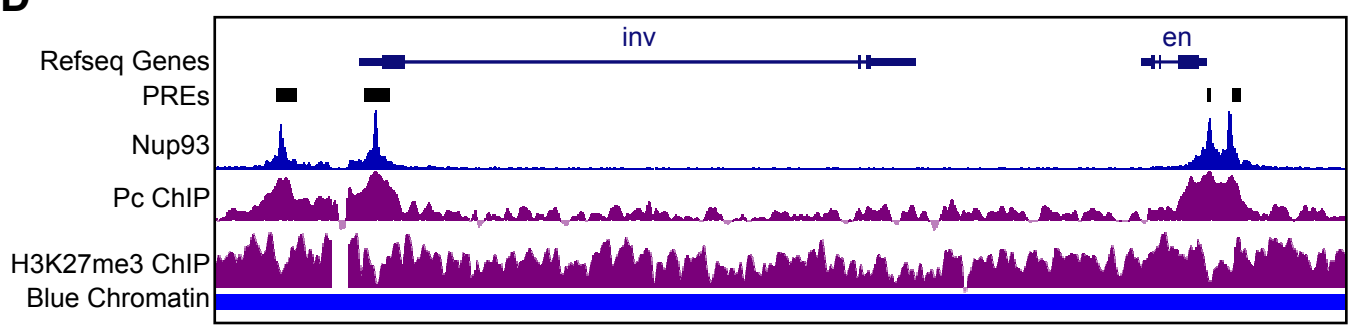


Figure S2

Supplemental Figure S2, related to Figure 2.

(A) P-values of enrichment of chromatin states (according to 5-state model (top) and 9-state model (bottom)) for each Nup peaks set, as labeled, calculated by Permutation test, which involves comparison to 100 random shuffles of the same number of peaks on the genome. **(B)** Box plots of ChIP signal of selected data sets as labeled, at Nup93 peaks and its random shuffled control (left 3 plots) and at Nup107 peaks and its random shuffled control (right 3 plots). P-values were calculated using two-tailed nonparametric t test (Mann Whitney test). The null hypothesis assumes that the Polycomb change may occur in both the same and opposite directions as the Nup change. **(C)** Top 20 GO Analysis terms of genes co-bound by Nup93 and Pc (GO terms sorted by fold enrichment). **(D)** Genome browser tracks showing previously defined Polycomb Response Elements (PREs) (work of Kassis lab, as referenced in text), relative to Nup93, Pc, H3K27Me3 ChIP-seq and Blue chromatin state.

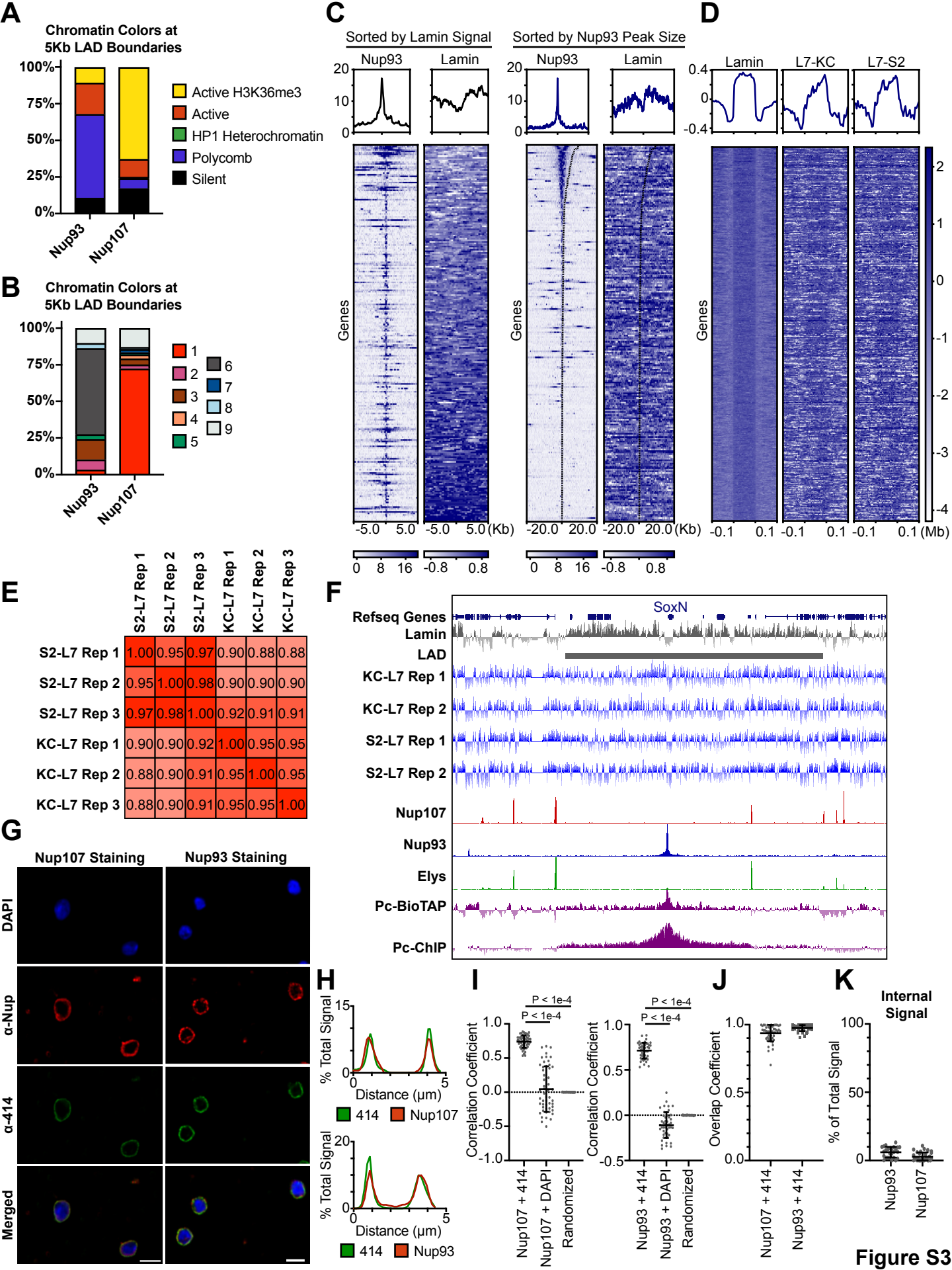

A
Chromatin Colors at 5Kb LAD Boundaries
100%
75%
50%
25%
0%
Nup93
Nup107
Active H3K36me3
Active
HP1 Heterochromatin
Polycomb
Silent
B
Chromatin Colors at 5Kb LAD Boundaries
100%
75%
50%
25%
0%
Nup93
Nup107
1
2
3
4
5
6
7
8
9
C
Sorted by Lamin Signal
Sorted by Nup93 Peak Size
Nup93
Lamin
Genes
-5.0
5.0
(Kb)
-20.0
20.0
0
8
16
-0.8
0.8
D
Lamin
L7-KC
L7-S2
0.4
0
-0.4
-0.1
0.1
(Mb)
2
1
0
-1
-2
-3
-4
E
S2-L7 Rep 1
S2-L7 Rep 2
S2-L7 Rep 3
KC-L7 Rep 1
KC-L7 Rep 2
KC-L7 Rep 3
1.00 0.95 0.97 0.90 0.88 0.88
0.95 1.00 0.98 0.90 0.90 0.90
0.97 0.98 1.00 0.92 0.91 0.91
0.90 0.90 0.92 1.00 0.95 0.95
0.88 0.90 0.91 0.95 1.00 0.95
0.88 0.90 0.91 0.95 0.95 1.00
F
SoxN
Refseq Genes
Lamin
LAD
KC-L7 Rep 1
KC-L7 Rep 2
S2-L7 Rep 1
S2-L7 Rep 2
Nup107
Nup93
Elys
Pc-BioTAP
Pc-ChIP
G
Nup107 Staining
Nup93 Staining
DAPI
α-Nup
α-414
Merged
H
% Total Signal
15
0
5
Distance (μm)
414
Nup107
20
414
Nup93
I
P < 1e-4
P < 1e-4
Correlation Coefficient
1.0
0.5
0.0
-0.5
-1.0
Nup107 + 414
Nup107 + DAPI
Randomized
Nup93 + 414
Nup93 + DAPI
Randomized
J
Overlap Coefficient
1.0
0.5
0.0
Nup107 + 414
Nup93 + 414
K
Internal Signal
% of Total Signal
100
50
0
Nup93
Nup107

Figure S3

Supplemental Figure S3, related to Figure 3.

(A-B) Distribution of chromatin colors (5 color model (A) or 9 color model (B)) among Nup93 or Nup107 ChIP-seq peaks within 5 Kb boundaries of previously defined LADs. **(C)** Enrichment heatmaps of Lamin DamID signal at ChIP-seq identified peaks of Nup93. Peaks are sorted by Lamin signal (left) and Nup93 peak size/width (right). **(D)** Enrichment heatmaps showing comparison of previously defined LADs, derived from Lamin DamID in Kc cells (described in text), to ChIP-seq of Lamin in Kc and S2 cells, using the L7 anti-Lamin antibody (cited in text). For both (C) and (D), y-axis units correspond to ChIP-seq/DamID signal intensity (rpm/bp) and the x-axis corresponds to distance from the peak (Kb (C), Mb (D)). **(E)** Heatmap table plotting Pearson correlations between S2 and Kc cell Lamin ChIP-seq replicates (3 replicates per cell type). **(F)** Representative GB snapshots of LADs defined by Lamin DamID along with Kc and S2 cell Lamin ChIP-seq replicates, and other tracks as indicated, at the *Soxn* locus (330 Kb). **(G)** Representative IF images of WT S2 cells stained against DNA (DAPI, blue), Nup93 (left column)/Nup107 (right column) and mAb414 (green). **(H)** Representative line graphs of florescence intensity of 414 and Nup93 or Nup107 across the diameter of an S2 cell nucleus, represented as fraction of total intensity signal. **(I)** Plot of Pearson correlation coefficients (PCCs) between 414 signal and Nup107 (left) or Nup93 (middle) (PCCs for Nup107/414 mean=0.74, Nup107/DAPI mean=0.04, and randomized pixels mean=0, n=52, 2 biological replicates; PCCs for Nup93/414 mean=0.71, Nup93/DAPI mean=-0.11, and randomized pixels mean=0, n=47, 2 biological replicates). **(J)** Plot of Manders overlap coefficients (MOCs) for fraction of Nup107 or Nup93 that overlaps with 414 signal (Nup107 MOC mean=0.94, n=52, 2 biological replicates; Nup93 MOC mean=0.98, n=45, 2 biological replicates). Middle line demarcates mean ± SD. P values are from a two tailed unpaired non-parametric t test. **(K)** Quantification of internal florescence signal of Nup93 or Nup107 as a fraction of total florescence signal calculated from line graphs in H using 414 signal to define periphery (for Nup93 mean=6.0%, n=26; for Nup107 mean=2.7%, n=32).

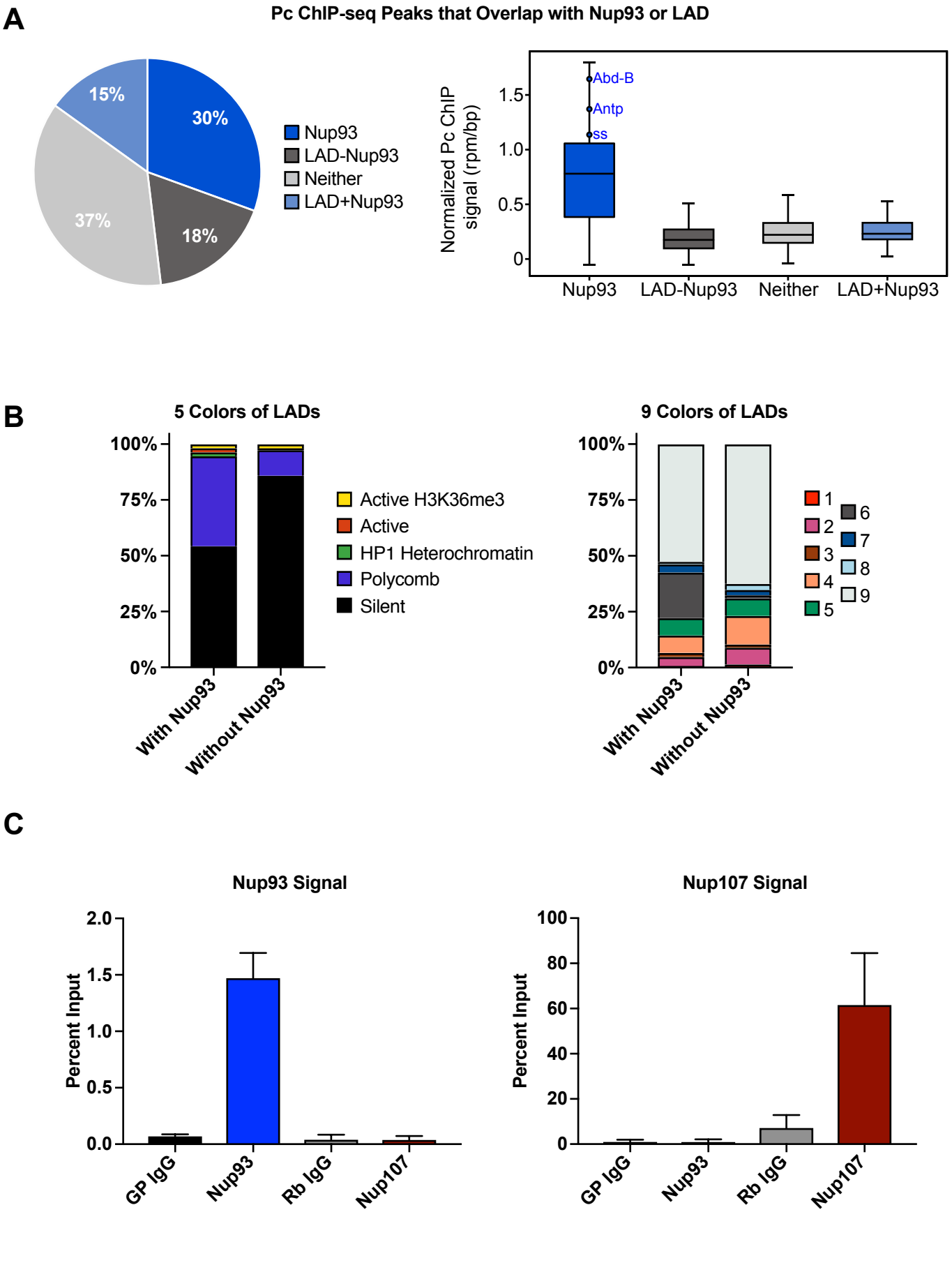

A
Pc ChIP-seq Peaks that Overlap with Nup93 or LAD
15%
30%
37%
18%
Nup93
LAD-Nup93
Neither
LAD+Nup93
Normalized Pc ChIP signal (rpm/bp)
1.5
1.0
0.5
0
Abd-B
Antp
ss
Nup93
LAD-Nup93
Neither
LAD+Nup93
B
5 Colors of LADs
100%
75%
50%
25%
0%
With Nup93
Without Nup93
Active H3K36me3
Active
HP1 Heterochromatin
Polycomb
Silent
9 Colors of LADs
1
2
3
4
5
6
7
8
9
C
Nup93 Signal
Percent Input
2.0
1.5
1.0
0.5
0.0
GP IgG
Nup93
Rb IgG
Nup107
Nup107 Signal
100
80
60
40
20
0

Figure S4

Supplemental Figure S4, related to Figure 4.

(A) Distribution of Pc ChIP-seq peaks (described in text) containing Nup93 ChIP-seq peaks (Nup93), LADs with Nup93 ChIP-seq signal within or at 5 Kb boundaries (LAD+Nup93), LADs without Nup93 ChIP-seq signal within or at 5 Kb boundaries (LAD-Nup93), or none of these (pie chart at left), and averaged Pc ChIP-seq signal intensities among the four groups (box and whisker plot at right). Nup93 ChIP-seq signal at peaks of three well-characterized PcG targets *Abd-B*, *Antp* and *ss* are depicted by the dots imbedded in the Nup93 box and whisker plot. **(B)** Predominant chromatin colors of LADs with and without Nup93 within or at 5 Kb boundaries. **(C)** Quantification of Nup93 (left) and Nup107 (right) IP efficiency, from Co-IPs depicted in figure 4E. Bars represent mean percentages of IP signal relative to 100% input (calculated from 5% input signal), +/- SEM (3 biological replicates).

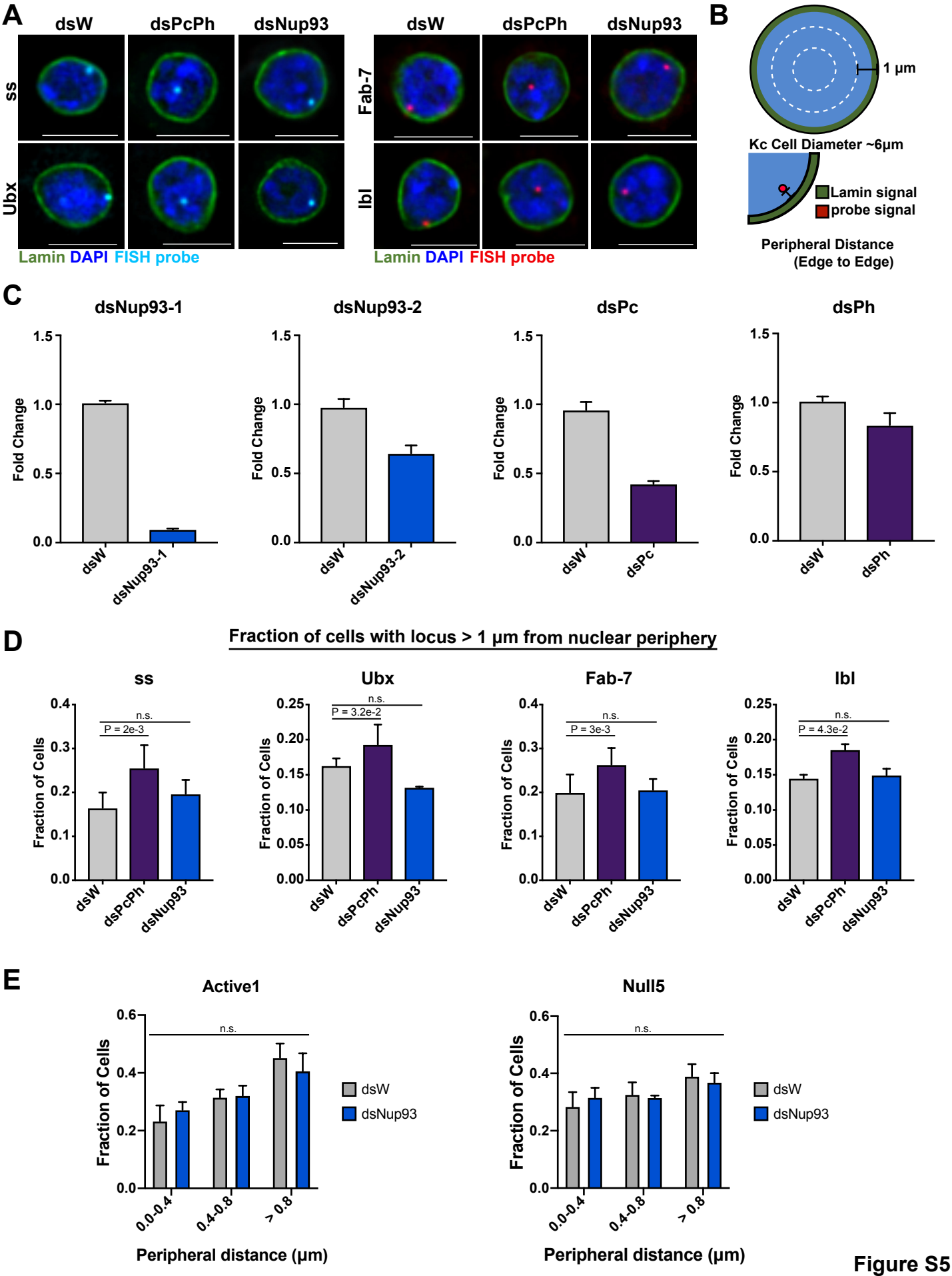

A
dsW
dsPcPh
dsNup93
ss
Ubx
Fab-7
lbl
Lamin DAPI FISH probe
B
1 µm
Kc Cell Diameter ~6µm
Lamin signal
probe signal
Peripheral Distance
(Edge to Edge)
C
dsNup93-1
dsNup93-2
dsPc
dsPh
Fold Change
D
Fraction of cells with locus > 1 µm from nuclear periphery
Fraction of Cells
n.s.
P = 2e-3
P = 3.2e-2
P = 3e-3
P = 4.3e-2
E
Active1
Null5
0.0-0.4
0.4-0.8
> 0.8
Peripheral distance (µm)

Figure S5

Figure S5, related to Figure 5.

(A) Representative images of Kc cells treated with RNAi against control (dsW), Pc/Ph (dsPcPh) or Nup93 (dsNup93) as described in Figure 5. Cells are co-labeled with Oligopaints FISH probes (red or cyan, probes as labeled), and IF for Lamin (green) and DNA (DAPI, blue). **(B)** Schematic of quantification scheme depicting defined distances between probe signal and the nuclear periphery. Average nuclear size is shown, with lines delimiting 1 μm intervals. Mean diameter for Kc cell nuclei demarcated by Lamin = 6.02μm, calculated from >400 cells per replicate, 4 biological replicates. **(C)** Normalized RT-qPCR analysis of Nup93-1, Nup93-2, Ph and Pc expression in Kc cells treated for 4 days with RNAi against indicated genes, relative to control cells treated with RNAi against dsWhite (dsW). Bars represent mean + SEM. **(D)** Fraction of cell population with a probe to periphery distance larger than 1 μm, for cells treated with RNAi described in 5F and 5G, for the 4 utilized probes. **(E)** Distributions of measured probe to periphery distances, for the 2 indicated control probes, in cells treated with control (dsW) and Nup93 RNAi (dsNup93), using DAPI to define the nuclear periphery, plotted as Fraction of cell population with the distance within an assigned bin (labeled on the bottom axis). Bars represent mean + SEM (3 biological replicates). P values are from a two-tailed unpaired non-parametric t test. Null hypothesis is “not being further away from periphery”.

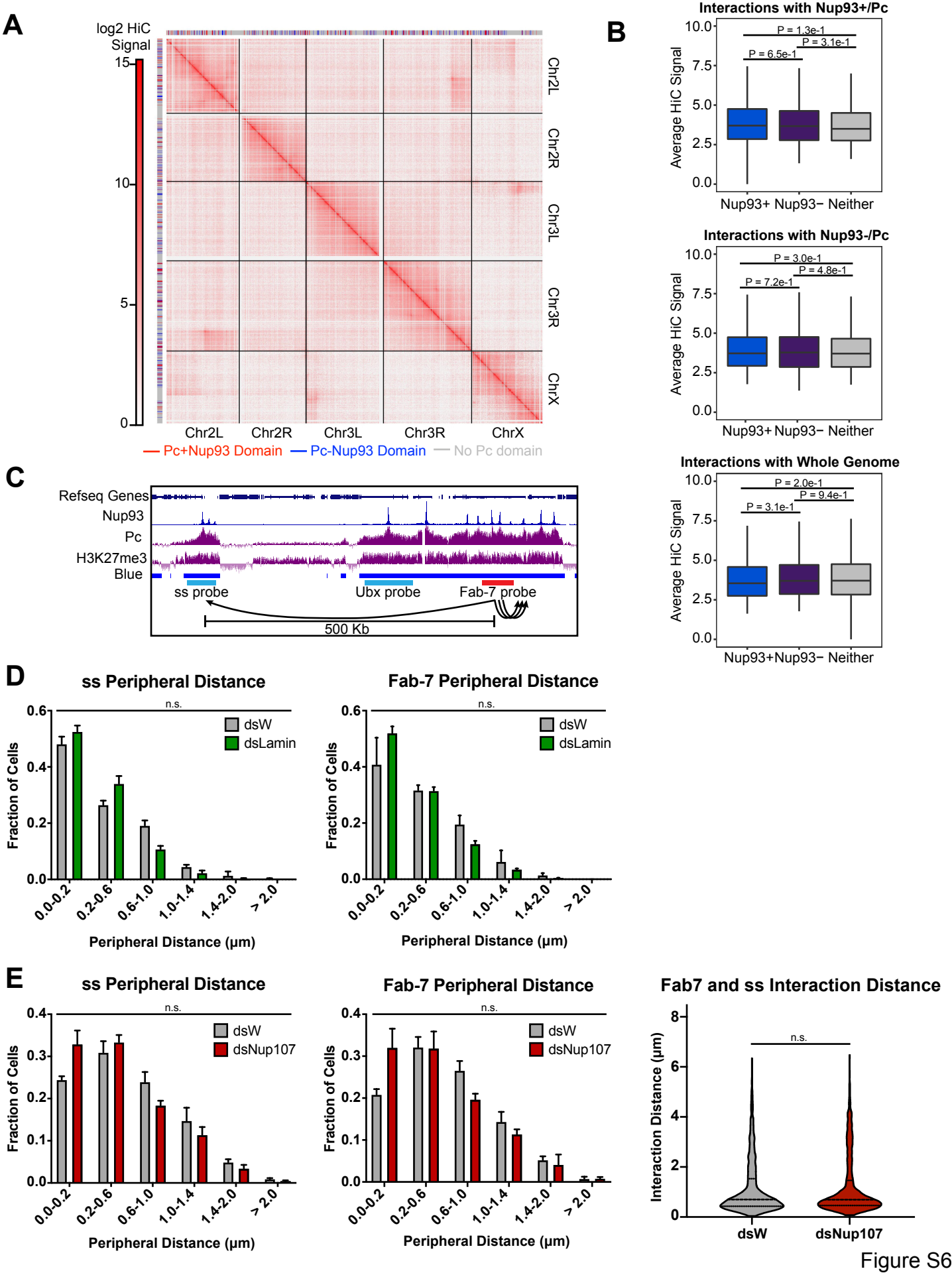

A
log2 HiC Signal
15
10
5
0
Chr2L
Chr2R
Chr3L
Chr3R
ChrX
Pc+Nup93 Domain
Pc-Nup93 Domain
No Pc domain
B
Interactions with Nup93+/Pc
P = 1.3e-1
P = 3.1e-1
P = 6.5e-1
Average HiC Signal
Nup93+ Nup93− Neither
Interactions with Nup93-/Pc
P = 3.0e-1
P = 4.8e-1
P = 7.2e-1
Interactions with Whole Genome
P = 2.0e-1
P = 9.4e-1
P = 3.1e-1
C
Refseq Genes
Nup93
Pc
H3K27me3
Blue
ss probe
Ubx probe
Fab-7 probe
500 Kb
D
ss Peripheral Distance
Fab-7 Peripheral Distance
n.s.
dsW
dsLamin
Fraction of Cells
0.0-0.2
0.2-0.6
0.6-1.0
1.0-1.4
1.4-2.0
> 2.0
Peripheral Distance (μm)
E
ss Peripheral Distance
Fab-7 Peripheral Distance
dsW
dsNup107
Fab7 and ss Interaction Distance
Interaction Distance (μm)
dsW
dsNup107

Figure S6

Supplemental Figure S6, related to Figure 6.

(A) Hi-C interaction map of *D. melanogaster* genome (see text for reference), with locations of Pc regions bound by Nup93 (red, Pc+Nup93), not bound by Nup93 (blue, Pc-Nup93), and of non-Pc regions (grey). **(B)** Averaged amounts of inter-chromosomal Hi-C interactions between Pc domains that contain Nup93 ChIP-seq peaks (Nup93+), Pc domains that do not (Nup93-) and regions that do not contain Pc. Average inter-chromosomal Hi-C interactions between Pc domains that do not have Nup93 ChIP-seq peaks (Nup93-) and the whole genome were also analyzed (plotted below). P-values were calculated using two-tailed nonparametric t test (Mann Whitney test). **(C)** GB snapshot of the Bithorax/*Fab-7* and ss loci, with arrows representing previously identified log-range interactions (see text). ChIP-seq of Nup93, Pc, and H3K27Me3, and Blue chromatin color domains are shown above. **(D)** Distributions of measured probe to periphery distances, for *Fab-7* and *ss* loci, in cells treated with control (dsW) and lamin B/C RNAi (dslamin) using DAPI to define the periphery. Distributions are plotted as fractions of the cell population within assigned bins. Bars represent mean + SEM (> 300 cells per condition, 2 biological replicates). P values are from two-tailed unpaired non-parametric t test. Null hypothesis is “not being further away from nuclear periphery”. RNAi treatments were performed for 2 rounds, 96 hours total **(E)** Distributions of measured probe to periphery distances, for *Fab-7* and *ss* loci, in Kc cells treated with control (dsW) and Nup107 RNAi (dsNup107) using lamin IF to define the nuclear periphery. RNAi treatments were performed for 3 rounds, 144 hours total. Distributions are plotted as fractions of the cell population within assigned bins (left and middle). Violin plot of measured distances between centers of *Fab-7* and *ss* Oligopaint probes in KC cells, treated with RNAi against control and Nup107 RNAi (right). Bars represent Mean + SEM (left and middle) and, lines depict median and quartiles (right). P values are from a two-tailed unpaired non-parametric t test. Measurements are from >300 cells per condition; 4 biological replicates. In case of periphery distance measurements, null hypothesis is “not being further away from nuclear periphery”.

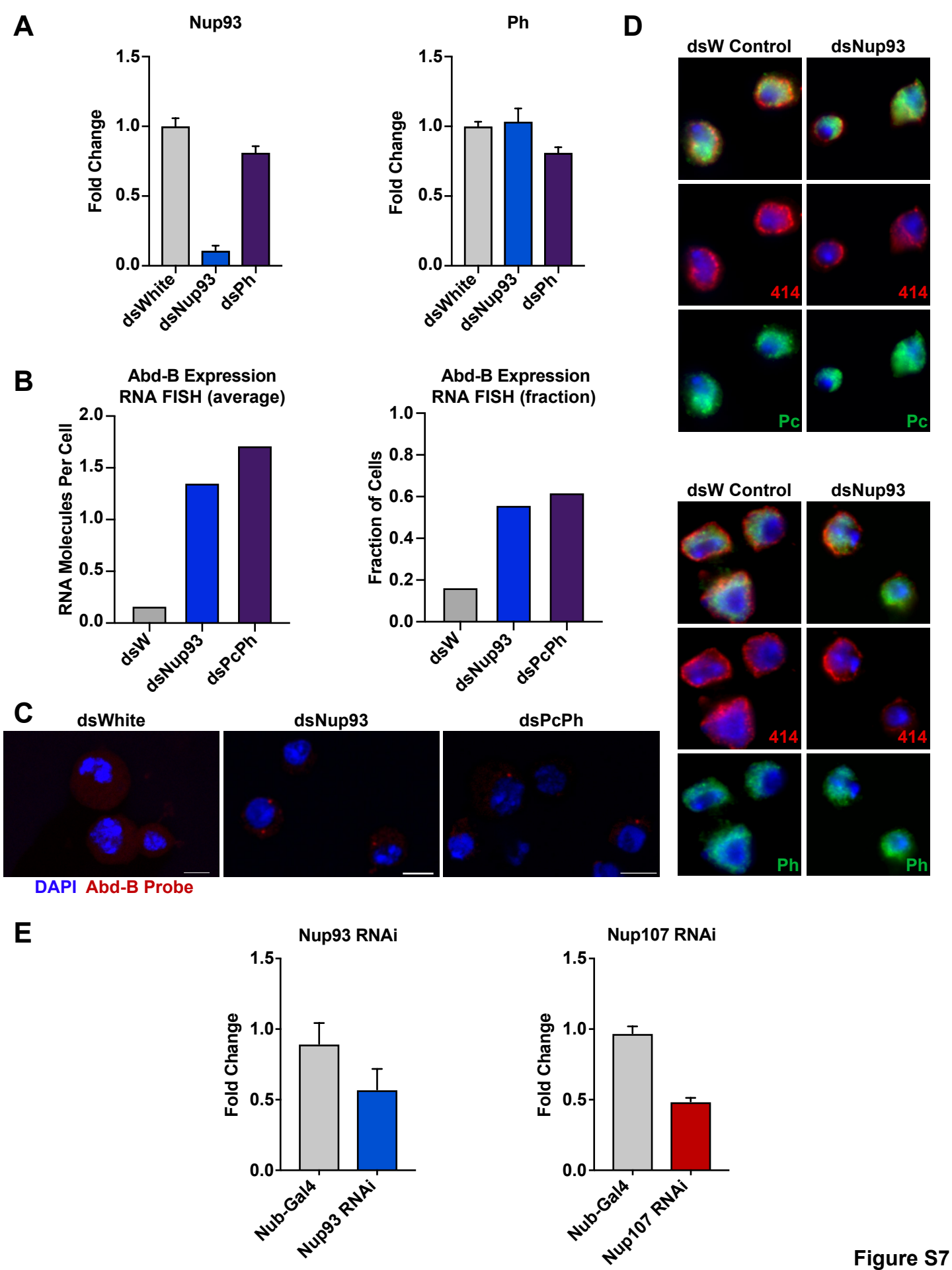

A
Nup93
Ph
Fold Change
1.5
1.0
0.5
0.0
dsWhite
dsNup93
dsPh
B
Abd-B Expression
RNA FISH (average)
RNA Molecules Per Cell
2.0
1.5
1.0
0.5
0.0
dsW
dsNup93
dsPcPh
Abd-B Expression
RNA FISH (fraction)
Fraction of Cells
1.0
0.8
0.6
0.4
0.2
0.0
C
dsWhite
dsNup93
dsPcPh
DAPI Abd-B Probe
D
dsW Control
dsNup93
414
Pc
Ph
E
Nup93 RNAi
Nup107 RNAi
Nub-Gal4
Nup93 RNAi
Nup107 RNAi

Figure S7

Supplemental Figure S7, related to Figure 7.

(A) Normalized RT-qPCR analysis of Nup93 and Ph expression in S2 cells treated with RNAi against control (dsWhite), Nup93 (dsNup93) or Ph (dsPh), for 2 rounds/96 hours total. Bars depict mean + SEM (5 biological replicates). **(B)** Average RNA FISH signal for *Abd-B* probe in S2 cells, calculated as an average number of probe-labeled dots per cell in indicated RNAi treatments (plot at left), and fraction of cells with at least one RNA FISH dot of *Abd-B* present in the 3 RNAi treatments (plot at right). 50 cells analyzed per condition. **(C)** Representative images of S2 cells treated with RNAi against control (dsW), Pc/Ph (dsPcPh) or Nup93 (dsNup93). Cells are labeled with Abd-B RNA FISH probes (red) and stained DNA (DAPI, blue). **(D)** IF of S2 cells treated with RNAi against control (dsW) or Nup93 (dsNup93), as described for Figure 7, using antibodies to nuclear pore marker mAb414 (red) and Pc or Ph (green). **(E)** Normalized RT-qPCR analysis of Nup93 and Nup107 expression from wing discs of wandering 3rd instar larvae with Nubbin-Gal4 driven RNAi against Nup93 or Nup107, relative to control Nubbin-Gal4 lines. Bars represent mean + SEM (3 biological replicates, 25+ animals each).