SUPPLEMENTAL FIG.LEGENDS

Fig.S1: Generation of FKBP^{degron} lines. (Related to Fig.1)

(A) Outline of protocol used to generate RAD21 and WAPL-FKBP^{degron} mESCs

(B) PCR amplifying knock-in site of parental TST mESC lines (WT) and RAD21 and WAPL-FKBP^{degron} clones. One PCR primer was located outside of the homology arms to ensure that only the endogenous locus was amplified. FKBP^{degron} tag is ~400bp.

(C) Western blots for RAD21 and GADPH loading control in RAD21-FKBP^{degron} lines at day 7 of differentiation. Samples treated with DMSO vehicle control (D) or 500nM dTAG-13 (13).

(D) Western blots for WAPL and GADPH loading control in WAPL-FKBP^{degron} lines at day 7 of differentiation. Samples treated with DMSO vehicle control (D) or 500nM dTAG-13 (13).

(E) Sanger sequencing of RAD21-FKBP^{degron} and WAPL-FKBP^{degron} lines.

(F) Comparison of genomic DNA (input DNA) in WAPL-FKBP^{degron} lines at chromosome 14. Sharp dip in WAPL-FKBP^{degron} , clone H7 input corresponds to the end of the right 900bp homology arm used for knock-in of the tag. Reads mapping to the plasmid backbone used for the homology repair template were also found here, although they did not appear to be incorporated into the main Wapl transcript.

(G) Immunofluorescence of WAPL-HA in WAPL-FKBP^{degron} lines at day 7 of differentiation. Samples treated with DMSO vehicle control (D) or 500nM dTAG-13 (13).

Fig.S2: Cell cycle profiling of FKBP^{degron} lines. (Related to Fig.1)

(A) DNA content profiles via propidium iodide staining of RAD21-FKBP^{degron} lines at day 7 of differentiation. Samples treated with DMSO vehicle control (D) or 500nM dTAG-13 (13) as indicated.

(B) DNA content profiles via propidium iodide staining of RAD21-FKBP^{degron} lines at day 10 of differentiation.

(C) DNA content profiles via propidium iodide staining of WAPL-FKBP^{degron} lines at day 7 of differentiation.

(D) DNA content profiles via propidium iodide staining of WAPL-FKBP^{degron} lines at day 7 of differentiation.

(E) DNA content profiles via propidium iodide staining of WAPL-FKBP^{degron} lines at day 10 of differentiation.

Fig.S3. Replication of architectural changes across FKBP^{degron} clones. (Related to Fig. s 1 and 2)

(A) Metaplot and heatmap of RAD21 binding by cChIP-seq in WAPL-FKBP^{degron} clone H2.

(B) Frequency versus distance plots for Hi-C interaction maps across individual FKBP^{degron} clones at all time points used in this study. Samples treated with DMSO vehicle control 500nM dTAG-13 as indicated. Saddle plot analysis of compartment strength in RAD21-FKBP^{degron} Hi-C maps at day 7 (clones merged) also shown. Scores represent B-B, B-A, and A-A compartment interaction strength.Representative sections of day 7 Hi-C maps at different resolutions for RAD21-FKBP^{degron} clones.

(C) Representative sections of day 10 Hi-C maps at different resolutions for RAD21-FKBP^{degron} clones.

(D) Section of day 7 RAD21-FKBP^{degron} Hi-C maps (clones merged) displaying example of cohesin-independent loops not anchored by H3K27me3 (arrows).

(E) Representative sections of day 7 Hi-C maps at different resolutions for WAPL-FKBP^{degron} clones.

(F) Aggregate Peak Analysis plots of aggregate RAD21-FKBP^{degron} Hi-C signal at day 7 (clones merged) over regions anchored by Hox clusters.

(G) Aggregate Peak Analysis plots of aggregate RAD21-FKBP^{degron} Hi-C signal at day 10 (clones merged) over regions anchored by Hox clusters.

(H) Aggregate Peak Analysis plots of aggregate WAPL-FKBP^{degron} Hi-C signal at day 7 (clones merged) over regions anchored by Hox clusters.

Fig.S4: Additional analysis of cChIP after short term WAPL degradation and gene expression changes after 3 day degradation. (Related to Fig.3)

(A) Log2-fold (dTAG-13/DMSO) change in FKBP^{degron} lines of genes overlapping RAD21-bound anchors of loops. Loops called in respective DMSO control samples. Significant p-values by Wilcoxon rank test indicated by * ($p<2.772e-06$). N.S., not significant.

(B) Metaplot and heatmaps of RAD21 binding by H3K27me3 cChIP-seq after 8 hours of WAPL degradation genome-wide (left) and on the active X chromosome (right).

(C) Metaplot and heatmaps of RAD21 binding by H3K27me3 cChIP after 8 hours of WAPL on the centromeric 30Mb, middle 100Mb, and telomeric 30Mb of the inactive X chromosome.

(D) Tracks of H3K27me3 by cChIP-seq on the X chromosome after 8 hours of WAPL degradation or DMSO control treatment. Log fold ratio tracks (dTAG-13/DMSO) are also shown (Δ).

(E) Genes upregulated or downregulated upon WAPL degradation from days 7-10 (relative to DMSO control) overlapping with H3K27me3 cChIP-seq peaks. All genes shown for reference.

(F) RNA-seq and H3K27me3 ChIP-seq over the *Usp17* and *Zscan4* gene clusters in CTCF-AID degron lines after 4 days of auxin treatment or untreated cells (Nora et al., 2017) or after 3 days of DMSO or dTAG-13 treatment (500nM) in WAPL-FKBP^{degron} cells early in differentiation (days 4-7). Note that cohesin was recently implicated in regulation of such 2-cell genes (Zhang et al., 2020).

Fig.S5. Cohesin significantly contributes to proper upregulation of proximal super-enhancer targets without affecting long-range super-enhancer interactions.

(A) Distance to nearest super-enhancer (Whyte et al. 2013) for expressed genes upregulated or downregulated upon RAD21 degradation (relative to DMSO). Expressed genes shown for reference. Significant p-values by Wilcoxon rank test indicated by * ($p<0.006057$), ** ($p<0.01569$). N.S., not significant.

(B) Day 7 RAD21-FKBP^{degron} PRO-seq and RAD21 cChIP-seq signal over representative super-enhancer targets downregulated upon 8 hours RAD21 degradation (relative to DMSO).

(C) Aggregate Peak Analysis plots of aggregate RAD21-FKBP^{degron} Hi-C signal (clones merged) over regions anchored by super-enhancers. Super-enhancers segregated by distance into 5 quantiles.

Fig.S6. Additional biological replicate for Xist CHART-seq and H3K27me3 cChIP-seq to examine spreading defects. (Related to Fig.6).

(A) Tracks of CHART-seq (Xist) and H3K27me3 cChIP-seq over the X chromosome and allelic RAD21 cChIP over the Xa and Xi in WAPL-FKBP^{degron} lines treated from days 4-7 of differentiation with DMSO (D) or 500nM dTAG-13 (13). Log fold ratio tracks (dTAG-13/DMSO) are also shown (Δ). Principal component corresponding to compartments on

the Xi in same samples shown for reference (PC1). Hi-C contacts to the 1Mb bin containing the Xist transcription locus in same conditions also shown.

(B) Boxplot showing Xist coverage over 100kb bins in the centromeric most 30Mb, middle 100Mb, and telomeric most 30Mb of the X chromosome in WAPL-FKBP^{degron} lines treated as in (A). Significant p-values by Wilcoxon signed rank test indicated by * (p<2.2e-16).

(C) Boxplots showing allelic H3K27me3 coverage over 100kb bins in the centromeric most 30Mb, middle 100Mb, and telomeric most 30Mb of the X chromosome in WAPL-FKBP^{degron} lines treated as in (A). Significant p-values by Wilcoxon signed rank test indicated by * (p<2.2e-16), ** (p<0.03), and *** (p<2.2e-05). N.S., not significant.

(D) Boxplot showing average Xist density over different classes of genes on the X chromosome in WAPL-FKBP^{degron} lines treated as in (A). Significant p-values by Wilcoxon signed rank test indicated by * (p<2.2e-16) and ** (p<1.049e-14). N.S., not significant.

(E) Boxplot showing average allelic H3K27me3 density over different classes of genes on the X chromosome in WAPL-FKBP^{degron} lines treated as in (A). Significant p-values by Wilcoxon signed rank test indicated by * (p< 2.2e-16), ** (p< 2.79e-15), *** (p< 9.04e-13), **** (p< 0.001573), ***** (p< 0.001954), ****** (p< 2.239e-05), ******* (p< 1.308e-05). N.S., not significant.

(F) Boxplot showing Xist average density over 100kb bins in S1 and S2 compartments. Significant p-values by Wilcoxon signed rank test indicated by * (p<2.2e-16) and ** (p<4.13e-09).

(G) Boxplot showing average allelic H3K27me3 density over 100kb bins in S1 and S2 compartments. Significant p-values by Wilcoxon signed rank test indicated by * (p<2.2e-16), **(p<0.0082), *** (p< 0.005), ****(p<1.86e-10), ***** (p<1.89e-08), ****** (p<2.127e-05), ******* (p<0.0008859).

(H) Boxplots showing average Xist and allelic H3K27me3 density over 100kb within 10Mb of the Xist transcription locus. Significant p-values by Wilcoxon signed rank test indicated by * (p<1.543e-05), **(p<4.026e-13).

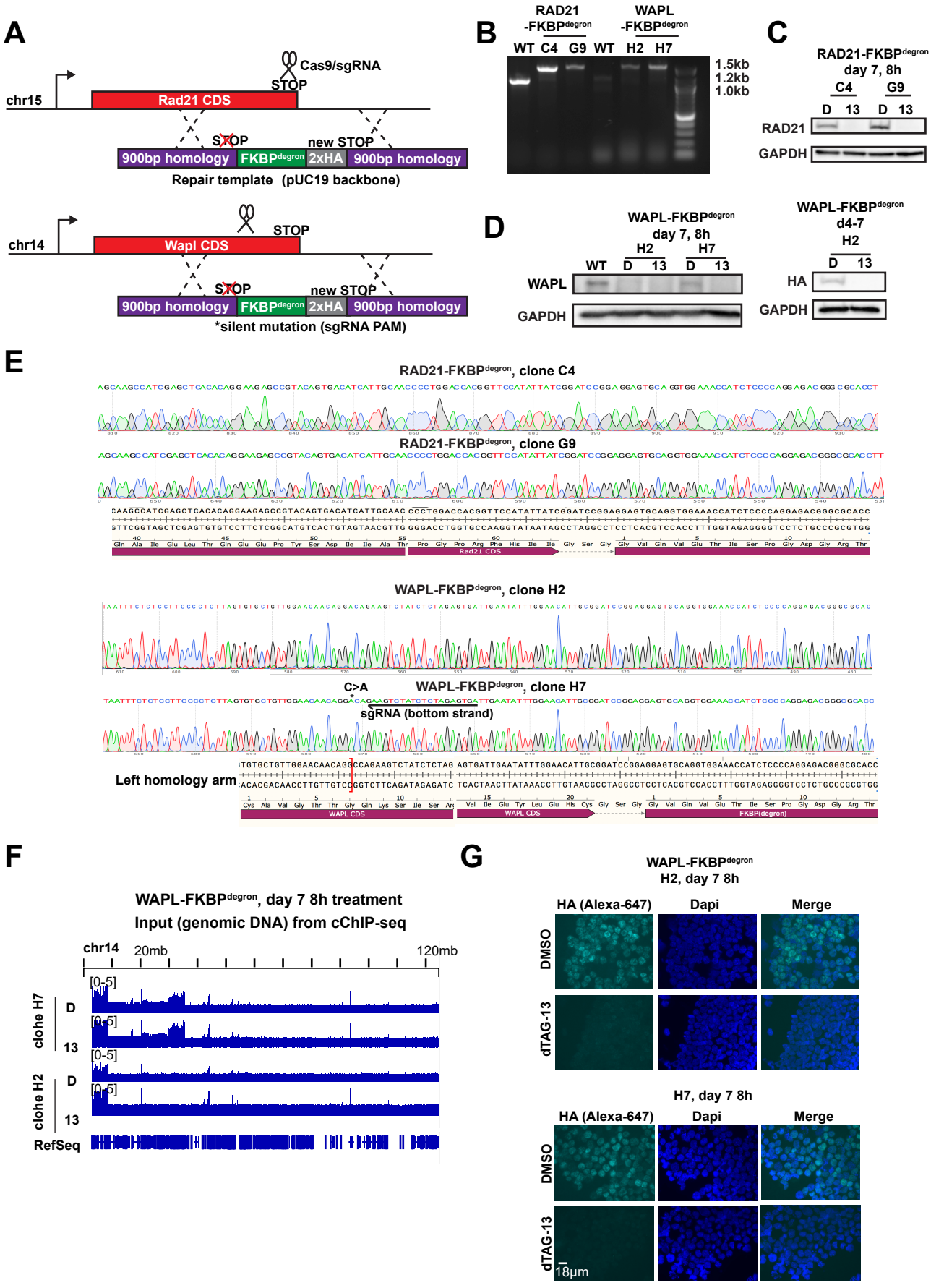
A
chr15
Rad21 CDS
Cas9/sgRNA
STOP
STOP
new STOP
900bp homology
FKBPdegron
2xHA
900bp homology
Repair template (pUC19 backbone)
chr14
Wapl CDS
STOP
STOP
new STOP
900bp homology
FKBPdegron
2xHA
900bp homology
*silent mutation (sgRNA PAM)
B
RAD21 -FKBPdegron
WAPL -FKBPdegron
WT C4 G9 WT H2 H7
1.5kb
1.2kb
1.0kb
C
RAD21-FKBPdegron
day 7, 8h
C4 G9
D 13 D 13
RAD21
GAPDH
D
WAPL-FKBPdegron
day 7, 8h
H2 H7
WT D 13 D 13
WAPL
GAPDH
WAPL-FKBPdegron
d4-7
H2
D 13
HA
GAPDH
E
RAD21-FKBPdegron, clone C4
RAD21-FKBPdegron, clone G9
Rad21 CDS
WAPL-FKBPdegron, clone H2
C>A
WAPL-FKBPdegron, clone H7
sgRNA (bottom strand)
Left homology arm
WAPL CDS
WAPL CDS
FKBP(degron)
F
WAPL-FKBPdegron, day 7 8h treatment
Input (genomic DNA) from cChIP-seq
chr14
20mb
120mb
clone H7
D
13
clone H2
D
13
RefSeq
G
WAPL-FKBPdegron
H2, day 7 8h
HA (Alexa-647)
Dapi
Merge
DMSO
dTAG-13
H7, day 7 8h
HA (Alexa-647)
Dapi
Merge
DMSO
dTAG-13
18μm

Figure S1

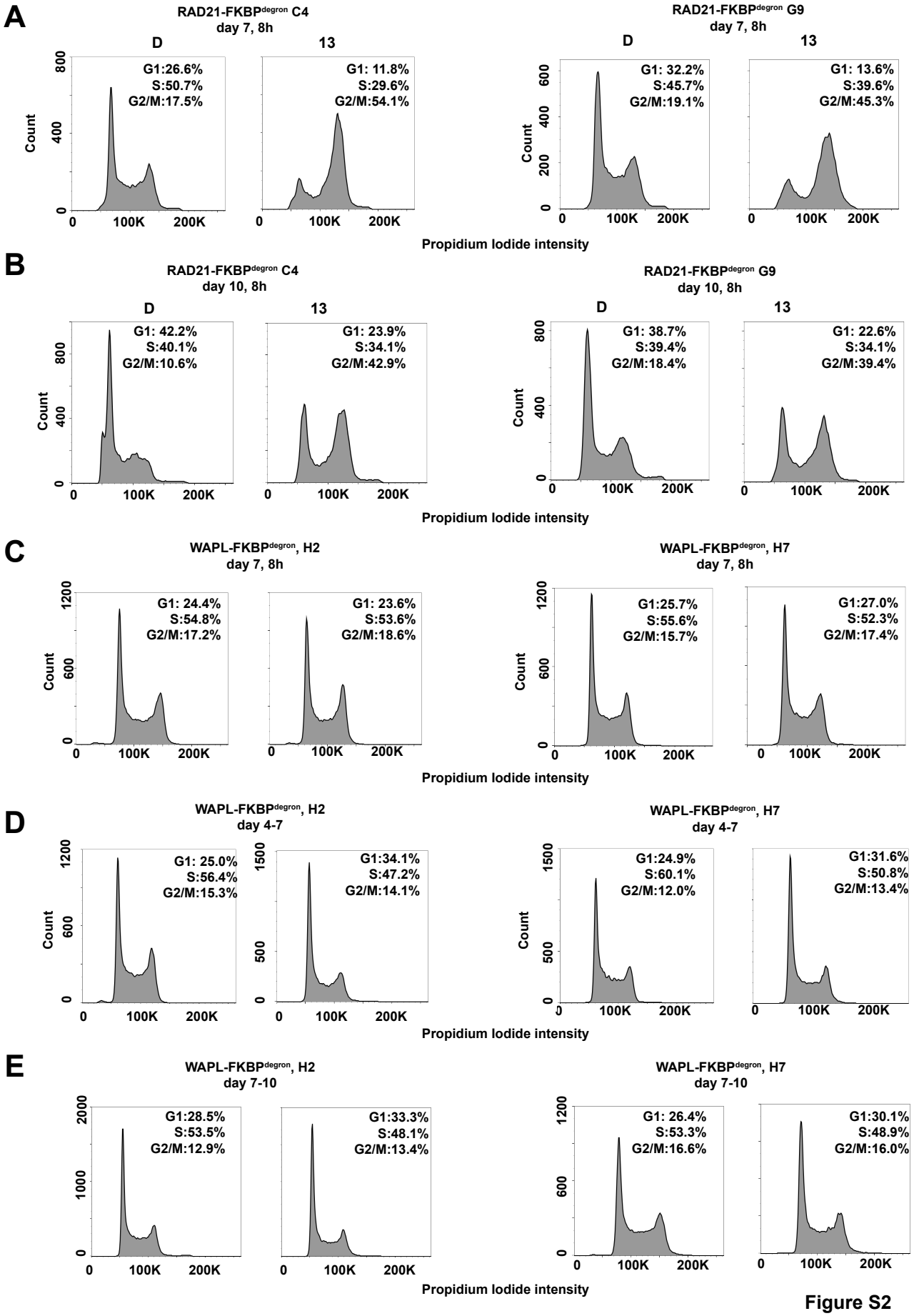
A
RAD21-FKBP^degron C4
day 7, 8h
D
13
G1:26.6%
S:50.7%
G2/M:17.5%
G1: 11.8%
S:29.6%
G2/M:54.1%
RAD21-FKBP^degron G9
day 7, 8h
G1: 32.2%
S:45.7%
G2/M:19.1%
G1: 13.6%
S:39.6%
G2/M:45.3%
Count
Propidium Iodide intensity
B
RAD21-FKBP^degron C4
day 10, 8h
G1: 42.2%
S:40.1%
G2/M:10.6%
G1: 23.9%
S:34.1%
G2/M:42.9%
RAD21-FKBP^degron G9
day 10, 8h
G1: 38.7%
S:39.4%
G2/M:18.4%
G1: 22.6%
S:34.1%
G2/M:39.4%
C
WAPL-FKBP^degron, H2
day 7, 8h
G1: 24.4%
S:54.8%
G2/M:17.2%
G1: 23.6%
S:53.6%
G2/M:18.6%
WAPL-FKBP^degron, H7
day 7, 8h
G1:25.7%
S:55.6%
G2/M:15.7%
G1:27.0%
S:52.3%
G2/M:17.4%
D
WAPL-FKBP^degron, H2
day 4-7
G1: 25.0%
S:56.4%
G2/M:15.3%
G1:34.1%
S:47.2%
G2/M:14.1%
WAPL-FKBP^degron, H7
day 4-7
G1:24.9%
S:60.1%
G2/M:12.0%
G1:31.6%
S:50.8%
G2/M:13.4%
E
WAPL-FKBP^degron, H2
day 7-10
G1:28.5%
S:53.5%
G2/M:12.9%
G1:33.3%
S:48.1%
G2/M:13.4%
WAPL-FKBP^degron, H7
day 7-10
G1: 26.4%
S:53.3%
G2/M:16.6%
G1:30.1%
S:48.9%
G2/M:16.0%

Figure S2

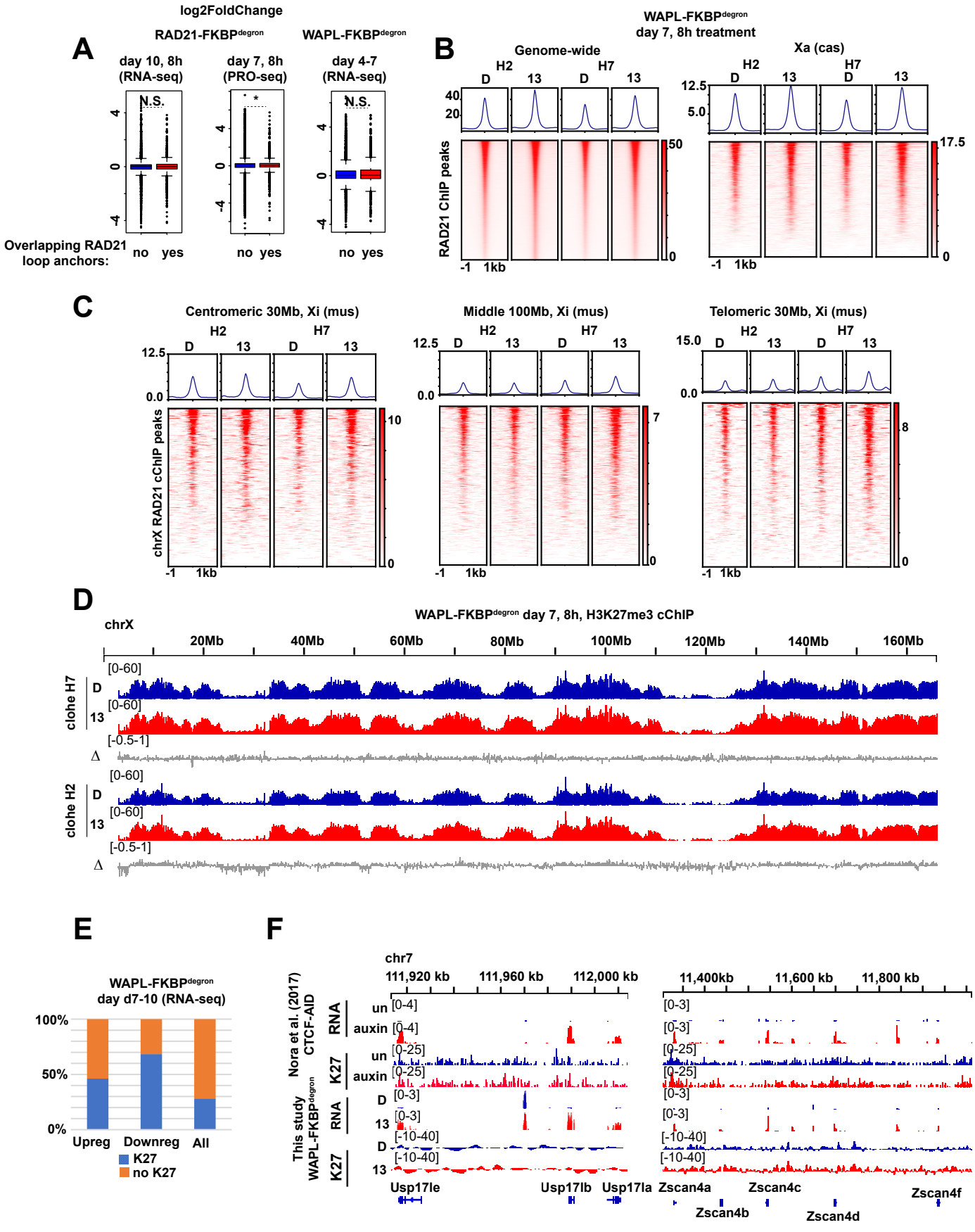
A
log2FoldChange
RAD21-FKBP^degron
WAPL-FKBP^degron
day 10, 8h (RNA-seq)
day 7, 8h (PRO-seq)
day 4-7 (RNA-seq)
N.S.
*
N.S.
Overlapping RAD21 loop anchors:
no yes
no yes
no yes
B
WAPL-FKBP^degron day 7, 8h treatment
Genome-wide
Xa (cas)
H2
H7
D
13
RAD21 ChIP peaks
-1 1kb
C
Centromeric 30Mb, Xi (mus)
Middle 100Mb, Xi (mus)
Telomeric 30Mb, Xi (mus)
chrX RAD21 cChIP peaks
D
WAPL-FKBP^degron day 7, 8h, H3K27me3 cChIP
chrX
20Mb
40Mb
60Mb
80Mb
100Mb
120Mb
140Mb
160Mb
clone H7
clone H2
E
WAPL-FKBP^degron day d7-10 (RNA-seq)
100%
50%
0%
Upreg
Downreg
All
K27
no K27
F
chr7
111,920 kb
111,960 kb
112,000 kb
11,400kb
11,600 kb
11,800 kb
Nora et al. (2017) CTCF-AID
This study WAPL-FKBP^degron
RNA
K27
un
auxin
Usp17le
Usp17lb
Usp17la
Zscan4a
Zscan4b
Zscan4c
Zscan4d
Zscan4f

Figure S4

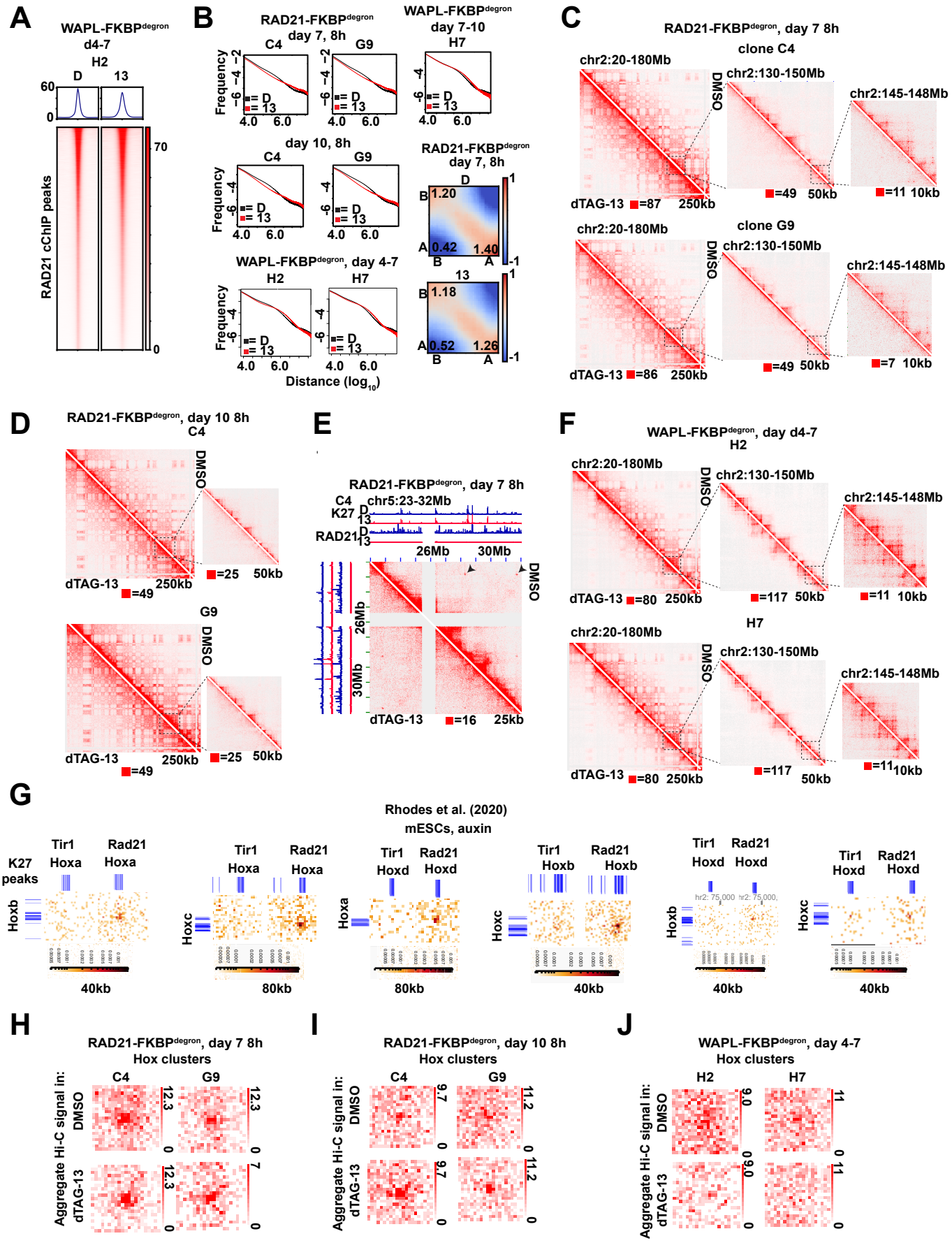
A
WAPL-FKBP^degron
d4-7
H2
D
13
60
0
70
0
RAD21 cChIP peaks
B
RAD21-FKBP^degron
day 7, 8h
C4
G9
WAPL-FKBP^degron
day 7-10
H7
day 10, 8h
RAD21-FKBP^degron
day 7, 8h
WAPL-FKBP^degron, day 4-7
H2
H7
Frequency
D
13
4.0
6.0
1.20
0.42
1.40
1.18
0.52
1.26
Distance (log10)
C
RAD21-FKBP^degron, day 7 8h
clone C4
chr2:20-180Mb
chr2:130-150Mb
chr2:145-148Mb
DMSO
dTAG-13
=87
250kb
=49
50kb
=11
10kb
clone G9
=86
=7
D
RAD21-FKBP^degron, day 10 8h
C4
G9
=25
=16
E
RAD21-FKBP^degron, day 7 8h
C4
chr5:23-32Mb
K27
RAD21
26Mb
30Mb
25kb
F
WAPL-FKBP^degron, day d4-7
H2
H7
=80
=117
=11
G
Rhodes et al. (2020)
mESCs, auxin
Tir1
Rad21
Hoxa
Hoxd
Hoxb
K27
peaks
Hoxb
Hoxc
Hoxa
40kb
80kb
H
RAD21-FKBP^degron, day 7 8h
Hox clusters
Aggregate Hi-C signal in:
DMSO
dTAG-13
12.3
I
RAD21-FKBP^degron, day 10 8h
Hox clusters
9.7
11.2
J
WAPL-FKBP^degron, day 4-7
Hox clusters
9.0
11

Figure S3

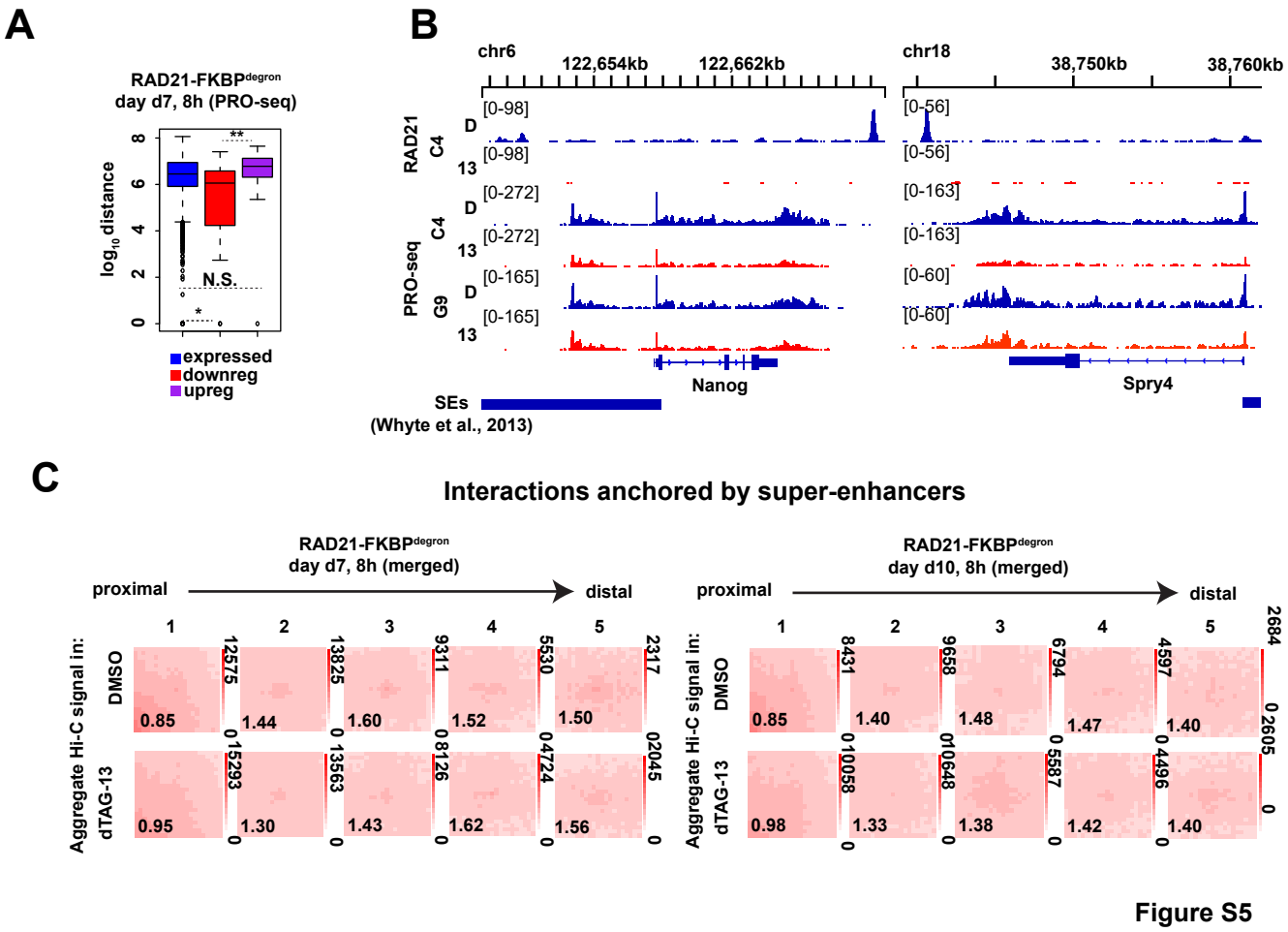
A
RAD21-FKBP^degron
day d7, 8h (PRO-seq)
log10 distance
N.S.
expressed
downreg
upreg
B
chr6
122,654kb
122,662kb
chr18
38,750kb
38,760kb
RAD21
PRO-seq
Nanog
Spry4
SEs
(Whyte et al., 2013)
C
Interactions anchored by super-enhancers
RAD21-FKBP^degron
day d7, 8h (merged)
day d10, 8h (merged)
proximal
distal
Aggregate Hi-C signal in:
DMSO
dTAG-13

Figure S5

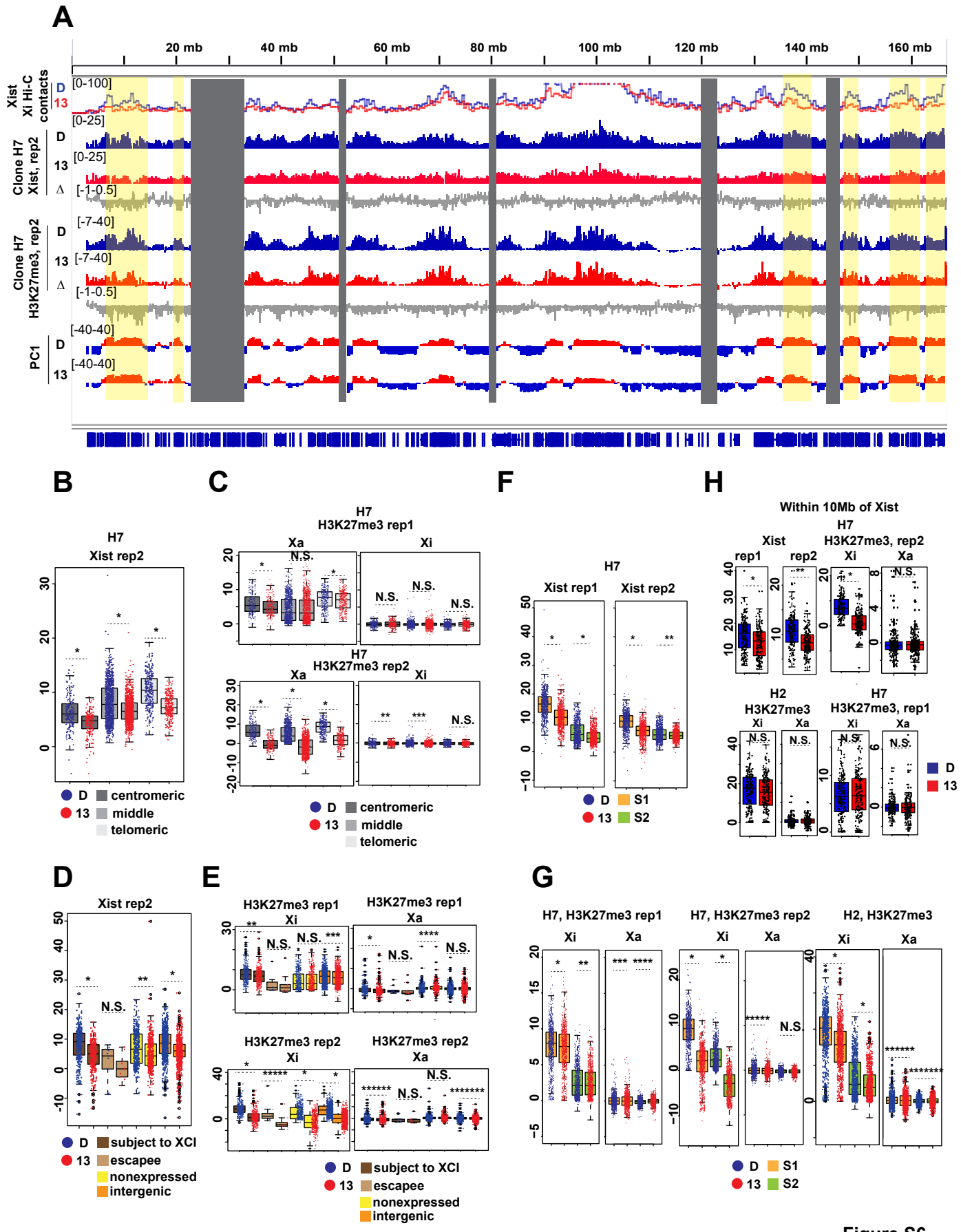
A
20 mb
40 mb
60 mb
80 mb
100 mb
120 mb
140 mb
160 mb
Xist Xi Hi-C contacts
D
13
[0-100]
Clone H7 Xist, rep2
[0-25]
Δ
[-1-0.5]
Clone H7 H3K27me3, rep2
[-7-40]
PC1
[-40-40]
B
H7
Xist rep2
D
13
centromeric
middle
telomeric
C
H7
H3K27me3 rep1
Xa
Xi
N.S.
H7
H3K27me3 rep2
F
H7
Xist rep1
Xist rep2
S1
S2
H
Within 10Mb of Xist
H7
Xist
rep1
rep2
H7
H3K27me3, rep2
H2
H3K27me3
H7
H3K27me3, rep1
D
E
H3K27me3 rep1
H3K27me3 rep2
subject to XCI
escapee
nonexpressed
intergenic
G
H7, H3K27me3 rep1
H7, H3K27me3 rep2
H2, H3K27me3

Figure S6