Supplemental Information

Supplementary Figures

Figure S1

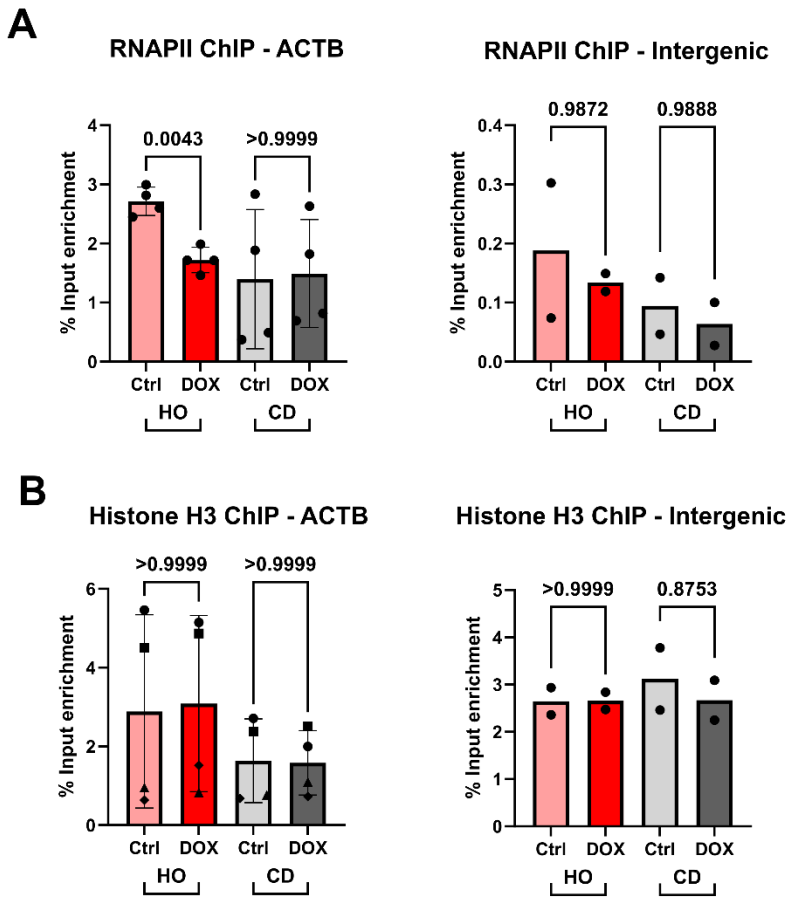


Figure S1. Control loci do not exhibit changes in nucleosome occupancy upon TRC induction on the episomal reporter system *in vivo*, Related to Figure 1

A) ChIP-qPCR analysis for RNAPII levels at beta-Actin (ATCB) (n=4) and Intergenic control locus (n=2) of mAIRN HO and CD cells treated with 1 µg/mL DOX for 24 h. Bars indicate mean values with standard deviations (SD) Ordinary one-way ANOVA with Tukey's multiple comparison test.

B) ChIP-qPCR analysis for histone H3 at ACTB (n=4) and Intergenic (n=2) in the same conditions as in **A)**.

Figure S2

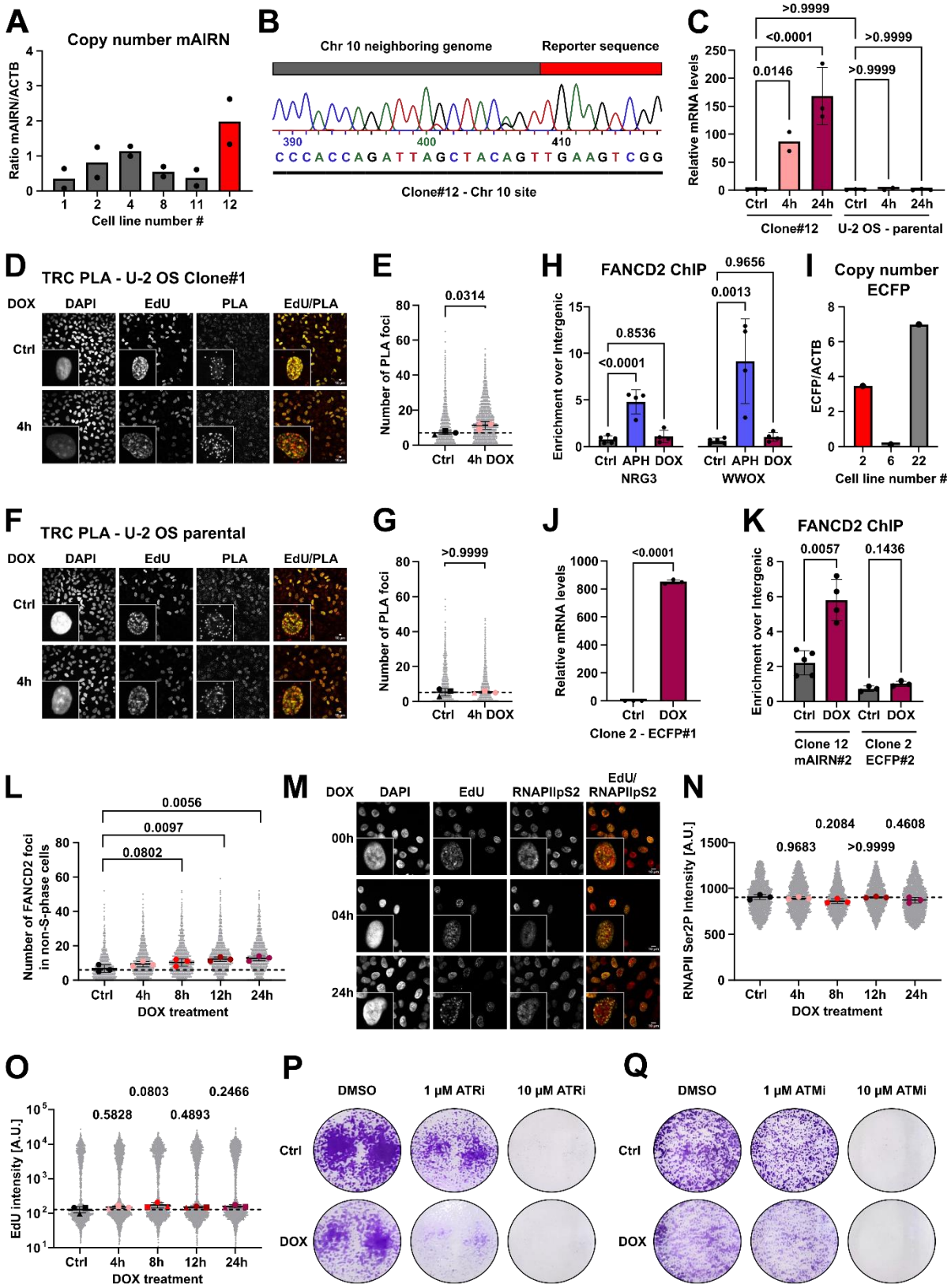

A
Copy number mAIRN
Ratio mAIRN/ACTB
Cell line number #
B
Chr 10 neighboring genome
Reporter sequence
390
400
410
C C C A C C A G A T T A G C T A C A G T T G A A G T C G G
Clone#12 - Chr 10 site
C
Relative mRNA levels
>0.9999
<0.0001
>0.9999
0.0146
>0.9999
Ctrl 4h 24h Ctrl 4h 24h
Clone#12
U-2 OS - parental
D
TRC PLA - U-2 OS Clone#1
DOX DAPI EdU PLA EdU/PLA
Ctrl
4h
E
Number of PLA foci
0.0314
Ctrl 4h DOX
H
FANCD2 ChIP
Enrichment over Intergenic
0.8536
<0.0001
0.9656
0.0013
Ctrl APH DOX
NRG3
Ctrl APH DOX
WWOX
I
Copy number ECFP
ECFP/ACTB
2 6 22
Cell line number #
F
TRC PLA - U-2 OS parental
DOX DAPI EdU PLA EdU/PLA
Ctrl
4h
G
Number of PLA foci
>0.9999
Ctrl 4h DOX
J
Relative mRNA levels
<0.0001
Ctrl DOX
Clone 2 - ECFP#1
K
FANCD2 ChIP
Enrichment over Intergenic
0.0057
0.1436
Ctrl DOX Ctrl DOX
Clone 12 mAIRN#2
Clone 2 ECFP#2
L
Number of FANCD2 foci in non-S-phase cells
0.0056
0.0097
0.0802
Ctrl 4h 8h 12h 24h
DOX treatment
M
DOX DAPI EdU RNAPIIpS2 EdU/ RNAPIIpS2
00h
04h
24h
N
RNAPII Ser2P Intensity [A.U.]
0.2084
0.4608
0.9683
>0.9999
Ctrl 4h 8h 12h 24h
DOX treatment
O
EdU intensity [A.U.]
0.0803
0.2466
0.5828
0.4893
Ctrl 4h 8h 12h 24h
DOX treatment
P
DMSO 1 μM ATRi 10 μM ATRi
Ctrl
DOX
Q
DMSO 1 μM ATMi 10 μM ATMi
Ctrl
DOX

Figure S2. Characterization of monoclonal cell lines with integrated chromosomal TRC reporter genes, Related to Figure 2

A) qPCR based DNA copy number analysis of several monoclonal cell lines generated by Sleeping Beauty-based integration of the mAIRN reporter sequence into the genome of U-2 OS cells. Signal is expressed as a ratio of mAIRN over ACTB (n=2).

B) Sanger-sequencing verification of integration site on chr 10 in Clone#12 cells. The displayed fragment of a Sanger-sequencing read shows overlap with both the 5'-region of the reporter construct and the upstream genomic sequence of chr 10.

C) RT-qPCR analysis of mAIRN RNA expression using primer pair mAIRN#1 in Clone#12 or parental U-2 OS cells treated with 0 or 1 µg/mL DOX for 4 h or 24 h. Error bars indicate mean values with standard deviations (SD). Ordinary one-way ANOVA with Tukey's multiple comparison test.

D) Representative images of TRC PLA with RNAPII Ser2P and PCNA antibodies in U2OS Clone#1 cells (Ctrl and 4 h time points). Additional EdU click-it staining was performed to label S-phase cells. Cells were treated with 0 or 1 µg/mL DOX for TRC induction. Scale bar 10 µm.

E) Quantification of TRC PLA foci number in S-phase cells from D) (n=3). Bars indicate mean values with standard deviations (SD). Unpaired t-test.

F) Representative images of TRC PLA with RNAPII Ser2P and PCNA antibodies in parental U-2 OS cells without any integrations (Ctrl and 4 h time points). Additional EdU click-it staining was performed to label S-phase cells. Cells were treated with 0 or 1 µg/mL DOX for TRC induction. Scale bar 10 µm.

G) Quantification of TRC PLA foci number in S-phase cells from D) (n=3). Bars indicate mean values with standard deviations (SD). Unpaired t-test.

H) ChIP-qPCR analysis showing FANCD2 levels in asynchronous cells using primer pairs targeting the common fragile site (CFS) genes NRG3 and WWOX. Cells were treated with 0 or 1 µg/mL DOX or alternatively 0.4 µM Aphidicolin (APH) for 24 h to induce FANCD2 at CFS genes (n=4). Error bars indicate SD. Ordinary one-way ANOVA with Tukey's multiple comparison test.

I) qPCR based DNA copy number analysis of several monoclonal cell lines generated by Sleeping Beauty-based integration of the ECFP reporter sequence into the genome of U-2 OS cells. Signal is expressed as a ratio of ECFP over ACTB (n=1).

J) RT-qPCR analysis of mAIRN RNA expression using primer pair ECFP#1 in Clone#2 cells treated with 0 or 1 µg/mL DOX for 4 h or 24 h. Error bars indicate SD. Student's t-test.

K) ChIP-qPCR analysis showing FANCD2 levels in Clone#12 and Clone#2 cells at the mAIRN#2 and ECFP#2 loci, respectively. Cells were treated with 0 or 1 µg/mL DOX 24 h (n=3). Error bars indicate SD. Ordinary one-way ANOVA with Tukey's multiple comparison test.

L) Quantification of FANCD2 foci number in non-S-phase cells (related to Figure 2G and 2H) (n=3). Bars indicate mean values with standard deviations (SD). Ordinary one-way ANOVA with Tukey's multiple comparison test.

M) Representative images of RNAPII pS2 immunofluorescence staining (Ctrl, 4 h and 24 h time points). Additional EdU click-it staining was performed to label S-phase cells. Cells were treated with 0 or 1 µg/mL DOX for TRC induction. Scale bar 10 µm.

N) Quantification of RNAPII Ser2P intensity in arbitrary units (A.U.) in all cells from J) as well as additional time points (n=3). Bars indicate mean values with standard deviations (SD). Ordinary one-way ANOVA with Tukey's multiple comparison test.

O) Quantification of EdU intensity in A.U. in all cells from J) as well as additional time points (n=3). Bars indicate mean values with standard deviations (SD). Ordinary one-way ANOVA with Tukey's multiple comparison test.

P) Crystal violet staining after proliferation assay (168 h) of cells treated with 0 or 1 µg/mL DOX combined with 1 or 10 µM ATRi or DMSO control treatment. Related to Figure 2K.

Q) Crystal violet staining after proliferation assay (168 h) of cells treated with 0 or 1 µg/mL DOX combined with 1 or 10 µM ATMi or DMSO control treatment. Related to Figure 2L.

Figure S3

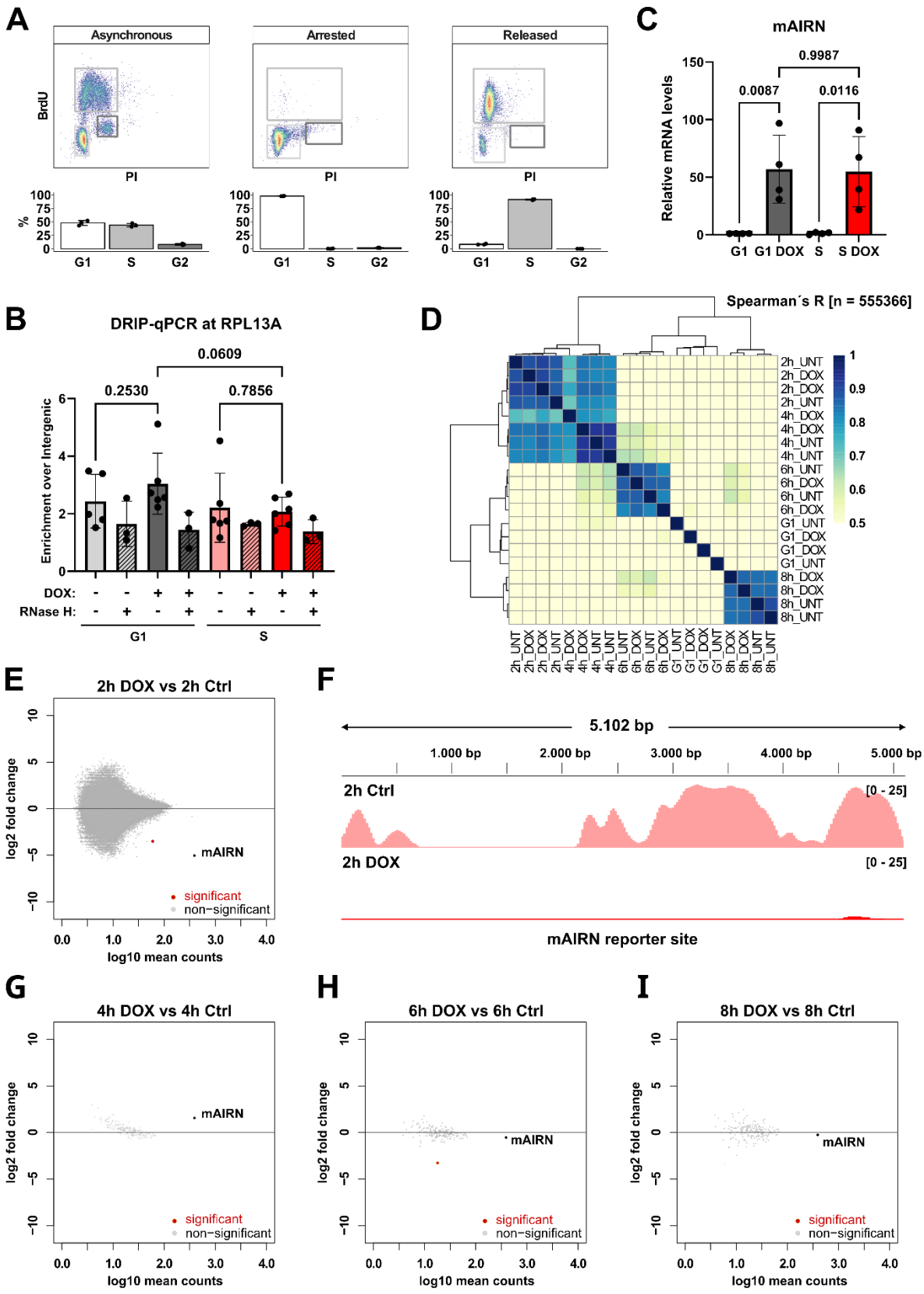

A
Asynchronous
Arrested
Released
BrdU
PI
%
G1
S
G2
C
mAIRN
Relative mRNA levels
0.9987
0.0087
0.0116
G1
G1 DOX
S
S DOX
B
DRIP-qPCR at RPL13A
0.0609
0.2530
0.7856
Enrichment over Intergenic
DOX:
RNase H:
G1
S
D
Spearman´s R [n = 555366]
E
2h DOX vs 2h Ctrl
log2 fold change
log10 mean counts
mAIRN
significant
non−significant
F
5.102 bp
1.000 bp
2.000 bp
3.000 bp
4.000 bp
5.000 bp
2h Ctrl
[0 - 25]
2h DOX
[0 - 25]
mAIRN reporter site
G
4h DOX vs 4h Ctrl
H
6h DOX vs 6h Ctrl
I
8h DOX vs 8h Ctrl

Figure S3. TRC-driven DNA replication fork progression impairments strictly match the replication timing of the chromosomal reporter sites, Related to Figure 3

A) BrdU flow cytometry analysis of asynchronous, double thymidine block arrested, and 4 h S-phase released cells. Frames in the cell distribution graphs show gating of G1, S, and G2 cell populations. Quantification of percentage of cells in each cell cycle phase shown below (n=3).

B) DRIP-qPCR analysis showing R-loop levels at the RPL13A positive control site in G1 or 4 h released S-phase cell treated with 0 or 1 µg/mL DOX. For RNase H conditions, isolated genomic DNA from cells was treated with E. coli RNase H1 overnight to degrade R-loops (n≥3). Error bars indicate SD. Ordinary one-way ANOVA with Tukey's multiple comparison test.

C) RT-qPCR analysis of mAIRN gene expression levels (mAIRN#1) in G1 or 4 h released S-phase cell treated with 0 or 1 µg/mL DOX (n=4). Error bars indicate SD. Ordinary one-way ANOVA with Tukey's multiple comparison test.

D) Heatmap showing replicate correlation (Spearman's R) of both BrdU-seq replicates for all treatment conditions and time points. ´n´ indicates the number of genomic bins.

E) MA plot showing differential regulation of global BrdU-seq signal, 5 kb bins. Significant bins are highlighted in red, not significantly changed bins are shown in grey.

F) Genome browser snapshot of BrdU-seq signal at the integrated mAIRN reporter construct at the 2 h S-phase timepoint in 0 or 1 µg/mL DOX treated cells, replicate 2.

G) MA plot showing differential regulation of BrdU-seq signal at the 4 h S-phase release time point comparing DOX vs Ctrl conditions in the +/- 100 kb regions around the integration sites shown in Fig. 3D, 5 kb bins. Significant bins are highlighted in red, not significantly changed bins are shown in grey.

H) Same as G) for the 6 h S-phase release time point.

I) Same as G) for the 8 h S-phase release time point.

Figure S4

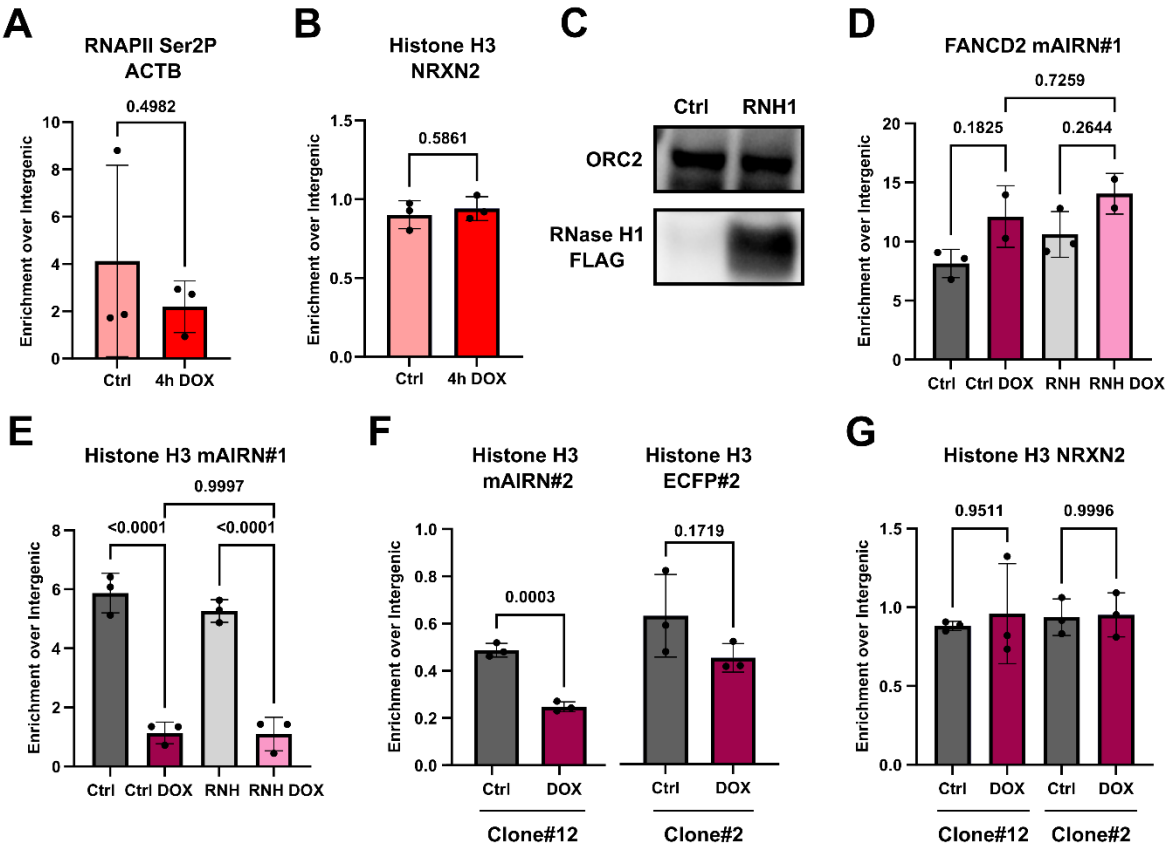

A
RNAPII Ser2P
ACTB
0.4982
Enrichment over Intergenic
Ctrl
4h DOX
B
Histone H3
NRXN2
0.5861
Enrichment over Intergenic
Ctrl
4h DOX
C
Ctrl
RNH1
ORC2
RNase H1
FLAG
D
FANCD2 mAIRN#1
0.7259
0.1825
0.2644
Enrichment over Intergenic
Ctrl
Ctrl DOX
RNH
RNH DOX
E
Histone H3 mAIRN#1
0.9997
<0.0001
<0.0001
Enrichment over Intergenic
Ctrl
Ctrl DOX
RNH
RNH DOX
F
Histone H3
mAIRN#2
Histone H3
ECFP#2
0.0003
0.1719
Enrichment over Intergenic
Ctrl
DOX
Ctrl
DOX
Clone#12
Clone#2
G
Histone H3 NRXN2
0.9511
0.9996
Enrichment over Intergenic
Ctrl
DOX
Ctrl
DOX
Clone#12
Clone#2

Figure S4. Fork stalling and nucleosome disruption are driven by stable RNase H1 resistant mAIRN R-loops, Related to Figure 4

A) ChIP-qPCR analysis showing RNAPII Ser2P levels at the ACTB control locus in synchronized S-phase cells 4 h after release from double thymidine block. Cells were treated with 0 or 1 µg/mL DOX for 4 h (n=3). Error bars indicate SD. Welch's t-test.

B) ChIP-qPCR analysis showing histone H3 levels at the NRXN2 control locus in the same conditions as in **A)** (n=3).

C) Representative Western Blot showing RNase H1-FLAG levels in Clone#12 upon overexpression of RNase H1 (RNH1) or an empty vector plasmid (Ctrl) for 24h. ORC2 loading control.

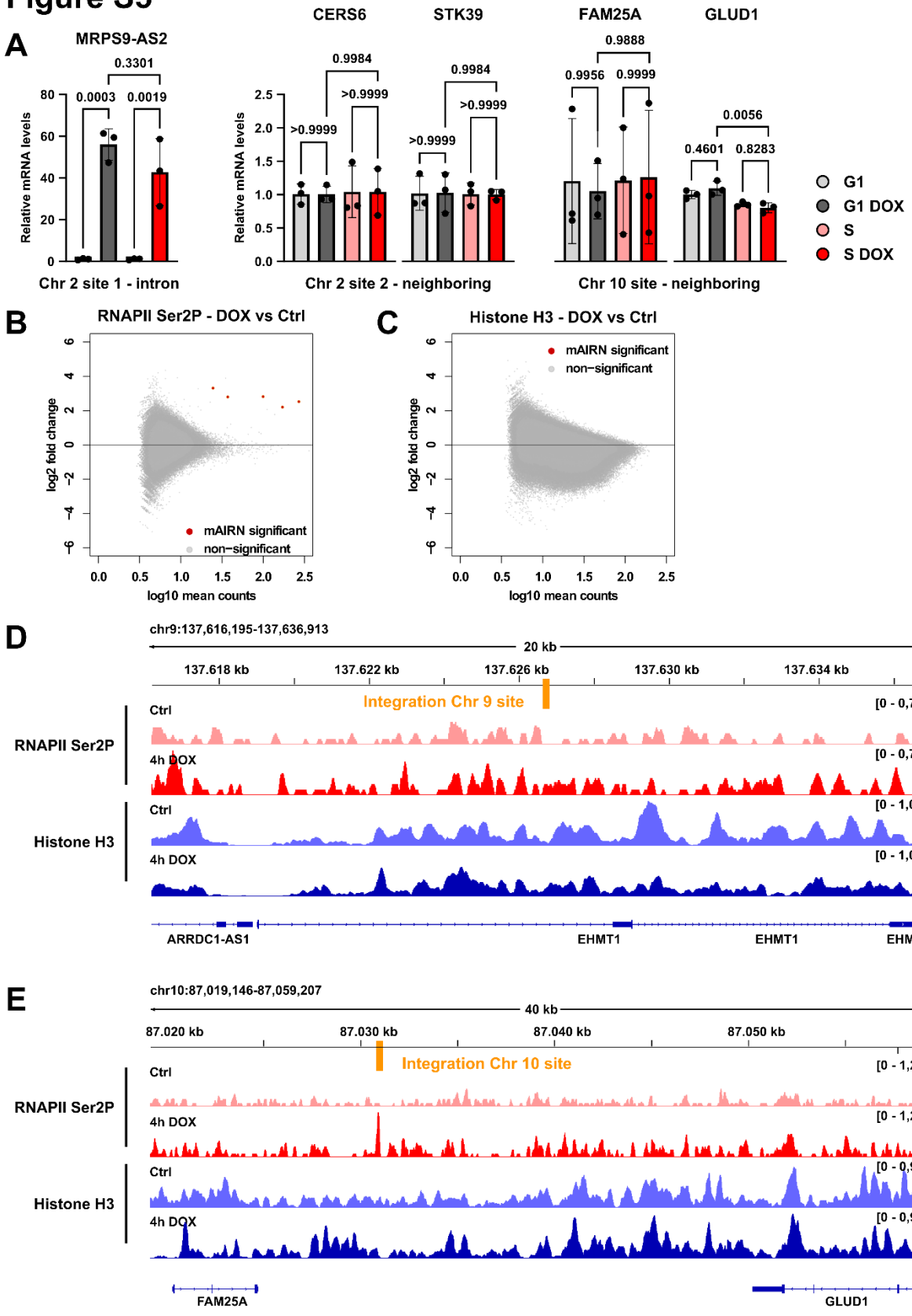
D) ChIP-qPCR analysis of FANCD2 levels in Clone#12 cells at the mAIRN#1 locus upon overexpression of RNase H1 or an empty vector plasmid for 24h. Cells were treated with 0 or 1 µg/mL DOX for 24 h (n≥2). Error bars indicate SD. Ordinary one-way ANOVA with Tukey's multiple comparison test.

E) ChIP-qPCR analysis showing histone H3 levels at the mAIRN#1 locus in the same conditions as in **D)** (n=3).

F) ChIP-qPCR analysis of Histone H3 levels in Clone#12 and Clone#2 cells at the mAIRN#2 and ECFP#2 loci, respectively. Cells were treated with 0 or 1 µg/mL DOX for 24 h (n=3). Error bars indicate SD. Student's test.

G) ChIP-qPCR analysis for NRXN2 control locus in Clone#12 and Clone#2 cells in the same conditions as in **F)**. Error bars indicate SD. Ordinary one-way ANOVA with Tukey's multiple comparison test.

Figure S5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MRPS9-AS2
CERS6
STK39
FAM25A
GLUD1
Relative mRNA levels
0.3301
0.0003
0.0019
0.9984
>0.9999
>0.9999
>0.9999
0.9984
>0.9999
0.9888
0.9956
0.9999
0.0056
0.4601
0.8283
Chr 2 site 1 - intron
Chr 2 site 2 - neighboring
Chr 10 site - neighboring
G1
G1 DOX
S
S DOX
B
RNAPII Ser2P - DOX vs Ctrl
log2 fold change
log10 mean counts
mAIRN significant
non−significant
C
Histone H3 - DOX vs Ctrl
mAIRN significant
non−significant
log2 fold change
log10 mean counts
D
chr9:137,616,195-137,636,913
20 kb
137.618 kb
137.622 kb
137.626 kb
137.630 kb
137.634 kb
Integration Chr 9 site
RNAPII Ser2P
Ctrl
4h DOX
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Histone H3
Ctrl
4h DOX
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[0 - 1,07]
ARRDC1-AS1
EHMT1
EHMT1
EHMT1
E
chr10:87,019,146-87,059,207
40 kb
87.020 kb
87.030 kb
87.040 kb
87.050 kb
Integration Chr 10 site
RNAPII Ser2P
Ctrl
4h DOX
[0 - 1,25]
[0 - 1,25]
Histone H3
Ctrl
4h DOX
[0 - 0,91]
[0 - 0,91]
FAM25A
GLUD1

Figure S5. TRC induction does not disrupt global transcription and nucleosome dynamics, Related to Figure 4

A) RT-qPCR analysis of gene expression levels of genes containing or neighboring different reporter integration sites. Synchronized G1 or 4 h released S-phase cells were treated with 0 or 1 µg/mL DOX for 4 h. (n=3). Error bars indicate SD. Ordinary one-way ANOVA with Tukey's multiple comparison test.

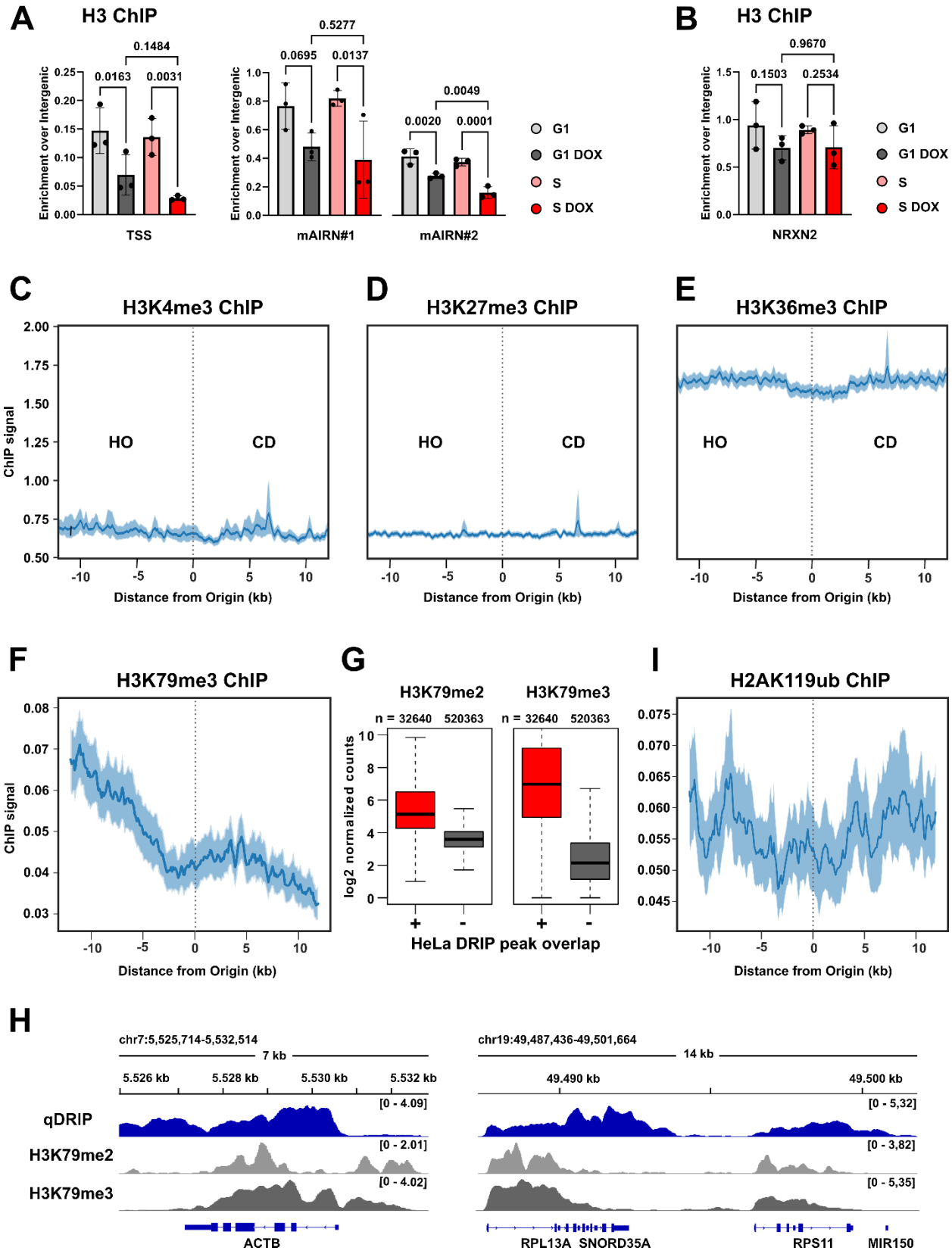
B) MA plot showing differential regulation of global RNAPII Ser2P ChIP signal, 1 kb bins. Significant mAIRN bins are highlighted in red, not significantly changed bins are shown in grey.

C) MA plot showing differential regulation of global histone H3 ChIP signal, 1 kb bins. Significant mAIRN bins are highlighted in red, not significantly changed bins are shown in grey.

D) Representative genome browser snapshot of a 20 kb region around the Chr 9 integration site (highlighted bar) showing RNAPII Ser2P and histone H3 occupancy in DOX treated or untreated conditions in synchronized S-phase cells.

E) Representative genome browser snapshot of a 40 kb region around the Chr 10 integration site (highlighted bar) showing RNAPII Ser2P and histone H3 occupancy in DOX treated or untreated conditions in synchronized S-phase cells.

Figure S6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H3 ChIP
Enrichment over Intergenic
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0.0163
0.0031
0.5277
0.0695
0.0137
0.0049
0.0020
0.0001
TSS
mAIRN#1
mAIRN#2
G1
G1 DOX
S
S DOX
B
H3 ChIP
0.9670
0.1503
0.2534
NRXN2
C
H3K4me3 ChIP
ChIP signal
HO
CD
Distance from Origin (kb)
D
H3K27me3 ChIP
E
H3K36me3 ChIP
F
H3K79me3 ChIP
G
H3K79me2
H3K79me3
n = 32640 520363
log2 normalized counts
HeLa DRIP peak overlap
I
H2AK119ub ChIP
H
chr7:5,525,714-5,532,514
7 kb
5.526 kb
5.528 kb
5.530 kb
5.532 kb
qDRIP
H3K79me2
H3K79me3
[0 - 4.09]
[0 - 2.01]
[0 - 4.02]
ACTB
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14 kb
49.490 kb
49.500 kb
[0 - 5,32]
[0 - 3,82]
[0 - 5,35]
RPL13A SNORD35A
RPS11
MIR150

Figure S6. H3K79 methylation is enriched at genomic TRC and R-loop sites, Related to Figure 5

A) Histone H3 ChIP-qPCR in G1 or 4 h released S-phase cell treated with 0 or 1 µg/mL DOX. H3 levels were tested at the reporter sequence with primers at TSS, mAIRN#1, or mAIRN#2 (n=3). Error bars indicate SD. Ordinary one-way ANOVA with Tukey's multiple comparison test.

B) Same as in A) with primers at the NRXN2 control locus.

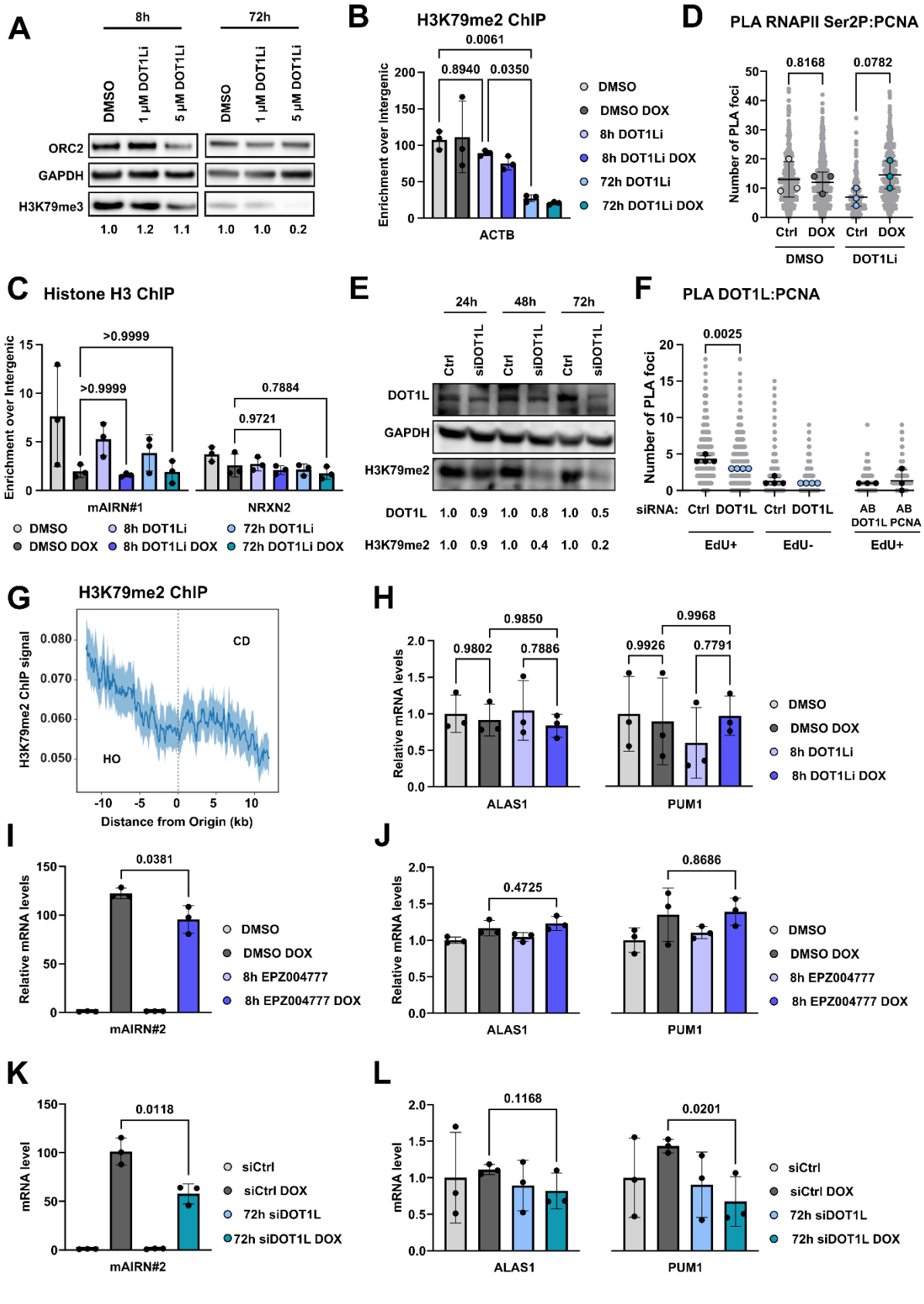
C) Analysis of H3K4me3, **D)** H3K27me3, **E)** H3K36me3, and **F)** H3K79me3 ChIP signal from HeLa cells at genic origins within actively transcribed genes. The analysis windows around the regions are 24 kb in size and at least 5 kb away from promoters and terminators. Error bands represent a 95 % confidence interval as determined by a bootstrap of the mean.

G) Box-plot comparing H3K79me2 and H3K79me3 ChIP signal at sites overlapping (+) or not overlapping (-) with R-loops. Bin size 5 kb. ´n´ indicates number of genomic bins.

H) Genome browser snapshot of two genomic example regions with R-loop-prone genes from chr 7 and chr 19 showing overlapping qDRIP-seq, H3K79me2, and H3K79me3 ChIP-seq signal in HeLa cells.

I) Same as in **C)** for H2AK119ub in MCF7 cells.

Figure S7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72h
DMSO
1 μM DOT1Li
5 μM DOT1Li
ORC2
GAPDH
H3K79me3
1.0 1.2 1.1 1.0 1.0 0.2
B
H3K79me2 ChIP
0.0061
0.8940
0.0350
Enrichment over Intergenic
ACTB
DMSO
DMSO DOX
8h DOT1Li
8h DOT1Li DOX
72h DOT1Li
72h DOT1Li DOX
D
PLA RNAPII Ser2P:PCNA
0.8168
0.0782
Number of PLA foci
Ctrl DOX Ctrl DOX
DMSO
DOT1Li
C
Histone H3 ChIP
>0.9999
>0.9999
0.7884
0.9721
Enrichment over Intergenic
mAIRN#1
NRXN2
DMSO
DMSO DOX
8h DOT1Li
8h DOT1Li DOX
72h DOT1Li
72h DOT1Li DOX
E
24h
48h
72h
Ctrl
siDOT1L
DOT1L
GAPDH
H3K79me2
DOT1L 1.0 0.9 1.0 0.8 1.0 0.5
H3K79me2 1.0 0.9 1.0 0.4 1.0 0.2
F
PLA DOT1L:PCNA
0.0025
Number of PLA foci
siRNA: Ctrl DOT1L Ctrl DOT1L AB DOT1L AB PCNA
EdU+
EdU-
EdU+
G
H3K79me2 ChIP
H3K79me2 ChIP signal
CD
HO
Distance from Origin (kb)
H
0.9850
0.9802
0.7886
0.9968
0.9926
0.7791
Relative mRNA levels
ALAS1
PUM1
DMSO
DMSO DOX
8h DOT1Li
8h DOT1Li DOX
I
0.0381
Relative mRNA levels
mAIRN#2
DMSO
DMSO DOX
8h EPZ004777
8h EPZ004777 DOX
J
0.4725
0.8686
Relative mRNA levels
ALAS1
PUM1
DMSO
DMSO DOX
8h EPZ004777
8h EPZ004777 DOX
K
0.0118
mRNA level
mAIRN#2
siCtrl
siCtrl DOX
72h siDOT1L
72h siDOT1L DOX
L
0.1168
0.0201
mRNA level
ALAS1
PUM1
siCtrl
siCtrl DOX
72h siDOT1L
72h siDOT1L DOX

Figure S7. Effect of DOT1L inhibition on H3K79 methylation and transcription at control sites, Related to Figure 6

A) Representative Western blot of H3K79me3 upon 1 µM and 5 µM DOT1L inhibition (EPZ-5676) or DMSO control treatment for 8 h or 72 h. GAPDH and ORC2 loading controls. Quantification of H3K79me3 signal shown below.

B) H3 normalized H3K79me2 ChIP-qPCR in cells treated with 5 µM DOT1L inhibition (EPZ-5676) for 8 h and 72 h as well as 0 or 1 µg/mL DOX. H3K79me2 levels at the control site were tested with the ACTB intron 3 (ACTB#in3) primers (n=3). Error bars indicate SD. Ordinary one-way ANOVA with Tukey's multiple comparison test.

C) Histone H3 ChIP-qPCR at the mAIRN#1 and NRXN2 control locus in the same conditions as in **B)**.

D) Quantification of TRC PLA foci (RNAPII Ser2P and PCNA antibodies) in S-phase cells. Cells were treated with DMSO or 720 or 1 µg/mL DOX for 4 h (n=3). Bars indicate mean values with standard deviations (SD). Unpaired t-test.

E) Representative Western Blot of DOT1L and H3K79me2 levels upon knockdown of DOT1L (siDOT1L) for 24 h, 48 h, and 72 h compared to non-targeting siRNA control (Ctrl). GAPDH loading control. Quantification of DOT1L and H3K79me2 signal shown below.

F) Quantification of PLA foci (DOT1L and PCNA antibodies) in EdU+ or EdU- cells. Cells were treated with Ctrl or siDOT1L (n=4). Single antibody controls for PCNA and DOT1L show the level of background signal (n=3). Bars indicate mean values with standard deviations (SD). Unpaired t-test.

G) Analysis of H3K79me2 ChIP signal from MOLM13 cells at intragenic origins within actively transcribed genes previously defined in HeLa cells. The analysis windows around the regions are 24 kb in size and excluded from the analysis if positioned within 5 kb from promoters and terminators. H3K79me2 signal accumulates at HO side of origins in gene bodies compared to the CD side. Error bands represent a 95 % confidence interval as determined by a bootstrap of the mean.

H) RT-qPCR analysis of RNA expression using primer pair ALAS1 and PUM1 in cells treated with 5 µM DOT1L inhibition (EPZ-5676) or DMSO control treatment for 8h. Additionally, 0 or 1 µg/mL DOX were added for 4 h (n=3). Error bars indicate mean

values with standard deviations (SD). Ordinary one-way ANOVA with Tukey's multiple comparison test.

I) RT-qPCR analysis of RNA expression at mAIRN#2 in cells treated with 25 µM DOT1L inhibition (EPZ-004777) or DMSO control treatment for 8h. Additionally, 0 or 1 µg/mL DOX were added for 4 h (n=3). Error bars indicate SD. Student's t-test.

J) Same as in **I)** for control loci ALAS1 and PUM1.

K) RT-qPCR analysis of RNA expression at mAIRN#2 in cells treated with siCtrl or siDOT1L for 72h. Additionally, 0 or 1 µg/mL DOX were added for 4 h (n=3). Error bars indicate SD. Student's t-test.

L) Same as in **I)** for control loci ALAS1 and PUM1.

Table S1: Integration site positions

CHROMOSOME	START LOCATION	END LOCATION	NAME	PHASE	STRAND
2	104941714	104941978	Chr 2 site 1	0	.
2	168374974	168375175	Chr 2 site 2	0	.
5	134521192	134521451	Chr 5 site	0	.
9	137626501	137626702	Chr 9 site	0	.
10	87030690	87030891	Chr 10 site	0	.

Table S2: Experimental model systems

REAGENT or RESOURCE	SOURCE	IDENTIFIER
U-2 OS Tet-ON	Takara Clontech	Michael Kirsch; Cat# 630919
HEK293 Tet-ON	Takara Clontech	Cat# 631182

Table S3: Antibodies

Antibody Name	SOURCE	IDENTIFIER
Monoclonal mouse IgM anti RNA polymerase II RPB1 (H5)	BioLegend	Cat#920204; RRID:AB_2616695
Polyclonal rabbit IgG anti PCNA	Abcam	Cat#ab18197; RRID:AB_444313
Polyclonal goat anti mouse IgG Alexa Fluor Plus 488 labeled	Invitrogen	Cat#A-32723, RRID:AB_2633275
Polyclonal goat anti rabbit IgG Alexa Fluor Plus 488 labeled	Invitrogen	Cat#A-11008; RRID:AB_143165
Polyclonal goat anti mouse IgG Alexa Fluor Plus 594 labeled	Invitrogen	Cat#A-11032; RRID:AB_2534091
Polyclonal goat anti rabbit IgG Alexa Fluor Plus 594 labeled	Invitrogen	Cat#A-11037; RRID:AB_2534095
Polyclonal goat anti mouse IgG Alexa Fluor Plus 647 labeled	Invitrogen	Cat#A-32728; RRID:AB_2866490
Polyclonal goat anti rabbit IgG Alexa Fluor Plus 647 labeled	Invitrogen	Cat#A-21245; RRID:AB_2535813
Monoclonal mouse IgG1 anti BrdU (B44)	BD bioscience	Cat#347580, RRID:AB_400326
Mouse monoclonal anti-RNA Polymerase II CTD Antibody (8WG16)	Merck	Cat#05-952; RRID: AB_11213782
Polyclonal rabbit anti human FANCD2	NovusBio	Cat#NB100-182; RRID:AB_10002867
Polyclonal rabbit IgG anti ORC2	Thermo Fisher Scientific	Cat#PA5-67313; RRID:AB_2663245
Monoclonal mouse IgG anti GAPDH (6C5)	Merck	Cat#CB1001-500UG; RRID:AB_2107426
Monoclonal rabbit IgG anti yH2A.X (Ser139) (20E3)	Cell Signaling Technology	Cat#9718S; RRID:AB_2118009

Monoclonal mouse IgG anti DNA-RNA-hybrid (S9.6)	Merck	Cat#MABE1095; RRID:AB_2861387
Polyclonal RNA polymerase II CTD repeat YSPTSPS (phospho S2)	Abcam	Cat#ab5095; RRID:AB_304749
Polyclonal rabbit anti Histone H3	Abcam	Cat#ab1791; RRID:AB_302613
Polyclonal rabbit anti Histone H3K4me3	Abcam	Cat#ab8580; RRID:AB_306649
Monoclonal rabbit anti Ubiquityl-Histone H2AK119ub (D27C4)	Cell Signaling Technology	Cat#8240S; RRID:AB_10891618
Polyclonal rabbit anti Histone H3K79me2	Active Motif	Cat#39143; RRID:AB_2561018
Polyclonal rabbit anti Histone H3K79me3	Abcam	Cat#ab195500; RRID:AB_2888917
Monoclonal rabbit IgG anti DOT1L (D1W4Z)	Cell Signaling Technology	Cat#77087; RRID:AB_2799889
Goat Anti-Rabbit IgG (H+L), Horseradish peroxidase conjugate	Invitrogen	Cat#G21234; RRID:AB_2536530
Goat Anti-mouse IgG (H+L), Horseradish peroxidase conjugate	Invitrogen	Cat#G21040; RRID:AB_2536527
DYKDDDDK (FLAG) Tag Polyclonal Antibody	Thermo Fisher Scientific	Cat# PA1-984B RRID:AB_347227

Table S4: Bacterial Strains

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Escherichia coli DH5alpha	Thermo Fisher Scientific	Cat#18265017

Table S5: Chemicals and recombinant proteins

REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Puromycin	Sigma Aldrich	Cat#P9620-10ML
Thymidine	Sigma-Aldrich	Cat#T9250-5G
5-Ethynyl-2'-deoxyuridine (EdU)	Carl Roth	Cat#7845.3
5-Bromo-2'-Deoxy-Uridine (BrdU)	Sigma Aldrich	Cat#B5002-1G
Lipofectamine 3000	Thermo Scientific	Cat#L3000001
Dulbecco's Modified Eagle Medium (DMEM)	Gibco	Cat# 41966-029
Penicillin-Streptomycin-Glutamin (100x) (PSG)	Gibco	Cat# 10378-016
Fetal bovine serum for cell culture (tetracycline-free) (FBS tet-)	Takara	Cat# 631106
Dulbecco's phosphate-buffered saline (PBS)	Gibco	Cat# 14190-094
0.25 % Trypsin-EDTA (1x)	Gibco	Cat# 25200-072
Opti-MEM I (1X) + GlutaMAX -I	Gibco	Cat# 51985-034
RNase A	Thermo Scientific	Cat#EN0531

E. coli RNase H	New England Biolabs	Cat#M0297S
DNase I	New England Biolabs	Cat# M0303S
MNase	New England Biolabs	Cat#M0247S
Proteinase K	SERVA	Cat#33756
TRIzol	Invitrogen	Cat#15596-026
phenol:chlorophorm:isoamyl alcohol (25:24:1; v/v)	Invitrogen	Cat# 15593-031
OmniPur Phenol:Chloroform:Isoamyl Alcohol, 25:24:1	Millipore	Cat#6805-100ML
Glycogen	Thermo Fisher Scientific	Cat#AM9510
Ampure XP beads	Beckman Coulter	Cat#A63880
Protein A/G agarose	Thermo Scientific	Cat#20421
Pierce Protein G Magnetic Beats	Thermo Scientific	Cat# 88847
Halt Protease-Inhibitor-Cocktail (100x)	Thermo Scientific	Cat#78429
Halt Protease and Phosphatase Inhibitor Cocktail (100x)	Thermo Scientific	Cat# 78446
Trizol reagent	Invitrogen	Cat# 15596018
Propidiumiodid	Invitrogen	Cat# P3566
SiR-DNA	Tebu-bio	Cat#SC007
4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI)	Sigma Aldrich	Cat# 32670
AlexaFluor 594 azide	ThermoFisher Scientific	Cat# A10270
AlexaFluor 488 azide	ThermoFisher Scientific	Cat# A10266
Ascorbic acid	Sigma-Aldrich	Cat#A7506-100G
BSA Fraction V	Sigma-Aldrich	Cat#10735078001
Hydroxyurea (HU)	Biomol	Cat#H9120.10
VE-821 (ATR inhibitor)	Biomol	Cat#Cay17587-5
KU-60019 (ATM inhibitor)	Sigma Aldrich	Cat#SML1416-5MG
EPZ-5676 (DOT1L inhibitor)	BPS Bioscience	Cat#27625
EPZ 004777	Tocris	Cat#5567
Aphidicolin (APH)	Santa Cruz	Cat#SC-201535
Doxycycline hydrochloride (DOX)	Sigma-Aldrich	Cat#D3447
Igepal CA-630	Sigma-Aldrich	Cat#I3021-100ML
Tween-20	Kraft	Cat#21440.2000
Triton X-100	Sigma-Aldrich	Cat#X100-100ML
Deoxycholic acid	Santa Cruz	Cat#sc-214865A
[α−32P] dATP	Hartmann Analytik	Cat#SCP-203

Table S6: Critical commercial assays

REAGENT or RESOURCE	SOURCE	IDENTIFIER
SuperSignal™ West Pico PLUS Chemiluminescent Substrate	ThermoFisher	Cat#34580

iTaq Universal SYBR Green Supermix	Bio-Rad; REF	Cat#1725121
Proteinase K	SERVA	Cat#33756
SuperScript III First-Strand Synthesis System	Thermo Fisher Scientific	Cat#18080-051
Invitrogen™ Click-iT™ EdU Cell Proliferation Kit for Imaging, Alexa Fluor 488 dye	Invitrogen	Cat#C10337
Invitrogen™ Click-iT™ EdU Cell Proliferation Kit for Imaging, Alexa Fluor 594 dye	Invitrogen	Cat#C10339
Duolink detection reagents (ligation/amplification) - FarRed	Sigma Aldrich	Cat#DUO92013
Duolink detection reagents (ligation/amplification)- Green	Sigma Aldrich	Cat# DUO92014
Duolink *In Situ* PLA Probe Anti-Mouse PLUS	Sigma Aldrich	Cat#DUO92001
Duolink *In Situ* PLA Probe Anti-Rabbit PLUS	Sigma Aldrich	Cat#DUO92002
Duolink *In Situ* PLA Probe Anti-Mouse MINUS	Sigma-Aldrich	Cat#DUO92004
Duolink *In Situ* PLA Probe Anti-Rabbit MINUS	Sigma Aldrich	Cat#DUO92005
RadPrime DNA labeling Kit	Thermo Fisher Scientific	Cat#18428011
NEBNext Ultra II DNA Library Kit with Purification Beads	New England Biolabs	Cat#E7103S
Qubit 1X dsDNA HS Kit	Thermo Fisher Scientific	Cat#Q33230
Gibson Assembly Cloning Kit	New England Biolabs	Cat# E5510S

Table S7: Oligonucleotides

PRIMER	**SOURCE**	**SEQUENCE 5'-3'**	**APPLICATION**
TSS FWD	This paper	ATGTCGAGGTAGGCGTGTAC	ChIP-qPCR
TSS REV	This paper	TGAAGCCTCGGGTACCGAGCTCGAATTC	ChIP-qPCR
mAIRN#1 FWD	This paper	TAGAGGATTCCGCAAAGGAA	ChIP-qPCR, DRIP, RT-qPCR, Copy number analysis
mAIRN#1 REV	This paper	TTCACCCTAGCGCTGAATCT	ChIP-qPCR, DRIP, RT-qPCR, Copy number analysis
mAIRN#2 FWD	This paper	CGAGAGAGGCTAAGGGTGAA	ChIP-qPCR, DRIP, RT-qPCR, Copy

			number analysis
mAIRN#2/ ECFP#2 REV	This paper	ACATGGTCCTGCTGGAGTTC	ChIP-qPCR, DRIP, RT-qPCR, Copy number analysis
ECFP#1 FWD	This paper	ACGTAAACGGCCACAAGTTC	ChIP-qPCR, RT-qPCR, Copy number analysis
ECFP#1 REV	This paper	AAGTCGTGCTGCTTCATGTG	ChIP-qPCR, RT-qPCR, Copy number analysis
ECFP#2 FWD	This paper	TGGTTTGTCCAAACTCATCAA	ChIP-qPCR, RT-qPCR, Copy number analysis
NRXN2 FWD	This paper	CGCAAAGCCCAGTTGTTCTG	ChIP-qPCR
NRXN2 REV	This paper	TTAAATTGGGGTTGCCGTGC	ChIP-qPCR
Intergenic FWD	This paper	CCAGGTGGGTCTCGAACTTC	ChIP-qPCR, DRIP
Intergenic REV	This paper	CAGGCTGGGCAACATACTGA	ChIP-qPCR, DRIP
ACTB FWD	This paper	CGGGGTCTTTGTCTGAGC	ChIP-qPCR, Copy number analysis
ACTB REV	This paper	CAGTTAGCGCCCAAAGGAC	ChIP-qPCR, Copy number analysis
ACTB#in3 FWD	(1)	TAACACTGGCTCGTGTGACAA	ChIP-qPCR
ACTB#in3 REV	(1)	AAGTGCAAAGAACACGGCTAA	ChIP-qPCR
M13 FWD	This paper	GTAAAACGACGGCCAGT	ChIP-qPCR, DRIP
M13 REV	This paper	CAGGAAACAGCTATGAC	ChIP-qPCR, DRIP
RPL13A FWD	This paper	AGGTGCCTTGCTCACAGAGT	DRIP
RPL13A REV	This paper	GGTTGCATTGCCCTCATTAC	DRIP
ACTB RT-qPCR	This paper	CCTGGCACCCAGCACAAT	RT-qPCR
ACTB RT-qPCR	This paper	GGGCCGGACTCGTCATACT	RT-qPCR
ALAS1 FWD	(2)	GGCAGCACAGATGAATCAGA	RT-qPCR

ALAS1 REV	(2)	CCTCCATCGGTTTTCACACT	RT-qPCR
PUM1 FWD	(2)	CAGGCTGCCTACCAACTCAT	RT-qPCR
PUM1 REV	(2)	GTTCCCGAACCATCTCATTC	RT-qPCR
MRSP9-AS2 FWD	This paper	GCTTGTGAGCAACCCAAGAA	RT-qPCR
MRSP9-AS2	This paper	CGGCATCTCGTTAGCTCTGA	RT-qPCR
CERS6 FWD	This paper	AAGCTGGGAGATCGTTGGAC	RT-qPCR
CERS6 REV	This paper	CATCCTTGGACACCTTGCCT	RT-qPCR
STK39 FWD	This paper	AGGAGGTTATCGGCAGTGGA	RT-qPCR
STK39 REV	This paper	CTGGTCTGGCATTTTTCCAAGT	RT-qPCR
FAM25A FWD	This paper	ATCCTAGTTCACCACTGTCTGC	RT-qPCR
FAM25A REV	This paper	CTTCCACGGCATGAATGGCTC	RT-qPCR
GLUD1 FWD	This paper	TGCAAGGGAGGTATCCGTTA	RT-qPCR
GLUD1 REV	This paper	CAAACGGCACATCAACCACT	RT-qPCR
MT073_gib_ins_FWD	This paper	CTATCTAGACGCCATATTAGTCATTGGTTATATAGCATAAATCAATATTGGCT	Cloning
MT073_gib_ins_REV	This paper	ATTGTTGTTGTTAGGTTTTACTTGCTTTAAAAAACCTCCCAC	Cloning
MT073_gib_bb_FWD	This paper	AAGTAAAACCTAACAACAACAATTGCATTCATTTTATGTTTCAGGT	Cloning
MT073_gib_bb_TEV	This paper	ACTAATATGGCGTCTAGATAGCGGACCCC	Cloning
Int_site chr10 FWD	This paper	CCACCCACATCCTGCTGATT	Sanger sequencing
siPOOL 2 - siDOT1L	siTOOLs Biotech	Cat#si-G020-84444	siRNA KD
siPOOL 2 - negative control	siTOOLs Biotech	Cat# si-C002	siRNA KD

Table S8: Plasmids

PLASMID	INSERT	CONSTRUCTION/SOURCE
K031_pSH26 1x LEXA	-	(3)
K069_pSH36 1xLEXA	-	(3)
K072_pSH37 1xLEXA	-	(3)
K191_pSB100	-	Tomas Zikmund

K192_pSBtet_DNMT3A_P2A_dsRed2	-	Tomas Zikmund
K275_pMT03_sb_mAIRN_puroR	Tight-TRE promoter_mAIRN-reporter_SV40-polyA	Tight-TRE promoter_mAIRN-reporter_SV40-polyA insert was amplified from K069_pSH36 1x LEXA with primers MT073_gib_ins_FWD and MT073_gib_ins_REV, Sleeping Beauty backbone from K192_pSBet_DNMT3A_P2A-dsRed2 was amplified using primers MT073_gib_bb_FWD and MT073_gib_bb_REV, insert and backbone were joined using Gibson assamebly
K276_pMT04_sb_ECFP_puroR	Tight-TRE promoter_ECFP - reporter_SV40-polyA	Tight-TRE promoter_ECFP-reporter_SV40-polyA insert was amplified from K031_pSH26 1x LEXA with primers MT073_gib_ins_FWD and MT073_gib_ins_REV, Sleeping Beauty backbone from K192_pSBet_DNMT3A_P2A-dsRed2 was amplified using primers MT073_gib_bb_FWD and MT073_gib_bb_REV, insert and backbone were joined using Gibson assamebly
K016_pcDNA3.1(+) ΔN1-27 RNAseH1-FLAG	-	(3)
K206_ pcDNA3.1(+)	-	(3)

Table S9: Software and algorithms

REAGENT or RESOURCE	**SOURCE**	**WEB LINK**
ImageJ	NIH	https://imagej.net/ij/index.html
FlowJo (version 10)	BD Bioscience	
bwa (version 0.7.17)	(4)	https://github.com/lh3/bwa
samtools (version 1.16.1 and 1.17)	(5)	https://www.htslib.org/doc/samtools.html
tiddit (version 3.3.2)	(6)	https://github.com/SciLifeLab/TIDDIT/releases/tag/TIDDIT-3.3.2
bedtools (version 2.31.0)	(7)	https://github.com/arq5x/bedtools2/releases/tag/v2.31.0
rtracklayer (version 1.54.0)	R Core Team	https://bioconductor.org/packages/3.18/bioc/html/rtracklayer.html
GenomicRanges (version 1.46.1)	(8)	https://bioconductor.org/packages/release/bioc/html/GenomicRanges.html
R v4.1.2	R Core Team	https://www.r-project.org
trim_galore (version 0.6.10)	(9)	https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/
bowtie2 (version 2.5.1)	(5)	https://bowtie-bio.sourceforge.net/bowtie2/index.shtml
picard MarkDuplicates (version 3.0.0)	Broad Institute	https://broadinstitute.github.io/picard/

deeptools (version 3.5.2)	(10)	https://deeptools.readthedocs.io/en/develop/index.html
DESeq2 package (version 1.34.0)	(11)	https://github.com/thelovelab/DESeq2
sva package (version 3.42.0)	(12)	https://bioconductor.org/packages/release/bioc/html/sva.html
pheatmap (version 1.0.12)	Raivo Kolde	https://CRAN.R-project.org/package=pheatmap
macs2 (version 2.2.9.1)	(13)	https://pypi.org/project/MACS2/
Fusion (version 2.3)	Andor Oxford Instruments	https://andor.oxinst.com/downloads/view/fusion-release-2.3

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