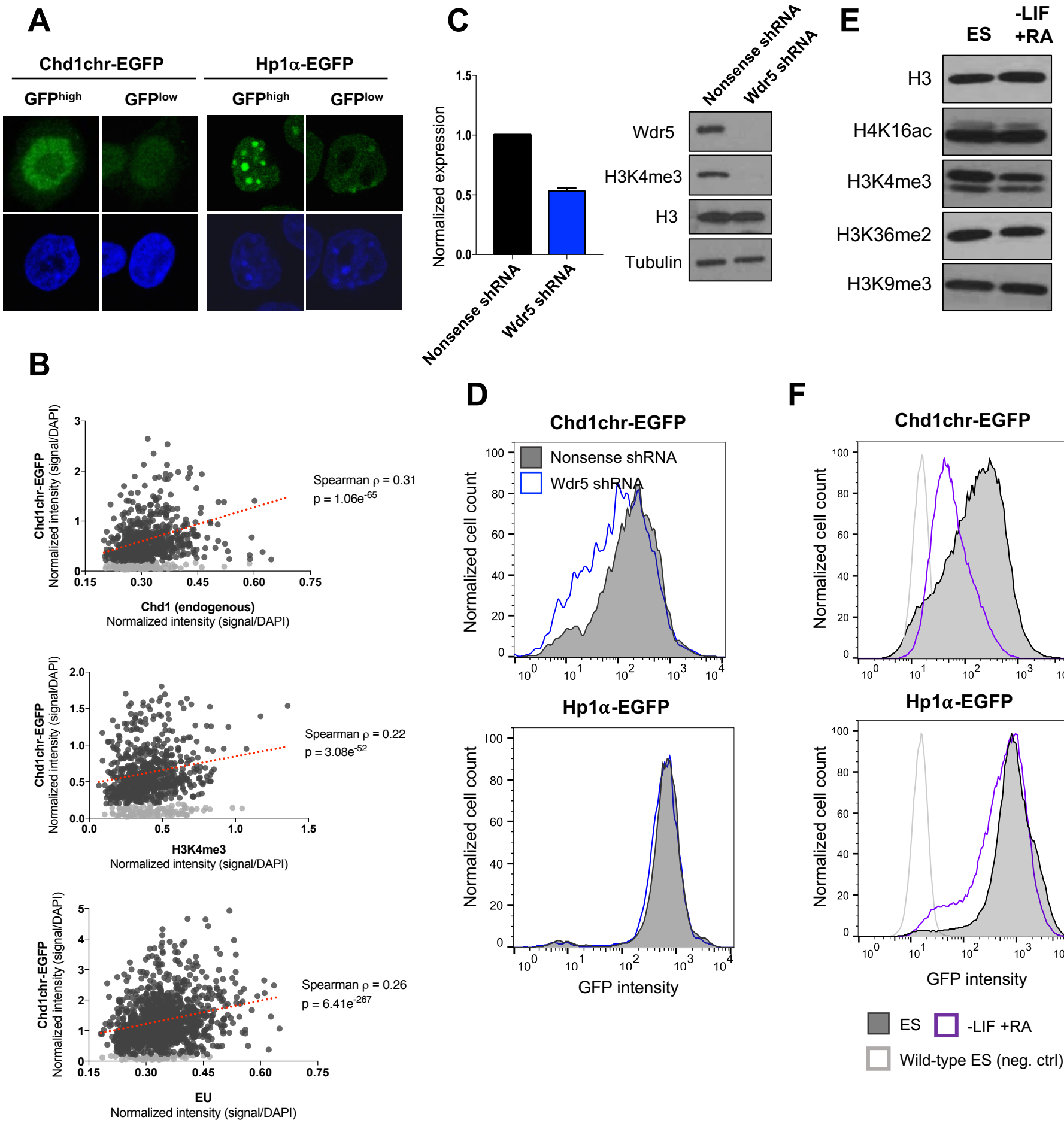

A
Chd1chr-EGFP
Hp1α-EGFP
GFPhigh
GFPlow
GFPhigh
GFPlow
B
Chd1chr-EGFP
Normalized intensity (signal/DAPI)
Spearman ρ = 0.31
p = 1.06e-65
Chd1 (endogenous)
Normalized intensity (signal/DAPI)
Chd1chr-EGFP
Normalized intensity (signal/DAPI)
Spearman ρ = 0.22
p = 3.08e-52
H3K4me3
Normalized intensity (signal/DAPI)
Chd1chr-EGFP
Normalized intensity (signal/DAPI)
Spearman ρ = 0.26
p = 6.41e-267
EU
Normalized intensity (signal/DAPI)
C
Normalized expression
Nonsense shRNA
Wdr5 shRNA
Nonsense shRNA
Wdr5 shRNA
Wdr5
H3K4me3
H3
Tubulin
D
Chd1chr-EGFP
Nonsense shRNA
Wdr5 shRNA
Normalized cell count
Hp1α-EGFP
Normalized cell count
GFP intensity
E
ES
-LIF +RA
H3
H4K16ac
H3K4me3
H3K36me2
H3K9me3
F
Chd1chr-EGFP
Normalized cell count
Hp1α-EGFP
Normalized cell count
GFP intensity
ES
-LIF +RA
Wild-type ES (neg. ctrl)

Figure S1, related to Figure 1. Characterization of Chd1chr-EGFP and Hp1α-EGFP reporters in ES cells.

(A) Fluorescence imaging of the Chd1chr-EGFP and Hp1α-EGFP reporters.

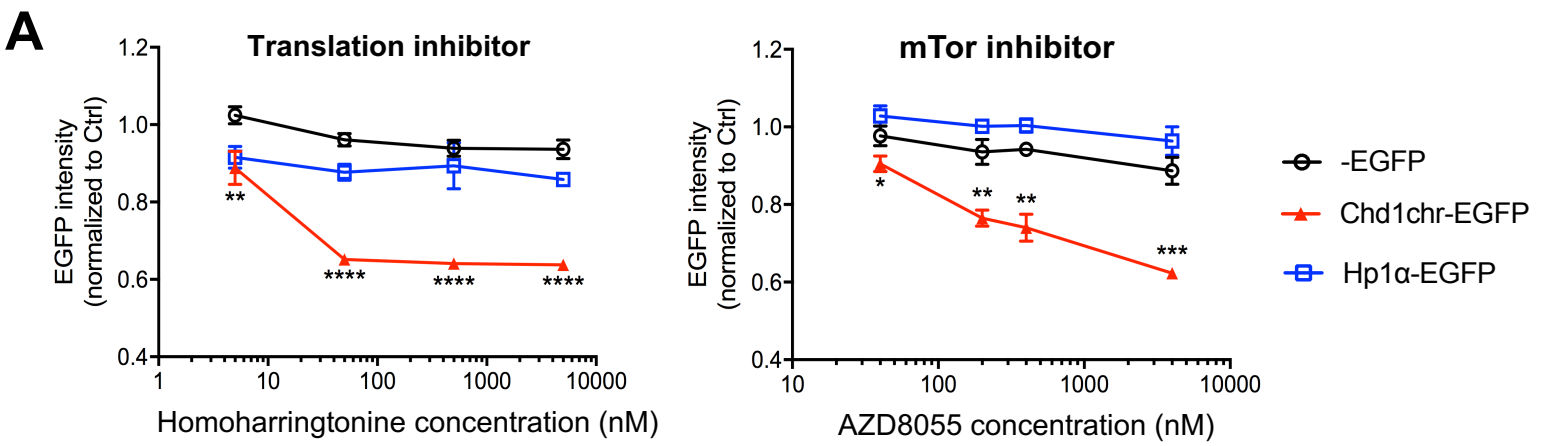
(B) Correlation of Chd1chr-EGFP reporter signal with endogenous Chd1, H3K4me3 and nascent transcription (EU). single-cell quantification of immunofluorescence for the indicated markers was performed. Cells with background levels of EGFP signal (grey points) were removed from the analysis.

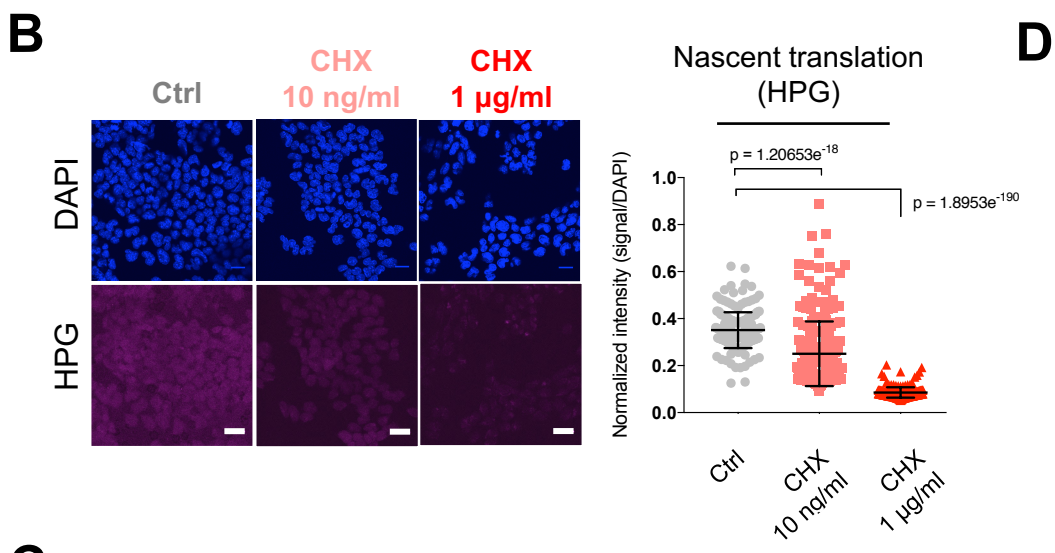
(C) mRNA and protein expression levels of Wdr5, a component of the MLL1 complex that deposits H3K4 methylation, upon transduction of ES cells with non-targeting or Wdr5-specific shRNAs.

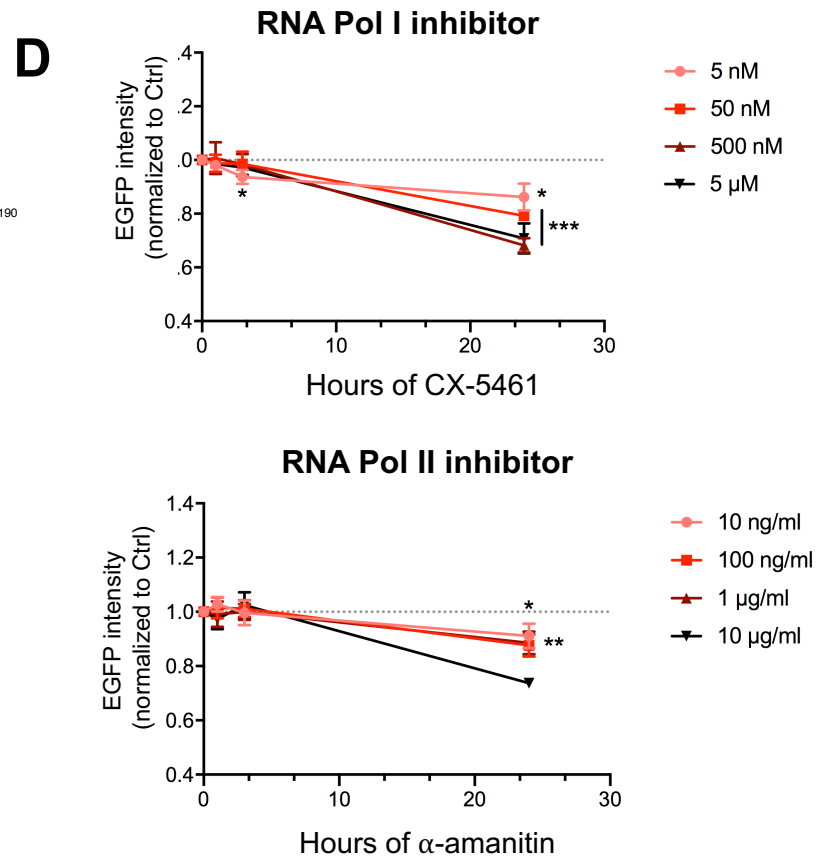
(D) Flow cytometry analysis of Chd1chr-EGFP and Hp1α-EGFP reporter fluorescence levels upon knock-down of Wdr5. Fluorescence was assayed 3 days post-transduction.

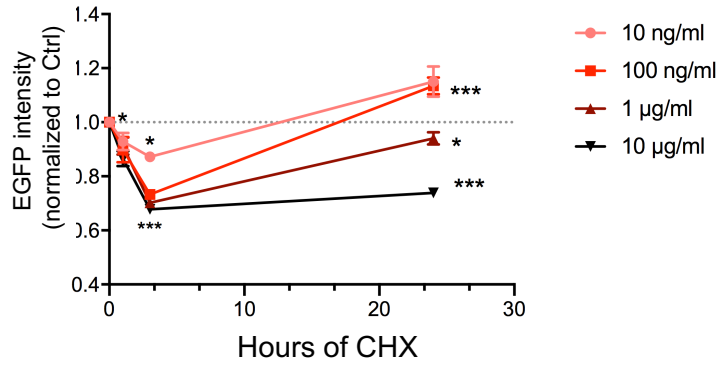
(E) Analysis of chromatin marks upon RA-mediated differentiation of ES cells for 2 days. ES cells grown in serum/LIF were used as control.

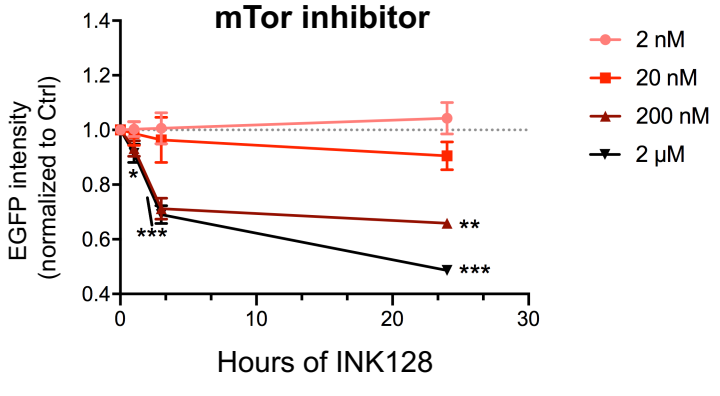
(F) Analysis of reporter fluorescence upon RA-mediated differentiation of ES cells for 2 days. ES cells grown in serum/LIF were used as control. Wild-type, non-fluorescent ES cells were used as negative controls for flow cytometry. A minimum of two biological replicates were performed for all experiments.

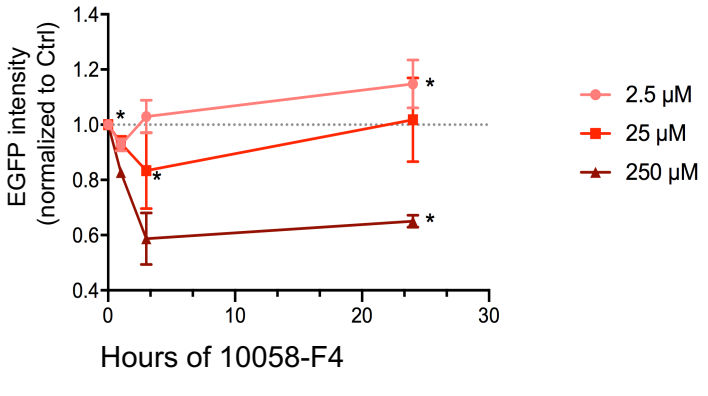

A
Translation inhibitor
EGFP intensity (normalized to Ctrl)
Homoharringtonine concentration (nM)
mTor inhibitor
AZD8055 concentration (nM)
-EGFP
Chd1chr-EGFP
Hp1α-EGFP

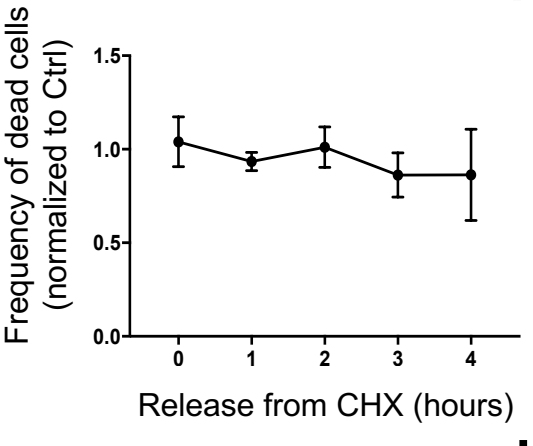

B
Ctrl
CHX 10 ng/ml
CHX 1 µg/ml
DAPI
HPG
Nascent translation (HPG)
Normalized intensity (signal/DAPI)
p = 1.20653e-18
p = 1.8953e-190
Ctrl
CHX 10 ng/ml
CHX 1 µg/ml

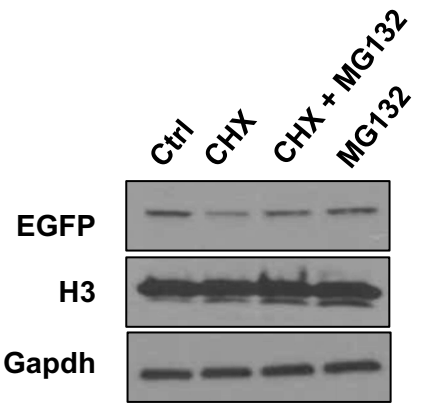

D
RNA Pol I inhibitor
EGFP intensity (normalized to Ctrl)
Hours of CX-5461
5 nM
50 nM
500 nM
5 µM
RNA Pol II inhibitor
Hours of α-amanitin
10 ng/ml
100 ng/ml
1 µg/ml
10 µg/ml

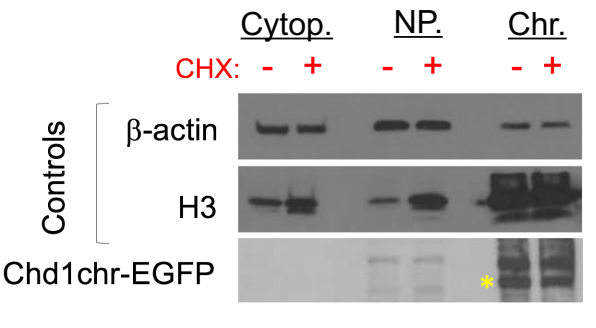

C
Translation inhibitor
EGFP intensity (normalized to Ctrl)
Hours of CHX
10 ng/ml
100 ng/ml
1 µg/ml
10 µg/ml


mTor inhibitor
EGFP intensity (normalized to Ctrl)
Hours of INK128
2 nM
20 nM
200 nM
2 µM


Myc inhibitor
EGFP intensity (normalized to Ctrl)
Hours of 10058-F4
2.5 µM
25 µM
250 µM


E
Frequency of dead cells (normalized to Ctrl)
Release from CHX (hours)


F
Ctrl
CHX
CHX + MG132
MG132
EGFP
H3
Gapdh


G
Cytop.
NP.
Chr.
CHX: - + - + - +
Controls
β-actin
H3
Chd1chr-EGFP

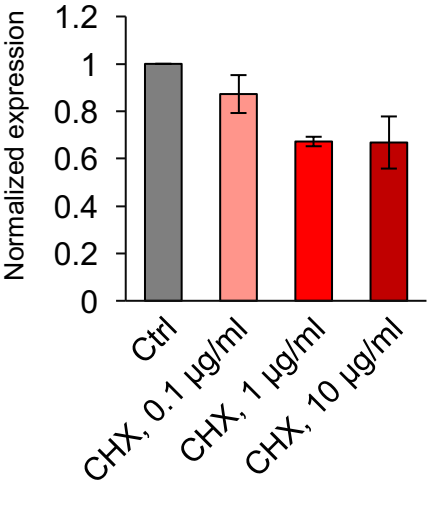

H
Normalized expression
Ctrl
CHX, 0.1 µg/ml
CHX, 1 µg/ml
CHX, 10 µg/ml

Figure S2, related to Figure 2. Characterization of the reporter response to small molecule-mediated inhibition of indicated cellular pathways.

(A) Response of the Chd1chr-EGFP, Hp1α-EGFP and control EGFP ES cells to inhibition of translation or mTor for 3 hours using independent inhibitors from those in Figure 2. Cells were treated with DMSO as control. Graphs show mean ± SD of at least 3 technical replicates and are representative of 2 biological replicates. Statistical significance was determined by a two-tailed Student's t-test.

(B) Fluorescence imaging of nascent translation by HPG incorporation upon DMSO or CHX treatment. Scale bars represent 20 μm. Right panel shows quantification of HPG signal. Statistical analysis performed is Mann Whitney *U* test. Error bars represent mean ± SD of at least 3 technical replicates.

(C) Chd1chr-EGFP reporter fluorescence levels upon treatment with varying doses of translation, mTor and Myc inhibitors for up to 24 hours. Cells were treated with DMSO as control.

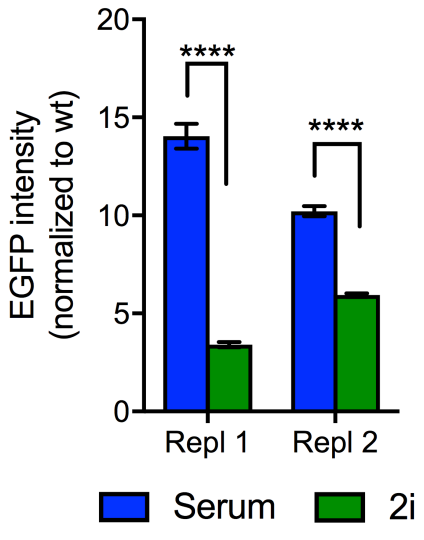
(D) Chd1chr-EGFP reporter fluorescence levels upon treatment with varying doses of Pol I and Pol II inhibitors for up to 24 hours. Cells were treated with DMSO as control.

(E) Assessment of cell death of CHX-treated ES cells by SYTOX Blue incorporation. Error bars show mean ± SD of 4 technical replicates and are representative of at least 3 biological replicates.

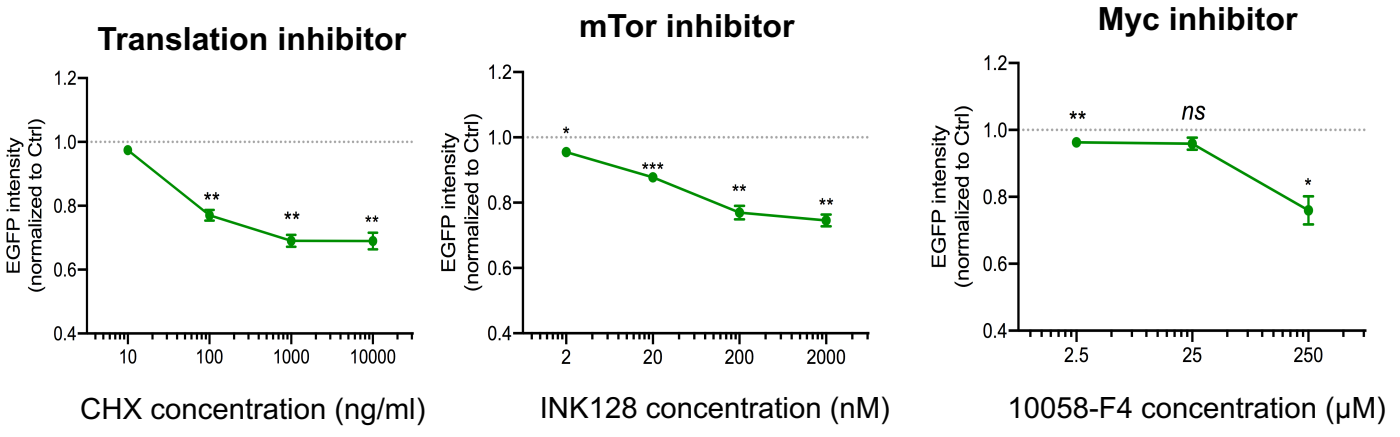
(F) Partial rescue of Chd1chr-EGFP fusion protein levels in whole-cell extracts upon inhibition of translation (CHX) ± inhibition of the proteasome (MG132). Error bars show mean ± SD of 2 biological replicates. Statistical tests are two-tailed t-test with Welch's correction when applicable. **, ***, **** = $p<0.01$, 0.001, 0.0001.

(G) Chd1chr-EGFP protein levels in the cytoplasm, nucleoplasm and chromatin upon DMSO or CHX treatment (1 mg/ml) for 3 hours. Asterisk denotes the specific band with correct molecular weight. (H) Chd1chr-EGFP mRNA expression levels upon DMSO or CHX treatment for 3 hours. Error bars show mean ± SD of 3 technical replicates. Graph is representative of 2 biological replicates.

A


EGFP intensity (normalized to wt)
20
15
10
5
0
****
****
Repl 1
Repl 2
Serum
2i

B


Translation inhibitor
EGFP intensity (normalized to Ctrl)
1.2
1.0
0.8
0.6
0.4
**
**
**
10
100
1000
10000
CHX concentration (ng/ml)
mTor inhibitor
EGFP intensity (normalized to Ctrl)
*
***
**
**
2
20
200
2000
INK128 concentration (nM)
Myc inhibitor
EGFP intensity (normalized to Ctrl)
**
ns
*
2.5
25
250
10058-F4 concentration (μM)

C

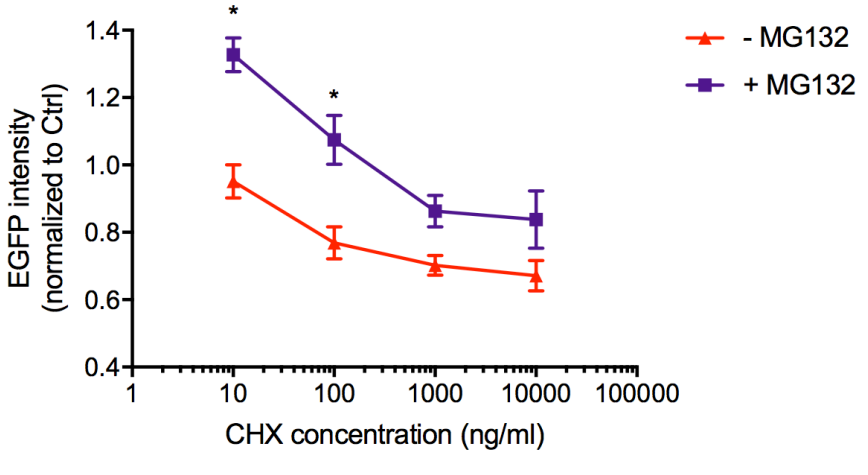

*
*
1.4
1.2
1.0
0.8
0.6
0.4
EGFP intensity (normalized to Ctrl)
1
10
100
1000
10000
100000
CHX concentration (ng/ml)
- MG132
+ MG132

Figure S3, related to Figure 2. Reporter expression and sensitivity to inhibition of translation and growth pathways in 2i conditions.

(A) EGFP reporter expression in cells cultured in 2i or serum conditions. Fluorescence signal was normalized to wild-type (non-fluorescent) E14 cells. Error bars show mean ± SD of at least 8 technical replicates.

(B) Normalized fluorescence levels of the Chd1chr-EGFP reporter in 2i/LIF upon small molecule-mediated inhibition of indicated pathways for 3 hours.

(C) Normalized fluorescence levels of the Chd1chr-EGFP reporter in 2i/LIF upon partial rescue of the effects of CHX ± proteasome inhibition by MG132. Data represent mean ± SD of 2 biological replicates. *, **, *** = $p<0.01$, 0.001, 0.0001; ns = not significant.

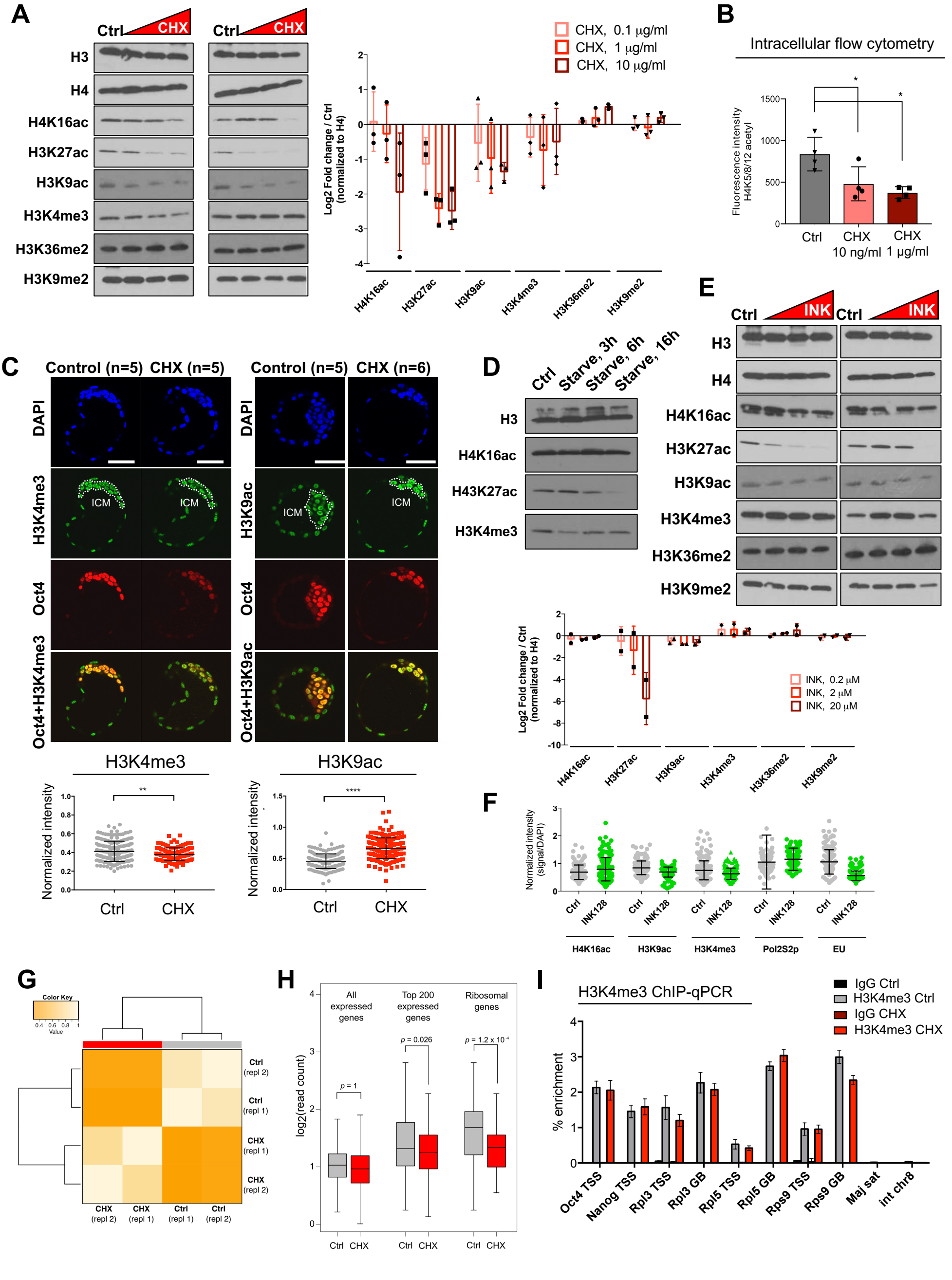

A
Ctrl
CHX
H3
H4
H4K16ac
H3K27ac
H3K9ac
H3K4me3
H3K36me2
H3K9me2
CHX, 0.1 μg/ml
CHX, 1 μg/ml
CHX, 10 μg/ml
Log2 Fold change / Ctrl (normalized to H4)
B
Intracellular flow cytometry
Fluorescence intensity H4K5/8/12 acetyl
Ctrl
CHX 10 ng/ml
CHX 1 μg/ml
C
Control (n=5)
CHX (n=5)
Control (n=5)
CHX (n=6)
DAPI
H3K4me3
H3K9ac
Oct4
Oct4+H3K4me3
Oct4+H3K9ac
ICM
H3K4me3
H3K9ac
Normalized intensity
Ctrl
CHX
D
Ctrl
Starve, 3h
Starve, 6h
Starve, 16h
H3
H4K16ac
H43K27ac
H3K4me3
E
Ctrl
INK
H3
H4
H4K16ac
H3K27ac
H3K9ac
H3K4me3
H3K36me2
H3K9me2
Log2 Fold change / Ctrl (normalized to H4)
INK, 0.2 μM
INK, 2 μM
INK, 20 μM
F
Normalized intensity (signal/DAPI)
Ctrl
INK128
H4K16ac
H3K9ac
H3K4me3
Pol2S2p
EU
G
Color Key
Value
Ctrl (repl 2)
Ctrl (repl 1)
CHX (repl 1)
CHX (repl 2)
H
All expressed genes
Top 200 expressed genes
Ribosomal genes
p = 1
p = 0.026
p = 1.2 x 10-4
log2(read count)
Ctrl
CHX
I
H3K4me3 ChIP-qPCR
IgG Ctrl
H3K4me3 Ctrl
IgG CHX
H3K4me3 CHX
% enrichment
Oct4 TSS
Nanog TSS
Rpl3 TSS
Rpl3 GB
Rpl5 TSS
Rpl5 GB
Rps9 TSS
Rps9 GB
Maj sat
int chr8

Figure S4, related to Figure 3. Chromatin response to inhibition of translation in ES cells and blastocysts.

(A) Biological replicates of the western blot analysis shown in Figure 3A. Right panel shows quantification of the 3 biological replicates.

(B) Intracellular flow cytometry analysis of H4 acetylation (H4K5,8,12) in DMSO- or CHX-treated (3 hours) ES cells. Data shown are representative of 2 biological replicates. Statistical significance was determined by Mann Whitney *U* test. **** = $p<0.0001$.

(C) Immunofluorescent detection of H3K4me3 and H3K9ac in control or CHX-treated (3 hours, 1 mg/ml) E4.5 blastocysts. Scale bars denote 50 μm. Bottom panels show quantification of the H3K4me3 or H3K9ac signal in each Oct4+ cell. Statistical significance was determined by Welch's two tailed t-test. **, *** = $p<0.01$, 0.001.

(D) Western blot analysis of euchromatin marks in response to serum starvation for the indicated durations. Histone extracts from unstarved cells were used as controls. Figure represents two biological replicates.

(E) Western blot analysis of euchromatin and heterochromatin marks in response to 3h treatment with the mTor inhibitor INK128. Data are quantified and reported as in (A).

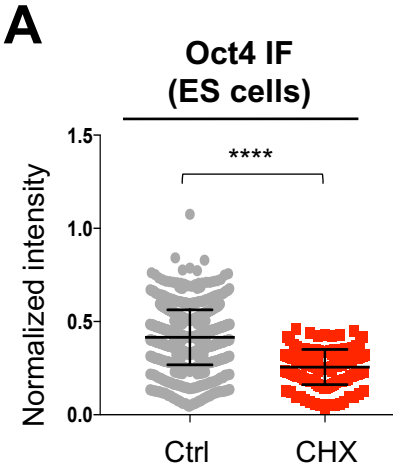
(F) Quantification of immunofluorescence staining of chromatin marks and nascent transcription (EU) in E4.5 blastocysts incubated with INK128. Blastocysts were treated as in Figure 3C.

(G) Heatmap of H4K16ac ChIP-seq replicate correlation at the top 1000 most highly expressed genes in ES cells.

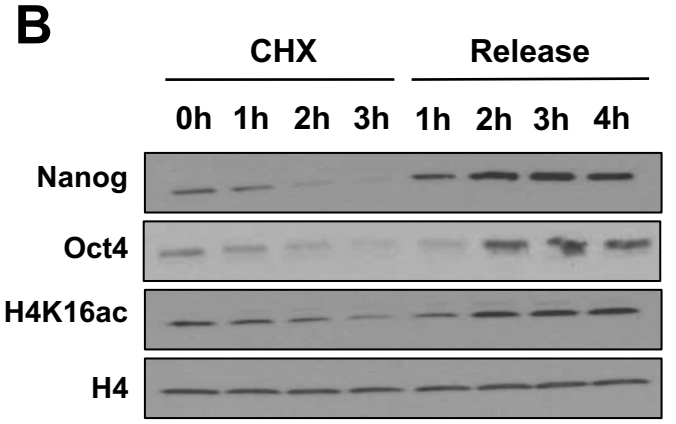
(H) H4K16ac ChIP-seq read abundance over all expressed genes or gene subsets.

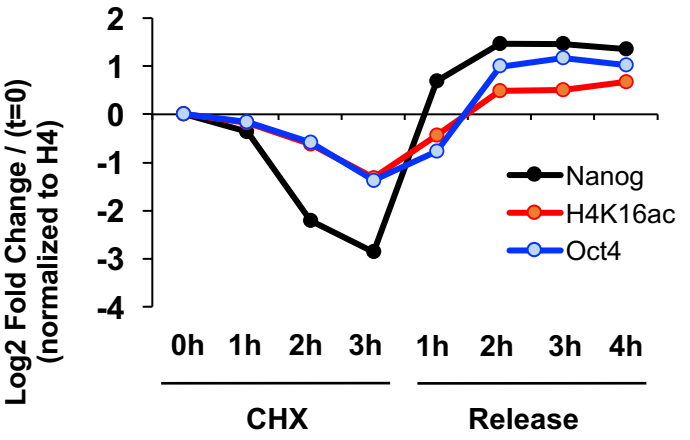
(I) ChIP-qPCR for H3K4me3 enrichment over TSSs and gene bodies in DMSO- or CHX-treated cells (1 μg/ml, 3 hrs). Error bars show mean ± SD of 3 technical replicates. Graph is representative of 2 biological replicates.

A

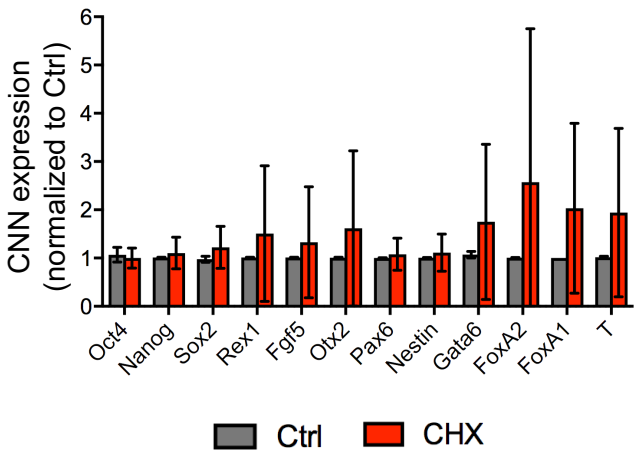


B

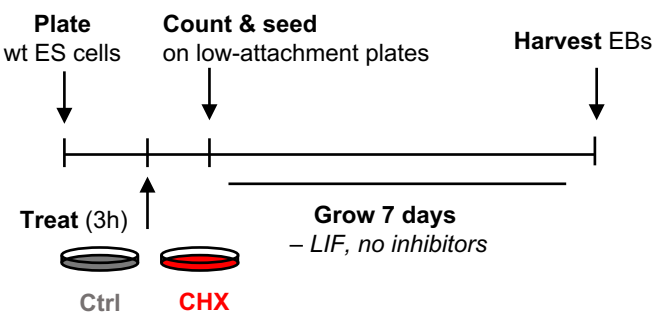


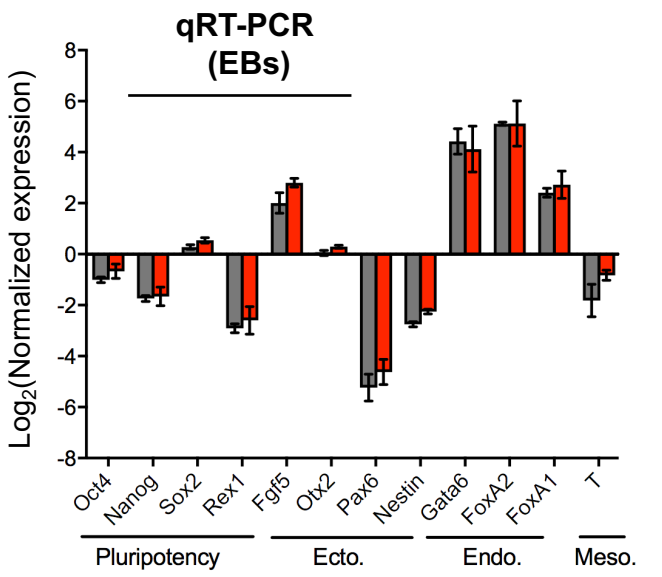


C

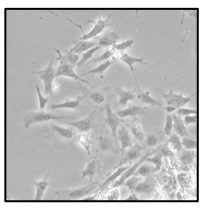


D

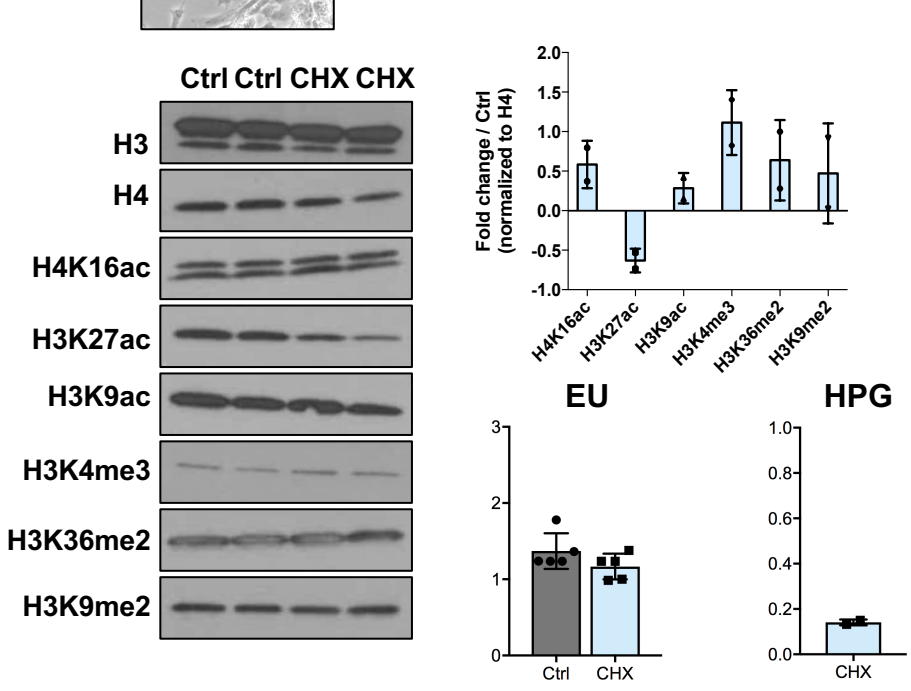




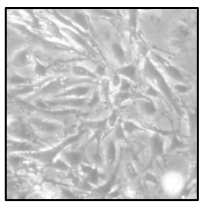
E



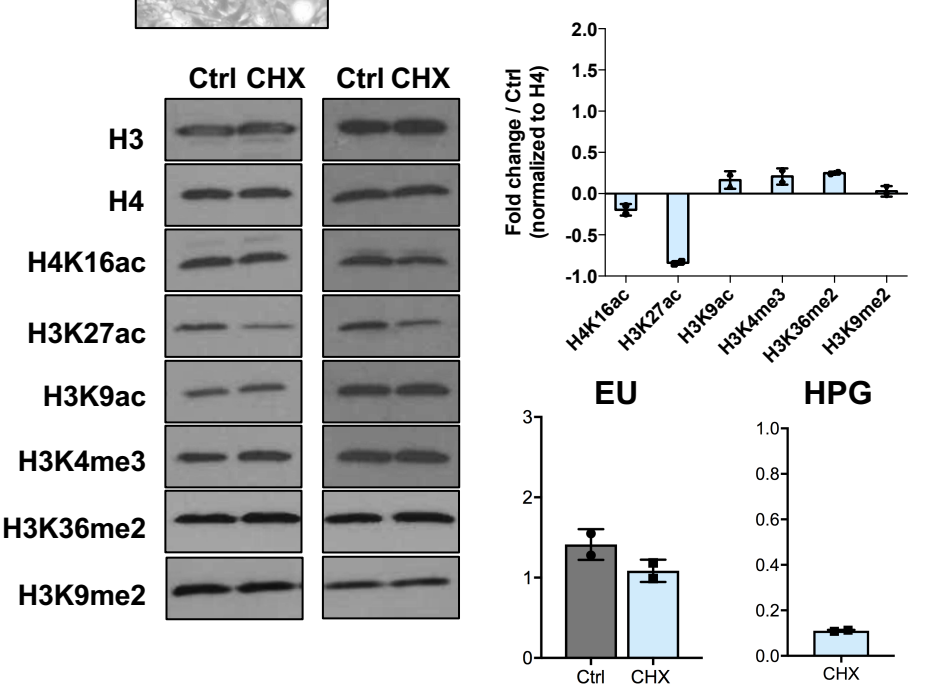
RA-treated cells



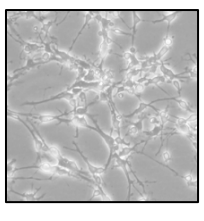
F

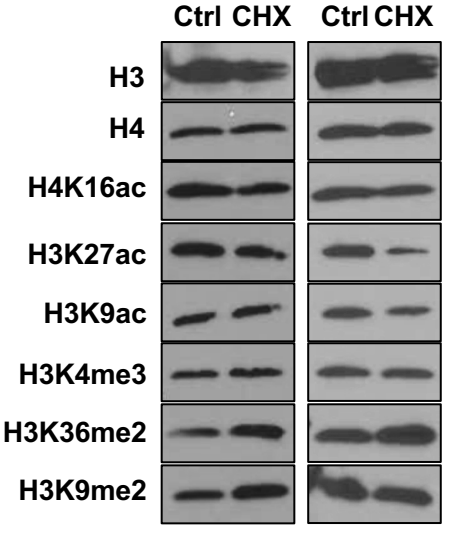


Mouse embryonic fibroblasts (MEFs)



G





Neural stem/progenitor cells (NSPCs)

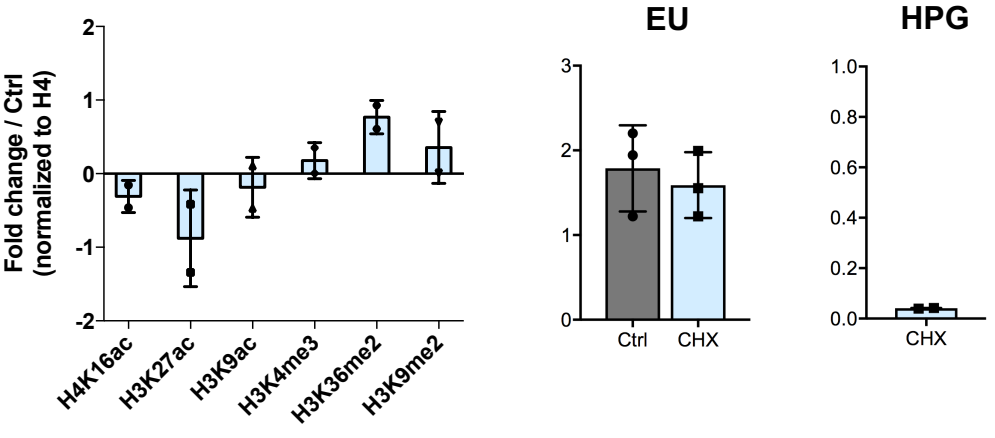


Figure S5, related to Figure 5. Effects of translation inhibition on ES cell pluripotency and on non-pluripotent cells.

(A) Quantification of Oct4 immunofluorescence in wild-type ES cells treated with DMSO or 1 μg/ml CHX for 3 hours.

(B) Western blot analysis and quantification of Nanog, Oct4 and H4K16ac levels during addition of and release from CHX.

(C) Cell number normalized qRT-PCR analysis of pluripotency and lineage markers in ES cells upon 3h of CHX. Data were normalized to Ctrl (DMSO). Error bars show mean ± SD of at least 2 biological replicates, each the mean of 3 technical qPCR replicates. No significant differences were detected by Student's t-test with multiple testing correction.

(D) Schematic and results of acute CHX treatment and differentiation of wild-type ES cells into Embryoid Bodies (EBs). qRT-PCR analysis revealing no differences in pluripotency gene repression and lineage marker induction in EBs derived from ES cells treated for 3h with DMSO or CHX (Ecto. = ectoderm, Endo. = endoderm, Meso. = mesoderm). Data were normalized to the average of *Ubb* and *Rpl7* and are reported as $\log_2$-fold change relative to wild-type ES cells. Error bars show mean ± SD of 2 biological replicates, each the mean of 3 technical qPCR replicates. No significant differences were detected by Student's t-test with multiple testing correction.

(E) Analysis of the chromatin, transcriptional, and translational responses to CHX in RA-treated ES cells.

(F) Analysis of the chromatin, transcriptional, and translational responses to CHX in primary mouse embryonic fibroblasts (MEFs).

(G) Analysis of the chromatin, transcriptional, and translational responses to CHX in neural stem/progenitor cells (NSPCs) isolated from E12.5 mouse cortex.

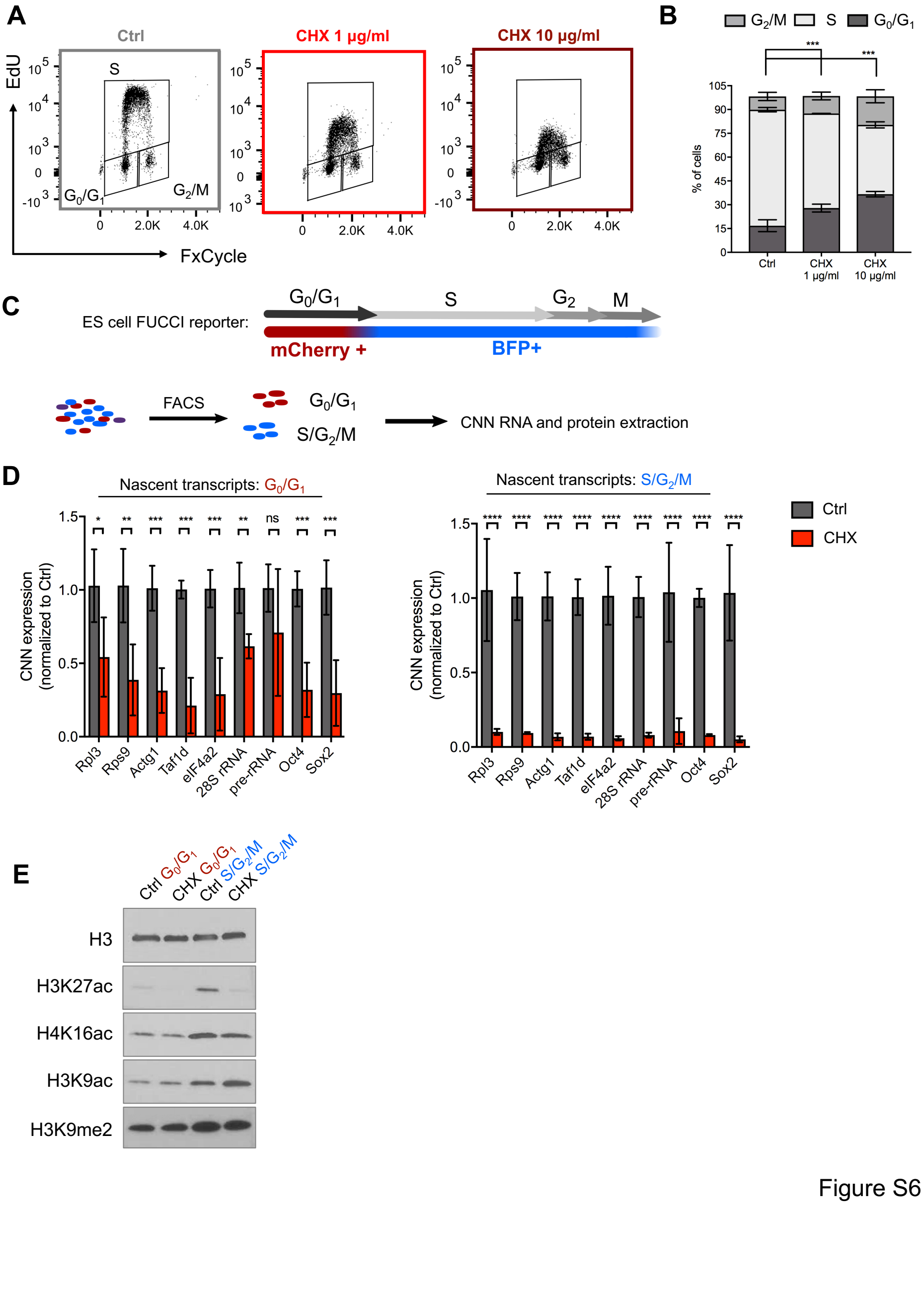

A
Ctrl
CHX 1 µg/ml
CHX 10 µg/ml
EdU
S
G0/G1
G2/M
FxCycle
B
G2/M
S
G0/G1
% of cells
Ctrl
CHX 1 µg/ml
CHX 10 µg/ml
C
ES cell FUCCI reporter:
G0/G1
S
G2
M
mCherry +
BFP+
FACS
G0/G1
S/G2/M
CNN RNA and protein extraction
D
Nascent transcripts: G0/G1
Nascent transcripts: S/G2/M
CNN expression (normalized to Ctrl)
Ctrl
CHX
Rpl3
Rps9
Actg1
Taf1d
eIF4a2
28S rRNA
pre-rRNA
Oct4
Sox2
E
Ctrl G0/G1
CHX G0/G1
Ctrl S/G2/M
CHX S/G2/M
H3
H3K27ac
H4K16ac
H3K9ac
H3K9me2

Figure S6

Figure S6, related to Figure 5. Impact of acute inhibition of translation on the cell cycle in ES cells.

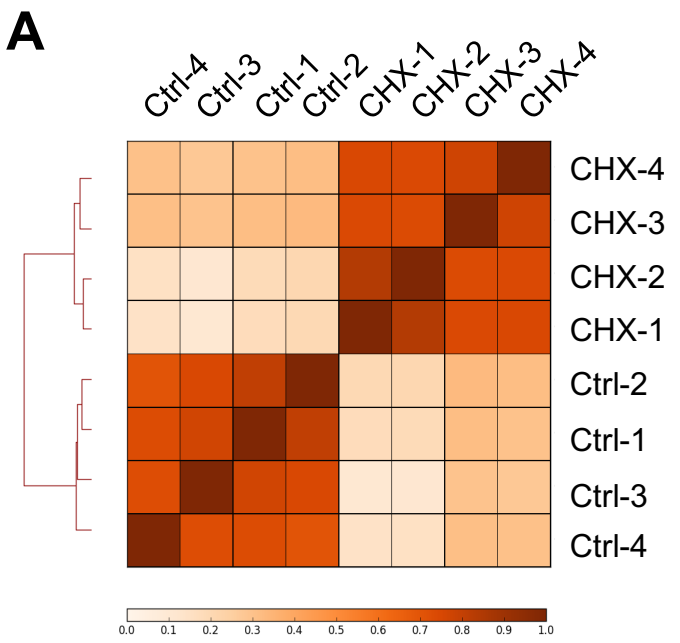
(A) Representative flow cytometry plots depicting cell cycle distributions of wild-type ES cells upon DMSO or CHX treatment.

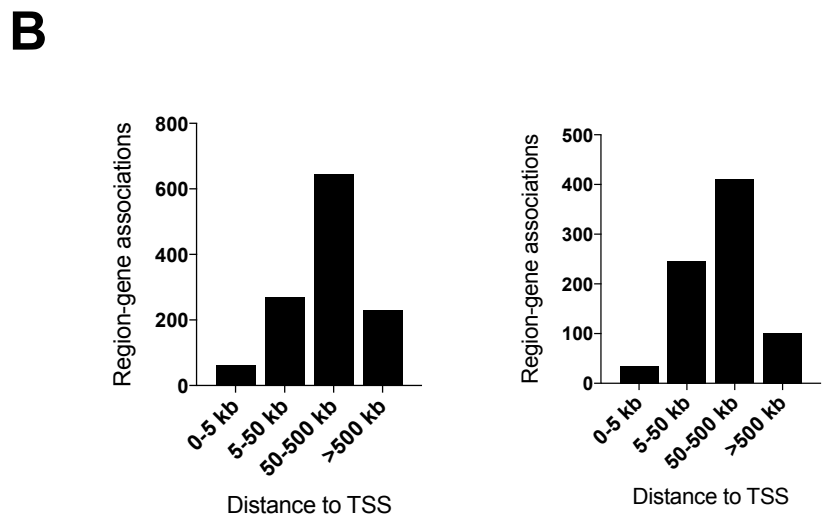
(B) Quantification of cell cycle stage distributions in DMSO- or CHX-treated ES cells. Error bars show mean ± SD of 2 biological replicates. Statistical significance was assessed by Chi-square test. **p<0.01.

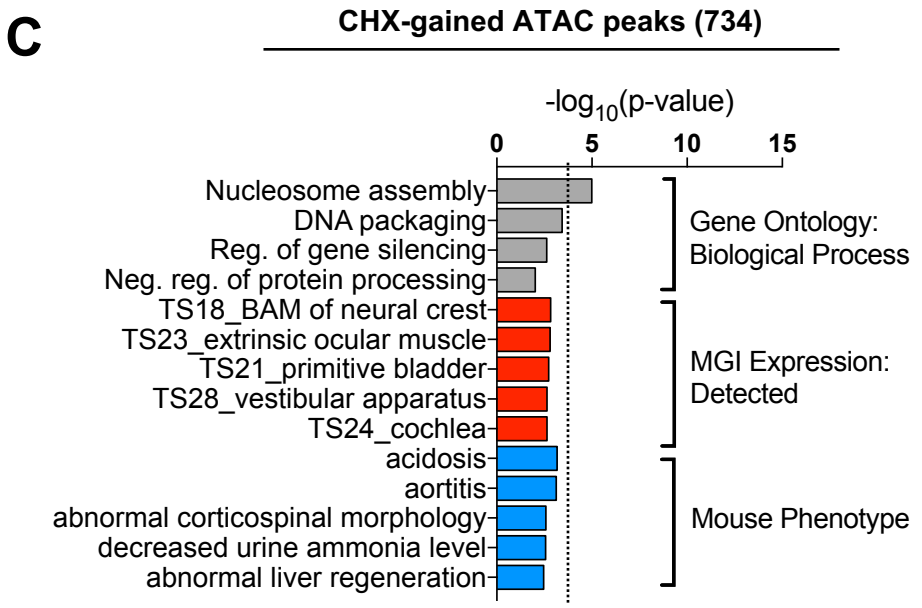
(C) Schematics of the FUCCI cell line used in this study.

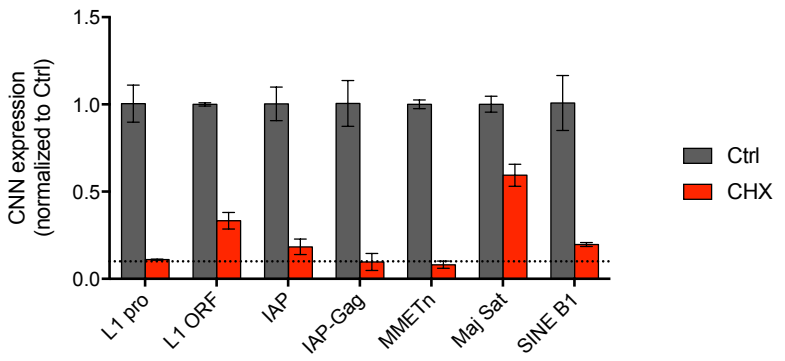
(D) Nascent RNA capture followed by qRT-PCR in the indicated FACS-isolated populations of DMSO- or CHX-treated (1 μg/ml, 3h) FUCCI. Error bars show mean ± SD of 2 biological replicates. Statistical test performed was two-tailed t-test. *** = p<0.001.

(E) Levels of indicated histone modifications in FACS-isolated populations of DMSO- or CHX-treated (1 μg/ml, 3h) FUCCI cells. Blots show 2 biological replicates.


A
Ctrl-4
Ctrl-3
Ctrl-1
Ctrl-2
CHX-1
CHX-2
CHX-3
CHX-4
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0


B
Region-gene associations
800
600
400
200
0
0-5 kb
5-50 kb
50-500 kb
>500 kb
Distance to TSS
Region-gene associations
500
400
300
200
100
0
0-5 kb
5-50 kb
50-500 kb
>500 kb
Distance to TSS


C
CHX-gained ATAC peaks (734)
-log10(p-value)
0
5
10
15
Nucleosome assembly
DNA packaging
Reg. of gene silencing
Neg. reg. of protein processing
TS18_BAM of neural crest
TS23_extrinsic ocular muscle
TS21_primitive bladder
TS28_vestibular apparatus
TS24_cochlea
acidosis
aortitis
abnormal corticospinal morphology
decreased urine ammonia level
abnormal liver regeneration
Gene Ontology: Biological Process
MGI Expression: Detected
Mouse Phenotype


D
CNN expression (normalized to Ctrl)
1.5
1.0
0.5
0.0
L1 pro
L1 ORF
IAP
IAP-Gag
MMETn
Maj Sat
SINE B1
Ctrl
CHX

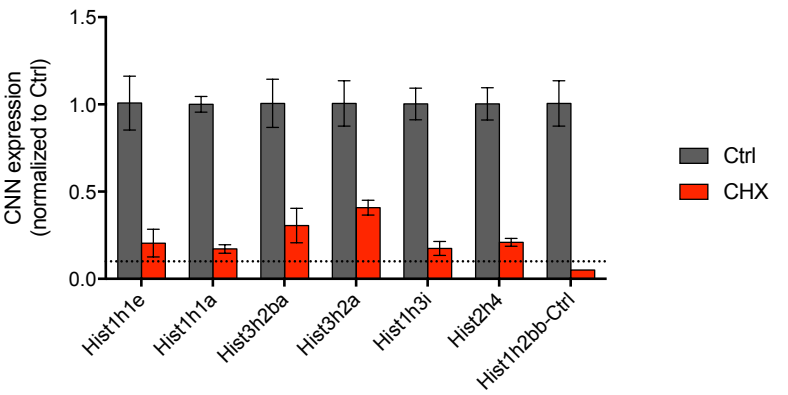

E
CNN expression (normalized to Ctrl)
1.5
1.0
0.5
0.0
Hist1h1e
Hist1h1a
Hist3h2ba
Hist3h2a
Hist1h3i
Hist2h4
Hist1h2bb-Ctrl
Ctrl
CHX

Figure S7

Figure S7, related to Figure 6. Characterization of chromatin accessibility and expression changes upon inhibition of translation in ES cells.

(A) Unsupervised clustering of individual ATAC-seq replicates upon DMSO or CHX treatment for 3 hours. The top 10,787 most variable regions, as determined by Macs14 algorithm, were used for clustering analysis.

(B) Distance of CHX-gained or CHX-lost regions from transcription start sites (TSS).

(C) Functional annotation of ATAC-seq peaks lost upon CHX treatment for 3 hours. See Table S9 for the full list of terms.

(D) Levels of nascent transcription of indicated transposable elements in 3h DMSO- or CHX-treated ES cells, assessed by EU labeling followed by capture and qRT-PCR. Dotted lines represent the average level of downregulation for mRNAs depicted in Figure 4B.

(E) Levels of nascent transcription of indicated histone genes in 3h DMSO- or CHX-treated ES cells, assessed by EU labeling followed by capture and qRT-PCR. Dotted lines represent the average level of downregulation for mRNAs depicted in Figure 4B.