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## Supplemental Information

# Gene-Specific Transcriptional Mechanisms at the Histone Gene Cluster Revealed by Single-Cell Imaging

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Figure S1

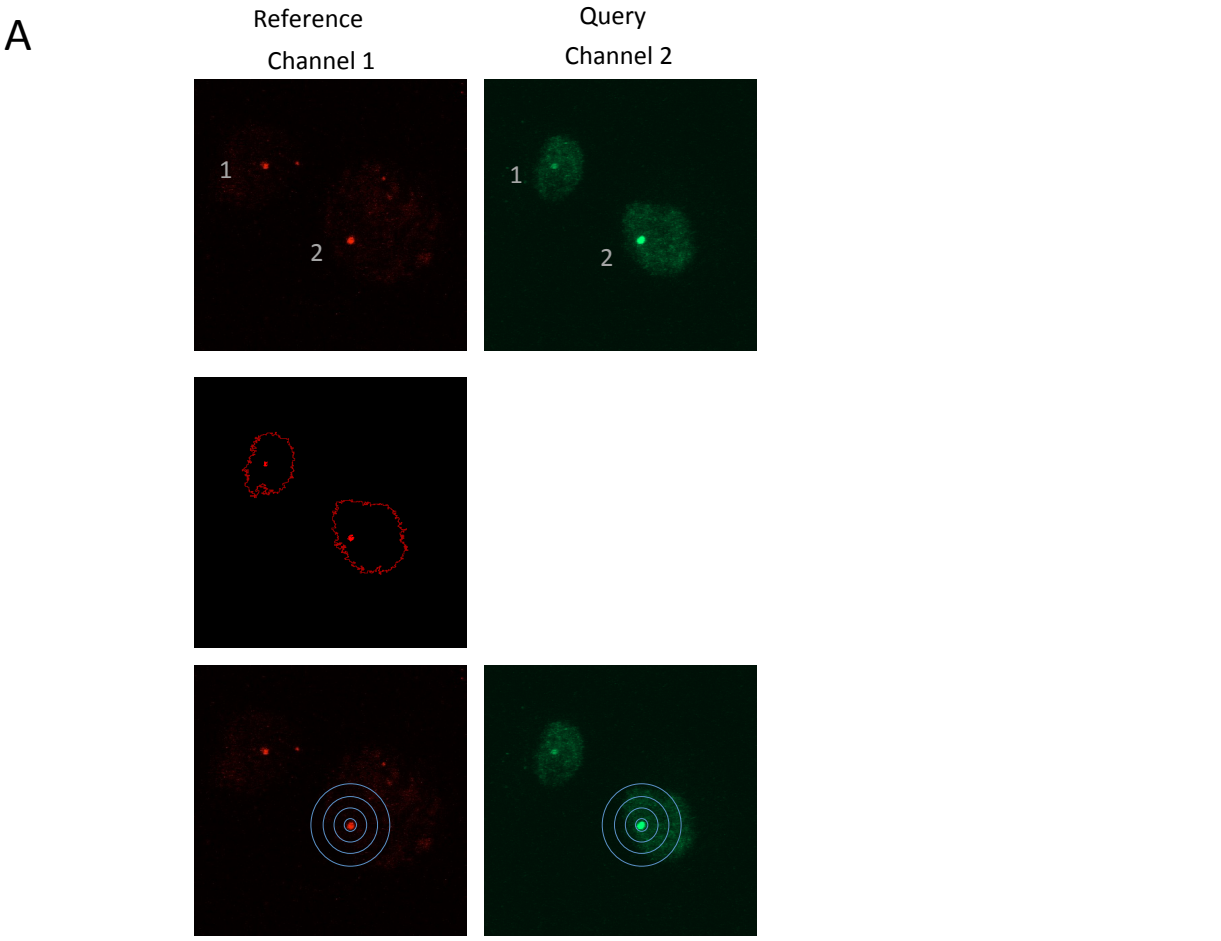

A
Reference
Channel 1
Query
Channel 2
1
2
1
2
B
Reference
Channel 1
Query
Channel 2
2
1
2
1
250
200
150
100
50
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Distance from center in µm
Distance from center in µm
Fluorescence intensity in arbitrary units

Figure S1. Schematic of the strategy for co-localization analysis at His-C (see supplemental methods for full description), related to Figure 1. (A) Upper panel: Cells are imaged in both a reference and a query channel for subsequent analysis. Two cells with a signal at His-C are shown (staining is Lsm11 in red and TBP in green). Middle panel: The nuclear signal provided by the immunostaining or an independent DNA staining is used to segment the nucleus (red border). Inside the nucleus, the signal is segmented using intensity, shape and size to identify His-C (red dot) in the reference channel. Lower panel: Using the signal center of mass in the reference channel as a starting point, the radial profile of the intensity is measured over a 5 μm radius (blue circles) in both channel. (B) Radial profiles are analyzed to quantify colocalization of signals in both channels (see supplemental methods for details).

Figure S2

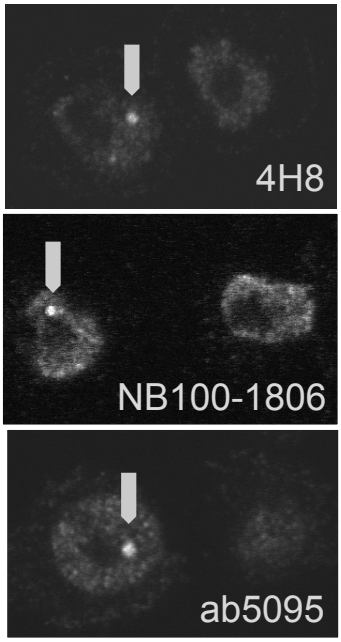


Figure S2: Immunofluorescence staining of S2 cells with three different antibodies recognizing various CTD phosphorylation, related to Figure 1. 4H8 is a mouse monoclonal antibody targeting CTD PSer5. NB100-1806 (Novus) is a rabbit polyclonal antibody targeting CTD PSer5. Ab5095 is a rabbit polyclonal antibody targeting CTD PSer2. All staining show the recognizable dot-like structure of Pol II associated with His-C.

Figure S3

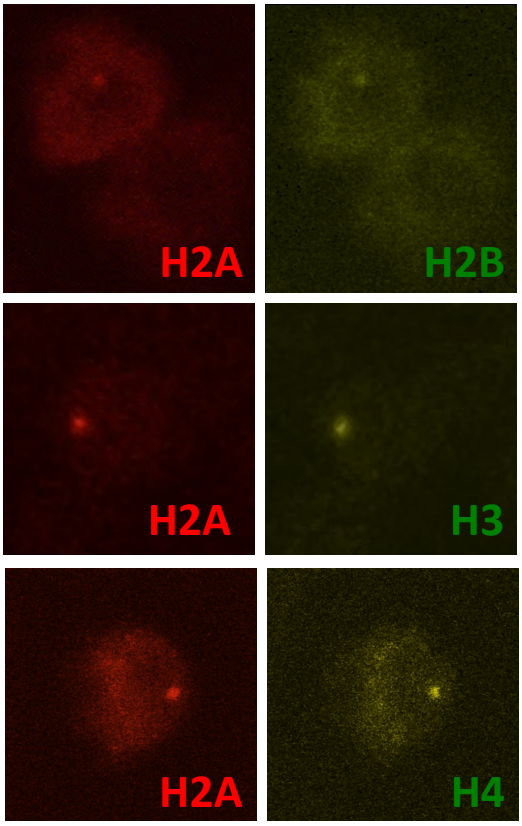
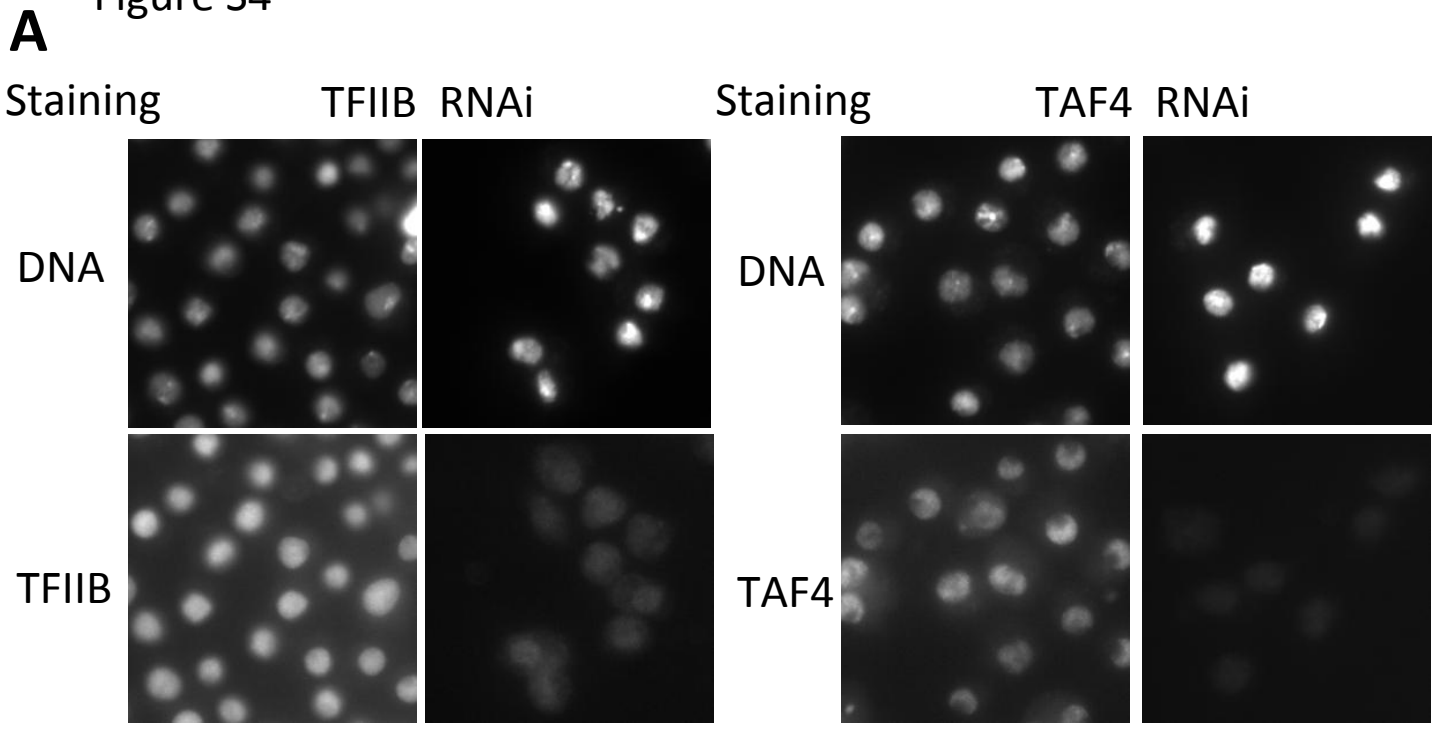


Figure S3: Representative FISH of H2A mRNA with H2B, H3 and H4 mRNA in S2 cells, related to Figure 2. Nascent RNAs appear as a more intense dot-like signal in the nucleus. H2A nascent RNA colocalize with H2B, H3 and H4.

Figure S4


A
Staining
TFIIB RNAi
DNA
TFIIB
Staining
TAF4 RNAi
DNA
TAF4

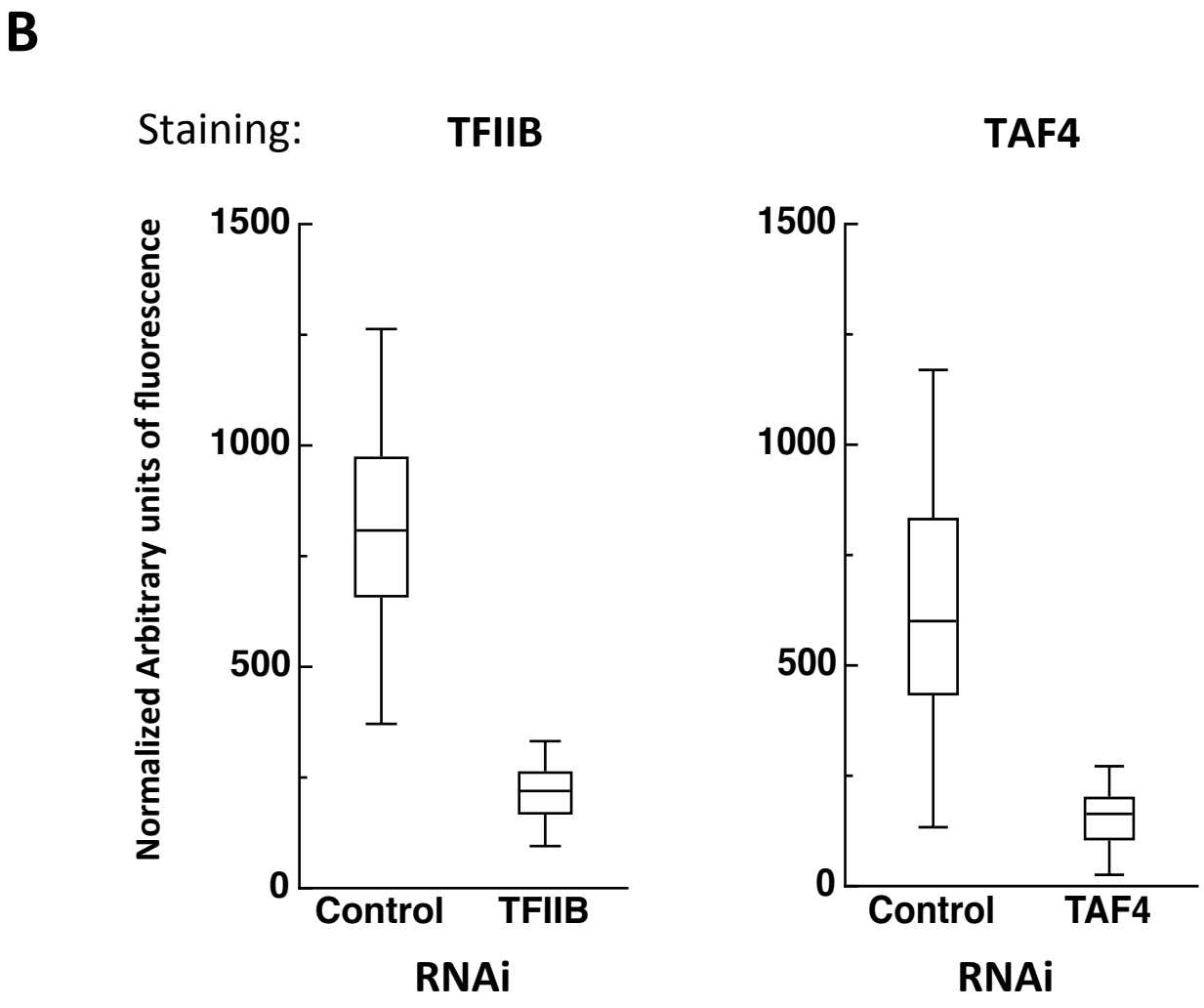
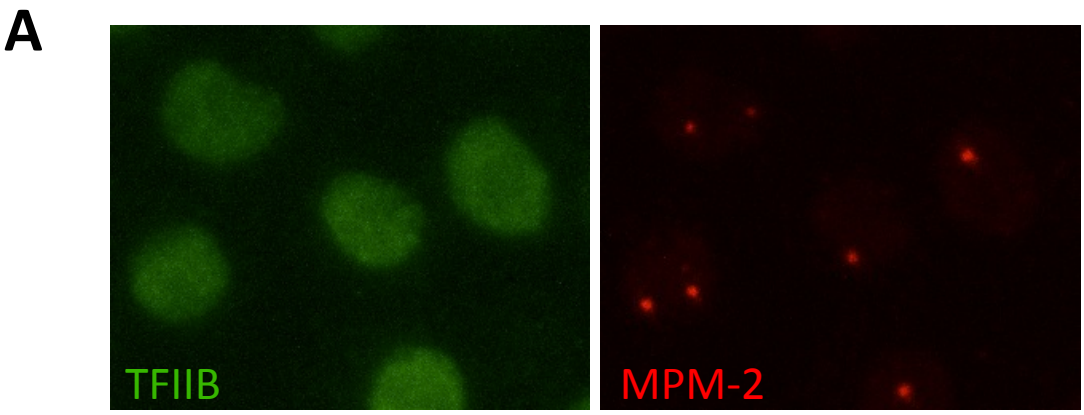

B
Staining:
TFIIB
TAF4
Normalized Arbitrary units of fluorescence
1500
1000
500
0
Control
TFIIB
RNAi
Control
TAF4
RNAi

Figure S4: Test of TFIIB and TAF4 antibodies specificity for immunostaining, related to Figure 4. (A) Staining of S2 cells with TFIIB and TAF4 antibodies +/- RNAi of TFIIB or TAF4. (B) Quantification of the immunofluorescent signal for TFIIB and TAf4 +/- RNAi of TFIIB or TAF4.

Figure S5



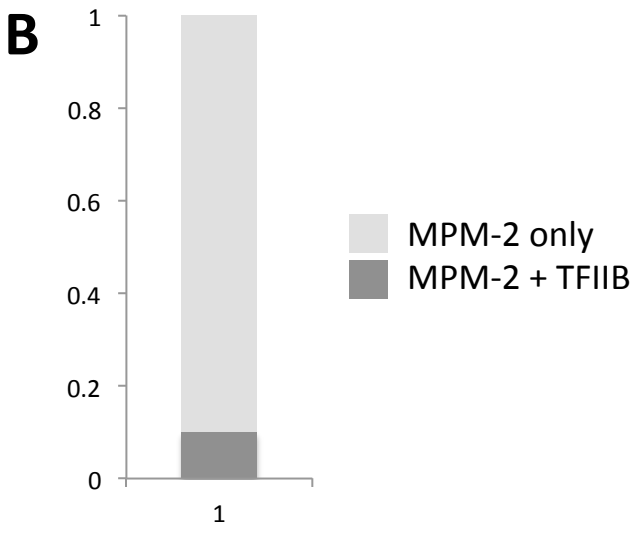



Figure S5: Localization of TFIIB through immunostaining, related to Figure 4. (A) Immunostaining of TFIIB and MPM-2 in S2 cells. (B) Quantification of the colocalized signal of TFIIB at MPM-2 foci.

Figure S6

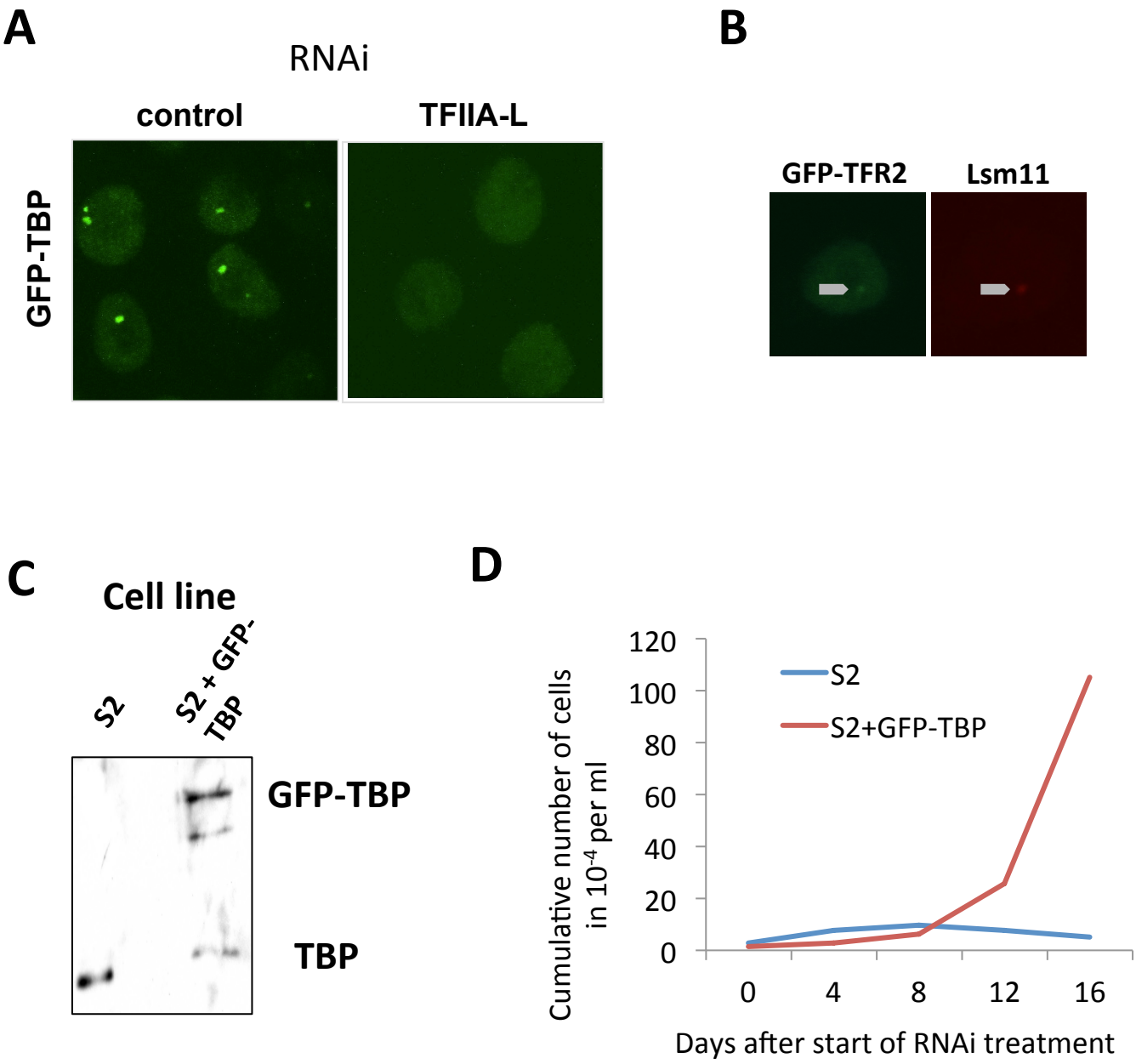

A
RNAi
control
TFIIA-L
GFP-TBP
B
GFP-TFR2
Lsm11
C
Cell line
S2
S2 + GFP-TBP
GFP-TBP
TBP
D
Cumulative number of cells in $10^{-4}$ per ml
120
100
80
60
40
20
0
S2
S2+GFP-TBP
0
4
8
12
16
Days after start of RNAi treatment

Figure S6: Expression of GFP-TBP in S2 cells, related to Figure 7. (A) (B) Costaining of GFP-TRF2 and Lsm11 in S2 cells. (C) Western blot of S2 cells whole cell extract, with a TBP antibody (3C3). Left lane, S2 untransformed S2 cells. Right lane, stable cell line expressing GFP-TBP.
Growth curve of untransformed S2 cells and a stable cell line expressing GFP-TBP, in the presence of Ds RNA against the endogenous TBP. Cells were split every 4 days, and dsRNA was added again in the new medium.

Figure S7

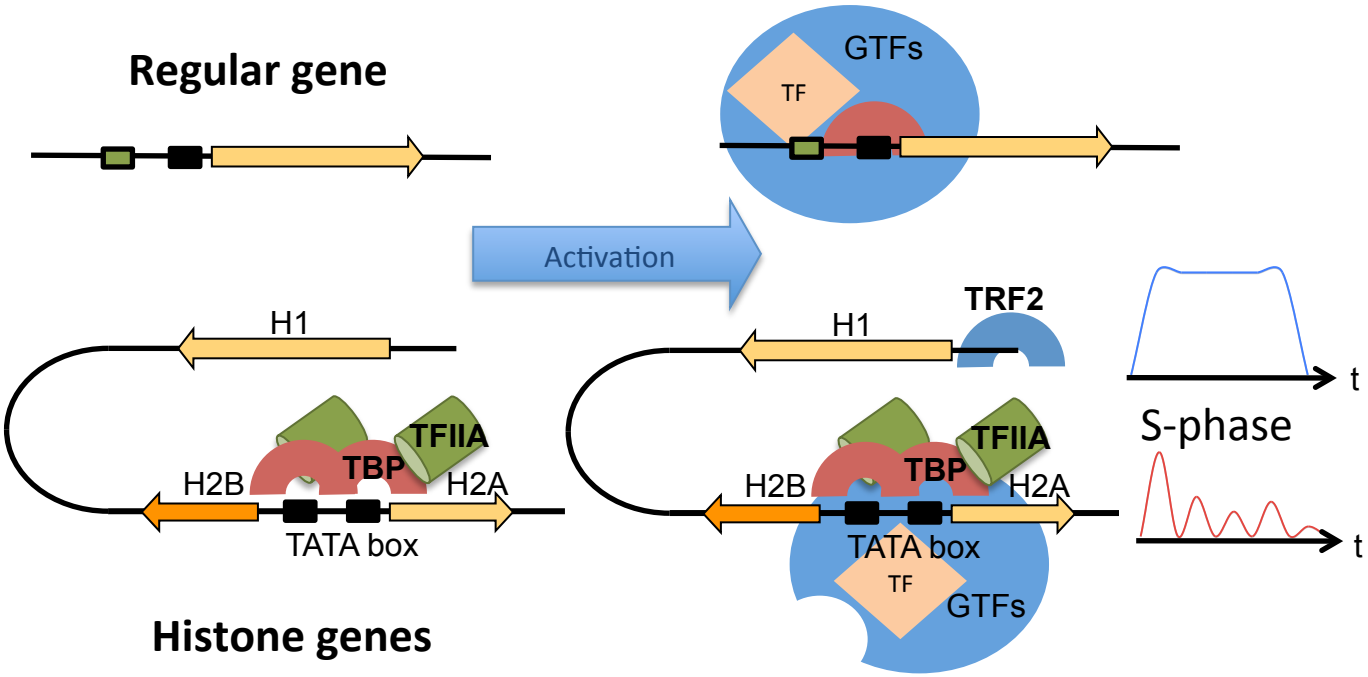

Regular gene
GTFs
TF
Activation
H1
TRF2
H1
t
S-phase
TFIIA
TBP
H2B
H2A
TFIIA
TBP
H2B
H2A
TATA box
TATA box
t
TF
GTFs
Histone genes

Figure S7: Schematic diagram of the core transcriptional machinery and gene activation at a standard promoter (upper part) and at the promoters of the histone gene cluster (lower part), related to Figure 7. The histone genes are dependent on an unusual preloaded TBP-TFIIA complex. The core H2A H2B histone genes show a peak of expression during early S-phase while histone H1 is transcribed throughout S-phase.

# Supplemental Experimental Procedures

## RNAi depletion

dsRNA was produced using T7 transcription of PCR amplicons as in (as in RNAi (Worby, 2001)).

List of template and primers:

Target, Template, 5' Primer, 3' Primer (uppercase is the T7 promoter)

Control (Luciferase), pGL3, TTAATACGACTCACTATAGGGAGAggagagcaactgcataaggc, TTAATACGACTCACTATAGGGAGAcatcgactgaaatccctggt

TAF4, SD04735, TTAATACGACTCACTATAGGGAGAagaagaatgtgcccaccaac, TTAATACGACTCACTATAGGGAGAttgaatctgcgtaatctgcg

TBP, LD44083, TTAATACGACTCACTATAGGGAGAtagcattgcatgcgagaaac, TTAATACGACTCACTATAGGGAGAacgaagatgaggagcacgat

TFIIB, LD24035, TTAATACGACTCACTATAGGGAGAggagcacccaagtaccaaaa, TTAATACGACTCACTATAGGGAGAacgtcccggtacaatatcca

Lsm11, SD11312, TTAATACGACTCACTATAGGGAGAGAgatcaggggacatcgaaa, TTAATACGACTCACTATAGGGAGAcctgggaagagtgataccca

TFIIAL, LD24213, TTAATACGACTCACTATAGGGAGAcatcacaccaacatatcggc, TTAATACGACTCACTATAGGGAGAgagcactcgagattccgaac

## RNA FISH

For RNA FISH, we used tiling probes crosslinked to single fluorophores in 3' (Raj, van den Bogaard, Rifkin, Van Oudenaarden, & Tyagi, 2008; Zenklusen & Singer, 2010).

Oligonucleotide with a 3' primary amino group (IDT) were cross-linked to either Cy3 or Cy5 fluorophores following instructions from Cy3/Cy5 mono-Reactive Dye Pack (GE Healthcare). Probes were designed with http://www.biosearchtech.com/stellarisdesigner/.

Probes sequences:

H1 (18 probes) gcaactgcagaatcagacat, agccactggggaagcggacg, tctcaactgtcgctggtggg, gccttttttggaccacttt, ctttgtgccagcagatccag, gcgtcgcagaggctttcttt, tgctgagttggcggatgtga, tttaatggaagcgtccacca, aaccgccacgttcctttaaa, tttttgattgccagaagtga, tttataagtggcagtgatat, gcgctaacttttgggcgtcg, tttaagtacttcttgatgaa, ctttccattgaccacggccg, cctttcccttagtttgaata, agtttgaaagatccagatgc, ttccttcttggcagaggccg, tcgactttgccttcggatcc

H2A (16 probes) tgcctttcccttcactttgc, cggcacggtttgagcgggac, cggcccacagggaattgaag, cttccggagcaaacggtgaa, caacacgctctgcgtagttt, aggtaaactggagcgcctgc, cagatattccattacggcag, ccaactcgagaacctcagcg, ttgtcacgagcagcattgcc, cggaataattctagtcttct, ggatggccagttgcagatga, ttgtttaactcctcgtcgtt, aattgtgacgccggagagca, taggcaacacgccaccttgt, ggcaacagaacagcctgtat, ggccttcttctcggtcttct

H2B (16 probes) tttccactagttttcggagg, tgagccttgccagccttctt, gtcggtcttggtgatgttct, tccttttgcgcttcttttttc, atgtagatggcatagctctc, gacctgcttgagaaccttgt, acgaaattccggtgtcagga, gttcattatgctcatcgcct, cgctcgaaaatatcatttac, acgagacgcttcggcagcaa, agcgcttgttgtagtgagct, atctcccgactggtgatggt, aagcaggcgaacagccgttt, atgcttggccaactctccag, ccttggttccctcactgaca, ttagagctggtgtacttggt

H3 (18 probes) gtttgcttggtacgagccat, ttccaccagtcgatttgcga, tagtagccagttgtttgcgt, tggagcactcttgcgagcgg, gcttcttcacacctccggtg, gttccagggcgatagcggtg, acgaatttcacgcaaggcca, agctcggtgctcttttggta, gaaaggcagcttgcggatta, agcgatttcacgcaccagac, gcaagtccgtcttaaagtcc, ataaccgccgagctctggaa, ttcgctagcttcctgcagag, tcgaagagaccaaccaggta, gaatggcacacaagttggta, attatggtgatacgcttggc, cgctaactggatgtctttgg, cacgctcgccgcgaatgcgt

H4 (14 probes) cctttaccacgaccagtcat, acccctttcccaagcctttgc, ctttgcgatgacgcttggcg, ccttggatgttatcacgcag, gcggatagcaggcttcgtga, caccgcctcgacgggccaaa, atgagtccagatatgcgctt, aacgccacgcgtttcctcgt, cgttctccaagaaaaccttc, taggtcacggcatcacgaat, cttcctcttggcgtgttccg, caacatccatggctgtaact, ccttgcctcttcagagcgta, gccaaatccgtagagggtgc

**Live cell imaging, FRAP acquisition and analysis**

Cells were grown on Mattek Glass bottom culture dish and imaged with a Zeiss 510 confocal microscope using a 488 nm laser with a *63X* Plan-Apo lens 1.4NA with a fully open pinhole. For imaging, laser power was 1% of bleaching intensity. For Pol

II FRAP, 10 pre-bleach images were acquired. Photo-bleaching was done in a ROI of 1µm diameter centered on the transcription site localized by GFP-Pol II accumulation and/or RFP-Lsm11 aggregation. Post-bleaching images were acquired every 700 msec in one single plane. For TBP FRAP in the nucleoplasm, the same conditions were used but with 5 pre-bleaching images and post-bleaching images acquired every 250 msec. For GFP-TBP FRAP at the histone locus, images were acquired in 10 z-stacks with a 1µm step, a maximum projection was used for measurements. Post-bleaching, an image was acquired every 5 minute. For FRAP of histone gene locus associated factors (Pol II, TBP), recovery was tracked with the spot detection algorithm Localize (Trcek et al., 2012).

**Colocalization analysis at the histone gene locus**

We assessed the co-localization of two factors stained independently by using one factor as reference, and checking the presence of the other factor at the same location. S2 drosophila cells were grown , fixed and stained on coverslips. DNA was stained with Hoechst, secondary antibody used for co-staining were Alexa 488 and Alexa 546 (Invitrogen). Images were acquired with a zeiss Lsm 510 or 710 confocal microscope. For each sample, multiple fields of view were imaged with a *63X* Plan-Apo lens 1.4NA in confocal slices over 20 µm in z-stacks. Image manipulations and analysis were done with ImageJ (Schneider, Rasband, & Eliceiri, 2012). For a typical analysis, a maximum projection of the z-stacks was used. Imaging produced three images, reference channel, Query channel and DNA staining. Nuclei were segmented using the DNA staining channel with thresholding and object analysis. Inside the border of the nuclei, Histone locus specific staining was segmented in the reference channel, using Maximum Entropy thresholding and object analysis, keeping only object between 0.2 and 1 µm in diameter. The list of objects was checked manually for quality control. The coordinates of the center of each object were computed. The pixel intensity radial profile was plotted in both the reference and the query channel with for origin the center of the object from the reference channel. The signal was considered positive at the histone locus if the first derivative of the radial profile in the first 0.5 µm was significantly superior to the rest of the profile.

**Double staining EdU – BrdU**

**Overview of the procedure.** Cells grown on coverslips are incubated with two different nucleotide analogs, EdU (Invitrogen) and BrdU (Sigma), with a chase time of varying length between the two incubations. Each nucleotide analog is detected in fixed cells in a different color. The relative level and pattern of incorporation of EdU and BrdU is compared in a population of asynchronous cells.

For chase experiments, an asynchronous population of cells was incubated for 15 minutes in the presence of 50 mM BrdU then washed 3 times in culture medium and incubated for variables amount of time in M3BPYE, after which they were incubated for 15 minutes with 50 mM EdU washed and fixed as for immunofluorescence. Edu was detected using the Click-It chemistry protocol from Invitrogen using Alexa fluorochrome (Invitrogen). BrdU was detected using 3D4 antibody (BD Biosciences) after DNase treatment of the fixed cells (protocol modified from (Easwaran, Leonhardt, & Cardoso, 2007)). The secondary antibody was Alexa conjugated (Invitrogen). DNA is stained with Hoechst (Sigma).

**Analysis of BrdU, EdU incorporation patterns.** Images were acquired with a zeiss Lsm 510 or 710 confocal microscope. For each sample, multiple large fields of view (140µm x 140 µm) containing multiple cells were imaged with a *63X* Plan-Apo lens 1.4NA with a wide-open pinhole (confocal slice around 10 µm). Image manipulations and analysis were done with ImageJ. For Edu staining pattern analysis, images were segmented using the DNA staining channel as described. Then two properties were measured inside the border of the nucleus in the Edu channel, the mean pixel intensity and the skewness of the pixel intensity distribution. The distribution of the EdU signal intensity across nuclei was brought to the same scale for all samples to compensate for variation in staining efficiency. Cells with a Edu signal over background were deemed positively stained. EdU positively stained population was separated between heterochromatin replicating cells and euchromatin replicating cells based on skewness of the pixel intensity distribution,

and checked manually. In both categories cells showed a range of staining intensity. The same analysis was performed for BrdU stained cells.
The EdU stained cells were split in regularly spaced increment of signal intensity. In each category we computed the fraction of nuclei that are also positively stained for BrdU.

**Analysis of Histone genes transcription foci together with EdU incorporation patterns.** EdU staining was carried as described, followed by RNA FISH. Edu incorporation patterns were determined as described before, transcription foci were detected as described for the co-localization procedure, and checked manually for quality control.

## Supplemental References


Easwaran, H. P., Leonhardt, H., & Cardoso, M. C. (2007). Distribution of DNA replication proteins in Drosophila cells. *BMC Cell Biology*, *8*(1), 42. doi:10.1186/1471-2121-8-42

Raj, A., van den Bogaard, P., Rifkin, S. A., Van Oudenaarden, A., & Tyagi, S. (2008). Imaging individual mRNA molecules using multiple singly labeled probes. *Nature Methods*, *5*(10), 877–879. doi:10.1038/nmeth.1253

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*.

Trcek, T., Chao, J. A., Larson, D. R., Park, H. Y., Zenklusen, D., Shenoy, S. M., & Singer, R. H. (2012). Single-mRNA counting using fluorescent in situ hybridization in budding yeast. *Nature Protocols*, *7*(2), 408–419. doi:10.1038/nprot.2011.451

Worby, C. A. (2001). RNA Interference of Gene Expression (RNAi) in Cultured Drosophila Cells. *Science's STKE*, *2001*(95), 1pl–1. doi:10.1126/stke.2001.95.pl1

Zenklusen, D., & Singer, R. H. (2010). Analyzing mRNA expression using single mRNA resolution fluorescent in situ hybridization. *Methods in Enzymology*, *470*, 641–659. doi:10.1016/S0076-6879(10)70026-4