Quantitative imaging of chromatin decompaction in living cells

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Abbreviations:

TSA – trichostatin A

ORF – open reading frame

qPCR – quantitative PCR

SE – standard error

SPB – spindle pole body

GST – Glutathione S transferase

smFISH – single molecule fluorescence *in situ* hybridization

Pol II – RNA polymerase II

Abstract

Chromatin organization is highly dynamic and regulates transcription. Upon transcriptional activation, chromatin is remodeled and referred to as "open", but quantitative and dynamic data of this decompaction process are lacking. Here, we have developed a quantitative superresolution microscopy assay in living yeast cells to visualize chromatin decompaction using the *GAL7-10-1* locus as a model system. Upon transcriptional activation of these three clustered genes, we detect an increase of the mean distance across this locus by ~100 nm. This decompaction is linked to active transcription but is not sensitive to the histone deacetylase inhibitor trichostatin A or to deletion of the histone acetyl transferase Gcn5. By contrast, the deletion of *SNF2* (encoding the ATPase of the SWI/SNF chromatin remodeling complex) or the deactivation of the histone chaperone complex FACT lead to a strongly reduced decompaction. Our assay quantifies and visualizes for the first time the chromatin conformation changes brought about by transcriptional activation and suggests that nucleosome remodeling and eviction activities are major contributors to chromatin reorganization.

Introduction

DNA in the cell nucleus is present as chromatin in a tight complex with histones and other proteins. This complex is central to spatially organize the DNA strand by balancing the negative charges of the phosphate backbone and is also crucial for gene regulation. The three-dimensional chromatin conformation is highly dynamic and remodeled continuously as cells change their physiological state or their transcriptional programs. This remodeling is orchestrated by histone modifiers and chromatin remodelers, which change the interaction between nucleosomes as well as the interaction between histones, DNA and the protein complement present at a chromatin site, thereby affecting the spatial packing of nucleosomes, their location, mobility and density.

Activation of transcription typically leads to a change in chromatin conformation manifested in higher accessibility of the DNA to digestion or transposon integration (Tsompana and Buck, 2014). Although the changes in histone occupancy and accessibility have been studied extensively, the quantitative structural changes of chromatin on a single cell level remain poorly understood, and it is still largely unclear how the activities of chromatin remodeling complexes are spatially and temporally integrated in living cells. The chromatin 'opening' associated with transcriptional activation consists of two distinct changes in chromatin structure: spatial decompaction by changes in nucleosome-nucleosome interactions, and linear decompaction by changes in nucleosome density (Even-Faitelson *et al.*, 2016). Both of these processes are thought to be tightly linked to the posttranslational modifications of histones, which characterize transcriptionally active and transcriptionally silent chromatin. For example, histone tail acetylation is generally associated with active chromatin (Li *et al.*, 2007). *In vitro*, specific histone modifications directly influence the spatial organization of chromatin by mediating or restricting nucleosome-nucleosome interactions. For example, the acetylation of histone H4 at lysine 16 prevents the interaction between the acetylated tail and a neighboring nucleosome, lowering the propensity to form a compact 30 nm fiber (Shogren-Knaak *et al.*, 2006). However, no effect on linear chromatin compaction was observed on autosomes in mammalian cells and *C. elegans* upon loss of H4K16 modification during differentiation (Taylor *et al.*, 2013; Lau *et al.*, 2017). In addition, evidence is accumulating that extended regular higher order chromatin structures like a 30 nm fiber do not form *in vivo,* and that chromatin fibers are present mostly in dispersed states in living cells (Fussner *et al.*, 2012; Hsieh *et al.*, 2015; Chen *et al.*, 2016). This is corroborated by recent visualizations of chromatin chains in intact mammalian nuclei, showing that the locally appearance of the chromatin fiber is similar in regions of eu- and heterochromatin (Ou *et al.*, 2017). It is therefore unclear how local chromatin folding is influenced by histone modifications *in vivo*.

In addition to their potential to mediate inter- and intrachromosomal interactions and thus to shape the longer-range organization of the chromatin fiber, nucleosomes constitute direct obstacles to the passage of RNA Polymerase II (Pol II) during transcription. Histones and entire nucleosomes are therefore evicted from the DNA during transcription by the concerted action of chromatin remodelers like the SWI/SNF and SWR1 complexes and histone chaperones like Asf1 and FACT (reviewed in Venkatesh and Workman, 2015). This nucleosome eviction (leading to a lower nucleosome density) would be expected to cause an increase in the effective length of the chromatin fiber, but this has not been investigated *in vivo*.

In order to study chromatin dynamics and organization *in vivo*, we took advantage of the budding yeast *GAL* locus, a highly regulated gene cluster which has served as a paradigm for inducible gene expression. The *GAL* locus comprises the *GAL7*, *GAL10* and *GAL1* genes located next to each other on chromosome II. These three genes, encoding enzymes required for the metabolism of galactose, are highly regulated depending on the carbon sources present in the growth medium. The genes are repressed in the presence of glucose, active in the presence of galactose (but the absence of glucose) and 'derepressed' in the absence of both glucose and galactose (e.g. in raffinose). Intricate regulation of carbon metabolic genes allows *S. cerevisiae* to adapt to and successfully compete with other organisms for various sugars present in the environment (New *et al.*, 2014).

GAL gene activation involves the recruitment of several histone modifying enzymes (Carrozza *et al.*, 2002; Wang *et al.*, 2002; Govind *et al.*, 2007) and the nucleosome occupancy at the locus is dramatically reduced (Schwabish and Struhl, 2004; Govind *et al.*, 2007; Bryant *et al.*, 2008). How this affects the chromatin conformation *in vivo* and to which extent chromatin decompaction and transcriptional activation are interdependent remains unclear. To address these important questions, we developed an assay to visualize chromatin compaction in living cells. Monitoring the distance between two chromosomal loci on either side of the *GAL* locus revealed a drastic linear decompaction upon activation of the *GAL* gene cluster. This decompaction was tightly coupled to transcriptional activity. Furthermore, the observed opening was not regulated by histone acetylation but depended on the activity of nucleosome-evicting chromatin remodelers.

Results

An assay to quantitatively analyze transcription-induced chromatin decompaction in living cells

To probe chromatin decompaction during transcription in a quantitative manner in living cells, we developed a microscopy-based assay to follow chromatin conformation in *S. cerevisiae* over time. We chose the *GAL7-GAL10-GAL1* gene cluster as a model system, since it is very well studied and the presence of three co-regulated genes spanning ~ 5.8 kb is expected to give a clear decompaction response. LacO and TetO repeats were introduced on either side of the *GAL* gene cluster or in a control region and visualized with LacI-GFP and TetR-mCherry (Figure 1A). Bright green and red dots were readily detected in all cells (Figure 1A), and their distance was analyzed in cells grown in glucose (repressed), raffinose (derepressed) or galactose (active). In a control region adjacent to but not spanning the *GAL* locus (18 kb between LacO- and TetO-repeat insertion sites), no change in the distribution of measured distances was observed between the different carbon sources (Figure 1B). In contrast, in strains with a distance of 14 or 31 kb between the insertion points of the repeats across the *GAL* locus, the distribution clearly shifted towards larger distances in activated cultures (Figure 1B and C) increasing from a mean distance of 255±13 nm in glucose to 310±7 nm in galactose in the 14 kb reporter strain or from 327±12 nm to 391±14 nm in the 31 kb reporter strain (2D distances measured on single imaging plates; values are means of three biological replicates with standard error of the mean). As expected, the deletion of the transcriptional activator Gal4 abolished

the decompaction response, while deletion of the transcriptional repressor Gal80 led to decompaction already in raffinose-grown cells (Supplemental Figure 1). Thus, we have developed a microscopy assay that can readily detect chromatin decompaction upon transcriptional activation in living cells.

To analyze the dynamics of decompaction, we acutely induced the *GAL* genes by adding galactose to cells growing in raffinose. This led to an increase in the population mean distance over the course of 20 minutes (Figure 1D) mimicking the kinetics of transcriptional activation as seen e.g. by quantitative PCR (qPCR) analysis (Green *et al.*, 2012). Similarly, the addition of glucose to cells growing in galactose resulted in fast compaction kinetics (Figure 1E) corresponding to the rapid shut-down of transcription due to glucose-induced repression. Decompaction could also be induced with faster kinetic within less than five minutes if cells were pregrown in galactose and then repressed with glucose for only 1 h before re-induction with galactose (Figure 1F). In this case, the presence of elevated levels of Gal1 and Gal3 proteins induce a 'transcriptional memory' and lead to faster activation (Kundu and Peterson, 2010). Thus, the kinetics of chromatin compaction closely follows transcriptional activity.

Active transcription correlates with chromatin decompaction in single cells

To directly correlate transcriptional activity with distance measurements on a single cell level, we applied single molecule fluorescent *in situ* hybridization (smFISH) to visualize transcripts as they are produced from the *GAL* locus in our distance reporter strains. We simultaneously used FISH probes for all three *GAL* genes *GAL1*, *GAL10* and *GAL7*, all labeled with the same fluorophore (Qasar670). While cytoplasmic mRNAs are visible as individual spots, or hazy signal due to the large number of molecules in induced cells, the transcription site is visible as a bright focus in the nucleus close to the repeat-marked gene loci (Figure 2A). Transcription spots and cytoplasmic mRNA foci are absent in cells grown in raffinose (Figure 2A, first panel). Upon induction with galactose, the fraction of cells with transcription spots increased over the course of 30 min paralleling the increase in the mean distance in the population after induction (Figure 2B). After 60 minutes or longer, a transcription spot can be seen in virtually every cell (Figure 2A), but since our automatic detection routine does not detect all transcription spots, the maximum measured percentage of cells with transcription spot is ~ 80 % (Figure 2B).

To correlate transcription with distance, we analyzed early time points after induction, when only a fraction of the cells shows a transcription spot (Figure 2B). We separately determined the distance between the LacO- and TetO-repeat markers in cells with or without a detectable transcription spot. Importantly, distances were measured in 3D on image stacks to provide additional resolution, since fixation and FISH procedures led to an overall shrinkage of the sample. While only few cells with a clear transcription spot were observed after 5 min and decompaction was still minimal, after 10 and 30 min the distances in cells with a transcription spots were significantly larger than in cells without a transcription spot (Figure 2C and D). Thus, decompaction correlates with transcription also on a single cell level.

Histone acetylation is dispensable for chromatin decompaction at the *GAL* locus

The increased distance distribution observed in our assay, which is indicative of decompaction of chromatin during transcription, could result from reduced internucleosomal interactions, from the eviction of nucleosomes, or both. Internucleosomal interactions are often mediated by histone tails and are thought to be regulated by posttranslational modification. Histones H3 and H4 contribute most to direct internucleosomal interactions but are also required for the recruitment of transcriptional activators. Acetylation of H3 *in vivo* occurs predominantly through Gcn5 (Grant *et al.*, 1997). This histone acetyl transferase is a component of the SAGA complex, which is recruited to the

GAL locus by the transcriptional activator Gal4 (Carrozza *et al.*, 2002; Govind *et al.*, 2007). However, the deletion of neither the SAGA histone acetyl transferase Gcn5 nor its activating subunit Ada2 influenced chromatin decompaction across the *GAL* locus (Figure 3A). These mutations also do not affect pre-initiation complex formation at the *GAL* promoter (Bhaumik and Green, 2001) or mRNA production (Stafford and Morse, 2001; Green *et al.*, 2012), suggesting that acetylation is dispensable for transcriptional activation and chromatin decompaction induced by Gal4. While dispensable for activation, Gcn5 activity is crucial for the association of the *GAL* locus with the nuclear periphery upon activation (Dultz *et al.*, 2016). Therefore, our results rule out that the observed decompaction response is a consequence of relocalization of the *GAL* locus to the nuclear pore complex, which might, for example, generate pulling forces that could lead to stretching of the chromatin fiber.

We also tested whether the inhibition of histone deacetylases using trichostatin A (TSA) would affect chromatin decompaction. TSA treatment neither changed the steady state distance distributions (Figure 3B) nor did it affect induction or repression kinetics (Figure 3C). Since both the deletion of *GCN5* and the inhibition of histone deacetylases targeted by TSA have been shown to globally affect acetylation levels of histones (Vogelauer *et al.*, 2000), these results indicate that the global acetylation state of histones *per se* does not play a major role in regulating chromatin compaction at the *GAL* locus.

Mutants that affect transcription also affect decompaction

Although Gcn5 was dispensable for chromatin decompaction, deletion of the SAGA component Spt20, which is required for the stability of the complex (Grant *et al.*, 1997) and for its recruitment by Gal4 (Bhaumik and Green, 2001; Larschan and Winston, 2001; Bryant and Ptashne, 2003), led to strongly reduced decompaction of the *GAL* locus (Figure 4 A and C). As previously reported (Bhaumik and Green, 2001), Spt20 mutants also show strongly reduced *GAL* gene transcription (Figure 4B).

Whereas SAGA is thought to be required to stabilize the general transcriptional coactivator Mediator at the *GAL* promoter, Gal4 is also able to directly recruit this complex (Traven *et al.*, 2006). Indeed, cells with a deletion of *GAL11,* a Mediator component that mediates direct interaction with Gal4, or expressing a temperature sensitive mutant of the Mediator protein Med7 (*med7-163*) phenocopied *spt20Δ* cells by exhibiting reduced overall decompaction in galactose as well as slowed decompaction kinetics (Figure 4 A and C and Supplemental Figure 2).

To analyze the effect of *SPT20* or *GAL11* deletions on a single cell level, we carried out single molecule FISH. After overnight growth in galactose, most cells displayed cytoplasmic mRNA signals, but the signal was strongly reduced compared to wildtype cells (Figure 4D). In addition, the intensity of transcription spots, which were still present in most cells, were reduced on average to ~ 30 % of the wildtype intensity (Figure 4D and E). Thus, also in the mutant cells, reduced transcription correlates with reduced decompaction of the *GAL* locus. Our results reveal a close linkage between transcriptional activation and decompaction, but they are not sufficient to establish a causality or temporal order between the two processes.

Nucleosome remodelers are required for decompaction

Next, we examined the function of nucleosome remodelers in our decompaction assay. As demonstrated by chromatin immunoprecipitation experiments, histone occupancy in the promoter of transcribed genes – but also in the open reading frame (ORF) of highly transcribed Pol II genes including the *GAL* genes – is reduced several fold upon activation (Lee *et al.*, 2004; Schwabish and Struhl, 2004). Nucleosome eviction is mediated by the SWI/SNF chromatin remodeling complex and the histone chaperones Asf1 (Antisilencing function 1) and FACT (FAcilitates Chromatin Transcription), a heterodimeric complex consisting of Pob3 and Spt16 (Schwabish and Struhl, 2006, 2007; Venkatesh and Workman, 2015). If the observed decompaction across the *GAL* locus is due to

nucleosome eviction, depletion of either of these factors should lead to a reduction of decompaction.

SWI/SNF is a chromatin remodeling complex with nucleosome sliding and nucleosome eviction activities. At the *GAL* locus, it is required for rapid Pol II recruitment and activation (Kundu and Peterson, 2010). We found that deletion of *SNF2*, encoding the ATPase subunit of the complex, slowed down the kinetics of decompaction and strongly reduced the degree of final decompaction as well (Figure 4A and C) consistent with a reduction in nucleosome eviction across the locus (Schwabish and Struhl, 2006). Although inactivation of SWI/SNF has previously been reported not to alter steady state *GAL* gene expression (Lemieux and Gaudreau, 2004), we observed strongly reduced mRNA levels both by qPCR and sm FISH in snf2Δ cells (Figure 4B and D). In contrast to the SWI/SNF complex, the chromatin remodeling complexes INO80 (represented by arp6Δ) and SWR1 (represented by swr1Δ) were not required for chromatin decompaction at the *GAL* locus (Figure 4A).

Histone chaperones play an important role in nucleosome eviction and deposition. Therefore, we tested the involvement of the histone chaperones Asf1 and FACT, which have both been implicated in the regulation of the *GAL* genes (Schwabish and Struhl, 2006; Xin *et al.*, 2009). Deletion of *ASF1* did not prevent chromatin decompaction in our assay (Figure 5A), which is consistent with previous findings that showed only a small decrease in histone H3 (but not H2B) removal at *GAL* promoters and ORFs (Schwabish and Struhl, 2006). In contrast, cells carrying temperature sensitive alleles for either component of the FACT complex (pob3-7 or spt16ts) did not show decompaction one hour after a shift to activating conditions at the restrictive temperature (Figure 5B). Together, these findings indicate that the eviction of nucleosomes mediated by SWI/SNF and FACT is the major source of decompaction observed in our assay.

Decompaction of the *GAL* locus is transcription dependent

If the observed decompaction is due to the removal of nucleosomes at the promoter and the ORF, the degree of decompaction will be expected to scale with the length of the ORF. To test this prediction, we introduced *GAL* promoter driven reporter genes at a different genomic site ~ 10 kb telomeric of the native *GAL* locus and measured changes in chromatin distance in the presence or absence of galactose (Figure 6A). The *GAL* promoter immediately followed by a terminator sequence did not exhibit a significant decompaction response. In contrast, robust decompaction was observed in a strain where the entire *GAL1* gene including promoter and downstream sequences was inserted. Decompaction, although to a weaker extent than for the entire *GAL1* gene, was also observed in a strain where we introduced a *GAL1* promoter-driven ORF coding for Glutathione S transferase (GST). Since the ORF of a single *GST* is shorter than the one of *GAL1* (700 bp versus 1 kb), we also examined the response in a reporter strain carrying two consecutive copies of the *GST* ORF (*2xGST*). However, the presence of a second *GST* ORF only slightly increased the decompaction, indicating that the *GAL1* ORF or 3'UTR have specific properties that enhance the observed decompaction response. However, the *2xGST* construct also contained MS2 stem loop sequences in the 5'UTR that might affect transcription (Heinrich *et al.*, 2017) and thus decompaction.

Decompaction leads to an untypical open chromatin state at the *GAL* locus

Our data provide the first quantitative measurement of transcription-dependent decompaction in living cells. To understand the changes in chromatin structure underlying the observed decompaction response, we more closely analyzed the distance distributions of the populations. The 3D distance of genomic loci on the same chromosome has been shown to scale with the distance in base pairs (van den Engh *et al.*, 1992). This is true also on chromosome II of budding yeast in both glucose and galactose grown cells as shown previously by us (Dultz *et al.*, 2016 and Figure 6B grey and black datapoints). One explanation for the observed changes in compaction at the *GAL* locus could be that

the *GAL* locus – compared to bulk chromatin – is *hyper*-compacted in the repressed state. However, this is not the case: in the repressed or derepressed state (glucose or raffinose growth), the median distances of TetO and LacO marker pairs across the *GAL* locus (Figure 6B open red circles, data for raffinose grown cells not shown) are in line with the distances of other pairs of loci on chromosome II. This indicates that the compaction state of the locus is comparable to other regions on the same chromosome. In contrast, in galactose-grown cells, the median distance increased far above the distance expected from the linear distance on the chromosome (Figure 6B, filled red circles), showing that the *GAL* locus is strongly *hypo*-compacted in its active state. This is consistent with the interpretation that linear decompaction at the *GAL* locus upon transcriptional activation is due to the eviction of nucleosomes, and that the high transcriptional activity over three clustered genes at the *GAL* locus leads to a very low histone density and thus hypo-compaction.

Since our data provide quantitative measurements of this hypo-compaction, we applied computational modeling to predict the level of histone loss that could lead to such a distance increase. To this end, we adapted a previously developed polymer model of the yeast genome, which accurately recapitulates several experimentally determined features (Tjong *et al.*, 2012; Dultz *et al.*, 2016), by increasing the resolution of the model in the proximity of the *GAL* locus. In this model, the compaction of the chromatin fiber in the *GAL* locus had to be reduced by ~60% in order to recapitulate the observed increase in distances (Figure 6C and D), which corresponds to a 1.7-fold increase in linear extension. Since the wrapping of DNA on nucleosomes leads to an approximately 6-fold compaction, this suggests that about 30 % of the nucleosomes at the *GAL* locus are evicted upon transcriptional activation. This is in accordance with nucleosome occupancy measurements (Schwabish and Struhl, 2006) and, at a density of 162bp/nucleosome (Horz and Zachau, 1980), corresponds to the eviction of approximately 11 of 36 nucleosomes across the locus.

Discussion

Recently, the development of chromosome conformation capture techniques has revolutionized the characterization of three-dimensional interaction landscapes of chromatin and has led to many important discoveries and new models of chromatin organization (reviewed in Pombo and Dillon, 2015; Bonev and Cavalli, 2016). However, these techniques require the fixation of cells and are usually applied to cell populations. Furthermore, it is not possible to directly convert the interactions detected by chromosome conformation capture into 3D distances in the cell nucleus. In fact, a direct comparison of chromosome capture analyses with DNA fluorescence *in situ* hybridisation results has revealed that the two techniques do not always result in congruent findings (Williamson *et al.*, 2014). It is therefore crucial to analyze chromatin conformation *in situ* and also *in vivo*.

Chromatin decompaction upon transcriptional activation has been visualized in mammalian cells using large repeat arrays (Tumbar *et al.*, 1999; Dietzel *et al.*, 2004; Verschure *et al.*, 2005; Hu *et al.*, 2009). FISH analysis has also been employed to obtain distance measurements at activated loci (Chambeyron and Bickmore, 2004; Chambeyron *et al.*, 2005; Morey *et al.*, 2007). Although making important contributions to our understanding of changes in chromatin conformation upon transcriptional activation, quantitative interpretation is limited because fixation and DNA FISH protocols introduce modifications to the cell ultrastructure and repeat arrays may themselves influence the local chromatin environment. Therefore, we have used the budding yeast *Saccharomyces cerevisiae* to generate a system where live cell analysis is possible in a quantitative manner at an endogenous gene locus. Although we initially use a 3-gene system to establish our assay, we also show that this assay is sensitive enough to visualize decompaction of a single activated gene. Furthermore, the use of budding yeast allows us to study the basic level of chromatin decompaction in the absence of confounding changes in chromatin conformation like enhancer-promoter looping or looping out from a chromosome territory. Thus, our data yield quantitative

measurements of chromatin decompaction upon transcriptional activation and allow us to model the changes in chromatin properties at an activated locus.

The role of acetylation

Interestingly, we found that deletion of the lysine acetyl transferase gene *GCN5* or inhibition of lysine deacetylation by trichostatin A had no observable effect on the distance distribution in our compaction assay. In contrast, the treatment of mammalian cells with TSA was previously reported to affect chromatin organization globally and to lead to decompaction of heterochromatic regions (Toth *et al.*, 2004; Lleres *et al.*, 2009). However, these studies used global histone signals (intensity or FRET-FLIM) to assess compaction levels. These analyses thus do not give sequence-specific readout and report on spatial rather than on linear compaction. In agreement with our findings, DNA FISH analysis in mouse cells, which also tested linear compaction between two loci, showed decompaction in polycomb silenced regions but not elsewhere (Eskeland *et al.*, 2010). Thus, very low acetylation levels may be crucial for the formation of certain subnuclear compartments in heterochromatic regions of the nucleus but may play a less prominent role in linear decompaction in the relatively open bulk yeast chromatin. However, it cannot be excluded that specific acetylation added by acetyl transferases other than Gcn5 or removed by non-TSA sensitive deacetylases contributes to linear chromatin decompaction. Furthermore, acetylation could facilitate the binding of factors to specific DNA sequences by rendering the interactions between histones and DNA less tight. This could allow nucleosomes to slide more easily or create binding sites for bromodomain-containing chromatin effector proteins (Filippakopoulos and Knapp, 2014). In contrast to the *GAL* genes, other budding yeast genes were shown to fully depend on the HAT activity of Gcn5 for transcriptional activation. For example, this is true for a heterologous β-estradiol responsive transcriptional activator based on the viral VP16 transactivation domain (Stafford and Morse, 2001). Intriguingly, we found that upon activation via a β-estradiol-inducible VP16 fusion protein (Louvion *et al.*, 1993), Gcn5 is essential for decompaction (Supplemental Figure 3) and neither *gcn5Δ* nor *spt20Δ* cells were able to decompact the *GAL* locus upon addition of β-estradiol (Supplemental Figure 3). This observation also corroborates the conclusion that transcriptional activation precedes decompaction.

The interplay of transcription and decompaction

Our mutant analysis shows that transcriptional activation and chromatin decompaction are tightly coupled. To directly address the role of Pol II in decompaction, we carried out experiments with cells expressing a temperature-sensitive mutant allele of the largest subunit of RNA Pol II, *rpb1-1*. *rpb1-1* cells were induced with galactose one hour after a shift to the restrictive temperature. Both expression of *GAL10* mRNA and decompaction were reduced but not abolished (Supplemental Figure 4A and B). Thus, it appears that induction at this locus is so strong that the temperature-sensitive *rpb1-1* mutant is not fully effective to suppress decompaction and transcription. Nevertheless, together with the observation that an ectopic *GAL* promoter in the absence of an ORF is not sufficient to confer decompaction (Figure 6A), our results suggest that ORF transcription by Pol II is required for decompaction of the chromatin at the *GAL* locus. This is consistent with the observation in mammalian cells that only initial decompaction is mediated by transcriptional activation but for complete decompaction elongation by RNA polymerase is necessary (Hu *et al.*, 2009). We hypothesize that the removal of nucleosomes at the promoter presents a first stage of decompaction, but elongation and the concomitant eviction of nucleosomes along the ORF constitutes the major contribution to chromatin opening. This interpretation is in line with the observation that opening of the *GAL* locus is not detected when both glucose and galactose are present (Figure 4C Galactose+Glucose condition), a condition where SWI/SNF activity has been reported to evict nucleosomes at the promoter (Bryant *et al.*, 2008). However, in addition to the length of the ORF, the strength of activation and possibly the composition of the 3’ and 5’ UTRs may

also contribute to the level of nucleosome eviction and thus decompaction. Importantly, the absence of a decompaction response from the promoter alone also indicates that longer range interactions mediated by the promoter, for example promoter-enhancer loops, do not play a role in our system allowing us to focus on the local changes in chromatin organization. Such effects or the release from higher spatial compaction states may have contributed to the transcription-independent decompaction that were observed previously (Hu *et al.*, 2009).

After transcriptional repression by the addition of glucose, the signal of *GAL* mRNAs in the cytoplasm quickly diminishes. Although the nuclear FISH signal at the site of transcription is reduced as well, a weak spot can still be detected in many cells even 60 minutes after glucose addition (Supplemental Figure 5A and C). This most likely corresponds to polyadenylated *GAL* mRNAs previously reported to persist at the *GAL* genes upon transcriptional shut-off (Abruzzi *et al.*, 2006). Interestingly, cells with a brighter RNA dots showed a distance distribution shifted to longer distances compared to cells with a weaker or absent RNA dot signal (Supplemental Figure 5B). This could indicate that transcription is still ongoing in those cells and thus the brighter RNA signal would stem from nascent transcripts. Alternatively, long-lived, chromatin-associated RNA dots might contribute to keeping chromatin in a partially decompacted state.

The role of nucleosome eviction

Histones are evicted during transcriptional elongation but repositioned after the passage of Pol II. Both eviction and deposition require the activity of histone chaperones like FACT. In highly transcribed genes like the *GAL* genes, individual transcription events follow closely after each other (Lenstra *et al.*, 2015). In this case, deposition of histones between rapid rounds of transcription may not be possible, and thus a net loss of histones and nucleosomes occurs. Such a response would explain the dramatic unfolding of chromatin across the *GAL* locus observed in our assay. This is consistent with our observation that mutants with reduced transcriptional activity display a reduced decompaction. Here, initiation and polymerase II progression occurs at a lower frequency, which could allow for more time to deposit histones after the passage of Pol II. This interpretation is also in agreement with the observed correlation between ORF length and decompaction. However, for the moment it remains unclear why the *GAL1* ORF and its native 3'UTR confer higher levels of decompaction compared to a GST ORF terminated by an ADH1 terminator. Of note, the presence of MS2 stem loops in our 2xGST construct may present a serious obstacle to Pol II (Heinrich *et al.*, 2017) and therefore could lead to reduced Pol II density on the body of the gene.

Outlook

Transcription initiation displays large cell-to-cell variability, and the same can be expected also for decompaction. Unfortunately, we were not able to reliably measure decompaction kinetics on a single cell level, due to the large fluctuations in distances measured over time even for cells in steady state growth conditions. Therefore, further developments of reporter systems with reduced biological noise in combination with improved superresolution microscopy approaches will be needed to enable the analysis of decompaction kinetics on the single cell level. For example, the contribution of DNA sequences that are not involved in the observed response could be minimized by placing fluorescent reporters directly adjacent to the gene of interest. Adaptions of our system thus have the potential to shed further light on the dynamics of chromatin decompaction by allowing to study this process quantitatively in single cells under physiological conditions. The data from live cell microscopy experiments are highly complementary to data obtained from chromosome capture techniques and are very valuable to better understand chromatin organization and its dynamics *in vivo*. Furthermore, using CRISPR/Cas technologies, our assay could be readily transferred to higher eukaryotic systems as well. Optimized cassettes for the integration of LacO repeats by CRISPR/Cas have recently been created (Tasan *et al.*, 2017) and the targeting of labelled CIRSPR/Cas complexes

(Chen *et al.*, 2013; Chen and Huang, 2014; Ma *et al.*, 2016) will in the future provide a powerful and flexible tool to dissect the contribution of individual players to chromatin compaction in various model systems.

Methods

Plasmid construction

Plasmid were constructed using standard molecular biology techniques. pKW1008 was constructed by ligating annealed oligos UC586/867 into pFA6-GFP(s65t)-kan (Longtine *et al.*, 1998) using PacI/AscI to replace GFP with a FLAG-tag. pKW2695, pKW2704 and pKW3264 were constructed by ligating PCR products of primers CH4505/CH4506, CH4513/CH4514 or CH6/CH106 on genomic DNA into pKW1689 (Green *et al.*, 2012) after cutting vector and insert with XhoI and SacI. pKW3035 was constructed from a PCR product of UC5681/UC5682 on pKW3010 (Backlund *et al.*, 2014) cut with DraIII and ligated into pAFS135 (Straight *et al.*, 1996) also cut with DraIII. pKW3681 and pKW3682 were constructed by inserting fragments generated by primers CH1211/CH1213 or CH1211/CH1177 on genomic DNA into the pFA6a backbone containing a NatMx resistance cassette (cut with PacI/XhoI). pKW3683 was constructed by inserting into the same vector a stitched PCR product of the *GAL1* promoter (CH1211/CH1218) and GST (CH1214/CH1215) with PacI/XhoI. pKW3999 was constructed in multiple steps. The final construct contains homology regions for a region on chromosome II which were amplified from genomic DNA using primers CH1795/CH1796 and CH1797/CH1798. Between the homology sites, the *GAL1* promoter amplified by CH1211/CH2043 drives a construct with 24 MS2 stem loops (Grunwald and Singer, 2010) in the 5' UTR and encoding 2xGST (CH1789/CH1794 and CH1791/CH1794) tagged with V5. It also contains the NatNT2 resistance cassette for selection and can be integrated after cutting NotI/AscI. The Gal4DBD-ER-VP16 activation construct was transferred from the construct published in (Louvion *et al.*, 1993) into a Ylplac204 backbone with an additional NatMx cassette added for selection in TRP+ strains (pKW3504). All plasmids used in this study are listed in Supplementary Table 1. Primers are listed in Supplementary Table 2.

Yeast strain construction

S. cerevisiae strains were constructed in the background of BY4741 and BY4742 (Brachmann *et al.*, 1998) using standard yeast genetic techniques either by transformation of a linearized plasmid or of a PCR amplification product with homology to the target site (Baudin *et al.*, 1993). The mutants *spt20Δ, gal80Δ, gcn5Δ, asf1Δ, snf2Δ, gal11Δ, pob3-7, spt16ts, med7-163, rpb1-1, gal4Δ, swr1Δ* and *arp6Δ* were constructed by mating of the wildtype strains with the corresponding strains from the MATa, MATα deletion collections (Winzeler *et al.*, 1999) or the MATa temperature sensitive collection (Li *et al.*, 2011) followed by sporulation and tetrad dissection. *ADA2* was deleted by PCR-directed mutagenesis using primers CH395/CH396 on pKW1008 to generate KWY5104. Reporter strains for the experiments shown in Figure 6A were generated by transformation of KWY4067 with the PCR product of CH1272/CH1273 on pKW3681 (=> KWY6245), pKW3682 (=> KWY6247) or pKW3683 (=> KWY6252) respectively. Genotypes were confirmed by PCR. All yeast strains used in this study are listed in Supplementary Table 3.

Yeast culture conditions

Yeast cells were cultured in complete synthetic medium with 2 % of either glucose, galactose or raffinose as indicated at 30 ˚C. Temperature sensitive mutants were grown at 25 ˚C. Some kinetic experiments with mutants showing significant growth defects were also carried out at 25 ˚C. All experiments were carried out with cells in exponential growth phase. For microscopy experiments, cells were usually inoculated from saturated cultures into fresh medium and grown overnight to OD 0.5-0.8 and then imaged. Alternatively, cells were diluted in the morning and grown for additional 2-

3 cell cycles before the start of the experiment. Trichostatin A (Sigma Catalog #:T8552) was used at 50 µM.

Microscopy

Cells were pregrown in raffinose-containing medium and inoculated in 1 ml in 24 well plates overnight so that they reached an OD of 0.5-0.8 in the morning. Cells were then transferred to concanavalin A coated 384 well plates (Matrical) and imaged on a temperature controlled Nikon Ti Eclipse equipped with a Spectra LED lamp using a Apochromat VC 100x objective NA 1.4 (Nikon) (filters: Spectra emission filters 475/28 & 542/27 and DAPI/FITC/Cy3/Cy5 Quad HC Filterset with 410/504/582/669 HC Quad dichroic and a 440/521/607/700 HC quadband filter (Semrock)) with exposure times of 50-200 ms. For timelapse experiments, induction with different sugars or trichostatin A was carried out on stage after the first imaging timepoint by mixing with an equal volume of trichostatin or sugar containing medium. Different fields of view were imaged at each timepoint to prevent negative effects of phototoxicity or bleaching on the analysis. 3D microscopy of living cells was carried out on a SpinningDisk microscope (Yokogawa Confocal Scanner Unit CSU-W1-T2) built on a Nikon TiE body and controlled with the VisiVIEW software using dual camera acquisition mode and a 100x NA 1.49 CFI Apo TIRF objective. Cameras were Orca Flash 4.0 V2, excitation lasers were a DPSS 488 nm (200mW) and a diode 561 nm (200 mW) laser. Z scanning was performed in streaming mode with a LUDL BioPrecision2 Piezo Stage using 2-by-2 binning with 100 ms exposure times per frame. Filters were: Dichroic quad-band DAPI/GFP/RFP/CY5, splitting filter to camera ports: 561LP, emission filters GFP/ET525/50 and mCherry ET630/75 respectively.

Single molecule fluorescence in situ hybridization

Single molecule fluorescence *in situ* hybridization was carried out according to (Mugler *et al.*, 2016) with slight adaptations. The indicated strains were inoculated in synthetic medium containing 2 % raffinose and grown overnight to saturation. The next day, cells were diluted into fresh synthetic complete media containing 2 % raffinose or 2 % galactose and grown overnight at 30 °C to exponential growth phase (OD600 = 0.6-0.8). Cells were then induced by addition of glucose or galactose to final concentration of 2 % and fixed after the indicated timepoints for 15 min at 30 °C and for 15 min at 25 °C with 4 % paraformaldehyde (EM grade 32 % paraformaldehyde acqueous solution electron Microscopy Sciences 15714), washed with buffer B (1.2 M sorbitol, 100 mM $KHPO_4$ at pH 7.5, 4 °C) and stored at 4 °C overnight. Cells were then spheroplasted for 20 min using 1 % 20T zymolyase in 1.2 M sorbitol, 100 mM $KHPO_4$ at pH 7.5, 20 mM vanadyl ribonuclease complex and 20 µM β-mercaptoethanol, washed with buffer B to stop the spheroplasting reaction and then washed into 10 % formamide (Merck Millipore S4117) in 2x SSC.

Mixtures of DNA probes coupled to Quasar670 (Stellaris, LGC Biosearch, Novato, CA; probes were synthesized by BioCat, Heidelberg, Germany) were used for smFISH, targeting the *GAL1*, *GAL7* and *GAL10* ORFs (Supplementary Table 4). Per sample, 0.5 µl of each probe mix (stock 25 uM) was mixed with 2 µl of salmon-sperm DNA (10 mg ml−1, Life Technologies, 15632-011) and 2 µl yeast transfer RNA (10 mg/ml, Life Technologies, AM7119). The probe mix was denatured in 50 µl per sample of hybridization buffer F (20 % formamide, 10 mM $NaHPO_4$ at pH 7.0) for 3 min at 95 °C and then mixed with 50 µl per sample hybridization buffer H (4× SSC, 4 mg/ml BSA (acetylated) and 20 mM vanadyl ribonuclease complex). Cells (approximately corresponding to pellet of 5 ml initial culture) were resuspended in the hybridization mix and incubated for 8-12 hours at 37 °C. After four washing steps (10 % formamide/2× SSC; 0.1 % Triton/2x SSC; 2x SSC/DAPI; 2x SSC), cells were stored at 4 °C. Cells were imaged in concanavaline A coated 384 wells. Microscopy was performed with an inverted epi-fluorescence microscope (Nikon Ti) equipped with a Spectra X LED light source and a Hamamatsu Flash 4.0 sCMOS camera using a PlanApo 100 x NA 1.4 oil-immersion objective and the NIS Elements software. 31 z planes were acquired. The stack of the Qasar670 channel was acquired first due to

significant bleaching. mCherry and GFP channels were acquired plane by plane to minimize shift between channels.

Image analysis

Images were processed using FIJI (NIH ImageJ 1.51p and previous) and Diatrack (Vallotton *et al.*, 2017). For 2D analysis of distances, position of chromosome location marked with LacI or TetR were detected by a 2D Gaussian fit using custom written scripts in Matlab (Dultz *et al.*, 2016). Distances between detected spots were calculated. Due to negligible shift between the two channels in these datasets, correction was not required. For 3D analysis of distances (data acquired on dual camera spinning disk microscope), positions of chromosome location marked with LacI or TetR were detected in Diatrack in 3D using across-color tracking to pair corresponding spots from the same cell (parameters used: 'exclude blurred' 0.07-0.09, 'exclude dim' 20-200, full width half maximum for Gaussian fitting 2.5). Subsequently, positions were corrected for shift between the two channels using bead images (1 µm TetraSpeck fluorescent microspheres, ThermoFisher Scientific) acquired under identical imaging conditions. For shift correction, the field of view was subdivided into 150x150 pixel large squares and the mean shift for beads acquired in this region was applied to correct the positions of chromosome locations acquired in this region of the camera. The distances between corrected positions were calculated and plotted in Matlab as boxplots. For FISH analysis, position of chromosome location marked with LacI or TetR were detected in Diatrack in 3D using across-color tracking to pair corresponding spots from the same cell. Positions of transcriptions spots were detected on maximum intensity projections of the Qasar670 channel in Diatrack and used to categorize cells as having or not having a transcription spot for separate analysis of the two populations. For analysis of transcription spot intensities, transcription spots were detected on 3D stacks in Diatrack and intensity values were obtained from the Diatrack session file. Only the intensity of those transcription spots for which corresponding chromosome locations marked with LacI or TetR could be detected in Diatrack were included in the analysis. Bootstrapping was used to obtain 95 % confidence intervals of the medians and mean. Typically 200-400 cells (but at least 90) were analyzed per condition in each experiment.

Quantitative real-time PCR

qPCR was performed as described previously (Dultz *et al.*, 2016): 1 ml of cells at OD600 0.8-1 was harvested by centrifugation and snap frozen in liquid nitrogen. RNA was extracted using the RNeasy kit from Qiagen via mechanical disruption. 300 ng of total extracted RNA was used for reverse transcription. The RNA was first treated with DNase I using the DNA-free kit from Ambion according to the protocol of the manufacturer. Reverse transcription was performed according to the protocol of the manufacturer using Superscript II reverse transcriptase (Invitrogen) with random hexamer primers. Quantitative Real Time PCR was performed on a StepOnePlus Instrument (Invitrogen) using Absolute Blue QPCR Mix with SYBR Green and ROX (ThermoFisher Scientific). All experiments were carried out in three technical replicates and three biological replicates. Data was analyzed by the comparative CT method using *ACT1* as endogenous control. Primers used for qPCR are listed in Supplementary Table 2.

Computational modeling

Average spatial distances were calculated from ensembles containing 1000 structure models of the entire haploid yeast genome. Ensembles were generated for each of the two *GAL* locus activation states (active/galactose and inactive/glucose), as described in (Tjong *et al.*, 2012; Dultz *et al.*, 2016). All chromosomes were modeled as chains of connecting beads subject to a number of spatial restraints. In particular, all chromosomes were confined inside a nucleus with a radius of 1 µm; the nucleolus and spindle pole body (SPB) are placed on opposite sides of the nucleus along the central nuclear axis; all non-ribosomal-DNA gene regions are excluded from the nucleolar volume;

centromeric regions are proximal to the SPB, whereas telomeric regions are tethered to the nuclear envelope (allowing a maximal distance between telomeres and the nuclear envelope of 50 nm). Each bead accommodated ~3.2kb of genome sequence as described in (Tjong *et al.*, 2012).

To reproduce the experimental data, we modeled a gradual decrease in chromatin compaction in the proximity of the *GAL* locus. For the 60 kb region starting at the position of the *GAL* locus, the compaction ratio was set to 1.6 kb per bead. We then determined the optimal chromatin compaction for the 6.2 kb *GAL* locus so that the models reproduce most closely the experimentally observed 3D fluorophore distances in both activation states. The *GAL* locus compaction in the inactive glucose state was found to be ~375 bp/bead whereas in the active galactose state it was set to ~200 bp/bead.

Each ensemble of genome structures was generated from 1000 independent simulations, each starting from random configurations. The optimization procedure consisted of a simulated annealing Molecular Dynamics run followed by Conjugate Gradients score minimization, both performed using IMP (Russel *et al.*, 2012).

Author contributions

Conception and design of the work: ED and KW; Data collection: ED and RM; Data analysis and interpretation: ED and RM; development of code for image analysis: ED and PV; modelling: GP & FA Drafting the article: ED; critical revision of the article: ED, RM, PV, GP, FA & KW.

Acknowledgements

We are grateful to members of the Weis laboratory for critical discussions and suggestions on the manuscript. We thank the Scientific Center for Optical and Electron Microscopy of ETHZ (ScopeM) for support with microscopy. This work was supported by the Swiss National Fond (SNF 159731) and NIH (U54DK107981 to F.A).

References

Abruzzi, K.C., Belostotsky, D.A., Chekanova, J.A., Dower, K., and Rosbash, M. (2006). 3'-end formation signals modulate the association of genes with the nuclear periphery as well as mRNP dot formation. The EMBO journal *25*, 4253-4262.

Backlund, M.P., Joyner, R., Weis, K., and Moerner, W.E. (2014). Correlations of three-dimensional motion of chromosomal loci in yeast revealed by the double-helix point spread function microscope. Molecular biology of the cell *25*, 3619-3629.

Baudin, A., Ozier-Kalogeropoulos, O., Denouel, A., Lacroute, F., and Cullin, C. (1993). A simple and efficient method for direct gene deletion in Saccharomyces cerevisiae. Nucleic acids research *21*, 3329-3330.

Bhaumik, S.R., and Green, M.R. (2001). SAGA is an essential in vivo target of the yeast acidic activator Gal4p. Genes & development *15*, 1935-1945.

Bonev, B., and Cavalli, G. (2016). Organization and function of the 3D genome. Nature reviews. Genetics *17*, 661-678.

Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P., and Boeke, J.D. (1998). Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast *14*, 115-132.

Bryant, G.O., Prabhu, V., Floer, M., Wang, X., Spagna, D., Schreiber, D., and Ptashne, M. (2008). Activator control of nucleosome occupancy in activation and repression of transcription. PLoS biology *6*, 2928-2939.

Bryant, G.O., and Ptashne, M. (2003). Independent recruitment in vivo by Gal4 of two complexes required for transcription. Molecular cell *11*, 1301-1309.

Carrozza, M.J., John, S., Sil, A.K., Hopper, J.E., and Workman, J.L. (2002). Gal80 confers specificity on HAT complex interactions with activators. The Journal of biological chemistry *277*, 24648-24652.

Chambeyron, S., and Bickmore, W.A. (2004). Chromatin decondensation and nuclear reorganization of the HoxB locus upon induction of transcription. Genes & development *18*, 1119-1130.

Chambeyron, S., Da Silva, N.R., Lawson, K.A., and Bickmore, W.A. (2005). Nuclear re-organisation of the Hoxb complex during mouse embryonic development. Development *132*, 2215-2223.

Chen, B., Gilbert, L.A., Cimini, B.A., Schnitzbauer, J., Zhang, W., Li, G.W., Park, J., Blackburn, E.H., Weissman, J.S., Qi, L.S., and Huang, B. (2013). Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system. Cell *155*, 1479-1491.

Chen, B., and Huang, B. (2014). Imaging Genomic Elements in Living Cells Using CRISPR/Cas9. Methods in enzymology *546*, 337-354.

Chen, C., Lim, H.H., Shi, J., Tamura, S., Maeshima, K., Surana, U., and Gan, L. (2016). Budding yeast chromatin is dispersed in a crowded nucleoplasm in vivo. Molecular biology of the cell.

Dietzel, S., Zolghadr, K., Hepperger, C., and Belmont, A.S. (2004). Differential large-scale chromatin compaction and intranuclear positioning of transcribed versus non-transcribed transgene arrays containing beta-globin regulatory sequences. Journal of cell science *117*, 4603-4614.

Dultz, E., Tjong, H., Weider, E., Herzog, M., Young, B., Brune, C., Müllner, D., Loewen, C., Alber, F., and Weis, K. (2016). Global reorganization of budding yeast chromosome conformation in different physiological conditions. The Journal of cell biology, jcb.201507069.

Eskeland, R., Freyer, E., Leeb, M., Wutz, A., and Bickmore, W.A. (2010). Histone acetylation and the maintenance of chromatin compaction by Polycomb repressive complexes. Cold Spring Harbor symposia on quantitative biology *75*, 71-78.

Even-Faitelson, L., Hassan-Zadeh, V., Baghestani, Z., and Bazett-Jones, D.P. (2016). Coming to terms with chromatin structure. Chromosoma *125*, 95-110.

Filippakopoulos, P., and Knapp, S. (2014). Targeting bromodomains: epigenetic readers of lysine acetylation. Nature reviews. Drug discovery *13*, 337-356.

Fussner, E., Strauss, M., Djuric, U., Li, R., Ahmed, K., Hart, M., Ellis, J., and Bazett-Jones, D.P. (2012). Open and closed domains in the mouse genome are configured as 10-nm chromatin fibres. EMBO reports *13*, 992-996.

Govind, C.K., Zhang, F., Qiu, H., Hofmeyer, K., and Hinnebusch, A.G. (2007). Gcn5 promotes acetylation, eviction, and methylation of nucleosomes in transcribed coding regions. Molecular cell *25*, 31-42.

Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F., Berger, S.L., and Workman, J.L. (1997). Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. Genes & development *11*, 1640-1650.

Green, E.M., Jiang, Y., Joyner, R., and Weis, K. (2012). A negative feedback loop at the nuclear periphery regulates GAL gene expression. Molecular biology of the cell *23*, 1367-1375.

Grunwald, D., and Singer, R.H. (2010). In vivo imaging of labelled endogenous beta-actin mRNA during nucleocytoplasmic transport. Nature *467*, 604-607.

Heinrich, S., Sidler, C.L., Azzalin, C.M., and Weis, K. (2017). Stem-loop RNA labeling can affect nuclear and cytoplasmic mRNA processing. Rna *23*, 134-141.

Horz, W., and Zachau, H.G. (1980). Deoxyribonuclease II as a probe for chromatin structure. I. Location of cleavage sites. J Mol Biol *144*, 305-327.

Hsieh, T.H., Weiner, A., Lajoie, B., Dekker, J., Friedman, N., and Rando, O.J. (2015). Mapping Nucleosome Resolution Chromosome Folding in Yeast by Micro-C. Cell *162*, 108-119.

Hu, Y., Kireev, I., Plutz, M., Ashourian, N., and Belmont, A.S. (2009). Large-scale chromatin structure of inducible genes: transcription on a condensed, linear template. The Journal of cell biology *185*, 87-100.

Kundu, S., and Peterson, C.L. (2010). Dominant role for signal transduction in the transcriptional memory of yeast GAL genes. Molecular and cellular biology *30*, 2330-2340.

Larschan, E., and Winston, F. (2001). The S. cerevisiae SAGA complex functions in vivo as a coactivator for transcriptional activation by Gal4. Genes & development *15*, 1946-1956.

Lau, M.S., Schwartz, M.G., Kundu, S., Savol, A.J., Wang, P.I., Marr, S.K., Grau, D.J., Schorderet, P., Sadreyev, R.I., Tabin, C.J., and Kingston, R.E. (2017). Mutation of a nucleosome compaction region disrupts Polycomb-mediated axial patterning. Science *355*, 1081-1084.

Lee, C.K., Shibata, Y., Rao, B., Strahl, B.D., and Lieb, J.D. (2004). Evidence for nucleosome depletion at active regulatory regions genome-wide. Nature genetics *36*, 900-905.

Lemieux, K., and Gaudreau, L. (2004). Targeting of Swi/Snf to the yeast GAL1 UAS G requires the Mediator, TAF IIs, and RNA polymerase II. The EMBO journal *23*, 4040-4050.

Lenstra, T.L., Coulon, A., Chow, C.C., and Larson, D.R. (2015). Single-Molecule Imaging Reveals a Switch between Spurious and Functional ncRNA Transcription. Molecular cell *60*, 597-610.

Li, B., Carey, M., and Workman, J.L. (2007). The role of chromatin during transcription. Cell *128*, 707-719.

Li, Z., Vizeacoumar, F.J., Bahr, S., Li, J., Warringer, J., Vizeacoumar, F.S., Min, R., Vandersluis, B., Bellay, J., Devit, M., Fleming, J.A., Stephens, A., Haase, J., Lin, Z.Y., Baryshnikova, A., Lu, H., Yan, Z., Jin, K., Barker, S., Datti, A., Giaever, G., Nislow, C., Bulawa, C., Myers, C.L., Costanzo, M., Gingras, A.C., Zhang, Z., Blomberg, A., Bloom, K., Andrews, B., and Boone, C. (2011). Systematic exploration of essential yeast gene function with temperature-sensitive mutants. Nature biotechnology *29*, 361-367.

Lleres, D., James, J., Swift, S., Norman, D.G., and Lamond, A.I. (2009). Quantitative analysis of chromatin compaction in living cells using FLIM-FRET. The Journal of cell biology *187*, 481-496.

Longtine, M.S., McKenzie, A., 3rd, Demarini, D.J., Shah, N.G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J.R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast *14*, 953-961.

Louvion, J.F., Havaux-Copf, B., and Picard, D. (1993). Fusion of GAL4-VP16 to a steroid-binding domain provides a tool for gratuitous induction of galactose-responsive genes in yeast. Gene *131*, 129-134.

Ma, H., Tu, L.C., Naseri, A., Huisman, M., Zhang, S., Grunwald, D., and Pederson, T. (2016). Multiplexed labeling of genomic loci with dCas9 and engineered sgRNAs using CRISPRainbow. Nature biotechnology *34*, 528-530.

Morey, C., Da Silva, N.R., Perry, P., and Bickmore, W.A. (2007). Nuclear reorganisation and chromatin decondensation are conserved, but distinct, mechanisms linked to Hox gene activation. Development *134*, 909-919.

Mugler, C.F., Hondele, M., Heinrich, S., Sachdev, R., Vallotton, P., Koek, A.Y., Chan, L.Y., and Weis, K. (2016). ATPase activity of the DEAD-box protein Dhh1 controls processing body formation. eLife *5*.

New, A.M., Cerulus, B., Govers, S.K., Perez-Samper, G., Zhu, B., Boogmans, S., Xavier, J.B., and Verstrepen, K.J. (2014). Different levels of catabolite repression optimize growth in stable and variable environments. PLoS biology *12*, e1001764.

Ou, H.D., Phan, S., Deerinck, T.J., Thor, A., Ellisman, M.H., and O'Shea, C.C. (2017). ChromEMT: Visualizing 3D chromatin structure and compaction in interphase and mitotic cells. Science *357*.

Pombo, A., and Dillon, N. (2015). Three-dimensional genome architecture: players and mechanisms. Nature reviews. Molecular cell biology *16*, 245-257.

Russel, D., Lasker, K., Webb, B., Velazquez-Muriel, J., Tjioe, E., Schneidman-Duhovny, D., Peterson, B., and Sali, A. (2012). Putting the pieces together: integrative modeling platform software for structure determination of macromolecular assemblies. PLoS biology *10*, e1001244.

Schwabish, M.A., and Struhl, K. (2004). Evidence for eviction and rapid deposition of histones upon transcriptional elongation by RNA polymerase II. Molecular and cellular biology *24*, 10111-10117.

Schwabish, M.A., and Struhl, K. (2006). Asf1 mediates histone eviction and deposition during elongation by RNA polymerase II. Molecular cell *22*, 415-422.

Schwabish, M.A., and Struhl, K. (2007). The Swi/Snf complex is important for histone eviction during transcriptional activation and RNA polymerase II elongation in vivo. Molecular and cellular biology *27*, 6987-6995.

Shogren-Knaak, M., Ishii, H., Sun, J.M., Pazin, M.J., Davie, J.R., and Peterson, C.L. (2006). Histone H4-K16 acetylation controls chromatin structure and protein interactions. Science *311*, 844-847.

Stafford, G.A., and Morse, R.H. (2001). GCN5 dependence of chromatin remodeling and transcriptional activation by the GAL4 and VP16 activation domains in budding yeast. Molecular and cellular biology *21*, 4568-4578.

Straight, A.F., Belmont, A.S., Robinett, C.C., and Murray, A.W. (1996). GFP tagging of budding yeast chromosomes reveals that protein-protein interactions can mediate sister chromatid cohesion. Current biology : CB *6*, 1599-1608.

Tasan, I., Sustackova, G., Zhang, L., Kim, J., Sivaguru, M., HamediRad, M., Wang, Y., Genova, J., Ma, J., Belmont, A.S., and Zhao, H. (2017). CRISPR/Cas9-mediated Knock-in of an Optimized TetO Repeat for Live Cell Imaging of Endogenous Loci. bioRxiv.

Taylor, G.C., Eskeland, R., Hekimoglu-Balkan, B., Pradeepa, M.M., and Bickmore, W.A. (2013). H4K16 acetylation marks active genes and enhancers of embryonic stem cells, but does not alter chromatin compaction. Genome research *23*, 2053-2065.

Tjong, H., Gong, K., Chen, L., and Alber, F. (2012). Physical tethering and volume exclusion determine higher-order genome organization in budding yeast. Genome research *22*, 1295-1305.

Toth, K.F., Knoch, T.A., Wachsmuth, M., Frank-Stohr, M., Stohr, M., Bacher, C.P., Muller, G., and Rippe, K. (2004). Trichostatin A-induced histone acetylation causes decondensation of interphase chromatin. Journal of cell science *117*, 4277-4287.

Traven, A., Jelicic, B., and Sopta, M. (2006). Yeast Gal4: a transcriptional paradigm revisited. EMBO reports *7*, 496-499.

Tsompana, M., and Buck, M.J. (2014). Chromatin accessibility: a window into the genome. Epigenetics & chromatin *7*, 33.

Tumbar, T., Sudlow, G., and Belmont, A.S. (1999). Large-scale chromatin unfolding and remodeling induced by VP16 acidic activation domain. The Journal of cell biology *145*, 1341-1354.

Vallotton, P., van Oijen, A.M., Whitchurch, C.B., Gelfand, V., Yeo, L., Tsiavaliaris, G., Heinrich, S., Dultz, E., Weis, K., and Grunwald, D. (2017). Diatrack particle tracking software: review of applications and performance evaluation. Traffic.

van den Engh, G., Sachs, R., and Trask, B.J. (1992). Estimating genomic distance from DNA sequence location in cell nuclei by a random walk model. Science *257*, 1410-1412.

Venkatesh, S., and Workman, J.L. (2015). Histone exchange, chromatin structure and the regulation of transcription. Nature reviews. Molecular cell biology *16*, 178-189.

Verschure, P.J., van der Kraan, I., de Leeuw, W., van der Vlag, J., Carpenter, A.E., Belmont, A.S., and van Driel, R. (2005). In vivo HP1 targeting causes large-scale chromatin condensation and enhanced histone lysine methylation. Molecular and cellular biology *25*, 4552-4564.

Vogelauer, M., Wu, J., Suka, N., and Grunstein, M. (2000). Global histone acetylation and deacetylation in yeast. Nature *408*, 495-498.

Wang, A., Kurdistani, S.K., and Grunstein, M. (2002). Requirement of Hos2 histone deacetylase for gene activity in yeast. Science *298*, 1412-1414.

Williamson, I., Berlivet, S., Eskeland, R., Boyle, S., Illingworth, R.S., Paquette, D., Dostie, J., and Bickmore, W.A. (2014). Spatial genome organization: contrasting views from chromosome conformation capture and fluorescence in situ hybridization. Genes & development *28*, 2778-2791.

Winzeler, E.A., Shoemaker, D.D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J.D., Bussey, H., Chu, A.M., Connelly, C., Davis, K., Dietrich, F., Dow, S.W., El Bakkoury, M., Foury, F., Friend, S.H., Gentalen, E., Giaever, G., Hegemann, J.H., Jones, T., Laub, M., Liao, H., Liebundguth, N., Lockhart, D.J., Lucau-Danila, A., Lussier, M., M'Rabet, N., Menard, P., Mittmann, M., Pai, C., Rebischung, C., Revuelta, J.L., Riles, L., Roberts, C.J., Ross-MacDonald, P., Scherens, B., Snyder, M., Sookhai-Mahadeo, S., Storms, R.K., Veronneau, S., Voet, M., Volckaert, G., Ward, T.R., Wysocki, R., Yen, G.S., Yu, K., Zimmermann, K., Philippsen, P., Johnston, M., and Davis, R.W. (1999). Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science *285*, 901-906.

Xin, H., Takahata, S., Blanksma, M., McCullough, L., Stillman, D.J., and Formosa, T. (2009). yFACT induces global accessibility of nucleosomal DNA without H2A-H2B displacement. Molecular cell *35*, 365-376.

Figure legends

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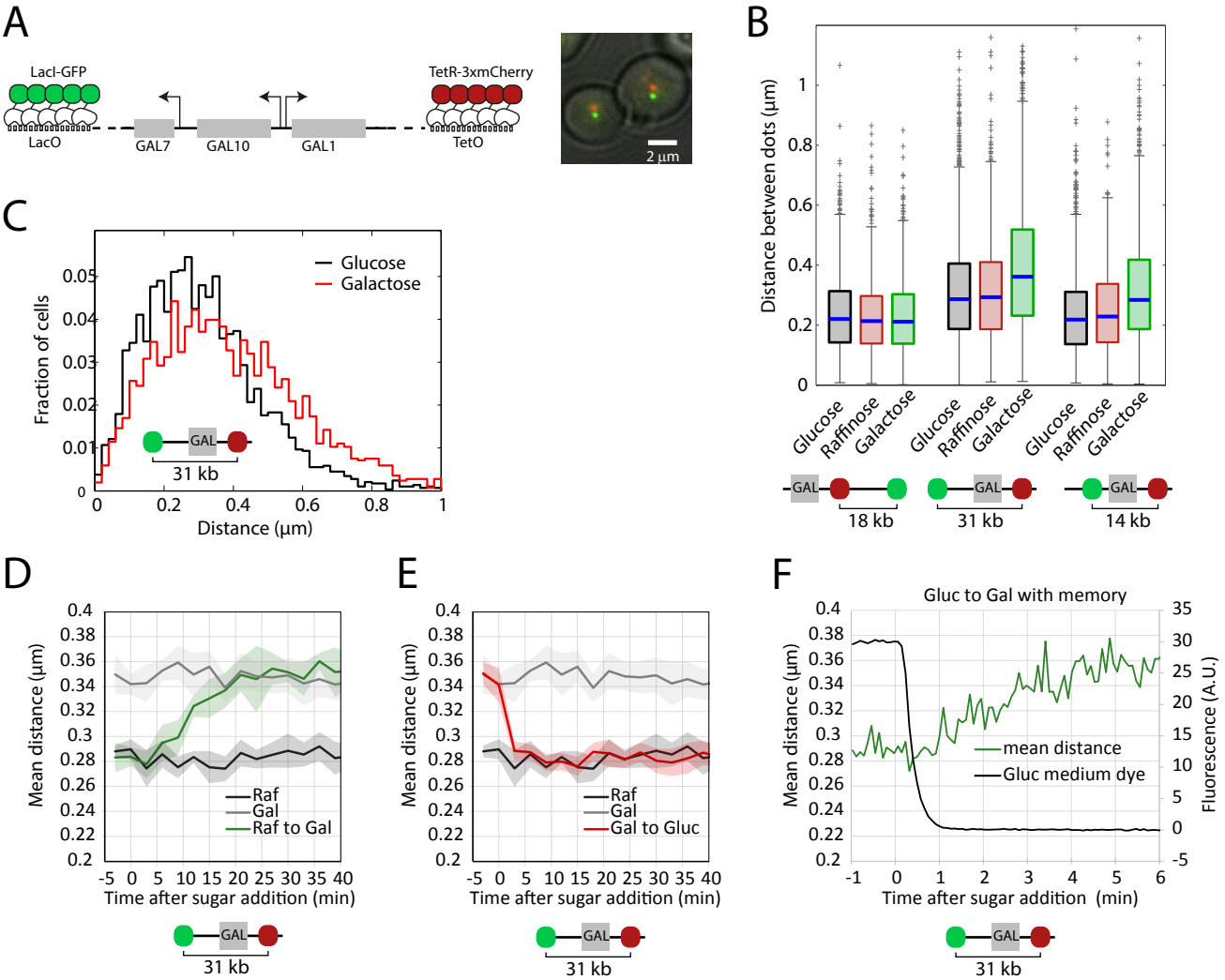
Figure 1



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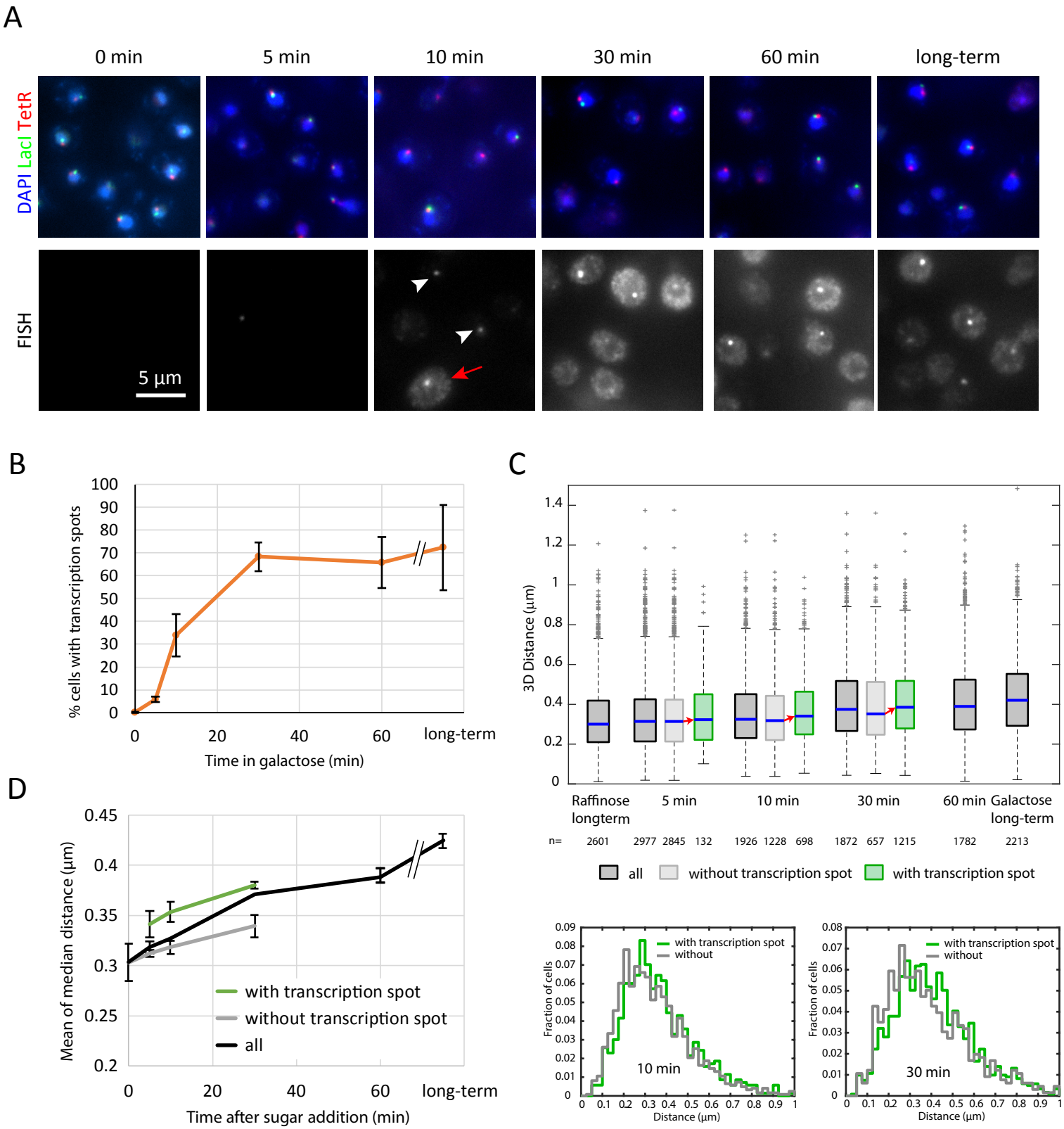

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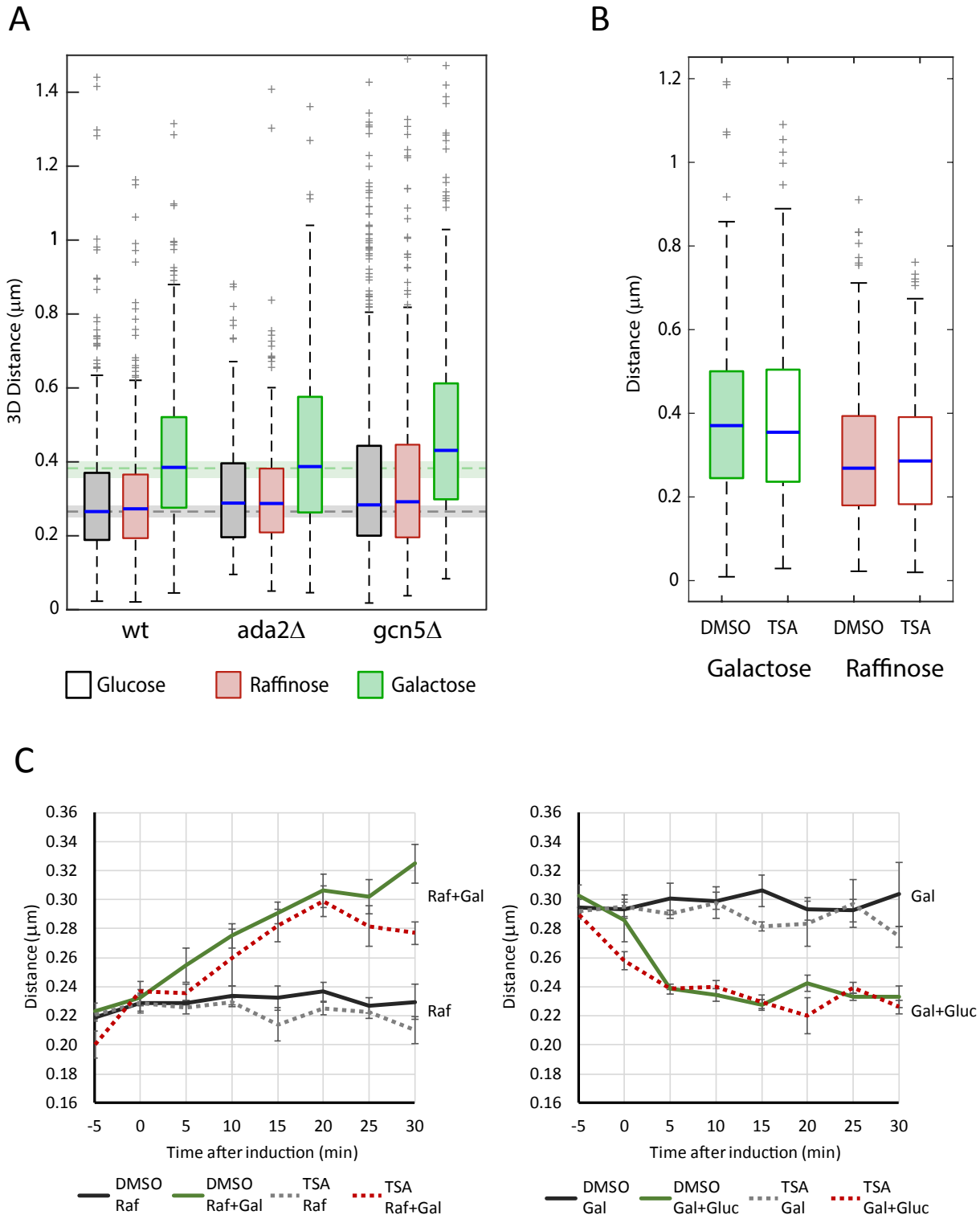

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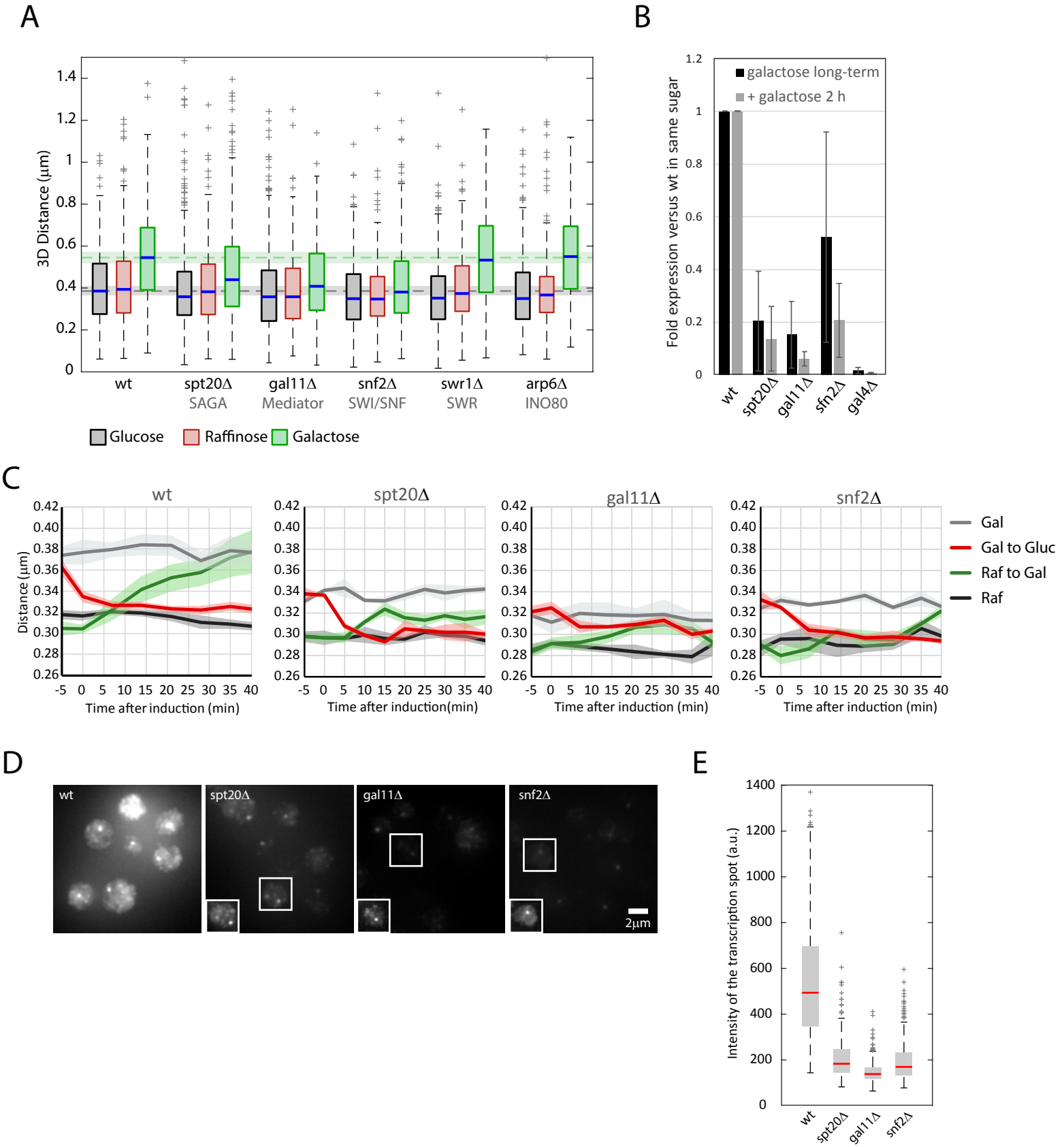

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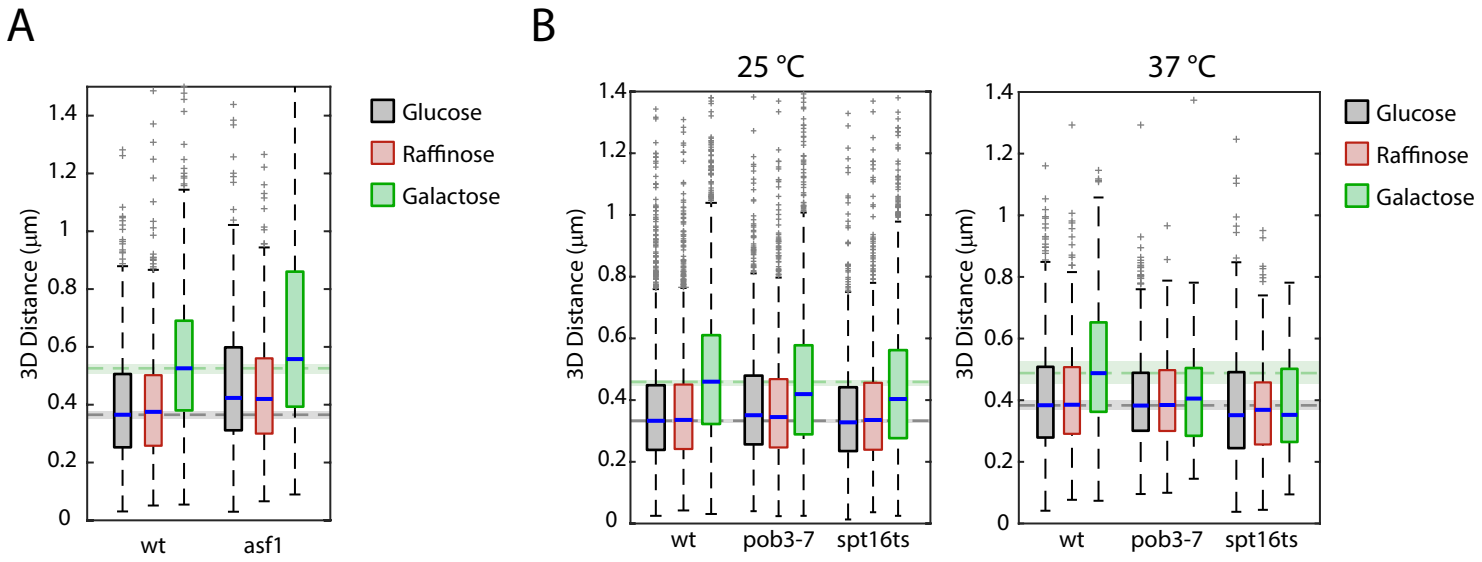

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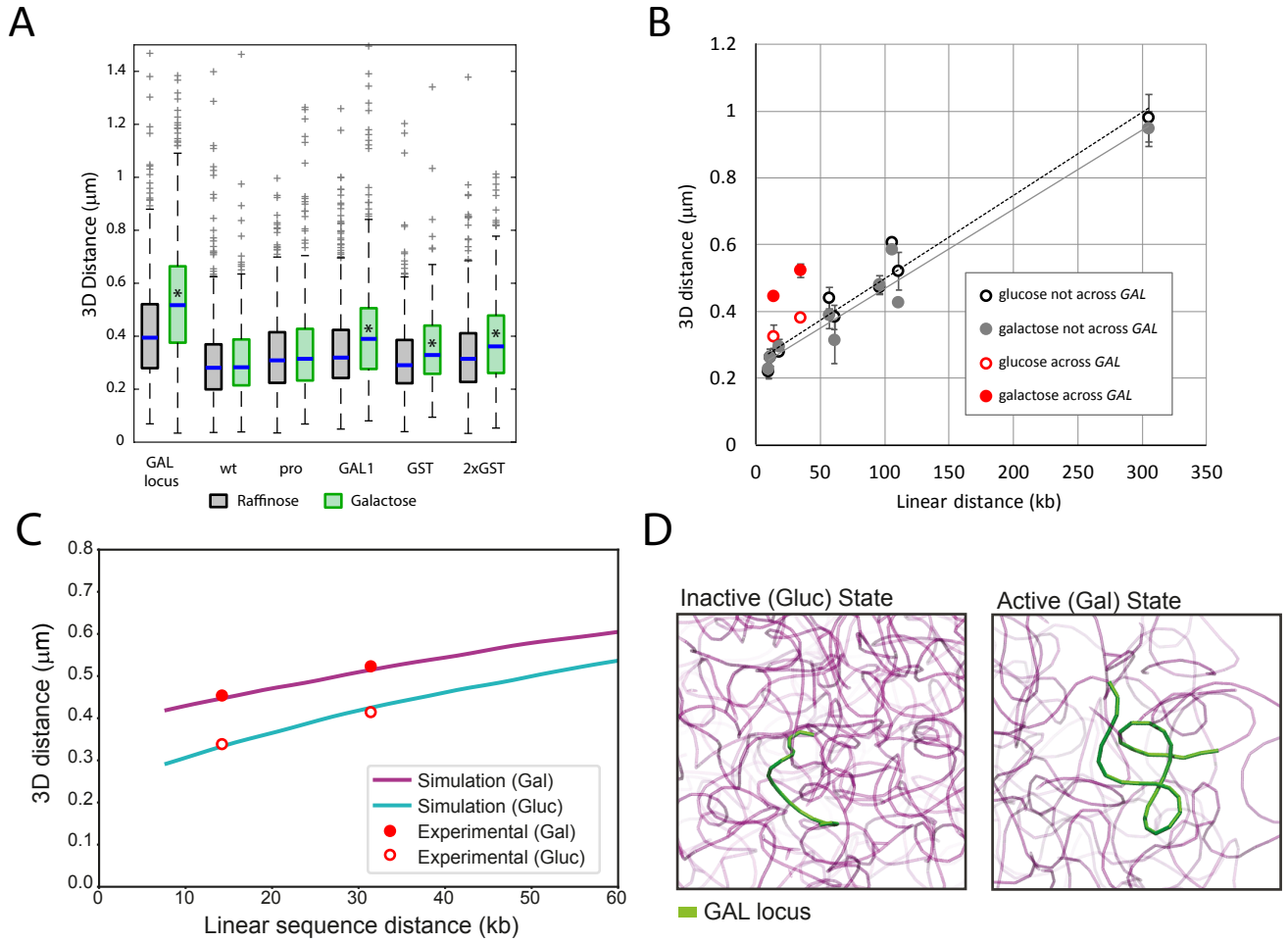

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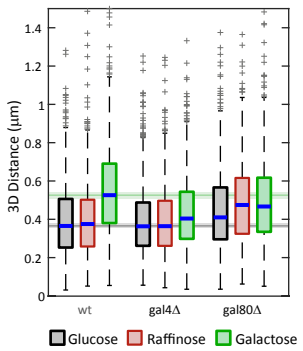

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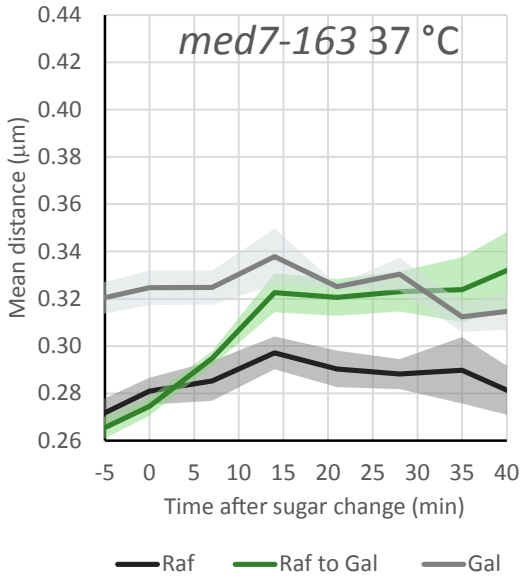

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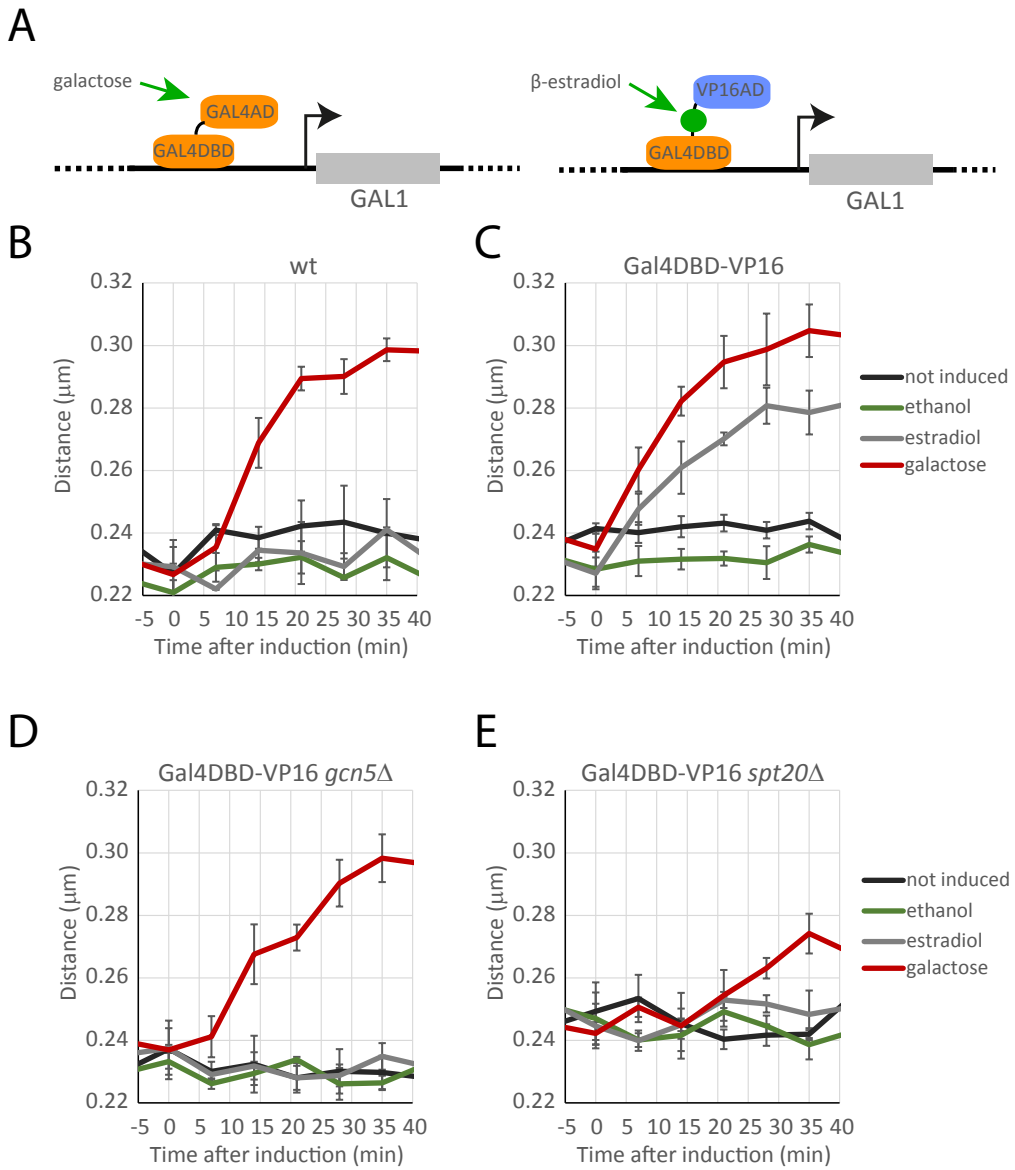

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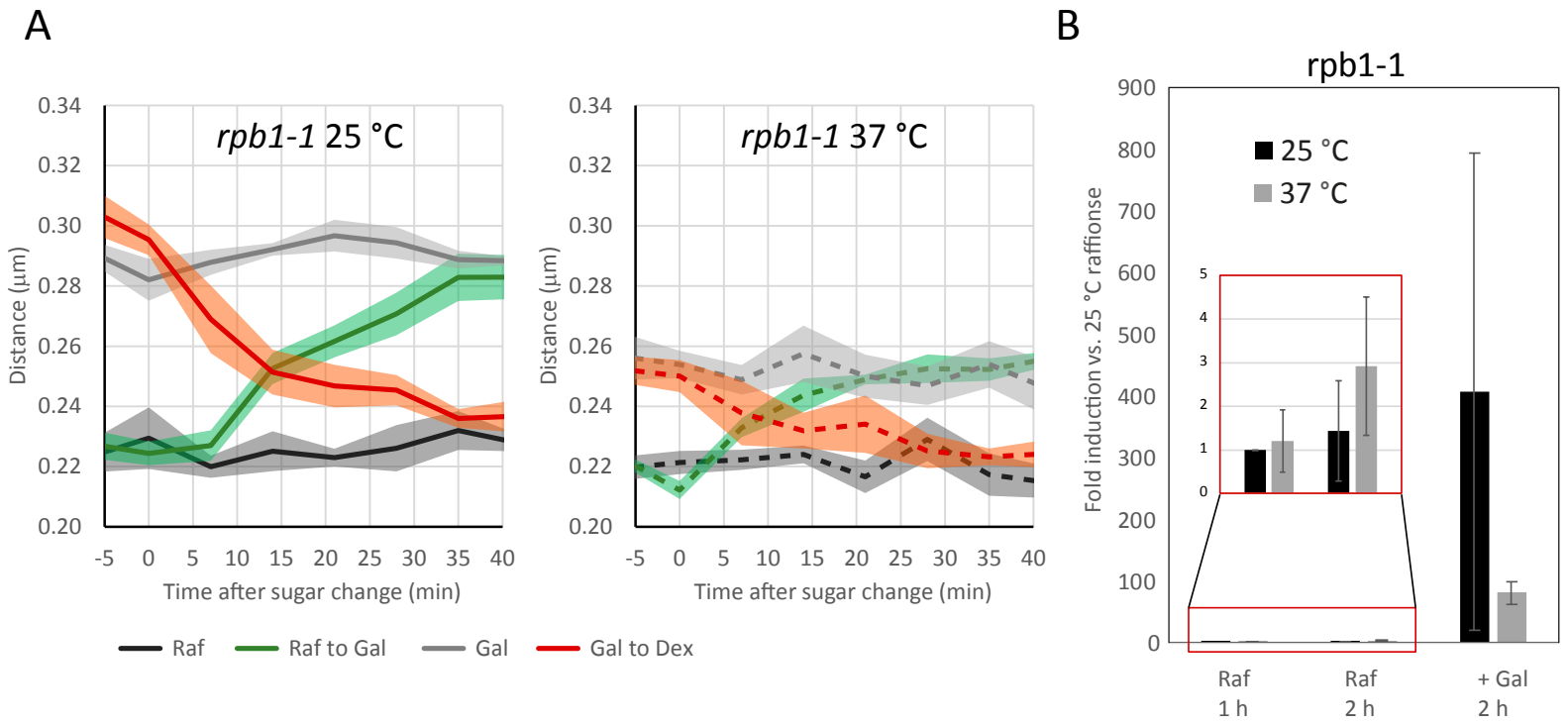

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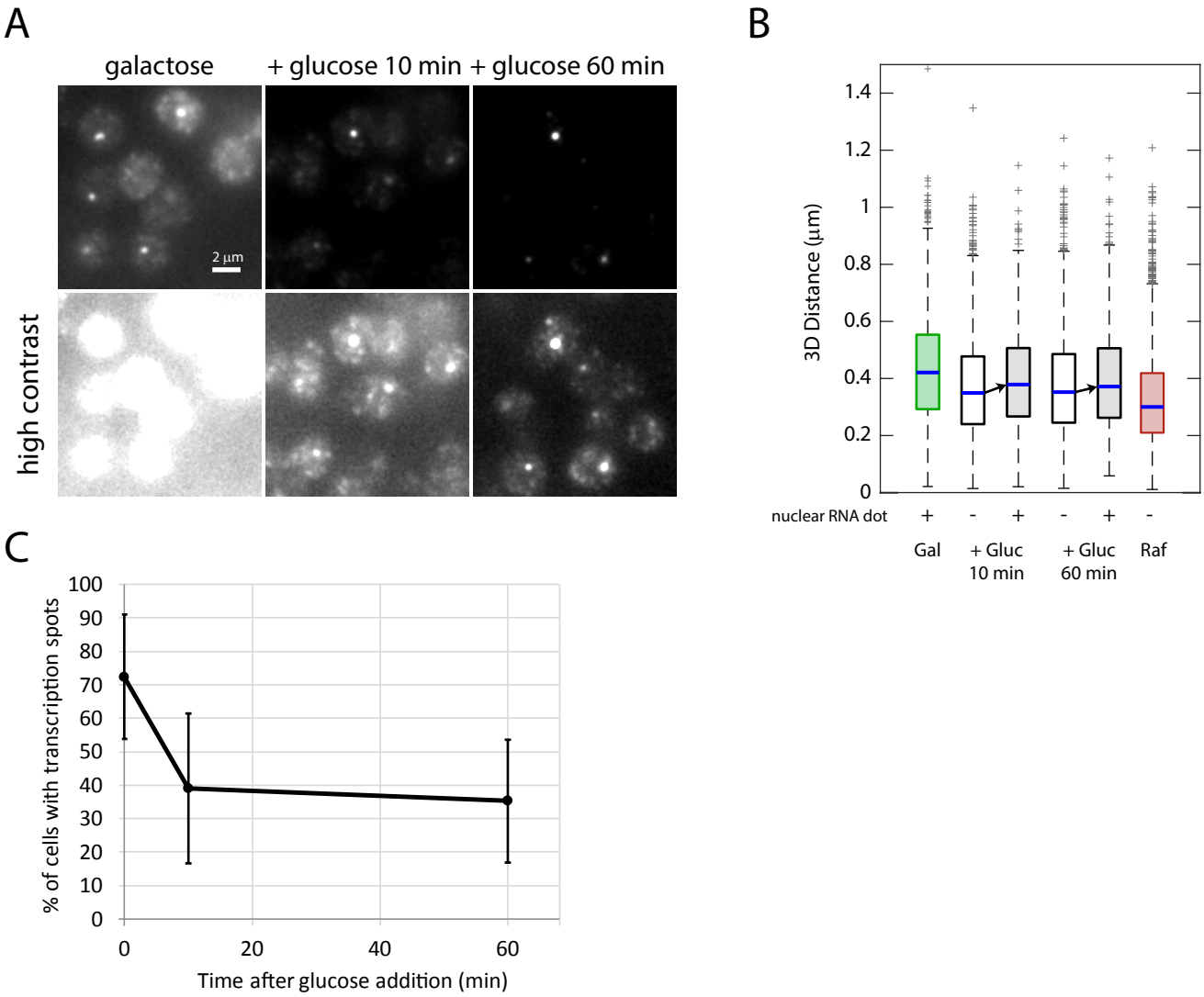

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