

# 1 **Cyclin CLB2 mRNA localization determines efficient protein synthesis** 2 **to orchestrate bud growth and cell cycle progression**

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## 1 **Abstract**

2 mRNA localization to subcellular compartments has been reported across all kingdoms of life and  
3 it is generally believed to promote asymmetric protein synthesis and localization. In striking  
4 contrast to previous observations, we show that in *S. cerevisiae* the B-type cyclin *CLB2* mRNA is  
5 localized and translated in the yeast bud, while the Clb2 protein, a key regulator of mitosis  
6 progression, is concentrated in the mother nucleus. Using single-molecule RNA imaging in fixed  
7 (smFISH) and living cells (MS2 system), we show that the *CLB2* mRNA is transported to the yeast  
8 bud by the She2-She3 complex, via an mRNA ZIP-code situated in the coding sequence. In *CLB2*  
9 mRNA localization mutants, Clb2 protein synthesis in the bud is decreased resulting in changes  
10 in cell cycle distribution and genetic instability. Altogether, we propose that *CLB2* mRNA  
11 localization acts as a sensor for bud development to couple cell growth and cell cycle progression,  
12 revealing a novel function for mRNA localization.

## 1 Introduction

2 Over the past decades, RNA imaging technologies revealed that hundreds of mRNAs localize to  
3 various subcellular compartments, from bacteria to multicellular eukaryotic organisms, suggesting  
4 that mRNA trafficking is a conserved and integral part of gene expression regulation<sup>1-7</sup>. However,  
5 for many mRNAs, the physiological function of their localization remains uncertain.

6 Current studies suggest that the primary role of mRNA trafficking is to control asymmetric protein  
7 distribution to sustain local functions such as cell migration and polarity<sup>5,8</sup>. Even in the single-cell  
8 organism *S. cerevisiae*, dozens of mRNAs localize to the endoplasmic reticulum, mitochondria,  
9 and the growing bud<sup>9</sup>. The best-characterized localized mRNA is *ASH1*, which is transported to  
10 the yeast bud on actin filaments by the She2-She3 complex and the type V myosin motor Myo4<sup>10-</sup>  
11 <sup>17</sup>. The RNA binding proteins (RBP) Khd1 and Puf6 bind the *ASH1* mRNA and inhibit its translation  
12 until the bud-localized kinases Yck1 and CK2 phosphorylate Khd1 and Puf6 and thereby release  
13 the inhibition and allow local translation to occur<sup>18-23</sup>. The Ash1 protein is subsequently  
14 asymmetrically segregated into the daughter nucleus, where it controls the mating-type switching  
15 program<sup>11,24,25</sup>. An additional kinase-RBP pair, Cbk1-Ssd1, has been shown to localize to the  
16 bud<sup>26</sup> and tune the translation of specific mRNA targets<sup>27-29</sup>. The coordination between these  
17 translation regulators remains unclear.

18 Besides *ASH1*, multiple mRNAs have been found to interact with the She2-She3-Myo4 complex<sup>30</sup>.  
19 Among these mRNAs is *CLB2*, which encodes a conserved nuclear-localized B-type cyclin,  
20 interacting with and controlling the substrate specificity of the cyclin-dependent kinase Cdk1<sup>31-41</sup>.  
21 Clb2-Cdk1 regulates entry and progression throughout mitosis in a threshold-dependent  
22 manner<sup>32,42-45</sup>, by phosphorylating transcriptional and post-transcriptional regulators<sup>34,46,47</sup>. This  
23 triggers a positive feedback loop leading to the transcription of the *CLB2* cluster<sup>36,48-50</sup>, a set of 35  
24 genes including *CLB2*, expressed during the G2/M phase transition<sup>50</sup>. Furthermore, Cdk1-Clb2  
25 controls spindle pole bodies elongation and in turn chromosome segregation and genome  
26 stability<sup>51,52</sup>. Aberrant Clb2 expression -depletion or over-expression- results in abnormal mitotic

1 progression and cell size alteration<sup>31,32,34,52</sup>. To achieve accurate periodic Clb2 expression, cells  
2 combine cell-cycle-dependent mRNA synthesis<sup>53,54</sup>, controlled mRNA decay<sup>55</sup>, and proteasome-  
3 dependent protein degradation<sup>56,57</sup>. While the molecular events controlling *CLB2* transcription and  
4 protein degradation are well characterized, as well as Clb2 function during cell cycle progression,  
5 it remains unclear whether and how *CLB2* mRNA translation and Clb2 protein levels are  
6 modulated in response to changes in cell growth that require adaptation of cell cycle progression.  
7 To address this question, we combined single-molecule mRNA fluorescence *in situ* hybridization  
8 (smFISH)<sup>58,59</sup> and immunofluorescence (IF)<sup>60–64</sup> to simultaneously detect *CLB2* mRNA and its  
9 protein product in individual cells. Furthermore, to study dynamic gene expression changes in  
10 intact living cells<sup>65,66</sup>, we utilized the MS2 system (MBSV6) optimized to endogenously tag  
11 unstable mRNAs in *S. cerevisiae*<sup>67–69</sup>. Our work shows that *CLB2* mRNAs are efficiently localized  
12 in the bud during the G2/M phase, while the Clb2 protein is localized to the mother nucleus. *CLB2*  
13 mRNAs are transported to the bud by the She2-She3 complex recognizing a single ZIP-code in  
14 the coding sequence necessary for localization. We find that the *CLB2* mRNA is preferentially  
15 translated in the bud, and that this localized translation does not require translation inhibition by  
16 Puf6, Khd1 or Ssd1 during transport. Consistent with these observations, lack of *CLB2* mRNA  
17 localization results in reduced Clb2 protein synthesis, leading to cell cycle and growth defects.  
18 Altogether, we propose that *CLB2* mRNA localization regulates protein synthesis and acts as a  
19 cellular timer to couple bud growth and cell cycle progression.

## 1 **Results**

### 2 *CLB2 mRNAs localize in the bud from S phase to Mitosis*

3 To precisely quantify *CLB2* mRNA expression throughout the *S. cerevisiae* cell cycle, we  
4 combined smFISH and IF<sup>64,70,71</sup>. To monitor cell cycle progression, nuclear localization of the  
5 transcription factor Whi5 was used to classify early G1 phase<sup>72</sup>, while G2 and mitotic cells were  
6 identified by staining tubulin (Tub1) and monitoring microtubules stretching between the mother  
7 and the daughter mitotic spindles<sup>73</sup> (**Fig. 1a**). *CLB2* smFISH revealed that mRNAs are detected  
8 from late S phase, when the bud emerges from the mother cell, until the end of anaphase.  
9 Quantification of *CLB2* mRNA spots showed that *CLB2* mRNAs are found in 60.7% of cells in an  
10 unsynchronized population (**Fig. 1Sa**). The expression peak occurred during G2 (average  $10.2 \pm$   
11  $5.7$  mRNAs/cell) when about 50% of the cells showed an active transcription site (**Fig. 1b-c**) with  
12 on average  $2.9 \pm 1.5$  nascent mRNAs per transcription site, similar to previous studies<sup>55</sup>(**Fig.**  
13 **1Sb**). Furthermore, in expressing cells, the *CLB2* gene showed Poissonian transcription kinetics  
14 typical of constitutive genes<sup>74,75</sup>, suggesting that this cell-cycle regulated gene is likely transcribed  
15 in a single activation event with a fixed initiation rate. From late S phase until anaphase, we  
16 observed that *CLB2* mRNAs localize to the bud from the first stages of bud formation. Throughout  
17 the budded phases, we measured up to 65.6% of mRNA in the bud, as compared to the  
18 distribution of the control mRNA *MDN1*, where only 17.2% of mRNAs are found in the bud (**Fig.**  
19 **1b, d, 1Sc-d**). *CLB2* mRNA bud localization is independent of the *S. cerevisiae* background since  
20 we observed it both in BY4741, used throughout this study, as well as in the W303 background  
21 (**Fig. 1Se**).

### 22 *CLB2 mRNAs efficiently localize in the bud of living S. cerevisiae cells*

23 To investigate *CLB2* mRNA localization dynamics in living cells, we used an MS2 system  
24 optimized for yeast mRNA tagging (MS2 binding sites V6, MBSV6)<sup>67,68,76</sup>. We inserted 24xMBSV6  
25 in the 3' UTR of the endogenous *CLB2* locus (**Fig. 2Sa**). To confirm that mRNA tagging with

1 MBSV6 did not alter *CLB2* mRNA expression and degradation, unlike with previous MS2  
2 variants<sup>67,77,78</sup>, we performed two-color smFISH with probes targeting either the coding sequence  
3 (CDS) or the MBSV6 loops<sup>67,68</sup>, to compare the expression of the endogenous and the tagged  
4 *CLB2* mRNA. This confirmed that MS2-tagged mRNAs are full-length and correctly localized in  
5 the bud (**Fig. 2Sb**). Furthermore, comparable mRNA levels were observed whether the mRNA  
6 was MS2-tagged, with or without GFP-tagged MS2 coat protein (MCP-GFP), which is used to  
7 detect mRNAs in living cells (**Fig. 2Sc-e**).

8 To monitor cell cycle progression and bud emergence in living cells, we endogenously tagged the  
9 bud neck protein Cdc10 with tdTomato in the *CLB2*-MS2-tagged strain (**Fig. 2a**). We performed  
10 time-lapse imaging every 2 minutes and measured *CLB2* mRNA expression throughout the cell  
11 cycle by acquiring z-stacks encompassing the cell volume (**Video 1**). To reduce perturbations in  
12 gene expression due to synchronization protocols<sup>79,80</sup>, we quantified *CLB2* mRNA expression in  
13 unsynchronized cells, using the bud neck marker expression to compare cells. This revealed that  
14 up to 62.9% of *CLB2* mRNAs localized in the bud (**Fig. 2b-c**), consistent with the smFISH  
15 quantifications (**Fig. 1e**). Furthermore, mRNAs are degraded before the end of mitosis with a half-  
16 life of  $3.8 \pm 1.4$  min, similar to previous measure performed for untagged *CLB2* mRNA<sup>55</sup>,  
17 demonstrating that the MS2 system does not affect *CLB2* mRNA stability (**Fig. 2b-d; 2Sf**).

18 Interestingly, imaging of mother-daughter pairs for more than one cell cycle showed that the  
19 daughter cell initiated *CLB2* mRNA expression about 20 minutes after the mother (**Fig. 2d**). This  
20 observation is consistent with previous evidence showing that *S. cerevisiae* daughter cells are  
21 born significantly smaller than mothers and that cell size control occurring during G1 regulates  
22 the entry into the next cell cycle<sup>34,81,82</sup>. High frame-rate imaging every 100 ms revealed that, as  
23 the bud grows, the number of *CLB2* mRNAs localized in the bud rapidly increases (**Fig 2e, Video**  
24 **2**). Altogether, these results show that *CLB2* mRNAs are efficiently transported to the bud,  
25 consistent with previous measurements estimating directed mRNA transport velocity in eukaryotic  
26 cells to be about  $1 \mu\text{m/s}$ <sup>83</sup>, suggesting that *CLB2* mRNAs reach the bud within seconds (**Fig 2e**).

1 *The She2-She3 complex independently transport the CLB2 and ASH1 mRNAs to the bud*

2 To elucidate the function of *CLB2* mRNA localization, we first investigated *CLB2* mRNA transport.  
3 We performed smFISH-IF throughout the cell cycle for the *CLB2* mRNA in *SHE2* or *SHE3* gene  
4 deletion strains to test whether the She2-She3 complex, required for *ASH1* mRNA transport<sup>10-17</sup>,  
5 is also involved in *CLB2* mRNA localization. This revealed that in  $\Delta she2$  and  $\Delta she3$  strains,  
6 localization is strongly affected (**Fig. 3a, 3Sa**). We also observed that during mitosis, when the  
7 bud reaches its maximum size, only up to 24.5% and 23.6% of *CLB2* mRNAs are found in the  
8 bud of the  $\Delta she2$  and  $\Delta she3$  strains, respectively (**Fig. 3Sb-c**). Even though the *CLB2* and *ASH1*  
9 mRNAs are transported by the same complex, we do not observe co-transport, possibly because  
10 *CLB2* expression peak precedes *ASH1* occurrence during late anaphase (**Fig. 3Sd-g** and **Online**  
11 **Methods**). Furthermore, we observed that *CLB2* mRNAs are mostly single-molecules (**Fig. 3Sh**),  
12 suggesting that *CLB2* and *ASH1* mRNAs are independently localized to the bud by the She2-  
13 She3 complex.

14 *The CLB2 mRNA has a conserved ZIP-code in the coding sequence*

15 As the She2-She3 complex is required for *CLB2* mRNA localization, we hypothesized that the  
16 *CLB2* mRNA might possess a ZIP-code akin to the *ASH1* ZIP-code. Previous work defined the  
17 sequence and structure of the *ASH1* mRNA ZIP-code bound by She2<sup>13,84-86</sup>. Based on sequence  
18 and structure similarity, a pattern search was performed to predict occurrences within the *CLB2*  
19 mRNA (see **Online Methods**). We identified one high-confidence site in the CDS at position  
20 1111-1145 (**Fig. 3b-c**). To test the role of the predicted site, we generated a *CLB2* synonymized  
21 mutant whereby the CDS was mutagenized at nine bases to destroy the ZIP-code structure, while  
22 keeping the protein sequence and the codon optimization index unaltered (*ZIP mut*, **Fig. 3d**). A  
23 pattern search confirmed that the ZIP-code was destroyed upon synonymization. smFISH  
24 revealed that the *CLB2* mRNA bud localization was lost in the *CLB2* ZIP-code mutant (**Fig. 3e**).  
25 This was further confirmed by quantifying the *CLB2* mRNA bud-mother distribution (**Fig. 3f**),

1 thereby demonstrating that the ZIP-code in the CDS of the *CLB2* mRNA is sufficient to control  
2 bud mRNA localization, possibly by recruiting the She proteins. To further characterize *CLB2*  
3 mRNA localization, we quantified the mRNA peripheral distribution index (PDI) in budded cells  
4 using the RNA Distribution Index Calculator<sup>87</sup> (see **Online Methods**). The PDI measures the  
5 location of the mRNA in relation to the nucleus and it allows to compare the localization of multiple  
6 mRNA species. An index value equals 1 for diffusely distributed mRNAs or >1 if the mRNA has a  
7 polarized pattern<sup>87,88</sup> (**Fig 3g**). This analysis revealed a PDI of  $1.9 \pm 0.42$  for the *CLB2* mRNA,  
8 similar to the index value of the control mRNA *ASH1* (PDI =  $2.2 \pm 0.43$ ) (**Fig 3h**). The PDI value  
9 was significantly reduced for *CLB2* in the  $\Delta she2$  (PDI =  $0.5 \pm 0.2$ ),  $\Delta she3$  (PDI =  $0.4 \pm 0.13$ ) and  
10 *CLB2* ZIP-code mutant strain (PDI =  $0.5 \pm 0.16$ ) (ANOVA statistical test:  $F(4, 185) = 15.74$ ,  $p <$   
11  $0.0001$ ), with PDI values similar to the non-localized mRNA *MDN1* (PDI =  $0.6 \pm 0.18$ ) (**Fig. 3h,**  
12 **1Sb**). Thus, the She2-3 complex is required to transport *CLB2* mRNAs to the bud via a conserved  
13 ZIP-code sequence.

#### 14 *Lack of CLB2 mRNA localization affects Clb2 protein expression*

15 To elucidate whether *CLB2* mRNA localization influences its expression, we measured *CLB2*  
16 mRNA and protein levels in the localization mutants. Using smFISH, we found no significant  
17 difference in the number of mature or nascent RNAs in the  $\Delta she2$ ,  $\Delta she3$ , or *CLB2* ZIP-code  
18 mutant strains compared to WT cells (**Fig. 4a-b**). Conversely, a western blot of the endogenously  
19 modified myc-tagged Clb2 protein showed that the protein expression in  $\Delta she2$  or  $\Delta she3$  and,  
20 even more, in the *CLB2* ZIP-code mutant was strongly reduced compared to WT cells (**Fig. 4c-**  
21 **d, 4Sa**). To test whether the decrease in protein expression was due to a change in protein  
22 degradation, we performed stability assays by treating WT and localization mutants with the  
23 translation inhibitor cycloheximide and measured the protein abundance over time (**Fig. 4e-f,**  
24 **4Sb-c**). No significant difference was observed in the stability of the localization mutants



1 compared to WT cells, suggesting that *CLB2* mRNA localization controls Clb2 protein synthesis,  
2 rather than the stability of *CLB2* mRNA or protein.

### 3 *Lack of CLB2 mRNA localization does not affect Clb2 protein localization*

4 Next, we investigated whether mRNA localization affected Clb2 protein localization. To this end,  
5 *CLB2* was endogenously tagged with yeast optimized GFP (yeGFP<sup>89</sup>) in WT and *CLB2* mRNA  
6 localization mutants. We observed that Clb2 is predominantly found in the nucleus in WT and  
7 mRNA localization mutants (**Fig. 4g**), as previously reported<sup>38,39,41</sup>. Consistent with the western  
8 blot results (**Fig. 4c-d**), the fluorescence of the *CLB2* ZIP-code mutant was below detection level  
9 by live imaging (**Fig. 4g**). Furthermore, comparing Clb2 expression during a complete cell cycle  
10 in living WT and  $\Delta she2$  cells (**Fig. 4Sd-e**), revealed that in contrast to WT cells, where a rapid  
11 Clb2 increase proportional to bud growth is observed, in  $\Delta she2$  cells a slower protein  
12 accumulation was measured, accounting for the decrease in Clb2 expression. In addition, Clb2  
13 was observed in the mother nucleus already during the G2 phase (**Fig. 4g**, top panels), when the  
14 mRNAs are already localized to the bud (**Fig. 1b, 2b, 3a**). Altogether, these results suggest that  
15 *CLB2* mRNA localization is not used to segregate the Clb2 protein in the daughter cell, as instead  
16 is observed for Ash1<sup>11,24,25</sup>, but rather to control *CLB2* mRNA translation efficiency in the bud  
17 before the protein is imported back to the mother nucleus.

### 18 *CLB2 mRNAs and protein co-localization suggests preferential translation in the bud.*

19 To detect *CLB2* mRNAs and their site of translation in single cells, we generated a yeast strain  
20 where 25 Myc tags were inserted at the N-terminus of the *CLB2* endogenous gene (**Fig. 5Sa-b**).  
21 This amplification strategy increases the fluorescent signal of Clb2 proteins without affecting the  
22 strain growth (**Fig. 4Sa**). Next, we combined smFISH and IF to simultaneously detect *CLB2*  
23 mRNAs and proteins in fixed cells. In addition, IF against tubulin was used to score the cell cycle  
24 phases. This approach revealed that the bulk of Clb2 proteins accumulated in the mother (M)  
25 nucleus from G2 to mitosis (**Fig. 5a-b**), while the mRNA was preferentially found in the bud (B)

1 (Fig. 5a, c), suggesting that Clb2 proteins were efficiently imported back to the mother nucleus,  
2 as observed by live imaging (Fig. 4g), and as shown previously<sup>38-41</sup>. Interestingly, from G2 to  
3 mitosis, Clb2 protein foci were also found in the bud in close proximity of *CLB2* mRNAs,  
4 suggesting that these foci may represent the site of mRNA translation (Fig. 5a, yellow  
5 arrowheads). Quantification of co-localized single mRNAs and protein foci within 250 nm distance  
6 (i.e. the resolution of our system), revealed that in WT cells, more mRNA-protein foci were found  
7 in the bud, while in the localization mutant  $\Delta she2$ , mRNA-protein foci are preferentially found in  
8 the mother cell where the bulk of mRNAs is localized (Fig. 5d-e, Fig. 3a). Furthermore, we found  
9 a reduction of the percentage of bud-localized mRNAs co-localized with protein foci in  $\Delta she2$  cells  
10 compared to WT cells (Fig. 5f), suggesting that in localization mutants *CLB2* mRNA translation  
11 efficiency may be reduced. This analysis could not be performed with the *CLB2* ZIP-code mutant  
12 because the protein signal was too weak (Fig. 4g). It is interesting to note that even in WT cells,  
13 only about 25% of the bud-localized mRNAs are found in close proximity to protein foci (Fig. 5f),  
14 suggesting that *CLB2* mRNAs are poorly translated, as previously reported<sup>90</sup>.

15 Finally, we quantified the accumulation of mRNA-protein foci in WT cells exposed for a short  
16 period of time to the translation elongation inhibitor cycloheximide (CHX, 20 minutes at 100  
17  $\mu\text{g/ml}$ ). This revealed that translation inhibition leads to an increase in *CLB2* mRNA levels  
18 accompanied by an accumulation of mRNAs in the mother cell (Fig. 5Sc-e). Furthermore, we  
19 observed a reduction of the Clb2 protein foci in the bud (Fig. 5Sf), compared to control conditions  
20 (Fig. 5b), consistent with a decrease in protein synthesis. Furthermore, the increased amount of  
21 *CLB2* mRNAs found in the mother upon CHX treatment suggested that mRNA translation may  
22 play a role in the asymmetric distribution of *CLB2* mRNAs in the bud, possibly by slowing down  
23 the diffusion kinetics of mRNAs bound to ribosomes, as previously shown in mammalian  
24 cells<sup>61,91,92</sup>. To test this hypothesis, we simulated the distribution of *CLB2* mRNAs upon their  
25 localization in the bud of a G2 cell, with bud and mother volumes based on our measurements.  
26 We included measured *CLB2* mRNA decay rates and assumed an apparent mRNA diffusion

1 coefficient based on previously reported measurements performed in eukaryotic cells<sup>91</sup> (see  
2 **Online Methods**). A fast coefficient of  $0.4 \mu\text{m}^2/\text{s}$  was previously measured for non-translated  
3 mRNAs, and a slower coefficient of  $0.1 \mu\text{m}^2/\text{s}$  was measured for translated mRNAs<sup>91</sup>.  
4 Interestingly, our model suggests that if we assume either a slow or a fast apparent mRNA  
5 diffusion coefficient of  $0.1 \mu\text{m}^2/\text{s}$  or  $0.4 \mu\text{m}^2/\text{s}$ , respectively, we do not obtain the expected  
6 enrichment of the *CLB2* mRNA in the bud (**Fig. 5Sg-h**). To predict the accumulation of about 65%  
7 of the mRNA in the bud observed during the G2 phase, we need to include in the simulation the  
8 presence of a high-affinity anchoring factor promoting *CLB2* mRNA segregation in the bud (**Fig.**  
9 **5Si**). Altogether, these data suggest that in WT cells, *CLB2* mRNAs are preferentially translated  
10 in the bud where the mRNA is actively transported and localized via an unknown anchoring  
11 mechanism, which may include the association with ribosomes and other yet unidentified factors.  
12 Furthermore, both in  $\Delta she2$  cells and in translationally repressed cells (CHX), we observed an  
13 increase of protein foci in the mother cell (**Fig. 5d, 5Sf**), despite protein levels being decrease  
14 under these conditions (**Fig. 4c, 4e-f**), suggesting that translation in the mother cell may be less  
15 efficient, resulting in reduced Clb2 protein levels in the localization mutants.

16 *Clb2 protein expression is not affected by the translation regulators Puf6, Ssd1 and Khd1*

17 To investigate whether *CLB2* mRNAs are preferentially translated in the bud as a result of  
18 translation repression prior to localization, we tested if the RNA binding proteins Puf6<sup>18,19,23</sup>,  
19 Khd1<sup>20-22</sup> and Ssd1<sup>27-29</sup>, previously shown to inhibit translation of bud-localized mRNAs,  
20 influenced Clb2 protein levels. We performed a western blot of Myc-Clb2 in strains lacking *SSD1*,  
21 *KHD1* or *PUF6* genes, to investigate whether an increase in Clb2 protein could be observed (**Fig.**  
22 **6a**). This revealed no significant difference in protein levels between the WT and the mutant  
23 strains (ANOVA statistical test:  $F(3, 8) = 0.7677$ ,  $p = 0.3$ ; **Fig. 6b**). Furthermore, we analyzed by  
24 smFISH whether *CLB2* mRNA localization or abundance were affected in  $\Delta ssd1$ ,  $\Delta khd1$  or  $\Delta puf6$   
25 strains. This showed that the *CLB2* mRNAs are still localized in the bud, suggesting that these  
26 RBPs are not required for transport nor bud localization (**Fig. 6c**). Interestingly, we found a

1 significant increase of *CLB2* mRNA counts in the  $\Delta puf6$  strain compared to WT ( $6.86 \pm 1.23$  and  
2  $4.27 \pm 0.52$  mRNA/cell, respectively,  $p < 0.0001$ ) (**Fig. 6d, 6Sa**). This rise was not caused by an  
3 increased RNA synthesis, measured by the number of nascent RNAs per transcription site (**Fig.**  
4 **6e**), but by a 25% increase in the number of cells with two active transcription sites (**Fig. 6Sb-c**).  
5 Since in WT haploid cells, we only observed one *CLB2* allele transcribed during the G2/M phase,  
6 when two copies of the gene are present, this indicated that Puf6 may regulate *CLB2* gene dosage  
7 by repressing the transcription of *CLB2* second allele. Altogether, these results suggest that *CLB2*  
8 mRNAs are not translationally repressed by factors controlling *ASH1* mRNA expression. While it  
9 is possible that other, yet unidentified factors exist, our data suggest the possibility that *CLB2*  
10 mRNA may be translated outside of the bud, albeit at a reduced rate.

#### 11 *CLB2* mRNA localization mutants display cell cycle progression defects

12 Our results so far indicated that the yeast bud promotes *CLB2* mRNA translation and a rapid  
13 increase of Clb2 protein levels proportional to bud growth (**Fig. 4c-d, 4Sb**). This suggested that  
14 *CLB2* mRNA localization may act as a cellular signal reporting on bud growth to the mother  
15 nucleus and coordinating cell growth and division. To test whether the decrease of Clb2 protein  
16 levels observed in *CLB2* mRNA localization mutants affected cell cycle progression and growth,  
17 we performed growth assays under different nutrient availabilities. In rich media (Synthetic  
18 complete medium with 2% glucose), the *CLB2* ZIP-code mutant clones did not show a growth  
19 defective phenotype. However, in the presence of a limiting carbon source (Synthetic complete  
20 medium with 0.1% glucose), it became apparent that the *CLB2* ZIP code mutant showed varying  
21 growth phenotypes with a marked clonal variability (**Fig. 7a**), suggesting incomplete phenotypic  
22 penetrance of the mutation. We proceeded with the analysis of three independently isolated  
23 clones (*ZIP cl1*, *ZIP cl2*, *ZIP cl3*).

24 We first tested by spot assay whether the localization mutants showed a growth defect at different  
25 temperatures (26°C, 30°C, 37°C) and growth limiting conditions set by the presence of different  
26 carbon sources (0.1% glucose or 2% glycerol/ethanol compared to 2% glucose). While the She

1 mutants grew like the WT strain, the ZIP-code mutants showed a clone-dependent growth defect,  
2 with the *ZIP cl1* showing reduced growth in carbon-limiting conditions when compared to the other  
3 clones and WT cells (**Fig. 7b**). Dynamic growth measurements of cells transitioned from rich liquid  
4 media to either liquid media containing 0.1% glucose or 2% glycerol/ethanol (**Fig. 7c**),  
5 demonstrated a complete growth arrest for *ZIP cl1*, while the other ZIP clones (**Fig. 7c**) and the  
6 She mutants (**Fig. 7Sa**) did not show reduced growth.

7 Finally, to evaluate the impact of the ZIP-code mutation on cell cycle progression, we performed  
8 a cell cycle distribution analysis by DNA staining with Sytox Orange coupled with flow cytometry  
9 (see **Online Methods**). Analysis of cells grown in rich media revealed a reduction of the G2/M  
10 population for *ZIP cl2*, *ZIP cl3*, but not *ZIP cl1* compared to the WT cells (**Fig. 7d**), suggesting  
11 that in rich conditions, even when growth was not affected for the ZIP-code mutants, the  
12 distribution of cell through the cell cycle was altered. Furthermore, cell cycle analysis of cells  
13 grown for 20 hours in presence of 0.1% glucose or 2% glycerol/ethanol, revealed that all the ZIP-  
14 code clones behaved differently from WT cells, mostly displaying a G2/M phase delay,  
15 demonstrated by a relative increase in the number of cells in this phase (**Fig. 7e-f**). On the other  
16 hand, *ZIP cl1* grown on 2% glycerol/ethanol displayed an arrest in G1 (**Fig. 7f**) and an increase  
17 in cell size (**Fig. 7Sb**), consistent with the growth defect observed both on agar plates and in liquid  
18 cultures (**Fig. 7b-c**). Thus, the ZIP-code mutants demonstrated complex clonal phenotypes,  
19 possibly triggered by critically low Clb2 protein levels. In most of the cases, the ZIP-code mutants  
20 behaved like *ZIP cl2* and *cl3*, and displayed altered cell cycle distribution but not a growth defect,  
21 possibly due to compensatory effects played by G1 growth checkpoints<sup>34,93</sup>. However, at least  
22 three clones were independently isolated behaving like *ZIP cl1*, suggesting that critically low levels  
23 of Clb2 may trigger the accumulation of secondary mutations and a loss of coordination between  
24 cell cycle and cell size control. To test this hypothesis, we investigated whether the *ZIP cl1* could  
25 be rescued by the presence of a WT copy of the *CLB2* gene. To this end, we generated diploid

1 strains where the WT or the *ZIP cl1* mutant were crossed with a WT strain (**Fig. 7Sc**). Liquid  
2 growth in presence of 0.1% glucose or 2% glycerol/ethanol revealed a growth rescue for *ZIP cl1*.  
3 Altogether, our data suggest that in growth-limiting conditions, when bud growth is slowed down  
4 by the presence of suboptimal nutrients and in turn ribosomes and other key metabolites may be  
5 limiting in this compartment, *CLB2* mRNA localization and protein transport back to the nucleus  
6 may act as a biochemical signal adjusting the cell cycle in response to cell growth changes.

## 7 **Discussion**

8 Numerous instances in prokaryotic and eukaryotic organisms revealed that subcellular  
9 localization of mRNAs regulates the synthesis and asymmetric localization of proteins<sup>8</sup>. Here, we  
10 show that the B-type cyclin *CLB2* mRNA is efficiently localized to the yeast bud in a cell cycle-  
11 dependent manner, while the Clb2 protein is imported back to the mother nucleus, demonstrating  
12 the first example of mRNA and protein localization uncoupling. We characterized a new function  
13 for mRNA localization, which is to coordinate cell growth and cell cycle progression, possibly by  
14 sensing the bud translation capacity via the transport and local translation of the *CLB2* mRNA.  
15 We propose that by shuttling back to the mother nucleus, Clb2 signals to the mother cell when  
16 the bud is ready for mitosis, establishing a biochemical-based communication between distinct  
17 subcellular compartments.

18 Here, we combined single molecule RNA FISH-IF in fixed cells and a yeast-optimized MS2  
19 tagging system for single RNA visualization in living cells to quantify, for the first time, the  
20 complete lifecycle of the *CLB2* mRNA and its protein product in intact cells. This approach  
21 revealed that *CLB2* mRNAs are transported to the bud as soon as this compartment is formed  
22 during late S phase (**Fig. 1,2,3**). Interestingly, previous reports showed that the B-type cyclin B1  
23 is also asymmetrically localized in higher eukaryotes such as in the *Xenopus*<sup>94</sup> and *Zebrafish*<sup>95,96</sup>  
24 oocytes as well as in *Drosophila* embryos<sup>1</sup>, suggesting that the spatiotemporal regulation of Clb2  
25 expression is a conserved mechanism.

1 The She2-She3 complex, previously shown to localize other mRNAs such as *ASH1*<sup>10-17</sup>, *IST2*,  
2 *TCB2*, and *TCB3*<sup>12,30</sup>, is also required to localize *CLB2* mRNAs to the bud (**Fig. 3**). Furthermore,  
3 by combining imaging and modelling, we suggested that the efficient asymmetric localization of  
4 *CLB2* mRNAs is likely produced by a combination of active transport and bud anchoring via yet  
5 unknown RBPs (**Fig. 5S**). To elucidate the function of *CLB2* mRNA localization we identified the  
6 cis-acting element required for transport. A single RNA ZIP-code in the mRNA CDS is sufficient  
7 to ensure localization in the bud (**Fig. 3**). This ZIP-code has a sequence and predicted structure  
8 similar to the *ASH1* ZIP-code, supporting the notion that cis-elements bound by the She2 RBP  
9 are conserved<sup>25,30,84</sup>. Mutation of the ZIP-code RNA sequence caused an mRNA localization  
10 defect and correlated with a strong decrease in Clb2 protein expression (**Fig. 4**). Simultaneous  
11 visualization of the *CLB2* mRNA and proteins in fixed cells using the myc-tag reporter suggested  
12 that *CLB2* mRNAs are preferentially translated in the bud (**Fig. 5**). While this compartment  
13 promotes *CLB2* mRNA translation, we could not demonstrate that known mRNA translation  
14 inhibitors such as Puf6, Ssd1 and Khd1, prevent *CLB2* mRNA translation prior localization (**Fig.**  
15 **6**). Unlike the Ash1 protein, the Clb2 protein is not restricted in the bud, possibly explaining why  
16 *CLB2* may not require translation inhibition. Yet unidentified translation inhibitors may also be  
17 involved in this process.

18 Interestingly, the *CLB2* ZIP-code mutant showed a further reduction in protein expression  
19 compared to the single SHE mutants, even though mRNA localization was equally impaired (**Fig.**  
20 **4**). This suggested that the destruction of the ZIP-code may prevent not only the binding of the  
21 She proteins required for transport, but also the recruitment of translation factors that may travel  
22 together with the mRNA to the bud. Further investigations will be focused on identifying factors  
23 involved in controlling *CLB2* mRNA local translation.

24 We found that before the nucleus is divided during yeast closed mitosis, most of the Clb2 protein  
25 is efficiently imported back to the mother nucleus (**Fig. 4**), consistent with previous reports<sup>38-41</sup>.

26 The best characterized function of Clb2 is to trigger mitotic entry via the phosphorylation of key

1 targets controlling transcription of mitotic genes, including *CLB2* itself, and spindle pole bodies  
2 formation. In line with these observations, *CLB2* deletion mutants have been shown to be  
3 defective in DNA repair, genome stability and cell size control<sup>31,32,52</sup>. Consistently, *CLB2* ZIP-code  
4 mutants display phenotypes including altered cell cycle distribution, the inability to grow under  
5 suboptimal nutrient conditions combined with changes in cell size. The strong clonal variability  
6 observed in our study also suggests that critical low levels of Clb2 may trigger genome instability  
7 and secondary mutations. Previous work showed that, when overexpressed<sup>38,39</sup> or when nuclear  
8 import is blocked<sup>41</sup>, Clb2 can be found also at the bud neck. In our experimental settings, both in  
9 living and fixed cells and without overexpression, we did not observe Clb2 at this site.  
10 Nevertheless, we cannot exclude that a transient localization of Clb2 at the bud neck may have  
11 spatially-defined functions that may contribute to the observed phenotypes.  
12 Altogether, our results suggest that *CLB2* mRNA localization in the bud regulates Clb2 protein  
13 synthesis to ensure coupling between cellular growth and cell cycle. An elegant mathematical  
14 model previously predicted a similar outcome, and suggested that *CLB2* mRNA localization may  
15 act as a bud sizer during the G2/M phase checkpoint<sup>97</sup>. Even though we did not observe specific  
16 bud size defects in the *CLB2* localization mutants, future studies may elucidate the role of *CLB2*  
17 localization during yeast cell size and cell cycle coordination. Thanks to improved imaging  
18 technologies and the advent of spatial transcriptomic, thousands of localized mRNAs have been  
19 identified in many model organisms<sup>4</sup>. We predict that additional functions for mRNA localization  
20 will emerge, revealing the importance of this process in controlling the spatiotemporal synthesis  
21 of proteins, from single-cell to multicellular organisms.

22



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## 1 **Online methods**

### 2 *Yeast strains construction*

3 Yeast strains were constructed in the BY4741 background as detailed in<sup>68</sup>. All strains where a  
4 gene of interest was tagged with MBSs in the 3'-UTR right after the STOP codon, were prepared  
5 as follow: PCR amplification of the MBS insert (see plasmids in Resource Table) followed by the  
6 kanamycin resistance gene, flanked by LoxP sequences, was performed with oligos (see  
7 Resource Table) containing homology sequences (70 nt) for the specific gene. For all strains, the  
8 Kanamycin resistance gene was removed by expressing the CRE recombinase under the control  
9 of the *GAL1* promoter (Resource Table, plasmids). Genomic DNA was extracted using standard  
10 techniques and PCR amplification of the 3'-UTR was loaded on a gel and sent for sequencing to  
11 verify the size and the sequence of the insert.

### 12 *Plasmids construction*

13 The synonymized *CLB2* region was synthesized as a DNA fragment by Genescript® with  
14 restriction endonuclease sites BglII and ClaI restriction sites. This fragment was used to replace  
15 the *CLB2* WT sequence in a plasmid encoding the *CLB2* promoter, CDS and UTRs. The promoter  
16 was preceded by the *URA3* marker flanked by LoxP sites, which were used to remove the marker  
17 upon integration into the yeast genome (pET531). 70-100 nucleotides *CLB2* homology sequences  
18 for insertion into the genome were cloned as well in the vector. Insertion of 5-Myc (synonymized  
19 multimer) or 25-Myc tags in the *CLB2* coding sequence was performed by restriction digestion  
20 into the BamHI site placed after the ATG codon.

### 21 *Yeast cell cultures*

22 All strains described are derived either by the *S. cerevisiae* background BY4741 (MATa; his3Δ1;  
23 leu2Δ0; met15Δ0; ura3Δ0) or W303 (MATa; ura3-1; trp1Δ 2; leu2-3,112; his3-11,15; ade2-1;  
24 can1-100). Strains are listed in the Resource Table. Yeast cultures were exponentially grown in  
25 6.7 g/L Yeast Nitrogen Base medium (YNB) with 2% glucose and the appropriate amino acids to

1 complement auxotrophies (either Synthetic Complete (SC), or Drop-Out (DO) media). Cells were  
2 grown at the indicated temperature using constant shaking at 210 rpm. For spot-test experiments  
3 (Fig. 7), cells were grown overnight in SC or DO media with 2% glucose (see figure legends). In  
4 the morning cells were diluted to an OD<sub>600</sub> of 0.8. Five ten-fold dilutions in water were prepared  
5 for each strains. 7 µL were spotted for each dilution on the indicated agar plates (20 g/L agar in  
6 the specific medium). For smFISH and live imaging, the details of the cell cultures are described  
7 in the Method sections below.

#### 8 *smFISH probes design*

9 *CLB2* probes were designed using the Stellaris™ Probe Designer by LGC Biosearch  
10 Technologies and purchased from Biosearch Technologies. *ASH1*, *MDN1* and *MBSV6* probes  
11 design was previously described in<sup>67,68</sup>. Probes sequence and fluorophores are provided in the  
12 Resource Table.

#### 13 *Single molecule fluorescence in situ hybridization (smFISH)*

14 Single-molecule FISH (smFISH) was performed as follows. Yeast strains were grown overnight  
15 at 26°C in synthetic medium with 2% glucose and containing the appropriate amino acids to  
16 complement the strain auxotrophies. In the morning, cells were diluted to OD<sub>600</sub> 0.1 and allowed  
17 to grow until OD<sub>600</sub> 0.3-0.4. Cells were fixed by adding paraformaldehyde (32% solution, EM  
18 grade; Electron Microscopy Science #15714) to a final concentration of 4% and gently shaken at  
19 room temperature for 45 minutes. Cells were then washed three times with buffer B (1.2 M sorbitol  
20 and 100 mM potassium phosphate buffer pH=7.5) and resuspended in 500 µL of spheroplast  
21 buffer (buffer B containing 20 mM VRC (Ribonucleoside–vanadyl complex NEB #S1402S), and  
22 25 U of Lyticase enzyme (Sigma #L2524) per OD of cells (~10<sup>7</sup> cells) for about 7-8 minutes at  
23 30°C. Digested cells were washed once with buffer B and resuspended in 1 mL of buffer B. 150  
24 µL of cells were seeded on 18 mm poly-L-lysine treated coverslips and incubated at 4°C for 30  
25 minutes. Coverslips were washed once with buffer B, gently covered with ice-cold 70% ethanol

1 and stored at -20°C. For hybridization, coverslips were rehydrated by adding 2xSSC at room  
2 temperature twice for 5 minutes. Coverslips were pre-hybridized with a mix containing 10%  
3 formamide (ACROS organics #205821000)/2xSSC, at room temperature for 30 minutes. For each  
4 coverslip, the probe mix (to obtain a final concentration in the hybridization mix of 125 nM) was  
5 added to 5 µL of 10 mg/mL *E. coli* tRNA/ ssDNA (1:1) mix and dried with a speed-vac. The dried  
6 mix was resuspended in 25 µL of hybridization mix (10% formamide, 2×SSC, 1 mg/ml BSA, 10  
7 mM VRC, 5 mM NaHPO<sub>4</sub> pH 7.5) and heated at 95°C for 3 minutes. Cells were then hybridized  
8 at 37°C for 3 hours in the dark. Upon hybridization, coverslips were washed twice with pre-  
9 hybridization mix for 30 minutes at 37°C, once with 0.1% Triton X-100 in 2xSSC for 10 minutes  
10 at room temperature, once with 1xSSC for 10 minutes at room temperature. Coverslips were  
11 mounted on glass slides using ProLong Gold antifade (4',6-diamidino-2-phenylindole) DAPI to  
12 counterstain the nuclei (Thermofisher).

### 13 *smFISH-IF*

14 smFISH-IF was performed as previously described in<sup>70,71</sup>. In brief, smFISH-IF was performed in  
15 a similar way as smFISH, described above. After the last 1xPBS wash of the smFISH, IF was  
16 performed on the same coverslips. The smFISH was fixed in 4% PFA in PBS for 10 minutes at  
17 room temperature and then washed for 5 min at room temperature with 1x PBS. The coverslips  
18 were blocked with 1xPBS, 0.1% RNase-free Bovine Serum Albumin for 30 minutes at room  
19 temperature before being incubated with primary antibodies (Thermofisher, mouse anti-tubulin,  
20 1:1000; Sigma mouse monoclonal anti-Myc clone 9E10, 1:1000; Covance, mouse monoclonal  
21 anti-HA, 1:1000) in 1xPBS, 0.1% RNase-free Bovine Serum Albumin for 45 minutes. After being  
22 washed with 1xPBS for 5 minutes at room temperature, the coverslips were incubated with the  
23 secondary antibody (goat anti-mouse Alexa 647 1:1500, or goat anti-mouse Alexa 488 1:1500) in  
24 1xPBS, 0.1% RNase-free Bovine Serum Albumin for 45 minutes at room temperature. Next, the  
25 coverslips were washed with 1x PBS three times for 5 minutes to remove excess antibody.

1 Coverslips were dehydrated by dipping them into 100% ethanol and letting them dry before being  
2 mounted onto glass slides using ProLong Gold antifade mounting solution with DAPI.

### 3 *smFISH/ smFISH-IF image acquisition and analysis*

4 Images were acquired using an Olympus BX63 wide-field epi-fluorescence microscope with a  
5 100X/1.35NA UPlanApo objective. Samples were visualized using an X-Cite 120 PC lamp (EXFO)  
6 and the ORCA-R2 Digital CCD camera (Hamamatsu). Image pixel size: XY, 64.5 nm. Metamorph  
7 software (Molecular Devices) was used for acquisition. Z-sections were acquired at 200 nm  
8 intervals over an optical range of 8.0  $\mu\text{m}$ . FISH images were analyzed using FISHQUANT<sup>98</sup>.  
9 Briefly, after background subtraction, the FISH spots in the cytoplasm were fit to a three-  
10 dimensional (3D) Gaussian to determine the coordinates of the mRNAs. The intensity and width  
11 of the 3D Gaussian were thresholded to exclude nonspecific signal. The average intensity of all  
12 the mRNAs was used to determine the intensity of each transcription site.

### 13 *Quantification of peripheral distribution index*

14 The peripheral distribution index (PDI) was quantified as described in<sup>87</sup>. Briefly, the Matlab-based  
15 software RDI (RNA dispersion index) calculator was used to calculate the peripheral distribution  
16 index for each cell by identifying cellular RNAs and describing their distribution in relation to the  
17 nucleus. Prior to analysis with the RDI calculator, the RNA channel was processed using a 3D  
18 Laplacian of Gaussian filter of radius=5 and standard deviation=1. The cell and nucleus channels  
19 were processed using the brightness/contrast function in ImageJ to enhance the contrast between  
20 the object and the background, as advised in<sup>87</sup>.

### 21 *Co-localization analysis*

22 The RNA-RNA co-localization analysis reported in **Fig. 3S f-g** was performed using FISH-quant  
23 as described in<sup>99</sup>. Briefly, FISH-quant performs the co-localization analysis in each cell separately  
24 by treating the assignment as a Linear Assignment Problem (LAP). The two spot detection results  
25 (x, y, z positions) are considered as 3D point clouds. The Hungarian algorithm solving the LAP

1 finds the best possible global assignment between these two points-clouds such that for each  
2 point in the first channel, the closest point in the second channel is found. LAP has the important  
3 property that assignment is exclusive; one point from the first channel can be linked to at most  
4 one point from the other channel, and conversely. The linking is also globally optimal because the  
5 sum of the squared distance is minimized. This analysis is implemented using the Matlab  
6 functions `hungarianlinker2` and `munkres3`.

7 For the co-localization of *CLB2* mRNA and protein foci, the FISH-quant data for the individual  
8 molecules were used as x, y, z coordinates and euclidean distances for all protein - mRNA  
9 molecule combinations were calculated in the mother and daughter cells. Protein and mRNA  
10 molecules closer than 250 nm were considered to be in a translation complex. Multiple protein  
11 molecules can be within 250 nm of a single mRNA molecule, and this would still be considered a  
12 single translation complex.

### 13 *PDE solution for mRNA diffusion*

14 We use a modified diffusion equation at steady state to model the mRNA movement in terms of  
15 diffusion of a concentration  $c(x,y,z)$  in 3 spatial dimensions, and include binding to ribosomes  
16 (uniformly spread) leading to the formation of complexes  $b(x,y,z)$ :

$$17 \quad 0 = D \cdot \nabla^2 c(x, y, z) - k_d \cdot c(x, y, z) + k_p - k_{on} \cdot c(x, y, z) + k_{off} \cdot b(x, y, z)$$

$$18 \quad 0 = k_{on} \cdot c(x, y, z) - k_{off} \cdot b(x, y, z) - k_d \cdot b(x, y, z)$$

19 With decay constant  $k_d = \text{Ln}(2)/t_{0.5} = \text{Ln}(2)/240 \text{ s}^{-1}$ ,  $k_{on} = 0.25 (k_{off} + k_d) = 0.0035$  and  $k_{off} = 1/90 =$   
20  $0.011$  chosen to reflect a half-life of 240 s, a 90 s mean lifetime of ribosome-bound complexes,  
21 and that approximately 20% of mRNAs appear bound at steady state. In the high-binding scenario  
22  $k_{on}$  was increase by a factor of 125. For the numerical implementation, the production constant is  
23 represented by a small non-zero spread around the bud centre using a smooth step-function of  
24 which the volume integral is normalised to 1:

1 
$$k_p = \frac{k_{max} \cdot e^{s \cdot m}}{e^{m \cdot r} + e^{s \cdot m}}$$

2 For the simulation results shown in Fig. 5S, the values chosen are  $s = 5$  and  $m = 10$ , and the  
3 normalization results in  $k_{max} = 0.0019$ . Although the exact value of this constant affects the  
4 absolute concentration of mRNA it does not affect the ratio of mother to bud RNA. The PDE is  
5 solved in three Cartesian coordinates using the FEM implementation in Wolfram Mathematica  
6 (Wolfram Research, Inc., Mathematica, Version 12.3.1, Champaign, IL).

7 *Ellipsoid fitting to mother and bud DIC images*

8 Differential interference contrast (DIC) images were analysed in Mathematica to fit 3D ellipsoids  
9 to mother and bud models. X- and y-axes length were measured for the cells and the short axis  
10 was used as estimation of the z-axis. The z-axis origin value was aligned with the z-stack images  
11 by maximising the Fish-Quant mRNA and protein point inclusions.

12 *Pattern search to predict ZIP-codes and synonymization*

13 To identify potential ZIP-codes in the *CLB2* mRNA, we performed a targeted pattern search  
14 (Seiler et al., manuscript in preparation). In the first step, we leniently screened for two nested  
15 pairs of inverted repeats with a minimal length of four nucleotides that framed an asymmetric  
16 bulge region as found in the E3 ZIP-code in the *ASH1* mRNA<sup>84</sup>. We also checked for the presence  
17 of a CGA motif and a singular cytosine on the opposite strand with a defined distance of six  
18 nucleotides<sup>100</sup>. The search was performed on the complete *CLB2* mRNA with 1476 nt of CDS  
19 (YPR119W; genomic coordinates: chromosome XVI, 771653-773128, +, genome version S288C;  
20 *Saccharomyces* Genome Database, <https://www.yeastgenome.org/locus/S000006323>). We  
21 further added 366 nt 3' UTR and 346 nt 5' UTR as previously determined by<sup>55</sup>. In the second step,  
22 the minimum free energy (MFE) folds of all initial instances were analyzed using RNAfold with  
23 and without including a constraint on the nested pairs of inverted repeats<sup>101,102</sup>. Fold prediction  
24 was performed at 28°C with 80 nt RNA sequence fragments centered on each instance. Instances

1 were only kept if (i) at least one of inverted repeat pairs was present in the MFE structure without  
2 constraints, and (ii) the free energy ( $\Delta G$ ) of the constraint structure did not differ by more than  
3 20% from the MFE structure without constraint. The latter accounts for energetic benefits from  
4 interaction with the She2-She3 proteins. The pattern search predicted a single ZIP-code at  
5 nucleotide positions +1111 to +1145 of the CDS (genomic coordinates: chromosome XVI,  
6 772763-772797, +). **Fig. 4c** displays the predicted fold with constraint using RNAfold of ZIP-code  
7 instance plus  $\pm 5$  nt flanking sequence. Visualization of the predicted structure in dot-bracket  
8 notation was generated using VARNA<sup>103</sup>. Repeating the pattern search described above on the  
9 synonymized ZIP-code mutant did not retrieve any hits. The complete sequences of the  
10 synonymized ZIP-code mutant are provided in the Resource Table.

#### 11 *Sample preparation for live yeast fluorescence imaging*

12 Yeast cells were grown at 26°C in synthetic selective medium. Exponentially growing cells ( $OD_{600}$   
13 0.2-0.4) were plated on coated Delta-T dishes (Biotech 04200417C). The dishes coating was  
14 done by incubating with Concanavalin A 1mg/ml (Cayman chemical company) for 10 minutes at  
15 room temperature. Excess liquid was aspirated and dishes were dried at room temperature. To  
16 activate Concanavalin A, dishes were incubated for 10 minutes at room temperature with a 50  
17 mM  $CaCl_2$  50 mM  $MnCl_2$  solution. Excess was removed and dishes dried at room temperature.  
18 Finally, dishes were washed once with ultrapure water (Invitrogen) and completely dried at room  
19 temperature. Cell attachment was performed by gravity for 20 minutes at room temperature,  
20 excess liquid removed and substitution with fresh media. Cells were diluted to  $OD_{600}$  0.1 and  
21 grown until  $OD_{600}$  0.3-0.4. before being plated on Concanavalin A coated dish.

#### 22 *Live cell fluorescence imaging and image analysis*

23 The two-color simultaneous imaging of mRNAs and the appropriate cellular marker was  
24 performed on a modified version of the home-built microscope described in<sup>67,68</sup>. Briefly, the  
25 microscope was built around an IX71 stand (Olympus). For excitation, a 491 nm laser



1 (Calypso<sup>TM</sup>, Cobolt) and a 561 nm laser (Jive<sup>TM</sup>, Cobolt) were combined and controlled by an  
2 acoustic-optic tunable filter (AOTF, AOTFnC-400.650-TN, AA Opto-electronic) before coupled  
3 into a single mode optical fiber (Qioptiq). The output of the fiber was collimated and delivered  
4 through the back port of the microscope and reflected into an Olympus 150x 1.45 N.A. Oil  
5 immersion objective lens with a dichroic mirror (zt405/488/561rpc, 2mm substrate, Chroma). The  
6 tube lens (180 mm focal length) was removed from the microscope and placed outside of the right  
7 port. A triple band notch emission filter (zet405/488/561m) was used to filter the scattered laser  
8 light. A dichroic mirror (T560LPXR, 3mm substrate, Chroma) was used to split the fluorescence  
9 onto two precisely aligned EMCCDs (Andor iXon3, Model DU897) mounted on alignment stages  
10 (x, y, z,  $\theta$ - and  $\phi$ - angle). Emission filters FF03-525/50-25 and FF01-607/70-25 (Semrock) were  
11 placed in front of green and red channel cameras, respectively. The two cameras were triggered  
12 for exposure with a TTL pulse generated on a DAQ board (Measurement Computing). The  
13 microscope was equipped with a piezo stage (ASI) for fast z-stack and a Delta-T incubation  
14 system (Biotech) for live-cell imaging. The microscope (AOTF, DAQ, Stage and Cameras) was  
15 automated with the software Metamorph (Molecular Devices). For two-color live-cell imaging,  
16 yeast cells were streamed at 50 ms, Z plane was streamed, and z-stacks acquired every 0.5  $\mu\text{m}$ .  
17 Single-molecule analysis was done on maximal projected images using Fiji. Maximally projected  
18 images were filtered using the Maxican Hat filter (Radius=2) in Fiji. Spots were identified and  
19 counted using the spot detection plugin integrated in TrackMate. LoG detector was used for the  
20 spot identification, object diameter= 3 and Quality threshold = 2500. Files were exported as csv  
21 files and plotted using GraphPad Prism.

## 22 *Deconvolution algorithm*

23 To reduce imaging artifacts arising from noise and optics of the microscope, we used the Huygens  
24 software v3.6, where a Classic Maximum Likelihood Estimation (CMLE) algorithm was applied as  
25 a restoration method to deconvolve the images used for protein-mRNA foci co-localization (**Fig.**

1 5). CMLE assumes the photon noise to be governed by Poisson statistics and optimizes the  
2 likelihood of an estimate of an object in the input 3D image while taking the point spread function  
3 into consideration. The CMLE deconvolution method was chosen since it is suited for images with  
4 low signal-to-noise ratio and to restore point-like objects. The result is a more accurate  
5 identification of the location of the object, which in our case is the fluorescently labeled mRNA  
6 and protein molecules. The restoration parameters used with the CMLE deconvolution algorithm  
7 was 99 iterations, a quality stop criterion of 0.01, and a signal-to-noise ratio of 15.

#### 8 *CLB2 mRNA bud localization quantification in living cells*

9 For the analysis reported in **Fig. 2e**, the ImageJ plugin Labkit (<https://imagej.net/Labkit>) was  
10 manually used to segment cells and RNAs. Segmented cells were used as input for training the  
11 deep learning program Stardist in 2 dimensions. Stardist was used to automatically detect and  
12 segment cells and single mRNAs from live imaging movie frames (Cell Detection with Star-convex  
13 Polygons, <https://arxiv.org/pdf/1806.03535.pdf>). Cell and RNA segmentation was imported into R  
14 using the RImageJROI package. In R, the cell size, number of mRNAs in the bud and the distance  
15 of each bud localized mRNA to the periphery was calculated and plotted over time using the R  
16 packages Spatial Data and PBSmapping<sup>104</sup>. The Stardist segmentations were used to plot the  
17 RNAs and the cell's periphery onto the live imaging movie using the FFmpeg wrapper function for  
18 the FFmpeg multimedia framework (<https://ffmpeg.org/>).

#### 19 *Protein extraction and Western blot*

20 Yeast strains were grown overnight at 26°C in yeast extract peptone dextrose (YEPD) medium  
21 with 2% glucose. In the morning, cells were diluted to OD<sub>600</sub> 0.1 and allowed to grow until OD<sub>600</sub>  
22 0.5-1. Cell lysis was performed by adding 1 ml H<sub>2</sub>O with 150 µl of Yex-lysis buffer (1.85 M NaOH,  
23 7.5% 2-mercaptoethanol) to the pellet of 3-5 ODs of cells (~3x10<sup>7</sup>) and kept 10 minutes on ice.  
24 Proteins were precipitated by the addition of 150 µl of TCA 50% for 10 minutes on ice. Cells were  
25 pelleted and resuspended in 100 µl of 1X sample buffer (1 M Tris-HCl pH 6.8, 8 M Urea, 20%

1 SDS, 0.5 M EDTA, 1% 2-mercaptoethanol, 0.05% bromophenol blue). Total protein extracts were  
2 fractionated on SDS-PAGE and examined by Western blot with mouse anti-Myc (Sigma), mouse  
3 anti-Pgk1 (ThermoFisher). For quantitative Western blot analyses, fluorescent secondary  $\alpha$ -Mouse  
4 (IRDye 800CW) and  $\alpha$ -Rabbit (IRDye 680RD) antibodies were used. The signals were revealed  
5 using the LYCOR<sup>®</sup> scanner and quantified using LITE<sup>®</sup> Software.

#### 6 *Growth curves setup and analysis*

7 Cells were grown overnight at 30°C in SC-complete or Drop-out medium with 2% glucose. Cells  
8 in mid-log phase were spun down, the supernatant was removed and cells were resuspended at  
9 a final OD<sub>600</sub> of about 0.1 in test medium containing different carbon sources, as indicated in the  
10 figure legend. In 48-well plates with flat bottom, 400  $\mu$ l were plated per well. At least 3 well  
11 replicates were done per experiment. Cells were grown for the indicated time, at 30°C. OD<sub>600</sub>  
12 measurements were taken every 5 minutes, with 700 rpm shaking between time-points using a  
13 CLARIOstar<sup>®</sup> plate reader (BMG Labtech). Growth curves analysis was performed using an  
14 adaptation of the R package Growthcurver<sup>105</sup> and plotted using the R package ggplot2<sup>106</sup>,  
15 tidyverse<sup>107</sup>, RColorBrewer<sup>108</sup>, dplyr<sup>109</sup>. Growthcurver fits a basic form of the logistic equation to  
16 experimental growth curve data. The logistic equation gives the number of cells  $N_t$  at time  $t$ .

17

$$N_t = \frac{K}{1 + \left(\frac{K - N_0}{N_0}\right) e^{-rt}}$$

18 The population size at the beginning of the growth curve is given by  $N_0$ . The maximum possible  
19 population size in a particular environment, or the carrying capacity, is given by  $K$ . The intrinsic  
20 growth rate of the population,  $r$ , is the growth rate that would occur if there were no restrictions  
21 imposed on total population size. Growthcurver finds the best values of  $K$ ,  $r$ , and  $N_0$  for the growth  
22 curve data using the implementation of the non-linear least-squares Levenberg-Marquardt  
23 algorithm. The carrying capacity and growth rate values ( $K$  and  $r$ ) are used to compare the growth  
24 dynamics of strains.

## 1 *Flow cytometry sample preparation and analysis*

2 Cells were grown overnight at 30°C in SC-complete medium with 2% glucose. Cells were grown  
3 to mid-log phase (OD<sub>600</sub> 0.3-0.4) with constant shaking (220 pm) at 30°C. Next, they were spun  
4 down, the supernatant was removed and cells were resuspended at a final OD<sub>600</sub> of about 0.1 in  
5 test medium containing different carbon sources, as indicated in the figure legend. At the indicated  
6 time-points, 1 mL of culture was transferred to a 1.5 mL Eppendorf tube and centrifuged for 3  
7 minutes at 3000 rpm. The supernatant was removed and cells were resuspended in 70% ethanol  
8 and incubated overnight at 4°C. Cells were washed once with 1xPBS pH 7.4 and resuspended in  
9 500 µl of 1xPBS with 1 µl of RNase A 1 mg/mL and incubated at 37°C for at least 3 hours. Cells  
10 were then washed with 1 mL of 1xPBS and resuspended in 500 µl of 1xPBS. 100 µl of cells were  
11 then incubated with 3 µl of a 5 µM solution of Sytox Orange and incubated in a water bath at 37°C  
12 for 3 hours covered from the light. Cells were then washed 3 times with 1xPBS and resuspended  
13 in 500 µl of 1xPBS. The cells were then analyzed with the Beckman Coulter Flow cytometer  
14 CytoFLEX S System (B2-R0-V2-Y2). A 561 nm laser was used to excite the fluorescent dye and  
15 a band pass filter was used to filter the emitted fluorescence. 50'000 cells were collected per  
16 sample. Analysis and plotting was performed using R Studio and the following R packages:  
17 ggplot2<sup>106</sup>; tidyverse<sup>107</sup>, RColorBrewer<sup>108</sup>, dplyr<sup>109</sup>, mixtools<sup>110</sup>.

## 18 *Quantifications and statistical analysis*

19 FISH-quant was used to quantify single mRNA molecules and protein foci in fixed samples. Fiji  
20 was used to quantify single mRNA molecules in living cells. GraphPad Prism was used to  
21 calculate the mean and the standard deviation (SD) of all the data and perform statistical analysis.  
22 Flow cytometry data, growth curves analysis was performed in R Studio, as detailed in previous  
23 paragraphs. For each experiment, the number of biological replicates, the number of cells  
24 analyzed (n), statistical analysis applied and significance (P<0.05 for significant differences) is  
25 indicated in the figure legend or in the main text.

## 1 **Figure Legends**

2 *Figure 1. CLB2 mRNAs localize to the bud in a cell-cycle dependent manner.*

3 **(a)** Schematic of *CLB2* mRNA expression during the cell cycle. Green dots represent *CLB2*  
4 mRNAs. The Whi5 protein (cyan) accumulates in the nucleus during early G1 phase<sup>72,111,112</sup>.  
5 Tubulin (magenta) is a major component of microtubules and the mitotic spindle. Bud emergence  
6 starts during S phase and ends with the formation of the daughter cell. During anaphase, the  
7 microtubules stretch between the mother and the daughter cell. **(b)** Top panels: MERGE Maximal  
8 projections of IF anti-HA (Whi5) (cyan) and DAPI (blue) merged to a single differential interference  
9 contrast (DIC) section (grey). Bottom panels: MERGE Maximal projections of *CLB2* mRNA  
10 smFISH (green), anti-tubulin IF (magenta) and DAPI (blue) merged to a single DIC section (grey).  
11 The corresponding cell cycle phase is indicated on the panels. Scale bars 3  $\mu$ m. **(c)** smFISH  
12 quantifications of *CLB2* mRNA expression during the different cell cycle phases determined using  
13 the markers shown in (b). Dots correspond to individual cells (2083 cells, from two replicates).  
14 The black bar indicates the average (G1 early=  $1.2 \pm 1.9$ ; G1 late=  $1.2 \pm 1.9$ ; S=  $3.8 \pm 3.0$ ; G2=  
15  $10.3 \pm 5.7$ ; M=  $8.0 \pm 6.5$  mRNAs/cell mean  $\pm$  standard deviation [SD]). **(d)** Relative bud vs mother  
16 distribution of the *CLB2* mRNA in WT budded cells based on the smFISH-IF data shown in (b)  
17 (mean  $\pm$  SD).

18 *Supplementary Figure 1. CLB2 mRNAs are synthesized and localized in the bud in a cell-cycle*  
19 *dependent manner.*

20 **(a)** Quantification of *CLB2* mRNA smFISH shown in Figure 1b reported as relative frequency  
21 distribution of mature mRNAs per cell. Data from two replicates (n=2083). **(b)** Quantification of  
22 *CLB2* nascent RNAs at transcriptions sites (TS) from smFISH shown in Figure 1b reported as  
23 relative frequency distribution of nascent RNAs per TS. Data from two replicates (n=2083). **(c)**  
24 MDN1 smFISH maximal projection (green), and DAPI merged to a single DIC section (grey).  
25 Scale bar 5  $\mu$ m **(d)** Relative bud vs mother distribution of *MDN1* mRNA in budded cells based on

1 the smFISH data shown in (b). Data from two replicates (n=2011; mean  $\pm$  SD). **(e)** smFISH in the  
2 *S. cerevisiae* background W303. MERGE Maximal projections of *CLB2* mRNA smFISH (green)  
3 and DAPI (blue) merged to a single DIC section (grey). Scale bar 3  $\mu$ m.

4 *Figure 2. CLB2 mRNA imaging in living cells reveals a rapid mRNA synthesis and degradation*  
5 *and localization to the bud.*

6 **(a)** Schematic of *CLB2* localization during the cell cycle. The *CLB2* mRNA (green) is expressed  
7 from late S-phase and localizes to the emerging bud. Bud emergence as well as the separation  
8 of the bud from the mother can be identified by endogenously tagging the bud neck protein Cdc10  
9 with tdTomato (magenta). The *CLB2* mRNA is rapidly degraded during mitosis. **(b)** *CLB2*  
10 endogenously tagged with 24xMBSV6 to enable visualization of the mRNA (black) in live cells.  
11 The bud neck protein Cdc10 is tagged with tdTomato (magenta). Cell A is the mother of cell B.  
12 Time point from start of acquisition is indicated in the upper left corner of each time frame. Scale  
13 bar 3  $\mu$ m. **(c)** Percentage of *CLB2* mRNAs localized in the bud over time from bud appearance.  
14 **(d)** Number of *CLB2* mRNAs per cell over time in cell A and cell B. **(e)** Snap shots from live cell  
15 imaging of *CLB2* mRNA endogenously tagged with 24xMBSV6 (green). Approximate cell outline  
16 identified from fluorescent background (orange). A single Z plane was acquired every 100 ms.  
17 Scale bars 3  $\mu$ m **(f)** Average cell area monitored over time in live cells (n=3). A single Z plane was  
18 acquired every 100 ms. Lower right insert shows cell size of a single cell acquired every 2 min.  
19 Red line in insert indicates when the 100 ms images were acquired. Pink, purple and green lines  
20 represent time points at which the snapshots in (e) were taken. **(g)** Average number of bud  
21 localized mRNAs per cell over time as monitored from 3 single cells. Pink, purple and green lines  
22 represent the time points at which the snapshots in (e) were taken.

1 *Supplementary Figure 2. CLB2 mRNAs endogenously tagged with the MBSV6 reporter*  
2 *recapitulate the expression of the endogenous CLB2 mRNA.*

3 **(a)** Schematic of *CLB2* locus endogenously tagged with 24xMBSV6 inserted in the 3' UTR after  
4 the STOP codon. Dotted lines represent smFISH probe positions targeting the CDS (green) or  
5 MBS sequences (magenta). **(b)** Two-color smFISH for cells expressing tagged *CLB2* mRNAs.  
6 Top panels (MBSV6), cells expressing the control vector (YcpLac111). Bottom panels  
7 (MBSV6+MCP) cells expressing MCP (YcpLac111 CYC1p-MCP-NLS-2xyeGFP). DIC/MERGE  
8 shows the overlap of the DAPI signal in the nucleus (blue), smFISH for the *CLB2* CDS (green)  
9 and the MBS (magenta) with the differential interference contrast (DIC) image. Scale bars 3  $\mu$ m.  
10 **(c)** Quantification of smFISH represented in 4b as well as untagged WT cells, with CDS probes  
11 (green plots) or MBS probes (magenta) reported as distribution of mature mRNAs per cell. Mean  
12 of three biological replicates, (*CLB2* MBSV6: *CLB2* probes n= 862, mean  $\pm$  SD 4.9  $\pm$  5.8  
13 mRNA/cell; MBSV6 probes n=738, mean  $\pm$  SD 5.1  $\pm$  5.6 mRNA/cell; *CLB2* MBSV6+MCP: *CLB2*  
14 probes n = 752, mean  $\pm$  SD 4.7 $\pm$  5.3 mRNA/cell, MBSV6 probes n=974, mean  $\pm$  SD 4.7 $\pm$ 4.9  
15 mRNA/cell; WT cells: *CLB2* probes n = 791). **(d-e)** Correlation between the number of single CDS  
16 and MBSV6 molecules per cell in presence or absence of MCP. Pearson *r* values calculated by  
17 combining two independent experiments (n=1908 and 2284, respectively). **(f)** Average number of  
18 *CLB2* mRNA per cell tagged with 24xMBSV6 monitored over time. Grey represents SD from  
19 average mRNA per cell (purple).

20 *Figure 3. The She2-3 complex and an RNA ZIP code in CLB2 mRNA CDS are required for bud*  
21 *localization.*

22 **(a)** smFISH-IF in WT (top row),  $\Delta$ *she2* (middle row), and  $\Delta$ *she3* (bottom row) cells. MERGE  
23 Maximal projections of *CLB2* mRNA smFISH (green), anti-tubulin IF (magenta) and DAPI (blue)  
24 merged to a single DIC section (grey). The corresponding cell cycle phase is indicated on top of  
25 the panel. Scale bars 3  $\mu$ m. **(b)** Schematic of *CLB2* mRNA coding sequence. The blue box

1 represents the ZIP-code at nucleotides 1111-1145 (relative to START codon). **(c)** Predicted  
2 secondary structure of ZIP-code (blue box) with flanking sequence (nt 1089- 1168). The free  
3 energy ( $\Delta G$ ) of the mRNA folding is indicated. **(d)** Synonymized ZIP code (*ZIP-mut*). Top  
4 sequence is *CLB2* WT. Bottom sequence is the synonymized sequence. Mutated nucleotides are  
5 indicated in red. Below is the corresponding Clb2 protein amino acid sequence, which is identical  
6 for both the WT and synonymized strains. Rare amino acid codons were avoided to maintain the  
7 same codon optimization index = 0.74. **(e)** smFISH in WT and ZIP-code mutant strain. Maximal  
8 projections of smFISH with *CLB2* probes (green) and DAPI (blue) and fluorescence images  
9 overlapped to a single DIC section (MERGE). Scale bars 3  $\mu\text{m}$ . **(f)** Relative bud vs mother  
10 distribution of the *CLB2* mRNA in WT and ZIP-code mutant strain based on smFISH-IF data  
11 shown in (e). **(g)** Schematic representation of mRNA peripheral distribution index (PDI). Black  
12 dots represent mRNA. Blue is the nucleus. A PDI close to 0 indicates that the RNA of interest is  
13 localized near the nucleus. A PDI of 1 indicates that the RNA is diffusely dispersed throughout  
14 the cell. As the PDI value grows  $> 1$ , the polarization of the mRNA increases. **(h)** PDI in WT cells  
15 for *ASH1*, *CLB2* and *MDN1* mRNAs and in  $\Delta she2$ ,  $\Delta she3$  and ZIP-code mutant strains for the  
16 *CLB2* mRNA. Index values are calculated from smFISH-IF experiments shown in (e) and **Fig.**  
17 **3Sa**.

18 *Supplementary Figure 3. CLB2 is not co-localized to the bud together with the ASH1 mRNA.*

19 **(a)** WT,  $\Delta she2$  and  $\Delta she3$  cells: Left panels, MERGE Maximal projections of *CLB2* mRNA  
20 smFISH (green), anti-tubulin IF (magenta) and DAPI (blue) merged to a single DIC section (grey).  
21 Right panels, MERGE Maximal projections of IF anti-HA (Whi5) (cyan) and DAPI (blue) merged  
22 to a single DIC section (grey). Scale bars 5  $\mu\text{m}$ . **(b)** Relative bud vs mother distribution of the  
23 *CLB2* mRNA in  $\Delta she2$  budded cells based on the smFISH-IF data shown in **Fig.3a** and **Fig.3Sa**.  
24 **(c)** Relative bud vs mother distribution of the *CLB2* mRNA in  $\Delta she3$  budded cells based on the  
25 smFISH-IF data shown in **Fig. 3a** and in **Fig. 3Sa**. **(d)** Schematic of *CLB2* and *ASH1* mRNAs



1 expression during the cell cycle. Green dots represent *CLB2* mRNAs. Magenta dots represent  
2 *ASH1* mRNAs. Tubulin (grey) co-localizes with the spindle pole body which is duplicated during  
3 S phase. The bud emergence starts during S phase and ends with the formation of the daughter  
4 cell. During anaphase, the microtubules stretch between the mother and the daughter cell **(e)**  
5 MERGE Maximal projections of *CLB2* mRNA smFISH (green), *ASH1* mRNA smFISH (magenta),  
6 anti-tubulin IF (white) and DAPI (blue) merged to a single DIC section (grey). The corresponding  
7 cell cycle phase is indicated on top of the panel. Scale bars 3  $\mu\text{m}$ . **(f)** Percentage of co-localized  
8 *ASH1* and *CLB2* mRNAs (<250 nm) over total amount of *ASH1* and *CLB2* mRNA, from S phase  
9 to mitosis (in budded cells co-expressing both mRNAs) (mean  $\pm$  SD). We found 10.3% of *ASH1*  
10 mRNA and 7.7% of *CLB2* mRNAs to co-localize, suggesting that *CLB2* and *ASH1* mRNAs are  
11 not co-transported. **(g)** Example of 2-color smFISH colocalization analysis performed using FISH-  
12 quant V3, FQ\_DualColor\_colocalization function<sup>99</sup>. Colocalization between the two channels  
13 was calculated as a linear assignment problem (LAP) solved with the Hungarian algorithm using  
14 the Matlab function "hungarianlinker" and "munkres". We defined the maximum 3D allowed  
15 distance between two spots to be considered co-localized to 250 nm (~3 pixels). Left, MERGE  
16 maximal projection of filtered smFISH channels, *CLB2* (green) and *ASH1* (magenta). Right, color-  
17 coded localization of mRNAs by smFISH: Blue, co-localized (co-loc); Red, not-co-localized  
18 mRNAs; Yellow, not-co-localized in the other channel. **(h)** Previous structural work demonstrated  
19 that the She2-She3 complex comprises two She2 and She3 dimers, which can bind two mRNA  
20 targets simultaneously<sup>84</sup>. Comparison of RNA spots intensities distributions by smFISH in WT or  
21  $\Delta\text{she2}$  and  $\Delta\text{she3}$  mutants was used to examine whether *CLB2* mRNAs are transported to the  
22 bud two-by-two by. Two mRNAs in close proximity (<250 nm) would be detected as a single bright  
23 spot due to the microscope diffraction limit. A drop in *CLB2* mRNA spot intensities in the She  
24 mutants would indicate co-transported mRNAs. Relative frequency distribution of *CLB2* mRNA  
25 spot intensities in WT (n= 3004),  $\Delta\text{she2}$  (n= 2619) and  $\Delta\text{she3}$  (n= 2731) mutant strains (median  
26 WT = 45.7, lower 95% CI=45.0, upper 95% CI=46.8;  $\Delta\text{she2}$ =40.4, lower 95% CI=39.1, upper 95%

1 CI=41.2;  $\Delta she3$ =42.65, lower 95% CI=41.8, upper 95% CI=43.3). Results are shown for a  
2 representative experiment where the three strains were collected, processed and imaged side by  
3 side. A 3D Laplacian of Gaussian filter was applied to all the images using FISH-quant. The mean  
4 and SD of spot intensities are expressed as arbitrary units (a.u.).

5 *Figure 4. CLB2 mRNA mislocalization affects Clb2 protein expression but not mRNA levels,*  
6 *protein stability or localization.*

7 **(a)** smFISH quantifications of *CLB2* mRNA expression in WT and localization mutants. Dots  
8 correspond to individual cells (~1000 cells per replicate, from at least 2 replicates). The black bar  
9 indicates the average (mRNAs/cell mean  $\pm$  SD). **(b)** Quantification of nascent *CLB2* RNAs at  
10 transcription sites (TS) from smFISH. Dots correspond to individual cells (~1000 cells per  
11 replicate, from at least 2 replicates). Visualization as in (a). **(c)** Western blot analysis using anti-  
12 myc antibody against Clb2 protein endogenously tagged with 5 myc tags in WT,  $\Delta she2$ ,  $\Delta she3$   
13 and ZIP-code mutant cells. First lane is the control untagged strain. Endogenous Pgk1 was used  
14 as loading control **(d)** Quantification of western blot in (c). Myc signal normalized to Pgk1 loading  
15 control. Protein levels relative to WT indicated in lower bar. Mean  $\pm$  SD from 3-7 replicates. **(e)**  
16 Clb2 protein stability assay in WT,  $\Delta she2$  and  $\Delta she3$  cells. Western blot was performed using an  
17 anti-myc antibody to target Clb2 protein tagged with 5 myc tags in cells treated with 100  $\mu$ g/ml  
18 cycloheximide for 0, 5, 10, 20, 30 45, or 60 minutes. Pgk1 was used as loading control. **(f)** Clb2  
19 protein stability assay in WT and ZIP-code mutant. Western blot was performed in the same way  
20 as in (c). **(g)** Clb2 fused to yeGFP in WT,  $\Delta she2$ ,  $\Delta she3$  and ZIP code mutant cells. Maximal  
21 projections of Clb2-GFP (green) overlapped to a single DIC section. Scale bars 2  $\mu$ m.

22 *Supplementary Figure 4. CLB2 mRNA mislocalization is not affecting protein stability but it*  
23 *correlates with delayed Clb2 protein accumulation.*

24 **(a)** Growth curves of strains with Myc-tagged Clb2 protein performed in SC-complete 2% glucose  
25 at 26°C. Growth rates (r), calculated by fitting the data to a logistic curve, are provided in the

1 associate table. **(b)** Example of western blot of Clb2 protein stability assay in WT,  $\Delta$ she2 and  
2  $\Delta$ she3 cells. Western blot was performed using an anti-myc antibody to target Myc-tagged Clb2  
3 protein tagged in cells treated with 100  $\mu$ g/ml cycloheximide for 0, 5, 10, 20, 30 45, or 60 minutes.  
4 Pgk1 was used as loading control. Quantifications are reported in **Fig. 4e**. **(c)** Example of western  
5 blot of Clb2 protein stability assay in WT and ZIP-code mutant cells. Western blot was performed  
6 using an anti-myc antibody to target Clb2 protein tagged with 5 myc tags in cells treated with 100  
7  $\mu$ g/ml cycloheximide for 0, 5, 10, 20, 30 45, or 60 minutes. Pgk1 was used as loading control.  
8 Quantifications are reported in **Fig. 4f**. **(d)** Quantification of Clb2-GFP expression measured in  
9 living WT and  $\Delta$ she2 cells. Cells were grown in SC-complete 2% glucose at 26°C. Images were  
10 collected every 5 minutes, 13 Z planes were acquired every 0.5  $\mu$ m, with an exposure time of 50  
11 ms. Maximally projected images were used for cell segmentation and quantification of GFP  
12 integrated intensity (expressed as arbitrary units a.u.). **(e)** Correlation of Clb2-GFP expression  
13 and bud volume measured in living WT and  $\Delta$ she2 cells.

14 *Figure 5. CLB2 mRNA and protein colocalization indicates preferential translation in the bud.*

15 **(a)** smFISH-IF in WT cells. Top panels: MERGE Maximal projections of IF anti-tubulin (cyan) and  
16 DAPI (blue) merged to a single DIC section (grey). Second panel row from the top: MERGE  
17 Maximal projections of IF anti-myc-Clb2 protein (magenta), *CLB2* mRNA smFISH (green) and  
18 DAPI (blue) merged to a single DIC section (grey). The third and fourth panels from the top are  
19 the *CLB2* mRNA smFISH and Clb2 protein IF, respectively. Scale bars 3  $\mu$ m. **(b)** Quantification  
20 of protein clusters in WT bud (B) and mother (M) cells from IF experiments shown in (a). Cell  
21 cycle classification was performed using tubulin and DAPI as markers. **(c)** Quantification of mRNA  
22 in WT bud (B) and mother (M) cells from smFISH experiments shown in (a). Cell cycle  
23 classification was performed as in (b). **(d)** smFISH-IF in  $\Delta$ she2 cells. Maximal projections of IF  
24 anti-myc-Clb2 protein (magenta), *CLB2* mRNA smFISH (green) and DAPI (blue) merged to a  
25 single DIC section (grey). Panels description as in (a). Scale bars 2  $\mu$ m. **(e)** Quantification of *CLB2*

1 mRNA-protein clusters found in close proximity (<250 nm) by smFISH-IF experiments performed  
2 in bud (B) and mother (M) cells of WT and  $\Delta$ she2 cells shown in panels (a) and (d). **(f)**  
3 Quantification of bud-localized mRNAs co-localizing with a protein cluster (<250 nm) in WT and  
4  $\Delta$ she2 cells shown in panels (a) and (d).

5 *Supplementary Figure 5. Translation inhibition with cycloheximide causes CLB2 mRNA*  
6 *stabilization and correlates with reduced mRNA localization in the bud*

7 **(a)** Schematic of the *CLB2* endogenous gene tagging to simultaneously visualize single mRNA  
8 by smFISH and Clb2 proteins by IF in fixed cells. 25 myc tags are inserted into the 5' end of the  
9 *CLB2* CDS. smFISH is performed against the *CLB2* CDS (top panel). As the mRNA is being  
10 translated the myc tags will be first to emerge from the ribosome and will be detectable with IF  
11 (bottom panel). By performing smFISH against the *CLB2* mRNA and IF against the 25myc-  
12 tagged Clb2 protein, translation can be detected by the co-localization of the two signals. **(b)**

13 Western blot analysis of *CLB2* Myc tagged strains. Top row is the myc signal. Bottom row is the  
14 P<sub>gk1</sub> loading control. First lane is untagged cells. Second and third lanes are the Clb2 protein  
15 tagged with 5 and 25 myc tags, respectively. **(c)** smFISH-IF in WT cells treated with 100  $\mu$ g/mL  
16 of translation elongation inhibitor cycloheximide (CHX) for 20 min. Top panels: MERGE Maximal  
17 projections of IF anti-tubulin (cyan) and DAPI (blue) merged to a single DIC section (grey). Second  
18 panel row from the top: MERGE Maximal projections of IF anti-myc-Clb2 protein (magenta), *CLB2*  
19 mRNA smFISH (green) and DAPI (blue) merged to a single DIC section (grey). The third and  
20 fourth panels from the top are the *CLB2* mRNA smFISH and Clb2 protein IF, respectively. Scale

21 bars 3  $\mu$ m. **(d)** smFISH quantifications of *CLB2* mRNA expression of cells exposed to CHX shown  
22 in (b). Dots correspond to individual cells (46 cells, from two replicates). **(e)** smFISH  
23 quantifications of *CLB2* mRNA expression in bud (B) and mother (M) cells exposed to CHX shown  
24 in (b). Dots correspond to individual cells (46 cells, from two replicates). **(f)** Quantification of *CLB2*  
25 mRNA-protein clusters found in close proximity (<250 nm) by smFISH-IF experiments performed

1 in buds (B) and mothers (M) of WT cell exposed to CHX shown in (b). **(f)** PDE simulation results  
2 of ribosome binding scenarios using concentration distribution at steady state for normal binding  
3 at diffusion  $D = 400'000 \text{ nm}^2/\text{s}$ ; **(g)** at  $D = 100'000 \text{ nm}^2/\text{s}$ . **(h)** Concentration distribution at steady  
4 state for high binding and  $D = 100'000 \text{ nm}^2/\text{s}$ . Units: length nm, time s, concentration  $1/\text{nm}^3$ . Note  
5 that the colors have been log-scaled.

6 *Figure 6. Known translation inhibitors are not involved in regulating Clb2 protein expression*

7 **(a)** Western blot analysis using anti-myc antibody against Clb2 protein tagged with 25 myc tags  
8 in WT,  $\Delta\text{ssd1}$ ,  $\Delta\text{khd1}$  and  $\Delta\text{puf6}$  cells. Pkg1 was used as loading control. First lane is untagged  
9 cells. **(b)** Quantification of (a). Mutant strain signal is normalized to WT signal. Each color  
10 corresponds to one replicate experiment. Black bar indicates mean  $\pm$  SD (WT= 100;  $\Delta\text{ssd1}$ =  
11  $88.3 \pm 15.6$ ;  $\Delta\text{khd1}$ =  $98.8 \pm 13.5$ ;  $\Delta\text{puf6}$ =  $83.9 \pm 10.4$ ). **(c)** Merge maximal projections of *CLB2*  
12 smFISH (green), DIC (gray), and DAPI (blue) in WT,  $\Delta\text{ssd1}$ ,  $\Delta\text{khd1}$  and  $\Delta\text{puf6}$  cells. Scale bars 5  
13  $\mu\text{m}$ . **(d)** Quantification of *CLB2* smFISH in WT,  $\Delta\text{ssd1}$ ,  $\Delta\text{khd1}$  and  $\Delta\text{puf6}$  cells. Each dot  
14 corresponds to a single cell (500-1500 cells from two to four replicates). Each color corresponds  
15 to one replicate. Black bar indicates the average (WT=  $4.3 \pm 0.5$ ;  $\Delta\text{ssd1}$ =  $4.8 \pm 0.8$ ;  $\Delta\text{khd1}$ =  $4.2 \pm 0.7$ ;  
16  $\Delta\text{puf6}$ =  $6.9 \pm 1.2$  mRNAs/cell mean  $\pm$  SD). **(e)** smFISH quantification of nascent *CLB2* RNA  
17 expression per transcription site in WT,  $\Delta\text{ssd1}$ ,  $\Delta\text{khd1}$  and  $\Delta\text{puf6}$  cells. Dots correspond to  
18 individual cells (500-1500 cells from two to four replicates). Each color corresponds to one  
19 replicate. Black bar indicates the average (WT=  $2.4 \pm 1.1$ ;  $\Delta\text{ssd1}$ =  $2.6 \pm 1.0$ ;  $\Delta\text{khd1}$ =  $2.1 \pm 0.7$ ;  
20  $\Delta\text{puf6}$ =  $2.5 \pm 1.1$  mRNAs/transcription site  $\pm$  SD).

21 *Supplementary Figure 6. Known translation inhibitors are not involved in regulating CLB2 local*  
22 *translation, but Puf6 regulates CLB2 transcription.*

23 **(a)** Relative frequency distribution of mature *CLB2* mRNAs per cell. **(b)** Merge maximal projection  
24 of *CLB2* smFISH (green), DIC (gray), and DAPI (blue) in a  $\Delta\text{puf6}$  cell. Yellow arrows indicate

1 transcription sites. Scale bar 2  $\mu\text{m}$ . **(c)** Quantification of transcription sites in transcriptionally  
2 active WT and  $\Delta puf6$  cells ( $n = 1340$  and  $1583$  cells, respectively) based on *CLB2* mRNA smFISH.

3 *Figure 7. The CLB2 mRNA ZIP code mutants show clone-dependent growth defect and cell cycle*  
4 *defect.*

5 **(a)** Top, single *CLB2* ZIP-code mutant clones grown on SC-complete 2% glucose at 30°C for 3  
6 days. Bottom, *CLB2* ZIP-code mutant clones from top plate were grown on SC-complete 0.1%  
7 glucose at 30°C for 3 days. **(b)** Tenfold serial dilutions of the indicated strains were spotted onto  
8 SC-complete plates at the indicated carbon sources and temperatures. **(c)** Growth curves of *CLB2*  
9 ZIP-code mutant clones (*ZIP cl1*, *cl2* and *cl3*) performed at 30°C in SC-complete at the indicated  
10 carbon sources. Optical density measurements ( $\text{OD}_{600}$ ) were collected every 5 min. Triplicate  
11 experiments are shown for each strain. **(d)** Cell cycle analysis by DNA content estimation with  
12 flow cytometry in the ZIP-code mutants grown at the indicated conditions. DNA was stained with  
13 Sytox Orange. Mixed Gaussian fitting (black lines) was used to estimate the indicated G1 and  
14 G2/M components. Percentages are reported for one representative experiment.

15 *Supplementary Figure 7. CLB2 ZIP code mutant cells shows a growth defect and clonal variability*  
16 *under nutrient limited conditions*

17 **(a)** Growth curves of *SHE* deleted strains ( $\Delta she2$ ,  $\Delta she3$ ) performed at 30°C in SC-complete at  
18 the indicated carbon sources. Optical density measurements ( $\text{OD}_{600}$ ) were collected every 5 min.  
19 Triplicate experiments are shown for each strain. **(b)** Left, Bright-field images of WT or *CLB2* ZIP-  
20 *cl1* mutant grown in SC-complete 2% glucose at 30°C. Right, quantification of WT and *CLB2* ZIP-  
21 code *cl1* mutant cell areas. **(c)** Growth curves of Diploid strains generated by crossing WT cells  
22 (mating type *alpha*) with either (mating type *a*) WT or the *CLB2* ZIP-code *cl1* mutant, performed  
23 at 30°C in SC-complete at the indicated carbon sources. Triplicate experiments are reported for  
24 each strain.

## 1 References

- 2 1. Lecuyer, E. *et al.* Global analysis of mRNA localization reveals a prominent role in organizing  
3 cellular architecture and function. *Cell* **131**, 174–87 (2007).
- 4 2. Nevo-Dinur, K., Nussbaum-Shochat, A., Ben-Yehuda, S. & Amster-Choder, O. Translation-  
5 Independent Localization of mRNA in *E. coli*. *Science* **331**, 1081–1084 (2011).
- 6 3. Martin, K. C. & Ephrussi, A. mRNA localization: gene expression in the spatial dimension. *Cell*  
7 **136**, 719–30 (2009).
- 8 4. Das, S., Vera, M., Gandin, V., Singer, R. H. & Tutucci, E. Intracellular mRNA transport and  
9 localized translation. *Nature Reviews Molecular Cell Biology* 1–22 (2021) doi:10.1038/s41580-  
10 021-00356-8.
- 11 5. Kejiou, N. S. & Palazzo, A. F. mRNA localization as a rheostat to regulate subcellular gene  
12 expression. *Wiley Interdiscip Rev RNA* **8**, (2017).
- 13 6. Becalska, A. N. & Gavis, E. R. Lighting up mRNA localization in *Drosophila* oogenesis.  
14 *Development (Cambridge, England)* **136**, 2493–503 (2009).
- 15 7. Cui, X. A. & Palazzo, A. F. Localization of mRNAs to the endoplasmic reticulum. *WIREs RNA*  
16 **5**, 481–492 (2014).
- 17 8. Buxbaum, A. R., Haimovich, G. & Singer, R. H. In the right place at the right time: visualizing  
18 and understanding mRNA localization. *Nature reviews. Molecular cell biology* **16**, 95–109  
19 (2015).
- 20 9. Singer-Krüger, B. & Jansen, R.-P. Here, there, everywhere. mRNA localization in budding  
21 yeast. *RNA Biol* **11**, 1031–1039 (2014).
- 22 10. Shen, Z., Paquin, N., Forget, A. & Chartrand, P. Nuclear shuttling of She2p couples ASH1  
23 mRNA localization to its translational repression by recruiting Loc1p and Puf6p. *Mol Biol Cell*  
24 **20**, 2265–75 (2009).
- 25 11. Long, R. M. *et al.* Mating type switching in yeast controlled by asymmetric localization of  
26 ASH1 mRNA. *Science* **277**, 383–7 (1997).

- 1 12. Takizawa, P. A. & Vale, R. D. The myosin motor, Myo4p, binds Ash1 mRNA via the  
2 adapter protein, She3p. *Proc Natl Acad Sci U S A* **97**, 5273–8 (2000).
- 3 13. Long, R. M. *et al.* Characterization of transport and localization of ASH1 mRNA in yeast.  
4 *Mol Biol Cell* **8**, 2060–2060 (1997).
- 5 14. Bertrand, E. *et al.* Localization of ASH1 mRNA particles in living yeast. *Mol Cell* **2**, 437–  
6 45 (1998).
- 7 15. Niessing, D., Huttelmaier, S., Zenklusen, D., Singer, R. H. & Burley, S. K. She2p is a novel  
8 RNA binding protein with a basic helical hairpin motif. *Cell* **119**, 491–502 (2004).
- 9 16. Long, R. M., Gu, W., Lorimer, E., Singer, R. H. & Chartrand, P. She2p is a novel RNA-  
10 binding protein that recruits the Myo4p-She3p complex to ASH1 mRNA. *Embo J* **19**, 6592–601  
11 (2000).
- 12 17. Böhl, F., Kruse, C., Frank, A., Ferring, D. & Jansen, R.-P. She2p, a novel RNA-binding  
13 protein tethers ASH1 mRNA to the Myo4p myosin motor via She3p. *EMBO J* **19**, 5514–5524  
14 (2000).
- 15 18. Deng, Y., Singer, R. H. & Gu, W. Translation of ASH1 mRNA is repressed by Puf6p-  
16 Fun12p/eIF5B interaction and released by CK2 phosphorylation. *Genes & development* **22**,  
17 1037–50 (2008).
- 18 19. Gu, W., Deng, Y., Zenklusen, D. & Singer, R. H. A new yeast PUF family protein, Puf6p,  
19 represses ASH1 mRNA translation and is required for its localization. *Genes & development*  
20 **18**, 1452–65 (2004).
- 21 20. Irie, K. *et al.* The Khd1 protein, which has three KH RNA-binding motifs, is required for  
22 proper localization of ASH1 mRNA in yeast. *The EMBO journal* **21**, 1158–67 (2002).
- 23 21. Paquin, N. *et al.* Local activation of yeast ASH1 mRNA translation through phosphorylation  
24 of Khd1p by the casein kinase Yck1p. *Mol Cell* **26**, 795–809 (2007).
- 25 22. Hasegawa, Y., Irie, K. & Gerber, A. P. Distinct roles for Khd1p in the localization and  
26 expression of bud-localized mRNAs in yeast. *RNA* **14**, 2333–2347 (2008).



- 1 23. Shahbadian, K., Jeronimo, C., Forget, A., Robert, F. & Chartrand, P. Co-transcriptional  
2 recruitment of Puf6 by She2 couples translational repression to mRNA localization. *Nucleic*  
3 *Acids Res* **42**, 8692–8704 (2014).
- 4 24. Heym, R. G. & Niessing, D. Principles of mRNA transport in yeast. *Cell Mol Life Sci* **69**,  
5 1843–53 (2012).
- 6 25. Niessing, D., Jansen, R.-P., Pohlmann, T. & Feldbrügge, M. mRNA transport in fungal top  
7 models. *Wiley Interdiscip Rev RNA* **9**, (2018).
- 8 26. Wanless, A. G., Lin, Y. & Weiss, E. L. Cell Morphogenesis Proteins Are Translationally  
9 Controlled through UTRs by the Ndr/LATS Target Ssd1. *PLOS ONE* **9**, e85212 (2014).
- 10 27. Jansen, J. M., Wanless, A. G., Seidel, C. W. & Weiss, E. L. Cbk1 Regulation of the RNA-  
11 Binding Protein Ssd1 Integrates Cell Fate with Translational Control. *Current Biology* **19**, 2114–  
12 2120 (2009).
- 13 28. Bayne, R. A. *et al.* Yeast Ssd1 is a non-enzymatic member of the RNase II family with an  
14 alternative RNA recognition interface. *bioRxiv* 2020.10.22.350314 (2020)  
15 doi:10.1101/2020.10.22.350314.
- 16 29. Ballou, E. R., Cook, A. G. & Wallace, E. W. J. Repeated evolution of inactive  
17 pseudonucleases in a fungal branch of the Dis3/RNase II family of nucleases. *bioRxiv*  
18 2020.07.30.229070 (2020) doi:10.1101/2020.07.30.229070.
- 19 30. Shepard, K. A. *et al.* Widespread cytoplasmic mRNA transport in yeast: Identification of  
20 22 bud-localized transcripts using DNA microarray analysis. *PNAS* **100**, 11429–11434 (2003).
- 21 31. Surana, U. *et al.* Destruction of the CDC28/CLB mitotic kinase is not required for the  
22 metaphase to anaphase transition in budding yeast. *The EMBO journal* **12**, 1969–78 (1993).
- 23 32. Fitch, I. *et al.* Characterization of four B-type cyclin genes of the budding yeast  
24 *Saccharomyces cerevisiae*. *Molecular biology of the cell* **3**, 805–18 (1992).

- 1 33. Amon, A., Irniger, S. & Nasmyth, K. Closing the cell cycle circle in yeast: G2 cyclin  
2 proteolysis initiated at mitosis persists until the activation of G1 cyclins in the next cycle. *Cell*  
3 **77**, 1037–50 (1994).
- 4 34. Örd, M. & Loog, M. How the cell cycle clock ticks. *MBoC* **30**, 169–172 (2019).
- 5 35. Surana, U. *et al.* The role of CDC28 and cyclins during mitosis in the budding yeast *S.*  
6 *cerevisiae*. *Cell* **65**, 145–161 (1991).
- 7 36. Amon, A., Tyers, M., Futcher, B. & Nasmyth, K. Mechanisms that help the yeast cell cycle  
8 clock tick: G2 cyclins transcriptionally activate G2 cyclins and repress G1 cyclins. *Cell* **74**, 993–  
9 1007 (1993).
- 10 37. Bloom, J. & Cross, F. R. Multiple levels of cyclin specificity in cell-cycle control. *Nature*  
11 *reviews. Molecular cell biology* **8**, 149–60 (2007).
- 12 38. Hood, J. K., Hwang, W. W. & Silver, P. A. The *Saccharomyces cerevisiae* cyclin Clb2p is  
13 targeted to multiple subcellular locations by cis- and trans-acting determinants. *Journal of Cell*  
14 *Science* **114**, 589–597 (2001).
- 15 39. Bailly, E., Cabantous, S., Sondaz, D., Bernadac, A. & Simon, M.-N. Differential cellular  
16 localization among mitotic cyclins from *Saccharomyces cerevisiae*: a new role for the axial  
17 budding protein Bud3 in targeting Clb2 to the mother-bud neck. *Journal of Cell Science* **116**,  
18 4119–4130 (2003).
- 19 40. Hahn, S., Maurer, P., Caesar, S. & Schlenstedt, G. Classical NLS Proteins from  
20 *Saccharomyces cerevisiae*. *Journal of Molecular Biology* **379**, 678–694 (2008).
- 21 41. Eluère, R. *et al.* Compartmentalization of the functions and regulation of the mitotic cyclin  
22 Clb2 in *S. cerevisiae*. *J Cell Sci* **120**, 702–711 (2007).
- 23 42. Coudreuse, D. & Nurse, P. Driving the cell cycle with a minimal CDK control network.  
24 *Nature* **468**, 1074–1079 (2010).
- 25 43. Stern, B. & Nurse, P. A quantitative model for the cdc2 control of S phase and mitosis in  
26 fission yeast. *Trends in Genetics* **12**, 345–350 (1996).

- 1 44. Novak, B., Tyson, J. J., Gyorffy, B. & Csikasz-Nagy, A. Irreversible cell-cycle transitions  
2 are due to systems-level feedback. *Nat Cell Biol* **9**, 724–728 (2007).
- 3 45. Harvey, S. L. *et al.* A phosphatase threshold sets the level of Cdk1 activity in early mitosis  
4 in budding yeast. *MBoC* **22**, 3595–3608 (2011).
- 5 46. Kõivomägi, M. *et al.* Dynamics of Cdk1 Substrate Specificity during the Cell Cycle. *Mol*  
6 *Cell* **42**, 610–623 (2011).
- 7 47. Darieva, Z. *et al.* Cell cycle-regulated transcription through the FHA domain of Fkh2p and  
8 the coactivator Ndd1p. *Curr Biol* **13**, 1740–1745 (2003).
- 9 48. Enserink, J. M. & Kolodner, R. D. An overview of Cdk1-controlled targets and processes.  
10 *Cell Div* **5**, 11 (2010).
- 11 49. Zhu, G. *et al.* Two yeast forkhead genes regulate the cell cycle and pseudohyphal growth.  
12 *Nature* **406**, 90–94 (2000).
- 13 50. Spellman, P. T. *et al.* Comprehensive Identification of Cell Cycle-regulated Genes of the  
14 Yeast *Saccharomyces cerevisiae* by Microarray Hybridization. *Mol Biol Cell* **9**, 3273–3297  
15 (1998).
- 16 51. Rahal, R. & Amon, A. Mitotic CDKs control the metaphase-anaphase transition and trigger  
17 spindle elongation. *Genes & development* **22**, 1534–48 (2008).
- 18 52. Machu, C. *et al.* Spatially distinct functions of Clb2 in the DNA damage response. *Cell*  
19 *Cycle* **13**, 383–398 (2014).
- 20 53. Veis, J., Klug, H., Koranda, M. & Ammerer, G. Activation of the G2/M-specific gene CLB2  
21 requires multiple cell cycle signals. *Molecular and cellular biology* **27**, 8364–73 (2007).
- 22 54. Maher, M., Cong, F., Kindelberger, D., Nasmyth, K. & Dalton, S. Cell cycle-regulated  
23 transcription of the CLB2 gene is dependent on Mcm1 and a ternary complex factor. *Mol Cell*  
24 *Biol* **15**, 3129–3137 (1995).

- 1 55. Trcek, T., Larson, D. R., Moldon, A., Query, C. C. & Singer, R. H. Single-Molecule mRNA  
2 Decay Measurements Reveal Promoter-Regulated mRNA Stability in Yeast. *Cell* **147**, 1484–  
3 1497 (2011).
- 4 56. Visintin, R. & Amon, A. Regulation of the mitotic exit protein kinases Cdc15 and Dbf2.  
5 *Molecular biology of the cell* **12**, 2961–74 (2001).
- 6 57. Hotz, M. & Barral, Y. The Mitotic Exit Network: new turns on old pathways. *Trends in Cell*  
7 *Biology* **24**, 145–152 (2014).
- 8 58. Femino, A. M., Fay, F. S., Fogarty, K. & Singer, R. H. Visualization of single RNA  
9 transcripts in situ. *Science* **280**, 585–90 (1998).
- 10 59. Raj, A., van den Bogaard, P., Rifkin, S. A., van Oudenaarden, A. & Tyagi, S. Imaging  
11 individual mRNA molecules using multiple singly labeled probes. *Nat Methods* **5**, 877–9 (2008).
- 12 60. Eliscovich, C., Shenoy, S. M. & Singer, R. H. Imaging mRNA and protein interactions  
13 within neurons. *Proceedings of the National Academy of Sciences of the United States of*  
14 *America* **114**, E1875–E1884 (2017).
- 15 61. Wu, B., Eliscovich, C., Yoon, Y. J. & Singer, R. H. Translation dynamics of single mRNAs  
16 in live cells and neurons. *Science* **352**, 1430–5 (2016).
- 17 62. Tutucci, E. & Singer, R. H. Simultaneous Detection of mRNA and Protein in *S. Cerevisiae*  
18 by Single Molecule FISH and Immunofluorescence. *Methods in Molecular Biology*.
- 19 63. Wu, B. *et al.* Synonymous modification results in high-fidelity gene expression of repetitive  
20 protein and nucleotide sequences. *Genes & development* **29**, 876–86 (2015).
- 21 64. Tutucci, E., Maekiniemi, A. & Singer, R. H. Single molecule mRNA Fluorescent In Situ  
22 Hybridization combined to Immunofluorescence in *S. cerevisiae*: Dataset and quantification.  
23 *Mendeley repository* doi:<http://dx.doi.org/10.17632/bcmn9cxyzs.2>.
- 24 65. Tutucci, E., Livingston, N. M., Singer, R. H. & Wu, B. Imaging mRNA in vivo, from Birth to  
25 Death. *Annu Rev Biophys* **47**, (2018).

- 1 66. Vera, M., Biswas, J., Senecal, A., Singer, R. H. & Park, H. Y. Single-Cell and Single-  
2 Molecule Analysis of Gene Expression Regulation. *Annu Rev Genet* **50**, 267–291 (2016).
- 3 67. Tutucci, E. *et al.* An improved MS2 system for accurate reporting of the mRNA life cycle.  
4 *Nat Methods* **15**, 81–89 (2018).
- 5 68. Tutucci, E., Vera, M. & Singer, R. H. Single-mRNA detection in living *S. cerevisiae* using  
6 a re-engineered MS2 system. *Nat Protoc* **13**, 2268–2296 (2018).
- 7 69. Pichon X, Robert MC, Bertrand E, Singer RH, & Tutucci E. New generations of MS2  
8 variants and MCP fusions to detect single mRNAs in living eukaryotic cells. **Methods in**  
9 **Molecular Biology**, (In Press).
- 10 70. Maekiniemi, A., Singer, R. H. & Tutucci, E. Single molecule mRNA fluorescent in situ  
11 hybridization combined with immunofluorescence in *S. cerevisiae*: Dataset and quantification.  
12 *Data in Brief* **30**, 105511 (2020).
- 13 71. Tutucci, E. & Singer, R. H. Simultaneous Detection of mRNA and Protein in *S. cerevisiae*  
14 by Single-Molecule FISH and Immunofluorescence. *Methods Mol. Biol.* **2166**, 51–69 (2020).
- 15 72. Taberner, F. J., Quilis, I. & Igual, J. C. Spatial regulation of the Start repressor Whi5. *Cell*  
16 *Cycle* **8**, 3013–3022 (2009).
- 17 73. Kilmartin, J. V. & Adams, A. E. Structural rearrangements of tubulin and actin during the  
18 cell cycle of the yeast *Saccharomyces*. *J Cell Biol* **98**, 922–933 (1984).
- 19 74. Zenklusen, D., Larson, D. R. & Singer, R. H. Single-RNA counting reveals alternative  
20 modes of gene expression in yeast. *Nat Struct Mol Biol* **15**, 1263–71 (2008).
- 21 75. Sanchez, A. & Golding, I. Genetic determinants and cellular constraints in noisy gene  
22 expression. *Science* **342**, 1188–93 (2013).
- 23 76. Pichon, X., Robert, M.-C., Bertrand, E., Singer, R. H. & Tutucci, E. New Generations of  
24 MS2 Variants and MCP Fusions to Detect Single mRNAs in Living Eukaryotic Cells. *Methods*  
25 *Mol. Biol.* **2166**, 121–144 (2020).

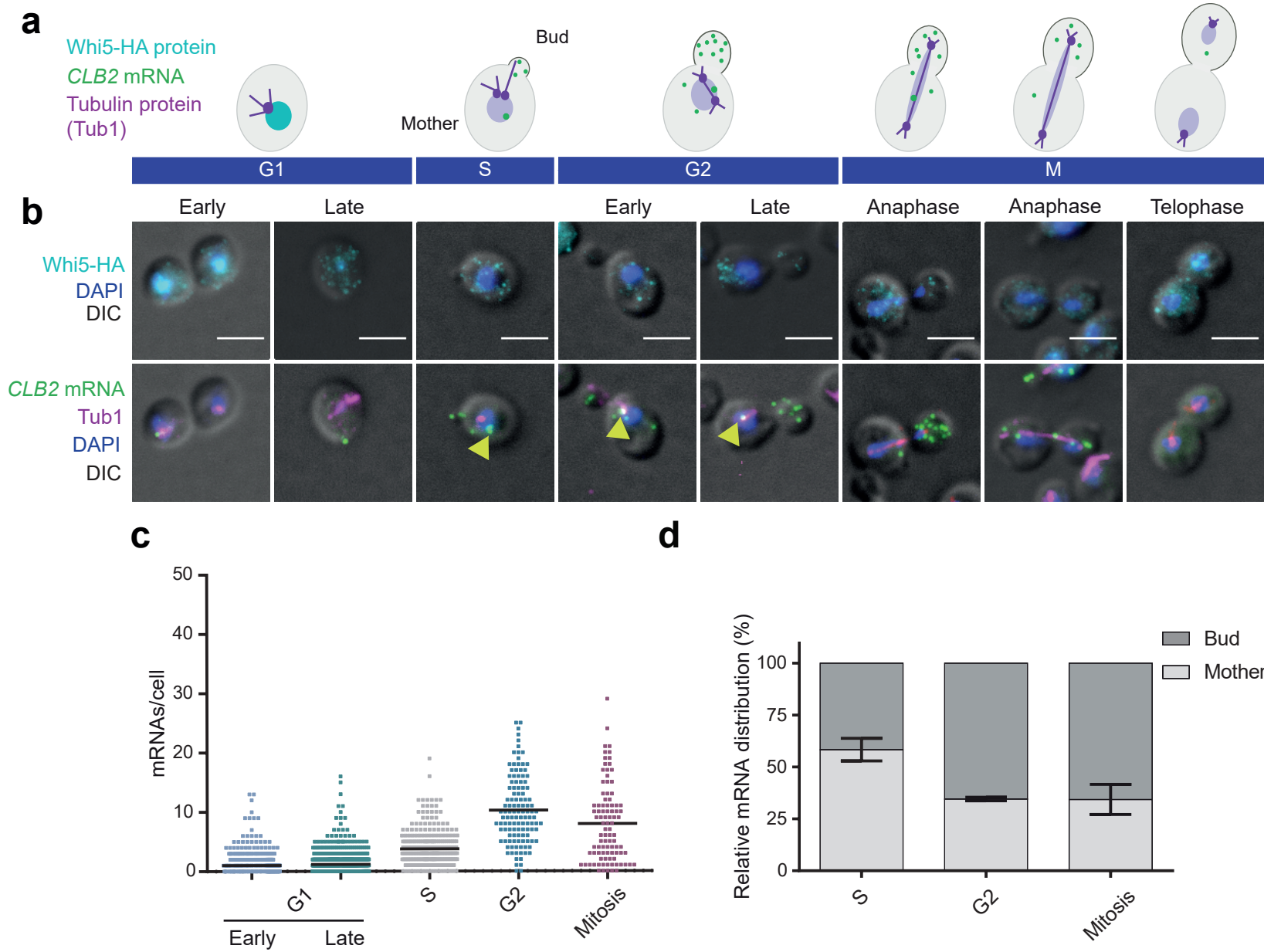
- 1 77. Haimovich, G. *et al.* Use of the MS2 aptamer and coat protein for RNA localization in  
2 yeast: A response to 'MS2 coat proteins bound to yeast mRNAs block 5' to 3' degradation and  
3 trap mRNA decay products: implications for the localization of mRNAs by MS2-MCP system'.  
4 *RNA* **22**, 660–6 (2016).
- 5 78. Garcia, J. F. & Parker, R. MS2 coat proteins bound to yeast mRNAs block 5' to 3'  
6 degradation and trap mRNA decay products: implications for the localization of mRNAs by  
7 MS2-MCP system. *RNA* **21**, 1393–5 (2015).
- 8 79. Schlichting, J. K. Modeling synchronization effects in the yeast cell cycle. (2019)  
9 doi:<http://dx.doi.org/10.18452/19835>.
- 10 80. Amoussouvi, A. *et al.* Transcriptional timing and noise of yeast cell cycle regulators—a  
11 single cell and single molecule approach. *npj Systems Biology and Applications* **4**, 1–10 (2018).
- 12 81. Johnston, G. C., Pringle, J. R. & Hartwell, L. H. Coordination of growth with cell division in  
13 the yeast *Saccharomyces cerevisiae*. *Experimental cell research* **105**, 79–98 (1977).
- 14 82. Leitao, R. M. & Kellogg, D. R. The duration of mitosis and daughter cell size are modulated  
15 by nutrients in budding yeast. *J Cell Biol* **216**, 3463–3470 (2017).
- 16 83. Park, H. Y. *et al.* Visualization of dynamics of single endogenous mRNA labeled in live  
17 mouse. *Science* **343**, 422–4 (2014).
- 18 84. Edelmann, F. T. *et al.* Molecular architecture and dynamics of ASH1 mRNA recognition  
19 by its mRNA-transport complex. *Nature Structural & Molecular Biology* **24**, 152–161 (2017).
- 20 85. Chartrand, P., Meng, X.-H., Singer, R. H. & Long, R. M. Structural elements required for  
21 the localization of ASH1 mRNA and of a green fluorescent protein reporter particle in vivo.  
22 *Current Biology* **9**, 333–338 (1999).
- 23 86. Gonzalez, I., Buonomo, S. B. C., Nasmyth, K. & Ahsen, U. von. ASH1 mRNA localization  
24 in yeast involves multiple secondary structural elements and Ash1 protein translation. *Current*  
25 *Biology* **9**, 337–340 (1999).

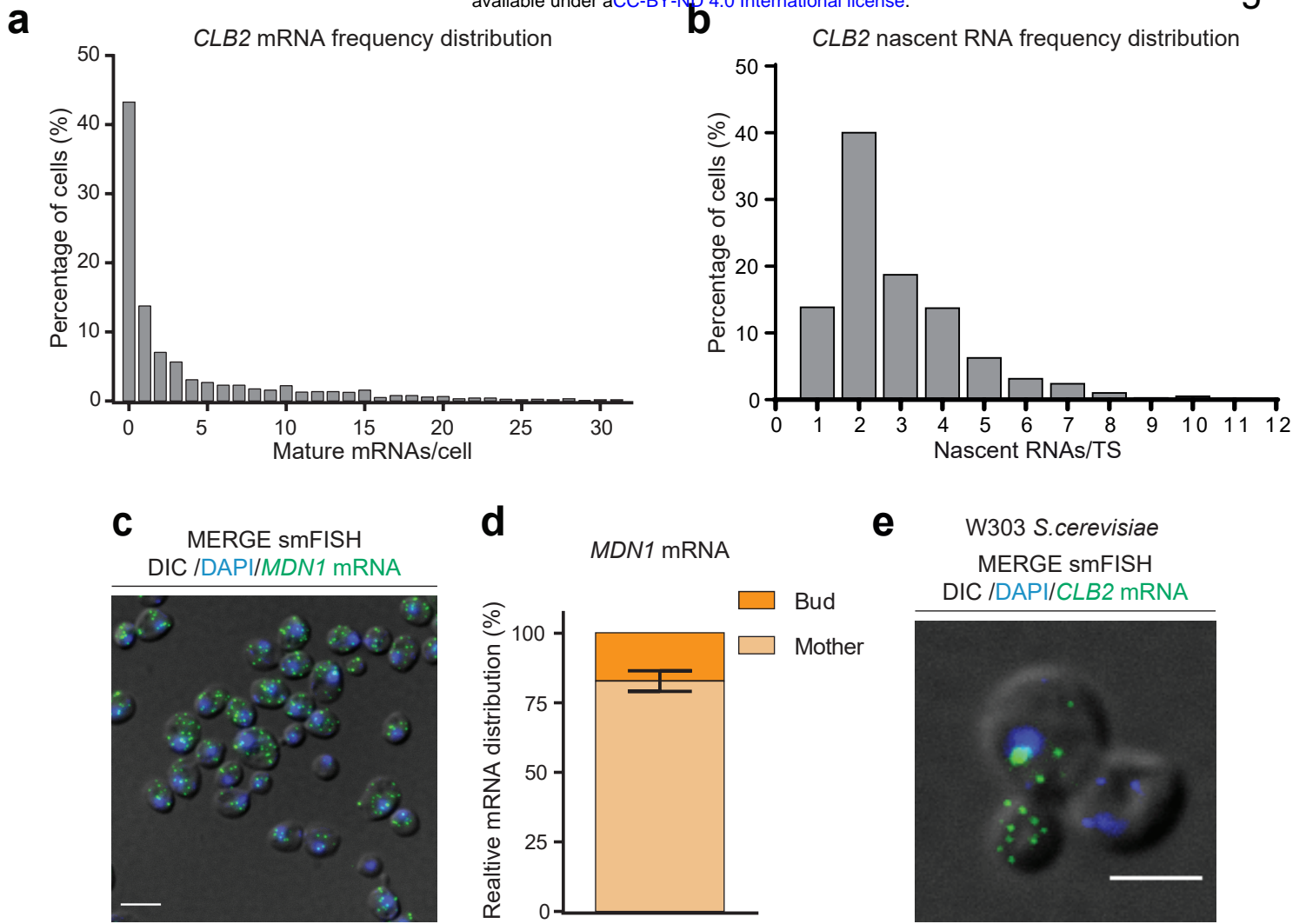
- 1 87. Stueland, M., Wang, T., Park, H. Y. & Mili, S. RDI Calculator: An Analysis Tool to Assess  
2 RNA Distributions in Cells. *Sci Rep* **9**, 1–10 (2019).
- 3 88. Moissoglu, K., Yasuda, K., Wang, T., Chrisafis, G. & Mili, S. Translational regulation of  
4 protrusion-localized RNAs involves silencing and clustering after transport. *eLife* **8**,
- 5 89. Cormack, B. P. *et al.* Yeast-enhanced green fluorescent protein (yEGFP): a reporter of  
6 gene expression in *Candida albicans*. *Microbiology (Reading)* **143 ( Pt 2)**, 303–311 (1997).
- 7 90. Arava, Y. *et al.* Genome-wide analysis of mRNA translation profiles in *Saccharomyces*  
8 *cerevisiae*. *Proc Natl Acad Sci U S A* **100**, 3889–3894 (2003).
- 9 91. Katz, Z. B. *et al.* Mapping translation ‘hot-spots’ in live cells by tracking single molecules  
10 of mRNA and ribosomes. *Elife* **5**, (2016).
- 11 92. Biswas, J., Liu, Y., Singer, R. H. & Wu, B. Fluorescence Imaging Methods to Investigate  
12 Translation in Single Cells. *Cold Spring Harb Perspect Biol* **11**, (2019).
- 13 93. Turner, J. J., Ewald, J. C. & Skotheim, J. M. Cell Size Control in Yeast. *Current Biology*  
14 **22**, R350–R359 (2012).
- 15 94. Groisman, I. *et al.* CPEB, maskin, and cyclin B1 mRNA at the mitotic apparatus:  
16 implications for local translational control of cell division. *Cell* **103**, 435–447 (2000).
- 17 95. Yasuda, K., Kotani, T. & Yamashita, M. A cis-acting element in the coding region of cyclin  
18 B1 mRNA couples subcellular localization to translational timing. *Developmental Biology* **382**,  
19 517–529 (2013).
- 20 96. Kotani, T., Yasuda, K., Ota, R. & Yamashita, M. Cyclin B1 mRNA translation is temporally  
21 controlled through formation and disassembly of RNA granules. *J Cell Biol* **202**, 1041–1055  
22 (2013).
- 23 97. Spiesser, T. W., Kühn, C., Krantz, M. & Klipp, E. Bud-Localization of CLB2 mRNA Can  
24 Constitute a Growth Rate Dependent Daughter Sizer. *PLOS Computational Biology* **11**,  
25 e1004223 (2015).

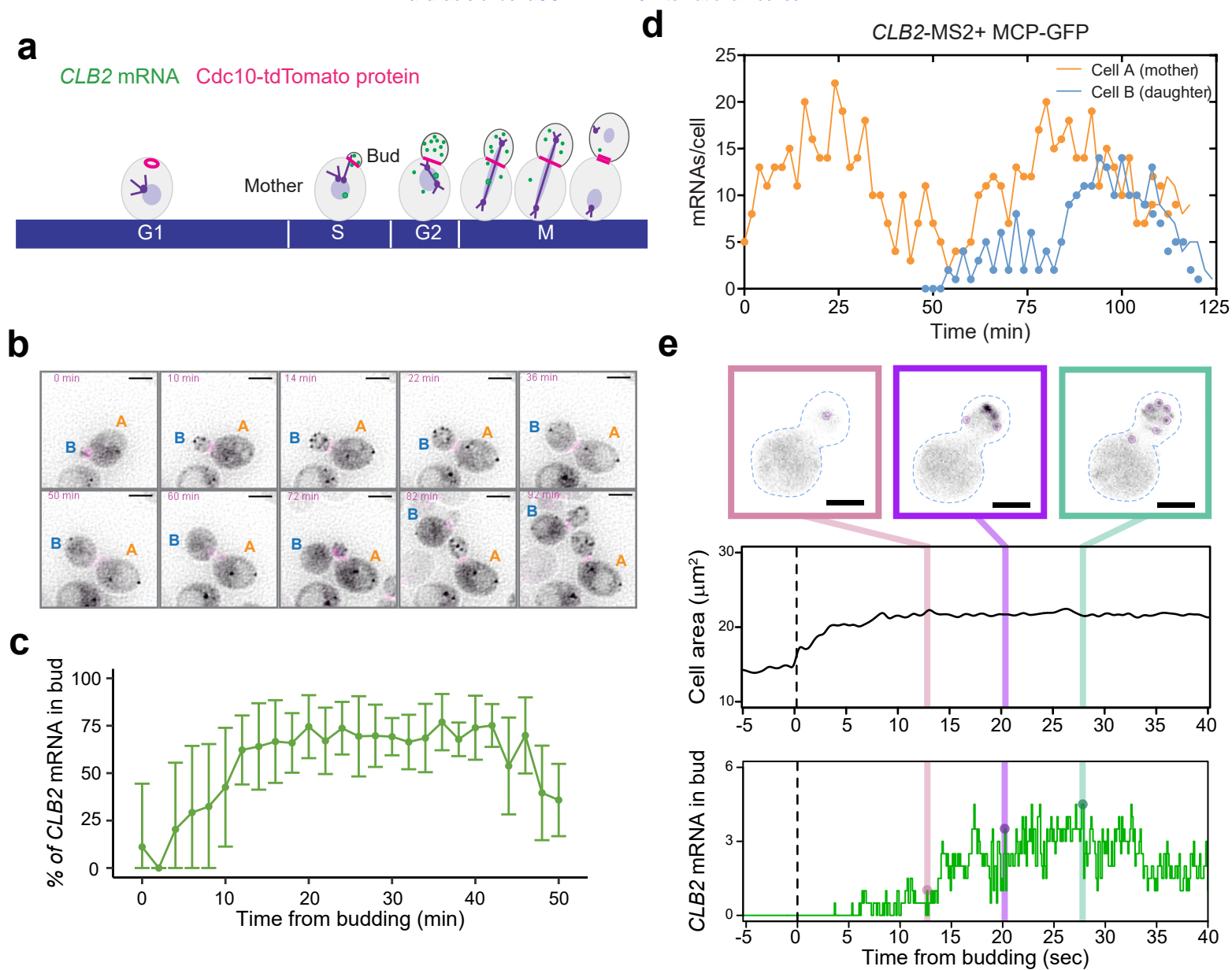
- 1 98. Mueller, F. *et al.* FISH-quant: automatic counting of transcripts in 3D FISH images. *Nat*  
2 *Methods* **10**, 277–8 (2013).
- 3 99. Kamenova, I. *et al.* Co-translational assembly of mammalian nuclear multisubunit  
4 complexes. *Nat Commun* **10**, 1740 (2019).
- 5 100. Olivier, C. *et al.* Identification of a conserved RNA motif essential for She2p recognition  
6 and mRNA localization to the yeast bud. *Mol Cell Biol* **25**, 4752–66 (2005).
- 7 101. Lorenz, R. *et al.* ViennaRNA Package 2.0. *Algorithms Mol Biol* **6**, 26 (2011).
- 8 102. Lorenz, R., Hofacker, I. L. & Stadler, P. F. RNA folding with hard and soft constraints.  
9 *Algorithms Mol Biol* **11**, 8 (2016).
- 10 103. Darty, K., Denise, A. & Ponty, Y. VARNA: Interactive drawing and editing of the RNA  
11 secondary structure. *Bioinformatics* **25**, 1974–1975 (2009).
- 12 104. Bivand, R. S., Pebesma, E. & Gómez-Rubio, V. *Applied Spatial Data Analysis with R*.  
13 (Springer-Verlag, 2013). doi:10.1007/978-1-4614-7618-4.
- 14 105. Sprouffske, K. & Wagner, A. Growthcurver: an R package for obtaining interpretable  
15 metrics from microbial growth curves. *BMC Bioinformatics* **17**, 172 (2016).
- 16 106. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*. (Springer International  
17 Publishing, 2016). doi:10.1007/978-3-319-24277-4.
- 18 107. Wickham, H. *et al.* Welcome to the Tidyverse. *Journal of Open Source Software* **4**, 1686  
19 (2019).
- 20 108. RColorBrewer: ColorBrewer Palettes version 1.1-2 from CRAN.  
21 <https://rdrr.io/cran/RColorBrewer/>.
- 22 109. dplyr: A Grammar of Data Manipulation — dplyr-package.  
23 <https://dplyr.tidyverse.org/reference/dplyr-package.html>.
- 24 110. Benaglia, T., Chauveau, D., Hunter, D. R. & Young, D. S. mixtools: An R Package for  
25 Analyzing Mixture Models. *Journal of Statistical Software* **32**, 1–29 (2010).

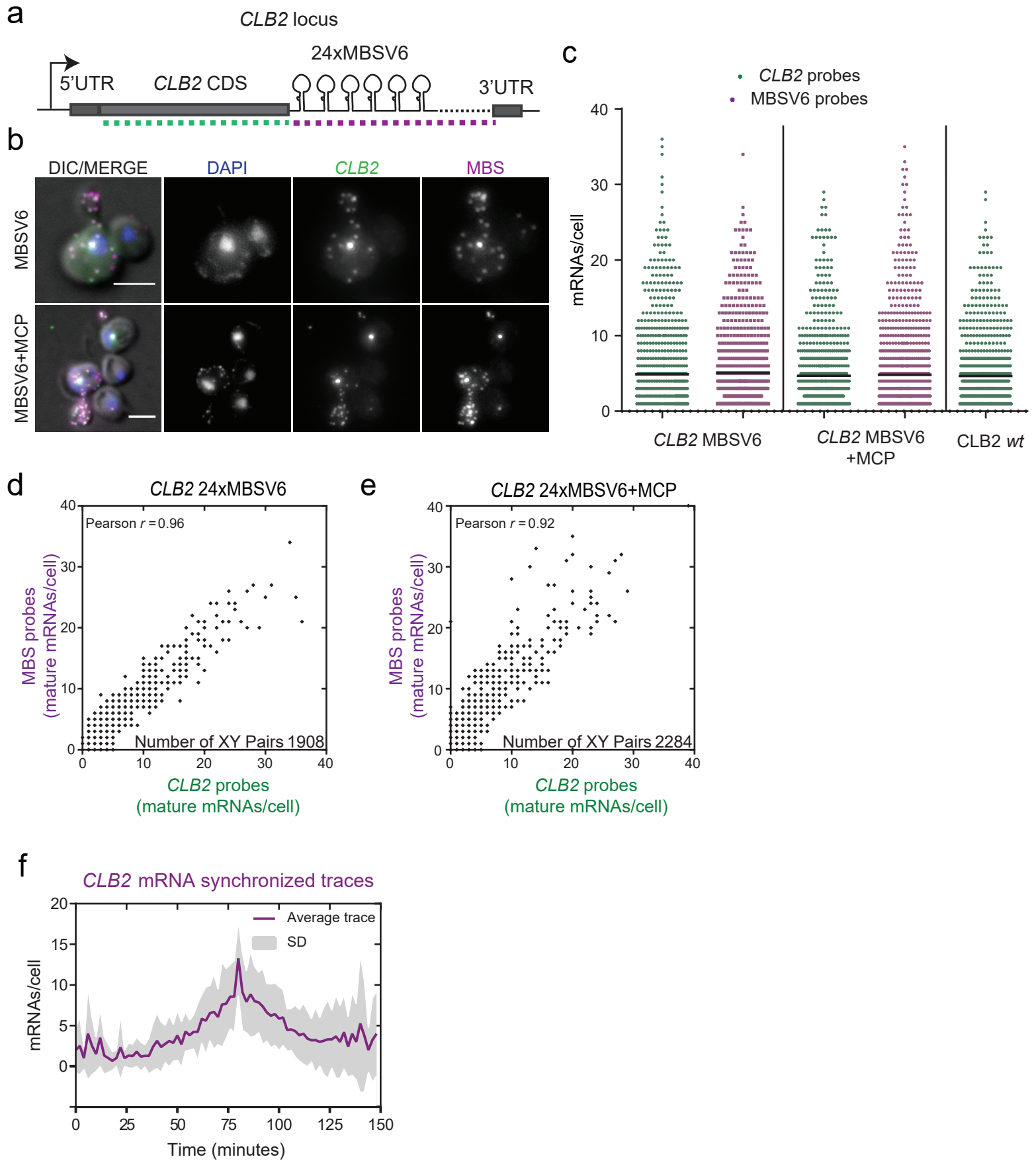


- 1 111. Costanzo, M. *et al.* CDK Activity Antagonizes Whi5, an Inhibitor of G1/S Transcription in  
2 Yeast. *Cell* **117**, 899–913 (2004).
- 3 112. Wagner, M. V. *et al.* Whi5 regulation by site specific CDK-phosphorylation in  
4 *Saccharomyces cerevisiae*. *PLoS ONE* **4**, e4300 (2009).
- 5

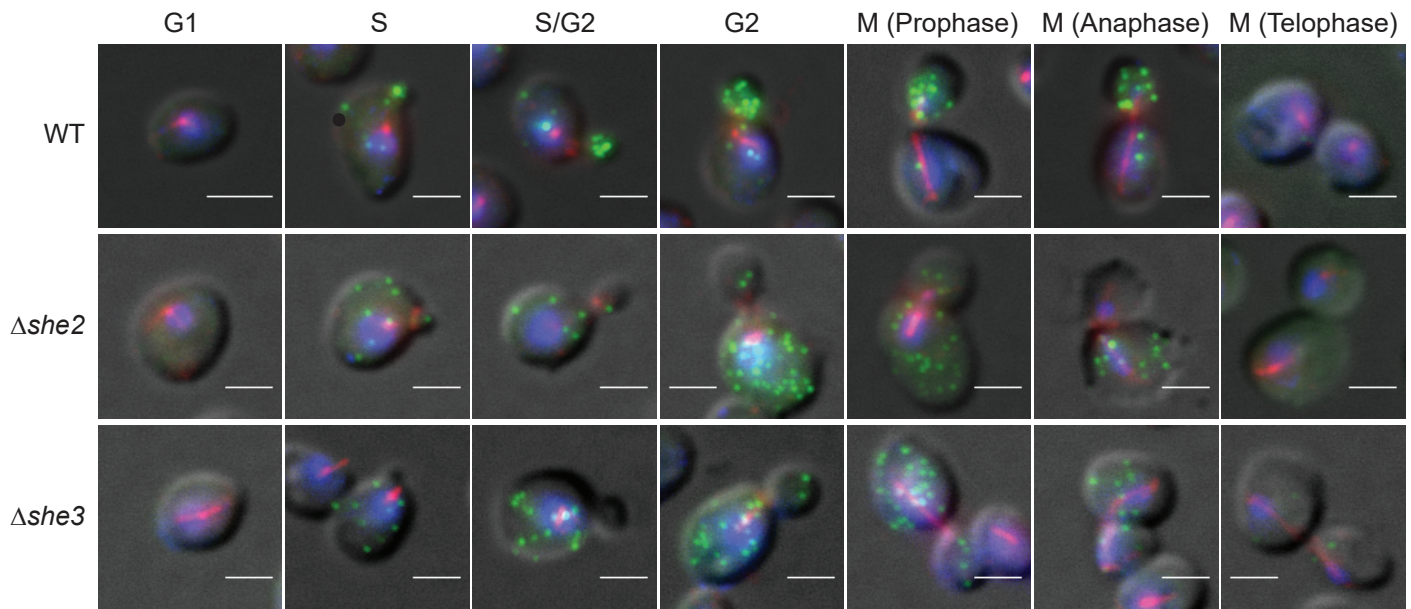




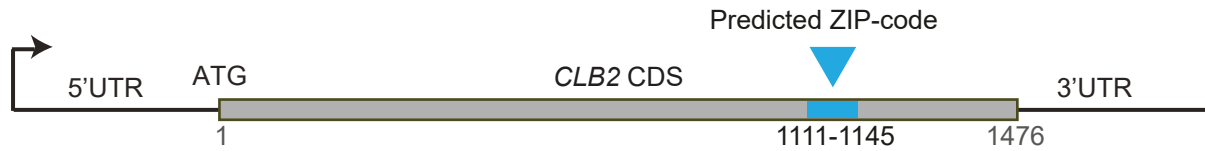




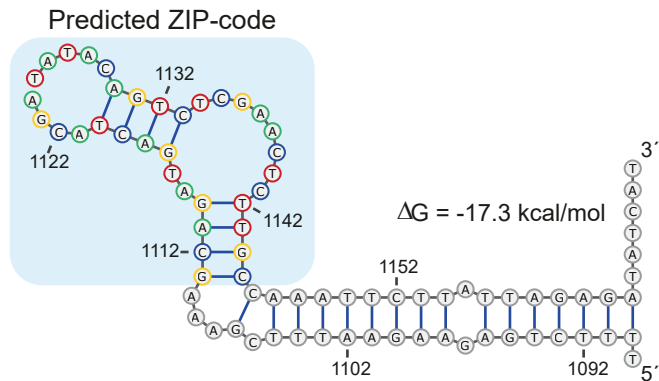
**a**



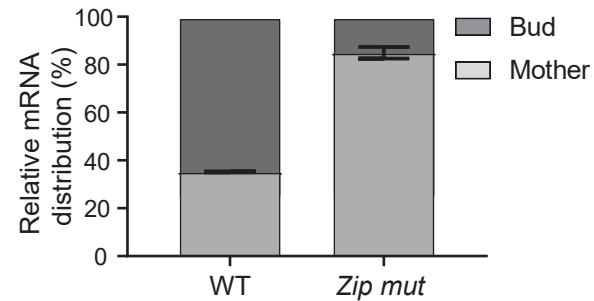
**b**



**c**



**f**

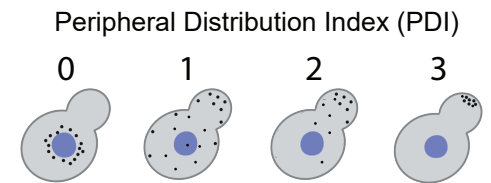


**d**

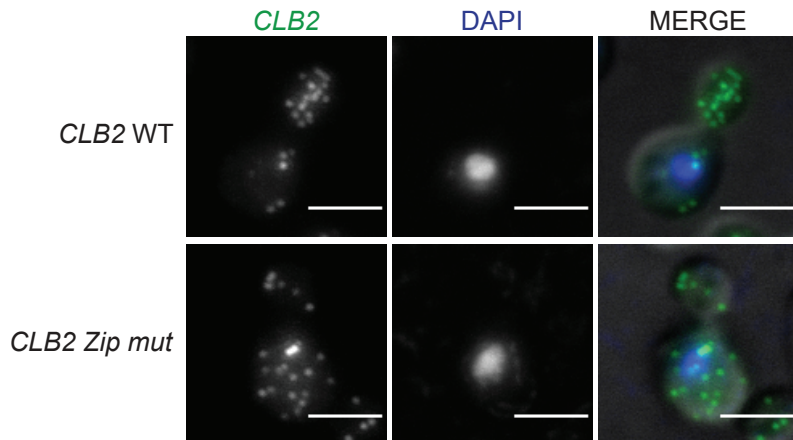
Synonymized ZIP-code mutant

Position	1111	1145
CLB2 WT	GCAGATGACTACGATATACAGTCTCGAACTCTTGC	
CLB2 Zip Mut	GCCGATGATTATGATATACAGAGCGAACCCTAGC	
Clb2 protein	A D D Y D I Q S R T L A	

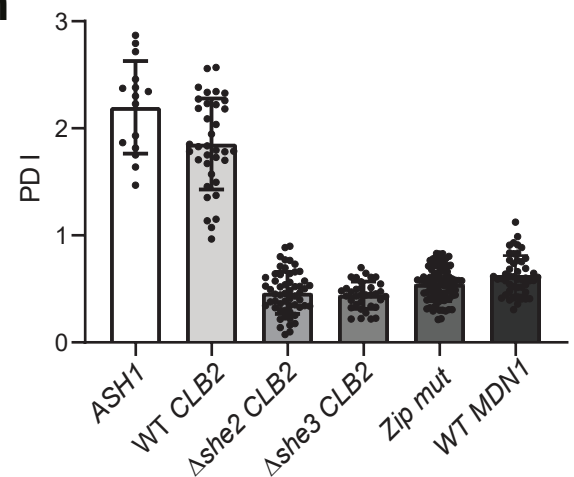
**g**



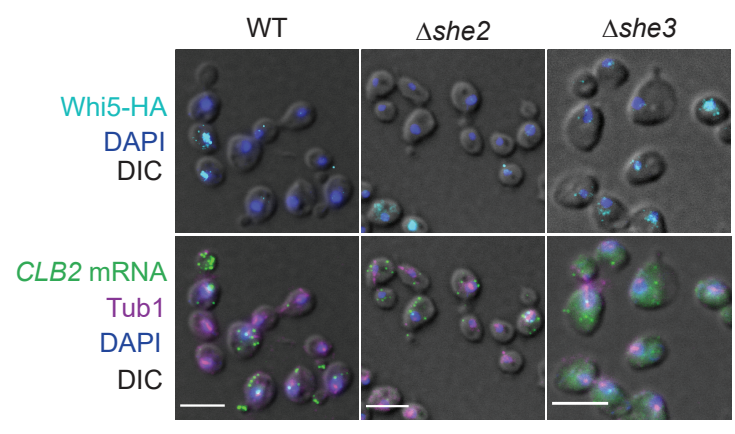
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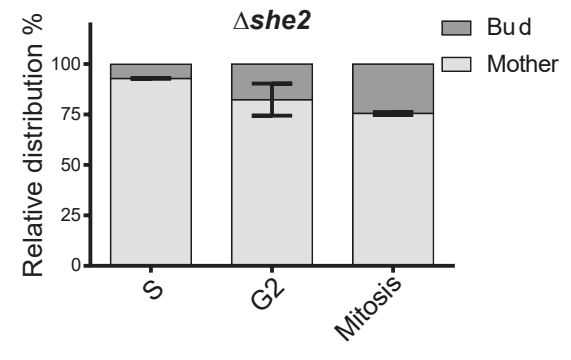
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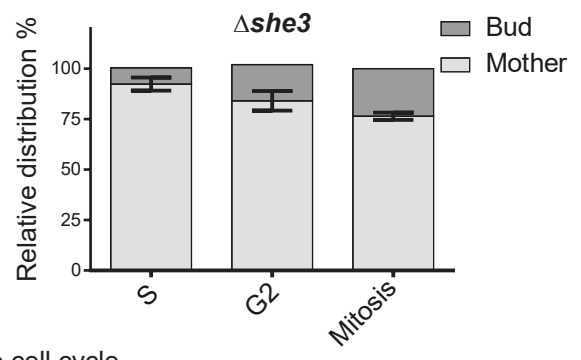
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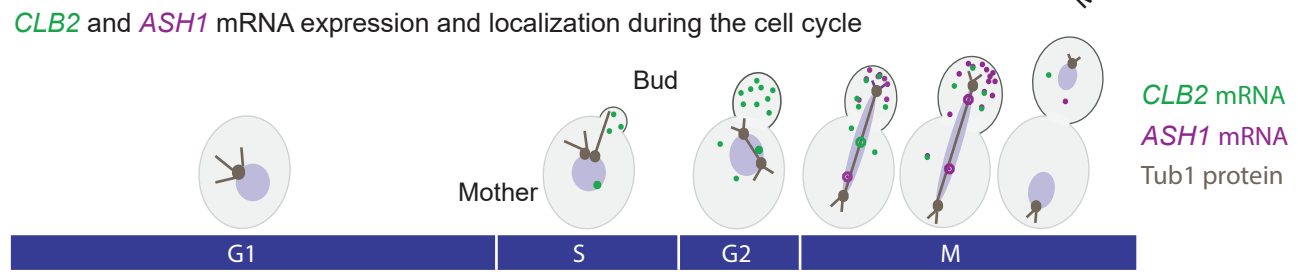
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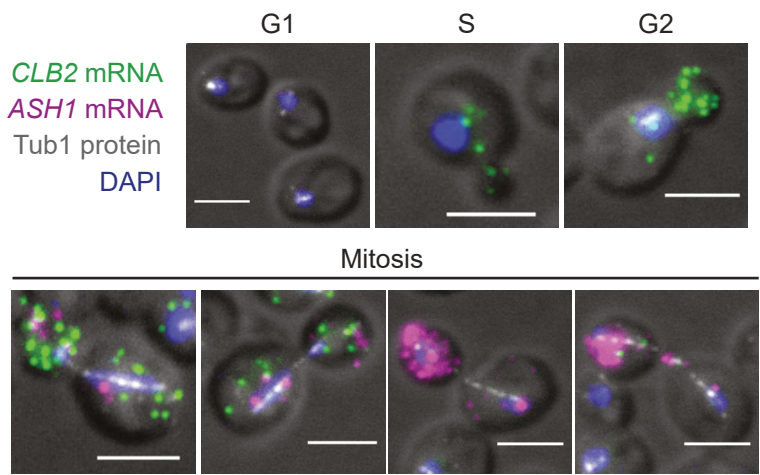
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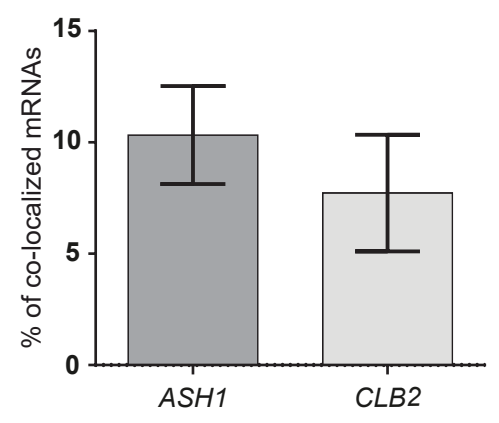
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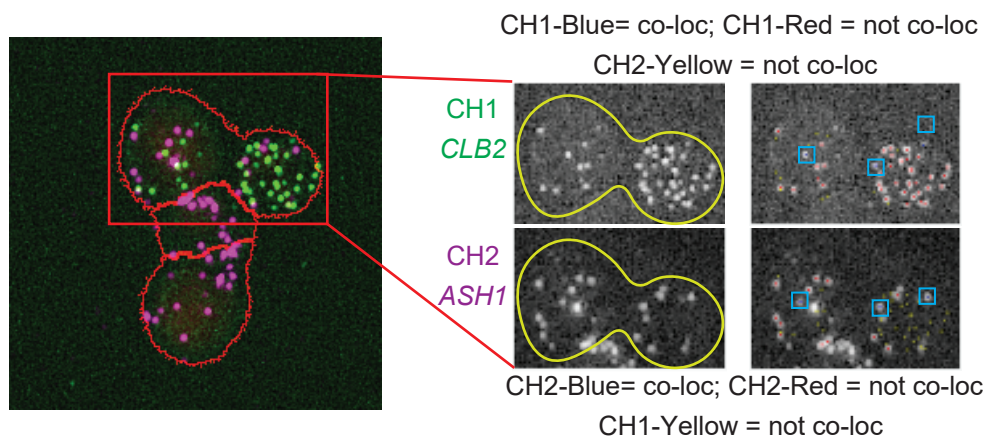
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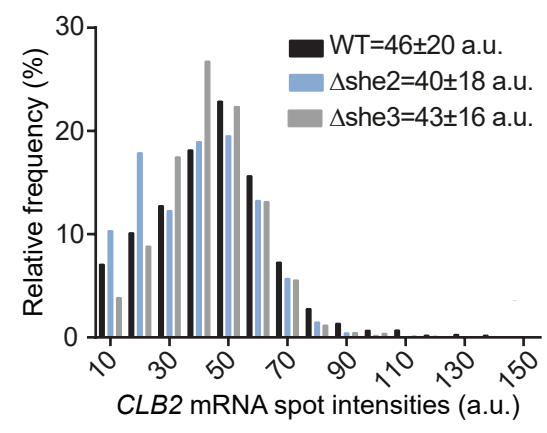
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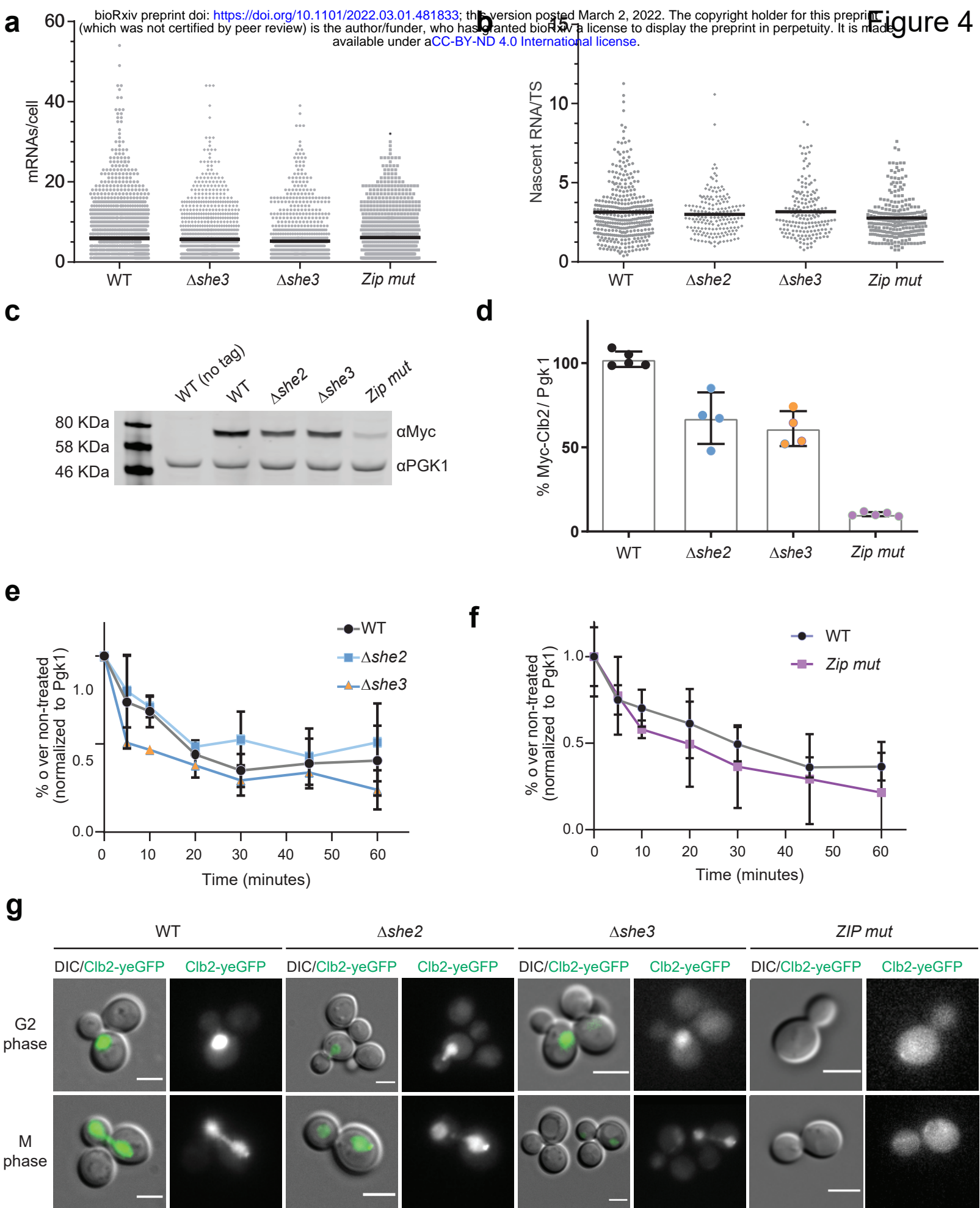


**g**

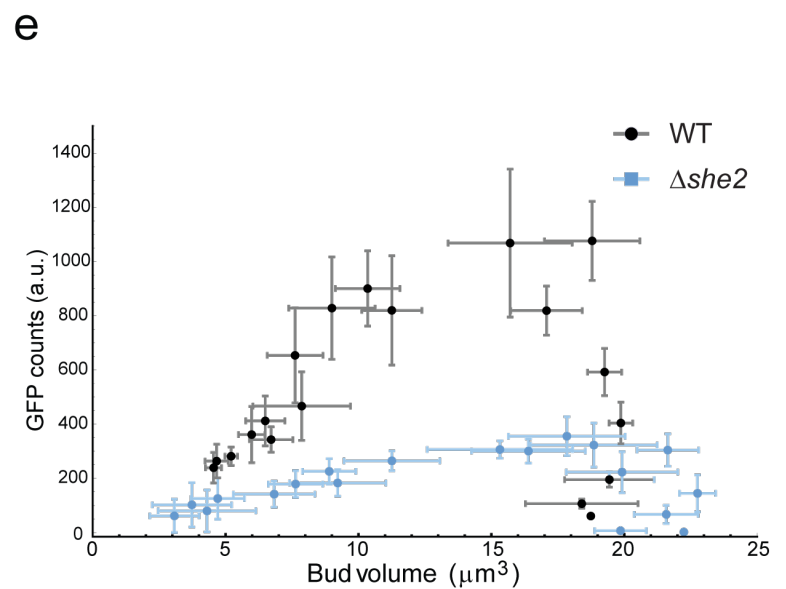
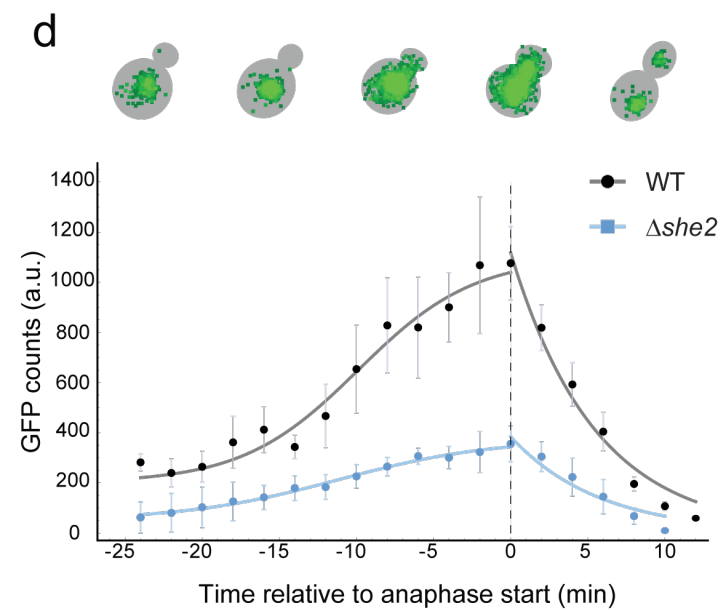
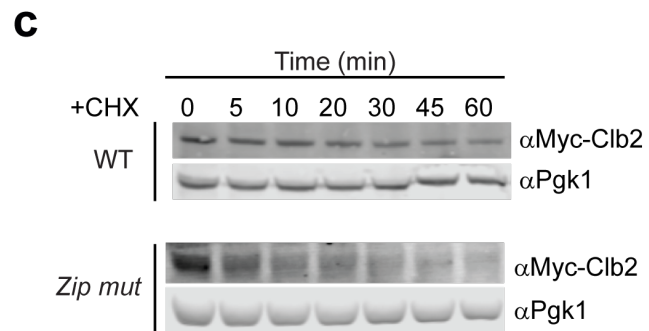
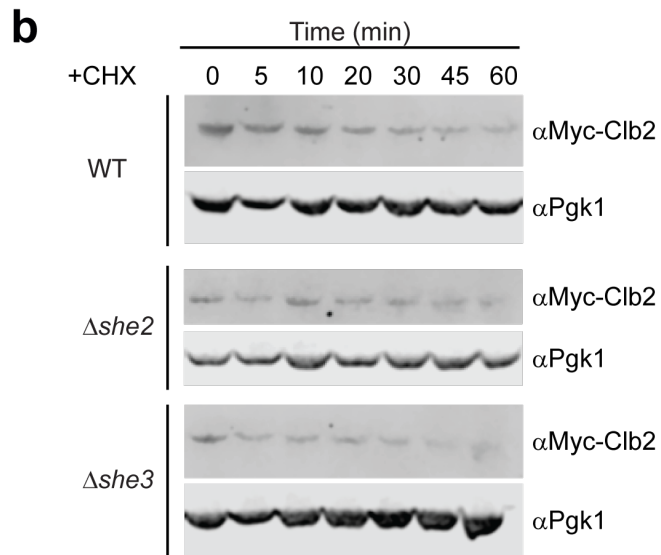
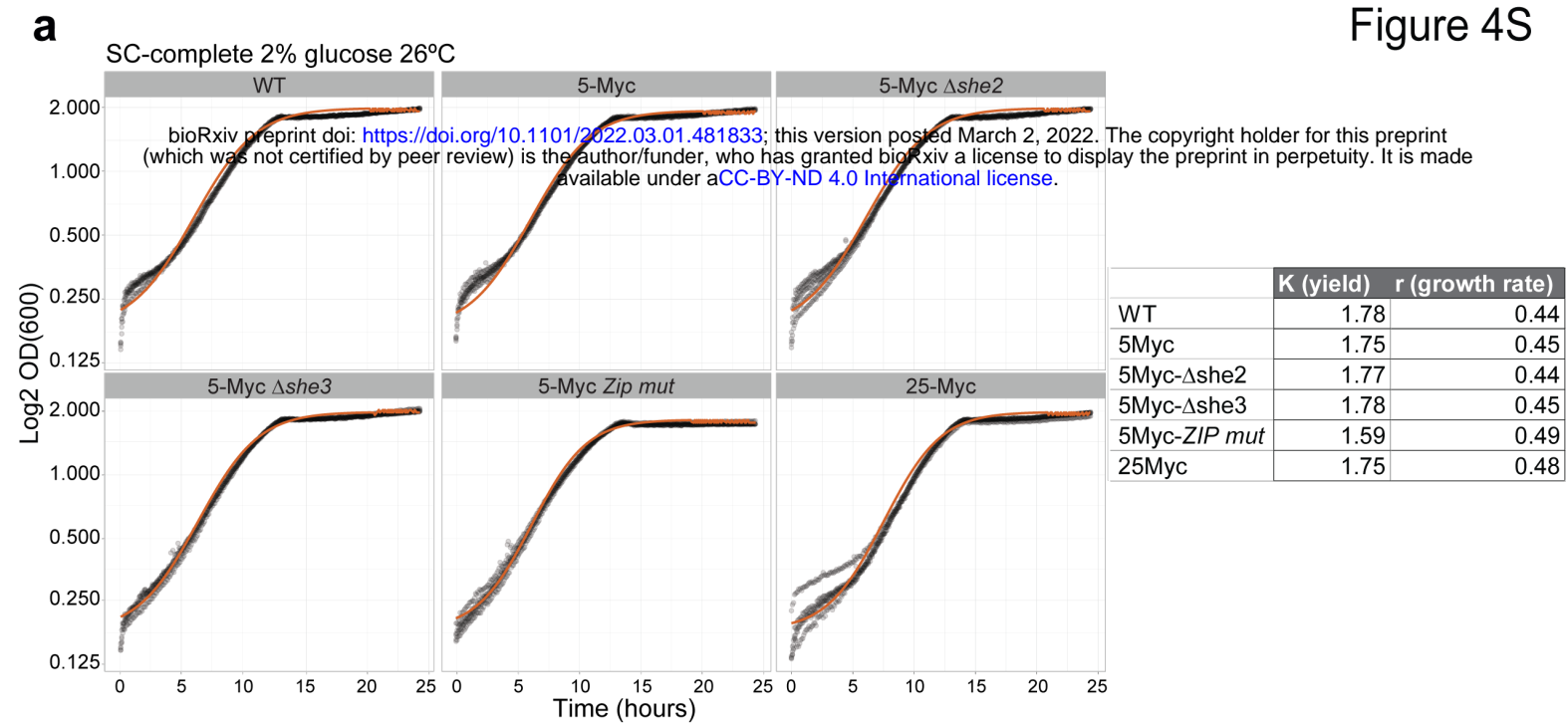


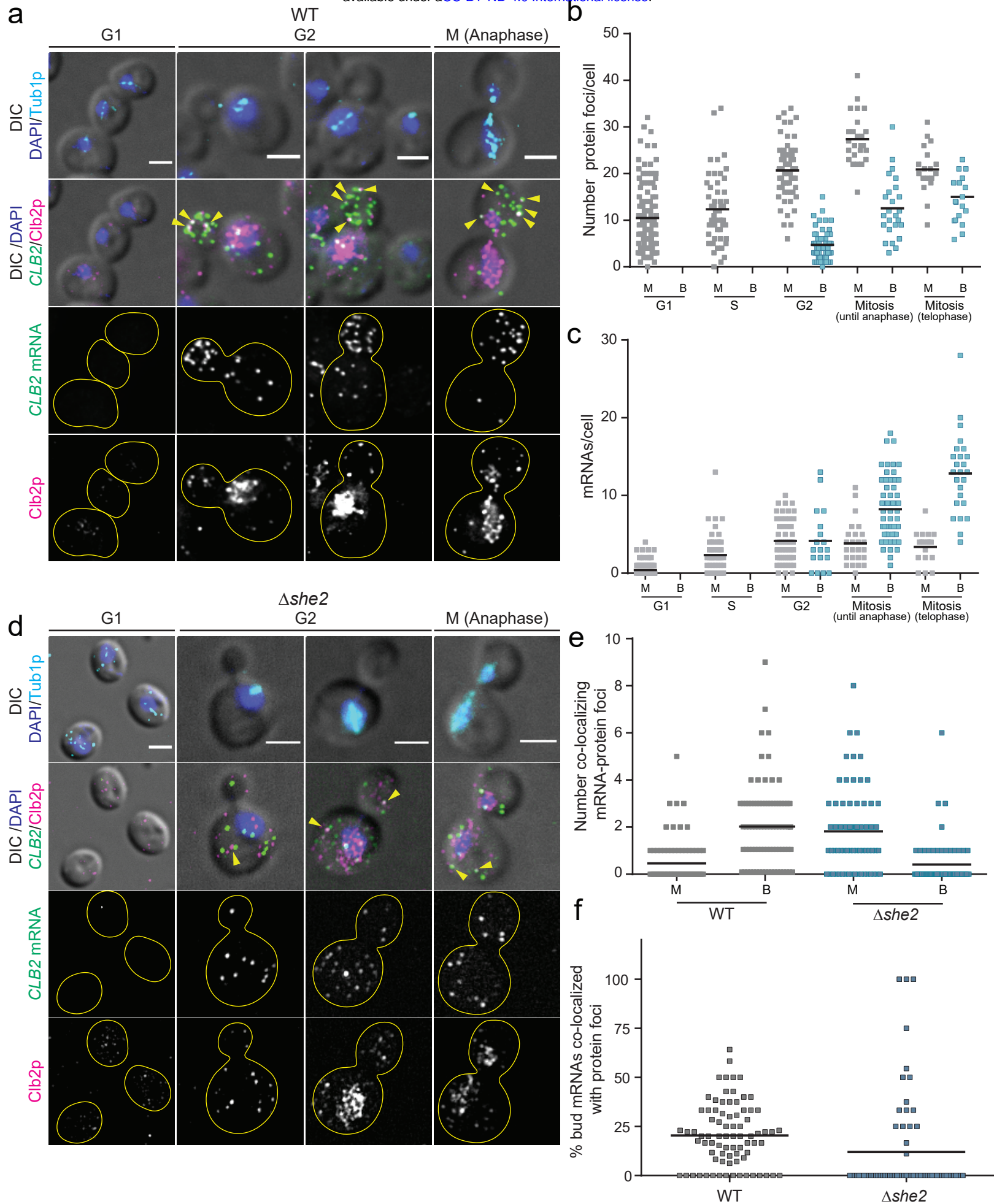
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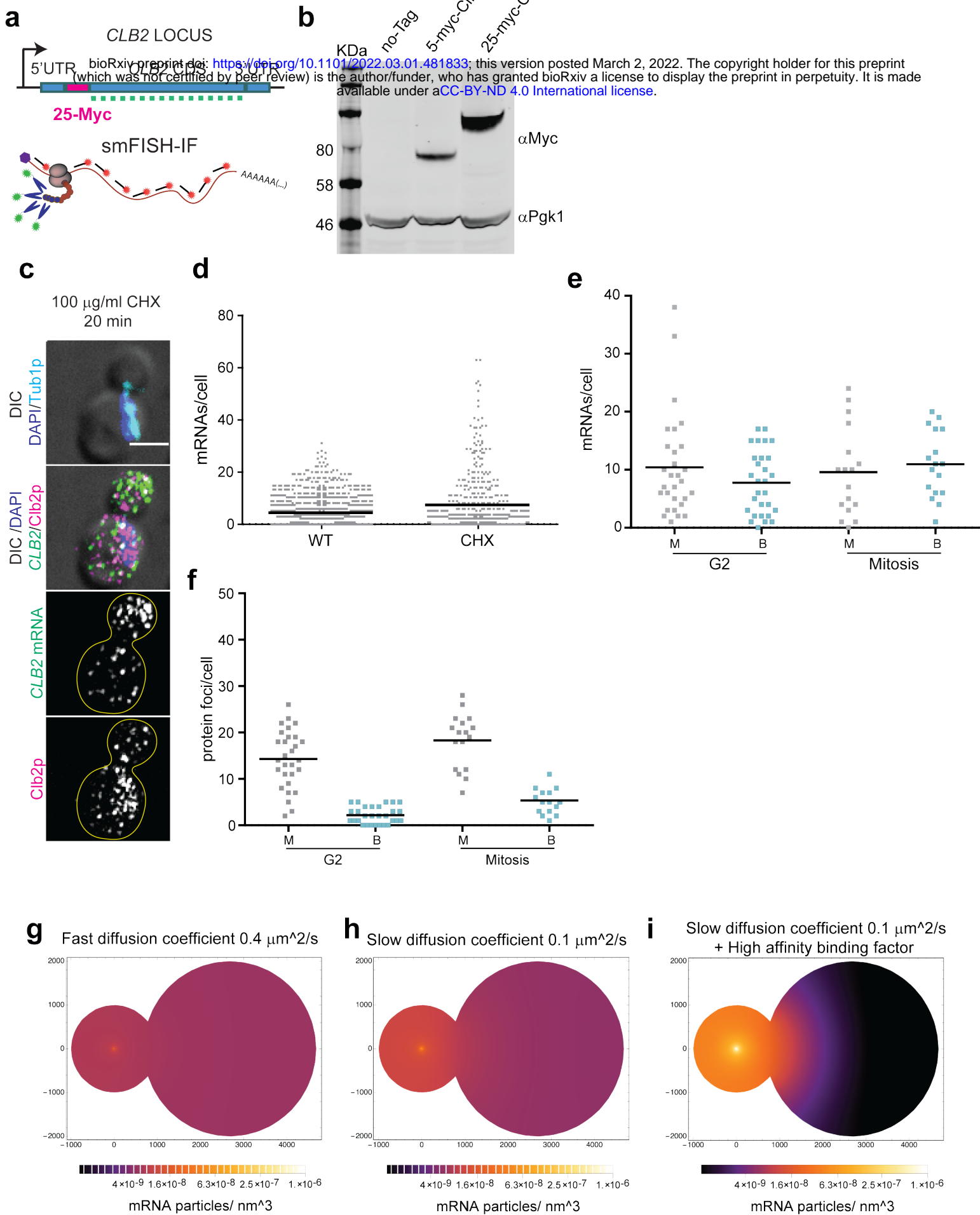


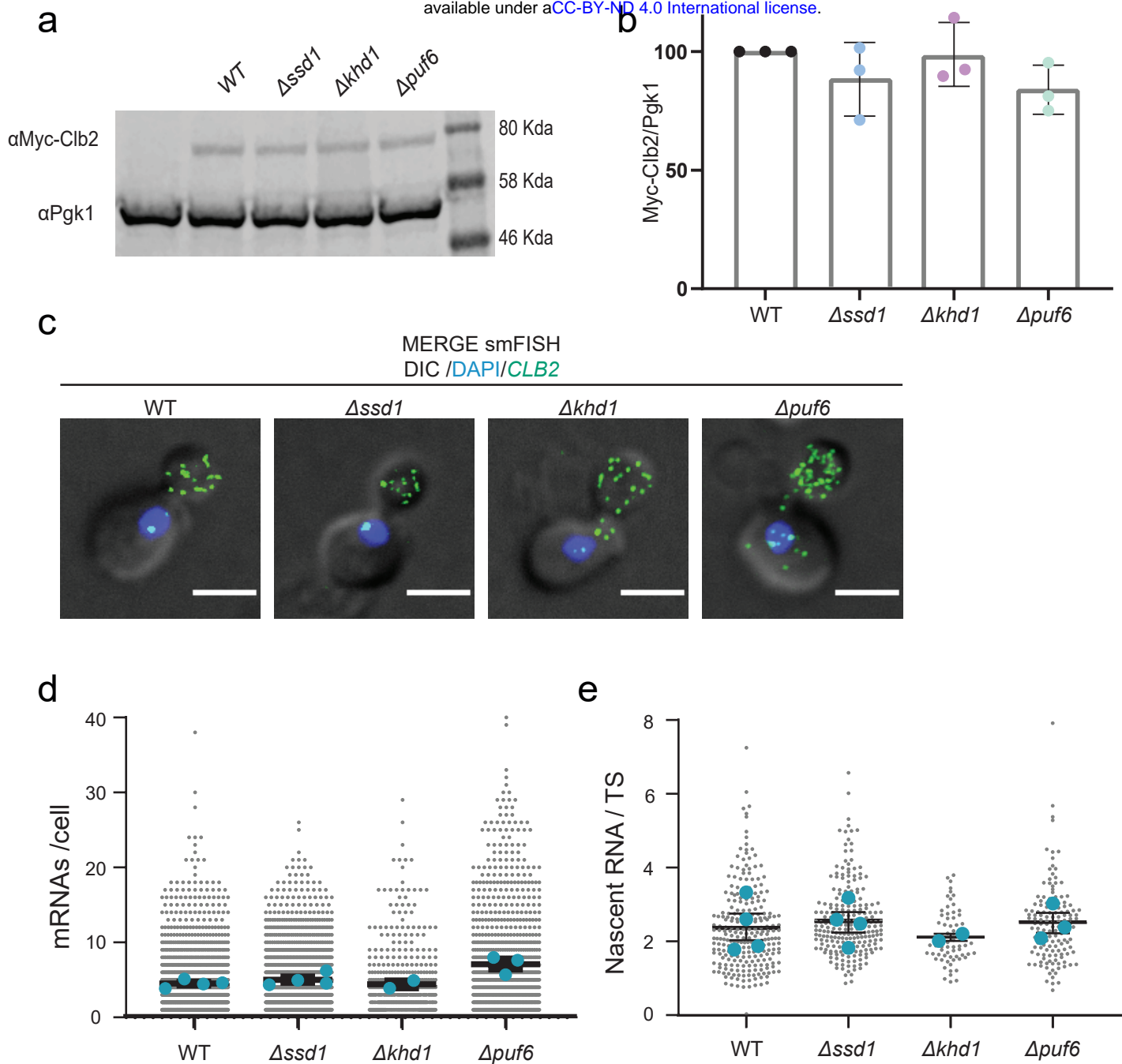




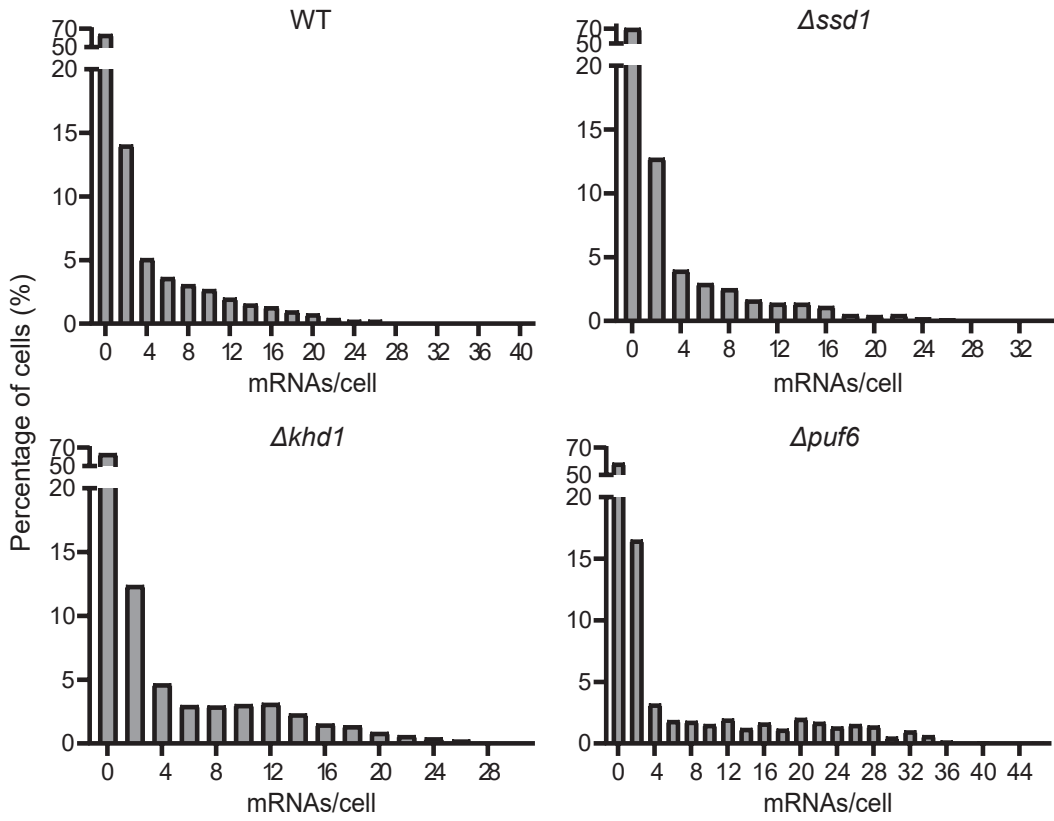




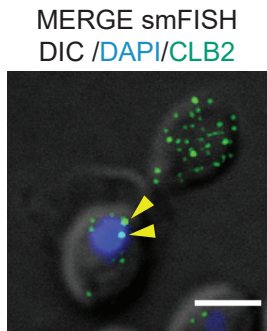




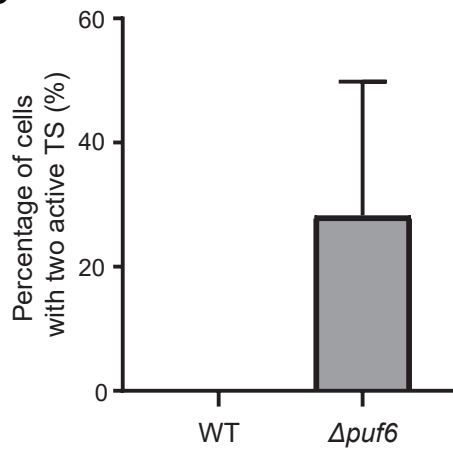
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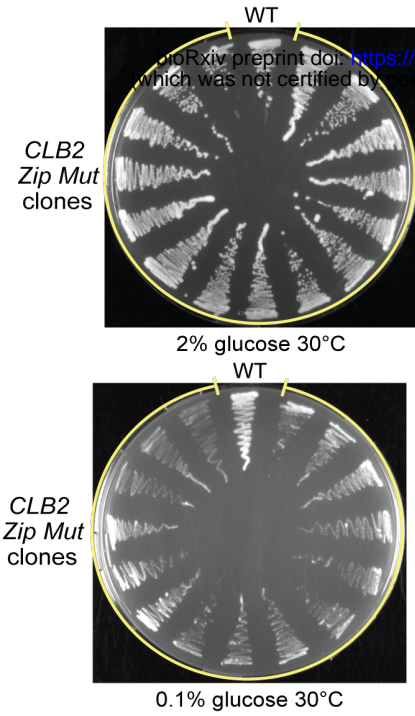
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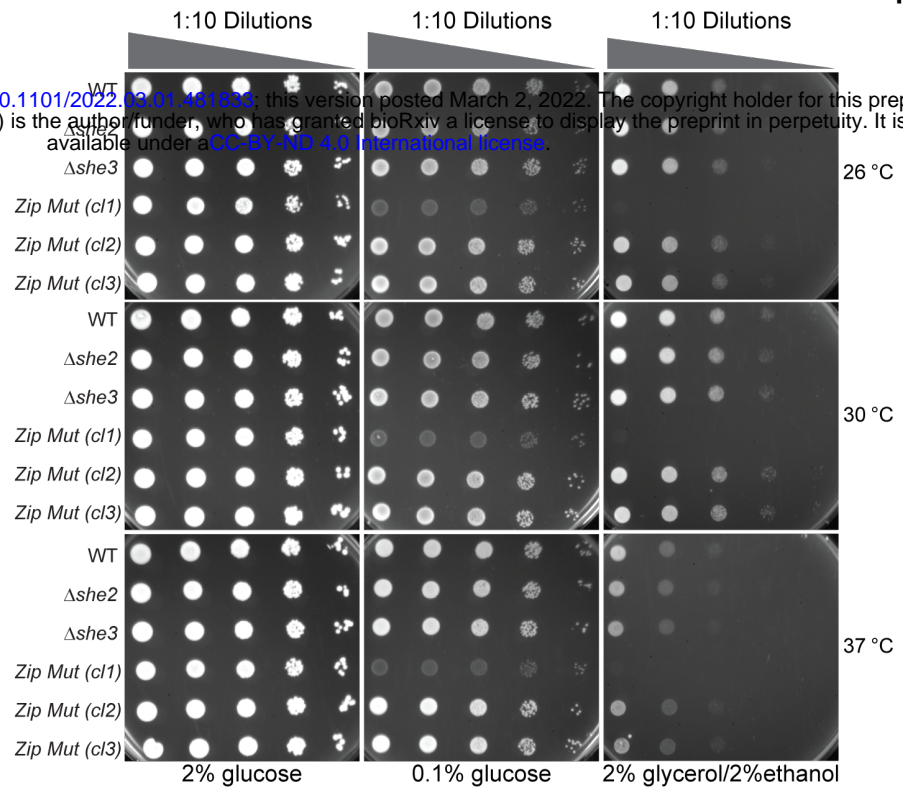
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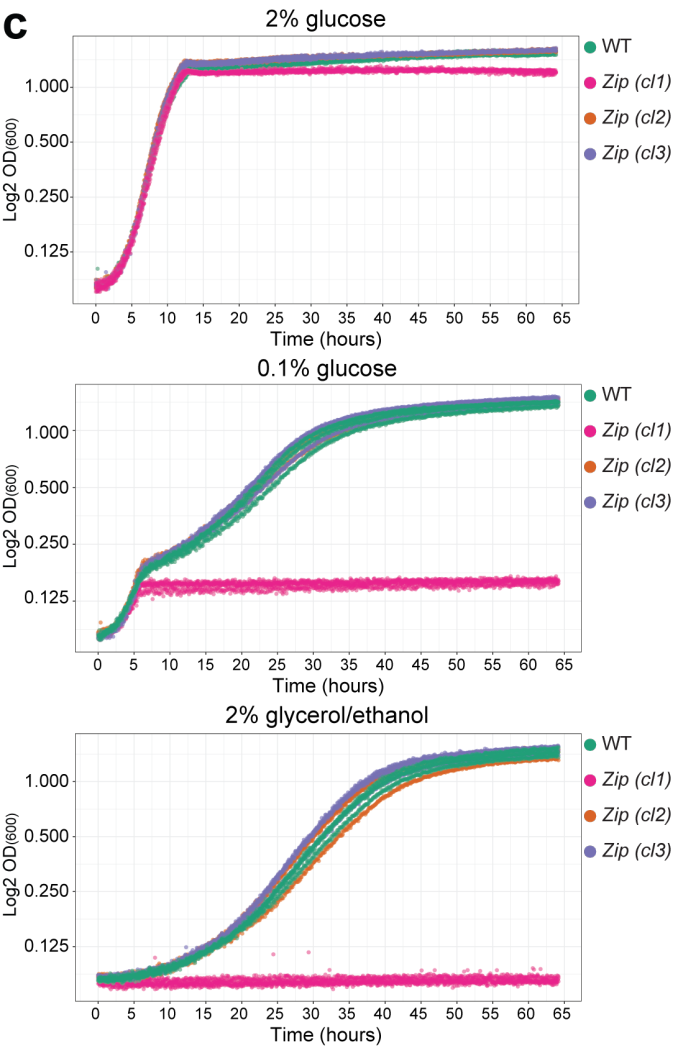
**a**



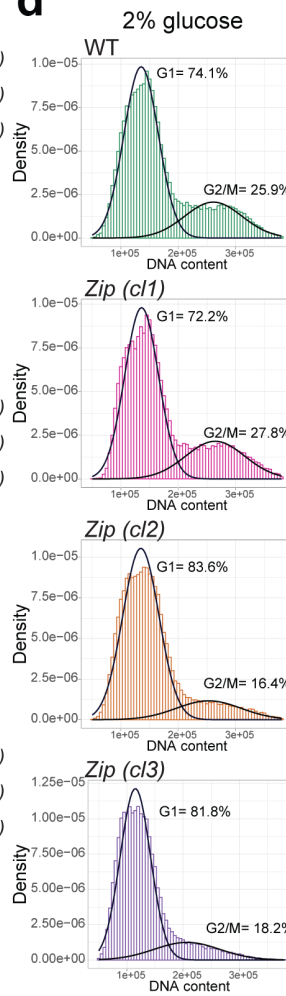
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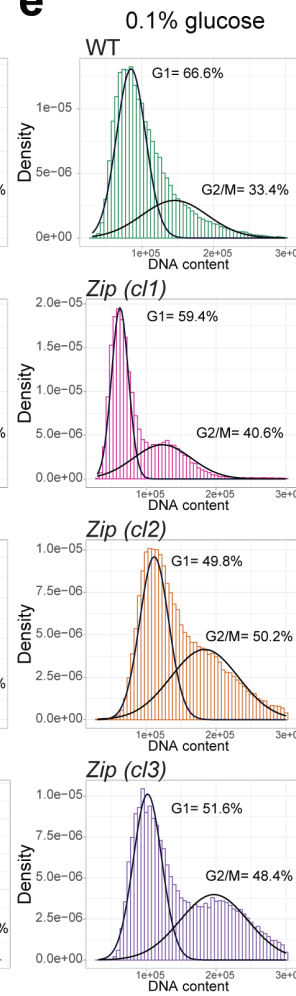
**c**



**d**



**e**



**f**

