Bimodal expression of *PHO84* is modulated by early termination of antisense transcription

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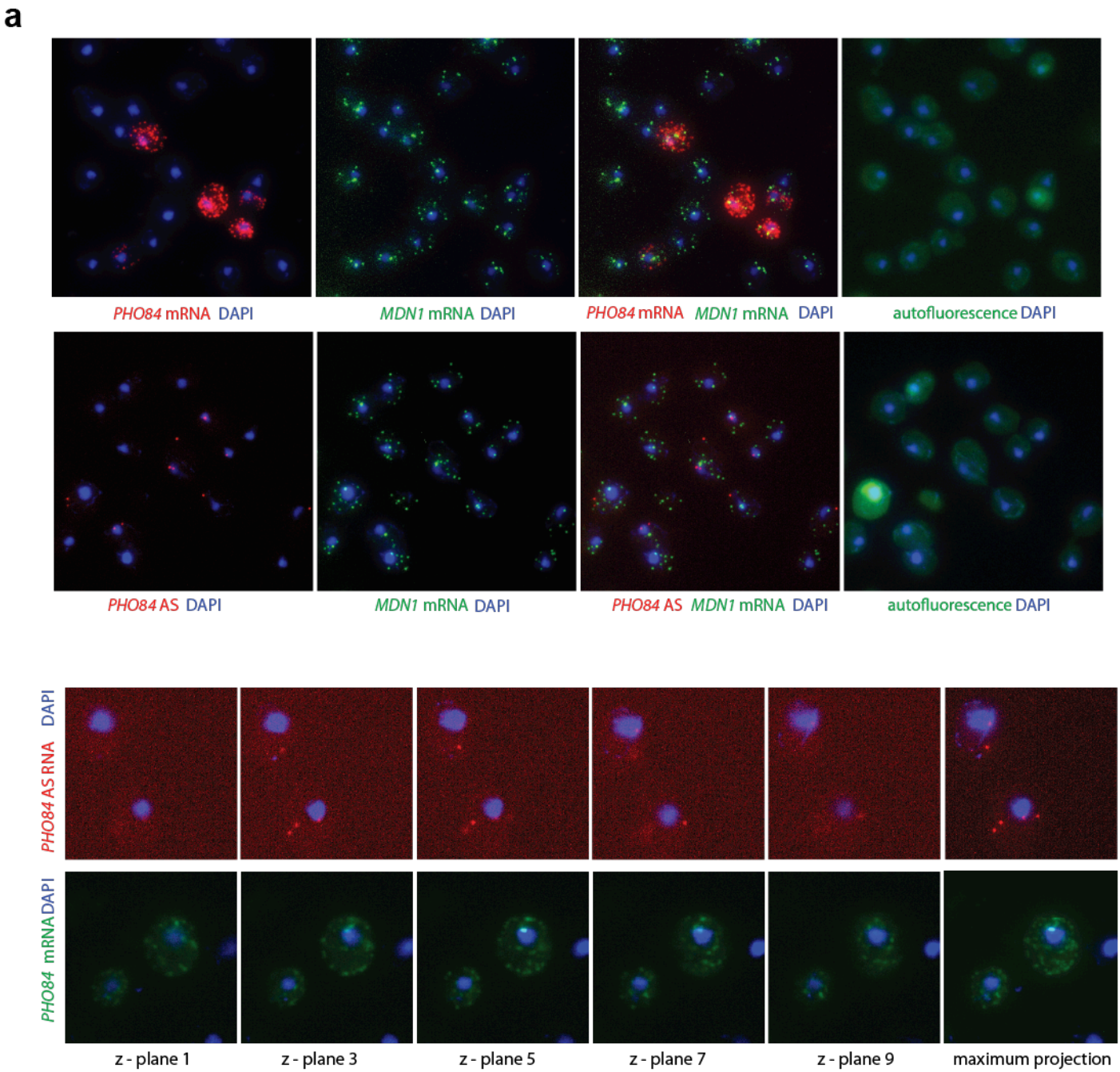
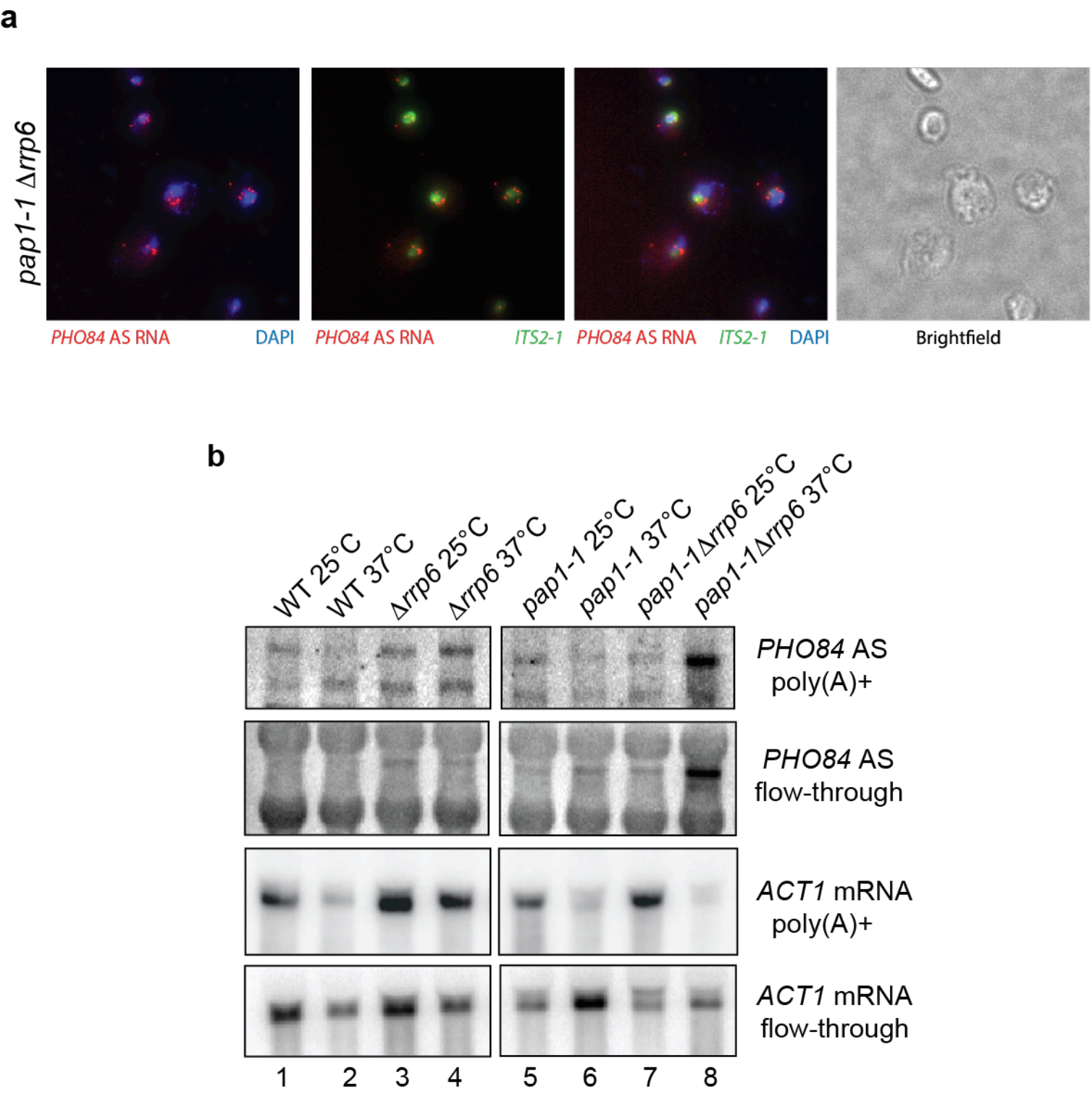
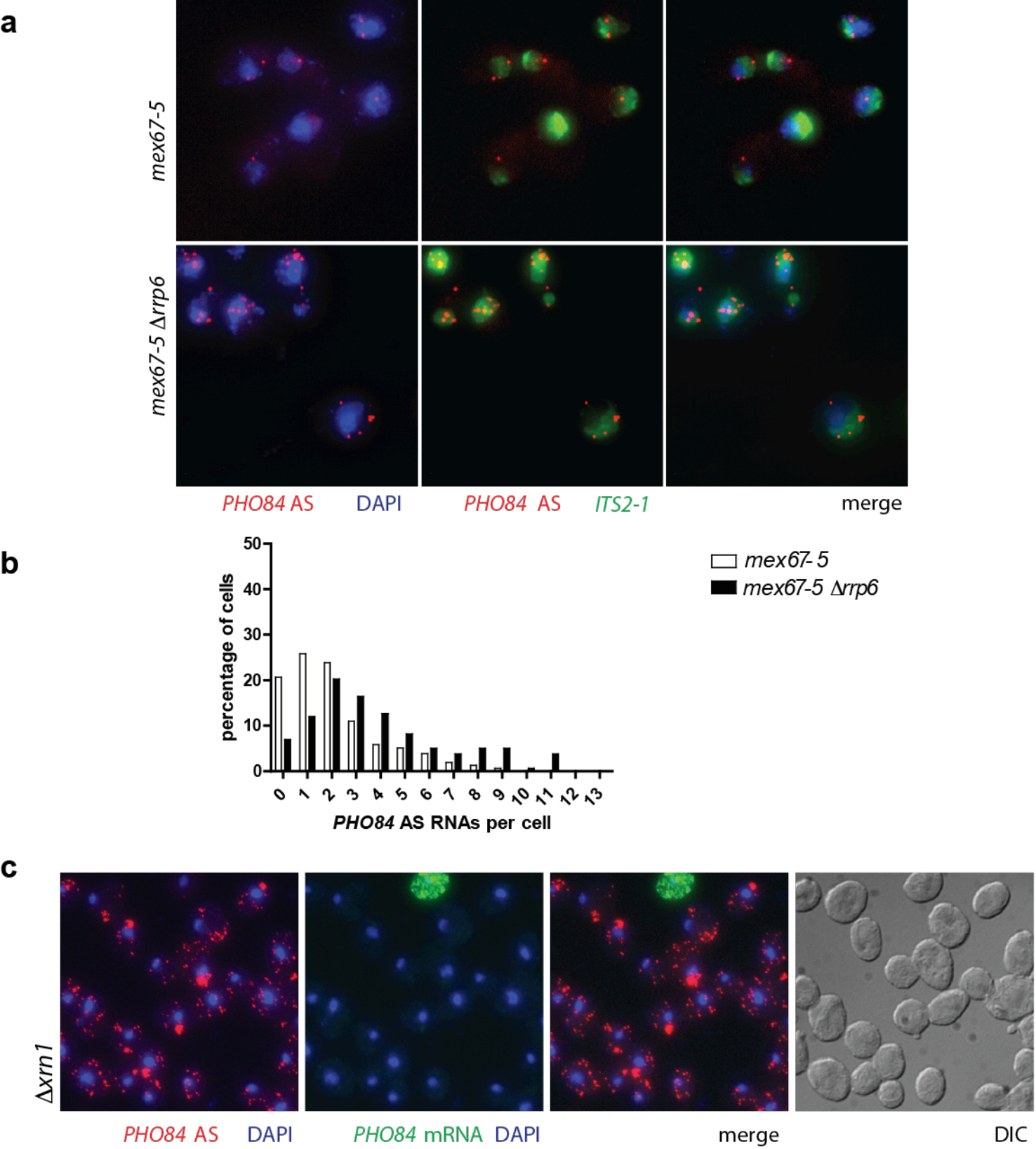
Supplementary Information



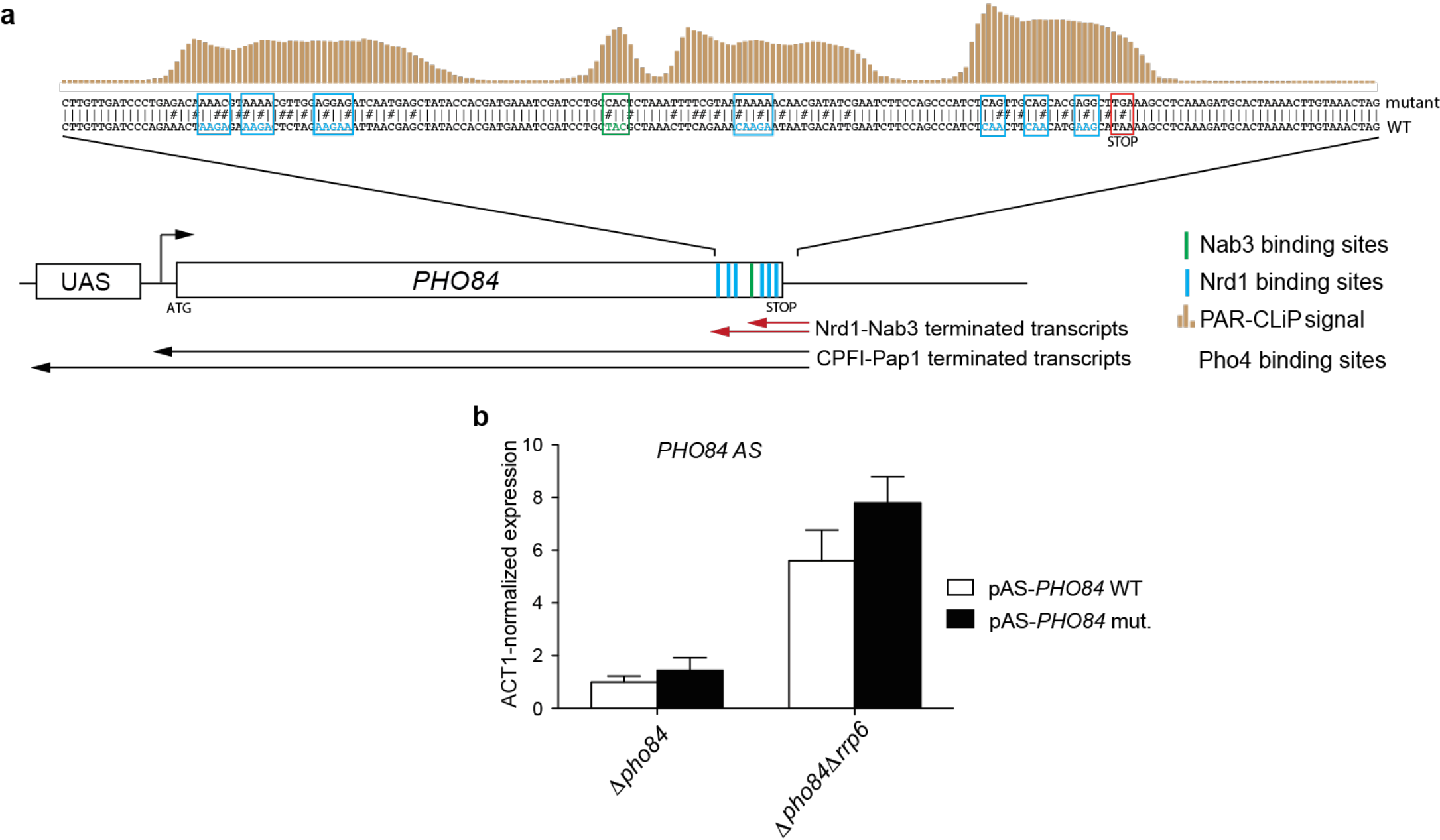
Supplementary Figure 1: smFISH of *MDN1* mRNAs and *PHO84* nascent transcripts.
a) *MDN1* mRNAs are detected in all cells, also in *PHO84* sense and/or *PHO84* AS RNA negative cells. smFISH using probes against *PHO84* sense (upper panel) or *PHO84* AS RNAs (lowe panel) and *MDN1* mRNAs were performed as shown in Figure 1. Nuclear DNA is stained using DAPI, cellular autofluorescence in the GFP chanel is used to show the cellular boundaries. **(b)** *PHO84* sense and AS RNA loci are always located at nuclear periphery, consistent with the location of the of the *PHO84* gene close to the subtelomeric region of Chromosome XIII. Different z-sections as well as the maximum projected image are shown. Images were acquired in 200nm steps, only every second z-plane is shown. Importantly, RNA signals are rarely observed in the nucleus, except at the site of transcription, suggesting that RNA nuclear export is fast.



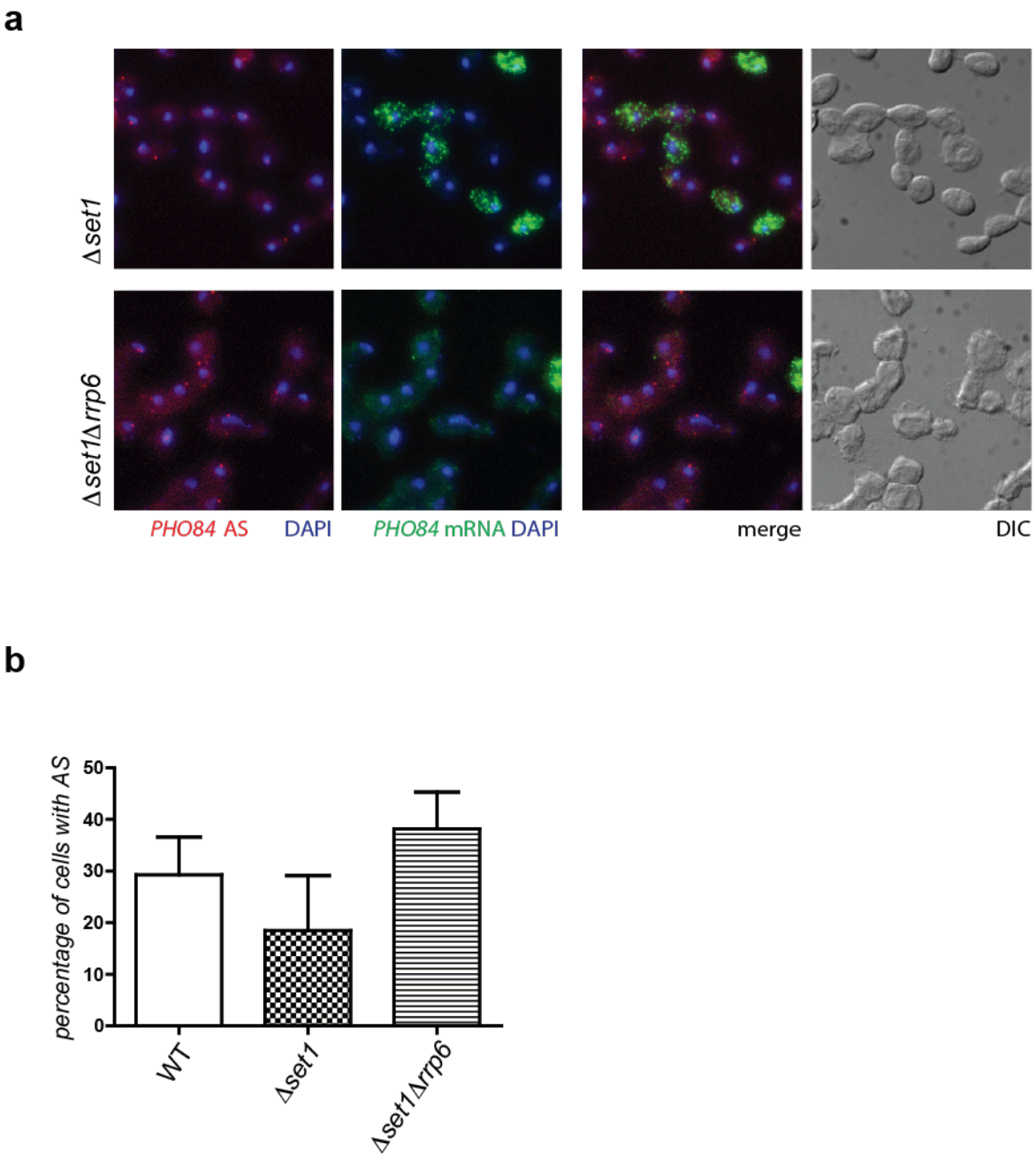
Supplementary Figure 2: *PHO84* AS RNAs are polyadenylated by Pap1.
(a) *PHO84* AS RNAs frequently accumulate in the nucleolus in a *pap1-1Δrrp6* strain after 1h at 37°C. *pap1-1Δrrp6* cells were grown at 25°C in SD complete and shifted to 37°C for 1h prior to fixation followed by hybridization of *PHO84* AS (red) and probes against the ribosomal pre-rRNA spacer sequence *ITS2-1* (green) as a molecular marker. Nuclear DNA was stained using DAPI (blue) and cellular outlines were visualized by brightfield imaging.
(b) In the absence of Pap1, *PHO84* AS RNAs are polyadenylated and degraded by the surveilling exosome, presumably following polyadenylation by Trf4/5. Northern blot membranes with oligo dT purified poly(A)+ RNA or polyA minus (flow-through) from the indicated strains grown at 25°C or shifted to 37°C for 1h were hybridized with *PHO84* AS specific probes as described in Figure 1. Membranes were rehybridized with *ACT1* random labelled probes to control for equal loading and for phenotype. In *pap1-1* at 37°C, *ACT1* mRNA is reduced in level probably due to the defect in 3' end formation.



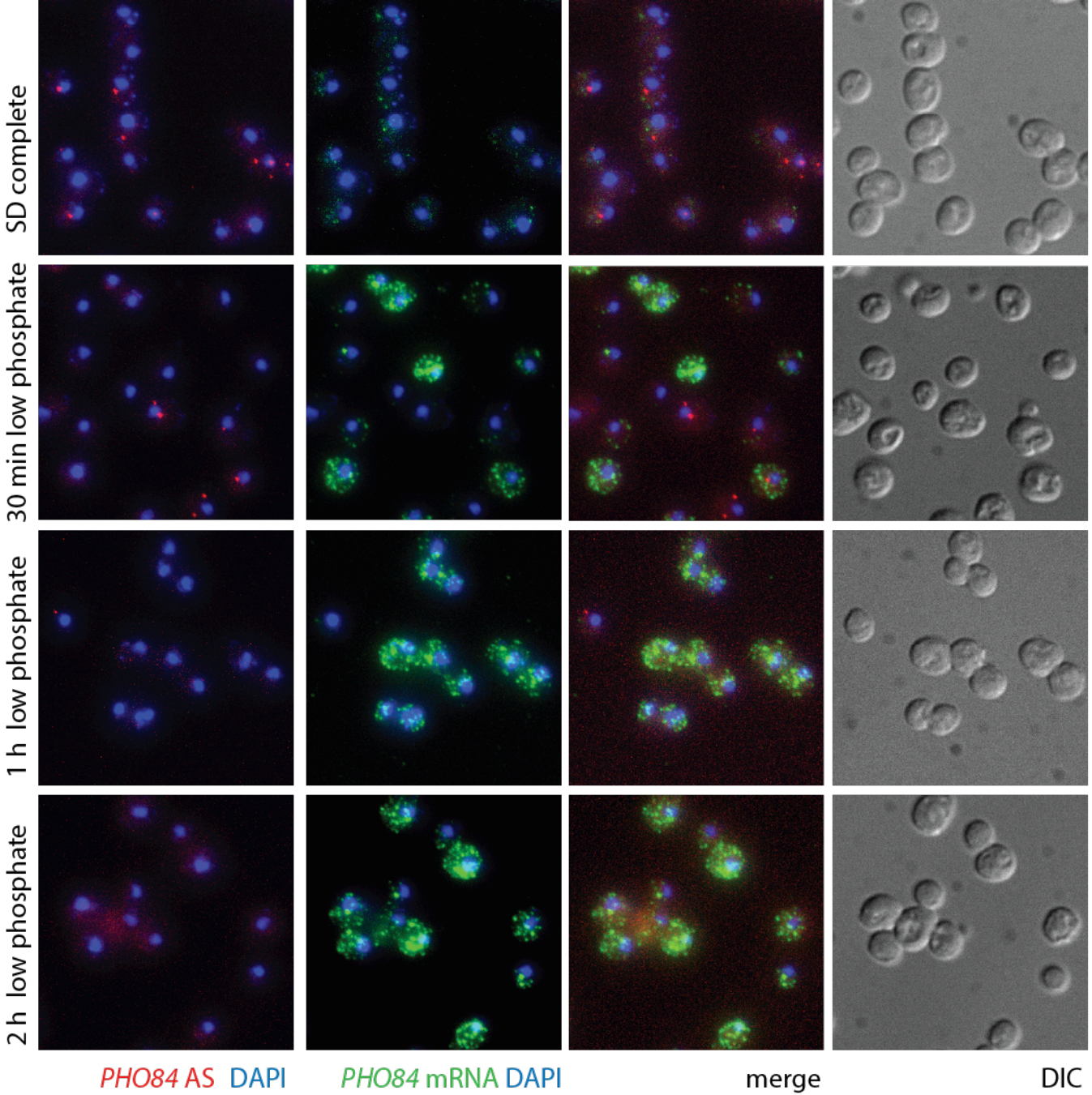
Supplementary Figure 3: *PHO84* AS RNAs have mRNA-like properties.
(a) *PHO84* AS RNAs are exported to the cytoplasm by the mRNA export receptor Mex67. *mex67-5 and mex67-5 Δrrp6* cells were grown at 25°C in SD complete and shifted to 37°C for 1 hour prior to fixation followed by hybridization with *PHO84* AS probes as shown in Figure 1c. The nucleolus was stained using a *ITS2-1* probe (green), and nuclear DNA visualized by DAPI stain (blue).
(b) Frequency disbribution of *PHO84* AS RNA expression levels after 1 hour heat shock at 37°C in *mex67-5* and *mex67-5 Δrrp6* cells. The numbers of *PHO84* AS RNAs were determined for > 100 cells.
(c) *PHO84* AS RNAs are degraded in the cytoplasm in an Xrn1 dependent manner. Δ*xrn1* cells were grown in SD complete medium at 25°C, fixed and hybridized with FISH probes complementary to *PHO84* sense and AS RNAs as shown in Figure 1c.



Supplementary Figure 4: Nrd1-Nab3 binding site mutantions increase AS RNA production.
(a) Schematic view of the mutagenesis of Nrd1 and Nab3 binding sites at the *PHO84* 3' end. WT and mutated sequences are reported in the scheme. The Nrd1 PAR-CLiP signal detected over the same region (Creamer et al., 2011) [45] is indicated above. The 3' ends of early terminated *PHO84* AS transcripts have been described (Neil et al., 2009) [1]. **(b)** Analysis of *PHO84* AS RNA levels in a Δ*pho84* strain transformed with a wild-type *PHO84* plasmid (pAS-*PHO84* WT) or a mutant *PHO84* plasmid (pAS-*PHO84* mut.) on which Nrd1-Nab3 motifs on the AS orientation are mutagenized. Plasmid encoded *PHO84* AS transcripts were measured by RT-qPCR with specific primers as described in Methods.



Supplementary Figure 5: Opposite effects of Set1 and Rrp6 on *PHO84* AS RNA levels.
(a) smFISH for *PHO84* sense and AS RNA in Δ*set1* and Δ*set1*Δ*rrp6* cells. Cells were grown, fixed and hybridized to probes specific for *PHO84* sense (green) and AS RNAs (red) as shown in Figure 1c.
(b) Percentage of cells expressing *PHO84* AS RNA in individual cells of the indicated strains.



Supplementary Figure 6: Low phosphate medium prevents *PHO84* AS RNA expression.
Strong induction of *PHO84* sense transcription in low phosphate medium prevents *PHO84* antisense expression. Cells were grown in SD complete medium and shifted to low phosphate medium for 0.5, 1 and 2 hours prior to fixation. smFISH is shown in green for *PHO84* sense and red for *PHO84* AS RNAs. DNA was stained using DAPI (blue) and cellular outlines were visualized using DIC optics.

	Mean number of total *PHO84* AS RNAs per cell	Half-life (min) measured by 1,10 Phenanthroline induced transcription inhibition	Transcription frequency (RNAs/hour)
Wild type	0.36±0.098	11.4±3.9	1.31
Δ*rrp6*	0.82±0.18	12±2.8	2.84

Supplementary Table 1: Transcription frequency in wild-type and *Δrrp6* cells. mRNA half-life was calculated using mean *PHO84* AS expression levels in wild-type and *Δrrp6* strains measured by smFISH and decay rates measured by qRT-PCR after transcription shutoff by 1,10 Phenanthroline. Assuming mRNA decay follows first-order kinetics, transcription frequency can be calculated using (Holstege et al. 1998; Wang et al. 2002) [56,57] :

Transcription frequency = Ln2 x (steady state RNA level)/half life (min)

Supplementary Table 2

STRAINS USED IN THIS STUDY

Code	Name	Genotype	Reference
W303 background			
FSY1742	WT	*MATa ade2 his3 leu2 trp1 ura3*	
FSY3117	*Δrrp6*	*MATa ade2 his3 leu2 trp1 ura3 Δrrp6::Kanr*	Camblong et al., 2007 [34]
FSY3518	*Δhda2*	*MATa ade2 his3 leu2 trp1 ura3 Δhda2::TRP1*	Camblong et al., 2007 [34]
FSY3018	*Δhda2Δrrp6*	*MATa ade2 his3 leu2 trp1 ura3 Δrrp6::Kanr Δhda2::TRP1*	this study
FSY3517	*Δset1*	*MATa ade2 his3 leu2 trp1 ura3 Δset1::TRP1*	this study
FSY3833	*Δset1Δrrp6*	*MATa ade2 his3 leu2 trp1 ura3 Δrrp6::Kanr Δset1::TRP1*	this study
FSY1982	*mex67-5*	*MATa ade2 his3 leu2 trp1 ura3 mex67-5 integrated*	Jimeno et al., 2002 [58]
FSY1985	*mex67-5 Δrrp6*	*MATa ade2 his3 leu2 trp1 ura3 mex67-5 integrated Δrrp6::Kanr*	this study
FSY2078	*Δxrn1*	*MATa ade2 his3 leu2 trp1 ura3 Δxrn1::RP1*	Jensen T.H. lab
FSY1968	*pap1-1*	*MATa ade2 his3 leu2 trp1 ura3 pap1-1*	Jensen T.H. lab
FSY1988	*pap1-1 Drrp6*	*MATa ade2 his3 leu2 trp1 ura3 pap1-1 Drrp6::Kanr*	Libri D. lab
FSY4838	*pap1-1 Dtrf4*	*MATa ade2 his3 leu2 trp1 ura3 pap1-1 Δtrf4::*	Libri D. lab
FSY4275	*GAL-NRD1*	*MATa ade2 his3 leu2 trp1 ura3 HisMX6-pGAL-NRD1*	Thiebaut et al., 2006 [7]
FSY4282	*GAL-NRD1 Drrp6*	*MATa ade2 his3 leu2 trp1 ura3 HisMX6-pGAL-NRD1 Drrp6::Kanr*	Thiebaut et al., 2006 [7]
FSY2527	*Dpho4*	*MATa ade2 his3 leu2 trp1 ura3 Dpho4::Kanr*	this study
FSY3313	*Dpho4 Drrp6*	*MATa ade2 his3 leu2 trp1 ura3 Dpho4::Kanr Drrp6::TRP1*	this study
FSY4265	*Dpho4 Dset1*	*MATa ade2 his3 leu2 trp1 ura3 Dpho4::Kanr Dset1::HIS3*	this study
FSY4266	*Dpho4 Dset1 Drrp6*	*MATa ade2 his3 leu2 trp1 ura3 Dpho4::Kanr Dset1::HIS3 Drrp6::TRP*	this study
FSY4911	*NRD1-HA Dpho4*	*MATa ade2 his3 leu2 trp1 ura3 NRD1-HA-HIS3 Dpho4::Kanr*	this study
FSY4918	*NRD1-HA Dpho4 Drrp6*	*MATa ade2 his3 leu2 trp1 ura3 NRD1-HA-HIS3 Dpho4::Kanr Drrp6::TI*	this study
FSY4912	*NRD1-HA Dpho4 Dset1*	*MATa ade2 his3 leu2 trp1 ura3 NRD1-HA-HIS3 Dpho4::Kanr Dset1::TI*	this study
FSY4841	*rpb1-1*	*MATa ade2 his3 leu2 trp1 ura3 rpb1-1*	Libri D. lab
FSY4842	*rpb1-1 Drrp6*	*MATa ade2 his3 leu2 trp1 ura3 rpb1-1 Drrp6::URA3*	Libri D. lab
FSY3799	*Dpho84*	*MATa ade2 his3 leu2 trp1 ura3 Dpho84::Kanr*	Camblong et al., 2009 [48]
FSY3811	*Dpho84 Drrp6*	*MATa ade2 his3 leu2 trp1 ura3 Dpho4::Kanr Drrp6::TRP1*	Camblong et al., 2009 [48]
BY4741 background			
FSY3158	*WT*	*MATa his3 leu2 lys2 ura3*	Euroscarf
FSY4747	*Drrp6*	*MATa his3 leu2 lys2 ura3 Drrp6::NatMX*	Houseley J. & Tollervey D. (2006) [9]
FSY3182	*Dtrf4*	*MATa ade2 his3 leu2 ura3 Dtrf4::Kanr*	"
FSY4748	*Dtrf4 Drrp6*	*MATa ade2 his3 leu2 ura3 Dtrf4::Kanr Drrp6::NatMX*	"
FSY4749	*Dtrf4 GAL-TRF5*	*MATa ade2 his3 leu2 ura3 Dtrf4::Kanr HisMX6-pGAL-3HA::trf5*	"

PRIMERS USED IN THIS STUDY

Code	Name	Sequence
OFS1741	*ACT1* F Mid	5'-TTCCAGCCTTCTACGTTTCCATC-3'
OFS1742	*ACT1* R Mid	5'-CGTGAGGTAGAGAGAAACCAGC-3'
OFS735	*ACT1* F 3'	5'-TACTCCGTCTGGATTGGTGGTT-3'
OFS736	*ACT1* R 3'	5'-GGTGAACGATAGATGGACCACTT-3'
OFS737	*ACT1* F 5'	5'-TGGTATGTTCTAGCGCTTGCAC-3'
OFS738	*ACT1* R 5'	5'-GTCAATATAGGAGGTTATGGGAGAGTG-3'
OFS1077	*PHO84* F 3'	5'-GAAATTAACGAGCTATACCACGATGAAATC-3'
OFS1078	*PHO84* R 3'	5'-CATGTTGAAGTTGAGATGGGCTGG-3'
OFS1158	*PHO84* F M	5'-CTGCCGCACAAGAACAAGATGG-3'
OFS1159	*PHO84* F M	5'-TTTGGAGGATGATTGTCAAGAGATTCG-3'
OFS1075	*PHO84* F 5'	5'-CCGTCAATAAAGATACTATTCATGTTGCTG-3'
OFS1076	*PHO84* F 5'	5'-AAAATCATTCAAATGGTTGTGGAAGGC-3'
OFS1717	*SCR1* F	5'-AACCGTCTTTCCTCCGTCGTAA-3'
OFS1718	*SCR1* R	5'-CTACCTTGCCGCACCAGACA-3'
OFS1249	*PHO84 -1000 SalI F*	5'-GGGGGGGGGGGTCGACCGAGAGTGATAAAGAAGAGGCGGT-3'
OFS1363	*PHO84 +355 SalI R*	5'-GGGGGGGGGGGTCGACGTCTCAAGTCGCTTGCTTAGTCGA-3'

smFISH probes used in this study

PHO84 mRNA, *PHO84* AS and *MDN1* probes were labeled in a single position with eigther Dylight 549 or DyLight 594
ITS2-1 probe was labeled in 3 positions with cy5 (Zenklusen et al., 2008). Labeled positions are shown in bold.

PHO84 sense	*PHO84* AS	*ITS2-1*	*MDN1*
ccttcaqtaaaqtqttcttt	cccatctcaacttcaacatq	GA**T** ATG CTT AAG TTC AGC GGG **T**AC TCC TAC CTG ATT TGA GG**T** C	caqaqqqaaaaqcaqaattq
aaatggttgtggaaggccat	ctgctacgctaaacttcaga		ttgttgctaaagtggaaggg
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