Saroufim et al., http://www.jcb.org/cgi/content/full/jcb.201503070

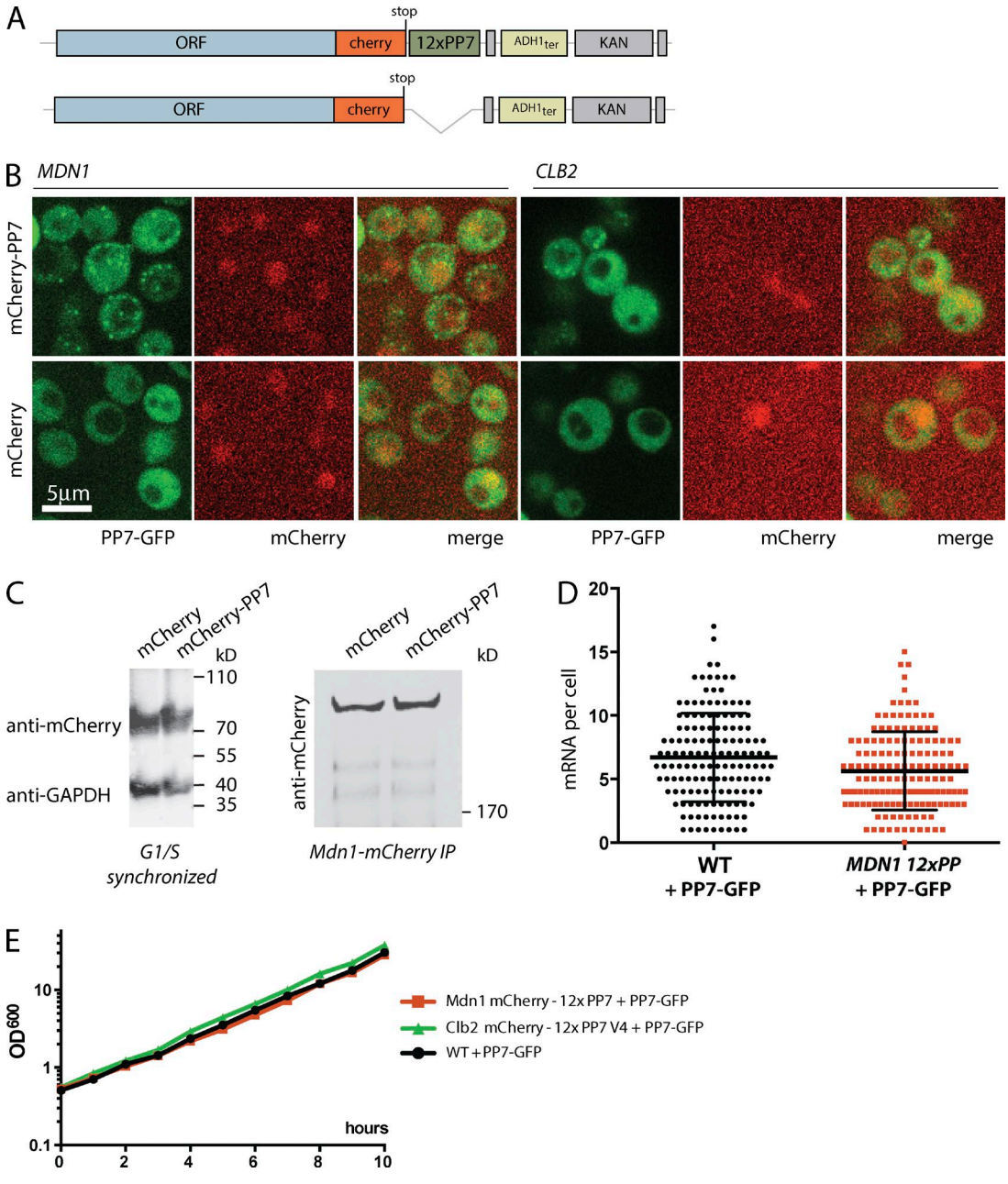


Figure S1. **Insertion of PP7 stem loops does not alter *MDN1* and *CLB2* mRNA and protein expression levels.** (A) Cartoon describing integration cassettes for simultaneous protein and mRNA detection. (B) Single plane images of PP7-GFP (green) and mCherry (red) in strains where mCherry or mCherry-12xPP7 sequences were integrated to the 3′ UTR of the *MDN1* and *CLB2* genes. (C) Western blot for Clb2 and Mdn1-mCherry protein fusions in the presence and absence of 12xPP7 stem loops and PP7-GFP. Both proteins are expressed at low levels. For Clb2 detection, cells were synchronized in G1/S using α-factor. To detect Mdn1, Mdn1-mCherry was enriched using an anti-mCherry antibody. (D) Quantification of *MDN1* mRNA levels by smFISH for *MDN1* strains shown in B. 148 cells were analyzed. Error bars show standard deviation. (E) Growth rate of PP7-tagged strains from B compared with a WT strain.

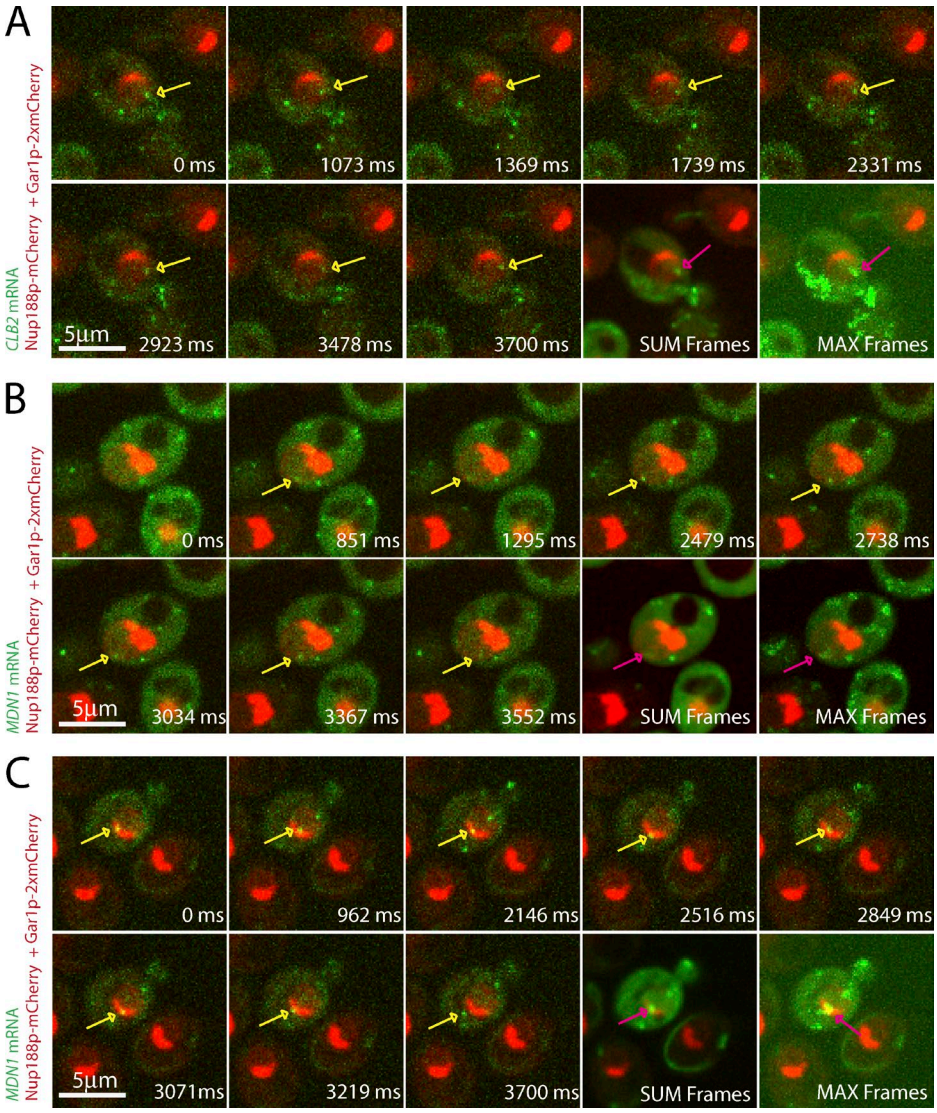


Figure S2. **mRNAs scan the nuclear periphery, excluding the nucleolus.** (A) Live cell imaging of *CLB2* mRNA in a strain where nuclear pores and the nucleolus are labeled. Individual frames from video acquired in 37-ms intervals. *CLB2* mRNAs are not observed in the nucleolus. (B) Live cell imaging showing *MDN1* mRNA scanning the periphery, excluding the nucleolus. (C) *MDN1* mRNAs can get trapped in the nucleolus and exit the nucleolus through pores at the nucleolus. Individual frames from video acquired in 37-ms intervals. Nucleolus and nuclear pores are marked in red by labeling Gar1 and Nup188 with mCherry. SUM and MAX show summary and maximum intensity projection of all frames. Yellow and purple arrows indicate tracked mRNAs in individual and in SUM and MAX projected images, respectively.

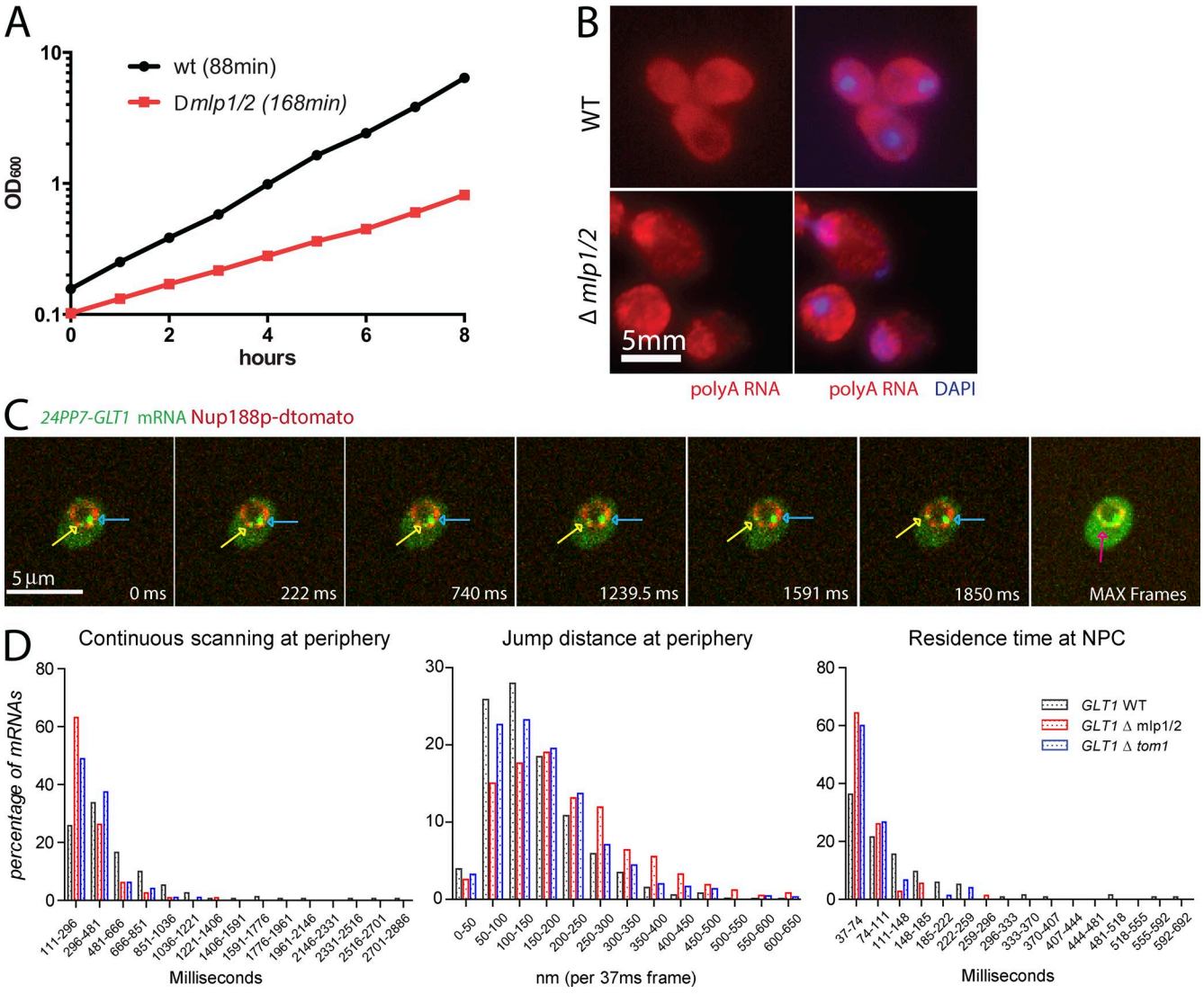
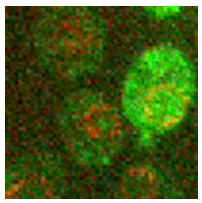
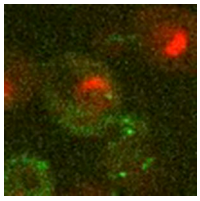


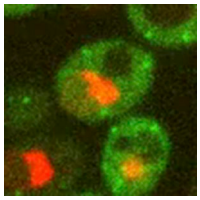
Figure S3. ***MLP1/2* deletion growth and mRNP export phenotype and mRNP behavior at the nuclear periphery in a *TOM1* deletion.** (A) Growth rate of *MLP1/2* deletion in diploid w303 compared with a WT strain. (B) Weak RNA export phenotype of a diploid *mlp1/2* double deletion strain shown by in situ hybridization visualizing using an oligo dT probe to visualize total polyA RNA. Only a subset of cells show nuclear mRNA accumulation. (C) Live cell imaging of GAL1pro-*24PP7-GLT1* mRNA. Individual frames from video acquired in 37-ms intervals in *TOM1* deletion background. MAX shows maximum intensity projection of all frames. Blue arrows show sites of transcription, and yellow and purple arrows show the tracked mRNA in individual frames and MAX projected image, respectively. (D) Timescales of continuous mRNP scanning (left), jump distance at the periphery (middle), and timescales of restricted movements (right) are shown. 156 (WT), 105 (Δ*MLP1/2*), and 95 (Δ*TOM1*) tracks were analyzed.



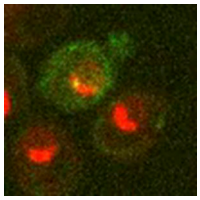
Video 1. **Nuclear scanning of *CLB2* mRNA.** Diploid WT yeast cells where 12xPP7 stem loops were inserted into the 3′ UTR of one allele of the *CLB2* gene and the nucleopore protein Nup188 was C-terminally tagged with mCherry (red). *CLB2* mRNAs were visualized by transforming a plasmid expressing PP7-2xGFP (green). Images were acquired using a spinning disk confocal microscope (Observer; Carl Zeiss), and frames were taken every 37 ms. The video is played at 10 frames per second.



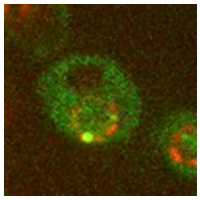
Video 2. **Nuclear scanning of *CLB2* mRNA occurs outside of the nucleolus.** Diploid WT yeast cells where 12xPP7 stem loops were inserted into the 3′ UTR of one allele of the *CLB2* gene, and the nucleopore protein Nup188 and the nucleolar marker Gar1p were C-terminally tagged with mCherry (red). *CLB2* mRNAs were visualized by transforming a plasmid expressing PP7-2xGFP (green). Images were acquired using a spinning disk confocal microscope, and frames were taken every 37 ms. The video is played at 10 frames per second.



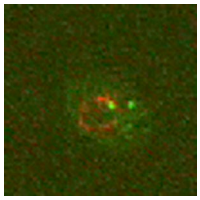
Video 3. **Nuclear scanning of *MDN1* mRNA occurs outside of the nucleolus.** Diploid WT yeast cells where 12xPP7 stem loops were inserted into the 3′ UTR of one allele of the *MDN1* gene, and the nucleopore protein Nup188 and the nucleolar protein Gar1p were C-terminally tagged with mCherry (red). *MDN1* mRNAs were visualized by transforming a plasmid expressing PP7-2xGFP (green). Images were acquired using a spinning disk confocal microscope, and frames were taken every 37 ms. The video is played at 10 frames per second.



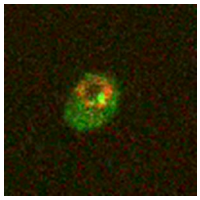
Video 4. ***MDN1* mRNA trapped and exported through the nucleolus.** Diploid WT yeast cells where 12xPP7 stem loops were inserted into the 3′ UTR of one allele of the *MDN1* gene, and the nucleopore protein Nup188 and the nucleolar protein Gar1p were C-terminally tagged with mCherry (red). *MDN1* mRNAs were visualized by transforming a plasmid expressing PP7-2xGFP (green). Images were acquired using a spinning disk confocal microscope, and frames were taken every 37 ms. The video is played at 10 frames per second.



Video 5. **Nuclear scanning of galactose-induced *GLT1* mRNA.** Diploid WT yeast cells containing a dTomato-tagged Nup188 (red) and where 24xPP7 stem loops were inserted to the 5′ UTR of one allele of the galactose-inducible *GLT1* reporter gene. *GLT1* mRNAs were visualized by transforming a plasmid expressing PP7-2xGFP (green) and inducing transcription by galactose. mRNAs were analyzed during the early time of induction. Images were acquired using a spinning disk confocal microscope, and frames were taken every 37 ms. The video is played at 10 frames per second.



Video 6. **Behavior of nuclear *GLT1* mRNAs in a Δmlp1/2 strain after galactose induction.** Diploid WT yeast cells containing a dTomato-tagged Nup188 (red) and where 24xPP7 stem loops were inserted to the 5′ UTR of one allele of the galactose-inducible *GLT1* reporter gene. *GLT1* mRNAs were visualized by transforming a plasmid expressing PP7-2xGFP (green) and inducing transcription by galactose. mRNAs were analyzed during the early time of induction. Images were acquired using a spinning disk confocal microscope, and frames were taken every 37 ms. The video is played at 10 frames per second.



Video 7. **Behavior of nuclear *GLT1* mRNAs in a *Δtom1* strain after galactose induction.** Haploid WT yeast cells containing a dTomato-tagged Nup188 (red) and where 24xPP7 stem loops were inserted to the 5′ UTR of the galactose-inducible *GLT1* reporter gene. *GLT1* mRNAs were visualized by transforming a plasmid expressing PP7-2xGFP (green) and inducing transcription by galactose. mRNAs were analyzed during the early time of induction. Images were acquired using a spinning disk confocal microscope, and frames were taken every 37 ms. The video is played at 10 frames per second.

Table S1. **Strains used in this study**

Background	Description	Genotype
BY4743	WT GAL1p-24PP7-*GLT1* + *Nup188*-dTomato + *Met25*p-PP7-GFP	MATα/α-GAL1p-24PP7-*GLT1*:HIS, *NUP188*-dTomato:KANMX, pURA-PP7-GFP
W303	WT *MDN1*-12PP7 + *Nup188*-2xmCherry + *ADE3*p-PP7-GFP	MATα/α-*MDN1*-12PP7:KANMX (loxp), *NUP188*-2xmCherry:KANMX, pURA-PP7-GFP
W303	WT *CLB2*-12PP7 + *Nup188*-2xmCherry + *ADE3*p-PP7-GFP	MATα/α-*CLB2*-12PP7:KANMX(loxp), *NUP188*-2xmCherry:KANMX, pURA-PP7-GFP
W303	WT *MDN1*-mCherry-12xPP7 + *ADE3*p-PP7-GFP	MATα *MDN1*-mCherry-12xPP7:KANMX, pURA ADE3-PP7-GFP
W303	WT *MDN1*-mCherry + *ADE3*p-PP7-GFP	MATα *MDN1*-mCherry-12xPP7:KANMX, pURA ADE3-PP7-GFP
W303	WT *CLB2*-mCherry-12xPP7 + *ADE3*p-PP7-GFP	MATα *CLB2*-mCherry-12xPP7:KANMX, pURA ADE3-PP7-GFP
W303	WT *CLB2*-mCherry + *ADE3*p-PP7-GFP	MATα *CLB2*-mCherry-12xPP7:KANMX, pURA ADE3-PP7-GFP
W303	*Δmlp1/2* GAL1p-24PP7-*GLT1* + *Nup188*-dTomato + *Met25*p-PP7-GFP	MATα/α-GAL1p-24PP7-*GLT1*:HIS, *NUP188*-dTomato:KANMX, pTRP-PP7-GFP (pDZ529)
BY4743	*Δsto1* + GAL1p-24PP7-*GLT1* + *Nup188*-dTomato + *Met25*p-PP7-GFP	MATα/α-GAL1p-24PP7-*GLT1*:HIS, *NUP188*-dTomato:KANMX,*STO1* ::HPH, pURA-PP7-GFP
BY4743	*Δpml1* + GAL1p-24PP7-*GLT1* + *Nup188*-dTomato + ADE3p-PP7-GFP	MATα/α-GAL1p-24PP7-*GLT1*:HIS, *NUP188*-dTomato:KANMX, *PML1*::HPH, pURA-PP7-GFP
BY4743	*Δpml39* + GAL1p-24PP7-*GLT1* + *Nup188*-dTomato + *ADE3*p-PP7-GFP	MATα/α-GAL1p-24PP7-*GLT1*:HIS, *NUP188*-dTomato:KANMX, *PML39*::HPH, pURA-PP7-GFP
BY4742	*Δnup60* + GAL1p-24PP7-*GLT1* + *Nup188*-dTomato + *ADE3*p-PP7-GFP	MATα/GAL1p-24PP7-*GLT1*:HIS, *NUP188*-dTomato:KANMX, *NUP60*::HPH, pURA-PP7-GFP
W303	ΔC-term *mlp1* + GAL1p-24PP7-*GLT1* + *Nup188*-dTomato + *ADE3*p-PP7-GFP	MA α/α-GAL1p-24PP7-*GLT1*:HIS, *NUP188*-dTomato:KANMX, C-term *MLP1*::NLS-2xmcherry-TRP, pURA-PP7-GFP
BY4741	*nab2F73D* + GAL1p-24PP7-*GLT1* + *Nup188*-dTomato + *ADE3*p-PP7-GFP	MAT α/α-GAL1p-24PP7-*GLT1*:HIS, *NUP188*-dTomato:KANMX, *nab2F73D*:HPH, pURA-PP7-GFP
BY4742	*Δtom1* + GAL1p-24PP7-*GLT1* + *Nup188*-dTomato + *ADE3*p-PP7-GFP	MATα GAL1p-24PP7-*GLT1*:HIS, *NUP188*-dTomato:KANMX, *TOM1*::HPH, pURA-PP7-GFP

Table S2 is provided as an Excel spreadsheet and summarizes the proteins identified by mass spectrometry purified using Mlp1-ProtA or Mlp1ΔC-ProtA as baits (Fig. 4, B and C).
Table S3 is provided as an Excel spreadsheet and lists the smFISH probes.