Smith et al., http://www.jcb.org/cgi/content/full/jcb.201503135/DC1

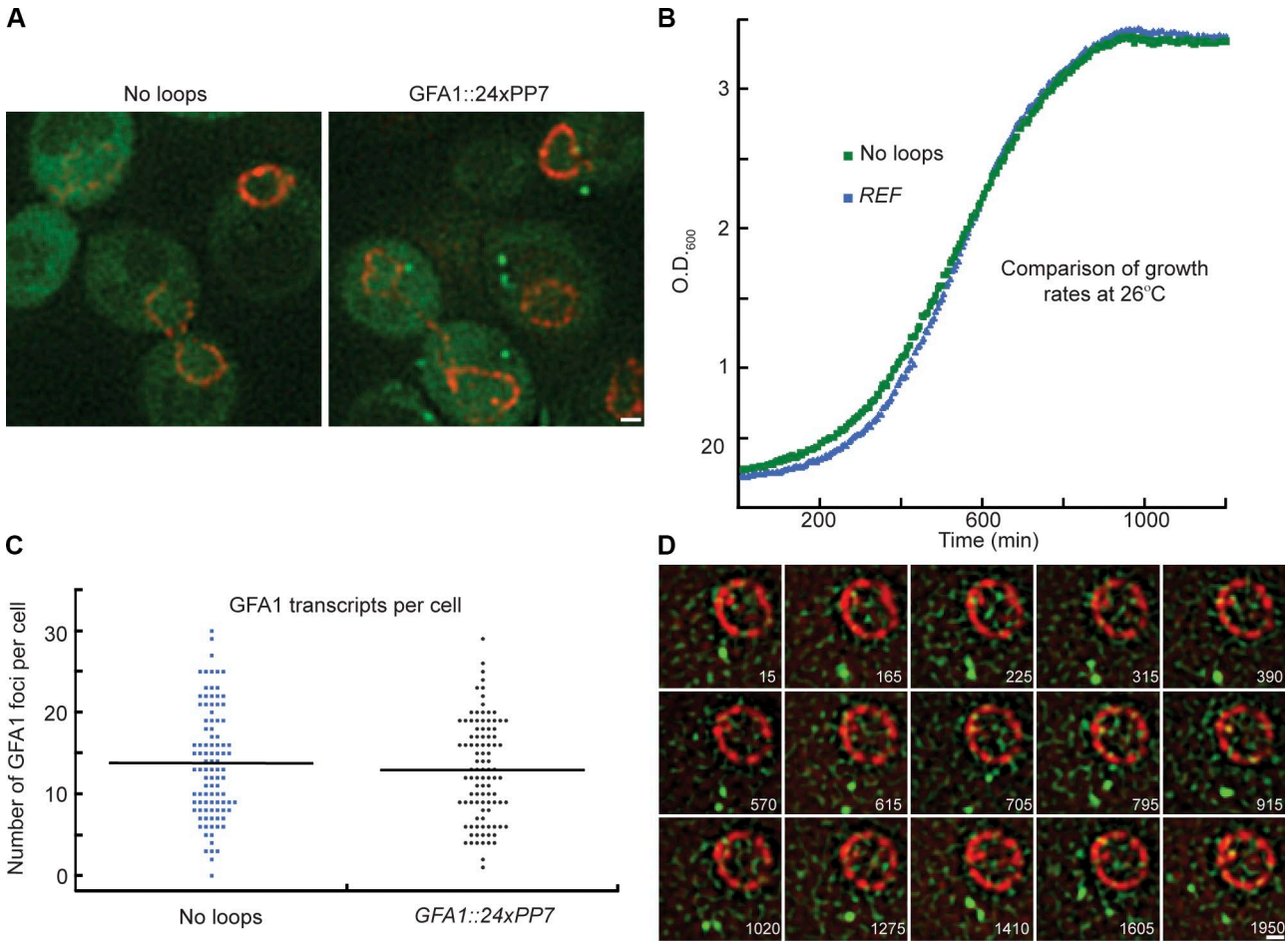


Figure S1. ***REF* strain characterization.** (A) Comparison of PP7-CP-3xYFP localization in a strain with no PP7 stem loops (BMY642) and the *REF* strain with *GFA1*-24xPP7. (B) Growth curves of a control strain with no PP7 stem loops (BMY642) and the *REF* strain with *GFA1*-24xPP7 at 26°C. The data shown are from a single representative experiment out of three repeats. (C) Dot plot shows the number of *GFA1* mRNAs per cell observed using single-molecule FISH probes against GFA1 in a strain with no PP7 stem loops (BMY642) and the *REF* strain at 26°C, with the mean denoted by a black line (n = 100 cells). (D) Selected nonconsecutive frames showing the splitting and merging of cytoplasmic particles in a *REF* cell (see Video 1) with the time from the start of the event given in the bottom right of each image in milliseconds. Bars, 1 µm.

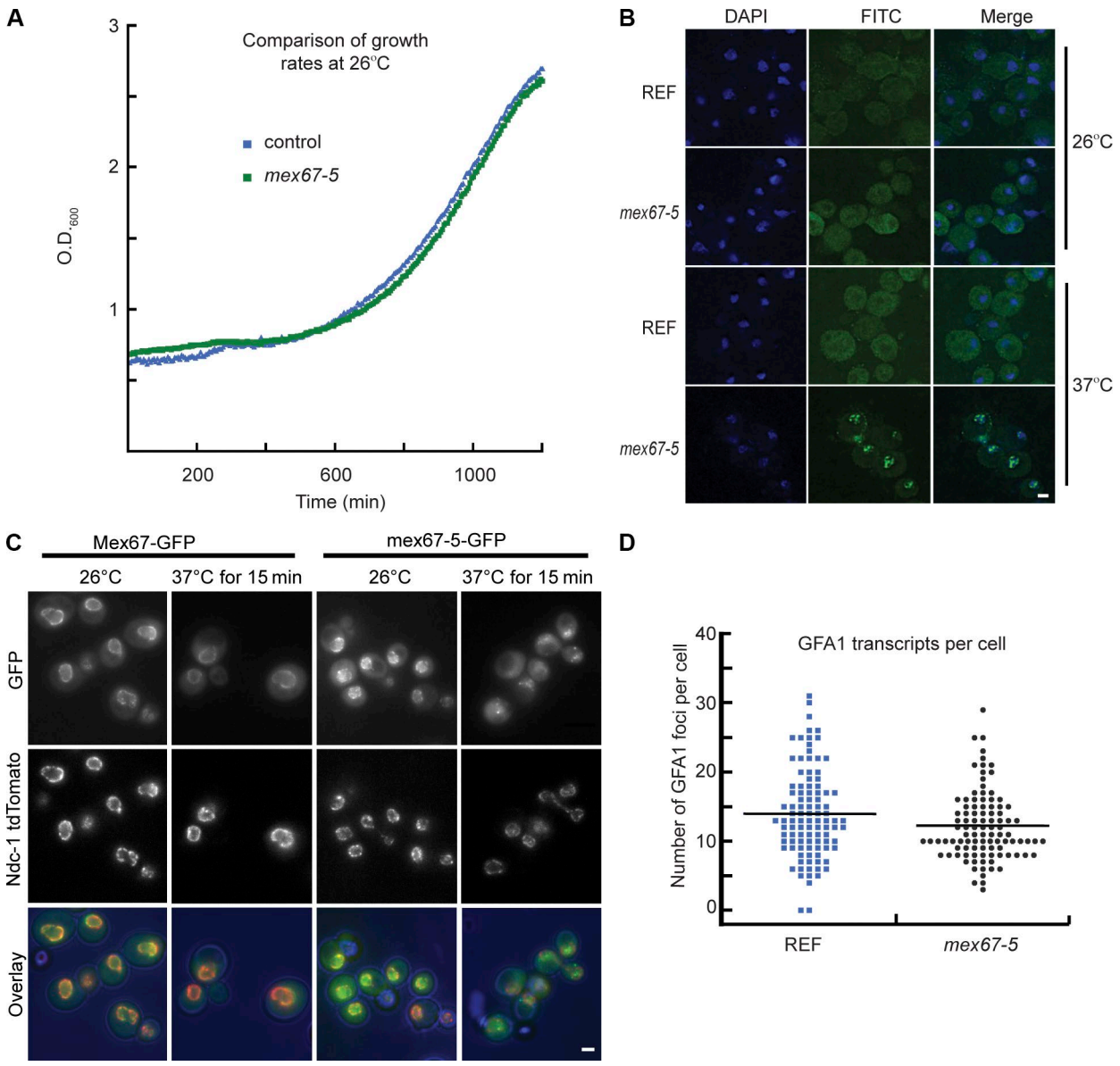
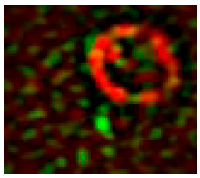
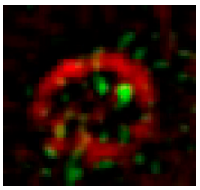


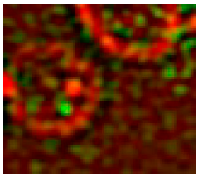
Figure S2. ***mex67-5* strain characterization.** (A) Growth curves of control (BMY129) and *mex67-5* (BMY135) strains at 26°C. The data shown are from a single representative experiment out of five repeats. (B) Representative images showing poly(A)-RNA localization in the *REF* and *mex67-5* strains at 26°C and 37°C. FISH was performed using a FITC-labeled oligo-(dT) probe, and DNA was stained with DAPI. (C) GFP-tagged Mex67 localization in control (KWY5566) and *mex67-5* (KWY5567) strains at 26°C and 37°C as compared with Ndc1-tdTomato. Overlay displays the green and red channels as well as the bright field image. (D) Dot plot shows the number of *GFA1* mRNAs per cell in logarithmically growing *REF* (14 ± 6) and *mex67-5* (12 ± 5) strains at 26°C determined by single-molecule FISH with the mean denoted by a black line (n = 100 cells). Bars, 1 µm.



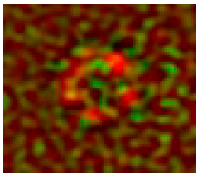
Video 1. ***GFA1* mRNP observed to split and merge.** Video shows a cytoplasmic *GFA1* mRNP with 24xPP7 loops in the 3′ UTR bound by PP7-CP-3xYFP (green) in *REF* cells. The particle splits and merges, suggesting that multiple *GFA1* mRNAs can assembly together. NPCs are marked by Ndc1-tdTomato (red). Images were acquired using a custom dual channel setup (see Materials and methods section Live cell imaging of mRNP export and image processing) at a frame rate of 67 Hz, equaling a time resolution of 15 ms. Video is provided at a 5× reduced rate (13 Hz). Image processing for visual analysis was performed using Fiji. Data for Fig. S1 D were taken from this event.



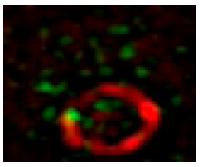
Video 2. **Successful *GFA1* mRNP export event in *REF* cells.** Video shows an example of a successful export event based on tracking of a *GFA1* mRNP with 24xPP7 loops in the 3′ UTR bound by PP7-CP-3xYFP (green). NPCs are marked by Ndc1-tdTomato (red). Images were acquired using a custom dual channel setup (see Materials and methods section Live cell imaging of mRNP export and image processing) at a frame rate of 67 Hz, equaling a time resolution of 15 ms. Video is provided at a 5× reduced rate (13 Hz). Image processing for visual analysis was performed using Fiji. Data for Fig. 2 A were taken from this event.



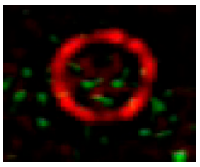
Video 3. **Successful *GFA1* mRNP export event in *REF* cells.** Video shows an example of a successful export event based on tracking of a *GFA1* mRNP with 24xPP7 loops in the 3′ UTR bound by PP7-CP-3xYFP (green). NPCs are marked by Ndc1-tdTomato (red). Images were acquired using a custom dual channel setup (see Materials and methods section Live cell imaging of mRNP export and image processing) at a frame rate of 67 Hz, equaling a time resolution of 15 ms. Video is provided at a 5× reduced rate (13 Hz). Image processing for visual analysis was performed using Fiji.



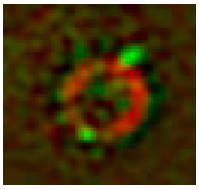
Video 4. **Successful *GFA1* mRNP export event in *REF* cells.** Video shows an exampleof a successful export event based on tracking of a *GFA1* mRNP with 24xPP7 loops in the 3′ UTR bound by PP7-CP-3xYFP (green). NPCs are marked by Ndc1-tdTomato (red). Images were acquired using a custom dual channel setup (see Materials and methods section Live cell imaging of mRNP export and image processing) at a frame rate of 67 Hz, equaling a time resolution of 15 ms. Video is provided at a 5× reduced rate (13 Hz). Image processing for visual analysis was performed using Fiji.



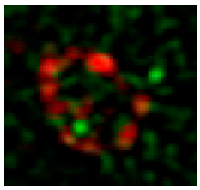
Video 5. **Local NE scanning.** Video shows an example of a *GFA1* mRNP with 24xPP7 loops in the 3′ UTR bound by PP7-CP-3xYFP (green) docking with the same local area of the NE. NPCs are marked by Ndc1-tdTomato (red). Images were acquired using a custom dual channel setup (see Materials and methods section Live cell imaging of mRNP export and image processing) at a frame rate of 67 Hz, equaling a time resolution of 15 ms. Video is provided at a 5× reduced rate (13 Hz). Image processing for visual analysis was performed using Fiji. Data for Fig. 2 B were taken from this event.



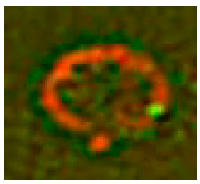
Video 6. **Distributive NE scanning.** Video shows an example of a *GFA1* mRNP with 24xPP7 loops in the 3′ UTR bound by PP7-CP-3xYFP (green) docking with the NE distributed over a large area. NPCs are marked by Ndc1-tdTomato (red). Images were acquired using a custom dual channel setup (see Materials and methods section Live cell imaging of mRNP export and image processing) at a frame rate of 67 Hz, equaling a time resolution of 15 ms. Video is provided at a 5× reduced rate (13 Hz). Image processing for visual analysis was performed using Fiji. Data for Fig. 2 C were taken from this event.



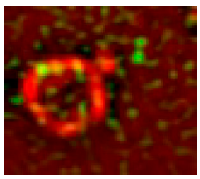
Video 7. **Extended mRNP interactions with the NE in *mex67-5*.** Video shows two *GFA1* mRNPs with 24xPP7 loops in the 3′ UTR bound by PP7-CP-3xYFP (green) engaged with the NE that persists for the entire length of the 7.5-s video in a *mex67-5* cell. NPCs are marked by Ndc1-tdTomato (red). Images were acquired using a custom dual channel setup (see Materials and methods section Live cell imaging of mRNP export and image processing) at a frame rate of 67 Hz, equaling a time resolution of 15 ms. Video is provided at a 5× reduced rate (13 Hz). Image processing for visual analysis was performed using Fiji. Data for Fig. 3 B were taken from this event.



Video 8. **Successful *GFA1* mRNP export event in *mex67-5* cells.** Video shows an example of a successful export event based on tracking of a *GFA1* mRNP with 24xPP7 loops in the 3′ UTR bound by PP7-CP-3xYFP (green) from the nucleus to the cytoplasm. NPCs are marked by Ndc1-tdTomato (red). Images were acquired using a custom dual channel setup (see Materials and methods section Live cell imaging of mRNP export and image processing) at a frame rate of 67 Hz, equaling a time resolution of 15 ms. Video is provided at a 5× reduced rate (13 Hz). Image processing for visual analysis was performed using Fiji. Data for Fig. 4 A were taken from this event.



Video 9. **Successful *GFA1* mRNP export event in *mex67-5* cells.** Video shows an example of a successful export event based on tracking of a *GFA1* mRNP with 24xPP7 loops in the 3′ UTR bound by PP7-CP-3xYFP (green) from the nucleus to the cytoplasm. NPCs are marked by Ndc1-tdTomato (red). Images were acquired using a custom dual channel setup (see Materials and methods section Live cell imaging of mRNP export and image processing) at a frame rate of 67 Hz, equaling a time resolution of 15 ms. Video is provided at a 5× reduced rate (13 Hz). Image processing for visual analysis was performed using Fiji.



Video 10. **Failed mRNP export in a *mex67-5* cell.** Video shows a retrograde transport event based on tracking of a *GFA1* mRNP with 24xPP7 loops in the 3′ UTR bound by PP7-CP-3xYFP (green) from the cytoplasmic side of the NE back to the nucleus. NPCs are marked by Ndc1-tdTomato (red). Images were acquired using a custom dual channel setup (see Materials and methods section Live cell imaging of mRNP export and image processing) at a frame rate of 67 Hz, equaling a time resolution of 15 ms. Video is provided at a 5× reduced rate (13 Hz). Image processing for visual analysis was performed using Fiji. Data for Fig. 4 C were taken from this event.

Table S1. **Summary of dwell time analysis for successful mRNP export events**

	***REF* (*n* = 43)**	***mex67-5* (*n* = 9)**
Mean export time—dwell time analysis	188 ± 27	ND
Mean export time—MLE	215 ± 33	ND
Mean nuclear docking time during export—dwell time analysis	32 ± 5	362 ± 121
Mean nuclear docking time during export—MLE	39 ± 6	202 ± 67
Mean transition time during export—dwell time analysis	87 ± 13	406 ± 135
Mean transition time during export—MLE	99 ± 15	383 ± 128
Mean cytoplasmic docking time during export—dwell time analysis	62 ± 10	1,258 ± 419
Mean cytoplasmic docking time during export—MLE	77 ± 12	943 ± 314

All times reported in milliseconds. Reported errors are the SEM.

Table S2. **Yeast strains used in this study**

Name	Genotype	Reference
BMY008	*BY4743 (MATa/Matα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0)*	Brachmann et al., 1998
BMY083	*BY4743 (MATa/Matα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0) GFA1-24xPP7/GFA1-24xPP7 NDC1-tdTomato::KanMX/NDC1-tdTomato::KanMX + [pBM242]*	This study
BMY129	*BY4743 (MATa/Matα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0) NAT MX::DBP5/NATMX::DBP5 GFA1-24xPP7/GFA1-24xPP7 NDC1-tdTomato::KanMX/NDC1-tdTomato::KanMX + [pBM242])*	This study
BMY135	*BY4743 (MATa/Matα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0) mex67-5::NATMX/mex67-5::NATMX GFA1-24xPP7/GFA1-24xPP7 NDC1-tdTomato::KanMX/ NDC1-tdTomato::KanMX + [pBM242])*	This study
BMY642	*BY4743 (MATa/Matα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0) NDC1-tdTomato::KanMX/NDC1-tdTomato::KanMX + [pBM242]*	This study
KWY5566	*BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) MEX67-EGFP::HIS3MX GFA1-24xPP7 NDC1-tdTomato::KanMX*	This study
KWY5567	*BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) mex67-5EGFP::HIS3MX GFA1-24xPP7 NDC1-tdTomato::KanMX*	This study

Table S3. **Plasmids used in this study**

Name	Description	Reference
pKT178	*pFA6a–link–tdimer2–KanMX* (integrative plasmid, SP6 promoter for C-terminal tdimer2 protein fusion with KanMX-based selection)	Sheff and Thorn, 2004
pBM242	*pRS313-P_{MET}PP7-CP-3xYFP* (HIS3 CEN plasmid, MET17 promoter for PP7-CP-3xYFP expression)	This study
pDZ417	*pDZ417-24xPP7-loxP-KanMX-loxP* (integrative plasmid, T7 promoter for 24xPP7-loxP-KanMX-loxP cassette integration)	Hocine et al., 2013
pYM28	*pFA6-yEGFP-HIS3MX* (integrative plasmid, SP6 promoter for C-terminal yEFG protein fusion with HIS3MX-based selection)	Janke et al., 2004
pSH47	pRS416-GAL1-Cre (URA3 CEN plasmid, GAL1 promoter for Cre recombinase expression)	Güldener et al., 1996

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