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Supplemental Information

**A Nuclear Export Block Triggers the Decay
of Newly Synthesized Polyadenylated RNA**

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Figure S1

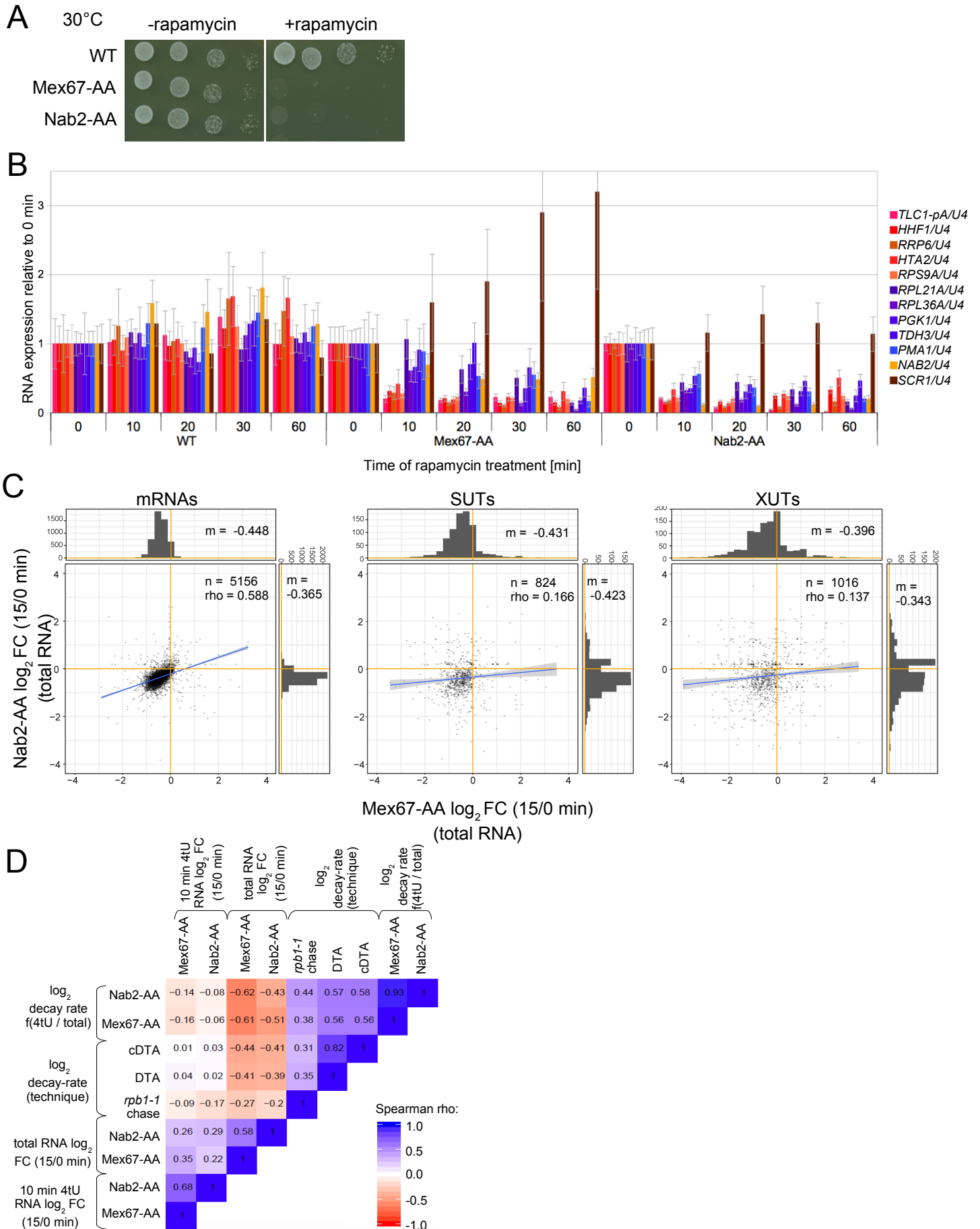


Figure S1. Related to Fig. 1

(A) Spot tests of 10x serial dilutions of WT, Mex67-AA and Nab2-AA cells plated and grown at 30°C without or with rapamycin as indicated.

(B) RT-qPCR analysis of selected RNAs, color-coded to the right of the graph, from wild-type (WT), Mex67-AA and Nab2-AA cells subjected to the indicated periods of rapamycin treatments at 30°C. RNAs that were rapidly or more slowly down-regulated in Mex67p-depleted cells are colored red and blue, respectively. Shown values were normalized to levels of *U4* RNA. *SCR1* RNA was used as a non-adenylated control. Error bars indicate standard deviations from three PCR technical replicates. The result is representative of additional biological repeats.

(C) Rapamycin-dependent changes of mRNA, SUT and XUT levels as depicted in Figure 1C, but for total RNA samples. Observed transcript changes were more modest than previously reported (Schmid et al. 2015). This is likely due to a prolonged doubling time of cells grown here in minimal media lacking uracil (to increase 4tU uptake) relative to rich media used in the previous study and in Fig. S1B. See also Table S1.

(D) Spearman correlation coefficient matrix comparing mRNA log₂FC of 15/0 min rapamycin treatment in Mex67- and Nab2-AA cells as in Fig. 1C and S1C against various estimates of decay rates. These were derived from the following published data: rpb1-1 chase experiments using total RNA (Presnyak et al., 2015), 4tU-based methods - Dynamic Transcriptome Analysis (DTA; Miller et al., 2011) and comparative DTA (cDTA; Sun et al. 2012). DRs estimated by comparing 4tU to total RNA, using established methods (Miller et al., 2011 ; Sun et al. 2012), from the data presented herein are included for comparison.

Figure S2

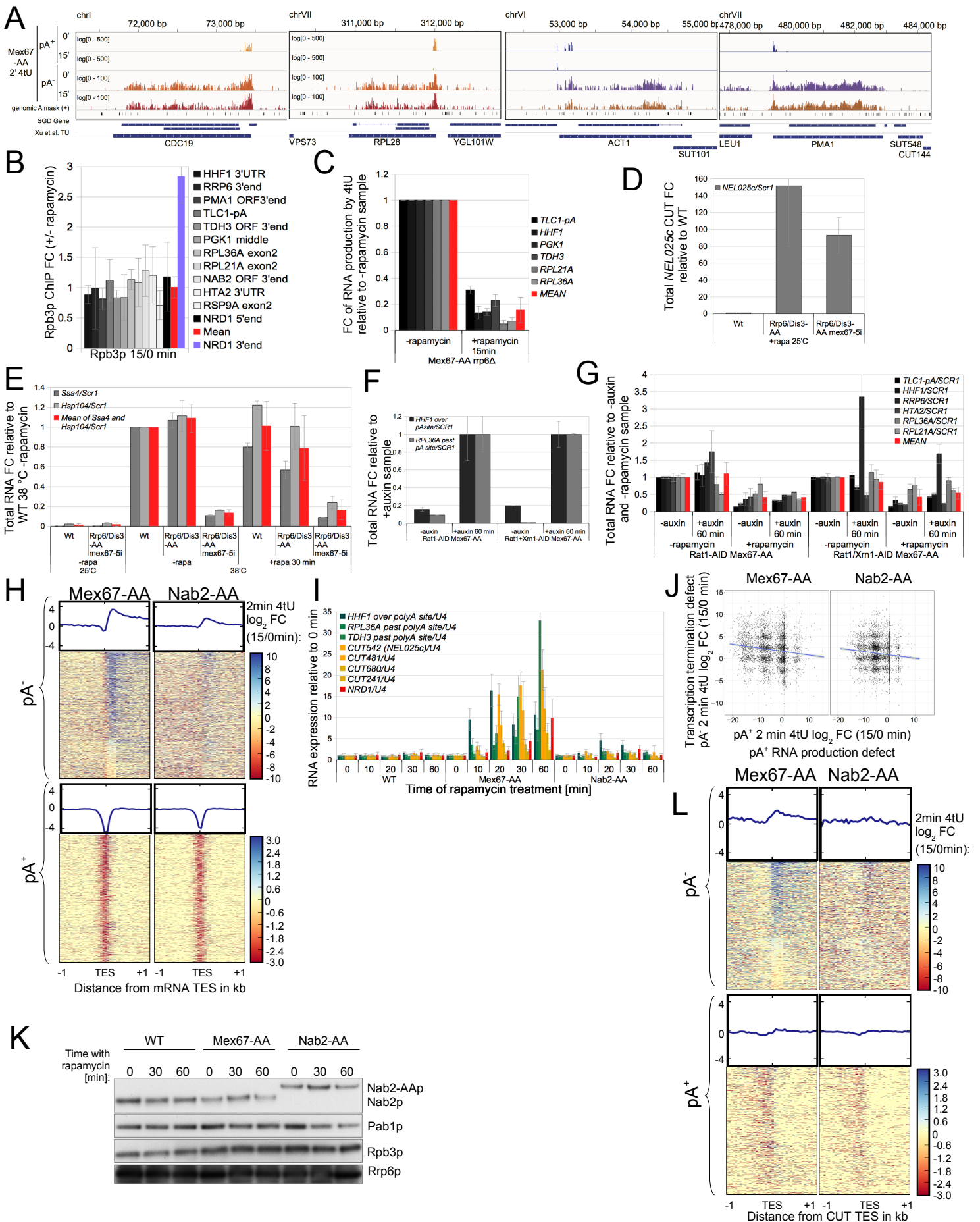
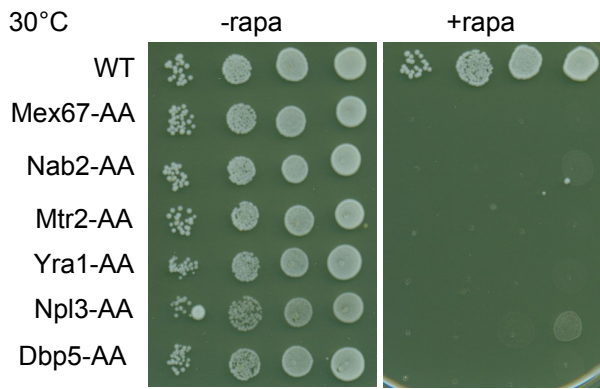


Figure S2. Related to Fig. 2.

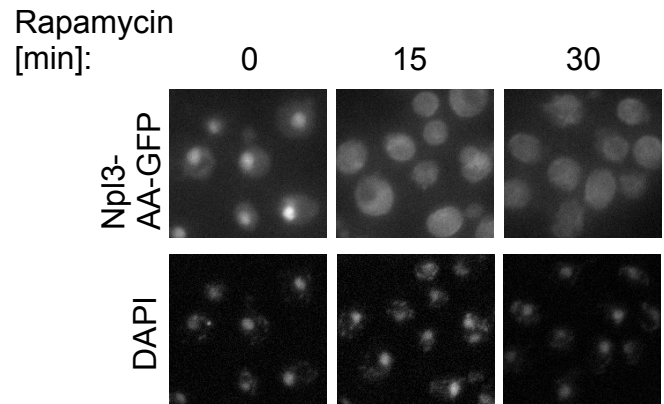
- (A)** Genome browser views of pA⁻ and pA⁺ RNA 3'end reads, derived from 2 min 4tU labeling experiments of the Mex67-AA strain, spanning across the indicated regions of chromosomes I, VI and VII. Depicted as in Fig. 2C.
- (B)** RNAPII ChIP (anti-Rpb3p) analysis of indicated genic regions in Mex67-AA cells treated for 15 min with rapamycin relative to non-rapamycin treated cells and corrected for background. Values and error bars shown for specific loci are mean RNAPII occupancies and standard deviations from three replicate IPs. Value and error bar of mean indicate mean and standard deviation of RNAPII occupancy on all tested loci from the grey series (excluding *NRD1* 3'end; blue series).
- (C)** RT-qPCR analysis of new RNAs from Mex67-AA/*rrp6*Δ cells treated with rapamycin for a total of 15 min and subjected to 10 min of 4tU-labeling. The values displayed are a mean of triplicate IPs, which were background subtracted and normalized to spike-ins and total RNA input. Values are plotted relative to the -rapamycin control. See Fig. 3C for average result in Mex67-AA *RRP6* background.
- (D)** RT-qPCR analysis of *NEL025c* CUT levels from WT, Rrp6-AA/Dis3-AA and Rrp6-AA/Dis3-AA/*mex67-5i* cells after a 30 min rapamycin treatment. Error bars represent standard deviations of PCR technical replicates from one representative experiment.
- (E)** RT-qPCR analysis of fold changes in *SSA4* and *HSP104* RNA levels in cells nuclear-depleted for 30 minutes of Rrp6p and Dis3p, and containing, or not, the *mex67-5i* mutated gene relative to a wild-type strain. Cells were incubated for 12 min at 38 °C. Shown values are means of two independent experiments and error bars represent median absolute deviation.
- (F)** RT-qPCR analysis showing levels of read-through transcripts deriving from impaired transcription termination at *HHF1* and *RPL36A* loci in Rat1-AID/Mex67-AA and Rat1-AID/Xrn1-AID/Mex67-AA cells treated, or not, with auxin for 1 hour. Values represent the means of duplicate samples prepared in parallel and error bars are median absolute deviations.
- (G)** RT-qPCR analysis of indicated transcripts from Rat1-AID/Mex67-AA and Rat1-AID/Xrn1-AID/Mex67-AA cells treated, or not, with auxin for 1 hour prior to a 15 min rapamycin exposure. Values represent means of duplicate samples prepared in parallel and error bars are median absolute deviations. The mean series represents an average of all tested transcripts. For technical reasons the *RRP6* mRNA values was excluded from the mean in Rat1-AID/Xrn1-AID Mex67-AA series.
- (H)** Heat maps and metagene analysis displaying log₂FC densities of 2 min labeled 4tU RNA 1 kb up- and downstream of protein coding TESs in the pA⁻ (top) and pA⁺ (bottom) fractions, between Mex67-AA (left) and Nab2-AA (right) cells treated with rapamycin for 15 min relative to untreated cells. Transcript orders are the same in all heat maps and based on the strength of the termination defect in the Mex67-AA strain, estimated by the total log₂FC of pA⁻ reads in the 250bp downstream the TES.
- (I)** RT-qPCR analysis of selected RNAs from WT, Mex67-AA and Nab2-AA cells subjected to the indicated periods of rapamycin treatments at 30°C. PCR amplicons spanned the regions around or past the pA sites of three mRNAs (green series), the mid regions of four CUTs (orange series) and the 5'end of the *NRD1* RNA (red bar; note a similar result was obtained using a 3'end amplicon (data not shown)) as indicated to the right of the image. Error bars indicate standard deviations from three PCR technical replicates. The result is representative of additional biological repeats.
- (J)** Scatter plot showing the relationship between the strength of the transcription termination defect (y-axis), defined as in Fig. S2H as the mean log₂FC in the region 250bp downstream the TES, and the decrease in RNA pA⁺ 3'ends (x-axis) as mean log₂FC in the region +/-50bp around the TES. Log₂FC values were derived from the 2 min 4tU labeled fractions of Mex67-AA and Nab2-AA cells treated for 15 relative to 0 min rapamycin.
- (K)** Western blotting analysis showing levels of Nab2p (endogenous or AA-tagged), Pab1p, Rpb3p and Rrp6p in the indicated strains and growth conditions.
- (L)** Heat maps and metagene analysis displaying log₂FC of 15/0 min rapamycin ratios in Mex67- (left) and Nab2-AA (right) cells of 2 min labeled 4tU RNA. Shown is the region 1 kb up- and downstream of CUT loci TESs in the pA⁻ (upper) and pA⁺ (lower) fractions. Transcript orders are the same in all heat maps and based on the strength of the termination defect in the Mex67-AA strain, estimated by the total log₂FC of pA⁻ reads in the 250bp downstream the TES.

Figure S3

A



B



C

38°C, 12 min

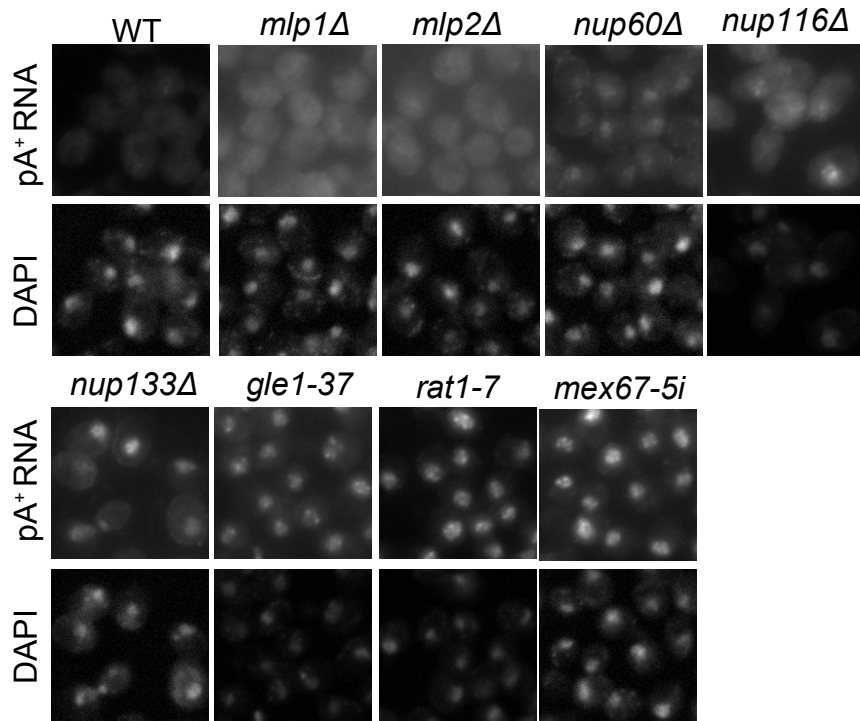


Figure S3. Related to Fig. 3.

(A) Spot tests of 10x serial dilutions of WT, Mex67-, Nab2-, Mtr2- Yra1-, Npl3- and Dbp5-AA cells plated and grown at 30°C without or with rapamycin as indicated.

(B) Sub-cellular localization of GFP-tagged Npl3-AA protein at 0, 15 and 30 min after rapamycin addition. Images were adjusted and stained with DAPI as in Fig. 1A.

(C) FISH analysis of pA+ RNA, as in Fig. 1A, on WT, *mlp1Δ*, *mlp2Δ*, *nup60Δ*, *nup116Δ*, *nup133Δ*, *gle1-37*, *rat7-1* and *mex67-5i* cells subjected to 12 min of incubation at 38°C before fixation. Note that *nup60Δ* is in the BY background as opposed to the remaining strains, which are W303. FISH images were adjusted and DAPI stained as in Fig. 1A.

Figure S4

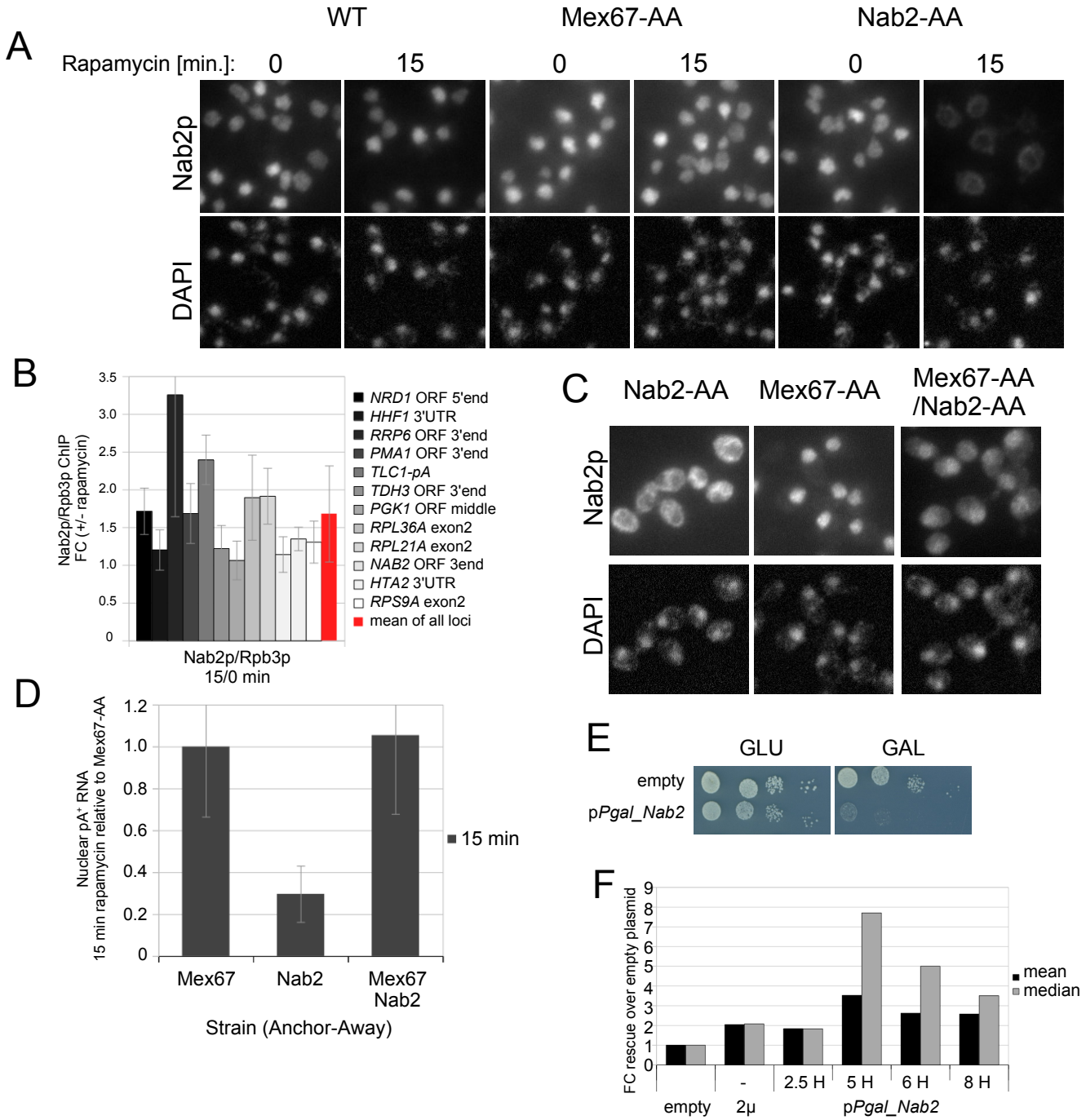


Figure S4. Related to Fig. 4.

- (A)** Immuno-localization of Nab2p without or 15 min after rapamycin treatment of WT, Mex67-AA and Nab2-AA cells as indicated. Images were adjusted and stained with DAPI as in Fig. 1A.
- (B)** ChIP of Nab2p at the indicated genic regions in Mex67-AA cells treated for 0 or 15 min with rapamycin. Values were corrected for mock IPs (no antibody) and are displayed relative to control values and normalized to Rpb3 ChIP signal. The Rpb3p levels are shown in Fig. 2D. Values and error bars shown are mean Nab2p/Rpb3 ratios and standard deviations from three replicate IPs.
- (C)** Immuno-localization of Nab2p in Nab2-, Mex67- and Nab2-/Mex67-AA cells treated with rapamycin for 15 min. Images were adjusted and stained with DAPI as in Fig. 1A.
- (D)** Quantification of nuclear pA⁺ RNA signals from AA cells employed in Fig. S4C. Values are shown relative to mean values of Mex67-AA cells and corrected for background signals. Error bars at individual time points indicate standard deviations calculated from nuclear pA⁺ RNA levels of a minimum of 1000 cells counted for each strain.
- (E)** Spot tests of 10x serial dilutions of the Mex67-AA strain containing empty or *Pgal_Nab2* plasmids on media containing glucose or galactose as indicated.
- (F)** Graph showing the mean and median fold rescue of net RNA production in Mex67-AA depletion conditions with overexpression of Nab2p, using either a 2 μ or a *pPgal_Nab2* plasmid, relative to cells containing an empty plasmid.

Table S1. Related to Figure 1. Overview of RNA-seq libraries.

Sample name (Strain_ fraction_ rapamycin[min]_ repeat)	Raw		Quality filtering	Uniquely mapped reads mapped to <i>S. cerevisiae</i> – <i>S.</i> <i>pombe</i> joint genome		Final <i>S.</i> <i>cerevisiae</i> reads after filtering for genomic A- rich positions
	Reads	Bases	Output reads	Mapped to <i>S. cerevisiae</i>	Mapped to <i>S. pombe</i>	
Nab_in_0_1	7273102	363655100	6979478	5396295	106881	4763184
Nab_in_15_1	3281586	164079300	3089524	2357069	71017	2076661
Nab_in_0_2	7891737	394586850	7538600	5482125	147565	4779771
Nab_in_15_2	7667549	383377450	7317712	5483223	133231	4762969
Nab_in_0_3	8925940	446297000	8624626	6450346	184617	5636310
Nab_in_15_3	8889698	444484900	8587558	6574692	197926	5763307
Nab_in_neg	5723825	286191250	5348882	4077028	109698	3607338
Mex_in_0_1	7452143	372607150	7052033	5942399	137163	5210550
Mex_in_15_1	7029611	351480550	6654170	5541109	176583	4851797
Mex_in_0_2	6075011	303750550	5671419	4707318	112692	4141477
Mex_in_15_2	5530398	276519900	5249025	4402660	81556	3843771
Mex_in_0_3	11875239	593761950	1,10E+007	10113976	235737	9052288
Mex_in_15_3	9698340	484917000	9247959	8171197	273885	7285130
Mex_in_neg	6039488	301974400	5824464	5261497	72637	4687659
Nab_ip_0_1	7538811	376940550	6704203	5876655	192255	5137191
Nab_ip_15_1	5565816	278290800	4908625	3924799	390638	3346347
Nab_ip_0_2	5684152	284207600	5308706	4597870	193963	3982006
Nab_ip_15_2	5152085	257604250	4487987	3665898	260786	3105436
Nab_ip_0_3	6918132	345906600	6731904	5694365	284033	4916991
Nab_ip_15_3	5857971	292898550	5234941	4191233	411475	3565773
Nab_ip_neg	9371130	468556500	8990840	5521766	1494515	4786756
Mex_ip_0_1	8246616	412330800	7843586	7150452	165124	6143287
Mex_ip_15_1	6647995	332399750	6077795	4930616	508465	3982991
Mex_ip_0_2	8144473	407223650	7785564	7054968	196736	6060297
Mex_ip_15_2	5758583	287929150	5091018	4323077	256616	3535866
Mex_ip_0_3	6176401	308820050	5675320	5173699	138931	4553450
Mex_ip_15_3	5313483	265674150	4598710	3742082	431698	3114382
Mex_ip_neg	7863817	393190850	7409785	5603811	851599	4910928

Table S2. Related to Figure 2. Overview of RNA-seq libraries.

Sample name (Papreatment_ Strain_ fraction_ rapamycin[min])_ repeat)	Raw		Quality filtering	Uniquely mapped reads mapped to <i>S. cerevisiae</i> – <i>S. pombe</i> joint genome		Final <i>S.</i> <i>cerevisiae</i> reads after filtering for genomic A- rich positions
	Reads	Bases	Output reads	Mapped to <i>S.</i> <i>cerevisiae</i>	Mapped to <i>S. pombe</i>	
noPap_Nab2AA_input_0_1	3334641	166732050	3282063	2595050	42095	2313940
noPap_Nab2AA_input_15_1	4873541	243677050	4804672	3634113	74302	3235115
noPap_Mex67AA_input_0_1	3054292	152714600	3002568	2467734	41709	2202503
noPap_Mex67AA_input_15_1	4199856	209992800	4155534	3352405	74880	2963985
noPap_Nab2AA_ip_0_1	3056716	152835800	2929431	2015728	377298	1769572
noPap_Nab2AA_ip_15_1	3666193	183309650	3552089	2195539	591965	1905171
noPap_Mex67AA_ip_neg0_1	5253477	262673850	5060704	3517377	795545	3116325
noPap_Mex67AA_ip_0_1	4038165	201908250	3883937	2949235	456822	2579454
noPap_Mex67AA_ip_15_1	2973551	148677550	2929123	2110422	519671	1837564
xPap_Nab2AA_input_0_1	5162819	258140950	4787399	3944905	40380	3822077
xPap_Nab2AA_input_15_1	4548001	227400050	4315658	3626890	35202	3530828
xPap_Mex67AA_input_0_1	4916981	245849050	4442576	3781260	35565	3654903
xPap_Mex67AA_input_15_1	6722820	336141000	6608844	5519178	59857	5390170
xPap_Nab2AA_ip_0_1	3933492	196674600	3394680	2057164	434311	1888025
xPap_Nab2AA_ip_0_2	4866432	243321600	4448755	3040507	192401	2815418
xPap_Nab2AA_ip_0_3	4189502	209475100	3675178	2446311	163220	2214857
xPap_Nab2AA_ip_15_1	5306967	265348350	4974073	2196866	138905	1901498
xPap_Nab2AA_ip_15_2	4571542	228577100	4215557	2547243	160215	2208254
xPap_Nab2AA_ip_15_3	3587820	179391000	3279510	2104394	144518	1902227
xPap_Mex67AA_ip_neg0_1	5649907	282495350	5045569	3579615	435360	3153307
xPap_Mex67AA_ip_0_1	4690443	234522150	4153541	2855569	151361	2648241
xPap_Mex67AA_ip_0_2	5035947	251797350	4645361	3156133	175134	3022976
xPap_Mex67AA_ip_0_3	3847037	192351850	3658720	2459241	132147	2310471
xPap_Mex67AA_ip_15_1	4738414	236920700	4308097	3044177	174875	2904716
xPap_Mex67AA_ip_15_2	6222728	311136400	5579940	3668144	213321	3379732
xPap_Mex67AA_ip_15_3	4208805	210440250	3326805	2260905	140711	2072147

Table S3. Related to Key Resource Table. Oligonucleotides used in this study.

Oligonucleotide name and sequence	Source	Cat. no.
Act1_S.pombe_fwd CCATTCTTGCTTCTCTTTCTACTTTCC	Sigma	custom-made
Act1_S.pombe_rev CGCTCTCATCATACTCTTGCT	Sigma	custom-made
CUT241_fwd ACCAACATAAGATATAGCTAGCAATTC	Sigma	custom-made
CUT241_rev CTCTCCAAAACAGACCAAACA	Sigma	custom-made
CUT481_fwd AGGTCAATAACGCAGACACA	Sigma	custom-made
CUT481_rev TGCTGAATCTCCAACGCT	Sigma	custom-made
CUT542 (NEL025c)_fwd ACTCTGCGATCCAAATTCTACT	Sigma	custom-made
CUT542 (NEL025c)_rev ATGCGTCTTTCCTGTTTATGAG	Sigma	custom-made
CUT680_fwd CTTTTGTTTGTGGTTGTTGTCG	Sigma	custom-made
CUT680_rev ATCGTCTTTCCTAATGGTCTTG	Sigma	custom-made
DT18 TTTTTTTTTTTTTTTTTT	Sigma	custom-made
dT18 LNA (FISH probe) TTTTTTTTTTTTTTTTTT	Thomsen et al., 2005	N/A
Hhf1_fwd ACTGCCCGTTTTTCTTCT	Sigma	custom-made
Hhf1_rev CCTAAACCCGCTATAATACACTCAT	Sigma	custom-made
Hhf1_past_polyAsite_rev TCCCTATTCCATGCAAGTTC	Sigma	custom-made
Hsp104_3'end_fwd AGCTGAAGAATGTCTGGAAGT	Sigma	custom-made
Hsp104_3'end_rev CGTCATCACCTAACGTGTCA	Sigma	custom-made
Hta2_fwd GTCATTTGGGGTTTTAAAGTAGGT	Sigma	custom-made
Hta2_rev ACCGTAAACTTGATACGTTTTTTATTTT	Sigma	custom-made
Nrd1_5'end_fwd CATAATGCAGCAGGACGACG	Sigma	custom-made
Nrd1_5'end_rev TGTGATCAAGTGCCTAAGTG	Sigma	custom-made
Nrd1_3'end_fwd AGGAGATGCCAATGGTGC	Sigma	custom-made
Nrd1_3'end_rev GGTAATGGTTGGTTGGGGG	Sigma	custom-made
Pgk1_fwd AGGTCGATGGTCAAAGG	Sigma	custom-made
Pgk1_rev CGGCAGCACGTTGT	Sigma	custom-made
Pma1_fwd TACTGTGTCCTCGTCTGGATCT	Sigma	custom-made
Pma1_rev CCTTCATTGGCTTACCGTTCA	Sigma	custom-made
random hexamers	Invitrogen	48190-011
Rpl21A_exon2_fwd ACAGATCTCGTACACGTTACA	Sigma	custom-made
Rpl21A_exon2_rev CGACAATGTCACCAACCT	Sigma	custom-made
Rpl36A_exon2_fwd AAGGTAAGAAGGTCACTAGCA	Sigma	custom-made
Rpl36A_exon2_rev GTTGAAGCAGCACCTTT	Sigma	custom-made
Rpl36A_pastPolyAsite_fwd GCCCATGTACTGAGCTGATAG	Sigma	custom-made
Rpl36A_pastPolyAsite_rev CGGGTAACTTATATTGCTTCAG	Sigma	custom-made
Rrp6_fwd ACGGAAAAAGATGCTGTGGATT	Sigma	custom-made
Rrp6_rev CTTTTTAGCTGCCCTTGGT	Sigma	custom-made
Rrp47_fwd AACCAATTCGAGCCCTCTAT	Sigma	custom-made
Rrp47_rev CTTCTCCCTCCTTTCTTTTTTCC	Sigma	custom-made
Scr1_fwd CCCGGCTATAATAAATCGATCT	Sigma	custom-made
Scr1_rev GCTGACGCTGGATAAAACT	Sigma	custom-made
Ssa4_fwd AAATTGTACTTGTGGTGGTTCA	Sigma	custom-made
Ssa4_rev GGGTTAATCGAACGGTTTGG	Sigma	custom-made
Tdh3_fwd CTCTCACTCTCCATCTTCGAT	Sigma	custom-made
Tdh3_rev CGTACCAGGAGACCAACTT	Sigma	custom-made
Tdh3_pastPolyAsite_fwd CTTGATGCGCTATTGCATTG	Sigma	custom-made
Tdh3_pastPolyAsite_rev TGTATCAGGTATCTACTACAG	Sigma	custom-made
Tlc1_pA_fwd GCCTTCGATGCATTTAGATAATTTT	Sigma	custom-made
Tlc1_pA_rev ACGCGCGATTTCTACAATAC	Sigma	custom-made
U4_fwd AGTAACCCTTCGTGGACAT	Sigma	custom-made
U4_rev AGGGGAACGCTGATCAT	Sigma	custom-made