Appendix

Nuclear Pore Complex Acetylation Regulates mRNA Export and Cell Cycle Commitment in Budding Yeast

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Appendix Figure S1.

*nup60-KN* mutation partially rescues the delay in synthesis of the G1/S cyclin Cln2 in *esa1-ts* cells.

Independent biological replicates of the experiment shown in Figure 2D. Cells of the indicated strains were arrested in G1 by treatment with α-factor for 2.5 h at 25 °C, shifted to 37 °C for 1 h and released from the G1 arrest at 37 °C. Samples for total protein extracts were collected at the indicated times after α-factor washout and the amount of Cln2-HA protein was assessed by western blot. G6PDH was used as loading control.
Appendix Figure S2.

**Whi5-mCherry nuclear export and budding for cells in Figure 2E.**

Whi5-mCherry nuclear export was scored in the fluorescence channel, and budding was scored in bright-field images (maximum projections of 3 z-confocal slices spaced 0.5 µm).
Appendix Figure S3.

Overexpression of the TREX-2 complex component Sac3 rescues the toxicity of HOS3-NLS overexpression.

(A) Toxicity of full-length SAC3 overexpression. 10-fold serial dilutions of wild-type (WT), HOS3-NLS-GFP and GAL1pr:HOS3-NLS-GFP cultures transformed with an empty vector or the GAL1pr:SAC3-HA plasmid were spotted onto SC-Glu and SC-Gal medium and incubated at 25 °C for 3 days.

(B) List of high-copy (2µ) plasmids from a tiling genome library (Jones et al., 2008) containing TREX-2 complex genes (SAC3, SUS1, CDC31, SEM1 and THP1) together with
neighboring genes. Asterisks (*) indicate that the corresponding ORFs are incomplete. Nucleotides of \( SAC3 \) (full length 3905 nt) included in each plasmid are indicated.

(C) A high-copy plasmid containing \( SAC3(464-3905) \) is not toxic and relieves the toxicity of \( HOS3-NLS \) over-expression. 10-fold serial dilutions of wild-type (\( WT \)) and \( GAL1pr:HOS3-NLS-GFP \) cultures, transformed with an empty vector or the indicated multicopy plasmids, were spotted onto SC-Glu and SC-Gal medium and incubated at 25 °C for 3 days. Note that \( SAC3(464-3905) \) rescues growth of \( GAL1pr:HOS3-NLS \) but that the overlapping plasmid \( SAC3(3860-3905) \), lacking all of \( SAC3 \) ORF but 45 nucleotides at its 3’, does not. The “vector” and \( SAC3(464-3905) \) sections of the left image are also shown in Figure 3C for simplicity.

(D) Overexpression of \( MEX67 \) and \( MTR2 \) does not rescue the growth defect of \( esa1-ts \) and \( esa1-ts \) \( gcn5Δ \) cells. 10-fold serial dilutions of the indicated strains transformed with the indicated plasmids spotted onto SC-Glu and SC-Gal medium. Due to poor growth at 37 °C on SC-gal, plates were pre-incubated for 24 or 48h at 25 °C and later incubated at indicated temperatures for up to 5 days from plating.
Appendix Figure S4.

Hos3-NLS overexpression or depletion of Esa1 impairs export of poly(A) RNA.

(A) Overexpression of Hos3-NLS promotes nuclear accumulation of mRNA. Cultures of the indicated strains were treated with β-estradiol (90 nM) to induce Hos3-NLS. After induction overnight, cells were fixed and FISH was performed using a Cy3-Oligo(dT) probe. Arrows point to polyadenylated RNA in the nucleus, which was visualized by DAPI staining (left). The fraction of cells with nuclear mRNA accumulation was determined for the indicated strains and conditions (right).

(B) Representative images of cells processed for poly(A) FISH as in (A) after incubation in the indicated conditions. Associated with Figure 3E.
Appendix Figure S5.

Depletion of Esa1 or Gcn5 does not affect export of rRNA.

(A) Wild-type, gcn5Δ, esa1-ts and gcn5Δ esa1-ts cultures were incubated at 25°C or 37°C at the indicated times. In all cases, cells were fixed and in situ hybridization was performed using Cy3-TXGTTCCCTCGTTAAGGXATTTACATTGTXCC-Cy3 to target 18S rRNA and monitor ribosomal 40S subunit nucleo/cytoplasmic distribution. DNA was visualized by DAPI staining. Cells from stationary cultures exposed to heat shock during 4h were used as positive control for nuclear accumulation of 18S rRNA.

(B) Wild-type, gcn5Δ, esa1-ts and gcn5Δ esa1-ts cultures transformed with an Rpl25-GFP plasmid as a reporter for ribosomal 60S subunit nucleo/cytoplasmic distribution were incubated at 25°C or 37°C for 7h and 30 min and imaged at the indicated conditions using fluorescence microscopy.
Appendix Figure S6.

Sac3 protein levels are not significantly affected by HOS3 deletion, nup60-KN mutation and Esa1 inactivation.

Cells of the indicated strains were grown at 25 °C (and shifted to 37 °C for 2 h when indicated), and then collected for total protein extraction. Experiment was repeated three times, the result of one representative western blot is shown (left). The amount of Sac3-GFP protein was assessed by western blot, normalized to Cdc28 (PSTAIR) and further normalized to the WT at the corresponding temperature (right). Exact p-values from one sample t-test are given.
Appendix Figure S7.

Bud emergence defects in the mex67-5 thermosensitive mutant.

(A) Bright field (BF) images of wild type (WT) and mex67-5 cells at the indicated times after the α-factor washout. Cells were arrested in G1 by treatment with α-factor for 2.5 h at 25 °C, shifted to 37 °C for 1 h and released from the G1 arrest at 37 °C. The DNA was visualized by DAPI staining.

(B) Cells were fixed at the indicated times and the presence of buds was assessed by microscopy. At least 200 cells were scored for each strain and time point.
Appendix Figure S8.

Daughter-cell specific Nup60 deacetylation inhibits GAL1 expression.

*(Top)* The GAL1pr:sfGFP reporter was integrated on Chr. II between the GAL1 and FUR4 loci. *(Bottom)* Time Lapse microscopy of WT, hos3Δ, nup60-KN hos3Δ and nup60-KN cells expressing GAL1pr:sfGFP and Nup60-mCherry at the indicated times of galactose induction. Scale bar, 4 µm.
Appendix Figure S9.

Nup60 protein levels upon galactose induction of GAL1pr: sfGFP are not changed by the acetyl-mimic allele of Nup60 (nup60-KN) or Hos3 and Sac3 anchoring to NPCs. (A) Cultures of WT, hos3Δ, nup60-KN and nup60-KN hos3Δ were shifted to galactose and imaged by Time Lapse microscopy to monitor Nup60-mCherry fluorescence during 7 hours of galactose induction of GAL1pr: sfGFP expression. Nuclear fluorescence was scored by segmentation of the nuclear area in the mCherry channel and total fluorescence of Nup60-mCherry was quantified as in Figure 6B. At least 200 cells were scored for each strain and time point. Shaded areas indicate the SEM. (B) Cells expressing either Nup60-mCherry-FKBP GAL1pr: sfGFP or Nup60-mCherry-FKBP Sac3-FRB GAL1pr: sfGFP were incubated with rapamycin for FRB-FKBP heterodimerization or DMSO as control as in Figure 7A. Cells were imaged upon rapamycin and galactose addition and the Nup60-mCherry fluorescence over time was monitored as in A.
Appendix Figure S10.

The β-estradiol-dependent GAL4-VP16 transactivator does not increase the perinuclear localization of GAL1,10 locus.

GAL1 locus localization in cells incubated with 2% glucose (repression), or 30 minutes after addition of 2% galactose or 90 μM β-estradiol (induction). Localisation was scored by time-lapse microscopy of GAL10::LacO cells expressing LacI-GFP and the β-estradiol-dependent transactivator (GEV). Gene localization was scored as “perinuclear” (arrows) when the nuclear focus was in contact with the nuclear periphery signal (Nup49-GFP). n indicates the number of cells scored, which were pooled from two independent experiments.
Appendix Figure S11.

Independent biological replicate of the microfluidics time lapse microscopy experiment shown in Figure 5E.

(A) GFP images, and composite of bright field and GFP, from time-lapse microscopy of WT, nup60-KN and hos3A cells expressing GAL1pr:sfGFP at the indicated times after addition of galactose (left panels) or β-estradiol (right panels). Scale bar, 4 µm.

(B) sfGFP expression of WT, hos3A and nup60-KN strain after switching to 2% galactose
(top) or estradiol (bottom) containing media was monitored. Mean intensity of the sfGFP signal quantified from sum projections for each strain (WT, hos3Δ and WT, nup60-KN – left and right panel correspondingly) and the difference of mean intensity in between the strains (Δ WT-hos3Δ and Δ WT-nup60-KN) is displayed. At least 450 cells have been quantified for each strain and time point. Shaded areas indicate the SEM.
Appendix Figure S12.
Mother/daughter pairs were quantified as in Figure 7B at 200 min after galactose addition.
Boxes include 50% of data points, the line represents the median and whiskers extend to maximum and minimum values. ***, p ≤ 0.001; **, p ≤ 0.01; *, p ≤ 0.05; ns, p > 0.05, two-tailed paired t-test for M-D comparisons, unpaired for comparisons between strains.
Appendix Figure S13.
Gating FACS strategy used in Figure 1D.
Shown are wild-type cells 240 minutes after the release from alpha factor block.
Appendix Table S1.

*Saccharomyces cerevisiae* strains used in this work.

| Name     | Strain          | Genotype                                                                 | Genetic background | Source               |
|----------|-----------------|---------------------------------------------------------------------------|--------------------|----------------------|
| YMM1     | wild type (WT)  | MATα ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63                       | S288c              |                      |
| YMM5088  | wild type (WT)  | MATα his3 leu2 met15 ura3                                                 | BY4741             |                      |
| YMM5737  | gen5A           | MATα ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 gen5Δ::kanMX6         | S288c              | This study           |
| YMM5671  | esa1-ts         | MATα ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 esa1-L254P::KANMX      | S288c              | This study           |
| YMM5686  | gen5A esa1-ts   | MATα ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 esa1-L254P::KANMX gen5Δ::kanMX6 | S288c              | This study           |
| YMM2936  | *HOS3*-NLS-GFP  | MATα ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63                      | S288c              | Kumar et al., 2018   |
| YMM3073  | GAL1pr:*HOS3*-NLS-GFP MYO1-mCherry | MATα ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 natNT2::GAL1pr:*HOS3*-NLS-GFP::KAN MYO1-mCherry::hphNT1 | S288c              | Kumar et al., 2018   |
| YMM5121  | GAL1pr:*HOS3*(EN)-NLS-GFP | ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 natNT2::GAL1pr:*HOS3*(EN)(H196E D231A)-NLS-GFP::KAN | S288c              | This study           |
| YMM5123  | GAL1pr:*HOS3*(EN)-NLS-GFP | ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 natNT2::GAL1pr:*HOS3*(EN)(H196E D231A)-NLS-GFP::KAN | S288c              | This study           |
| YMM3861  | GAL1pr:*HOS3*-NLS-GFP MYO1-mCherry ADGEV | MATα ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 natNT2::GAL1pr:*HOS3*-NLS-GFP::KAN MYO1-mCherry::hphNT1 ADHpr::GAL4-ER-VP16::URA3 (ADGEV) | S288c              | Kumar et al., 2018   |
| YMM5761  | NUP60-GFP       | MATα ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 NUP60-GFP::HIS3MX6    | S288c              | This study           |
| YMM5763  | nup60-KN-GFP    | MATα ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 nup60(K467N)-GFP::HIS3MX6 | S288c              | This study           |
| YMM5769  | esa1-ts NUP60-GFP | ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 NUP60-GFP::HIS3MX6 esal-L254P::kanMX | S288c              | This study           |
| YMM5771  | esa1-ts nup60-KN-GFP | ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 nup60(K467N)-GFP::HIS3MX6 esal-L254P::kanMX | S288c              | This study           |
| YMM5027  | CLN2-HA         | MATα ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 CLN2-6xHA::HIS3        | S288c              | This study           |
| JCY2452  | esa1-ts NUP60-GFP CLN2-HA | MATα ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 NUP60-GFP::HIS3MX6 esal-L254P::kanMX CLN2-6xHA::hphNT1 | S288c              | This study           |
| YMM5844 | SAC3-mCherry-FKB P NUP60-FRB WHIS-GFP esa1-ts | MATa his3Δ1 leu2Δ0 ura3Δ0 LYS+, Can1::Ste2pr-Leu2, Lypl:: tor1-1, Fpr1::Ura SAC3-mCherry-FKBP::natNT2 NUP60-FRB::hphNT1 esa1-L254P::kanMX WHIS-GFP::HIS3MX6 | BY4742 This study |
| YMM5848 | SAC3-mCherry-FKB P NUP60-FRB WHIS-GFP | MATa his3Δ1 leu2Δ0 ura3Δ0 LYS+, Can1::Ste2pr-Leu2, Lypl:: tor1-1, Fpr1::Ura SAC3-mCherry-FKBP::natNT2N2 NUP60-FRB::hphNT1 WHIS-GFP::HIS3MX6 | BY4742 This study |
| YMM5850 | NUP60-GFP WHIS-mCherry | MATa ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trplΔ63 NUP60-GFP::HIS3MX6 WHIS-mCherry::hphNT1 | S288c This study |
| YMM5854 | NUP60-GFP WHIS-mCherry esa1-ts | MATa ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trplΔ63 esa1-L254P::kanMX NUP60-GFP::HIS3MX6 WHIS-mCherry::hphNT1 | S288c This study |
| YMM5860 | nup60-KN-GFP WHIS-mCherry esa1-ts | MATa ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trplΔ63 nup60(K467N)-GFP::HIS3MX6 WHIS-mCherry::hphNT1 esa1-L254P::kanMX | S288c This study |
| YMM5773 | gcg5Δ esa1-ts NUP60-GFP | MATa ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trplΔ63 NUP60-GFP::HIS3MX6 esa1-L254P::kanMX gcg5Δ::kanMX | S288c This study |
| YMM5775 | gcg5Δ esa1-ts nup60-KN-GFP | MATa ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trplΔ63 nup60-KN-GFP::HIS3MX6 esa1-L254P::kanMX gcg5Δ::kanMX | S288c This study |
| YMM5019 | CLN2-PP7 NLS-PCP-EGFP WHIS-tdTomato | MATa, ADE2, leu2-3, ura3, Lys2-1, His3-11,15, can1-100, psi¹, WHIS-tdTomato::KAN, URA::NAT::pCYC1-NLS-PCP-GFP-ADH1ter m::ura3 CLN2-24xPP7SL::loxP | W303 Neurohr et al., 2018 |
| YMM6020 | CLN2-PP7 NLS-PCP-EGFP WHIS-tdTomato NUP60-6xHA | MATa, ADE2, leu2-3, ura3, Lys2-1, His3-11,15, can1-100, psi¹, WHIS-tdTomato::KAN, URA::NAT::pCYC1-NLS-PCP-GFP-ADH1ter m::ura3 CLN2-24xPP7SL::loxP NUP60-6xHA::HIS3MX6 | W303 This study |
| YMM6022 | CLN2-PP7 NLS-PCP-EGFP WHIS-tdTomato NUP60-6xHA esa1-ts | MATa, ADE2, leu2-3, ura3, Lys2-1, His3-11,15, can1-100, psi¹, WHIS-tdTomato::KAN, URA::NAT::pCYC1-NLS-PCP-GFP-ADH1ter m::ura3 CLN2-24xPP7SL::loxP NUP60-6xHA::HIS3MX6 esa1-ts::hphNT1 | W303 This study |
| YMM6026 | CLN2-PP7 NLS-PCP-EGFP WHIS-tdTomato nup60-KN-6xHA esa1-ts | MATa, ADE2, leu2-3, ura3, Lys2-1, His3-11,15, can1-100, psi¹, WHIS-tdTomato::KAN, URA::NAT::pCYC1-NLS-PCP-GFP-ADH1ter m::ura3 CLN2-24xPP7SL::loxP nup60(K467N)-6xHA::HIS3MX6 esa1-ts::hphNT1 | W303 This study |
| YMM6077 | NUP60-GFP | MATa ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trplΔ63 NUP60-GFP::HIS3MX6 | S288c This study |
| Code     | Description              | Strain Details                                                                 | Reference |
|----------|--------------------------|-------------------------------------------------------------------------------|-----------|
| YMM6081  | nup60-KR-GFP             | MATa ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 nup60(K467R)-GFP::HIS3MX6 | S288c     |
| YMM6085  | NUP60-GFP esa1-ts        | MATa ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 NUP60-GFP::HIS3MX6 esa1-ts::kanMX | S288c     |
| YMM6087  | nup60-KR-GFP esa1-ts     | MATa ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 nup60(K467R)-GFP::HIS3MX6 esa1-ts::kanMX | S288c     |
| YMM6070  | hat1Δ                    | MATa ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 hat1Δ::natNT2             | S288c     |
| YMM6072  | gcn5Δ hat1Δ             | MATa ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 gcn5Δ::kanMX6 hat1Δ::natNT2 | S288c     |
| YMM6070  | esa1-ts hat1Δ            | MATa ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 hat1Δ::natNT2 esa1-ts::kanMX | S288c     |
| YMM3836  | Gal10-LacO LacI-GFP NUP49-GFP | MATa ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 LacI-GFP::HIS Gal10-LacO::TRP NUP49-GFP | W303      |
| YMM6104  | Gal10-LacO LacI-GFP NUP49-GFP Gal4-ER-VP16 (ADEGV) | MATa ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 LacI-GFP::HIS Gal10-LacO::TRP NUP49-GFP Adh1pr-Gal4-ER-VP16::URA | W303      |
| YMM6101  | Gal4-ER-VP16 (ADE GV)   | MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 Gal4-ER-VP16::ADE | W303      |
| YMM6140  | sfGFP-CLN2PEST Nup60-mCherry Gal4-ER-VP16 (ADE GV) | MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 GAL1pr: sfGFP-CLN2PEST::KAN Nup60mCherry:: hphNT1 Gal4-ER-VP16::ADE | W303      |
| YMM6142  | sfGFP-CLN2PEST Nup60KN-mCherry Gal4-ER-VP16 (ADE GV) | MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 GAL1pr: sfGFP-CLN2PEST::KAN Nup60(K467N)mCherry:: hphNT1 Gal4-ER-VP16::ADE | W303      |
| YMM6144  | sfGFP-CLN2PEST hos3Δ Nup60-mCherry Gal4-ER-VP16 (ADE GV) | MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 hos3Δ::natNT2 GAL1pr: sfGFP-CLN2PEST::KAN Nup60mCherry:: hphNT1 Gal4-ER-VP16::ADE | W303      |
| YMM6138  | NUP60-GFP gcn5Δ          | MATa ura3-52 his3Δ200 leu2 lys2-801 ade2-101 trp1Δ63 NUP60-GFP::HIS3 gcn5Δ::kanMX6 | S288c     |