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Supplemental information

Regulation of translation

by methylation multiplicity of 18S rRNA

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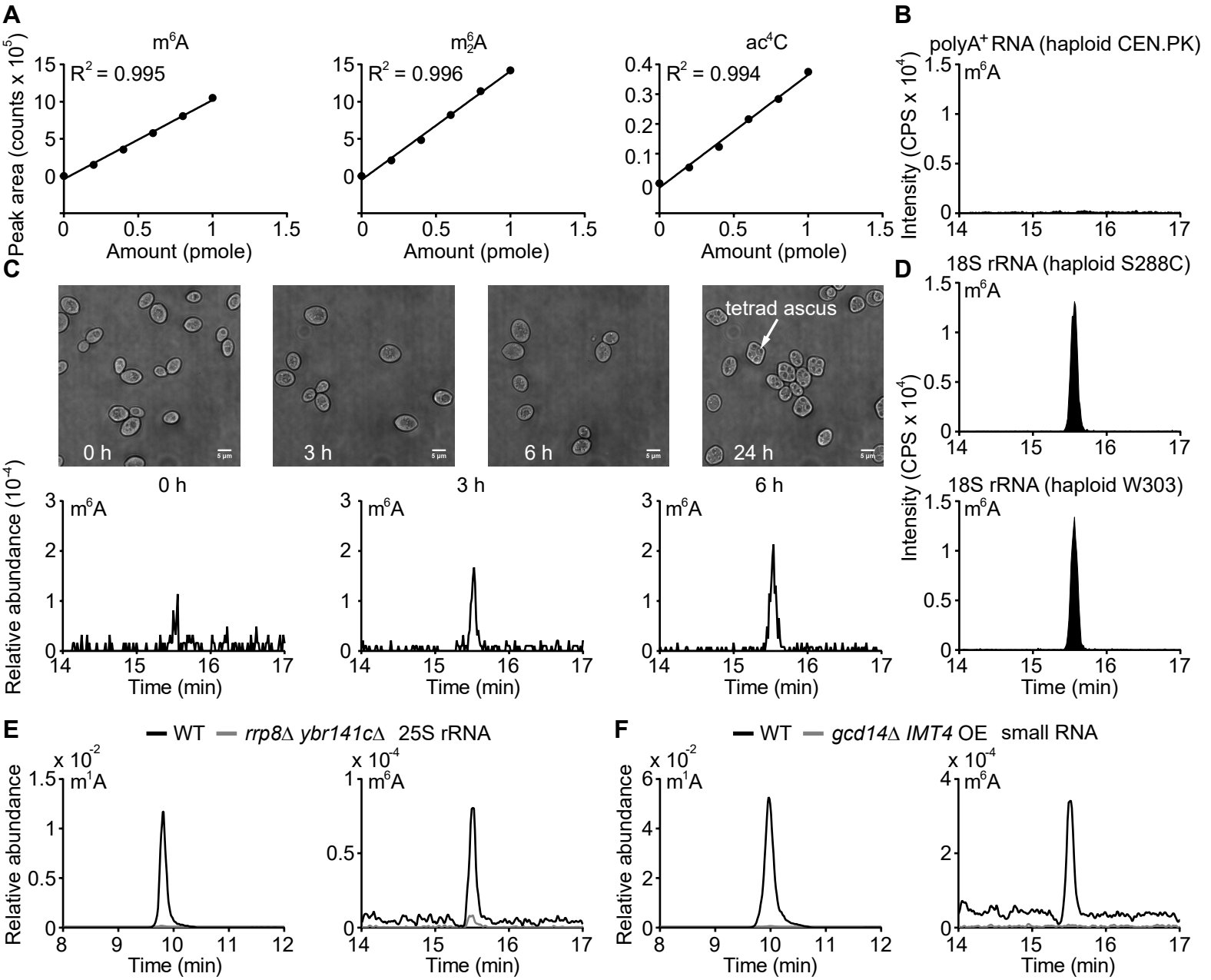


Figure S1. Detection of m^{6}A in 18S rRNA from vegetatively growing haploid yeast cells. Related to Figure 1.
(A) Standard curves for m^{6}A, m$_2^6$A, and ac^{4}C. **(B)** m^{6}A is not detected in polyA$^+$ RNA from vegetatively growing haploid yeast (CEN.PK). **(C)** m^{6}A levels in polyA$^+$ RNA increase during sporulation. KL139 (CEN.PK diploid) was induced to sporulate as described exactly by Agarwala et al. (Agarwala et al. 2012). Samples were collected, immediately (0 h), three (3 h), and six hours (6 h) after cells were resuspended in sporulation medium (0.3% potassium acetate). Total RNA was isolated as described in STAR METHODS and polyA$^+$ RNA was purified from total RNA using the Dynabeads mRNA kit (Invitrogen). Approximately 1 µg polyA$^+$ RNA was digested and analyzed by LC-MS/MS. Each chromatogram was normalized by the abundance of adenosine to allow for comparison between time points. Scale bar = 5 µm. **(D)** m^{6}A is detected in 18S rRNA isolated from vegetatively growing haploid yeast cells (strains: S288C and W303). m^{6}A detected in 25S **(E)** and small RNA **(F)** is likely derived from m^{1}A via Dimroth rearrangement. OE: overexpression. m^{1}A and m^{6}A can be detected by the same MRM transition (282/150), but are eluted at different times. Each chromatogram was normalized by the abundance of adenosine to allow for comparison between different conditions. The essential tRNA m^{1}A methyltransferase Gcd14p becomes dispensable when the initiator methionyl tRNA gene *IMT4* is overexpressed (Calvo O, et al. 1999).

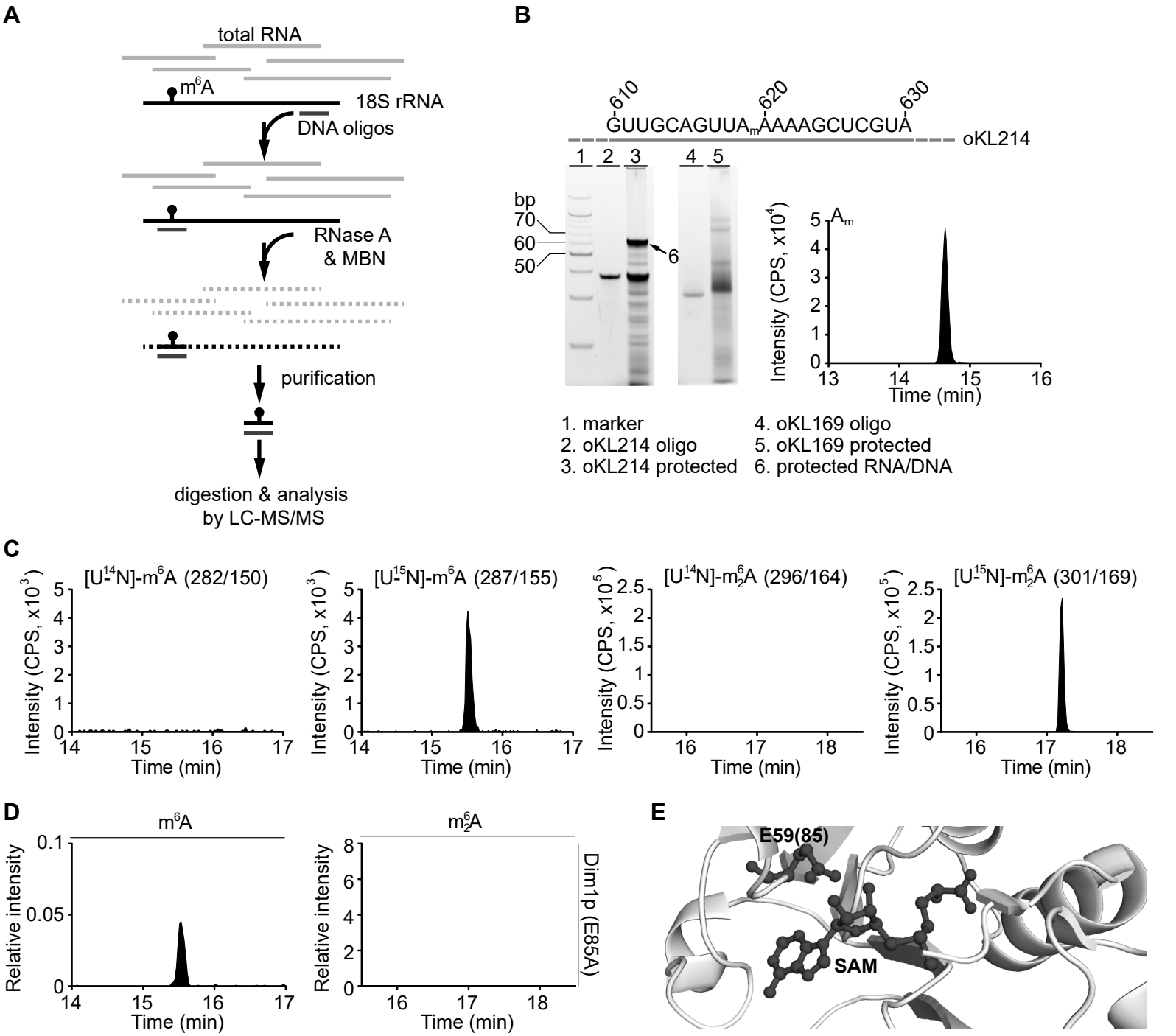


Figure S2. Validation of m^{6}A in yeast 18S rRNA. Related to Figure 1.
(A) Schematic of the mung bean nuclease (MBN) protection assay. **(B)** Detection of the expected 2'-O-methyladenosine(A_m) at 619 in 18S rRNA. oKL169 is not complimentary to any yeast RNA and thus did not yield a RNA/DNA hybrid after digestion by RNase A and MBN. **(C)** m^{6}A and m$^6_2$A are derived from growing yeast cells, not from contamination during sample processing. WT cells were grown from a single colony in [^{15}N] SD (1.7 g L^{-1} yeast nitrogen base without ammonium sulfate and amino acids (BD Difco), 2% glucose, and 5 g L^{-1}(^{15}NH$_4$)$_2$SO$_4$ (ISOTEC). Cells were diluted in the same medium with a starting OD_{600} ~0.01 and grown to saturation. Cells were then diluted in the same medium with a starting OD_{600} ~0.1 and grown to log phase before harvest. Total RNA was isolated and the 3' fragment of 18S rRNA was isolated using the MBN protection assay with oKL204 and analyzed by LC-MS/MS.
(D) The Dim1p (E85A) mutant is still able to synthesize m^{6}A, but not m$^6_2$A. **(E)** Active site of *Methanocaldococcus jannaschi* Dim1. The structure was adapted from PDB#3GRY (O'Farrell et al. 2010) and prepared using Pymol (https://pymol.org). The number in parentheses corresponds to the position in *S. cerevisiae* Dim1p.

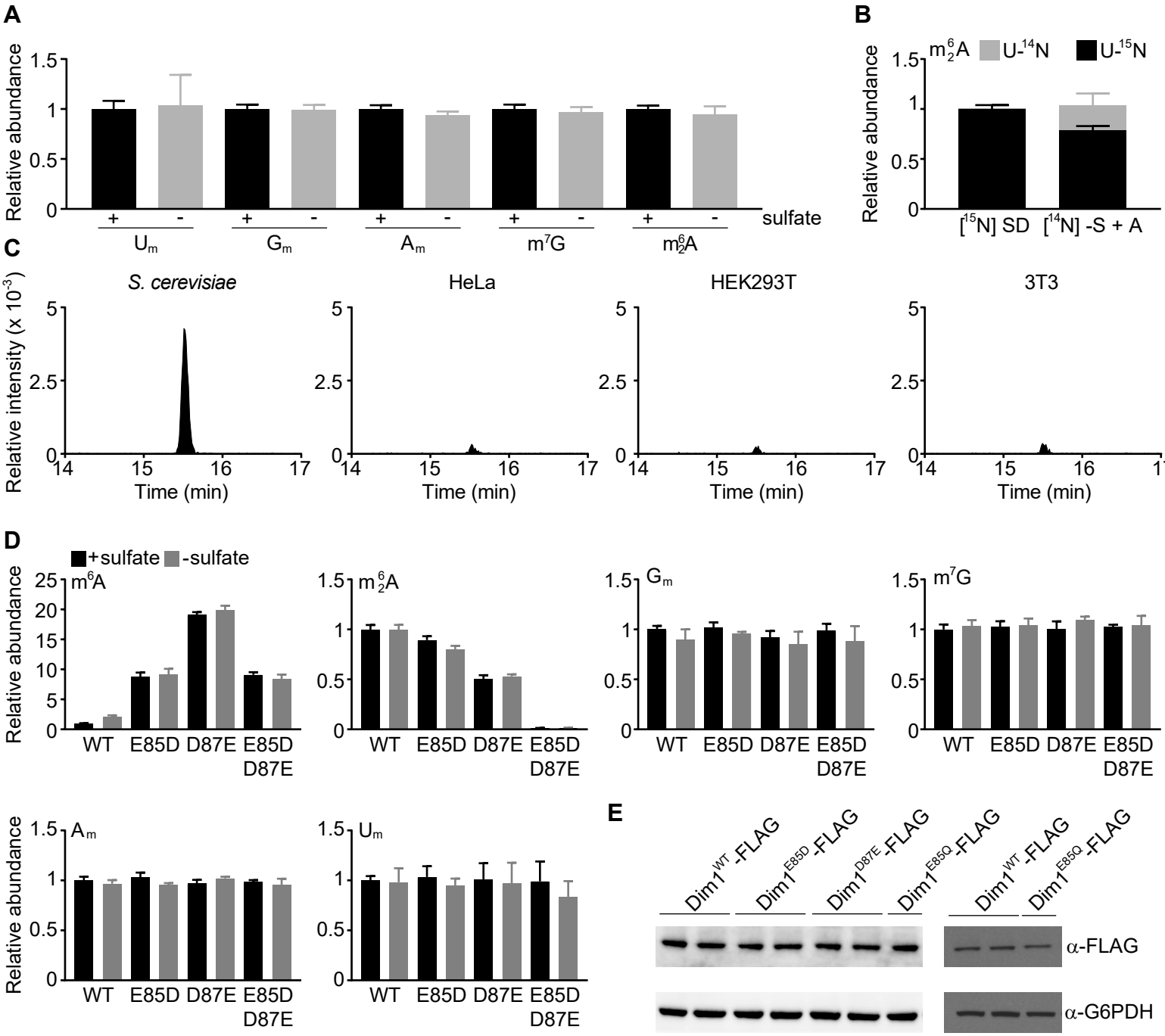


Figure S3. Examination of rRNA modifications in yeast and mammalian cell lines. Related to Figures 2 and 3.
(A) Sulfate starvation does not result in obvious changes in other methylated nucleosides in 18S rRNA. Cells were grown in complete medium to log phase and starved in sulfur free medium (-S) for 2 h. Total RNA was isolated and 18S rRNA was purified, digested, and analyzed by LC-MS/MS. Peak area of each modified nucleoside was first normalized to that of ac^4C and to that of complete medium. Mean ± s.d. (n = 14 biological replicates). **(B)** Synthesis of m_2^6A under sulfate starvation. Cells were grown from a single colony in [^{15}N] SD (1.7 g L^{-1} yeast nitrogen base without amino acids and ammonium sulfate (BD Difco), 5 g L^{-1} $(^{15}NH_4)_2SO_4$ (ISOTEC), and 2% glucose) to saturation and the next day cells were diluted into fresh [^{15}N] SD with a starting OD_{600} ~0.01. Cells were allowed to grow to saturation again, diluted to [^{15}N] SD with a starting OD_{600} ~0.1, and then were grown to log phase. Cells were shifted to pre-warmed [^{14}N] sulfur free (-S) medium supplemented with 50 mg L^{-1} adenine (A) and were collected before and 2 h after the switch. Peak areas of [U-^{14}N] and [U-^{15}N] labeled ac^4C for each condition were summed and used to normalize differentially labeled m_2^6A. Normalized abundance was further divided by that of [^{15}N] SD samples. Mean ± s.d. (n =3 biological replicates). **(C)** In the presence of sulfur, yeast cells bear more m^6A in the last 37 nucleotides of 18S rRNA than mammalian cell lines. Total RNA was isolated and the 3' end of 18S rRNA was isolated using oKL204 by the MBN protection assay, digested, and analyzed by LC-MS/MS. Chromatograms were normalized by the peak area of m_2^6A to allow for comparison between samples. **(D)** Changes in modified nucleosides in 18S rRNA of *dim1* mutants under sulfate starvation. Cells were grown in complete medium and shifted to sulfur free medium (-S) for 2 h. Cells were collected and 18S rRNA was isolated, digested, and analyzed by LC-MS/MS as described in STAR METHODS. Peak area of modified nucleosides was first normalized to that of ac^4C, and to WT grown in complete medium. Mean ± s.d. (n = 3-7 biological replicates). **(E)** Abundance of Dim1p is not discernibly impacted by the E85D, E85Q, or D87E mutations.

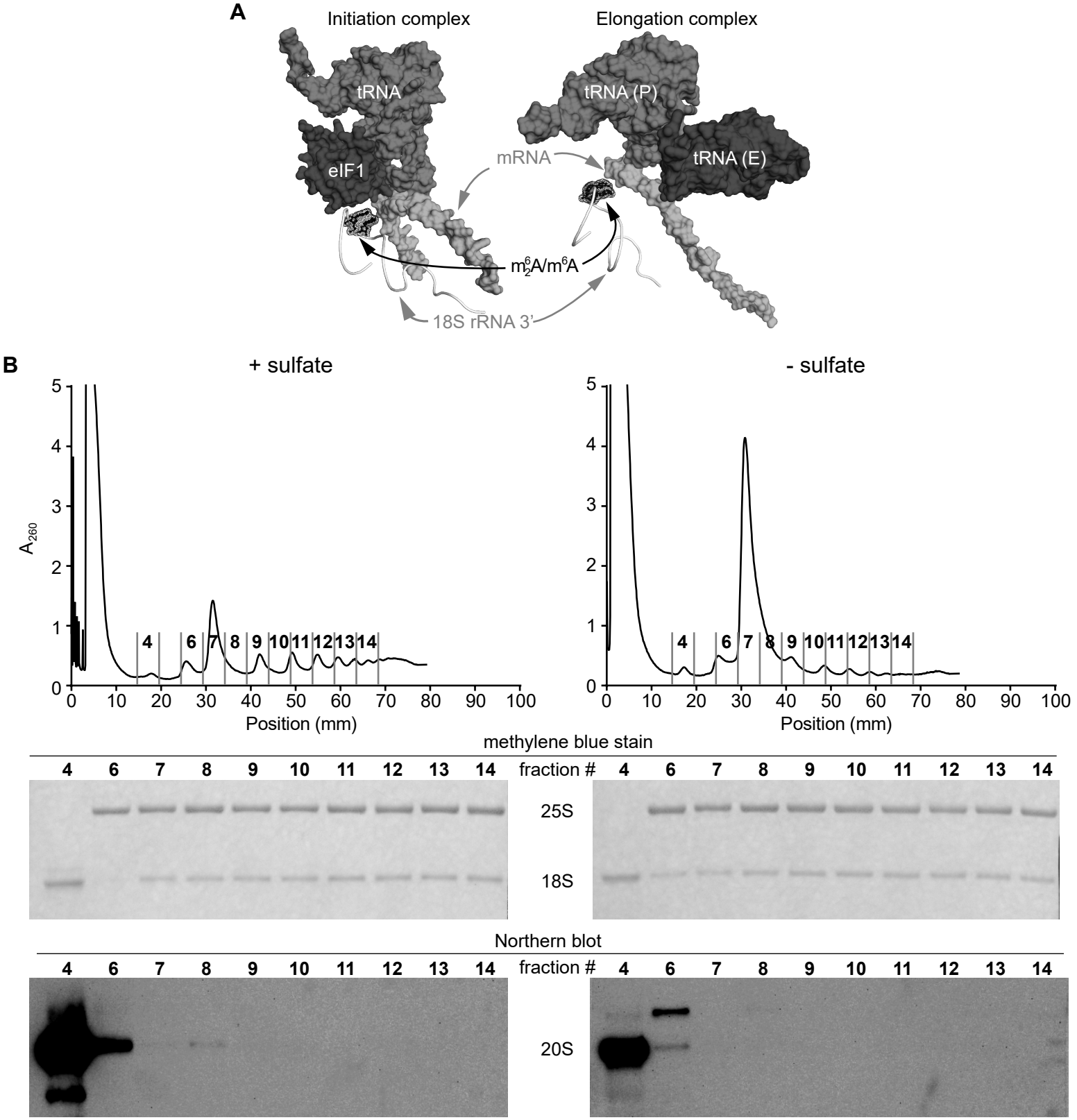


Figure S4. m^{6}A-bearing ribosomes engage in active translation. Related to Figure 3.
(A) m^{6}A/m$^6_2$A resides close to the ribosome P-site. Structures of initiation and elongation complexes were adapted from PDB#3J81 (Hussain et al. 2014). and PDB#6Q8Y (Tesina et al. 2019), respectively, and prepared using Pymol (https://pymol.org). tRNA (P) and tRNA (E) occupy the P- and E-sites of the ribosome, respectively. **(B)** 20S rRNA is absent from polysome fractions under sulfate-replete and -starvation conditions. Cells were grown in complete medium to log phase and starved of sulfate for two hours. Samples were collected for polysome profiling as described in STAR METHODS. Sucrose fractions were extracted with an equal volume of phenol (pH 4.3)/chloroform and nucleic acids were precipitated with an equal volume of isopropanol. Northern blot was performed as described in STAR METHODS.

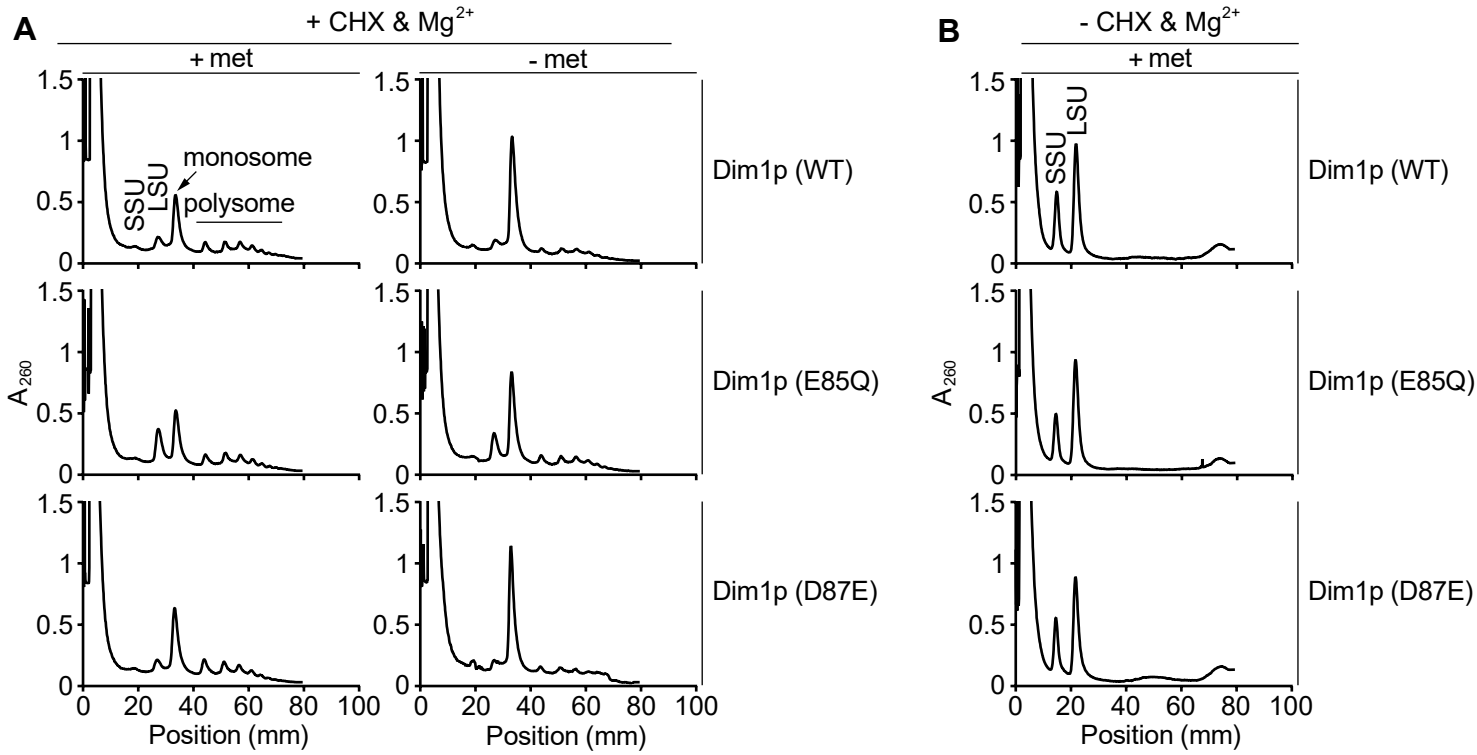


Figure S5. Analysis of ribosome biogenesis in *dim1* mutants. Related to Figure 4.
(A) Polysome profiling. SSU: small subunit (40S); LSU: large subunit (60S). Cells were grown in SFM and starved of methionine in SF for 2 h. Samples were collected for polysome profiling as described in STAR METHODS. **(B)** Ribosome subunit profiling. Cells were grown in SFM and collected without cycloheximide (CHX), and were lysed in the absence of magnesium to separate the two subunits.

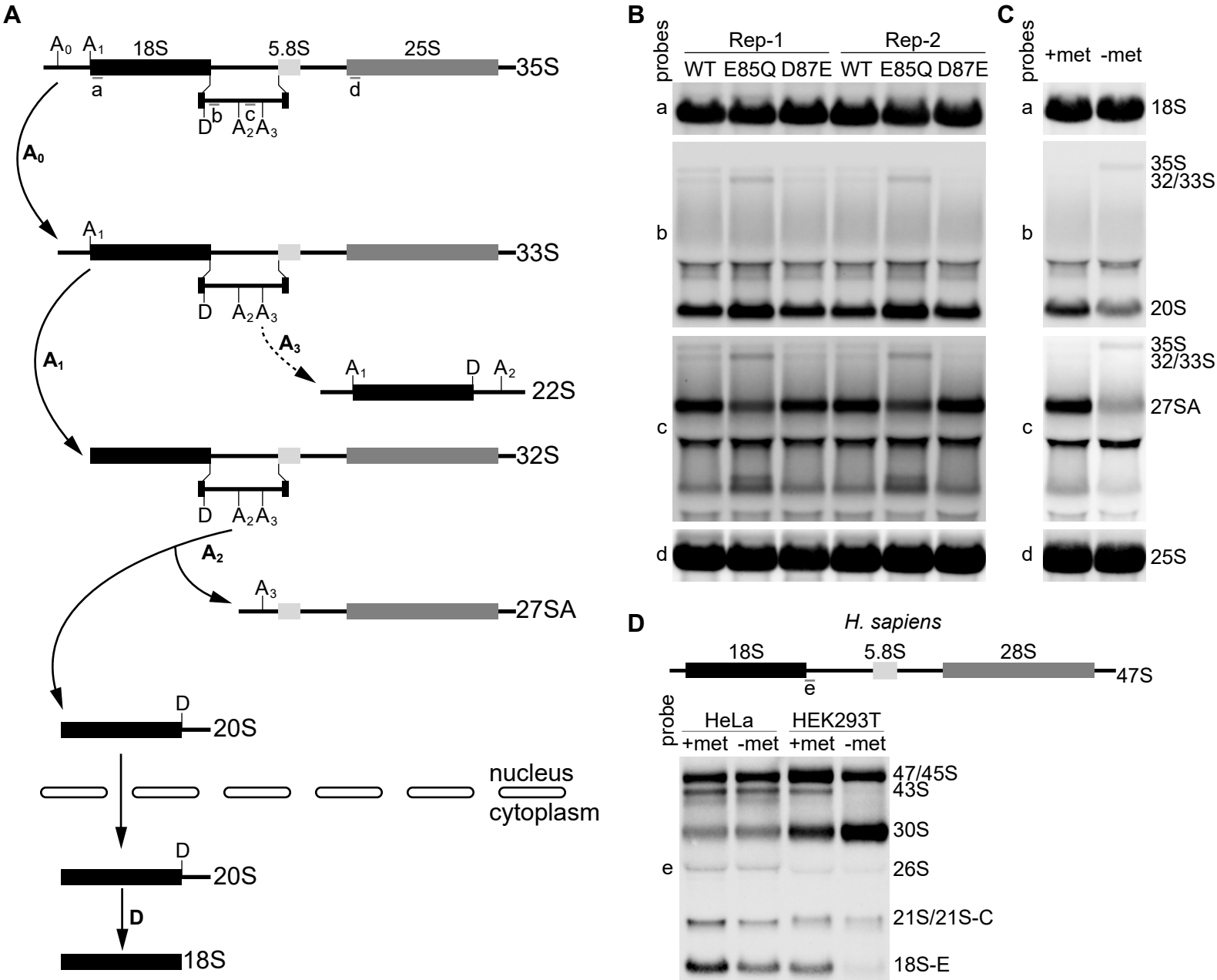


Figure S6. Analysis of rRNA processing in yeast and human cell lines. Related to Figure 4.
(A) Simplified schematic of rRNA processing in yeast. Only relevant processing intermediates and cleavage sites were shown. Probes used for Northern blot were also indicated. The 22S pre-rRNA is thought to arise from cleavage at A3 of the 33S pre-rRNA, observed when Dim1p is depleted (Lafontaine et al., 1995). **(B)** The E85Q mutation, but not the D87E mutation causes rRNA processing defects. **(C)** Examination of rRNA processing in WT yeast cells grown with methionine and without methionine (2 h) using Northern blot. **(D)** Examination of rRNA processing in mammalian cell lines grown with and without methionine (6 h) using Northern blot.

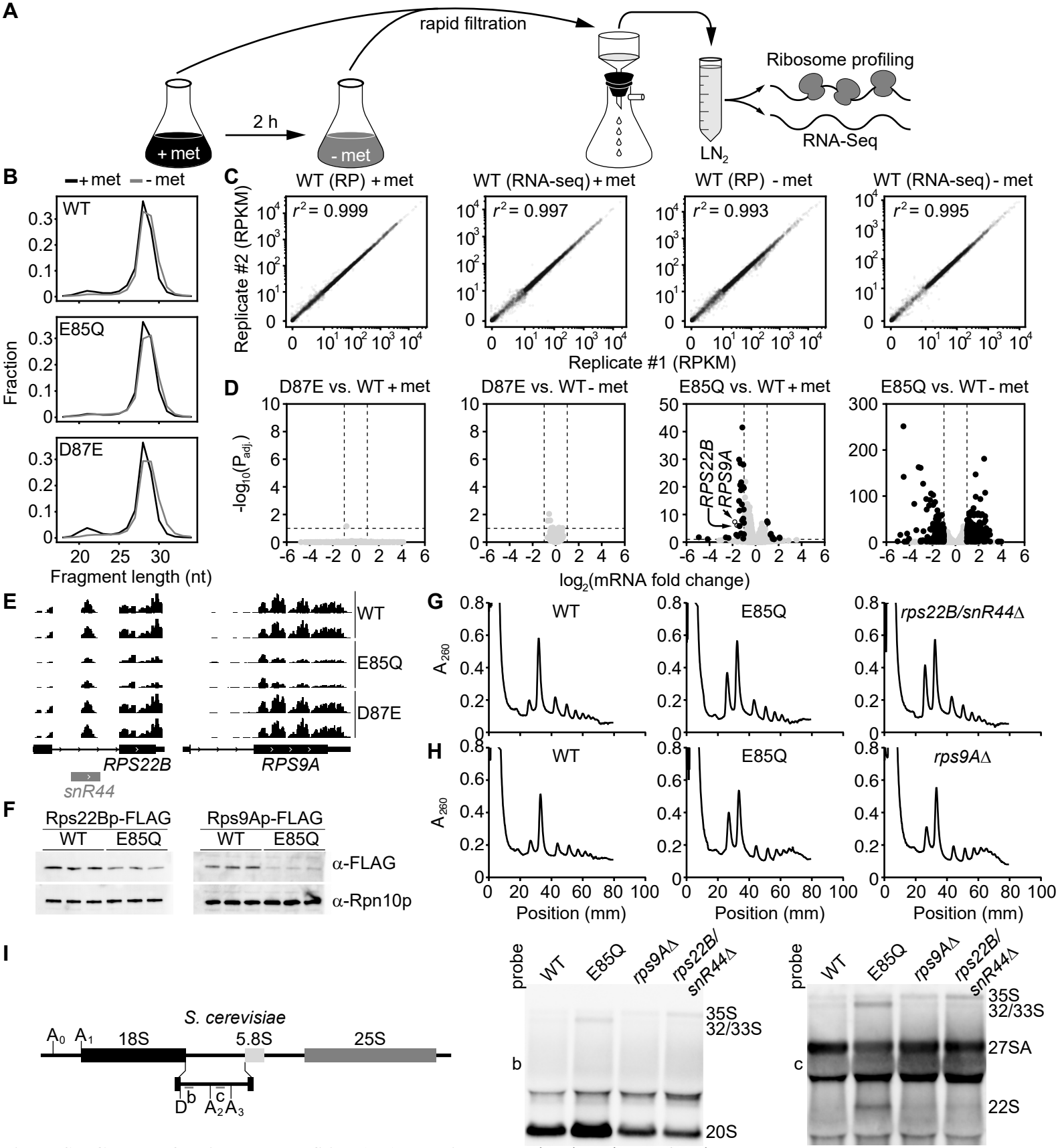


Figure S7. Controls for ribosome profiling (RP) experiments. Related to Figures 4 and 5.
(A) Schematic of ribosome profiling experiment. Cells were grown in SFM and starved of methionine in SF for 2 h. Samples were collected by rapid filtration followed by immersion in liquid nitrogen. See STAR METHODS for details. **(B)** Size distribution of ribosome-protected RNA fragments. One replicate is shown for each strain. **(C)** RP and RNA-seq data are highly reproducible between replicates. WT is shown as an example. **(D)** Impact of *dim1* mutations on the transcriptome under methionine-replete and -starvation conditions. Shown are relative mRNA changes compared to WT. A 10% false discovery rate ($-\log_{10}(P_{adj}) \geq 1$) and 2-fold change were considered significant (highlighted in black). **(E)** Transcripts of *RPS22B*/*snR44* and *RPS9A* are lower in the E85Q mutant. Shown are RNA-seq tracks under methionine-replete conditions (two replicates for each genotype). **(F)** Rps22Bp and Rps9Ap are lower in the E85Q mutant. Cells were grown in SFM and collected for Western blot as described in STAR METHODS. The *rps22B/snR44*Δ mutant **(G)** and *rps9A*Δ mutant **(H)** exhibit defects in SSU biogenesis. Cells were grown in SFM and collected for polysome profiling as described in STAR METHODS. **(I)** Examination of rRNA processing in *rps9A*Δ and *rps22B/snR44*Δ using Northern blot. Cells were grown in SFM and collected for Northern blot as described in STAR METHODS.

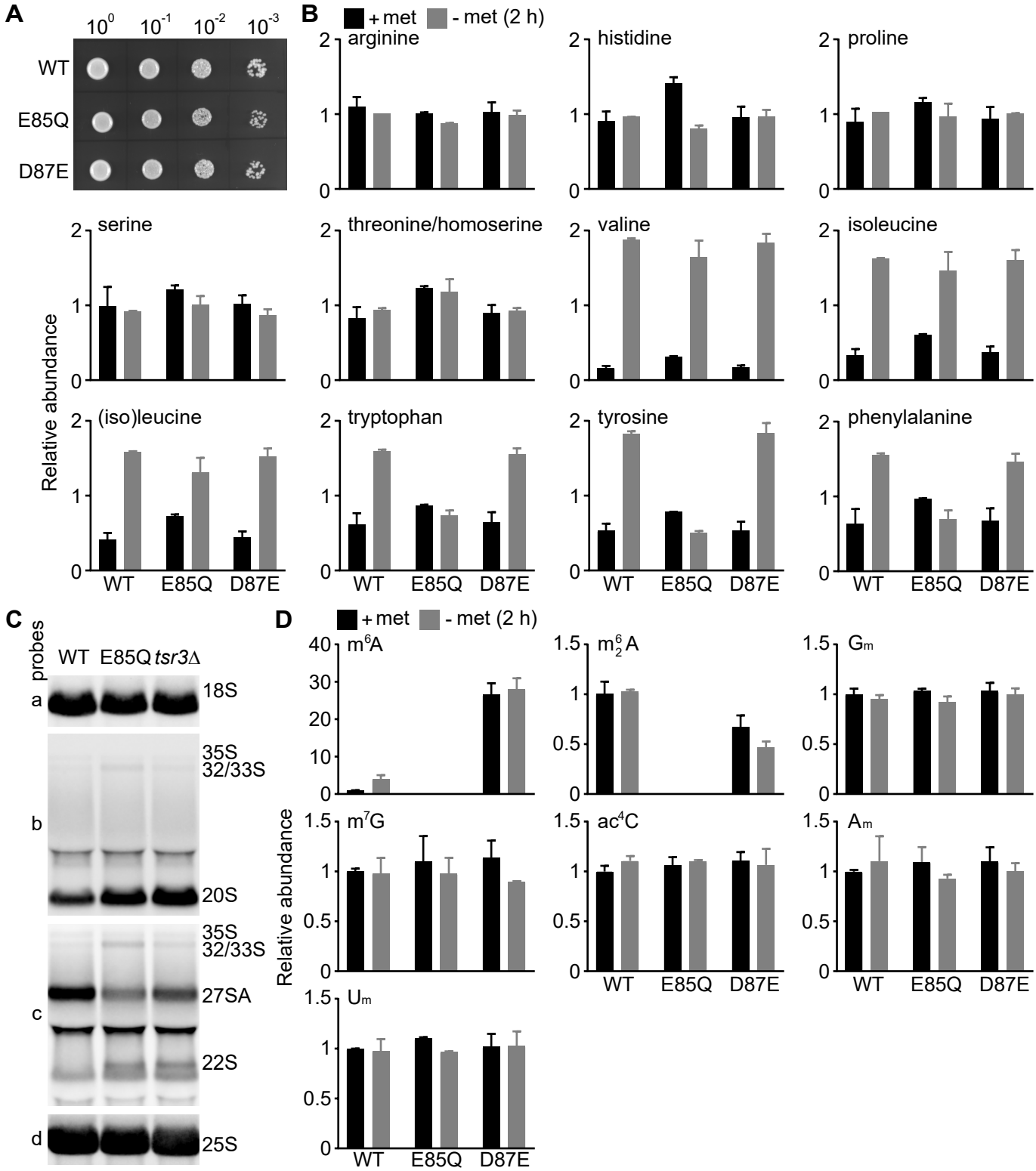
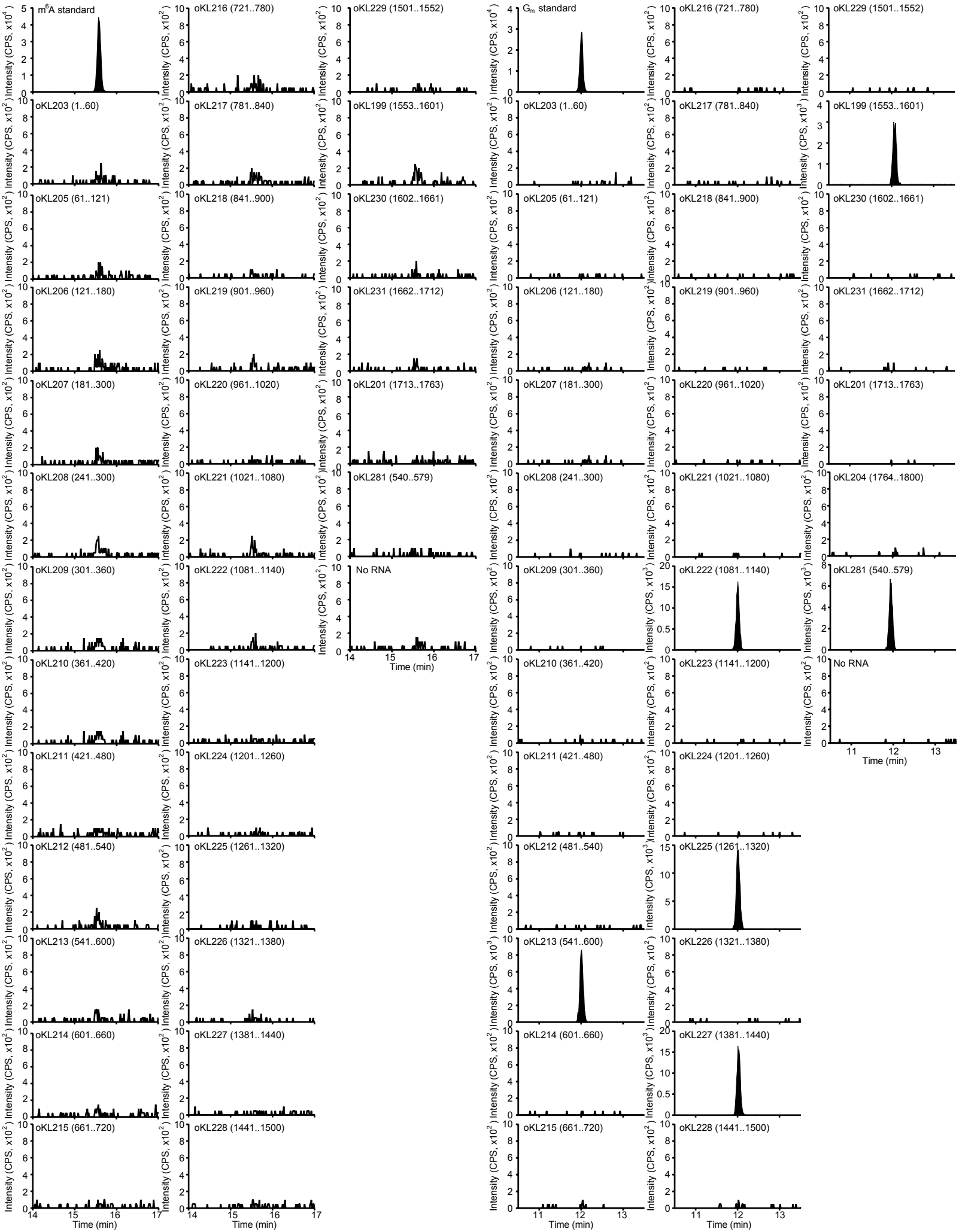


Figure S8. Characterization of *dim1* mutants. Related to Figures 3 and 5.

(A) The E85Q mutant grows slightly more slowly under nutrient replete condition. Cells were grown in SFM medium to log phase and washed with sterile water. Cells were adjusted to a final OD_{600} of 0.5 and serially diluted in water, before being plated onto an SD agar plate. **(B)** Levels of amino acids in *dim1* mutants under methionine-replete and -starvation conditions. Mean ± s.d. (n = 2 biological replicates). **(C)** Examination of rRNA processing in *tsr3*Δ mutant using Northern blot. Cells were grown in SFM and collected for Northern blot as described in STAR METHODS. **(D)** Changes in modified nucleosides in 18S rRNA. Cells were grown in SFM and shifted to SF for 2 h. 18S rRNA was isolated, digested, and analyzed by LC-MS/MS as described in STAR METHODS. Peak area of modified nucleosides was first normalized to that of adenosine and to WT with methionine. m^6A and m^6_2A from the E85Q mutant were at background levels and thus not shown. Mean ± s.d. (n = 2 biological replicates).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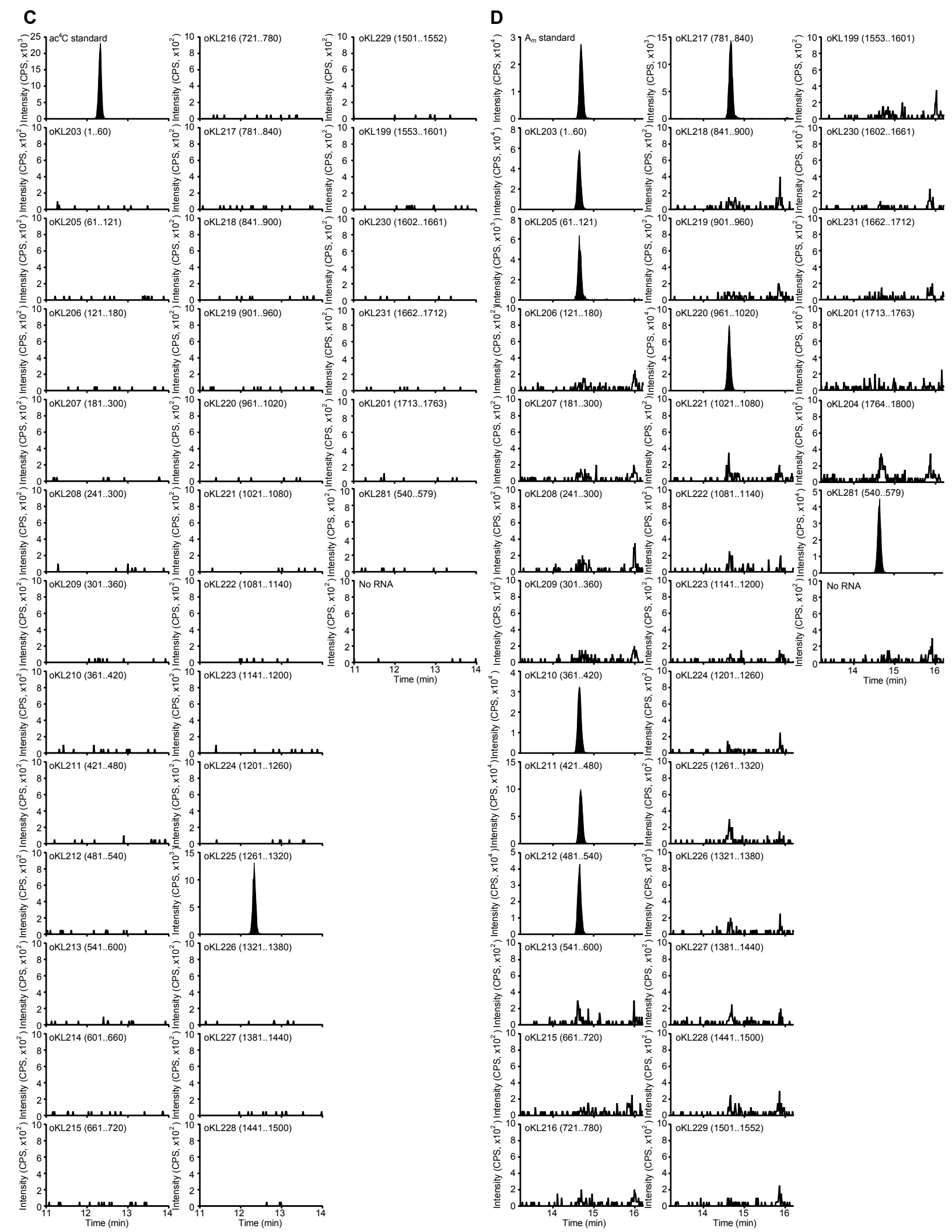
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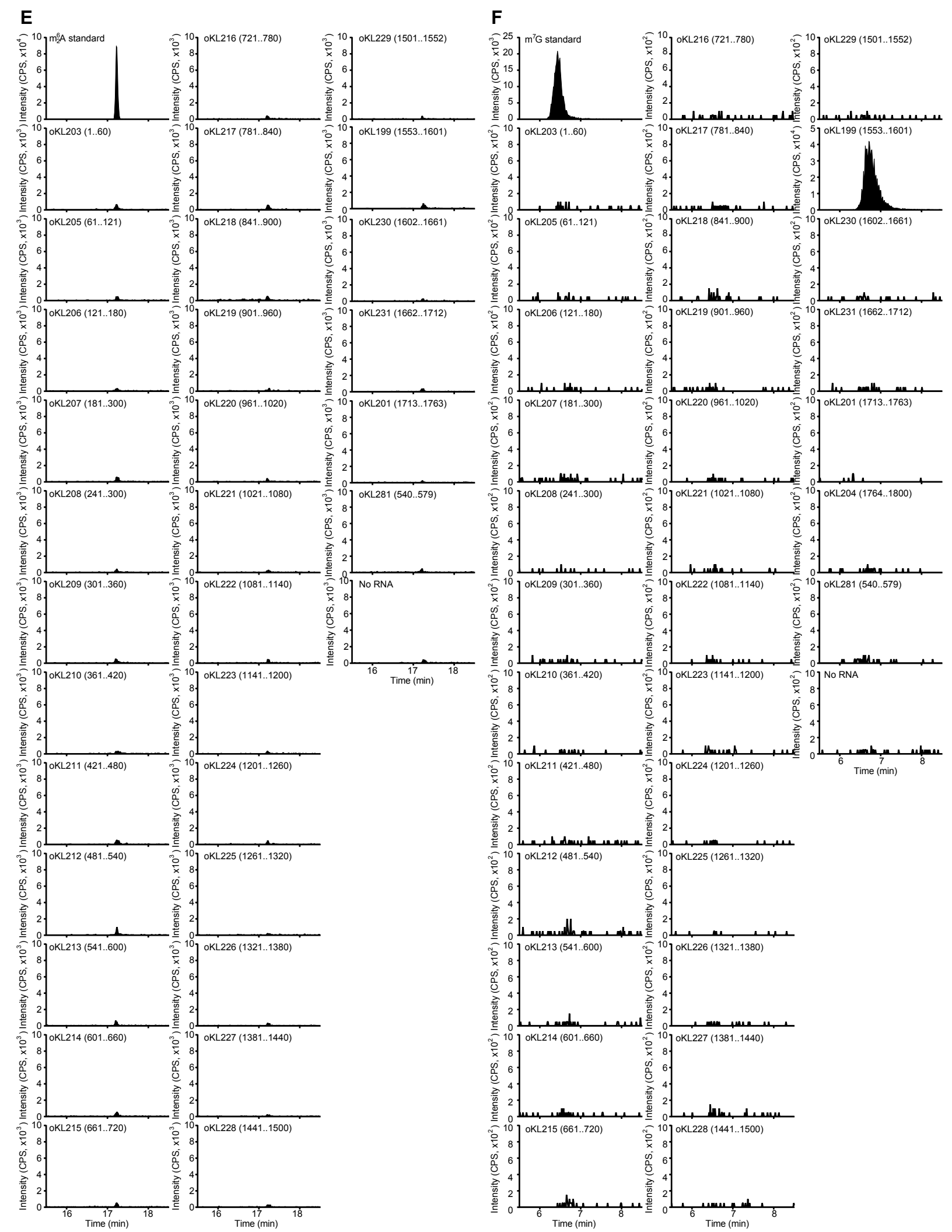
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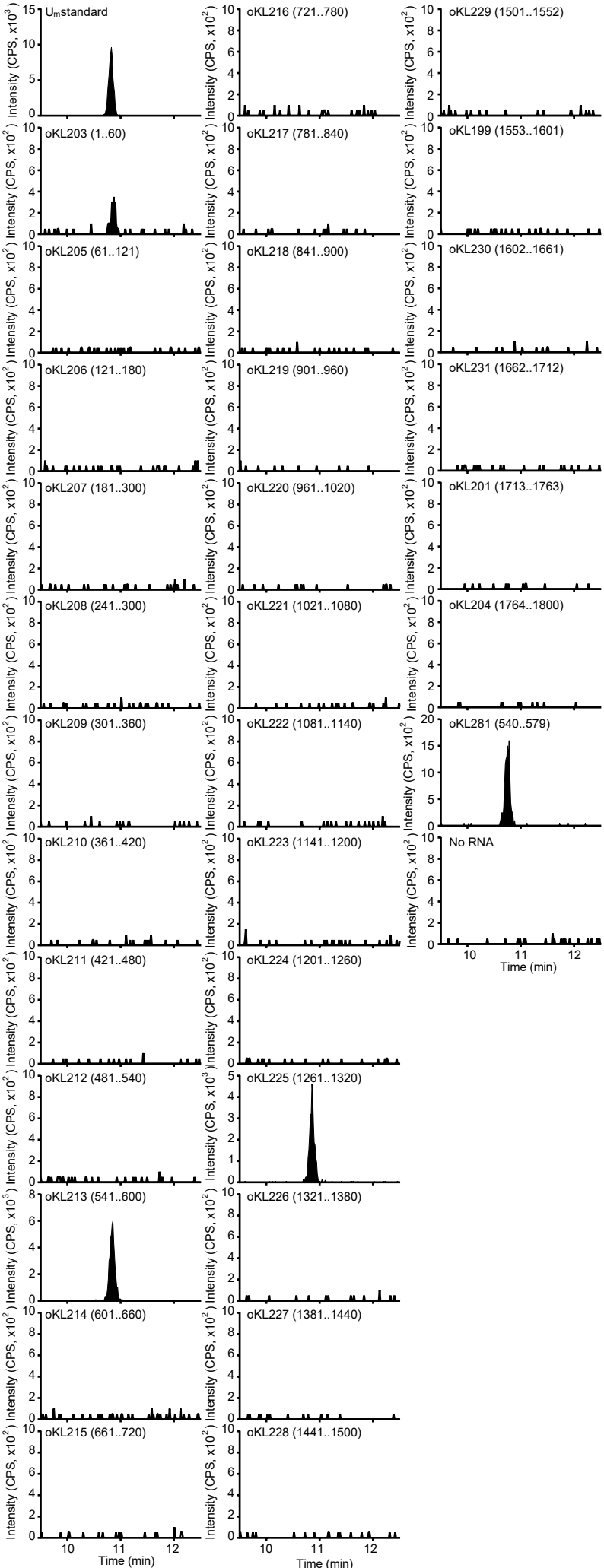

Data S1. Chromatograms of MBN protection assay targeting m^6A (A), G_m (B), ac^4C (C), A_m (D), m^6_2A (E), m^7G (F), and U_m(G). Related to Figure 1. All the modified nucleosides were detected in the expected regions of 18S rRNA according to Taoka et al. (2016). It should be noted that the A_m modification at 541 was not detected in oKL213-protected region (541..600), but in oKL212-protected region (481..540) and oKL281-protected region (540..579). This discrepancy is likely due to the imprecise trimming of the corresponding RNA/DNA ends in MBN protection assay.

Table S4. Strains, plasmids, antibodies, and primers. Related to START METHODS.

Strains

Name	Genotype	References
WT[a]	*MATa* CEN.PK	(van Dijken et al., 2000)
KL139	*MATa/α* CEN.PK	(van Dijken et al., 2000)
S288C	*MATalpha SUC2 mal mel gal2 CUP1 flo1 flo8-1 hap1*	ATCC®204508
W303	*MATa bud4Δ::BUD4*(S288C) *can1-100*	(Korolev et al., 2012)
WT[b]	*MATa ho:: kanMX6-DIM1 dim1:: hygMX6*	This work
E85Q	*MATa ho:: kanMX6-dim1^{E85Q} dim1:: hygMX6*	This work
E85D	*MATa ho:: kanMX6-dim1^{E85D} dim1:: hygMX6*	This work
D87E	*MATa ho:: kanMX6-dim1^{D87E} dim1:: hygMX6*	This work
E85D D87E	*MATa ho:: kanMX6-dim1$^{E85D\ D87E}$ dim1:: hygMX6*	This work
E85A	*MATa ho:: kanMX6-dim1^{E85A} dim1:: hygMX6*	This work
E85W	*MATa ho:: kanMX6-dim1^{E85W} dim1:: hygMX6*	This work
E85F	*MATa ho:: kanMX6-dim1^{E85F} dim1:: hygMX6*	This work
E85Y	*MATa ho:: kanMX6-dim1^{E85Y} dim1:: hygMX6*	This work
E85C	*MATa ho:: kanMX6-dim1^{E85C} dim1:: hygMX6*	This work
E85G	*MATa ho:: kanMX6-dim1^{E85G} dim1:: hygMX6*	This work
E85V	*MATa ho:: kanMX6-dim1^{E85V} dim1:: hygMX6*	This work
E85I	*MATa ho:: kanMX6-dim1^{E85I} dim1:: hygMX6*	This work
E85L	*MATa ho:: kanMX6-dim1^{E85L} dim1:: hygMX6*	This work
E85S	*MATa ho:: kanMX6-dim1^{E85S} dim1:: hygMX6*	This work
E85T	*MATa ho:: kanMX6-dim1^{E85T}dim1:: hygMX6*	This work
E85N	*MATa ho:: kanMX6-dim1^{E85N} dim1:: hygMX6*	This work
E85K	*MATa ho:: kanMX6-dim1^{E85K} dim1:: hygMX6*	This work
E85R	*MATa ho:: kanMX6-dim1^{E85R} dim1:: hygMX6*	This work
E85P	*MATa ho:: kanMX6-dim1^{E85P} dim1:: hygMX6*	This work
E85M	*MATa ho:: kanMX6-dim1^{E85M} dim1:: hygMX6*	This work
E85H	*MATa ho:: kanMX6-dim1^{E85H} dim1:: hygMX6*	This work
sam1Δ sam2Δ	*MATa sam1::kanMX6 sam2::hygMX6*	This work
WT-*lacZ*	*MATa ho::kanMX6-DIM1 dim1::hygMX6 x2::Ptef1-Ec lacZ-Tcyc1-natMX6*[c]	This work
E85D-*lacZ*	*MATa ho::kanMX6-dim1^{E85D} dim1::hygMX6 x2::Ptef1-Ec lacZ-Tcyc1-natMX6*[c]	This work
E85Q-*lacZ*	*MATa ho::kanMX6-dim1^{E85Q} dim1::hygMX6 x2::Ptef1-Ec lacZ-Tcyc1-natMX6*[c]	This work
D87E-*lacZ*	*MATa ho::kanMX6-dim1^{D87E} dim1::hygMX6 x2::Ptef1-Ec lacZ-Tcyc1-natMX6*[c]	This work
WT-FLAG	*MATa ho:: kanMX6-DIM1-FLAG dim1::hygMX6*	This work
E85D-FLAG	*MATa ho:: kanMX6-dim1^{E85D}-FLAG dim1:: hygMX6*	This work
D87E-FLAG	*MATa ho:: kanMX6-dim1^{D87E}-FLAG dim1:: hygMX6*	This work
E85Q-FLAG	*MATa ho:: kanMX6-dim1^{E85Q}-FLAG dim1:: hygMX6*	This work
gcd14Δ IMT4 OE	*MATa gcd14::hygMX6 2 micron-IMT4-kanMX6*	This work
rrp8Δ ybr141cΔ	*MATa rrp8::kanMX6 ybr141c::hygMX6*	This work
WT *rps22B-3×FLAG*	*MATa ho:: kanMX6-DIM1 dim1:: hygMX6 rps22B::3×FLAG-natMX6*	This work
E85Q *rps22B-3×FLAG*	*MATa ho:: kanMX6-dim1^{E85Q} dim1:: hygMX6 rps22B::3×FLAG-natMX6*	This work
WT *rps9A-3×FLAG*	*MATa ho:: kanMX6-DIM1 dim1:: hygMX6 rps9A::3×FLAG-natMX6*	This work
E85Q *rps9A-3×FLAG*	*MATa ho:: kanMX6-dim1^{E85Q} dim1:: hygMX6 rps9A::3×FLAG-natMX6*	This work
rps22BΔ pKL23 (H245R)	*MATa ho:: kanMX6-DIM1 dim1:: hygMX6 rps22B::natMX6 ura3:: Sh ble pKL23*	This work
rps22BΔ pKL24 (control)	*MATa ho:: kanMX6-DIM1 dim1:: hygMX6 rps22B::natMX6 ura3:: Sh ble pKL24*	This work
rps9AΔ pKL23 (H245R)	*MATa ho:: kanMX6-DIM1 dim1:: hygMX6 rps9A::natMX6 ura3:: Sh ble pKL23*	This work
rps9AΔ pKL24 (control)	*MATa ho:: kanMX6-DIM1 dim1:: hygMX6 rps9A::natMX6 ura3:: Sh ble pKL24*	This work
WT pKL23 (H245R)	*MATa ho:: kanMX6-DIM1 dim1:: hygMX6 ura3:: natMX6 pKL23*	This work
E85Q pKL23 (H245R)	*MATa ho:: kanMX6-dim1^{E85Q} dim1:: hygMX6 ura3:: natMX6 pKL23*	This work
D87E pKL23 (H245R)	*MATa ho:: kanMX6-dim1^{D87E} dim1:: hygMX6 ura3:: natMX6 pKL23*	This work
WT pKL24 (control)	*MATa ho:: kanMX6-DIM1 dim1:: hygMX6 ura3:: natMX6 pKL24*	This work
E85Q pKL24 (control)	*MATa ho:: kanMX6-dim1^{E85Q} dim1:: hygMX6 ura3:: natMX6 pKL24*	This work
D87E pKL24 (control)	*MATa ho:: kanMX6-dim1^{D87E} dim1:: hygMX6 ura3:: natMX6 pKL24*	This work
tsr3Δ pKL23 (H245R)	*MATa ho:: kanMX6-DIM1 dim1:: hygMX6 tsr3::natMX6 ura3:: Sh ble pKL23*	This work
tsr3Δ pKL24 (control)	*MATa ho:: kanMX6-DIM1 dim1:: hygMX6 tsr3::natMX6 ura3:: Sh ble pKL24*	This work

Plasmids

Name	Description	References
pFA6a-*hphMX6*	To replace a gene of interest with the *hphMX6* gene	(Longtine et al., 1998)
pFA6a-*kanMX6*	To replace a gene of interest with the *kanMX6* gene	(Longtine et al., 1998)

pFA6a-*natMX6*	To replace a gene of interest with the *natMX6* gene	(Longtine et al., 1998)
pUG66	To replace a gene of interest with the *Sh ble* gene	(Gueldener et al., 2002)
HO-kanMX6-HO	To ectopically express a gene of interest at the *HO* locus	(Voth et al., 2001)
Control (aka. pDB722 or pKL24)	Control Dual-Luciferase reporter	(Keeling et al., 2004)
H245R (aka. pDB868 or pKL23)	Dual-Luciferase reporter for miscoding	(Salas-Marco and Bedwell, 2005)
2 micron *IMT4-kanMX6*	To express *IMT4* with its 5' (~500 bp) and 3' (~300 bp) UTRs on a 2 micron plasmid	This work
HO-kanMX6-DIM1-HO	To ectopically express *DIM1* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO-kanMX6-dim1^{E85A}-HO	To ectopically express *dim1^{E85A}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85G} -HO	To ectopically express *dim1^{E85G}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85D}-HO	To ectopically express *dim1^{E85D}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85V}-HO	To ectopically express *dim1^{E85V}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85R}-HO	To ectopically express *dim1^{E85R}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85S}-HO	To ectopically express *dim1^{E85S}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85K}-HO	To ectopically express *dim1^{E85K}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85N}-HO	To ectopically express *dim1^{E85N}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85T}-HO	To ectopically express *dim1^{E85T}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85M}-HO	To ectopically express *dim1^{E85M}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85I}-HO	To ectopically express *dim1^{E85I}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85Q}-HO	To ectopically express *dim1^{E85Q}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85H}-HO	To ectopically express *dim1^{E85H}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85P}-HO	To ectopically express *dim1^{E85P}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85L}-HO	To ectopically express *dim1^{E85L}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85C}-HO	To ectopically express *dim1^{E85C}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85Y}-HO	To ectopically express *dim1^{E85Y}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85F}-HO	To ectopically express *dim1^{E85F}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85W}-HO	To ectopically express *dim1^{E85W}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1$^{E85D\ D87E}$-HO	To ectopically express *dim1$^{E85D\ D87E}$* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{D87E}-HO	To ectopically express *dim1^{D87E}* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-DIM1-FLAG-HO	To ectopically express *DIM1-FLAG* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85D}-FLAG-HO	To ectopically express *dim1^{D85D}-FLAG* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{E85Q}-FLAG-HO	To ectopically express *dim1^{D85Q}-FLAG* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
HO- kanMX6-dim1^{D87E}-FLAG-HO	To ectopically express *dim1^{D87E}-FLAG* with its 5' (~500 bp) and 3' (~300 bp) UTRs	This work
p417-P_{TEF1}-*lacZ*-T_{CYC1}-*natMX6*	To express *Escherichia coli lacZ*	This work

Antibodies

Name	Description	Cat. #
α-FLAG	To detect the FLAG epitope	Sigma F1804
α-G6pdh	To detect G6pdhp	Sigma A9521
α-Rpn10	To detect Rpn10p	Abcam ab98843

Primers

Name	Sequence (5' to 3')	Note
oKL169	ATCCCCGGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCC	
oKL203	AGACATGCATGGCTTAATCTTTGAGACAAGCATATGACTACTGGCAGGATCAACCAGATA	1..60[d]
oKL205	ATAAACGATAACTGATTTAATGAGCCATTCGCAGTTTCACTGTATAAATTGCTTATACTT	61..120[d]
oKL206	TTAAGCATGTATTAGCTCTAGAATTACCACAGTTATACCATGTAGTAAAGGAACTATCAA	121..180[d]
oKL207	AGAGTCCGAAGACATTGATTTTTTATCTAATAAATACATCTCTTCCAAAGGGTCGAGATT	181..240[d]
oKL208	TTGAATGAACCATCGCCAGCACAAGGCCATGCGATTCGAAAAGTTATTATGAATCATCAA	241..300[d]
oKL209	TTACCCGTTGAAACCATGGTAGGCCACTATCCTACCATCGAAAGTTGATAGGGCAGAAAT	301..360[d]
oKL210	TCCTTGGATGTGGTAGCCGTTTCTCAGGCTCCCTCTCCGGAATCGAACCCTTATTCCCCG	361..420[d]
oKL211	CGTTATTTATTGTCACTACCTCCCTGAATTAGGATTGGGTAATTTGCGCGCCTGCTGCCT	421..480[d]
oKL212	CCTCGTTAAGGTATTTACATTGTACTCATTCCAATTACAAGACCCGAATGGGCCCTGTAT	481..540[d]
oKL213	ATACGCTATTGGAGCTGGAATTACCGCGGCTGCTGGCACCAGACTTGCCCTCCAATTGTT	541..600[d]
oKL214	CGGACCGGCCAACCGGGCCCAAAGTTCAACTACGAGCTTTTTAACTGCAACAACTTTAAT	601..660[d]
oKL215	CAAGGACTCAAGGTTAGCCAGAAGGAAAGGCCCCGTTGGAAATCCAGTACACGAAAAAAT	661..720[d]
oKL216	TACGCCTGCTTTGAACACTCTAATTTTTTCAAAGTAAAAGTCCTGGTTCGCCAAGAGCCA	721..780[d]
oKL217	AACCAACAAAATAGAACCAAACGTCCTATTCTATTATTCCATGCTAATATATTCGAGCAA	781..840[d]
oKL218	TCTGACAATTGAATACTGATGCCCCCGACCGTCCCTATTAATCATTACGATGGTCCTAGA	841..900[d]
oKL219	AAACGTCCTTGGCAAATGCTTTCGCAGTAGTTAGTCTTCAATAAATCCAAGAATTTCACC	901..960[d]
oKL220	TTAAGACTACGACGGTATCTGATCATCTTCGATCCCCTAACTTTCGTTCTTGATTAATGA	961..1020[d]
oKL221	AAGGTGCCGAGTGGGTCATTAAAAAAACACCACCCGATCCCTAGTCGGCATAGTTTATGG	1021..1080[d]
oKL222	CTTTAAGTTTCAGCCTTGCGACCATACTCCCCCCAGAACCCAAAGACTTTGATTTCTCGT	1081..1140[d]
oKL223	CCCGTGTTGAGTCAAATTAAGCCGCAGGCTCCACTCCTGGTGGTGCCCTTCCGTCAATTC	1141..1200[d]

oKL224	AAAATCAAGAAAGAGCTCTCAATCTGTCAATCCTTATTGTGTCTGGACCTGGTGAGTTTC	1201..1260[d]
oKL225	ATCGCAATTAAGCAGACAAATCACTCCACCAACTAAGAACGGCCATGCACCACCACCCAC	1261..1320[d]
oKL226	AGAAGTGGATAACCAGCAAATGCTAGCACCACTATTTAGTAGGTTAAGGTCTCGTTCGTT	1321..1380[d]
oKL227	GGCATCACAGACCTGTTATTGCCTCAAACTTCCATCGGCTTGAAACCGATAGTCCCTCTA	1381..1440[d]
oKL228	GCCAAGGTTAGACTCGCTGGCTCCGTCAGTGTAGCGCGCGTGCGGCCCAGAACGTCTAAG	1441..1500[d]
oKL229	AATGCTCTATCCCCAGCACGACGGAGTTTCACAAGATTACCAAGACCTCTCG	1501..1552[d]
oKL199	CTGATGACTTGCGCTTACTAGGAATTCCTCGTTGAAGAGCAATAATTAC	1553..1601[d]
oKL230	ATTCAATCGGTACTAGCGACGGGCGGTGTGTACAAAGGGCAGGGACGTAATCAACGCAAG	1602..1661[d]
oKL231	TGAGATGGAGTTGCCCCCTTCTCTAAGCAGATCCTGAGGCCTCACTAAGCC	1662..1712[d]
oKL201	TTACGACTTTTAGTTCCTCTAAATGACCAAGTTTGTCCAAATTCTCCGCTC	1713..1763[d]
oKL204	TAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTG	1764..1800[d]
oKL235	CCTACGGAAACCTTGTTACGACTTTTAGTTCCTCTAAATGACCAAGTTTG	1729..1778[d]
oKL236	TCACCTACGGAAACCTTGTTACGACTTTTAGTTCCTCTAAATGACCAAGTTTG	1729..1781[d]
oKL237	TTCACCTACGGAAACCTTGTTACGACTTTTAGTTCCTCTAAATGACCAAGTTTG	1729..1782[d]
oKL242	TCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTAGTTCCTCTAAATGACCAAGTTTG	1729..1790[d]
oKL243	TTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTAGTTCCTCTAAATGACCAAGTTTG	1729..1791[d]
oKL246	TAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTAGTTCCTCTAAATGACCAAGTTTG	1729..1800[d]
oKL261	GCAAAAAACGTAGTGGCAGTAGCAATGGATCCCAGAATGGCTGC[e]	*dim1*^{E85A}(f)
oKL262	GCAGCCATTCTGGGATCCATTGCTACTGCCACTACGTTTTTTGC	*dim1*^{E85A}(r)
oKL506	AACGTAGTGGCAGTATGGATGGATCCCAGAATG[e]	*dim1*^{E85W}(f)
oKL507	CATTCTGGGATCCATCCATACTGCCACTACGTT	*dim1*^{E85W}(r)
oKL625	AACGTAGTGGCAGTAGGTATGGATCCCAGAATG[e]	*dim1*^{E85G}(f)
oKL626	CATTCTGGGATCCATACCTACTGCCACTACGTT	*dim1*^{E85G}(r)
oKL627	AACGTAGTGGCAGTAGATATGGATCCCAGAATG[e]	*dim1*^{E85D}(f)
oKL628	CATTCTGGGATCCATATCTACTGCCACTACGTT	*dim1*^{E85D}(r)
oKL629	AACGTAGTGGCAGTAGTTATGGATCCCAGAATG[e]	*dim1*^{E85V}(f)
oKL630	CATTCTGGGATCCATAACTACTGCCACTACGTT	*dim1*^{E85V}(r)
oKL631	AACGTAGTGGCAGTAAGAATGGATCCCAGAATG[e]	*dim1*^{E85R}(f)
oKL632	CATTCTGGGATCCATTCTTACTGCCACTACGTT	*dim1*^{E85R}(r)
oKL633	AACGTAGTGGCAGTATCTATGGATCCCAGAATG[e]	*dim1*^{E85S}(f)
oKL634	CATTCTGGGATCCATAGATACTGCCACTACGTT	*dim1*^{E85S}(r)
oKL635	AACGTAGTGGCAGTAAAAATGGATCCCAGAATG[e]	*dim1*^{E8K}(f)
oKL636	CATTCTGGGATCCATTTTTACTGCCACTACGTT	*dim1*^{E85K}(r)
oKL637	AACGTAGTGGCAGTAAATATGGATCCCAGAATG[e]	*dim1*^{E85N}(f)
oKL638	CATTCTGGGATCCATATTTACTGCCACTACGTT	*dim1*^{E85N}(r)
oKL639	AACGTAGTGGCAGTAACTATGGATCCCAGAATG[e]	*dim1*^{E85T}(f)
oKL640	CATTCTGGGATCCATAGTTACTGCCACTACGTT	*dim1*^{E85T}(r)
oKL641	AACGTAGTGGCAGTAATGATGGATCCCAGAATG[e]	*dim1*^{E85M}(f)
oKL642	CATTCTGGGATCCATCATTACTGCCACTACGTT	*dim1*^{E85M}(r)
oKL643	AACGTAGTGGCAGTAATTATGGATCCCAGAATG[e]	*dim1*^{E85I}(f)
oKL644	CATTCTGGGATCCATAATTACTGCCACTACGTT	*dim1*^{E85I}(r)
oKL645	AACGTAGTGGCAGTACAAATGGATCCCAGAATG[e]	*dim1*^{E85Q}(f)
oKL646	CATTCTGGGATCCATTTGTACTGCCACTACGTT	*dim1*^{E85Q}(r)
oKL647	AACGTAGTGGCAGTACATATGGATCCCAGAATG[e]	*dim1*^{E85H}(f)
oKL648	CATTCTGGGATCCATATGTACTGCCACTACGTT	*dim1*^{E85H}(r)
oKL649	AACGTAGTGGCAGTACCAATGGATCCCAGAATG[e]	*dim1*^{E85P}(f)
oKL650	CATTCTGGGATCCATTGGTACTGCCACTACGTT	*dim1*^{E85P}(r)
oKL651	AACGTAGTGGCAGTATTGATGGATCCCAGAATG[e]	*dim1*^{E85L}(f)
oKL652	CATTCTGGGATCCATCAATACTGCCACTACGTT	*dim1*^{E85L}(r)
oKL653	AACGTAGTGGCAGTATGTATGGATCCCAGAATG[e]	*dim1*^{E85C}(f)
oKL654	CATTCTGGGATCCATACATACTGCCACTACGTT	*dim1*^{E85C}(r)
oKL655	AACGTAGTGGCAGTATATATGGATCCCAGAATG[e]	*dim1*^{E85Y}(f)
oKL656	CATTCTGGGATCCATATATACTGCCACTACGTT	*dim1*^{E85Y}(r)
oKL657	AACGTAGTGGCAGTATTTATGGATCCCAGAATG[e]	*dim1*^{E85F}(f)
oKL658	CATTCTGGGATCCATAAATACTGCCACTACGTT	*dim1*^{E85F}(r)
oKL700	GTGGCAGTAGAAATGGAACCCAGAATGGCTGCA[e]	*dim1*^{D87E}(f)
oKL701	TGCAGCCATTCTGGGTTCCATTTCTACTGCCAC	*dim1*^{D87E}(r)
oKL698	GTGGCAGTAGATATGGAACCCAGAATGGCTGCA[e]	*dim1*^{E85D,D87E} (f)
oKL699	TGCAGCCATTCTGGGTTCCATATCTACTGCCAC	*dim1*^{E85D,D87E} (r)

Probes for Northern blot

Name	Sequence (5' to 3')	Target region
a	[btn]TACTTAGACATGCATGGCTTAATCTTTGAGACAAGCATATGACTACTGGC	yeast 18S rRNA

b	[btn]GACTCTCCATCTCTTGTCTTCTTGCCCAGTAAAAGCTCTCATGCTCTTGC	yeast 20S pre-rRNA
c	[btn]CTCTGGGCCCCGATTGCTCGAATGCCCAAAGAAAAAGTTGCAAAGATATG	yeast ITS1 (A2&A3)
d	[btn]GTTACTAAGGCAATCCCGGTTGGTTTCTTTTCCTCCGCTTATTGATATGC	yeast 25S rRNA
e	[btn]CCTCGCCCTCCGGGCTCCGTTAATGATCCT	Human ITS1

[a]This strain was used throughout this work except when mutant *dim1* strains were investigated.

[b]This strain was used only for comparison with mutant *dim1* strains.

[c]The *x2* site is the integration site for the *E. coli lacZ* gene on chromosome X, between *NCA3* and *ASF1*, precisely encompassing from 605 to 646 nucleotides upstream of the *NCA3* start codon. It was previously examined by Mikkelsen et al., who reported that ectopic expression at this site did not cause growth defects (Mikkelsen et al., 2012).

[d]Numbers correspond to nucleotide positions in yeast 18S rRNA.

[e]The mutated codon is underlined in the forward primer. (f): forward primer; (r): reverse primer

Table S5. Medium formula[a]. Related to STAR METHODS.

	complete	-C	-N	-P	-S (sulfur free)
salts (g L^{-1})					
$CaCl_2•2H_2O$	0.1	0.1	0.1	0.1	0.1
NaCl	0.1	0.1	0.1	0.1	0.1
$MgCl_2•6H_2O$	0.412	0.412	0.412	0.412	0.412
$(NH_4)_2SO_4$	5	5	0	5	0
Na_2SO_4	0	0	5.4	0	0
NH_4Cl	0	0	0	0	4.05
KH_2PO_4	1	1	1	0	1
KCl	0	0	0	0.55	0
metals (mg L^{-1})					
boric acid	0.5				
$CuCl_2•2H_2O$	0.0273				
KI	0.1				
$FeCl_3•6H_2O$	0.2				
$MnCl_2•4H_2O$	0.4684				
$Na_2MoO_4•2H_2O$	0.2				
$ZnCl_2•H_2O$	0.1895				
vitamins (mg L^{-1})					
biotin	0.002				
calcium pantothenate	0.4				
folic acid	0.002				
inositol	2				
niacin	0.4				
4-aminobenzoic acid	0.2				
pyridoxine HCl	0.4				
riboflavin	0.2				
thiamine-HCl	0.4				

[a]Formula is based on Miller et al. (Miller et al., 2013) with sulfate ions replaced by chloride ions.

Table S6. MRM transitions for nucleosides and metabolites. Related to STAR METHODS.

Compounds	Q1	Q3	[U-^{15}N]-Q1	[U-^{15}N]-Q3
N^6-methyladenosine (m^6A)	282	150	287	155
N^4-acetylcytidine (ac^4C)	286	154	289	157
N^6, N^6-dimethyladenosine ($m^6{}_2A$)	296	164	301	169
N^1-methyladenosine (m^1A)	282	150		
2'-O-methyladenosine (A_m)	282	136		
2'-O-methylguanosine (G_m)	298	152		
2'-O-methyluridine (U_m)	259	113		
N^7-methylguanosine (m^7G)	298	166		
adenosine	268	136		
cytidine	244	112		
uridine	245	113		
guanosine	284	152		
cysteine	122	59		
methionine	150	104		
homocysteine	136	90		
cystathionine	223	134		
SAM	399	250		
SAH	385	136		
GSH	308	179		
GSSG	613	355		
proline	116	70		
arginine	175	116		
histidine	156	110		
serine	106	60		
threonine/homoserine	120	74		
isoleucine	132	69		
(iso)leucine	132	86		
valine	118	55		
tryptophan	205	188		
phenylalanine	166	103		
tyrosine	182	136		