Supplementary information for:

Nonsense-mediated mRNA decapping occurs on polyribosomes in *Saccharomyces cerevisiae*

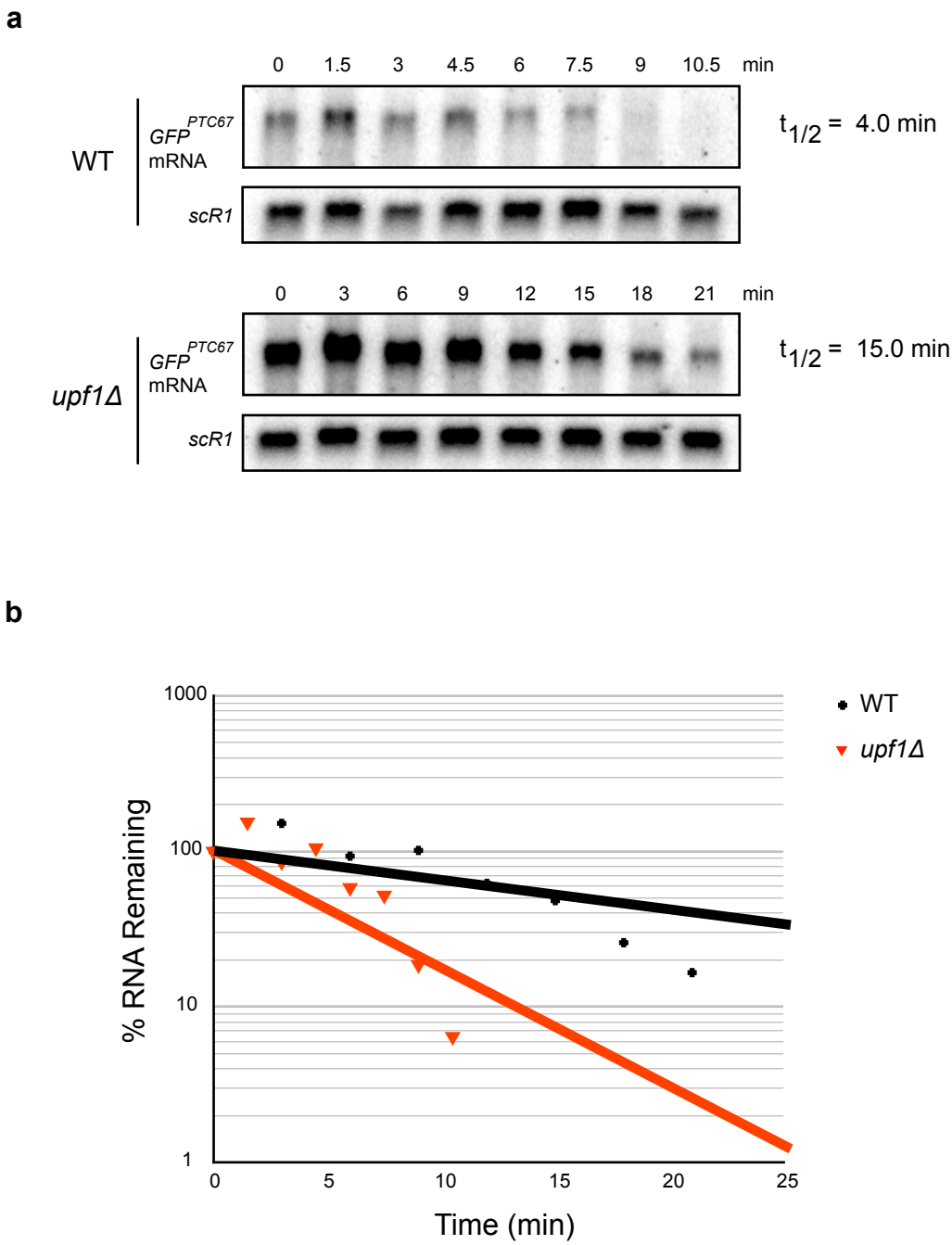
Wenqian Hu, Christine Petzold, Jeff Coller*, and Kristian E. Baker*

Center for RNA Molecular Biology, Case Western Reserve University, Cleveland, OH 44106

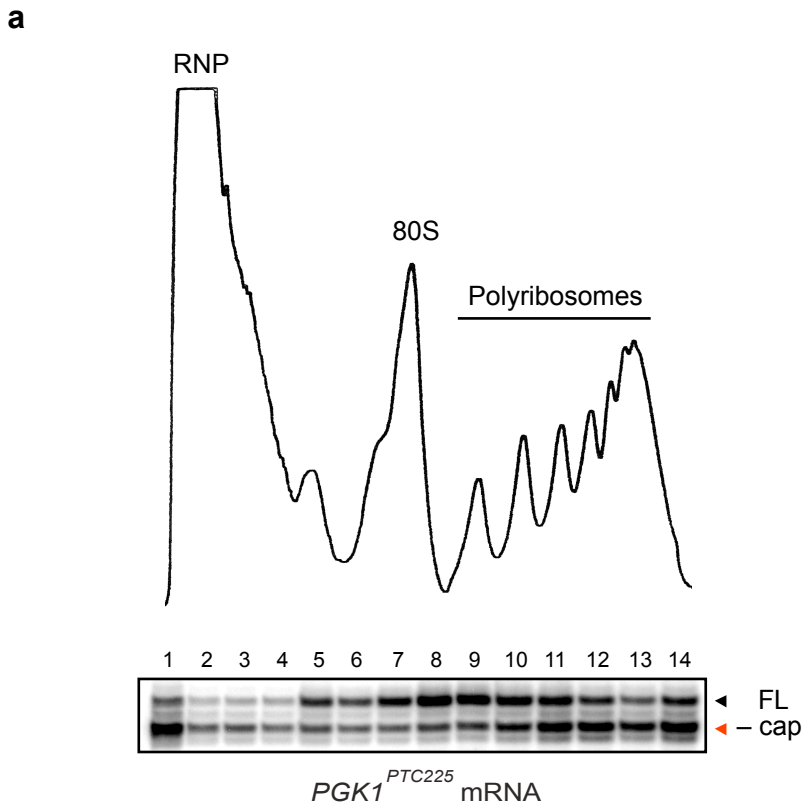
*To whom correspondence should be addressed: K. Baker (keb22@case.edu) or

J. Coller (jmc71@case.edu)

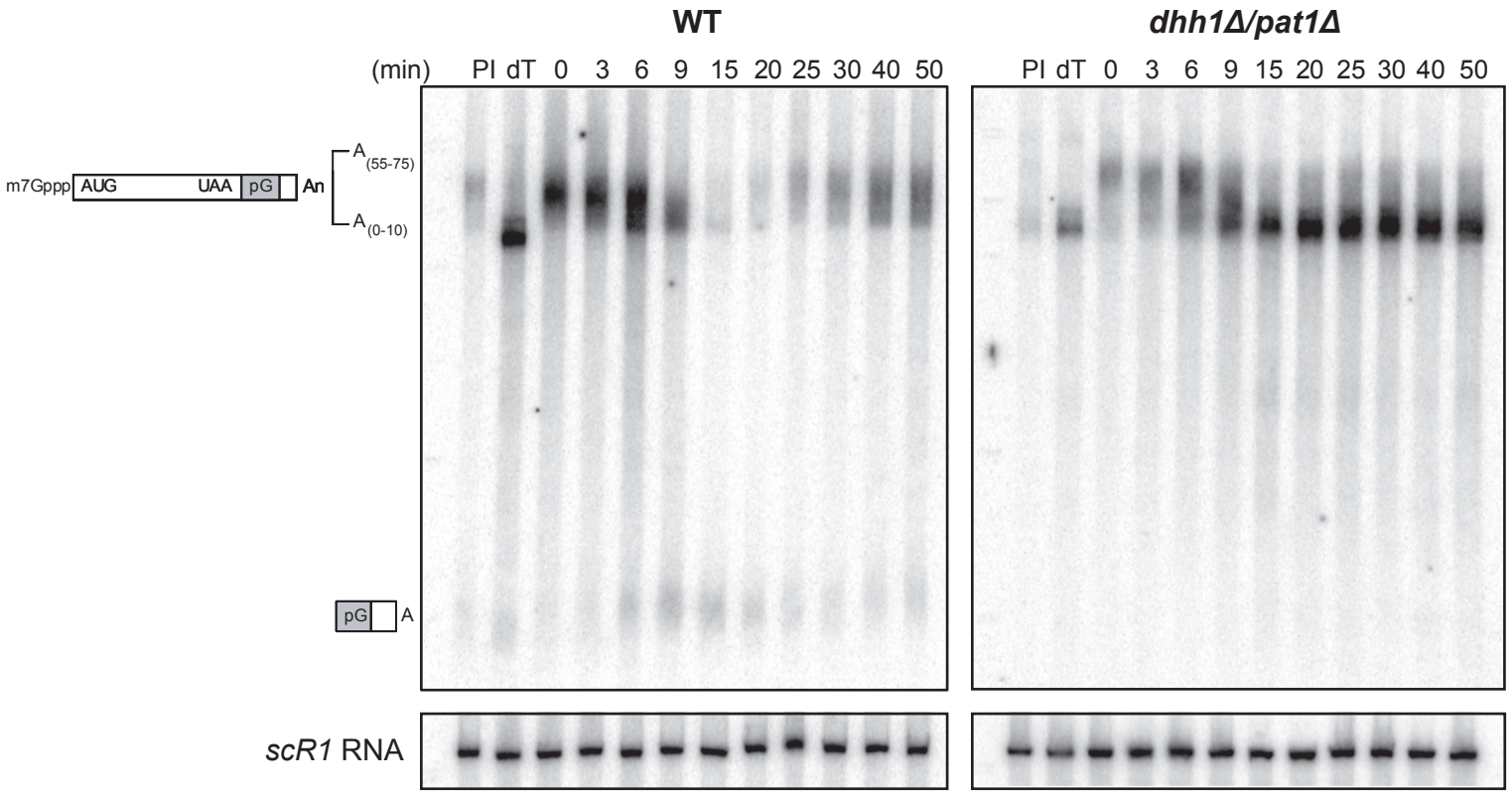
Nature Structural & Molecular Biology: doi:10.1038/nsmb.1734



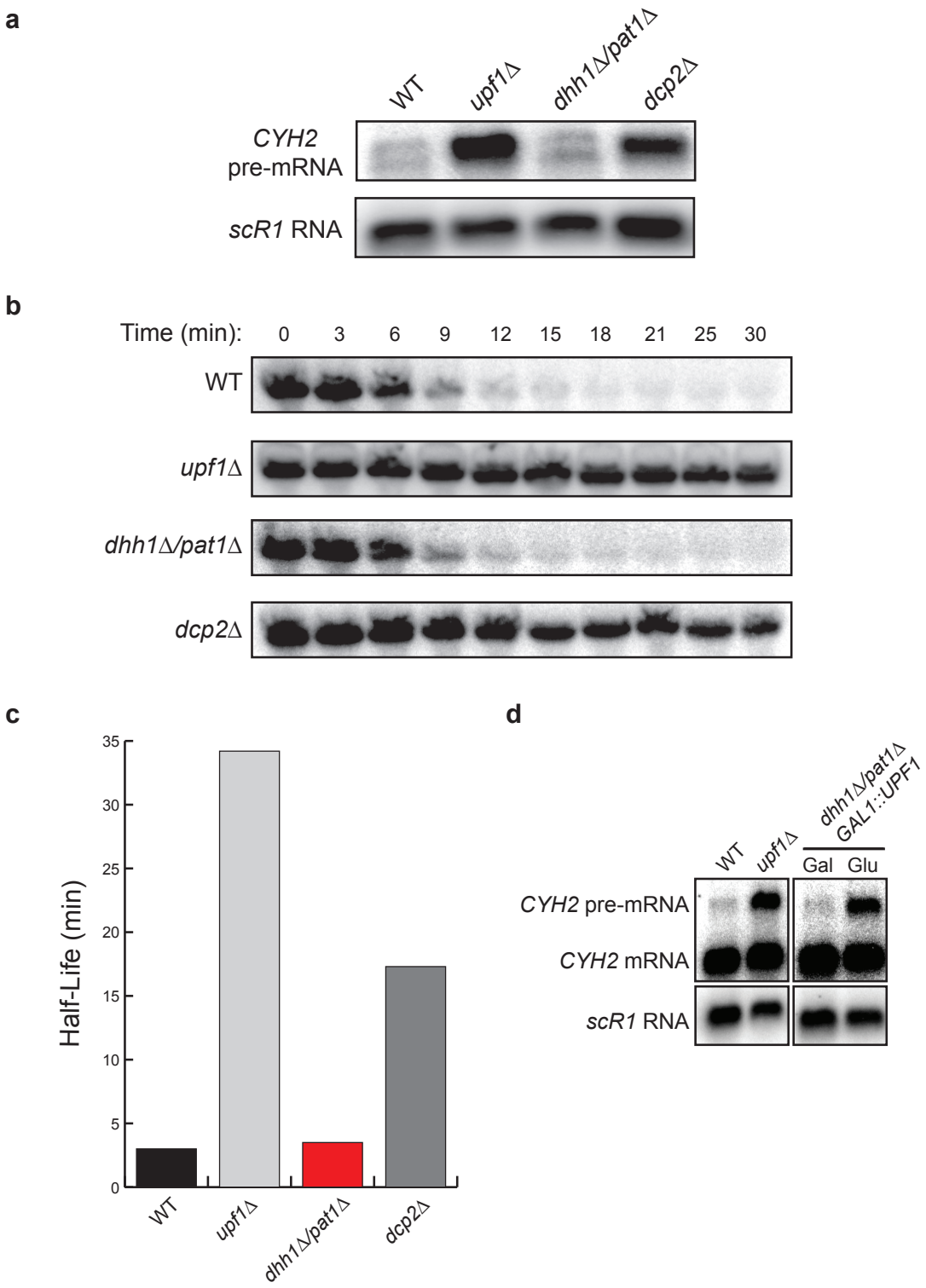
Supplementary Figure 1 *GFP*^{PTC67} mRNA is a substrate for nonsense-mediated mRNA decay. **(a)** Transcription shut-off analysis of *GFP*^{PTC67} reporter mRNA in wild-type and *upf1Δ* cells. RNA (30 μg) isolated from cell aliquots removed at various time points after inhibition of transcription were subject to Northern blot analysis. *GFP*^{PTC67} mRNA and a loading control, *scR1* RNA, were detected using radiolabeled complementary oligonucleotides. **(b)** Quantification of mRNA levels, after normalization, measured in (a).



Supplementary Figure 2 Decapped, nonsense-containing mRNA is associated with polyribosomes in *xrn1Δ* cells. **(a)** Primer extension analysis for *PGK1*^{PTC225} mRNA was performed on RNA recovered from sucrose gradient fractions after centrifugation of lysates from *xrn1Δ* cells. RNP, 80S, and polyribosome sedimentation profiles are indicated. FL: full length mRNA; – cap: decapped mRNA. **(b)** Quantification of decapped *PGK1*^{PTC225} mRNA in RNP versus polyribosome fractions.



Supplementary Figure 3 *dhh1Δ/pat1Δ* cells exhibit a strong defect in decapping of normal mRNA. Transcription pulse-chase analysis for *MFA2pG* reporter mRNA in wild-type and *dhh1Δ/pat1Δ* cells. Reporter gene expression was induced for 7 minutes and followed by inhibition of transcription. RNA, isolated from time points after inhibition of transcription, was analyzed by high resolution polyacrylamide gel electrophoresis and Northern blotting. *MFA2pG* reporter mRNA was detected using an oligonucleotide complementary to the poly(G) tract in the 3' UTR. *scR1* RNA served as a control for RNA loading. PI: pre-induction; dT: RNA treated with oligo d(T)/RNase H to delineate the migration pattern of deadenylated mRNA.



Supplementary Figure 4 Degradation of nonsense-containing mRNA is unaffected in *dhh1Δ/pat1Δ* cells and dependent upon NMD. **(a)** Steady-state *CYH2* pre-mRNA levels in wild-type, *upf1Δ*, *dhh1Δ/pat1Δ*, and *dcp2Δ* cells were detected by Northern blot. *scR1* RNA levels serve as a control for loading. **(b)** Transcription shut-off analysis of $PGK1^{PTC225}$ mRNA in wild-type, *upf1Δ*, *dhh1Δ/pat1Δ*, and *dcp2Δ* cells. $PGK1^{PTC225}$ mRNA half-lives after normalization to *scR1* RNA (c). **(d)** Steady-state *CYH2* mRNA and pre-mRNA levels in wild-type, *upf1Δ*, and *dhh1Δ/pat1Δ/GAL1*::*UPF1* cells (*UPF1* gene under control of the galactose-inducible, *GAL1* promoter). Gal: cell grown in galactose media; Glu: cells grown in glucose media.

Table 1. Yeast Strains, Plasmids and Oligonucleotides

Name	Description	Reference
yJC151	MATa, *ura3Δ, leu2Δ, his3Δ, met15Δ*	EUROSCARF
yJC182	MATa, *ura3Δ, leu2Δ, his3Δ, met15Δ, xrn1::KanMX6*	EUROSCARF
yJC287	CB012: MATa, *ade2-1, his3, leu2, trp1, ura3, pep4::HIS3, prb::HIS3, pre1::HIS3*	1
yJC288	YIT613: MATa, ad*e2-1, his3, leu2, trp1, ura3, pep4::HIS3, prb::HIS3, pre1::HIS3, rpl25::LEU2* [pRPL25-Flag-*URA3*-CEN]	1
yJC324	MATa, *ura3Δ, leu2Δ, his3Δ, met15Δ, dhh1::KanMX6, pat1::HIS3*	This study
yJC327	MATa, *ura3Δ, leu2Δ, his3Δ, met15Δ, dcp2::KanMX6*	2
yJC443	MATa, *ura3Δ, leu2Δ, his3Δ, met15Δ, upf1::KanMX6*	EUROSCARF
yJC751	MATa, *ura3Δ, leu2Δ, his3Δ, met15Δ, dhh1::KanMX6, pat1::HIS3, URA3::GAL1-HA-UPF1*	This study
pKB290	GFP WT	3
pKB303	GFP-PTC at codon 67	3
pJC331	PGK1pG	This study
pJC364	PGK1-PTC225	This study
pRP469	MFA2pG	4
oJC591	5'-CGGATAAGAAAGCAACACCTGGC-3'	This study
oJC620	5'-GATCAATTCGTCGTCGTCGAATAAAGAAGACAA-3'	This study
oJC652	5'-ACCAAGGAGTTTGCATCAATGAC-3'	This study
oJC706	5'-GCUGAUGGCGAUGAAUGAACACUGCGUUUGCUGGCUUUGAUGAAA-3'	This study
oJC707	5'GCTGATGGCGATGAATGAACACTG-3'	This study
oJC809	5'-ACAAGATTCAATTGATTGACAACTAGTTGGACAAGGTCGACTCTATCATCA-3'	This study
oJC810	5'-TGATGATAGAGTCGACCTTGTCCAACTAGTTGTCAATCAATTGAATCTTGT-3'	This study
oJC826	5'-ATTGTTTTATATTTGTTGTAAAAAGTAGATAATTACTTCCTT TTTCATCAAAGCCAGCAAACGCAGTGTTCATTCATCGCCATCAGC-3'	This study
oJC834	5'-AAAGTCACCGTCTTGGTTCTTTCATTCCCT-3'	This study
oJC836	5'-TGGAAGGCATTCTTGATTAGTTGGATGA TTTCATCAAAGCCAGCAAACGCAGTGTTCATTCATCGCCATCAGC-3'	This study
oJC838	5'-GCTCTCATTTCGATTGAATCGATGTGGTCT TTTCATCAAAGCCAGCAAACGCAGTGTTCATTCATCGCCATCAGC-3'	2
oJC839	5'-CTGGCTCTCACCTTCCGTCTCTTTCTCTTA-3'	2
oKB118	5'-GGAGAAGAACTCTTCACTGGAGTTGTCCC-3'	This study
oKB132	5'-GGGCAGATTGTGTGGACAGGTAATGGTTGTCTG-3'	This study
oKB347	5'-CATAACCTTCGGGCATGGCACTCTTG-3'	This study
oRP100	5'-GTCTAGCCGCGAGGAAGG-3'	4
oRP121	5'-AATTCCCCCCCCCCCCCCCCCCA-3'	4

SUPPLEMENTARY DATA

GFP^{PTC67} reporter mRNA is degraded by NMD

To demonstrate *GFP* reporter mRNA containing a PTC at codon 67 (*GFP*^{PTC67}) is targeted to degradation by NMD, a transcription shut-off analysis was performed on wild-type and *upf1Δ* cells (Supplementary Fig. 1). *GFP*^{PTC67} reporter mRNA was stabilized ~4-fold in *upf1*Δ cells (cf. 4 min versus 15 min in wild-type versus *upf1*Δ cells).

Decapping of normal mRNA is strongly inhibited in *dhh1Δ/pat1Δ* cells

Deletion of the decapping activators *DHH1* and *PAT1* leads to a defect in mRNA decapping and a dramatic stabilization of normal mRNA[18]. To confirm that decapping of mRNA is inhibited in *dhh1Δ/pat1Δ* cells, transcription pulse-chase analysis was performed for *MFA2pG* reporter mRNA in wild-type and *dhh1Δ/pat1Δ* cells (Supplementary Fig. 2). Specifically, a brief induction of reporter mRNA transcription followed by inhibition of transcription produces a homogenous population of *MFA2pG* mRNA and decay of the mRNA is monitored over time. In wild-type cells, *MFA2pG* mRNA is deadenylated prior to the disappearance of the full-length mRNA and appearance of a decay fragment (generated by the block of 5'→3' exonucleolytic RNA digestion by the 18 nucleotide G track in the mRNA 3' UTR)[18]. In *dhh1Δ/pat1Δ* cells, reporter mRNA is deadenylated, however, the deadenylated mRNA is stable and accumulates over the time course and no decay fragment is detected. The stabilization of deadenylated mRNA is indicative of a defect in mRNA decapping.

Degradation of NMD substrates is unaffected in *dhh1Δ/pat1Δ* cells

Three lines of evidence were used to demonstrate that recognition and degradation of NMD substrates are not affected in *dhh1Δ/pat1Δ* cells. First, the steady-state level of the endogenous NMD substrate, *CYH2* pre-mRNA, was unchanged in *dhh1Δ/pat1Δ* cells (as compared to wild-type cells; Supplementary Fig. 3a). In contrast, *CYH2* pre-mRNA levels increased in both NMD mutants (*upf1Δ*) and decapping defective cells (*dcp2Δ*). Second, transcription shut-off analysis for a PTC-containing reporter ($PGK1^{PTC225}$ mRNA) revealed that the mRNA half-life was identical in wild-type versus *dhh1Δ/pat1Δ* cells (Supplementary Fig. 3b & c). Inhibition of NMD (*upf1Δ*) or mRNA decapping (*dcp2Δ*) lead to dramatic stabilization of $PGK1^{PTC225}$ mRNA, demonstrating that the reporter is indeed sensitive to NMD and degraded by a decapping-dependent mechanism. Third, when UPF1 was depleted in *dhh1Δ/pat1Δ/UPF1::GAL1* cells, the level of *CYH2* pre-mRNA increased dramatically (Supplementary Fig. 3d), indicating that degradation of nonsense-containing mRNA is dependent upon UPF1 and decayed predominantly by NMD in *dhh1Δ/pat1Δ* cells. Together, these results demonstrate that the degradation of nonsense-containing mRNA in *dhh1Δ/pat1Δ* cells is unaffected and dependent upon NMD.

SUPPLMENTARY LITERATURE CITED

1 Inada, T. *et al*. *RNA* **8**, 948-958 (2002).

2 Hu, W., Sweet, T.J., Chamnongpol, S., Baker, K.E., & Coller, J. *Nature* **461**, 225-229 (2009).

3 Baker, K.E. & Parker, R. *RNA* **12**, 1441-1445 (2006).

4 Muhlrad, D., Decker, C.J., & Parker, R. *Mol Cell Biol* **15**, 2145-2156 (1995).