Table I. Strains in this study

Stocks name	Strains name	Genotype	Source
SPJK029	*upf2*Δ	*h⁻ leu1-32 ura4-D18 upf2::ura4+*	(Mendell et al., 2000)
SPJK033	MR3571	*h⁻ leu1-32 ura4-D18 ade6-M26 upf1::kanMX6*	(Rodriguez-Gabriel et al., 2006)
SPJK039	GP937	*h⁻ his3D leu1-23 ura4-D18 ade6-M216*	(Szankasi and Smith, 1996)
SPJK045	*sppab1*Δ	*h⁻ leu1-32 ura4-D18 ade6-M216 sppab1::ura4*	(Thakurta et al., 2002)
SPJK049	*rnps1*Δ	*h⁻ leu1-32 ura4-D18 ade6-M216 rnps1::kanMX6*	This study
SPJK051	*spbc3b9.08c*	*h⁻ leu1-32 ura4-D18 ade6-M210 mago::kanMX6*	(Decottignies et al., 2003)
SPJK039-1	Int-GFP	*h⁻ leu1-32:: [GFP leu1⁺] ura4-D18 ade6-M216* (p*GFP*-wt integrant)	This study
SPJK039-2	Int-PTC6	*h⁻ leu1-32:: [GFP-PTC6 leu1⁺] ura4-D18 ade6-M216* (p*GFP*-PTC6 integrant)	This study
SPJK039-3	Int-PTC27	*h⁻ leu1-32:: [GFP-PTC27 leu1⁺] ura4-D18 ade6-M216* (p*GFP*-PTC27 integrant)	This study
SPJK039-4	Int-PTC140	*h⁻ leu1-32:: [GFP-PTC140 leu1⁺] ura4-D18 ade6-M216* (p*GFP*-PTC27 integrant)	This study
SPJK039-5	Int-GFP$_{ivs}$	*h⁻ leu1-32:: [GFP$_{ivs}$ leu1⁺] ura4-D18 ade6-M216* (p*GFP$_{ivs}$*-wt integrant)	This study
SPJK039-6	Int-PTC6$_{ivs}$	*h⁻ leu1-32:: [GFP$_{ivs}$-PTC6 leu1⁺] ura4-D18 ade6-M216* (p*GFP$_{ivs}$*-PTC6 integrant)	This study
SPJK039-7	Int-PTC27$_{ivs}$	*h⁻ leu1-32:: [GFP$_{ivs}$-PTC27 leu1⁺] ura4-D18 ade6-M216* (p*GFP$_{ivs}$*-PTC27 integrant)	This study
SPJK039-8	Int-PTC140$_{ivs}$	*h⁻ leu1-32:: [GFP$_{ivs}$-PTC140 leu1⁺] ura4-D18 ade6-M216* (p*GFP$_{ivs}$*-PTC140 integrant)	This study
SPJK029-1	upf2-GFP	*h⁻ leu1-32::[GFP leu1⁺] ura4-D18 upf2::ura4+* (p*GFP*-wt integrant)	This study
SPJK029-2	upf2-PTC6	*h⁻ leu1-32::[GFP-PTC6 leu1⁺] ura4-D18 upf2::ura4+* (p*GFP*-PTC6 integrant)	This study
SPJK029-3	upf2-PTC27	*h⁻ leu1-32::[GFP-PTC27 leu1⁺] ura4-D18 upf2::ura4+* (p*GFP*-PTC27 integrant)	This study
SPJK029-4	upf2-GFP$_{ivs}$	*h⁻ leu1-32::[GFP$_{ivs}$ leu1⁺] ura4-D18 upf2::ura4+* (p*GFP$_{ivs}$*-wt integrant)	This study
SPJK029-5	upf2-PTC6$_{ivs}$	*h⁻ leu1-32::[GFP$_{ivs}$-PTC6 leu1⁺] ura4-D18 upf2::ura4+* (p*GFP$_{ivs}$*-PTC6 integrant)	This study
SPJK029-6	upf2-PTC27$_{ivs}$	*h⁻ leu1-32::[GFP$_{ivs}$-PTC27 leu1⁺] ura4-D18 upf2::ura4+* (p*GFP$_{ivs}$*-PTC27 integrant)	This study

Supplementary Figure 1. Homologs of putative EJC components in *S. pombe*. (A-D) Protein sequence alignment of *S. pombe* eIF4AIII (A), RNPS1 (B), MAGO (C) and Y14 (D) with the corresponding proteins of *S. cerevisiae*, *D. melanogaster*, *C. elegans*, *A. thaliana*, human, and *Xenopus laevis*. Identical residues are highlighted in red, and similar residues are in green.
The accession numbers of the proteins are: *S. cerevisiae* eIF4AIII, NP_010304; *S. pombe* eIF4AIII, NP_592863; *X. laevis* eIF4AIII, AAH84859; human eIF4AIII, NP_055555; *D. melanogaster* eIF4AIII, NP_649788; *C. elegans* eIF4AIII, AAK29954; *A. thaliana* eIF4AIII, NP_188610; *S. pombe* RNPS1, NP_596549; *X. laevis* RNPS1, Q5XG24; human RNPS1, NP_006702; *D. melanogaster* RNPS1, NP_649903; *C. elegans* RNPS1, AAK21429; *A. thaliana* RNPS1, NP_173107; *S. pombe* MAGO, NP_596666; *X. laevis* MAGO, NP_001079724; human MAGO, NP_002361; *D. melanogaster* MAGO, NP_476636; *C. elegans* MAGO, P49029; *A. thaliana* MAGO, NP_171716; *S. pombe* Y14, NP_594439; *X. laevis* Y14, NP_001083872; human Y14, NP_005096; *D. melanogaster* Y14, NP_610454; *C. elegans* Y14, CAA83626; and *A. thaliana* Y14, NP_564591.

Supplementary Figure 2. NMD is not affected by the level of expression of the reporter. (A-B) Northern blotting analysis of total-RNA from wild-type (A) and *upf2Δ* (B) cells, transformed with the plasmids indicated; WT-nmt41 is the same construct as WT in Fig 1A and is regulated by the nmt41 derivative of the nmt1 promoter. Top panels show hybridization with a GFP specific probe; the bottom panel shows hybridization with a probe specific for the large ribosomal protein 32 (*Rpl32*) mRNA, as a loading control. The quantification is based on two independent experiments.

Supplementary Figure 3. Lengthening the distance between stop codon and a 3' UTR intron inhibits NMD. (A) Diagrams of plasmid GFP reporters used; the top shows a PTC-less reporter with an insertion of 147 bp in the 3'UTR, adjacent to the GFP stop codon; the bottom shows a similar reporter with an intron located just after the 147 bp insertion (more information in Material and Methods); PA and PB indicate the primers used for the PCR. (B) Real-time qRT-PCR analysis (using SYBER green PCR master mix) of total-RNA extracted from in wild-type cells transformed with the plasmids indicated. The level of GFP mRNA was normalized that of *Rpl32* mRNA, which was assayed in parallel using specific primers.

Supplementary Figure 4. The entire picture file of the Northern membrane used to generate Fig. 2D, which corresponds to the boxed region. The noticeable line just below the bands is an artifact of our phosphoroimager which occasionally automatically readjusts the contrast when moving from regions of high to low signal. This bug on the system does not affect quantification of the signal.

Supplementary references

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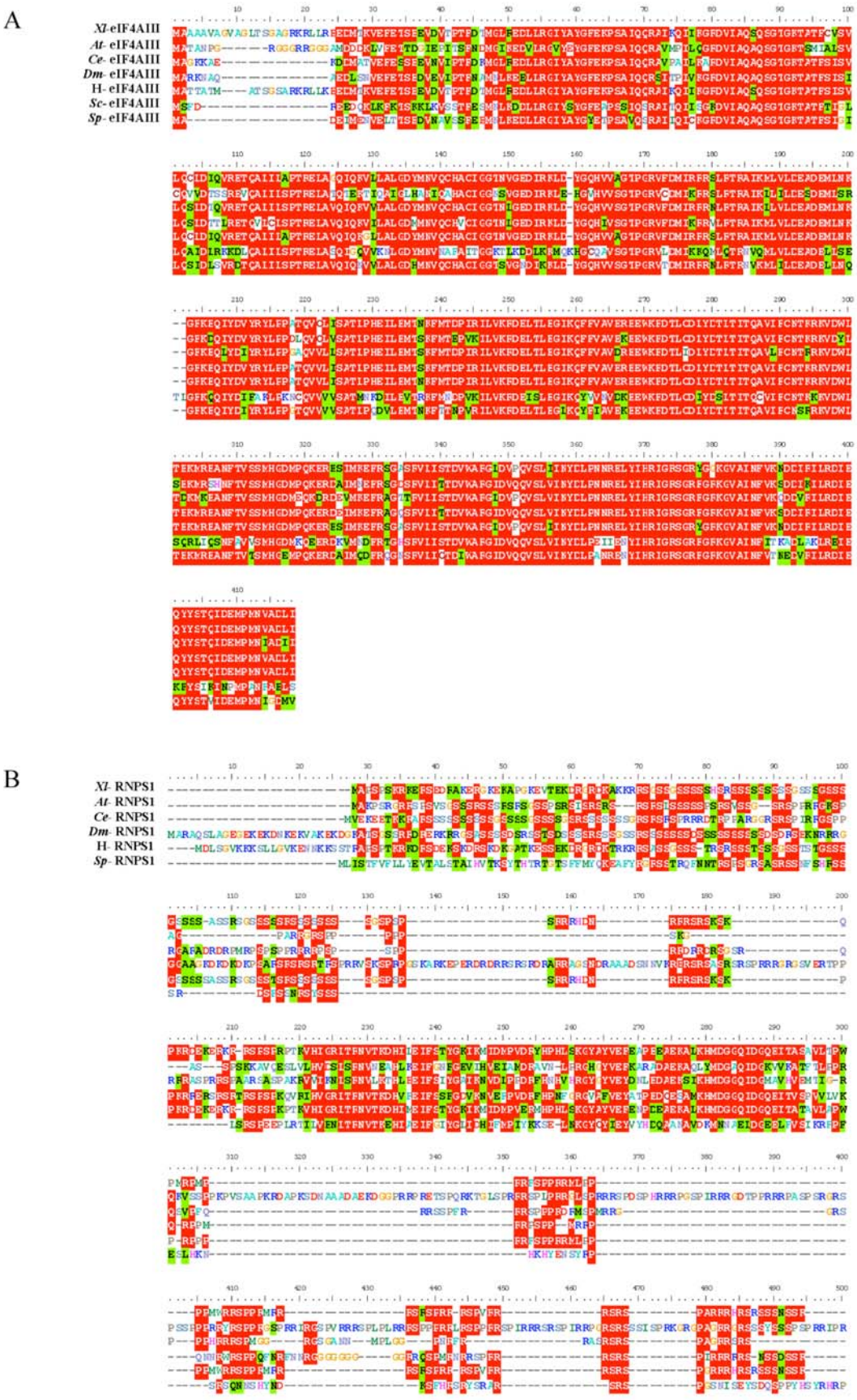
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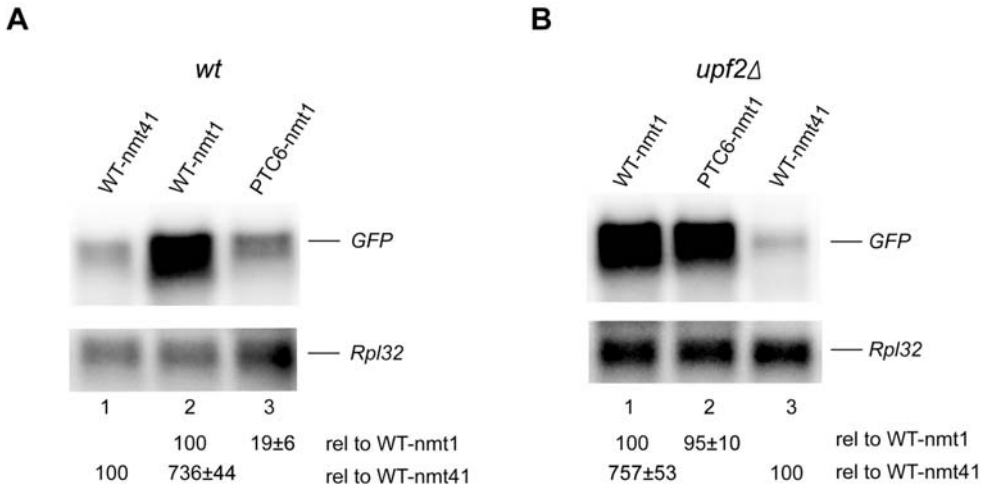
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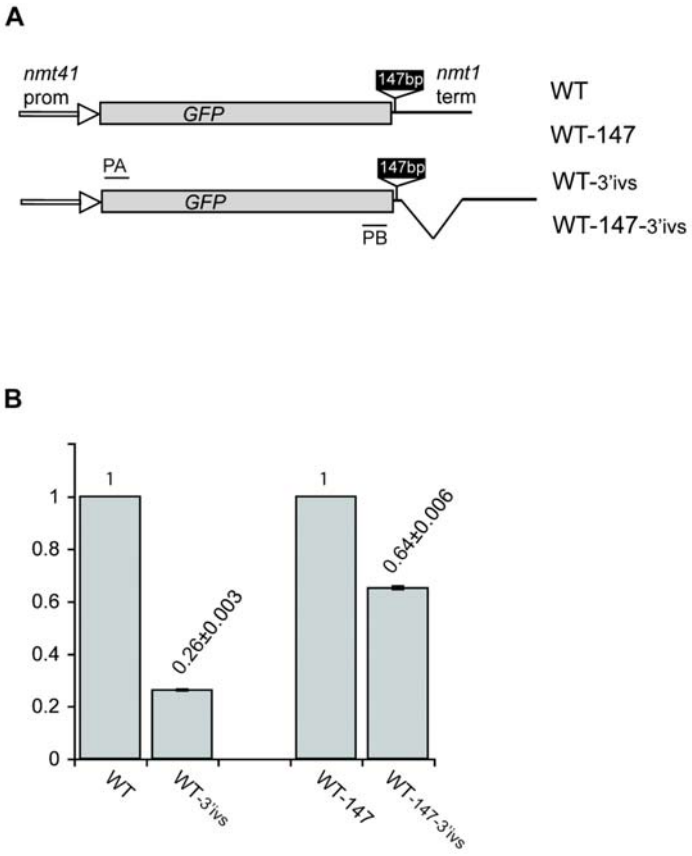
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GFP
PB
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WT-147-3'ivs
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1
0.26±0.003
0.64±0.006
0
0.2
0.4
0.6
0.8
1
WT
WT-3'ivs
WT-147
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