Supplemental Information: Control of mRNA stability in fungi by NMD, EJC and CBC factors through 3'UTR introns

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Supplemental Tables

Table S1 Homologs of NMD, EJC, CBC factors and other components related to these pathways.

The *N. crassa* homologs identified represent the best bidirectional hits for each organism as determined using BLASTP at the NCBI website.

Exceptions are indicated.

Protein Name	Organism	NCBI Reference Sequence[a]	Reciprocal BLAST[b]	Ref/Note
Upf1	*N. crassa*	XP_961233.1		NCU04242
	S. pombe	NP_593080.1	XP_961233.1	Upf1
	S. cerevisiae	NP_013797.1	XP_961233.1	NAM7
	H. sapiens	NP_001284478.1	XP_961233.1	regulator of nonsense transcripts 1 isoform 1
	C. elegans	NP_490829.1	XP_961233.1	SMG-2
Upf2	*N. crassa*	XP_961757.2		NCU05267
	S. pombe	NP_593784.1	XP_961757.2	Upf2
	S. cerevisiae	NP_011944.2	XP_961757.2	Nmd2p
	H. sapiens	NP_056357.1	XP_961757.2	regulator of nonsense transcripts 2
	C. elegans	NP_500974.2	XP_961757.2	SMG-3
Upf3	*N. crassa*	XP_956721.1		NCU03435
	S. cerevisiae	NP_011586.1	XP_956721.1	Upf3p
	H. sapiens	NP_075386.1	XP_956721.1	regulator of nonsense transcripts 3B isoform 2
	C. elegans	(NP_741600.1)[c]	XP_956721.1	SMG-4, isoform b
Xrn1	*N. crassa*	XP_960925.2		NCU06678
	S. pombe	NP_593482.1	XP_960925.2	exonuclease II Exo2
	S. cerevisiae	NP_011342.1	XP_960925.2	Xrn1p
	H. sapiens	NP_001269786.1	XP_960925.2	5'-3' exoribonuclease 1 isoform c
	C. elegans	NP_496945.3	XP_960925.2	XRN-1
Y14	*N. crassa*	XP_965326.1		NCU03226
	S. pombe	NP_594439.1	XP_965326.1	
	S. cerevisiae	not detected		
	H. sapiens	NP_005096.1	XP_965326.1	
	C. elegans	NP_497891.1	XP_965326.1	RNP-4
	X. laevis	NP_001079905.1	XP_965326.1	RNA-binding protein 8A-B

	D. melanogaster	NP_610454.2	XP_965326.1	tsunagi
	A. thaliana	NP_564591.1	XP_965326.1	
Mago	*N. crassa*	XP_957482.1		NCU04405
	S. pombe	NP_596666.1	XP_957482.1	mago nashi
	S. cerevisiae	not detected		
	H. sapiens	NP_060518.1	XP_957482.1	mago nashi
	C. elegans	NP_493025.1	XP_957482.1	MAG-1
	X. laevis	NP_001079724.1	XP_957482.1	mago nashi
	D. melanogaster	NP_476636.1	XP_957482.1	mago nashi
	A.thaliana	NP_171716.1	XP_957482.1	mago nashi
eIF4AIII	*N. crassa*	XP_961600.1		NCU01234
	S. pombe	NP_592863.1	XP_961600.1	
	S. cerevisiae	NP_010304.3	XP_961600.1	FAL1
	H. sapiens	NP_055555.1	XP_961600.1	eIF4AIII
	C. elegans	NP_490761.2	XP_961600.1	
	X. laevis	NP_001084200.1	XP_961600.1	eIF4A-III-B
	D. melanogaster	NP_649788.2	XP_961600.1	eIF4AIII
	A.thaliana	NP_188610.1	XP_961600.1	DEAD-box ATP-dependent RNA helicase 2
Btz[d]				
RNPS1	*N. crassa*	XP_958016.1		NCU09901
	S. pombe	NP_596549.2	XP_958016.1	RNA-binding protein (predicted)
	S. cerevisiae	NP_015147.1[e]	XP_956595.1	Cbc2p
	H. sapiens	NP_001273556.1	XP_958016.1	RNA-binding protein with serine-rich domain 1 isoform c
	C. elegans	NP_497276.2	XP_958016.1	Protein RNP-5
CBP20	*N. crassa*	XP_956595.1		NCU00210
	S. pombe	NP_596414.1	XP_956595.1	nuclear cap-binding complex small subunit (predicted)
	S. cerevisiae	NP_015147.1	XP_956595.1	Cbc2p
	H. sapiens	NP_031388.2	XP_956595.1	nuclear cap-binding protein subunit 2 isoform 1
	C. elegans	NP_001250552.1	XP_956595.1	NCBP-2, isoform a
CBP80	*N. crassa*	XP_961147.1		NCU04187
	S. pombe	NP_594104.1	XP_961147.1	nuclear cap-binding complex large subunit (predicted)
	S. cerevisiae	NP_013844.2	XP_961147.1	Sto1p
	H. sapiens	NP_002477.1	XP_961147.1	nuclear cap-binding protein subunit 1
	C. elegans	NP_491850.2	XP_961147.1	NCBP-1
eRF1	*N. crassa*	XP_957296.1		NCU00410
	S. pombe	NP_594680.1	XP_957296.1	eRF1
	S. cerevisiae	NP_009701.3	XP_957296.1	eRF1
	H. sapiens	NP_004721.1	XP_957296.1	eukaryotic peptide chain release factor subunit 1 isoform 1
	C. elegans	NP_001024107.2	XP_957296.1	ETF-1

eIF4A[f]	*N. crassa*	XP_958421.2		NCU07420
	S. pombe	NP_594854.1	XP_958421.2	eIF4A
	S. cerevisiae	NP_012397.1	XP_958421.2	eIF4A
	H. sapiens	NP_001407.1	XP_958421.2	eIF4A-I isoform 1
	C. elegans	NP_001022623.1	XP_958421.2	INF-1, isoform a

[a]. The N. crassa protein was used in BLAST and the entry represents the top hit

[b]. The top hit identified in each organism was used to BLAST N. crassa proteins and the entry represents the top hit to evaluate best bidirectional hits

[c]. N. crassa Upf3 did not give a significant hit when BLASTed against C. elegans, but C. elegans SMG4 identifies N. crassa NCU03435

[d]. No Btz homolog was identified in N. crassa

[e]. The protein identified as N. crassa RNPS1 has a best hit to S. cerevisiae CBP20 (which reciprocally hits N. crassa CBP20)

[f]. eIF4A is distinct from eIF4AIII and the homologs are included here for reference

Table S2 Phenotypes of selected *N. crassa* deletion strains.

Phenotypes of the wild-type strain (wt), and indicated mutant strains were assessed as described in Materials and Methods.

FGSC #	Genotype	Gene Name	Colony Growth and Morphology (Growth Pattern)				Colony Growth and Morphology (Plate Pigmentation)				25°C Growth in Slant Cultures			25°C Growth Rate of Basal Hyphae (mm/hr)	25°C Aerial Hyphae Height (mm/day)		Female Sexual Development		
			VM		VM+YE		VM		VM+YE		Slant Pigmen-tation	Conid-iation	Aerial Hyphae Forma-tion		VM	VM +YE	Protoper-ithecium Forma-tion	Perithe-cium Forma-tion	Asco-spore Forma-tion
			25	37	25	37	25	37	25	37									
2489	wild-type		+	+	+	+	+	+	+	+	+	+	+	2.8	20-25	20-25	+	+	+
11230	ΔNCU04242	upf1	-	-	-	+	r	r	r	r	r	r	r	2.2	15-20	15-20	+	+	+
15706	ΔNCU05267	upf2	-	-	-	-	r	r	r	r	r	r	short	2	5-10	10-15	r	-	-
11679	ΔNCU03435	upf3	+	+	+	+	+	+	+	+	r	r	r	2.9	20-25	15-20	+	+	+
13031	ΔNCU04405	mago	-	+	-	+	r	r	r	r	r	+	r	1.7	15-20	15-20	-	-	-
15492	ΔNCU03226	y14	-	-	-	-	r	r	r	r	r	r	+	0.4	0-5	0-5	-	-	-
19228	ΔNCU06678	xrn1	-	-	-	-	+	r	+	r	+	r	r	1.6	10-15	10-15	+	+	r
17986	ΔNCU04270	btz	+	+	+	+	+	+	+	+	+	+	+	2.9	20-25	20-25	r	+	r
12342	ΔNCU09901	rnps1	+	+	+	+	+	+	+	+	+	+	+	3	20-25	25-30	+	+	+
18692	ΔNCU00210	cbp20	+	+	+	+	-	-	-	+	+	+	+	2.2	20-25	20-25	+	+	+
22440	ΔNCU04187	cbp80	+	+	-	+	+	+	+	+	+	+	+	2.8	20-25	20-25	+	+	+
20906	ΔNCU05889	eif3e	-	-	-	-	-	-	-	-	r	-	-	0.8	5-10	0-5	-	-	-

"+" : normal
"-" : not formed
"r" : reduced formation

Table S3 Identified *N. crassa* transcripts with at least one spliced 3'UTR intron.

The *Neurospora crassa* gene models from release 7 indicates 321 of the 9728 predicted protein coding genes in *N. crassa* potentially have 3'UTR introns. For 237, the 3'UTR-intron was present in all expected forms of the transcript were further examined according to our recently published RNA-seq data for wt (Wu *et al.* 2014). 31 mRNAs with spliced 3'UTR introns were identified and their predicted 3'UTR and 3'UTR intron-lengths were calculated based on *N. crassa* RNA-seq data; additional information about these genes is also listed.

Locus	Predicted 3'UTR Left [intron] Right	Gene Symbol or Description	Protein
NCU00261	471 [64] 327	pyr-7	CTP synthase
NCU00410	235 [55] 366	erf1	eukaryotic release factor 1
NCU00778	53 [57] 412	sed5 vesicle protein	sed5 vesicle protein
NCU00854	131 [58] 21	hypothetical protein	hypothetical protein
NCU01234	139 [62] 93 or 180	eif4a3	eukaryotic initiation factor 4A-12
NCU01312	102 [62] 380	rca-1	regulator of conidiation in Aspergillus-1
NCU01888	98 [69] 321	tRNA-specific adenosine deaminase	tRNA-specific adenosine deaminase
NCU01907	92 [68] 148	syntaxin 5	syntaxin 5
NCU02174	9 [170] 501	hypothetical protein	hypothetical protein
NCU02249	337 [63] 784	hat-5	histone acetyltransferase
NCU02423	36 [93] 880	mic-12	mitoferrin-1
NCU02885	104 [63] 1253	stk-20	hypothetical protein
NCU02948	12 [78] 277	ncw-4	non-anchored cell wall protein-4
NCU03226	394 [60] 375	y14	Y14 protein
NCU03491	43 [56] 704	pad-1	Paddle-1
NCU03682	184 [71] 250	endonuclease/exonuclease/phosphatase	endonuclease/exonuclease/phosphatase
NCU04650	194 [75] 562	hypothetical protein	hypothetical protein
NCU04699	104 [63] 599	chol-2	methylene-fatty-acyl-phospholipid synthase
NCU04986	131 [60] 71	hypothetical protein	hypothetical protein
NCU05243	14 [69] 174	hypothetical protein	hypothetical protein
NCU05964	69 [65] 741	developmental regulator VosA	developmental regulator VosA
NCU06322	359 [131] 231	drc-2/DNA replication complex GINS protein psf-2	DNA replication complex GINS protein psf-2
NCU06869	541 [55] 436	paa-9	cleavage and polyadenylylation specificity factor
NCU07408	220 [215] 109	po	60S ribosomal protein P0
NCU07587	382 [60] 182	div-16/Swi6	Swi6
NCU08026	71 [63] 697	3' exoribonuclease	3' exoribonuclease
NCU08727	289 [59] 261	hypothetical protein	hypothetical protein
NCU09615	197 [60] 345	hypothetical protein	hypothetical protein
NCU10500	118 [77] 492	F-box domain-containing protein	F-box domain-containing protein
NCU10572	70 [75] 484	short chain oxidoreductase	short chain oxidoreductase
NCU11426	535 [107] 342	nuc-2	nuc-2 protein

Table S4 FunCat analysis for identified *N. crassa* transcripts with at least one spliced 3'UTR intron.

The FunCat database (Ruepp *et al.* 2004) was used to calculate functional enrichments for the products specified by the 31 identified mRNAs with spliced 3'UTR introns as described in Materials and Methods.

FUNCTIONAL CATEGORY	abs SET	rel SET	genes SET	abs GENOME	rel GENOME	rel SET/rel GENOME	P-VALUE
01.03 nucleotide/nucleoside/nucleobase metabolism	8	28.5	NCU00261 NCU02423 NCU01234 NCU11426 NCU01888 NCU07408 NCU08026 NCU03226	745	7.4	3.851	0.001
01.03.04 pyrimidine nucleotide/nucleoside/nucleobase metabolism	3	10.7	NCU00261 NCU11426 NCU01888	159	1.57	6.815	0.009
01.03.04.01 pyrimidine nucleotide/nucleoside/nucleobase catabolism	2	7.14	NCU11426 NCU01888	29	0.28	25.500	0.003
01.03.16 polynucleotide degradation	5	17.8	NCU01234 NCU11426 NCU07408 NCU08026 NCU03226	220	2.18	8.165	0.000
01.03.16.01 RNA degradation	4	14.2	NCU01234 NCU07408 NCU08026 NCU03226	153	1.51	9.404	0.001
01.06.02 membrane lipid metabolism	3	10.7	NCU00261 NCU04699 NCU11426	262	2.6	4.115	0.035
01.06.02.01 phospholipid metabolism	3	10.7	NCU00261 NCU04699 NCU11426	189	1.87	5.722	0.015
10 CELL CYCLE AND DNA PROCESSING	9	32.1	NCU02249 NCU01312 NCU01234 NCU04650 NCU11426 NCU06322 NCU07587 NCU02885 NCU03226	1715	17	1.888	0.038
10.01 DNA processing	8	28.5	NCU02249 NCU01312 NCU01234 NCU11426 NCU06322 NCU07587 NCU02885 NCU03226	1117	11	2.591	0.009
10.01.03 DNA synthesis and replication	4	14.2	NCU02249 NCU01234 NCU06322 NCU07587	365	3.62	3.923	0.017
10.01.05 DNA recombination and DNA repair	5	17.8	NCU02249 NCU01234 NCU11426 NCU07587 NCU02885	558	5.54	3.213	0.017
10.01.05.01 DNA repair	4	14.2	NCU02249 NCU01234 NCU11426 NCU02885	477	4.73	3.002	0.041
10.03.01.01.03 G1/S transition of mitotic cell cycle	3	10.7	NCU01312 NCU07587 NCU02885	159	1.57	6.815	0.009
10.03.04 nuclear and chromosomal cycle	3	10.7	NCU04650 NCU11426 NCU02885	289	2.87	3.728	0.045
11 TRANSCRIPTION	15	53.5	NCU02249 NCU02423 NCU01312 NCU01234 NCU04986 NCU11426 NCU01888 NCU06869 NCU07408 NCU07587 NCU08026 NCU02948 NCU02885 NCU09615 NCU03226	1759	17.4	3.075	0.000
11.02 RNA synthesis	10	35.7	NCU02249 NCU01312 NCU01234 NCU04986 NCU11426 NCU06869 NCU07587 NCU02948 NCU09615 NCU03226	1489	14.7	2.429	0.005
11.02.03 mRNA synthesis	10	35.7	NCU02249 NCU01312 NCU01234 NCU04986 NCU11426 NCU06869 NCU07587 NCU02948 NCU09615 NCU03226	1426	14.1	2.532	0.004
11.02.03.04 transcriptional control	9	32.1	NCU02249 NCU01312 NCU01234 NCU11426 NCU06869 NCU07587 NCU02948 NCU09615 NCU03226	1279	12.7	2.528	0.006

11.04 RNA processing	9	32.1	NCU02423 NCU01312 NCU01234 NCU11426 NCU06869 NCU07408 NCU08026 NCU02885 NCU03226	732	7.27	4.415	0.000
11.04.01 rRNA processing	4	14.2	NCU01234 NCU07408 NCU08026 NCU03226	363	3.6	3.944	0.017
11.04.03 mRNA processing (splicing, 5^-, 3^-end processing)	9	32.1	NCU02423 NCU01312 NCU01234 NCU11426 NCU06869 NCU07408 NCU08026 NCU02885 NCU03226	538	5.34	6.011	0.000
11.04.03.01 splicing	5	17.8	NCU02423 NCU01312 NCU01234 NCU06869 NCU03226	437	4.34	4.101	0.006
11.04.03.05 3^-end processing	2	7.14	NCU06869 NCU03226	84	0.83	8.602	0.023
11.04.03.11 control of mRNA stability	3	10.7	NCU01234 NCU02885 NCU03226	95	0.94	11.383	0.002
12 PROTEIN SYNTHESIS	6	21.4	NCU00410 NCU01234 NCU07408 NCU08026 NCU02885 NCU03226	753	7.47	2.865	0.016
12.04 translation	4	14.2	NCU00410 NCU01234 NCU07408 NCU03226	460	4.56	3.114	0.037
16 PROTEIN WITH BINDING FUNCTION OR COFACTOR REQUIREMENT (structural or catalytic)	17	60.7	NCU00410 NCU02249 NCU02423 NCU01312 NCU01234 NCU00778 NCU04650 NCU11426 NCU06322 NCU01888 NCU01907 NCU06869 NCU07408 NCU07587 NCU08026 NCU02885 NCU03226	3639	36.1	1.681	0.007
16.01 protein binding	13	46.4	NCU00410 NCU02249 NCU01312 NCU01234 NCU00778 NCU04650 NCU11426 NCU01907 NCU06869 NCU07408 NCU07587 NCU02885 NCU03226	2333	23.1	2.009	0.006
16.03 nucleic acid binding	10	35.7	NCU02249 NCU01312 NCU01234 NCU04650 NCU11426 NCU06322 NCU06869 NCU07587 NCU08026 NCU03226	1290	12.8	2.789	0.002
16.03.01 DNA binding	7	25	NCU02249 NCU01312 NCU01234 NCU11426 NCU06322 NCU07587 NCU03226	854	8.48	2.948	0.007
16.03.03 RNA binding	6	21.4	NCU01234 NCU04650 NCU11426 NCU06869 NCU08026 NCU03226	552	5.48	3.905	0.004
16.07 structural protein binding	2	7.14	NCU04650 NCU07408	94	0.93	7.677	0.028
18.02.01.01 enzyme activator	4	14.2	NCU00410 NCU11426 NCU06869 NCU02885	376	3.73	3.807	0.019
18.02.01.02.05 kinase inhibitior	2	7.14	NCU11426 NCU02885	103	1.02	7.000	0.033
20 CELLULAR TRANSPORT, TRANSPORT FACILITIES AND TRANSPORT ROUTES	11	39.2	NCU02423 NCU01312 NCU01234 NCU00778 NCU04650 NCU11426 NCU01907 NCU07587 NCU08026 NCU02948 NCU02885	2165	21.5	1.823	0.025
20.01.13 lipid/fatty acid transport	3	10.7	NCU02423 NCU11426 NCU07587	206	2.04	5.245	0.019
20.09 transport routes	8	28.5	NCU02423 NCU01234 NCU00778 NCU04650 NCU11426 NCU01907 NCU07587 NCU02885	1454	14.4	1.979	0.040
20.09.07 vesicular transport (Golgi network, etc.)	6	21.4	NCU00778 NCU04650 NCU11426 NCU01907 NCU07587 NCU02885	521	5.17	4.139	0.003
20.09.07.05 intra Golgi transport	2	7.14	NCU00778 NCU01907	74	0.73	9.781	0.018
30.01.09.03 Ca2+ mediated signal transduction	2	7.14	NCU11426 NCU02885	125	1.24	5.758	0.047
32.01.06 cold shock response	2	7.14	NCU01234 NCU11426	65	0.64	11.156	0.014

34.11.09 temperature perception and response	2	7.14	NCU02423 NCU11426	110	1.09	6.550	0.037
42.04.03 actin cytoskeleton	3	10.7	NCU11426 NCU07587 NCU02885	275	2.73	3.919	0.040

[a]The comparison is done to p3_p13841_Neu_crass_MIPS containing 10067 annotated genes.
28 out of 31 genes are found:
NCU00261 NCU00410 NCU00778 NCU00854 NCU01234 NCU01312 NCU01888 NCU01907 NCU02174 NCU02249 NCU02423 NCU02885 NCU02948 NCU03226 NCU03682 NCU04650 NCU04699 NCU04986 NCU05243 NCU05964 NCU06322 NCU06869 NCU07408 NCU07587 NCU08026 NCU08727 NCU09615 NCU11426

Table S5 *N. crassa* strains used in this study.

Strain	Genotype	Reference
FGSC2489	*mat A (74-OR23-1VA)*	FGSC
FGSC4200	*mat a (ORS-SL6a)*	FGSC
FGSC6103	*mat A, his-3*$^{-}$	FGSC
RANCR6A	*mat a, his-3, inl*	(Pratt and Aramayo 2002)
FGSC16561	*mat a, Δuc-4(NCU01446) ::hph*	(Colot *et al.* 2006)
FGSC11230	*mat A, Δupf1::hph*	(Colot *et al.* 2006)
FGSC15706	*mat a, Δupf2::hph*	(Colot *et al.* 2006)
FGSC11679	*mat a, Δupf3::hph*	(Colot *et al.* 2006)
FGSC19228	*mat a, Δxrn1::hph*	(Colot *et al.* 2006)
FGSC15492	*mat a, Δy14::hph*	(Colot *et al.* 2006)
FGSC13031	*mat A, Δmago::hph*	(Colot *et al.* 2006)
FGSC22440	*mat A, Δcbp80::hph*	(Colot *et al.* 2006)
FGSC18692	*mat a, Δcbp20::hph*	(Colot *et al.* 2006)
NZ060	*mat A, his-3+::upf1*$_P$*-upf1, Δupf1::hph*	microconidiation
NZ070	*mat A, his-3+::upf1*$_P$*-upf1-Gly-3xFLAG, Δupf1::hph*	microconidiation
NZ100	*mat a, his-3+::upf2*$_P$*-upf2-Gly3xFLAG, Δupf2::hph*	microconidiation
NZ110	*mat a, his-3+::upf2*$_P$*-upf2-GlyHATFLAG, Δupf2::hph*	microconidiation
NZ130	*mat a, y14*$_P$*-y14-Bar, Δy14::hph*	microconidiation
NZ140	*mat A, mago*$_P$*-mago-Bar, Δmago::hph*	microconidiation
NZ150	*mat a, xrn1*$_P$*-xrn1-Bar, Δxrn1::hph*	microconidiation
NZ160	*mat A, cbp80*$_P$*-cbp80-Bar, Δcbp80::hph*	microconidiation
NZ170	*mat a, cbp20-cbp20-Bar, Δcbp20::hph*	microconidiation
Luciferase reporter strains		
NZ1000	*mat A; his-3+::cox-5*$_P$*|cox-5|luc|cox-5*	microconidiation
NZ1001	*mat A; his-3+::cox-5*$_P$*|cox-5|luc|cox-5; Δupf1:: hph*	microconidiation
NZ1005	*mat A; his-3+::cox-5*$_P$*|cox-5|luc|eIF4A3+i*	microconidiation
NZ1006	*mat A; his-3+::cox-5*$_P$*|cox-5|luc|eIF4A3+i; Δupf1:: hph*	microconidiation
NZ1011	*mat A; his-3+::cox-5*$_P$*|cox-5|luc|eIF4A3-i*	microconidiation
NZ1012	*mat A; his-3+::cox-5*$_P$*|cox-5|luc|eIF4A3-i; Δupf1:: hph*	microconidiation
NZ1017	*mat A; his-3+::cox-5*$_P$*|AAP|luc|cox-5*	cross
NZ1018	*mat A; his-3+::cox-5*$_P$*|AAP|luc|cox-5; Δupf1:: hph*	cross
NZ1021	*mat a; his-3+::cox-5*$_P$*::AAP*$_{D12N}$*::Luc::cox-5*	cross
NZ1030	*mat A; his-3+::cox-5*$_P$*::AAP*$_{D12N}$*::Luc::cox-5; Δupf1:: hph*	cross
NZ1031	*mat A; csr-1::cox-5*$_P$*|cox-5|luc|cox-5*	cross
NZ1036	*mat a; csr-1::cox-5*$_P$*|cox-5|luc|cox-5; Δy14:: hph*	cross
NZ1037	*mat A; csr-1::cox-5*$_P$*|cox-5|luc|eIF4A3+i*	cross
NZ1040	*mat a csr-1::cox-5*$_P$*|cox-5|luc|eIF4A3+i; Δy14::hph*	cross
NZ1041	*mat A; csr-1::cox-5*$_P$*|cox-5|luc|eIF4A3-i*	cross
NZ1046	*mat a; csr-1::cox-5*$_P$*|cox-5|luc|eIF4A3-i; Δy14::hph*	cross
NZ1047	*mat A; his-3+::cox-5*$_P$*|cox-5|luc|eRF1+i*	cross
NZ1052	*mat A; his-3+::cox-5*$_P$*|cox-5|luc|eRF1+i; Δupf1:: hph*	cross
NZ1053	*mat A; his-3+::cox-5*$_P$*|cox-5|luc|eRF1-i*	cross
NZ1058	*mat A; his-3+::cox-5*$_P$*|cox-5|luc|eRF1-i; Δupf1:: hph*	cross
NZ1059	*mat A; csr-1::cox-5*$_P$*|cox-5|luc|cox-5; Δcbp80:: hph*	cross
NZ1062	*mat A csr-1::cox-5*$_P$*|cox-5|luc|eIF4A3+i; Δcbp80::hph*	cross
NZ1063	*mat A csr-1::cox-5*$_P$*|cox-5|luc|eIF4A3-i;Δcbp80::hph*	cross
NZ1065	*mat A; csr-1::cox-5*$_P$*|cox-5|luc|cox-5; Δcbp20:: hph*	cross
NZ1066	*mat A csr-1::cox-5*$_P$*|cox-5|luc|eIF4A3+i; Δcbp20::hph*	cross
NZ1067	*mat A csr-1::cox-5*$_P$*|cox-5|luc|eIF4A3-i; Δcbp20::hph*	cross
NZ1068	*mat A; csr-1::cox-5*$_P$*|cox-5|luc|cox-5; Δcbp20:: hph;Δcbp80:: hph*	cross
NZ1069	*mat A; csr-1::cox-5*$_P$*|cox-5|luc|eIF4A3+i; Δcbp20:: hph;Δcbp80:: hph*	cross
NZ1070	*mat A; csr-1::cox-5*$_P$*|cox-5|luc|eIF4A3-i; Δcbp20:: hph;Δcbp80:: hph*	cross

Table S6 Plasmids used for rescuing *N. crassa* deletion mutants.

Plasmid	Promoter-coding region	PCR oligo	Oligonucleotide sequence, 5′ → 3′	Cloning site	Vector
pZY05	his3::upf1$_P$-upf1	oYZ165	CTTGGCGGCCGCTTTCTAGAATACAG	NotI	pBM61
		oYZ166	CGGAATTCGACCGCCAGCTAACCCAAC	EcoRI	
pZY43	his3::upf1$_P$-upf1-Gly-3xFLAG	oYZ165	CTTGGCGGCCGCTTTCTAGAATACAG	NotI	pCCGC-Gly3xFLAG (FJ457001)
		oYZ194	CGTTAATTAAATCGAAACCTGTCCCGATCTGACTGGC	PacI	
pZY68	his3::upf2$_P$-upf2-Gly3xFLAG	oYZ219	GGGCGGCCGCAGCTCAAGGGAAGCCAAC	NotI	pCCGC-Gly3xFLAG (FJ457001)
		oYZ220	CCACTAGTACTGGGCGATGAGAATC	SpeI	
pZY70	his3::upf2$_P$-upf2-GlyHATFLAG	oYZ221	GGACTAGTGGATGGATCGCCAAAG	SpeI	pCCGC-GlyHATFLAG (FJ457003)
		oYZ222	GGTTAATTAAAGTCCAATCAACATCAC	PacI	
pZY125	xrn1$_P$-xrn1-[Bar^r]	oYZ477	GGGCATATGTGTTGTCTTTATTTAGAGCCG	NdeI	pBARGPE1 (DBP 357)
		oYZ478	CATACGCGTCATCAACTTCTCCCTCG	MluI	
pZY120	y14$_P$-y14-[Bar^r]	oYZ435	CTTCCATATGAACCGCAACGATTGC	NdeI	pBARGPE1 (DBP 357)
		oYZ436	GCTACGCGTTTTCGTCACAGCCTAC	MluI	
pZY104	mago$_P$-mago-[Bar^r]	oYZ437	CCTCATATGTACTCGTTCGGGTGTTG	NdeI	pBARGPE1 (DBP 357)
		oYZ438	GTTCACGCGTCAACAGTCAAGTTGTATC	MluI	
pZY128	cbp20$_P$-cbp20-[Bar^r]	oYZ501	GGCCATATGGAGGTCTTAACGGAGAC	NdeI	pBARGPE1 (DBP 357)
		oYZ502	GTTAAACGCGTCGTCGCCACCATTGAG	MluI	
pZY126	cbp80$_P$-cbp80-[Bar^r]	oYZ479	GGGCATATGATGGATTATGGCATATAGGG	NdeI	pBARGPE1 (DBP 357)
		oYZ481	CATACGCGTGGTGAAGGATGTTGG	MluI	

Table S7 Vectors and oligonucleotides used to construct luciferase reporters.

Plasmid	Description	Insert Oligo	Oligonucleotide sequence, 5′ → 3′	Cloning site	Vector(s)
pZY78	his-3::cox-5_P|cox-5|luc|	oYZ335	GCTCTAGAGTTACGCGTGCTTGTGATTGGAAG	XbaI	pRMP57
		oYZ336	CGACTAGTTACCGGTAGGAGTGAAGTCTTGCAAAG	SpeI	
pZY82	his-3::cox-5_P|cox-5|luc|cox-5	oYZ339	GCCATTAATTAAGTTTCGCATACTTTTATTGG	PacI	pZY78
		oYZ340	GCTTAATTAATGCAATAGATCACACGTC	PacI	
pZY118	his-3::cox-5_P|AAP|luc|cox-5	oYZ169	CTGGGATCCAACTTGTCTTGTCGC	BamHI	pZY82[2], pPR101[3]
		oYZ170	CGCCCGGGCTTGACTTGAATGGT	XmaI	
pZY119	his-3::cox-5_P|D12N|luc|cox-5	oYZ169	CTGGGATCCAACTTGTCTTGTCGC	BamHI	pZY82, D12N (pPS101)
		oYZ170	CGCCCGGGCTTGACTTGAATGGT	XmaI	
pZY92	his-3::cox-5_P|cox-5|luc|eIF4A3+I	oYZ330	TTATTAATTAAGAGGCTAGCGCGGGG	PacI	pZY78
		oYZ332	CCACTTAATTAACACCTGCGCGGG	PacI	
pZY94	his-3::cox-5_P|cox-5|luc|eIF4A3-I	oYZ330	TTATTAATTAAGAGGCTAGCGCGGGG	PacI. Overlapping PCR	pZY78
		oYZ334	CAATCTGTAAAGCGTCCTTAAGGCCGACATGC		
		oYZ333	GCATGTCGGCCTTAAGGACGCTTTACAGATTG		
		oYZ332	CCACTTAATTAACACCTGCGCGGG		
pZY84	his-3::cox-5_P|cox-5|luc|eRF1+I	oYZ181	GCCTTAATTAACAAGTTGAATCCCTCGCCG	PacI	pZY78
		oYZ329	CGCGTTAATTAATCTCATGAACACTACTGG	PacI	
pZY86	his-3::cox-5_P|cox-5|luc|eRF1-I	oYZ181	GCCTTAATTAACAAGTTGAATCCCTCGCCG	PacI	pZY78
		oYZ182	GGCCTTAATTAAACAAAGACAGAATGAGCG		
		oYZ329	CGCGTTAATTAATCTCATGAACACTACTGG	PacI	
pZY122	csr-1::cox-5_P|cox-5|luc|cox-5	NotI/ClaI fragment from pZY82		NotI	pCSR1
				ClaI	
pZY123	csr-1::cox-5_P|cox-5|luc|eIF4A3+I	NotI/ClaI fragment from pZY96		NotI	pCSR1
				ClaI	
pZY124	csr-1::cox-5_P|cox-5|luc|eIF4A3-I	NotI/ClaI fragment from pZY98		NotI	pCSR1
				ClaI	

Table S8 Oligonucleotide primers used for 3'RACE.

Oligonucleotide for 3'RACE			
PCR oligo			Oligonucleotide sequence, 5′ → 3′
3'–RACE CDS Primer A		oYZ291	AAGCAGTGGTATCAACGCAGAGTAC(T)30VN
Nested Universal Primer A		oYZ294	AAGCAGTGGTATCAACGCAGAGT
luc-cox-5	Nest oligo1	oYZ365	CGTCTTCGTCGACGAGGTCC
	Nest oligo2	oYZ287	GGCCAAGAAGGGCGGCAAGATCGCCGTC
luc-eIF4A3	Nest oligo1	oYZ365	CGTCTTCGTCGACGAGGTCC
	Nest oligo2	oYZ287	GGCCAAGAAGGGCGGCAAGATCGCCGTC
eIF4A3	Nest oligo1	oYZ027	CCCGCGGTATCGATGTTC
	Nest oligo2	oYZ330	CCCGCGGTATCGATGTTC
Oligonucleotide for 3´UTR intron (+/-) detection			
PCR oligo			Oligonucleotide sequence, 5′ → 3′
eIF4A3 (411/349)	oYZ330		TTATTAATTAAGAGGCTAGCGCGGGG
	oYZ482		GCGAAATAAACCCCGGTAGGGG
eRF1 (667/611)	oYZ181		GCCTTAATTAACAAGTTGAATCCCTCGCCG
	oYZ182		GGCCTTAATTAAACAAAGACAGAATGAGCG
Y14 (458/399)	oYZ300		CGACCTCCTGCACTCAACAA
	oYZ301		CCTCAAGCGTCGGATACTCAA

Table S9 Oligonucleotide primers and templates used to generate probes for northern and Southern analyses.

Genes	Template	Fragment (bp)	Oligo	Oligonucleotide sequence, 5′ → 3′
arg-2 (NCU07732)	cDNA*	997	MB001	GTTATAGGAATTCGCGCTTCCACCATGCCCATCTCC
			MB002	CTCTATTCCGCTCGAGTTGTAGCCTTCGGAGGTGAGACC
eif5 (NCU00366)		1278	oYZ405	GCTCTAGATCAATACCGCCAAAATGGG
			oYZ406	GCTTAATTAACTCATCGTCCTCCTCCTCAG
eif4a3 (NCU01234)		1150	oYZ195	GATCTAGACAGAATCGCCCACCATG
			oYZ196	GCTTAATTAAGGAGATAAGGTCGGCAACATTCATGGGC
eRF1 (NCU00410)		757	oYZ121	ACTTCGTCTTCCTCATCTTCTTCAC
			oYZ120	AGGCGTTCAGGTCGTTCTTG
cox-5 (NCU05457)		396	MB005	GTTATAGGAATTCGCGCTTCCACCATGCCCATCTCC
			MB006	CTCTATTCCGCTCGAGTTGTAGCCTTCGGAGGTGAGACC
luc	pRMP57	663	oYZ167	ACATCACCTACGCCGAGTACTTC
			oYZ188	TCCTCCTCGAAGCGGTACA
his-3 (NCU03139)	cDNA*	1761	oYZ069	CCACCGTTGTCGTTGATGAG
			oYZ068	GAGTAATCGCCAACGGACTCA
bar	pBARGPE1	300	oYZ487	CAACCACTACATCCAGACAAGCA
			oYZ486	ACAGCGACCACGCTCTTGA
mago (NCU04405)	cDNA*	602	oYZ377	GCTCTAGAAGCAACAAACAGCCATGTCAGC
			oYZ378	CCTTAATTAAAATGGGCTTGATCTTGAAGTGC
y14 (NCU03226)	cDNA*	519	oYZ375	GCTCTAGAACGTAGAAGCTGCTAAAATG
			oYZ376	CCTTAATTAACTCGTCCCTGCCAACG

*DNase I treated total RNA was used as template to synthesize first-strand cDNA with a combination of oligo$(dT)_{18}$ and random hexamer primers as described in Materials and Methods

Table S10 List of transcripts and oligonucleotide primers used for qPCR.

Genes	Oligo	Position	Oligonucleotide sequence, 5′ → 3′
25S	oYZ029	1794	CCGCGGGAGGGAATAATT
	oYZ030	1849	GGAGACCTGCTGCGGTTATG
luc	oYZ187	787	ACGGCTTCGGCATGTTCA
	oYZ188	865	TCCTCCTCGAAGCGGTACA
upf1 (NCU04242)	MSS169	1284	CAAGCTGCTGGGACACGAA
	MSS170	1347	GTGGAACTTCTTGGGCATCGTA
upf2 (NCU05267)	oYZ055	3514	CCGGGAGAACGATGATTATGA
	oYZ056	3578	GATCGGAGCGGTTATGGTGAT
upf3 (NCU03435)	oYZ061	167	TCGGCGATGAATGGAAAGTC
	oYZ062	227	TTGCCAGGCCAGTATGAAAAC
mago (NCU04405)	oYZ009	260	AATGGCCGACCAAAAACAAA
	oYZ010	314	TTCCCGAGGCGGATCTC
y14 (NCU03226)	oYZ299	429	ACAGGCGAAGCGGTTACG
	oYZ300	485	CCTCAAGCGTCGGATACTCAA
cbp20 (NCU00210)	oYZ379	450	TCARGGARCGCRTCTRCGARTTGRC
	oYZ380	508	TGARTGCRGCTRCGTRCCARACTRT
cbp80 (NCU04187)	oYZ459	470	TGGRTGTRCTTRCCCRCGTRGTTRG
	oYZ460	533	GGARACTRGGCRAGTRCTGRCAARGTC
arg-2 (NCU07732)	MB019	315	CACCCAGCCCTTGATTGG
	MB020	373	GGTTGAACTCGTCACGCTCAT
eif5 (NCU00366)	oYZ251	17	TCAACGTTCGTCGGGACAA
	oYZ252	79	TGGTCTGGATACGCTCCATCT
inl (NCU06666)	oYZ271	1336	CTTGCCTCGCCCCTGAT
	oYZ272	1395	CTGGATGCGGGTCATGATC
eif4a3 (NCU01234)	oYZ023	486	TCATCTCCGCACCAGACACA
	oYZ024	543	GAGGAGCTCATCGGCTTCAT
eRF1 (NCU00410)	oYZ119	727	CGTTGCCGGTCTCGTTTT
	oYZ120	782	AGGCGTTCAGGTCGTTCTTG
xrn1(NCU06678)	oYZ357	1454	AGCRCGTRTCTRCCGRTATRCATRCAA
	oYZ358	1516	TCGRGCCRGCARGTTRAAARTCC
cox-5 (NCU05457)	MSS177	402	GCAAGAGGCTACCAACGAGTTC
	MSS178	469	CTTCGGAGGTGAGACCAGTGA
uc-4 (NCU01446)	MB063	91	GCCGTCAACTTCGACAACGT
	MB064	156	AATGCTGAGGAGAGCGATGAG

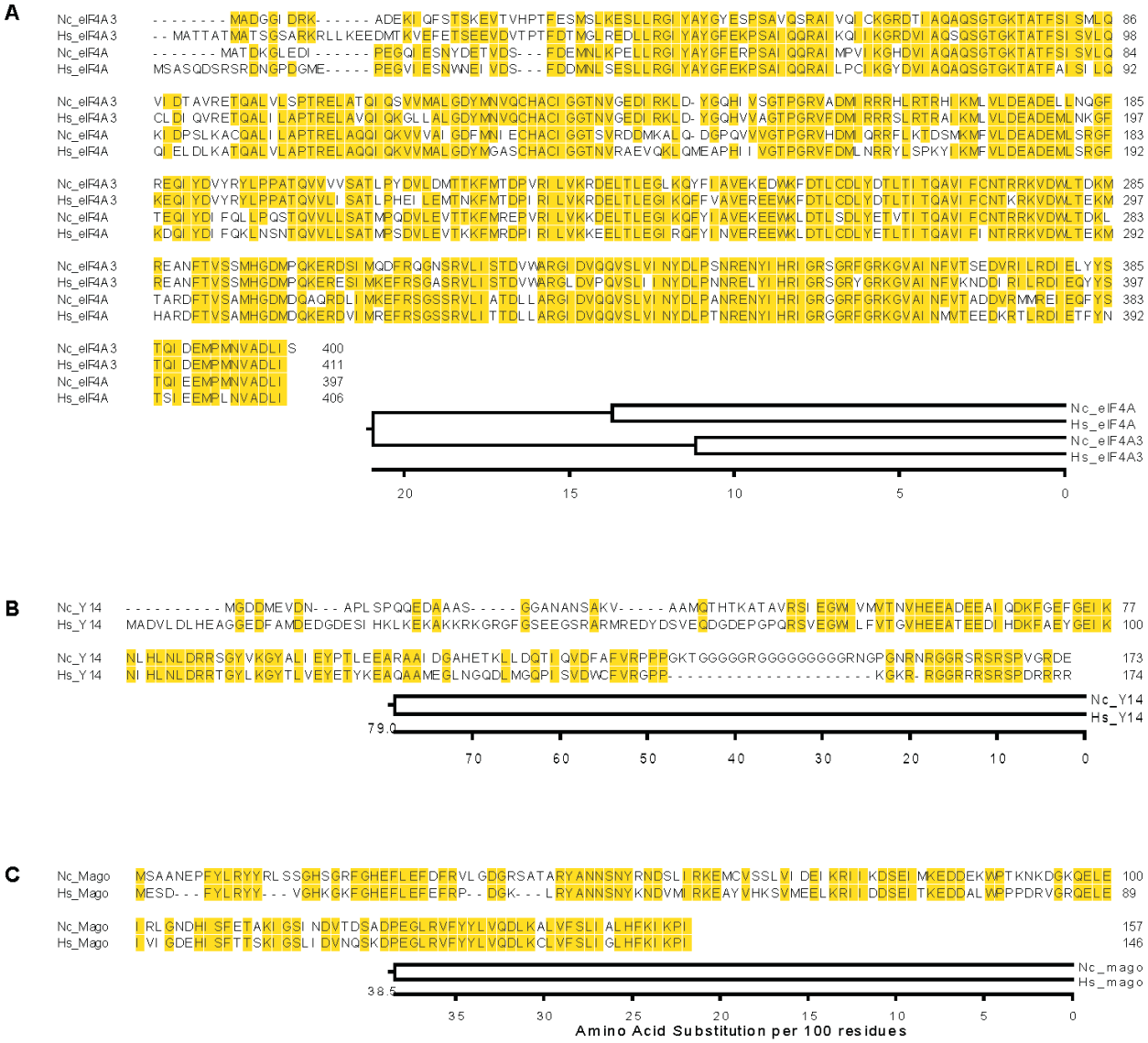

Figure S1 Proteins sequence alignments of eIF4A3, Y14 and Mago.

Protein sequence alignment showing sequence conservation of (A) eIF4A3 and eIF4A, (B) Y14, (C) Mago; alignments and phylogenetic trees were constructed with CLUSTALW using DNASTAR® (version 11.2.1). Completely conserved residues are highlighted in yellow.

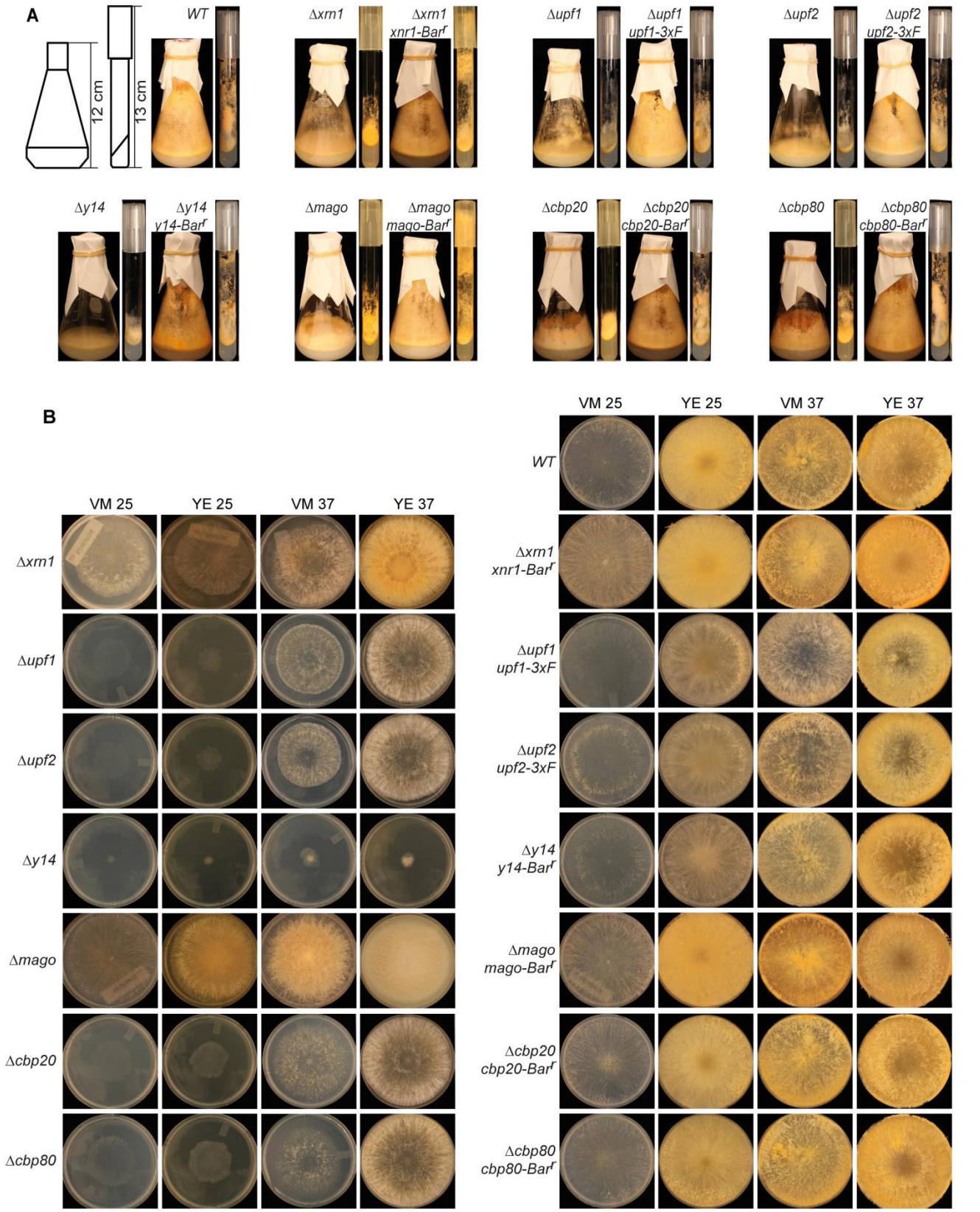
A
12 cm
13 cm
WT
Δxrn1
Δxrn1 xnr1-Barr
Δupf1
Δupf1 upf1-3xF
Δupf2
Δupf2 upf2-3xF
Δy14
Δy14 y14-Barr
Δmago
Δmago mago-Barr
Δcbp20
Δcbp20 cbp20-Barr
Δcbp80
Δcbp80 cbp80-Barr
B
VM 25
YE 25
VM 37
YE 37
Δxrn1
Δupf1
Δupf2
Δy14
Δmago
Δcbp20
Δcbp80
WT
Δxrn1 xnr1-Barr
Δupf1 upf1-3xF
Δupf2 upf2-3xF
Δy14 y14-Barr
Δmago mago-Barr
Δcbp20 cbp20-Barr
Δcbp80 cbp80-Barr

Figure S2 Phenotypes of mutant strains.

(A) *N. crassa* wild type (wt, FGSC2489), and the indicated deletion and corrected-deletion strains Δ*xrn1* (NCU06678 FGSC19228), Δ*xnr1 xrn1*-Bar^r, Δ*upf1* (NCU04242, FGSC11230), Δ*upf1 his-3*::*upf1*-3XFLAG, Δ*upf2* (NCU05267, FGSC15706), Δ*upf2 his-3*::*upf2*-3XFLAG, *Δy14* (NCU03226, FGSC15492), *Δy14 y14*-Bar^r, *Δmago* (NCU04405, FGSC13031), *Δmago mago-Bar^r*, *Δcbp20* (NCU00210, FGSC18692), *Δcbp20 cbp20*-Bar^r , *Δcbp80* (NCU04187, FGSC22440), and *Δcbp80 cbp80*-Bar^r were grown in 1X VM/1.5% sucrose/2% agar at 25°C for 4 days in 125 ml flasks containing 25 ml medium or for 4 days in 16x125 mm culture tubes containing 3 ml medium. (B) The strains shown in panel (A) were grown by inoculating the centers of 100 mm Petri plates containing 1X VM/1.5% sucrose/2% agar with or without 2% yeast extract (YE) and incubating the plates at 25°C or 37°C for 48 hr.

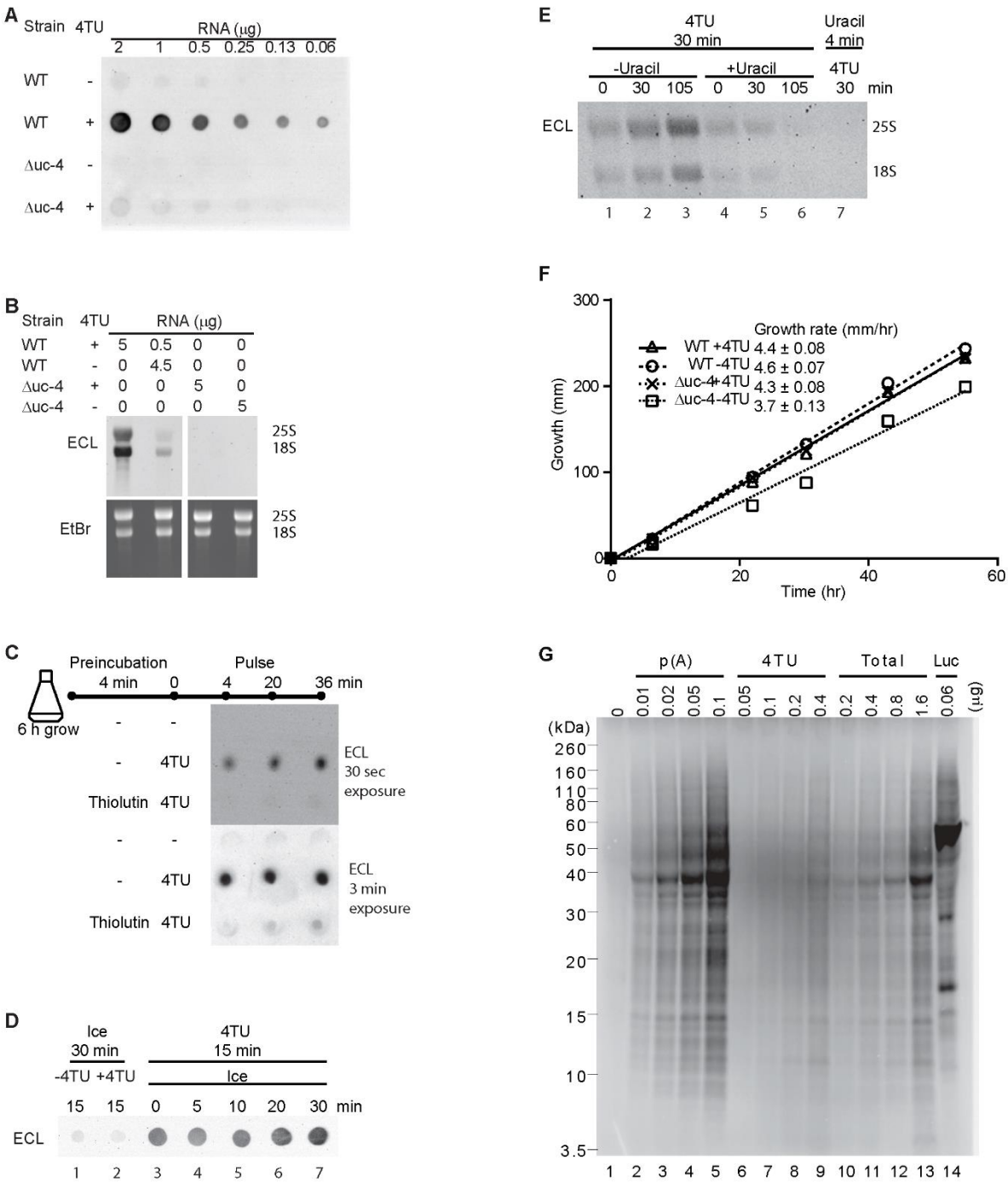

Figure S3 Validation of the 4TU pulse-chase protocol.

(A) ECL detection of biotinylated RNA prepared from wt or *Δuc-4 N. crassa* cultures germinated for 6 hr and incubated for 15 min with 0.2 mM 4TU (+) or without 4TU (-). Total RNA was prepared and biotinylated. The biotinylation probe is designed to react with the thio-group in 4TU-labeled

RNA. Serial dilutions of biotinylated RNA were spotted directly on nylon membrane at the indicated amounts. (B) Biotinylated RNA (5 μg/lane) was subjected to denaturing agarose gel electrophoresis and then transferred to membrane. The presence of biotinylated-4TU RNA was assessed using streptavidin coupled to horseradish peroxidase and incubation with ECL reagent. *25S* and *18S* rRNA bands stained with ethidium bromide are shown below. (C) Thiolutin blocks incorporation of 4TU into RNA. *N. crassa* wt cultures were germinated for 6 hr and treated with thiolutin (0.5 μg/ml) or not treated. Then 0.2 mM 4TU was added to cultures and samples were taken 4, 20 and 36 min following addition of 4TU. Total RNA was prepared and biotinylated; 1 μg of biotinylated RNA was spotted on nylon membrane and biotinylated 4TU RNA detected by ECL. Top panel, 30 sec exposure; bottom panel, 3 min exposure. (D) Chilling of cultures on ice reduces incorporation of 4TU. Lanes 1 and 2: 6 hr germinated wt cultures were placed on ice for 30 min; then 0.2 mM 4TU was added (lane 2) or not added (lane 1) and incubation on ice continued for 15 min; incorporation of 4TU into RNA was examined by dot-blotting and ECL. Lanes 3-7: a 6 hr germinated wt culture was incubated with shaking for 15 min at 30°C with 0.2 mM 4TU; then uracil was added to 10 mM and the culture quick-chilled by mixing with 7 ml VM/2% sucrose/10 mM uracil that had been pre-frozen in a 50 ml conical centrifuge tubes. The chilled culture was placed on ice and cells were harvested by filtration at 0, 5, 10, 20 and 30 min time points. For ECL analyses in lanes 1-7, total RNA was prepared and biotinylated; 1 μg of biotinylated RNA was spotted on nylon membrane and biotinylated 4TU RNA detected by ECL. (E) Lanes 1-6: Cultures of conidia from wt were germinated for 5.5 hr and then were treated with 0.2 mM 4TU for 30 min. Then 10 mM uracil was added to cultures using a 0.25M stock prepared in DMSO (+uracil, lanes 4-6) or the equivalent volume of DMSO was added (- uracil, lanes 1-3). Cells were harvested 0, 30 and 105 min later by filtration. In a separate analysis (lane 7), a 7 hr germinated culture were pretreated with 10 mM uracil for 4 min, then incubated with 0.2 mM 4TU for an additional 30 min, and then harvested by filtration. For lanes 1-7, total RNA was prepared and biotinylated; 2.5 μg of biotinylated RNA was subjected to denaturing agarose gel electrophoresis and then transferred to membrane. The presence of biotinylated-4TU *25S* and *18S* RNA was detected with ECL reagent. (F) Effects of 4TU on growth in race tubes. wt and *Δuc-4* strains were grown in race tubes containing VM/1.5% sucrose/2% agar with or without 0.2mM 4TU at 30°C with 12:12 hr L:D for 60 hr. The growth front was marked twice daily and the growth rate was calculated from triplicate experiments. (G) *In vitro* translation of 4TU RNA. Indicated amounts of *N. crassa* poly(A) RNA (Lanes 2-5), 4TU RNA (Lanes 6-9), total RNA (Lanes 10-13) and *in vitro* transcribed synthetic *luc* mRNA that was capped and polyadenylated (Lane 14) were used as templates for *in vitro* translation in micrococcal nuclease-treated wt *N.crassa* cell extracts (Wu *et al.* 2012). A translation reaction with no added template is shown in Lane 1. [^{35}S]Met-labeled translation products were separated on a 12% NuPAGE Bis-Tris Gel (Invitrogen NP0343) and detected by phosphorimaging.

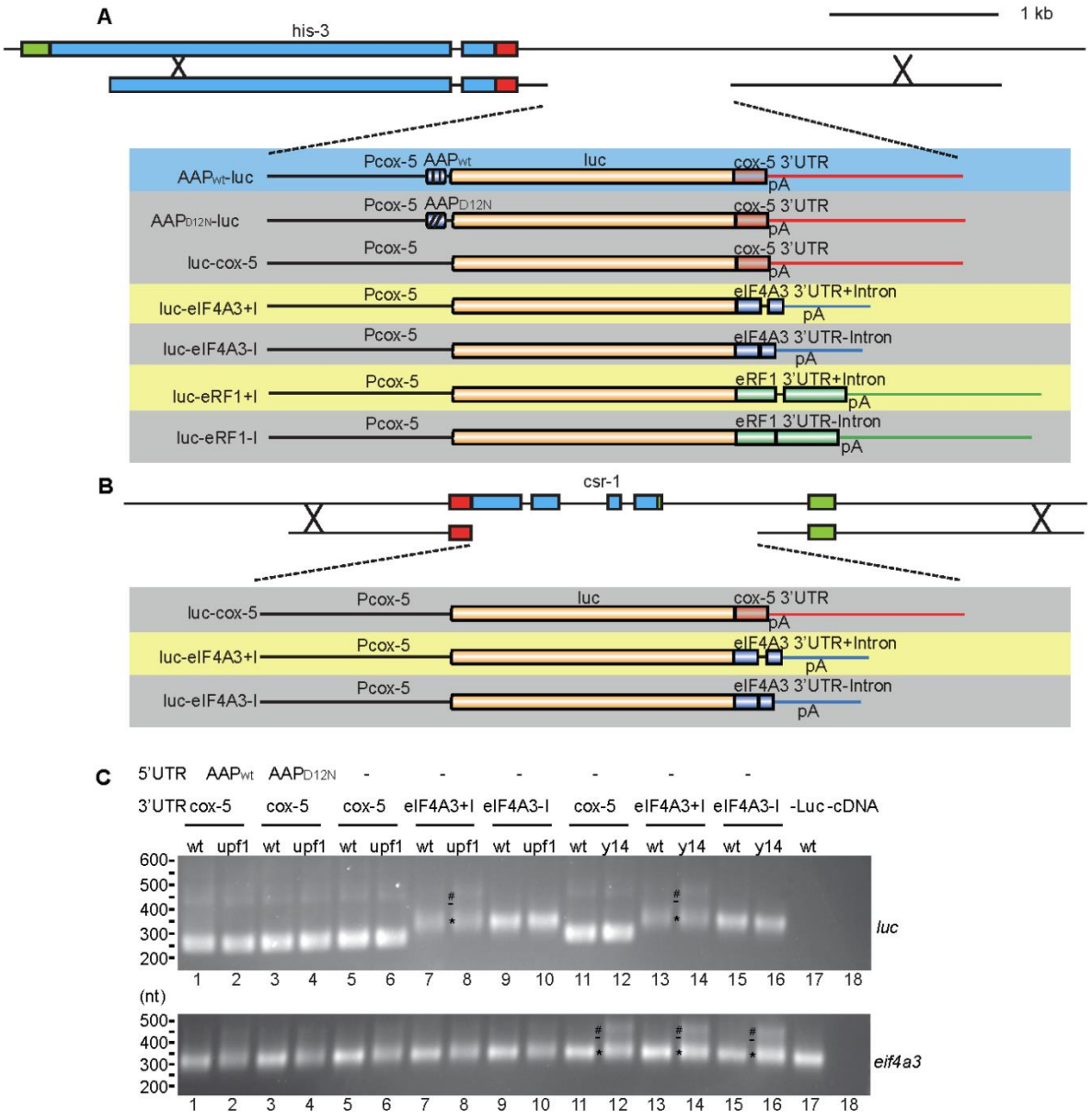

Figure S4 *In vivo* codon-optimized luciferase (*luc*) constructs integrated at *his-3* or *csr-1* for assessing uORF and 3'UTR intron effects on mRNA stability.

Schematic representation of the reporters integrated at (A) *his-3* locus or (B) *csr-1* locus**.** For both recipient loci, boxes indicate mRNA exons; green indicates the 5'UTR, blue indicates coding sequences, and red indicates the 3'UTR. All reporters contained the *cox-5* promoter. Constructs contained the wild-type arginine attenuator peptide (AAP) or the mutated or D12N AAP (AAP_{wt}–luc or AAP_{D12N}–luc) for analyzing the role of the *arg-2*-encoded uORF. To examine the roles of 3'UTR introns, the *cox-5* 3'UTR (luc-cox-5) served as a negative control. The shaded boxes surrounding the constructs indicate the reporter mRNAs contain specific features: blue, uORFs that trigger NMD; yellow, spliced 3'UTRs that trigger NMD; gray, no NMD-triggering features. The functions of 3'UTR introns were examined by comparing intron-containing or intronless *eif4a3* 3'UTRs

(luc-eIF4A3+I or luc-eIF4A-I) or *erf1* 3'UTRs (luc-eRF1+I or luc-eRF1-I). (C) 3'RACE analyses of *luc* and *eif4a3* mRNA 3'UTRs in wt and mutant strains. 3'RACE analysis was performed with specific oligos as described in Materials and Methods. The major and minor bands were excised and sequenced. The major 3'RACE fragment from *luc-cox-5* with oligos oYZ287~oYZ294 was 282 bp (top panel, lanes 1-6, 11,12). The minor larger band was 437 bp and represents an mRNA whose 3'UTR was 155-nt longer. The major fragment from *luc-eif4a3* (spliced-intron or intronless reporters) using oligos oYZ287~oYZ294 was 328 bp (top panel, lanes 7-10, 13-16, position indicated by "*"; the position for unspliced mRNA is 390 bp and indicated by "-"). No fragments were amplified with these *luc*-specific oligo sets from the wt strain lacking a *luc* reporter or from a no-cDNA control (top panel, lanes 17-18). The same cDNAs were used to obtain the endogenous *eif4a3* 3'RACE fragment of 300 bp using oYZ330~oYZ294 (bottom panel, lanes 1~17, position indicated by "*"; the position for unspliced mRNA is 362 bp and indicated by "-"). The larger fragment indicated by "#" observed for *eif4a3* in the *Δy14* strain (387 bp) represents a 3' extension of the spliced mRNA. This 3' extension (415 bp fragment, indicated by "#") is also observed for the spliced-intron *luc-eif4a3* reporter mRNA in the *Δupf1* and *Δy14* strains as determined by sequencing these fragments.

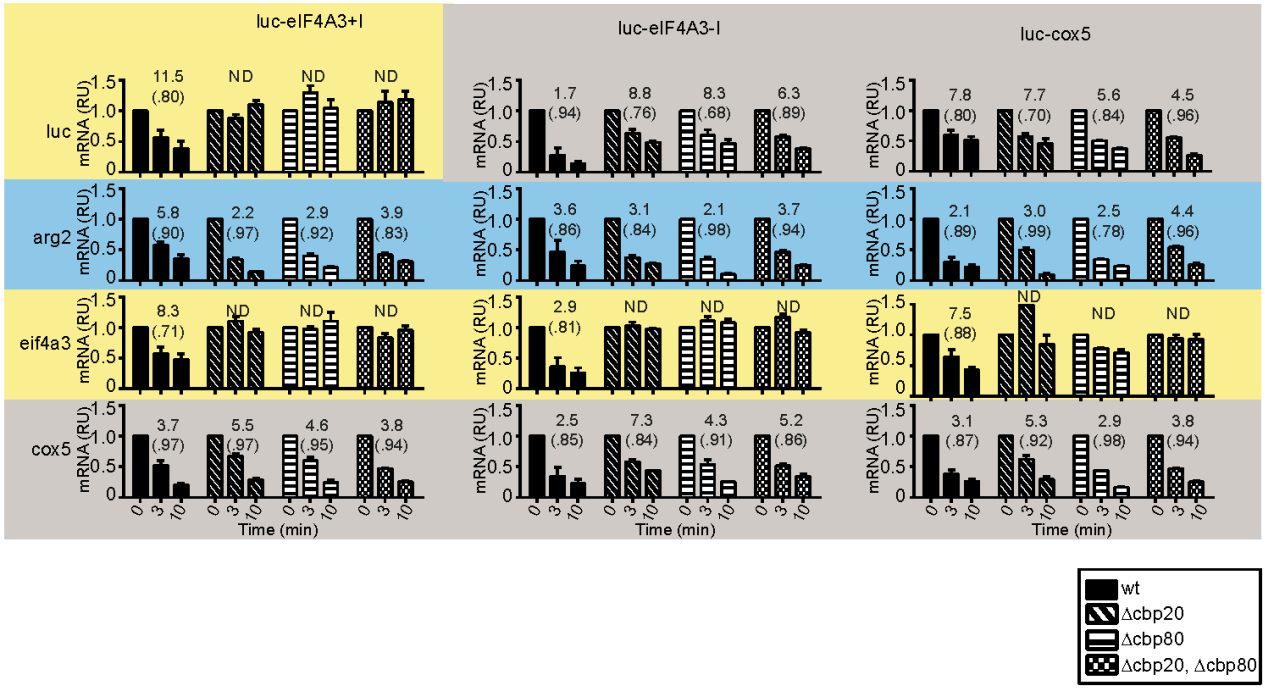

Figure S5 Stability of 3'UTR-intron containing reporters in *Δcbp20*, *Δcbp80* and *Δcbp20 Δcbp80* strains.

Half-lives of mRNA were measured by 4TU RNA pulse-chase (as described in Figure 2) to analyze the effects of three different 3'UTRs on reporter mRNA stability: *eif4a3* 3'UTR intron (left), intronless *eif4a3* 3'UTR (middle); *cox-5* 3'UTR (right), indicated as luc-eIF4A3+I, luc-eIF4A3-I and luc-cox5, respectively. The different reporters were placed in wt, *Δcbp80, Δcbp20* and *Δcbp20Δcbp80* double mutant strains as indicated.

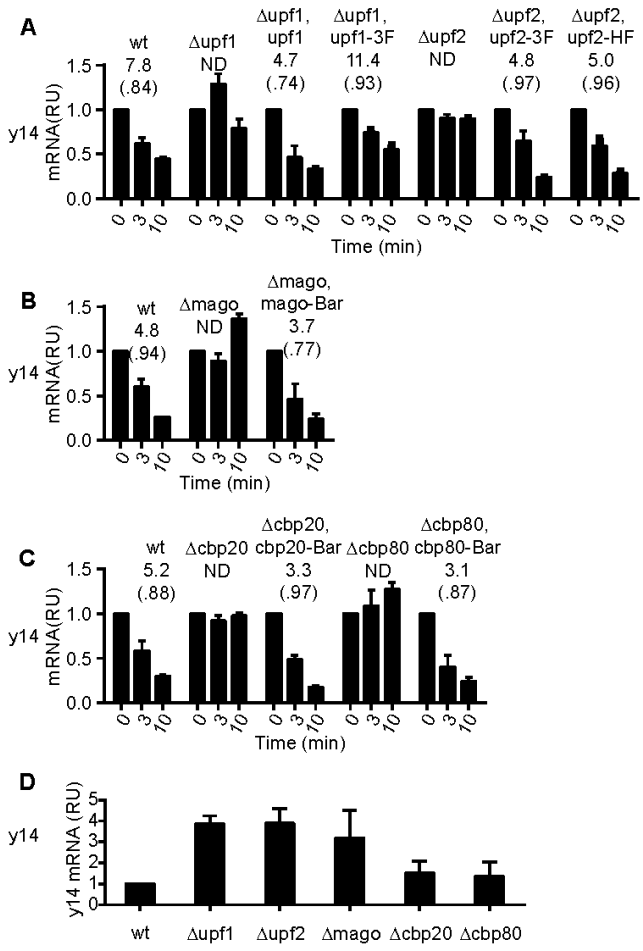

Figure S6 The stability of the spliced 3'UTR intron-containing *y14* mRNA is affected by NMD, EJC and CBC mutations.

(A-C) Half-lives of mRNA were measured by 4TU RNA pulse-chase as described in Figure 2 to analyze the effects of NMD, EJC and CBC mutations on *y14* transcript stability. 4TU RNA was from the same samples used for analyses of other RNAs in other figures (A) wt, Δ*upf1,* Δ*upf1 his-3*::*upf1,* Δ*upf1 his-3*::*upf1*-3XFLAG , Δ*upf2*, Δ*upf2 his-3*::*upf2*-3XFLAG and Δ*upf2*, *his-3*::*upf2*-HAT-FLAG strains (B) wt, *Δmago* and *Δmago mago[Bar^r]* strains (C) wt, *Δcbp20, Δcbp20 cbp20[Bar^r], Δcbp80, Δcbp80 cbp80[Bar^r]* strains. (D) Measurement of mRNA levels of *y14*, *eif4a3*, e*rf1*, *arg-2*, *eif5* and *cox-5* in total RNA in wt, Δ*upf1,* Δ*upf2*, *Δy14, Δmago,Δcbp20* and *Δcbp80* strains. The data for total RNA levels show the average and standard deviations from four independent RNA preparations (mRNA levels were normalized to 25S rRNA levels in each case and all then all calculated relative to the normalized level in wt).

File S1

Supplemental movie

Comparisons of asexual growth and development of wt vs *Δupf1* vs *Δupf1 upf1-3XFLAG:* wt vs *Δupf2,* vs *Δupf2 upf2-3xFLAG;* wt vs*Δy14* vs *Δy14 y14-Bar^r;* wt vs *Δmago* vs *Δmago mago-Bar^r* ;wt vs *Δcbp80* vs *Δcbp80 cbp80-Bar^r;* wt vs *Δcbp20* vs *Δcbp20 cbp20-Bar^r*

10^5 conidia in 5 μl sterile water obtained from the indicated strains were inoculated on a sterile Whatman 451 filter disk placed in the center of a VM/1.5% sucrose/2% agar plate and incubated at room temperature with constant light. Images of growing cultures were taken every 3 min or 5 min for a given experiment, and images collected over an approximately 70-h period of time. Time lapse movies were created at 30 images/sec.

Available for download as an mp4 file at www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.176743/-/DC1