SUPPLEMENTAL MATERIAL

Supplemental figure legends

Figure S1. (A) The levels of the truncated Dcp2 proteins. Wild-type cells (yRP840) expressing Dcp2 (1-300), Dcp2 (1-247), Dcp2 (102-300) or Dcp2 (102-247)-GFP from the plasmid pRP1892, pRP1893, pRP1904 or pRP1905 were used. pRP250 was used as a control vector (no GFP). Cells were cultured as described in the figure legend to Fig. 1C. Western blot analysis was performed as described in the figure legend to Fig. 1C. Arrowheads indicate GFP-tagged proteins. Asterisks indicate non-specific bands. (B) Localization of truncated Dcp2 proteins. The same strains as described in the figure legend to Fig. S1 were used. Imaging was performed as described in the figure legend to Fig. 1B. Bar, 5 μm.

Figure S2. (A) Coomassie blue-stained SDS-PAGE of recombinant purified proteins used in *in vitro* decapping assays. Representative decapping time courses for (B) 50 nM GB1-Dcp1:Dcp2 (1-245), (C) 50 nM GB1-Dcp1:Dcp2 (1-245) with 500nM Edc3, (D) 50 nM GB1-Dcp1:Dcp2 (1-315), and (E) 50 nM GB1-Dcp1:Dcp2 (1-315) with 500nM Edc3.

Figure S3. (A) The *RPS28B* mRNA was analyzed in strains lacking C-terminal regions of the chromosomal *DCP2* gene (yRP2749, yRP2745, yRP2746, yRP2751, yRP2752 and yRP2753). Cells were grown in YP media containing 2% galactose to mid-log phase at 24˚C. The mRNA level normalized to the *SCR1* RNA level relative to the normalized mRNA level in yRP2749 (full-length Dcp2 without tag) was indicated below each lane. The average and standard deviation of values obtained with three independent strains of each genotype are shown. (B) The protein levels of Dcp2 (1-300)-3HA and Dcp2 (1-247)-3HA. Cells of the strains yRP2745, yRP2746, yRP2752 and yRP2753 were grown in YP media containing 2% galactose to mid-log phase at 24˚C. Anti-HA antibody and anti-Pgk1 antibody were used in Western blot to detect HA-tagged proteins and the endogenous Pgk1 proteins as a loading control. (C) Analysis of the *YRA1* pre-mRNA. The

same RNA samples as described in the figure legend to Fig. S3A were used. The *YRA1* pre-mRNA level normalized to the *SCR1* RNA level relative to the normalized pre-mRNA level in yRP2749 (full-length Dcp2 without tag) was indicated below each lane. (D) The *MFA2pG* mRNA was analyzed in the same strains as described in the figure legend to Fig. S3A. Cells were grown in YP media containing 2% galactose to mid-log phase at 24˚C to express the *MFA2pG* mRNA from the *GAL* promoter. The ratio of the full-length mRNA (FL) to the decay intermediate (pG) relative to the ratio in yRP2749 (full-length Dcp2 without tag) was indicated below each lane. The average and standard deviation of values obtained with three independent strains of each genotype are shown. (E) Growth phenotype of strains lacking C-terminal regions of the chromosomal *DCP2* gene (yRP2749, yRP2745, yRP2746, yRP2751, yRP2752 and yRP2753) was analyzed by serial dilution spotting. Cells were incubated on YP media containing 2% glucose for 2 days at 24˚C.

Figure S4. (A) The *RPS28B* mRNA was analyzed in the strains yRP2793 (*dcp2*Δ) and yRP2794 (*dcp2*Δ *edc3*Δ) expressing the full-length or truncated Dcp2 protein from the plasmid pRP1207, pRP1453, pRP1450 or pRP1449. pRP10 was used as a control vector. The mRNA level normalized to the *SCR1* RNA level relative to the normalized mRNA level in yRP2793 (*dcp2*Δ) expressing full-length Dcp2 was indicated below each lane. The average and standard deviation of values obtained with three independent strains of each genotype are shown. (B) The *RPS28B* mRNA was analyzed in the strains yRP2793 (*dcp2*Δ) and yRP2794 (*dcp2*Δ *edc3*Δ) expressing Dcp2 (1-300)-GFP or Dcp2 (1-300) L255A K256A-GFP from a plasmid pRP1892 or pRP1896. The mRNA level normalized to the *SCR1* RNA level relative to the normalized mRNA level in yRP2793 (*dcp2*Δ) expressing wild-type Dcp2 was indicated below each lane.

Figure S5. (A) Growth phenotype of the same strains as described in the figure legend to Fig. S4A was analyzed by serial dilution spotting. Cells were incubated on SC media containing 2% glucose for 2 days at 30˚C. (B) Analysis of the *MFA2pG* mRNA. The same RNA samples described as in the figure legend to Fig. S4A were used. The ratio of the full-length mRNA (FL) to the decay intermediate (pG) relative to the ratio in yRP2793

(*dcp2*Δ) expressing full-length Dcp2 was indicated below each lane.

Figure S6. (A) Growth phenotype of the same strains as described in the figure legend to Fig. S4B was analyzed by serial dilution spotting. Cells were incubated on SC media containing 2% glucose for 2 days at 30˚C. (B) Analysis of the *MFA2pG* mRNA. The same RNA samples described as in the figure legend to Fig. S4B were used. The ratio of the full-length mRNA (FL) to the decay intermediate (pG) relative to the ratio in yRP2793 (*dcp2*Δ) expressing wild-type Dcp2 was indicated below each lane.

Figure S7. Deletion of the region 248-300 of Dcp2 shows a synthetic effect with the *dcp1-2* mutation. Cells of the strains yRP2745 (*DCP1 dcp2(*Δ*301-970)*), yRP2746 (*DCP1 dcp2(*Δ*248-970)*), yRP2747 (*dcp1-2 dcp2(*Δ*301-970)*) and yRP2748 (*dcp1-2 dcp2(*Δ*301-970)*) were grown in YP media containing 2% galactose to mid-log phase at 24˚C, which is permissive for the *dcp1-2* temperature sensitive allele. The ratio of the full-length mRNA to the poly(G) decay intermediate in each strain relative to the ratio in the *DCP1 dcp2(*Δ*301-970)* strain is indicated. Three independent strains of each genotype were analyzed.

Table S1. Oligonucleotides used in this study

Number	Sequences	Description	Sources
oRP1440	CGGGAGCTCTCGTCGTAAGGCTGACACTGCA	To create pRP1891, pRP1892, pR1893, pRP1896, pRP1903, pRP1904, pRP1905, pRP1906, pRP1907, pRP1908, pRP1909 and pRP1910	This study
oRP1458	CAGTCGCGATGTCTAATGCGAAGGTACTTTTATTTTTTTCAGAT	To create pRP1903	This study
oRP1441	GGGGATCCGCTTCCTATGCAAAATGCTTAATAATTCATTAGACC	To create pRP1891	This study
oRP1442	GGGGATCCGCTCTTGGCTCGAGGGTACCTGT	To create pRP1892, pRP1896, pRP1904, pRP1906, pRP1907, pRP1908, pRP1909 and pRP1910	This study
oRP1443	GGGGATCCGCAATTGATCTTCATTTTTTATTTGCCTCTGATGCCT	To create pRP1893 and pRP1905	This study
oRP1459	ACATCGCGAATGAATGAAGATCAATTGAAATCCTATGCGGAA	To create pRP1894	This study
oRP1460	ACATCGCGAATGCCTTCAATATTTCCATCTCTTTCTGAACCG	To create pRP1895	This study
oRP1444	CAGTCGCGACATATGTCACTGCCGCTACGACA	To create pRP1897 and pRP1898	This study
oRP1445	CAGACGGGATCCTCATCACTCTTGGCTCGAGGGTACCTGT	To create pRP1897	This study
oRP1446	CAGACGGGATCCTCATCACAATTGATCTTCATTTTTTATTTGCCTCTGATGCCT	To create pRP1898	This study
oRP1447	AATCCTATGCGGAAGAACAAGCGGCATTGTTGTTGGGTATCACTAA	To create pRP1896	This study
oRP1448	TTAGTGATACCCAACAACAATGCCGCTTGTTCTTCCGCATAGGATT	To create pRP1896	This study
oRP1465	ATCAATTGAAATCCTATGCGGCAGAACAATTGAAATTGTTGTT	To create pRP1908	This study
oRP1466	AACAACAATTTCAATTGTTCTGCCGCATAGGATTTCAATTGAT	To create pRP1908	This study
oRP1467	TCACTAAGGAGGAGCAGATTGCTCCCGGTAGAGAGTTGCTGAA	To create pRP1909	This study

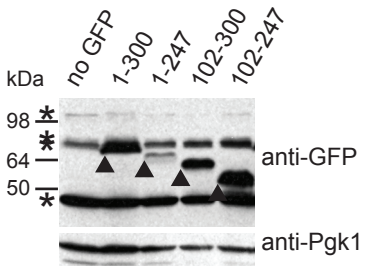
oRP1468	TTCAGCAACTCTCTACCGGGAGCAATCTGCTCCTCCTTAGTGA	To create pRP1909	This study
oRP1469	AGGAGCAGATTGATCCCGGTGCAGAGGCGCTGAATATGTTACATACTGC	To create pRP1906	This study
oRP1470	GCAGTATGTAACATATTCAGCGCCTCTGCACCGGGATCAATCTGCTCCT	To create pRP1906	This study
oRP1471	GGAATTCGAGCTCCAAGATCTAGTGTTATACGTTTTATAGACACACTGTAAATG	To create pRP1454	This study
oRP1472	TGATATCGAATTCCTGCAGCCCGGGGGATCCACTAGTCCGCAGATAAATTGACGCACC	To create pRP1454	This study
oRP1473	CGAATTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGACGTCGTAAGGCTGACACTGCAGAAG	To create pRP1449, pRP1450 and pRP1453	This study
oRP1474	CGGGATCCAGATCTTTCACTCTTGGCTCGAGGGTACCTGTCCGTTGGA	To create pRP1453	This study
oRP1475	CGGGATCCAGATCTTTCACAATTGATCTTCATTTTTTATTTGCCTCTG	To create pRP1450	This study
oRP1476	CGGGATCCAGATCTTTCACATGGAATTAATCAGATAATACTTGATATT	To create pRP1449	This study
oRP1449	GTTAAGGCATCAGAGGCAAATAAAAAATGAAGATCAATTGTGAGGCGCGCCACTTCTAAA	To create yRP2746 and yRP2748	This study
oRP1450	TAATGCGGTCTCCAACGGACAGGTACCCTCGAGCCAAGAGTGAGGCGCGCCACTTCTAAA	To create yRP2745 and yRP2747	This study
oRP1451	CATTTACAGTGTGTCTATAAAACGTATAACACTTATTCTTGAATTCGAGCTCGTTTAAAC	To create yRP2745, yRP2746, yRP2747, yRP2748, yRP2749, yRP2751, yRP2752 and yRP2753	This study
oRP1461	TTCAGGGTCTAATGAATTATTAAGCATTTTGCATAGGAAGTGAGGCGCGCCACTTCTAAA	To create yRP2749	This study
oRP1462	GTTAAGGCATCAGAGGCAAATAAAAAATGAAGATCAATTGCGGATCCCCGGGTTAATTAA	To create yRP2753	This study
oRP1463	TTCAGGGTCTAATGAATTATTAAGCATTTTGCATAGGAAGCGGATCCCCGGGTTAATTAA	To create yRP2751	This study
oRP1464	TAATGCGGTCTCCAACGGACAGGTACCCTCGAGCCAAGAGCGGATCCCCGGGTTAATTAA	To create yRP2752	This study
oRP1452	GCCCAAGCCTCTTAATGATGG	To verify integration in the *DCP2* locus	This study
oRP1453	CGGTGATGTCAAATTGTGTTATGG	To verify integration in the *DCP2* locus	This study
oRP1454	ATGGGCTCGCGATAATGTCG	To verify integration with the kanMX casette	This study

oRP1455	GCGATTCCGACTCGTCCAAC	To verify integration with the kanMX casette	This study
oRP140	ATATTGATTAGATCAGGAATTCC	Probe for *MFA2pG* mRNA	Caponigro and Parker, 1995
oRP100	GTCTAGCCGCGAGGAAGG	Probe for *SCR1* RNA	Caponigro *et al.*, 1993
oRP1300	GCTTTCTGTGCTTACCGATACGACCTTTACCGG	Probe for *CYH2* mRNA	This study
oRP1456	TTAGCAGGCTTTTCTCTCTTTGGTT	Probe for *YRA1* mRNA	This study
oRP1439	CATCATTGAGTATTTCTACGCATTTG	Probe for *RPS28B* mRNA	This study
oRP831	ACTTAGAGCTCACTAGTGCGGCCGCGTCGTAAGGCTGAC ACTGC	To verify the sequence of *DCP2*	T. Dunckley
oRP842	GAGATTAATTCCGATGGGG	To verify the sequence of *DCP2*	T. Dunckley
oRP843	CATCATTAAGAGGCTTGGGC	To verify the sequence of *DCP2*	T. Dunckley
oRP844	TTGTTGTTGATGATAACCCG	To verify the sequence of *DCP2*	T. Dunckley
oRP845	CCACATTGATAAGGGTCTC	To verify the sequence of *DCP2*	T. Dunckley
oRP846	CGATAAATCTTCATTCGGAC	To verify the sequence of *DCP2*	T. Dunckley
oRP864	CCTTCTGTTGGTTGTGTTCTCC	To verify the sequence of *DCP2*	T. Dunckley
oRP865	CGGACAGGTACCCTCGAGCCAAGAGC	To verify the sequence of *DCP2*	T. Dunckley
oRP1457	CCATGATGAGACCCTTATCAATG	To verify the sequence of *DCP2*	T. Dunckley
oRP1477	GAAGGATTCCACAGTTGTTTAATCCTCC	To verify the sequence of *DCP2*	This study
oRP1478	CCTTCACCCTCTCCACTGACAG	To verify sequence uptream of the GFP gene	This study

Caponigro, G., and R. Parker. 1995. Multiple functions for the poly(A)-binding protein in mRNA decapping and deadenylation in yeast. *Genes Dev.* 9:2421-2432

Caponigro, G., D. Muhlrad, and R. Parker. 1993. A small segment of the MAT alpha 1 transcript promotes mRNA decay in *Saccharomyces cerevisiae*: a stimulatory role for rare codons. *Mol. Cell Biol.* 13: 5141-5148

(A)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98
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(B)

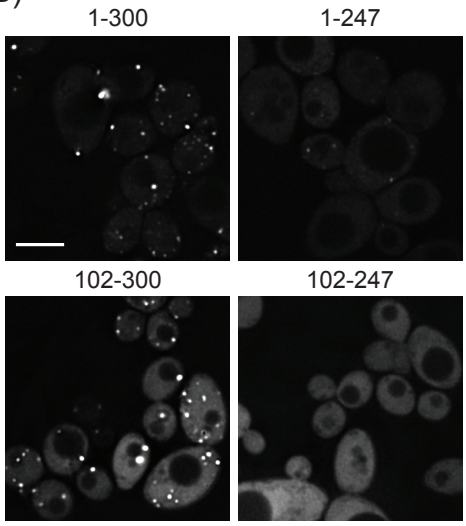
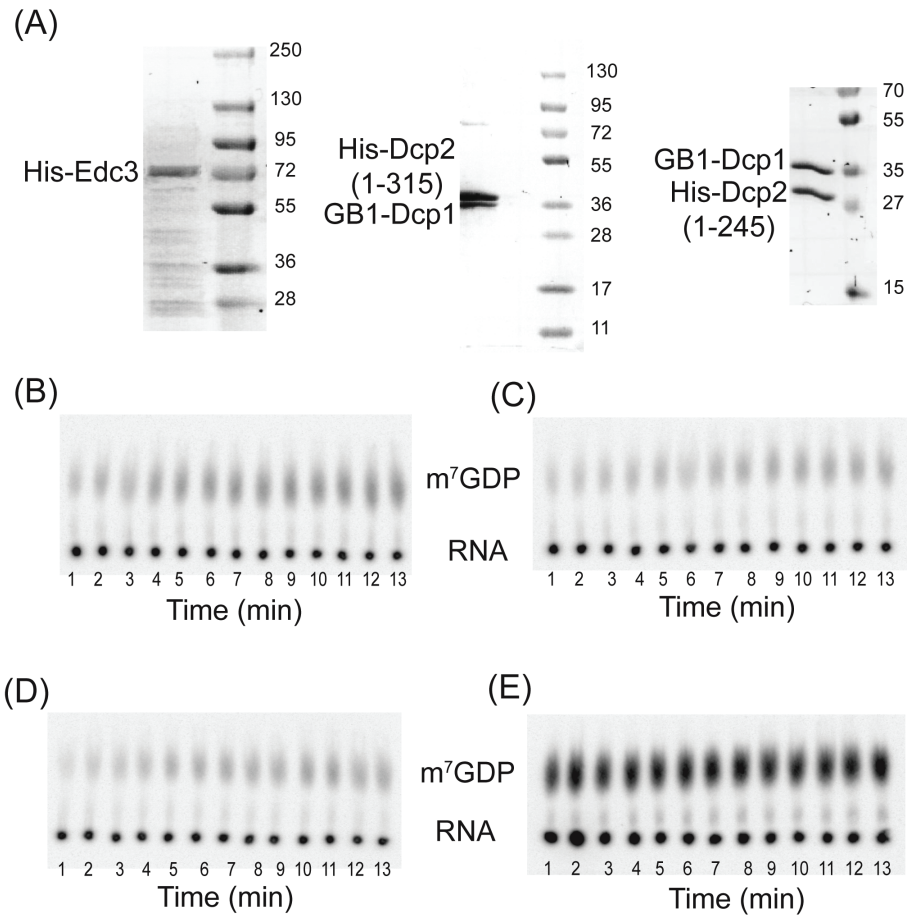

1-300
1-247
102-300
102-247

Figure S1


(A)
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130
95
72
55
36
28
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(1-315)
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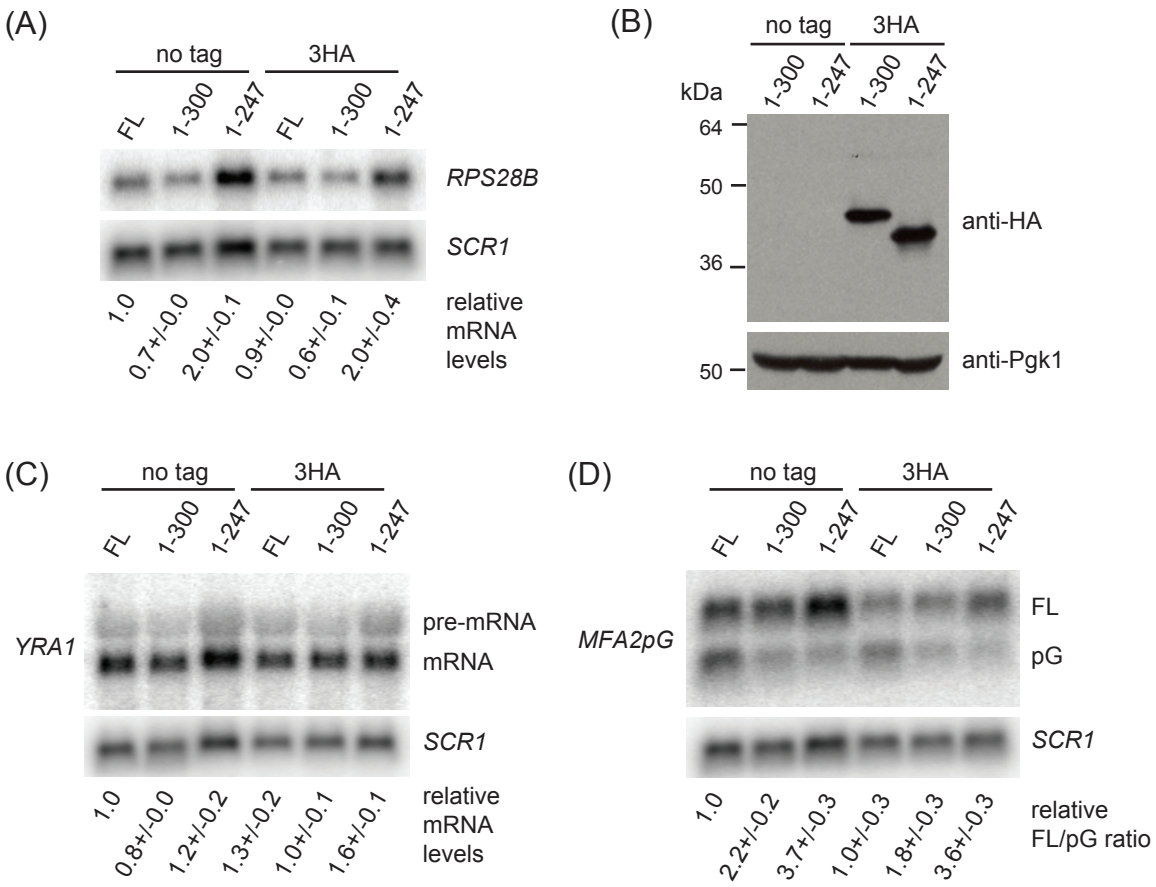
95
72
55
36
28
17
11
GB1-Dcp1
His-Dcp2
(1-245)
70
55
35
27
15
(B)
m7GDP
RNA
1 2 3 4 5 6 7 8 9 10 11 12 13
Time (min)
(C)
1 2 3 4 5 6 7 8 9 10 11 12 13
Time (min)
(D)
m7GDP
RNA
1 2 3 4 5 6 7 8 9 10 11 12 13
Time (min)
(E)
1 2 3 4 5 6 7 8 9 10 11 12 13
Time (min)

Figure S2


(A)
no tag
3HA
FL
1-300
1-247
FL
1-300
1-247
RPS28B
SCR1
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2.0+/-0.1
0.9+/-0.0
0.6+/-0.1
2.0+/-0.4
relative mRNA levels
(B)
no tag
3HA
kDa
1-300
1-247
1-300
1-247
64
50
36
50
anti-HA
anti-Pgk1
(C)
no tag
3HA
FL
1-300
1-247
FL
1-300
1-247
YRA1
pre-mRNA
mRNA
SCR1
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1.2+/-0.2
1.3+/-0.2
1.0+/-0.1
1.6+/-0.1
relative mRNA levels
(D)
no tag
3HA
FL
1-300
1-247
FL
1-300
1-247
MFA2pG
FL
pG
SCR1
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2.2+/-0.2
3.7+/-0.3
1.0+/-0.3
1.8+/-0.3
3.6+/-0.3
relative FL/pG ratio
(E)
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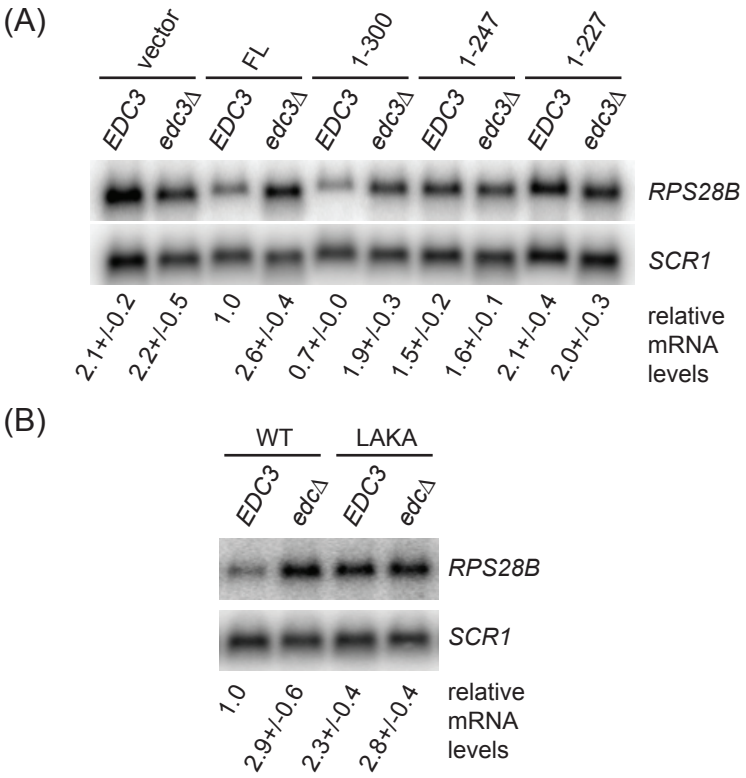
1-300
1-247
3HA
1-970
1-300
1-247

Figure S3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1-247
1-227
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edc3Δ
RPS28B
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2.2+/-0.5
1.0
2.6+/-0.4
0.7+/-0.0
1.9+/-0.3
1.5+/-0.2
1.6+/-0.1
2.1+/-0.4
2.0+/-0.3
relative mRNA levels
(B)
WT
LAKA
EDC3
edcΔ
RPS28B
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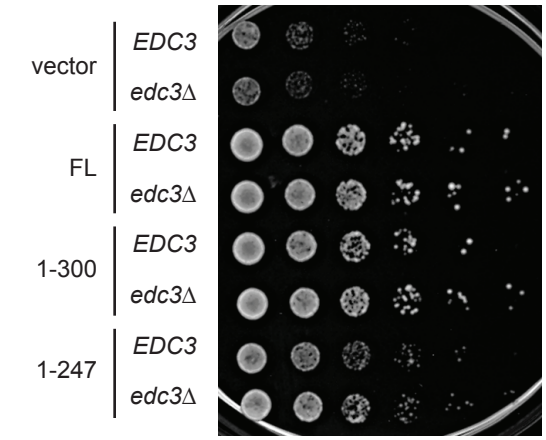
2.3+/-0.4
2.8+/-0.4
relative mRNA levels

Figure S4

(A)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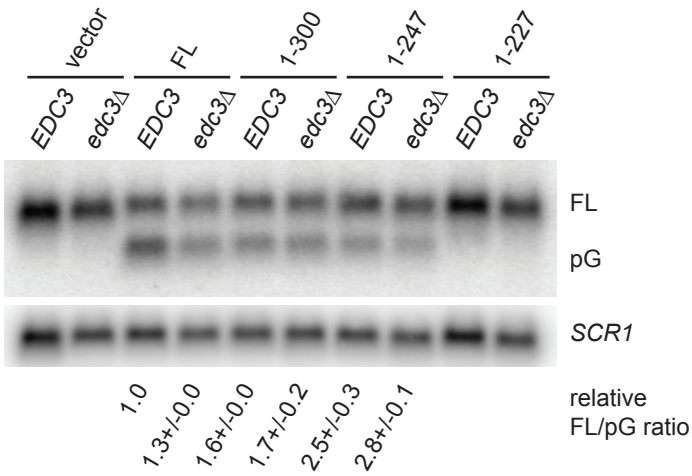
EDC3
edc3Δ

(B)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1-227
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edc3Δ
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edc3Δ
EDC3
edc3Δ
EDC3
edc3Δ
EDC3
edc3Δ
FL
pG
SCR1
1.0
1.3+/-0.0
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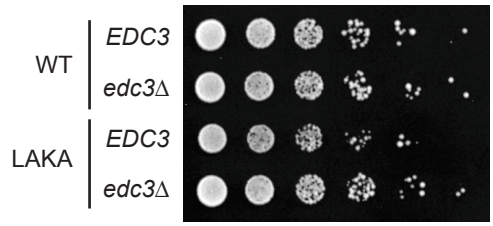
1.7+/-0.2
2.5+/-0.3
2.8+/-0.1
relative
FL/pG ratio

Figure S5

(A)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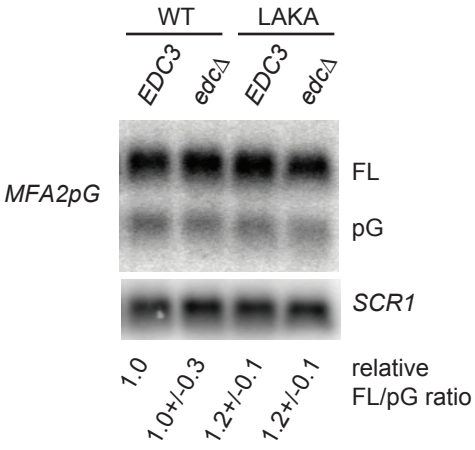
edc3Δ
LAKA
EDC3
edc3Δ

(B)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EDC3
edcΔ
MFA2pG
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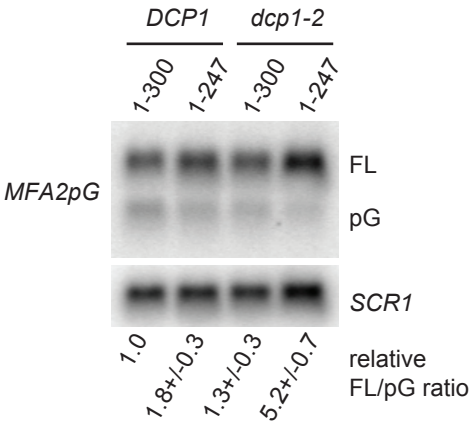
1.2+/-0.1
relative
FL/pG ratio

Figure S6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dcp1-2
1-300
1-247
1-300
1-247
MFA2pG
FL
pG
SCR1
1.0
1.8+/-0.3
1.3+/-0.3
5.2+/-0.7
relative
FL/pG ratio

Figure S7