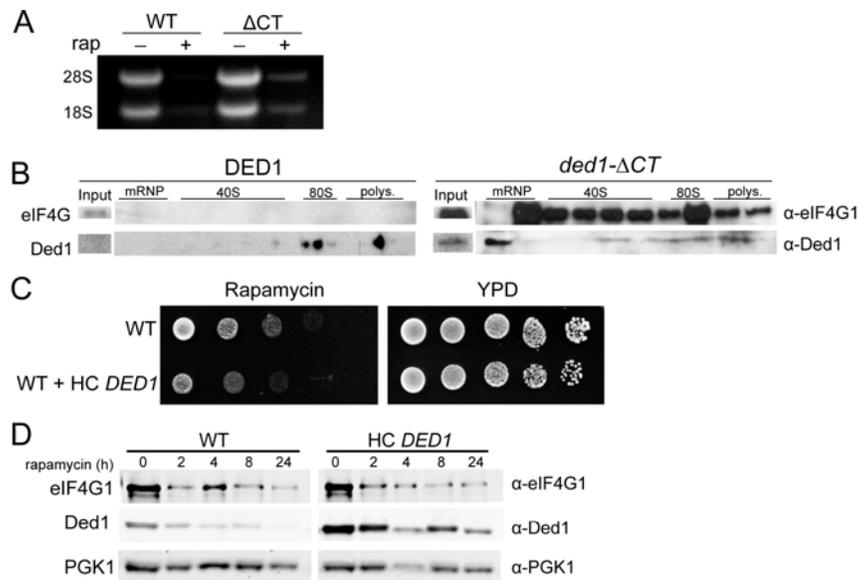


Supplemental Materials

Molecular Biology of the Cell

Aryanpur et al.



Supplementary Figure S1:

(A) *DED1* or *ded1- ΔCT* cells were treated with rapamycin for 18 hours, RNA extraction was performed, and 18S and 28S ribosomal RNA was analyzed by gel electrophoresis. (B) *DED1* and *ded1- ΔCT* cells were grown in the presence of rapamycin for 18 hours. Polysome profiles were generated by subjecting cell lysates to 7.5–30% sucrose density centrifugation and OD254 analysis. Fractions were collected for western blotting using antibodies against *TIF4631* (eIF4G1) and Ded1. (C) Wild-type cells with an empty vector (WT), or cells containing high-copy (2 μ) plasmid with *DED1* (WT + HC-*DED1*) were serially diluted and plated on selective media +/- rapamycin (100 ng/ml), and incubated at 30°C. (D) WT + vector or WT + HC-*DED1* cells were subjected to a time course of rapamycin treatment, and cell extracts were prepared and blotted for the indicated translation initiation factors (and PGK1 as a loading control) as in Figure 5.

A

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Ded1 (Saccharomyces cerevisiae) RAGNTGLATAFFNSENINIVKGLHEILTEANQEVPSFLKDAMMSAPGSR-----NS 543
DDX3/Belle (Drosophila melanogster) RMGNLGVATSTFFNEKRNINICSDLLELLIETKQEIIPSFMEDMSSDRGHGGAKRAGRGGGGR 711
DDX3X (Mus musculus) RVGNLGLATSTFFNERNINITKDLLDLLVEAKQEVPSWLENMAFEHHYKGS-SRGRSKSSR 592
DDX3X (Homo sapien) RVGNLGLATSTFFNERNINITKDLLDLLVEAKQEVPSWLENMAYEHHYKGS-SRGRSKSSR 592
* ** *:*:*:*:*:*..* ** ..* ::* *:*:*:*:*:*: . . :
.

Ded1 (Saccharomyces cerevisiae) RRGGFGRNNRDYRKAGGASAGGWGSSRSRDN-----SFRGG----SGW 583
DDX3/Belle (Drosophila melanogster) YGGGFG---SRDYRQSSGGGGGRSGPPRSGGSGSGGGGGSYKSNNGSYGGNSGGGGYY 768
DDX3X (Mus musculus) FSGGFG---ARDYRQSSGASSSSFSRRASSRSRGGGGHGGSRGFGG-----GGY 639
DDX3X (Homo sapien) FSGGFG---ARDYRQSSGASSSSFSRRASSRSRGGGGHGGSRGFGG-----GGY 639
**** *:*:*:*:*:*..* ** ..* ::* *:*:*:*:*:*: . . :
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Ded1 (Saccharomyces cerevisiae) GSDSKSSGWGNS-----GGSNSSWW-- 604
DDX3/Belle (Drosophila melanogster) GGGAGGGSYGGSYGGGSAHSSNAPDWWAQ 798
DDX3X (Mus musculus) GGFYNSDGYGGN-----YNSQGVDDWGN 662
DDX3X (Homo sapien) GGFYNSDGYGGN-----YNSQGVDDWGN 662
* ** * **

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B

536 591 604
 PGRSRSNRRGGFGRNNNRD YRKAGGASAGGWGSSRSRDNSFRGGSGWGSDSKSSGWGNSGGSNSS **WW**

Supplementary Figure S2: Multiple sequence alignment of the C-terminus of Ded1 orthologs. (A) The sequence alignment was performed using Clustal Omega with Ded1 orthologs ranging from yeast to humans. Accession numbers for sequences are, NP_015206.1 (*S. cerevisiae*), Q9VHP0 (*Drosophila Belle/DDX3* variant), NP_034158.1 (mouse DDX3 variant), and NP_001347.3 (human DDX3 variant). Conservation is shown by (*) for the same residue, (:) for strong conservation, and (.) for weak conservation of residues as defined by Clustal Omega default parameters. Note the C-terminal tryptophans are conserved. (B) The Ded1 C-terminal domain (aa's# 536-604) is shown, marked with the location of amino acids 591-604. C-terminal tryptophans are shown in red.

Supplemental Table S1: Yeast strains used in this study

Strain Name	Genotype	Background	Source
SWY4093	<i>MAT</i> <i>ded1::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100</i> <i>+pCEN/URA3/DED1</i>	W303	Bolger and Went, 2011
TBY5	<i>MAT</i> <i>ded1::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100</i> <i>+pCEN/HIS3/DED1</i>	W303	This study
TBY21	<i>MAT</i> <i>ded1::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100</i> <i>+pCEN/HIS3/ded1-ΔCT</i>	W303	This study
TBY118	<i>MAT</i> <i>ded1::KAN NIP1-PROTA::spHIS5 ade2-1, ura3-1, his3-11,15 leu2-3,112 trp1-1 can1-100</i> <i>+pCEN/LEU2/DED1</i>	W303	This study
TS161	<i>MATα ura3-52</i>	S288C	Takahara and Maeda, 2012
TS184	<i>MATα ura3-52TOR1^{L2134M}</i>	S288C	Takahara and Maeda, 2012
TBY130	<i>MAT</i> <i>ded1::KAN NIP1-PROTA::spHIS5</i>	W303	This study

	<i>ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/LEU2/ded1-ΔCT</i>		
TBY120	<i>MAT^{ded1}::KAN TIF1:3XHA:TRP1 ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/HIS3/DED1</i>	W303	This study
TBY131	<i>MAT^{ded1}::KAN TIF1:3XHA:TRP1 ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/HIS3/ded1-ΔCT</i>	W303	This study
SWY4277	<i>MAT^{ded1}::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/LEU2/ded1-120</i>	W303	Bolger and Went, 2011
TBY52	<i>MAT^{ded1}::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/LEU2/DED1</i>	W303	This study
TBY121	<i>MAT^{ded1}::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/LEU2/ded1-ΔCT</i>	W303	This study
TBY104	<i>MAT^{ded1}::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/LEU2/ded1-Δ535-548</i>	W303	This study
TBY105	<i>MAT^{ded1}::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/LEU2/ded1-Δ549-562</i>	W303	This study
TBY106	<i>MAT^{ded1}::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/LEU2/ded1-Δ563-576</i>	W303	This study
TBY107	<i>MAT^{ded1}::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/LEU2/ded1-Δ577-590</i>	W303	This study
TBY108	<i>MAT^{ded1}::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/LEU2/ded1-Δ591-604</i>	W303	This study
TBY110	<i>MAT^{ded1}::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/HIS3/ded1-W604A</i>	W303	This study
TBY111	<i>MAT^{ded1}::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/HIS3/ded1-W603/604A</i>	W303	This study
TBY132	<i>MAT^{ded1}::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/LEU2/ded1-4SA</i>	W303	This study
TBY114	<i>MAT^{ded1}::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/LEU2/ded1-4SD</i>	W303	This study
TBY101	<i>MAT^{ded1}::KAN SCH9:3XHA:TRP1 ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/HIS3/DED1</i>	W303	This study
TBY103	<i>MAT^{ded1}::KAN SCH9:3XHA:TRP1 ade2-</i>	W303	This study

	<i>1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/HIS3/ded1-ΔCT</i>		
TBY133	<i>MATα ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +2μ/URA3/DED1</i>	W303	This study
TBY134	<i>MATα tif4631::HYG ded1::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/HIS3/DED1</i>	W303	This study
TBY135	<i>MATα tif4631::HYG ded1::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/HIS3/ded1-ΔCT</i>	W303	This study
TBY136	<i>MATα tif4631::HYG ded1::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/HIS3/ded1-Δ591-604</i>	W303	This study
TBY137	<i>MATα tif4631::HYG ded1::KAN ade2-1 ura3-1 his3-11,15 leu2-3,112 trp1-1 can1-100 +pCEN/HIS3/ded1-W603/604A</i>	W303	This study

Supplemental Table S2: Plasmids used in this study

Plasmid Name	Description	Source
<i>DED1</i>	<i>CEN/HIS3/DED1</i>	Chuang <i>et al.</i> , 1997
pRP2044	<i>CEN/HIS3/ded1-ΔCT</i>	Hilliker <i>et al.</i> , 2011
pSW3619	<i>CEN/LEU2/DED1</i>	Bolger and Wentle, 2011
<i>ded1-120</i>	<i>CEN/LEU2/ded1-120</i>	Chuang <i>et al.</i> , 1997
pTB136	<i>CEN/LEU2/ded1-ΔCT</i>	This study
pTB111	<i>CEN/LEU2/ded1-Δ535-548</i>	This study
pTB112	<i>CEN/LEU2/ded1-Δ549-562</i>	This study
pTB113	<i>CEN/LEU2/ded1-Δ563-576</i>	This study
pTB114	<i>CEN/LEU2/ded1-Δ577-590</i>	This study
pTB115	<i>CEN/LEU2/ded1-Δ591-604</i>	This study
pTB123	<i>CEN/HIS3/ded1-W604A</i>	This study
pTB124	<i>CEN/HIS3/ded1-W603/604A</i>	This study
pTB104	<i>CEN/LEU2/ded1-4SA</i>	This study
pTB122	<i>CEN/LEU2/ded1-4SD</i>	This study
pTB105	<i>2μ/URA3/DED1</i>	This study
pSW3576	<i>pET28a-DED1</i>	Bolger and Wentle, 2011
pTB6	<i>pET28a-ded1-Δ536-604</i>	This study
pTB160	<i>pET28a-ded1-Δ591-604</i>	This study
pTB161	<i>pET28a-ded1-W603/604A</i>	This study