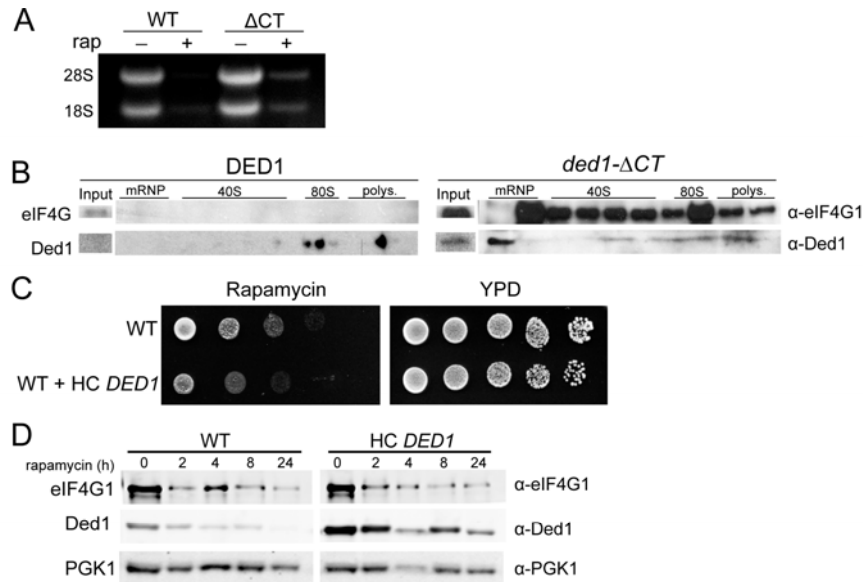


Supplemental Materials

Molecular Biology of the Cell

Aryanpur et al.



Supplementary Figure S1:

(A) *DED1* or *ded1*- Δ CT 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*- Δ CT 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.

	<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