

Supplementary Material:

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. *DHH1* deletion partially suppressed the growth defect by overexpression of Rbp1p.

We generated BY4741 wild-type, *dhh1Δ*, *pat1Δ*, or *lsm1Δ* strains whose expression of *RBPI* is under control of galactose-inducible promoter. (A) These yeast strains were grown in glucose to log phase, tenfold serial dilutions were plated onto YPD, YPGal, or YPGal plus 0.02% raffinose plates, and were grown at 30°C for 2~3 day. (B) The growth curves of these yeast strains in liquid culture were determined by measuring optical density at 600 nm. Yeasts were logarithmically grown in YPGal plus 0.02% raffinose medium with starting density $OD_{600} = 0.05$. The growth optical density (600 nm) was measured in aliquots from cultures at indicated time points. (C) Quantification of differential growth after 25 h incubation in panel B. The *bars* represent the means of three independent cultures.

Figure S2. Rbp1p-dNMP lost its interaction with Dhh1p, but not Rbp1p-rrm1.

(A) Rbp1p interacts with Dhh1p through its C-terminal NMP-rich region and (B) Rbp1p-rrm1 interacts with Dhh1p in yeast two-hybrid assays. β -galactosidase reporter assays were performed in YEM1 α cells expressing fusion protein LexA-lamin, -Rbp1p, -Rbp1p-dNMP, or -Rbp1p-rrm1 with Gal4AD-Dhh1p. Immunoblotting using anti-LexA and anti-HA show the expression level of indicated proteins.

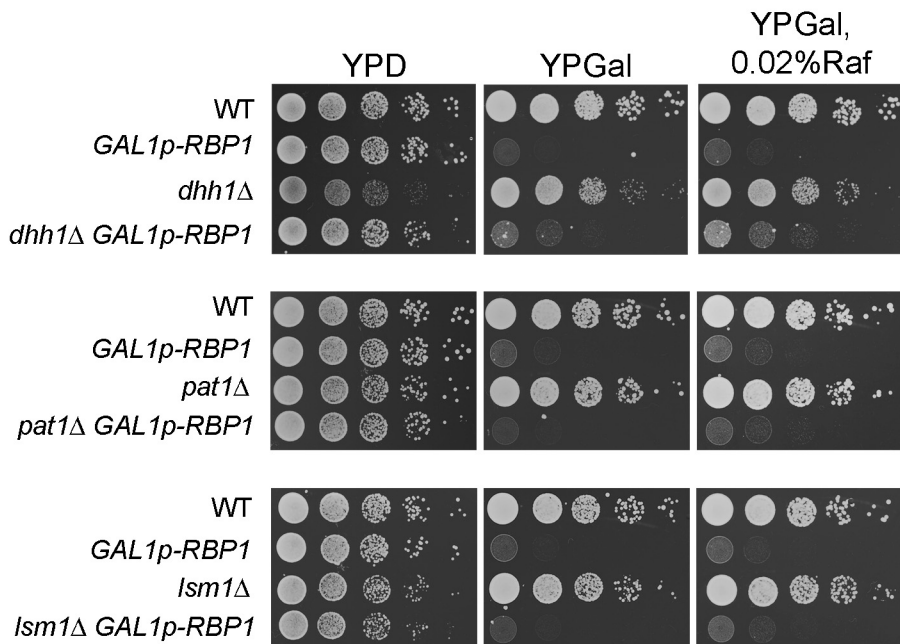
Figure S3. RecA-like domain II of Dhh1p or DDX6 is needed for the nonconserved C-terminal domain of Dhh1 to interact with Rbp1p.

(A) Schematic representation of the C-terminal variants of Dhh1p, DDX6, and DDX6 chimera protein domain structures used in yeast two-hybrid. (B) Dhh1p-Ct or DDX6-Ct-C85 is sufficient for Rbp1p interaction. Yeast cotransformants and β -galactosidase reporter assays was performed as described in Materials and Methods. Immunoblotting using anti-LexA and anti-HA show the expression level of indicated proteins.

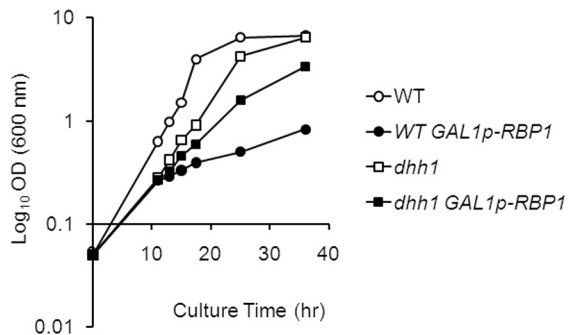
Figure S4. Dhh1p or DDX6-C85 interacts with Rbp1p in RNA-independent manner.

Immunoprecipitation was performed as described in Figure 6E and Materials and Methods, except the input lysate was pretreated with 100 mg/ml RNase A to digest RNA.

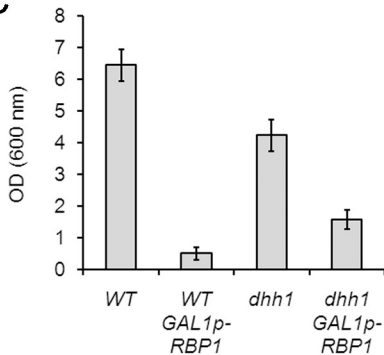
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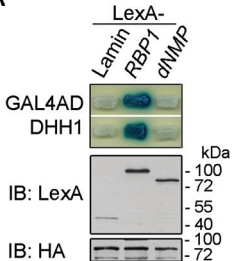
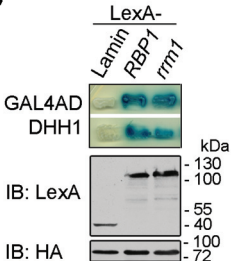


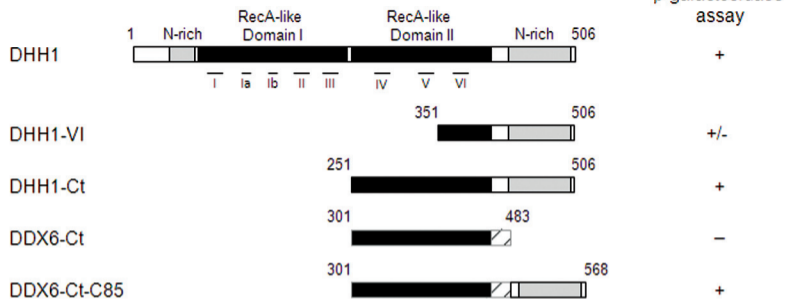
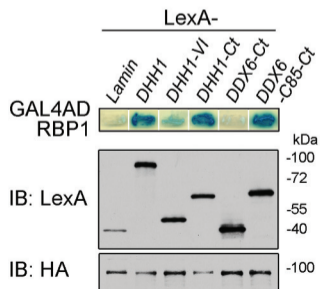
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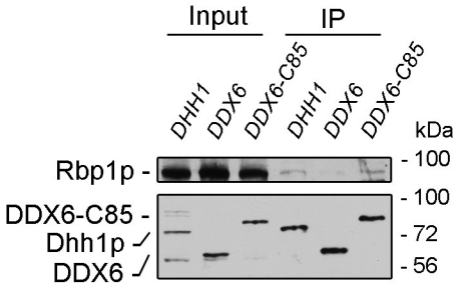


C



A**B**

A**B**



Supplementary Table I. Yeast strains used in this study

Strain	Genotype	Source
YEM1 α	<i>MATa his3 trp1 leu2 6ops-LEU2 2ops-LacZ</i>	
BY4741	<i>MATa his3 leu2 ura3 met15</i>	
BY4741 <i>dhh1</i> Δ	BY4741 except <i>dhh1::KanMX6</i>	ResGen (Invitrogen)
BY4741 <i>pat1</i> Δ	BY4741 except <i>pat1::KanMX6</i>	ResGen (Invitrogen)
BY4741 <i>lsm1</i> Δ	BY4741 except <i>lsm1::KanMX6</i>	ResGen (Invitrogen)
BY4741 <i>xrn1</i> Δ	BY4741 except <i>xrn1::KanMX6</i>	ResGen (Invitrogen)
BY4741 <i>rbp1</i> Δ	BY4741 except <i>rbp1::KanMX6</i>	ResGen (Invitrogen)
BY4741 <i>rbp1</i> Δ <i>dhh1</i> Δ	BY4741 except <i>rbp1::KanMX6 dhh1::HisMX6</i>	This study
BY4741 <i>DCP2-mCh</i> <i>DHH1-GFP</i>	BY4741 except <i>DCP2-mCherry::KanMX6</i> <i>DHH1-GFP::HisMX6</i>	This study
BY4741 <i>DCP2-mCh</i> <i>DHH1-dC81-GFP</i>	BY4741 except <i>DCP2-mCherry::KanMX6</i> <i>DHH1-dC81-GFP::HisMX6</i>	This study
BY4741 <i>DCP2-mCh</i> <i>DHH1-dC106-GFP</i>	BY4741 except <i>DCP2-mCherry::KanMX6</i> <i>DHH1-dC106-GFP::HisMX6</i>	This study
BY4741 <i>DHH1-3HA</i>	BY4741 except <i>DHH1-3HA::HisMX6</i>	This study
BY4741 <i>DHH1-dC81-3HA</i>	BY4741 except <i>DHH1-dC81-3HA::HisMX6</i>	This study
BY4741 <i>rbp1</i> Δ <i>DHH1-3HA</i>	BY4741 except <i>rbp1::KanMX6</i> <i>DHH1-3HA::KanMX6</i>	This study
BY4741 <i>RBPI-3HA</i>	BY4741 except <i>RBPI-3HA::HisMX6</i>	This study
BY4741 <i>dhh1</i> Δ <i>RBPI-3HA</i>	BY4741 except <i>dhh1::KanMX6</i> <i>RBPI-3HA::HisMX6</i>	This study
BY4741 <i>GAL1p-RBPI</i>	BY4741 except <i>GAL1p-RBPI::HisMX6</i>	This study
BY4741 <i>dhh1</i> Δ <i>GAL1p-RBPI</i>	BY4741 except <i>dhh1::KanMX6</i> <i>GAL1p-RBPI::HisMX6</i>	This study
BY4741 <i>pat1</i> Δ <i>GAL1p-RBPI</i>	BY4741 except <i>pat1::KanMX6</i> <i>GAL1p-RBPI::HisMX6</i>	This study
BY4741 <i>lsm1</i> Δ <i>GAL1p-RBPI</i>	BY4741 except <i>lsm1::KanMX6</i> <i>GAL1p-RBPI::HisMX6</i>	This study
YTC345	<i>MATa rpb1-1ura3 leu2</i>	Dr. Tien-Hsien Chang
YTC345 <i>dhh1</i> Δ	YTC345 except <i>dhh1::KanMX6</i>	This study

Supplementary Table II. Plasmids used in this study

Plasmid	Features	Source
pVT101U	<i>URA3</i> , 2 μ m, <i>ADH1p</i>	(1)
pVT101U-HA-RBP1	<i>URA3</i> , 2 μ m, <i>ADH1p-HA-RBP1</i>	(2)
pVT101U-RBP1	<i>URA3</i> , 2 μ m, <i>ADH1p-RBP1</i>	This study
pVT101U-RBP1-dNMP	<i>URA3</i> , 2 μ m, <i>ADH1p-RBP1-dNMP</i>	This study
pVT101U-RBP1-rrm1	<i>URA3</i> , 2 μ m, <i>ADH1p-RBP1-rrm1</i>	This study
pVT101U-DHH1	<i>URA3</i> , 2 μ m, <i>ADH1p-DHH1</i>	This study
pVT101U-DHH1dC81	<i>URA3</i> , 2 μ m, <i>ADH1p-DHH1-dC81</i>	This study
pVT101U-DHH1dC106	<i>URA3</i> , 2 μ m, <i>ADH1p-DHH1-dC106</i>	This study
pVT101U-DDX6	<i>URA3</i> , 2 μ m, <i>ADH1p-DDX6</i>	This study
pVT101U-DDX6-C85	<i>URA3</i> , 2 μ m, <i>ADH1p-DDX6-C85^{Dhh1p}</i>	This study
pVT101U-DHH1-2HA	<i>URA3</i> , 2 μ m, <i>ADH1p-DHH1-2HA</i>	This study
pVT101U-DDX6-2HA	<i>URA3</i> , 2 μ m, <i>ADH1p-DDX6-2HA</i>	This study
pVT101U-DDX6-C85-2HA	<i>URA3</i> , 2 μ m, <i>ADH1p-DDX6-C85^{Dhh1p}-2HA</i>	This study
YEplac181	<i>LEU2</i> , 2 μ m	(3)
YEplac181-HA-RBP1	<i>LEU2</i> , 2 μ m, <i>ADH1p-HA-RBP1</i>	This study
YCplac111	<i>LEU2</i> , <i>CEN4</i>	(3)
YCplac111-DHH1	<i>LEU2</i> , <i>CEN4</i> , <i>ADH1p-DHH1</i>	This study
YCplac111-DHH1-dC81	<i>LEU2</i> , <i>CEN4</i> , <i>ADH1p-DHH1-dC81</i>	This study
YCplac111-DHH1-dC106	<i>LEU2</i> , <i>CEN4</i> , <i>ADH1p-DHH1-dC106</i>	This study
pEG202	<i>HIS3</i> , 2 μ m, <i>ADH1p</i>	(4)
pEG202-DHH1	<i>HIS3</i> , 2 μ m, <i>ADH1p-DHH1</i>	This study
pEG202-DHH1-dC81	<i>HIS3</i> , 2 μ m, <i>ADH1p-DHH1-dC81</i>	This study
pEG202-DHH1-dC106	<i>HIS3</i> , 2 μ m, <i>ADH1p-DHH1-dC106</i>	This study
pEG202-DHH1-VI	<i>HIS3</i> , 2 μ m, <i>ADH1p-DHH1-VI</i>	This study
pEG202-DHH1-Ct	<i>HIS3</i> , 2 μ m, <i>ADH1p-DHH1-Ct</i>	This study
pEG202-DDX6	<i>HIS3</i> , 2 μ m, <i>ADH1p-DDX6</i>	This study
pEG202-DDX6-Ct	<i>HIS3</i> , 2 μ m, <i>ADH1p-DDX6-Ct</i>	This study
pEG202-DDX6-C85	<i>HIS3</i> , 2 μ m, <i>ADH1p-DDX6-C85^{Dhh1p}</i>	This study
pEG202-DDX6-C85-Ct	<i>HIS3</i> , 2 μ m, <i>ADH1p-DDX6-C85^{Dhh1p}-Ct</i>	This study
pEG202-RBP1	<i>HIS3</i> , 2 μ m, <i>ADH1p-RBP1</i>	This study
pEG202-RBP1-dNMP	<i>HIS3</i> , 2 μ m, <i>ADH1p-RBP1-dNMP</i>	This study
pEG202-RBP1-rrm1	<i>HIS3</i> , 2 μ m, <i>ADH1p-RBP1-rrm1</i>	This study
pJG4-5	<i>TRP1</i> , 2 μ m, <i>GAL1p</i>	(4)
pJG4-5-RBP1	<i>TRP1</i> , 2 μ m, <i>GAL1p-RBP1</i>	Our Lab
pJG4-5-DHH1	<i>TRP1</i> , 2 μ m, <i>GAL1p-DHH1</i>	Our Lab

1. Vernet, T., Dignard, D. and Thomas, D.Y. (1987) A family of yeast expression vectors containing the phage f1 intergenic region. *Gene*, **52**, 225-233.
2. Buu, L.M., Jang, L.T. and Lee, F.J. (2004) The yeast RNA-binding protein Rbp1p modifies the stability of mitochondrial porin mRNA. *J Biol Chem*, **279**, 453-462.
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4. Gyuris, J., Golemis, E., Chertkov, H. and Brent, R. (1993) Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. *Cell*, **75**, 791-803.