

## Supplementary Information

### **DEAD-box ATPases are global regulators of phase-separated organelles**

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**Supplementary Table 1: Yeast strains used in this study**

KWY 1973	<i>MAT a ade2-1 can1-100 GAL phi+ his3-11,15 ura3-1 leu2-3, 112 trp1-1 dhh1::DHH1-HisMX6 GFP dcp2::DCP2-mCherry-NatMX6</i>
KWY7681	<i>MAT a ade2-1 can1-100 GAL phi+ his3-11,15 ura3-1 leu2-3, 112 trp1-1 dhh1::DHH1 Δ NΔ C HisMX6 yEGFP dcp2::DCP2-mCherry-NatMX6</i>
KWY7906	<i>Nup60-3xmKate2::CaURA3 HIS::pGPD1-LexA-B112(pNH604) Chromosome2(CHS3-SCO2 intergenic region)::p(LexA(4)-pCYC1min)_3xGST-V5_24xPP7sl_Tcyc1_NatNT2 LEU2::pMET25-PP7CP-yEGFP Sub2-V5-IAA7::KAN</i>
KWY7910	<i>Nup60-3xmKate2::CaURA3 HIS::pGPD1-LexA-B112(pNH604) Chromosome2(CHS3-SCO2 intergenic region)::p(LexA(4)-pCYC1min)_3xGST-V5_24xPP7sl_Tcyc1_NatNT2 LEU2::pMET25-PP7CP-yEGFP Sub2-V5-IAA7::KAN</i>
KWY7913	<i>Nup60-3xmKate2::CaURA3 HIS::pGPD1-LexA-B112(pNH604) Chromosome2(CHS3-SCO2 intergenic region)::p(LexA(4)-pCYC1min)_3xGST-V5_24xPP7sl_Tcyc1_NatNT2 LEU2::pMET25-PP7CP-yEGFP Sub2-V5-IAA7::KAN TRP1::pGPD-osTIR-hphNT1</i>
KWY7914	<i>Nup60-3xmKate2::CaURA3 HIS::pGPD1-LexA-B112(pNH604) Chromosome2(CHS3-SCO2 intergenic region)::p(LexA(4)-pCYC1min)_3xGST-V5_24xPP7sl_Tcyc1_NatNT2 LEU2::pMET25-PP7CP-yEGFP Sub2-V5-IAA7::KAN TRP1::pGPD-osTIR-hphNT1</i>
KWY8254	<i>his3_1 leu2_0 met15_0 ura3_0 FBA1::24 X PP7 loops DED1-yEGFP-Y66L-IAA7-KAN TRP1::YIPlac-Gal4BD-ER_BD-VP16-hphNT1 ChrII-HR1_pGal1_Ded1-mCherry_cyc1T_NATNT2_ChrII-HR2</i>
KWY8255	<i>his3_1 leu2_0 met15_0 ura3_0 FBA1::24 X PP7 loops DED1-yEGFP-Y66L-IAA7-KAN pNH603-pGPD1-osTIR::HIS TRP1::YIPlac-Gal4BD-ER_BD-VP16-hphNT1 ChrII-HR1_pGal1_Ded1-mCherry_cyc1T_NATNT2_ChrII-HR2</i>
KWY8256	<i>his3_1 leu2_0 met15_0 ura3_0 FBA1::24 X PP7 loops DED1-yEGFP-Y66L-IAA7-KAN TRP1::YIPlac-Gal4BD-ER_BD-VP16-hphNT1 ChrII-HR1_pGal1_Ded1-DQAD-mCherry_cyc1T_NATNT2_ChrII-HR2</i>
KWY8257	<i>his3_1 leu2_0 met15_0 ura3_0 FBA1::24 X PP7 loops DED1-yEGFP-Y66L-IAA7-KAN pNH603-pGPD1-osTIR::HIS TRP1::YIPlac-Gal4BD-ER_BD-VP16-hphNT1 ChrII-HR1_pGal1_Ded1-DQAD-mCherry_cyc1T_NATNT2_ChrII-HR2</i>
KWY8275	<i>MAT a ade2-1 can1-100 GAL phi+ his3-11,15 ura3-1 leu2-3, 112 trp1-1 ded1::DED1-yEGFP-HisMX6</i>
KWY8278	<i>MAT a ade2-1 can1-100 GAL phi+ his3-11,15 ura3-1 leu2-3, 112 trp1-1 dhh1::dqad ded1::DED1-yEGFP-HisMX6</i>
KWY8281	<i>MAT a ade2-1 can1-100 GAL phi+ his3-11,15 ura3-1 leu2-3, 112 trp1-1 dhh1Δ::KanMX6 ded1::DED1-yEGFP-HisMX6</i>
KWY8283	<i>MAT a ade2-1 can1-100 GAL phi+ his3-11,15 ura3-1 leu2-3, 112 trp1-1 pab1::PAB1-yEGFP-HisMX6</i>
KWY8286	<i>MAT a ade2-1 can1-100 GAL phi+ his3-11,15 ura3-1 leu2-3, 112 trp1-1 dhh1::dqad pab1::PAB1-yEGFP-HisMX6</i>
KWY8289	<i>MAT a ade2-1 can1-100 GAL phi+ his3-11,15 ura3-1 leu2-3, 112 trp1-1 dhh1Δ::KanMX6 pab1::PAB1-yEGFP-HisMX6</i>

**Supplementary Table 2: Plasmids used in this study**

pKW891	in vitro transcription, CY5-labeled 1329nt RNA	pAS575
pKW967	bacterial expression, GFP	pET-GFP
pKW2613	bacterial expression, eIF4G	pSV272-eIF4G(572-952)
pKW2922	bacterial expression, GFP	pET_2xGFP-6xHis
pKW3469	bacterial expression, Not1-MIF4G	pETMCN_His-TEV_V5-Not1-MIF4G (754-1000)
pkw3570	plasmid for endogenous tagging	pFA6a-yEGFP-HIS3MX
pKW3631	bacterial expression, Dhh1 FL	pETMCN_His-TEV_V5-Dhh1-mCherry
pKW3640	bacterial expression, Dhh1-GFP	pETMCN_His-TEV_V5-Dhh1-GFP(A206K)
pKW3714	bacterial expression, Dbp5-mCherry	pETMCN_His-TEV_V5-Dbp5-mCherry
pKW4063	bacterial expression, Dhh1 core	pETMCN_His-TEV_V5-Dhh1[48-425]-mCh
pKW4080	bacterial expression, Sub2-mCherry	pETMCN_His-TEV_V5-Sub2-mCherry
pKW4084	bacterial expression, Ded1-mCherry	pETMCN_His-TEV_V5-Ded1-mCherry
pKW4085	bacterial expression, eIF4A-mCherry	pETMCN_His-TEV_V5-eIF4A-mCherry
pKW4289	bacterial expression, Dbp1-mCherry	pETMCN_His-TEV_V5-Dbp1-mCherry
pKW4293	bacterial expression, Dbp2-mCherry	pETMCN_His-TEV_V5-Dbp2-mCherry
pKW4360	bacterial expression, DbpA_mCherry	pETMCN_His-TEV_V5-DbpA_mCherry
pKW4361	bacterial expression, RhlB-mCherry	pETMCN_His-TEV_V5-RhlB-mCherry
pKW4379	bacterial expression, DeaD-mCherry	pETMCN_His-TEV_V5-DeaD-mCherry
pKW4380	bacterial expression, SrmB-mCherry	pETMCN_His-TEV_V5-SrmB-mCherry
pKW4382	bacterial expression, RhlE-mCherry	pETMCN_His-TEV_V5-RhlE-mCherry
pKW4383	bacterial expression, Ded1 <sup>DQAD</sup> -mCherry	pETMCN_His-TEV_V5-Ded1 <sup>DQAD</sup> -mCh
pKW4406	bacterial expression, Ded1-GFP	pETMCN_His-TEV_V5-Ded1-yEGFP(A206K)
pkw4519	bacterial expression, DDX6-mCherry	pETMCN_HisMBP_V5-DDX6-mCherry
pKW4557	bacterial expression, DDX3X-mCherry	pETMCN_His-TEV_V5-DDX3X-mCherry

**Supplementary Table 3: Oligonucleotides used in this study**

CH3566	Fw, Ded1 GFP tagging	AGCGATTCCAAGTCTTCTGGCTGGGGTAACAGCGGT GGTCAAACAACACTCTTCTTGGTGG CGGATCCCCGGGTTAATTA
CH3567	Rev, Ded1 GFP tagging	AGAAAATATAAGACATGCTAGAGCAGAAAACGAAGA ATCCTCACCTAGTTTGTCTGAAA GAATTCGAGCTCGTTTAAAC
CH3568	Fw, Pab1 GFP tagging	GAAGCTTCTGCTGCCTATGAGTCTTTCAAAAAGGAGC AAGAACAACAACACTGAGCAAGCT CGGATCCCCGGGTTAATTA
Ch3569	Rev, Pab1 GFP tagging	AAGGGAGAAAAAAAAAAGATGATAAGTTTGTGAGTAG GGAAGTAGGTGATTACATAGAGCA GAATTCGAGCTCGTTTAAAC

**Supplementary Table 4: setup conditions for *in vitro* droplet assays**

	<b>storage buffer</b>	<b>droplet setup conditions</b>	<b>final concentration</b>
Dhh1 FL pKW3631	MH200G	5.25 $\mu$ l 50 $\mu$ M Dhh1-mCherry 15.75 $\mu$ l LSB-150 1 $\mu$ l CKM 1 $\mu$ l 10 mg/ml BSA 1 $\mu$ l Hepes pH 6.4 0.25 $\mu$ l 10 mg/ml polyU	10.5 $\mu$ M
Dhh1 core pKW4063	MH200G	5.25 $\mu$ l 50 $\mu$ M Dhh1-core-mCherry 15.75 $\mu$ l LSB-150 1 $\mu$ l CKM 1 $\mu$ l 10 mg/ml BSA 1 $\mu$ l Hepes pH 6.4 0.25 $\mu$ l 10 mg/ml polyU	10.5 $\mu$ M
Ded1-mCherry pKW4084	MH20G	2.5ul 50uM Ded1-mCherry in MH200G 13ul LSB-50 1ul CK mix 2ul Hepes pH 6.8 1ul 10mg/ml BSA 1.5ul polyU 1mg/ml	6.25 $\mu$ M
Dbp1-mCherry pKW4289	MH200G	3 $\mu$ l 50uM Dbp1-mCherry in MH200G 14 $\mu$ l LSB-50 1 $\mu$ l CKM 2 $\mu$ l Hepes pH 6.4 1 $\mu$ l 10mg/ml BSA 1 $\mu$ l 1mg/ml polyU	7.5 $\mu$ M
Dbp2-mCherry pKW4293	MH300G	2.5 $\mu$ l 50 $\mu$ M mCherry-Dbp2 in MH200G 11.5 $\mu$ l MH200G 1 $\mu$ l 1M phosphate pH 6.0 1 $\mu$ l CKM 2 $\mu$ l Hepes pH 6.0 1 $\mu$ l 10mg/ml BSA 1 $\mu$ l 1mg/ml polyU	6.25 $\mu$ M
eIF4A-mCherry pKW4085	MH200G	2 $\mu$ l eIF4A-mCherry at 500 $\mu$ M 9 $\mu$ l LSB-150 2 $\mu$ l CKM 1 $\mu$ l BSA 10mg/ml 1 $\mu$ l Hepes pH 6.6 3 $\mu$ l 1x ATPase buffer 2 $\mu$ l 1 mg/ml polyU	50 $\mu$ M
Sub2-mCherry pKW4080	MH200G	2 $\mu$ l Sub2-mCherry at 500 $\mu$ M 9 $\mu$ l LSB-150 2 $\mu$ l CKM 1 $\mu$ l BSA 10mg/ml 1 $\mu$ l Hepes pH 6.6 3 $\mu$ l 1x ATPase buffer 2 $\mu$ l 1mg/ml polyU	50 $\mu$ M
Dbp5-mCherry pKW3714	MH200G	2 $\mu$ l Dbp5-mCherry at 500 $\mu$ M 9 $\mu$ l LSB-150 2 $\mu$ l CKM 1 $\mu$ l BSA 10mg/ml 1 $\mu$ l Hepes pH 6.6 3 $\mu$ l 1x ATPase buffer 2 $\mu$ l 1mg/ml polyU	50 $\mu$ M
DDX6-mCherry pKW4519	MH200G	2.5 $\mu$ l DDX6-mCherry at 50 $\mu$ M 14.5 $\mu$ l LSB-50 2 $\mu$ l CKM 1 $\mu$ l BSA 10mg/ml 1 $\mu$ l Hepes pH 6.4 3 $\mu$ l 2 mg/ml polyU	6.4 $\mu$ M

	<b>storage buffer</b>	<b>droplet setup conditions</b>	<b>final concentration</b>
DDX3X-mCherry pKW4557	MH300G	3 $\mu$ l DDX3X-mCherry at 50 $\mu$ M 14 $\mu$ l LSB-50 2 $\mu$ l CKM 1 $\mu$ l BSA 10mg/ml 1 $\mu$ l Hepes pH 6.8 2 $\mu$ l 2 mg/ml polyU	7.5 $\mu$ M
DbpA-mCherry pKW4360	MH200G	3 $\mu$ l DbpA-mCherry at 50 $\mu$ M 16 $\mu$ l LSB-100 2 $\mu$ l CKM 1 $\mu$ l BSA 10 mg/ml 1 $\mu$ l 1M Hepes pH 6.4 2 $\mu$ l 2 mg/ml polyU	6 $\mu$ M
RhlB-mCherry pKW4361	MH200G	3 $\mu$ l RhlB-mCherry at 50 $\mu$ M 16 $\mu$ l LSB-100 2 $\mu$ l CKM 1 $\mu$ l BSA 10 mg/ml 1 $\mu$ l 1M Hepes pH 6.4 2 $\mu$ l 2 mg/ml polyU	6 $\mu$ M
DeaD-mCherry pKW4379	MH200G	3 $\mu$ l DeaD-mCherry at 50 $\mu$ M 17.5 $\mu$ l LSB-100 1 $\mu$ l CKM 1 $\mu$ l BSA 10mg/ml 1 $\mu$ l Hepes pH 6.4 1.5 $\mu$ l 2 mg/ml polyU	6 $\mu$ M
SrmB-mCherry pKW4380	MH200G	3 $\mu$ l SrmB-mCherry at 50 $\mu$ M 18.5 $\mu$ l LSB-50 1 $\mu$ l CKM 1 $\mu$ l BSA 10mg/ml 1.5 $\mu$ l 2 mg/ml polyU	6 $\mu$ M
RhlE-mCherry pKW4382	MH200G	2 $\mu$ l RhlE-mCherry at 50 $\mu$ M 18.5 $\mu$ l LSB-100 1 $\mu$ l CKM 1 $\mu$ l BSA 10mg/ml 1 $\mu$ l Hepes 1M pH 6.8 1.5 $\mu$ l 2 mg/ml polyU	4 $\mu$ M

**Supplementary Table 5: setup conditions for *in vitro* Dhh1 phase diagram reactions**

	<b>droplet setup conditions</b>
pH titration	13.4 $\mu$ l LSB-100 2.1 $\mu$ l 0.5M KCl (final KCl concentration 100mM) 1 $\mu$ l CKM mix 1.25 $\mu$ l 100mM ATP/MgCl <sub>2</sub> (final concentration 5mM) 1 $\mu$ l 10 mg/ml BSA, 2 $\mu$ l 1M Hepes of the respective pH 1.25 $\mu$ l 1mg/ml polyU (final concentration 0.05 mg/ml) 3 $\mu$ l mix of MH200G and Dhh1 stock to the final concentration as indicated
ATP titration	13.4 $\mu$ l LSB-100 2.1 $\mu$ l 0.5M KCl (final KCl concentration 100mM) 1 $\mu$ l CKM mix 1.5 $\mu$ l mix of ATP/MgCl <sub>2</sub> (250mM stock) and H <sub>2</sub> O to the final concentration as indicated 1 $\mu$ l 10 mg/ml BSA 2 $\mu$ l 1M Hepes of the respective pH 1.25 $\mu$ l 1mg/ml polyU (final concentration 0.05 mg/ml) 3 $\mu$ l mix of MH200G and Dhh1 stock to the final concentration as indicated
salt titration	12.5 $\mu$ l LSB-100 3 $\mu$ l mix of 0.5M KCl / water to the final KCl concentration as indicated 1 $\mu$ l CKM mix 1.25 $\mu$ l 100mM ATP/MgCl <sub>2</sub> (final concentration 5 mM) 1 $\mu$ l 10 mg/ml BSA, 2 $\mu$ l 1M Hepes of the respective pH 1.25 $\mu$ l 1mg/ml polyU (final concentration 0.05 mg/ml) 3 $\mu$ l mix of MH200G and Dhh1 stock to the final concentration as indicated
pU titration	13.4 $\mu$ l LSB-100 2.1 $\mu$ l 0.5M KCl (final KCl concentration 100 mM) 1 $\mu$ l CKM mix 1.25 $\mu$ l 100mM ATP/MgCl <sub>2</sub> (final concentration 5 mM) 1 $\mu$ l 10 mg/ml BSA, 2 $\mu$ l 1M Hepes of the respective pH 1.25 $\mu$ l mix of water / 10mg/ml polyU to the final concentration as indicated 3 $\mu$ l mix of MH200G and Dhh1 stock to the final concentration as indicated

# Supplementary Figure 1

