

## **Supplemental Information**

### **Condensation of Ded1p Promotes a Translational Switch from Housekeeping to Stress Protein Production**

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**Table S1: Overview of 5'UTR characteristics of differentially translated genes. Related to Figures 1 and 6 and Figures S1 and S6.** Median and means of PARS scores, calculated MFEs and length of all known yeast 5'UTRs in comparison to the PARS scores, calculated MFEs and length of 5'UTRs of differentially translated genes between wildtype Ded1p and Ded1-IDR<sub>m</sub> at 30°C, 40°C and 42°C. Significance of differences between the conditions were determined using a two-sided Wilcoxon test.

comparison	PARS adj p value	PARS mean	PARS median	gene count*	MFE adj p value	MFE mean	MFE median	length adj p value	length mean	length median	gene count**
All genes		7.78	3.77	2674		-11.41	-5.00		81	53	4358
WT_40_<_WT_42	0.868	6.56	6.84	99	3.93E-06	-6.32	-1.61	8.46E-07	53	34	118
WT_40_>_WT_42	3.68E-04	20.84	10.00	177	8.19E-04	-17.11	-7.10	2.29E-10	123	78	240
WT_40_<_IDRm_40	0.934	13.89	3.01	71	0.659	-12.31	-5.50	0.059	100	64	93
WT_40_>_IDRm_40	0.064	14.14	8.44	96	0.002	-17.30	-8.40	2.67E-04	110	72	110
WT_42_<_IDRm_42	0.209	11.45	6.93	138	0.091	-10.88	-3.79	0.482	89	55	235
WT_42_>_IDRm_42	0.009	15.15	6.34	190	3.59E-06	-14.84	-9.30	4.01E-05	96	74	249

\* the gene count corresponds to the number of genes differentially expressed for which the PARS score was experimentally determined.

\*\* the gene count corresponds to the number of genes differentially expressed for which the 5'UTR sequence is known.

**Table S2: Overview of *in vitro* translation assay reporter transcripts. Related to Figure 6.** The likelihood of an mRNA to fold was measured with the program RNAfold from the ViennaRNA Package (Lorenz et al., 2011) by calculating the minimum free energy and the partition function. The more negative the free energy for a given RNA, the more likely it is to be folded. The *GIS2* 5'UTR was used as the unstructured baseline control for the *in vitro* translation assays. The “high” Ded1p induction is defined as a greater than 10-fold increase in the translation of the respective mRNA upon addition of 0.8  $\mu$ M Ded1p in the *in vitro* translation reaction after 90 minutes. A “low” induction is defined as a less than 10-fold increase in the *in vitro* translation of the respective mRNA.

Gene	Description	Minimum free energy (kcal/mol)	Ensemble free energy (kcal/mol)	Centroid free energy (kcal/mol)	5'UTR length (nt)	Ded1p induction
<b><i>GIS2</i></b>	IRES activator	0	0	0	14	Low
<b><i>HSP12</i></b>	Heat induced small Hsp	0	-0.01	0	39	Low
<b><i>HSP104</i></b>	Heat induced disaggregase	0	-0.4	0	40	Low
<b><i>SSA4</i></b>	Heat induced Hsp70	0	-0.38	0	55	Low
<b><i>DED1</i></b>	RNA helicase	-0.8	-1.13	-0.8	21	Low
<b><i>SSA1</i></b>	Housekeeping Hsp70	-2.7	-3.95	-0.3	64	High
<b><i>SBE22</i></b>	Involved in bud growth	-12.84	-13.72	-12.84	81	High
<b><i>PAB1</i></b>	Poly(A)-binding protein	-12.5	-15.46	-9.3	133	High
<b><i>SSK2</i></b>	MAPKKK of HOG1 signaling pathway.	-16.83	-19.55	-4.18	98	High

**Table S3: Overview of the maximum growth temperature of fungi species and  $T_{\text{onset}}$  for Ded1p phase separation. Related for Figure 7. The  $T_{\text{onset}}$  of purified Ded1p homologues was assessed using DLS.**

<b>Species</b>	<b>Max. growth temperature (°C)</b>	<b><math>T_{\text{onset}}</math> (°C)</b>
<i>S. kudriavzevii</i>	36 (Salvadó et al., 2011)	34
<i>S. cerevisiae</i> (W303)	42 (Observation, growth on plates)	40
<i>T. terrestris</i>	50 (Samson et al., 1977)	46