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Supplemental Information

Global Analysis of Eukaryotic mRNA Degradation Reveals Xrn1-Dependent Buffering of Transcript Levels

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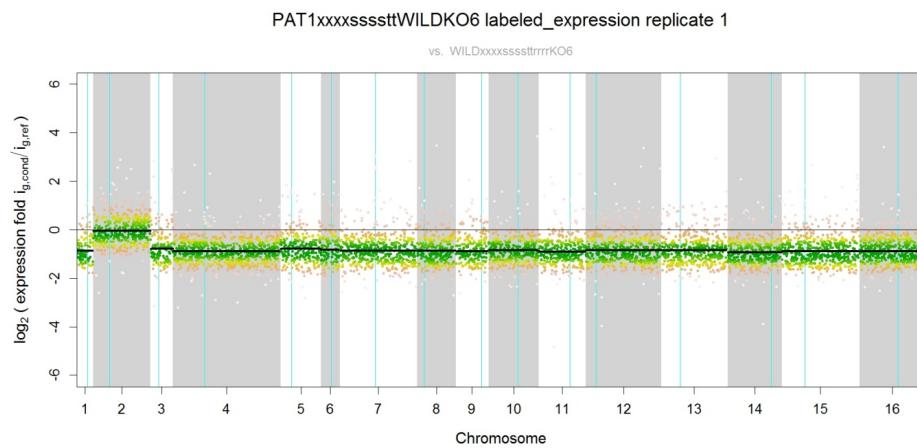
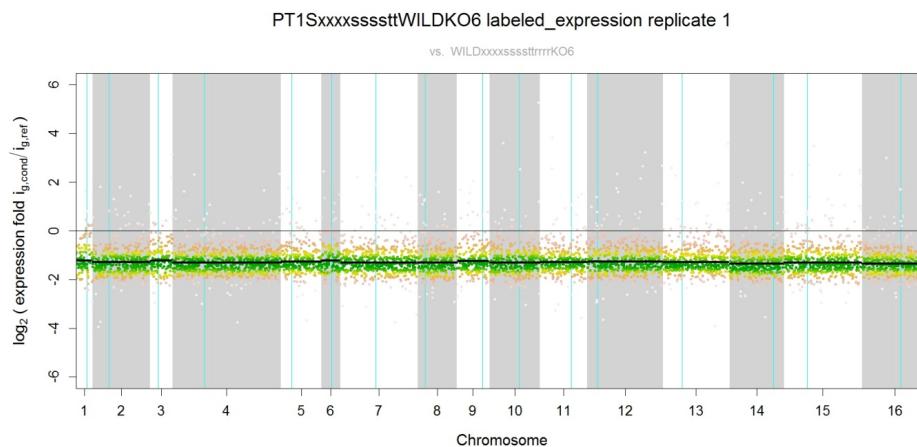
A**B**

Figure S1 Expression level of genes in *pat1Δ* plotted against their position in chromosomes. Related to **Figure 1**

A. An aneuploid strain variant shows two-fold overexpression of genes on Chr II compared to the median level of all other genes.

B. The strain used in this study with normal ploidy. Such ploidy analysis was carried out for all strains used in this study. The analysis revealed that the published strain *pop2Δ* (Sun, M., et.al. 2012 *Genome Research*) has a chromosome II X aneuploidy. A new knock-out strain was therefore generated by homologous recombination and measured by cDTA. The new data however does not change any of the conclusions in our previous work.

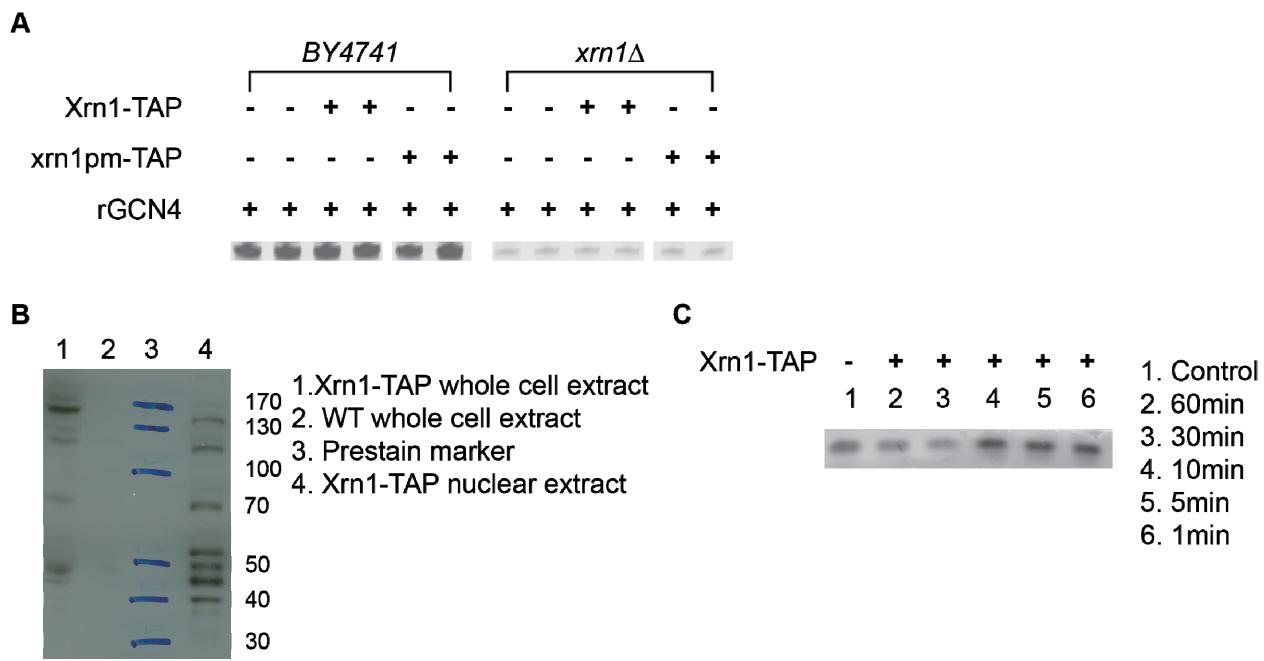


Figure S2 *In vitro* transcription assay. Related to **Figure 2**

- A.** The assay was carried out using nuclear extract from BY4741 (left) and *xrn1Δ* (right). TAP-purified Xrn1 and Xrn1pm has been added to the assay separately. RNase inhibitor Ribolock (Fermentas) was added to inhibit non-specific RNA degradation by enzymes present in the extract. The inhibitor is necessary to observe RNA synthesis and according to the manufacturer does not inhibit Xrn1. No difference was observed in the GCN4 activator dependent transcription activity *in vitro* from HIS4 promoter.
- B.** To check whether a nuclear extract prepared according to our protocol contains Xrn1 protein, we repeated extract preparation from a yeast strain carrying a TAP tag on Xrn1 that enabled protein monitoring in the absence of an antibody against yeast Xrn1. Western blotting revealed a specific protein degradation pattern that arises from the proteolytically sensitive Xrn1 protein (lane 4). A very similar pattern is observed in whole cell extracts (lane 1) but not in a control extract preparation from a strain that lacks the TAP tag (lane 2).
- C.** To test whether the recombinant Xrn1 protein we added to the assay was active as a nuclease, we incubated it with a 30-mer RNA substrate with 5' monophosphate group (sequence: 5'-Phos/CGGC GGUCUUAUGUAACGGC GGUGAAAUCUU-3') and observed partial degradation of the RNA by non-specific staining after 30-60 min of incubation (lanes 2, 3).

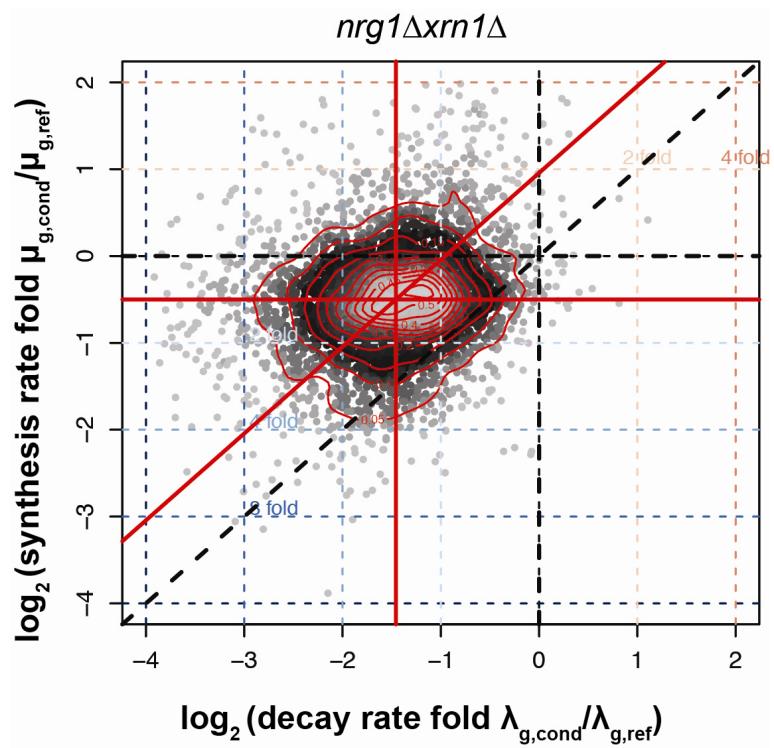


Figure S3 Scatter plot of cDTA analysis on *nrg1Δ* and *xrn1Δ* double knock out strain

Scatter plot show the SR and DR changes in *nrg1Δxrn1Δ* double mutant (Related to **Figure 3**)

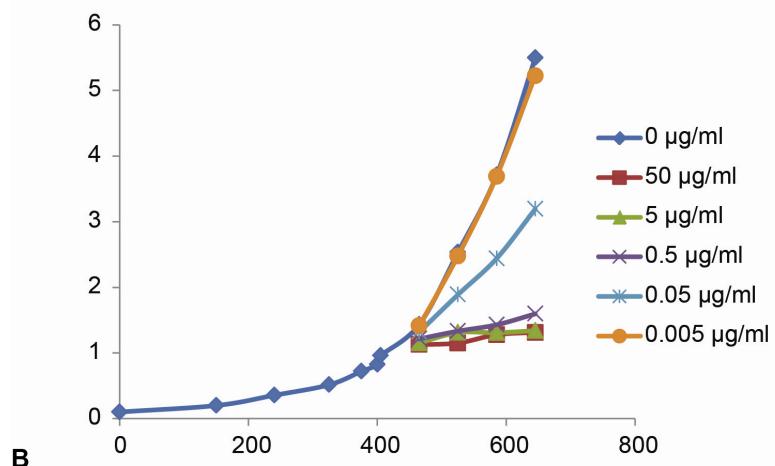
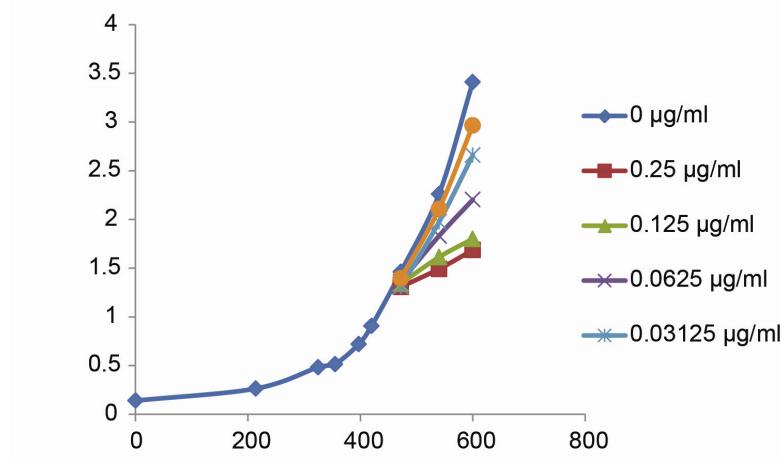
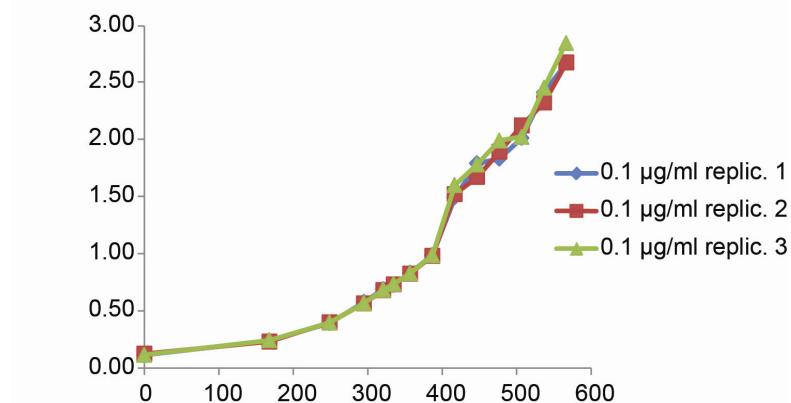
A**B****C**

Figure S4 Growth analysis during cycloheximide perturbation. Related to **Figure 4**

(A, B) Growth effect of cycloheximide on wild-type BY4741 cells. Cycloheximide was added at OD₆₀₀=0.8, and a concentration of 0-50 µg/ml was tested.

(C) Replicates experiments with the concentration used in this study.

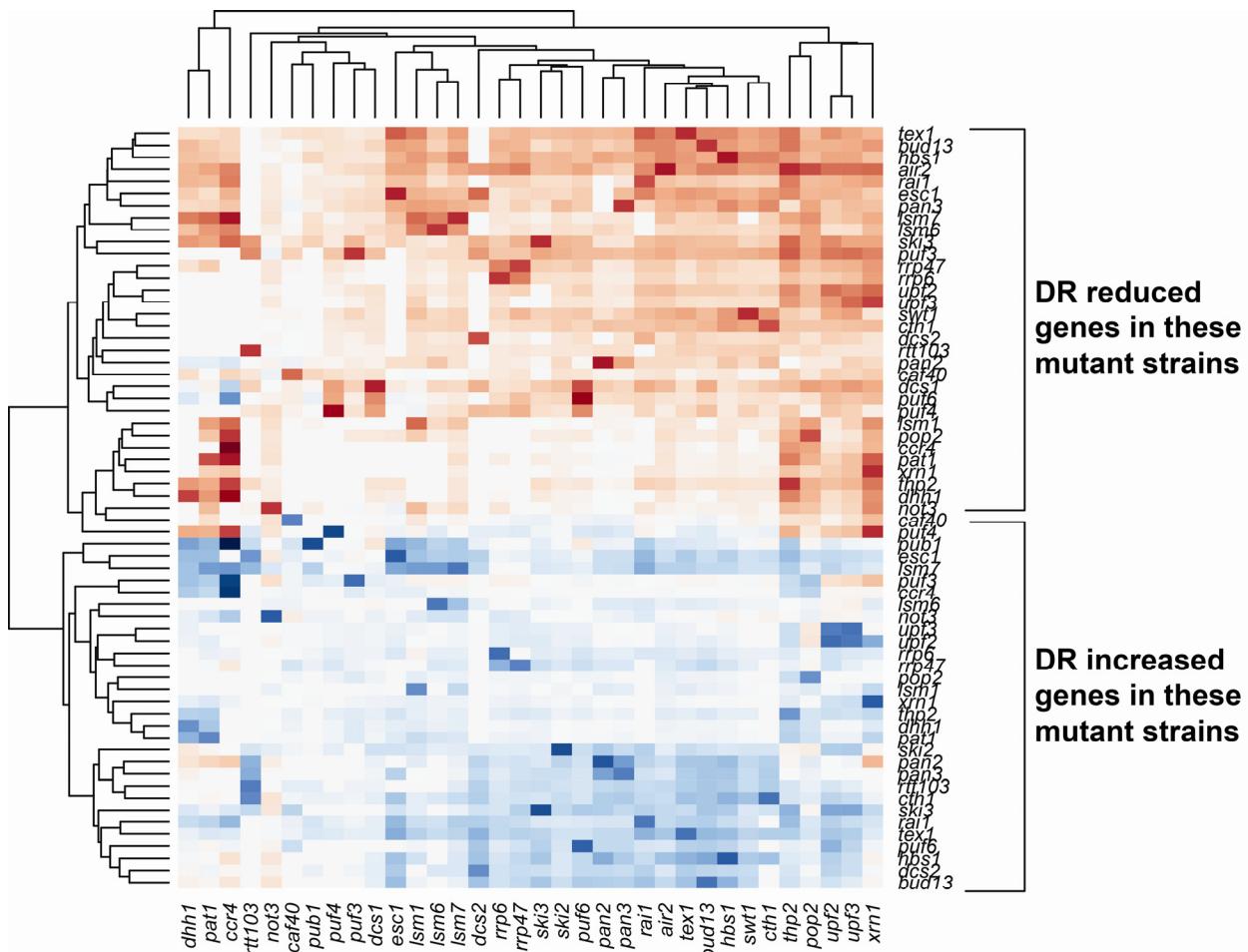


Figure S5. Cluster analysis provides evidence for a general mRNA degradation machinery.

The plot shows the color-coded median t-statistics of DRs of mRNAs that show significantly decreased or increased DRs in the gene deletion strains shown on the x-axis. The t-statistics gives the ratio of the difference in mean DR and its standard errors as a measure for differential stabilization (decreased/increased DR). Destabilization (increased DR) is shown in red, stabilization (decreased DR) in blue. The overall directionality of perturbed degradation indicates a general mRNA degradation machinery. (Related to **Figure 6**)

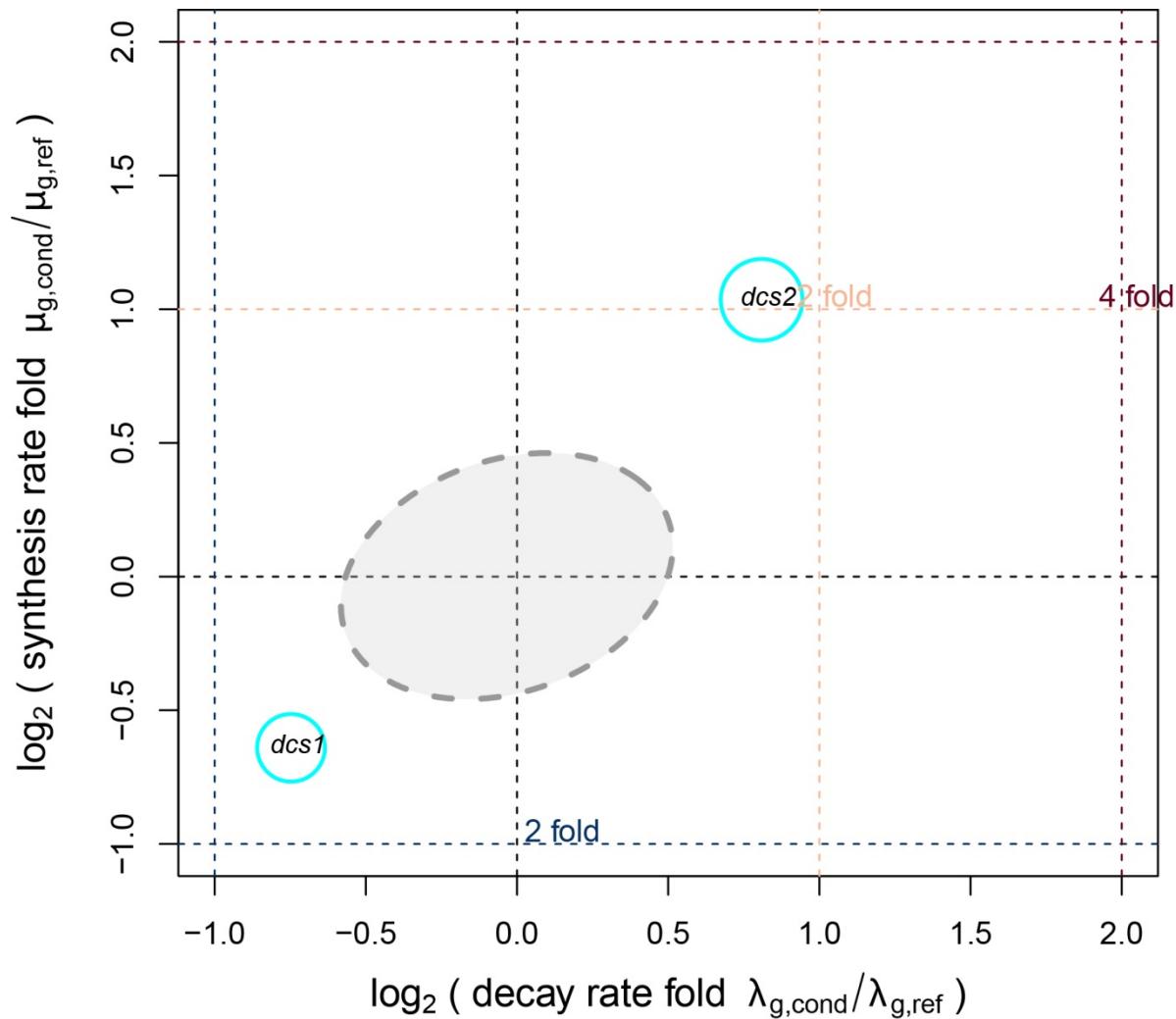


Figure S6 Antagonistic effect in *dcs1* and *dcs2*. Related to **Figure 6**

Scatter plots show global changes in mRNA DRs (log fold, x-axis) vs. the global changes in SRs (log fold, y-axis). The global changes of *dcs1* and *dcs2* are plotted. The coordinate of the center of each circle is determined by the median DR and SR of the mutant. The diameters of the circles represent the relative comparison of the fold of RNA amount over wild-type level.

Table S1 Strains used in this study

1	BY4743	MAT a/a ; <i>his3Δ1/his3Δ1; leu2Δ0/leu2Δ0;</i> <i>lys2Δ0/LYS2; MET15/met15Δ0;</i> <i>ura3Δ0/ura3Δ0</i>	OpenBiosystems
2	BY4741	MAT a <i>his3Δ1; leu2Δ0; met15Δ0;</i> <i>ura3Δ0</i>	OpenBiosystems
3	air1	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; yil079c::kanMX4</i>	OpenBiosystems
4	air2	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ydl175c::kanMX4</i>	OpenBiosystems
5	bud13	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ygl174w::kanMX4</i>	OpenBiosystems
6	caf40	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ynl288w::kanMX4</i>	OpenBiosystems
7	ccr4	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; yal021c::kanMX4</i>	Generated in the lab
8	cth1	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ydr151c::kanMX4</i>	OpenBiosystems
9	dcs1	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ylr270w::kanMX4</i>	OpenBiosystems
10	dcs2	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; yor173w::kanMX4</i>	OpenBiosystems
11	dhh1	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ydl160c::kanMX4</i>	Generated in the lab
12	dom34	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ynl001w::kanMX4</i>	OpenBiosystems
13	edc1	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ygl222c::kanMX4</i>	OpenBiosystems
14	edc2	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; yer035w::kanMX4</i>	Generated in the lab
15	edc3	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; yel015w::kanMX4</i>	OpenBiosystems
16	esc1	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ymr219w::kanMX4</i>	OpenBiosystems
17	hbs1	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ykr084c::kanMX4</i>	OpenBiosystems
18	lsm1	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; yjl124c::kanMX4</i>	OpenBiosystems
19	lsm6	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ydr378c::kanMX4</i>	OpenBiosystems
20	lsm7	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ynl147w::kanMX4</i>	OpenBiosystems
21	not3	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; yil038c::kanMX4</i>	Generated in the lab
22	pan2	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ygl094c::kanMX4</i>	OpenBiosystems
23	pan3	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ykl025c::kanMX4</i>	OpenBiosystems

24	pat1	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ycr077c::kanMX4</i>	Generated in the lab
25	pop2	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ynr052c::kanMX4</i>	Generated in the lab
26	pub1	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ynl016w::kanMX4</i>	OpenBiosystems
27	puf1	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yjr091c::kanMX4</i>	OpenBiosystems
28	puf2	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ypr042c::kanMX4</i>	OpenBiosystems
29	puf3	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yll013c::kanMX4</i>	OpenBiosystems
30	puf4	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ygl014w::kanMX4</i>	OpenBiosystems
31	puf5	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ygl178w::kanMX4</i>	OpenBiosystems
32	puf6	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ydr496c::kanMX4</i>	OpenBiosystems
33	rai1	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ygl246c::kanMX4</i>	OpenBiosystems
34	rrp47	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yhr081w::kanMX4</i>	Generated in the lab
35	rrp6	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yor001w::kanMX4</i>	Generated in the lab
36	rtt103	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ydr289c::kanMX4</i>	OpenBiosystems
37	scd6	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ypr129w::kanMX4</i>	OpenBiosystems
38	ski2	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ylr398c::kanMX4</i>	Generated in the lab
39	ski3	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ypr189w::kanMX4</i>	OpenBiosystems
40	ski7	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yor076c::kanMX4</i>	OpenBiosystems
41	ski8	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ygl213c::kanMX4</i>	OpenBiosystems
42	swt1	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yor166c::kanMX4</i>	OpenBiosystems
43	tex1	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ynl253w::kanMX4</i>	OpenBiosystems
44	thp2	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yhr167w::kanMX4</i>	OpenBiosystems
45	tpa1	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yer049w::kanMX4</i>	OpenBiosystems
46	upf2	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yhr077c::kanMX4</i>	OpenBiosystems
47	upf3	BY4741 MATa <i>his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; ygr072w::kanMX4</i>	OpenBiosystems
48	xrn1	BY4741 MATa <i>his3Δ1; leu2Δ0;</i>	Generated in the lab

		<i>met15Δ0; ura3Δ0; ygl173c::kanMX4</i>	
49	Y40343	W303 MAT alpha <i>tor1-1 fpr1::NAT</i> RPL13A-2×FKBP12::TRP1	Euroscarf
50	XRN1AA	W303 MAT alpha <i>tor1-1 fpr1::NAT</i> RPL13A-2×FKBP12::TRP1 <i>YGL173C::YGL173C-FRB-GFP-KanMX4</i>	Generated in the lab
51	xrn1pm	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ygl173c:: pFA6a-</i> <i>ygl173c-D206A,D208A-3HA-His3MX</i>	Generated in the lab
52	XRN1-WT	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; YGL173C:: pFA6a-</i> <i>YGL173C-3HA-His3MX</i>	Generated in the lab
53	nrg1	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ydr043c::kanMX4</i>	Generated in the lab
54	nrg1xrn1	BY4741 MAT a <i>his3Δ1; leu2Δ0;</i> <i>met15Δ0; ura3Δ0; ydr043c::kanMX4;</i> <i>ygl173c::natMX</i>	Generated in the lab