

### **Description of Supplementary Files**

File Name: Supplementary Information

Description: Supplementary Figures, Supplementary Tables and Supplementary References

File Name: Supplementary Data 1

Description: RNAseq data related to Figure 1

File Name: Supplementary Data 2

Description: GO term enrichment data related to Figure 1

File Name: Supplementary Data 3

Description: HU sensitivity suppressor screen data related to Figure 2

File Name: Supplementary Data 4

Description: RNAseq data related to Figure 6

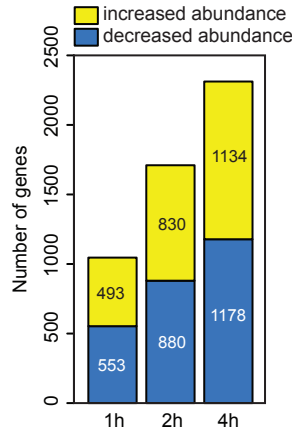
File Name: Supplementary Data 5

Description: Fitness data related to Figure 6.

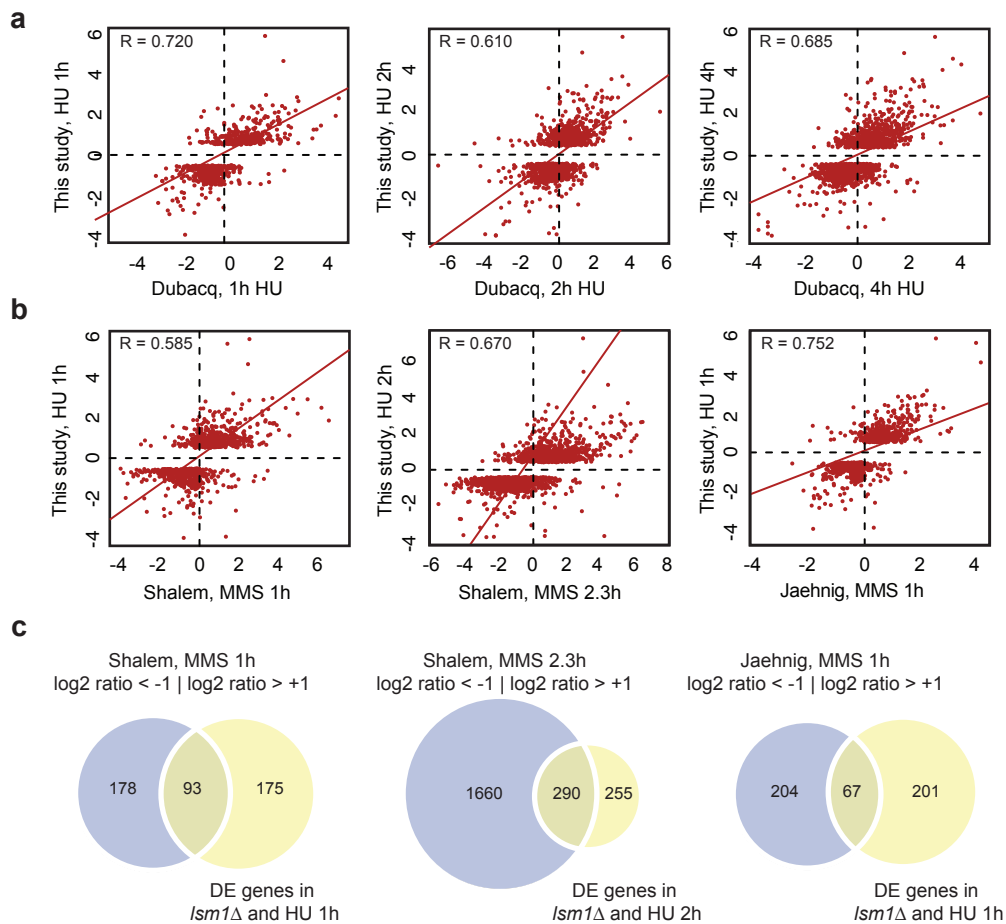
File Name: Supplementary Software 1

Description: R scripts and Cellprofiler pipelines used for the analysis of the data generated in this manuscript

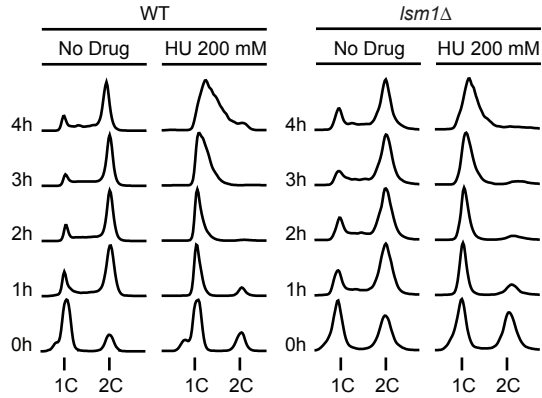
File Name: Peer Review File



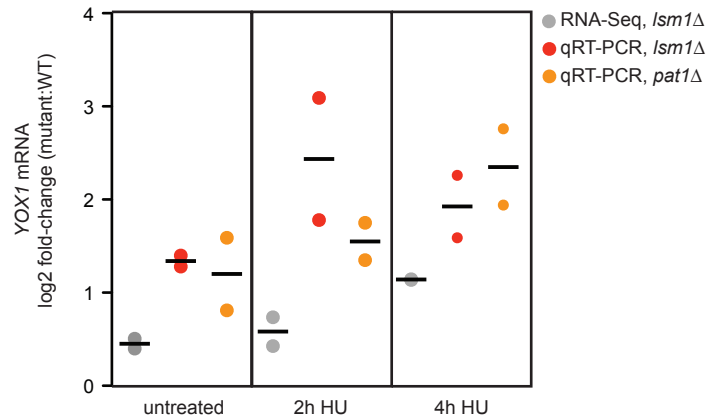
**Supplementary Fig. 1. The number of genes induced or repressed upon HU exposure in WT cells.** The number of differentially expressed genes was determined using the Tuxedo protocol.



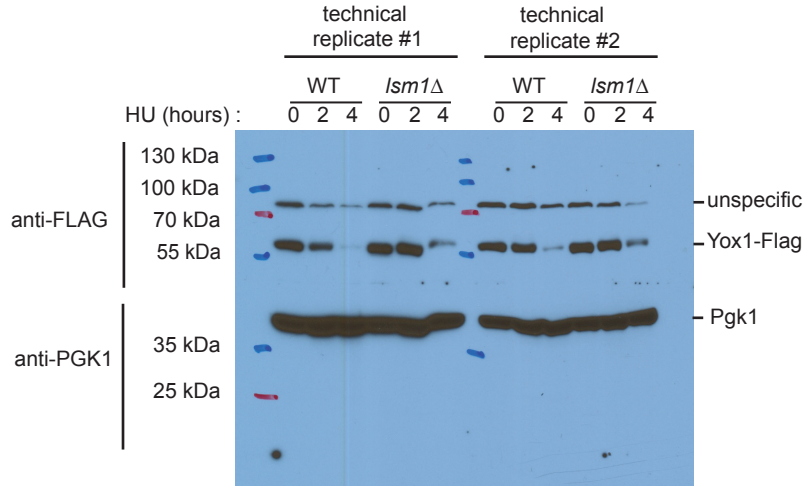
**Supplementary Fig. 2. Lsm1 regulates RNA abundance of HU-responsive genes as well as more general DNA replication stress genes.** (a) Correlation between the expression ratios for the differentially expressed genes identified in WT cells in this study upon HU exposure and the study of Dubacq et al. (2006) at 1, 2 and 4 hours in HU. (b) Correlation between the expression ratios for the differentially expressed genes identified in WT cells in this study upon HU exposure and WT cells in MMS in the study of Shalem et al. (2008) or Jaehnig et al. (2013). (c) Venn Diagram representing the overlap between the genes showing  $\log_2$  expression ratios inferior to -1 or superior to +1 in the study of Shalem et al. (2008) or Jaehnig et al. (2013) and the genes showing differential expression upon HU exposure and in *lsm1Δ* cells in this study. DE: Differentially Expressed



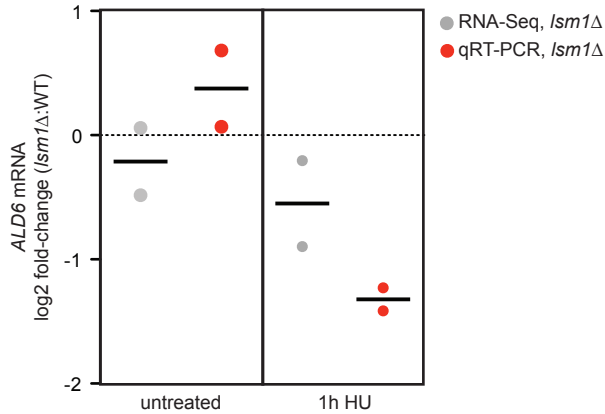
**Supplementary Fig. 3. Cell-cycle progression of the WT and *Ism1* $\Delta$  strain under normal growth or in presence of HU.** Flow cytometry histograms of DNA contents in wild type and *Ism1* $\Delta$  cells. Cells were arrested in G1 and then released into S phase in the absence or presence of HU. The positions of cells with 1C and 2C DNA contents are indicated.



**Supplementary Fig. 4. *YOX1* RNA abundance increases in *Ism1* $\Delta$  and *pat1* $\Delta$  cells.** log<sub>2</sub> expression ratios comparing *YOX1* mRNA levels in WT and *Ism1* $\Delta$  cells obtained by RNA-Seq (grey) or qRT-PCR (red) or log<sub>2</sub> expression ratios comparing *YOX1* mRNA levels in WT and *pat1* $\Delta$  cells obtained by qRT-PCR (orange). The means and two biological replicates are shown. Each qRT-PCR biological replicate included at least 2 technical replicates.



**Supplementary Fig. 5. Full immuno-blot image related to Fig. 5b.** Yox1-FLAG protein abundance was probed using anti-FLAG antibody. Pgk1 was used as loading control.



**Supplementary Fig. 6. ALD6 mRNA abundance decreases in *Ism1Δ* cells.** log<sub>2</sub> expression ratios comparing *ALD6* mRNA levels in WT and *Ism1Δ* cells obtained by RNA-Seq (grey) or qRT-PCR (red). The means and two biological replicates are shown. Each qRT-PCR biological replicate included at least 2 technical replicates.

**Supplementary Table 1. Comparison of RNA-seq results obtained with Cuffdiff, EBSeq or edgeR**

method	condition	down-regulated genes		up-regulated genes	
		number of DE genes identified	overlap with cuffdiff (%)	number of DE genes identified	overlap with cuffdiff (%)
cuffdiff	untreated	258	NA	333	NA
EBSeq		564	198 (35%)	521	208 (40%)
edgeR		703	236 (34%)	800	305 (38%)
cuffdiff	1h HU	332	NA	499	NA
EBSeq		682	265 (39%)	867	382 (44%)
edgeR		762	319 (42%)	911	455 (50%)
cuffdiff	2h HU	686	NA	911	NA
EBSeq		817	514 (63%)	959	697 (73%)
edgeR		1509	666 (44%)	1578	888 (57%)
cuffdiff	4h HU	1051	NA	1203	NA
EBSeq		1026	793 (77%)	1085	860 (79%)
edgeR		1753	1024 (58%)	1887	1176 (62%)

DE : Differentially Expressed

**Supplementary Table 2. Expression data from cuffdiff, EBSeq and edgeR for the genes identified in Fig. 2**

condition	gene	cuffdiff			EBSeq		edgeR		
		log2 FC	p-value	FDR corrected p-value	log2 FC	posterior probability <sup>1</sup>	log2 FC	p-value	FDR corrected p-value
untreated	<i>ACF4</i>	0,185	3,70E-01	6,76E-01	0,186	1,18E-12	0,190	2,09E-01	3,99E-01
	<i>ARL3</i>	0,497	1,62E-02	1,12E-01	0,497	1,38E-08	0,499	8,44E-04	6,61E-03
	<i>HHT1</i>	1,010	5,00E-05	1,63E-03	1,011	1,00E+00	1,014	2,30E-14	3,00E-12
	<i>RRS1</i>	0,682	1,50E-04	3,99E-03	0,683	1,00E+00	0,686	1,26E-08	5,08E-07
	<i>TMA16</i>	0,941	5,00E-05	1,63E-03	0,942	1,00E+00	0,946	1,65E-14	2,19E-12
	<i>YOX1</i>	0,454	1,88E-02	1,22E-01	0,454	1,21E-07	0,458	9,92E-04	7,47E-03
1h HU	<i>ACF4</i>	0,336	1,33E-01	3,57E-01	0,336	2,68E-12	0,325	4,93E-02	1,39E-01
	<i>ARL3</i>	0,437	4,29E-02	1,76E-01	0,437	1,05E-09	0,426	1,04E-02	4,34E-02
	<i>HHT1</i>	1,801	5,00E-05	1,11E-03	1,802	1,00E+00	1,789	5,49E-23	1,01E-20
	<i>RRS1</i>	0,386	7,17E-02	2,46E-01	0,387	1,78E-01	0,377	9,04E-02	2,17E-01
	<i>TMA16</i>	1,079	5,00E-05	1,11E-03	1,079	1,00E+00	1,068	1,86E-09	7,75E-08
	<i>YOX1</i>	0,958	5,00E-05	1,11E-03	0,956	1,00E+00	0,947	7,49E-07	1,53E-05
2h HU	<i>ACF4</i>	0,510	5,60E-03	2,74E-02	0,500	7,25E-07	0,500	2,94E-04	1,06E-03
	<i>ARL3</i>	0,645	4,50E-04	3,60E-03	0,635	3,07E-01	0,631	1,37E-08	1,12E-07
	<i>HHT1</i>	1,321	5,00E-05	5,58E-04	1,313	1,00E+00	1,309	7,11E-40	1,24E-37
	<i>RRS1</i>	0,647	1,50E-04	1,47E-03	0,638	1,00E+00	0,634	7,96E-12	1,07E-10
	<i>TMA16</i>	1,034	5,00E-05	5,58E-04	1,024	1,00E+00	1,021	5,04E-25	3,22E-23
	<i>YOX1</i>	0,572	1,25E-03	8,27E-03	0,563	4,38E-04	0,560	1,26E-05	6,01E-05
4h HU	<i>ACF4</i>	0,630	1,45E-03	6,30E-03	0,623	1,44E-04	0,631	2,10E-06	8,16E-06
	<i>ARL3</i>	0,425	1,25E-02	3,77E-02	0,420	3,81E-07	0,427	2,66E-04	7,54E-04
	<i>HHT1</i>	1,356	5,00E-05	3,47E-04	1,351	1,00E+00	1,355	3,19E-55	4,82E-53
	<i>RRS1</i>	0,746	5,00E-05	3,47E-04	0,741	1,00E+00	0,745	9,25E-17	1,19E-15
	<i>TMA16</i>	1,233	5,00E-05	3,47E-04	1,226	1,00E+00	1,231	4,60E-32	1,89E-30
	<i>YOX1</i>	1,141	5,00E-05	3,47E-04	1,134	1,00E+00	1,139	1,64E-23	4,00E-22

FC : Fold-Change; Red shading indicates statistically supported differential expression

<sup>1</sup> Values superior or equal to 0.95 are considered significant for posterior probability in EBSeq

Supplementary Table 3. YETFASCO scanning results for *ALD6* and *ICS2* promoter regions

Gene promoter scanned	Transcription factor	Coordinates (relative to START codon)		Strand	% of Max score	Motif	YETFASCO motif ID
<i>ALD6</i>	Yox1	-987	-978	-	76,8	GTTGATTGGG	#2196
		-855	-846	-	75,4	GTTAATGGTG	#2196
		-660	-651	+	77	CTAAATGACT	#2196
		-298	-289	-	81,6	AGTAATTGGC	#2196
		-296	-289	+	100	CAATTACT	#453
<i>ICS2</i>	Yox1	-162	-155	-	97,3	TAATTACA	#453
		-545	-536	-	76,7	GTTAATGACT	#2196
		-303	-296	-	76,3	TAATTAGG	#453
		-302	-295	-	80,6	ATAATTAG	#498
		-268	-259	-	78,3	GTTTATTACG	#2196
<i>ALD6</i>	Mcm1	-823	-816	-	84,2	TTCGTCAA	#1369
		-384	-375	+	80,2	CCCCAAACGG	#2139
		-383	-374	+	93,8	CCCCAAACGGG	#2139
		-344	-329	-	79,9	CTTCCCAAAGAGGAAA	#1015
		-344	-329	-	78,2	CTTCCCAAAGAGGAAA	#1485
		-344	-330	-	86	TTCCCAAAGAGGAAA	#1298
		-344	-329	-	78,7	CTTCCCAAAGAGGAAA	#1544
		-341	-330	-	86,2	TTCCCAAAGAGG	#1543
		-341	-332	-	85,1	CCCAAAGAGG	#2139
-337	-330	-	78,6	TTCCCAA	#1369		
<i>ICS2</i>	Mcm1	-993	-986	+	80,5	TCCGCAAT	#1369
		-924	-917	-	91,1	TTCGCAAT	#1369
		-580	-573	+	82,9	AAAATAGG	#1368
		-304	-297	-	82,9	AATTAGGT	#1367
		-303	-296	-	85,4	TAATTAGG	#1368
		-40	-33	+	79,6	TTCGTTAT	#1369
		-303	-296	-	85,4	TAATTAGG	#1368
		-40	-33	+	79,6	TTCGTTAT	#1369

**Supplementary Table 4. Strains used in this study.**

Lab ID	Strain name	Genotype	Source
BY4741	WT	<i>MATa leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	1
RLKY6	<i>lsm1Δ</i>	<i>MATα lsm1Δ::kanMX can1Δ::STE2pr-his5 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0</i>	C. Boone
JTY16	<i>lsm1Δ</i>	<i>MATa leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	2
RLKY15	<i>pat1Δ</i>	<i>MATα pat1Δ::natMX can1Δ::STE2pr-his5 lyp1Δ::STE3pr-LEU2</i>	C. Boone
RLKY24	WT + pBY011	<i>MATa pBY011[GAL1-10pr-] leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	this study
RLKY27	WT + pBY011-YOX1	<i>MATa pBY011[GAL1-10pr-YOX1] leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	this study
RLKY53	<i>lsm1Δ</i> + pBY011	<i>MATa lsm1Δ::kanMX pBY011[GAL1-10p-] leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	this study
RLKY54	<i>lsm1Δ</i> + pBY011-YOX1	<i>MATa lsm1Δ::kanMX pBY011[GAL1-10pr-YOX1] leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	this study
RLKY55	<i>pat1Δ</i> + pBY011	<i>MATa pat1Δ::kanMX pBY011[GAL1-10pr-] leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	this study
RLKY56	<i>pat1Δ</i> + pBY011-YOX1	<i>MATa pat1Δ::kanMX pBY011[GAL1-10p-YOX1] leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	this study
RLKY57	<i>yox1Δ</i>	<i>MATa yox1Δ::kanMX leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	3
RLKY58	<i>yhp1Δ</i>	<i>MATa yhp1Δ::kanMX leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	3
RLKY60	<i>lsm1Δ yox1Δ</i>	<i>MATa lsm1Δ::natMX yox1Δ::kanMX can1Δ::STE2pr-his5 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0 lys2Δ0</i>	this study
RLKY61	<i>lsm1Δ yhp1Δ</i>	<i>MATa lsm1Δ::natMX yhp1Δ::kanMX can1Δ::STE2pr-his5 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0 lys2Δ0</i>	this study
RLKY63	<i>pat1Δ yox1Δ</i>	<i>MATa pat1Δ::natMX yox1Δ::kanMX can1Δ::STE2pr-his5 lyp1Δ::STE3pr-LEU2 leu2Δ0 his3Δ1 ura3Δ0 lys2Δ0</i>	this study
RLKY64	<i>pat1Δ yhp1Δ</i>	<i>MATa pat1Δ::natMX yhp1Δ::kanMX can1Δ::STE2pr-his5 lyp1Δ::STE3pr-LEU2 leu2Δ0 his3Δ1 ura3Δ0 lys2Δ0</i>	this study
RLKY66	<i>xrn1Δ</i>	<i>MATα xrn1Δ::natMX can1Δ::STE2pr-his5 lyp1Δ::STE3pr-LEU2 leu2Δ0 his3Δ1 ura3Δ0</i>	C. Boone
RLKY81	<i>xrn1Δ yox1Δ</i>	<i>MATα xrn1Δ::natMX yox1Δ::kanMX can1Δ::STE2pr-his5 lyp1Δ::STE3pr-LEU2 leu2Δ0 his3Δ1 ura3Δ0 lys2Δ0</i>	this study
RLKY82	<i>xrn1Δ yhp1Δ</i>	<i>MATα xrn1Δ::natMX yhp1Δ::kanMX can1Δ::STE2pr-his5 lyp1Δ::STE3pr-LEU2 leu2Δ0 his3Δ1 ura3Δ0 lys2Δ0</i>	this study
RLK43	YOX1-MS2 <i>Lsm1-mCherry</i>	<i>MATa /MATα YOX1-MS2/YOX1 LSM1/LSM1-mCherry-natMX pMS2-CP-GFP(x3) leu2Δ0/leu2Δ0 his3Δ1/his3Δ1 ura3Δ0/ura3Δ0 lys2Δ0/LYS2 met15Δ0/MET15</i>	this study
RLK45	YOX1-MS2 <i>Pat1-mCherry</i>	<i>MATa /MATα YOX1-MS2/YOX1 PAT1/PAT1-mCherry-natMX pMS2-CP-GFP(x3) leu2Δ0/leu2Δ0 his3Δ1/his3Δ1 ura3Δ0/ura3Δ0 lys2Δ0/LYS2 met15Δ0/MET15</i>	this study
RLKY72	<i>xrn1Δ</i> YOX1-MS2 <i>Lsm1-mCherry</i>	<i>MATa /MATα xrn1Δ::kanMX/xrn1Δ::kanMX YOX1-MS2/YOX1 LSM1/LSM1-mCherry-natMX pMS2-CP-GFP(x3) leu2Δ0/leu2Δ0 his3Δ1/his3Δ1 ura3Δ0/ura3Δ0 lys2Δ0/LYS2 met15Δ0/MET15</i>	this study
RLKY71	<i>xrn1Δ</i> YOX1-MS2 <i>Pat1-mCherry</i>	<i>MATa /MATα xrn1Δ::kanMX/xrn1Δ::kanMX YOX1-MS2/YOX1 PAT1/PAT1-mCherry-natMX pMS2-CP-GFP(x3) leu2Δ0/leu2Δ0 his3Δ1/his3Δ1 ura3Δ0/ura3Δ0 lys2Δ0/LYS2 met15Δ0/MET15</i>	this study
RLKY74	YOX1 <i>Lsm1-mCherry</i>	<i>MATa /MATα YOX1/YOX1 LSM1/LSM1-mCherry-natMX pMS2-CP-GFP(x3) leu2Δ0/leu2Δ0 his3Δ1/his3Δ1 ura3Δ0/ura3Δ0 lys2Δ0/LYS2 met15Δ0/MET15</i>	this study



RLKY75	YOX1 Pat1-mCherry	<i>MATa</i> / <i>MATα</i> <i>YOX1/YOX1 PAT1/PAT1-mCherry-natMX pMS2-CP-GFP(x3)</i> <i>leu2Δ0/leu2Δ0 his3Δ1/his3Δ1 ura3Δ0/ura3Δ0 lys2Δ0/LYS2 met15Δ0/MET15</i>	this study
RLKY76	<i>xrn1Δ</i> YOX1 Lsm1-mCherry	<i>MATa</i> / <i>MATα</i> <i>xrn1Δ::kanMX/xrn1Δ::kanMX YOX1/YOX1 LSM1/LSM1-mCherry-natMX pMS2-CP-GFP(x3)</i> <i>leu2Δ0/leu2Δ0 his3Δ1/his3Δ1 ura3Δ0/ura3Δ0 lys2Δ0/LYS2 met15Δ0/MET15</i>	this study
RLKY77	<i>xrn1Δ</i> YOX1 Pat1-mCherry	<i>MATa</i> / <i>MATα</i> <i>xrn1Δ::kanMX/xrn1Δ::kanMX YOX1/YOX1 PAT1/PAT1-mCherry-natMX pMS2-CP-GFP(x3)</i> <i>leu2Δ0/leu2Δ0 his3Δ1/his3Δ1 ura3Δ0/ura3Δ0 lys2Δ0/LYS2 met15Δ0/MET15</i>	this study
RLKY80	HTA2-mCherry	<i>MATα</i> HTA2-mCherry-hphMX <i>can1Δ::STE2pr-LEU2 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0</i>	this study
RLKY86	YOX1-GFP HTA2-mCherry	<i>MATa</i> HTA2-mCherry-hphMX YOX1-GFP- GFP-HIS3MX <i>can1Δ::STE2pr-LEU2 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0</i>	this study
RLKY87	<i>lsm1Δ</i> YOX1-GFP HTA2-mCherry	<i>MATa</i> <i>lsm1Δ::kanMX</i> HTA2-mCherry-hphMX YOX1-GFP- GFP-HIS3MX <i>can1Δ::STE2pr-LEU2 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0</i>	this study
NTY13	RPL39pr-tdTomato	<i>MATα</i> <i>can1Δ::RPL39pr-tdTomato-CaURA3::STE2pr-LEU2 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0</i>	4
RLKY83	YOX1-GFP RPL39pr-tdTomato	<i>MATa</i> YOX1-GFP-HIS3MX <i>can1Δ::RPL39pr-tdTomato-CaURA3::STE2pr-LEU2 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0</i>	this study
RLKY84	<i>lsm1Δ</i> YOX1-GFP RPL39pr-tdTomato	<i>MATa</i> <i>lsm1Δ::kanMX</i> YOX1-GFP-HIS3MX <i>can1Δ::RPL39pr-tdTomato-CaURA3::STE2pr-LEU2 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0</i>	this study
RLKY104	<i>pry3Δ</i> (query)	<i>MATα</i> <i>pry3Δ::natMX can1Δ::STE2pr-his5 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	C. Boone
RLKY105	<i>ald6Δ</i> (query)	<i>MATα</i> <i>ald6Δ::natMX can1Δ::STE2pr-his5 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	C. Boone
RLKY106	<i>rad54Δ</i> (query)	<i>MATα</i> <i>rad54Δ::natMX can1Δ::STE2pr-his5 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	C. Boone
RLKY107	<i>yml131WΔ</i> (query)	<i>MATα</i> <i>yml131wΔ::natMX can1Δ::STE2pr-his5 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	C. Boone
RLKY108	<i>mnr1Δ</i> (query)	<i>MATα</i> <i>mnr1Δ::natMX can1Δ::STE2pr-his5 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	C. Boone
RLK109	<i>ura3Δ</i> (query)	<i>MATα</i> <i>ura3Δ::natMX can1Δ::STE2pr-his5 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	C. Boone
RLKY110	<i>ald6Δ</i>	<i>MATa</i> <i>ald6Δ::kanMX leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	3
RLKY111	YOX1-FLAG	<i>MATa</i> YOX1-FLAG-KanMX <i>leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	this study
RLKY112	<i>lsm1Δ</i> YOX1-FLAG	<i>MATa</i> <i>lsm1Δ::URA3MX</i> YOX1-FLAG-KanMX <i>leu2Δ0 his3Δ1 ura3Δ0 met15Δ0</i>	this study
	<b>All strains in Figure 2 have the following genotypes:</b> <i>MATa</i> <i>lsm1Δ::natMX xxxΔ::kanMX can1Δ::STE2pr-his5 lyp1Δ leu2Δ0 his3Δ1 ura3Δ0 lys2Δ0</i> <i>MATa</i> <i>pat1Δ::natMX xxxΔ::kanMX can1Δ::STE2pr-his5 lyp1Δ::STE3pr-LEU2 leu2Δ0 his3Δ1 ura3Δ0 lys2Δ0</i>		this study

	<p><b>All strains in Figure 6 have the following genotypes:</b>  <i>MATa xxxΔ::kanMX yyyΔ::natMX leu2Δ0 his3Δ1 ura3Δ0 lyp1Δ0 can1Δ0::STE2pr-SpHIS5</i></p>	this study
	<p><b>All strains in Figure 7a-b have the following genotypes:</b>  <i>MATa lsm1Δ::kanMX leu2Δ0 his3Δ1 ura3Δ0 met15Δ0 pBY011[GAL1-10pr-]</i>  <i>MATa lsm1Δ::kanMX leu2Δ0 his3Δ1 ura3Δ0 met15Δ0 pBY011[GAL1-10pr-XXX]</i></p>	this study
	<p><b>All strains in Figure 7f-g have the following genotypes:</b>  <i>MATa xxxΔ::NatMX RNR3-GFP-HIS3MX can1pr::RPL39pr-tdTomato-CaURA3</i>  <i>can1Δ::STE2pr-LEU2 leu2Δ0 his3Δ1 ura3Δ0 met15Δ0 lyp1Δ0</i></p>	this study

## References

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2. Tkach, J. M. *et al.* Dissecting DNA damage response pathways by analysing protein localization and abundance changes during DNA replication stress. *Nat. Cell Biol.* **14**, 966–76 (2012).
3. Giaever, G. *et al.* Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* **418**, 387–391 (2002).
4. Hendry, J. A., Tan, G., Ou, J., Boone, C. & Brown, G. W. Leveraging DNA damage response signaling to identify yeast genes controlling genome stability. *G3 (Bethesda)*. **5**, 997–1006 (2015).