## SUPPLEMENTARY FIGURES



**Supplementary Figure 1. HU sensitivity assays in double deletion** *sus1<sup>Δ</sup>* **strains with TREX-2 mutants.** The indicated mutants were spotted onto YPD plates with or without 150 mM HU, as described in Figure 1E.



Α

В



WT sus1∆ sgf11∆  $ubp8\Delta$ sgf73∆ sir2∆ 12 % marker loss 10 8 6 4 2 SOLITA 0 Ubp8A SUSTA 59173A sir2A NY NY

**Supplementary Figure 2.** *SUS1* deletion does not affect rDNA silencing and recombination. (A) Schematic diagram (top) showing an rDNA unit embedded within a tandem array on chromosome XII with the position of *mURA3* reporters inserted into NTS1 or NTS2. The 35S pre-rRNA encoding the 18S, 5.8S, and 25S rRNAs is separated by NTS1 and NTS2. The locations of RFB (double triangle), ARS replication origin (oval), 5S rRNA gene (triangle), and 35S transcription start site (bent arrow) are represented. *URA3*-based rDNA silencing assays (bottom) were carried out in DMY2798 (*leu2::mURA3*), DMY2804 (*RDN1-NTS1::mURA3*), or DMY2800 (*RDN1-NTS2::mURA3*) strains with the indicated deletions. (B) The frequency of unequal rDNA crossovers was monitored by loss of the *ADE2* gene located within the rDNA array for the WT (W303R) strain and the indicated deletion strains. Pictures of plates are shown in the top panels. The percentage of *ADE2* gene loss (% marker loss) was calculated as the ratio of red-sectored colonies to the total number of colonies and is shown in the bottom panel. Completely red colonies were excluded. Error bars indicate the SD from two repetitions, and asterisks indicate statistically significant; \**P* < 0.05).

	sus1∆, 30°C	sus1∆, 37°C	Genes in pGP564
PRS316- <i>SUS1</i> pGP564 YGPM25d21 YGPM11d19 YGPM11d24 YGPM3j23 YGPM20a24 YGPM20m13			- YAL004W EFB1 SNR18 VPS8 TFC3 <b>NUP60</b> <b>NUP170</b> ATG8 YBL077W ILS1 UGA4 CWC2 NHP2 <b>GLE1</b> YDL206W HEM3 YDL121C YFH1 YDL119C YDL118W CYK3 <b>NUP84</b> IWR1 YDL114W-A YDL114W <b>ASM4</b> LUC7 YDL086W YDL086C-A YDL085C-A NDE2 SUB2 RPS16B SLY1 RVB1 HST4 <b>NUP42</b> YDR193W
pRS316- <i>SUS1</i> pGP564 YGPM8e02 YGPM17c06 YGPM11n21 YGPM33c11 YGPM17e06 YGPM28l21	<ul> <li>●●●●●</li> <li>●●●●●</li> <li>●●●●</li> <li>●●●●</li> <li>●●●</li> <li>●●</li> <li>●●<td></td><td>- ARO80 SIP1 CAD1 <b>DYN2</b> RTT105 <b>NUP157</b> MAM1 GLE2 YER107W-A SPB4 DEG1 LOC1 <b>NIC96</b> YPI1 RPN11 KEM1 <b>NUP49</b> ROK1 SP074 tK(CUU)G2 MLC1 ARC1 VPS73 RPL28 YGL102C YGL101W <b>SEH1</b> SPC105 <b>NUP145</b> NBP35 LIF1</td></li></ul>		- ARO80 SIP1 CAD1 <b>DYN2</b> RTT105 <b>NUP157</b> MAM1 GLE2 YER107W-A SPB4 DEG1 LOC1 <b>NIC96</b> YPI1 RPN11 KEM1 <b>NUP49</b> ROK1 SP074 tK(CUU)G2 MLC1 ARC1 VPS73 RPL28 YGL102C YGL101W <b>SEH1</b> SPC105 <b>NUP145</b> NBP35 LIF1
pRS316- <i>SUS1</i> pGP564 YGPM11j07 YGPM2k17 YGPM13j07 YGPM13p14 YGPM29i02 YGPM18m04			- YGR117C <i>RPS23A</i> <b>NUP57</b> COG2 IN(GUU)G MEP1 <b>MLP2</b> RPL40A SLN1 ECM37 HIS5 YIL115W-A <b>NUP159</b> POR2 SDP1 MPM1 DL51 YJL064W MRPL8 YJL062W-A LAS21 <b>NUP82</b> YJL043W MHP1 <b>NSP1</b> <b>NUP192</b> ID(GUC)J3 IR(UCU)J2 YJL038C YJL037W IV(AAC)J
pRS316- <i>SUS1</i> pGP564 YGPM29m05 YGPM15p13 YGPM29n02 YGPM19j13 YGPM31a08 YGPM28j24		<ul> <li>●●●</li> <li>●●●</li> <li>●●●</li> <li>●●</li> <li>●</li> <li>●<td>- GEF1 URB2 <b>NUP85</b> YKL070W YKL069W <i>IV(AAC)K1</i> YKL068W-A <b>NUP100</b> <i>IH(GUG)K</i> YNK1 YKL066W YET1 MTD1 RPF2 <b>NUP133</b> DAD2 HBS1 MRPL20 RPL40B <b>MLP1</b> PCC1 <b>POM33</b> HIF1 SPA2 YLL020C GAT3 PPR1 BRE2 YLR016C MEU1 <b>POM34</b></td></li></ul>	- GEF1 URB2 <b>NUP85</b> YKL070W YKL069W <i>IV(AAC)K1</i> YKL068W-A <b>NUP100</b> <i>IH(GUG)K</i> YNK1 YKL066W YET1 MTD1 RPF2 <b>NUP133</b> DAD2 HBS1 MRPL20 RPL40B <b>MLP1</b> PCC1 <b>POM33</b> HIF1 SPA2 YLL020C GAT3 PPR1 BRE2 YLR016C MEU1 <b>POM34</b>

	sus1∆, 30°C	sus1∆, 37°C	Genes in pGP564
pRS316-SUS1 pGP564 YGPM16d05 YGPM2m11 YGPM3j10 YGPM21b06 YGPM25c21 YGPM5f02	0087 0087 0087 0087 0087 0087 0087 0087		- SEC13 PNP1 CLB4 YLR211C REC102 CHS5 JIP3 MID2 ID(GUC)L2 SNR61 SNR55 SNR57 RPS25B YLR334C IE(UUC)L <b>NUP2</b> NUP18B CAC2 YML101C-A CUE4 YML100W-A AMD1 YML034C-A SRC1 RAD52 YML031C-A <b>NDC1</b> YML030W USA1 POM152 YMR130W RR81 YMR2 YMR130W RR81 YMR2 YMR1 NUP53 YMR153C-A RIM13 YMR155W TPP1 FMP39 MRPS8 IM(CAU)M YMR158C-A YMR158W-B ATG16
pRS316-SUS1 pGP564 YGPM10e17 YGPM27n09 YGPM17n15 YGPM8d07 YGPM14m17 YGPM14h07			RPS7A YOR097C NUP1 KTR1 CRC1 RAS1 YOR102W OST2 IT(AGU)B YBR014C MNN2 YBR016W KAP104 GAL7 GAL10 RMD1 NTH1 YRB1 RCR2 YDR003W-A RAD57 MAF1 SXM1 YDR396W NCB2 UTP5 HPT1 YER107W-A FLOB KAP123 GUS1 RTF1 TAD1 YGL242C KAP114 DOC1 YGL239C
pRS316-SUS1 pGP564 YGPM11b07 YGPM32o15 YGPM9h05 YGPM26i03 YGPM31j13 YGPM6e10			YILLOBCC YILLOBGW-A RNR3 FIST YILLOB4W YRB2 ARC15 YKL206C LOST EAP1 YLR346C KAP95 YLR347W-A DICT YLR349W ORM2 NIT3 REX2 FRST RPL228 BUD28 YLR0B3W PER33 YLR0B5C SPC3 YMR253C YMR254C GFD1 COX7 PET111 YMR258C SNR62 WHI2 IRC23 TOM6 DBP5 STD1
pRS316- <i>SUS1</i> pGP564 YGPM2p09 YGPM20m03 YGPM5d22 YGPM2h11			- OSW1 YOR256C <b>CDC31</b> HNT3 RPT4 GCD1 RPN8 YOR262W YOR263C DSE3 YOR111W <b>CEX1</b> AZF1 YOR114W TRS33 <b>MEX67</b> MRX4 REV3 ATG29 SET6 ALG1 YSA1 <b>SUS1</b> CYC8 YBR113W RAD16

**Supplementary Figure 3. Screening of NPC-related genes for the suppression of growth defects in** *sus1* $\Delta$  cells. Growth analysis of *sus1* $\Delta$  strains, including the indicated plasmids, as described in Figure 1E. Genes on the plasmids (pGP564) are listed on the right of each panel.





**Supplementary Figure 4. The mRNA export defect induced by** *sus1* $\Delta$  was observed at both 30°C and 37°C. (A) Poly(A)<sup>+</sup> RNA FISH analysis of the WT, *sus1* $\Delta$ , and *sus1* $\Delta$  containing pRS316-SUS1 strains, as described in Figure 5A. (B) Violin plot of poly(A)<sup>+</sup> RNA FISH results for the strains used in Figure 5A at both 30°C and 37°C. The medians and quartiles are marked as thick and dotted lines, respectively. \*\*\*\*P < 0.0001; \*\*P < 0.01; \*P < 0.05 (Student's *t*-test between the indicated pairs of values).



В

Experiments		Cell numbers	Mean (Nuclear/cytoplasmic poly(A)+ intensity at 37°C)
	WT	103	1.176154
	sus1∆	112	1.376510
1	<i>sus1∆</i> pSUS1	100	1.167284
	sus1∆pMEX67	104	1.294548
	sus1∆pDBP5	103	1.304344
	WT	102	1.182704
	sus1∆	105	1.337783
2	sus1∆pSUS1	105	1.188931
	sus1∆pMEX67	103	1.218946
	sus1∆pDBP5	104	1.309805
	WT	103	1.107127
	sus1∆	102	1.369916
3	sus1∆pSUS1	102	1.192707
	sus1∆ pMEX67	110	1.231505
	sus1∆pDBP5	104	1.229178

**Supplementary Figure 5. Additional copies of** *MEX67* or *DBP5* rescued the mRNA export defect in sus1 $\Delta$  cells. (A) Violin plot of poly(A)<sup>+</sup> RNA FISH results presented in Figure 5A, as described in Supplementary Figure 4B. The nuclear/cytoplasmic poly(A)<sup>+</sup> intensity of each replicate is plotted. (B) The cell numbers and mean nuclear/cytoplasmic poly(A)<sup>+</sup> intensity ratio in (A) are shown.