

Supplemental Materials

Genome-wide screen uncovers novel pathways for tRNA processing and nuclear-cytoplasmic dynamics

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Oligonucleotides

Probe 1 (JW0048, tRNA^{Ile}_{UAU} 5' exon and intron; employed for Northern analyses):
5'- GGCACAGAAACTCGGAAACCGAATGTTGCTATAAGCACGAAGCTCTAACCC
ACTGAGCTACACGAGC -3'

Probe KC031 (tRNA^{Tyr} complementary to 5' and 3' exons and intron; employed for Northern analyses):

5'-CCCGATCTCAAGATTCTGTAGTGATAAATTACAGTCTTGCCTAAACC-3'

Probe SRIM15 (tRNA^{Tyr} mature sequence; employed for FISH):

5'- GCGAGTCGAACGCCGATCTCAAGATTACAGTCTTGCCTAAACCAAC
TTGGCTACC-3'

Probe SRIM04 (tRNA^{Ile}_{UAU}; mature sequences employed for FISH):

5'GTGGGGATTGAACCCACGACGGTCGCGTTATAAGCACGAAGCTCTAACCAACTGAGCT
ACA-3'

Probe SRIM03 (tRNA^{Ile}_{UAU}; intron-specific employed for FISH):

5'CGTTGCTTTAAAGGCCTGTTGAAAGGTCTTGGCACAGAAACTCGGAAACCGAAT
GTTGCTAT-3'

Table S1. Mutations of unessential genes affecting tRNA biology in the MAT α deletion collection

Gene Name	Systemic Name	Phenotypes ¹	Northern data location (Figure #, gel#)	Confirmation
AMD1	YML035C	Type 1/2	S1-3, JW 196	MAT α vs. MAT α
AMD2	YDR242W	Type 1/2	S1-3, JW 198	MAT α vs. MAT α
APL5	YPL195W	Type 1/2	S1-2, JW 190	MAT α vs. MAT α
ARA1	YBR149W	Type 1/2	S1-3, JWI 196	MAT α vs. MAT α
ARL1	YBR164C	Type 1/2	S1-3, JW 196	MAT α vs. MAT α
ATG15	YCR068W	Type 1/2	S1-2, JW 194	MAT α vs. MAT α
BDF1	YLR399C	Type 1/2	S1-3, JW 198	MAT α vs. MAT α
BMH1	YER177W	Type 1/2	S1-3, JW 197	MAT α vs. MAT α
BRE1	YDL074C	Type 1/2	S1-3, JW 198	MAT α vs. MAT α
BRE2	YLR015W	Type 1/2	S1, AB 119	MAT α vs. MAT α
BUD26	YDR241W	Type 1/2	S1-2, JW 191	MAT α vs. MAT α
BUD31	YCR063W	Type 4/5/6	S1-3, JW 198	MAT α vs. MAT α
BUL2	YML111W	Type 1/2	S1-3, JW 198	MAT α vs. MAT α
CKA2	YOR061W	Type 1/2	S1-1, AB 117	MAT α vs. MAT α
COA1	YIL157C	Type 1/2	S1-3, JW 195	MAT α vs. MAT α
COX5A	YNL052W	Type 1/2	S1-3, JW 195	MAT α vs. MAT α
CPT1	YNL130C	Type 1/2	S1-3, JW 195	MAT α vs. MAT α
CTP1	YBR291C	Type 1/2	S1-2, JW 193R	MAT α vs. MAT α
DUG2	YBR281C	Type 1/2	S1-2, JW 193R	MAT α vs. MAT α
DUS2	YNR015W	Type 1/2	S1-2, JW 194	MAT α vs. MAT α
DUS3	YLR401C	Type 9	2I; S1, AB 119	MAT α vs. MAT α
EAF7	YNL136W	Type 1/2	S1-3, JW 195	MAT α vs. MAT α
ECM33	YBR078W	Type 1/2	S1-3, JW 199	MAT α vs. MAT α
FMP21	YBR269C	Type 1/2	S1-2, JW 193R	MAT α vs. MAT α
FUN12	YAL035W	Type 1/2	S1, AB 119	MAT α vs. MAT α
GCN5	YGR252W	Type 1/2	2A; S1-3, JW 199	Complementation
GDS1	YOR355W	Type 1/2	S1-1, AB 117	MAT α vs. MAT α
GLN3	YER040W	Type 1/2 and an extra band below I	2B; S1-2, JW 190	Complementation
GLO3	YER122C	Type 1/2	S1-3, JW 199	MAT α vs. MAT α
GPB1	YOR371C	Type 1/2	S1-1, AB 117	MAT α vs. MAT α
HLR1	YDR528W	Type 1/2	S1-3, JW 197	MAT α vs. MAT α
HOS2	YGL194C	Type 1/2	S1-2, JW 192	MAT α vs. MAT α
HXT12	YIL170W	Type 1/2	S1-3, JW 195	MAT α vs. MAT α
ISC10	YER180C	Type 1/2	S1-3, JW 197	MAT α vs. MAT α

<i>ISR1</i>	YPR106W	Type 1/2	S1-2, JW 191	MATa vs. MATa
<i>IST1</i>	YIR005W	Type 1/2	S1-3, JW 195	MATa vs. MATa
<i>LEM3</i>	YNL323W	Type 1/2	S1-1, AB 116	MATa vs. MATa
<i>LOS1</i>	YKL205W	Type 4/5/6	NA	MATa vs. MATa
<i>LSM1</i>	YJL124C	Type 4/5/6	S1-2, JW 193L	MATa vs. MATa
<i>MAL33</i>	YBR297W	Type 1/2	S1-2, JW 193R	MATa vs. MATa
<i>MET10</i>	YFR030W	Type 1/2	S1-3, JW 196	MATa vs. MATa
<i>MET17</i>	YLR303W	Type 1/2	S1-2, JW 192	MATa vs. MATa
<i>MNN2</i>	YBR015C	Type 1/2	S1-3, JW 195	MATa vs. MATa
<i>MRPL35</i>	YDR322W	Type 1/2	S1-2, JW 192	MATa vs. MATa
<i>NCS2</i>	YNL119W	Type 1/2	S1-3, JW 195	MATa vs. MATa
<i>NGG1</i>	YDR176W	Type 1/2	S1-2, JW 192	MATa vs. MATa
<i>NPP1</i>	YCR026C	Type 1/2	S1-2, JW 194	MATa vs. MATa
<i>NUP133</i>	YKR082W	Type 4/5/6	S1-2, JW 192	MATa vs. MATa
<i>NUP170</i>	YBL079W	Type 4/5/6	S1-2, JW 191	MATa vs. MATa
<i>NUP2</i>	YLR335W	Type 1/2	S1-2, JW 191	MATa vs. MATa
<i>OCA1</i>	YNL099C	Type 1/2	S1-3, JW 195	MATa vs. MATa
<i>OCA2</i>	YNL056W	Type 1/2	S1-3, JW 195	MATa vs. MATa
<i>OCA4</i>	YCR095C	Type 1/2	S1-3, JW 196	MATa vs. MATa
<i>OPT3</i>	YGL114W	Type 1/2	S1-3, JW 197	MATa vs. MATa
<i>PCA1</i>	YBR295W	Type 1/2	S1-2, JW 193R	MATa vs. MATa
<i>PEX32</i>	YBR168W	Type 1/2	S1-3, JW 196	MATa vs. MATa
<i>PPM1</i>	YDR435C	Type 1/2	S1-2, JW 190	MATa vs. MATa
<i>PSP1</i>	YDR505C	Type 1/2	S1-3, JW 197	MATa vs. MATa
<i>RBK1</i>	YCR036W	Type 1/2	S1-2, JW 194	MATa vs. MATa
<i>REX1</i>	YGR276C	Type 2*	2C; S1-3, JW 198	MATa vs. MATa
<i>RNP1</i>	YLL046C	Type 1/2	S1, AB 119	MATa vs. MATa
<i>RPB4</i>	YJL140W	Type 4/5/6	S1-3, JW 199	Complementation
<i>RPL34B</i>	YIL052C	Type 4/5/6	S1-2, JW 192	MATa vs. MATa
<i>RPL40A</i>	YIL148W	Type 1/2	S1-3, JW 195	MATa vs. MATa
<i>RPS21B</i>	YJL136C	Type 1/2	S1-3, JW 196	MATa vs. MATa
<i>RPS6A</i>	YPL090C	Type 1/2	S1-2, JW 191	MATa vs. MATa
<i>RPS8A</i>	YBL072C	Type 4/5/6	S1-2, JW 191	MATa vs. MATa
<i>RRT12</i>	YCR045C	Type 1/2	S1-2, JW 194	MATa vs. MATa
<i>SAM37</i>	YMR060C	Type 4/5/6	S1-2, JW 193L	Complementation
<i>SAP155</i>	YFR040W	Type 1/2	S1-2, JW 192	MATa vs. MATa
<i>SAP190</i>	YKR028W	Type 1/2	S1-3, JW 196	MATa vs. MATa
<i>SET3</i>	YKR029C	Type 1/2	S1-3, JW 196	MATa vs. MATa
<i>SHG1</i>	YBR258C	Type 1/2	S1-2, JW 190	MATa vs. MATa
<i>SPT8</i>	YLR055C	Type 1/2	S1, AB 119	MATa vs. MATa
<i>SSA2</i>	YLL024C	Type 1/2	S1, AB 119	MATa vs. MATa

<i>SWI4</i>	YER111C	Type 1/2	S1-3, JW 197	MAT α vs. MAT α
<i>TOM70</i>	YNL121C	Type 4/5/6	2G,H; S1-3, JW 195	Complementation
<i>TRK1</i>	YJL129C	Type 1/2	S1-2, JW 190; S1-3, JW 196	MAT α vs. MAT α
<i>TYR1</i>	YBR166C	Type 1/2	S1-3, JW 196	MAT α vs. MAT α
<i>UBX3</i>	YDL091C	Type 1/2	S1-3, JW 197	MAT α vs. MAT α
<i>UME6</i>	YDR207C	Type 1/2	S1-2, JW 192	MAT α vs. MAT α
<i>URA7</i>	YBL039C	Type 1/2	S1-2, JW 191	MAT α vs. MAT α
<i>VBA1</i>	YMR088C	Type 1/2	S1-2, JW 193L	MAT α vs. MAT α
<i>VPS55</i>	YJR044C	Type 1/2	S1-3, JW 197	MAT α vs. MAT α
<i>XRN1</i>	YGL173C	Type 7	Wu & Hopper, 2014	Complementation
<i>YAF9</i>	YNL107W	Type 1/2	S1-3, JW 195	MAT α vs. MAT α
<i>YAP1</i>	YML007W	Type 1/2	S1-1, AB 116	MAT α vs. MAT α
	YCR025C	Type 1/2	S1-2, JW 194	MAT α vs. MAT α
	YCR050C	Type 1/2	S1-2, JW 194	MAT α vs. MAT α
	YCR061W	Type 1/2	S1-2, JW 194	MAT α vs. MAT α
	YCR087C-A	Type 1/2	S1-2, JW 194	MAT α vs. MAT α
	YCR087W	Type 1/2	S1-2, JW 194	MAT α vs. MAT α
	YDR417C	Type 4/5/6	S1-3, JW 199	MAT α vs. MAT α
	YDR444W	Type 1/2	S1-3, JW 198	MAT α vs. MAT α
	YER152C	Type 1/2	S1-3, JW 197	MAT α vs. MAT α
	YER181C	Type 1/2	S1-3, JW 197	MAT α vs. MAT α
	YIR043C	Type 1/2	S1-3, JW 196	MAT α vs. MAT α
	YLR012C	Type 1/2	S1, AB 119	MAT α vs. MAT α
	YLR111W	Type 1/2	S1, AB 119	MAT α vs. MAT α
	YML002W	Type 1/2	S1-1, AB 116	MAT α vs. MAT α
	YML083C	Type 1/2	S1, AB 119	MAT α vs. MAT α
	YMR254C	Type 1/2	S1-1, AB 117	MAT α vs. MAT α
	YNR014W	Type 1/2	S1-1, AB 116	MAT α vs. MAT α
	YOR072W	Type 1/2	S1-1, AB 117	MAT α vs. MAT α
	YOR186W	Type 1/2	S1-2, JW 190	MAT α vs. MAT α

¹. See Fig. 1 for an explanation of the various types of mutants; NA, not applicable as *los1Δ* is a control for most gels.

Table S2. ts Mutations from Boone collection affecting tRNA biology

Gene/allele	Systemic name	Phenotypes ¹	Northern data location (Figure #, gel#)	Confirmation
<i>abf1-102</i>	YKL112W	Type 1/2	S2, AB 74	Boone vs. Hieter
<i>abf1-103</i>	YKL112W	Type 1/2	S2-1, AB 83	Boone vs. Hieter
<i>act1-101</i>	YFL039C	Type 4/5/6	2F; S2, AB 73; S2-1, AB 92	Complementation
<i>arp4-G161D</i>	YJL081C	Type 1/2	S2-1, AB 118	Boone vs. Hieter
<i>bos1-1</i>	YLR078C	Type 4/5/6	S2, AB 73	Boone vs. Hieter
<i>brl1-2221</i>	YHR036W	Type 4/5/6	S2-1, AB 118	Boone vs. Hieter; Multiple alleles
<i>brl1-3231</i>	YHR036W	Type 4/5/6	S2-1, AB 118	Boone vs. Hieter; Multiple alleles
<i>brl1-C371R</i>	YHR036W	Type 4/5/6	S2-1, AB 118	Boone vs. Hieter; Multiple alleles
<i>brl1-C371S</i>	YHR036W	Type 4/5/6	S2-1, AB 118	Boone vs. Hieter; Multiple alleles
<i>cca1-1</i>	YER168C	Type 3	2D; S3	Boone vs. Hieter
<i>cdc28-4</i>	YBR160W	Type 1/2	S2-1, AB 92	Complementation
<i>cdc34-1</i>	YDR054C	Type 4/5/6	S2, AB 72	Boone vs. Hieter
<i>cdc60-ts</i>	YPL160W	Type 4/5/6	S2, AB 81	Boone vs. Hieter
<i>cks1-38</i>	YBR135W	Type 1/2	S2-1, AB 83	Repetition
<i>crm1-1</i>	YGR218W	Type 4/5/6	S2-1, AB 83	Boone vs. Hieter
<i>dbf4-ts</i>	YDR052C	Type 4/5/6	S2, AB 73	Boone vs. Hieter
<i>dcp2-7Δ</i>	YNL118C	Type 4/5/6	S2, AB 80; S2-1, AB 118; S2-1, AB 97	Complementation
<i>dna2-2</i>	YHR164C	Type 1/2	S2, AB 74; S2-1, AB 97	Complementation
<i>hrp1-4</i>	YOL123W	Type 1/2	S2, AB 81; S2-1, AB 118; S2-1, AB 92	Complementation
<i>hyp2-1</i>	YEL034W	Type 4/5/6	S2, AB 80	Boone vs. Hieter; Multiple alleles
<i>hyp2-ts</i>	YEL034W	Type 4/5/6	S2, AB 80	Boone vs. Hieter; Multiple alleles
<i>lcb2-19</i>	YDR062W	Type 4/5/6	S2, AB 79	Boone vs. Hieter
<i>ils1-1</i>	YBL076C	Type 4/5/6	S2-1, KC	Repetition
<i>mex67-ts5</i>	YPL169C	Type 4/5/6	2E; S2, AB 81	Boone vs. Hieter; Complementation
<i>myo2-14</i>	YOR326W	Type 4/5/6	S2, AB 73; S2-1, AB 92	Complementation

<i>ndc1-4</i>	YML031W	Type 4/5/6	S2, AB 73; S2-1, AB 92	Complementation
<i>nog2-1</i>	YNR053C	Type 4/5/6	S2-1, AB 83; S2-1, AB 97	Complementation
<i>nop4-3</i>	YPL043W	Type 4/5/6	S2, AB 80	Boone vs. Hieter
<i>nup159-1</i>	YIL115C	Type 4/5/6	S2, AB 79; S2-1, AB 92	Complementation
<i>nup192-15</i>	YJL039C	Type 4/5/6	S2, AB 74	Boone vs. Hieter
<i>pfy1-13</i>	YOR122C	Type 4/5/6	S2, AB 73	Boone vs. Hieter; Multiple alleles
<i>pfy1-4</i>	YOR122C	Type 4/5/6	S2, AB 73	Boone vs. Hieter; Multiple alleles
<i>prp43-ts2</i>	YGL120C	Type 1/2	S2, AB 79	Boone vs. Hieter
<i>prt1-1</i>	YOR361C	Type 4/5/6	S2, AB 72	Boone vs. Hieter
<i>rap1-1</i>	YNL216W	Type 4/5/6	S2, AB 79	Boone vs. Hieter
<i>rap1-2</i>	YNL216W	Type 4/5/6	S2, AB 79	Boone vs. Hieter
<i>rmp1-ts</i>	YLR145W	Type 4/5/6	S2, AB 81	Boone vs. Hieter
<i>rna1-1</i>	YMR235C	Type 4/5/6	S2, AB 73	Boone vs. Hieter
<i>rpc34-1</i>	YNR003C	Type 1/2	S2-1, AB 118; S2-1, AB 97	Complementation
<i>rpg1-1</i>	YBR079C	Type 4/5/6	S2-1, AB 120	Boone vs. Hieter
<i>rpn1-821</i>	YHR027C	Type 4/5/6	S2, AB 73	Repetition
<i>rpo31-698</i>	YOR116C	Type 1/2	S2, AB 81; S2-1, AB 92	Complementation
<i>rsp5-1</i>	YER125W	Type 4/5/6	S2, AB 73	Boone vs. Hieter
<i>sec13-1</i>	YLR208W	Type 4/5/6	S2, AB 72	Boone vs. Hieter
<i>sec18-1</i>	YBR080C	Type 4/5/6	S2, AB 72	Boone vs. Hieter
<i>sec20-1</i>	YDR498C	Type 4/5/6	S2, AB 72	Boone vs. Hieter
<i>sec22-1</i>	YLR268W	Type 4/5/6	S2, AB 74	Complementation
<i>sec5-24</i>	YDR166C	Type 4/5/6	S2-1, AB 92	Complementation
<i>sen2-1</i>	YLR105C	Type 4/5/6	S2, AB 80	Boone vs. Hieter
<i>snu114-40</i>	YKL173W	Type 1/2	S2, AB 80	Boone vs. Hieter; Multiple alleles
<i>snu114-60</i>	YKL173W	Type 1/2	S2, AB 80	Boone vs. Hieter; Multiple alleles
<i>srm1-ts</i>	YGL097W	Type 4/5/6	S2, AB 81	Boone vs. Hieter
<i>sth1-2</i>	YIL126W	Type 1/2	S2, AB 74	Complementation
<i>sup35-td</i>	YDR172W	Type 4/5/6	S2, AB 72; S2-1, AB 92	Complementation
<i>sup45-ts</i>	YBR143C	Type 4/5/6	S2-1, AB 120	Boone vs. Hieter
<i>ura6-4</i>	YKL024C	Type 1/2	S2-1, AB 118	Boone vs. Hieter; Multiple alleles
<i>ura6-5</i>	YKL024C	Type 1/2	data not shown	Boone vs. Hieter; Multiple alleles

<i>ura6-6</i>	YKL024C	Type 1/2	data not shown	Boone vs. Hieter; Multiple alleles
<i>yef3-F650S</i>	YLR249W	Type 4/5/6	S2, AB 79; S2-1, AB 118; S2-1, AB 92	Complementation
<i>yrb1-51</i>	YDR002W	Type 4/5/6	S2, AB 79	Boone vs. Hieter

¹. See Fig. 1 for an explanation of the various types of mutants

Table S3. ts Mutations from Hieter collection affecting tRNA biology

Gene	Systemic name	Phenotypes ¹	Northern data location (Figure #, gel#)	Confirmation
<i>ALG14</i>	YBR070C	Type 1/2	S3	Complementation
<i>CCA1</i>	YER168C	Type 3	2D; S3	Complementation
<i>CSE1</i>	YGL238W	Type 1/2	S3	Complementation
<i>ERG27</i>	YLR100W	Type 4/5/6	S3	Complementation
<i>FRS2</i>	YFL022C	Type 4/5/6	S3	Complementation
<i>GLN4</i>	YOR168W	Type 4/5/6	S3	Complementation
<i>GPN2</i>	YOR262W	Type 1/2	S3	Complementation
<i>GUK1</i>	YDR454C	Type 1/2	S3	Complementation
<i>MTR2</i>	YKL186C	Type 4/5/6	S3	Complementation
<i>NUP57</i>	YGR119C	Type 4/5/6	S3	Complementation
<i>POP3</i>	YNL282W	Type 1/2	S3	Repetition
<i>POP7</i>	YBR167C	Type 1/2	S3	Repetition
<i>PRP46</i>	YPL151C	Type 1/2	S3	Complementation
<i>RPC11</i>	YDR045C	Type 1/2	S3	Complementation
<i>RPC25</i>	YKL144C	Type 1/2	S3	Complementation
<i>RRN5</i>	YLR141W	Type 1/2	S3	Complementation
<i>RSC6</i>	YCR052W	Type 1/2	S3	Complementation
<i>SNP1</i>	YIL061C	Type 1/2	S3	Complementation
<i>TFC8</i>	YPL007C	Type 1/2	S3	Complementation
<i>THS1</i>	YIL078W	Type 4/5/6	S3	Complementation

¹. See Fig. 1 for an explanation of the various types of mutants

Supplementary figures

Fig. S1. Verification of the mutants from *MAT α* deletion collection by Northern analysis utilizing probe 1. The *MAT α* candidates (a) were compared with the strains containing a deletion of the same gene in the independently constituted *MAT α* deletion collection (α). The verified mutants are highlighted in red. Different exposure times of each gel are shown. In S1, the top band represents the primary tRNA transcript, second band the end-matured intron-containing tRNA and the third band the mature tRNA. In S1-1, S1-2 and S1-3, the top band represents the primary tRNA transcript, and the bottom the end-matured intron-containing tRNA.

Fig. S2. Verification of the mutants from C. Boone ts collection by Northern analysis utilizing probe 1. RNAs were isolated from candidate mutants shifted to at 37°C for 2 hr. The C. Boone ts candidates (B.) were compared with the strains containing the same allele in the P. Hieter ts collection (H.). The remaining mutants which do not have either the same allele in different genetic backgrounds or multiple alleles were assessed by complementation assays utilizing the vectors from the yeast genomic tiling collection (T) or the MoBY-ORF collection (M) and empty vector (EV). The verified mutants are highlighted in red. The top band represents the primary tRNA transcript and the bottom the end-matured intron-containing tRNA. Different exposure times of each gel are shown.

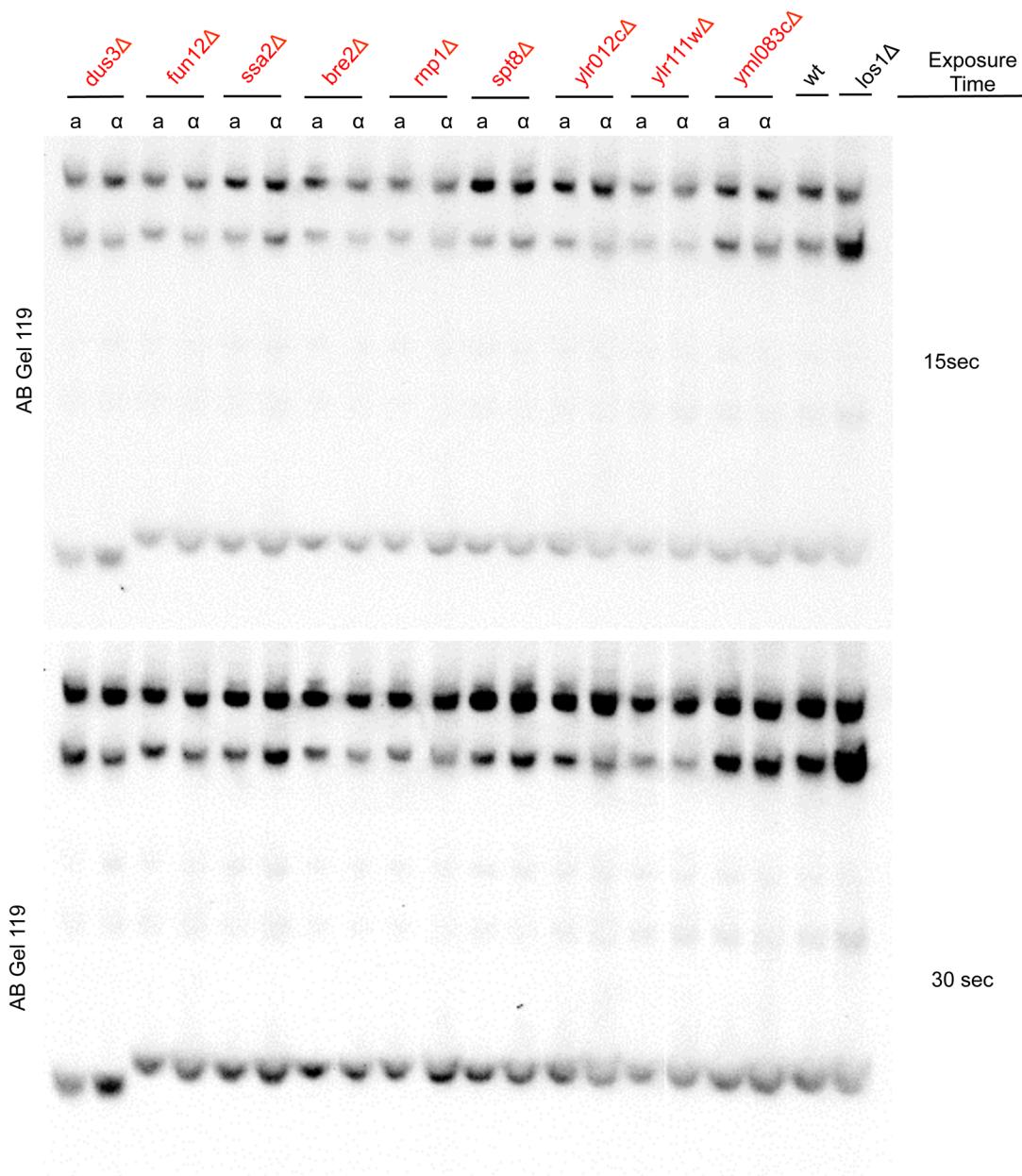
Fig. S3 Mutants from P. Heiter ts collection. A. Repeated Northern analyses of RNAs isolated from candidate mutants incubated for 2 hr at 37°C utilizing probe 1. B. Example Northern analyses of complementation confirmation. EV, cells contain vector only; M, cells contain a plasmid from the MoBY-ORF collection that encodes the wt version of the relevant gene. P, primary tRNA transcript; I, end-matured intron-containing tRNA; M, mature tRNA^{le}. Equal

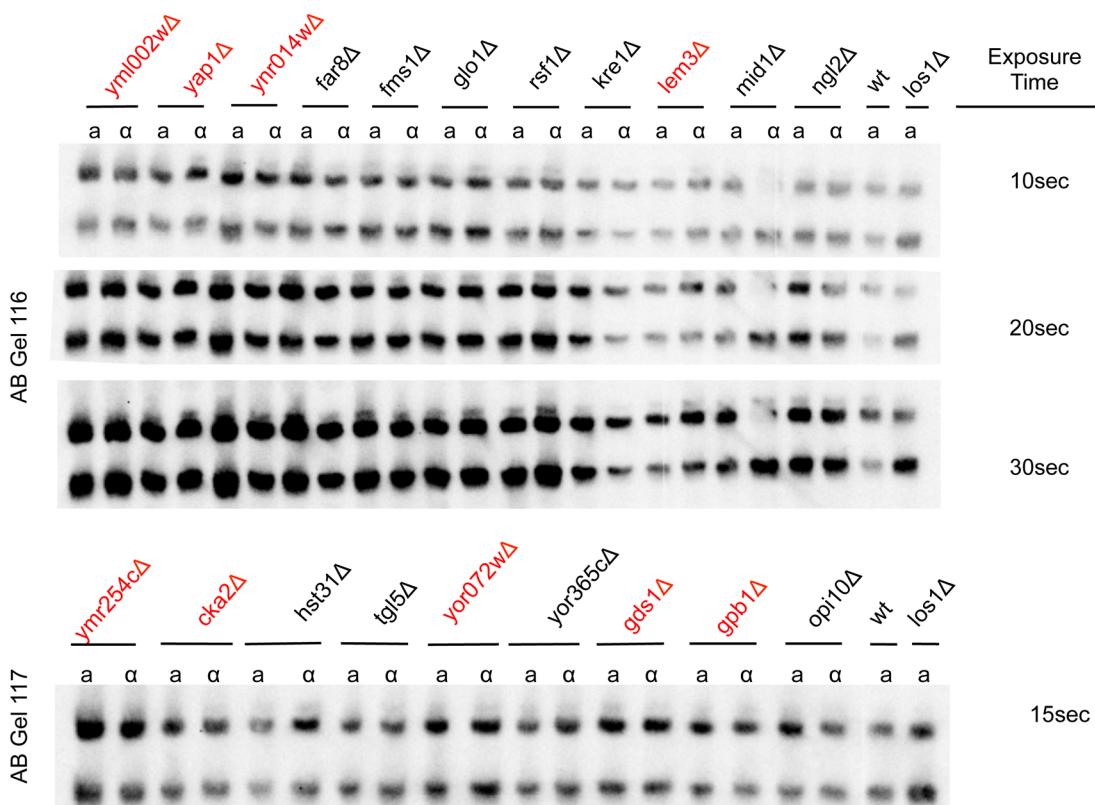
loading for each lane was assessed by EtBr staining of 5S rRNA. The verified mutants are highlighted in red. The red asterisks highlight aberrant species.

Fig. S4. The growth defect of *crm1-1* is suppressed when Crm1 is exogenously expressed from a multi-copy plasmid. Serial dilutions of *crm1-1* containing empty vector and *crm1-1* containing plasmid encoded Crm1 grown on selective media at 23°C and 37°C for 2 days.

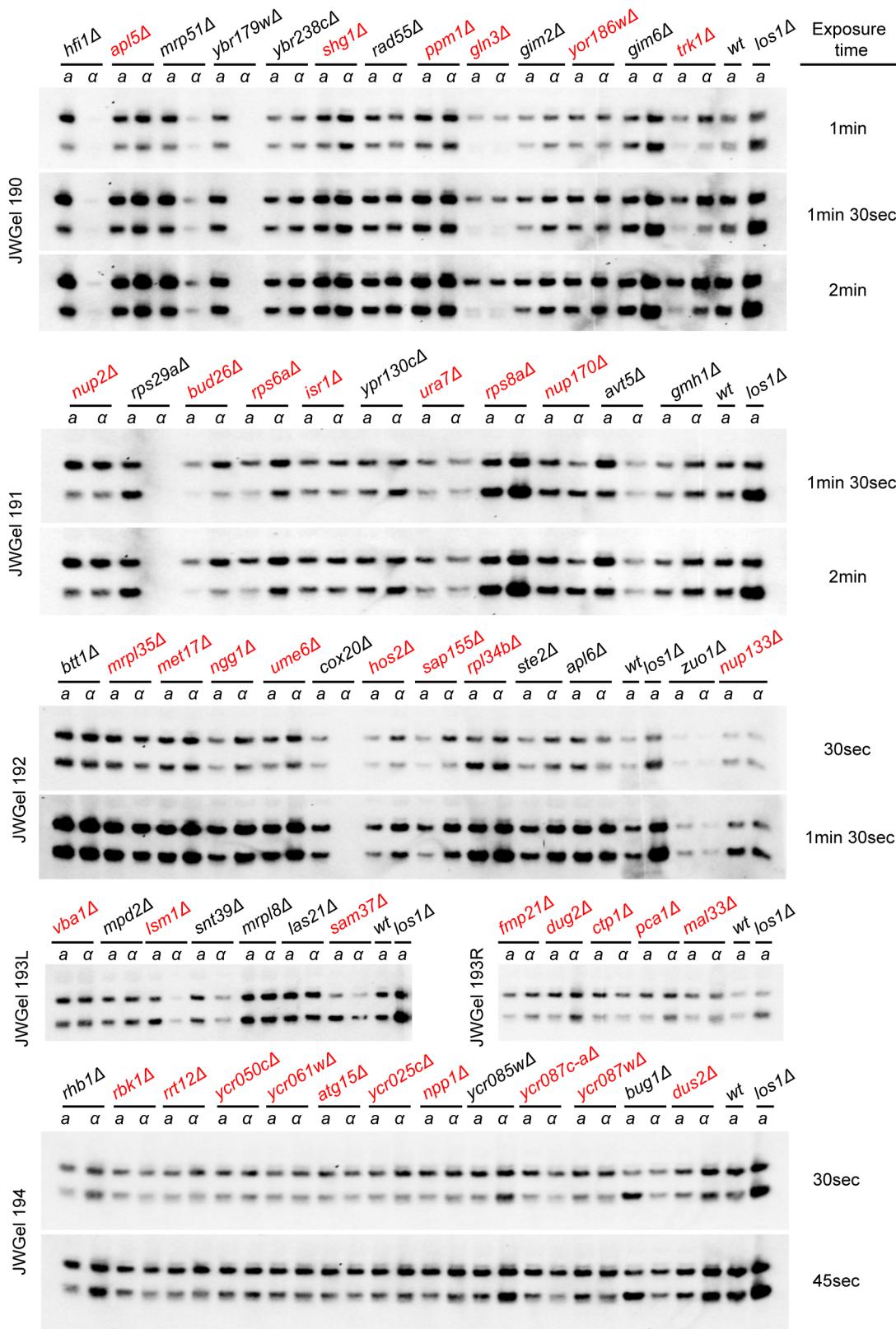
Fig. S5. tRNA^{Ile}_{UAU} accumulate at the nuclear periphery in *crm1-1* cells. A. FISH analyses employing probe SRIM04 (tRNA^{Ile}_{UAU}; mature sequences). *rna1-1* and *crm1-1* cells were cultured at 23°C, shifted to 37°C, and harvested at 0 and 4 hr after the temperature shift. Nuclei were visualized by DAPI staining of DNA highlighted in blue in the overlay panels. Arrowheads highlight example cells with tRNA signal at the nuclear periphery. B. The same cells as for A. were employed and FISH analyses were conducted employing tRNA^{Ile}_{UAU} intron-specific probe SRIM03.

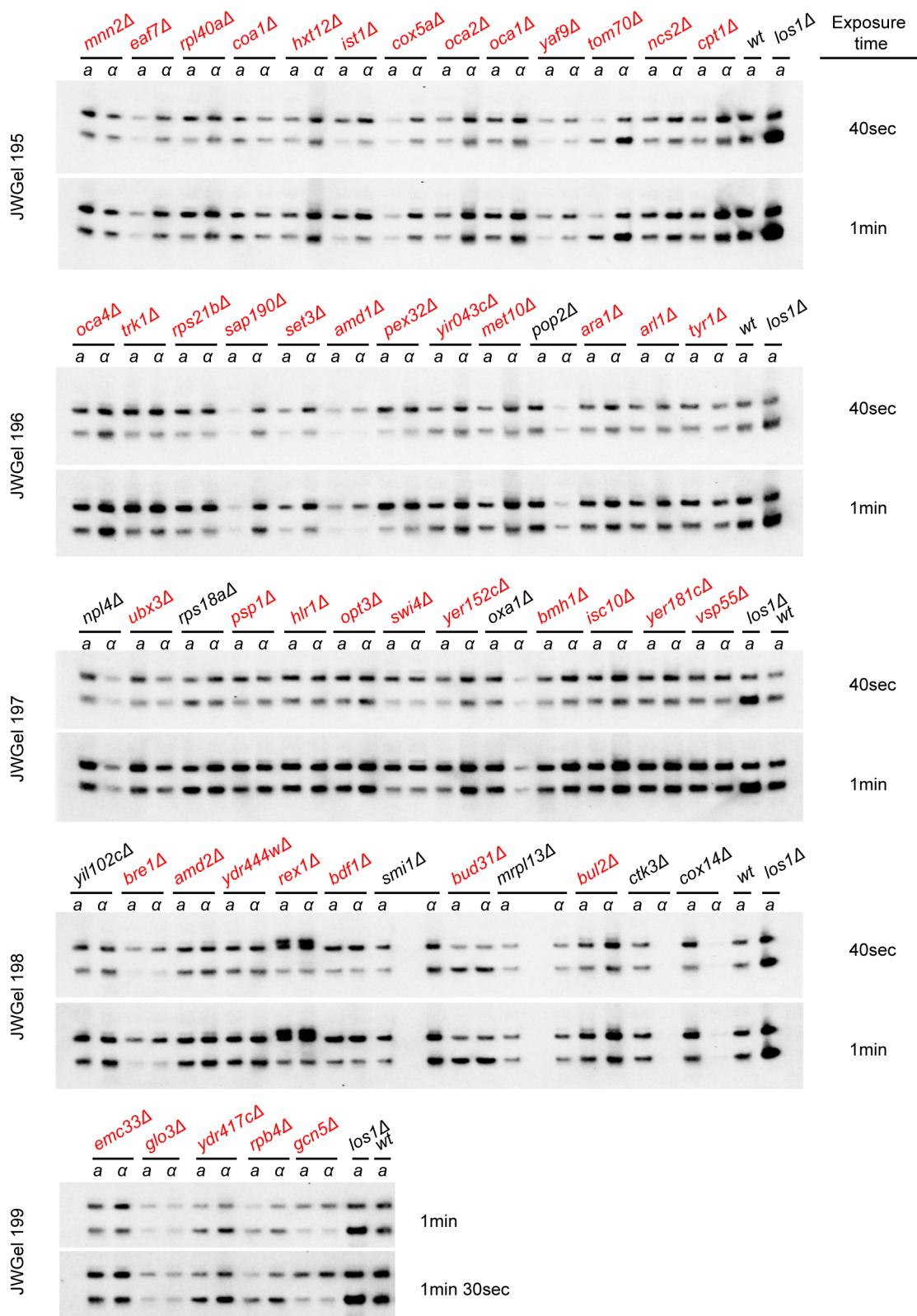
Wu et al., Fig. S1

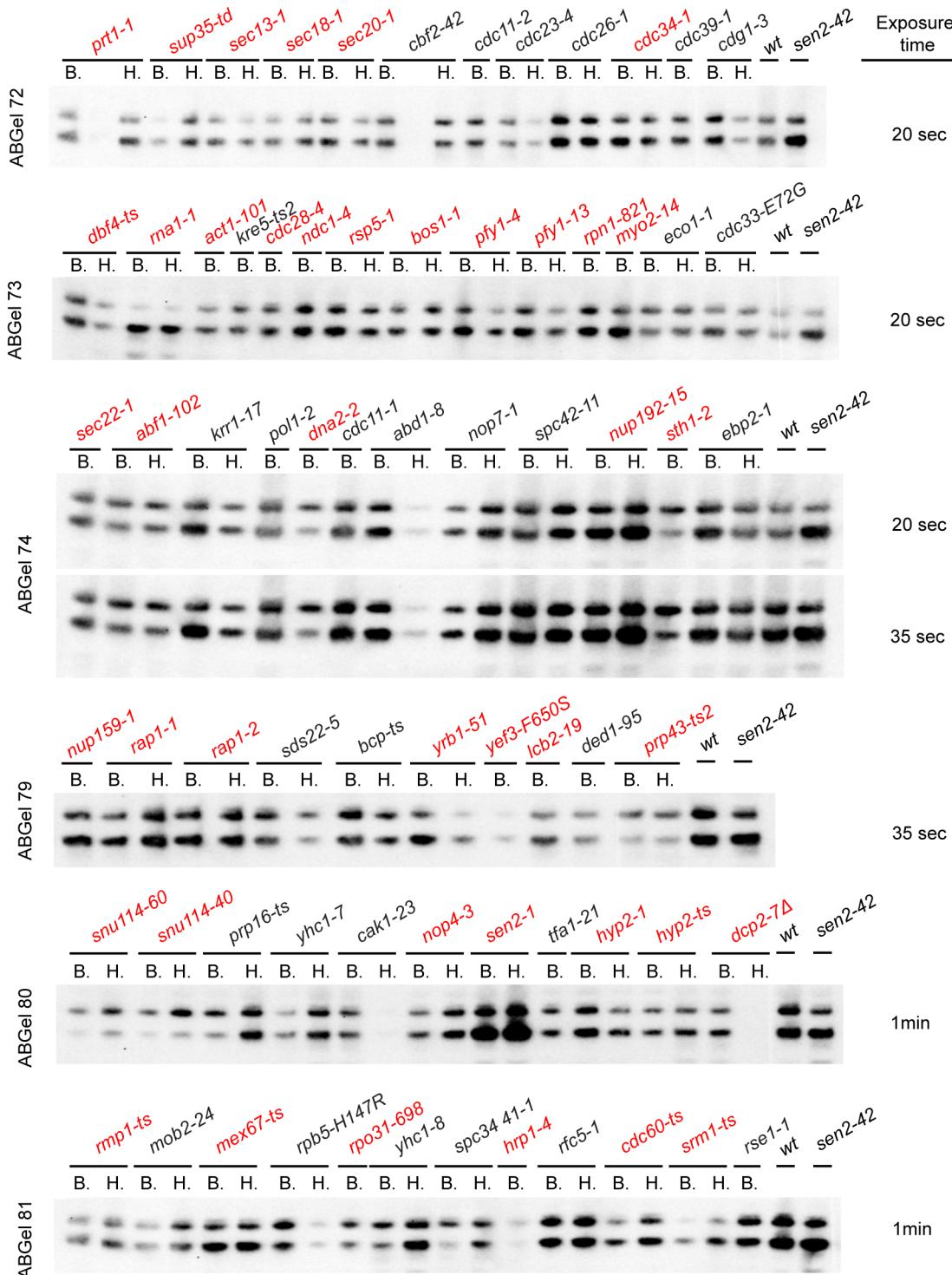




Wu et al., Fig. S1-2







Wu *et al.*, Fig. S2-1

