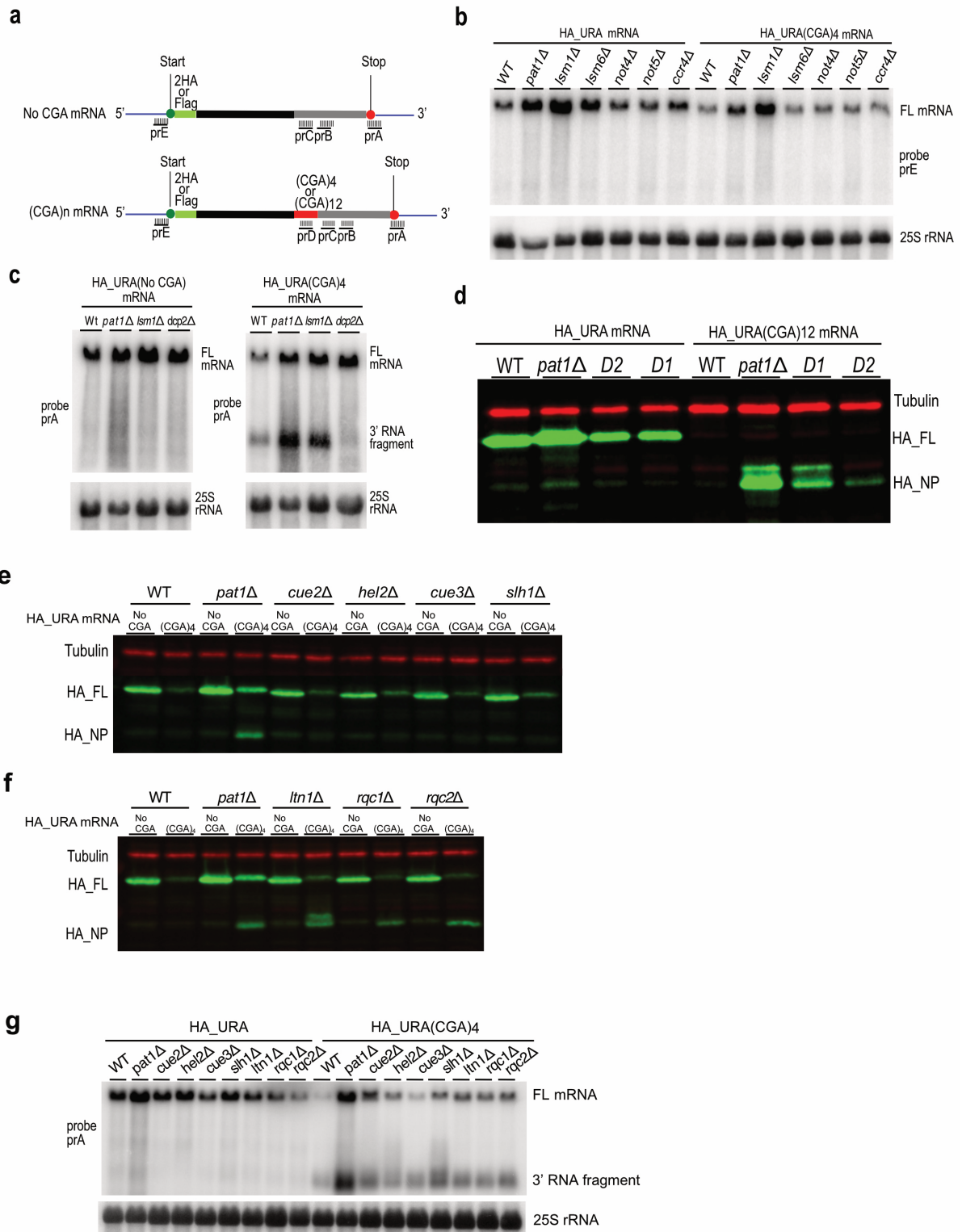


SUPPLEMENTARY INFORMATION

Pat1 Couples mRNA Decay with Ribosome-associated Quality Control

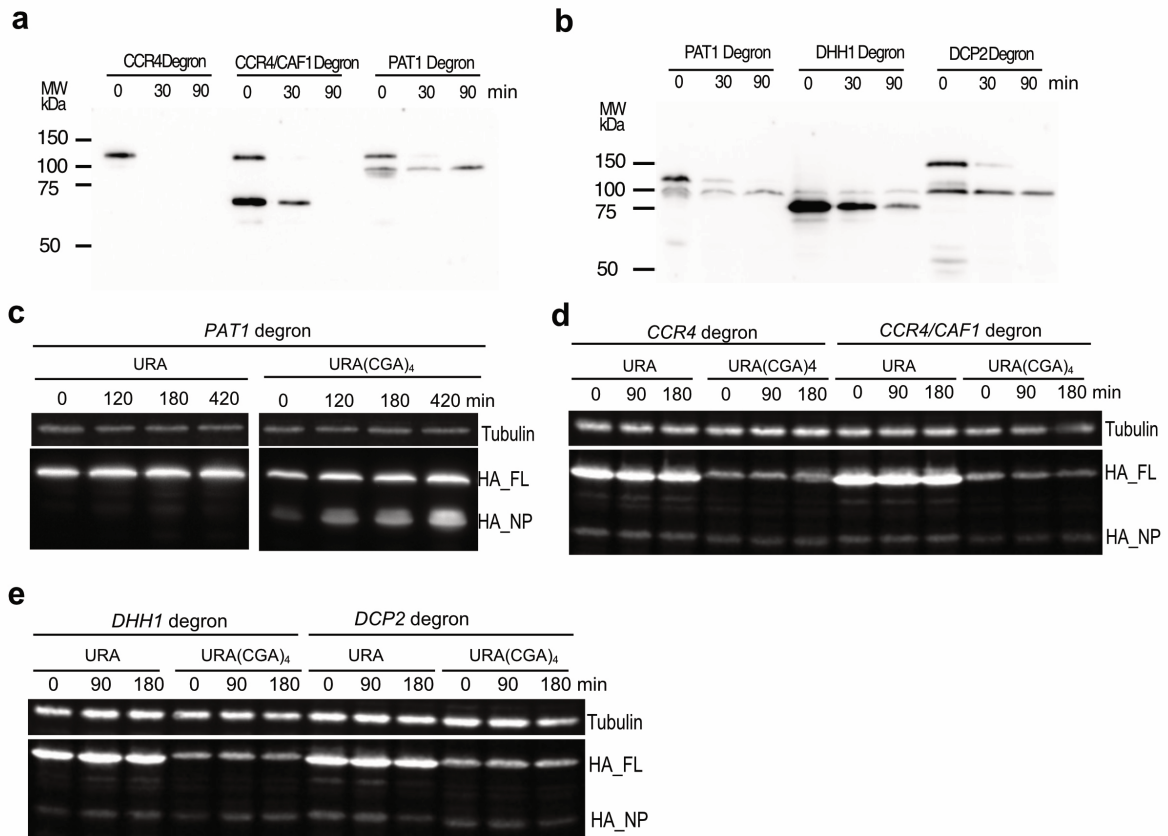
Ferrand, G., Bouez, T., Torchet*, C. and L., Benard*

This file comprises five Supplementary Figures, three Supplementary Tables, detailed sequences of constructs generated in this study and four supplementary references

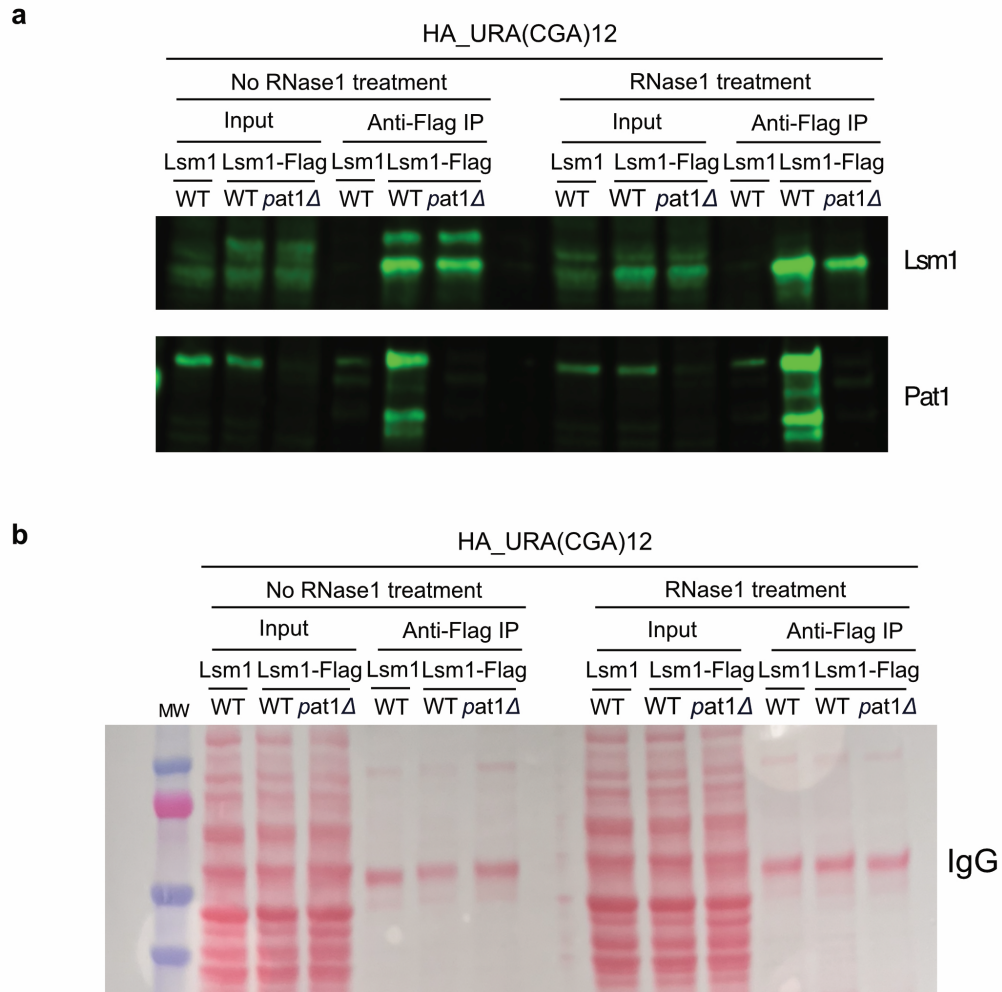


Supplementary Figure 1. Characterization of reporter constructs and analysis of mRNA and nascent peptide accumulation in mRNA decay, RQT, and RQC mutants. **a** Schematic representation of the *Candida albicans* *URA3*-derived reporter mRNAs used throughout this study. The HA_URA reporter (No CGA) serves as a non-stalling control, whereas HA_URA(CGA)₄ and HA_URA(CGA)₁₂ contain four or twelve consecutive CGA codons

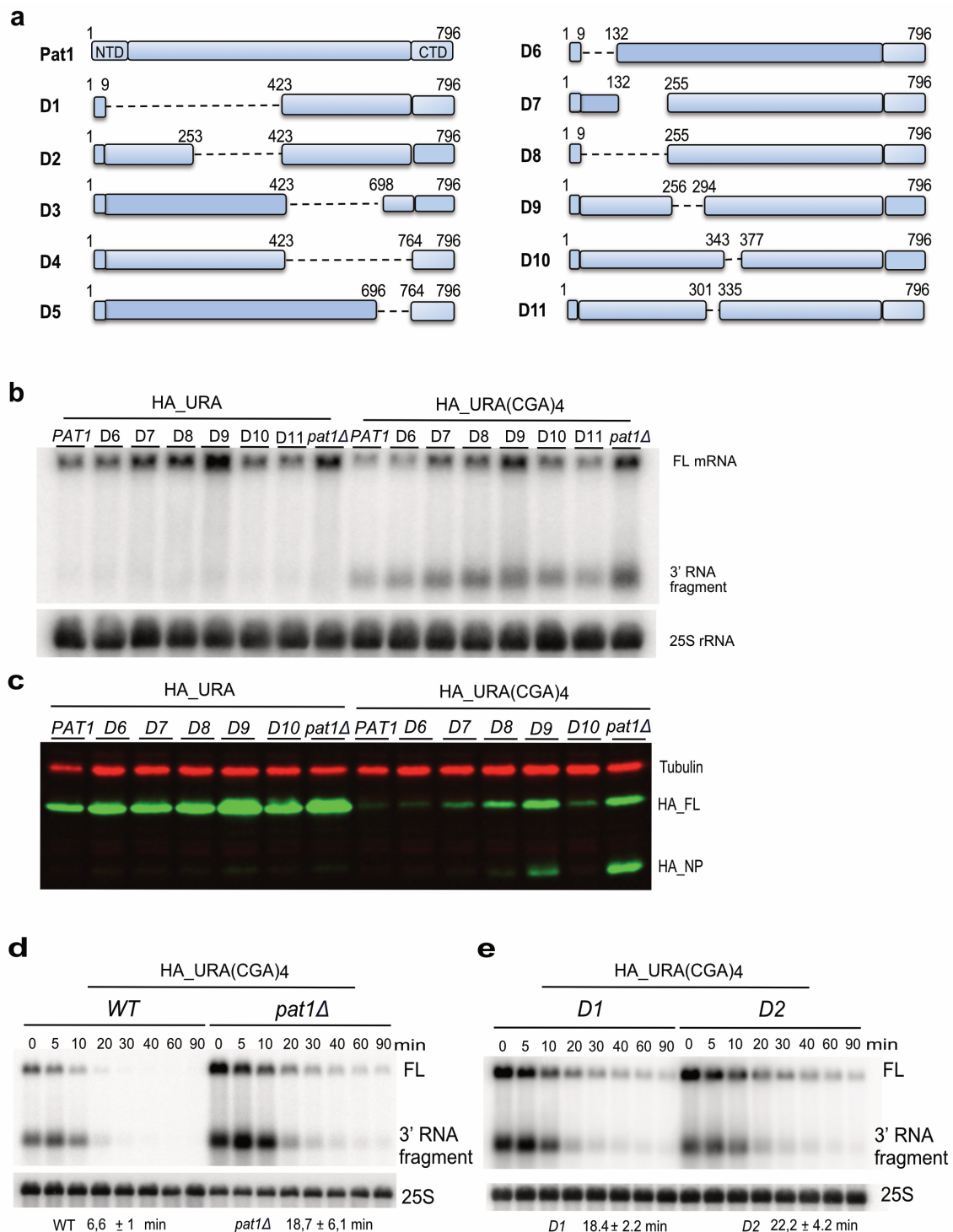
(red), which induce ribosome stalling during translation. Reporters carry either an N-terminal HA or Flag epitope tag, allowing detection of the full-length protein (FL) and the nascent peptide (NP). The positions of the northern blot probes (prA–prE) used throughout this study are indicated (see supplementary Table 3 and additional supplementary information for construct sequences). **b** Northern blot analysis of HA_URA and HA_URA(CGA)₄ reporter mRNAs expressed in WT and mRNA decay mutants (*pat1Δ*, *lsm1Δ*, *lsm6Δ*, *not4Δ*, *not5Δ*, and *ccr4Δ*). RNAs were detected using probe prE, which hybridizes to the 5' region of the reporter mRNAs. Full-length reporter mRNAs are shown. 25S rRNA serves as a loading control. **c** Northern blot analysis of HA_URA and HA_URA(CGA)₄ reporter mRNAs expressed in WT, *pat1Δ*, *lsm1Δ*, and *dcp2Δ* cells. RNAs were detected using probe prA, which hybridizes near the translation termination codon. 25S rRNA serves as a loading control. **d** Western blot analysis of HA-tagged reporter proteins expressed from the HA_URA and HA_URA(CGA)₁₂ reporters in WT, *pat1Δ*, *D1*, and *D2* cells. Proteins were detected using an anti-HA antibody. Tubulin serves as a loading control. **e** Western blot analysis of HA-tagged reporter proteins expressed from the control (HA_URA) and stalled (HA_URA(CGA)₄) reporters (designated No CGA and (CGA)₄, respectively) in WT, *pat1Δ*, and mutants affecting ribosome collision recognition or RQT function (*cue2Δ*, *hel2Δ*, *cue3Δ*, and *slh1Δ*). Proteins were detected using an anti-HA antibody. Tubulin serves as a loading control. **f** Western blot analysis of reporters as in (e) expressed in WT, *pat1Δ*, and mutants defective in the ribosome-associated quality control (RQC) pathway (*ltn1Δ*, *rqc1Δ*, and *rqc2Δ*). Tubulin serves as a loading control. **g** Northern blot analysis of HA_URA and HA_URA(CGA)₄ reporter mRNAs expressed in WT, mRNA decay mutants, and mutants affecting the RQT or RQC pathways. RNAs were detected using probe prA, which hybridizes near the translation termination codon and detects both the full-length reporter mRNA and the 3' RNA decay intermediate. 25S rRNA serves as a loading control.



Supplementary Figure 2. Depletion of Pat1, but not other mRNA decay factors, promotes nascent peptide accumulation. **a–b** Validation of auxin-induced depletion of mRNA decay factors using the auxin-inducible degron (AID) system. Degron-tagged versions of *Ccr4*, *Ccr4/Caf1*, *Pat1* (**a**), *Dhh1*, and *Dcp2* (**b**) were used to conditionally deplete these mRNA decay factors. The auxin-inducible degron (AID) system functions by fusing the protein of interest to an AID tag that is recognized by the *Oryza sativa* TIR1 F-box protein in the presence of auxin (indole-3-acetic acid, IAA). TIR1 expression is driven by the Z3EV synthetic transcription system and induced by β -estradiol¹. **c–e** Western blot analysis of HA-tagged reporter proteins expressed from the control (HA_URA) and stalled (HA_URA(CGA)₄) reporters following depletion of the indicated mRNA decay factors. **c** Pat1 depletion. **d** Ccr4 or Ccr4/Caf1 depletion. **e** Dhh1 or Dcp2 depletion. Protein extracts were collected at the indicated time points after addition of β -estradiol and auxin. Full-length (HA_FL) and nascent peptide (HA_NP) products were detected using an anti-HA antibody. Tubulin, detected with an anti-tubulin antibody, serves as a loading control.

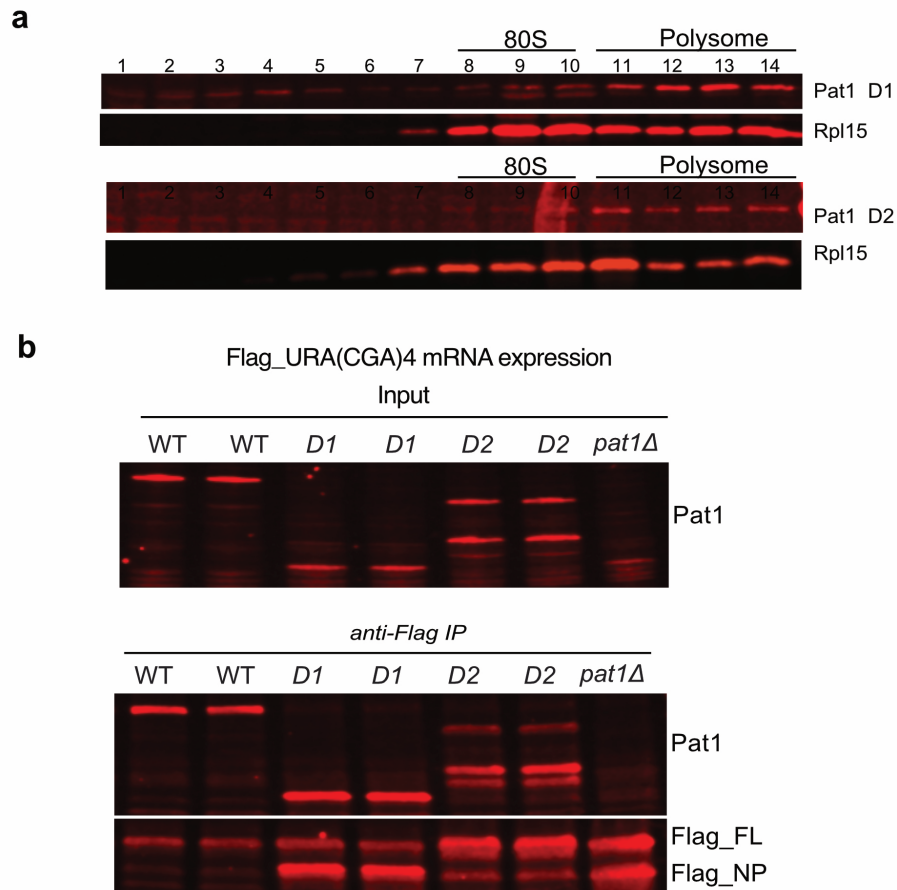


Supplementary Figure 3. Validation that Pat1 co-immunoprecipitates with Lsm1 independently of RNA. **a**, Western blot analysis of total (Input) and anti-Flag immunoprecipitated (anti-Flag IP) protein extracts from WT and *pat1Δ* cells expressing Lsm1-Flag, prepared in the presence or absence of RNase I treatment. Proteins were detected using anti-Flag and anti-Pat1 antibodies to monitor Lsm1-Flag and endogenous Pat1, respectively. **b** Ponceau S staining of the membrane shown in (a).



Supplementary Figure 4. Pat1 truncations and analysis of additional *pat1* mutants for 3' RNA fragment and NP accumulations . **a** Schematic representation of the Pat1 truncation mutants used in this study. Full-length Pat1 (amino acids 1–796) is shown at the top, with the N-terminal domain (NTD) and C-terminal domain (CTD) indicated, and deletion mutants (*D1*–*D11*) are shown below. Solid boxes represent retained regions, whereas dashed lines indicate deleted sequences. Numbers correspond to amino acid positions. **b** Northern blot analysis of

1 full-length (FL) mRNA and the 3' RNA decay intermediate derived from the HA_URA and
2 HA_URA(CGA)₄ reporters expressed in WT, *pat1Δ*, and cells expressing the Pat1 truncation
3 mutants *D6–D11*. RNAs were detected using probe prA, which hybridizes near the translation
4 termination codon. 25S rRNA serves as a loading control. **c** Western blot analysis of HA-tagged
5 full-length (HA_FL) and nascent peptide (HA_NP) products expressed from the HA_URA and
6 HA_URA(CGA)₄ reporters in the same strains as in (b). Proteins were detected using an anti-
7 HA antibody. Tubulin, detected with an anti-tubulin antibody, serves as a loading control. **d**
8 Representative northern blot for analysis of mRNA decay kinetics derived from the
9 HA_URA(CGA)₄ reporter following transcriptional shutoff in WT and *pat1Δ* cells.
10 Transcription was repressed by glucose addition, RNA samples were collected at the indicated
11 time points, and analyzed by northern blot using probe prA. The calculated half-lives of the full
12 length (FL) RNA fragment are indicated below each blot. 25S rRNA serves as a loading control.
13 **e** Representative northern blot analysis of mRNA decay kinetics derived from the
14 HA_URA(CGA)₄ reporter following transcriptional shutoff in *D1* and *D2* cells. RNA samples
15 were analyzed as in (d). The calculated half-lives of the full length (FL) RNA are indicated
16 below each blot. 25S rRNA serves as a loading control.



Supplementary Figure 5. Pat1 D1 and D2 factors co-sediment with polysomes and interact with stalled translation complexes. **a** Western blot analysis of sucrose gradient fractions prepared from *D1* and *D2* cell extracts. Fractions were collected following polysome profiling by sucrose gradient ultracentrifugation and analyzed by western blot using an anti-Pat1 antibody recognizing the C-terminal region of Pat1. The large ribosomal subunit protein Rpl15 was detected as a ribosomal marker. The positions of the 80S monosome and polysome fractions are indicated. **b** Co-immunoprecipitation analysis of Pat1 variants with Flag-tagged translation products derived from the Flag_URA(CGA)₄ reporter. Total (Input) and anti-Flag immunoprecipitated (IP) protein fractions from WT, *D1*, *D2*, and *pat1Δ* cells were analyzed by western blot using an anti-Pat1 antibody to detect endogenous Pat1 or the Pat1 truncation variants. Polyclonal anti-Pat1 antibody detects the C-terminal region of Pat1. Translation products were detected using an anti-Flag antibody, revealing the full-length protein (Flag_FL) and the nascent peptide (Flag_NP).

Supplementary Table 1 : strains used in this study

Strain	Other Name	Genotype	Source or Reference	gene targeting	used in experiment shown in
WT	BY4741	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Euroscarf		
<i>pat1Δ</i>	Y05797	<i>MATalpha his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pat1::kanMX4</i>	Euroscarf		
<i>lsm1Δ</i>	Y01301	<i>MATalpha his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 lsm1::kanMX4</i>	Euroscarf		
<i>hel2Δ</i>	Y03625	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hel2::kanMX4</i>	Euroscarf		
<i>not4Δ</i>	Y00207	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 not4::kanMX4</i>	Euroscarf		
<i>rqc1Δ</i>	Y03692	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rqc1::kanMX4</i>	Euroscarf		
<i>ltn1Δ</i>	Y00834	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ltn1::kanMX4</i>	Euroscarf		
<i>rqc2Δ</i>	Y02819	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rqc2::kanMX4</i>	Euroscarf		
<i>cue3Δ</i>	YLB387	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 cue3::kanMX4</i>	Euroscarf		
<i>cue2Δ</i>	YLB302	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 cue2::kanMX4</i>	Euroscarf		
<i>slh1Δ</i>	YLB514	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 slh1::kanMX4</i>	Euroscarf		
<i>lsm6Δ</i>	YLB480	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 lsm6::kanMX4</i>	Euroscarf		
<i>not5Δ</i>	YLB353	<i>MATalpha his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 not5::kanMX4</i>	Euroscarf		
<i>ccr4Δ</i>	YLB464	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ccr4::kanMX4</i>	Euroscarf		
<i>dcp2Δ</i>	YJC432	<i>As BY4741 but dcp2::neoMX</i>	2		
LMA5419	LMA5419	<i>Mat a tao3(E1493Q) rme1 insert A-308trp1-1::TRP1-prURA3-Z3EVtf-tURA3-Z3EV-TIR1</i>	1		
DHH1-degron	YLB588	<i>As LMA5419 but dhh1-polyG-3FLAG-AID::KANMX6</i>	1		
LSM1-degron	YLB589	<i>As LMA5419 but lsm1-polyG-3FLAG-AID::KANMX6</i>	Gift from C. Saveanu		Fig. 2c and supp Figs. 3a-b
CCR4-degron	YLB585	<i>As LMA5419 but AID-3FLAG-ccr4::KANMX6</i>	1		
CCR4-degron CAF1-degron	YLB587	<i>As LMA5419 but AID-3FLAG-ccr4 (caf1)pop2-AID-polyG-3FLAG-AID::KANMX6</i>	1		
DCP2-degron	YLB584	<i>As LMA5419 but dcp2-polyG-3FLAG-AID::KANMX6</i>	1		
PAT1-degron	YLB613	<i>As LMA5419 but pat1-polyG-3FLAG-AID::KANMX6</i>	This study	Cas9 mediated	Fig. 2c, supp Figs. 2a-b-c
<i>pat1Δ rqc2Δ</i>	YLB658	<i>As BY15797 but rqc2::NatMX6</i>	This study		
<i>pat1Δ CUE3myc</i>	YLB568	<i>As BY15797 but Cue3-Cter-9myc-kNatMX6</i>	This study		
<i>pat1Δ SLH1myc</i>	YLB568	<i>As BY15797 but slh1-Cter-9myc-kNatMX6</i>	This study		
<i>D1 or pat1-D1</i>	YLB661	<i>As BY15797 but pat1::D1 mutant</i>	This study	Cas9 mediated	Fig. 8
<i>D2 or pat1-D2</i>	YLB669	<i>As BY15797 but pat1::D2 mutant</i>	This study	Cas9 mediated	Fig. 8
<i>D1-3FLAG</i>	YLB744	<i>As BY15797 but pat1::D1-3FLAG</i>	This study		Fig. 6
<i>D2-3FLAG</i>	YLB747	<i>As BY15797 but pat1::D2-3FLAG</i>	This study		Fig. 6
LSM1-degron in <i>pat1Δ</i>	YLB761	<i>As YLB589 but pat1Δ0</i>	This study	Cas9 mediated	Supp Fig. 3
<i>D1</i>		<i>As pat1Δ + p428</i>	This study		Figs. 4a-b-c, 5 d-e, 7 and supp Figs. 4 b- and 6
<i>D2</i>		<i>As pat1Δ + p429</i>	This study		Figs. 4a-b-c, 5 d-e, 7 and supp Figs. 4 b-c and 6
<i>D3</i>		<i>As pat1Δ + p430</i>	This study		Figs. 4a-b-c
<i>D4</i>		<i>As pat1Δ + p431</i>	This study		Figs. 4a-b-c
<i>D5</i>		<i>As pat1Δ + p432</i>	This study		Fig. 4a-b-c
<i>D6</i>		<i>As pat1Δ + p440</i>	This study		Supp Figs. 4 b-c
<i>D7</i>		<i>As pat1Δ + p441</i>	This study		Supp Figs 4 b-c
<i>D8</i>		<i>As pat1Δ + p442</i>	This study		Supp Figs. 4 b-c
<i>D9</i>		<i>As pat1Δ + p443</i>	This study		Supp Figs. 4 b-c
<i>D10</i>		<i>As pat1Δ + p444</i>	This study		Supp Figs. 4 b-c
<i>D11</i>		<i>As pat1Δ + p445</i>	This study		Supp Figs. 4 b-c
<i>D1 rqc2Δ</i>		<i>As pat1Δ rqc2Δ + p428</i>	This study		Figs. 5 d-e
<i>D2 rqc2Δ</i>		<i>As pat1Δ rqc2Δ + p429</i>	This study		Figs. 5 d-e

1 Supplementary Table 2: plasmids used in this study

Plasmid Name	Alias in this study	Short Description	Marker	Reference
p397	HA_URA	p415pTDH3_2HA_caURAmut_SeqNter_tTDH3 ampR	LEU2	This study
p398		p415pTDH3_2HA_caURAmut_CGA4_SeqNter_tTDH3 ampR	LEU2	This study
p448	HA_URA_CGA12	p415pTDH3_2HA_caURAmut_CGA12_SeqNter	LEU2	This study
p468	Flag_URA	p415 pTDH3_3Flag_TEV_caURAmut_SeqNter	LEU2	This study
p469	Flag_URA_CGA4	p415 pTDH3_3Flag_TEV_caURAmut_CGA4_SeqNter	LEU2	This study
p470	Flag_URA_CGA12	p415 pTDH3_3Flag_TEV_caURAmut_CGA12_SeqNter	LEU2	This study
p422	mCherry_P2A_URA	pTEF1_mCherry_P2A_caURAmut_SeqNter	URA3	This study
p423	mCherry_P2A_URA_CGA4	pTEF1_mCherry_P2A_caURAmut_CGA4_SeqNter	URA3	This study
p463	pGAL_FLAG_mcherry pGAL_HA_URA	pGAL_FLAG_mcherry pGAL_HA_ca mut_SeqNter	LEU2	This study
p464	pGAL_FLAG_mcherry pGAL_HA_URA_CGA4	pGAL_FLAG_mcherry pGAL_HA_ca mut_CGA4_SeqNter	LEU2	This study
p404	PAT1	pRS313 PAT1 Full Length ampR	HIS3	This study
p428	D1	PAT1_D1_D(deletion codons 10-422) ampR	HIS3	This study
p429	D2	PAT1_D2_D(deletion codons 254-422) ampR	HIS3	This study
p430	D3	PAT1_D3_D(deletion codons 422-697) ampR	HIS3	This study
p431	D4	PAT1_D4_D(deletion codons 422-763) ampR	HIS3	This study
p432	D5	PAT1_D5_D(deletion codons 697-763) ampR	HIS3	This study
p440	D6	PAT1_D6_D(deletion codons 10-131) ampR	HIS3	This study
p441	D7	PAT1_D7_D(deletion codons 133-254) ampR	HIS3	This study
p442	D8	PAT1_D8_D(deletion codons 10-254) ampR	HIS3	This study
p443	D9	PAT1_D9(deletion codons257-293) ampR	HIS3	This study
p444	D10	PAT1_D10(deletion codons 344-376) ampR	HIS3	This study
p445	D11	PAT1_D11(deletion codons 302-334) ampR	HIS3	This study
p546	Flag_PAT1	Pat1 FL-Flag Cter ampR	HIS3	This study
p529	Flag_PAT1 D1	PRS313_PAT1(D1)_CterFlag	HIS3	This study
p530	Flag_PAT1 D2	PRS313_PAT1(D2)_CterFlag	HIS3	This study
p524		PLB Crispr Pat1-MID_LEU ampR LEU2	LEU2	This study
p521		CRISPR KAN_LEU ampR LEU2	LEU2	This study
p547	HA_SDD1	p415pTDH3_2HA_SDD1_tTDH3 ampR	LEU2	This study
pRS313		CEN AmpR	HIS3	2
pRS315		CEN AmpR	LEU2	2
pRS316		CEN AmpR	URA3	2
pRS415		CEN AmpR	LEU2	3
p545		p1451 polyG_3Flag_miniAID_kanMX6 ampR	///	1
p526		pFA6a-6xGLY-3xFLAG-HIS3MX6 ampR HIS3	HIS3	4

2

3

1 Supplementary Table 3: oligonucleotides used in this study

Name	Alias	used for	Sequence 5' to 3'
1308	prE	Probe of all 5' URA(CGA)0-12 mRNA	CCAAACAATCCTCTACCAACAATG
1471	prA	Probe of all 3' URA(CGA)0-12 mRNA	GGTTGTCTCATTAGGCGGC
1776	pRC	For detecting RNA after Rnase H treatment	GTTGCGACGAAGATCTGC
1779	pRB	For cleaving RNA by using Rnase H	GAGTTATGGTGGCACC
1886	prD	probe for detecting GCAn containing mRNA	GTTGCGACGAAGATCTGC
olb25S	25S	detection of 25S rRNA	ATCCGCTAAGGAGTGTGTAACAACCTACC
olb18S	18S	detection of 18S rRNA	AGCCATTGCGAGTTTCACTG
olb959	5S	detection of 5S rRNA	CTACTCGGTCAGGCTCTTAC
1017		PAT1 deletion by natMX6	GTACACCTTGAAGGAAGCAAGGTTTTAACCGGAAGTAAGAGCAGCAAGAAGCACTAGCAcagctgaagcttcgtacgc
1018		PAT1 deletion by natMX6	GGAAATAAAAAATAAGGGGAGAAAAAATACATGCGTAAGTACATTAAATACAGGAAAAATCgcatagccactagtg gatctg
1315		PAT1 deletion verification	CCCTATTGTCTTCTCGCATTAC
1316		PAT1 deletion verification	GGTGGTATTATTGATGCTAATACGG
1271		HEL2 deletion by natMX6	ATTTTGATACAGTCTCTTCGTCGAAAAAATAGTGGCTATCTCTTTCAAGAATTAGGCAGCTGAAGCTTCGTACGC
1272		HEL2 deletion by natMX6	ATTTAAGACTTTCATTTCTCTAATGCTATTGTCAGTTACAGGTTAGAAATATATTTCCAAGCATAGGCCACTAGTGGATC TG
1273		HEL2 deletion verification	GCATCTGAACGACTGCCAC
1274		HEL2 deletion verification	CGCAGACGCTGAGCAGGAG
1458		RQC2 deletion by natMX6	AAAGAAAGTTGCTACTTATTATCCGGTCTAAGAAGTCAGGCAGGCAAGAGATTAATAGCGCAGCTGAAGCTTCGTACGC C
1459		RQC2 deletion by natMX6	GTGATGAAAAATAATGAAAAAATATAATTGCTGTCTATTTCTTTTCATCTCATATGATCATAGGCCACTAGTGGATCT G
1449		RQC2 deletion verification	GTTTTAAAGAATCCGATGGC
1450		RQC2 deletion verification	CATGCTAATCATTTTATTCTCTCG
1311		CUE2 deletion by natMX6	TCAGTATATCAATATAAGCAATTATGACACCTCATTATCGTGCATATAAGATCATGCACAGCTGAAGCTTCGTACGC
1312		CUE2 deletion by natMX6	ACCCAATGCAGTTTAAAGAAGCCTAGCGTTTATAACCTTTATAGAAAAATCAGATAGGTTTCATAGGCCACTAGTGGATC TG
1313		CUE2 deletion verification	CAAATTTCAACAGCGATATTCGCC
1314		CUE2 deletion verification	GTGAAGTGTCTCTGTGC
1650		Construction of CUE3myc strain	AACCATAACCGTAAGAAGGGGCACGATAAAAAATTGGCTCGTGGGAAATAACGCTATTCTGTACGCTGCAGGTC
1651		Construction of CUE3myc strain	CACCTTCTGAACGCGACGCTGAATAACTTATAATACGTTACATAATTGTATACCGTCTAGCATCGATGAATTCGAGCTC G
1660		CUE3myc verification	CGGATGAAAAATCGAAGGTTGG
1661		CUE3myc verification	GGCAAAGTTTACTAGTCGGC
1654		Construction of SLH1myc strain	TTTTCCCTTATTAATGACGCTTTAGGATTACGTTATGATATGGTCCATAAACTATCTCTGTACGCTGCAGGTC
1655		Construction of SLH1myc strain	GAAAAATATACGAAGATGTATACTAGATAGATTCCACTGTTATCTTTCAAAAATATCATCGATGAATTCGAGCTC G
1659		SLH1myc verification	CGAGGTGTTAATCTTTATAGCAAGC
CtermTA G		SLH1myc verification	CGAGCTCGAATTCATCGAT
1688		Construction of Pat1-degron strain	CTAAACGTTATGGGGTTGGTGATCGCGATGGTGAAATATCAGAACTAAAGCGGATCCCCGGGTTAATTA

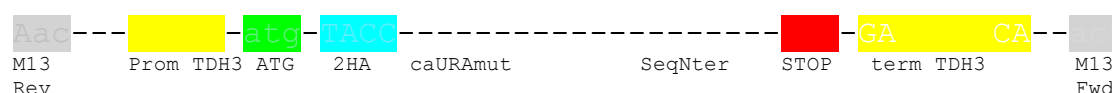
1689	Construction of Pat1-degron strain	AGTTCTGCTACCGATTCAACCTTATTCTCAATCATTGTTAACTCTTGCGGAATTCGAGCTCTTTAAAC
1558	Construction of p428 (pat1_D1_deletion)	AGTCAGTGACGTCGCTATTTTCTAACCCAAAGAAGG
1559	Construction of p428 (pat1_D1_deletion)	GTTAGAAAAAGCGACGCTACTGACTCTCT
1560	Construction of p429 (pat1_D2_deletion)	AGTCAGTGACGTCATCTCTCTTCGATCTGCG
1561	Construction of p429 (pat1_D2_deletion)	ATCGAAGAGAGATGACGCTACTGACTCTCT
1562	Construction of p430 (pat1_D3_deletion)	CTTCATCCATAAATAGCGCATCGGGG
1563	Construction of p430 (pat1_D3_deletion)	CCCCGATGCGCTATTTATGGATGAAGCATACATTTGG
1564	Construction of p431 (pat1_D4_deletion)	TATAAATAATAGATAGCGCATCGGGG
1565	Construction of p431 (pat1_D4_deletion)	CCCCGATGCGCTATCTATTATTATAAAGAGAACTATACCAAGATTGG
1566	Construction of p432 (pat1_D5_deletion)	TATAAATAATAGAGTCATTTTGTAAACGCATGATG
1567	Construction of p432 (pat1_D5_deletion)	TTTACAAAATGACTCTATTATTATAAAGAGAACTATACCAAGATTGGAATTG
1584	Construction of p440 (pat1_D6_deletion)	CCATTGCTGCGGGCTATTTTCTAACCCAAAGAAGGAC
1585	Construction of p440 (pat1_D6_deletion)	GTTAGAAAATAGCCCCGAGCAAAATGGCCC
1586	Construction of p441 (pat1_D7_deletion)	GCTCTTCAGGCGAAGGAGCGGAGCCATTG
1587	Construction of p441 (pat1_D7_deletion)	GGCTCCGGCTCCTTCGCTGAAGAGCAAAGACG
1588	Construction of p442 (pat1_D8_deletion)	GCTCTTCAGGCGAGCTATTTTCTAACCCAAAGAAGGAC
1589	Construction of p442 (pat1_D8_deletion)	GTTAGAAAATAGCTCGCTGAAGAGCAAAGACG
1590	Construction of p443 (pat1_D9_deletion)	CAGTGACAATTTGCGACAAATCTCTTCGATC
1591	Construction of p443 (pat1_D9_deletion)	GAGAGATTTGTCGCAAATTGTCAGTGAAGACC
1592	Construction of p444 (pat1_D10_deletion)	AGTTACCAGCAGCATAGCGACCACCGAGTCTG
1593	Construction of p444 (pat1_D10_deletion)	CGGTGGTCGCTATGCTGCTGGAATCTAG
1594	Construction of p445 (pat1_D11_deletion)	GTCTGTGCCAGAGTAAGGGTCTCAGTGACAA
1595	Construction of p445 (pat1_D11_deletion)	TGAGGACCCTTACTCTGGACACAGACTCGGTG
1576	To clone CGA construct	CGACGACGACGAATGC
1577	To clone CGA construct	GTCGTCGTCGGGATG
1578	To clone CGA construct	GTCGTCGCCAGC
1579	To clone CGA and noCGA sequences	GCACCAGCACCTGCTCC
1522	Construction of p448 CGA12	GCTGGGCGACGACGACGACGACGACGACGACGACGACGACGAATGC
1523	CGA12 to clone	CTGCATTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCC
1524	CGA12 to clone	GCATCCCGACGACGACGACGACGACGACGACGACGACGACGAATGC
1525	CGA12 to clone	CTGCATTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTCGGG
1895	to built CGA12 plasmid	AATTTGATTTTTCAG GGTGAGCTACCGC GCTTGCGCAACACG AAGCCCGACGACGA CGAATGCAGATC
1896	to built CGA12 plasmid	AATTTGATTTTTCAG GGTGAGCTACCGC GCTTGCGCAACACG AAGCCGCTGGGCGA CGACGACGACGA
1678	to built flagTev CGA and noCGA plasmids	GTCATCCTTATAATCATGATGTCatGTCCTTATAGTACCATCatGATCCTTATAATCAGACATCGTggatcCtctagttc
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1683	to built flagTev CGA and noCGA plasmids	ATAGTCACCATCatgATCCTTATAATCAGACATCGTggatcCtctagttctagaTTTGTTGTTTATGTG
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1686	to built Flag CGA and noCGA plasmids	ACAAGGGTGCTGGAGAAAACCTGTATTTTCAGGGCGGTtcaGCTActagTATCacagtcaacactaatacctataG
1781	to built 2HA_SDD1 plasmid	CAA GAT TTA AAG TAA ATT CCC GGG CTT AGA CTT TGG TTT GGT AAT TGA AAT TCA TGG
1832	gRNAcrispr pat1 to delete PAT1	ACG TGG ACT TGG CCT CTT CAG GTT TT
1833	gRNAcrispr pat1 to delete PAT1	TGA AGA GGC CAA GTC CAC GTG ATC A
1919	gRNA_ pat1 MID to insert D1 and D2 mutants	CAT TCT GGA CAC AGA CTC GGG TTT T
1920	gRNA_ pat1 MID to insert D1 and D2 mutants	CCG AGT CTG TGT CCA GAA TGG ATC A
1921	Donor Pat1 crispr for deletion	AAGGAAGCAAAGGTTTTTAACCGGAAGTAAGAGCAGCAAGAAGCACTAGCAGAT TTTTCTGTAAATTTTAATGTACTTACGCATGTATTTTTTTTCTCC C
1943	to built Pat1_Flag plasmid from P404, P526	GAT TTT TCC TGT AAT TTT AAT GTA CTT ACG
1944	to built Pat1_Flag plasmid from P404, P526	TTA AAA TTA CAG GAA AAA TCC TTA TTT AGAAGT GGC GCG C
1945	to built Pat1_Flag plasmid from P404,P526	CTT TAG TTC TGA TAT TTC ACC ATC GC
1946	to built Pat1_Flag plasmid from P404,P526	GTG AAA TAT CAG AAC TAA AGC CCG GGT TAATTA ACG GG

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Sequences of inserts in the newly constructed plasmids described in Supplementary Table 2, as well as the genomic insertions present in the strains generated for this study and listed in Supplementary Table 1.

Construction 2HA_URA

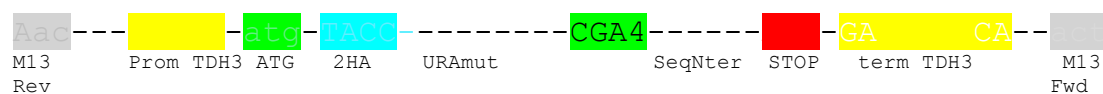


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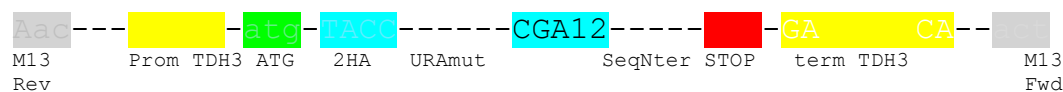


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Construction 2HA_URA_CGA12



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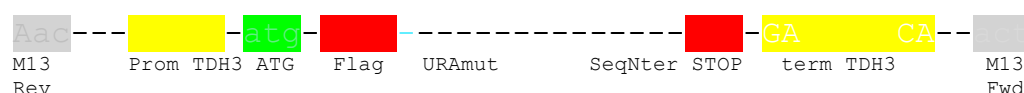


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Construction Flag_URA

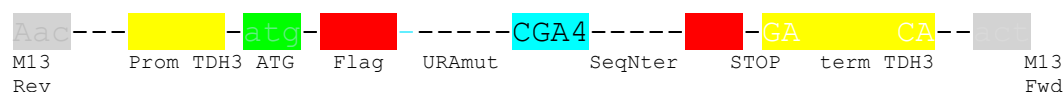


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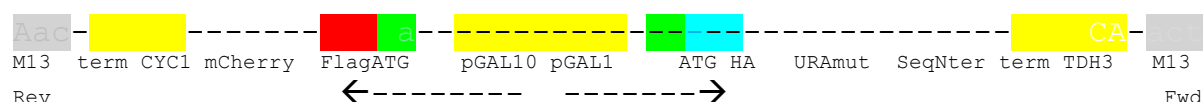
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M13 Rev

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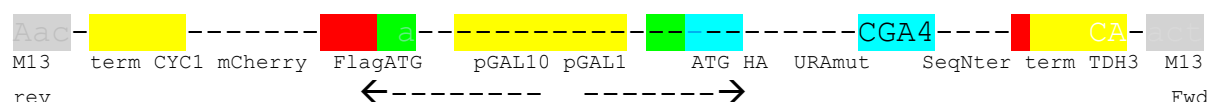


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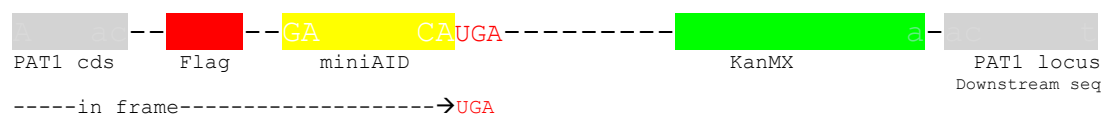
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Construction PAT1_FLAG_Degron_KanMX



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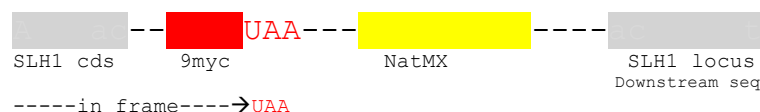
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1 Construction Flag-Pat1 (Flag tag insertion in Cter region)
2 Or Construction Flag-D1
3 Or Construction Flag-D2



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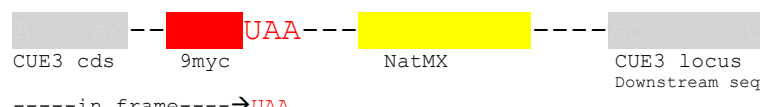


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Construction CUE3-Myc (9Myc tag insertion in Cter region)



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15 TGATTTCTGAAGAAGATTTGAACGGTGAACAAAAGCTAATCTCCGAGGAAGACTTGAACGGTGAACAAAATTA
16 TCTCAGAAGAAGACTTGAACGGATCCACTAGCTAAAAAAGTGGAACGATCATTCAAGAGATCCCCGGGTTAATTA
17 GGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTACCCCGCCAGCGACATGGAGGCCCAGAATACCC
18 TCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTGCGCCGTACATTTAGCCCATACATCCCC
19 ATGTATAATCATTTCATCCATACATTTTGATGGCCGCACGGCGCAAGCAAAAATACGGCTCCTCGCTGCAGA
20 CCTGCGAGCAGGGAAACGCTCCCCTCACAGACGCGTTGAATTGTCCCCACGCCGCGCCCTGTAGAGAAATATAA
21 AAGGTTAGGATTTGCCACTGAGGTTCTTCTTTTCATATACTTCCTTTTAAATCTTGCTAGGATACAGTTCTCACA
22 TCACATCCGAACATAAACAACCATGGGTACCACTCTTGACGACACGGCTTACCGGTACCGCACCAGTGTCCCGGG
23 GGACGCCGAGGCCATCGAGGCACTGGATGGGTCTTCACCACCGACACCGTCTTCCGCGTCACCGCCACCGGGGA
24 CGGCTTCACCCTGCGGGAGGTGCCGGTGACCCGCCCTGACCAAGGTGTTCCCCGACGACGAATCGGACGACGA
25 ATCGGACGACGGGGAGGACGGCGACCCGGACTCCCGGACGTTTCGTGCGGTACGGGGACGACGGCGACCTGGCGGG
26 CTTCGTGGTCATCTCGTACTCGGCGTGGAACCGCCGGCTGACCGTCGAGGACATCGAGGTGCGCCCGGAGCACCG
27 GGGGCACGGGGTCGGGCGCGCGTTGATGGGGCTCGCGACGGAGTTCGCCGGCGAGCGGGGCGCCGGGCACCTCTG
28 GCTGGAGGTACCAACGTCAACGCACCGGCGATCCACGCGTACCGGCGGATGGGGTTCACCTCTGCGGCCTGGA
29 CACCGCCCTGTACGACGGCACCGCCTCGGACGGCGAGCGGCAGGCGCTCTACATGAGCATGCCCTGCCCTAATC
30 TCGAGGCGAATTTCTTATGATTTATGATTTTTATTATTAAATAAGTTATAAAAAAATAAGTGATACAAATTTT
31 AAAGTGAATCTTAGGTTTTTAAACGAAAATTCCTTGTTCTTGAGTAACCTCTTTCCTGTAGGTGAGTTGCTTCTC
32 AGGTATAGCATGAGGTGCGCTCTTATTGACCACACCTCTACCGGCATGCCGAGCAAATGCCTGCAAATCGCTCCCC
33 ATTTACCCCAATTGTAGATATGCTAACTCCAGCAATGAGTTGATGAATCTCGGTGTGTATTTTATGTCTCAGAG
34 GACAACACCTGTTGTAATCGAGCTCGAATTCATCGATCTAGACGGTATACAATTATGTAACGTATTATAAGTTAT
35 TCAGCGTCGCGTTTCAGGAAGTGCTTTTTTTAATA

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