# SUPPLEMENTARY METHODS

### *Construction of plasmids and strains*

**NMD_control(HIS3).** Control plasmid for histidine reporters. Constructed by ligating the *HIS3* promoter into the EcoRI/BamHI site of NMDv9. *The HIS3* promoter was amplified from BY4741 genomic DNA with proper restriction sites using primers EcorI_pHIS3_F and BamHI_pHIS3_R.
**NMD_control(LEU2).** Control plasmid for leucine reporter aka NMD_control(LEU2)v2. Constructed by three-way ligation of the *HIS3* promoter digested with EcoRI/BamHI, *LEU2* ORF digested with BamHI/PstI and pCM188 digested with EcoRI/PstI. The *HIS3* promoter was amplified from BY4741 genomic DNA using primers EcorI_pHIS3_F and BamHI_pHIS3_R; the *LEU2* ORF was amplified from CENPK2 genomic DNA using primers leu2_orf_bamhi_f and leu2_orf_psti_r.
**NMDv9.** *ALR1* promoter-based plasmid with *HIS3* auxotrophy reporter, constructed in two steps. In the first step, the *HIS3* ORF was amplified from CENPK2 genomic DNA using primers HIS3_ORF_BAMHI_F and HIS3_ORF_PstI_R then digested with BamHI/PstI and ligated into BamHI/PstI pCM188 to create an intermediary plasmid. In the second step, the *ALR1* promoter was amplified from BY4741 genomic DNA using primers pALR1_insert_EcoRI_F and pALR1_insert_BamHI_R, then digested with EcoRI/BamHI and ligated to EcoRI/BamHI digested intermediary plasmid.
**NMDv12.** *DAL2* promoter-based plasmid with *HIS3* auxotrophy reporter. Constructed by ligating the *DAL2* promoter into the EcoRI/BamHI site of NMDv9. The *DAL2* promoter was amplified from BY4741 genomic DNA with proper restriction sites using primers EcoRI_pDAL4_F and BamHI_pDAL4_R.
**NMDv14.** *STN1* promoter-based plasmid with *HIS3* auxotrophy reporter. Constructed by using the NEBuilder® HiFi DNA Assembly. The *STN1* promoter fragment was amplified from BY4741 genomic DNA using primers nmdv14_ga_stn1_F and nmdv14_ga_stn1_R, while the *HIS3* fragment was amplified from CENPK2 genomic DNA using primers nmdv14_ga_HIS3_F and nmdv14_ga_HIS3_R. The fragments were assembled into EcoRI/PstI digested NMDv9 per manufacturer's instructions.
**NMDv9.2.** *ALR1* promoter-based plasmid with *HIS3* auxotrophy reporter; terminated with the *PGA1* 3'UTR. Constructed by three-way ligation of the *HIS3* ORF digested with BamHI/PstI, *PGA1* 3' UTR digested with PstI/HindIII and NMDv9 digested with BamHI/HindIII. The *HIS3* ORF was amplified from CENPK2 genomic DNA using primers HIS3_ORF_BAMHI_F and HIS3_ORF_PstI_R. The *PGA1* 3'-UTR was amplified from BY4741 genomic DNA using primers PGA1_3UTR_pstI_F and PGA1_3UTR_hindIII_R.
**NMDv15.** *HIS3* promoter-based plasmid with *HIS3* auxotrophy reporter; terminated with the *PGA1* 3'-UTR. Constructed by ligating the *HIS3* promoter into the EcoRI/BamHI site of NMDv9.2. The *HIS3* promoter was amplified from BY4741 genomic DNA with proper restriction sites using primers EcorI_pHIS3_F and BamHI_pHIS3_R.
**NMDv16.** *Ashbya gossypii TEF1* (agTEF1) promoter-based plasmid with *HIS3* auxotrophy reporter; terminated with the *PGA1* 3'-UTR. Constructed by ligating the *agTEF1* promoter into the

EcoRI/BamHI site of NMDv9.2. The *agTEF1* promoter was amplified from plasmid pFA6-hph-nt1 with proper restriction sites using primers agTEFp_Insert_ecoRI_F and agTEFp_Insert_BamHI_R.

**NMDv17.** *TDH3* promoter-based plasmid with HIS3 auxotrophy reporter; terminated with the *PGA1* 3'-UTR. Constructed by ligating the *TDH3* promoter into the EcoRI/BamHI site of NMDv9.2. The *TDH3* promoter was amplified from BY4741 genomic DNA with proper restriction sites using primers TDH3p_Inset_ecorI_F and TDH3p_Inset_bamhi_R.

**NMDv17.1.** *TDH3* promoter-based plasmid with *LEU2* auxotrophy reporter; terminated with the *PGA1* 3'-UTR. Constructed by ligating the *LEU2* ORF into the BamHI/PstI site of NMDv17. The *LEU2* ORF was amplified from CENPK2 genomic DNA with proper restriction sites using primers leu2_orf_bamhi_f and leu2_orf_psti_r.

**NMDv18.** *ALR1* promoter-based plasmid with *LEU2* auxotrophy reporter; terminated with the *PGA1* 3'UTR. Constructed by ligating the *ALR1* promoter into the EcoRI/BamHI site of NMDv17.1. The *ALR1* promoter was amplified from BY4741 genomic DNA using primers pALR1_insert_EcoRI_F and pALR1_insert_BamHI_R.

**NMDv19.** *HIS3* promoter-based plasmid with *LEU2* auxotrophy reporter; terminated with the *PGA1* 3'UTR. Constructed by ligating the *HIS3* promoter into the EcorRI/BamHI site of NMDv17.1. The *HIS3* promoter was amplified from BY4741 genomic DNA with proper restriction sites using primers EcorI_pHIS3_F and BamHI_pHIS3_R.

**pALR1_LEU2_PGA1 .** Plasmid identical to NMDv18 with the exception of the changing the *URA3* marker to a HIS3MX6 maker. Used for the primary library screening. Constructed by homologous recombination of the HIS3MX6 marker into BY4741 strain carrying NMDv18. HIS3MX6 maker was amplified from plasmid pYM28 (1) using primers his3mx6_18v2_F and his3mx6_18v2_R.

**pTDH3_HIS3_PGA1.** Plasmid identical to NMDv17 with the exception of the changing the *URA3* marker to a *LEU2* marker. Used for the tertiary library screening. Constructed by ligating the *LEU2* marker to AatII/NsiI site of plasmid NMDv17. The *LEU2* marker was amplified from CENPK2 genomic DNA with proper restriction sites using primers NsiI_Leu2_F and AatII_Leu2_R.

**pHA_URA2deltaCPaseV3.** pHA_URA2deltaCPaseV1 was constructed by using the NEBuilder® HiFi DNA Assembly. *URA2* fragments were amplified from BY4741 genomic DNA using primers URA2_ga_frag1_F with URA2_ga_frag1_R and URA2_ga_frag2_F with URA2_ga_frag2_R. The fragments were then assembled into AatII site of NMDv18 to create a form of *URA2* that lacks the CPase domain. pHA_URA2deltaCPaseV2 was constructed by NEBuilder® HiFi DNA Assembly. The *URA2* form from pHA_URA2deltaCPaseV1 was assembled into PvuII/BamHI digested pHLUMv2 (Addgene#64166) after its amplification with primers URA2deltaCPaseV2_GA_F and URA2deltaCPaseV2_GA_R. Finally, pHA_URA2deltaCPaseV3 was constructed by recombinational cloning to retain only the ACTase domain. The cloning was done using primers URA2_cpasedeltav3_oligo1 and URA2_cpasedeltav3_oligo2.

**URA2 deletion strains.** *URA2* was deleted in both BY4741 and *upf3Δ* using the his3mx6 marker that was amplified using primers URA2_HISMX_KO_F and URA2_HISMX_KO_R.

**RLI1-SMASh** strain. A DNA fragment containing 50 bp of homology 5' of the stop codon of *RLI1* fused in frame to an ORF encoding a HCV NS3 protease cleavage site, the HCV NS3 protease, and a degron sequence (2) terminated the *CYC1* terminator and was followed by the *URA3* marker in addition to 50 bp of homology 3' to the stop codon of *RLI1* was amplified using primers RLI1_SMASh_tag_F and RLI1_SMASh_tag_R from plasmid pAG426GPD-ccdB-YFP-yeastSMASh (Addgene #68855). The DNA fragment above was transformed into strain BY4741 to create strain RLI1-SMAsh. The correctness of the strain was verified with PCR and sequencing.

The transformation of cassettes and plasmids into each strain was done using the standard lithium acetate protocol.

***Primers***

| Primer | Sequence 5' to 3' |
|---|---|
| EcorI_pHIS3_F | GGTACGAATTCCTAGTACACTCTATATTTTTTTATGCC |
| BamHI_pHIS3_R | CGGAGGGATCCCTTTGCCTTCGTTTATCTTGC |
| leu2_orf_bamhi_f | GGTACGGATCCATGTCTGCCCCTAAGAAGAT |
| leu2_orf_psti_r | CGGAGCTGCAGTTAAGCAAGGATTTTCTTAACTTCTTCG |
| HIS3_ORF_BAMHI_F | GGTACGGATCCATGACAGAGCAGAAAGCCCT |
| HIS3_ORF_PstI_R | CGGAGCTGCAGCTACATAAGAACACCTTTGGTGGA |
| pALR1_insert_EcoRI_F | GGTACGAATTCGAAATTAGAACCGAATCAAAATAGTTCC |
| pALR1_insert_BamHI_R | CGGAGGGATCCGGTAAAATGCTTTTACGCTTTCT |
| EcoRI_pDAL4_F | GGTACGAATTCATAAAAATCCCAATTTATTCTCTTACCA |
| BamHI_pDAL4_R | CGGAGGGATCCTTTTCTAAAGAAGAGACCGATTAG |
| nmdv14_ga_stn1_F | GTAAAACGACGGCCAGTGAATTCGTGCCTCTCCGCCATTAGACA |
| nmdv14_ga_stn1_R | TTTCTGCTCTGTCTTATCCATGGCTCCAACCCAT |
| nmdv14_ga_HIS3_F | CCATGGATAAGACAGAGCAGAAAGCCCTAGTAAAG |
| nmdv14_ga_HIS3_R | TGCGGCCCTCCTGCAGCTACATAAGAACACCTTTGGTGGAGGGAAC |
| PGA1_3UTR_pstI_F | GGTACCTGCAGTATGGAAGCCTGAGCCCCC |
| PGA1_3UTR_hindIII_R | GTACCAAGCTTGCATCTGCATTGGGGTGGG |
| agTEFp_Insert_ecoRI_F | GGTACGAATTCAGCTTGCCTCGTCCCCG |
| agTEFp_Insert_BamHI_R | CGGAGGGATCCGGTTGTTTATGTTCGGATGTGATGT |
| TDH3p_Inset_ecorI_F | GGTACGAATTCCAGTTCGAGTTTATCATTATCAATACT |
| TDH3p_Inset_bamhi_R | CGGAGGGATCCTTTGTTTGTTTATGTGTGTTTATTCGAA |

| | |
|---|---|
| his3mx6_18v2_F | TAAGAAACCATTATTATCATGACATTAACCTATAAAGC TTGCCTCGTCCCCG |
| his3mx6_18v2_R | TTAAATTGAAGCTCTAATTTGTGAGTTTAGTATACACT GGATGGCGGCGTTAGT |
| NsiI_Leu2_F | GGTACATGCATGTGTTTTTTATTTGTTGTATTTTTTTTT TTTTAGAG |
| AatII_Leu2_R | CGGAGGACGTCAACTGTGGGAATACTCAGGTAT |
| URA2_ga_frag1_F | AAAAGTGCCACCTGACGTCATATATACTCATTCTTTAG AAATAGGTTTTCGTACTCAATATTTAAG |
| URA2_ga_frag1_R | GAAATGGCTTCAACCTTCTTCGCTTCGATTCTAGG |
| URA2_ga_frag2_F | CGAAGAAGGTTGAAGCCATTTCAAGAAATATCACTTT AGATGTTTCTGAAC |
| URA2_ga_frag2_R | TGTCATGATAATAATGGTTTCTTAGACGTATCCACGGA CCTGATGTTACCT |
| URA2deltaCPaseV2_GA_F | TTGGCCGATTCATTAATGCAGCATATATACTCATTCTT TAGAAATAGGTTTTCGTACTCAATATT |
| URA2deltaCPaseV2_GA_R | GCCTTACGACGTAGTCGAGATCCACGGACCTGATGTT ACCT |
| URA2_cpasedeltav3_oligo1 | TACCTAATAGAATATAACAATCATAATATGGAATTAG TTTCACCAGGCGCCAAAGGAAAA |
| URA2_cpasedeltav3_oligo2 | TTTTCCTTTGGCGCCTGGTGAAACTAATTCCATATTAT GATTGTTATATTCTATTAGGTA |
| URA2_HISMX_KO_F | TGCAGCATACAACACATAAACCTTACCTAATAGAATA TAACAATCATAATGACATGGAGGCCCAGAATAC |
| URA2_HISMX_KO_R | AAGTTTAATAATAGATTATAAATTTAAAATACGGATA GGTCTCTTATCATCAGTATAGCGACCAGCATTC |
| RLI1_SMASh_tag_F | AAGAACAAAAATCATCAGGAAACTACTTTTTCTTGGA TAACACCGGTATTgacgaaatggaagaatgtag |
| RLI1_SMASh_tag_R | AACCCAAAAATAAAACAATCGTCCTCTTGGTTCTCCGA ATCCCAAGATGCagagtgcaccataccacctt |

**References for Supplementary Methods:**


1. Janke C., Magiera M.M., Rathfelder N., Taxis C., Reber S., Maekawa H., *et al.* (2004) A versatile toolbox for PCR-based tagging of yeast genes: new fluorescent proteins, more markers and promoter substitution cassettes. *Yeast* **21**: 947–62.

2. Chung, H.K., Jacobs, C.L., Huo, Y., Yang, J., Krumm, S.A., Plemper, R.K., Tsien, R.Y. and Lin, M.Z. (2015) Tunable and reversible drug control of protein production via a self-excising degron. *Nature Chem Biol* **11**: 713-720