**Supplemental file 1A. Detailed genotypes for the strains used in this study.**

|  |  |
| --- | --- |
| **Strain** | **Genotype** |
| SK1  wild-type | *ho::LYS2 lys2 ura3 leu2::hisG his3::hisG trp1::hisG* |
| UB494 | *MAT***a** *ndc80-1* |
| UB877 | *MAT***a** */MATalpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2::pURA3-TetR-GFP::LEU2 CENV::TetOx224::HIS3 Ndc80-3V5:KanMX pCUP-CLB3::KANMX* |
| UB880 | *MAT***a** */MATalpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2::pURA3-TetR-GFP::LEU2 CENV::TetOx224::HIS3 KanMX:pCUP-Ndc80-3V5:CNAT pCUP-CLB3::KANMX* |
| UB885 | *MAT***a** */MATalpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2::pURA3-TetR-GFP::LEU2 CENV::TetOx224::HIS3 Ndc80-3V5:KanMX pCUP-CLB3::KANMX HISMX:pCUP-Spc25* |
| UB980 | *MAT***a** */MATalpha GAL-NDT80::TRP1/GAL-NDT80::TRP1 ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 leu2::pURA3-TetR-GFP::LEU2 CENV::TetOx224::HIS3 Ndc80-3V5:KanMX pCUP-CLB3::KANMX pCUP-SPC24::KANMX* |
| UB1051 | *MAT***a** */MATalpha GAL-NDT80::TRP1/GAL-NDT80::TRP1 ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 Spc25-3V5:HisMX/Spc25-3V5:HisMX* |
| UB1217 | *MAT***a** *HISMX:pGAL-Ndc80-3V5:KanMX ura3::pGPD1-GAL4(848).ER::URA3*  ***pGAL integrated 536 bp upstream of NDC80 AUG*** |
| UB1240 | *MAT***a** *Ndc80-3V5:KanMX ura3::pGPD1-GAL4(848).ER::URA3* |
| UB1323 | *MAT***a** *KanMX:pGAL-Δ9AUG-5'UTR-Ndc80-3V5:CNAT ura3::pGPD1-GAL4(848).ER::URA3*  ***pGAL integrated 536 bp upstream of NDC80 AUG***  ***ATG-ATC mutation in 9 of the 9 potential upstream start codons within NDC80-5'UTR. The leader sequence contains 2 SNPs from S288C introduced by a gene block and a third mutation (T🡪C) 8 bp after the 6th ATG.*** |
| UB1337 | *MAT***a** */MATalpha pCUP-IME1::NAT/pCUP-IME1::NAT pCUP-IME4::NAT/pCUP-IME4::NAT Ndc80-3V5:KanMX/Ndc80-3V5:KanMX* |
| UB2388 | *MAT***a** *amn1::KanMX6 ura3::pGPD1-GAL4(848).ER::URA3 HISMX:pGAL-Ndc80-3V5:KanMX* ***pGAL integrated 536 bp upstream of NDC80 AUG*** |
| UB2389 | *MAT***a** *amn1::KanMX6 Ndc80-3V5:KanMX ura3::pGPD1-GAL4(848).ER::URA3* |
| UB2531 | *MAT***a** */MATalpha irt1:cup1::Hphmx/irt1:cup1::Hphmx ime4::cup1::NAT/ime4::cup1::NAT* |
| UB2936 | *MAT***a** */MATalpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2::pURA3-TetR-GFP::LEU2 CENV::TetOx224::HIS3 HisMX:Δ9AUG-5'UTR-Ndc80-3V5:CNAT/HisMX:Δ9AUG-5'UTR-Ndc80-3V5:CNAT pCUP-CLB3::KANMX*  ***ATG-ATC mutation in 9 of the 9 potential upstream start codons within NDC80-5'UTR. The leader sequence contains 2 other point mutations, in addition to the ATCs.*** |
| UB2940 | *MAT***a** */MATalpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2::pURA3-TetR-GFP::LEU2 CENV::TetOx224::HIS3 pCUP-CLB3::KANMX HIS3MX::(Δ-600 to -400)-NDC80-3V5::KanMX/HIS3MX::(Δ-600 to -400 bp)-NDC80-3V5::KanMX* |
| UB2942 | *MAT***a** */MATalpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2::pURA3-TetR-GFP::LEU2 CENV::TetOx224::HIS3 Ndc80-3V5:KanMX/Ndc80-3V5:KanMX* |
| UB3262 | *MAT***a** *ndc80Δ:KanMX4 leu2::NDC80-3V5:LEU2* |
| UB3301 | *MAT***a** */MATalpha UME6-3V5::His3MX/UME6-3V5::His3MX irt1:cup1::Hphmx/irt1:cup1::Hphmx ime4::cup1::NAT/ime4::cup1::NAT* |
| UB3392 | *MAT***a** */MATalpha GAL-NDT80::TRP1/GAL-NDT80::TRP1 ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 ndc80∆(-1000 and ORF):KanMX4/ndc80∆(-1000 and ORF):KanMX4 leu2::mse-NDC80-3V5:LEU2/leu2::mse-NDC80-3V5:LEU2* |
| UB4074 | *MAT***a** */MATalpha GAL-NDT80::TRP1/GAL-NDT80::TRP1 ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 ndc80Δ(-1000 and ORF):KanMX4/ndc80Δ(-1000 and ORF):KanMX4 leu2::NDC80-3V5:LEU2/leu2::NDC80-3V5:LEU2* |
| UB4212 | *MAT***a** *leu2::urs1Δ-NDC80-3V5:LEU2 ndc80Δ::KanMX* |
| UB4361 | *MAT***a** */MATalpha GAL-NDT80::TRP1/GAL-NDT80::TRP1 ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 SPC24-3V5:KanMX/SPC24-3V5:KanMX NUF2-3V5:HisMX/NUF2-3V5:HisMX NDC80-3V5:CNAT/NDC80-3V5:CNAT* |
| UB4432 | *MAT***a** */MATalpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 CENV::TetOx224::HIS3 leu2::pURA3-TetR-GFP::LEU2 NDC80-3V5:KanMX* |
| UB4434 | *MAT***a** */MATalpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 CENV::TetOx224::HIS3 leu2::pURA3-TetR-GFP::LEU2 NDC80-3V5:KanMX pCUP-CLB3::KANMX* |
| UB4436 | *MAT***a** */MATalpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 CENV::TetOx224::HIS3 leu2::pURA3-TetR-GFP::LEU2 Ndc80-3V5:KanMX pCUP-Nuf2::KANMX pCUP-CLB3::KANMX* |
| UB5101 | *MAT***a** */MATalpha pCUP-IME1::NAT/pCUP-IME1::NAT pCUP-IME4::NAT/pCUP-IME4::NAT nuf2::KanMX/nuf2::KanMX leu2::NDC80(-1000 to -1)-NUF2-3V5:LEU2/leu2::NDC80(-1000 to -1)-NUF2-3V5:LEU2* |
| UB5103 | *MAT***a** */MATalpha pCUP-IME1::NAT/pCUP-IME1::NAT pCUP-IME4::NAT/pCUP-IME4::NAT nuf2::KanMX/nuf2::KanMX leu2::NUF2-3V5:LEU2/leu2::NUF2-3V5:LEU2* |
| UB5437 | *MAT***a** */MATalpha ndc80Δ(-1000 and ORF):KanMX4/ndc80Δ(-1000 and ORF):KanMX4 leu2::mse-NDC80-3V5:LEU2/leu2::mse-NDC80-3V5:LEU2 CENV::tetOx224::HIS3/CENV::tetOx224::HIS3 his3::pURA3-TetR-GFP::HIS3/his3::pURA3-TetR-GFP::HIS3* |
| UB5473 | *MAT***a** */MATalpha leu2::urs1Δ-NDC80-3V5:LEU2/leu2::urs1Δ-NDC80-3V5:LEU2 ndc80Δ(-1000 and ORF):KanMX4/ndc80Δ(-1000 and ORF):KanMX4* |
| UB5875 | *MAT***a** */MATalpha leu2::NDC80-3V5:LEU2/leu2::NDC80-3V5:LEU2 ndc80Δ(-1000 and ORF):KanMX4/ndc80Δ(-1000 and ORF):KanMX4* |
| UB5876 | *MAT***a** */MATalpha ndc80∆(-1000 and ORF):KanMX4/ndc80∆(-1000 and ORF):KanMX4 leu2::NDC80-3V5:LEU2/leu2::NDC80-3V5:LEU2 CENV::tetOx224::HIS3/CENV::tetOx224::HIS3 his3::pURA3-TetR-GFP::HIS3/his3::pURA3-TetR-GFP::HIS3* |
| UB6075 | *MAT***a** */Matalpha irt1:cup1::Hphmx/irt1:cup1::Hphmx ime4::cup1::NAT/ime4::cup1::NAT leu2::urs1Δ-NDC80-3V5:LEU2/leu2::urs1Δ-NDC80-3V5:LEU2 ndc80∆(-1000 and ORF):KanMX4/ndc80∆(-1000 and ORF):KanMX4* |
| UB6077 | *MAT***a** */Matalpha irt1:cup1::Hphmx/irt1:cup1::Hphmx ime4::cup1::NAT/ime4::cup1::NAT leu2::(-295::ADH1)-NDC80-3V5:LEU2/leu2::(-295::ADH1)-NDC80-3V5:LEU2 ndc80Δ(-1000 and ORF):KanMX4/ndc80Δ(-1000 and ORF):KanMX4* |
| UB6079 | *MAT***a** */Matalpha irt1:cup1::Hphmx/irt1:cup1::Hphmx ime4::cup1::NAT/ime4::cup1::NAT leu2::(Δ-600 to -479)-NDC80-3V5:LEU2/leu2::(Δ-600 to -479)-NDC80-3V5:LEU2 ndc80∆(-1000 and ORF):KanMX4 /ndc80∆(-1000 and ORF):KanMX4* |
| UB6181 | *MAT***a** */Matalpha irt1:cup1::Hphmx/irt1:cup1::Hphmx ime4::cup1::NAT/ime4::cup1::NAT leu2::Δ6AUG-NDC80-3V5:LEU2/leu2::Δ6AUG-NDC80-3V5:LEU2 ndc80Δ(-1000 and ORF):KanMX4/ndc80Δ(-1000 and ORF):KanMX4*  ***ATG-ATC mutation in 6 of the 9 potential upstream start codons within NDC80-5'UTR*** |
| UB6183 | *MAT***a** */MATalpha irt1:cup1::Hphmx/irt1:cup1::Hphmx ime4::cup1::NAT/ime4::cup1::NAT leu2::Δ9AUG-NDC80-3V5:LEU2/leu2::Δ9AUG-NDC80-3V5:LEU2 ndc80Δ(-1000 and ORF):KanMX4 /ndc80Δ(-1000 and ORF):KanMX4*  ***ATG-ATC mutation in 9 of the 9 potential upstream start codons within NDC80-5'UTR*** |
| UB6190 | *MAT***a** */MATalpha irt1:cup1::Hphmx/irt1:cup1::Hphmx ime4::cup1::NAT/ime4::cup1::NAT ndc80Δ(-1000 and ORF):KanMX4 /ndc80Δ(-1000 and ORF):KanMX4 leu2::NDC80-3V5:LEU2/leu2::NDC80-3V5:LEU2* |
| UB6295 | *MAT***a** *HISMX:pGAL-Ndc80-3V5:KanMX trp1::pGAL-dCas9-MxiI::TRP1 ura3::pGPD1-GAL4(848).ER::URA3 2micron\_plasmid\_LEU2*  ***pGAL integrated 536 bp upstream of NDC80 AUG*** |
| UB6297 | *MAT***a** *HISMX:pGAL-Ndc80-3V5:KanMX trp1::pGAL-dCas9-MxiI::TRP1 ura3::pGPD1-GAL4(848).ER::URA3 2micron\_plasmid\_LEU2\_NDC80guideA*  ***pGAL integrated 536 bp upstream of NDC80 AUG*** |
| UB6299 | *MAT***a** *HISMX:pGAL-Ndc80-3V5:KanMX trp1::pGAL-dCas9-MxiI::TRP1 ura3::pGPD1-GAL4(848).ER::URA3 2micron\_plasmid\_LEU2\_NDC80guideB*  ***pGAL integrated 536 bp upstream of NDC80 AUG*** |
| UB6301 | *MAT***a** *HISMX:pGAL-Ndc80-3V5:KanMX trp1::pGAL-dCas9-MxiI::TRP1 ura3::pGPD1-GAL4(848).ER::URA3 2micron\_plasmid\_LEU2\_NDC80guideC*  ***pGAL integrated 536 bp upstream of NDC80 AUG*** |
| UB6302 | *MAT***a** *HISMX:pGAL-Ndc80-3V5:KanMX trp1::pGAL-dCas9-MxiI::TRP1 ura3::pGPD1-GAL4(848).ER::URA3 2micron\_plasmid\_LEU2\_NDC80guideD*  ***pGAL integrated 536 bp upstream of NDC80 AUG*** |
| UB6760 | *MAT***a** */MATalpha Ume6-3V5::His3MX/Ume6-3V5::His3MX irt1:cup1::Hphmx/irt1:cup1::Hphmx ime4::cup1::NAT/ime4::cup1::NAT leu2::urs1Δ-NDC80:LEU2/leu2::urs1Δ-NDC80:LEU2 ndc80Δ(-1000 and ORF):KanMX4/ndc80Δ(-1000 and ORF):KanMX4* |
| UB7496 | *MAT***a** */MATalpha TRP1::GAL-NDT80-3V5::KanMX/TRP1::GAL-NDT80-3V5::KanMX ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 ndc80Δ(-1000 and ORF):KanMX4/ndc80Δ(-1000 and ORF):KanMX4 leu2::mse-NDC80:LEU2/leu2::mse-NDC80:LEU2* |
| UB7997 | *MAT***a** */MATalpha GAL-NDT80::TRP1/GAL-NDT80::TRP1 ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 leu2::NDC80:LEU2/leu2::NDC80:LEU2 ndc80Δ(-1000 and ORF):KanMX4/ndc80Δ(-1000 and ORF):KanMX4* |
| UB7999 | *MAT***a** */MATalphaTRP1::GAL-NDT80-3V5::KanMX/TRP1::GAL-NDT80-3V5::KanMX ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 leu2::NDC80:LEU2/leu2::NDC80:LEU2 ndc80Δ(-1000 and ORF):KanMX4/ndc80Δ(-1000 and ORF):KanMX4* |
| UB8001 | *MAT***a** *HISMX:pGAL-Ndc80-3V5:KanMX ura3::pGPD1-GAL4(848).ER::URA3 leu2::NDC80-3V5:LEU2* ***pGAL integrated 536 bp upstream of Ndc80 AUG*** |
| UB8144 | *MAT***a** */MATalpha GAL-NDT80::TRP1/GAL-NDT80::TRP1 ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 ZIP1::GFP(700)/ZIP1::GFP(700)*  ***GFP is inserted internally of the coding region of ZIP1*** |
| UB8682 | *MAT***a** *ura3::pGPD1-GAL4(848).ER::URA3 amn1::HygB CENV::TetOx224::HIS3 leu2::pURA3-TetR-GFP::LEU2 SPC42-mCherry::NAT* |
| UB8684 | *MAT***a** *ura3::pGPD1-GAL4(848).ER::URA3 HISMX:pGAL-Ndc80-3V5:KanMX amn1::HygB CENV::TetOx224::HIS3 leu2::pURA3-TetR-GFP::LEU2 SPC42-mCherry::NAT*  ***pGAL integrated 536 bp upstream of Ndc80 AUG*** |
| UB9243 | *MAT***a** */MATalpha irt1:cup1::Hphmx/irt1:cup1::Hphmx ime4::cup1::NAT/ime4::cup1::NAT leu2::uORF(mini)-NDC80-3V5::LEU2/leu2::uORF(mini)-NDC80-3V5::LEU2 ndc80Δ(-1000 and ORF):KanMX4/ndc80Δ(-1000 and ORF):KanMX4* |
| UB12543 | *MAT****a*** */MATalpha ura3::pGPD1-GAL4(848).ER::URA3/ ura3::pGPD1-GAL4(848).ER::URA3 GALNDT80::TRP1/ GAL-NDT80::TRP1* ***CENV::TetOx224::HIS3 leu2::pURA3-TetR-GFP::LEU2*** *KANMX::pCUP-SPC24-3V5::HisMX pCUP-CLB3::KANMX* |
| UB12547 | *MAT****a*** */MATalpha ura3::pGPD1-GAL4(848).ER::URA3/ ura3::pGPD1-GAL4(848).ER::URA3 GALNDT80::TRP1/ GAL-NDT80::TRP1* ***CENV::TetOx224::HIS3 leu2::pURA3-TetR-GFP::LEU2*** *HISMX::pCUP-SPC25-3V5::KanMX pCUP-CLB3::KANMX* |
| UB12662 | *MAT****a*** */MATalpha ura3::pGPD1-GAL4(848).ER::URA3/ ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/ GAL-NDT80::TRP1* ***CENV::TetOx224::HIS3 leu2::pURA3-TetR-GFP::LEU2*** *KANMX::pCUP-NUF2-3V5::HISMX pCUP-CLB3::KANMX* |
| FW1208 | *MAT***a** */MATalpha UME6-3V5::His3MX/UME6-3V5::His3MX* |
| FW1511 | *MAT***a** */MATalpha* |
| FW1871 | *MAT***a** */MATalpha ime1::pCUP-IME1::NatMX/ime1::pCUP-IME1::NatMX ime4::pCUP-IME4::NatMX/ime4::pCUP-3HA-IME4::NatMX NDC80-3V5::KanMX/NDC80-3V5::KanMX (Δ-600 to -300)-NDC80::His3MX/(Δ-600 to -300)-NDC80::His3MX* |
| FW1899 | *MAT***a** */MATalpha ime1::pCUP-IME1::NatMX/ime1::pCUP-IME1::NatMX ime4::pCUP-IME4::NatMX/ime4::pCUP-IME4::NatMX NDC80-3V5:KanMX/(Δ-600 to -300)-NDC80::His3MX* |
| FW1900 | *MAT***a** */MATalpha ime1::pCUP-IME1::NatMX/ime1::pCUP-IME1::NatMX ime4::pCUP-IME4::NatMX/ime4::pCUP-IME4::NatMX NDC80-3V5:KanMX* |
| FW1902 | *MAT***a** */MATalpha ime1::pCUP-IME1::HphMX/ime1::pCUP-IME1:: HphMX ime4::pCUP-IME4::NatMX/ime4::pCUP-IME4:: NatMX NDC80-3V5::KanMX/ NDC80-3V5::KanMX* |
| FW1923 | *MAT***a** */MATalpha ime1::pCUP-IME1::NatMX/ime1::pCUP-IME1::NatMX ime4::pCUP-IME4::NatMX/ime4::pCUP-IME4::NatMX HisMX::(Δ-600 to -300)-NDC80-3V5:KanMX/NDC80* |
| FW3058 | *MAT***a** */MATalpha ime1::His3MX/ime1::His3MX ime4::pCUP-IME4::NatMX/ime4::pCUP-IME4::NatMX NDC80-3V5::KanMX/NDC80-3V5::KanMX* |

**Supplemental file 1B. Primers used for strain construction in this study.**

|  |  |  |
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| **Construct name** | **Forward primer** | **Reverse primer** |
| *NUF2-3V5* | TTAATAAATACATGAATGAAATGCTCGAATATATGCAAgcggccgctctagaactagtgg | CACAGAAGGGGGAGTAAAAATAAGTATACCGCTGCTAccccctcgaggtcgacggtatcg |
| *SPC24-3V5* | TCTATAAGACCAAATACATCTGGGAAAGATTAGGAAAGgcggccgctctagaactagtgg | TGCCACTGTAGTATTTTATTAATGATCTCAATTTTCAccccctcgaggtcgacggtatcg |
| *SPC25-3V5* | TTTTAGTCGTGGCCCGCGATATGCTTCTGGCATCTTTAgcggccgctctagaactagtgg | CCCAGAATAAACTGAACAGATGCGTATAAAGGCGTTAccccctcgaggtcgacggtatcg |
| *pCUP-NUF2* | TGGTAAAAAGCATGTACTGAGGAGAAAGGCTCCAGCATCCGAATTCGAGCTCGTTTAAAC | GTAGATCCAAAATGGGGAACACATCTTGATTCCTACTCATTTTATGTGATGATTGATTGATTGATTG |
| *pCUP-SPC24* | AAAGAAGTAACGAGGGAAGAGCAAGTGTAAAGGGAAGGAAGAATTCGAGCTCGTTTAAAC | ATTCAACCGGATTGTCGAGTAGGTTATCCTTTTGTGACATTTTATGTGATGATTGATTGATTGATTG |
| *pCUP-SPC25* | GATTCAATTAAAACCGCTCATACGTATACAACACATACACACATACAAGAgaattcgagctcgtttaaac | AACCCGTCCATCCGGCGCTCAAGGTCCGAAAATGCGTCTATGCTGGCCATtttatgtgatgattgattgattgattg |
| *ndc80∆(-1000 and ORF)* | AAGCAATTTGATGTCGTCTGGCAAAGTTCGAGGATAGTTAcggatccccgggttaattaa | TTGCTGTAGATTGCTCGGGTATTATATATCATTTATTTTAgaattcgagctcgtttaaac |
| *ndc80∆* | GGAGAGGTAGAATCGTCCCTG | GAGTGAGGGTGGAATTGAAC |
| *nuf2∆* | CGTATTGGGTTTATCGCTTTGG | CATCTTGTGTGACTTGCC |
| *pGAL-NDC80luti* | GATATCTGTTCAGCCAACATTATAAAAAAGATGGGCGCTT GAATTCGAGCTCGTTTAAAC | CTGATGTAAGTTAATTAGAAGGAGTGATATCTGTAAGAGTATTTTGAGATCCGGGTTTT |
| *(∆-600 to -300)* | AAAGTAACATTTACCCGGATATCTGTTCAGCCAACATTAT  CGGATCCCCGGGTTAATTAA | TATTTTAACCGCTAATCGCAATAGACTGCTTACATCTTTAGAATTCGAGCTCGTTTAAAC |

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| **LEU2 single integration plasmids** | **Plasmid number** |
| *NDC80* (untagged) | pUB872 |
| *NDC80* | pUB873 |
| *∆9AUG* | pUB880 |
| *∆6AUG* | pUB895 |
| *mini uORF* | pUB905 |
| *∆NDC80luti (∆-600 to -479)* | pUB883 |
| *NDC80luti-NUF2* | pUB665 |
| *NUF2* | pUB666 |
| *ndc80-∆urs1* | pUB882 |
| *ndc80-mse* | pUB875 |
| *NDC80luti-Ter* | pUB894 |

**Supplemental file 1C. Plasmids used for strain construction in this study.**

|  |  |  |
| --- | --- | --- |
| **2-micron plasmids** | **Guide RNA template** | **Plasmid number** |
| Guide A | AGCAACAATTCGCCACAAGA | pUB759 |
| Guide B | GCCACAAGAAGGTCTCTGTA | pUB760 |
| Guide C | TAGGAGCAGGGCTCTTGTCA | pUB761 |
| Guide D | CTGTGAATGCAAAGGAAAGA | pUB762 |
| Empty vector | N/A | pUB52 |

**Supplemental file 1D. Primers used for quantitative PCR and northern blotting in this study.**

|  |  |
| --- | --- |
| **Primer Name** | **Oligonucleotide sequence from 5’ to 3’** |
| *NDC80\_probe\_F* | GGAGAGGTAGAATCGTCCCTG |
| *NDC80\_probe\_R* | CTCCTCTTGAATAGCGCTTTGG |
| *NUF2\_probe\_F* | AACAGGGGATGGTCACTTACAGG |
| *NUF2\_probe\_R* | CCCACAAGTTCCGTTTCAGTTCG |
| *SCR1\_probe\_F* | GAAGTGTCCCGGCTATAATAAA |
| *SCR1\_probe\_R* | GACGCTGGATAAAACTCCCC |
| *CIT1\_probe\_F* | CCGTGTTAGACCCCGAAGAAG |
| *CIT1\_probe\_R* | GGGCAGAAACGTTACCACCTTC |
| *NDC80\_2\_F* | ACCCGGATATCTGTTCAGCC |
| *NDC80\_2\_R* | TGTGGCGAATTGTTGCTCTT |
| *NDC80\_6\_F* | GGTTGAGAGCCCCGTTAAGT |
| *NDC80\_6\_R* | TTGGCACTTTCAGTATGGGT |
| *NDC80\_7\_F* | CCCATACTGAAAGTGCCAAAAGA |
| *NDC80\_7\_R* | GGGACGATTCTACCTCTCCTGTG |
| *pNUF2\_F* | GTCGCTGCGTATTCAGCGTA |
| *pNUF2\_R* | GAACGCTGATATACTCGACTAAC |
| *ACT1\_F* | GTACCACCATGTTCCCAGGTATT |
| *ACT1\_R* | AGATGGACCACTTTCGTCGT |
| *NDC80\_9\_F* | TGCAAAGCTCAACAAGTACTGA |
| *NDC80\_9\_R* | TGCAGTTGGTATTTGGGACG |
| *NDC80\_ORF\_F* | ATCCGAGTGTGAACTGAAAGAAG |
| *NDC80\_ORF\_R* | GAACTGCTCAGTTGAAATTCCC |
| *IME2\_URS1\_F* | CCAAATACGCTTTTTAAACTTGG |
| *IME2\_URS1\_R* | CTCAAATAGCCGCCGTAAC |
| *MAM1\_MSE\_F* | CACAATTGAAATCCGAGCTGT |
| *MAM1\_MSE\_R* | CATCTGAATTTTGAATGGCTTT |

**Supplemental file 1E. smFISH oligonucleotide probes used in this study.**

The NDC80ORF (Q 670) probe set consists of a mixture of thirty 20-mer oligonucleotide probes that tile the common region shared between *NDC80luti* and *NDC80ORF*. Each individual probe is labeled with the Quasar 670® dye.

The NDC80Long (CF 590) probe set consists of a mixture of twenty 20-mer oligonucleotide probes that tile the unique 5’ region of *NDC80luti*. Each individual probe is labeled with the CAL Fluor Red 590® dye.

|  |  |  |  |
| --- | --- | --- | --- |
| **Probe name** | **Probe sequence** | **Probe name** | **Probe sequence** |
| NDC80ORF\_1 | tccatgtgatgtagcacatg | NDC80Long\_1 | ttttgctttcttactgatgt |
| NDC80ORF\_2 | cagttggtatttgggacgta | NDC80Long\_2 | tcttgtggcgaattgttgct |
| NDC80ORF\_3 | ctgtttcttctcctcaattg | NDC80Long\_3 | cctattgaccctacagagac |
| NDC80ORF\_4 | atgtcggttagaccttgatt | NDC80Long\_4 | tttccctgacaagagccctg |
| NDC80ORF\_5 | ttgtattcctggcaatactc | NDC80Long\_5 | tctttcctttgcattcacag |
| NDC80ORF\_6 | ttttatttatgcctcctgtg | NDC80Long\_6 | aatgcttttcggacctccaa |
| NDC80ORF\_7 | aatgctgtaccatttgtacc | NDC80Long\_7 | tctcttcaatcctaacatca |
| NDC80ORF\_8 | tgacgctgtttctactgttg | NDC80Long\_8 | ctggcacatagtacggtgaa |
| NDC80ORF\_9 | gctgccaagttgatttattg | NDC80Long\_9 | ttcaatgttcagttataacc |
| NDC80ORF\_10 | agtttttgtctcttagtggc | NDC80Long\_10 | cagcccataatcacgatatt |
| NDC80ORF\_11 | aatctcctcttgaatagcgc | NDC80Long\_11 | aatacttaacggggctctca |
| NDC80ORF\_12 | tagtaaagccgtaacctgga | NDC80Long\_12 | atagactgcttacatcttta |
| NDC80ORF\_13 | acctacagccgaaatttgtg | NDC80Long\_13 | ggtattttaaccgctaatcg |
| NDC80ORF\_14 | atgccaagaaatttgtgcca | NDC80Long\_14 | cagtatgggtaacccttgaa |
| NDC80ORF\_15 | gctggctcagaattgttatt | NDC80Long\_15 | tctttttttcttttggcact |
| NDC80ORF\_16 | ctgttcgtccaaagtcttta | NDC80Long\_16 | gactatatcattccatacgt |
| NDC80ORF\_17 | ttcagttcttgcatcgaagg | NDC80Long\_17 | ttttaggaaatattagtttt |
| NDC80ORF\_18 | tggttttgatcttttggctt | NDC80Long\_18 | ccatttttggtgttgtttgt |
| NDC80ORF\_19 | ctcagttgaaattccctttt | NDC80Long\_19 | gtgaatgtattccaattatt |
| NDC80ORF\_20 | tttatcaagttccctagtca | NDC80Long\_20 | cagggacgattctacctctc |
| NDC80ORF\_21 | ttccagctttctggatttaa |  |  |
| NDC80ORF\_22 | cgaatcgtattgcctcaacg |  |  |
| NDC80ORF\_23 | gactcgttaattccagatcc |  |  |
| NDC80ORF\_24 | gttgtctttctcaatggttt |  |  |
| NDC80ORF\_25 | ttcgttctcttgcttagaga |  |  |
| NDC80ORF\_26 | ctcaattctttgcgctacta |  |  |
| NDC80ORF\_27 | gaagttaccaattcctcagc |  |  |
| NDC80ORF\_28 | ttcttccaattttagttccg |  |  |
| NDC80ORF\_29 | cttgtatcgtttcctgttta |  |  |
| NDC80ORF\_30 | catgtatcacttgttggtgt |  |  |

**Supplemental file 1E (continued). smFISH oligonucleotide probes used in this study.**

The NDC80Odd (CF 590) probe set consists of a mixture of twenty-seven 20-mer oligonucleotide probes that tile *NDC80ORF*. Each individual probe is labeled with the CAL Fluor Red 590® dye.

The NDC80Even (Q 670) probe set consists of a mixture of twenty-seven 20-mer oligonucleotide probes that tile *NDC80ORF*. Each individual probe is labeled with the Quasar 670® dye.

|  |  |  |  |
| --- | --- | --- | --- |
| **Probe name** | **Probe sequence** | **Probe name** | **Probe sequence** |
| NDC80Odd\_1 | atttttcttgttccgtttca | NDC80Even\_1 | atagtacaccctaacgttta |
| NDC80Odd\_2 | acgggtatctcttatggaat | NDC80Even\_2 | acttgttgagctttgcattt |
| NDC80Odd\_3 | tgtgatgtagcacatgttga | NDC80Even\_3 | gacgtaaaccgatgagggtc |
| NDC80Odd\_4 | acgatgttgcagttggtatt | NDC80Even\_4 | ctgtttcttctcctcaattg |
| NDC80Odd\_5 | atgtcggttagaccttgatt | NDC80Even\_5 | ttgtattcctggcaatactc |
| NDC80Odd\_6 | ttttatttatgcctcctgtg | NDC80Even\_6 | aatgctgtaccatttgtacc |
| NDC80Odd\_7 | tgacgctgtttctactgttg | NDC80Even\_7 | gctgccaagttgatttattg |
| NDC80Odd\_8 | ctattgctcagatgttgctg | NDC80Even\_8 | agtttttgtctcttagtggc |
| NDC80Odd\_9 | aatctcctcttgaatagcgc | NDC80Even\_9 | aataaaccccttttgagtgg |
| NDC80Odd\_10 | tagtaaagccgtaacctgga | NDC80Even\_10 | acctacagccgaaatttgtg |
| NDC80Odd\_11 | atgccaagaaatttgtgcca | NDC80Even\_11 | tttgttcgtaccatccaatg |
| NDC80Odd\_12 | ttcaagcacatatccagttt | NDC80Even\_12 | gctggctcagaattgttatt |
| NDC80Odd\_13 | ctgttcgtccaaagtcttta | NDC80Even\_13 | gctcatatctttcttgtctt |
| NDC80Odd\_14 | attaacagtttctccaccat | NDC80Even\_14 | ttcagttcttgcatcgaagg |
| NDC80Odd\_15 | gattgtcattttgggtttgt | NDC80Even\_15 | tggttttgatcttttggctt |
| NDC80Odd\_16 | cttttcaaagccttccattt | NDC80Even\_16 | ttcttgactcttttgcttca |
| NDC80Odd\_17 | ggatttcatcttttccagtt | NDC80Even\_17 | tcttcttctttcagttcaca |
| NDC80Odd\_18 | ctcagttgaaattccctttt | NDC80Even\_18 | tttctctttcttggttttgt |
| NDC80Odd\_19 | tttatcaagttccctagtca | NDC80Even\_19 | ttccagctttctggatttaa |
| NDC80Odd\_20 | agcttttgaatattccctcg | NDC80Even\_20 | cgaatcgtattgcctcaacg |
| NDC80Odd\_21 | ccacgcgatctggttaaatt | NDC80Even\_21 | ctcgtaggagatagcttcat |
| NDC80Odd\_22 | gactcgttaattccagatcc | NDC80Even\_22 | gttgtctttctcaatggttt |
| NDC80Odd\_23 | attcgcttcggataattcca | NDC80Even\_23 | ttcgttctcttgcttagaga |
| NDC80Odd\_24 | ctcaattctttgcgctacta | NDC80Even\_24 | gaagttaccaattcctcagc |
| NDC80Odd\_25 | ttcttccaattttagttccg | NDC80Even\_25 | cttgtatcgtttcctgttta |
| NDC80Odd\_26 | catgtatcacttgttggtgt | NDC80Even\_26 | ttcgttttcagagttttcca |
| NDC80Odd\_27 | actcttcaatgacgtttcct | NDC80Even\_27 | atgttcagtttcaaactcca |

**Supplemental file 1F. Summarized smFISH results for this study.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Figure** | **Conditions** | **Median of the number of *NDC80luti* transcripts per cell** | **Median of the number of *NDC80ORF* transcripts per cell** |
| 2D | Vegetative growth | 0 | 4 |
| Meiotic prophase | 21 | 3 |
| Meiosis I | 6 | 24 |

Note: This strain (UB8144) harbored the *pGAL-NDT80* *GAL4-ER* system. Meiotic prophase cells were defined as Zip1-GFP positive cells, and meiosis I cells were defined as Zip1-GFP negative cells present after release from the *pGAL-NDT80* block.

|  |  |  |  |
| --- | --- | --- | --- |
| **Figure** | **Conditions** | **Median of the number of *NDC80luti* transcripts per cell** | **Median of the number of *NDC80ORF* transcripts per cell** |
| Fig. 2-S6 | wild-type  Pre-meiotic stage | 0 | 5 |
| Fig. 2-S6, 4C, 6H | wild-type  Meiotic prophase | 15 | 4 |
| 4C | *∆NDC80luti*  Meiotic prophase | 1 | 6 |
| 6H | *ndc80-urs1∆*  Meiotic prophase | 5 | 4 |

Note:Different from in the strain UB8144 listed above, these 3 strains (wild-type cells (UB6190), ∆*NDC80luti* (UB6079), and *ndc80-urs1∆* (UB6075)) harbored the *pCUP-IME1 pCUP-IME4* system. Meiotic prophase was staged as 2 hr after Cu addition, and pre-meiotic cells was staged as prior to Cu addition.

|  |  |  |  |
| --- | --- | --- | --- |
| **Figure** | **Conditions** | **Median of the number of *NDC80luti* transcripts per cell** | **Median of the number of *NDC80ORF* transcripts per cell** |
| 6E | wild-type  Vegetative growth | 0 | 3 |
| *ndc80-urs1∆*  Vegetative growth | 3 | 2 |

Note: These two strains (wild-type cells (UB5875) and *ndc80-urs1∆* (UB5473)) do not carry either the *pGAL-NDT80 GAL4-ER* or the *pCUP-IME1* *pCUP-IME4* system.

**Supplemental file 1G. Predicted peptide sequences for the putative AUG uORFs.**

|  |  |  |
| --- | --- | --- |
| Species | Number of uORFs | Predicted uORF peptide sequence |
| *S. bayanus* | 10 | MARRTEQDNKKKVTRQTNAG\*  MRADLMPFDSLW\*  MVMVFKYRKEHQRCKYVSRFVGTRWLRPVLT\*  MVFKYRKEHQRCKYVSRFVGTRWLRPVLT\*  MCLDLSVHGG\*  MI\*  MSDHGGNKHTARSGWLCSAVKGLSMALAT\*  MVVINIQLDRVGSARRLRGFQWHLLPNLLPSAAKLRYPASNTQKDAFLRTK\*  MIIHKTKISKINKRHQKWQKTKEIDSIEEVGIYHWLFRDRTVKK\*  MAKNKRNR\* |
| *S. kudriavzevii* | 8 | MRLVLE\*  MRTKRLRVLNQFRFIGAKIEQRLCCSAC\*  MLEPIAIVL\*  MS\*  MALSDS\*  MGYPSQEIQFNASPRTK\*  MIYPQNENTQNKQTTPKMVKQKR\*  MVKQKR\* |
| *S. mikatae* | 5 | MIQVLLVYYHQIVVRE\*  MLRKAKKTGGPKSIGVRIGEELYYTTGHI\*  MTTSRWDPCLVTLNI\*  MFSKVNKEHQK\*  MEIDNLGGG\* |
| *S. paradoxus* | 6 | MS\*  MHIGWEPC\*  MLTMVRM\*  MDHNV\*  MIYTKLNSPKINKQHQKWQELLE\*  MARIIGIDCVGEVQSVPSFCQKLKQSKKNN\* |
| *S. cerevisiae* | 9 | MQRKDGWRSEKHY\*  MLGLKRNSPYYVPDGYN\*  MCQMVITEH\*  MVITEH\*  MGWLRAPLSIVKDVSSLLRLAVKIPFQGLPILKVPKEKKITYGMI\*  M\*  ME\*  MI\*  MVEIIGIHSQER\* |