

Supplemental Text 1: Details of strain construction for Black and Johnson, 2021

AJY4281: Tag-swapping by homologous recombination of a *6xGly-FLAG-TEV-ProtA::KIURA3* cassette amplified from pAJ4028 into AJY3283 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ENP1-GFP::HIS3MX6*; (Huh et al. 2003)) to replace the *GFP::HIS3MX6* tag. Note that this strain grows faster than BY4741 for an unknown reason and was replaced with AJY4724.

AJY4283: Genomic integration of *ENP1-6xGly-FTP::KIURA3* amplified from AJY4281 into AJY3398 (Black et al. 2019).

AJY4285: Genomic integration of *ENP1-6xGly-FTP::KIURA3* amplified from AJY4281 into AJY3711 (Sardana et al. 2015).

AJY4295: Genomic integration of a *PTH-BUD23* amplicon, containing the native *BUD23* promoter, made by stitch PCR into AJY4293 (*bud23Δ::CloNAT*; A. Johnson).

AJY4705: Genomic integration of *ENP1-6xGly-FTP::KIURA3* amplified from AJY4283 into ToY256 (Ferreira-Cerca et al. 2005).

AJY4706: Genomic integration of *ENP1-6xGly-FTP::KIURA3* amplified from AJY4283 into ToY286 (Ferreira-Cerca et al. 2005).

AJY4707: Genomic integration of *ENP1-6xGly-FTP::KIURA3* amplified from AJY4283 into Y801 (Linnemann et al. 2019).

AJY4710: Genomic integration of *RIO2-AID-HA_PGAL1-OsTIR1_GAL4BD-ER-VP16::CloNAT* amplified from AJY4733 into BY4741.

AJY4717: ToY286 (Ferreira-Cerca et al. 2005) was transformed with an *RPS2 CEN URA3* plasmid and selected on SD Ura- until the *PGAL1-RPS2 CEN LEU2* vector was lost. The resultant strain was then transformed with pAJ4688 (*RPS2-GS-FLAG LEU2 CEN*) and grown on SD Leu-. The *URA3* vector was then evicted by selection on media containing 5-fluoroorotic acid (5FOA).

AJY4718: Like AJY4718, except pAJ2960 (*RPS2 LEU2 CEN*) was shuffled into the strain.

AJY4719: Genomic integration of a *6xGly-FLAG-TEV-ProtA::KIURA3* cassette, containing 50-bp of flanking homology to the 3'-end off *BUD23*, amplified from pAJ4028 into BY4741.

AJY4721: *UTP9-GFP::HIS3MX6* amplified from AJY3431 (Sardana et al. 2013) was then genomically integrated into AJY4717.

AJY4722: Like AJY4721, except *BUD23-GFP::HIS3MX6* amplified from a *BUD23-GFP::HIS3MX6* strain (Huh et al. 2003) was genomically integrated.

AJY4723: Like AJY4721, except *RIO2-GFP::HIS3MX6* amplified from AJY2582 (*MAT α his3 Δ 1 leu2 Δ 0 met15 Δ 0 ura3 Δ 0 RIO2-GFP::HIS3MX6*; (Huh et al. 2003)) was genomically integrated.

AJY4724: Genomic integration of *ENP1-FTP::KIURA3* amplified from AJY4705 into BY4741.

AJY4725: Genomic integration of *ENP1-FTP::KIURA3* amplified from AJY4705 into ToY327 (Ferreira-Cerca et al. 2005).

AJY4731: Y801 (Linnemann et al. 2019) was transformed with an *RPS21A CEN URA3* vector and selected on SD Ura- media until the *PGAL1-RPS21A CEN LEU2* vector was lost. The resultant strain was then transformed with pAJ4672 (*RPS21A LEU2 CEN*) and grown on SD Leu-. The *URA3* vector was then evicted by selection of media containing 5FOA.

AJY4732: Like AJY4731, except pAJ4693 (*RPS21A-GS-HA LEU2 CEN*) was shuffled into the strain.

AJY4733: Genomic integration of *AID-HA_PGAL1-OsTIR1_GAL4BD-ER-VP16::CloNAT* amplified from pAJ4686 into the *RIO2* locus of AJY4724.

AJY4734: Genomic integration of *AID-HA_PGAL1-OsTIR1_GAL4BD-ER-VP16::CloNAT* amplified from pAJ4392 into the *LTV1* locus of AJY4724.

AJY4735: Genomic integration of *AID-HA_PGAL1-OsTIR1_GAL4BD-ER-VP16::CloNAT* amplified from pAJ4686 into the *TSR4* locus of AJY4724.

AJY4743: Genomic integration of *ENP1-6xGly-FTP::KIURA3* amplified from AJY4724 into AJY4726 (*NOB1/nob1 Δ ::KanMX6*; Open Biosystems). The resultant strain was then transformed with pAJ4702 (*NOB1-AID-HA_PGAL1-OsTIR1_GAL4BD-ER-VP16 LEU2 CEN*), sporulated, and dissected.

AJY4745: ToY286 (Ferreira-Cerca et al. 2005) was transformed with an *RPS2 CEN URA3* plasmid and selected on SD Ura- until the *PGAL1-RPS2 CEN LEU2* vector was lost. The resultant strain was then transformed with pAJ2960 (*RPS2 CEN LEU2*) and grown on SD Leu-. The *URA3* vector was

then evicted by selection on media containing 5FOA. *ENP1-6xGly-FTP::KIURA3* amplified from AJY4724 was then genomically integrated into the resultant strain.

AJY4748: *RPS2-GS-FLAG* was genomically integrated into BY4741 using CRISPR-Cas9 as previously described (Akhmetov et al. 2018), and *ENP1-TAP::HIS3MX6* was amplified from AJY4384 (A. Johnson) and genomically integrated into the resultant *RPS2-GS-FLAG* strain.

AJY4749: *RPS21A-GS-HA* was genomically integrated into BY4741 using CRISPR-Cas9 as previously described (Akhmetov et al. 2018), and *ENP1-TAP::HIS3MX6* was amplified from AJY4384 (A. Johnson) and genomically integrated into the resultant *RPS21A-GS-HA* strain.

AJY4750: Genomic integration of *BUD23-AID-HA_OsTIR1::LEU2* amplified from AJY4395 (Black et al. 2020) into AJY4748.

AJY4751: Genomic integration of *BUD23-AID-HA_OsTIR1::LEU2* amplified from AJY4395 (Black et al. 2020) into AJY4749.

AJY4754: Genomic integration of *TSR1-TAP::KIURA3* amplified from LY193 (D. Lycan) into BY4741.

AJY4755: Genomic integration of *TSR1-TAP::KIURA3* amplified from LY193 (D. Lycan) into AJY3398 (Black et al. 2019).

AJY4757: Genomic integration of *TSR1-TAP::KIURA3* amplified from LY193 (D. Lycan) into AJY4710.

AJY4761: Genomic integration of *KanMX6::PGAL1-RPS2* amplified from AJY4755 into AJY2667.

AJY4766: Genomic integration of *UTP9-GFP::HIS3MX6* amplified from AJY4739 (A. Johnson) into AJY4732.

AJY4767: Genomic integration of *BUD23-GFP::HIS3MX6* amplified from AJY4737 (A. Johnson) into AJY4732.

AJY4768: Genomic integration of *RIO2-GFP::HIS3MX6* amplified from AJY4738 (A. Johnson) into AJY4732.

References:

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