

Fig. S1. Duc1 is conserved among other *Schizosaccharomyces* species. Sequence alignment of Duc1 related proteins from other *Schizosaccharomyces* species. *S. pombe* (S.p), *Schizosaccharomyces japonicus* (S.j), *Schizosaccharomyces octosporus* (S.o) and *Schizosaccharomyces cryophilus* (S.c). The DUF1769 domain segments are highlighted in gray and an FFAT-like sequence is highlighted in yellow. Residues conserved in all proteins are marked with an asterisk, similar residues are indicated with a colon and slightly similar residues are marked with a period.

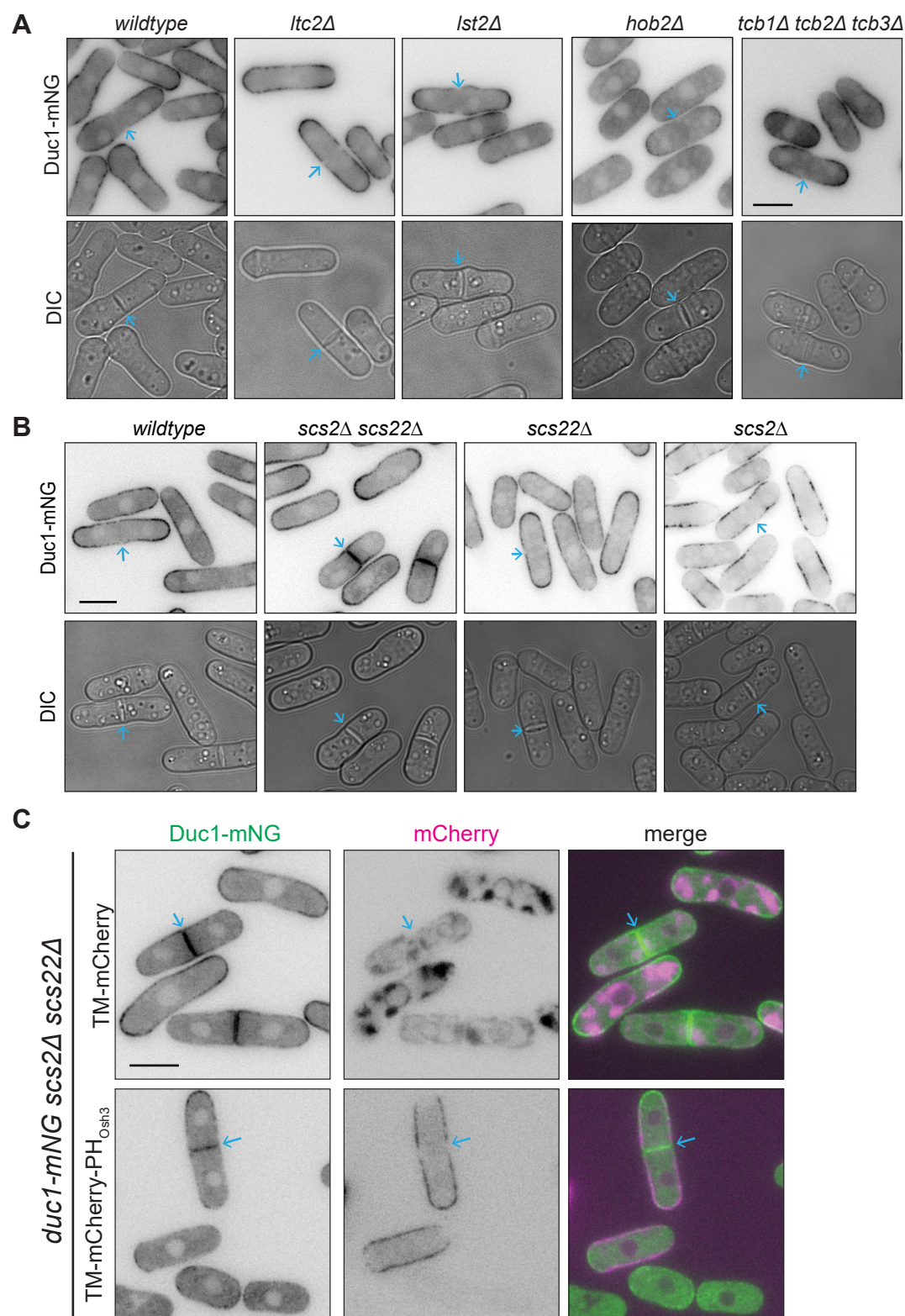


Fig. S2. Duc1 localization is not influenced by all ER-PM contact site proteins. (A-B) Live-cell imaging of cells expressing Duc1-mNG in the indicated genetic backgrounds. (C) Live-cell imaging of *duc1-mNG scs2Δ scs22Δ* cells expressing either TM-mCherry or TM-mCherry-PH_{Osh3} from the *nmt81* promoter. Expression was induced in media without thiamine for 24 hours prior to imaging. (A-C) Blue arrows indicated the position of a septum. Scale bars, 5 μm.

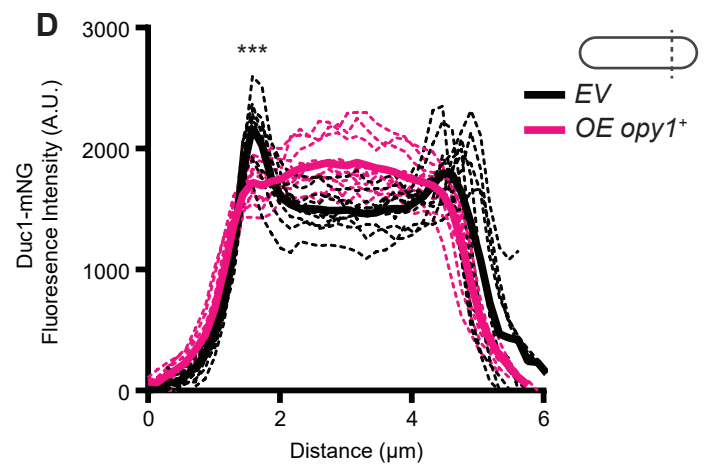
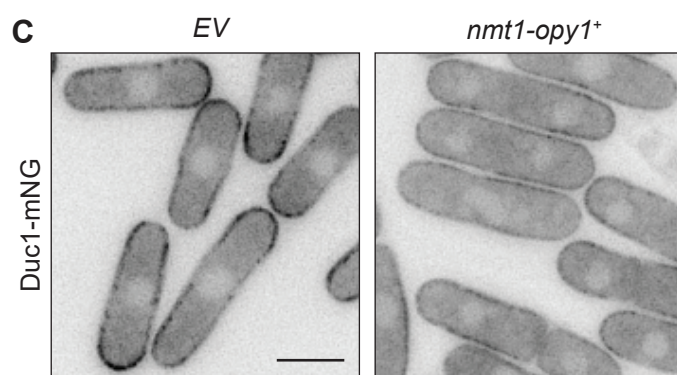
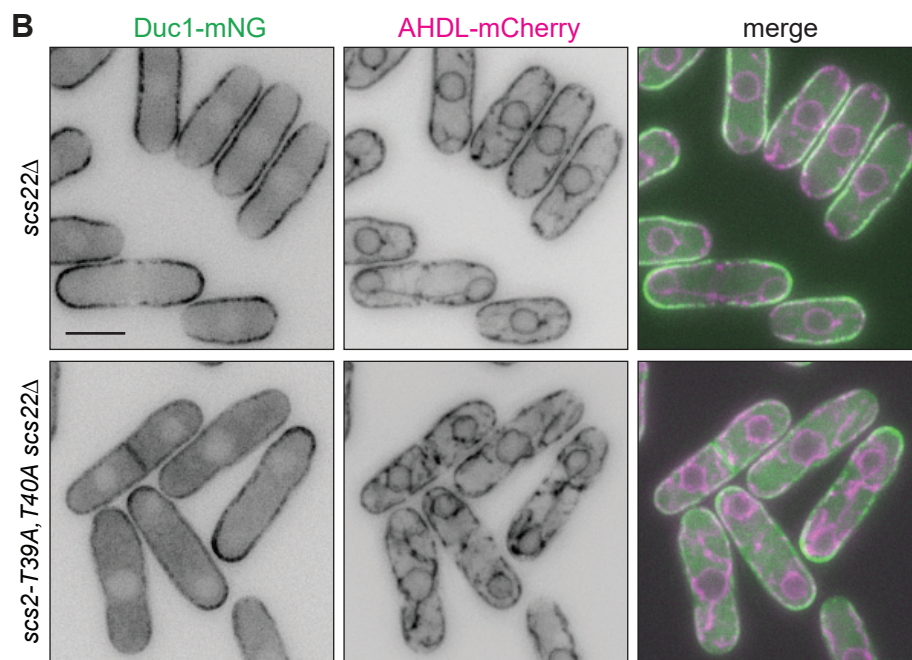
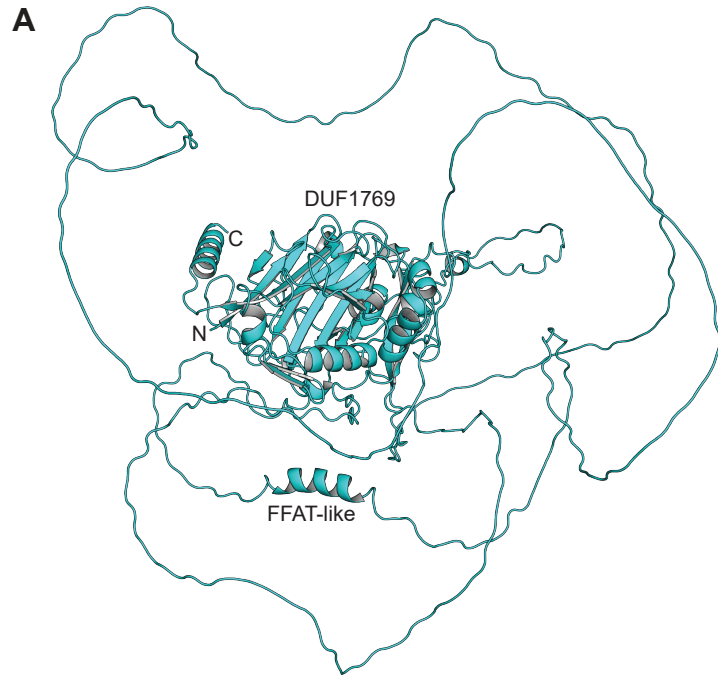


Fig. S3. Duc1 exclusion of the cell division site is not dependent on ER-PM contacts. (A) AF predicted structure of the full Duc1 protein. The N- and C-termini, the DUF1769 domain and the FFAT-like sequence are indicated. (B) Live-cell imaging of *duc1-mNG AHDL-mCherry scs22Δ* or *duc1-mNG AHDL-mCherry scs22Δ scs2-T39A, T40A* cells. (C) Live-cell imaging of cells expressing Duc1-mNG and expressing an empty vector (EV) or a vector with *nmt1-opy1+* induced for 24 hours prior to imaging. (D) Line scans of fluorescence intensity drawn across the short axis of 10 cells from each of the indicated strains. Solid lines represent the mean and dotted lines are the individual line traces. Data is from two biological replicates. For EV versus *nmt1-opy1+* expressing cells at first peak, 1.58 μm distance, $P < 0.001$ (unpaired, two-tailed Student's t-test). $***P < 0.001$. Scale bars, 5 μm.

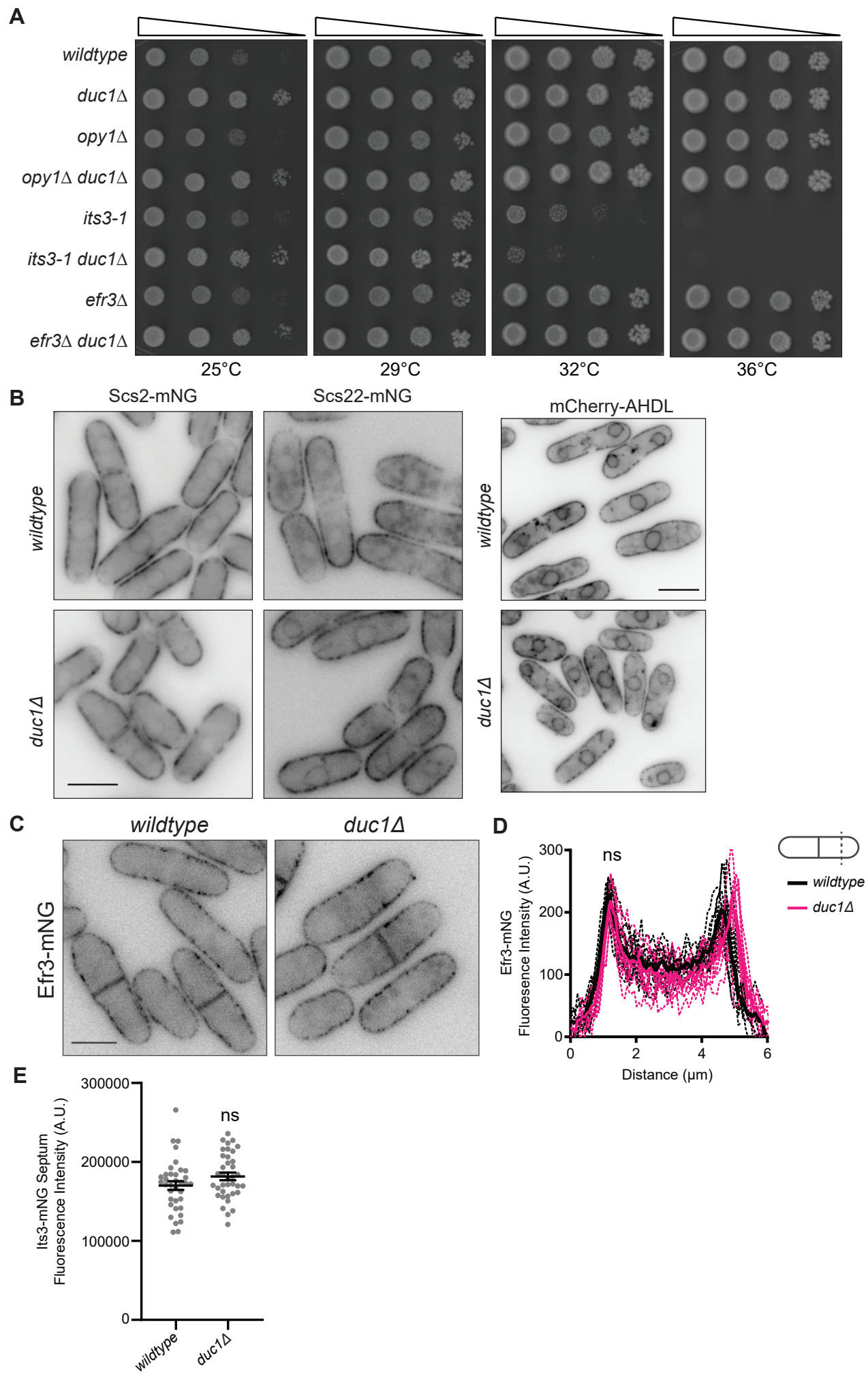


Fig. S4. Genetic and microscopic analysis of *duc1Δ*. (A) The indicated strains were serially diluted ten-fold and spotted on YE plates at the indicated temperatures for 3 days prior to imaging. (B) Live-cell imaging of wildtype or *duc1Δ* cells expressing Scs2-mNG, Scs22-mNG or mCherry-AHDL. (C) Representative live-cell images of Efr3-mNG in wildtype or *duc1Δ* cells. (D) Line scans of fluorescence intensity drawn across the short axis of 10 cells from each of the indicated strains. Solid lines represent the mean and dotted lines are the individual line traces. Data is from two biological replicates. For Efr3-mNG signal in wild-type versus *duc1Δ* cells at first peak, 1.15 μm distance, is $P=0.377$ (unpaired, two-tailed Student's t-test). (E) Quantification of the fluorescence intensity of its3-mNG at the septum in wildtype and *duc1Δ* cells. $n \geq 36$ cells for each from three independent replicates. $P=0.11$ (unpaired, two-tailed Student's t-test). Scale bars, 5 μm .

Table S1. Proteins identified by LC-MS/MS in Its3-TurboID, Opy1-TurboID, and Myo2-TurboID. Shown in the table are the ORF numbers (ID), protein names, and protein descriptions. Also indicated are the Total Spectral Counts (TSC) of proteins from both the bait protein-TurboID and the control-TurboID, Only proteins with a minimum of two spectral counts are shown and proteins identified in control-TurboID are listed at the end of the table in grey-shaded text.

Available for download at
<https://journals.biologists.com/jcs/article-lookup/doi/10.1242/jcs.262347#supplementary-data>

Table S2. *S. pombe* strains used in this study

Figure 1		
KGY19921	<i>its3-V5-TurboID:kanMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY19922	<i>opy1-V5-TurboID:kanMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY19896	<i>myo2-V5-TurboID:kanMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY4485-2	<i>duc1-mNG:kanMX6 rlc1-mCherry:natMX6 sad1-mCherry:natMX6 ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
KGY4564-2	<i>its3-mCherry:natMX6 duc1-mNG:kanMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY4563-2	<i>opy1-mCherry:natMX6 duc1-mNG:kanMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
Figure 2		
KGY6331-2	<i>duc1-mNG:kanMX6 leu1:pBip1-mCherry-AHDL ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
KGY6430-2	<i>duc1-mNG:kanMX6 leu1:pBip1-mCherry-AHDL scs2Δ::ura4⁺ scs22Δ::ura4⁺ ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
KGY7733-2	<i>duc1-mNG:kanMX6 rlc1-mCherry:natMX6 sad1-mCherry:natMX6 scs2Δ::ura4⁺ scs22Δ::ura4⁺ ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
Figure 3		
KGY8327-2	<i>scs22-mNG:hphMX6 duc1-mCherry:natMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY7909-2	<i>tcb1-mNG:hphMX6 duc1-mCherry:natMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY7810-2	<i>hob2-mNG:hphMX6 duc1-mCherry:natMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY7794-2	<i>ltc2-mNG:hphMX6 duc1-mCherry:natMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY7809-2	<i>ist2-mNG:hphMX6 duc1-mCherry:natMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY7579-2	<i>leu1:pBip1-mCherry-AHDL rlc1-mNG:hphMX6 sid4-mNG:kanMX6 ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
Figure 4		
KGY291-2	<i>duc1-mNG:kanMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY9128-2	<i>duc1-mNG:kanMX6 scs22Δ::ura4⁺ scs2-T39A,T40A ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY9028-2	<i>duc1-Y374A,F375A-mNG:kanMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
Figure 5		
KGY7582-2	<i>duc1-mNG:kanMX6 efr3Δ::kanMX6 ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
KGY581-2	<i>duc1-mNG:kanMX6 its3-1 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY1879-2	<i>opy1-mNG:kanMX6 ade6-M210 ura4-D18 leu1-32 h⁺</i>	Lab stock

KGY246	<i>ade6-M210 ura4-D18 leu1-32 h⁻</i>	Lab stock
Figure 6		
KGY338-3	<i>duc1Δ::ura4⁺ ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY269-2	<i>GFP-2xPH(Plc):leu1⁺ ade6-M210 ura4-D18 leu1-32 h⁻</i>	Lab stock
KGY19217	<i>GFP-P4C(SidC):leu1⁺ ade6-M210 ura4-D18 leu1-32 h⁺</i>	Lab stock
KGY1401-3	<i>GFP-2xPH(Plc):leu1⁺ duc1Δ::ura4⁺ ade6-x ura4-D18 leu1-32 h⁻</i>	This study
KGY4506-2	<i>GFP-P4C(SidC):leu1⁺ duc1Δ::ura4⁺ ade6-M21x ura4-D18 leu1-32 h⁺</i>	This study
KGY19002	<i>rlc1-mNG:hphMX6 sid4-mNG:kanMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	Lab stock
KGY7732-2	<i>duc1Δ::ura4⁺ rlc1-mNG:hphMX6 sid4-mNG:kanMX6 ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
KGY243-2	<i>its3-mNG:hphMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	Lab stock
KGY584-2	<i>its3-mNG:hphMX6 duc1Δ::ura4⁺ ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
Figure 7		
KGY488-2	<i>its3-mNG:hphMX6 scs2Δ::ura4⁺ scs22Δ::ura4⁺ ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY2839-2	<i>GFP-2xPH(Plc):leu1⁺ scs2Δ::ura4⁺ scs22Δ::ura4⁺ ade6-x ura4-D18 leu1-32 h⁻</i>	This study
KGY2825-2	<i>scs2Δ::ura4⁺ scs22Δ::ura4⁺ rlc1-mNG:hphMX6 sid4-mNG:kanMX6 ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
Figure S2		
KGY8139-2	<i>duc1-mNG:kanMX6 ltc2Δ::kanMX6 ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
KGY9177-2	<i>duc1-mNG:kanMX6 ist2Δ::kanMX6 ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
KGY8576-2	<i>duc1-mNG:kanMX6 hob2Δ::ura4⁺ ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
KGY8222-2	<i>duc1-mNG:kanMX6 tcb1Δ::kanMX6 tcb2Δ::ura4⁺ tcb3Δ::kanMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY7865-2	<i>duc1-mNG:kanMX6 scs2Δ::ura4⁺ ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
KGY7866-2	<i>duc1-mNG:kanMX6 scs22Δ::ura4⁺ ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
KGY6432-2	<i>duc1-mNG:kanMX6 scs2Δ::ura4⁺ scs22Δ::ura4⁺ ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
Figure S3		
KGY9217-2	<i>duc1-mNG:kanMX6 leu1:pBip1-mCherry-AHDL scs22Δ::ura4⁺ ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
KGY9227-2	<i>duc1-mNG:kanMX6 leu1:pBip1-mCherry-AHDL scs22Δ::ura4⁺ scs2-T39A,T40A ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
Figure S4		

KGY6369-2	<i>its3-1 ade6-M21X ura4-D18 leu1-32 h⁻</i>	Lab stock
KGY900-3	<i>duc1Δ::ura4⁺ its3-1 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY15603	<i>efr3Δ::kanMX6 ade6-M210 ura4-D18 leu1-32 h⁺</i>	Lab stock
KGY7582-2	<i>duc1Δ::ura4⁺ efr3Δ::kanMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY250-2	<i>opy1Δ::kanMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	Lab stock
KGY4510-2	<i>opy1Δ::kanMX6 duc1Δ::ura4⁺ ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
KGY7430-2	<i>scs2-mNG:kanMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY8326-2	<i>scs2-mNG:kanMX6 duc1Δ::ura4⁺ ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY7695-2	<i>scs22-mNG:kanMX6 ade6-M210 ura4-D18 leu1-32 h⁺</i>	This study
KGY8324-2	<i>scs22-mNG:kanMX6 duc1Δ::ura4⁺ ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY8325-2	<i>leu1:pBip1-mCherry-AHDL duc1Δ::ura4⁺ ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study
KGY17908	<i>efr3-mNG:kanMX6 ade6-M210 ura4-D18 leu1-32 h⁻</i>	Lab stock
KGY9032-2	<i>efr3-mNG:kanMX6 duc1Δ::ura4⁺ ade6-M210 ura4-D18 leu1-32 h⁻</i>	This study