

Supplementary Figure Legends

Fig. S1

Cells from Fig. 1 proceed synchronously through meiosis

(a) Cell-cycle stage quantification for Fig. 1a.

(b) Only the full-length Sld2 protein isoform is detectable during meiosis (yDP336). Top: Sld2-13myc immunoblot during meiosis with protein samples run on a 12% SDS-PAGE gel. Size markers are indicated to the right of the gel. These are the same protein samples as those in Fig. 1b. Bottom: Cell-cycle stage quantification for samples used in this figure and Fig. 1b.

(c) Cell-cycle stage quantification for Fig. 1c.

Fig. S2

Characterization of the *CDC6^{short}* and *SLD2^{short}* RNA's and *Cdc6^{short}* protein

(a) 5' RACE of *SLD2* shows heterogeneous 5' ends during the meiotic divisions. Left: Agarose gel of PCR products. Portions that were gel purified for ligation into a cloning vector are denoted by colored boxes, along with the range of 5' ends detected for each species. Right: Diagram of *SLD2* promoter, ORF, and 13myc tag with the relative location of *SLD2*-specific primers for the RT reaction and PCR reactions. Colored vertical lines and numbers indicate the 5' ends of individually sequenced clones from specific gel-purified sections.

(b) Neither full-length *Cdc6* nor *Cdc6^{short}* depend on Met120, Met261, or Met267. Plasmids containing P_{CDC6} -*CDC6*-3V5 with Met→Val start codon mutations were integrated into yeast, and the subsequent strains were assayed for the ability to produce *Cdc6^{short}* during meiosis. *CDC6^{Met-120-Val}* (yDP226, lanes 1-7); *CDC6^{Met-261-Val}* (yDP228, lanes 8-14); *CDC6^{Met-267-Val}* (yDP229, lanes 15-21); *CDC6^{Met-261/267-Val}* (yDP230, lanes 22-28). The time after transfer into sporulation medium is indicated above each lane. Protein was detected by V5-immunoblot.

Fig. S3

Cdc6^{short} does not form a stable complex with ORC, Cdt1, and Mcm2-7 in ATPγS

(a) WT *Cdc6* can form the OCCM complex (lanes 2-6), whereas *Cdc6^{short}* cannot form the OCCM complex (lanes 4-7) at all concentrations tested in ATPγS. Top: Total protein stain (Krypton stain). Bottom: FLAG-immunoblot to specifically examine *Cdc6* vs *Cdc6^{short}*, as well as Mcm3 and Orc1, all of which have a FLAG epitope tag.

Fig. S4

Cells from Fig. 4 complete both meiotic divisions

(a) Cells from Fig. 4c complete both MI and MII.

(a) Cells from Fig. 4d complete both MI and MII.

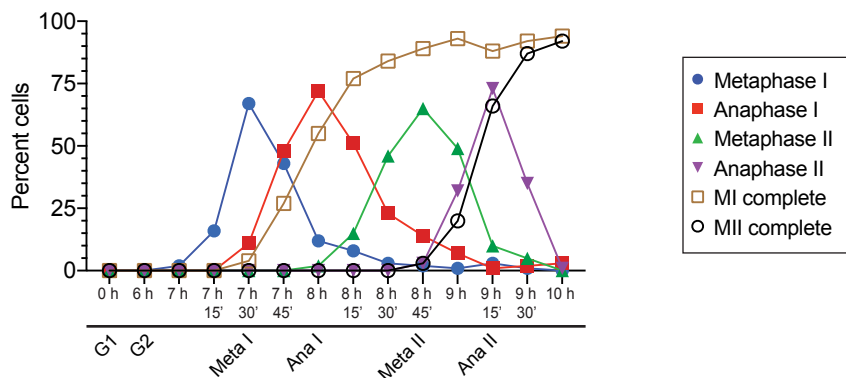
Fig. S5

Cells from Fig. 5 complete both meiotic divisions

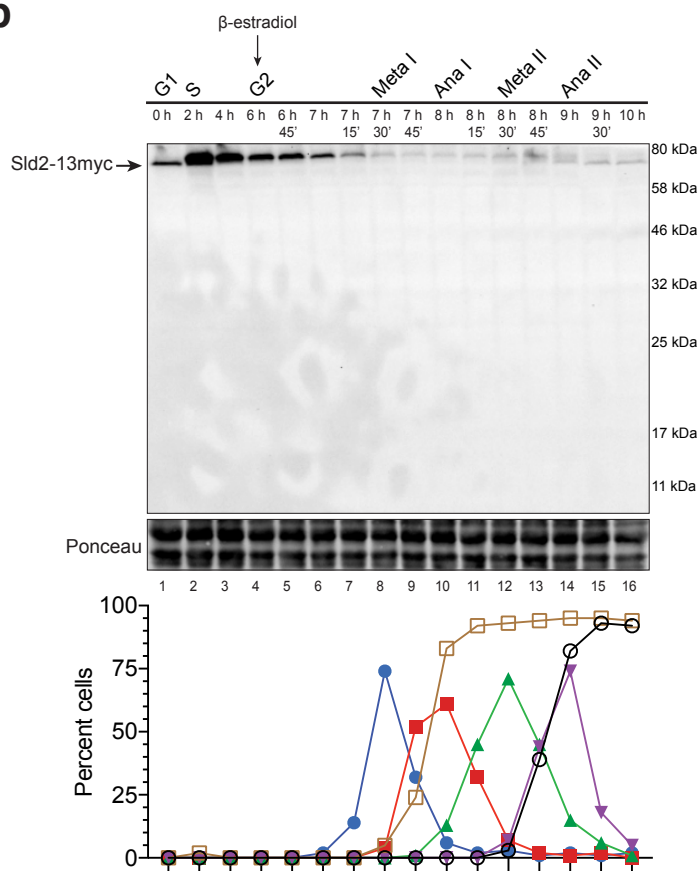
(a) Cells from Fig. 5b and 5c complete both MI and MII.

Supplementary Fig. S1

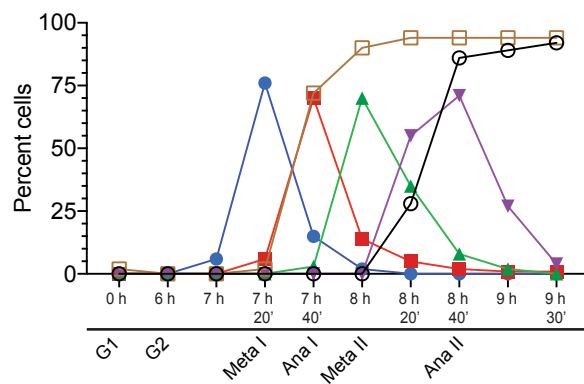
a



b

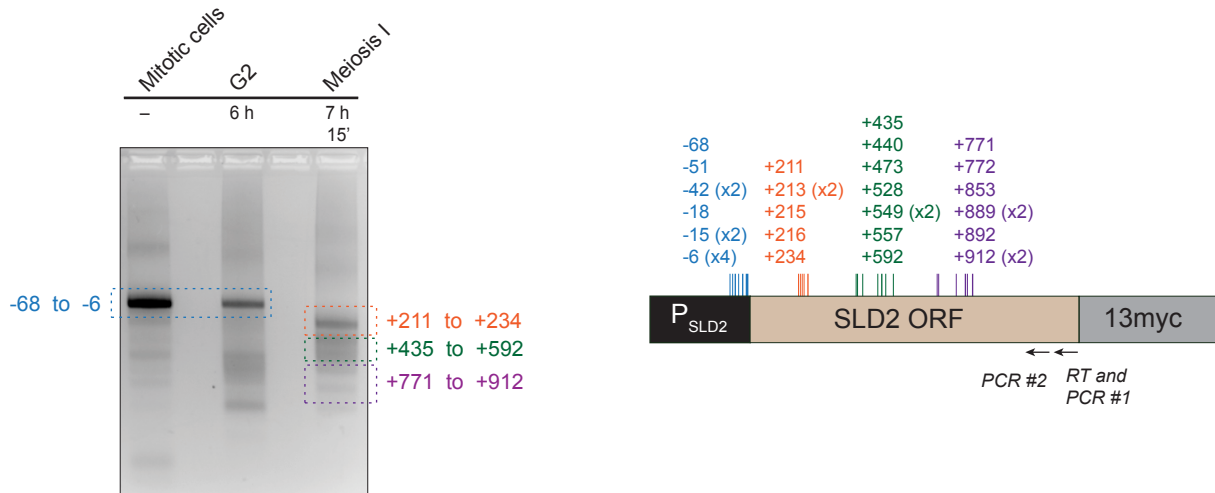


c

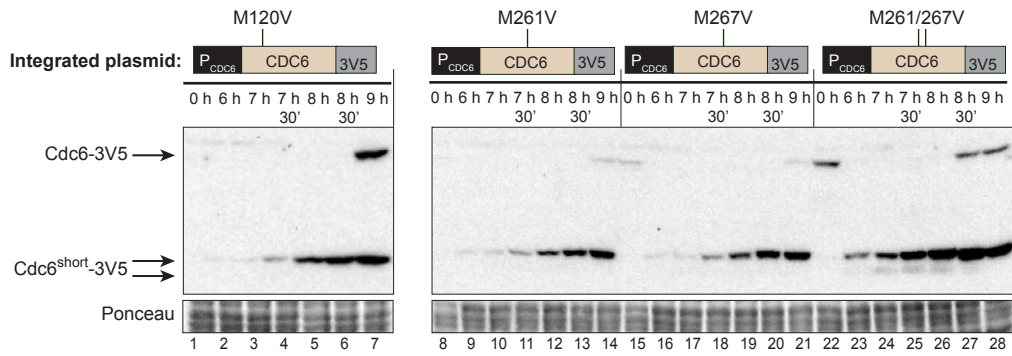


Supplementary Fig. S2

a

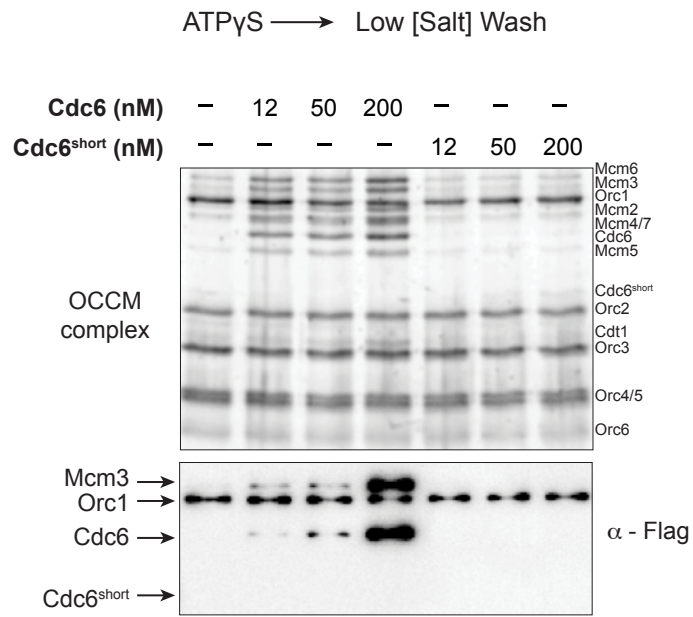


b

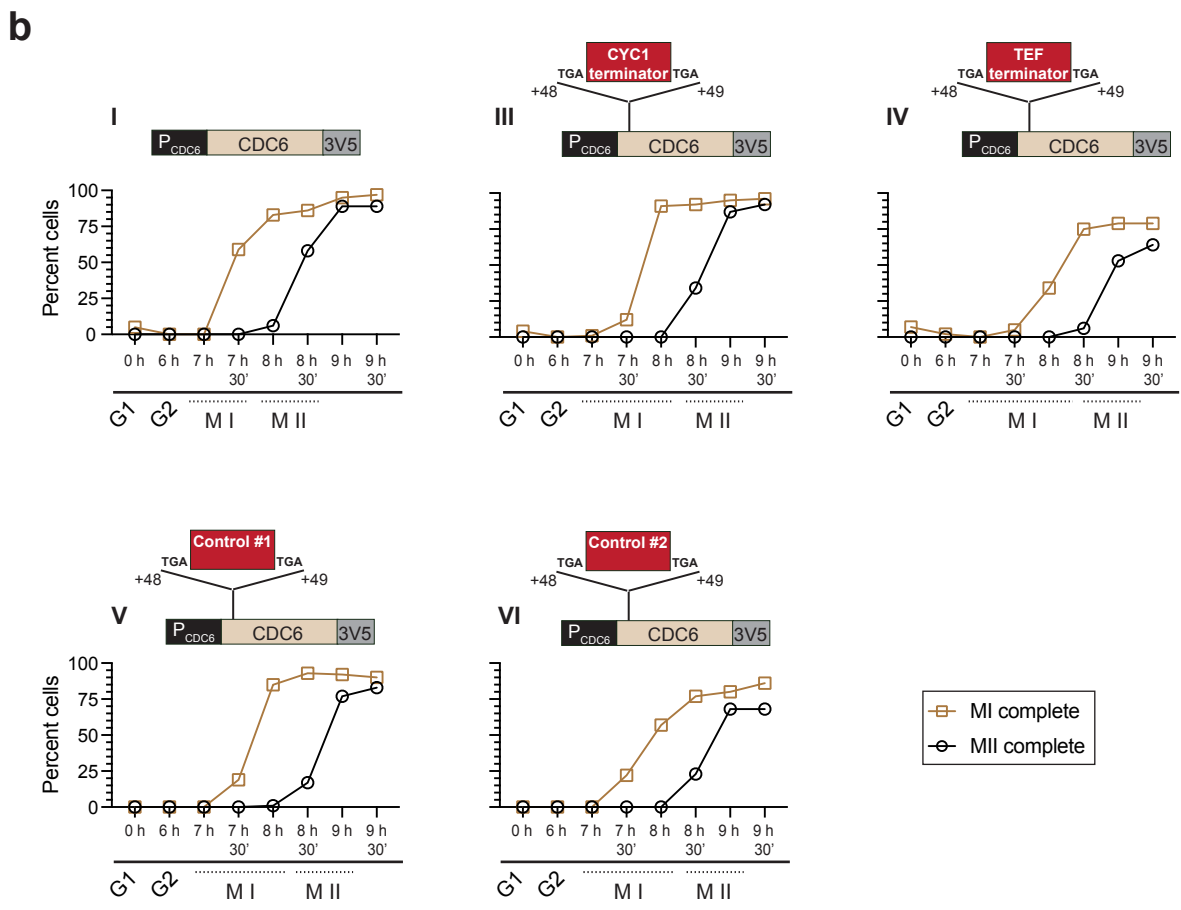
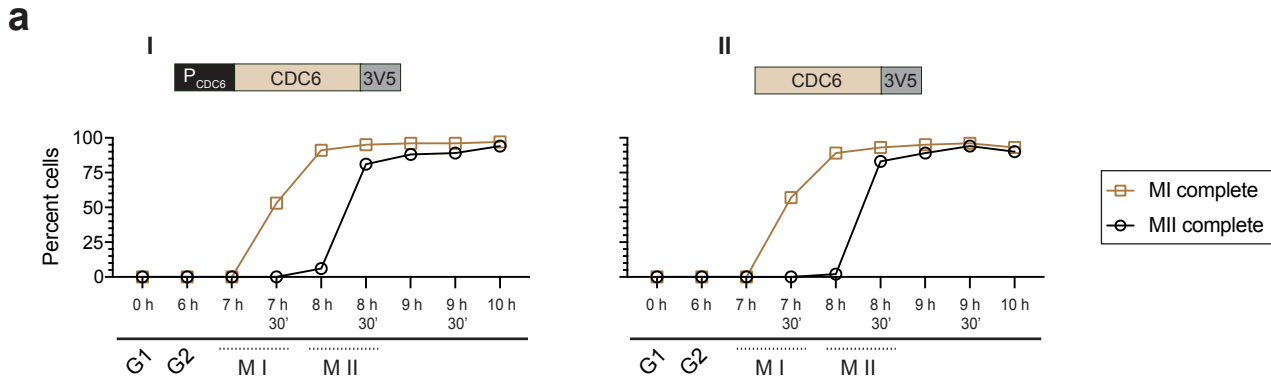


Supplementary Fig. S3

a



Supplementary Fig. S4



Supplementary Fig. S5

a

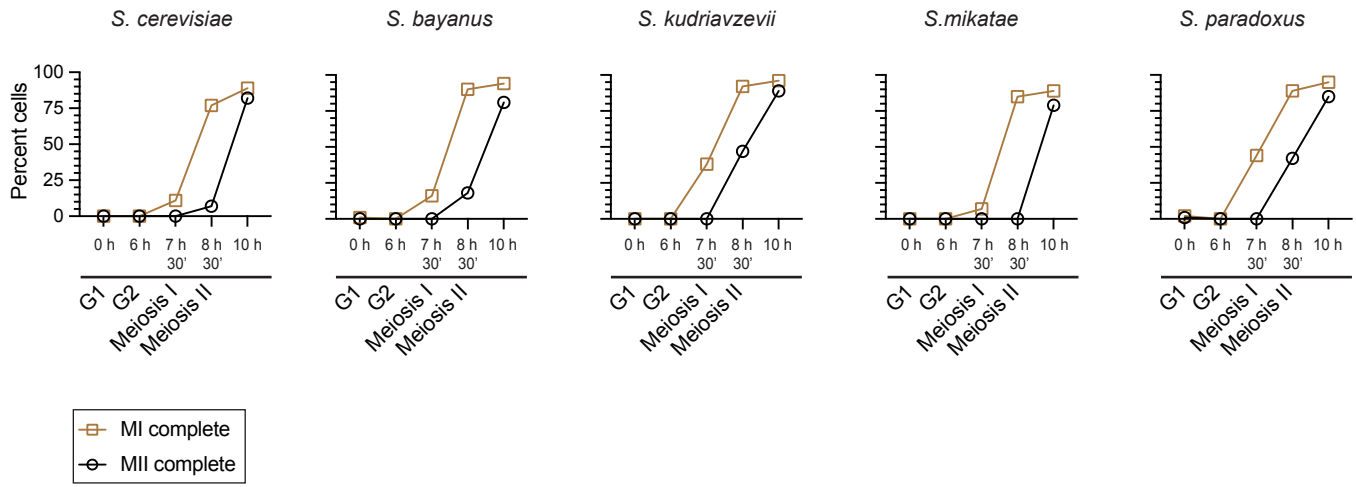


Table S1: Strains used in this study

Strain	Genotype	Source
yDP33	SK1 MATalpha CDC6-3V5::KANMX6	This study
yDP71	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 CDC6-3V5::KANMX6/CDC6-3V5::KANMX6	Phizicky <i>et al.</i> 2018
yDP198	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP31(PCDC6-CDC6-3V5)::LEU2	This study
yDP213	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP34(PCDC6-CDC6(Met-1-Val)-3V5)::LEU2	This study
yDP226	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP38(PCDC6-CDC6(Met-120-Val)-3V5)::LEU2	This study
yDP227	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP39(PCDC6-CDC6(Met-202/225-Val)-3V5)::LEU2	This study
yDP228	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP40(PCDC6-CDC6(Met-261-Val)-3V5)::LEU2	This study
yDP229	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP41(PCDC6-CDC6(Met-267-Val)-3V5)::LEU2	This study
yDP230	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP42(PCDC6-CDC6(Met-261/267-Val)-3V5)::LEU2	This study
yDP231	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP44(PCDC6-CDC6(Met-202-Val)-3V5)::LEU2	This study
yDP232	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP45(PCDC6-CDC6(Met-225-Val)-3V5)::LEU2	This study
yDP242	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP55(Promoter deletion, CDC6-3V5)::LEU2	This study
yDP245	SK1 MATa CDC6(Met-202/225-Val)-3V5::KANMX6	This study
yDP246	SK1 MATalpha CDC6(Met-202/225-Val)-3V5::KANMX6	This study
yDP249	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP63(PCDC6-CDC6(+48-CYC1 Terminator+49)-3V5)::LEU2	This study

Table S1: Strains used in this study

yDP250	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP64(PCDC6-CDC6(+48-TEF Terminator+49)-3V5)::LEU2	This study
yDP251	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP65(PCDC6-CDC6(+48-Internal GFP ORF+49)-3V5)::LEU2	This study
yDP252	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP66(PCDC6-CDC6(+48-Internal GST ORF+49)-3V5)::LEU2	This study
yDP259	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 CDC6(Met-202/225-Val)-3V5::KANMX6/CDC6(Met-202/225-Val)-3V5::KANMX6	This study
yDP336	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 SLD2-13myc::KANMX6/SLD2-13myc::KANMX6	Phizicky <i>et al.</i> 2018
yDP668	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP152(<i>S. bayanus</i> PCDC6-CDC6-3V5)::LEU2	This study
yDP670	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP153(<i>S. kudriavzevii</i> PCDC6-CDC6-3V5)::LEU2	This study
yDP671	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP155(<i>S. mikatae</i> PCDC6-CDC6-3V5)::LEU2	This study
yDP673	SK1 MATa/alpha ura3::pGPD1-GAL4(848).ER::URA3/ura3::pGPD1-GAL4(848).ER::URA3 GAL-NDT80::TRP1/GAL-NDT80::TRP1 leu2/leu2::pDP156(<i>S. paradoxus</i> PCDC6-CDC6-3V5)::LEU2	This study
yST144	W303 MATa bar1::hisG pep4::unmarked TRP1::pSKM003(pGAL1,10-MCM6,MCM7) HIS3::pSKM004-(pGAL1,10-MCM2,Flag-MCM3) LYS2::pSKM002-(pGAL1,10-MCM4,MCM5) URA3::pALS1(pGAL1,10-Cdt1,GAL4)	Ticau <i>et al.</i> , 2014
ySDORC	W303 MATa bar1::hyg pep4::kanMX TRP1::pGAL1,10-ORC5,ORC6 HIS3::pGAL1,10-ORC3,ORC4 URA3::pGAL1,10-CBP-TEV-ORC1,ORC2	John Diffley
YZBS-113	<i>S. bayanus</i> ho::HYGb lys2-1 ura3::clonat	Maitreya Dunham
FM527	<i>S. kudriavzevii</i>	Mark Johnston
FM356	<i>S. mikatae</i>	Mark Johnston
DBY10073	<i>S. paradoxus</i>	Maitreya Dunham

Table S2: Plasmids used in this study

Plasmid	Description	Purpose	Source
pDP69	pMAL-MBP-FLAG- <i>CDC6(Amino Acids 1-513)</i> -6xHIS	Cdc6 purification	This study
pDP70	pMAL-MBP-FLAG- <i>CDC6(Amino Acids 202-513)</i> -6xHIS	Cdc6-short purification	This study