

Supplementary Figure 1. Synchronous azygotic meiosis in non-repressive conditions in an *nmt41-cdc13* strain. Synchronous meiosis was chemically induced in (a) h+/h+ *pat1as2* (wt) and (b) h+/h+  $\triangle$ cdc13 *nmt41-cdc13 pat1as2 (nmt41-cdc13)* homozygous diploid strains. Pat1 inactivation was achieved by adding 30µM of Nm-PP1 at 28°C to the culture after 17 hours of nitrogen starvation (time 0). No thiamine was added to the media to allow *cdc13* expression under *nmt41* promoter. DNA content (left panel) and the number of nuclei (right panel) were followed every hour.



Supplementary Figure 2.  $\Delta rem1\Delta crs1$  mutants show multipolar divisions during zygotic meiosis. Time-lapse of  $h^{90}$  hhtc1-CFP atb1-mCherry sid4-mCherry rem1 $\Delta crs1\Delta$  strain (Pi275) after meiotic induction. Images show a representative example of multipolar division during meiosis II (left panels) and meiosis I (right panels). These aberrant divisions were more frequent during meiosis II (45.4%) than during meiosis I (36.4%), while 18.2% of these zygotes showed defects in both meiosis I and II. Scale bars, 5µm.



Supplementary Figure 3. Cdc13-Cdc2 fusion protein dynamics during synchronous meiosis. Cells from the experiment described in Figure 7a and 7b were collected every hour to follow protein dynamics. A diploidized wild type strain was used as a control (Pi263). Protein samples were analysed by Western blot using anti-Cdc13 (SP4), Cdc2 (PSTAIRE) and Cdc2-Tyrosine15 phospho-specific antibodies in the case of wt extracts and anti-Cdc13 and Cdc2-Tyrosine15 phospho-specific antibodies for *cdc13-cdc2* samples.  $\alpha$ -Tubulin was used in both cases as loading control.



**Supplementary Figure 4.** Characterization of the mitotic phenotype of *cdc13-cdc2* overexpression. a. qPCR quantification of *cdc13-cdc2* copy number in *h*+ (Pi46) and *h*- (Pi43) *cdc13-cdc2* over-expressing strains, normalized using the qPCR values of *h*+ and *h*- *cdc13cdc2* parental strains. Protein levels were analysed by Western blot in the same strains using anti-Cdc13 (SP4) and anti-tubulin (TAT1) antibodies, and including *h*- and *h*+ wild type strains as controls. **b.** Size at division, (n=80 cells per strain, boxes represent 25– 75% of the data and the central lines indicate the median. Whiskers show 5–95% of the data. Dots represent the outter 10%). **c.** FACS profiles. **d.** generation times (mean of three experiments ± standard deviation) of h+ (PN4) and h- (PN1) wt, *cdc13-cdc2*  $\Delta 2 \Delta 13 \Delta CCP$  (*cdc13-cdc2*, h+ DC235 and h- Pi4) and 4x *cdc13-cdc2*  $\Delta 2 \Delta 13 \Delta CCP$  (4x*cdc13-cdc2*, h+ Pi46 and h- Pi43) strains grown at 32°C in YE4S.

	wt	cdc13-cdc2	4x <i>c</i>	dc13-cdc2	2Δ2Δ13
		Δ2Δ13ΔССР	∆ССР	∆CCPR	∆CCPRC
> 4 DAPI stained bodies	1.8	1.2	3.5	2.6	3.7
	89.3	3.4	90.2	87	85.7
$\textcircled{\bullet} \textcircled{\bullet} \textcircled{\bullet} \textcircled{\bullet} \textcircled{\bullet} \textcircled{\bullet} \textcircled{\bullet} \textcircled{\bullet} $	0.9	2.2	-	2.6	0.5
$\bigcirc \bigcirc \bigcirc \bigcirc$	4.5	3.4	3.3	1	1.2
$\textcircled{\bullet}$	1.3	6.7	-	1.6	1.7
$\textcircled{\bullet} \textcircled{\bullet}$	2.2	82	3	4.1	5.6
$\textcircled{\bullet}$	-	-	-	1.1	0.5
$\textcircled{\bullet}$	-	1.1	-	-	1.1

b





Supplementary Figure 5. Frequency of aberrant nuclear divisions in Cdc13-Cdc2 fusion protein strains during meiosis. Asci from *azygotic* synchronous meiosis were stained with DAPI and observed by microscopy. **a**. Phenotipic defects considered (based on <sup>20</sup>) and percentage of asci in each of them. At least 400 asci were scored for each strain. b. Representative pictures of each strain after 14 hours of meiotic induction. Scale bars, 5µm.

## Supplementary Table 1. Strains used in this study

Name	Genotype	Source
PN1	h <sup>-</sup> 972	Lab collection
PN4	h <sup>+</sup> 975	Lab collection
PN71	h <sup>-</sup> ade6-M210 leu1-32	Lab collection
PN1394	$h^{-} \Delta cig1$ :: $ura4 + \Delta cig2$ :: $ura4 + ade6 - leu1 - 32 ura4 - D18$	Lab collection
PN1400	$h^{-} \Delta cig1$ :: $ura4 + \Delta cig2$ :: $ura4 + ura4 - D18$	Lab collection
PN1401	$h^+ \Delta cig1$ :: $ura4 + \Delta cig2$ :: $ura4 + ura4 - D18$	Lab collection
PN1926	$h^+ \Delta cig2$ :: $ura4 + ura4 - D18$	Lab collection
PN1931	$h^{-} \Delta cig2$ ::ura4+ leu1-32 ura4-D18	Lab collection
PN1942	$h^{-} \Delta cig2$ ::ura4+ ura4-D18	Lab collection
DC177	h <sup>-</sup> leu1 ::Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+ Δcdc2 ::kanMX6 Δcdc13::natMX6 ura4-D18	[4]
DC210	$h^+$ leu1 ::Pcdc13::cdc13ts-L-cdc2as::cdc13 3'UTR::ura4+ $\Delta$ cdc2 ::kanMX6 $\Delta$ cdc13::natMX6 ura4-D18	[4]
DC235	$h^+$ leu1 ::Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+ $\Delta$ cdc2 ::kanMX6 $\Delta$ cdc13::natMX6 $\Delta$ cig1 ::ura4+ $\Delta$ cig2 ::ura4+ $\Delta$ puc1 ::ura4+ ura4-D18	[4]
Pi1	h <sup>-</sup> Δcig1::ura4 Δcig2::ura4 Δpuc1::ura4 ura4-D18	This work
Pi4	h <sup>-</sup> leu1 ::Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+ Δcdc2 ::kanMX6 Δcdc13::natMX6 Δcig1 ::ura4+ Δcig2 ::ura4+ Δpuc1 ::ura4+ ura4-D18	This work
Pi5	$h^+ \Delta cig1$ ::ura4 $\Delta cig2$ ::ura4 $\Delta puc1$ ::ura4 ura4-D18	This work
Pi43	h- leu1 ::Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+ Δcdc2 ::kanMX6 Δcdc13::natMX6 Δcig1 ::ura4+ Δcig2 ::ura4+ Δpuc1 ::ura4 3x(ura4+:: Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+::Scleu2) ura4-D18	This work
Pi46	$h^+$ leu1 ::Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+ $\Delta$ cdc2 ::kanMX6 $\Delta$ cdc13::natMX6 $\Delta$ cig1 ::ura4+ $\Delta$ cig2 ::ura4+ $\Delta$ puc1 ::ura4 3x(ura4+:: Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+::Scleu2) ura4-D18	This work
Pi130	h <sup>-</sup> leu1 ::Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+ Δcdc2 ::kanMX6 Δcdc13::natMX6 Δcig1 ::ura4+ Δcig2 ::ura4+ Δpuc1 ::ura4 Δrem1::hphNT1 3x(ura4+:: Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+::Scleu2) ura4-D18	This work
Pi132	$h^+$ leu1 ::Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+ $\Delta$ cdc2 ::kanMX6 $\Delta$ cdc13::natMX6 $\Delta$ cig1 ::ura4+ $\Delta$ cig2 ::ura4+ $\Delta$ puc1 ::ura4 $\Delta$ rem1::hphNT1 3x(ura4+:: Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+::Scleu2) ura4-D18	This work
Pi155	$\begin{array}{c} h^{-} leu1 :: Pcdc13::cdc13-L-cdc2::cdc13 \; 3'UTR::ura4+ \; \Delta cdc2 ::kanMX6 \\ \Delta cdc13::natMX6 \; \Delta cig1 ::ura4+ \; \Delta cig2 ::ura4+ \; \Delta puc1 ::ura4 \; \Delta rem1::hphNT1 \\ \Delta crs1::hphNT1 \; 3x(ura4+:: Pcdc13::cdc13-L-cdc2::cdc13 \\ \end{array}$	This work

	3'UTR::ura4+::Scleu2) ura4-D18	
Pi158	h <sup>+</sup> leu1 ::Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+ Δcdc2 ::kanMX6 Δcdc13::natMX6 Δcig1 ::ura4+ Δcig2 ::ura4+ Δpuc1::ura4 Δrem1::hphNT1 Δcrs1::hphNT1 3x(ura4+:: Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+::Scleu2) ura4-D18	This work
163	$h + \Delta rem 1:: hphNT1$	This work
166	h- Δcrs1::hphNT1	This work
Pi175	h <sup>-</sup> Δrem1::hphNT1 Δcrs1::hphNT1	This work
Pi176	$h^+ \Delta rem1::hphNT1 \Delta crs1::hphNT1$	This work
Pi211	h <sup>+</sup> Δcig1::ur4 Δcig2::ura4 Δpuc1::ura4 Δrem1::natNT2 Δcrs1::hphNT1 ura4- D18	This work
Pi230	$h^+/h^- \Delta rem1::hphNT1/\Delta rem1::hphNT1 \Delta crs1::hphNT1/\Delta crs1::hphNT1 cut11-GFP::kanmx6/cut11-mCherry::natmx6$	This work
Pi234	h <sup>90</sup> hht1-CFP:his+Pnda3-mCherry-atb2:aur1R Sid4-mCherry::Scleu2 leu1-32 his3-D1 ura4-D18	This work
Pi236	$h^+ \Delta cig1::ura4 \Delta cig2::ura4 \Delta puc1::ura4 \Delta rem1::natNT2 ura4-D18$	This work
Pi237	h- Δcig1::ur4 Δcig2::ura4 Δpuc1::ura4 Δcrs1::hphNT1	This work
Pi240	$h^+ \Delta cig1::ura4 \Delta cig2::ura4 \Delta puc1::ura4 \Delta rem1::natNT2 \Delta pat1::pat1as2::hph$	This work
Pi242	h <sup>-</sup> <i>Acig1::ur4 Acig2::ura4 Apuc1::ura4 Arem1::natNT2 Acrs1::hphNT1 ura4-D18</i>	This work
Pi251	h <sup>-</sup> Δcig1::ur4 Δcig2::ura4 Δpuc1::ura4 Δrem1::natNT2 ura4-D18	This work
Pi253	h <sup>+</sup> leu1 ::Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+ Δcdc2 ::kanMX6 Δcdc13 ::natMX6 Δcig1 ::ura4+ Δcig2 ::ura4+ Δpuc1 ::ura4+ Δpat1::pat1as2::hph ura4-D18	This work
Pi259	$h^+ \Delta cig1::ura4 \Delta cig2::ura4 \Delta puc1::ura4 \Delta pat1::pat1as2::hph ura4-D18$	This work
Pi260	$h^+$ leu1 ::Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+ $\Delta$ cdc2 ::kanMX6 $\Delta$ cdc13 ::natMX6 $\Delta$ cig1 ::ura4+ $\Delta$ cig2 ::ura4+ $\Delta$ puc1 ::ura4 3x(ura4+:: Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+::Scleu2) $\Delta$ pat1::pat1as2::hph ura4-D18	This work
Pi263	$h^+ \Delta pat1::pat1as2::hph$	This work
Pi275	h <sup>90</sup> hht1-CFP:his+ Pnda3-mCherry-atb2:aur1R Sid4-mCherry::Scleu2 <i>Δrem1::natNT2 Δcrs1::natNT2 leu1-32 his3-D1 ura4-D18</i>	This work
Pi278	$h^+ \Delta cig1::ur4 \Delta cig2::ura4 \Delta puc1::ura4 \Delta rem1::natNT2 \Delta crs1::hphNT1 \Delta pat1::pat1as2::hph$	This work
Pi279	h <sup>-</sup> leu1 ::Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+ Δcdc2::kanMX6 Δcdc13::natMX6 Δcig ::ura4+ Δcig2::ura4+ Δpuc1::ura4 Δrem1::hphNT1	This work

	<i>3x(ura4+:: Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+::Scleu2)</i> <i>Δpat1::pat1as2::hph ura4-D18</i>	
Pi283	h <sup>+</sup> Δrem1::hphNT1 Δcrs1::hphNT1 Δpat1::pat1as2::hph	This work
Pi285	h <sup>+</sup> leu1::Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+ Δcdc2 ::kanMX6 Δcdc13::natMX6 Δcig1 ::ura4+ Δcig2 ::ura4+ Δpuc1::ura4 Δrem1::hphNT1 Δcrs1::hphNT1 3x(ura4+::Pcdc13::cdc13-L-cdc2::cdc13 3'UTR::ura4+::Scleu2) Δpat1::pat1as2::hph	This work
Pi292	$h^+ \Delta cig1::ur4 \Delta cig2::ura4 \Delta puc1::ura4 \Delta crs1::hphNT1$	This work
Pi296	$h^+ \Delta cig1::ur4 \Delta cig2::ura4 \Delta puc1::ura4 \Delta crs1::hphNT1 \Delta pat1::pat1as2::hph$	This work
Pi313	$h^+ \Delta cdc13$ ::ura4 intREP41-cdc13::Scleu2 $\Delta pat1$ ::pat1as2::hph	This work
Pi327	h- <i>Acrs1::hphNT1 ade6M26</i>	This work
Pi328	$h+\Delta crs1::hphNT1\ leu1-32\ ade6M210$	This work
Pi333	h+ade6M26	This work
Pi334	h- Δcig1::ur4 Δcig2::ura4 Δpuc1::ura4 leu1-32	This work
Pi335	$h + \Delta cig1::ur4 \Delta cig2::ura4 pat1as2::hph$	This work
Pi336	$h+ \Delta cig2::ura4 pat1as2::hph$	This work
Pi337	h- Δrem1::hphNT1	This work
Pi339	$h + \Delta crs1::hphNT1$	This work
Pi340	$h+\Delta rem1::hphNT1$ ade6M26	This work
Pi344	h- ∆rem1::hphNT ade6M210 leu1-32	This work
Pi346	h- ade6M210 leu1-32 Δcrs1::hphNTΔrem1::hphNT1	This work
Pi348	$h+ade6M26\ leu1+\Delta crs1::hphNT\Delta rem1::hphNT1$	This work

Primmer name	Sequence	
fw-cdc13	ATCATTGCGGGTATGCTCTC	
rv-cdc13	CTGAAGGGCACATCACCTC	
fw-act1	CGTCGCTTTGGACTTTGAGC	
rv-act1	TACCAGGTCCGCTCTCATCA	

Supplementary Table 2. List of primers used for quantitative PCR.