

Supplementary Data

The global role for CDC13 and Yku70 in preventing telomere resection across the genome

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Supplementary Table S1

Strains

Strain Background	Relevant genotype	Strain Name	Full Genotype
CG379	WT	DAG635	MATalpha <i>ade5-1 his7-2 leu2-3,112 trp1-289 ura3Δ can1Δ</i>
	<i>yku70Δ</i>	JWW2033.1	MATalpha <i>ade5-1 his7-2 leu2-3,112 trp1-289 ura3Δ can1Δ yku70::NAT</i>
	<i>cdc13-1</i>	DAG760	MATalpha <i>ade5-1 his7-2 leu2-3,112 trp1-289 ura3Δ can1Δ cdc13-1</i>
	<i>yku70Δ cdc13-1</i>	JWW1859.2	MATalpha <i>ade5-1 his7-2 leu2-3,112 trp1-289 ura3Δ can1Δ cdc13-1 yku70Δ</i>
	<i>yku70Δ cdc13-1</i>	JWW2036.1	MATalpha <i>ade5-1 his7-2 leu2-3,112 trp1-289 ura3Δ can1Δ cdc13-1 yku70Δ</i>
	<i>cdc13-1 exo1Δ</i>	JWW1830.1	MATalpha <i>ade5-1 his7-2 leu2-3,112 trp1-289 ura3Δ can1Δ cdc13-1 exo1::HYG</i>
	<i>yku70Δ cdc13-1 exo1Δ</i>	JWW2035.1	MATalpha <i>ade5-1 his7-2 leu2-3,112 trp1-289 ura3Δ can1Δ cdc13-1 yku70Δ exo1::HYG</i>
W303	WT	KBY583-1D	MAT <i>a ADE2 his3-1 bar1::LEU2 trp1-1 lys2Δ ura3-1 Rad52-YFP Rfa1-CFP RAD5</i>
	<i>yku70Δ</i>	KBY647-2D	MATa <i>ADE2 bar1::LEU2 yku70-HIS3 Rad52-YFP Rfa1-CFP</i>
	<i>cdc13-1</i>	KBY823-6C	MAT alpha <i>ADE2 his3-1 leu2-3 trp1-1 LYS2 ura3-1 cdc13-1 Rad52-YFP Rfa1-CFP RAD5</i>
	<i>yku70Δ cdc13-1</i>	JWW 2037.1	MAT alpha <i>ADE2 his3-1 leu2-3 trp1-1 LYS2 ura3-1 cdc13-1 yku70::NAT Rad52-YFP Rfa1-CFP RAD5</i>
	<i>cdc13-F688S</i>	JWW 2024.1	MATa <i>ADE2 his3-1 bar1::LEU2 trp1-1 lys2Δ ura3-1 Rad52-YFP Rfa1-CFP RAD5 cdc13-F684S</i>
	<i>yku70Δ cdc13-F688S</i>	JWW 2027.2	MATa <i>ADE2 his3-1 bar1::LEU2 trp1-1 lys2Δ ura3-1 Rad52-YFP Rfa1-CFP RAD5 cdc13-F684S yku70::NAT</i>

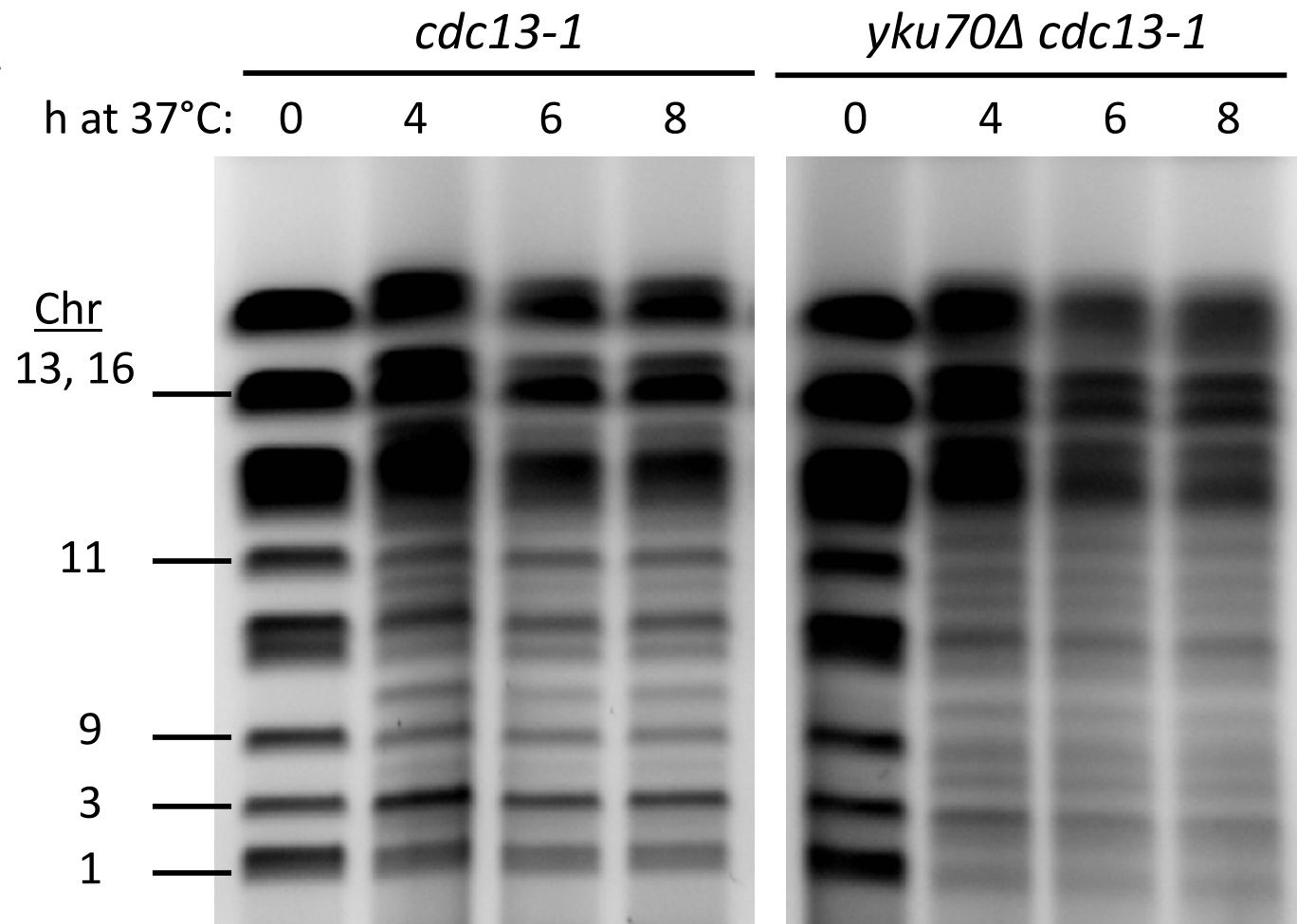
Supplementary Table S2

Primers used in preparation of probes for Southern hybridizations

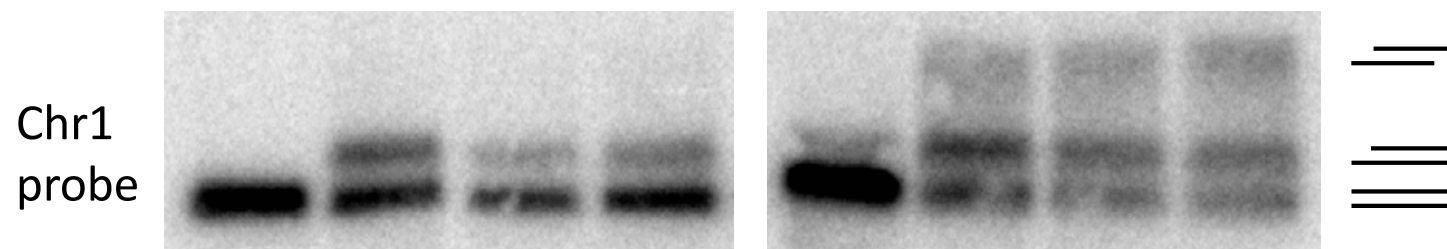
Probe	Primer name	Sequence
Chr 1	ADE1-5' probe	TGACGAAAACATCTCTCCTGC
Chr 1	ADE1-3' probe	CCTGTCAATGTTTCATAAGCC
Chr 3	CHA1-5' probe	AACGGCCGTGATCTCTAATC
Chr 3	CHA1-3' probe	TCCAACGCTTCTTCCAAGTC
Chr 9	IX408-fwd	TTTTCTCCATAACCACGGAGC
Chr 9	IX408-rev	ATTTTGTATGCGACAGCGAG
Chr 11 Left NotI fragment	XI46-fwd	AGAAAGCCACATTACTGGCA
Chr 11 Left NotI fragment	XI46-rev	AATTACACGCTGCTTCGCA
Chr 11 Right NotI fragment	XI616-fwd	TTCTCCTACTACGGGCTTTCC
Chr 11 Right NotI fragment	XI616-rev	GAACCGACATTGATCATGAAA

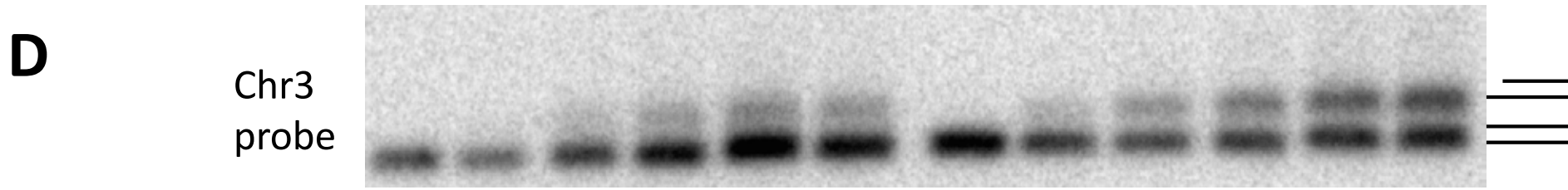
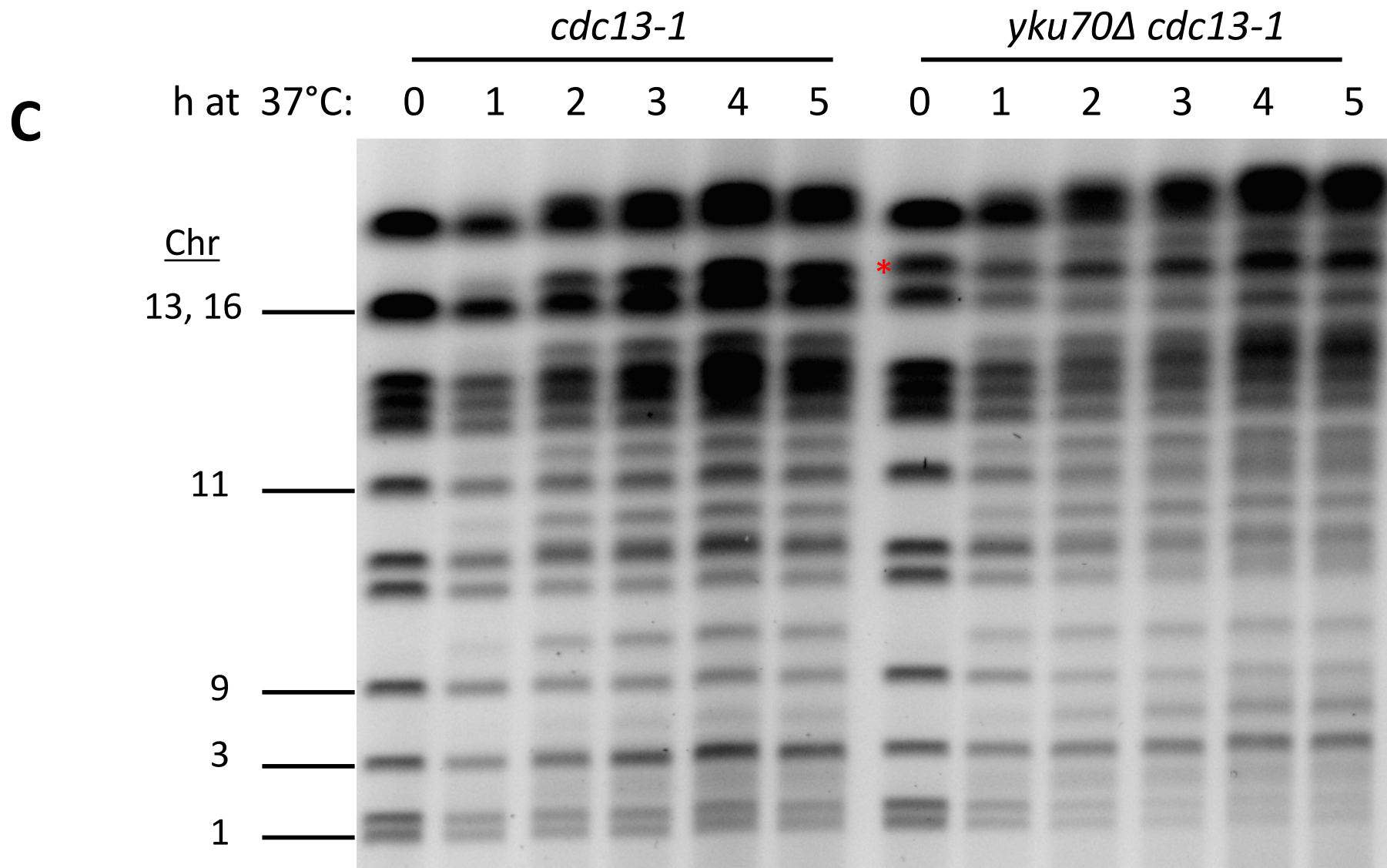
S1

A



B





Supplementary Figure S1. Global resection of telomeres detected by PFGE-shift and protection provided by Cdc13 and Yku70. **(A)** Cells in the CG379 strain background grown to late log at 20°C (YPDA+SRB) were diluted to fresh medium and shifted to 37°C. Chromosomal DNA was examined using the PFGE protocols in Material and Methods. The PFGE-shift was detected as bands above the main chromosomal bands obtained with cells that were not raised to the higher temperature. **(B)** Southern blots of Chr1 with ³²P-labeled probes. The line diagrams correspond to the relative positions of the bands. For example, the lowest band for the double mutant is unresected DNA, the next band up is for molecules with one or the other end resected, and the upper band corresponds to molecules with both telomeres resected. In **(C)** and **(D)** there were comparable but separate experiments as **(A)** and **(B)** except that there was a probe for Chr3 in **(D)**. The red asterisk in **(C)** indicates a GCR in Chr13 of the *yku70Δ cdc13-1* double mutant (see JWW2036.1 in Supplementary Table S1).

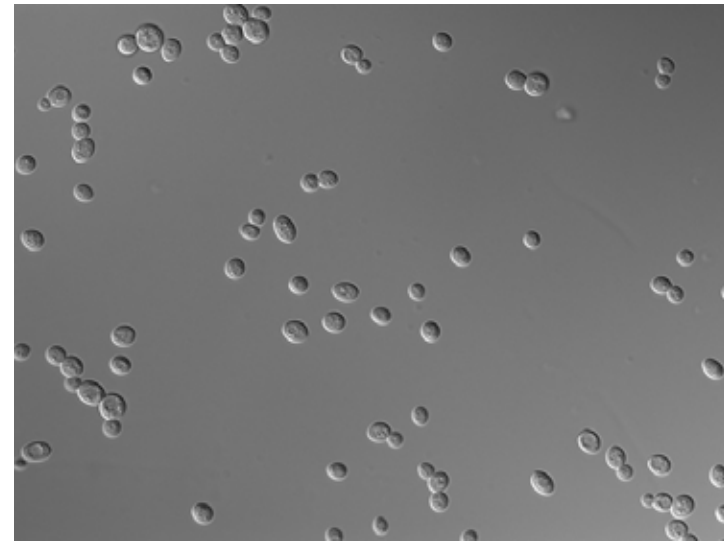
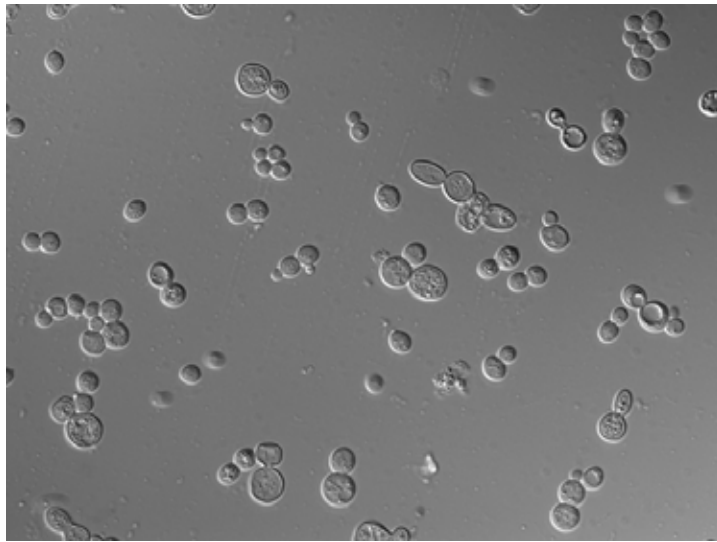
S2

yku70 cdc13-1

no sorbitol

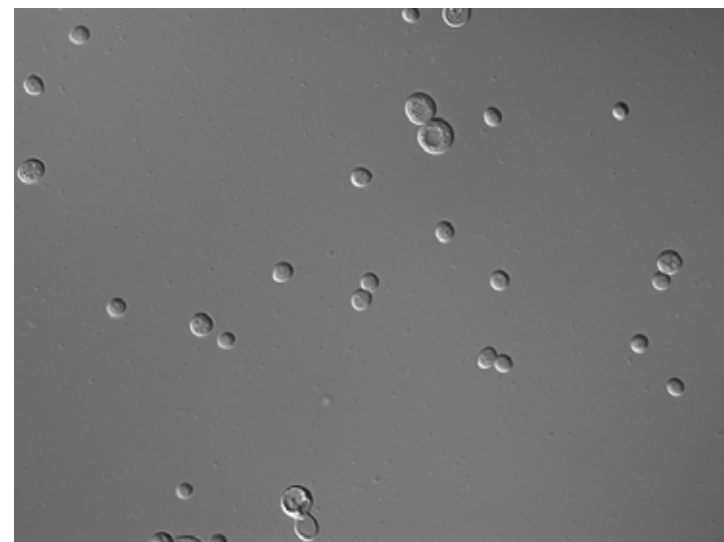
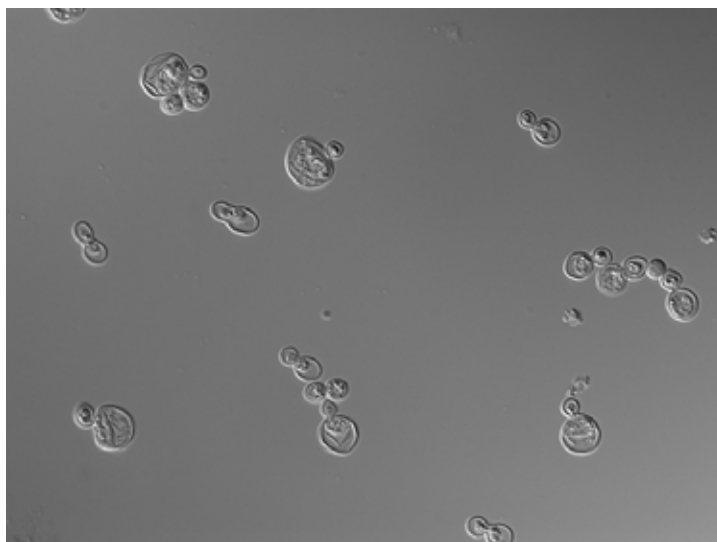
1M sorbitol

20° C



20° C

23° C



23° C

no sorbitol

1M sorbitol

Supplementary Figure S2. Relief of stress of W303 background *yku70Δ cdc13-1* mutants by including 1M sorbitol in YPDA medium. A frozen stock of the double mutant which had always been maintained on YPDA+SRB was patched to a YPDA±SRB plate and incubated for 2 days at 20°C before inoculating to liquid media at the indicated permissive conditions.

S3

Permissive temperature:

20°C

23°C

+sorb

-sorb

+sorb

-sorb

A

h at 37°C: 0 4 0 4 0 4 0 4

Chr

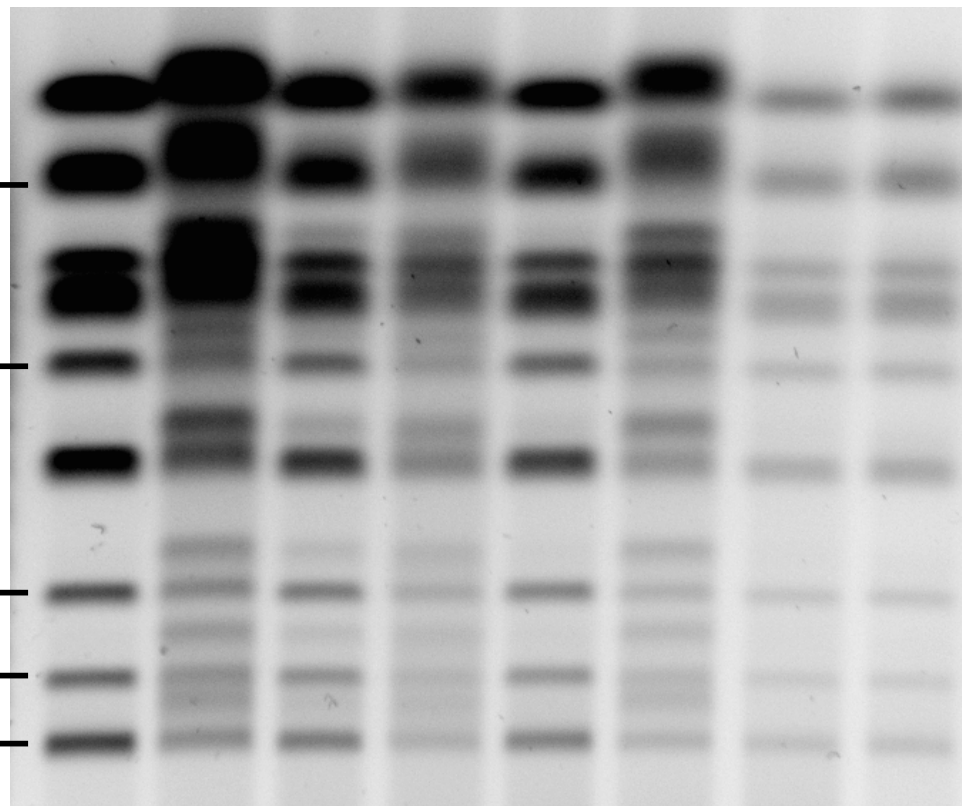
13, 16

11

9

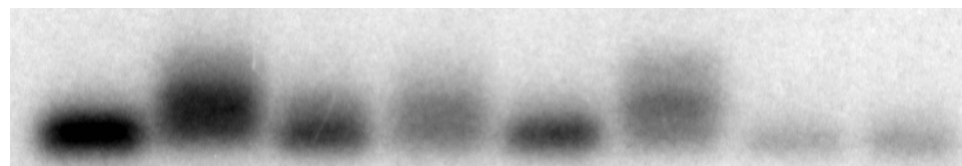
3

1

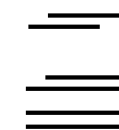
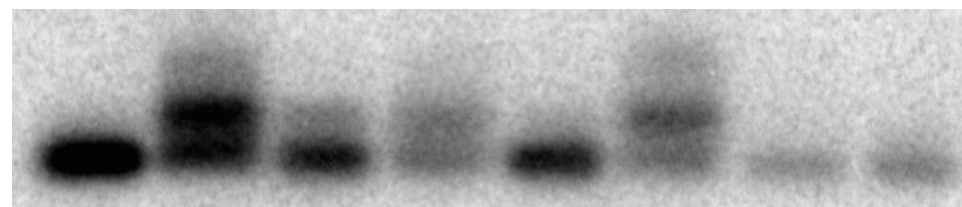


B

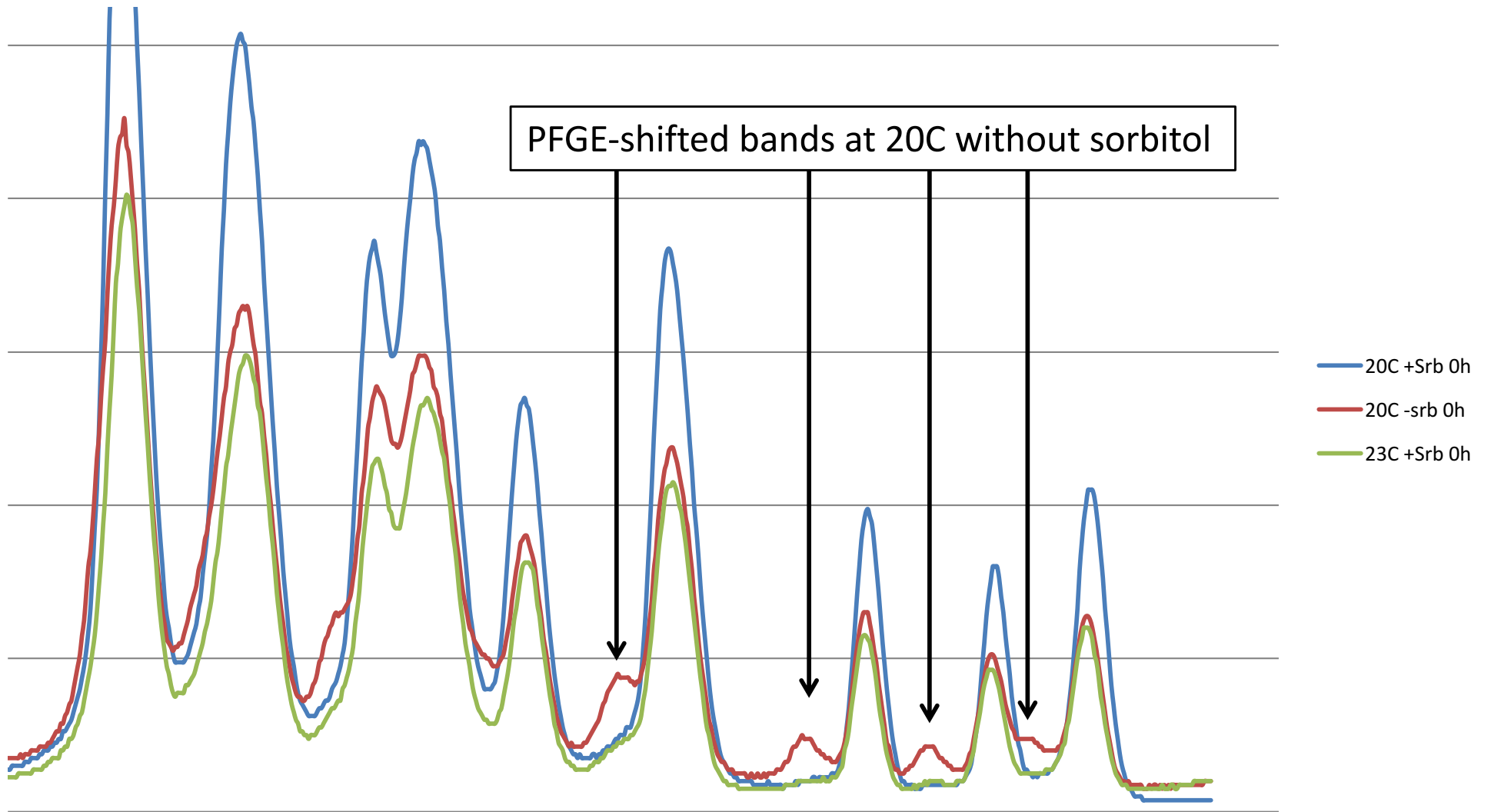
Chr11
probe



Chr9
probe



C

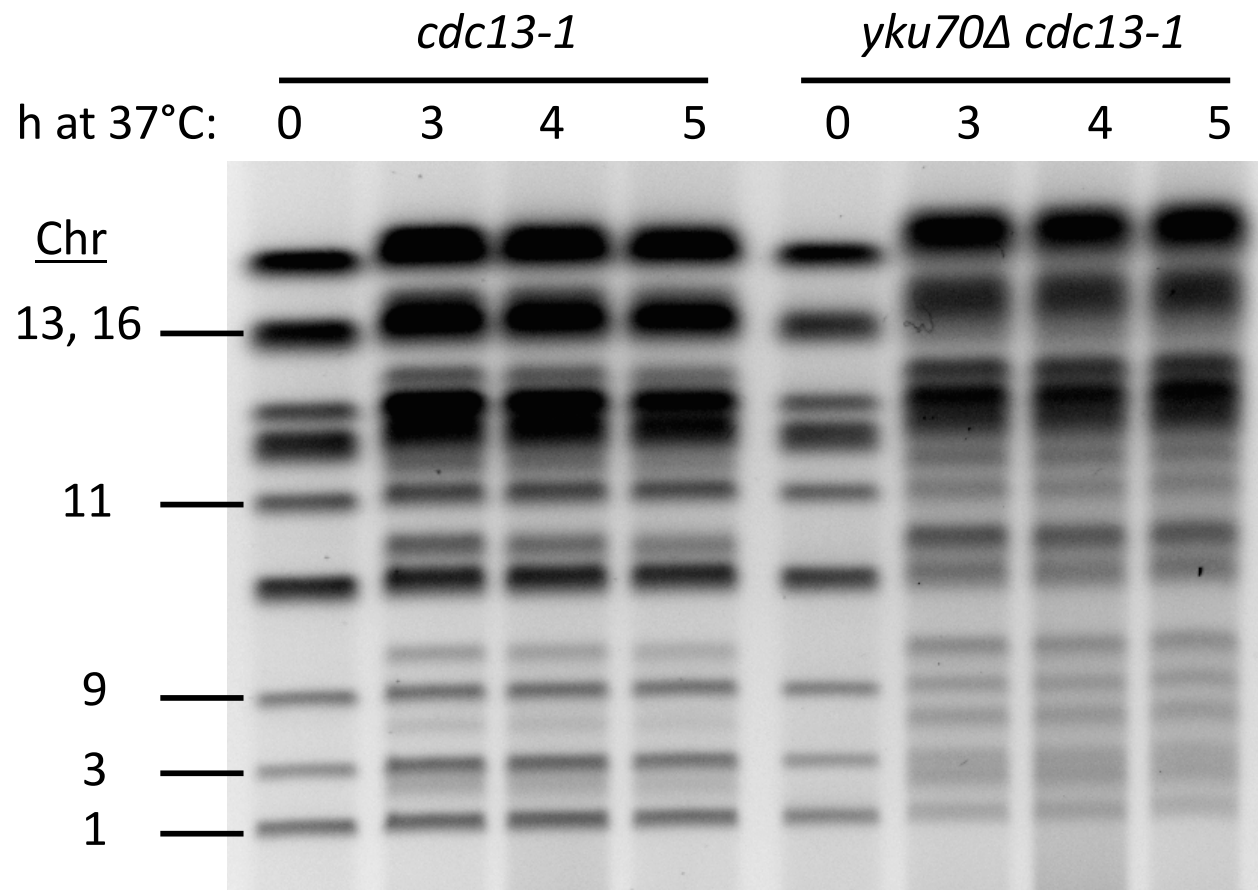


Supplementary Figure S3. Global resection of telomeres detected by PFGE-shift in *yku70Δ cdc13-1* W303 background cells at permissive temperatures 20°C (**A**) and 23°C (**B**) is prevented by inclusion of sorbitol in the medium. Conditions were the same as those in Figure 1C and 1D except for YPDA±SRB medium.

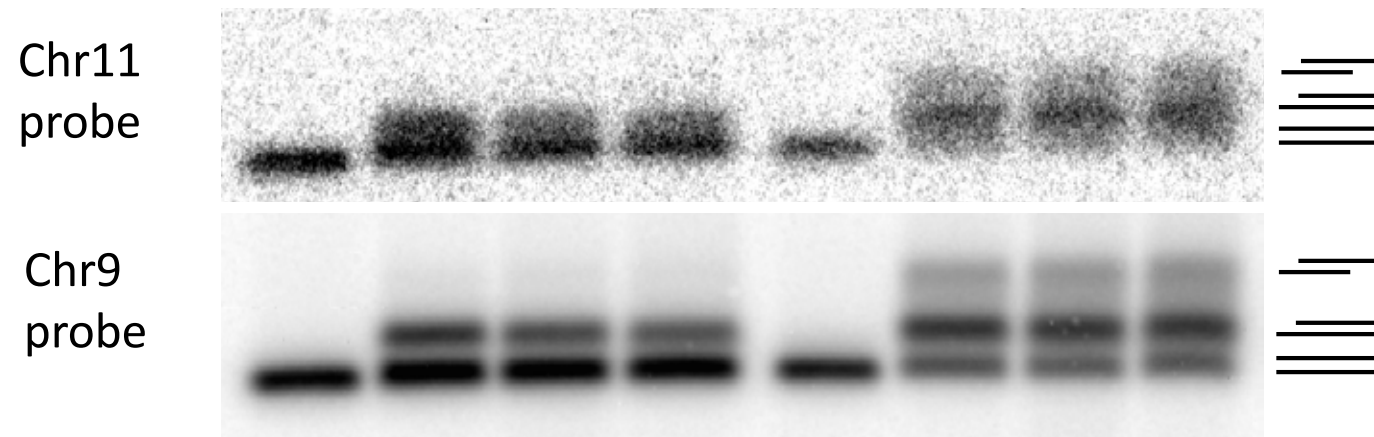
(C) Densitometry tracing of 0 h (permissive temperature) lanes from stained gel in **(A)**.

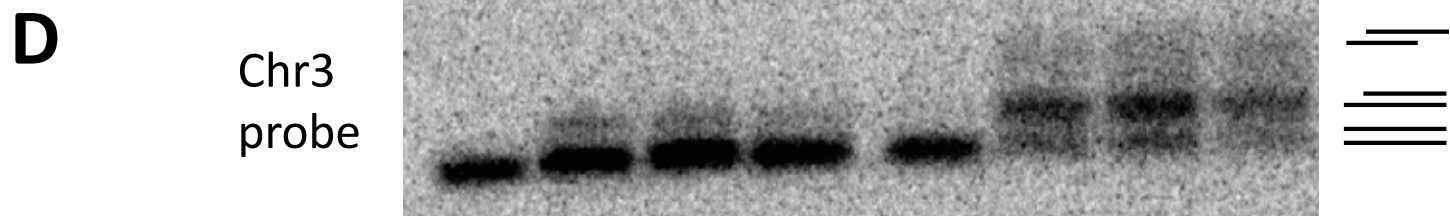
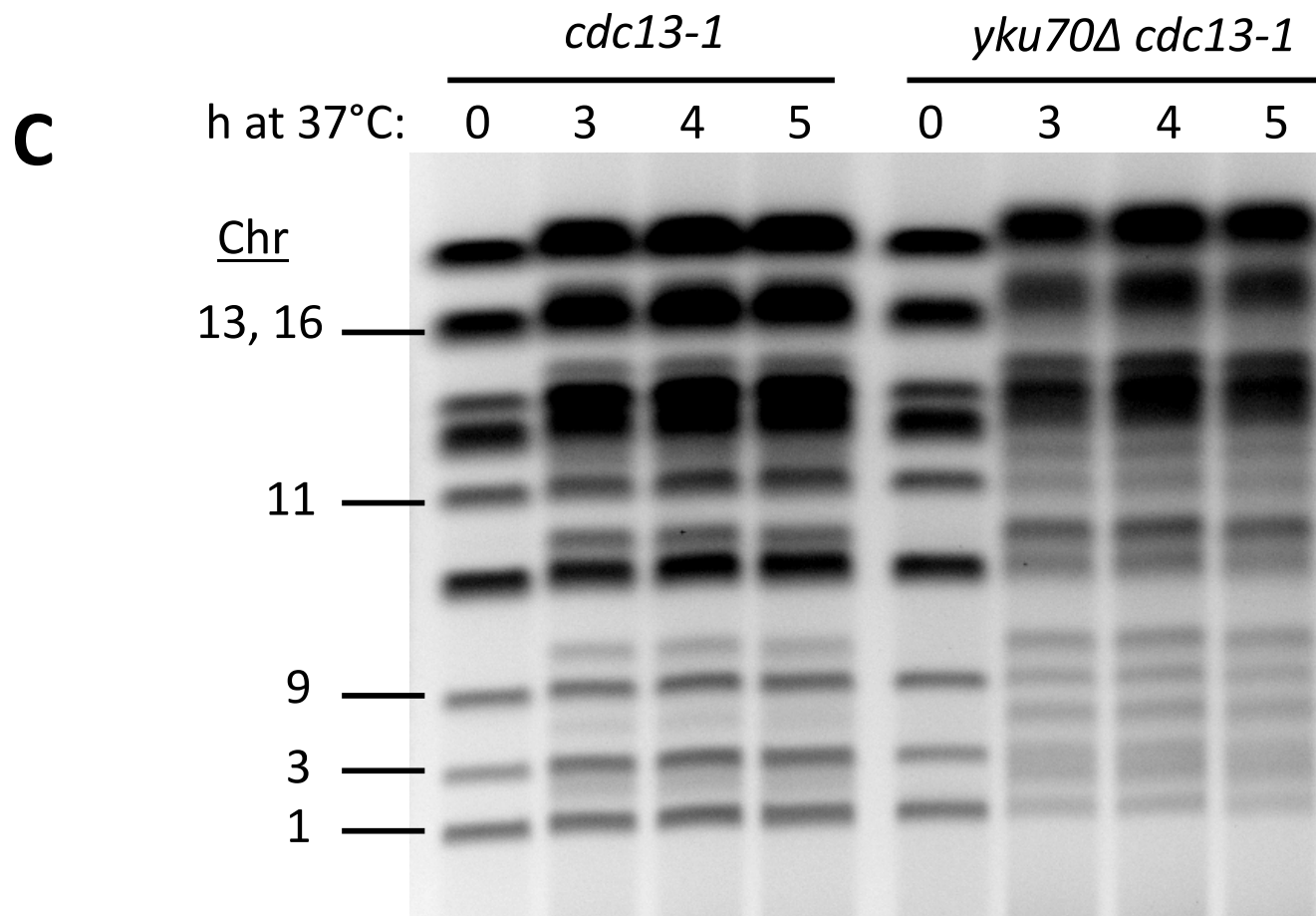
S4

A



B

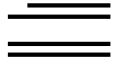
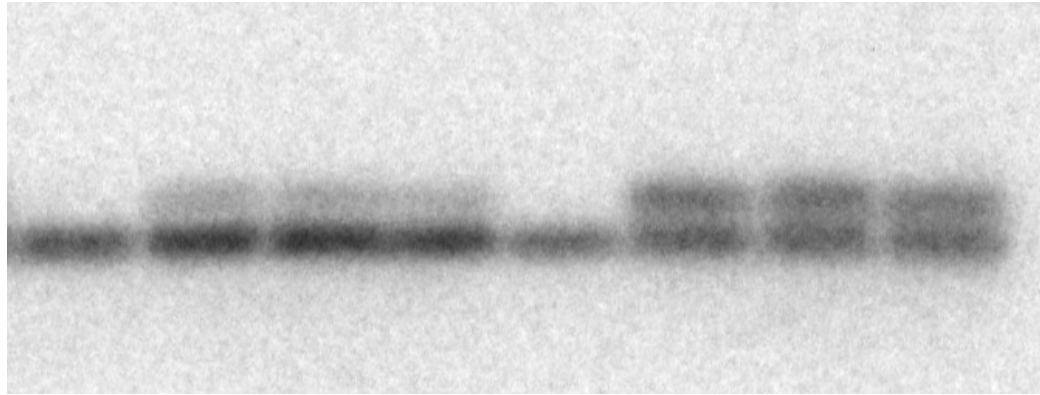




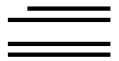
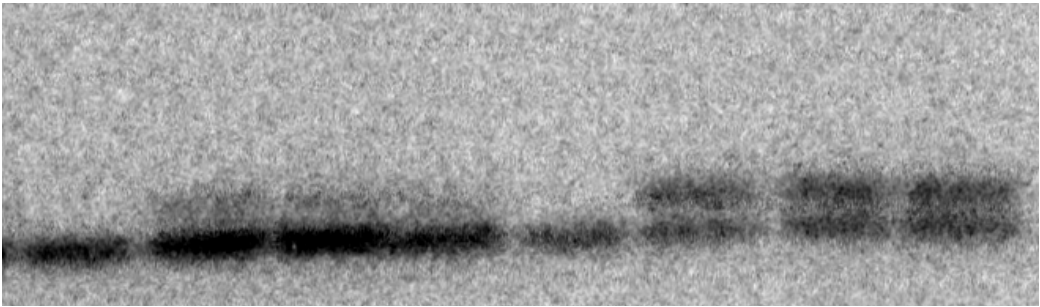
E

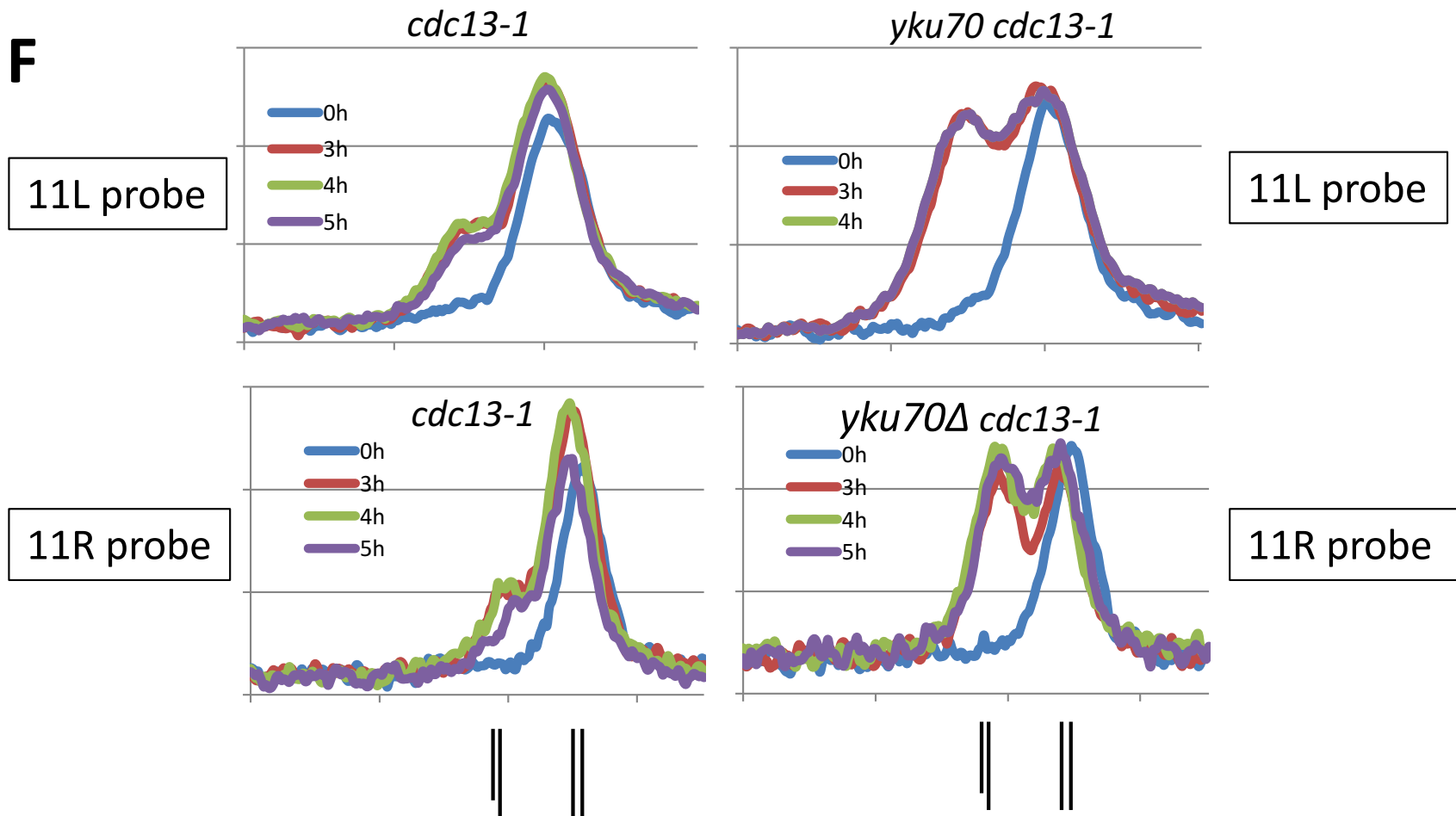
cdc13-1 *yku70Δ cdc13-1*
h at 37°C: 0 3 4 5 0 3 4 5

11L probe
Left telomere of Chr11
(351 kb NotI fragment)



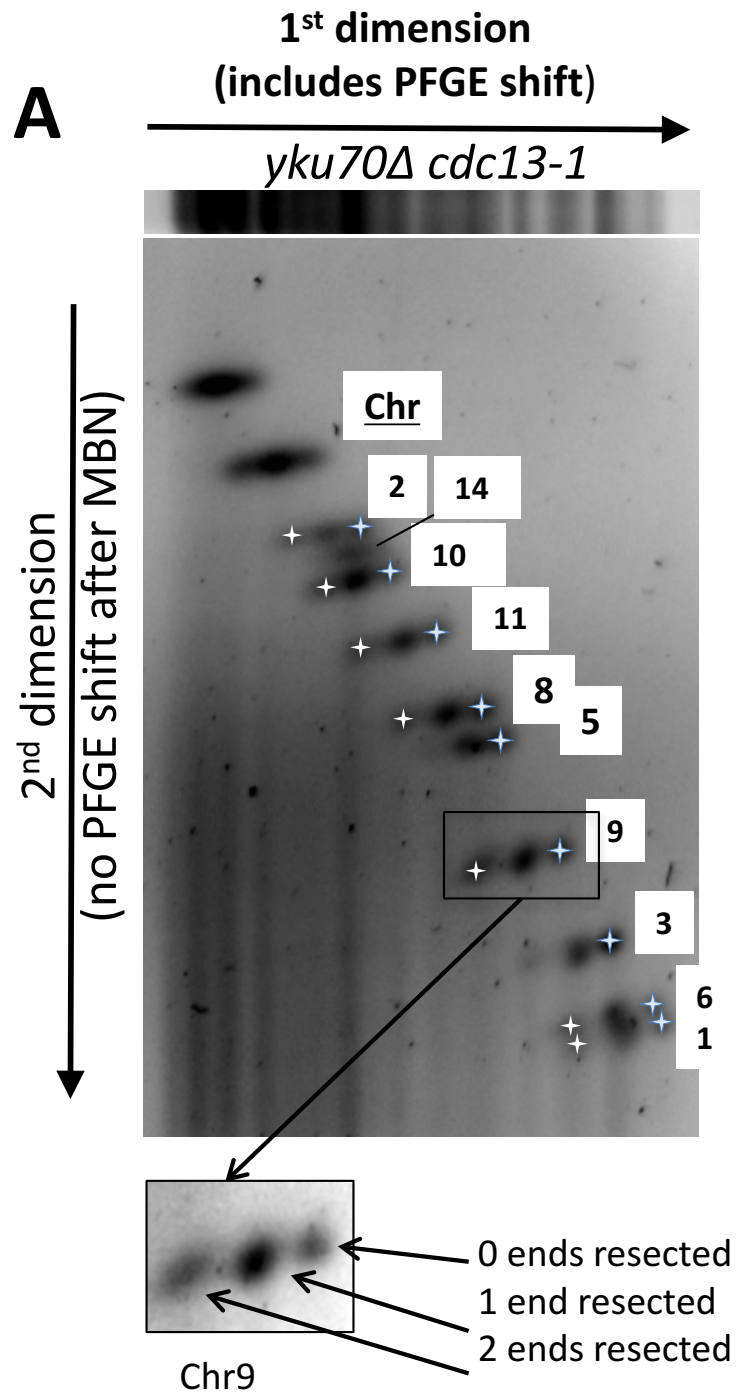
11R probe
Right telomere of Chr11
(286 kb NotI fragment)



F

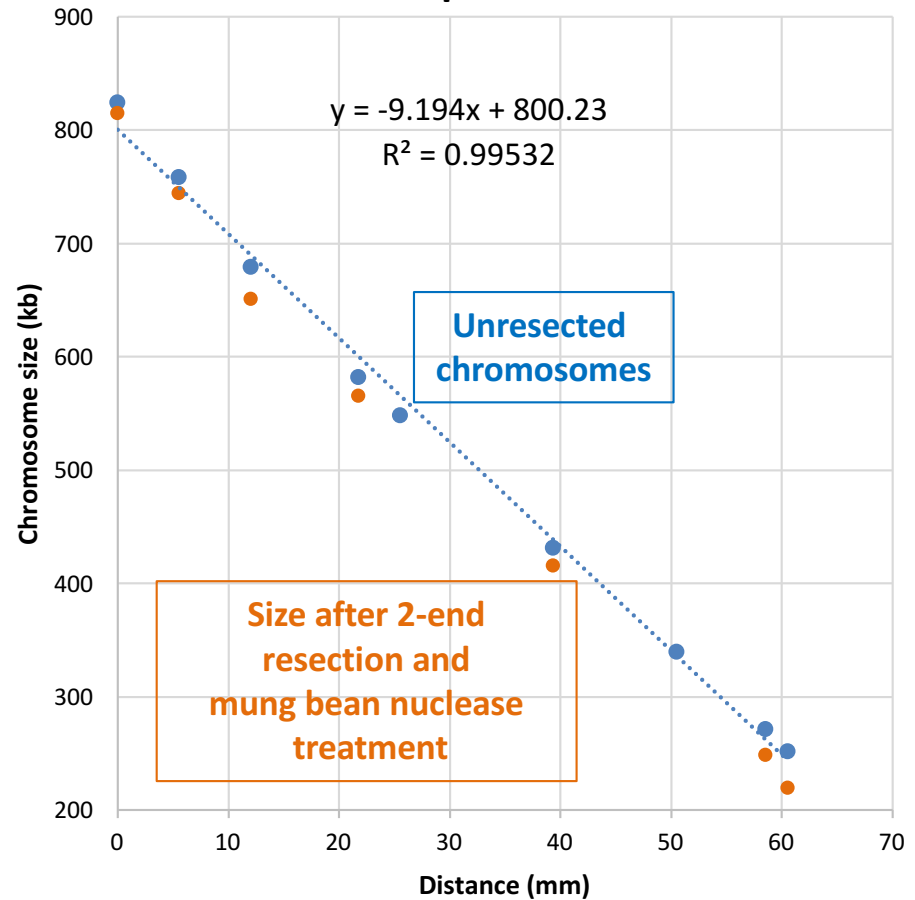
Supplementary Figure S4. Global telomere and chromosome telomere resection of telomeres detected by PFGE-shift in *cdc13-1* and *yku70Δ cdc13-1* W303 background cells. **(A)** Stained pulse-field gel of *cdc13-1* mutant and *yku70Δ cdc13-1* double mutant; **(B)** Southern probing for Chr9 and Chr11 of the gel shown in **(A)**; **(C)** separate experiment with *cdc13-1* and *yku70Δ cdc13-1* mutants; **(D)** Southern of the stained gel in **(C)** with Chr3 probe. **(E)** Resection at the “left” and “right” ends of Chr11. Sample plugs were digested with restriction enzyme NotI before PFGE and Southern transfer. The blot was first probed (11L probe) for the fragment containing the left telomere of Chr11. It was then stripped and probed again (11R) for the right fragment of Chr11. (Note that NotI fragmentation of Chr11 eliminated the possibility of 2-end PFGE-shift that is found in the whole chromosome PFGE-shift assay in *yku70Δ cdc13-1* double mutants.) Presented in **(F)** are the densitometry profiles of **(E)**. Line diagrams at the bottom of **(F)** indicate relative positions of PFGE-shifted (1-end resected) and non-PFGE-shifted (0-ends resected) positions of the densitometry profiles.

S5



B

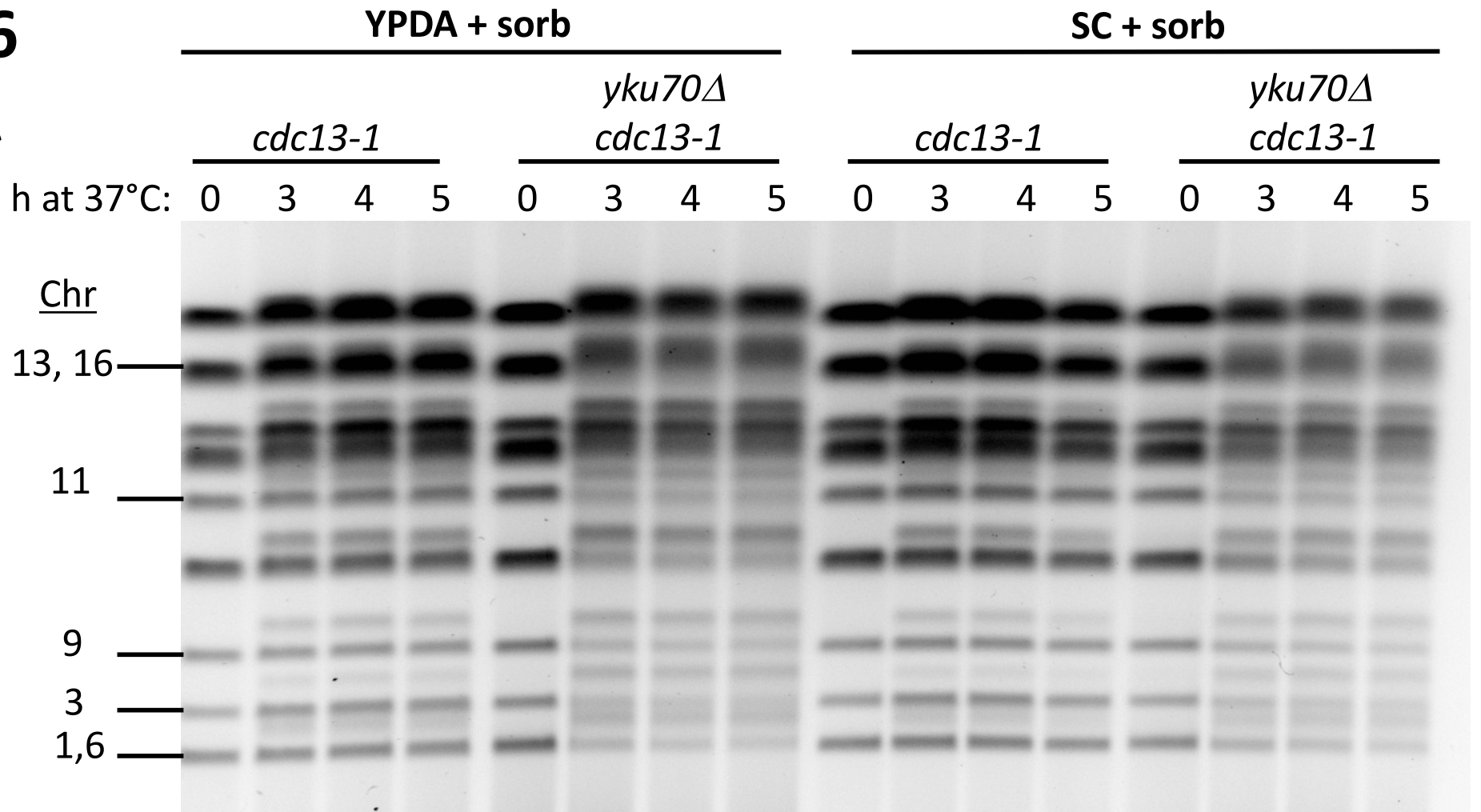
Linear relation between chromosome size and distance from the 825 kb (Chr2) position



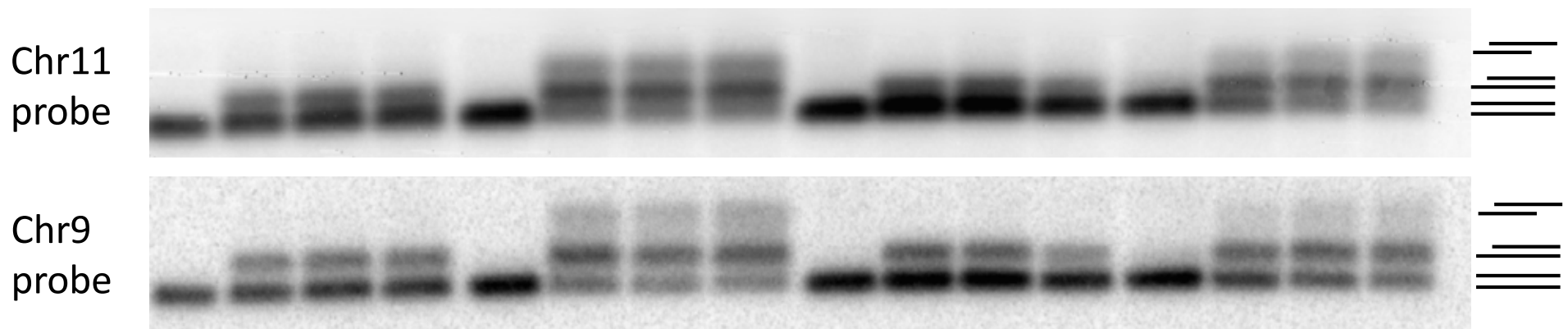
Supplementary Figure S5. Extent of resection determined from 2-D PFGE positions of chromosomes in Figure 3. (A) This corresponds to panel (C) of Figure 3, the *yku70Δ cdc13-1* cells from the 2-D PFGE at 4 h after shifting to 37°C. The starred markers identify the positions of the 0-end (blue) and the 2-end (white) resected chromosomes. (B) Presented is the size (in kb) of the unresected chromosomes (0-end) vs the vertical position of the chromosomal spot on the gel (i.e., vertical distance migrated from unresected Chr2). The actual sizes of the individual chromosomes in our CG379 background were determined using a different 1-D gel and an adjoining lane that had a lambda phage DNA ladder. Over the range of chromosomes that vary in size from 252 kb (Chr1) to 825 kb (Chr2), there is an approximate linear relationship between chromosome length and distance migrated. This enabled us to assess the size of the 2-end resected chromosomes after removal of the tails by the mung bean nuclease. The orange dots correspond to the sizes of the 2-end resected chromosomes after mung bean nuclease treatment. The resection length per 2-end resected chromosome appears to vary between 15-30 kb with an average of 20 kb, corresponding to 10 kb per telomere end.

S6

A



B



Supplementary Figure S6. Similarity in resection between cells incubated in YPDA+SRB and SC+AD+SRB medium. The experimental design is as described in supplementary Figures S4, panels A through D. Since experiments to characterize Rad52 GFP and Rfa1 CFP foci were done with nocadazole (as described in the text and in Figure 7), the SC+AD+SRB medium also contained nocadazole.

Supplementary Table S3

Frequency of cells with 7 or 8 Rfa1-CFP foci

Genotype	Time (h) at 37°C	% cells with 7 or 8 Rfa1-CFP foci per nucleus	Total budded cells examined
WT	4	0	369
<i>yku70Δ</i>	4	0	302
<i>cdc13-1</i>	4	1.5	267
<i>yku70Δ cdc13-1</i>	4	9.5	149
WT	5	0	289
<i>yku70Δ</i>	5	0	274
<i>cdc13-1</i>	5	3.9	237
<i>yku70Δ cdc13-1</i>	5	3.8	208
WT	4	0	479
<i>yku70Δ</i>	4	0	470
<i>cdc13-FS</i>	4	0.49	395
<i>yku70Δ cdc13-1</i>	4	4	431