Hotspot of *de novo* telomere addition stabilizes linear amplicons in yeast grown in sulfate-limiting conditions

Remington E. Hoerr[1], Alex Eng[2], Celia Payen[2,3], Sara C. Di Rienzi[2,4], M. K. Raghuraman[2], Maitreya J. Dunham[2], Bonita J. Brewer[2], Katherine L. Friedman[1]

[1] Department of Biological Sciences, Vanderbilt University, Nashville, TN, USA, Email: remington.hoerr@vanderbilt.edu; katherine.friedman@vanderbilt.edu

[2] Department of Genome Sciences, University of Washington, Seattle, WA, USA, Email: engal@uw.edu; raghu@uw.edu; bbrewer@uw.edu; maitreya@uw.edu;

[3] Current affiliation: IFF, Wilmington, Delaware, USA, Email: celia.e.payen@iff.com

[4] Current affiliation: Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston TX, USA, Email: sara.dirienzi@bcm.edu

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Abstract:

Evolution is driven by the accumulation of competing mutations that influence survival. A broad form of genetic variation is the amplification or deletion of DNA (≥50 bp) referred to as copy number variation. In humans, copy number variation may be inconsequential, contribute to minor phenotypic differences, or cause conditions such as birth defects, neurodevelopmental disorders, and cancers. To identify mechanisms that drive copy number variation, we monitored the experimental evolution of *Saccharomyces cerevisiae* populations grown under sulfate-limiting conditions. Cells with increased copy number of the gene *SUL1*, which encodes a primary sulfate transporter, exhibit a fitness advantage. Previously, we reported interstitial inverted triplications of *SUL1* as the dominant rearrangement in a haploid population. Here, in a diploid population, we find instead that small linear fragments containing *SUL1* form and are sustained over several generations. Many of the linear fragments are stabilized by *de novo* telomere addition within a telomere-like sequence near *SUL1* (within the *SNF5* gene). Using an assay that monitors telomerase action following an induced chromosome break, we show that this region acts as a hotspot of *de novo* telomere addition and that required sequences map to a region of <250 base pairs. Consistent with previous work showing that association of the telomere-binding protein Cdc13 with internal sequences stimulates telomerase recruitment, mutation of a four-nucleotide motif predicted to associate with Cdc13 abolishes *de novo* telomere addition. Our study suggests that internal telomere-like sequences that stimulate *de novo* telomere addition can contribute to adaptation by promoting genomic plasticity.

37 **Introduction:**

38 Genomic variation is the basis of evolution. Mutations, including both single-nucleotide
39 polymorphisms and larger genomic rearrangements, can rise in prevalence within a population if they
40 confer a fitness advantage. Copy number variation (CNV) refers to a genomic rearrangement that
41 results in loss or gain of DNA (Alkan et al. 2011; MacDonald et al. 2014). CNV is most likely to have a
42 phenotypic impact when the DNA that is duplicated or lost contains one or more genes that impact
43 cellular fitness. In humans, CNV is associated with a variety of disorders primarily (but not exclusively)
44 related to intellectual disabilities, developmental delays, and predisposition to cancers (reviewed in
45 Zhang *et al.*, 2009; Shaikh, 2017; Doran and Pennington, 2022; R. H. Song *et al.*, 2022).

46 CNV can arise through a variety of chromosomal rearrangements stemming from errors in
47 replication fork progression, recombination, or in DNA damage repair pathways (Hastings et al. 2009).
48 The frequency of CNVs is often increased in cancer cells due to dysregulation of DNA repair or
49 replication pathways (Doran and Pennington 2022). Gene amplification can present in a variety of
50 configurations including both direct and inverted tandem duplication (additional copies at the original
51 location), dispersed duplication (integration of additional copies elsewhere in the genome), or double
52 minute chromosomes that are small circular extrachromosomal fragments of DNA that lack a
53 centromere (Biedler et al. 1983; Chakraborty and Ay 2018; Doran and Pennington 2022).

54 The origin of CNVs is not well understood, in part because the initiating event may be replaced
55 over time by more beneficial secondary and tertiary events that confer increased growth advantages
56 (Ilić et al. 2022). For example, double minute chromosomes may in some cases serve as
57 recombinational intermediates in the formation of tandem or dispersed duplications (Turner et al. 2017;
58 Rosswog et al. 2021; K. Song et al. 2022). Since most studies of CNV in humans rely on samples from a
59 single point in time, they provide only a snapshot of the most dominant rearrangement (Chakraborty

60 and Ay 2018). This limitation highlights the need for experimental systems in which the temporal
61 relationship between events can be observed.

62 The budding yeast *Saccharomyces cerevisiae* utilizes DNA repair pathways similar to those of
63 human cells but is more easily manipulated and monitored for genomic rearrangements (Krogh and
64 Symington 2004; Wellinger and Zakian 2012). For example, growth under specific environmental
65 conditions selects for amplification of one of several genes (e.g., *HXT6/7*, *CUP1, GAP1,* or *SUL1*) (Brown
66 et al. 1998; Gresham et al. 2008; Kao and Sherlock 2008; Gresham et al. 2010; Zhang et al. 2013).
67 Growth of continuous yeast cultures in a chemostat allows the genetic composition of the population to
68 be monitored over time, providing an attractive system to monitor CNV formation and persistence in a
69 heterogenous population. Previously reported evolution experiments with *S. cerevisiae* describe several
70 different types of chromosomal rearrangements that increase gene copy number. Extrachromosomal
71 circular DNAs, similar to the double minute chromosomes described above, have been reported
72 (Gresham et al. 2010). Additionally, persistent linear fragments have also been described (Dorsey et al.
73 1992; Narayanan et al. 2006). Circular molecules lack DNA ends, but linear fragments must protect their
74 ends from nucleolytic degradation. In most cases, protection of the ends is afforded by the telomere, a
75 repetitive, protein-bound DNA sequence that resists nuclease action and engagement with the DNA
76 repair machinery (Blackburn 1991). Where such linear fragments have been described, they arise from
77 palindromic duplication, resulting in an isochromosomal fragment capped at both ends by copies of the
78 same pre-existing telomere (Dorsey et al. 1992; Narayanan et al. 2006).

79 In haploid laboratory yeast strains grown in sulfate-limiting chemostats, the fitter variants
80 almost always contain an inverted interstitial triplication of the primary sulfate transporter gene (*SUL1*)
81 and variable amounts of the flanking regions in an otherwise unrearranged chromosome (Araya *et al.*
82 2010; Payen *et al.* 2014; Brewer *et al.* 2015). Here, we report that diploids of the same laboratory strain
83 appear to follow a different path to *SUL1* amplification. Instead of chromosomally stabilized amplicons,

84 we find extrachromosomal linear fragments that contain *SUL1* and the nearby endogenous telomere at
85 one end and a new telomere added to a sequence centromere proximal to *SUL1* at the other end.
86 Similar fragments arose independently multiple times, suggesting that one or more sequences
87 centromere proximal to *SUL1* have a propensity to stimulate new telomere formation. Indeed, we show
88 that a sequence within the *SNF5* gene is sufficient to stimulate *de novo* telomere addition when moved
89 to an ectopic location. The ability of this sequence to stimulate *de novo* telomere addition requires at
90 least two short sequence motifs that are predicted to associate with the telomere binding protein Cdc13
91 (Eldridge et al. 2006) and can be functionally replaced by artificial recruitment of Cdc13.
92
93 **Methods and Materials:**
94 Strains and Plasmids
95 Eighteen sulfate-limited chemostat experiments (S701-S712, S10101-3 and S10105-7) were performed
96 on diploid strains of the S288c background (FY3 and FY5) (Gresham et al. 2008; Miller et al. 2013) that
97 were isogenic except for their ARS228 status- *ARS228*/*ARS228*, *ars228*/*ars228*, and *ARS228*/*ars228*.
98 Creation of the *ars228* mutation has been described (Brewer et al. 2015). All strains for SiRTA analyses
99 were derived from S288c and are listed in Supplementary File 1. Plasmid pAB180 (pRS414-
100 ADHpromoter-GBD-*CDC13*) and pAB220 (pRS414-ADHpromoter-GBD) were gifts of A. Bianchi. Strains for
101 the HO cleavage assay were generated as described in Ngo et al. 2020. In brief, the HO endonuclease
102 recognition sequence was integrated on chromosome VII by one-step gene replacement using plasmid
103 pHO invC (based on JH2017; gift of J. Haber). The *URA3* gene was integrated by one-step gene
104 replacement using pRS306 (Sikorski and Hieter 1989) as template. Putative SiRTAs were inserted on
105 chromosome VII using CRISPR/Cas9 as described (Anand et al. 2017) using gRNA sequence 5'-
106 TGCGGCAAGTTCATCTTCCA. Sequences of different length within *SNF5* were amplified from genomic
107 yeast DNA with appropriate primers (File S1) and additional sequences were added that lie upstream (5'-

108 TTTCTTTGGAAAACGTTGAAAATGAGGTTCTATGATCTAC) and downstream (5'-
109 AGAACATAGAATAAATTTGGTACTGGAACGTTGATTAACT) of the gRNA recognition site. *RAD52* was
110 replaced by one-step gene replacement using pFA6a-TRP1 (Longtine et al 1998) as template.
111
112 Contour-clamped homogeneous electric field (CHEF) gels
113 Agarose plugs for CHEF gel electrophoresis were generated using the method by Lucas Argueso
114 (described in Kwan et al., 2016). For separation of whole chromosomes, run conditions in the BioRad
115 CHEF-DRII were 1% LE agarose in 0.5XTBE in 2.3 L 0.5% TBE running buffer at 14˚C. Switch times were
116 either 47" to 170" at 165V for 39-62 hours or 300" to 900" at 100V for 68 hr. Linear fragments were
117 separated from chromosomes by CHEF gels with switch times 1.7" to 2.6" at 198 V for 20 hr. Restriction
118 analysis of linear fragments was performed by conventional agarose gel electrophoresis in 0.4% ME
119 agarose gels in 1XTBE at 1V/cm for 19 hr at room temperature. Standard protocols for restriction
120 digestion, Southern blotting and hybridization using ^{32}P-labeled PCR probes were used. Linear
121 fragments were concentrated from plug supernatants using Centricon Filter devices (Millipore) and then
122 ethanol precipitated.
123
124 Sequence analysis of linear fragments
125 Whole genome sequencing of fragments purified from plug supernatants and split read analysis were as
126 described (Payen et al. 2014). Briefly, sequencing libraries were generated using Nextera tagmentation
127 (Illumina). Libraries were sequenced on an Illumina MiSeq instrument with paired end 150 bp reads.
128 Number of reads obtained per sample ranged from 42,619 to 345,290 (exact reads for each sample can
129 be found on each data deposit record, see below). Reads were then mapped onto the S. cerevisiae
130 genome with BLAST and filtered for those mapping to chromosome II. Coordinates on chromosome II
131 with large differences in upstream and downstream read coverage were identified as putative junction

132 points. Reads that mapped to these coordinates were aligned to generate junction sequences. Raw
133 sequencing data are deposited at the NIH Sequence Read Archive (SRA) under BioProject ID
134 PRJNA909857.
135
136 HO cleavage assay
137 The HO cleavage assay was performed as described in Ngo et al. 2020. In brief, cells were grown in SD-
138 Ura medium (+2% raffinose) overnight to an optical density at 600 nm (OD600) of 0.6-0.9. Cells were
139 plated on yeast extract peptone medium with 2% galactose (YEPGal). Simultaneously, cells were serially
140 diluted in water and plated on yeast extract peptone medium with 2% dextrose (YEPD) to determine
141 total cell number. When plasmid selection was required (pRS414, pAB220, and pAB180), cells were
142 plated on synthetic medium lacking tryptophan and containing either 2% glucose or 2% galactose. After
143 3 days, colonies were counted and at least 100 individual colonies were transferred to plates containing
144 5-fluoroorotic acid (5-FOA) to determine the frequency of *URA3* loss. For each experiment, at least 30 5-
145 FOA resistant clones were grown in YEPD for DNA extraction by MasterPure™ Yeast DNA Purification Kit
146 (Lucigen). The relative location of each chromosome truncation event was determined using multiplex
147 PCR with primers that anneal centromere or telomere proximal to the SiRTA (File S1). *De novo* telomere
148 addition events were amplified using CHECKCHR7GUIDE1F and TelomereRev as primers. The purified
149 PCR product was sequenced using Sanger sequencing to determine the site of *de novo* telomere
150 addition. Full data sets corresponding to Fig. 3 and 4 are found in Supplementary File 2.
151
152 **Results:**
153 **Linear fragments containing *SUL1* accumulate in cells grown in sulfate-limiting conditions**
154 Under sulfate-limiting conditions, *S. cerevisiae* cells with increased capacity for sulfate transport
155 exhibit a selective growth advantage (Gresham et al. 2008). The most common genomic rearrangement

156 observed after prolonged competitive growth of haploid lab strains in low-sulfate conditions is an
157 interstitial, inverted triplication of a chromosomal region containing the primary sulfate-transporter
158 gene *SUL1* and its adjacent origin of replication *ARS228*, located ~25 kb from the right telomere of
159 chromosome II (Payen et al. 2014; Brewer et al. 2015). From the structure of these amplicons (the
160 central copy of the triplication is in inverted orientation) Brewer et al. (2011) proposed a model [Origin
161 Dependent Inverted Repeat Amplification (ODIRA)] involving template switching at preexisting short
162 interrupted inverted repeats that flank the *SUL1* gene and its adjacent origin of replication (*ARS228*) and
163 the *SUL1* gene. This model proposes that, following fork stalling and reversal, inverted repeats at the 3'
164 end of the leading strands anneal to the complementary repeats on the lagging strand template.
165 Extension of the 3' ends by DNA polymerase and ligation to the nearest Okazaki fragment creates a
166 closed loop that is subsequently displaced from the replicating chromosome structure (Brewer et al.
167 2011). Replication of this evicted molecule in the next cell cycle forms a dimeric, inverted circular DNA
168 molecule that can recombine at the *SUL1* locus to generate the interstitial triplication with the inverted
169 central copy (Brewer et al. 2011). The requirement for an origin of replication within the region that is
170 triplicated is supported by the observation that deletion of *ARS228* results in larger amplicons that
171 encompass the next nearest replication origin (Brewer et al. 2015).
172 A key feature of the ODIRA model is the generation of an extrachromosomal intermediate that
173 later recombines with the chromosomal locus (Brewer et al. 2011). In an attempt to gather evidence for
174 the unique extrachromosomal intermediate, we constructed a diploid heterozygous for the *ARS228*
175 deletion (*ARS228*/*ars228Δ*; Brewer et al. 2015) and two diploid control strains (*ARS228/ARS228* and
176 *ars228Δ/ars228Δ*) and carried out independent evolution experiments in sulfate-limited chemostats (n =
177 10, 4, and 4, respectively). We reasoned that if the intermediate arose from the *ARS228* chromosome
178 and subsequently recombined with the *ars228Δ* chromosome, the resulting amplicon would contain
179 copies of both *ARS228 and ars228Δ*.

180 Contrary to expectations, these diploids did not produce the same type of inverted interstitial
181 amplicons of *SUL1* that has been observed in haploid strains. Array comparative genome hybridization
182 (aCGH) of each of the diploid populations after ~180 generations (33-36 days) under sulfate-limitation
183 revealed consistent amplification of the *SUL1* region (e.g., Fig. 1a, Supplementary Fig. 1). However, in
184 each case the amplicon was not interstitial but continued through the right telomere. The amplified
185 sequences were limited to this region of chromosome II and the new junctions were narrowly clustered
186 in a small region of chromosome II near the *SNF5* gene.
187 To determine the genomic location of the amplicons, we analyzed samples taken every few days
188 through the 33 days of chemostat growth (each day is approximately 6 generations) and analyzed the
189 yeast karyotype by contour-clamped homogeneous electric field (CHEF) gels. Southern blotting and
190 hybridization with probes specific to *SUL1* detected chromosome II as expected, but also revealed the
191 generation of small (~30 kilobases) molecules that typically were detected at around days 14-17 and
192 often persisted through the end of the chemostat run (Fig. 1b, Supplementary Figs. 1 and 2). Similar
193 sized fragments were present in all samples from all three genotypes (e.g., Fig. 1c, Supplementary Figs. 1
194 and 2). Because they were generated in diploid cells with both copies of *ARS228* deleted
195 (Supplementary Fig. 1c) we propose that the adjacent origin of replication *ARS229* can support the
196 creation and maintenance of these fragments. The sizes of many of these molecules estimated from
197 their migration on CHEF gels were consistent with the sizes of the amplicon detected by aCGH
198 suggesting that they were acentric linear fragments.
199 It is notable that the linear fragments often persisted in the population through the end of the
200 experiment. Because the fragments lack a centromere, mitotic partitioning is likely asymmetric.
201 However, the fragments are presumably retained in the population due to the selective growth
202 advantage provided by additional copies of *SUL1,* similar to the maintenance of an ARS plasmid under
203 selection. In half of the cases, chromosomally integrated *SUL1* amplicons were the predominant form

204 by the end of the chemostat run. In many of these cultures, the linear fragments disappeared
205 concurrently with the emergence of new, slower-migrating *SUL1*-hybridizing bands (Supplementary Figs.
206 1 and 2), suggesting either a precursor-product relationship between the linear fragments and the
207 chromosomally integrated form or the independent occurrence of a second chromosome amplification
208 event conferring increased fitness. One of the most intriguing aspects of these free linear fragments is
209 their consistent small size—ranging between ~26 and 42 kb (Fig. 1c). Since all of the linear fragments
210 extend through the right telomere of chromosome II, the newly formed left ends of the linear fragments
211 appear to arise near or within *SNF5*, a gene centromere proximal to *SUL1*.
212
213 **Linear fragments are stabilized by addition of a new telomere**
214 While selective pressure can partially compensate for the lack of a centromere, persistence of a
215 linear fragment requires protection at both ends from exonucleases. Normal chromosome ends
216 (telomeres) in *S. cerevisiae* consist of a thymine and guanine (TG)-rich strand that extends 10-15 bases
217 past the complementary adenine and cytosine (AC)-rich strand to create a 3' overhang (Blackburn 1991).
218 This TG-rich single-stranded overhang interacts with the enzyme telomerase, which utilizes its reverse
219 transcriptase catalytic subunit and intrinsic AC-rich RNA template to add telomeric repeats to the
220 chromosome end (Singer and Gottschling 1994; Lingner et al. 1997). In yeast, the 3' terminating strand
221 consists of a heterogeneous sequence pattern with a single thymine followed by one to three guanines
222 (abbreviated TG_{1-3}) (Wellinger and Zakian 2012).
223 The aCGH analyses (Fig. 1a, Supplementary Fig. 1) suggested that the right end of the linear
224 fragment contained the endogenous right telomere, but the structure of the left end was initially
225 unclear. More detailed analysis of the linear fragments was facilitated by the development of a method
226 to purify these small molecules through passive diffusion from agarose CHEF gel plugs. Over the course
227 of several weeks, the small fragments diffuse out of the agarose plug and into the supernatant where

228 they can be recovered, while the intact chromosomes remain trapped in the plug (Supplementary Fig.
229 3). Purified linear fragments were digested with *Eco*NI, which cleaves three times in the terminal region
230 of chromosome II (Fig. 2a). Following separation by CHEF gel, the Southern blotted was probed
231 sequentially with sequences that hybridize to either *SUL1* or telomeric DNA (Fig. 2b). *Eco*NI digestion
232 separated *SUL1* from the right telomere and allowed us to query both ends of the linear fragments. The
233 restriction fragments containing the right telomere hybridized to telomeric DNA ($C_{1\text{-}3}A/G_{1\text{-}3}T$) and not to
234 *SUL1* (Fig. 2b, gold rectangles). However, fragments of multiple sizes (ranging from ~3-25 kb) hybridized
235 to both the *SUL1* and the telomeric probe (Fig. 2b), suggesting that the left end of each fragment was
236 also capped by telomeric DNA.

237 To identify the sites of telomeric repeat addition, we determined the sequence of fragments
238 purified from the plug supernatants by Illumina sequencing. While short read sequencing is limited in its
239 ability to identify large rearrangements— particularly in organisms with genomes rich in repeated
240 sequences— in yeast, it is perfectly suited for identifying split reads that mark rearrangement junctions.
241 The split reads were confined to the junction between the internal chromosome II sequence and a new
242 telomere— no other junctions were detected along the length of the linear fragment. In addition, the
243 Southern blots and aCGH data were consistent with the absence of large structural changes to the linear
244 fragments. The sequencing results revealed that nine of the 15 fragments contained telomeric repeats
245 clustered within a ~100 bp region of the gene *SNF5*, approximately ~7 kb from *SUL1* (Fig. 3). The
246 remaining six events were dispersed across four other regions between *SNF5* and *SUL1* (Fig. 3).

247 Among the 11 experiments for which we had junction data from both population aCGH and
248 sequence of the *de novo* sites of telomere addition, eight were in perfect agreement. The remaining
249 three identified sequenced junctions that were discordant with the location of the aCGH junction,
250 suggesting an amplicon larger than that predicted by the telomere addition site. These three cases were
251 unique in that the linear fragments had disappeared before the last day of growth—the day on which

252 aCGH was performed. These results are not consistent with the free linear fragments giving rise to the
253 integrated *SUL1* fragments. Rather, they support the idea that a second, independent, more stable
254 chromosomal amplification event (that encompassed a larger section of chromosome II) out competed
255 the linear fragments. Among all of the linear fragments that were sequenced, there was only one
256 example where the new left end had acquired subtelomeric sequences (S706). In all of the other
257 examples the telomeric DNA was appended directly to the chromosome II sequence, consistent with
258 addition of a new telomere to internal sequences by telomerase.
259

260 **Sequences in the *SNF5* gene stimulate unusually high levels of *de novo* telomere addition**

261 The observation that many of the telomere addition events associated with extrachromosomal
262 *SUL1* fragments cluster within a small (~100 bp) region is reminiscent of sequences in the yeast genome
263 that are hotspots of *de novo* telomere addition (termed SiRTAs or Sites of Repair-associated Telomere
264 Addition) (Stellwagen et al. 2003; Obodo et al. 2016; Hoerr et al. 2021). SiRTAs contain strand-specific,
265 TG-rich sequence motifs (Obodo et al. 2016; Ngo et al. 2020). These motifs can provide a substrate for
266 the acquisition of telomeric sequences if they become exposed in single-stranded DNA either as a result
267 of 5' end resection following a DSB or at nicks in single-stranded gaps. In either case, the resulting TG-
268 rich 3' overhang can invade a similar sequence and acquire a telomere through break-induced
269 replication or can undergo *de novo* telomere addition by direct action of telomerase (Putnam and
270 Kolodner 2017). Such events are most easily assayed in cases where the new telomere is added to the
271 centromere-proximal side of the break, causing a terminal truncation. In contrast, the putative SiRTA
272 within *SNF5* is oriented in the opposite orientation on the right arm of the chromosome. Rather than
273 stabilizing the centromere-containing region of the chromosome, *de novo* telomere addition stabilizes a
274 terminal acentric fragment containing *SUL1*.

275 Like telomeres, SiRTAs have an asymmetric sequence pattern in which one strand is relatively
276 rich in T and G nucleotides (Obodo et al. 2016; Ngo et al. 2020). For telomerase to act upon a SiRTA, this
277 TG-rich strand must be exposed in single-stranded DNA. SiRTAs have a bipartite structure consisting of
278 one TG-rich region that is most often the site of telomere addition (the "Core") and a second TG-rich
279 "Stim" sequence located 5' to the Core on the TG-rich strand (Obodo et al. 2016). The Stim sequence
280 itself is rarely targeted for telomere addition but is required to stimulate *de novo* telomere addition in
281 the Core (Obodo et al. 2016). Based on this known structure, we speculated that the ~100 bp region of
282 *SNF5* most frequently observed to undergo telomere addition during generation of the linear fragments
283 represents the Core sequence and that an adjacent sequence would provide Stim function. For a
284 telomere to be added to the left end of the acentric fragment of chromosome II, the TG-rich sequence
285 must be on the bottom or 3' to 5' strand (Fig. 3, Fig. 4a). Analysis of this strand showed that a 718
286 nucleotide (nt) region including and extending 5' to the putative Core sequence (chromosome II
287 coordinates 779784 to 780501) is unusually TG-rich. Sequences of 300 nt from the 3' to 5' strand
288 generated as 10 base sliding windows extending across this region contain, on average, 66% T or G
289 nucleotides [(T+G)/300 = 0.66 ±0.05 n= 42; Supplementary Fig. 4a). In contrast, 300 nt sequences
290 randomly selected from the yeast genome (n=493) contain, on average, 50% T or G nucleotides
291 [(T+G)/300 = 0.50±0.04], as expected from an unbiased distribution relative to each other and the two
292 strands (Supplementary Fig. 4a). To ensure that our analysis included any potential Stim sequence, we
293 initially tested this entire 718 nt sequence for its ability to stimulate *de novo* telomere addition.
294 To determine the ability of this 718 nt sequence to stimulate telomere addition, we took
295 advantage of an assay in which the sequence of interest is integrated on the left arm of chromosome VII,
296 replacing sequences between chromosome VII coordinates 18086 and 18108 (Ngo et al. 2020). This
297 strain (Lydeard et al. 2010; Obodo et al. 2016) contains a single recognition site for the yeast
298 homothallic switching (HO) endonuclease that allows regulated generation of a double-strand break

299 approximately 2 kb distal to the sequence being tested (Fig. 4a). Of note, the *SNF5* sequence is inserted
300 on chromosome VII in the inverted orientation relative to the centromere compared with its orientation
301 on chromosome II. However, because the endogenous location is on the right arm of chromosome II
302 and the insertion site is on the left arm of chromosome VII, the TG-rich sequence that will stimulate *de*
303 *novo* telomere addition is, in both cases, located on the bottom (3' to 5') strand. In both configurations,
304 exposure of the TG-rich strand in single-stranded DNA (for example, by resection of a DSB occurring to
305 the left of the sequence of interest) followed by telomerase action on that 3' overhang will stabilize the
306 chromosome fragment located to the right of the lesion. On chromosome II, *de novo* telomere addition
307 will create an acentric terminal fragment. On chromosome VII, *de novo* telomere addition will truncate
308 the chromosome. The site at which the putative SiRTA is integrated is distal to the last essential gene on
309 the chromosome arm (*BRR6*), ensuring that cells adding a *de novo* telomere within the sequence of
310 interest remain viable. Because the sequence being tested is present both at its endogenous location
311 and at the integrated site on chromosome VII, we deleted *RAD52* to prevent repair by ectopic
312 recombination.

313 Growth of this strain on media containing galactose induces expression of the HO endonuclease,
314 generating a double-strand break (DSB) distal to the putative SiRTA (Lydeard et al. 2010; Obodo et al.
315 2016). Correct repair restores the cleavage site, leading to a cycle of cleavage and repair that culminates
316 in cell death or mutation/loss of the HO site. Among the cells that survive HO cleavage, those that have
317 lost a *URA3* marker distal to the HO site are identified through selection for growth on media containing
318 5-fluoroorotic acid (5-FOA) and are subsequently screened through a combination of PCR and Sanger
319 sequencing to identify telomere addition events (Obodo et al. 2016). The fraction of 30 analyzed clones
320 that have undergone *de novo* telomere addition within the sequence of interest represents the
321 "relative" frequency of *de novo* telomere addition and is used as a measure of SiRTA function (Obodo et

322 al. 2016; Epum et al. 2020). Previously described SiRTAs display relative frequencies of *de novo*
323 telomere addition ranging from 10-35% (Obodo et al. 2016; Ngo et al. 2020).
324 In this case, analysis of 5-FOA resistant clones surviving HO cleavage from four independent
325 assays (30 clones each) showed that 30.8% ±5% had incurred truncation events mapping to the 718 bp
326 sequence (Fig. 4b). Each of the 51 5-FOA resistant clones was analyzed by PCR using one primer that
327 anneals to the AC-rich telomeric strand and a second primer that anneals centromere-proximal to the
328 718 bp sequence (Mangahas et al. 2001). In every case, a product of the expected size was produced
329 which, when sequenced, revealed the direct addition of telomeric DNA to the chromosome II sequence
330 (File S2). Taken together, these results indicate that this region functions as a SiRTA (subsequently
331 referred to as the "*SNF5*" SiRTA due to its location within the *SNF5* open reading frame). 50 of the 51
332 telomere addition events mapped to ~180 bases at the 3' end of the 718 base region, relative to the TG-
333 rich strand (Fig. 4a, Supplementary Fig. 5). This ~180 base region includes the ~100 base region in which
334 telomere addition was observed in the linear fragments and 69% of the HO-induced events map within 3
335 bases of a telomere-addition event observed on the linear molecules. However, six of nine telomere
336 additions in the linear fragments lie 3' to the vast majority of telomere additions observed after HO
337 induction (Fig. 3 and Supplementary Fig. 5). It is unclear why events observed in the linear fragments
338 from the sulfate limited chemostats are distributed differently than in the HO assay.
339
340 ***De novo* telomere addition requires specific motifs associated with binding of Cdc13**
341 To refine the location of sequences required for *de novo* telomere addition, we tested a series of
342 fragments that included the region within which telomere addition occurred but were progressively
343 truncated at the opposite (5') end of the TG-rich strand. Fragments of 456 and 226 bp stimulated *de*
344 *novo* telomere addition as strongly as the full 718 bp sequence (Fig. 4b). In contrast, no telomere
345 addition events were observed in a fragment of 175 bp, despite retention of virtually all sequences that

346 were targeted by telomerase in the context of the larger fragment (Fig. 4a, b). We conclude that the
347 *SNF5* SiRTA has a structure similar to other characterized SiRTAs, with stimulatory sequences that map
348 to the ~50 bp region delimited by the 226 and 175 bp fragments. We refer to this 50 bp region as the
349 *SNF5* SiRTA Stim. Although the fraction of T and G nucleotides is elevated throughout the 718 bp region
350 (Supplementary Fig. 4b, left axis), the ratio of G to T is notably higher in the centromere proximal 226
351 nucleotide region that is sufficient for *de novo* telomere addition than in the remainder of the region
352 (Supplementary Fig. 4b, right axis). Because telomeres conform to a pattern of TG_{1-3}, the higher G to T
353 ratio is consistent with a sequence that has the ability to associate with telomeric proteins and/or the
354 RNA template of telomerase.

355 In *S. cerevisiae*, telomerase is primarily recruited to the telomere by the single-stranded binding
356 protein Cdc13 (Pennock et al. 2001; Bianchi et al. 2004). Binding of Cdc13 to TG-rich Stim sequences
357 rendered single-stranded as a result of 5' end resection following a DSB has been implicated in the high
358 frequency of *de novo* telomere addition within a SiRTA (Obodo et al. 2016; Epum et al. 2020).
359 Therefore, we hypothesized that the 50 bp *SNF5* SiRTA Stim functions through recruitment of Cdc13.
360 The optimal Cdc13 binding site within telomeric repeats has been characterized (5'-GTGTGGGTGTG)
361 (Anderson et al. 2003; Eldridge et al. 2006), but single nucleotide substitutions within most positions are
362 well tolerated. The largest decrease in Cdc13 binding affinity is observed when G1, G3, or T4 nucleotide
363 positions are substituted (<u>G</u>T<u>GT</u>GGGTGTG), suggesting that a 5'-GxGT-3' motif may be necessary (but
364 not sufficient) for Cdc13 binding (Anderson et al. 2003; Eldridge et al. 2006). The *SNF5* SiRTA Stim
365 sequence defined above contains two 5'-GxGT-3' motifs on the TG-rich (bottom) strand (Fig. 5a). To
366 determine if these motifs contribute to the high frequency of *de novo* telomere addition within the
367 SiRTA, we mutated each 5'-GxGT-3' motif to its complementary CxCA in the context of the 718 bp
368 fragment. Remarkably, mutation of one or both motifs nearly eliminated *de novo* telomere addition
369 (Fig. 5a, b), suggesting that both are required for SiRTA function. This result is consistent with previous

370 reports that SiRTA activity likely requires more than one Cdc13 binding site (Strecker et al. 2017). We
371 hypothesize that the SiRTA sequence must reach a threshold of Cdc13 binding to stimulate *de novo*
372 telomere addition and this threshold is not met in the absence of one or both 5'-GxGT-3' motifs.
373 To further interrogate the requirement of Cdc13 binding to stimulate *de novo* telomere
374 addition, we replaced the 50 bp region of the *SNF5* SiRTA Stim sequence with two copies of the Gal4
375 upstream activating sequence (Stim::2xUAS) to allow specific recruitment of the Gal4 DNA binding
376 domain (GBD) (Bianchi et al. 2004; Zhang and Durocher 2010; Obodo et al. 2016). We introduced an
377 empty vector, a vector expressing GBD alone, or a vector expressing a fusion of GBD to full-length Cdc13
378 into cells with the *SNF5* SiRTA Stim::2xUAS fragment integrated at the chromosome VII test site. As
379 expected, *SNF5* SiRTA Stim::2xUAS did not support high frequency *de novo* telomere addition with
380 empty vector or GBD alone. Consistent with previous reports that artificial recruitment of Cdc13 to the
381 SiRTA supports *de novo* telomere addition (Bianchi et al. 2004; Zhang and Durocher 2010; Obodo et al.
382 2016), expression of the GBD-Cdc13 fusion protein supported *de novo* telomere addition in 24.4% ±8.3%
383 of clones tested from three independent assays and stimulation was dependent on the presence of the
384 UAS sequences (Fig. 5c). These results further support our hypothesis that a threshold of Cdc13 binding
385 must be met to stimulate *de novo* telomere addition and that without the 5'-GxGT-3' motif-containing
386 Stim sequence, this critical threshold is not satisfied.
387
388 **Discussion:**
389 In this work, we demonstrate that diploid laboratory yeast strains grown in sulfate-limiting
390 conditions generate small, extrachromosomal linear DNA fragments containing the primary sulfate
391 transporter *SUL1* that persist because they provide a selective growth advantage. The linear fragments
392 are capped at one end by sequences from the endogenous right telomere and at the other end by *de*
393 *novo* telomere addition events clustered in a region centromere proximal to *SUL1*. Fragments of similar,

394 but not identical, size arise in multiple independent cultures, suggesting that some sequence feature or
395 features in this region are responsible for the creation and/or stabilization of these linear fragments.
396 These results raise three intertwined questions: 1) What features of the region make it a hotspot for
397 telomere addition? 2) What process generates the substrate for telomere addition in the sulfate
398 limiting chemostats (in the absence of HO cleavage)? And 3) Why do the diploids produce a different
399 form of *SUL1* amplicon than their isogenic haploids?

400 Telomere addition: We find multiple sequence features within the *SNF5* gene that contribute to
401 the stabilization of linear fragments through *de novo* telomere addition. We demonstrate that a DNA
402 fragment from the *SNF5* gene that contains the common sites of telomere addition is sufficient to
403 stimulate *de novo* telomere addition at an ectopic location, similar to other characterized hotspots of *de*
404 *novo* telomere addition (SiRTAs) (Obodo et al. 2016; Ngo et al. 2020). Previous work demonstrated that
405 association of Cdc13 with a SiRTA is critical for the stimulation of telomere addition (Obodo et al. 2016;
406 Epum et al. 2020). However, the number and/or affinity of Cdc13 binding sites sufficient to support *de*
407 *novo* telomere formation are unclear. Strecker *et al.* (2017) placed differing lengths of telomeric repeats
408 adjacent to a double-strand break to determine a threshold length above which telomere addition is
409 stimulated. Telomere addition is strongly inhibited at sequences below that threshold length (Strecker
410 et al. 2017). Hypomorphic mutations in Cdc13 increase this threshold value, suggesting that the net
411 number and/or affinity of Cdc13 binding sites likely determines the threshold at which a sequence acts
412 to stimulate telomere addition (Strecker et al. 2017). Our results are consistent with this hypothesis.
413 Remarkably, mutation of just four nucleotides corresponding to a predicted binding site for Cdc13
414 (Anderson et al. 2003; Eldridge et al. 2006) nearly eliminates detectable *de novo* telomere addition at
415 the *SNF5* SiRTA in the ectopic assay. Our results suggest that there are likely two Cdc13 binding sites in
416 the ~50 bp fragment that together constitute the *SNF5* SiRTA Stim. Mutation of either site lowers the
417 total amount of Cdc13 binding below the threshold required for this sequence to function as a hotspot

418 of *de novo* telomere addition. Consistent with this interpretation, replacement of the 50 bp *SNF5* SiRTA
419 Stim sequence with two copies of the Gal4 upstream activating sequence supports *de novo* telomere
420 addition only when cells express the Gal4 DNA binding domain fused to Cdc13.

421 Substrate for telomere addition: We showed that *SNF5* SiRTA activity is supported by a minimal
422 fragment of 226 bp. This small region comprises approximately ~1% (226 bp/20.2 kb) of the total region
423 of chromosome VII in which a viable chromosome truncation could occur (between the HO site and the
424 most distal essential gene on this chromosome arm), and yet nearly one third of all truncation events
425 were mapped to the *SNF5* SiRTA. This clustering of *de novo* telomere additions in a small region of *SNF5*
426 could result from chromosome fragility in this region; however, observations from the ectopic assay
427 argue against this model. The *SNF5* SiRTA undergoes unusually high levels of *de novo* telomere addition
428 even when the initiating chromosome break is intentionally induced two kilobases distal to the eventual
429 site at which telomerase acts, suggesting that it is a site at which DNA ends are preferentially repaired.
430 We conclude that this region of *SNF5* is not necessarily more prone to double stranded breaks, but
431 rather has the fortuitous combination of features that permit telomere addition.

432 While we have not detected double stranded breaks in chemostat cultures, we have evidence
433 that replication errors, instead of double stranded breaks, may generate aberrant intermediates that are
434 subsequently targeted for repair (Brewer *et al.* 2015). The discontinuous nature of DNA replication forks
435 provides an opportunity for nascent leading strands to anneal to the lagging strand template during fork
436 reversal at sites of interrupted inverted repeats (described as ODIRA in Brewer et al. 2011). In a scan of
437 the region at the 5' end of the *SNF5* gene we find a ~5 fold increase in the density of short interrupted
438 inverted repeats (Supplemental Fig. 6a) resulting primarily from a sequence in the promoter region (5'-
439 TGTTGTTG-3') that is complementary to the several stretches of CAA triplet repeats within the N-
440 terminal coding region of *SNF5* (Fig. 6a). When compared to >10,000 regions across the genome, the
441 *SNF5* region is enriched for short interrupted inverted repeats (Supplemental Fig. 6b). The regression of

442 a replication fork proceeding toward the centromere from *ARS228* or another distal origin (e.g., *ARS229*)
443 could provide multiple opportunities for this sequence on the leading strand to find a match with a
444 complementary sequence in the single stranded gaps on the lagging strand and thereby create a
445 "closed" fork (Fig. 6b). After completion of replication by a fork established centromere proximal to the
446 *SNF5* region, the hairpin capped linear could be released (Fig. 6b) (Brewer et al. 2011). Processing of the
447 single-stranded loop by nucleases provides the appropriate substrate for telomere addition (Fig. 6c, d).
448 Ploidy specific amplification pathways: In previous experiments with haploid laboratory strains,
449 we have not observed the generation of linear fragments—essentially all events were interstitial
450 inverted triplications on chromosome II (Payen et al. 2014). In some cases, rearrangements that would
451 be lethal in haploids are tolerated in diploids by the presence of a second, unrearranged chromosome
452 (Zhang et al. 2013). It is unlikely that a similar phenomenon is affecting the nature of *SUL1* amplification
453 in diploid and haploid cells as there are no essential genes beyond *SNF5* on chromosome II, so loss of
454 this terminal fragment should be tolerable in haploids. In point of fact, we do not detect loss of the
455 distal markers on chromosome II in the diploids— both homologs of chromosome II remain intact. The
456 aCGH profiles indicated that there were no regions at the right end of chromosome II that were present
457 at fewer than two copies per diploid genome and CHEF gels did not reveal any truncated variants of
458 chromosome II (Fig. 1 and Supplemental Fig. 1, and 2). While these results are consistent with repair of
459 a broken chromosome II by homology directed repair off the homolog or sister chromatid (perhaps by
460 break-induced replication), it is also possible that a double stranded break was not the initiating event.
461 We would like to propose that the same type of ODIRA event that can explain the events that occur in
462 haploid cells is also occurring in the diploids, but that the resolution of the intermediate differs between
463 haploids and diploids. Differences in the regulation of repair pathways between haploid and diploid
464 cells in this laboratory strain may contribute to the different outcomes (Li and Tye 2011). There may be
465 a race between replication of the hairpin intermediate and the processing of the single stranded loop

466 that is influenced by the levels of repair enzymes. In support of this notion, we have recently observed
467 that in some haploid wild strains with varied genetic backgrounds, linear amplicons similar to those
468 described here, also arising from the vicinity of the *SNF5* gene, are the preferred amplicon (manuscript
469 in preparation). Analysis of the transcriptomes and proteomes of these strains could identify co-varying
470 RNA/proteins that influence the amplicon outcome. Regardless of mechanism, our observations in
471 yeast may provide a useful model for the types of rearrangements that occur in humans and other
472 organisms. Finally, results presented here suggest that interstitial TG-rich hotspots of *de novo* telomere
473 addition that are oppositely oriented relative to the chromosome arm can stabilize an acentric fragment
474 to provide, in some circumstances, a selective advantage. These observations highlight a possible
475 evolutionary consequence of SiRTA sequences regardless of orientation, by increasing adaptation and
476 genome plasticity.

477 **Data Availability**

478 Strains and plasmids are available upon request. Source data for Figs. 4, 5, and Supplemental Fig. 5 are
479 provided in Supplemental File 2. Sequencing data are available from the NIH Sequence Read Archive
480 (SRA) under BioProject ID PRJNA909857. aCGH data are available from the NIH Gene Expression
481 Omnibus (GEO) under accession GSE220549.

482

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486

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492

493 **Figure legends:**

494 Fig. 1. Amplification of *SUL1* chromosomal fragments in sulfate-limited chemostats. a) Array
495 Comparative Genomic Hybridization (aCGH) of the population of a diploid yeast strain (Chemostat run
496 S712) 33 days after continuous growth in a sulfate limited chemostat. The locations of *SNF5*, *SUL1*, and
497 *ARS228* are indicated in the diagram at the top. The Log2 hybridization ratio of day33/day0 indicates
498 that the right end of chromosome II, containing the gene for the primary sulfur transporter *SUL1*, is
499 present in 8 or more copies in excess of the genomic copies. The presence of two discontinuities at 782
500 kb and 788 kb suggests two discrete amplicons within the cell population. b) CHEF gel electrophoresis of

501 a single population (S712) sampled across the 33 days of continuous growth in sulfate limited medium.
502 The ethidium bromide-stained gel displays the total yeast karyotype. The CHEF gel was Southern
503 blotted and probed sequentially for *SUL1* and *CEN2* as indicated. One extrachromosomal band of low
504 molecular weight was detected by the *SUL1* probe at day 17 that persists in the population onward. A
505 second, faster migrating band appears at day 23 (more clearly resolved as 2 bands in Figure 1c) and
506 persists in the population onward. c) CHEF gel electrophoresis, under conditions to determine the sizes
507 of extrachromosomal amplicons, from specific days of 15 individual chemostat runs. The ethidium
508 bromide-stained gel and Southern blot hybridized with *SUL1* reveal multiple small extrachromosomal
509 fragments. The sizes of extrachromosomal amplicons vary from 26 kb to 40 kb with a recurring event
510 producing a fragment of approximately 33 kb (red arrow).
511
512 Fig. 2. Linear fragments are capped by new telomeric sequences. a) Top: Positions of the *Eco*NI
513 restriction sites at the right end of chromosome II (coordinates 770 to 813 kb). Chromosome II-R
514 telomeric sequences are indicated with gold triangles. Bottom: Structure of linear fragments. Proposed
515 telomeric sequences at variable positions at the left margin of the amplicons are indicated by the orange
516 triangles. Positions of two probes [$C_{(1-3)}A/G_{(1-3)}T$ and *SUL1* (indicated by the horizontal bars)]. b) Linear
517 fragments purified from the plug supernatant (see Supplemental Fig. 3) for five cultures (S708-S712)
518 were digested with *Eco*NI and separated on a CHEF gel. Southern blots were sequentially hybridized
519 with a *SUL1* probe and a telomere specific probe ($C_{(1-3)}A/G_{(1-3)}T$). Cultures S710 and S712 contain two
520 species of extrachromosomal molecules. Orange triangles indicate the fragments with *de novo*
521 telomeres. Fragments boxed in gold correspond to the native right telomere.
522
523 Fig. 3. Extrachromosomal fragments purified from plug supernatants were prepared for Illumina
524 sequencing. Split reads (red/black sequences) were aligned to chromosome II (black sequence with

525 chromosome II coordinates). The junctions of the left end of the extrachromosomal fragments each
526 contained a new telomeric sequence (red) and potential seed sequences for telomere addition
527 (blue/underline). The number in parentheses to the right of each sequence corresponds to the number
528 of split reads that mapped to that site. One of the 15 sequences (S706) was a junction with an internal
529 portion of a Y' element. The remaining 14 contained unique junctions with C(1-3)A/G(1-3)T telomere
530 sequences. The four nucleotide 5'-GxGT-3' motifs (see Fig. 5) are in bold italics in the (top) reference
531 sequence.
532
533 Fig. 4. The *SNF5* SiRTA is sufficient to stimulate *de novo* telomere addition at an ectopic location. a) Top
534 schematic: the endogenous right arm of chromosome II; distance between the endogenous *SNF5*
535 sequence and *SUL1* is shown. Triangles represent the endogenous telomere. Middle schematic: lines
536 representing the relative size of each fragment from *SNF5* that was integrated and tested (actual size in
537 bp shown). Black arrows above the 718 bp fragment depict approximate locations of *de novo* telomere
538 addition events stimulated by HO cleavage. Blue arrows below the 718 bp fragment depict approximate
539 locations of spontaneous *de novo* telomere addition events from the chemostat experiments. Arrow
540 size is proportional to the number of independent events at that location (see Supplemental Fig. 5 for
541 exact numbers and locations). Bottom schematic: modified left arm of chromosome VII. Distances
542 between the integrated *SNF5* sequence and the HO cleavage site or the most distal essential gene
543 (*BRR6*) on this chromosome arm are shown. b) Chromosome truncation events following HO cleavage
544 preferentially involve *de novo* telomere addition in the SiRTA. At least 30 clones that survived HO
545 cleavage and lost the *URA3* marker were assayed for each experiment. The percentage of clones that
546 incurred telomere addition within the sequence of interest is shown. Bar height is the average of three
547 to four independent experiments; error bars represent standard deviations. Significant difference is

548 indicated (* p<0.05) by unpaired Student's *t-test* comparing 226 bp to 175 bp. See File S2 for data used
549 to generate graph.
550
551 Fig. 5. The *SNF5* SiRTA requires Cdc13 binding to stimulate *de novo* telomere addition. a) Top: lines
552 representing 718, 175 bp, and *SNF5* SiRTA Stim::2XUAS fragments integrated and tested on
553 chromosome VII. Sites of *de novo* telomere addition are depicted as in Fig. 4. Bottom: Twenty-nine
554 nucleotides of the TG-rich strand of the *SNF5* SiRTA Stim sequence are shown from 3' to 5'. Note that a
555 double-strand break that occurs to the left of this sequence will expose the TG-rich sequence in single-
556 stranded DNA following 5' end resection. 5'-GxGT-3' motifs and corresponding mutations are
557 highlighted in gray. b) Percentage of 5-FOA resistant clones that incurred *de novo* telomere addition
558 within the 718 bp *SNF5* SiRTA in the presence of the indicated mutation. Wild-type (WT) samples are
559 repeated from Fig. 4 for comparison. Samples with statistically significant values by one-way ANOVA
560 with *post hoc* Dunnett's are indicated (**, p< 0.01). c) Percentage of 5-FOA resistant clones that
561 incurred *de novo* telomere addition within the 175 bp *SNF5* fragment when 2 copies of the Gal4 UAS are
562 added (Stim::2xUAS) or when the 175 bp fragment is present alone. Cells were transformed with
563 pRS414 (empty vector) or pRS414 expressing either Gal4 DNA binding domain (GBD) or GBD fused to the
564 N-terminus of Cdc13 as indicated. For b and c, at least 30 colonies were assayed for each experiment.
565 Bar height is the average of three to four independent experiments; error bars represent standard
566 deviations. Samples with statistically significant values by one-way ANOVA with *post hoc* Tukey's are
567 indicated (*, p< 0.01; ***, p<0.001). See Supplementary File 2 for data used to generate graph.
568
569 Figure 6. A model to explain how an ODIRA event may generate a substrate for *de novo* telomere
570 addition at the *SNF5* SiRTA. a) The endogenous right arm of chromosome II contains interrupted
571 inverted repeats (IR; orange arrows) flanking the *SNF5* SiRTA (star) and the TG-rich strand of the right

572 telomere (gold triangles). In the expanded schematic the relevant sequences are shown for one possible
573 pair of inverted repeats (orange arrows), the start codon of *SNF5* (ATG), the Core of the *SNF5* SiRTA, and
574 the two 5'-GxGT-3' motifs from Figure 5 (labeled Stim 1 &2). b) Ligation of the leading strand to the
575 lagging strand following fork reversal at the leftward moving fork and template switching at the inverted
576 repeats results in a "closed" fork with the rightward moving fork completing replication through the
577 right chromosomal telomere. When a replication fork from *ARS227*, proceeding towards the telomere,
578 encounters the closed fork structure, a hairpin capped linear is displaced (red curved arrow). c) In the
579 displaced hairpin-capped linear the inverted repeats form the base of the terminal single stranded loop
580 that contains the SiRTA Stim and Core sequences. Cleavage of the hairpin to the 3' side of the SiRTA (1)
581 and resection at the site of the break (dashed lines) exposes a 3' single stranded tail containing SiRTA
582 sequences which can be bound by Cdc13 (2) and telomerase (3) to form a *de novo* telomere. d) In this
583 model, after a single round of replication, the chromosome that experienced the closed fork produces
584 two unaltered sister chromatids and the linear fragment containing *SUL1* which is now protected by a *de*
585 *novo* telomere addition at the *SNF5* SiRTA. (Normal replication of the homolog in this diploid is not
586 shown.) Under sulfate-limiting conditions, the cells containing the linear fragment are retained in the
587 population due to the growth advantage provided by one or more extra copies of *SUL1*.
588

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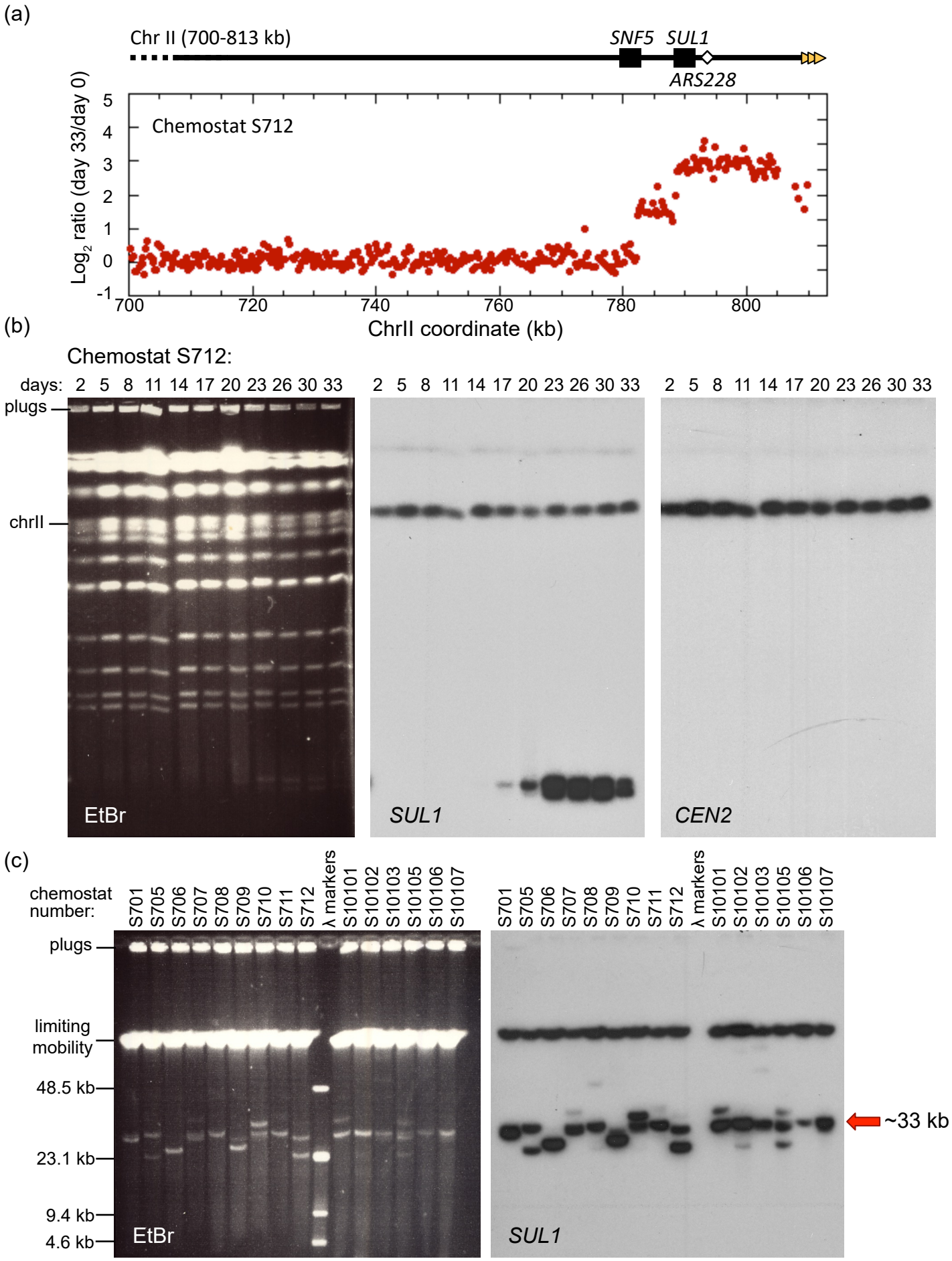
Figure 1


(a)
Chr II (700-813 kb)
SNF5
SUL1
ARS228
Chemostat S712
Log2 ratio (day 33/day 0)
5
4
3
2
1
0
-1
700
720
740
760
780
800
ChrII coordinate (kb)
(b)
Chemostat S712:
days: 2 5 8 11 14 17 20 23 26 30 33
2 5 8 11 14 17 20 23 26 30 33
2 5 8 11 14 17 20 23 26 30 33
plugs
chrII
EtBr
SUL1
CEN2
(c)
chemostat number:
S701 S705 S706 S707 S708 S709 S710 S711 S712 λ markers S10101 S10102 S10103 S10105 S10106 S10107
S701 S705 S706 S707 S708 S709 S710 S711 S712 λ markers S10101 S10102 S10103 S10105 S10106 S10107
plugs
limiting mobility
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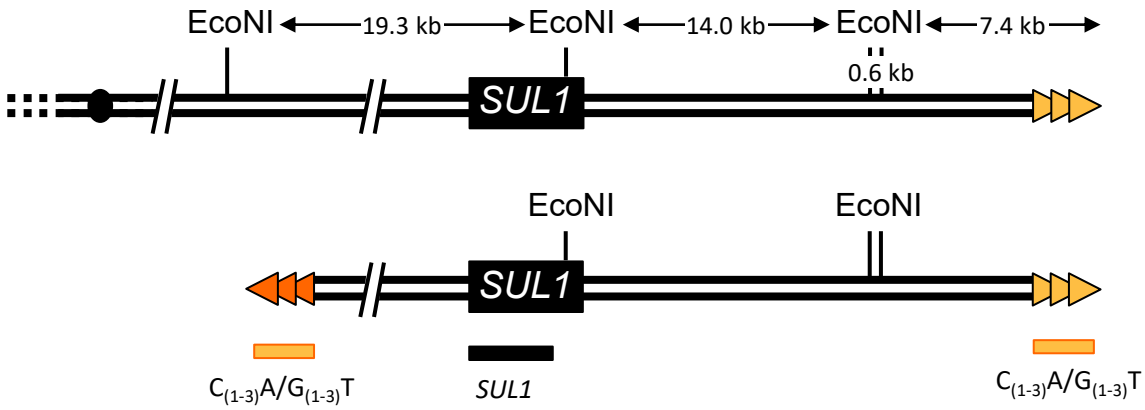
23.1 kb
9.4 kb
4.6 kb
EtBr
SUL1
~33 kb

Figure 2

(a)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SUL1
EcoNI
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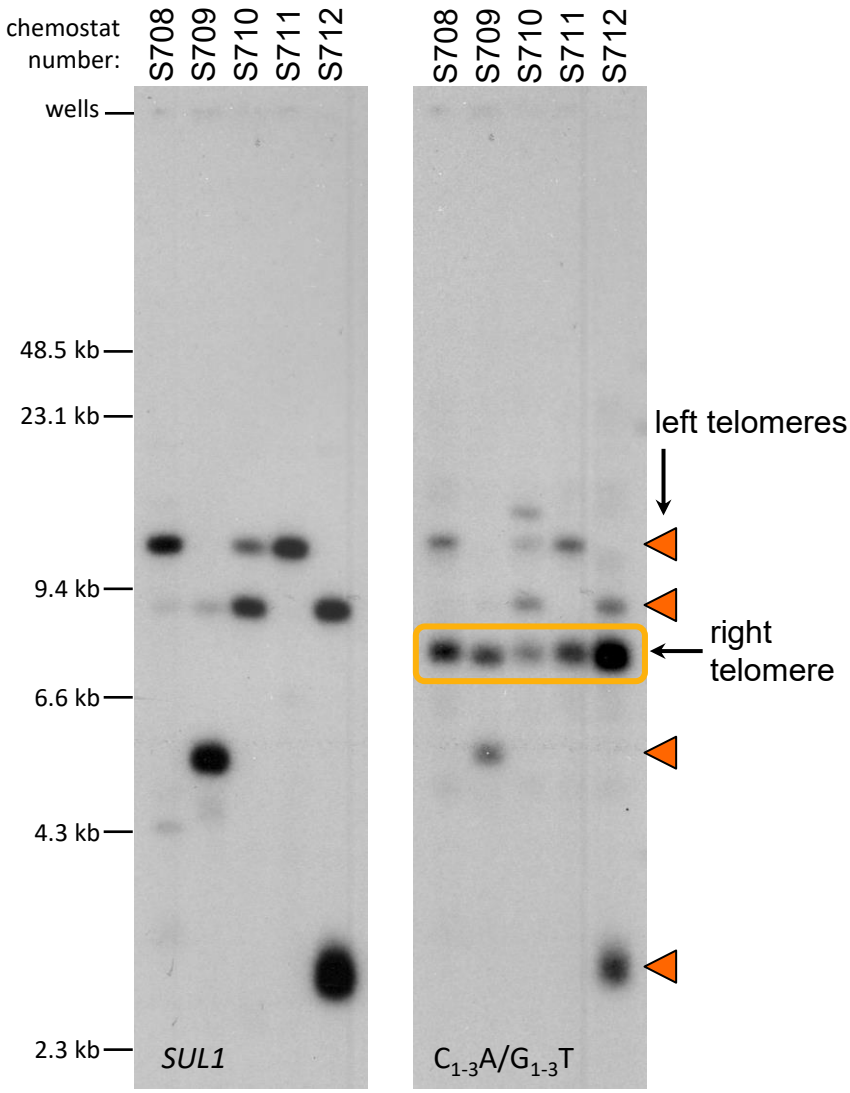
SUL1
C(1-3)A/G(1-3)T
SUL1
C(1-3)A/G(1-3)T

(b)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9.4 kb
6.6 kb
4.3 kb
2.3 kb
SUL1
C1-3A/G1-3T
left telomeres
right telomere

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  |                   |                   |                   |                   |                   |                   |                   |                   |
5’CACCAACAGTTACTGAGGAGTCGTCTACAACAACAACAACAACAACAACAACAACAAACTTCACCGCCACCGCAAACGCATCAATCTCCACCCCCTCCTCCGCAACAATCTCAACCCATTGCTAATCAATCAGCGACTTCTACCCCTCCTCCTCCTCCAGCACCACACAA
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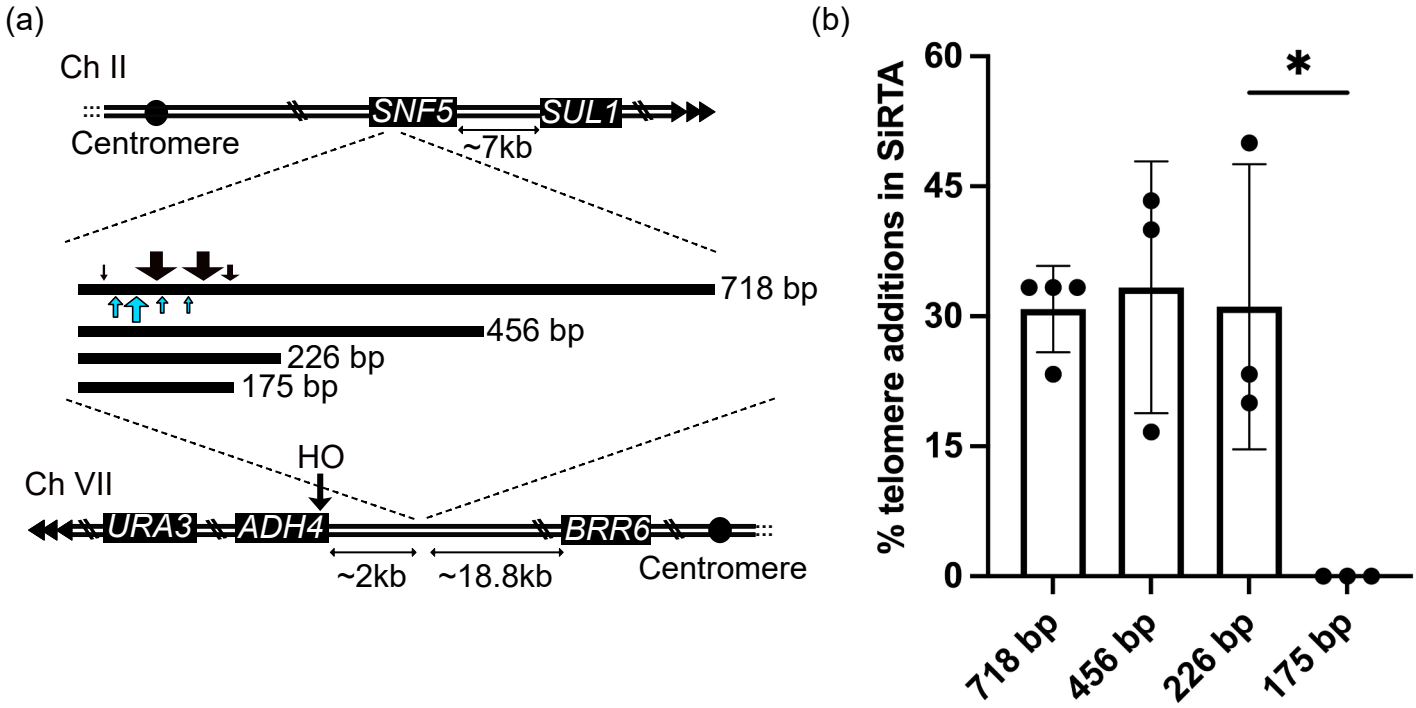
Figure 3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Ch II
Centromere
SNF5
SUL1
~7kb
718 bp
456 bp
226 bp
175 bp
HO
Ch VII
URA3
ADH4
BRR6
~2kb
~18.8kb
Centromere
(b)
% telomere additions in SiRTA
60
45
30
15
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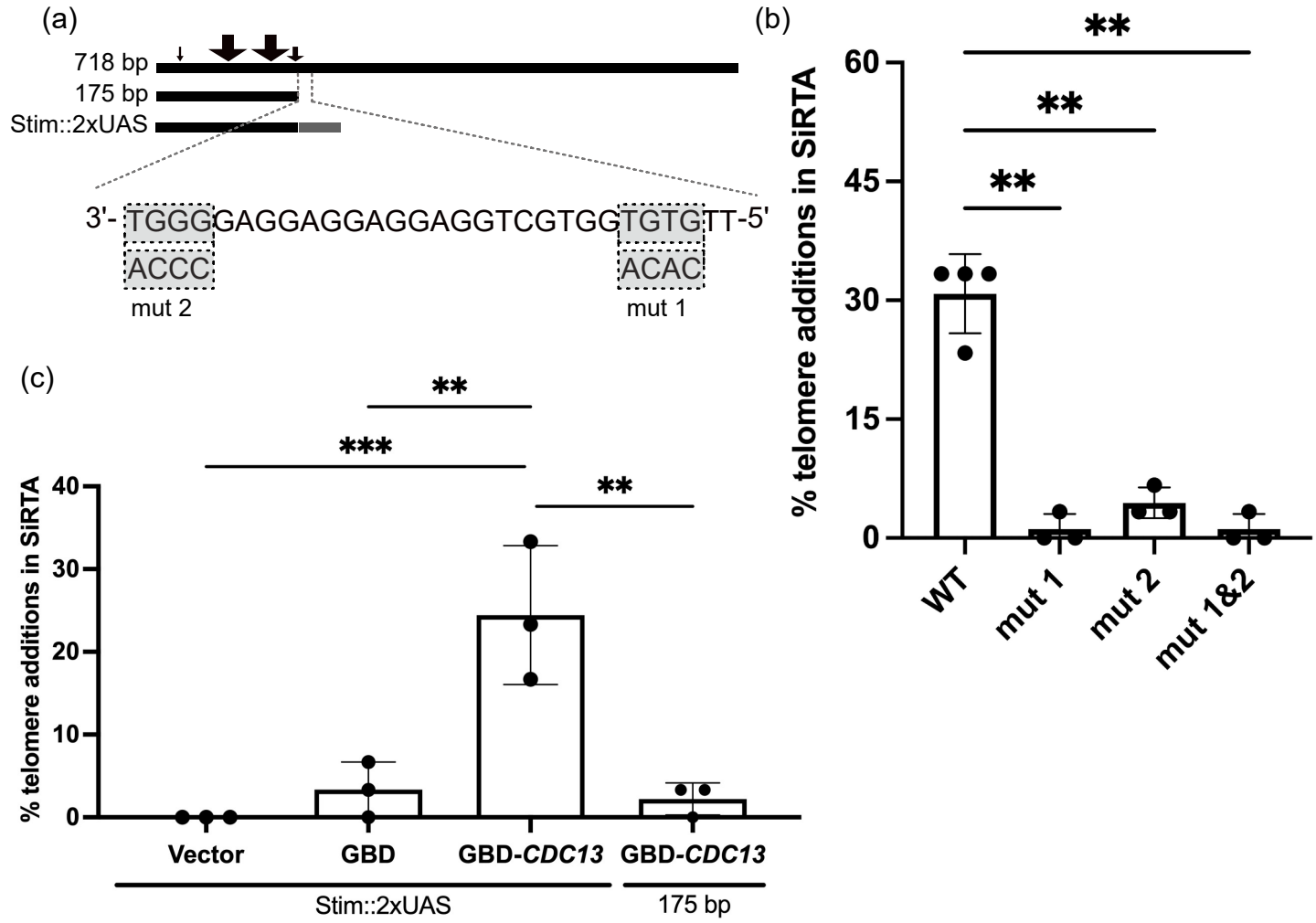
*
718 bp
456 bp
226 bp
175 bp

Figure 4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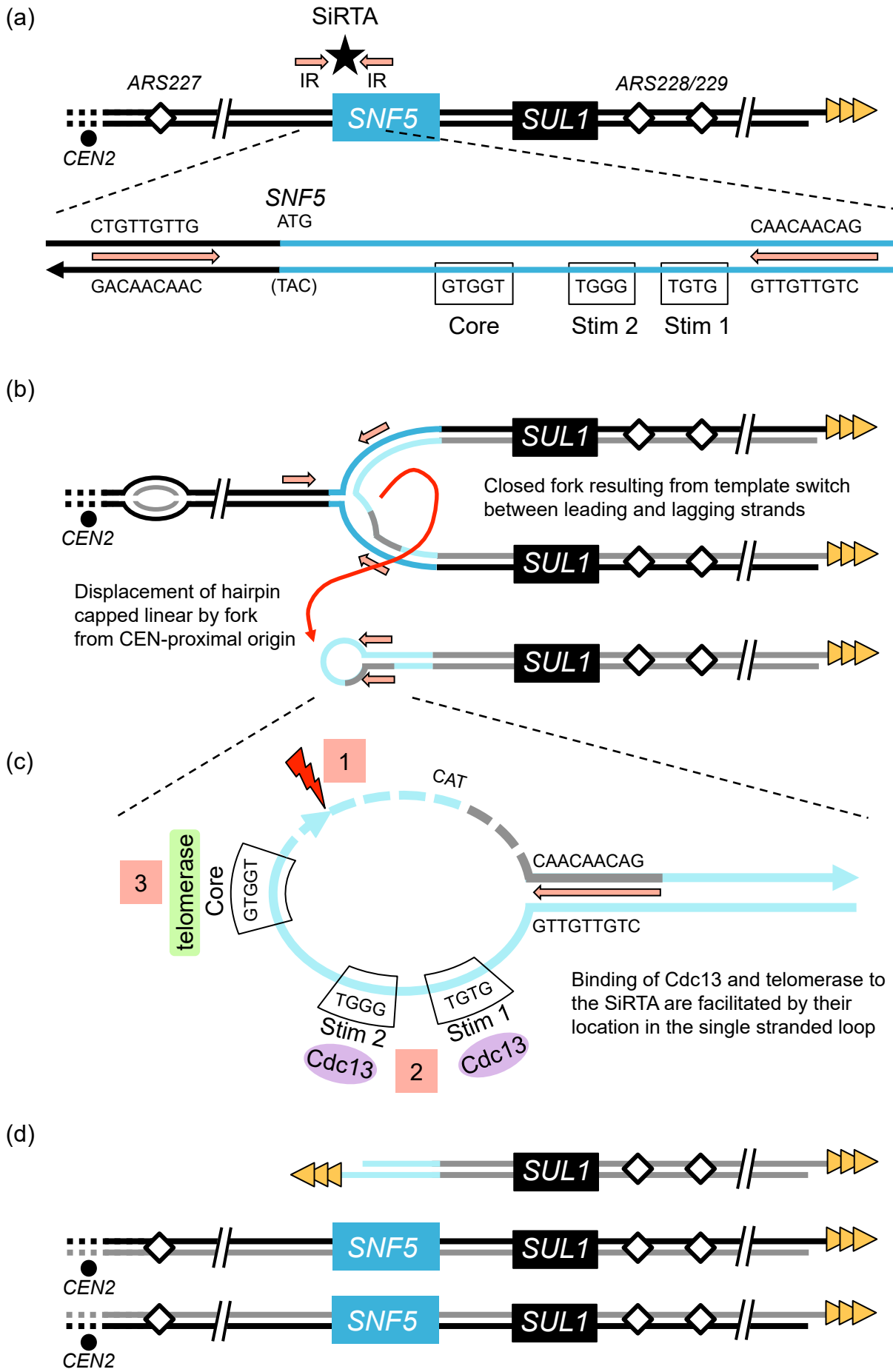
3'- TGGGGAGGAGGAGGAGGTCGTGGTGTGTT-5'
ACCC
ACAC
mut 2
mut 1
(b)
% telomere additions in SiRTA
60
45
30
15
0
**
**
**
WT
mut 1
mut 2
mut 1&2
(c)
% telomere additions in SiRTA
40
30
20
10
0
***
**
**
Vector
GBD
GBD-CDC13
GBD-CDC13
Stim::2xUAS
175 bp

Figure 5

Figure 6


(a)
SiRTA
IR
IR
ARS227
ARS228/229
SNF5
SUL1
CEN2
SNF5
ATG
CTGTTGTTG
CAACAACAG
GACAACAAC
(TAC)
GTGGT
TGGG
TGTG
GTTGTTGTC
Core
Stim 2
Stim 1
(b)
SUL1
CEN2
Closed fork resulting from template switch between leading and lagging strands
SUL1
Displacement of hairpin capped linear by fork from CEN-proximal origin
SUL1
(c)
1
CAT
3
telomerase
Core
GTGGT
CAACAACAG
GTTGTTGTC
TGGG
Stim 2
Cdc13
2
TGTG
Stim 1
Cdc13
Binding of Cdc13 and telomerase to the SiRTA are facilitated by their location in the single stranded loop
(d)
SUL1
SNF5
SUL1
CEN2
SNF5
SUL1
CEN2