

1 **Cytological and genetic consequences for the progeny of a mitotic catastrophe provoked**
2 **by Topoisomerase II deficiency.**

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25 **ABSTRACT**

26 Topoisomerase II (Top2) removes topological linkages between replicated chromosomes.
27 Top2 inhibition leads to mitotic catastrophe (MC) when cells unsuccessfully try to split their
28 genetic material between the two daughter cells. Herein, we have characterized the fate of
29 these daughter cells in the budding yeast. Clonogenic and microcolony experiments, in
30 combination with vital and apoptotic stains, showed that 75% of daughter cells become
31 senescent in the short term; they are unable to divide but remain alive. Decline in cell vitality
32 then occurred, yet slowly, uncoordinatedly when comparing pairs of daughters, and
33 independently of the cell death mediator Mca1/Yca1. Furthermore, we showed that
34 senescence can be modulated by ploidy, suggesting that gross chromosome imbalances
35 during segregation may account for this phenotype. Indeed, we found that diploid long-term
36 survivors of the MC are prone to genomic imbalances such as trisomies, uniparental disomies
37 and terminal loss of heterozygosity (LOH), the latter affecting the longest chromosome arms.

38

39 **Keywords:** Mitotic catastrophe, Top2, senescence, cell death, genomic instability.

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42 INTRODUCTION

43 Mitotic catastrophe (MC) is a class of cell death still poorly understood, and with a
44 conflictive definition among the scientific community [1–3]. In its most general acceptance,
45 we can consider MC as the cell-death-triggering event that follows an aberrant mitosis. MC is
46 presumed to be of the utmost importance in cancer biology, both as an oncosuppressive
47 barrier in carcinogenesis and as a mechanism of cell death after anti-cancer treatments. Many
48 antitumor drugs that damage the DNA or the microtubules lead to chromosome segregation
49 failures, provided that cells do not stop their division cycle in a timely fashion [4,5]. Human
50 cells make use of checkpoints to arrest the cell cycle in G₁/S or G₂/M following treatment
51 with these antitumor drugs, and tumour cells frequently lack one or several of these
52 checkpoints. When checkpoints are functional, cancer cells often die after a transient cell
53 cycle arrest through a regulated cell death (RCD) known as intrinsic apoptosis [6]. Apoptosis
54 causes the permeabilization of the mitochondrial outer membrane and the subsequent leakage
55 of pro-apoptotic factors into the cytosol. Execution of the intrinsic apoptosis is significantly
56 accelerated by the activation of the so-called caspase-mediated transduction cascade. When
57 checkpoints or apoptosis are non-functional, MC is expected to ensue. Therefore,
58 understanding MC is becoming increasingly important in cancer biology.

59 MC is expected to kill most of the progeny due to major genomic imbalances and massive
60 irreparable DNA damage. However, whether MC might also trigger a form of RCD or
61 accidental cell death (ACD) is still unclear. In addition, MC is reported to lead to senescence
62 in certain backgrounds [1,7]. Senescence refers to the irreversible cell cycle arrest of
63 otherwise live cells. Significant differences in the outcome are expected for MCs that result
64 from different sources. Thus, MC occurring upon microtubule damage likely leads to

65 missegregation of whole chromosomes prior to cell death, whereas DNA damage can give
66 rise to more complex outcomes. For instance, DNA damage may result in either breakage of
67 the DNA molecule (i.e, double strand breaks, DSBs) or replication stress (for example,
68 stalled replication forks). Attempts to segregate broken chromosomes would yield daughter
69 cells with irreparable damage. Attempts to segregate underreplicated chromosomes would
70 cause the formation of anaphase bridges, which could produce DSBs as a consequence of
71 cytokinesis [8–11]. Another condition that leads to MC concomitant with the appearance of
72 anaphase bridges occurs when catenations between sister chromatids persist until anaphase.
73 This happens when the catalytic action of topoisomerase II (Top2) is downregulated [12–14].
74 Top2 downregulation seems to pass undetected by cell cycle checkpoints in many cancer cell
75 lines but not in normal differentiated cells [15–18]. Consequently, catalytic inhibition of
76 Top2 offers a promising target to promote MC in cancer cells irrespective of the status of
77 DNA damage checkpoints [19]. Of note, Top2 is often downregulated during acquisition of
78 secondary resistance to chemotherapeutical regimes that comprise Top2 poisons, a major
79 class of antitumor drugs [20,21]. This observation implies that these resistant cancer cells
80 should become even more hypersensitive to inhibition of Top2.

81 Top2 is essential in all organisms. In *Saccharomyces cerevisiae*, inactivation of Top2 by
82 most thermosensitive (ts) alleles leads to MC in anaphase without previous checkpoint
83 activation [13,22–26]. In recent years, *S. cerevisiae* has also been a model to study both cell
84 death pathways and genomic instability footprints after environmental or genetic insults
85 [27,28]. Here, we have characterized the consequences for the offspring of the MC that
86 follows inactivating Top2 through the *top2-5* ts allele (hereafter refer to as *top2* MC). We
87 show that most of the *top2* MC progeny lose their ability to divide. Interestingly, these
88 daughter cells do not die abruptly but undergo a slow decline in cell vitality over several

89 hours. The patterns of cell death point towards an ACD, which was genetically corroborated
90 with mutants for the main apoptotic pathway. We have also used heterozygous diploids to
91 diagnose chromosome rearrangements in the surviving progeny, and we found genomic
92 footprints that include uniparental disomy and terminal loss of heterozygosity in the longest
93 chromosome arms. We conclude that (i) most *top2* daughter cells become senescent in the
94 short-term while eventually dying by ACD; and (ii) the surviving offspring frequently carry
95 genomic rearrangements expected from transiting through anaphase with intertwined sister
96 chromatids.

97

98

99 **RESULTS.**

100 **Seventy five percent of the progeny of a *top2-5* mitotic catastrophe is inviable.**

101 We have recently reported that the *top2-5* thermosensitive mutant undergoes timely
102 progression through the cell cycle until a MC occurs in late anaphase [25]. Importantly, *top2-*
103 *5* gives a clear point-of-no-return in the MC phenotype because cytokinesis makes the *top2-5*
104 anaphase bridges collapse irreversibly. In many ways, this MC is similar to other previously
105 studied *top2* conditional alleles [13,24], although *top2-5* provides a better synchrony for the
106 MC since a larger percentage of cells quickly sever the anaphase bridge [25].

107 We performed single-cell videomicroscopy on agar plates through long-range
108 objectives and found that mother and daughter cells struggled to rebud (the most obvious
109 yeast signal for a new cell cycle) without Top2 (Fig. 1A) [25]. Whereas *TOP2* unbudded
110 (G_1/G_0) cells were able to form microcolonies of around 10 cell bodies after 6 h at 37 °C,
111 *top2-5* cells stopped dividing at either 2 (~65%) or 3 (~20%) cell bodies (Fig. 1A). We
112 hereafter refer to cell bodies rather than cells or buds since it is difficult to conclude whether
113 a 3 cell-body is part of a single multi-budded cell, a budded mother with a daughter, or a
114 mother with two daughters. This 2-3 cell-body pattern was an end-point phenotype upon
115 continuous Top2 inactivation, since we observed the same proportions after 24 h at 37 °C
116 (Fig. 1B). Next, we investigated whether reactivation of Top2 by shifting the temperature
117 down to 25 °C would allow any of these bodies to form a viable population. In order to have
118 an overall picture of cell viability, we first determined clonogenic survival after different
119 incubation periods at 37 °C. Because of the complexity of the budding patterns after the MC,
120 we chose a solid medium-based clonogenic assay that allows to determine if at least one of
121 the cell bodies was still viable by the time of the temperature shift, no matter how many cells

122 are present in the progeny (Fig. 1C). We found that *top2-5* had a gradual loss of viability
123 (50% survival after ~ 4 h), and less than 5% clonogenic survival was obtained after 24 h at 37
124 °C (Fig. 1D); the *TOP2* isogenic strain retained the expected 100% clonogenic survival in this
125 assay (Fig. S1A).

126 Because in these clonogenic assays there is a mixture of budded (S/G₂/M) and
127 unbudded (G₁/G₀) cells at the time of plate seeding, we repeated the clonogenic survival after
128 6 h at 37° C, but photomicrographing the plate surface at different time points. Through this
129 analysis, we determined that at time 0 h the unbudded:budded ratio was 2:1; however, only
130 half of the surviving macrocolonies came from unbudded cells (Fig. 1E). This result implies
131 that the chance to become a macrocolony is doubled if the original cell was budded at the
132 time of the temperature upshift. The most likely explanation for this bias resides in the fact
133 that the *top2* MC is expected to be milder if cells are closer to anaphase onset when Top2 is
134 inactivated. Indeed, budded cells appeared to better complete the first two cell cycles at the
135 restrictive conditions (Fig. S2). A calculation based on the cell proportions at 0h (66% G₁/G₀;
136 33% S/G₂/M), overall macrocolony formation from the 37 °C for 6h regime (~25%), and the
137 origin of those macrocolonies (~50% from G₁/G₀; ~50% from S/G₂/M) led us to conclude
138 that around ~20% of the original G₁/G₀ cells gave rise to survivors after 6 h at 37 °C [$0.25 \times$
139 $0.5 / 0.66 = 0.19$]. This proportion increases to ~40% for the S/G₂/M cells at the time of the
140 temperature upshift [$0.25 \times 0.5 / 0.33 = 0.38$].

141

142 **Most of the inviable progeny of a *top2-5* mitotic catastrophe immediately stops dividing.**

143 Next, we tried to correlate the long-term clonogenic survival of the G_1/G_0 cells with
144 their ability to form microcolonies. We reasoned that it is possible that many MCs could
145 render viable progeny in the short-term (microcolony) but could not raise a visible colony
146 later (macrocolony). This difference could reflect a gradual loss of viability in the progeny as
147 a consequence of genomic imbalances acquired after the MC. To get further insights into this
148 possibility, we took pictures of the cells on the surface of Petri dishes at the time of seeding
149 (0h), right after the 37 °C incubations (6h or 24h), and 16 h or 24 h after the plates were
150 shifted back to 25 °C (Fig. 2A). As expected from above, most of the original G_1/G_0
151 (unbudded) cells did not go beyond the 2-3 bodies stage during the 37 °C incubations (Fig.
152 2A, B; inner circles in the sunburst plots). Restoring permissive conditions for Top2 activity
153 allowed very few of the cell progeny to divide again (Fig. 2A, B; outer circles in the sunburst
154 plots). This finding was true not only during the long (24 h) incubation at 37 °C, but also for
155 the 6 h incubation. Indeed, only ~15% of the 2-3 cell bodies observed after 6 h at 37 °C were
156 able to re-bud again once or more after shifting them back to 25 °C. Incidentally, a low but
157 significant proportion of G_1/G_0 cells did not bud during the 37 °C regimes. However, even in
158 these non-MC cases, cells did not divide after the Top2 reactivation, suggesting that this
159 G_1/G_0 subpopulation was already incapable of cell division following growth at 25 °C.
160 Longer incubations at 25 °C after the MC resulted in microcolonies of >50 cells that
161 eventually developed into macrocolonies. With our cell density settings, microcolonies of
162 more than 20-30 cells hindered us from raising conclusions about the fate of adjacent cells.
163 However, the position of the center in these microcolonies suggests that most, if not all, of
164 the G_1/G_0 cells that ended up as macrocolonies had re-budded again within the first 24 h that
165 followed the temperature downshift.

166 From previous analysis by videomicroscopy, we know that most *top2-5* doublets (2
167 bodies) and all triplets (3 bodies) have passed anaphase and thus completed a MC after 6 h at
168 37 °C [25]. Nevertheless, we decided to complete the analysis of the immediate progeny by
169 testing whether the observed cell bodies have accomplished cell separation. Our reasoning
170 was that separation by micromanipulation would demonstrate that cell bodies have become
171 individual daughter cells. Indeed, we could separate with the needle more than half of the
172 doublets and triplets (Fig. 2C, middle concentric circle in the sunburst chart). We next tried to
173 correlate the ability of all these cells to form macrocolonies but found that they were largely
174 unviable. The percentage of macrocolonies (~2%) was much lower than expected, indicating
175 that that micromanipulation killed cells that otherwise would have retained viability.

176 We noticed from the microcolony experiments performed above that cells swelled
177 after prolonged incubations at 37 °C (Fig. 2A). After 24 h at 37 °C, the volume occupied by
178 the original mother cell doubled (Fig. 2D). Downshift of the temperature to 25 °C only
179 modestly deflated these cells. In addition, a low proportion of cell bodies underwent lysis
180 (“0”; 2 → 1 and 3 → 2 categories in Fig. 2B sunburst charts). These findings, together with
181 the hypersensitivity to micromanipulation, led us to consider osmotic stress (due to the
182 continuous growth in size of the mother cell) as a possible cause underlying the long-term
183 inviability and/or short-term inability to divide of the *top2* progeny. However, addition of 1.2
184 M sorbitol, an osmotic stabilizer, neither prevented cells from swelling (Fig. 2D) nor
185 improved long-term viability (Fig. 2E; S1B) or short-term division capability (Fig. 2F; Table
186 S1).

187

188 **Cell death after prolonged absence of Top2 activity occurs slowly, asynchronously,**
189 **asymmetrically and is independent of the Yca1 metacaspase.**

190 Next, we analysed cell bodies in these microcolonies more closely, seeking other
191 morphological patterns of cell disease aside from swelling; for example, lysis, darkening and
192 loss of the rounded shape (Fig. 3A). Most of these morphological features have been
193 previously related to different forms of cell death. Because the *top2-5* strain also carries a
194 GFP-labeled H2A histone, we also monitored nuclear morphology and chromatin integrity
195 (i.e., GFP intensity). Cells still looked fully healthy after 6 h at 37 °C (no difference with 0 h),
196 despite the great loss in the ability of the progeny to divide. Only after prolonged 37 °C
197 incubations did the cells start to look clearly sick. Still, more than 50% of cell bodies
198 harboured a nuclear GFP signal even 24 h following the 37 °C upshift. This GFP signal co-
199 existed in cell progenies that showed unhealthy patterns in the bright field such as swelled,
200 darkened, or non-rounded cell bodies.

201 In order to better study cell death after *top2* MC, we employed other means that
202 required experiments to be performed in liquid media instead of Petri dishes. We first
203 quantified the rate of cell death and metabolic decline by staining with the vital dye
204 methylene blue (MB) (Fig. 3B). This dye stains dead cells blue, although it can also stain
205 cells that are alive but metabolically attenuated [29]. A time course after the 37 °C upshift
206 showed that there was not a major increase in MB positive cell bodies in the first 4 h (the
207 equivalent in liquid cultures to 6 h on solid medium; [25]). In general, the increase in MB+
208 bodies was linear, but even 24 h after the 37 °C upshift ~40% of cell bodies were not stained
209 by MB. These staining experiments uncovered two other properties of the *top2* MC: i) it was
210 common that only one cell body was MB+ in doublets and triplets (Fig. S3A); and ii) both

211 unbudded and budded cells were equally stained (Fig. S3B). Regarding the first observation,
212 the result suggests that loss of vitality is not coordinated between mother and daughter(s)
213 cells. This asymmetry also confirms that many doublets must have completed cytokinesis
214 despite remaining together. As for the second observation, the staining pattern suggests that
215 loss of vitality occurs in an asynchronous fashion in terms of any preference for a cell cycle
216 stage.

217 Because MB does not distinguish whether cell bodies are dead or simply
218 metabolically stressed, we next sought other more informative vital stains. First, we
219 employed the fluorescent vitality probe FUN1©. This probe stains metabolically active live
220 cells with red vacuolar aggregates [30]. FUN1© is considered more informative and reliable
221 than MB. With this probe we confirmed that only ~10% of cell bodies have lost vitality after
222 just 4 h at 37 °C (left chart of Fig. 3C; Fig. S4). It was also surprising that vitality decline still
223 affected no more than 40% of all cell bodies after 24 h at 37 °C.

224 We also used Propidium Iodide (PI) to monitor cell viability. Loss of plasma
225 membrane impermeability is considered a *bona fide* marker of cell death [27]. PI is only able
226 to fluorescently stain cells that have lost such impermeability. Anticipating some sort of RCD
227 after the *top2* MC, we decided to accompany PI staining in red with reporters for either
228 reactive oxygen species (ROS) or externalization of phosphatidylserine (PS) at the plasma
229 membrane; both in green (DCFH-DA and annexinV-FITC, respectively). Intrinsic ROS
230 production has been observed during RCD in all eukaryotes, including yeast, and is
231 considered one of most reliable RCD markers [31]. After overnight growth at 25 °C (0h), the
232 *top2-5* strain had neither dead cells nor cells with ROS (right part of Fig. 3C, Fig. S5). Four
233 hours after the 37 °C temperature shift, there was only a slight increase in dead cell bodies

234 (~6%) and almost no signs of ROS in the rest (~3%). Only after 24 h of incubating the cells
235 at 37 °C, we found a significant proportion of both ROS (~20%) and dead cells (~20%). It is
236 noteworthy that the percentage of PI+ cells was still relatively low after this 24 h incubation,
237 and 60% of all cell bodies were still resistant to PI and free of ROS. Similarly, PS
238 externalization is considered a conserved *bona fide* marker of RCD [32]. Double staining
239 with PI and annexin V-FITC in cells where the cell wall has been digested (a necessary step
240 for annexin V to reach externalized PS) showed less than 10% of cell bodies with the staining
241 expected for early apoptosis, even 24 h after the temperature upshift (Fig. S6; annexin V
242 positive plus PI negative). It is noteworthy that there were more dead cell bodies (PI positive)
243 in this assay than in the ROS/PI assay (~60% vs. ~20%). This observation is likely a
244 consequence of digesting the cell wall, since the plasma membrane of swollen cells at 24 h
245 might collapse during the treatment.

246 We next examined if the observed pattern of death after the *top2* MC was altered by a
247 mutation in caspase. We reasoned that this approach might shed more light on the RCD/ACD
248 nature of the observed death, considering the low presence of intrinsic ROS and the technical
249 caveats of the annexin V assay. Hence, we deleted the only caspase-like gene in yeast,
250 *YCA1/MCA1*. Yca1 is required for RCD in yeast in response to several environmental stresses
251 [33–35]. In addition to cell death, we checked whether Yca1 modulated the other behaviours
252 seen in the *top2* MC progeny (for example, the inability to divide and the slow decline in cell
253 vitality). The conclusions derived from comparing *top2-5 YCA1* and *top2-5 yca1Δ* were that
254 Yca1/Mca1: (i) had no influence in the percentage or rate of cells that end up dying (Fig. 3B,
255 C); (ii) neither accelerated nor slowed down the vitality decline (Fig. 3B, C); (iii) did not
256 modify the profile of clonogenic survival after the *top2* MC (Fig. 3D; S1C); and (iv) its

257 absence did not improve the ability of the immediate cell progeny to divide (Fig. 3E; Table
258 S1).

259 The overall conclusion from these experiments is that the immediate progeny of the
260 *top2* MC enter a senescent-like state as they retain vitality but loses their ability to re-bud.
261 Senescence is only a transient state that lasts several hours or days, until cells eventually die.
262 The pattern of cell death (morphologically diverse, slow, asynchronous and asymmetrical),
263 together with the lack of effect of Yca1, suggest that loss of Top2 leads to ACD.

264

265 **Chromosome ploidy modulates the ability of the progeny to divide.**

266 All the experiments described above were carried out in haploid yeast cells. In
267 haploids, MC associated with the presence of partly unresolved sister chromatids, as in the
268 *top2-5* mutant, is expected to be highly deleterious. Severing of these anaphase bridges result
269 in daughter cells that may lack several chromosome arms [11]. Based on this consideration,
270 one might expect that diploid cells would be more resistant to the consequences of *top2* MC
271 than haploid cells. Thus, we studied the *top2* MC in an isogenic homozygous *top2-5/top2-5*
272 diploid (2N) strain. Unlike its haploid counterparts, diploid cells were more often able to re-
273 bud at least once. In fact, ~50% of all 2-3 cell bodies originated from just after 6h at 37 °C
274 were able to do so after the 25 °C downshift (Fig. 4A; Table S1). This percentage dropped
275 considerably if the progeny was incubated 24 h at 37 °C. Strikingly, however, the increase in
276 the ability to divide again after the MC did not yield better clonogenic survival (Fig. 4B;
277 S1D), indicating that most of this viable progeny was competent to re-bud only in the first
278 generations. We also performed a microdissection analysis of the diploid *top2* progeny.

279 Unlike haploid *top2-5* cells, which was rather sensitive to micromanipulation, 13% of the
280 diploid progeny raised a macrocolony after the separation attempt (Fig. 4C). Altogether, we
281 conclude that a *top2* MC in diploids results in better short-term ability to divide.

282

283 **Genome instability footprints in the surviving progeny from the *top2*-mediated mitotic**
284 **catastrophe.**

285 Above, we have just shown that ~25% of the *top2-5* diploid cells still gave rise a
286 macrocolony after the 6h at 37°C regime. We next examined the genomes of these survivors
287 in search for specific genomic footprints of the *top2* MC. To accomplish this goal, instead of
288 using the homozygous isogenic diploid employed in the previous chapter, we generated a
289 highly heterozygous hybrid *top2-5* diploid. The hybrid diploid was generated by crosses of
290 two sequence-diverged *top2-5* haploids, derivatives of W303-1A and YJM789, which are
291 heterozygous for more than 55,000 single-nucleotide polymorphisms (SNPs) distributed
292 throughout the yeast genome (the yeast genome is 15 Mb). These heterozygous SNPs allow
293 the analysis of various types of genomic alterations by using SNP-specific microarrays
294 [36,37]. The hybrid diploid is also engineered to select various types of chromosome
295 alterations on chromosome V (Fig. 5A). It is homozygous for the *ade2-1* mutation on
296 chromosome XV (an ochre-suppressible allele) and heterozygous for the *SUP4-o* suppressor
297 gene in chromosome V [38]. Strains with the *ade2-1* mutation form red colonies in YPD in
298 the absence of the *SUP4-o* suppressor. Diploid strains with one or two copies of *SUP4-o* form
299 pink and white colonies, respectively [39]. Thus, loss of the *SUP4-o* gene by either mitotic
300 recombination or chromosome loss results in a red colony instead of the pink colonies
301 characteristic of the original strain. Colony colour changes are also expected if the copy

302 number of the *ade2-1* allele varies by loss or duplication of chromosome XV. In addition,
303 aneuploidy for other chromosomes sometimes alters the color of the colony.

304 Microcolony and clonogenic experiments performed in the *top2-5* hybrid diploid
305 FM1873 showed that this strain lost viability quicker than the isogenic homozygous diploid
306 in the S288C background (Fig. 5B, C; S1E). There was a steady rise in red and/or red/white
307 sectored colonies among the survivors during the 37 °C incubation, as expected if the *top2*
308 MC increases genome instability (Fig. 5D). We used microarrays to examine genomic
309 alterations in the control FM1873 isolate (no exposure to 37 °C) and in isolates exposed to 37
310 °C for 6 h, and then allowed to form colonies at 25 °C (Fig. 5E; see also [supplemental](#)
311 [information](#) for interpretation on the genomic alterations picked up by SNP array). It is
312 important to stress that the SNP microarrays allow analysis of genomic alterations throughout
313 the genome in addition to those changes that occur on chromosome V [36,37]. When the
314 control FM1873 strain was examined before exposure to the restrictive temperature,
315 surprisingly, we found that it was altered relative to an isogenic *TOP2/TOP2* hybrid [40].
316 More specifically, we realized that FM1873 carried a terminal loss of heterozygosity (T-
317 LOH) on the right arm of chromosome XII (the longest chromosome arm in yeast). In
318 addition, all isolates had three to four copies of chromosome XIV. We tried several times to
319 recreate the hybrid *top2-5* diploid but were unable to isolate a derivative that had only two
320 copies of XIV. Since this chromosome is the location of *top2-5*, it is likely that chromosome
321 XIV trisomes and tetrasomes have a selective growth advantage at the permissive
322 temperature over the diploids that have only two *top2-5* copies. We generated an isogenic
323 derivative of FM1873 (MD684) that lacked the T-LOH event on XII, although it still had
324 extra copies of XIV. For our subsequent genomic analyses, we studied both FM1873 and
325 MD684.

326 The experimental strains were exposed to the restrictive temperature of 37 °C by
327 incubating the cells for six hours either on plates or in liquid (details in Materials and
328 Methods); these two protocols resulted in similar levels of instability. A total of 27 isolates
329 were examined for FM1873 (13 experimental, 14 control), and 19 isolates of MD684 (10
330 experimental, 9 control). Somewhat surprisingly, the control single-colony isolates (cells not
331 exposed to 37 °C) also had high rates of instability (Table 1; “C” samples), indicating that the
332 Top2p encoded by *top2-5* does not have wild-type activity even at the permissive
333 temperature. Indeed, a previous biochemical study reported that the Top2-5 activity at 25 °C
334 is 33% of that of wild type Top2 [41]. Among all isolates examined, we found 76 T-LOH
335 events, 3 interstitial LOH (I-LOH) events, 31 trisomies, 2 monosomies, and 6 uniparental
336 disomies (UPDs) (Fig. 5E; Table 1). The average number of genetic changes per strain
337 (including all the data of Table 1) was 3.3 alterations/isolate. Since the strains were grown
338 approximately 40 generations before microarray analysis, the rate of alterations/cell
339 division/isolate is about 8.3×10^{-2} . In a previous microarray analysis of a wild-type diploid
340 isogenic with FM1873 and MD684, we found a rate of alterations of about 2×10^{-3} /division, a
341 rate about 40-fold less than for the *top2* strains. The most common alteration in the *top2*
342 strains was a T-LOH event (64% of the total events). These events likely reflect the repair of
343 a DSB by either a crossover or a break-induced replication (BIR) event (Fig. 5E). The
344 chromosomal distribution of the events is striking. The right arms of chromosome IV and XII
345 (the two longest arms in the yeast genome) had over 80% of the terminal LOH events
346 (60/76), although these arms represent less than 30% of the yeast genome. Other
347 chromosomes with T-LOH events (the number of events shown in parentheses) are: XIII (5),
348 XV (4), VII (3), XIV (2), VII (1), XI (1), and V (1). Chromosomes XIII, XV and XIV have
349 the third, fourth, and fifth longest chromosome arms in the genome, respectively; all of the

350 mapped events in these chromosomes are on the longest arms. Strikingly, the very large
351 (about 1.2 Mb) ribosomal RNA gene cluster (rDNA) on the right arm of chromosome XII
352 was not a preferred site for T-LOH events. Although the rDNA is about 60% of the right arm
353 of XII, only 30% (3 of 11) of the LOH events had a breakpoint in or near the rRNA genes.
354 We should point out that T-LOH events on XII could only be followed in the MD684 strains
355 (FM1873 already had a cXIIr T-LOH); hence, our estimate of the frequency of T-LOH events
356 on the right arm of XII is a minimal estimate.

357 In addition to LOH events, we observed 33 changes in chromosome number (31 trisomies
358 and 2 monosomies) (Table 1). The frequencies of trisomies are not simply related to the size
359 of the chromosomes. Chromosomes V, VIII, and XV were the most frequently-observed
360 trisomes; chromosomes V and VIII are medium-sized chromosomes (both about 550 kb),
361 whereas chromosome XV is large (1092 kb). Only one trisomy was observed involving one
362 of the three smallest chromosomes (I, VI, and III). Only three of the trisomies involved the
363 two largest chromosomes IV and XII.

364 We also observed six UPD events (Fig. 5E). In strains with these events, the homolog is
365 present in two copies, but both copies are derived from one of the original parental homologs.
366 There are two plausible pathways to generate UPD (Fig. S7): two non-disjunction events in
367 different cell cycles or reciprocal UPD (RUPD) in which one pair of homologs segregates
368 into one cell and the other pair segregates into the other cell. Although both pathways
369 probably contribute to UPD in yeast, at least some of the events in wild-type strains are
370 RUPD [42]. To determine whether RUPD events occurred frequently in the *top2* cells, we
371 used a protocol in which both daughter cells produced as a result of RUPD or a reciprocal
372 crossover (RCO) in chromosome V could be recovered in different sectors of a sectored

373 colony (Fig. S8). The sectored colonies were derived from the same strains (FM1873 and
374 MD684) used for our single-colony analysis. A crossover or RUPD can produce a red/white
375 sectored colony. However, to select for such events, both FM1873 and MD684 contained a
376 heterozygous *can1-100* marker located allelically to the *SUP4-o* insertion and a gene
377 encoding hygromycin resistance (*hph*) located distal to the *can1-100* insertion (Fig. 5A). The
378 *can1-100* mutation is a nonsense mutation and is suppressed by *SUP4-o*. Strains that lack the
379 suppressor are resistant to the drug canavanine and those with the suppressor are sensitive. A
380 crossover between *can1-100* and the centromere results in formation of a canavanine-
381 resistant red/white sectored colony (Fig. S8A); a sectored colony with the same phenotype
382 can also result from RUPD (Fig. S8B). RCO and RUPD events can be distinguished by
383 microarray analysis (bottom panels of Fig. S8).

384 Following exposure of FM1873 and MD684 to 37 °C, we found 242 red/white Can^R
385 sector colonies, 43 of which had the sectoring pattern for the *hph* marker suggestive of RCO
386 or RUPD events. We found both RCO and RUPD events in two-thirds of these colonies
387 (Table S2). The rate of RUPDs in cells of FM1873 and MD684 treated for 6 h at 37 °C was
388 the same, 1.1×10^{-5} /division; the rate of RUPD in cells of FM1873 that were not exposed to
389 37 °C was 1×10^{-6} . The rate of RCO in FM1873 cells treated at 37 °C was 2.7×10^{-6} ; no RCO
390 events were observed in MD684. In an isogenic wild-type strain, the rates of RUPD and RCO
391 for chromosome V were 10^{-7} and 1.6×10^{-6} , respectively [42]. These results support the
392 conclusion that the *top2* mutation results in a substantially elevated rate of RUPD (about 100-
393 fold) and has little effect on the rate of RCO.

394

395

396

397 **DISCUSSION**

398 The formation of anaphase chromosome bridges during the cell division is one of the
399 most dramatic sources of genetic instability. Such bridges are expected to cause a MC that
400 would kill most of the progeny. Downregulation of Top2 is likely the most common way of
401 generating large numbers of anaphase bridges. In addition, downregulation of Top2 has
402 important clinical implications during acquisition of resistance against cancer therapy that
403 comprises Top2 poisons (for example, etoposide and anthracyclines). In the present work, we
404 have studied the consequences of inactivating Top2 in yeast through the *top2-5*
405 thermosensitive allele. We show that the expected MC often leads to progeny unable to
406 divide; however, cell death is not immediate but the result of a decline in cell vitality that
407 takes hours to complete. We further propose that the irreversible genomic imbalances that
408 occur during chromosome segregation in the absence of Top2 explain the short-term
409 senescence observed in the immediate progeny. This hypothesis is strengthened by the
410 observation that diploid survivors of a *top2* MC often carry genomic footprints expected from
411 anaphase bridges such as UPDs and T-LOH. A step-by-step summary of the *top2* MC events
412 is shown in [Fig. 6](#).

413 We would like to point out there are many different strategies to abolish Top2
414 activity, from depleting the enzyme through degron systems to using catalytically dead
415 mutants. In between, there are a large collection of conditional thermosensitive alleles. Baxter
416 and Diffley [24] showed that depletion of Top2 had a different phenotype than expression of
417 a catalytically-dead enzyme. Depletion of Top2 did not lead to cell-cycle delays but did result
418 in lethal DNA damage during cytokinesis. In contrast, a mutation in the Top2 catalytic

419 domain resulted in arrest in G₂/M as a consequence of DNA damage (nicks and gaps). The
420 absence of a G₂/M arrest is also a common pattern of thermosensitive alleles [13,22–26], with
421 the remarkable exception of top2-B44 (F977L), which delays cells in G₂/M through
422 activation of the spindle assembly checkpoint [26]. In addition, we must consider post-G₂/M
423 delays since the NoCut checkpoint slows down cytokinesis under the presence of anaphase
424 bridges [43]. This was shown using the *top2-4* allele, and this delay affects the synchrony of
425 the MC. We previously examined the kinetics of both completion of cytokinesis and severing
426 of anaphase bridges in *top2-4* and *top2-5* [25]. We found that the *top2-5* strain severed the
427 bridge more efficiently and synchronously than *top2-4*, arguing that *top2-5* is a better allele to
428 model *top2* MC; hence, we used this allele to address the *top2* MC. It would be interesting,
429 though, to address the consequences of *top2* MC in other models, including degron-assisted
430 Top2 depletion, partial inactivation (semipermissive conditions) and *top2-ts* alleles that delay
431 either G₂/M or cytokinesis.

432

433 **On the short-term cytological consequences of the *top2* mitotic catastrophe.**

434 In this study we have adopted the term mitotic catastrophe (MC) in its broadest
435 cytological sense, referring to aberrant mitoses that are expected be deleterious for the
436 progeny based on the degree of observed abnormalities. It is worth mentioning that other
437 authors, especially those working with metazoans, restrict the MC term to death occurring in
438 mitosis after a mitotic insult [44,45]. With this restriction in mind, MC in metazoans is a sort
439 of mitotic RCD. In our yeast experimental model with the *top2-5* allele, death before
440 anaphase is insignificant since cells go through G₂/M and anaphase as quickly as their *TOP2*
441 counterparts [25]. In nocodazole-treated cells, which arrest cells in G₂/M, 40% cell death was

442 reported after 10 h in clonogenic assays [46]. This cell death was described to occur through
443 an RCD apoptosis-like mechanism. By contrast, our clonogenic assays showed that a sudden
444 drop in viability occurs between 3-6 h after Top2 inactivation (Fig 1C), about the time needed
445 for cells to complete telophase and cytokinesis on solid medium [25]. From the microcolony
446 experiments, we concluded that the ensuing *top2-5* progeny are largely impaired in entering a
447 second cell cycle (Fig 1A, B and 2). This impairment can be partly alleviated in diploids, yet
448 only in the short-term (Fig 4). This observation leads us to propose that gross genomic
449 imbalances prevent the immediate progeny from cell cycle progression. Taking into account
450 previous reports on the formation of anaphase bridges in *top2-ts* mutants [13,25] and high
451 levels of chromosome missegregation [23], it appears logical that many of the haploid
452 progeny lose entire chromosomes containing essential genes. In addition, loss of essential
453 genes may reflect breakage of chromosomes at the bridge followed by loss of the distal
454 chromosome regions [11].

455 Another possibility is that daughter cells immediately die upon the *top2* MC through
456 an RCD program. This would imply that either anaphase/telophase cells or their immediate
457 progeny sense the MC and execute an RCD. Our results, however, argue against this
458 possibility. Firstly, many cells stained negative for death (PI) and apoptotic (annexin V)
459 markers, whereas they stained positive for metabolic activity (FUN1©), even after 24 h at 37
460 °C. Secondly, the decline in cell vitality occurred slowly, linearly (asynchronously) and
461 asymmetrically (when comparing daughter cells that remained together after the MC).
462 Thirdly, Yca1/Mca1, the main RCD player in *S. cerevisiae*, does not regulate the vitality
463 declines. Even though there are other RCD proteins aside from Yca1/Mca1 [47,48], we point
464 out that that the pattern of cell death after the *top2* MC is better explained through ACD.
465 Although we found intrinsic ROS production in a minor subset of the *top2* MC progeny, and

466 this finding has been considered a marker of RCD [31,49], we hypothesize that, in our case,
467 ROS accumulation is a consequence of the steady decline of cell homeostasis. For instance,
468 ROS might arise from loss of nuclear genes involved in eliminating ROS in metabolically
469 active cells.

470 Comparing with previous studies, the events that lead to death after the *top2* MC
471 resemble those observed after prolonged G₂/M arrest in the *cdc13-1* mutant, which results in
472 irreversible DNA damage at chromosome ends. In arrested *cdc13* cells, there are cell markers
473 of RCD such as ROS production [50], although further biochemical assays and genetic
474 manipulation suggest ACD rather than RCD [47]. In *cdc13-1*, because cells get blocked in
475 G₂/M, there are no genetic imbalances prior to cell death. It was proposed that cell lysis,
476 resulting from cell growth without cell division, was the ultimate cause of death, a hypothesis
477 confirmed because sorbitol (an osmotic stabilizer) improved cell viability [47]. Although we
478 also observed oversized cells one day after the *top2* MC (Fig 2A and D), cell lysis was a rare
479 event and sorbitol did not improve cell viability (Fig 2D-F). Therefore, we propose that the
480 secondary ACD observed after the *top2* MC is the consequence of the steady decline of cell
481 homeostasis resulting from loss of essential genes.

482

483 **On the long-term genetic consequences of the *top2* mitotic catastrophe.**

484 We have also compared the genomes of surviving diploids after 6h of Top2
485 inactivation. Even though we did not prove that all survivors came from a MC (i.e., they went
486 through anaphase), the results shown in Figs 1-5, together with previous findings [13,24,25]
487 lead us to conclude that most of these survivors likely came from a *top2* MC. Indeed, many

488 of the observed chromosome variations and rearrangements can be best explained if cells go
489 through anaphase in the absence of proper sister chromatid disjunction [11]. One
490 interpretation of the strong bias for T-LOH events relative to I-LOH events (76 terminal and
491 3 interstitial) is that DSBs are repaired primarily through BIR (Fig.5E) [11,51]. In many
492 previous studies, I-LOH represented a very significant fraction of the total LOH events. For
493 example, in G₁-synchronized cells treated with ultraviolet light, we observed a 1:3 ratio
494 between T-LOH and I-LOH [40]. I-LOH requires both ends of the DSB to invade the other
495 homolog during repair through homologous recombination, a condition difficult to fulfil in
496 DSBs generated by cytokinesis [11].

497 According to a previous study [52], the frequency of DSBs in *top2* mutants is higher for
498 long chromosome arms than short chromosome arms. Spell and Holm [50] explained this
499 observation by suggesting that intertwinings on short chromosome arms could be resolved by
500 passive diffusion off the end, whereas such intertwinings on long chromosome arms required
501 Top2. Our results are also consistent with this interpretation. Chromosome IV and XII right
502 arms are the longest in the yeast genome and are overrepresented in the T-LOH events
503 (normalizing for the size of the arm). An unusual feature of the T-LOH data is the rDNA is
504 under-represented as a breakpoint in the cXIIr T-LOH events. It was previously shown, using
505 an assay that detects loss of inserted marker within the rDNA, that *top2* strains have
506 substantially elevated rates of mitotic recombination in the rDNA [53]. One possible
507 explanation of this discrepancy is that DSBs within the rDNA may be repaired by single-
508 strand annealing between flanking copies of the rDNA genes [54], an event that would result
509 in loss of an inserted marker without an interaction with the other homolog. Such an event
510 could not be detected by the microarray analysis.

511 Another genetic alteration that is consistent with sister chromatid non-disjunction at
512 the *top2* MC is the elevated presence of trisomies. A straightforward explanation for this is
513 that the intertwining of sister chromatids in the *top2* strains often results in their co-
514 segregation into one of the daughter cells (Fig. 5E). Although this type of non-disjunction
515 would be expected to create equal numbers of monosomic and trisomic strains, it is possible
516 that the monosomic strains have a competitive growth disadvantage and are, therefore,
517 selected against. In *tell mec1* diploids, expected to enter anaphase with broken and/or
518 underreplicated chromosomes, trisomies were five times more common than monosomies
519 [55]. A similar bias towards trisomies was observed in *cdc14-1* diploids [51]; *cdc14* results in
520 elevated levels of anaphase bridges because Cdc14 regulates condensin and Top2 actions in
521 anaphase [56–58].

522 Lastly, the 100-fold enrichment in RUPD after the *top2* MC is also in agreement with
523 models of genomic instability generated by sister chromatid non-disjunction. Two models are
524 proposed for the generation of RUPD. One model involves two independent missegregation
525 events occurring in successive divisions. In the other model, segregation of the chromosomes
526 occurs in a manner similar to meiosis-I (Figs. S7 and S8). In wild-type cells, we demonstrated
527 that the second model is correct [42]. From our data in the current study, we cannot
528 distinguish between these models. The rate of a single non-disjunction event of chromosome
529 V in a *top2* mutant is very high, about 3×10^{-3} /division [23]. Thus, the likelihood of two
530 independent non-disjunction events in a single division is $(3 \times 10^{-3})^2$ or 10^{-5} which is close to
531 our observed rate of RUPD in the *top2-5* diploid. It is possible that both mechanisms
532 contribute to the high rate of RUPD observed in the *top2-5* mutants. Another difference with
533 our earlier study [42] is the low frequency (18%) of sectors reflecting RUPD and RCO
534 recovered from the red/white Can^R sector assay. In our previous study, almost all sectored

535 colonies reflected RCOs or RUPD events. It is likely that the high levels of aneuploidy
536 observed in the *top2* mutants affects colony color by mechanisms unrelated to RCO or RUPD
537 events on chromosome V.

538 In conclusion, we have characterized the consequences for the progeny of depleting
539 yeast cells of Top2. We determined that the *top2* mitotic catastrophe leads to the sudden loss
540 of the capability to divide again. Nevertheless, restrictions for cell division are not a
541 consequence of immediate cell death as the progeny remain alive for several hours. In
542 addition, survivors of the *top2* mitotic catastrophe carry genomic footprints that point towards
543 sister chromatid non-disjunction and breakage of anaphase bridges as the source of the *top2*-
544 driven genome instability. Overall, the *top2*-mediated mitotic catastrophe is highly
545 deleterious for the cell progeny but it might also bring about highly unstable surviving clones.

546 MATERIALS AND METHODS

547 Strain construction.

548 All strains used in this work are listed in [Table S3](#). Gene deletions were achieved
549 using standard PCR and transformation methods [\[25,59\]](#). To obtain the transformation
550 products, genomic DNA of the corresponding strain in the Euroscarf yeast haploid *MATa*
551 knockout collection was used as the PCR template. Primers used in the PCR bind 100-400
552 bps upstream and downstream the deleted gene ORF ([Table S4](#)).

553 The *top2-5* isogenic homozygous diploid derivative was generated through a one-step
554 marker-free transformation approach that takes advantage of the α -factor hypersensitivity in
555 the haploid *MATa bar1* Δ genotype (a proof of concept is provided in [Fig. S9](#)). Further details
556 in [supplemental information](#).

557 The *top2-5* heterozygous diploids FM1873 and MD684 were obtained by crossing of
558 haploid strain *top2-5* derivatives of PSL2 and PSL5 (see [supplemental information](#)). These
559 two strains are isogenic with W303-1A and YJM789, respectively, and have been engineered
560 to select and visually detect chromosome V rearrangements ([Fig 5A](#)) [\[38\]](#). W303-1A and
561 YJM789 differ by about 55,000 SNPs. Thus, these hybrid heterozygous diploids were also
562 used for genome-scale detection of chromosome rearrangements by SNP arrays (see below).

563 Unless stated otherwise, all strains were grown overnight in air orbital incubators at
564 25 °C in rich YPD media (1% w/v of yeast extract, 2% w/v peptone and 2% w/v dextrose)
565 before every experiment.

566

567 **Assays to assess clone survivability and capability of single cells to divide.**

568 A modified clonogenic assay performed on agar plates was used to assess
569 survivability of the progeny after the mitotic catastrophe (details in [supplemental](#)
570 [information](#)). The purpose of this assay was to determine if at least one of the resulting cells
571 in the progeny was still able to raise a new cell population, irrespective of how many times
572 the cell has divided and how many cells in the progeny are viable. Half-life values ($t_{1/2}$), the
573 time in which the clone survival drops to 50%, were calculated adjusting the data to a four-
574 parameter model using Graphpad Prism 7.

575 The time-lapse microcolony analyses were performed with a 40x long-range objective
576 mounted on a Leica LMD6000 direct microscope (details in [supplemental information](#)). The
577 cell density on the agar plate surface was set to 25 cells per 10,000 μm^2 . The cell volume of
578 the original mother cell in all three frames was calculated assuming a perfect sphere and
579 taking the cell diameter for calculations.

580 The single cell analysis by micromanipulation was performed on YPD plates with a
581 Singer Sporeplay tetrad microdissector (details in [supplemental information](#)). Just 12 cells
582 were harvested per plate in order to avoid prolonged incubations at 25° C at the beginning of
583 the experiment.

584 The segregation and morphology of the histone-labelled nucleus (H2A-GFP) was
585 analyzed by wide-field fluorescence videomicroscopy as described before [\[25\]](#). Further
586 details in [supplemental information](#).

587

588 **Assays to assess cell vitality and cell death.**

589 All the colorimetric and fluorometric assays to assess metabolic competence, ROS
590 production, and plasma membrane permeability were carried out in asynchronous logarithmic
591 cultures grown overnight at 25 °C, adjusting the OD₆₀₀ to 0.2 and incubated them for 24 h at
592 37 °C. Samples were taken at 0, 4 and 24 h and directly observed under the microscope.
593 Fluorescence microscopy was used instead of flow cytometry because strains were already
594 fluorescent for H2A-GFP. ROS were visualized with 10 µg/ml of 2',7'-dichlorofluorescein
595 diacetate (DCFH-DA; Sigma-Aldrich; D6883); and 3 µg/ml PI (Fluka; #81845) was used to
596 count dead cells. Both dyes were directly mixed with a 200 µl aliquot of the culture and
597 incubated 15 min at 37 °C in the dark. Cell bodies were considered ROS positive when the
598 cytoplasm stained green in the absence of staining for PI. FUN® 1 (Invitrogen; F7030)
599 staining was done washing 200 µl of each sample with water containing 2% D-(+)-glucose
600 and 10 mM Na-HEPES, pH 7.2, resuspending the cells in the same buffer with 10 µM FUN®
601 1 and incubating 30 min at 37 °C in the dark. For MB (Sigma-Aldrich; M9140) staining, 1 µl
602 of each sample was mixed with 1 µl of a 0.04% w/v MB solution in water onto a microscope
603 slide and directly visualized (bright field). Staining with FITC-labeled annexin V was
604 undertaken in spheroplasts as previously described [32]. Minor modifications to this protocol
605 were employed; namely, we used the TACS Annexin V-FITC Apoptosis Detection Kit
606 (R&Dsystems; 4830-01-K) as well as 60 units/ml of zymolyase (ZymoResearch; E1005) for
607 cell was digestion.

608

609 **Analysis of genomic rearrangements using microarrays**

610 Two similar protocols were used to expose heterozygous diploids to the *top2*-
611 mediated MC. For both protocols, cells were grown in YPD to an optical density of 0.5-1. For

612 one set of experiments (marked as E1 in the tables in the text), the cells were struck for single
613 colonies on plates containing solid YPD medium, incubated for 6 hours at 37 °C, then
614 incubated at room temperature until colonies had formed. For the second protocol, the cells
615 grown at room temperature were harvested by centrifugation, and resuspended in 37 °C liquid
616 medium, followed by incubation for 6 hours at 37 °C. Following this incubation, they were
617 struck on YPD plates and incubated at room temperature until colonies were formed. For
618 both protocols, the control cells were struck to room temperature YPD plates without
619 incubating them at 37 °C. In experiments to detect sectored colonies, the YPD medium was
620 replaced with solid omission medium lacking arginine and containing 120 µg/ml canavanine
621 [39].

622 We detected LOH events, aneuploidy and UPD using SNP-specific microarrays
623 similar to those used by [60]. The details of this procedure can be found in the [supplemental](#)
624 [information](#) and have been described before [36,37]. In analysis of single-colony isolates,
625 whole-genome arrays were used (Gene Expression Omnibus [GEO] #GPL20144). For
626 analysis of sectored colonies, we used microarrays specific for the right arm of V, and
627 chromosomes I, III, and VIII (GEO #GPL21274).

628

629 **ABBREVIATIONS**

630 MC: mitotic catastrophe; RCD: regulated cell death; ACD: accidental cell death;
631 DSB: DNA double strand break; MB: methylene blue; PI: propidium iodide; ROS: reactive
632 oxygen species; PS, phosphatidylserine; SNP: single nucleotide polymorphism; LOH: loss of

633 heterozygosity; T-LOH: terminal LOH; I-LOH: interstitial LOH; UPD: uniparental disomy;
634 RUPD: reciprocal UPD; rDNA: ribosomal RNA gene cluster

635

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641

642 **CONFLICTS OF INTEREST**

643 The authors declare no conflict of interests.

644

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653

654 REFERENCES

- 655 1. Hayashi MT, Karlseder J. DNA damage associated with mitosis and cytokinesis
656 failure. *Oncogene*. 2013; 32: 4593–601.
- 657 2. Portugal J, Mansilla S, Bataller M. Mechanisms of drug-induced mitotic catastrophe in
658 cancer cells. *Curr Pharm Des*. 2010; 16: 69–78.
- 659 3. Galluzzi L, Bravo-San Pedro JM, Vitale I, Aaronson S a, Abrams JM, Adam D,
660 Alnemri ES, Altucci L, Andrews D, Annicchiarico-Petruzzelli M, Baehrecke EH,
661 Bazan NG, Bertrand MJ, et al. Essential versus accessory aspects of cell death:
662 recommendations of the NCCD 2015. *Cell Death Differ*. 2014; : 58–73.
- 663 4. Chan K-S, Koh C-G, Li H-Y. Mitosis-targeted anti-cancer therapies: where they stand.
664 *Cell Death Dis*. 2012; 3: e411.
- 665 5. Galluzzi L, Vitale I, Vacchelli E, Kroemer G. Cell Death Signaling and Anticancer
666 Therapy. *Front Oncol*. 2011; 1: 1–18.
- 667 6. Fulda S, Debatin KM. Extrinsic versus intrinsic apoptosis pathways in anticancer
668 chemotherapy. *Oncogene*. 2006; 25: 4798–811.
- 669 7. Vitale I, Galluzzi L, Castedo M, Kroemer G. Mitotic catastrophe: a mechanism for
670 avoiding genomic instability. *Nat Rev Mol Cell Biol*. 2011; 12: 385–92.
- 671 8. Hoffelder DR, Luo L, Burke NA, Watkins SC, Gollin SM, Saunders WS. Resolution
672 of anaphase bridges in cancer cells. *Chromosoma*. 2004; 112: 389–97.
- 673 9. Ganem NJ, Pellman D. Linking abnormal mitosis to the acquisition of DNA damage. *J*
674 *Cell Biol*. 2012; 199: 871–81.
- 675 10. Quevedo O, García-Luis J, Matos-Perdomo E, Aragón L, Machín F. Nondisjunction of
676 a single chromosome leads to breakage and activation of DNA damage checkpoint in
677 g2. Laceyfield S, editor. *PLoS Genet*. Public Library of Science; 2012; 8: e1002509.
- 678 11. Machín F, Quevedo O, Ramos-Pérez C, García-Luis J. Cdc14 phosphatase: warning,
679 no delay allowed for chromosome segregation! *Curr Genet*. Springer Berlin
680 Heidelberg; 2016; 62: 7–13.
- 681 12. Uemura T, Tanagida M. Mitotic spindle pulls but fails to separate chromosomes in
682 type II DNA topoisomerase mutants: uncoordinated mitosis. *EMBO J*. 1986; 5: 1003–
683 10.
- 684 13. Holm C, Goto T, Wang JC, Botstein D. DNA topoisomerase II is required at the time
685 of mitosis in yeast. *Cell*. 1985; 41: 553–63.
- 686 14. Gorbisky GJ. Cell cycle progression and chromosome segregation in mammalian cells
687 cultured in the presence of the topoisomerase II inhibitors ICRF-187 [(+)-1,2-bis(3,5-
688 dioxopiperazinyl-1-yl)propane; ADR-529] and ICRF-159 (Razoxane). *Cancer Res*.
689 1994; 54: 1042–8.
- 690 15. Damelin M, Bestor TH. The decatenation checkpoint. *Br J Cancer*. 2007; 96: 201–5.
- 691 16. Downes CS, Clarke DJ, Mullinger AM, Giménez-Abián JF, Creighton AM, Johnson
692 RT. A topoisomerase II-dependent G2 cycle checkpoint in mammalian cells. *Nature*.
693 1994; 372: 467–70.

- 694 17. Nakagawa T, Hayashita Y, Maeno K, Masuda A, Sugito N, Osada H, Yanagisawa K,
695 Ebi H, Shimokata K, Takahashi T. Identification of decatenation G2 checkpoint
696 impairment independently of DNA damage G2 checkpoint in human lung cancer cell
697 lines. *Cancer Res.* 2004; 64: 4826–32.
- 698 18. Brooks K, Chia KM, Spoorri L, Mukhopadhyay P, Wigan M, Stark M, Pavey S,
699 Gabrielli B. Defective decatenation checkpoint function is a common feature of
700 melanoma. *J Invest Dermatol.* Nature Publishing Group; 2014; 134: 150–8.
- 701 19. Jain CK, Roychoudhury S, Majumder HK. Selective killing of G2decatenation
702 checkpoint defective colon cancer cells by catalytic topoisomerase II inhibitor.
703 *Biochim Biophys Acta - Mol Cell Res.* Elsevier B.V.; 2015; 1853: 1195–204.
- 704 20. Nitiss JL. Targeting DNA topoisomerase II in cancer chemotherapy. *Nat Rev Cancer.*
705 2009; 9: 338–50.
- 706 21. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance:
707 an evolving paradigm. *Nat Rev Cancer.* 2013; 13: 714–26.
- 708 22. DiNardo S, Voelkel K, Sternglanz R. DNA topoisomerase II mutant of *Saccharomyces*
709 *cerevisiae*: topoisomerase II is required for segregation of daughter molecules at the
710 termination of DNA replication. *Proc Natl Acad Sci U S A.* 1984; 81: 2616–20.
- 711 23. Holm C, Stearns T, Botstein D. DNA topoisomerase II must act at mitosis to prevent
712 nondisjunction and chromosome breakage. *Mol Cell Biol.* 1989; 9: 159–68.
- 713 24. Baxter J, Diffley JFX. Topoisomerase II inactivation prevents the completion of DNA
714 replication in budding yeast. *Mol Cell.* 2008; 30: 790–802.
- 715 25. Ramos-Pérez C, Ayra-Plasencia J, Matos-Perdomo E, Lisby M, Brown GW, Machín
716 F. Genome-Scale Genetic Interactions and Cell Imaging Confirm Cytokinesis as
717 Deleterious to Transient Topoisomerase II Deficiency in *Saccharomyces cerevisiae*.
718 *G3 (Bethesda).* 2017; 7: 3379–91.
- 719 26. Andrews CA, Vas AC, Meier B, Giménez-Abián JF, Díaz-Martínez LA, Green J,
720 Erickson SL, Vanderwaal KE, Hsu W-S, Clarke DJ. A mitotic topoisomerase II
721 checkpoint in budding yeast is required for genome stability but acts independently of
722 Pds1/securin. *Genes Dev.* 2006; 20: 1162–74.
- 723 27. Carmona-Gutierrez D, Bauer MA, Zimmermann A, Aguilera A, Austriaco N,
724 Ayscough K, Balzan R, Bar-Nun S, Barrientos A, Belenky P, Blondel M, Braun RJ,
725 Breitenbach M, et al. Guidelines and recommendations on yeast cell death
726 nomenclature. *Microb Cell.* 2018; 5: 4–31.
- 727 28. Klein HL, Bačinskaja G, Che J, Cheblal A, Elango R, Epshtein A, Fitzgerald DM,
728 Gómez-González B, Khan SR, Kumar S, Leland BA, Marie L, Mei Q, et al. Guidelines
729 for DNA recombination and repair studies: Cellular assays of DNA repair pathways.
730 *Microb Cell.* 2019; 6: 1–64.
- 731 29. Kucsera J, Yarita K, Takeo K. Simple detection method for distinguishing dead and
732 living yeast colonies. *J Microbiol Methods.* 2000; 41: 19–21.
- 733 30. Millard PJ, Roth BL, Thi HPT, Yue ST, Haugland RP. Development of the FUN-1
734 family of fluorescent probes for vacuole labeling and viability testing of yeasts. *Appl*
735 *Environ Microbiol.* 1997; 63: 2897–905.

- 736 31. Madeo F, Fröhlich E, Ligr M, Grey M, Sigrist SJ, Wolf DH, Fröhlich KU. Oxygen
737 stress: A regulator of apoptosis in yeast. *J Cell Biol.* 1999; 145: 757–67.
- 738 32. Madeo F, Fröhlich E, Fröhlich KU. A yeast mutant showing diagnostic markers of
739 early and late apoptosis. *J Cell Biol.* 1997; 139: 729–34.
- 740 33. Carmona-Gutierrez D, Eisenberg T, Büttner S, Meisinger C, Kroemer G, Madeo F.
741 Apoptosis in yeast: triggers, pathways, subroutines. *Cell Death Differ.* 2010; 17: 763–
742 73.
- 743 34. Madeo F, Herker E, Maldener C, Wissing S, Lächelt S, Herlan M, Fehr M, Lauber K,
744 Sigrist SJ, Wesselborg S, Fröhlich KU. A caspase-related protease regulates apoptosis
745 in yeast. *Mol Cell.* 2002; 9: 911–7.
- 746 35. Mitsui K, Nakagawa D, Nakamura M, Okamoto T, Tsurugi K. Valproic acid induces
747 apoptosis dependent of Yca1p at concentrations that mildly affect the proliferation of
748 yeast. *FEBS Lett.* 2005; 579: 723–7.
- 749 36. St. Charles J, Hazkani-Covo E, Yin Y, Andersen SL, Dietrich FS, Greenwell PW,
750 Malc E, Mieczkowski P, Petes TD. High-resolution genome-wide analysis of
751 irradiated (UV and γ -Rays) diploid yeast cells reveals a high frequency of genomic
752 loss of heterozygosity (LOH) events. *Genetics.* 2012; 190: 1267–84.
- 753 37. St. Charles J, Petes TD. High-Resolution Mapping of Spontaneous Mitotic
754 Recombination Hotspots on the 1.1 Mb Arm of Yeast Chromosome IV. *PLoS Genet.*
755 2013; 9.
- 756 38. Lee PS, Greenwell PW, Dominska M, Gawel M, Hamilton M, Petes TD. A fine-
757 structure map of spontaneous mitotic crossovers in the yeast *Saccharomyces*
758 *cerevisiae*. *PLoS Genet.* 2009; 5: e1000410.
- 759 39. Barbera MA, Petes TD. Selection and analysis of spontaneous reciprocal mitotic cross-
760 overs in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A.* 2006; 103: 12819–24.
- 761 40. Yin Y, Petes TD. Genome-Wide High-Resolution Mapping of UV-Induced Mitotic
762 Recombination Events in *Saccharomyces cerevisiae*. *PLoS Genet.* 2013; 9.
- 763 41. Jannatipour M, Liu YX, Nitiss JL. The top2-5 mutant of yeast topoisomerase II
764 encodes an enzyme resistant to etoposide and amsacrine. *J Biol Chem.* 1993; 268:
765 18586–92.
- 766 42. Andersen SL, Petes TD. Reciprocal uniparental disomy in yeast. *Proc Natl Acad Sci.*
767 2012; 109: 9947–52.
- 768 43. Mendoza M, Norden C, Durrer K, Rauter H, Uhlmann F, Barral Y. A mechanism for
769 chromosome segregation sensing by the NoCut checkpoint. *Nat Cell Biol.* 2009; 11:
770 477–83.
- 771 44. Galluzzi L, Vitale I, Abrams JM, Alnemri ES, Baehrecke EH, Blagosklonny M V,
772 Dawson TM, Dawson VL, El-Deiry WS, Fulda S, Gottlieb E, Green DR, Hengartner
773 MO, et al. Molecular definitions of cell death subroutines: recommendations of the
774 Nomenclature Committee on Cell Death 2012. *Cell Death Differ.* 2012; 19: 107–20.
- 775 45. Burgess A, Rasouli M, Rogers S. Stressing Mitosis to Death. *Front Oncol.* 2014; 4: 1–
776 7.
- 777 46. Endo K, Mizuguchi M, Harata A, Itoh G, Tanaka K. Nocodazole induces mitotic cell

- 778 death with apoptotic-like features in *Saccharomyces cerevisiae*. *FEBS Lett.* Federation
779 of European Biochemical Societies; 2010; 584: 2387–92.
- 780 47. Wysocki R, Kron SJ. Yeast cell death during DNA damage arrest is independent of
781 caspase or reactive oxygen species. *J Cell Biol.* 2004; 166: 311–6.
- 782 48. Váchová L, Palková Z. Caspases in yeast apoptosis-like death: facts and artefacts.
783 *FEMS Yeast Res.* 2007; 7: 12–21.
- 784 49. Perrone GG, Tan S-X, Dawes IW. Reactive oxygen species and yeast apoptosis.
785 *Biochim Biophys Acta.* 2008; 1783: 1354–68.
- 786 50. Qi H, Li TK, Kuo D, Nur-E-Kamal A, Liu LF. Inactivation of Cdc13p triggers MEC1-
787 dependent apoptotic signals in yeast. *J Biol Chem.* 2003; 278: 15136–41.
- 788 51. Quevedo O, Ramos-Perez C, Petes TD, Machin F. The Transient Inactivation of the
789 Master Cell Cycle Phosphatase Cdc14 Causes Genomic Instability in Diploid Cells of
790 *Saccharomyces cerevisiae*. *Genetics.* 2015; 200: 755–69.
- 791 52. Spell RM, Holm C. Nature and distribution of chromosomal intertwining in
792 *Saccharomyces cerevisiae*. *Mol Cell Biol.* 1994; 14: 1465–76.
- 793 53. Christman MF, Dietrich FS, Fink GR. Mitotic recombination in the rDNA of *S.*
794 *cerevisiae* is suppressed by the combined action of DNA topoisomerases I and II. *Cell.*
795 1988; 55: 413–25.
- 796 54. Ozenberger BA, Roeder GS. A unique pathway of double-strand break repair operates
797 in tandemly repeated genes. *Mol Cell Biol.* 1991; 11: 1222–31.
- 798 55. McCulley JL, Petes TD. Chromosome rearrangements and aneuploidy in yeast strains
799 lacking both Tel1p and Mec1p reflect deficiencies in two different mechanisms. *Proc*
800 *Natl Acad Sci U S A.* 2010; 107: 11465–70.
- 801 56. D’Amours D, Stegmeier F, Amon A. Cdc14 and condensin control the dissolution of
802 cohesin-independent chromosome linkages at repeated DNA. *Cell.* 2004; 117: 455–69.
- 803 57. Sullivan M, Higuchi T, Katis VL, Uhlmann F. Cdc14 phosphatase induces rDNA
804 condensation and resolves cohesin-independent cohesion during budding yeast
805 anaphase. *Cell.* 2004; 117: 471–82.
- 806 58. Machín F, Torres-Rosell J, Jarmuz A, Aragón L. Spindle-independent condensation-
807 mediated segregation of yeast ribosomal DNA in late anaphase. *J Cell Biol.* 2005; 168:
808 209–19.
- 809 59. Smith JS, Burke DJ. *Yeast Genetics: Methods and Protocols.* Smith JS, Burke DJ,
810 editors. New York, NY: Springer New York; 2014. 1–375 p.
- 811 60. Gresham D, Curry B, Ward A, Gordon DB, Brizuela L, Kruglyak L, Botstein D.
812 Optimized detection of sequence variation in heterozygous genomes using DNA
813 microarrays with isothermal-melting probes. *Proc Natl Acad Sci.* 2010; 107: 1482–7.

814

815

816 **FIGURE LEGENDS.**

817 **Figure 1. Most progeny coming from a Top2-mediated mitotic catastrophe is inviable.**
818 (A) Haploid *TOP2* (WT) or *top2-5* cells were grown at 25 °C and spread on YPD agar plates.
819 Unbudded cells (G_1/G_0) were identified and photographed again after 6 h at 37 °C. Number of
820 cell bodies (buds) coming from these G_1/G_0 cells were then counted and plotted as indicated.
821 (B) The same analysis as in panel A but including data coming from independent experiments
822 as well as after 24 h incubation at 37 °C (mean \pm s.e.m., n=3). (C) The principle of the solid
823 medium-based clonogenic assay. Unlike the liquid medium-based clonogenic assay, cells are
824 spread on the Petri dish before the condition that challenges survivability is transiently
825 triggered (Top2 inactivation in our study). In the solid medium-based assay, the colony
826 forming unit (CFU) reading after the challenge is binary, irrespective of how far cells keep on
827 dividing during the challenge: “0” if all clonal cells are inviable (grey); “1” if at least one cell
828 from the clone stays viable (yellowish orange). (D) Time course of clonogenic survivability.
829 Asynchronous *top2-5* cultures growing at 25 °C were spread onto several YPD plates. The
830 plates were incubated at 37 °C for different periods before transferring them 25 °C. Four days
831 after the initial plating, visible colonies (macrocolonies) were counted and normalized to a
832 control plate which was never incubated at 37 °C (0h). (E) Analysis of the origin of
833 macrocolonies after the 6 h x 37 °C regime as determined after microscanning plates at the
834 time of seeding (N=33 macrocolonies; 2:1 unbudded:budded ratio at seeding).

835

836 **Figure 2. Most daughter cells coming from a Top2-mediated mitotic catastrophe are**
837 **unable to divide again.** (A) Haploid *top2-5* cells were spread at high cell density on two
838 Petri dishes. At the time of seeding, 0h (25 °C), several fields were photomicrographed before
839 incubating the plates at 37 °C during either 6 h or 24 h. After the 37 °C incubations, the same
840 fields were localized, photomicrographed again, and further incubated 16-24 h at 25 °C. An
841 example of a microscope field of a 37 °C x 24 h experiment. Three representative unbudded
842 cells at 0h (25 °C) are highlighted. In red, two cells that budded just once during the 37 °C x
843 24 h incubation (“2” cell bodies); one of them able to re-bud again a few times after the 25 °C
844 downshift (“m”) and the second one that remained stuck as “2”. In green, a cell that reached
845 “3” bodies at 37 °C and remained so after the final 25 °C x 24 h incubation. Scale bar
846 corresponds to 50 μ m. (B) Analysis of how far the *top2-5* MC progeny can go based on the
847 microcolony approach shown in panel A. Only unbudded (G_1/G_0) cells at 0h (25 °C) were
848 considered. The inner circle in the sunburst chart depicts proportions of cell bodies after the
849 37 °C incubation. The outer circle depicts the situation after the final 25 °C incubations (see
850 [supplemental information](#) for a detailed description). On the left are results from a 37 °C x 6 h
851 regime; on the right are results from a 37 °C x 24 h regime. Numbers point to the number of
852 cell bodies; “m” means microcolonies of 5 or more bodies. (C) Capability of the *top2-5*
853 progeny to split apart and relationship with overall survivability. Unbudded cells were
854 micromanipulated and arranged at defined plate positions before incubating them for 6 h at
855 37 °C. Then, those cells able to re-bud at least once were subjected to an attempt to physically
856 separate the cell bodies. The inner circle in the sunburst depicts the number of cell bodies
857 after the 37 °C incubation. The middle circle depicts the result of the separation attempt (“Y”
858 or “N”, successful or unsuccessful, respectively). The outer circle indicates if any of the

859 bodies was able to raise a macrocolony (Yes or No) after 4 d incubation at 25 °C. **(D)**
860 Progression of the size (volume) of the original G_1/G_0 cells (mother) after the *top2-5* mitotic
861 catastrophe with and without the osmotic stabilizer Sorbitol (Sorb, 1.2 M). **(E)** Time course
862 of clonogenic survivability in the presence of 1.2 M Sorbitol. The experiment was conducted
863 as in Figure 1D. **(F)** Sunbursts of microcolony analyses in the presence of 1.2 M Sorbitol
864 (Srb) at the 6 h and 24 h x 37 °C regimes. Interpretation as in panel B. In sunburst charts, N
865 indicates number of original unbudded cells which were followed; blue sectors depict G_1/G_0
866 cells that remained unbudded during the 37 °C incubations; red sectors, cells that budded
867 once at 37 °C; green sectors, cells that reached 3 bodies at 37 °C; orange sectors, cells that
868 reached 4 bodies at 37 °C; cyan sectors, cells that reached 5 or more bodies at 37 °C.

869

870 **Figure 3. Cell vitality remain high for several hours after the *top2* mitotic catastrophe**
871 **and is not modulated by *Yca1*.** **(A)** Morphological patterns of cell and nuclear sickness after
872 the *top2* MC. Haploid *top2-5 HTA2-GFP* cells were seeded onto agarose patches and the
873 same fields visualized under the fluorescence microscope at 0 h, 6 h and 24 h after the 37 °C
874 temperature upshift. White filled triangles point to darkened inclusion bodies, asterisks (*)
875 swelled cells, open circles (○) cells that has lost their rounded shape, and hash (#) points to
876 cells that have largely lost the H2A-GFP signal. BF, bright field. Scale bar corresponds to 20
877 μm. **(B)** Time course of cell vitality decline as reporter by methylene blue (MB) negative
878 staining. Asynchronous cultures of the *top2-5* and *top2-5 yca1Δ* strains were grown at 25 °C
879 before shifting the temperature to 37 °C. At the indicated time points (0, 2, 4, 6, 24 & 48 h),
880 samples were taken and stained with the vital dye MB. **(C)** Cell vitality decline as reported by
881 metabolic competence, intrinsic ROS generation, and loss of plasma membrane
882 impermeability. Cells were treated as in B and stained at the indicated time points with the
883 vital dye FUN1, the death marker propidium iodide (PI), and/or the ROS reporter DCFH-DA
884 (mean ± s.e.m., n=3). **(D)** Clonogenic survival profile of *top2-5 yca1Δ* as determined on the
885 low-density plates (mean ± s.e.m., n=3). The experimental procedure is described in Figure
886 1D. **(E)** Ability to re-bud of the *top2-5 yca1Δ* MC progeny as determined on the high-density
887 plates. The experimental procedure is described in Figure 2.

888

889 **Figure 4. Mitotic catastrophe in *top2-5* diploids leads to progeny with a greater capacity**
890 **for cell division than observed in the haploid.** Isogenic homozygous *top2-5* diploid cells
891 were grown and spread at either low or high cell density on Petri dishes. In addition, G_1/G_0
892 cells were micromanipulated, arrayed and treated as described in Figure 2C. **(A)** Ability to re-
893 bud after transient (6 h or 24 h) incubations at 37 °C of the high-density plates. The
894 experimental procedure is described in Figure 2. **(B)** Clonogenic survival profile as
895 determined on the low-density plates (mean ± s.e.m., n=3). The experimental procedure is
896 described in Figure 1D. **(C)** Capability of the progeny to split apart and relationship with
897 overall survivability. The experimental procedure is described in Figure 2C.

898

899 **Figure 5. Mitotic catastrophe in *top2-5* heterozygous diploids leads to survivors with**
900 **specific genetic instability footprints. (A)** Schematic of the engineered chromosome V (cV)
901 from the hybrid highly heterozygous (~55,000 SNPs) diploids used in this study. As
902 explained in the text, the genetic modifications applied in cV allowed for selection of
903 chromosome rearrangements. **(B)** G_1/G_0 cells from the hybrid highly heterozygous *top2-5*
904 diploid FM1873 strain were micromanipulated, arrayed and treated as described in Figure 2C.
905 The capability of the immediate progeny to split apart and its relationship with overall
906 survivability is shown in the sunburst chart. **(C)** Clonogenic survival profile of FM1873 as
907 determined on low-density plates (mean \pm s.e.m., n=3). The experimental procedure is
908 described in Figure 1D. **(D)** Percentage of red or sectored (either white:red or pink:red)
909 colonies in the surviving clones. Both outcomes often reflect genetic alterations on cV as
910 described in the text. **(E)** Results of SNP microarray analysis of colonies derived from
911 FM1873 or MD684. Microarray patterns showing specific chromosome rearrangements are
912 shown on the left side, and diagrams of the putative events producing these patterns are
913 shown on the right side. The number of specific events out of 118 total events is indicated.
914 For the microarray patterns, hybridization to SNPs specific to homologs derived from W303-
915 1A are shown in red, and hybridizations to SNPs specific to YJM789 are shown in blue. The
916 X-axis shows SGD coordinates for the chromosome, and the Y-axis shows the ratio of
917 hybridization normalized to a heterozygous diploid strain. The representative examples
918 correspond to (1) a T-LOH event on chromosome IV (MD684.1.1 (E1) in Table 1); (2) a I-
919 LOH event (marked with a green arrow) plus T-LOH event on chromosome IV (FM1873-15c
920 (C2) in Table 1); (3) a Trisomy for chromosome XIV (MD684.1.1 (E1) in Table 1); and (4) a
921 UPD for chromosome V (This isolate has two copies of the W303-1A-derived and no copies
922 of the YJM789-derived chromosome).

923

924 **Figure 6. Summary of the *top2*-mediated mitotic catastrophe and the fate of the**
925 **immediate progeny.** After inactivation of Top2, cells cannot resolve sister chromatids in
926 anaphase, leading to an anaphase bridge between the mother (M) and its daughter (D1).
927 These bridges are quickly severed (at least in the *top2-5* mutant [25]). The immediate
928 progeny coming from the *top2* mitotic catastrophes (MCs) is largely unable to enter a new
929 cell cycle (do not re-bud) despite remaining metabolically active for many hours; hence, these
930 cells become senescent. Only ~25% of the original mothers re-bud once (D2) after the *top2*
931 MC. The long-term fate of most daughter cells is death. They will eventually die through
932 accidental cell death (ACD), as deduced from both the asynchrony and asymmetry of death
933 events and the lack of regulation by the death modulator Yca1(Mca1). The inability to enter a
934 new cell cycle is likely a consequence of both the massive DNA damage as a consequence of
935 bridge severing, and the misdistribution of essential genetic material coded on the
936 chromosome arms between the daughter cells. A small proportion of the progeny, especially
937 those cell that underwent a milder *top2* MC (e.g., already in S/G₂ at the time of Top2
938 inactivation) survives to yield a population of cells with characteristic footprints of genomic
939 instability. Two of these footprints, terminal loss of heterozygosity (T-LOH) and uniparental
940 disomy (UPD) are expected outcomes from anaphase bridges.

941

942 **Table 1. Genomic changes in single-colony isolates of FM1873 and MD684.**

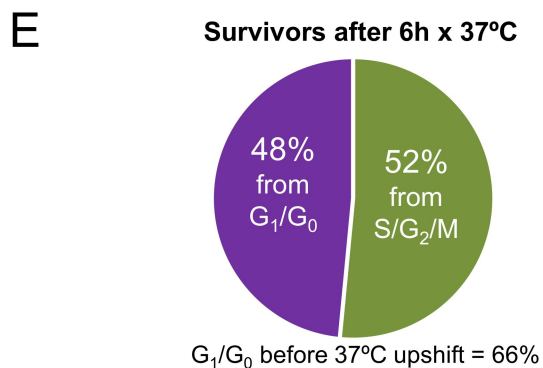
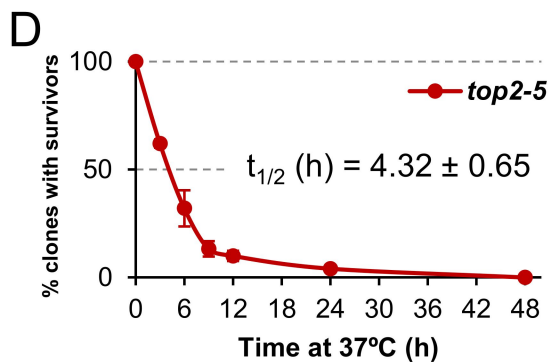
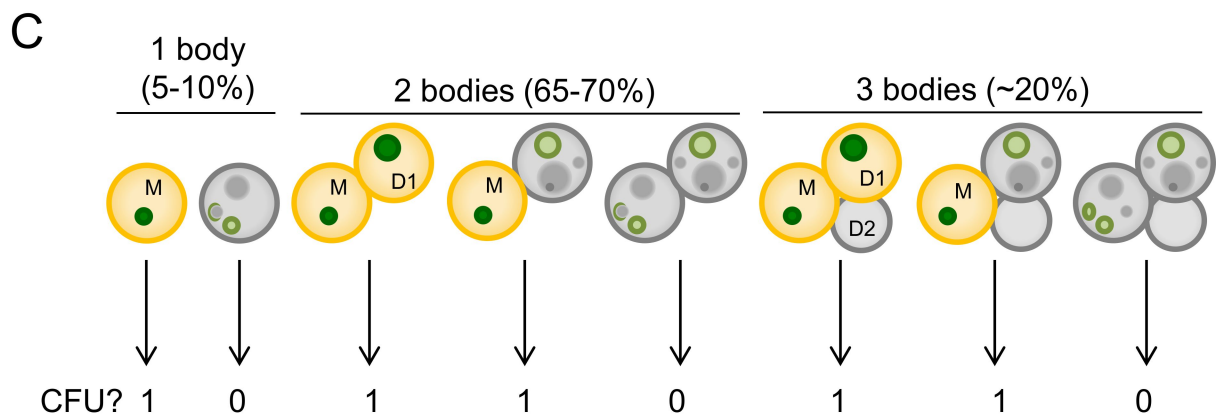
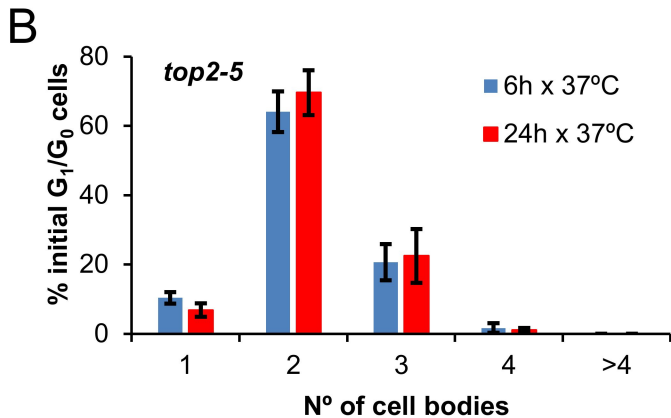
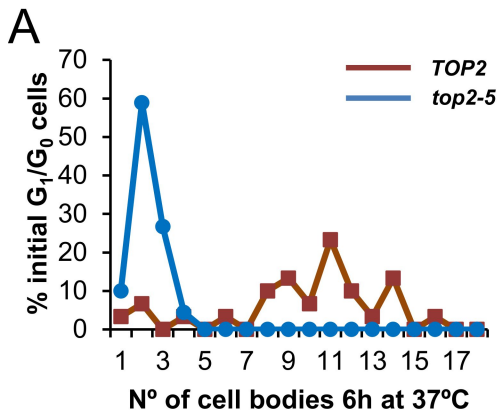
Strain name¹	Genomic alterations²
FM1873-01 (E1)	T-LOH (IV/966), Tri (V), Partial UPD (VII), Tri (X)
FM1873-04 (E1)	T-LOH (IV/471), UPD (VIII), 3 T-LOH (XIII/450/777/864)
FM1873-11 (E1)	UPD (IV), Tri (VIII), Mon (IX), Tri (X), Partial Tri (XV), UPD (XVI)
FM1873-1 (E2)	UPD (IV), Tri (XV)
FM1873-2 (E2)	T-LOH (IV/790)
FM1873-3 (E2)	T-LOH (IV/790)
FM1873-4 (E2)	T-LOH (IV/671), Tri (V), Tri (VII), T-LOH (XV/1002)
FM1873-5 (E2)	T-LOH (IV/755), T-LOH (XIII/450)
FM1873-11X (E2)	T-LOH (IV/958), Tri (VII)
FM1873-12 (E2)	Tri (VIII)
FM1873-13 (E2)	T-LOH (IV/668), Tri (V), T-LOH (XV/1002)
FM1873-14 (E2)	T-LOH (IV/1018)
FM1873-15 (E2)	T-LOH (IV/483)
FM1873-C1 (C1)	No additional alterations
FM1873-C2 (C1)	No additional alterations
FM1873-C3 (C1)	2 T-LOH (IV/485/820)
FM1873-C4 (C1)	T-LOH (IV/470), T-LOH (XIII/856), T-LOH (XIV/216)
FM1873-1c (C2)	2 T-LOH (IV/456/1440); T-LOH (VII/385), T-LOH (XIV/400)
FM1873-2c (C2)	2 T-LOH (IV/957/1440)
FM1873-3c (C2)	T-LOH (IV/935), Tri (V)
FM1873-4c (C2)	T-LOH (IV/858)
FM1873-5c (C2)	Tri and T-LOH (IV/1270), Tri (V), Partial Tri (XIII)
FM1873-11c (C2)	T-LOH (IV/1017), Tri (VIII), Tri (XV)
FM1873-12c (C2)	T-LOH (IV/1004), Tri (X), Tri (XV)
FM1873-13c (C2)	T-LOH (IV/1017), Tri (VIII)
FM1873-14c (C2)	2 T-LOH (IV/889/1221)
FM1873-15c (C2)	I-LOH (IV/1354-1357), T-LOH (IV/1474), Tri (V), UPD (VIII)
MD684.1.1 (E1)	T-LOH (IV/596), T-LOH (XI/7.5), T-LOH (XII/430)
MD684.1.2 (E1)	T-LOH (IV/808), Tri (VIII)
MD684.1.3 (E1)	T-LOH (IV/1002), T-LOH (XII/165)
MD684.1.4 (E1)	T-LOH (IV/458), Tri (XV)

MD684.1.5 (E1)	T-LOH (IV/1065), Tri (VIII), 2 T-LOH (XII/253/446)
MD684.1.6 (E2)	Partial Tri (II)
MD684.1.7 (E2)	T-LOH (IV/1028), T-LOH (VII/29), T-LOH (XII/175)
MD684.1.8 (E2)	3 T-LOH (IV/921/1050/1070), T-LOH (V/538), T-LOH (XII/450)
MD684.1.9 (E2)	Partial Mon (I), Tri and T-LOH (IV/486)
MD684.1.10 (E2)	2 T-LOH (IV/661/963), Tri and T-LOH (XV/626)
MD684 C1.1 (C1)	I-LOH (IV/747-760), T-LOH (IV/840), T-LOH (XII/176)
MD684 C1.2 (C1)	T-LOH (IV/458), T-LOH (XII/447)
MD684 C1.3 (C1)	2 T-LOH (IV/893/1472), Tri (V), T-LOH (XII/170)
MD684 C1.4 (C1)	Tri (I), T-LOH (IV/920)
MD684 C1.5 (C1)	T-LOH (IV/527), Tri (XII)
MD684 C1.6 (C2)	2 T-LOH (IV/680/1050), T-LOH (VII/760), T-LOH (XII/175)
MD684 C1.7 (C2)	T-LOH (IV/1008), I-LOH (XII/215-228), T-LOH (XII/277), Partial Tri (XVI)
MD684 C1.8 (C2)	T-LOH (IV/842), T-LOH (XV/657)
MD684 C1.9 (C2)	T-LOH (IV/1028)

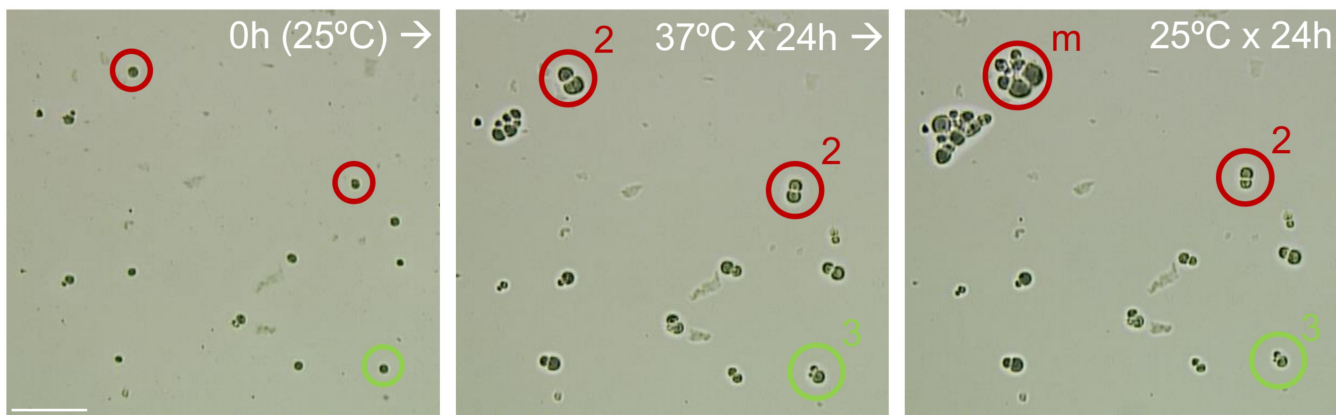
943 ¹ Parentheses after the strain name indicate whether the strain was experimental (E, incubated
944 for six hours at 37 °C) or control (C, not incubated at 37 °C). E1 and C1 indicates that the 37
945 °C incubation was done on plates; in E2 and C2 experiments, the 37 °C incubations were
946 done in liquid.

947 ² Strains derived from FM1873 (both experimental (incubated at 37 °C for six hours) and
948 control (not incubated at 37 °C) strains had three to four copies of chromosome XIV and a
949 terminal LOH event on the right arm of chromosome XII (breakpoint at 236 kb). These
950 alterations, therefore, are not listed in the FM1873 strains. The MD684 strain also had three
951 to four copies of chromosome XIV in isolates, and this alteration is not shown in the table.
952 Code: T-LOH (terminal LOH event), I-LOH (interstitial LOH event), Tri (trisomy), Mon
953 (monosomy), UPD (uniparental disomy). Between brackets: altered
954 chromosomes/breakpoints.

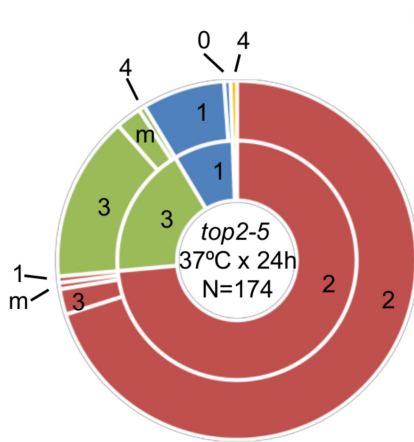
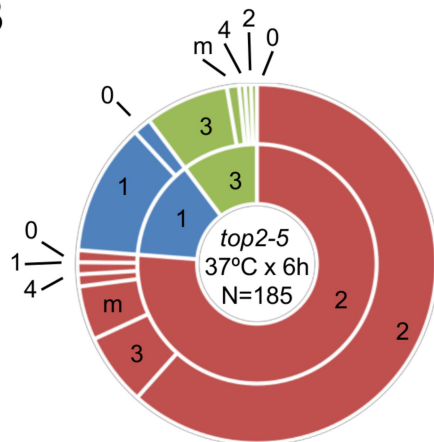
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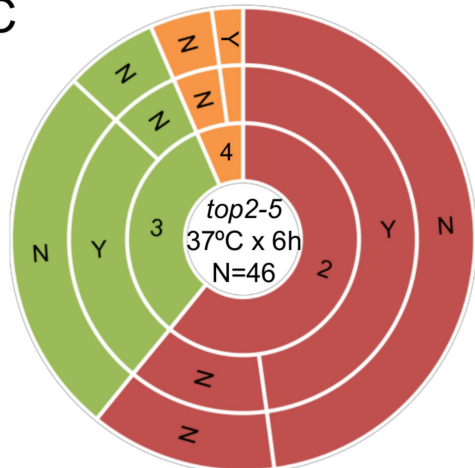
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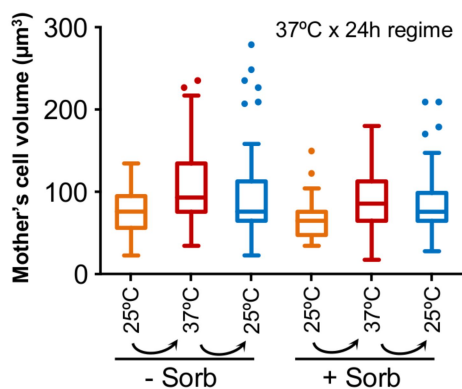
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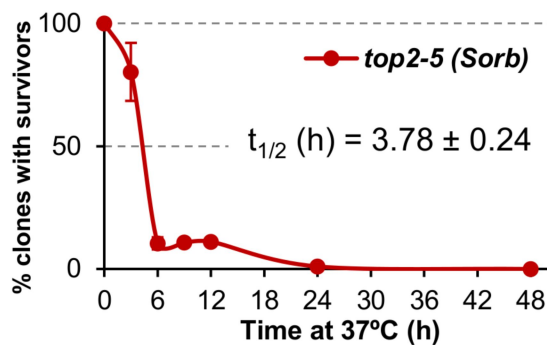
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D



E



F

