

1 **TITLE**

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3 The SUMO protease Ulp2 regulates genome stability and drug resistance in the
4 human fungal pathogen *Candida albicans*

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24 **Abstract**

25 Stress-induced genome instability in microbial organisms is emerging as a critical
26 regulatory mechanism for driving rapid and reversible adaption to drastic
27 environmental changes. In *Candida albicans*, a human fungal pathogen that causes
28 life-threatening infections, genome plasticity confers increased virulence and
29 antifungal drug resistance. Discovering the mechanisms regulating *C. albicans*
30 genome plasticity is a priority to understand how this and other microbial pathogens
31 establish life-threatening infections and develop resistance to antifungal drugs. We
32 identified the SUMO protease Ulp2 as a critical regulator of *C. albicans* genome
33 integrity through genetic screening. Deletion of *ULP2* leads to hypersensitivity to
34 genotoxic agents and increased genome instability. This increased genome diversity
35 causes reduced fitness under standard laboratory growth conditions but enhances
36 adaptation to stress, making *ulp2 Δ* cells more likely to thrive in the presence of
37 antifungal drugs. Whole-genome sequencing indicates that *ulp2 Δ* cells counteract
38 antifungal drug-induced stress by developing segmental aneuploidies of
39 chromosome R and chromosome I. We demonstrate that intrachromosomal
40 repetitive elements drive the formation of complex novel genotypes with adaptive
41 power.

42

43 **Introduction**

44 Understanding how organisms survive and thrive in changing environments is a
45 fundamental question in biology. Genetic variation is central to environmental
46 adaptation as it allows selection of certain genotypes better fit to grow in new
47 environments. Different types of genetic change contribute to genetic variability,
48 including (i) mutations such as single-base alteration and small (<100 bp) insertions
49 or deletions (indels), (ii) large (>1 kb) deletions and duplications, (iii) whole-
50 chromosome or segmental-chromosome aneuploidy and (iv) translocations and
51 complex genomic rearrangements [1]. Furthermore, diploid cells can undergo Loss
52 of Heterozygosity (LOH) driven by cross-overs or gene conversions between the two
53 homologous chromosomes [2]. Excessive genome instability is harmful in the
54 absence of selective pressure as it alters the copy-number of many genes, leading to
55 unbalanced protein levels [3]. However, an unstable genome can provide rapid
56 adaptive power in hostile environments [4,5] because it provides genetic diversity
57 upon which selection can act.

58 Genome plasticity – the ability to generate genomic variation – is emerging as a
59 critical adaptive mechanism in human microbial pathogens that need to adapt rapidly
60 to extreme environmental shifts, including changes in temperature, pH and nutrient
61 availability following colonisation of different host environments [6,7]. One such
62 organism is *Candida albicans*, the most common human fungal pathogen and the
63 most prevalent cause of death due to fungal infection. *C. albicans* is part of the
64 normal microflora of most healthy individuals where it colonises the skin, mucosal
65 surface, gastrointestinal and the female genitourinary tract. However, *C. albicans*
66 can become a dangerous pathogen causing a wide range of infections, from
67 superficial mucosal infections to life-threatening disseminated diseases [8]. Azole
68 antifungal agents, such as Fluconazole (FLC), are the most commonly prescribed
69 drugs for treating *Candida* infections [9–11]. FLC targets the enzyme lanosterol 14 α -
70 demethylase, encoded by *ERG11*, blocking biosynthesis of ergosterol, an essential
71 component of the fungal cell membrane [12,13]. As a result, FLC arrests *C. albicans*
72 cell growth without killing the fungus. This fungistatic, rather than fungicidal, mode of
73 action allows for the evolution of drug-resistant strains [14]. One primary mechanism
74 of drug resistance is an increased production of the FLC target, Erg11 enzyme,
75 diluting the activity of the drug [12]. This high target production is often due to

76 increased activity of the transcription factor Upc2 activating *ERG11* transcription [15–
77 18]. Overproduction of efflux pumps, such as the *C. albicans* proteins Cdr1, Cdr2 and
78 Mdr1, can also drive FLC resistance by decreasing intracellular FLC levels [19].
79 In recent years, genome plasticity has emerged as a critical adaptive mechanism
80 causing antifungal drug resistance. *C. albicans* is a diploid organism with a highly
81 heterozygous genome organised into 2×8 chromosomes ($2n = 16$) [20,21].
82 Population studies have identified a remarkable genomic variation among *C.*
83 *albicans* isolates and specific chromosomal variations are selected during host-niche
84 colonisation [22–28]. Indeed, many drug-resistant isolates exhibit karyotypic
85 diversity, including aneuploidy and gross chromosomal rearrangements that can
86 confer resistance due increased copy number of specific genes including *ERG11*,
87 and/or multidrug transporters [7,29,30].

88 *C. albicans* genome instability is not random: it occurs more frequently at specific
89 hotspots that are often repetitive [31–35]. Subtelomeric regions and the *rDNA* locus
90 are among the most unstable genomic sites [34,36]. Indeed, *C. albicans*
91 subtelomeric regions are enriched in transposons-derived repetitive sequences and
92 protein-coding genes [31,37]. Most notable are the telomere-associated *TLO* genes,
93 a family of 14 closely related paralogues encoding proteins similar to the Mediator 2
94 subunit of the mediator transcriptional regulator [38–40]. The majority of *TLO* genes
95 are located at subtelomeric regions except *TLO34*, located at an internal locus on
96 the left arm of Chr1 [38]. The number and position of *TLO* genes vary widely
97 between clinical isolates, indicating significant plasticity with potential consequences
98 for the fitness of the organism [34]. The *rDNA* locus consists of a tandem array of a
99 ~12 kb unit repeated 50 to 200 times on chromosome R; rDNA length
100 polymorphisms occur frequently [21,34]. In addition to these complex repetitive
101 elements, different types of Long Repeat Sequences (65 bp to 6.5 Kb) dispersed
102 across the *C. albicans* genomes have been shown to drive karyotype variation
103 during adaptation to antifungal drugs and passage through the mouse host [32,33].
104 *C. albicans* genome plasticity is regulated by environmental conditions: the genome
105 is relatively stable under optimal laboratory growth conditions but becomes more
106 unstable under stress conditions [41,42]. For example, FLC treatment drives a global
107 increase in LOH, chromosome rearrangements and aneuploidy [41,42]. This
108 increased genetic variation facilitates selection of fitter genotypes [28,29]. Similarly,

109 higher rates of genomic variation are detected following passage of *C. albicans* in
110 *vivo* relative to passage *in vitro* [35,43]. It is unknown if and how stress regulates
111 genome plasticity. The discovery of such regulatory mechanisms will be essential to
112 reveal how resistance to antifungal drugs emerges.

113 This study posits that gene deletions for critical regulators of *C. albicans* genome
114 integrity would cause higher genome variation and rapid adaptation to FLC. To test
115 this hypothesis, we performed a genetic screen to identify modulators of *C. albicans*
116 genome stability. The screen led to the identification of the SUMO protease Ulp2. In
117 the absence of stress, *ULP2* deletion leads to elevated genome instability causing
118 fitness defects and hypersensitivity to genotoxic agents. In contrast, the elevated
119 genome instability of the *ulp2* Δ/Δ strain is advantageous in the presence of high FLC
120 doses. This is because the increased genetic diversity expands the pool of
121 genotypes upon which selection can act, driving adaptation to a new stress
122 environment (FLC), concomitantly rescuing the fitness defects associated with *ULP2*
123 deletion. We also demonstrate that intrachromosomal repetitive elements are sites of
124 genetic diversity that drive the formation of complex novel genotypes with adaptive
125 potential.

126 **Results**

127 ***A systematic genetic screen identifies the Ulp2 as a regulator of C. albicans*** 128 ***genotoxic stress response***

129 To identify factors regulating *C. albicans* genome integrity, we utilised a deletion
130 library comprising a subset (674/3000) of *C. albicans* genes that are not conserved
131 in other organisms or have a functional motif potentially related to virulence [44]. As
132 defects in genome integrity lead to hypersensitivity to genotoxic agents [45], the
133 deletion library was screened for hypersensitivity to two DNA damaging agents:
134 Ultraviolet (UV) irradiation which induces formation of pyrimidine dimers [46], and
135 Methyl MethaneSulfonate (MMS), which leads to replication blocks and base
136 mispairing [47].

137 Genotoxic stress hypersensitivity was semi-quantitatively scored by comparing the
138 growth of treated versus untreated on a scale of 0 to 4, where 0 indicates no
139 sensitivity, and 4 specifies strong hypersensitivity (**Fig 1A**). The screen identified 32
140 gene deletions linked to DNA damage hypersensitivity (UV or MMS score ≥ 2).

141 Almost half of these hits (14/32; ~44%) are genes predicted to encode components
142 of the DNA Damage Response pathway (7/32; ~22%) or the cell division machinery
143 (7/32; ~22%) (**Table S1**). For example, the top 4 hits of the screen were *MEC3*,
144 *RAD18*, *GRR1* and *KIP3* genes. Although *C. albicans MEC3* and *RAD18* are
145 uncharacterised, they encode for proteins, conserved in other organisms, that are
146 universally involved in sensing DNA damage (Mec3) [48] and in DNA post-replication
147 repair (Rad18) [49]. *C. albicans GRR1* and *KIP3* are required for cell cycle
148 progression [50] and mitotic spindle organisation, respectively [51] (**Fig 1B** and
149 **Table S1**). ~25% (8/32) of the remaining hits are genes encoding proteins with no
150 apparent orthologous in the two well-studied yeast model systems (*S. cerevisiae* and
151 *S. pombe*). This high percentage is not surprising as one of the criteria used to select
152 target genes for the deletion library was the lack of conservation between *C. albicans*
153 and yeast model systems [44]. The remaining 10 hits are genes encoding for
154 proteins with diverse functions, including stress response (*HOG1*) [52],
155 transcriptional and chromatin regulation (*SPT8*, *SET3*) [53–55], transport (*YPT7*,
156 *DUR35*, *NPR2*, *FCY2*) [56–59], protein folding (*HCH1*) [60], MAP kinase pathway
157 (*STT4*) [61] and cell wall biosynthesis (*KRE5*) [62].
158 One of the highest-ranked genes on our screen is *ULP2* (CR_03820C/ *orf19.4353*:
159 EMS score:3, UV score:3) encoding for a SUMO protease (**Fig 1C**). SUMOylation is
160 a dynamic and reversible post-translation modification in which a member of the
161 SUMO family of proteins is conjugated to target proteins at lysine residues by E1
162 activating enzymes, E2 conjugating enzymes and E3 ligases [63–65]. SUMO
163 proteases remove the polypeptide SUMO from target proteins, regulating their
164 function, activity or localisation [66,67].
165 *C. albicans ULP2* is an excellent candidate for a modulator of stress-induced
166 genome plasticity for several reasons: (i) post-translation modifications (PTMs), such
167 as SUMOylation, are rapid and reversible. Consequently, PTMs can modulate
168 genome instability in response to rapid and transient environmental changes [68,69],
169 (ii) protein sumoylation is emerging as a critical stress response mechanism across
170 eukaryotes [66,70–73] (iii) *C. albicans* protein sumoylation levels change in response
171 to environmental stresses encountered in the host [74].
172 Colony-Forming Unit (CFU) assays of UV-treated cells confirm the importance of
173 *ULP2* in DNA damage resistance as UV treatment reduced the number of CFU in a
174 *ulp2 Δ/Δ* strain (~14.5% survival) compared to a wild-type (WT) strain (~33.7%

175 survival:) (**Fig 1D**). Furthermore, the *ulp2* Δ/Δ strain also displayed a reduced growth
176 rate in liquid media containing MMS or Hydroxyurea (HU), a chemotherapeutic agent
177 that challenges genome integrity by stalling replication forks [75] (**Fig 1E** and **1F**).
178 Thus, *ULP2* has a role in the response to a wide range of genotoxic agents.

179 ***ULP2* but not *ULP1* is required for survival under stress**

180 *C. albicans* contains three putative SUMO-deconjugating enzymes: Ulp1, Ulp2 and
181 Ulp3 (**Fig 2A**). Sequence comparison between the three *C. albicans* Ulp proteins
182 and the two *S. cerevisiae* Ulps (Ulp1 and Ulp2) reveals that although the *C. albicans*
183 proteins are poorly conserved, the amino acid residues essential for catalytic activity
184 are conserved. This analysis suggests that all *C. albicans* Ulps are active SUMO
185 proteases (**Fig 2A** and **2B**). Accordingly, recombinantly expressed *C. albicans* Ulp1,
186 Ulp2 and Ulp3 have SUMO-processing activity *in vitro* [76]. Similarly to *S. cerevisiae*
187 *ULP1*, *C. albicans ULP3* is an essential gene and was not investigated further in this
188 study [77].

189 Previous studies suggested that *C. albicans* Ulp2 is an unstable or a very low
190 abundant protein undetectable by Western blot analysis [76]. We reassessed *ULP2*
191 expression by generating strains expressing, at the endogenous locus, an epitope-
192 tagged Ulp2 protein (Ulp2-HA). Western analyses show that Ulp2-HA expression is
193 readily detected in extracts from independent integrant strains. (**Fig 2C**). Thus, a
194 stable Ulp2 protein is expressed in cells grown under standard laboratory growth
195 conditions (YPD, 30 °C). To assess whether *C. albicans ULP1* and *ULP2* gene share
196 a similar function, we engineered homozygous deletion strains for *ULP1* (*ulp1* Δ/Δ)
197 and *ULP2* (*ulp2* Δ/Δ). Growth analysis demonstrated that deletion of *ULP2* reduces
198 fitness as the newly generated *ulp2* Δ/Δ strain is viable, but cells are slow-growing
199 (**Fig 2D** and **2E**). In contrast, the *ulp1* Δ/Δ strain grows similarly to the WT control in
200 solid and liquid media (**Fig 2D** and **2E**). Phenotypic analysis confirms that *ULP2* is
201 an important regulator of *C. albicans* stress response as, similarly to the deletion
202 library mutant, the newly generated *ulp2* Δ/Δ strain is sensitive to different stress
203 conditions including treatment with DNA damaging agents (UV and MMS), DNA
204 replication inhibitor (HU), oxidative stress (H₂O₂) and high temperature (39°C) (**Fig**
205 **2E**) In contrast, deletion of *ULP1* did not cause any sensitivity to the tested stress
206 conditions (**Fig 2E**).

207 In summary, we could not detect any phenotype associated with deletion of *ULP1*,
208 while loss of *ULP2* leads to poor growth in standard laboratory growth conditions and
209 hypersensitivity to multiple stresses.

210 ***Loss of ULP2 leads to increased genome instability***

211 To assess whether the hypersensitivity to DNA damage agents observed in the
212 *ulp2Δ/Δ* strain was indeed due to enhanced genome instability, we deleted the *ULP2*
213 gene from a set of tester strains containing a heterozygous *URA3*⁺ marker gene
214 inserted in three different chromosomes (Chr 1, 3 and 7) [41]. We quantified the
215 frequency of *URA3*⁺ marker loss by plating on plates containing the *URA3* counter-
216 selective drug FOA and scoring the number of colonies able to grow on FOA-
217 containing media compared to non-selective (N/S) media. Deletion of *ULP2* leads to
218 a dramatic increase in LOH rate at all three chromosomes (Chr1: ~5000X, Chr3:
219 ~18X, Chr7: ~170X), indicating that *ULP2* is required for maintaining genome
220 stability across the *C. albicans* genome (**Fig 3A**).

221 In *C. albicans*, hypersensitivity to genotoxic stress often correlates with filamentous
222 growth [45,78–81]. Accordingly, and in agreement with a significant role for *ULP2* in
223 genotoxic stress response, the *ulp2Δ/Δ* strain displays a higher frequency of
224 abnormal morphologies than a WT strain, including filamentous pseudohyphal-like
225 and hyphal-like cells (**Fig 3B**). To assess whether the exacerbated *ulp2Δ/Δ* genome
226 instability is linked to defective chromosome segregation, we deleted the *ULP2* gene
227 in a reporter strain in which *TetO* sequences are integrated adjacent to the
228 centromere (*CEN7*) of one Chromosome 7 homolog and TetR-GFP fusion protein is
229 expressed from an intergenic region [82]. Binding of TetR-GFP to *tetO* sequences
230 allowed visualisation of Chr7 duplication and segregation during the cell cycle. We
231 found that deletion of *ULP2* leads to abnormal Chr7 segregation, including cells with
232 no TetR-GFP signals or multiple TetR-GFP-foci, which was ~5 fold higher in the *ulp2*
233 *Δ/Δ* strain than the WT control strain (**Fig 3C**).

234 Previous studies performed in the model system *S. cerevisiae* demonstrated that
235 loss of *ULP2* leads to the accumulation of a specific multichromosome aneuploidy
236 (amplification of both ChrI and ChrXII) that rescues the potential lethal defects of
237 *ulp2* deletion by amplification of specific genes on both chromosomes [83,84]. To
238 determine whether loss of *C. albicans ULP2* results in a specific aneuploidy, we

239 sequenced the genome of 3 randomly selected *ulp2* Δ/Δ colonies by whole genome
240 sequencing (WGS) and compared their genome sequences to the *C. albicans*
241 reference genome. This analysis demonstrates that deletion of *C. albicans ULP2*
242 does not select for specific chromosome rearrangements and identifies different
243 genomic variations that are not present in the parental WT strain (**Fig 3D** and **Table**
244 **S2**) [85]. While deletion of *ULP2* leads to very few (<10) *de novo* mutations (**Table**
245 **S2**), two of the three colonies underwent extensive LOH on different chromosomes
246 (**Fig 3D** and **Table S2**). For example, chromosome missegregation followed by
247 reduplication of the remaining homologue is detected on isolate C1 (C1: ChrR) and
248 the genome of C2 contains a long-track LOH (C2:Chr 3L) that occurred within 4.6 kb
249 of a repeat locus on Chr3L (*PGA18*, [32]) (**Fig 3D**). Our analysis collectively
250 demonstrates that deletion of *C. albicans ULP2* leads to increased genome instability
251 via the formation of extensive chromosomal variation.

252 ***Loss of ULP2 leads to drug resistance via selection of novel genotypes***

253 We hypothesised that the increased genome instability of the *ulp2* Δ/Δ strain would
254 facilitate adaptation to hostile environments via selection of fitter genotypes. To test
255 this hypothesis, we assessed whether WT and *ulp2* Δ/Δ strains differ in their ability to
256 overcome the stress imposed by low or high concentrations of 2 drugs: Fluconazole
257 (FLC) and caffeine (CAF). FLC was chosen because it is the most used antifungal
258 drug in the clinic. CAF was chosen because it is associated with well-known
259 resistance mechanisms [86,87]. Serial dilution analyses demonstrate that the
260 *ulp2* Δ/Δ strain is not sensitive to a low FLC (15 μ g/ml) dose while it is sensitive a low
261 CAFF (5mM) doses (**Fig 4A** and **4B**).

262 In contrast, deletion of *ULP2* increases adaptation to high doses FLC and CAF. On
263 plates containing an inhibitory concentration of FLC (128 μ g/ml), a WT strain
264 produced only tiny abortive colonies while the *ulp2* Δ/Δ strain produces colonies of
265 heterogenous size (Large and Small, **Fig 4C**). The starting *ulp2* Δ/Δ strain is highly
266 sensitive to 12 mM CAF (**Fig S1A**), and therefore a reduced number of *ulp2* Δ/Δ
267 colonies grew at this high drug concentration compared to the WT strain (**Fig 4D**).
268 Despite this difference, the *ulp2* Δ/Δ strain, but not the WT strain, produces large
269 colonies that can grow on high CAF concentration following passaging in the

270 absence of the drug, indicative of adaptation (**Fig 4D, Fig S1B**). Thus, deletion of
271 *ULP2* accelerates adaptation to lethal drug concentration.

272 To test whether enhanced drug adaption was linked with selection of novel
273 genotypes, we sequenced the genome of 4 independent *ulp2 Δ/Δ* FLC-adapted
274 isolates (*FLC-1, FLC-2, FLC-3* and *FLC-4*). *FLC-1, FLC-2* and *FLC-3* were randomly
275 selected from the High FLC plates and sequenced immediately. In contrast, *FLC-4*
276 was selected because this isolate was still able to grow on high FLC following
277 passaging in non-selective (N/S) media (**Fig S1C**). To assess for genotype
278 heterogeneity, three *FLC-4* derived single colonies (*FLC-4a, b* and *c*) were
279 sequenced (**Fig S2A and B**). The WGS analysis demonstrates that all FLC-adapted
280 colonies have a genotype that is distinct from the *ulp2 Δ/Δ* progenitor. We detected
281 very few (<10) *de novo* point mutations, and none of these are common among all
282 the sequenced FLC isolates (**Table S3**). In contrast, all colonies are marked by an
283 extensive segmental chromosome aneuploidy: a partial deletion (~ 388 Kb) of the
284 right arm of Chromosome R (ChrRR-Deletion). ChrRR-deletion occurs at the
285 ribosomal DNA (25S subunit) and it extends to the right telomere of ChrR
286 (ChrR:1,897,750 bp - 2,286,380 bp), reducing the dosage of 204 genes (**Fig 4E,**
287 **S2A and Table S4**). GO analysis revealed that ChrRR-Deletion leads to a reduced
288 dosage of 34/204 genes associated with the "response to stress" pathways and
289 18/204 genes linked to "response to drug" pathways (**Table S4**). We posit that this
290 reduced gene dosage enables growth in the presence of high FLC. For example,
291 *CKA1*, a gene whose deletion leads to FLC resistance [88], is located within the
292 ChrRR-deletion (**Fig 4G**).

293 Interestingly, we found that all three *FLC-4* sequences colonies (*FLC-4a, b* and *c*),
294 are marked by a second segmental aneuploidy: a partial Chr1 amplification (Chr1-
295 Duplication) (**Fig 4E and S2A**). This novel Chr1-Duplication amplifies a genomic
296 fragment of ~1.3 Mbp containing 535 protein-coding genes (**Table S4**). The Chr1-
297 Duplication starts and ends near two distinct DNA repeat sequences with high
298 sequence identity elsewhere in the genome: the 5' breakpoint is within the *TLO34*
299 and its 3' breakpoint is within 3 kb of a *Zeta-1a* Long Terminal Repeat (LTR) (**Fig 4G**
300 and **S3**) [32,33,89]. These WGS data led us to hypothesise that a chromosome-
301 chromosome fusion event occurred between the Chr1-Duplication and Chr6 within
302 homologous *TLO* sequences (**Fig 4G**). Indeed, the *TLO34* gene on Chr1 has high

303 sequence identity with a 380 bp region located at Chr6 (position: 6182-6562 bp). In
304 addition, sequence polymorphisms unique to Chr1-*TLO34* mapped to Chr6 in the
305 *FLC-4* isolate (but not in *FLC-1*, *FLC-2* and *FLC-3*), supporting a novel
306 interchromosomal recombination product between *TLO*-homologous sequences.
307 This model is supported by CHEF gel electrophoresis analyses as, when compared
308 to the *ulp2* Δ/Δ progenitor, the *FLC-4* genome lacks one band corresponding to the
309 shorter Chr6 homologue (blue asterisk), and it contains a new chromosome band of
310 ~2.2 Mb (magenta asterisk) (**Fig 4F**).

311 We posit that Chr1-Duplication provides a synergistic fitness advantage in response
312 to two independent stressors (the presence of FLC and lack of *ULP2*) by
313 simultaneously changing the dosage of several genes. Indeed, GO analyses
314 demonstrated that 41 genes present in the Chr1-Duplication are associated with a
315 "drug resistance" phenotypes (**Table S4**). Among these, amplification of *UPC2*
316 encoding for the Upc2 transcription factor is likely to be critical. Indeed, it is well
317 established that *UPC2* overexpression leads to FLC resistance by *ERG11*
318 upregulation [90,91]. Chr1-Duplication likely rescues the fitness defects of the *ulp2*
319 Δ/Δ strain by amplifying two key genes: *CCR4* and *NOT5* (**Fig 4G**). Ccr4 and Not5
320 are subunits of the evolutionarily conserved Ccr4-Not complex that modulate gene
321 expression at multiple levels, including transcription initiation, elongation, de-
322 adenylation and mRNA degradation [92]. It has been shown that *S. cerevisiae* *CCR4*
323 and *NOT5* overexpression rescue the lethal defects associated with a *ulp2* deletion
324 strain [83].

325 Collectively our data suggest that the combined selective pressure of two
326 independent stresses leads to selection of a chromosome aneuploidy that
327 overcomes both stresses by overexpressing two different sets of genes.

328 **Discussion**

329 In this study, we demonstrate that the SUMO protease Ulp2 is a critical regulator of
330 *C. albicans* genome plasticity and that the development of drug resistance is
331 accelerated in cells lacking *ULP2*. We unveil a striking flexibility of *C. albicans* cells
332 in their response to complex stresses caused by drug treatment and dysregulation of
333 the SUMO system, leading to the selection of extensive chromosome
334 rearrangements.

335 ***Ulp2 is a critical regulator of C. albicans genome stability***

336 Our study identifies protein SUMOylation as a critical regulatory mechanism of *C.*
337 *albicans* genome stability. SUMOylation is a dynamic and reversible post-translation
338 modification in which a member of the SUMO family of proteins is conjugated to
339 target proteins at lysine residues by E1 activating enzymes, E2 conjugating enzymes
340 and E3 ligases [63–65]. SUMO is removed from its target proteins by SUMO-specific
341 Ulp2 proteases [67]. Several observations are in agreement with our findings and
342 suggest that SUMOylation controls stress-induced genome plasticity. Firstly,
343 SUMOylation is a post-translational modification that is rapid and reversible, an
344 essential requirement for a regulator of stress-induced genome plasticity. Secondly,
345 *C. albicans* protein SUMOylation levels are different in normal and stress growth
346 conditions [74]. Thirdly, deletion of genes encoding other components of the *C.*
347 *albicans* SUMOylation machinery lead to filamentation, a phenotype often associated
348 with defective cell division and compromised genome integrity [74,93,94]. Finally, *C.*
349 *albicans* strains lacking the SUMO (Smt3) protein or the E3 ligase Mms21 display
350 nuclear segregation defects [74,93].

351 *C. albicans* Ulp2 likely controls genome plasticity by modulating SUMO levels of
352 several target proteins. SUMO proteases have a broad substrate specificity
353 catalysing SUMO deconjugation of several substrates [95]. In other organisms, it is
354 well known that SUMOylation modulates pathways ensuring genome integrity,
355 including the DNA damage-sensing and repair pathway and the cell division and
356 chromosome segregation pathway [63–66,96–98]. Despite the broad substrate
357 specificity, our data suggest that one significant function of *C. albicans* *ULP2* is to
358 ensure faithful chromosome segregation as high rates of chromosome
359 missegregation is detected in the *ulp2* Δ/Δ strain. Furthermore, the Illumina Genome
360 sequencing analyses demonstrated that lack of *ULP2* is associated with extensive
361 LOH events. Such extensive genomic changes are reminiscent of catastrophic
362 mitotic events associated with defective chromosome segregation [99,100]. The
363 targets of *C. albicans* Ulp2 are unknown, and it will be important to adopt proteomic
364 approaches to identify the entire repertoire of SUMO targets and determine how
365 *ULP2* contributes to *C. albicans* genome plasticity.

366 ***Complex chromosome rearrangements drive adaptation to multiple stress***
367 ***environments.***

368 Our data demonstrate that the *ulp2* Δ/Δ strain is more likely than the WT parental
369 strain to develop resistance to anti-fungal drugs by selecting specific segmental
370 aneuploidies on ChrR (ChrRR-deletion) and Chr1 (Chr1-duplication). These adaptive
371 genotypes confer a growth advantage in response to two independent stressors: the
372 absence of *ULP2* and drug treatment.

373 In agreement with the notion that repetitive elements play a significant role in
374 genome instability, we identified intrachromosomal repetitive elements as drivers of
375 genome instability. Indeed, all the sequenced *FLC*-adapted isolates carry a partial
376 deletion of ChrR originating within the rDNA locus. We have previously
377 demonstrated that the *C. albicans* rDNA locus is a hotspot for mitotic recombination
378 [36], and clinical isolates are often marked by chromosomal aberrations originating
379 from this locus [34]. This rDNA-driven chromosomal aberration leads to the deletion
380 of one copy of 204 genes. We hypothesise that this reduced gene dosage drives
381 *FLC* adaptation. For example, *CKA1*, one of the genes affected by ChrRR deletion,
382 encodes for one of the two *C. albicans* Casein Kinases (Cka1 and Cka2). Deletion of
383 these genes causes *FLC* resistance by controlling the expression of the efflux pump
384 *CDR1* and *CDR2* [88].

385 WGS analysis demonstrated that the *FLC-4* isolate, whose *FLC* resistance is
386 maintained followed by passaging on non-selective media, carries a second
387 segmental aneuploidy: a partial duplication of Chr1 with breakpoints at repetitive
388 elements. We provide evidence suggesting that Chr1 Duplication results from a
389 fusion event between Chr1 and Chr6 due to a novel interchromosomal
390 recombination product between *TLO* homologous sequences. We hypothesise that
391 Chr1-duplication leads to gene dosage changes that are critical for overcoming two
392 independent stresses: the presence of *FLC* and the absence of *ULP2*. Indeed, one
393 of the master regulators of *FLC* resistance, *UPC2*, is located on the Chr1-duplication
394 and its overexpression is likely to allow growth in the presence of *FLC*. *UPC2*
395 encodes a key transcription factor of *ERG11*, the target of *FLC* [91]. It is well
396 established that *UPC2* deletion leads to increased *FLC* susceptibility and that *UPC2*

397 overexpression causes FLC resistance [91,101]. Accordingly, *UPC2* gain-of-function
398 mutations are prevalent among FLC resistant clinical isolates [101].
399 The Chr1-duplication carries two key genes, *CCR4* and *NOT5*, likely to rescue the
400 fitness defects associated with the *ulp2* Δ/Δ strain. Indeed, it has been shown that
401 *CCR4* and *NOT5* overexpression rescues the fitness defects of a *ULP2* deletion
402 strain in *S. cerevisiae* [83]. Crr4 and Not5 are components of the evolutionarily
403 conserved Crr4-Not multiprotein complex that regulate gene expression at all steps
404 from transcription to translation and mRNA decay [102]. It is unknown why
405 overexpression of the Crr4-Not complex rescues the fitness defect of an *ulp2*
406 deletion strain, but it has been suggested that it might be linked to the transcriptional
407 regulation of snoRNA and rRNA genes [84]. Here, for the first time, we demonstrate
408 that segmental aneuploidy can lead to adaptation to different stressors by
409 overexpressing genes located in the same chromosome and independently rescue
410 the two stressors, leading to an overall fitness advantage.

411 **Material and Methods**

412 ***Yeast strains and Growth Conditions***

413 Strains used in this study are listed in **Table S5**. Routine culturing was performed at
414 30 °C in Yeast Extract-Peptone-D-Glucose (YPD) liquid and solid media containing
415 1% yeast extract, 2% peptone, 2% dextrose, 0.1 mg/ml adenine and 0.08 mg/ml
416 uridine, Synthetic Complete (SC-Formedium) or Casitone (5 g/L Yeast extract, 9 g/L
417 BactoTryptone, 20 g/L Glucose, 11.5 g/L Sodium Citrate dehydrate, 15 g/L Agar)
418 media. When indicated, media were supplemented with 1mg/ml 5-Fluorotic acid (5-
419 FOA, Melford), 200 µg/ml Nourseothricin (clonNAT, Melford), 5mM and 12 mM
420 Caffeine (Sigma #C0750), 15 mg/ml and 128 mg/ml Fluconazole (Sigma #F8929),
421 6m H₂O₂ (Sigma #H1009), 12 mM and 22 mM Hydroxyurea (Sigma #H8627),
422 0.005% MMS (Sigma #129925).

423 ***Genetic Screening***

424 The genetic screening was performed using a *C. albicans* homozygous deletion
425 library [44] arrayed in 96 colony format on YPD plates (145x20 mm) using a replica
426 plater (Sigma #R2508). Control N/S plates were grown at 30 °C for 48 hours. UV
427 treatment was performed using UVitec (Cambridge) with power density of
428 7.5µW/cm² (0.030 J for 4 seconds). Following UV treatment, plates were incubated
429 in the dark at 30°C for 48 hours. For MMS treatment, the library was spotted on YPD

430 plates (145x20mm) containing 0.05% MMS and incubated at 30°C for 48 hours. UV
431 and/or MMS sensitivity of selected strains was confirmed by serial dilution assays in
432 control (YPD) and stress (UV: power density of 7.5 μ W/cm², MMS: 0.05%) plates.
433 Correct gene deletions were confirmed by PCR using gene-specific primers (**Table**
434 **S6**).

435 ***Yeast strain construction***

436 Integration and deletion of genes were performed using long oligos-mediated PCR
437 for gene deletion and tagging [103]. Oligonucleotides and plasmids used for strain
438 constructions are listed in Supplementary **Table S6** and **S7**, respectively. For Lithium
439 Acetate transformation, overnight liquid yeast cultures were diluted in fresh YPD and
440 grown to OD₆₀₀ of 1.3. Cells were harvested by centrifugation and washed once with
441 dH₂O and once with SORB solution (100mM Lithium acetate, 10mM Tris-HCL pH
442 7.5, 1mM EDTA pH 7.5/8, 1M sorbitol; pH 8). The pellet was resuspended in SORB
443 solution containing single-stranded carrier DNA (Sigma-Aldrich) and stored -80 °C in
444 50 μ l aliquots. Frozen competent cells were defrosted on ice, mixed with 5 μ L of
445 PCR product and 300 μ L PEG solution (100mM Lithium acetate, 10mM Tris-HCL pH
446 7.5, 1mM EDTA pH 8, 40% PEG4000) and incubated for 21-24 hours at 30 °C. Cells
447 were heat-shocked at 44°C for 15 minutes and grown in 5mL YPD liquid for 6 hours
448 before plating on selective media at 30 °C.

449 ***UV survival quantification***

450 Following dilution of overnight liquid cultures, 500 cells were plated in YPD control
451 plates while 1500 cells were plated in YPD stress plates and UV irradiates with
452 power density of 7.5 μ W/cm² (0.030 J for 4 seconds). Plates were kept in the dark
453 and incubated at 30°C for 48 hours. Colonies were counted using a colony counter
454 (Stuart Scientific). Experiments were performed in 5 biological replicates, and violin
455 plots graphs were generated using R Studio (<http://www.r-project.org/>).

456 ***Growth curve***

457 Overnight liquid cultures were diluted to 60 cells/ μ L in 100 μ L YPD and incubated at
458 30 °C in a 96 well plate (Cellstar®, #655180) with double orbital agitation of 400 rpm
459 using a BMG Labtech SPECTROstar nanoplate reader for 48 hours. When indicated,
460 YPD media was supplemented with MMS (0.05%) and HU (22 mM). Graphs show
461 the average of 3 biological replicates and error bars show the standard deviation.

462 ***Serial dilution assay***

463 Overnight liquid cultures were diluted to an OD₆₀₀ of 4, serially diluted 1:5 and
464 spotted into agar plates with and without indicated additives using a replica plater
465 (Replica plater for 96-well plates, Sigma Aldrich, #R2383). Images of the plates were
466 then taken using Syngene GBox Chemi XX6 Gel imaging system. Experiments were
467 performed in 3 biological replicates

468 **Protein extraction and Western blotting**

469 Yeast extracts were prepared as described [104] using 1×10^8 cells from overnight
470 cultures grown to a final OD₆₀₀ of 1.5–2. Protein extraction was performed in the
471 presence of 2% SDS (Sigma) and 4 M acetic acid (Fisher) at 90°C. Proteins were
472 separated in 2% SDS (Sigma), 40% acrylamide/bis (Biorad, 161-0148) gels and
473 transfer into PVDF membrane (Biorad) by semi-dry transfer (Biorad, Trans Blot SD,
474 semi-dry transfer cell). Western-blot antibody detection was used using antibodies
475 from Roche Diagnostics Mannheim Germany (Anti-HA, mouse monoclonal primary
476 antibody (12CA5 Roche, 5 mg/ml) at a dilution of 1:1000, and anti-mouse IgG-
477 peroxidase (A4416 Sigma, 0.63 mg/ml) at a dilution of 1:5000, and Clarity™ ECL
478 substrate (Bio-Rad).

479 ***URA3⁺ marker loss quantification***

480 Strains were first streaked on –Uri media to ensure the selection of cells carrying
481 the *URA3⁺* marker gene. Parallel liquid cultures, grown for 16 hours at 30°C in YPD,
482 were plated on synthetic complete (SC) plates containing 1 □mg/ml 5-FOA (5-
483 fluorotic acid; Sigma) and on non-selective SC plates/. Colonies were counted after
484 2 □days of growth at 30°C, the frequency of the *URA3⁺* marker loss was calculated
485 using the formula $F = m/M$, where m represents the median number of colonies
486 obtained on 5-FOA medium corrected by the dilution factor used and the fraction of
487 culture plated and M the average number of colonies obtained on YPD corrected by
488 the dilution factor used and the fraction of culture plated [80]. Statistical differences
489 between results from samples were calculated using the Kruskal-Wallis test and the
490 Mann-Whitney U test for *post hoc* analysis. Statistical analysis was performed and
491 violin plots were generated using R Studio (<http://www.r-project.org/>).

492 ***Microscopy***

493 30 ml of yeast cultures (OD₆₀₀=1) grown in SC were centrifuged at 2000 rpm for 5
494 minute and washed once with dH₂O. Cells were fixed in 10ml of 3.7%
495 paraformaldehyde (Sigma #F8775) for 15 minutes, washed twice with 10ml of

496 KPO₄/Sorbitol (100 mM KPO₄, 1.2 M Sorbitol) and resuspended in 250 µl PBS
497 containing 10 µg of Dapi. Cells were then sonicated and resuspended in a 1% low
498 melting point agarose (Sigma Aldrich) before mounting under a 22mm coverslip of
499 0,17µm thickness. Samples were imaged on a Zeiss LSM 880 Airyscan with a
500 63x/1.4NA oil objective. Airyscan images were taken with a relative pinhole diameter
501 of 0.2 AU (airy unit) for maximal resolution and reduced noise. GFP was imaged with
502 a 488nm Argon laser and 495-550 nm bandpass excitation filter, RFP with a 546nm
503 solid-state diode laser and a 570nm long pass excitation filter. The Dapi channel was
504 imaged on a PMT with standard pinhole of 1AU and brightfield image were captured
505 on the trans-PMT with the same excitation laser of 405nm., Dapi and brightfield
506 images were taken with the same pixel size and bit depth (16bit) as the airyscan
507 images. Images were of a 42.7x42.7µm field of view and with a 33 nm pixel size
508 resolution. z-stacks were taken containing cells of z interval of 500nm. Airyscan
509 Veena filtering was performed with the inbuilt algorithms of Zeiss Zen Black 2.3. Fiji
510 scripts were written to automatically create a maximum intensity projection with
511 standardised intensity scaling for the fluorescence images and overlay them with the
512 best focus image of the brightfield picture. Experiments were performed in 3
513 biological replicates and >100 cells/replicate were counted.

514 ***Drug Selection***

515 Strains were incubated overnight in casitone liquid media at 30°C with shaking. 10⁴
516 cells were plated in small (10cm) casitone plates or plates containing: (i) 128 µg/mL
517 DMSO (Fluconazole Control), (ii) 128 µg/mL Fluconazole or (iii) 12 mM Caffeine.
518 Plates were incubated at 30°C for 7 days. Colonies able to grow on Fluconazole- or
519 Caffeine-containing plates were streaked in non-selective plates and tested by
520 spotting assay in casitone+ DMSO plates, casitone+Fluconazole or
521 casitone+Caffeine plates. Following incubation at 30°C, plates were imaged using
522 Syngene GBox Chemi XX6 Gel imaging system. Experiments were performed in 3
523 biological replicates.

524 ***Whole-genome sequence analysis***

525 All genome sequencing data have been deposited in the Sequence Read Archive
526 under BioProject PRJNA781758, Genomic DNA was isolated using a phenol-
527 chloroform extraction as previously described [29]. Paired-end (2 x 151 bp)
528 sequencing was carried out by the Microbial Genome Sequencing Center (MiGS) on

529 the Illumina NextSeq 2000 platform. Adaptor sequences and low-quality reads were
530 removed using Trimmomatic (v0.33 LEADING:3 Trailing:3 SLIDINGWINDOW:4:15
531 MINLEN:36 TOPHRED33) [105]. Trimmed reads were mapped to the *C. albicans*
532 reference genome (A21-s02-m09-r08) from the *Candida* Genome Database
533 (http://www.candidagenome.org/download/sequence/C_albicans_SC5314/Assembly_21/archive/C_albicans_SC5314_version_A21-s02-m09-r08_chromosomes.fasta.gz).
534 Reads were aligned to the reference using BWA-MEM (v0.7.17) with default
535 parameters [106]. The BAM files, containing aligned reads, were sorted and PCR
536 duplicates removed using Samtools (v1.10 samtools sort, samtools rmdup) [107].
537 Qualimap (v2.2.1) analysed the BAM files for mean coverage of the reference
538 genome; coverages ranged from 73.7x to 89.3x coverage [108]. Variant detection
539 was conducted using the Genome Analysis Toolkit (Mutect, v2.2-25) [109]. Variants
540 were annotated using SnpEff (V4.3) [110] using the SC5314 reference genome fasta
541 and gene feature file above. Parental variants were removed, and all remaining
542 variants were verified visually using the Integrative Genomic Viewer (IGV, v2.8.2)
543 [111].

545 **Read depth and breakpoint analysis**

546 Whole-genome sequencing data were analysed for copy number and allele ratio
547 changes as previously described [32,33]. Aneuploidies were visualised using the
548 Yeast Mapping Analysis Pipeline (YMAP, v1.0) [112]. BAM files aligned to the
549 SC5314 reference genome as described above were uploaded to YMAP and read
550 depth was determined and plotted as a function of chromosome position. Read
551 depth was corrected for both chromosome-end bias and GC-content. The GBrowse
552 CNV track and GBrowse allele ratio track identified regions of interest for CNV and
553 LOH breakpoints, and more precise breakpoints were determined visually using IGV.
554 LOH breakpoints are reported as the first informative homozygous position in a
555 region that is heterozygous in the parental genome. CNV breakpoints were identified
556 as described previously [32,33].

557 **Contour-clamped homogeneous electric field (CHEF) electrophoresis**

558 Intact yeast chromosomal DNA was prepared as previously described [113].
559 Briefly, cells were grown overnight, and a volume equivalent to an OD₆₀₀ of 7 was
560 washed in 50 mM EDTA and resuspended in 20 µl of 10 mg/ml Zymolyase 100T
561 (Amsbio #120493-1) and 300 µl of 1% Low Melt agarose (Biorad® # 1613112) in
562 100 mM EDTA. Chromosomes were separated on a 1% Megabase agarose gel (Bio-

563 Rad) in 0.5X TBE using a CHEF DRII apparatus. Run conditions as follows: 60-120s
564 switch at 6 V/cm for 12 hours followed by a 120-300s switch at 4.5 V/cm for 12
565 hours, 14 °C. The gel was stained in 0.5x TBE with ethidium bromide (0.5 µg/ml) for
566 30 minutes and destained in water for 30 minutes. Chromosomes were visualised
567 using a Syngene GBox Chemi XX6 gel imaging system.

568

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577 **References**

- 578 1. Sui Y, Qi L, Wu JK, Wen XP, Tang XX, Ma ZJ, et al. Genome-wide mapping of
579 spontaneous genetic alterations in diploid yeast cells. *Proc Natl Acad Sci U S*
580 *A*. 2020;117: 28191–28200. doi:10.1073/pnas.2018633117
- 581 2. Bennett RJ, Forche A, Berman J. Rapid Mechanisms for Generating Genome
582 Diversity: Whole Ploidy Shifts, Aneuploidy, and Loss of Heterozygosity. [cited
583 11 Aug 2021]. Available: <http://perspectivesinmedicine.cshlp.org/>
- 584 3. Pavelka N, Rancati G, Zhu J, Bradford WD, Saraf A, Florens L, et al.
585 Aneuploidy confers quantitative proteome changes and phenotypic variation in
586 budding yeast. *Nature*. 2010;468: 321–325. doi:10.1038/nature09529
- 587 4. Siegel JJ, Amon A, Koch DH. New Insights into the Troubles of Aneuploidy.
588 *Annu Rev Cell Dev Biol*. 2012;28: 189–214. doi:10.1146/annurev-cellbio-
589 101011-155807
- 590 5. Rancati G, Pavelka N, Fleharty B, Noll A, Trimble R, Walton K, et al.
591 Aneuploidy underlies rapid adaptive evolution of yeast cells deprived of a
592 conserved cytokinesis motor. *Cell*. 2008;135: 879–93.
593 doi:10.1016/j.cell.2008.09.039

- 594 6. Rancati G, Pavelka N. Karyotypic changes as drivers and catalyzers of cellular
595 evolvability: A perspective from non-pathogenic yeasts. *Semin Cell Dev Biol.*
596 2013;24: 332–338. doi:10.1016/j.semcdb.2013.01.009
- 597 7. Selmecki A, Forche A, Berman J. Genomic plasticity of the human fungal
598 pathogen *Candida albicans*. *Eukaryot Cell.* 2010;9: 991–1008.
599 doi:10.1128/EC.00060-10
- 600 8. Teoh F, Pavelka N. pathogens How Chemotherapy Increases the Risk of
601 Systemic Candidiasis in Cancer Patients: Current Paradigm and Future
602 Directions. doi:10.3390/pathogens5010006
- 603 9. Pfaller MA, Diekema DJ. Epidemiology of invasive mycoses in North America.
604 *Crit Rev Microbiol.* 2010;36: 1–53. doi:10.3109/10408410903241444
- 605 10. Berman J, Krysan DJ. Drug resistance and tolerance in fungi. *Nat Rev*
606 *Microbiol.* 2020;18: 319–331. doi:10.1038/s41579-019-0322-2
- 607 11. Fisher MC, Hawkins NJ, Sanglard D, Gurr SJ. Worldwide emergence of
608 resistance to antifungal drugs challenges human health and food security.
609 *Science (80-).* 2018;360: 739–742. doi:10.1126/science.aap7999
- 610 12. Berkow EL, Lockhart SR. Fluconazole resistance in *Candida* species: a current
611 perspective. *Infect Drug Resist.* 2017;10: 237. doi:10.2147/IDR.S118892
- 612 13. Heimark L, Shipkova P, Greene J, Munayyer H, Yarosh-Tomaine T,
613 DiDomenico B, et al. Mechanism of azole antifungal activity as determined by
614 liquid chromatographic/mass spectrometric monitoring of ergosterol
615 biosynthesis. *J Mass Spectrom.* 2002;37: 265–269. doi:10.1002/jms.280
- 616 14. Charlier C, Hart E, Lefort A, Ribaud P, Dromer F, Denning DW, et al.
617 Fluconazole for the management of invasive candidiasis: Where do we stand
618 after 15 years? *J Antimicrob Chemother.* 2006;57: 384–410.
619 doi:10.1093/JAC/DKI473
- 620 15. Heilmann CJ, Schneider S, Barker KS, Rogers PD, Morschhäuser J. An
621 A643T mutation in the transcription factor *Upc2p* causes constitutive *ERG11*
622 upregulation and increased fluconazole resistance in *Candida albicans*.
623 *Antimicrob Agents Chemother.* 2010;54: 353–359. doi:10.1128/AAC.01102-09

- 624 16. Whaley SG, Caudle KE, Vermitsky JP, Chadwick SG, Toner G, Barker KS, et
625 al. UPC2A is required for high-level azole antifungal resistance in *Candida*
626 *glabrata*. *Antimicrob Agents Chemother.* 2014;58: 4543–4554.
627 doi:10.1128/AAC.02217-13
- 628 17. Dunkel N, Liu TT, Barker KS, Homayouni R, Morschhäuser J, Rogers PD. A
629 gain-of-function mutation in the transcription factor Upc2p causes upregulation
630 of ergosterol biosynthesis genes and increased fluconazole resistance in a
631 clinical *Candida albicans* isolate. *Eukaryot Cell.* 2008;7: 1180–1190.
632 doi:10.1128/EC.00103-08
- 633 18. Hoot SJ, Smith AR, Brown RP, White TC. An A643V amino acid substitution in
634 Upc2p contributes to azole resistance in well-characterized clinical isolates of
635 *Candida albicans*. *Antimicrob Agents Chemother.* 2011;55: 940–942.
636 doi:10.1128/AAC.00995-10
- 637 19. Morschhäuser J. The development of fluconazole resistance in *Candida*
638 *albicans* – an example of microevolution of a fungal pathogen. *J Microbiol.*
639 2016;54: 192–201. doi:10.1007/s12275-016-5628-4
- 640 20. Jones T, Federspiel NA, Chibana H, Dungan J, Kalman S, Magee BB, et al.
641 The diploid genome sequence of *Candida albicans*. *Proc Natl Acad Sci U S A.*
642 2004;101: 7329–34. doi:10.1073/pnas.0401648101
- 643 21. van het Hoog M, Rast TJ, Martchenko M, Grindle S, Dignard D, Hogues H, et
644 al. Assembly of the *Candida albicans* genome into sixteen supercontigs
645 aligned on the eight chromosomes. *Genome Biol.* 2007;8: R52.
646 doi:10.1186/gb-2007-8-4-r52
- 647 22. Hirakawa MP, Martinez D a, Sakthikumar S, Anderson MZ, Berlin A, Gujja S,
648 et al. Genetic and phenotypic intra-species variation in *Candida albicans*.
649 2015; 1–13. doi:10.1101/gr.174623.114.
- 650 23. Tso GHW, Reales-Calderon JA, Tan ASM, Sem X, Le GTT, Tan TG, et al.
651 Experimental evolution of a fungal pathogen into a gut symbiont. *Science (80-*
652 *).* 2018;362: 589–595. doi:10.1126/SCIENCE.AAT0537
- 653 24. Forche A, Cromie G, Gerstein AC, Solis N V., Pisithkul T, Srifa W, et al. Rapid

- 654 Phenotypic and Genotypic Diversification After Exposure to the Oral Host
655 Niche in *Candida albicans*. *Genetics*. 2018;209: genetics.301019.2018.
656 doi:10.1534/genetics.118.301019
- 657 25. Forche A, Magee PT, Selmecki A, Berman J, May G. Evolution in *Candida*
658 *albicans* Populations During a Single Passage Through a Mouse Host.
659 *Genetics*. 2009;182: 799–811. Available:
660 <http://www.genetics.org/content/182/3/799.abstract>
- 661 26. Ene I V., Farrer RA, Hirakawa MP, Agwamba K, Cuomo CA, Bennett RJ.
662 Global analysis of mutations driving microevolution of a heterozygous diploid
663 fungal pathogen. *Proc Natl Acad Sci*. 2018;115: 201806002.
664 doi:10.1073/pnas.1806002115
- 665 27. Ropars J, Maufrais C, Diogo D, Marcet-houben M, Perin A, Sertour N, et al.
666 Gene flow contributes to diversification of the major fungal pathogen
667 *Candida albicans*. 2018. doi:10.1038/s41467-018-04787-4
- 668 28. Forche A, Solis N V., Swidergall M, Thomas R, Guyer A, Beach A, et al.
669 Selection of *Candida albicans* trisomy during oropharyngeal infection results in
670 a commensal-like phenotype. *PLoS Genet*. 2019;15.
671 doi:10.1371/journal.pgen.1008137
- 672 29. Selmecki A, Forche A, Berman J. Aneuploidy and isochromosome formation in
673 drug-resistant *Candida albicans*. *Science*. 2006;313: 367–70.
674 doi:10.1126/science.1128242
- 675 30. Selmecki A, Gerami-Nejad M, Paulson C, Forche A, Berman J. An
676 isochromosome confers drug resistance in vivo by amplification of two genes,
677 *ERG11* and *TAC1*. *Mol Microbiol*. 2008;68: 624–641. doi:10.1111/J.1365-
678 2958.2008.06176.X
- 679 31. Dunn MJ, Anderson MZ. To repeat or not to repeat: Repetitive sequences
680 regulate genome stability in *Candida albicans*. *Genes (Basel)*. 2019;10.
681 doi:10.3390/genes10110866
- 682 32. Todd RT, Wikoff TD, Forche A, Selmecki A. Genome plasticity in *Candida*
683 *albicans* is driven by long repeat sequences. *Elife*. 2019;8.

- 684 doi:10.7554/eLife.45954
- 685 33. Todd RT, Selmecki A. Expandable and reversible copy number amplification
686 drives rapid adaptation to antifungal drugs. *Elife*. 2020;9: 1–33.
687 doi:10.7554/eLife.58349
- 688 34. Hirakawa MP, Martinez DA, Sakthikumar S, Anderson MZ, Berlin A, Gujja S, et
689 al. Genetic and phenotypic intra-species variation in *Candida albicans*.
690 *Genome Res*. 2015;25: 413–25. doi:10.1101/gr.174623.114
- 691 35. Ene I V., Farrer RA, Hirakawa MP, Agwamba K, Cuomo CA, Bennett RJ.
692 Global analysis of mutations driving microevolution of a heterozygous diploid
693 fungal pathogen. *Proc Natl Acad Sci U S A*. 2018;115: E8688–E8697.
694 doi:10.1073/pnas.1806002115
- 695 36. Freire-Benítez V, Gourlay S, Berman J, Buscaino A. Sir2 regulates stability of
696 repetitive domains differentially in the human fungal pathogen *Candida*
697 *albicans*. *Nucleic Acids Res*. 2016;44. doi:10.1093/nar/gkw594
- 698 37. Buscaino. Chromatin-Mediated Regulation of Genome Plasticity in Human
699 Fungal Pathogens. *Genes (Basel)*. 2019;10: 855. doi:10.3390/genes10110855
- 700 38. Anderson MZ, Baller J a, Dulmage K, Wigen L, Berman J. The three clades of
701 the telomere-associated TLO gene family of *Candida albicans* have different
702 splicing, localization and expression features. *Eukaryot Cell*. 2012;11: 612–
703 625. doi:10.1128/EC.00230-12
- 704 39. Haran J, Boyle H, Hokamp K, Yeomans T, Liu Z, Church M, et al. Telomeric
705 ORFs (TLOs) in *Candida* spp. Encode Mediator Subunits That Regulate
706 Distinct Virulence Traits. *PLoS Genet*. 2014;10: e1004658.
707 doi:10.1371/journal.pgen.1004658
- 708 40. Zhang A, Petrov KO, Hyun ER, Liu Z, Gerber SA, Myers LC. The Tlo proteins
709 are stoichiometric components of *Candida albicans* mediator anchored via the
710 Med3 subunit. *Eukaryot Cell*. 2012;11: 874–84. doi:10.1128/EC.00095-12
- 711 41. Forche A, Abbey D, Pisithkul T, Weinzierl MA, Ringstrom T, Bruck D, et al.
712 Stress alters rates and types of loss of heterozygosity in *Candida albicans*.
713 *MBio*. 2011;2. doi:10.1128/mBio.00129-11

- 714 42. Harrison BD, Hashemi J, Bibi M, Pulver R, Bavli D, Nahmias Y, et al. A
715 tetraploid intermediate precedes aneuploid formation in yeasts exposed to
716 fluconazole. *PLoS Biol.* 2014;12: e1001815. doi:10.1371/journal.pbio.1001815
- 717 43. Forche A, Cromie G, Gerstein AC, Solis N V, Pisithkul T. Rapid Phenotypic
718 and Genotypic Diversification After. 2018;209: 725–741.
- 719 44. Noble SM, French S, Kohn LA, Chen V, Johnson AD. Systematic screens of a
720 *Candida albicans* homozygous deletion library decouple morphogenetic
721 switching and pathogenicity. *Nat Genet.* 2010;42: 590–598.
722 doi:10.1038/ng.605
- 723 45. Hakem R. DNA-damage repair; the good, the bad, and the ugly. *EMBO J.*
724 2008;27: 589–605. doi:10.1038/emboj.2008.15
- 725 46. Aboussekhra A, Wood RD. Repair of UV-damaged DNA by mammalian cells
726 and *Saccharomyces cerevisiae*. *Curr Opin Genet Dev.* 1994;4: 212–220.
727 doi:10.1016/S0959-437X(05)80047-4
- 728 47. Beranek DT. Distribution of methyl and ethyl adducts following alkylation with
729 monofunctional alkylating agents. *Mutat Res - Fundam Mol Mech Mutagen.*
730 1990;231: 11–30. doi:10.1016/0027-5107(90)90173-2
- 731 48. T W. DNA damage checkpoints update: getting molecular. *Curr Opin Genet*
732 *Dev.* 1998;8: 185–193. doi:10.1016/S0959-437X(98)80140-8
- 733 49. L di C, BS C. DNA synthesis in UV-irradiated yeast. *Mutat Res.* 1981;82: 69–
734 85. doi:10.1016/0027-5107(81)90139-1
- 735 50. Butler DK, All O, Govena J, Loveless T, Wilson T, Toenjes KA. The GRR1
736 gene of *Candida albicans* is involved in the negative control of pseudohyphal
737 morphogenesis. *Fungal Genet Biol.* 2006;43: 573–582.
738 doi:10.1016/j.fgb.2006.03.004
- 739 51. McCoy KM, Tubman ES, Claas A, Tank D, Clancy SA, O'Toole ET, et al.
740 Physical limits on kinesin-5-mediated chromosome congression in the smallest
741 mitotic spindles. *Mol Biol Cell.* 2015;26: 3999–4014. doi:10.1091/mbc.E14-10-
742 1454
- 743 52. Enjalbert B, Smith DA, Cornell MJ, Alam I, Nicholls S, Brown AJP, et al. Role

- 744 of the Hog1 Stress-activated Protein Kinase in the Global Transcriptional
745 Response to Stress in the Fungal Pathogen *Candida albicans*. *Mol Biol Cell*.
746 2006;17: 1018. doi:10.1091/MBC.E05-06-0501
- 747 53. Bhaumik SR, Green MR. Differential requirement of SAGA components for
748 recruitment of TATA-box-binding protein to promoters in vivo. *Mol Cell Biol*.
749 2002;22: 7365–7371. doi:10.1128/MCB.22.21.7365-7371.2002
- 750 54. Sterner DE, Belotserkovskaya R, Berger SL. SALSA, a variant of yeast SAGA,
751 contains truncated Spt7, which correlates with activated transcription. *Proc*
752 *Natl Acad Sci U S A*. 2002;99: 11622–11627. doi:10.1073/pnas.182021199
- 753 55. Hnisz D, Majer O, Frohner IE, Komnenovic V, Kuchler K. The Set3/Hos2
754 histone deacetylase complex attenuates cAMP/PKA signaling to regulate
755 morphogenesis and virulence of *Candida albicans*. *PLoS Pathog*. 2010;6:
756 e1000889. doi:10.1371/journal.ppat.1000889
- 757 56. Ferreira T, Brèthes D, Pinson B, Napias C, Chevallier J. Functional analysis of
758 mutated purine-cytosine permease from *Saccharomyces cerevisiae*. A
759 possible role of the hydrophilic segment 371-377 in the active carrier
760 conformation. *J Biol Chem*. 1997;272: 9697–9702.
761 doi:10.1074/jbc.272.15.9697
- 762 57. Rousselet G, Simon M, Ripoche P, Buhler JM. A second nitrogen permease
763 regulator in *Saccharomyces cerevisiae*. *FEBS Lett*. 1995;359: 215–219.
764 doi:10.1016/0014-5793(95)00038-B
- 765 58. ElBerry HM, Majumdar ML, Cunningham TS, Sumrada RA, Cooper TG.
766 Regulation of the urea active transporter gene (*DUR3*) in *Saccharomyces*
767 *cerevisiae*. *J Bacteriol*. 1993;175: 4688–4698. doi:10.1128/jb.175.15.4688-
768 4698.1993
- 769 59. Wichmann H, Hengst L, Gallwitz D. Endocytosis in yeast: Evidence for the
770 involvement of a small GTP-binding protein (*Ypt7p*). *Cell*. 1992;71: 1131–
771 1142. doi:10.1016/S0092-8674(05)80062-5
- 772 60. Talbert PB, Henikoff S. Histone variants--ancient wrap artists of the
773 epigenome. *Nat Rev Mol Cell Biol*. 2010;11: 264–75. doi:10.1038/nrm2861

- 774 61. Yoshida S, Ohya Y, Goebel M, Nakano A, Anraku Y. A novel gene, STT4,
775 encodes a phosphatidylinositol 4-kinase in the PKC1 protein kinase pathway of
776 *Saccharomyces cerevisiae*. *J Biol Chem*. 1994;269: 1166–1171.
777 doi:10.1016/s0021-9258(17)42237-x
- 778 62. Herrero AB, Magnelli P, Mansour MK, Levitz SM, Bussey H, Abeijon C. KRE5
779 gene null mutant strains of *Candida albicans* are avirulent and have altered
780 cell wall composition and hypha formation properties. *Eukaryot Cell*. 2004;3:
781 1423–1432. doi:10.1128/EC.3.6.1423-1432.2004
- 782 63. Garvin AJ, Morris JR. SUMO, a small, but powerful, regulator of double-strand
783 break repair. *Philos Trans R Soc B Biol Sci*. 2017;372.
784 doi:10.1098/RSTB.2016.0281
- 785 64. Watts FZ. The role of SUMO in chromosome segregation. *Chromosoma*.
786 2007;116: 15–20. doi:10.1007/s00412-006-0079-z
- 787 65. Cremona CA, Sarangi P, Zhao X. Sumoylation and the DNA Damage
788 Response. *Biomolecules*. 2012;2: 376. doi:10.3390/BIOM2030376
- 789 66. Ryu HY, Ahn SH, Hochstrasser M. SUMO and cellular adaptive mechanisms.
790 *Exp Mol Med*. 2020;52: 931–939. doi:10.1038/s12276-020-0457-2
- 791 67. Felberbaum R, Hochstrasser M. Ulp2 and the DNA damage response:
792 Desumoylation enables safe passage through mitosis. *Cell Cycle*. 2008;7: 52–
793 56. doi:10.4161/cc.7.1.5218
- 794 68. Jaiswal D, Turniansky R, Green EM. Choose your own adventure: The role of
795 histone modifications in yeast cell fate. *J Mol Biol*. 2017;429: 1946.
796 doi:10.1016/J.JMB.2016.10.018
- 797 69. LD V, K G, I DS. Protein Language: Post-Translational Modifications Talking to
798 Each Other. *Trends Plant Sci*. 2018;23: 1068–1080.
799 doi:10.1016/J.TPLANTS.2018.09.004
- 800 70. Morrell R, Sadanandom A. Dealing With Stress: A Review of Plant SUMO
801 Proteases. *Front Plant Sci*. 2019;10: 1–19. doi:10.3389/fpls.2019.01122
- 802 71. K D. Ubiquitin and SUMO Modifications in *Caenorhabditis elegans* Stress
803 Response. *Curr Issues Mol Biol*. 2020;35: 145–158.

- 804 doi:10.21775/CIMB.035.145
- 805 72. JS S, A D. SUMO and the robustness of cancer. *Nat Rev Cancer*. 2017;17:
806 184–197. doi:10.1038/NRC.2016.143
- 807 73. Brown AJP, Budge S, Kaloriti D, Tillmann A, Jacobsen MD, Yin Z, et al. Stress
808 adaptation in a pathogenic fungus. *J Exp Biol*. 2014;217: 144–155.
809 doi:10.1242/jeb.088930
- 810 74. Leach MD, Stead DA, Argo E, Brown AJP. Identification of sumoylation
811 targets, combined with inactivation of SMT3, reveals the impact of sumoylation
812 upon growth, morphology, and stress resistance in the pathogen *Candida*
813 *albicans*. *Mol Biol Cell*. 2011;22: 687–702. doi:10.1091/mbc.E10-07-0632
- 814 75. Bianchis V, Pontis E, Reichard P. THE JOURNAL OF BIOLOGICAL
815 CHEMISTRY Changes of Deoxyribonucleoside Triphosphate Pools Induced by
816 Hydroxyurea and Their Relation to DNA Synthesis*. *J Biol Chem*. 1986;261:
817 16037–16042. doi:10.1016/S0021-9258(18)66672-4
- 818 76. Huaping L, Jie L, Zhifeng W, Yingchang Z, Yuhuan L. Cloning and functional
819 expression of ubiquitin-like protein specific proteases genes from *Candida*
820 *albicans*. *Biol Pharm Bull*. 2007;30: 1851–1855. doi:10.1248/bpb.30.1851
- 821 77. Segal ES, Gritsenko V, Levitan A, Yadav B, Dror N, Steenwyk JL, et al. Gene
822 Essentiality Analyzed by In Vivo Transposon Mutagenesis and Machine
823 Learning in a Stable Haploid Isolate of *Candida albicans*. *MBio*. 2018;9:
824 e02048-18. doi:10.1128/MBIO.02048-18
- 825 78. O’Meara TR, Hay C, Price MS, Giles S, Alspaugh JA. *Cryptococcus*
826 *neoformans* histone acetyltransferase *Gcn5* regulates fungal adaptation to the
827 host. *Eukaryot Cell*. 2010;9: 1193–202. doi:10.1128/EC.00098-10
- 828 79. Berman J. Morphogenesis and cell cycle progression in *Candida albicans*
829 *Curr. Curr Opin Microbiol*. 2006;9: 595–601.
830 doi:10.1016/j.mib.2006.10.007.Morphogenesis
- 831 80. Loll-Krippleber R, d’Enfert C, Feri A, Diogo D, Perin A, Marcet-Houben M, et
832 al. A study of the DNA damage checkpoint in *Candida albicans*: uncoupling
833 of the functions of *Rad53* in DNA repair, cell cycle regulation and genotoxic

- 834 stress-induced polarized growth. *Mol Microbiol.* 2014;91: 452–471.
835 doi:10.1111/mmi.12471
- 836 81. Legrand M, Chan CL, Jauert PA, Kirkpatrick DT. The contribution of the S-
837 phase checkpoint genes MEC1 and SGS1 to genome stability maintenance in
838 *Candida albicans*. *Fungal Genet Biol.* 2011;48: 823–30.
839 doi:10.1016/j.fgb.2011.04.005
- 840 82. Burrack LS, Applen Clancey SE, Chacón JM, Gardner MK, Berman J.
841 Monopolin recruits condensin to organize centromere DNA and repetitive DNA
842 sequences. *Mol Biol Cell.* 2013;24: 2807–19. doi:10.1091/mbc.E13-05-0229
- 843 83. Ryu HY, Wilson NR, Mehta S, Hwang SS, Hochstrasser M. Loss of the SUMO
844 protease ULP2 triggers a specific multichromosome aneuploidy. *Genes Dev.*
845 2016;30: 1881–1894. doi:10.1101/gad.282194.116
- 846 84. Ryu HY, López-Giráldez F, Knight J, Hwang SS, Renner C, Kreft SG, et al.
847 Distinct adaptive mechanisms drive recovery from aneuploidy caused by loss
848 of the Ulp2 SUMO protease. *Nat Commun.* 2018;9. doi:10.1038/s41467-018-
849 07836-0
- 850 85. Ma Q, Ola M, Iracane E, Butler G. Susceptibility to Medium-Chain Fatty Acids
851 Is Associated with Trisomy of Chromosome 7 in *Candida albicans*. *mSphere.*
852 2019;4. doi:10.1128/MSPHERE.00402-19
- 853 86. Calvo IA, Gabrielli N, Iglesias-Baena I, García-Santamarina S, Hoe K-L, Kim
854 DU, et al. Genome-Wide Screen of Genes Required for Caffeine Tolerance in
855 Fission Yeast. *PLoS One.* 2009;4: e6619.
856 doi:10.1371/JOURNAL.PONE.0006619
- 857 87. Pfaller M a. Antifungal drug resistance: mechanisms, epidemiology, and
858 consequences for treatment. *Am J Med.* 2012;125: S3-13.
859 doi:10.1016/j.amjmed.2011.11.001
- 860 88. Bruno VM, Mitchell AP. Regulation of azole drug susceptibility by *Candida*
861 *albicans* protein kinase CK2. *Mol Microbiol.* 2005;56: 559–573.
862 doi:10.1111/j.1365-2958.2005.04562.x
- 863 89. Goodwin TJD, Poulter RTM. Multiple LTR-retrotransposon families in the

- 864 asexual yeast *Candida albicans*. *Genome Res.* 2000;10: 174–191.
865 doi:10.1101/gr.10.2.174
- 866 90. MacPherson S, Akache B, Weber S, De Deken X, Raymond M, Turcotte B.
867 *Candida albicans* zinc cluster protein Upc2p confers resistance to antifungal
868 drugs and is an activator of ergosterol biosynthetic genes. *Antimicrob Agents*
869 *Chemother.* 2005;49: 1745–1752. doi:10.1128/AAC.49.5.1745-1752.2005
- 870 91. Silver PM, Oliver BG, White TC. Role of *Candida albicans* transcription factor
871 Upc2p in drug resistance and sterol metabolism. *Eukaryot Cell.* 2004;3: 1391–
872 1397. doi:10.1128/EC.3.6.1391-1397.2004/ASSET/112DA7BA-82B3-42CB-
873 BDEB-198E91B9521D/ASSETS/GRAPHIC/ZEK0060423520006.JPEG
- 874 92. Miller JE, Reese JC. Ccr4-Not complex: the control freak of eukaryotic cells.
875 *Crit Rev Biochem Mol Biol.* 2012;47: 315. doi:10.3109/10409238.2012.667214
- 876 93. Islam A, Tebbji F, Mallick J, Regan H, Dumeaux V, Omran RP, et al. Mms21:
877 A putative SUMO E3 ligase in *Candida albicans* that negatively regulates
878 invasiveness and filamentation, and is required for the genotoxic and cellular
879 stress response. *Genetics.* 2019;211: 579–595.
880 doi:10.1534/genetics.118.301769
- 881 94. Omeara TR, Veri AO, Ketela T, Jiang B, Roemer T, Cowen LE. Global
882 analysis of fungal morphology exposes mechanisms of host cell escape. *Nat*
883 *Commun.* 2015;6: 1–10. doi:10.1038/ncomms7741
- 884 95. Hickey CM, Wilson NR, Hochstrasser M. Function and regulation of SUMO
885 proteases. *Nat Rev Mol Cell Biol.* 2012;13: 755–766. doi:10.1038/nrm3478
- 886 96. Suhandynata RT, Quan Y, Yang Y, Yuan W-T, Albuquerque CP, Zhou H.
887 Recruitment of the Ulp2 protease to the inner kinetochore prevents its hyper-
888 sumoylation to ensure accurate chromosome segregation. *PLOS Genet.*
889 2019;15: e1008477. doi:10.1371/JOURNAL.PGEN.1008477
- 890 97. Miyagawa K, Low RS, Santosa V, Tsuji H, Moser BA, Fujisawa S, et al.
891 SUMOylation regulates telomere length by targeting the shelterin subunit
892 Tpz1Tpp1 to modulate shelterin–Stn1 interaction in fission yeast. *Proc Natl*
893 *Acad Sci.* 2014;111: 5950–5955. doi:10.1073/PNAS.1401359111

- 894 98. Montpetit B, Hazbun TR, Fields S, Hieter P. Sumoylation of the budding yeast
895 kinetochore protein Ndc10 is required for Ndc10 spindle localization and
896 regulation of anaphase spindle elongation. *J Cell Biol.* 2006;174: 653–663.
897 doi:10.1083/jcb.200605019
- 898 99. Barra V, Fachinetti D. The dark side of centromeres: types, causes and
899 consequences of structural abnormalities implicating centromeric DNA. *Nat*
900 *Commun.* 2018;9. doi:10.1038/s41467-018-06545-y
- 901 100. Stephens PJ, Greenman CD, Fu B, Yang F, Bignell GR, Mudie LJ, et al.
902 Massive genomic rearrangement acquired in a single catastrophic event during
903 cancer development. *Cell.* 2011;144: 27–40. doi:10.1016/j.cell.2010.11.055
- 904 101. Flowers SA, Barker KS, Berkow EL, Toner G, Chadwick SG, Gyax SE, et al.
905 Gain-of-function mutations in UPC2 are a frequent cause of ERG11
906 upregulation in azole-resistant clinical isolates of *Candida albicans*. *Eukaryot*
907 *Cell.* 2012;11: 1289–1299. doi:10.1128/EC.00215-12
- 908 102. Collart MA. The Ccr4-Not complex is a key regulator of eukaryotic gene
909 expression. *Wiley Interdiscip Rev RNA.* 2016;7: 438–454.
910 doi:10.1002/WRNA.1332
- 911 103. Wilson RB, Davis D, Mitchell AP. Rapid hypothesis testing with *Candida*
912 *albicans* through gene disruption with short homology regions. *J Bacteriol.*
913 1999;181: 1868–74. Available: <http://www.ncbi.nlm.nih.gov/pubmed/10074081>
- 914 104. von der Haar T. Optimized protein extraction for quantitative proteomics of
915 yeasts. *PLoS One.* 2007;2: e1078. doi:10.1371/journal.pone.0001078
- 916 105. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina
917 sequence data. *Bioinformatics.* 2014;30: 2114–2120.
918 doi:10.1093/BIOINFORMATICS/BTU170
- 919 106. Li H. Aligning sequence reads, clone sequences and assembly contigs with
920 BWA-MEM. 2013 [cited 17 Nov 2021]. Available:
921 <https://arxiv.org/abs/1303.3997v2>
- 922 107. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler
923 transform. *Bioinformatics.* 2009;25: 1754–1760.

- 924 doi:10.1093/bioinformatics/btp324
- 925 108. Okonechnikov K, Conesa A, García-Alcalde F. Qualimap 2: advanced multi-
926 sample quality control for high-throughput sequencing data. *Bioinformatics*.
927 2016;32: 292–294. doi:10.1093/BIOINFORMATICS/BTV566
- 928 109. Cibulskis K, Lawrence MS, Carter SL, Sivachenko A, Jaffe D, Sougnez C, et
929 al. Sensitive detection of somatic point mutations in impure and heterogeneous
930 cancer samples. *Nat Biotechnol* 2013 313. 2013;31: 213–219.
931 doi:10.1038/nbt.2514
- 932 110. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, et al. A program
933 for annotating and predicting the effects of single nucleotide polymorphisms,
934 SnEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2;
935 iso-3. *Fly (Austin)*. 2012;6: 80–92. doi:10.4161/fly.19695
- 936 111. Thorvaldsdóttir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer
937 (IGV): high-performance genomics data visualization and exploration. *Brief*
938 *Bioinform*. 2013;14: 178–192. doi:10.1093/BIB/BBS017
- 939 112. Abbey D a, Funt J, Lurie-Weinberger MN, Thompson D a, Regev A, Myers CL,
940 et al. YMAP: a pipeline for visualization of copy number variation and loss of
941 heterozygosity in eukaryotic pathogens. *Genome Med*. 2014;6: 1–15.
942 doi:10.1186/s13073-014-0100-8
- 943 113. Schwartz DC, Cantor CR. Separation of yeast chromosome-sized DNAs by
944 pulsed field gradient gel electrophoresis. *Cell*. 1984;37: 67–75.
945 doi:10.1016/0092-8674(84)90301-5
- 946
- 947

948 **Figure Legends**

949 **Fig 1. *ULP2* is a regulator of *C. albicans* genotoxic stress response**

950 **(A)** Schematic representation of the screening strategy. 674 *C. albicans* deletion
951 strains were screened using a 96-plate format for hypersensitivity to UV and MMS.
952 Hypersensitivity was scored by comparing the growth of treated vs untreated on a
953 scale of 0 (white) to 4 (magenta). Black *: genes encoding for DNA damage and
954 sensing repair pathway components, Blue *: genes encoding for cell division and
955 chromosome segregation machinery, Green arrow: *ulp2* Δ/Δ **(B)** Data for a plate
956 containing *mec3* Δ/Δ strain (cyan circle). Growth on Non-selective (N/S) media or
957 following UV and MMS treatment is shown. **(C)** Data for a plate containing *ulp2* Δ/Δ
958 strain (magenta circle). Growth on Non-selective (N/S) media or following UV and
959 MMS treatment is shown **(D)** Colony-forming Unit assay of UV treated WT and *ulp2*
960 Δ/Δ strains. % survival is shown. **(E)** Growth curve on WT and *ulp2* Δ/Δ strains
961 grown in non-selective (N/S) liquid media and MMS-containing liquid media. Error
962 bars: standard deviation (SD) of three biological replicates **(F)** Growth curve on WT
963 and *ulp2* Δ/Δ strains grown in non-selective (N/S) liquid media and HU-containing
964 liquid media.

965 **Fig 2. *ULP2* is necessary for survival under stress**

966 **(A)** Schematic representation of Ulp1, Ulp2 and Ulp3 protein organisation. The
967 systematic name and the amino acid (aa) number is indicated for each protein. Blue
968 box: putative catalytic UD domain typical of Ulp SUMO proteases **(B)** Protein
969 alignments of the three *C. albicans* Ulp proteins (Ulp1, Ulp2 and Ulp3) and the two *S.*
970 *cerevisiae* proteins (Ulp1 and Ulp2). Magenta arrows: amino acids essential for
971 SUMO protease activity **(C)** HA Western Blot analysis of 4 independent ULP2-HA
972 integrants and the progenitor untagged control (No Tag). Magenta arrow: Ulp2-HA
973 (Magenta arrow). *: non-specific cross-reacting bands serving as a loading control
974 **(C)** Growth curves of WT, *ulp1* Δ/Δ and *ulp2* Δ/Δ strains grown in non-selective (N/S)
975 liquid media. Error bars: standard deviation (SD) of three biological replicates **(D)**
976 Serial dilution assay of WT, *ulp1* Δ/Δ and *ulp2* Δ/Δ strains grown in unstressed (N/S)
977 or stress (UV, MMS, HU, H2O2 and 39 °C) growth conditions.

978 **Fig 3. Loss of *ULP2* leads to increased genome instability**

979 **(A)** Quantification of loss of a heterozygous *URA3*⁺ marker gene inserted in Chr1,
980 Chr3 and Chr7 in WT and *ulp2* Δ/Δ strain. A fold difference of *URA3*⁺ marker loss
981 between *ulp2* Δ/Δ and WT strains is indicated. **: Chr1 (4.11E-07) and Chr7 (6.74E-
982 05) p-value, *: Chr3 (2.87E-02) p-value **(B)** *Top*: Representative images displaying
983 the morphologies of WT and *ulp2* Δ/Δ strains. *Bottom*: Quantification (%) of yeast
984 and filamentous (hyphae + pseudohyphae) cells in WT and *ulp2* Δ/Δ strains. Error
985 bar: Standard deviation of 3 biological replicates. **(C)** *Top*: schematics of the CEN7
986 TetO and TetR-GFP system. *Bottom*: nuclear morphology and segregation pattern of
987 centromere 7 (*CEN7*) in WT and *ulp2* Δ/Δ strain. Quantification (%) of abnormal
988 GFP-CEN7 patterns is indicated. Error bar: Standard deviation of 3 biological
989 replicates. **(D)** Whole genome sequencing analysis of the progenitor (SN152) and
990 three single colonies C1, C2, and C3. Data were plotted as the log₂ ratio and
991 converted to chromosome copy number (y-axis, 1-4 copies) as a function of
992 chromosome position (x-axis, Chr1-ChrR) using the Yeast Mapping Analysis Pipeline
993 (YMAP) [112]. Heterozygous (AB) regions are indicated with grey shading, and
994 homozygous regions (loss of heterozygosity) are indicated by shading of the
995 remaining haplotype, either AA (cyan) or BB (magenta). Two homozygous positions
996 are present in the progenitor (the left side of Chr2 and a small region near the
997 centromere of Chr3), while C1 and C2 underwent loss of heterozygosity of ChrR and
998 Chr3.

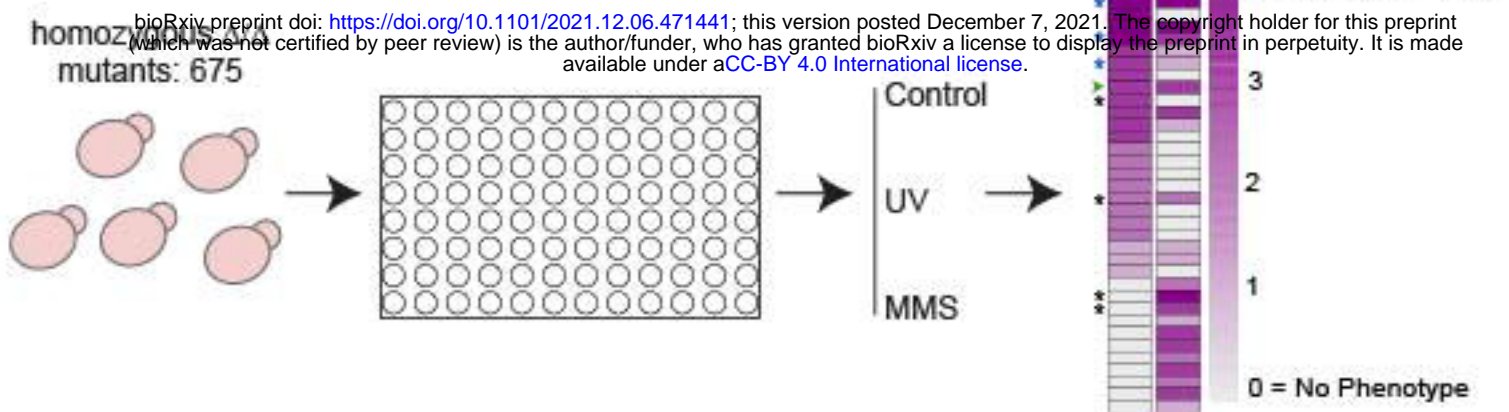
999 **Fig 4. Loss of ULP2 leads to drug resistance via selection of novel genotypes**

1000 **(A)** Serial dilution assay of WT and *ulp2* Δ/Δ strains grown in non-selective (N/S) or
1001 media containing low (15 μ g/ml) concentration of fluconazole (FLC). **(B)** Serial
1002 dilution assay of WT and *ulp2* Δ/Δ strains grown in non-selective (N/S) or media
1003 containing low (5 mM) Caffeine (CAF). **(C)** *Left*: Plating assay of *ulp2* Δ/Δ and WT
1004 strain in media containing high (128 μ g/ml) concentration of fluconazole (FLC) or
1005 non-selective (NS) media *Right*: Plating assay quantification. The number of large
1006 (L) and small (S) colonies recovered from fluconazole (FLC) containing media and
1007 non-selective (N/S) media is shown for WT and *ulp2* Δ/Δ strains. **(D)** *Left*: Plating
1008 assay of *ulp2* Δ/Δ and WT strain in media containing high (12 mM) concentration of
1009 caffeine (CAF) and non-selective (NS) media *Right*: Plating assay quantification. The
1010 number of large (L) and small (S) colonies recovered from caffeine (CAF)-containing

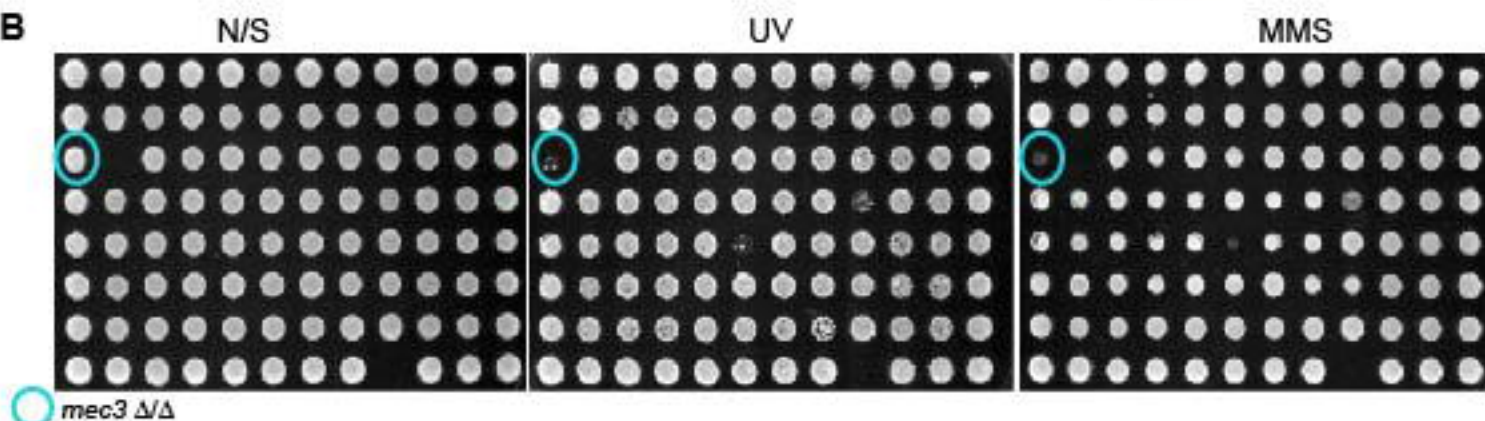
1011 media and non-selective (N/S) media is shown for WT and *ulp2* Δ/Δ strains. **(E)**
1012 Whole genome sequencing data plotted as in Figure 3D for four single colonies
1013 isolated from 128 μ g/ml fluconazole plates (*FLC1-FLC4*). The chromosome copy
1014 number is plotted along the y-axis (1-4 copies). All four single colonies have a
1015 recurrent segmental deletion of part of ChrRR. Colony *FLC-4* has an amplification of
1016 the middle part of Chr1. Copy number breakpoints and allele ratio changes in *FLC-4*
1017 are indicated in Figure S3. **(F)** CHEF karyotype gel stained with ethidium bromide of
1018 *ulp2* Δ/Δ progenitor and *FLC-4* isolate. A band (blue *) corresponding to Chr6 is
1019 present in the *ulp2* Δ/Δ progenitor and absent in the *FLC-4* isolate. Conversely, a
1020 new band (magenta *) is present in the *FLC-4* isolate but absent in the *ulp2*
1021 Δ/Δ progenitor. **(G)** Schematics of segmental aneuploidies detected in *FLC-1*, *FLC-2*,
1022 *FLC-3* and *FLC-4* isolates.
1023

Fig 1

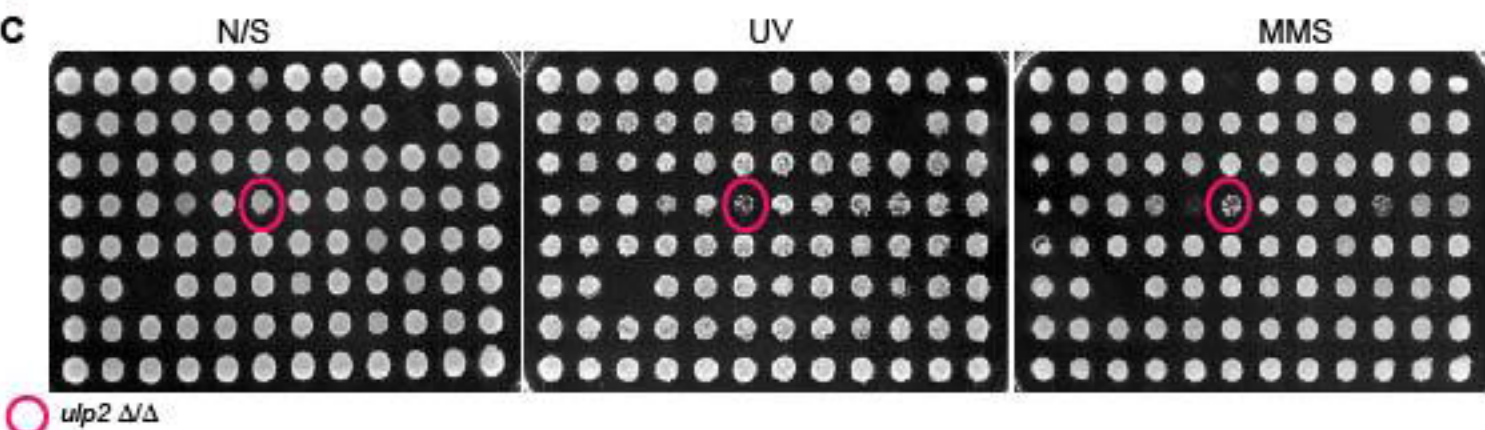
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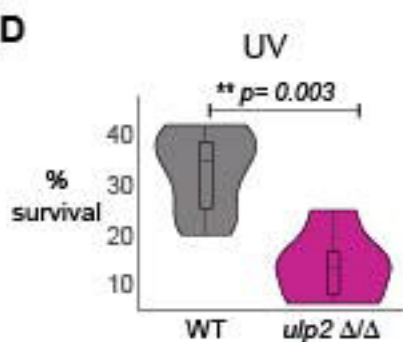
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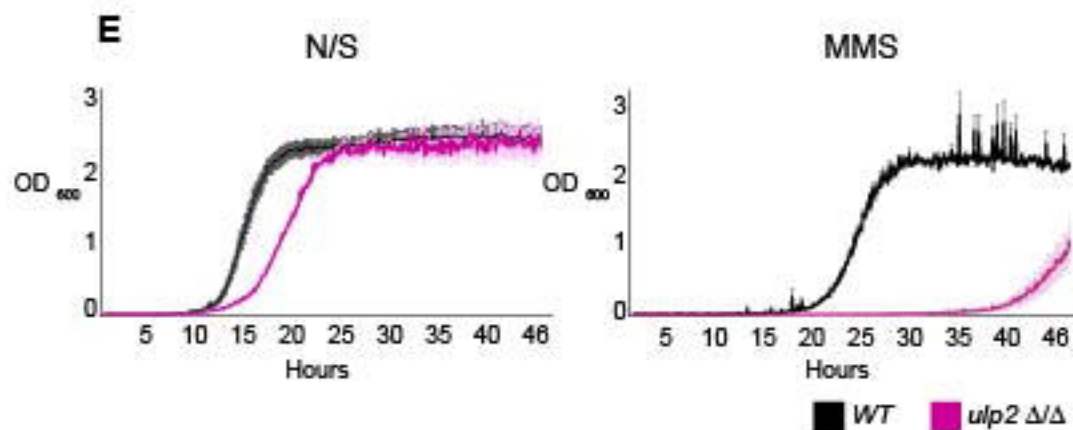
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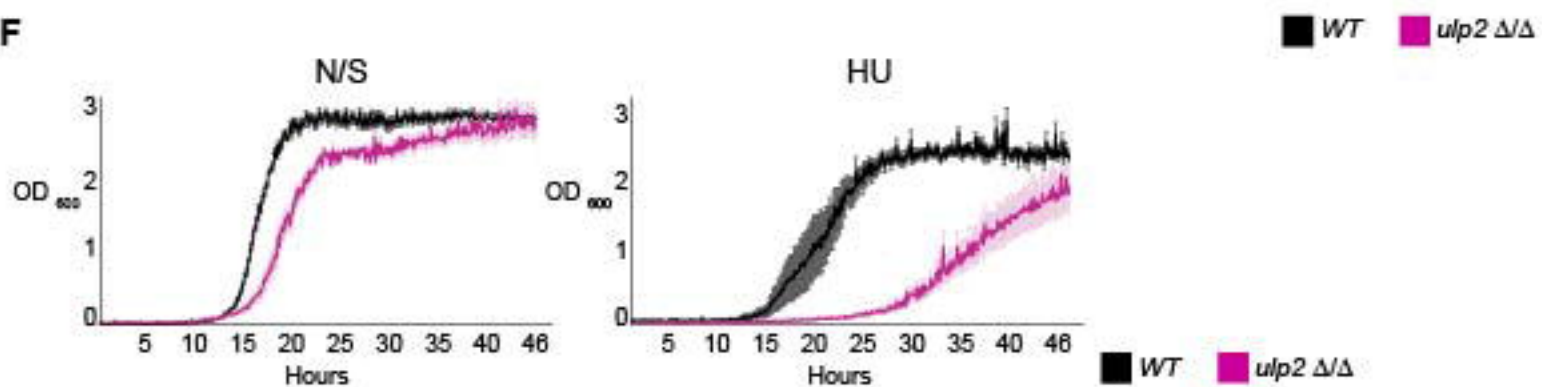
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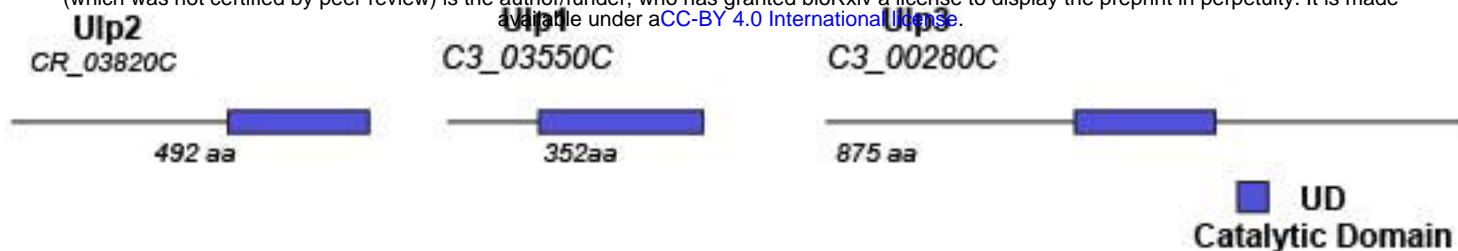


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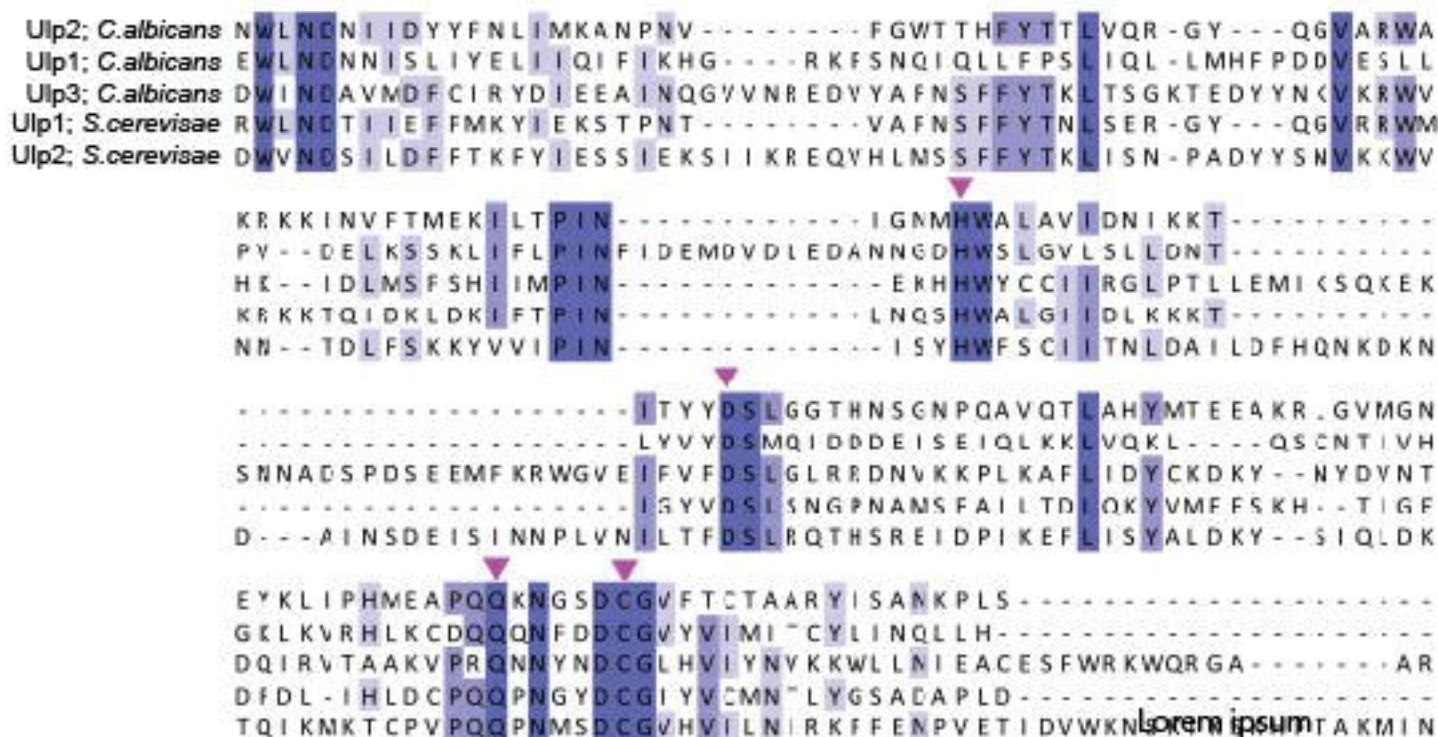


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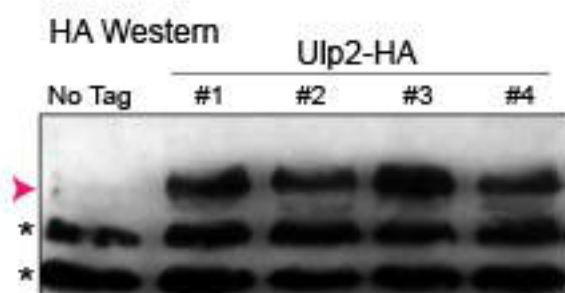
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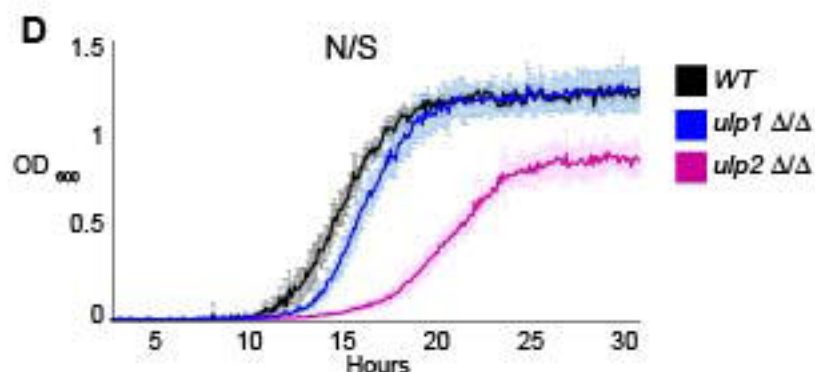
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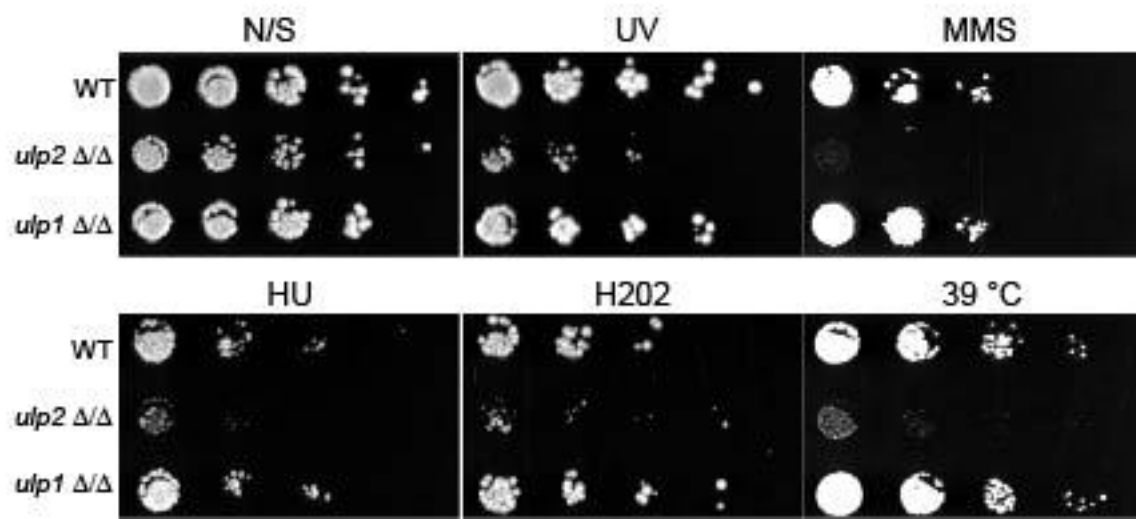
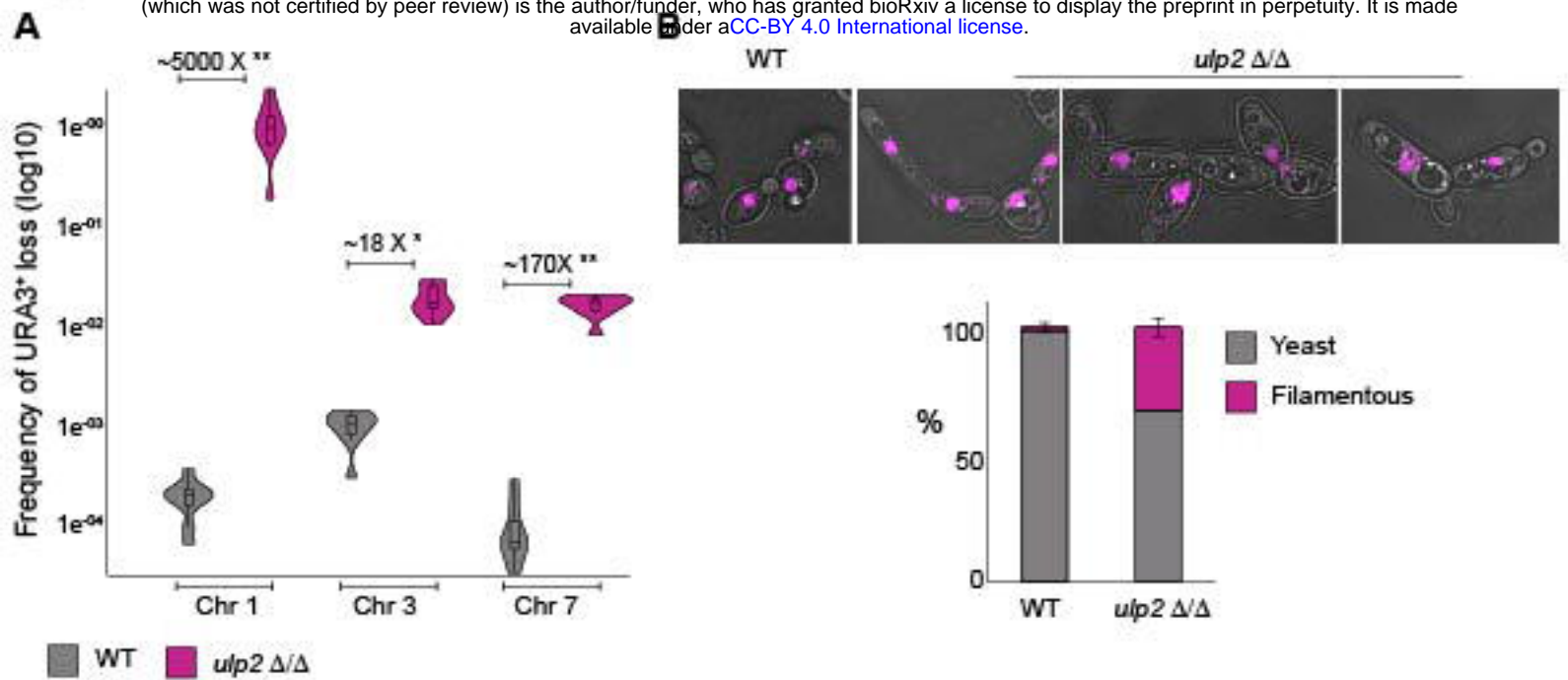
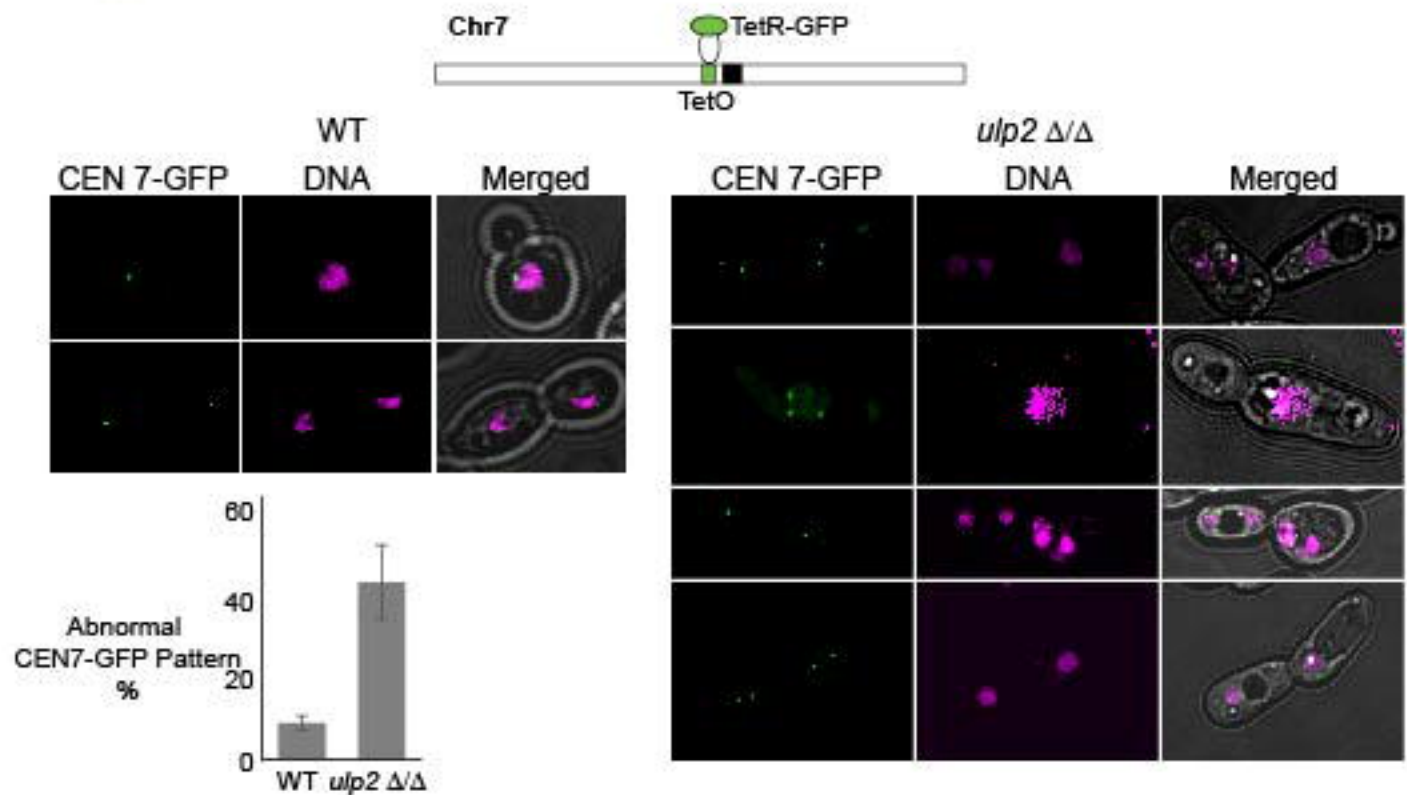


Fig 3

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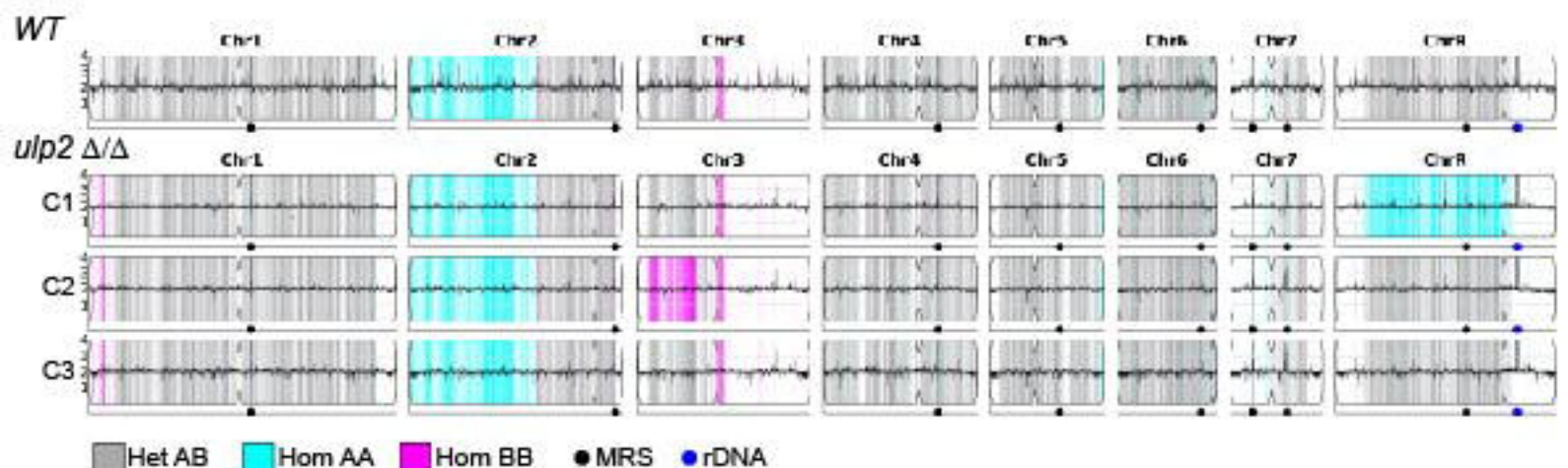


Fig 4

