

1 **A genome-wide screen identifies genes that suppress the accumulation of spontaneous**
2 **mutations in young and aged yeast cells**

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4 **Short Title: A screen for mutation suppression genes in young and aged yeast cells**

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14 Abstract

15 To ensure proper transmission of genetic information, cells need to preserve and faithfully
16 replicate their genome, and failure to do so leads to genome instability, a hallmark of both
17 cancer and aging. Defects in genes involved in guarding genome stability cause several human
18 progeroid syndromes, and an age-dependent accumulation of mutations has been observed in
19 different organisms, from yeast to mammals. However, it is unclear if the spontaneous mutation
20 rate changes during aging, and if specific pathways are important for genome maintenance in
21 old cells. We developed a high-throughput replica-pinning approach to screen for genes
22 important to suppress the accumulation of spontaneous mutations during yeast replicative
23 aging. We found 13 known mutation suppression genes, and 31 genes that had no previous link
24 to spontaneous mutagenesis, and all acted independently of age. Importantly, we identified
25 *PEX19*, encoding an evolutionarily conserved peroxisome biogenesis factor, as an age-specific
26 mutation suppression gene. While wild-type and *pex19Δ* young cells have similar spontaneous
27 mutation rates, aged cells lacking *PEX19* display an elevated mutation rate. This finding
28 suggests that functional peroxisomes are important to preserve genome integrity specifically
29 in old cells, possibly due to their role in reactive oxygen species metabolism.

30 Author Summary

31 Spontaneous mutations arise as a consequence of improper repair of DNA damage caused by
32 intracellular (i.e. toxic by-products of normal cellular metabolism or inaccurate DNA
33 replication) or external (e.g. UV light or chemotherapy) sources. Elevated mutagenesis is
34 implicated in tumorigenesis, and an age-dependent accumulation of mutations has been
35 observed in many organisms. However, it is still unclear how and at which rate mutations
36 accumulate during aging. It is also unknown if specific mechanisms exist that protect the
37 genome of aged cells. We developed a high-throughput, genome-wide approach to identify
38 genes that suppress the accumulation of mutations during yeast replicative aging. Yeast
39 replicative aging refers to the decline in viability a single cell experiences with increasing
40 number of mitotic divisions. We identified a number of new genes that counteract the
41 accumulation of mutations independently of age. Moreover, we discovered that *PEX19*, a gene
42 involved in the biogenesis of peroxisomes, is important to prevent the accumulation of
43 mutations in aged cells. Since *PEX19* is conserved in humans, our work might help understand
44 how human cells could better protect their genome from mutations during aging.

45 **Introduction**

46 Genomic instability, which refers to an increased rate of accumulation of mutations and other
47 genomic alterations, is a hallmark, and a likely driving force, of tumorigenesis [1]. Genomic
48 instability is also a hallmark of aging [2], as suggested by the age-related accumulation of
49 mutations observed in yeast, flies, mice and humans [3], and highlighted by the fact that defects
50 in DNA repair pathways result in human premature aging diseases [4]. However, whether
51 genome instability has a causative role in aging is still controversial [3], and it is not known if
52 aged cells rely more heavily on specific genome maintenance pathways.

53 *Saccharomyces cerevisiae* is a convenient model to study genomic instability and its
54 relationship with aging, since genome maintenance pathways are evolutionary conserved [5,6]
55 and a large number of genetic assays have been developed to study DNA repair and
56 mutagenesis in budding yeast [6,7]. Furthermore, most aging-related cellular pathways, as well
57 as lifespan-modulating environmental and genetic interventions, show a remarkable degree of
58 conservation from yeast to mammals [8–10]. There are two main *S. cerevisiae* aging models:
59 replicative aging refers to the decline in viability that a cell experiences with increasing number
60 of mitotic divisions (a model for aging of mitotically active cells), while chronological aging
61 refers to the decline in viability of a non-dividing cell as a function of time (a model for aging
62 of post-mitotic cells) [11].

63 To identify genes and pathways involved in mutagenesis during yeast replicative aging,
64 two main challenges need to be overcome. First, while *S. cerevisiae* is a leading model system
65 for genetic and genomic studies and the availability of the yeast deletion collection makes this
66 model organism particularly amenable for genome-wide genetic screens [12,13], cellular
67 processes involving low-frequency events, such as point mutations, recombination events or
68 gross chromosomal rearrangements, pose a specific technical challenge, since these events are
69 barely detectable with standard genome-wide screening methods [14]. In a pioneering study,

70 Huang and colleagues performed a genome-wide screen for yeast genes that suppress the
71 accumulation of spontaneous mutations in young cells by screening patches of large numbers
72 of cells on solid media [15]. Patches of each strain of the deletion collection were replica-plated
73 on media containing canavanine to detect canavanine-resistant (Can^R) colonies arising from
74 spontaneous mutations at the *CAN1* locus. A similar approach was subsequently used in other
75 screens for genes controlling genome integrity [5,16–18]. This strategy has proven to be
76 effective but is extremely laborious. To overcome these limitations, we developed a screening
77 strategy to detect low-frequency events, based on high-throughput replica pinning of high-
78 density arrays of yeast colonies.

79 The second challenge to identify genes involved in mutagenesis during yeast replicative
80 aging is the isolation aged cells, since they constitute a tiny fraction of an exponentially
81 growing cell population. To allow the study of a cohort of aging mother cells, Lindstrom and
82 Gottschling developed the Mother Enrichment Program (MEP), an inducible genetic system
83 that prevents the proliferation of daughter cells (Fig 1A) [19]. Upon activation by estradiol, the
84 Cre recombinase, which is under the control of a daughter-specific promoter, enters the nucleus
85 and disrupts two genes essential for cell cycle progression (namely *UBC9* and *CDC20*),
86 resulting in an irreversible arrest of daughter cells in G2/M, while mother cells are unaffected.
87 Thus, in the absence of estradiol, MEP cells grow exponentially and form normal colonies on
88 an agar plate, while upon addition of estradiol, linear growth occurs and microcolonies are
89 formed. Occasionally, due to spontaneous mutations, the MEP is inactivated and cells become
90 insensitive to estradiol: these cells are called “escapers” [19]. Escaper cells grow exponentially
91 and form normal sized colonies even in the presence of estradiol.

92 We combined the MEP system with a high-throughput replica-pinning strategy to
93 perform a genome-wide screen aimed at identifying genes important to suppress the
94 accumulation of spontaneous mutations in replicatively aging yeast cells, using escaper

95 formation as a readout for spontaneous mutagenesis events. With our approach, we identified
96 several new mutation suppression genes that act independently of age. We also found that
97 *PEX19*, involved in peroxisome biogenesis, is an age-specific mutation suppression gene:
98 while wild-type and *pex19A* young cells have similar spontaneous mutation rates, the absence
99 of *PEX19* causes an elevated mutation rate specifically in old cells. We suggest that functional
100 peroxisomes protect the genome of aged cells from spontaneous mutagenesis.

101 **Results**

102 **A high-throughput screen to identify genes important for suppressing spontaneous
103 mutations during yeast replicative aging**

104 We developed a high-throughput replica-pinning strategy that enables detection of low-
105 frequency events and used it to perform a genome-wide screen for genes important for
106 suppressing spontaneous mutations during yeast replicative aging (Fig 1). We introduced the
107 MEP system into the yeast knockout (YKO) collection via Synthetic Genetic Array (SGA)
108 technology [20]. The resulting MEP-YKO collection was pinned multiple times in parallel on
109 estradiol-containing plates (18 replicates per knockout strain) to activate the MEP, and grown
110 for one week (Fig 1C). An example of our experimental setting is shown in Fig 1B. If at any
111 time during aging a MEP-inactivating mutation occurs, an escaper colony is formed. Each plate
112 was then re-pinned on estradiol and grown for two days to detect escapers (escaper test). At
113 this point, all MEP-proficient mother cells that have exhausted their replicative potential are
114 not able to give rise to a colony; conversely, if escaper cells are present, a fully grown colony
115 can be observed (Fig 1B and C). Our high-throughput replica-pinning method allows the semi-
116 quantitative estimation of spontaneous mutation rates on the basis of escaper formation (Fig
117 1C). Since every plate of the MEP-YKO collection was pinned multiple times in parallel on
118 estradiol, it is possible to calculate the frequency of escaper formation for each deletion mutant
119 strain in the collection: an increased escaper frequency compared to wild-type control strains
120 is an indication of a high spontaneous mutation rate (Fig 1D).

121 To validate our assumption that the escaper frequency of each strain is a proxy for the
122 spontaneous mutation rate, we could make use of the fact that 72 strains from the YKO
123 collection were derived from a parental strain carrying an additional mutation in the mismatch
124 repair gene *MSH3* and are therefore expected to show increased spontaneous mutation rates,
125 independently of the identity of the knockout gene [21]. In addition, 340 empty positions

126 randomly dispersed over the 14 plates of the MEP-YKO library were manually filled in with a
127 wild-type MEP control. In Fig 1D, an overview of the escaper frequencies of the whole MEP-
128 YKO collection is shown. Most of the strains have an escaper frequency between 10% and
129 40% (median: 27.8%). The wild-type control strains show a similar behavior (median: 22.2%),
130 but with the important difference that the wild-type escaper frequency never exceeds 72.2%.
131 In contrast, the escaper frequency of most of the *msh3* strains falls between 50% and 80%
132 (median: 55.6%), validating the rationale of our screening method.

133 We then used Cutoff Linked to Interaction Knowledge (CLIK) analysis [22] to
134 determine the cutoff for validation in an unbiased manner. The CLIK algorithm identified an
135 enrichment of highly interacting genes at the top of our list (ranked according to escaper
136 frequency), confirming the overall high quality of our screen (Fig 1E). The cutoff suggested
137 by CLIK corresponds to an escaper frequency of 75%, which, not surprisingly, is slightly
138 higher than the maximum escaper frequency observed in the wild-type controls (72.2%). To
139 further explore the overall quality of the screen, we set the cutoff at 75% escaper frequency
140 and performed phenotypic enrichment analysis using ScreenTroll, which examines the
141 similarity between genome-scale screens [23]. Predictably, the first overlap with our gene list
142 is the mutator screen performed by Huang and colleagues [15]. Furthermore, most of the top
143 overlapping screens are related to genome instability and DNA damage sensitivity (S1 Table).
144 Based on the threshold determined by CLIK, we proceeded to direct validation of all hits with
145 an escaper frequency higher than 75%.

146

147 **Identification of new genes that suppress the accumulation of mutations independently
148 of replicative age**

149 We first discarded as false positive all hits where the escaper-causing mutation(s) had occurred
150 before the beginning of the aging experiment (i.e. during the generation of the MEP-YKO

151 library and before the subsequent high-throughput replica-pinning step) as in these cases, an
152 escaper frequency of 100% is not an indication of an extremely high mutation rate. By spotting
153 serial dilutions of strains from the MEP-YKO library on estradiol-containing plates (S1 Fig),
154 we found that 25/115 hits had escaped before the actual screen started (S1 File). We then set
155 out to validate the remaining 90 putative mutator strains.

156 Spontaneous mutations can occur at any moment of the replicative lifespan and our
157 experimental design does not allow us to discriminate if a high escaper frequency is an
158 indication of an increased mutation rate already in young cells, or of an elevated age-dependent
159 accumulation of mutations. To distinguish between these two possibilities, we performed
160 fluctuation tests to measure the forward mutation rate at the endogenous *CAN1* locus, where
161 any type of mutation that inactivates the *CAN1* gene confers canavanine resistance [24,25].
162 Since fluctuation tests are performed with logarithmically growing cultures, they measure the
163 spontaneous mutation rate in an age-independent fashion (i.e. in young cells). Twelve of our
164 hits had been previously validated [15], and therefore were not re-tested. Several genes
165 identified in the aforementioned study (namely *CSM2*, *SHU1*, *TSA1* and *SKN7*) fell just below
166 our 75% escaper frequency cutoff (S1 File). Importantly, we validated 13 new mutator mutants.
167 Our screening strategy thus enabled us to identify new genes important for the suppression of
168 spontaneous mutations independently of replicative age. The 26 genes whose deletion results
169 in an increased *CAN1* mutation rate of at least 1.8-fold compared to wild type are listed in
170 Table 1. We named this group of genes “general mutation suppression genes” because the
171 corresponding knockout strains, besides showing an elevated escaper frequency in our
172 screening setup, also display an increased mutation rate when tested in young cells with a
173 second assay for spontaneous mutagenesis at a different genetic locus. Of the general mutation
174 suppression genes identified, 16/26 have one or more human orthologs. As expected, these

175 genes are significantly enriched for Gene Ontology categories related to DNA damage
176 response, DNA repair and recombination (Fig 2 and S2 File).

177 **Table 1. List of validated general mutation suppression genes.**

Gene deleted	Can^R rate (x 10⁻⁷)	Function	Human ortholog(s)^a
1 <i>RAD27</i>	167.1 [#]	DNA replication and repair	<i>FEN1, GEN1</i>
2 <i>PMS1</i>	79.9 [#]	mismatch repair	<i>PMS1, PMS2</i>
3 <i>MSH2</i>	64.9 [#]	mismatch repair	<i>MSH2</i>
4 <i>MLH1</i>	53.0 [#]	mismatch repair	<i>MLH1</i>
5 <i>MME1</i>	45.1 [#]	magnesium ion export from mitochondrion	
6 <i>RAD54</i>	37.9 [#]	recombinational repair	<i>ATRX, RAD54B, RAD54L, RAD54L2</i>
7 <i>RAD57</i>	37.2 [#]	recombinational repair	<i>XRCC3</i>
8 <i>RAD55</i>	35.8 [#]	recombinational repair	
9 <i>MPH1</i>	29.5 ^b	error-free bypass of DNA lesions	<i>FANCM</i>
10 <i>MSH6</i>	27.7 [#]	mismatch repair	<i>MSH6</i>
11 <i>YGL177W</i>	27.6	dubious open reading frame (ovlp <i>MPT5</i>)	
12 <i>CRS5</i>	23.6	copper-binding metallothionein	
13 <i>VMA6</i>	22.5	V-ATPase	<i>ATP6V0D1, ATP6V0D2</i>
14 <i>GRX7</i>	21.7	oxidative stress response	
15 <i>PSY3</i>	20.4 [#]	error-free DNA lesion bypass	
16 <i>OGGI</i>	17.6 [#]	DNA repair	<i>OGGI</i>
17 <i>SHU2</i>	17.4 [#]	error-free DNA lesion bypass	
18 <i>NAT3</i>	17.0	NatB N-terminal acetyltransferase	<i>NAA20</i>
19 <i>APL1</i>	16.3	vesicle mediated transport	
20 <i>MET18</i>	8.1	Fe-S cluster assembly	<i>MMS19</i>
21 <i>YLR358C</i>	8.0	unknown (ovlp <i>RSC2</i>)	
22 <i>DSS4</i>	5.4	post-Golgi vesicle-mediated transport	
23 <i>RLF2</i>	5.2	chromatin assembly complex	<i>CHAF1A</i>
24 <i>SRP40</i>	5.0	preribosome assembly or transport	<i>NOLC1</i>
25 <i>NUP84</i>	4.9	nuclear pore complex	<i>NUP107</i>
26 <i>RAD10</i>	4.9	DNA repair	<i>ERCC1</i>
Wild type	2.6		

178
179 Genes in **bold** were newly identified; genes in regular font were previously identified [15,26]
180 [#] values from Huang et al. [15]; all other values were determined as described in the Materials
181 and Methods and are statistically supported (Student's t-test)

182 ^a Human orthologs of yeast genes are taken from the "*S. cerevisiae* to human ortholog pairs"

183 tool from the Rothstein Lab Tool Suite
184 (http://www.rothsteinlab.com/tools/scerevisiae_hsapien_orthologs)

185 ^b An elevated mutation rate for this mutant has been previously reported [26]

186

187 We then determined whether the 64 strains that do not show an elevated mutation rate
188 at the *CANI* locus would display an increase in the mutation rate if measured by escaper
189 formation in the MEP genetic background. To do so, we performed a slightly modified version
190 of the fluctuation test, where selective (i.e. estradiol-containing) plates are incubated for seven
191 days and colonies are counted after two and seven days. Escaper colonies appearing after two
192 days of incubation originate from mutations occurring prior to plating (i.e. in young cells),
193 while all colonies appearing between day 2 and day 7 originate from a mutation event that
194 occurred during replicative aging (see Materials and Methods for details). This experimental
195 setup mimics the conditions in which the initial screen was performed and allows us to
196 simultaneously measure the escaper formation rates in young cells and the age-dependent
197 escaper formation frequencies. With this assay we identified 18 genes whose deletion results
198 in an increased escaper formation rate of at least 1.8-fold compared to the wild type,
199 independently of replicative age (i.e. based on colonies counted at day 2). We named these
200 genes “MEP-specific mutation suppression genes”, since the spontaneous mutation rate
201 measured at the *CANI* locus in the corresponding knockout mutants is indistinguishable from
202 the wild type (Table 2). About half (8/18) of these genes have one or more human orthologs.
203 Intriguingly, MEP-specific mutation suppression genes are enriched for members of the
204 THO/TREX complex, which is involved in co-transcriptional mRNA export from the nucleus.
205 This process is important in the interplay between transcriptional elongation and R-loop
206 formation in yeast and mammalian cells [27,28] (Fig 3 and S2 File).

207 **Table 2. List of validated MEP-specific mutation suppression genes**

Gene deleted	Escaper rate (x 10 ⁻⁷) ^a	Function	Human ortholog(s) ^b
1 <i>XRN1</i>	84.1	exoribonuclease	<i>XRN1</i>
2 <i>COS10</i>	54.6	turnover of plasma membrane proteins	
3 <i>NIP100</i>	52.1	dynactin complex	<i>CEP350, CLIP1, CLIP2, CLIP3, CLIP4</i>
4 <i>RPP1A</i>	34.4	ribosomal protein	<i>RPLP1</i>

5	<i>FMP45</i>	29.7	mitochondrial membrane protein		
6	<i>RPL13B</i>	26.3	ribosomal protein	<i>RPL13</i>	
7	<i>HOP2</i>	25.2	meiosis		
8	<i>SAC3</i>	17.1	mRNA export (TREX complex)	<i>MCM3AP, SAC3D1</i>	
9	<i>HBT1</i>	14.2	polarized cell morphogenesis		
10	<i>NFT1</i>	12.2	putative ABC transporter		
11	<i>SNF2</i>	7.9	SWI/SNF chromatin remodeling complex	<i>SMARCA2, SMARCA4</i>	
12	<i>MFT1</i>	7.6	mRNA export (THO complex)		
13	<i>GTO3</i>	7.6	glutathione transferase		
14	<i>THP1</i>	7.2	mRNA export (TREX complex)		
15	<i>SEMI</i>	6.1	mRNA export / proteasome regulation	<i>SHFM1</i>	
16	<i>YPL205C</i>	6.0	dubious open reading frame		
17	<i>THP2</i>	5.9	mRNA export (THO/TREX complex)		
18	<i>UBA4</i>	5.6	thio-modification of tRNA	<i>MOCS3, UBA5</i>	
	Wild type	2.9			

208

209 ^a All values shown are statistically supported (Student's *t*-test)

210 ^b Human orthologs of yeast genes are taken from the "*S. cerevisiae* to human ortholog pairs"

211 tool from the Rothstein Lab Tool Suite
212 (http://www.rothsteinlab.com/tools/scerevisiae_hsapien_orthologs)

213

214 ***PEX19* suppresses age-dependent accumulation of mutations**

215 At the end of our validation pipeline, we were left with four gene knockout strains that display
216 no significant increase in forward mutation rate at the *CAN1* locus and in escaper formation
217 rate in young cells (colonies counted at day 2) but show a higher age-dependent escaper
218 frequency compared to wild type (colonies counted at day 7) (S2 Table). We were particularly
219 interested in these genes, since our observations might indicate an age-dependent mutator
220 phenotype. To validate these putative age-specific mutator mutants with an independent and
221 more accurate method, we mechanically isolated young and aged mother cells by biotinylation
222 and magnetic sorting and measured mutation frequencies at the *CAN1* locus in both cell
223 populations [29]. Based on the Can^R frequencies in young cells, the replicative age (assessed
224 by bud scar counting), and the mutation rate in young cells (previously determined by
225 fluctuation test), we could calculate the expected Can^R frequencies in aged cells under the
226 assumption that the mutation rate remains constant during replicative aging (see Materials and

227 Methods for details). By comparing the observed and the expected frequencies, it becomes
228 clear if the mutation rate of a given strain is constant or varies as cells age.

229 To establish a reference, we tested wild type cells. Strikingly, the observed mutation
230 frequency in aged cells (replicative age ~17) was lower than expected (Fig 4B and S2 Fig),
231 suggesting a decrease in the spontaneous mutation rate during replicative aging. We then
232 measured mutation frequencies in young and old cells from the four putative age-specific
233 mutator strains. After the first test, three of these strains did not show any increase in age-
234 dependent mutation frequency compared to the wild type and were therefore discarded as false
235 positives (S3 Fig and S2 Fig). Conversely, age-dependent mutation frequency in the absence
236 of *PEX19* was higher than in the wild type. We therefore repeated the test and confirmed that
237 *pex19Δ* aged cells (replicative age ~15.5) display much higher mutation frequencies than
238 expected (Fig 4 and S2 Fig). This result suggests that *PEX19*, encoding an evolutionarily
239 conserved factor required for peroxisome biogenesis [30], suppresses age-dependent
240 accumulation of mutations. We observed a similar effect after the deletion of *PEX3*, which
241 causes the same peroxisome biogenesis defect as observed in the absence of *PEX19*, namely
242 lack of detectable peroxisomal structures [31]. Mutation frequency in aged *pex3Δ* cells
243 (replicative age ~13) was much higher than expected (S4 Fig), supporting the notion that
244 functional peroxisomes contribute to genome maintenance during yeast replicative aging.

245 **Discussion**

246 *S. cerevisiae* is an outstanding model in which to perform genetic screens. However, there has
247 been a lack of genome-wide, high-throughput screening techniques to detect low-frequency
248 events (such as point mutations, recombination events and gross chromosomal
249 rearrangements). These technical limitations, in combination with the difficulty to isolate large
250 populations of replicative old cells, have so far hindered the study of genome maintenance
251 during replicative aging.

252

253 **Potential applications of the high-throughput replica-pinning methodology**

254 We developed a high-throughput replica-pinning approach to screen for cellular processes
255 involving low-frequency events, thus filling a technological gap in the yeast screening field.
256 We applied this strategy to screen for genes controlling the accumulation of spontaneous
257 mutations during yeast replicative aging, using the Mother Enrichment Program both as a tool
258 to induce replicative aging and as a reporter for spontaneous mutation events (Fig 1). Key
259 technical adjustments (see Material and Methods) were: a) the use of 1536 format pads to pin
260 the MEP-YKO collection in 384 format on estradiol, so that a smaller number of cells (~1.5 x
261 10^4) was deposited on the plate, to prevent nutrient limitation during replicative aging; b) the
262 MEP-YKO collection amplification by parallel high-throughput replica-pinning to analyze 18
263 colonies per strain; c) the one-week incubation time, which allowed accumulation of
264 spontaneous mutations throughout replicative lifespan. In this way, we were able to monitor
265 enough cell divisions to detect low-frequency mutation events. Furthermore, the analysis of 18
266 independent colonies allowed the use of escaper frequency as a proxy for the spontaneous
267 mutation rate. Bioinformatic analysis and experimental confirmation indicated the high quality
268 of the screen, thus validating our methodology.

269 It is worth noting that, even independently of the MEP and the replicative aging
270 perspective, a similar high-throughput replica-pinning approach can be used to screen for genes
271 involved in other genome stability-related processes. For instance, we recently applied this
272 strategy to study spontaneous homologous recombination events (manuscript in preparation).
273 Similarly, our replica-pinning strategy could be adapted to screen for genes controlling genome
274 integrity in the chronological lifespan model [32,33]. More generally, this technique can be
275 used to study any process involving low-frequency events for which genetically selectable
276 reporters exist or can be developed. Examples include transient (loss of) gene silencing [34],
277 transcription errors [35] and read-through at premature termination codons [36].
278

279 **New general mutation suppression genes identified**

280 Our screening setup was designed to allow simultaneous identification of age-independent and
281 age-specific mutator mutants. We identified 13 new genes that suppress the accumulation of
282 spontaneous mutations at the *CANI* locus independently of age (Table 1). Some of these
283 general mutation suppression genes (*RAD10* and *NUP84*) have defined roles in genome
284 integrity [37–39]. For some other well-characterized genes, their role in preventing
285 accumulation of mutations can be inferred from their molecular function. For instance, *MET18*
286 has a conserved role in iron-sulfur (Fe/S) cluster assembly and insertion in several proteins
287 involved in DNA replication and repair [40–42]. *RLF2/CAC1* encodes the largest subunit of
288 the Chromatin Assembly Factor-I (CAF-1) complex, for which a role in DNA replication and
289 repair of UV-induced DNA damage has been described [43–47].

290 For another group of new mutation suppression genes (*VMA6*, *GRX7*, *CRS5*, *NAT3*),
291 their role in preventing accumulation of mutations might be more indirect. *VMA6* encodes a
292 subunit of the evolutionary conserved vacuolar H⁺-ATPase (V-ATPase), responsible for
293 vacuole acidification and cellular pH regulation [48,49]. Defects in yeast V-ATPase result in

294 vacuole alkalinization and increased cytoplasm acidification [50], mitochondrial
295 depolarization and fragmentation [51,52], altered iron homeostasis [53] and chronic
296 endogenous oxidative stress [54]. This phenotype could potentially explain the elevated
297 spontaneous mutation rate of a *vma6Δ* mutant, due to compromised functioning of Fe/S cluster-
298 containing DNA replication and repair proteins as a consequence of mitochondrial
299 depolarization [42,55], and/or to DNA damage caused by increased endogenous oxidative
300 stress [56]. It would be interesting to test if disruption of other V-ATPase subunits causes the
301 same mutator phenotype.

302 The *CRS5* gene product is a copper- and zinc- binding metallothionein [57,58]. The
303 role of Crs5 in preventing spontaneous mutations is likely linked to its protective role against
304 endogenous oxidative stress, since scavenging of reactive oxygen species is a general function
305 of metallothioneins [59]. Crs5 might also directly protect DNA from copper-induced cleavage
306 [60]. The reported physical interaction between Crs5 and the peroxiredoxins Tsa1 and Tsa2,
307 responsible for preventing DNA damage and genome instability due to hydrogen peroxide and
308 organic peroxides generated during normal cell metabolism, further supports a role for Crs5 in
309 protecting the genome from oxidative damage [61,62].

310 Of particular interest is the mutation suppression genes *NAT3*, encoding the catalytic
311 subunit of the conserved NatB N-terminal acetyltransferase. *NAT3* was identified in a screen
312 for radiation sensitive mutants, and thereafter named *RAD56* [63,64]. Importantly, its human
313 homologue hNAT3 has been implicated in carcinogenesis [65,66]. Non-degradative protein N-
314 acetylation occurs co-translationally and can modulate protein folding, protein localization and
315 protein-protein interactions [67]. It is likely that Nat3 prevents the accumulation of
316 spontaneous mutations by ensuring the proper functioning of one (or more) of its targets.
317 Interestingly, among the identified substrates of yeast NatB, several are involved in DNA
318 metabolism, such as Pol31, Rnr4, Sml1, Nup84 [68–70]. Furthermore, many other factors

319 involved in DNA processing and repair contain the peptide sequence recognized by NatB and
320 are thus potential targets of Nat3 [71]. Further work will be needed to identify the relevant
321 target(s) for preventing accumulation of spontaneous mutations.

322 *GRX7* encodes a largely uncharacterized glutaredoxin localized in the cis-Golgi
323 [72,73]. How the disulfide bond-reducing activity of Grx7 in the Golgi affects spontaneous
324 mutation rate is unclear, even though functional links between the Golgi apparatus and genome
325 maintenance mechanisms have been suggested [74,75]. The *DSS4* gene product functions in
326 the post-Golgi secretory pathway, while *APL1* is involved in clathrin-mediated vesicle
327 transport. The elevated spontaneous mutation rate of *dss4Δ* and *apl1Δ* strains may indicate a
328 thus far unanticipated connection between genome stability and vesicle transport in the
329 secretory pathway. The remaining genes (*YGL177W*, *YLR358C*, and *SRP40*) are poorly
330 characterized and require further investigation.

331

332 **MEP-specific age-independent mutation suppression genes are enriched for genes related
333 to mRNA export**

334 We also identified 18 mutants that, despite an undetectable increase in the spontaneous
335 mutation rate at the *CANI* locus, display an age-independent elevated escaper formation rate
336 (Table 2). This observation hints at a locus-specific increase in mutagenesis for this group of
337 mutator strains. The observation that MEP-specific mutation suppression genes are enriched
338 for genes involved in mRNA export from the nucleus (Fig 3 and S2 File) suggests the
339 involvement of R-loop-dependent genome instability [27,28]. R-loops form preferentially at
340 specific genomic locations and can cause genomic instability by exposing single-stranded
341 DNA tracts, triggering hyper-recombination and interfering with DNA replication [76].
342 Intriguingly, the exoribonuclease encoded by *XRN1*, our top MEP-specific mutation
343 suppression gene, has also been implicated in preventing R-loop-dependent genome instability

344 [77]. To confirm this hypothesis, one would need to examine the genomic features of the locus
345 or loci where mutations that give rise to escapers happen. It is assumed that escaper-originating
346 mutations occur at the *cre-EBD78* locus (since inactivating the Cre recombinase results in a
347 disruption of the MEP system), but the creators of the MEP already suggested that this is not
348 always the case, and other unknown endogenous loci might be involved in escaper formation
349 [19]. Indeed, our genetic analysis of a few escapers originating from a wild-type MEP strain
350 showed that the escaper phenotype does not always co-segregate with the *cre-EBD78* locus,
351 confirming that mutations occurring at other genomic loci can result in MEP inactivation and
352 escaper formation (S3 Table).

353

354 **A decrease in the spontaneous mutation rate in aged yeast cells**

355 To investigate age-dependent spontaneous mutagenesis, we first compared mutation
356 frequencies at the *CAN1* locus in young and old wt cells. Interestingly, *CAN1* mutation
357 frequencies in aged cells are lower than predicted, indicating that spontaneous mutation rate
358 decreases during replicative aging (Fig 4B). This might occur, for instance, if the efficiency of
359 a mutagenic DNA repair pathway, such as translesion synthesis [78], is reduced in old cells.
360 The same effect would be observed if an error-free repair pathway is upregulated during aging.
361 A similar decrease in the spontaneous mutation rate at the *CAN1* endogenous locus has been
362 previously reported, although the same study suggested that this effect could be locus-specific
363 [29].

364

365 **A role for peroxisomes in suppressing age-dependent accumulation of spontaneous
366 mutations**

367 To better understand age-dependent mutagenesis, our screen aimed at identifying genes—if
368 they exist—that prevent accumulation of spontaneous mutations specifically in old cells. We

369 showed that *PEX19* is one of these genes, since its deletion has no effect on mutagenesis in
370 young cells, but causes an elevated accumulation of mutations in aged cells (Fig 4). To our
371 knowledge, this is the first described case of an age-dependent mutation suppression gene,
372 suggesting that some cellular pathways are particularly important in protecting the genome of
373 old cells.

374 *PEX19* is an evolutionary conserved gene which plays a key role in peroxisome
375 biogenesis, and whose absence results in the lack of detectable peroxisomes [30,31]. Deletion
376 of *PEX3*, another peroxisome biogenesis gene, causes the same age-specific mutator phenotype
377 (S4 Fig), implying that functional peroxisomes are important to prevent age-dependent
378 accumulation of mutations. The human orthologs of *PEX19* and *PEX3*, together with other
379 peroxins, are mutated in Zellweger syndrome, a severe cerebro-hepato-renal peroxisome
380 biogenesis disorder [79]. Peroxisomes are key organelles for the maintenance of the redox
381 balance of the cell. On the one hand, they generate H₂O₂ as a consequence of fatty acid
382 peroxidation; on the other hand, they contain a set of antioxidant enzymes and function
383 therefore as reactive oxygen species (ROS) scavenging organelles [80]. Interestingly, loss of
384 Pex19 in *D. melanogaster* causes ROS accumulation and mitochondrial damage, and a mouse
385 model of Zellweger syndrome displays a similar phenotype [81,82]. Elevated endogenous ROS
386 are known to induce genome instability [83,84].

387 How could peroxisomes potentially contribute to genome integrity maintenance in aged
388 yeast cells? Several studies report an asymmetric ROS distribution between mother and
389 daughter cell, resulting in ROS accumulation during replicative aging [85–87]. These elevated
390 ROS levels are accompanied by an age-dependent hyperoxidation and inactivation of the
391 peroxiredoxin Tsa1, a key antioxidant enzyme important for genome integrity and for
392 suppression of mutations [15,62,83,84,88]. In this context, the role of peroxisomes in
393 protecting the genome from endogenous oxidative stress might become crucial, due to the age-

394 dependent increase in ROS accumulation and the concomitant progressive failure of other
395 redundant antioxidant systems that are active in young cells. Our observations suggest that
396 deletion of *PEX19* has a synergistic effect with age, resulting in elevated spontaneous
397 mutagenesis. Given the evolutionary conservation of this and many other peroxisome
398 biogenesis factors, it would be interesting to test the contribution of peroxisomes in genome
399 maintenance during mammalian cell aging and cancer development. Indeed, several studies
400 have reported the absence of peroxisomes in cancer cells [89–91], suggesting a possible link
401 between peroxisome biogenesis defects and tumorigenesis [92].

402 **Materials and Methods**

403 **Yeast strains and growth conditions**

404 Standard yeast media and growth conditions were used [93,94]. All yeast strains used in this
405 study are derivatives of the BY4741 genetic background [95] and are listed in Table 3. DNY34
406 was obtained from Y7092 and UCC8773 by crossing and tetrad dissection. The *ice2Δ::kanMX*
407 strain from the deletion collection (EUROSCARF) strain was crossed with strain UCC8774 by
408 standard yeast genetics to create the strain DNY80. Strains DNY99, DNY101, DNY102 and
409 DNY105 were constructed by standard PCR-mediated gene deletion in strain UCC8773.

410

411 **Table 3. Yeast strains used in this study.**

Strain name	Relevant genotype	Source
BY4741	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0</i>	[95]
UCC8773	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 hoΔ::Pscw11-cre-EBD78-natMX loxP-CDC20-intron-loxP-hphMX loxP-UBC9-loxP-LEU2</i>	[96]
UCC8774	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 trp1Δ63 hoΔ::Pscw11-cre-EBD78-natMX loxP-CDC20-intron-loxP-hphMX loxP-UBC9-loxP-LEU2</i>	[96]
Y7092	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 can1Δ::STE2pr-Sp_his5 lyp1Δ met15Δ0</i>	[97]
DNY34	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 can1Δ::STE2pr-Sp_his5 lyp1Δ met15Δ0 hoΔ::PSCW11-cre-EBD78-natMX loxP-UBC9-loxP-LEU2 loxP-CDC20-Intron-loxP-hphMX</i>	This study
DNY80	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 met15Δ0 hoΔ::Pscw11-cre-EBD78-natMX loxP-CDC20-intron-loxP-hphMX loxP-UBC9-loxP-LEU2 ice2Δ::kanMX</i>	This study

DNY99	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 met15Δ0 hoΔ::Pscw11-cre-EBD78-natMX loxP-CDC20-intron-loxP-hphMX loxP-UBC9-loxP-LEU2 pex19Δ::kanMX</i>	This study
DNY101	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 met15Δ0 hoΔ::Pscw11-cre-EBD78-natMX loxP-CDC20-intron-loxP-hphMX loxP-UBC9-loxP-LEU2 rox3Δ::kanMX</i>	This study
DNY102	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 met15Δ0 hoΔ::Pscw11-cre-EBD78-natMX loxP-CDC20-intron-loxP-hphMX loxP-UBC9-loxP-LEU2 atg23Δ::kanMX</i>	This study
DNY105	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 met15Δ0 hoΔ::Pscw11-cre-EBD78-natMX loxP-CDC20-intron-loxP-hphMX loxP-UBC9-loxP-LEU2 pex3Δ::kanMX</i>	This study

412

413

414 **High-throughput replica-pinning screen**

415 High-throughput manipulation of high-density yeast arrays was performed with the RoToR-
416 HDA pinning robot (Singer Instruments). The Mother Enrichment Program (MEP) was
417 introduced into the *MATa* yeast deletion collection (EUROSCARF) through Synthetic Genetic
418 Array (SGA) methodology [97] using the DNY34 query strain. The procedure was performed
419 twice in parallel to generate two independent sets of MEP yeast deletion arrays in 384-colony
420 format. When a specific MEP mutant was missing in one of the arrays, it was manually pinned
421 over from the other set. Positions that were empty in both sets were filled with *his3Δ::kanMX*
422 control strains, unless they were kept empty for plate identification purposes. Colonies from
423 the two sets of MEP yeast deletion arrays were pinned onto YPD + G418 plates and incubated
424 for six hours at 30°C. Each plate of each set was then pinned onto nine YPD plates containing
425 1 μM estradiol (18 replicates in total). At this step, colonies were pinned in 384 format using
426 1536 format pads, so that a smaller number of cells was deposited to prevent nutrient limitation.

427 Plates were incubated for seven days at 30°C and then scanned with a flatbed scanner.
428 Subsequently, each plate was pinned onto one YPD plate containing 1 μ M estradiol and
429 incubated for two days at 30°C before scanning (“escaper test”). Colony area measurement
430 was performed using the ImageJ software package [98] and the ImageJ plugin ScreenMill
431 Colony Measurement Engine [99], to assess colony circularity and size in pixels. The data was
432 filtered to exclude artifacts by requiring a colony circularity score greater than 0.8. Colonies
433 with a pixel area greater than 200 were considered escapers, and for each deletion strain, the
434 ratio of escapers to total colonies in replica pinning experiments was used as the escaper
435 frequency score.

436

437 **Screen validation pipeline**

438 Putative hits were initially analyzed by fluctuation test to measure the forward mutation rate at
439 the endogenous *CANI* locus. To do so, we used the strains from the YKO collection, because
440 the strains from the MEP-YKO collection are *can1Δ*. At first, we performed one fluctuation
441 test per strain. If the mutation rate was higher than 1.5-fold of the wild-type mutation rate, the
442 test was repeated another two or three times.

443 For all the genes whose deletion does not cause an increase in the mutation rate at the
444 *CANI* locus, the corresponding knockout strains from the MEP-YKO collection were analyzed
445 by fluctuation test to measure the escaper formation rate in young cells and the escaper
446 formation frequency in replicatively aged cells. At first, we performed one fluctuation test per
447 strain. If the escaper formation rate was higher than 1.5-fold of the wild-type escaper formation
448 rate, the test was repeated another two or three times. In case no increase in escaper formation
449 rate was detected but elevated age-dependent escaper formation frequencies were observed,
450 the experiment was repeated another one or two times.

451 Strains that consistently displayed an elevated age-dependent escaper formation
452 frequency were further validated by construction of a new knockout strain in a MEP *CAN1*
453 background and by direct measurement of spontaneous mutation frequencies at the *CAN1* locus
454 in young and aged cells. Each knockout strain was tested once. If the age-dependent mutation
455 frequencies were not higher than the wild-type control, the strain was discarded as a false
456 positive; if the age-dependent mutation frequencies were increased compared to the wild-type
457 control, the experiment was repeated three times.

458 The identity of all validated strains from the YKO and MEP-YKO collections was
459 confirmed by barcode sequencing as previously described [100].

460

461 **Measurements of the spontaneous forward mutation rate at the *CAN1* locus**

462 Single colonies were inoculated in 5 ml YPD and grown up to saturation (two days at 30°C).
463 100 µl were plated onto canavanine-containing SD medium (50 µg/ml) to identify forward
464 mutations in *CAN1* and 50 µl of a 10⁵-fold dilution was plated onto SD medium to count viable
465 cells. Colonies were counted after two days of growth at 30°C and the spontaneous forward
466 mutation rate at the *CAN1* locus was determined by fluctuation test from nine independent
467 cultures using the method of the median [25,101]. Values represent the average of at least three
468 independent experiments.

469

470 **Measurements of spontaneous escaper formation rate and age-dependent escaper
471 formation frequencies**

472 Single colonies were inoculated in 5 ml YPD and grown up to saturation (two days at 30°C).
473 50 µl of a 50-fold dilution (or a higher dilution, when needed) were plated onto YPD plates
474 containing 1 µM estradiol to identify escaper occurrence in young cells (“young plates”), 50 µl
475 of a 500-fold dilution (or a higher dilution, when needed) were plated onto YPD plates

476 containing 1 μ M estradiol to identify escapers occurrence in aging cells (“old plates”), and 50
477 μ l of a 500000-fold dilution was plated onto YPD plates to count viable cell number. Colonies
478 were counted after two days of growth at 30°C (for “young plates” and YPD plates) or after
479 two and after seven days of growth at 30°C (for “old plates”). In “young plates”, colonies that
480 were smaller than the colonies growing on the corresponding YPD plate were not counted,
481 because for those colonies the escaper-causing mutation occurred after plating. The
482 spontaneous escaper formation rate in young cells was determined by fluctuation test from 7-
483 10 independent cultures using the MSS-maximum-likelihood estimator method from the
484 FALCOR fluctuation analysis calculator [102]. Values represent the average of at least three
485 independent experiments. Age-dependent escaper formation frequencies was calculated by
486 dividing the number of escaper colonies that appeared between day 2 and day 7 (on “old
487 plates”) by the number of viable cells plated (determined from the YPD plates).

488

489 **Measurements of spontaneous mutation frequencies at the *CAN1* locus in young and aged**
490 **cells**

491 Isolation of young and aged cells was performed essentially as previously described [19,103].
492 1.5×10^9 cells from a log-phase MEP culture were washed with cold phosphate buffered saline
493 (PBS), resuspended in cold PBS containing 7 mg/ml Sulfo-NHS-LC-Biotin (Thermo
494 Scientific) and incubated for 20 min at room temperature with gentle shaking. Biotinylated
495 cells were then washed with PBS, resuspended in 250 ml of pre-warmed YPD medium and
496 allowed to recover for 2 h at 30°C with shaking. Estradiol was added to a final concentration
497 of 1 μ M to induce the MEP (aging starts here). After 2 h of incubation at 30°C with shaking,
498 100 ml were harvested (young cells), while the rest of the culture (150 ml) was inoculated in a
499 total volume of 1 L YPD containing 1 μ M estradiol and 100 μ g/ml ampicillin (to discourage
500 bacterial contamination), and incubated at 30°C with shaking. Young cells were washed with

501 cold PBS, resuspended in 5 ml cold PBS and incubated with 100 μ l streptavidin-coated BioMag
502 beads (Qiagen) in a 5 ml LoBind tube (Eppendorf) at 4°C with gentle shaking for 30 min. Cells
503 were gently pelleted at 4°C (3 min 1800 \times g), resuspended in 7 ml cold YPD and transferred
504 to a glass test tube (Lab Logistics Group). The tube was placed in a magnet ("The Big Easy"
505 EasySep Magnet, Stemcell Technologies) for 5 min on ice. Cells were then washed three times
506 by removing supernatant by pipetting, resuspending them in 7 ml cold YPD and incubating for
507 5 min on ice in the magnet. Finally, cells were resuspended in 5.2 ml PBS and transferred in a
508 5 ml LoBind tube (Eppendorf). Of the 5.2 ml of purified mother cells, 100 μ l were stained for
509 bud scars counting (see below); 100 μ l were diluted 1000x and plated on SD medium to assess
510 cell viability; the remaining 5 ml were pelleted and plated on canavanine-containing SD
511 medium (50 μ g/ml) to identify forward mutations in *CANI*. After 20 h of MEP induction, the
512 entire aged 1 L culture was harvested. The aged cells were processed similarly to the young
513 cells, with slight modifications because of the higher number of cells due to the presence of
514 daughter cells. For beading, the cells were split into 4 different 5 ml LoBind tubes, and 50 μ l
515 streptavidin coated BioMag beads were added to each tube. For magnetic sorting, two glass
516 tubes were used and cells were washed four times.

517 For both young and aged samples, colonies were counted after 2 d of growth at 30°C
518 and the spontaneous forward mutation frequencies at the *CANI* locus were determined.
519 Expected mutation frequencies in aged cells were calculated as previously described [29].

520

521 **Bud scar detection and counting**

522 Purified mother cells (see above) were stained with propidium iodide (PI) (Sigma) to identify
523 viable cells and with Calcofluor White (Fluorescent Brightener 28, Sigma) to detect bud scars.
524 100 μ l of purified mother cells in PBS (\sim 5 \times 10⁵ cells) were stained with 2 μ l of a 2 mM PI
525 (Sigma) solution for 30 min at 30°C. Cells were then washed with ddH₂O, fixed in 500 μ l of

526 3.7% formaldehyde for 30 min at room temperature, washed with PBS, resuspended in 100 μ l
527 PBS and stored at 4°C. Just before imaging, cells were stained with Calcofluor White for 5 min
528 at room temperature, washed with PBS and resuspended in 5-10 μ l PBS. Images were acquired
529 using a DeltaVision Elite imaging system (Applied Precision (GE), Issaquah, WA, USA)
530 composed of an inverted microscope (IX-71; Olympus) equipped with a Plan Apo 100X oil
531 immersion objective with 1.4 NA, InsightSSITM Solid State Illumination, excitation and
532 emission filters for DAPI and A594, ultimate focus and a CoolSNAP HQ2 camera
533 (Photometrics, Tucson, AZ, USA). Stacks of 30 images with 0.2 μ m spacing were taken at an
534 exposure time of 5 ms at 10% intensity for DAPI (Calcofluor White staining) and 50 ms at
535 32% intensity for A594 (PI staining). Reference bright-field images were also taken.
536 Fluorescent images were subjected to 3D deconvolution using SoftWoRx 5.5 software
537 (Applied Precision). Processing of all images was performed using Fiji (ImageJ, National
538 Institute of Health) [98]. Bud scars from at least 50 PI-negative cells (which were alive after
539 magnetic sorting) were manually counted for each sample to determine the cells' replicative
540 age.

541

542 **Gene Ontology enrichment analysis and functional annotation**

543 GO enrichment analysis was performed with DAVID 6.8 (<https://david.ncifcrf.gov/home.jsp>)
544 using the Functional Annotation tool [104,105]. To reduce functional redundancy among GO
545 terms, we used the REVIGO Web server (<http://revigo.irb.hr/>) with a cutoff value C = 0.5
546 [106].

547 Functional enrichment within the yeast global genetic similarity network was performed and
548 visualized with TheCellMap.org (<http://thecellmap.org/>), using SAFE [107,108].

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867 **Figure captions**

868 **Fig 1. Combining the Mother Enrichment Program with high-throughput replica-**
869 **pinning to screen for genes that suppress spontaneous mutations during yeast replicative**
870 **aging.** (A) The Mother Enrichment Program (MEP). Estradiol induction causes irreversible
871 arrest of daughter cell proliferation, while growth of mother cells is unaffected. Inactivation of
872 the MEP due to spontaneous mutations results in estradiol-insensitive cells called escapers. (B)
873 Escaper formation is a readout for spontaneous mutation events during replicative aging. High-
874 density arrays of MEP colonies are pinned on estradiol and escapers are subsequently detected
875 by re-pinning on estradiol (big colonies). (C) Schematic of the screening procedure. The MEP
876 is introduced into the YKO collection via Synthetic Genetic Array (SGA) methodology. The
877 resulting MEP-YKO collection is amplified by high-throughput (HT) replica-pinning on
878 estradiol, which activates the MEP and triggers replicative aging. A second replica-pinning on
879 estradiol allows detection of escapers (escaper test). Escaper frequencies are calculated for each
880 strain of the MEP-YKO collection. (D) Comparison of escaper frequencies of the whole MEP-
881 YKO collection with escaper frequencies of wt and mutator (*msh3*) controls. (E) CLIK analysis
882 sets an unbiased cutoff for validation. Green and blue colors indicate regions of the plot
883 significantly enriched for physical and genetic interactions, while regions deprived of
884 significant enrichment are plotted in gray. The red dotted line marks the cutoff suggested by
885 the CLIK algorithm.

886

887 **Fig 2. Overview of the general mutation suppression genes.** (A) Functional enrichment in
888 the yeast genetic landscape. Dotted lines indicate functional domains within the yeast genetic
889 landscape, i.e. gene clusters enriched for a specific set of GO terms (the name of each functional
890 domain is indicated by a colored label). Regions of the global similarity network significantly

891 enriched for genes exhibiting genetic interactions with general mutation suppression genes
892 were mapped using SAFE and are indicated in blue. (B) Gene Ontology enrichment analysis.

893

894 **Fig 3. Overview of the MEP-specific mutation suppression genes.** (A) Functional
895 enrichment in the yeast genetic landscape. Regions of the global similarity network
896 significantly enriched for genes exhibiting genetic interactions with MEP-specific mutation
897 suppression genes were mapped using SAFE and are indicated in orange. See Legend to Fig
898 2A for details. (B) Gene Ontology enrichment analysis.

899

900 **Fig 4. *PEX19* suppresses age-dependent accumulation of mutations.** (A) *CAN1* forward
901 mutation rate in young wt and *pex19* Δ cells. Values from three independent experiments are
902 plotted. The thick dark bars represent the median values. ns: non-significant. (B) Age-
903 dependent mutation frequencies at the *CAN1* locus in wt (replicative age ~17) and *pex19* Δ
904 (replicative age ~15.5) cells. The difference between observed and expected mutation
905 frequencies from four independent experiments is plotted. The horizontal bars represent the
906 median values. p-value was determined by Student's *t*-test.

907 **Supporting information**

908 **S1 Fig. Exclusion of strains that escaped before the beginning of the screen.** When serial
909 dilutions of strains from the MEP-YKO collection are spotted in the presence of estradiol,
910 growth of MEP-proficient strains is restricted, while escaper strains grow normally. An
911 example of one MEP-proficient strain and one escaper is shown.

912

913 **S2 Fig. Raw data for all the age-dependent mutation frequency measurement**
914 **experiments.** (A) Mutation frequencies at the *CAN1* locus in young and old cells from the
915 indicated strains. In the case of old cells, both observed and expected mutation frequencies are
916 shown. For each individual experiment, the median replicative age of the young and old cell
917 populations is indicated. (B) Bud scars distribution of young and old cell populations from each
918 experiment.

919

920 **S3 Fig. *ICE2*, *ATG23* and *ROX3* did not validate as age-specific mutation suppression**
921 **genes.** (A) *CAN1* forward mutation rate in young wt, *ice2Δ*, *atg23Δ*, and *rox3Δ* cells. Mean
922 values from three independent experiments are plotted. Error bars represent standard error. ns:
923 non-significant. (B) Age-dependent mutation frequencies at the *CAN1* locus in wt (replicative
924 age ~17), *ice2Δ* (replicative age ~15), *atg23Δ* (replicative age ~15.5), and *rox3Δ* (replicative
925 age ~15) cells. The difference between observed and expected mutation frequency is plotted.
926 For the wt, the mean value from four independent experiments is plotted. Error bars represent
927 standard error. For the mutants, only one experiment is shown (see the Results and Material
928 and Methods sections for details).

929

930 **S4 Fig. *PEX3* deletion results in elevated spontaneous mutations in aged cells.** (A) *CAN1*
931 forward mutation rate in young wt, and *pex3Δ* cells. Mean values from three or four

932 independent experiments are plotted. Error bars represent standard error. ns: non-significant.
933 (B) Age-dependent mutation frequencies at the *CANI* locus in wt (replicative age ~17), and
934 *pex3Δ* (replicative age ~13) cells. The difference between observed and expected mutation
935 frequency is plotted. For the wt, the mean value from four independent experiments is plotted.
936 Error bars represent standard error. For *pex3Δ*, only one experiment is shown.

937

938 **S1 Table. ScreenTroll phenotypic enrichment analysis.** ScreenTroll analysis
939 (<http://www.rothsteinlab.com/tools/screenTroll>) identifies overlaps between published screens
940 and our gene dataset (cutoff 75% escaper frequency, determined by CLIK). The top overlaps
941 are related to genome instability and DNA damage sensitivity. “ORFs in screen” refers to the
942 total number of hits identified in the overlapping screen.

943

944 **S2 Table. List of putative age-specific mutation suppression genes.**

945

946 **S3 Table. Escaper formation is not always caused by mutations at the *cre-EBD78* locus.**

947

948 **S1 File. Complete escaper frequency data from the screen and validation of mutation
949 suppression genes.**

950

951 **S2 File. SAFE enrichments and GO enrichment analysis for general and MEP-specific
952 mutation suppression genes.**

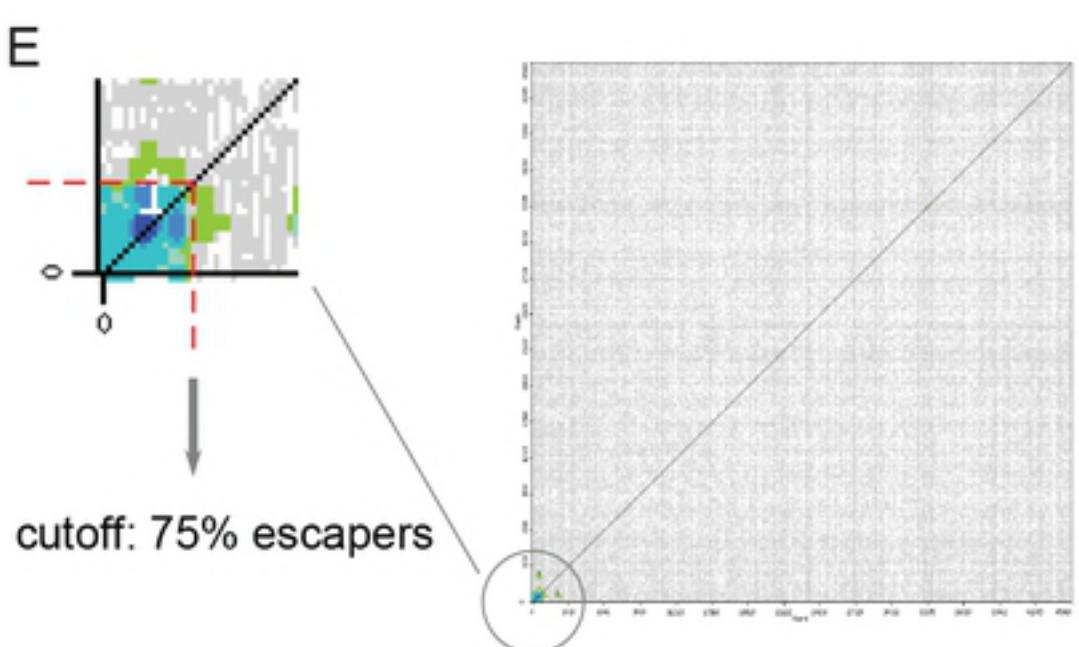
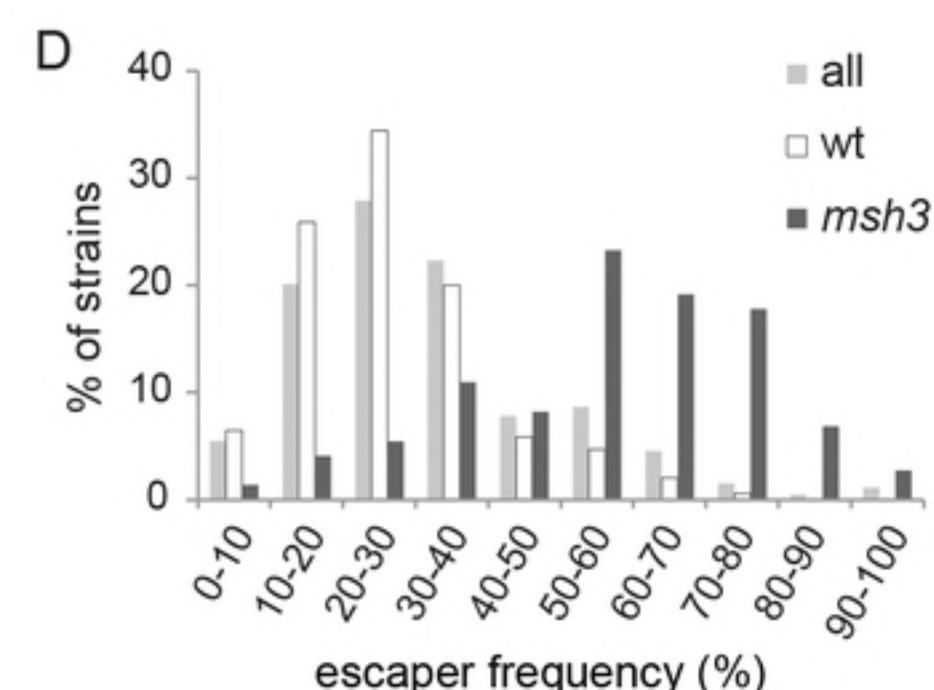
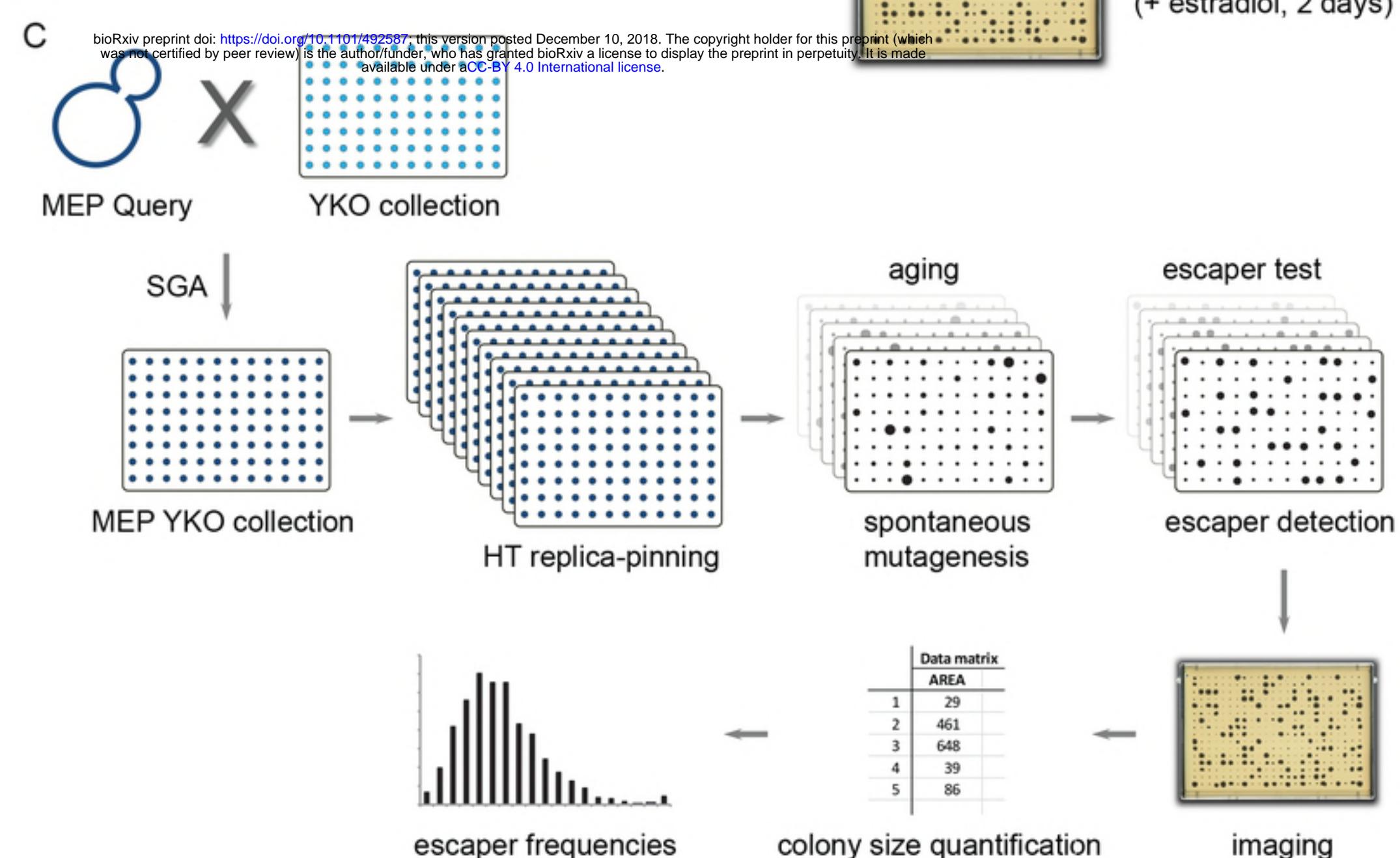
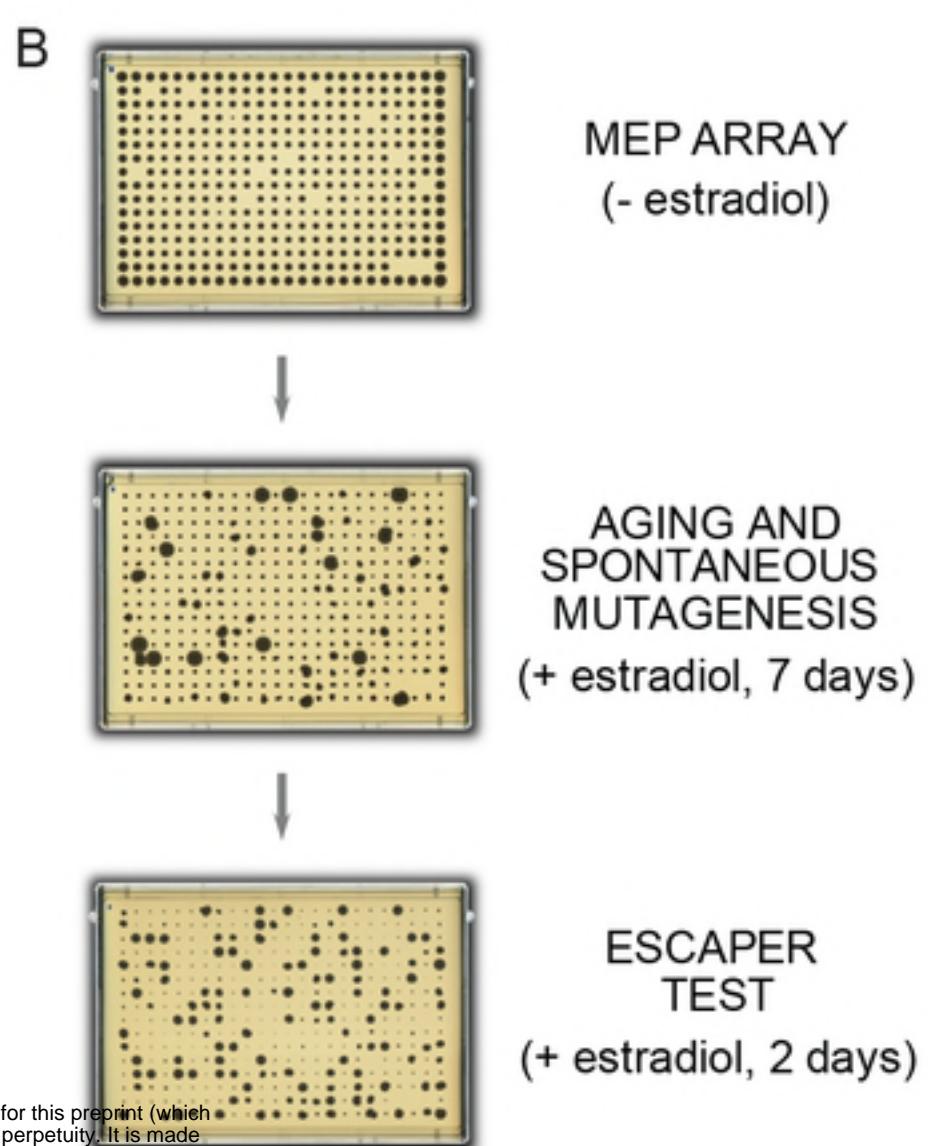
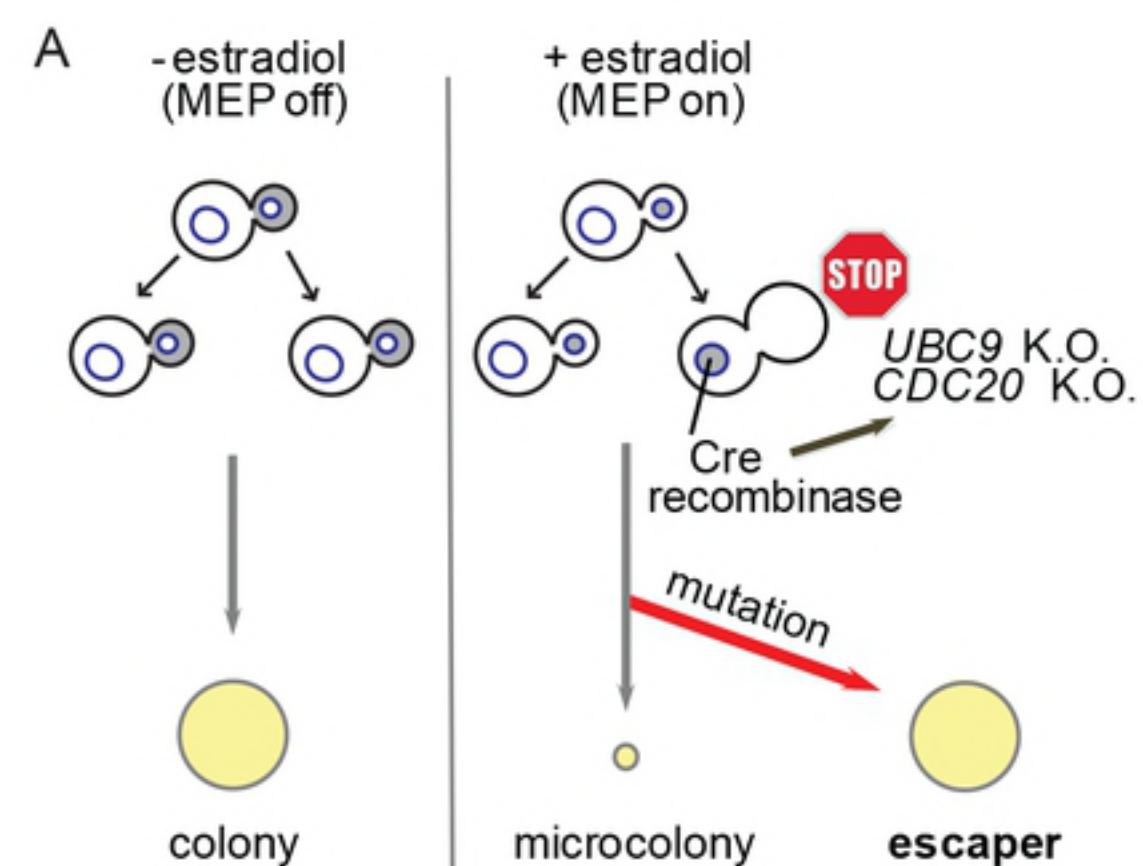
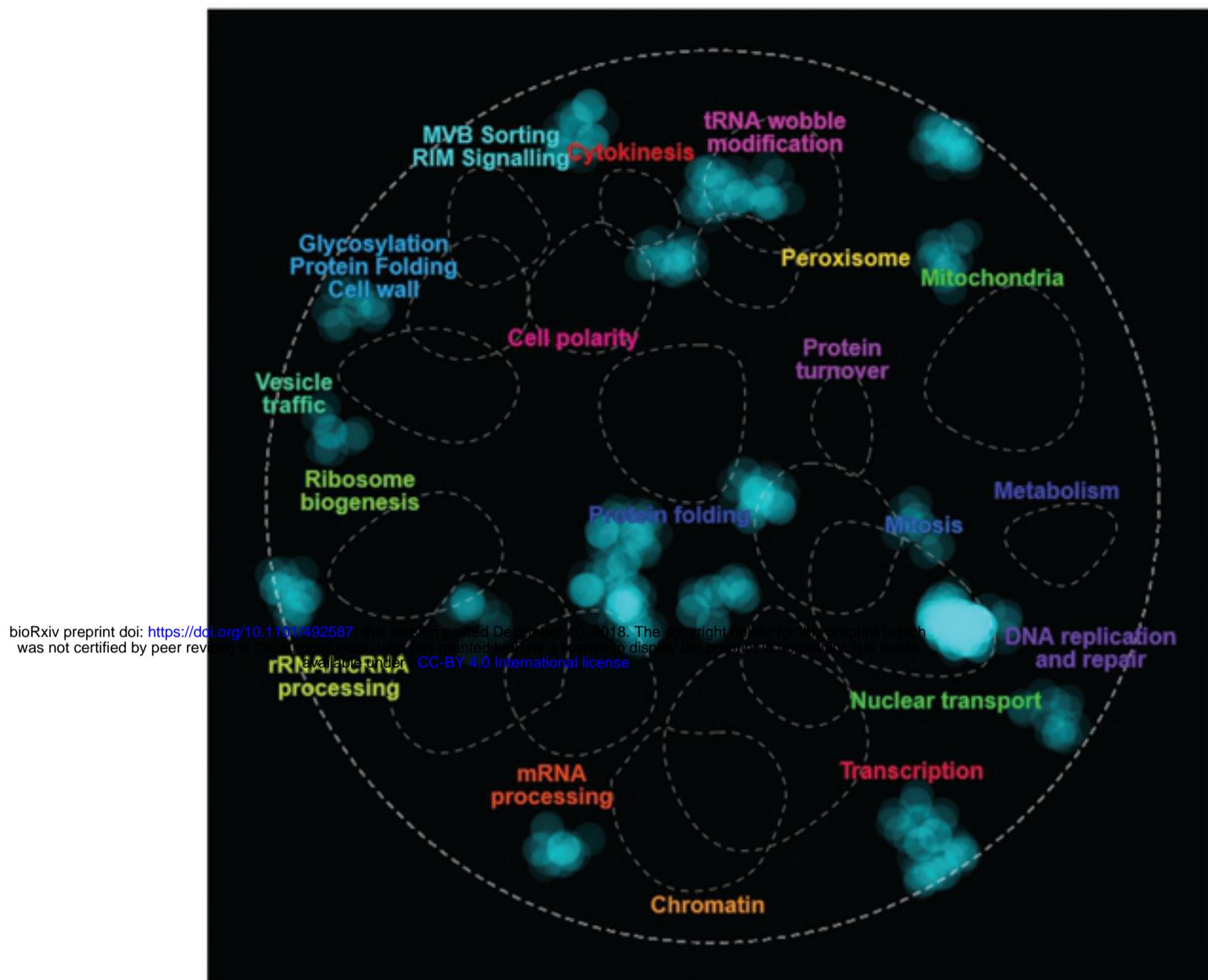


Figure 1

A



B

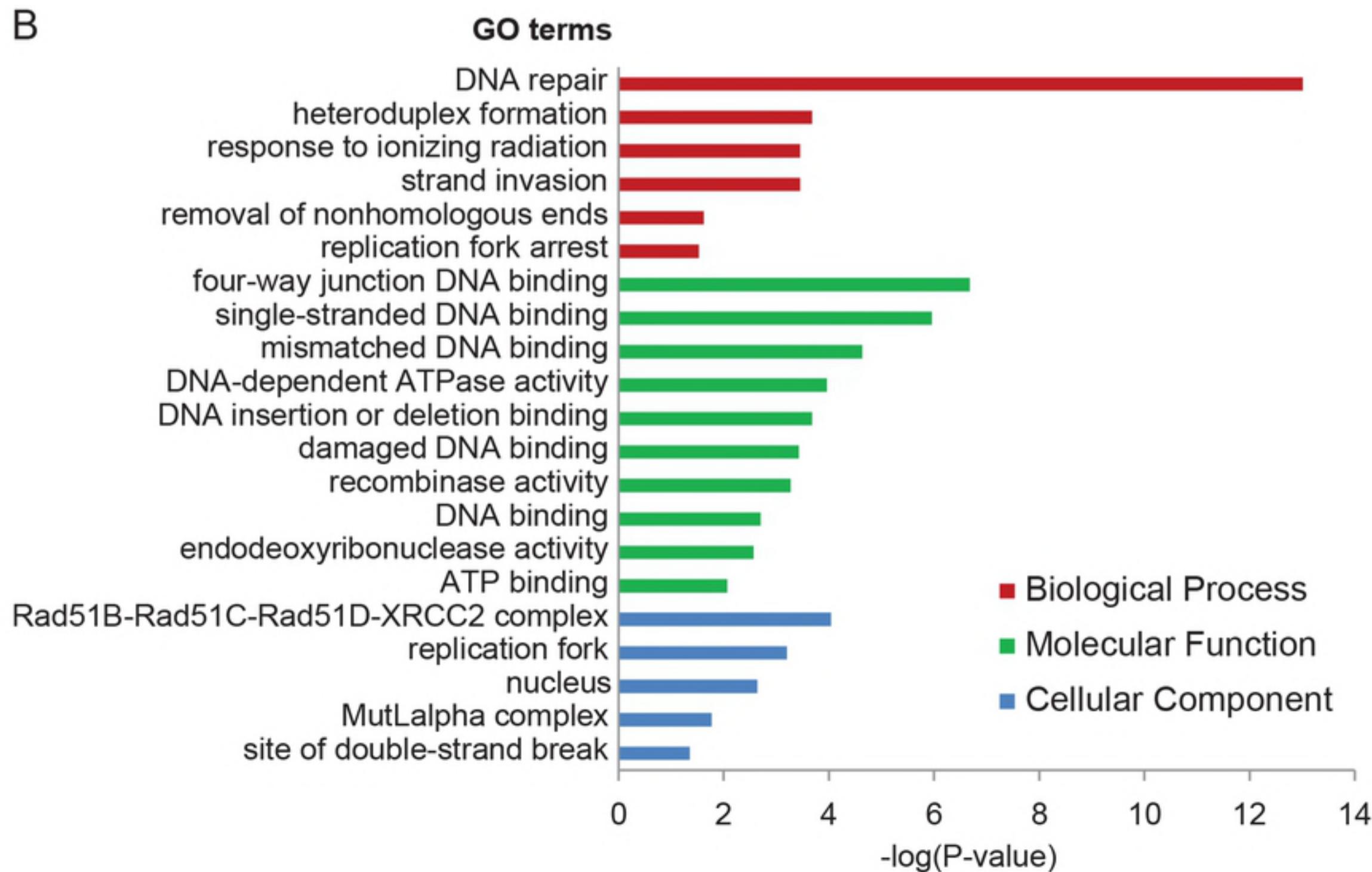
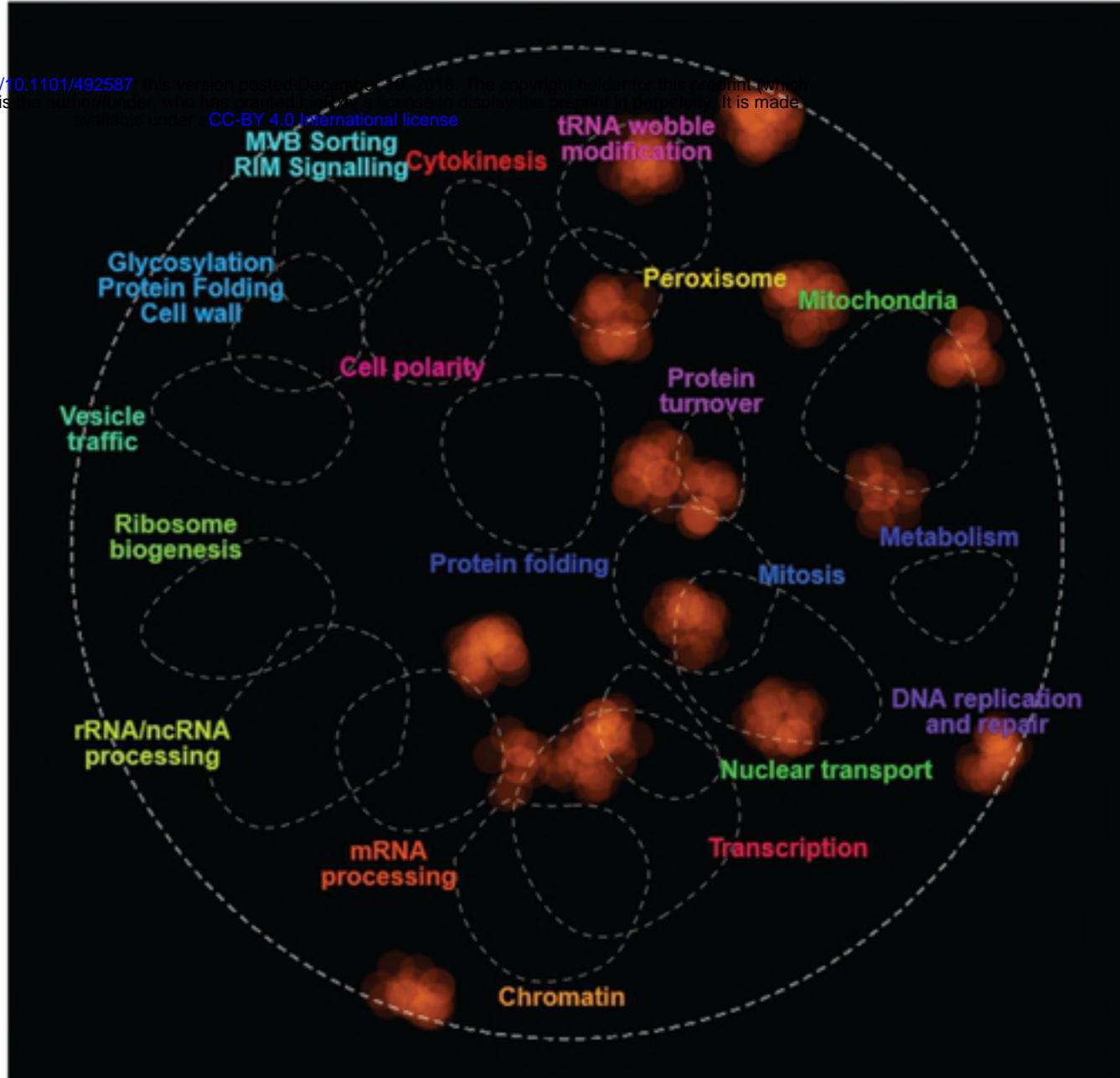


Figure 2

A

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B

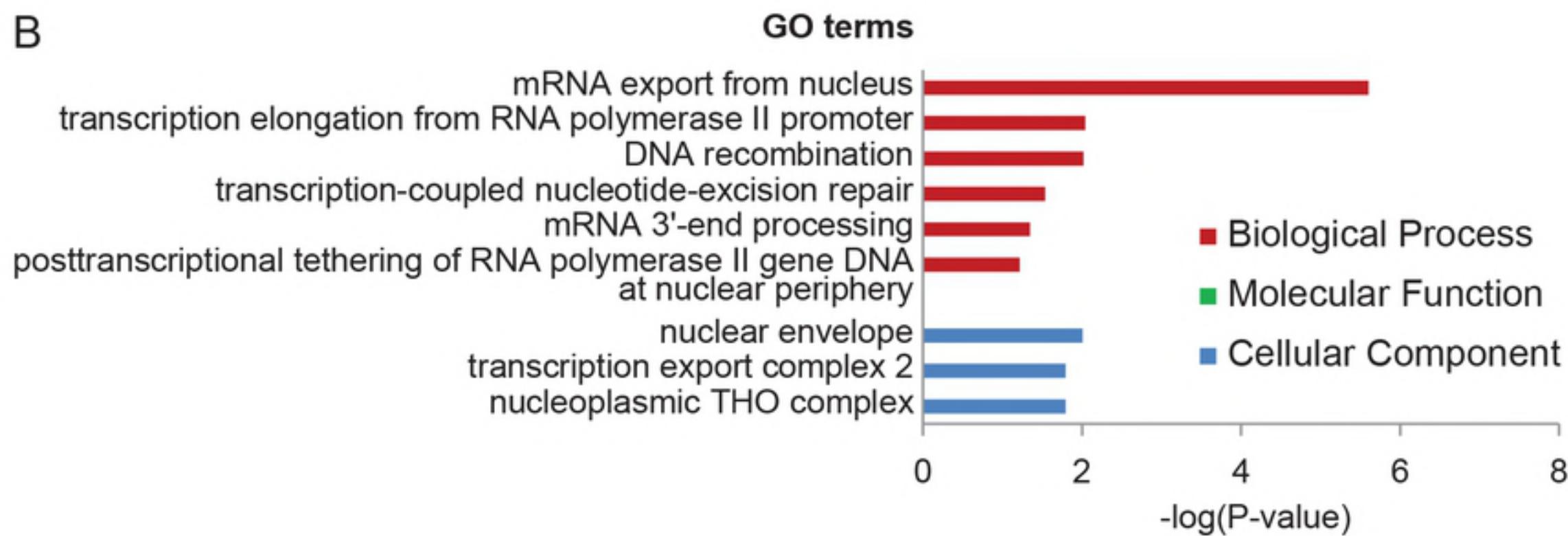


Figure 3

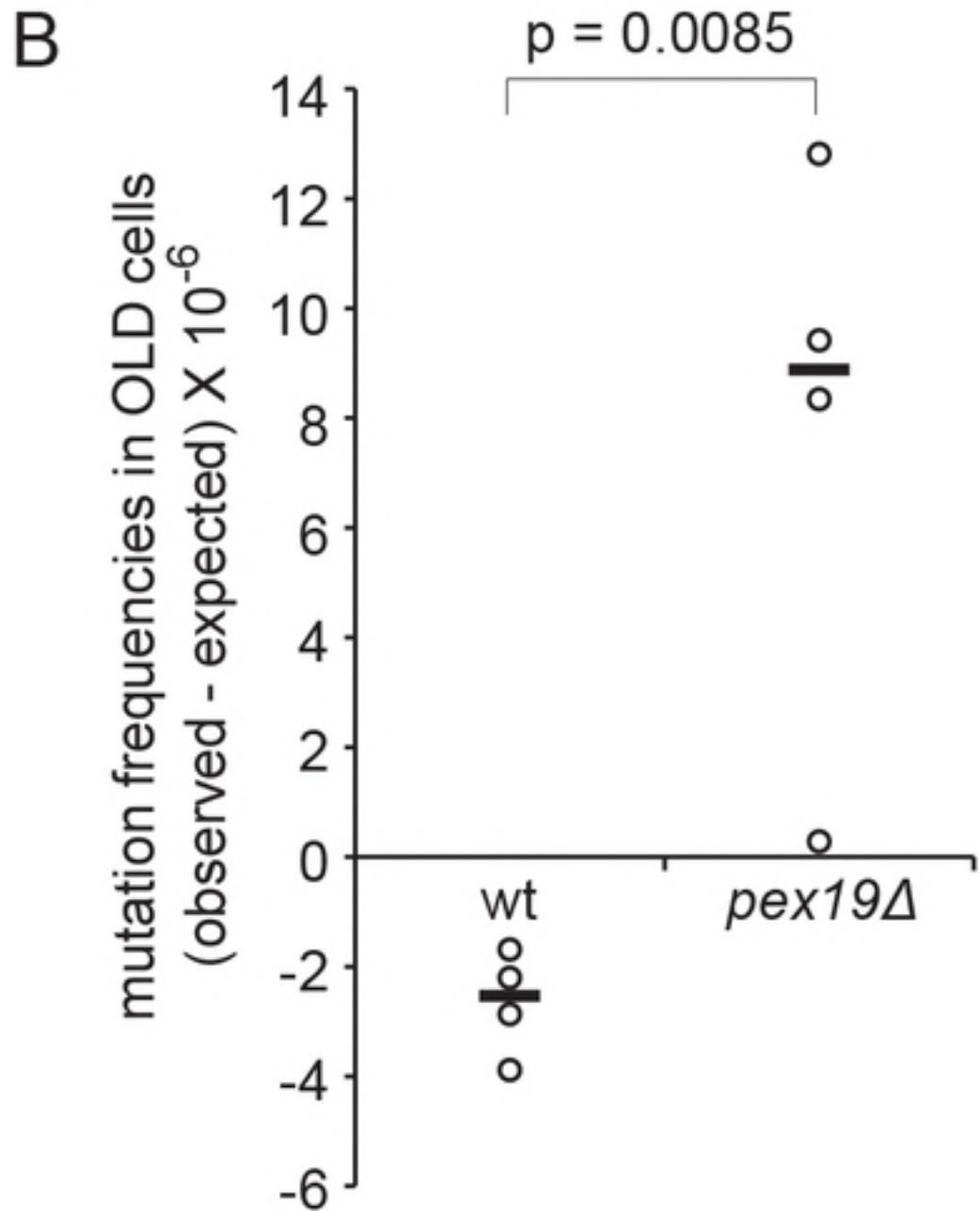
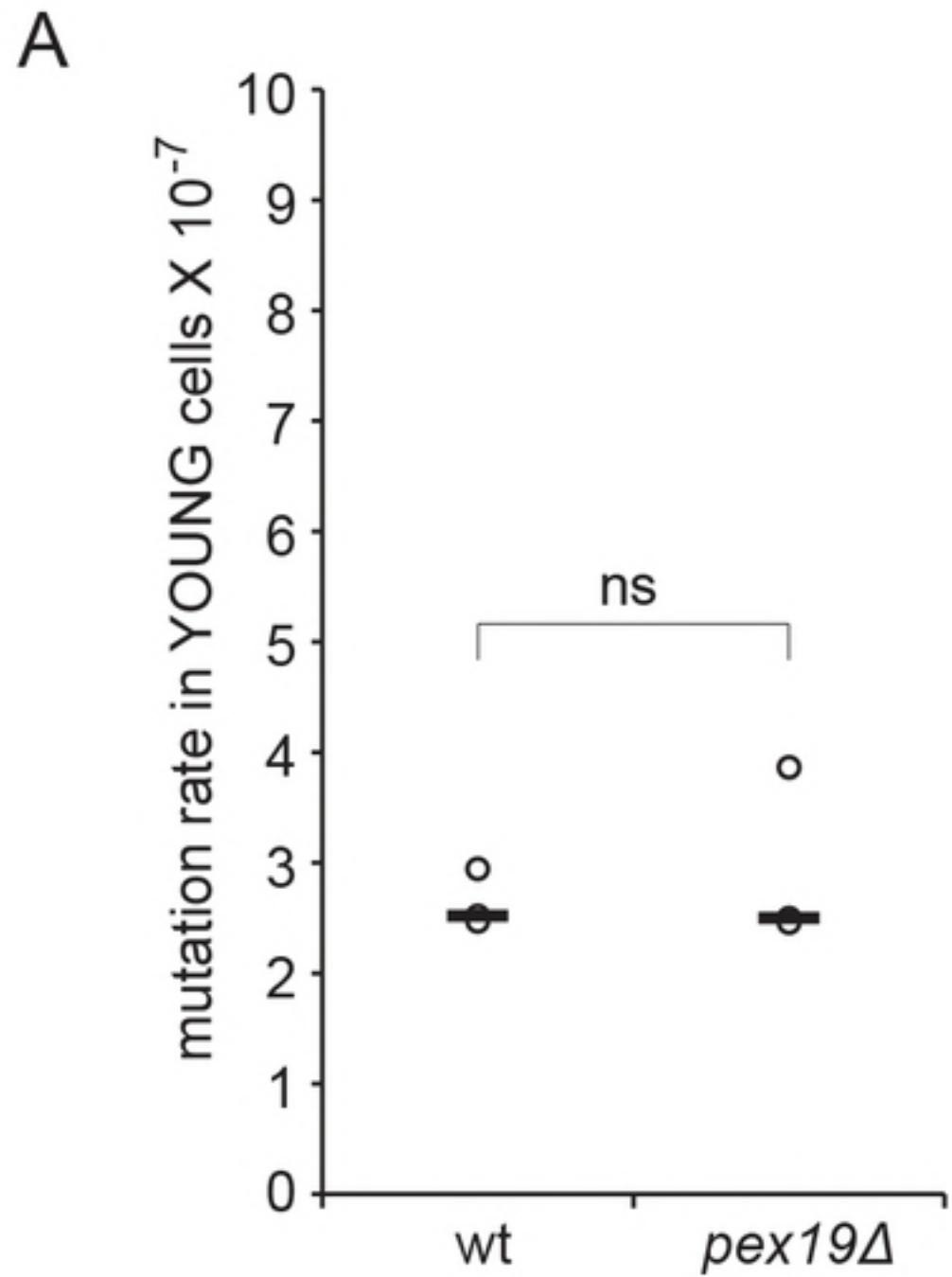


Figure 4