The *wtf* meiotic driver gene family has unexpectedly persisted for over 100 million years

Authors: Mickael De Carvalho[1,2]*, Guo-Song Jia[3,4]*, Ananya Nidamangala Srinivasa[1,5], R. Blake Billmyre[1], Yan-Hui Xu[4], Jeffrey J. Lange[1], Ibrahim M. Sabbarini[1], Li-Lin Du[4,6#], Sarah E. Zanders[1,5#]

* These authors contributed equally

Affiliations:

1 Stowers Institute for Medical Research, Kansas City, MO 64110, USA

2 Open University, Milton Keynes, MK7 6BJ, UK.

3 PTN Joint Graduate Program, School of Life Sciences, Tsinghua University, Beijing, China

4 National Institute of Biological Sciences, Beijing, China

5 Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160, USA

6 Tsinghua Institute of Multidisciplinary Biomedical Research, Tsinghua University, Beijing, China

Corresponding authors:

Li-Lin Du

Address: 7 Science Park Road, Zhongguancun Life Science Park, Beijing 102206, China

Email: dulilin@nibs.ac.cn

Phone: 86-10-80713938

Sarah E. Zanders

1000 E 50th Street

Stowers Institute for Medical Research

Kansas City, MO 64110, USA

Email: sez@stowers.org

Phone: (816) 926-4000

Abstract

Meiotic drivers are selfish elements that bias their own transmission into more than half of the viable progeny produced by a driver+/driver− heterozygote. Meiotic drivers are thought to exist for relatively short evolutionary timespans because a driver gene or gene family is often found in a single species or in a group of very closely related species. Additionally, drivers are generally considered doomed to extinction when they spread to fixation or when suppressors arise. In this study, we examine the evolutionary history of the *wtf* meiotic drivers first discovered in the fission yeast *Schizosaccharomyces pombe*. We identify homologous genes in three other fission yeast species *S. octosporus*, *S. osmophilus*, and *S. cryophilus*, which are estimated to have diverged over 100 million years ago from the *S. pombe* lineage. Synteny evidence supports that *wtf* genes were present in the common ancestor of these four species. Moreover, the ancestral genes were likely drivers as *wtf* genes in *S. octosporus* cause meiotic drive. Our findings indicate that active meiotic drive systems can be maintained for long evolutionary timespans.

Introduction

During meiosis, the two alleles at a given locus segregate from each other and are each transmitted into an equal number of the viable gametes produced by a heterozygous organism. This fundamental rule of genetics is known as Mendel's Law of Segregation (Abbott and Fairbanks, 2016). Most genetic loci follow this law, which facilitates natural selection by allowing alternate variants to compete on an even playing field (Crow, 1991). Meiotic drivers, however, are genetic loci that manipulate gametogenesis to gain an unfair transmission advantage into gametes. Rather than being transmitted to 50% of the gametes produced by a driver+/driver− heterozygote, meiotic drivers are transmitted to most or even all of the functional gametes (Sandler and Novitski, 1957; Zimmering et al., 1970).

Meiotic drivers are found in diverse eukaryotes including plants, fungi and animals (Bravo Núñez et al., 2018b; Burt and Trivers, 2006; Courret et al., 2019a; Lindholm et al., 2016). Despite their broad phylogenetic distribution, drivers in different systems are not thought to share common evolutionary origins. Instead, empirical observations combined with theoretical work have led to the expectation that drivers are evolutionarily short-lived (Burt and Trivers, 2006). Specifically, drivers are believed to have been born repeatedly, but each driver can only persist for a short evolutionary period before extinction, and as a result, drive systems are lineage-specific (Hatcher, 2000; Price et al., 2019).

Understanding the birth of a driver is conceptually straightforward: if a sequence acquires the ability to drive, it can spread in the population (Crow, 1991). The paths to driver extinction are more complex but one route to extinction is through suppression (Bastide et al., 2011; Bravo Núñez et al., 2018a; Carvalho and Vaz, 1999; Courret et al., 2019b; Tao et al., 2007; Unckless et al., 2015). Drive is generally costly to fitness, so natural selection is thought to favor the evolution of drive suppressors unlinked to the drive locus (Cazemajor et al., 1997; Crow, 1991; Finseth et al., 2021; Kumon et al., 2021; Zanders and Unckless, 2019). Suppressed drivers have no transmission advantage and are expected to accumulate inactivating mutations (Burt and Trivers, 2006). In a second path to driver extinction, the driver evades suppression and spreads to fixation. If the driver is on a sex chromosome or the driving haplotype acquires strongly deleterious mutations, driver fixation can lead to driver extinction via host extinction (Dyer et al., 2007; Hamilton, 1967). If the fixed driver is autosomal, it experiences no transmission advantage and can accumulate inactivating mutations, in a fate similar to that of suppressed drivers.

The molecularly identified meiotic drivers largely support the idea that drivers have limited evolutionary lifespans and confined species distribution, with a driver gene or gene family often

only found in a single species (Finseth et al., 2021; Lindholm et al., 2016; Lyon, 2003; Price et al., 2019; Zanders and Johannesson, 2021). In *Drosophila*, for example, the sister species *D. melanogaster* and *D. simulans* shared a common ancestor only 5.4 million years ago (Tamura, 2003), but they each contain distinct meiotic drive systems (Cazemajor et al., 1997; Helleu et al., 2016; Larracuente and Presgraves, 2012; Lin et al., 2018; Tao et al., 2007).

There are a few known exceptions where a drive gene is found in more than one species. For example, sequences homologous to the *Dox* driver of *Drosophila simulans* are also found in *D. mauritiana* and *D. sechellia* (Muirhead and Presgraves, 2021; Vedanayagam et al., 2021). Although there have been more recent introgressions involving *Dox* between *D. simulans* and *D. mauritiana*, sequences homologous to *Dox* appear to have existed 0.2 million years ago in the ancestor of the *D. simulans* clade (Meiklejohn et al., 2018; Muirhead and Presgraves, 2021; Vedanayagam et al., 2021). In rice (*Oryza*), many meiotic drive systems and potential meiotic drive loci have been mapped as sterility loci in crosses between domesticated varieties/species, or between domesticated and wild varieties/species (representing up to ~0.9 million years of divergence). Homologs of genes in these drive systems exist in more distantly related rice species, but whether they are meiotic drivers or precursors of drivers is unclear (Chen et al., 2008; Huang et al., 2015; Koide et al., 2018; Long et al., 2008; Sakata et al., 2021; Shen et al., 2017; Xie et al., 2019, 2017; Yang et al., 2012; Yu et al., 2016). Another crop drive system is the 'knobs' found in maize (*Zea mays*) and its wild relative *Tripsacum dactyloides*. These two species diverged about 1 million years ago (Ross-Ibarra et al., 2009), but drive of knobs has only been conclusively demonstrated in maize (Dawe et al., 2018; Kanizay et al., 2013). In fungi, the *Spok* genes first identified as drivers in *Podospora anserina* are found in several distantly related fungal species. However, horizontal gene transfer is a more likely explanation for the phylogenetic distribution of *Spok* genes than shared decent from a common ancestor (Grognet et al., 2014; Vogan et al., 2021).

In this work, we explore the long-term evolutionary dynamics of drive systems using the recently discovered *wtf* drivers as a model system. *wtf* driver genes are found in all sequenced isolates of the fission yeast *Schizosaccharomyces pombe*. Each sequenced *S. pombe* isolate contains between 4-14 distinct predicted *wtf* driver genes (Eickbush et al., 2019; Hu et al., 2017). The *wtf* drivers are killer meiotic drivers as they act by destroying the meiotic products (spores) that do not inherit the *wtf* driver from a *wtf*+/*wtf*− heterozygote (Figure 1A). To cause selective spore death, each *wtf* driver employs a poison protein and an antidote protein produced from two overlapping transcripts. All developing spores are exposed to the poison, while only spores that inherit the *wtf* drivers express the antidote and are rescued from destruction (Bravo Núñez et al., 2020a, 2020b, 2018a; Hu et al., 2017; Nuckolls et al., 2017).

Here, we analyzed the phylogenetic distribution of *wtf* genes and found highly diverged but homologous *wtf* genes in *Schizosaccharomyces octosporus*, *Schizosaccharomyces osmophilus* and *Schizosaccharomyces cryophilus*, three species that have diverged more than 100 million years ago from the *S. pombe* lineage (Brysch-Herzberg et al., 2019; Rhind et al., 2011). Analyses of synteny support that the *wtf* gene family existed in the common ancestor of *S. pombe* and these three other fission yeast species. Like the *S. pombe wtf* genes, the newly described *wtf* genes exhibit evolutionary signatures of genetic conflict, namely rapid evolution. Moreover, we demonstrate that at least one *wtf* gene in each of the species can encode poison and antidote proteins on overlapping coding sequences. We investigated a subset of the *S. octosporus wtf* genes further and found that some cause meiotic drive when heterozygous. We conclude that *wtf* drivers have been active for over 100 million years. Finally, we present a model in which *wtf* drivers outrun extinction through perpetual gene birth and renewal via recombination mechanisms.

Results

Genes with homology to *wtf* drivers are found in *S. octosporus*, *S. osmophilus* and *S. cryophilus*

As a first step in understanding the long-term evolution of the *wtf* meiotic drivers, we analyzed the phylogenetic distribution of the *wtf* gene family. There are four described *Schizosaccharomyces* species in addition to *S. pombe*: *S. octosporus*, *S. osmophilus, S. cryophilus* and *S. japonicus* (Figure 1B) (Brysch-Herzberg et al., 2019; Rhind et al., 2011). These species are thought to have shared a common ancestor around 200 million years ago. The amino acid divergence of 1:1 orthologs between *S. pombe* and *S. japonicus* is 55%, which is similar to that observed between humans and lancelets (a cephalochordate) (Rhind et al., 2011).

At the time this work was initiated, genome assemblies were available for all species except *S. osmophilus*, which was only recently described (Brysch-Herzberg et al., 2019). We therefore sequenced *S. osmophilus* using both Illumina paired-end reads and Oxford nanopore reads. We used these data to assemble a draft of the *S. osmophilus* genome consisting of 11 contigs. We then predicted the coding sequences of *S. osmophilus* genes using *S. octosporus* annotations as a guide (Figure 1-supplement 1-1 2) (Tong et al. 2019; Hoff & Stanke, 2018). We found that 1:1 orthologs between *S. osmophilus* and *S. octosporus* share 88.9% amino acid identity on average, while *S. osmophilus* and *S. cryophilus* orthologs share 85.2% amino acid identity on average (Xu et al., 2019) (Table S1). Our results are consistent with the previously proposed phylogeny of the fission yeasts that used limited sequencing data from *S. osmophilus* ((Brysch-Herzberg et al., 2019); Figure 1B).

We next searched for *wtf* gene homologs in the genome assemblies of *S. octosporus*, *S. osmophilus, S. cryophilus* and *S. japonicus*. Even within *S. pombe*, the *wtf* genes are diverse and a standard BLAST (Basic Local Alignment Search Tool) search using one *wtf* gene as a query does not identify all members of the family (Altschul et al., 1990). Because of this, we searched for homologs using PSI-BLAST (Position-Specific Iterated BLAST). PSI-BLAST uses the results from an initial search to create a profile of the multi-alignment between the query protein and the best hits. This profile is then used to find other proteins and the iterative process continues until no more significant hits are found (Altschul et al., 1997). Using the protein encoded by *S. pombe wtf4* as an initial query, we were able to find potential *wtf* homologs in *S. octosporus*, *S. osmophilus* and *S. cryophilus* but not *S. japonicus* (Figure 1B). We repeated PSI-BLAST searches using as queries proteins encoded by candidate *wtf* genes from non-*pombe* species (S. *octosporus wtf25, S. cryophilus wtf1 and S. osmophilus wtf14*). These searches all identified *S. pombe* Wtf proteins as hits. None of our PSI-BLAST searches found candidate *wtf* homologs in *S. japonicus* or outside of fission yeasts.

We then used the nucleotide sequences of candidate *wtf* genes as queries to perform additional BLASTn searches to find potential pseudogenes missed by our PSI-BLAST searches. For example, we used the nucleotide sequences of all the *S. octosporus wtf* genes identified by the PSI-BLAST search as queries to search for homologous pseudogenes within *S. octosporus*. Only hits more than 200 base pairs long were considered, although there were additional shorter hits that are likely homologous. We then used sequence alignments of candidate *wtf* genes within each species, and sometimes between species, to refine the predicted coding sequences. In *S. octosporus*, we also generated long-read RNA sequencing data from a meiotic sample to facilitate the delineation of exon-intron boundaries of *wtf* genes.

Overall, we identified 48 predicted *wtf* genes and 35 predicted *wtf* pseudogenes in *S. octosporus*; 31 predicted *wtf* genes and 11 predicted *wtf* pseudogenes in *S. osmophilus;* and 2 predicted *wtf* genes and 3 predicted *wtf* pseudogenes in *S. cryophilus* (Figure 1B; Figure 1-figure supplement 1; Table S2-S4). Previously, 16 intact *wtf* genes and 9 pseudogenes were described in the reference isolate of *S. pombe* (Bowen et al., 2003; Eickbush et al., 2019; Hu et al., 2017). We were concerned that the lack of PSI-BLAST hits in *S. japonicus* could have been due to extensive divergence rather than a lack of potential *wtf* gene homologs. However, a more extensive search not dependent on high sequence homology also failed to find potential *wtf* homologs in *S. japonicus* (see Methods).

Candidate *wtf* genes of *S. octosporus*, *S. osmophilus* and *S. cryophilus* share additional features with *S. pombe wtf* genes

The homology between the *S. pombe wtf* genes and those found in the other *Schizosaccharomyces* species is low (Figure 2-figure supplement 1). For example, the most similar *wtf* gene pair between *S. pombe* (FY29033 *wtf25*) and *S. octosporus* (*S. octosporus wtf56*) shares only 16% amino acid identity, compared to an average of 65.3% amino acid identity between orthologous gene pairs (Table S1 and S5). Given this high divergence, we examined features other than protein sequences to further test if the candidate *wtf* genes are truly members of the *wtf* gene family.

We first looked for similarities in overall gene structure between the *S. pombe wtf* genes and the candidate *wtf* genes in *S. octosporus*, *S. osmophilus* and *S. cryophilus*. The *wtf* genes of *S. pombe* have been classified into three broad categories: predicted meiotic drivers (4-14 per isolate), predicted suppressors of drive that encode only antidote proteins (9-17 per isolate) and genes of unknown function (4 in each isolate) (Bravo Núñez et al., 2020b, 2018a; Eickbush et al., 2019; Hu et al., 2017; Nuckolls et al., 2017). We found that the overall gene structure of the

candidate *wtf* genes in all three species was similar to that of the 5-exon *wtf* drivers and 5-exon *wtf* suppressors in *S. pombe* (Figure 2A). Moreover, the relative sizes of the corresponding exons and introns are remarkably similar between the species, even though the actual sequences are generally quite different (Figure 2-figure supplement 1).

We next looked for similarities between promoters controlling the transcription of the *S. pombe* *wtf* genes and the potential promoters of the candidate *wtf* genes in other species. The promoters of the *S. pombe wtf4* gene are representative of the promoters of *wtf* drivers in *S. pombe* (Nuckolls et al., 2021). The Wtf4^{antidote} protein is encoded on exons 1-6, with the promoter found upstream of exon 1. We found no shared homology between the *S. pombe* *wtf*^{antidote} promoter sequences and sequences upstream of exon 1 in the candidate *wtf* genes found in the other species.

The Wtf4^{poison} protein is encoded on exons 2-6 and the promoter is found within what is intron 1 of the *wtf4*^{antidote} transcript. The *S. pombe wtf4* poison promoter contains a cis-regulatory FLEX motif that is bound by the Mei4 master meiotic transcription factor and is essential for expression of the Wtf4^{poison} protein (Nuckolls et al., 2021). The consensus sequence of the FLEX motif has been defined as GTAAACAAACA(A/T)A(A/C), with the first 11 nucleotides being more invariant (Abe and Shimoda, 2001). All verified *S. pombe wtf* drivers contain in their intron 1 the 11-bp GTAAACAAACA FLEX motif sequence (Nuckolls et al., 2021).

To examine whether Mei4 also regulates the expression of the candidate *wtf* genes outside of *S. pombe*, we first analyzed the conservation of the Mei4-binding motif. We compiled a list of 49 *S. pombe* Mei4 target genes that have 1:1:1:1 orthologs in *S. octosporus*, *S. osmophilus* and *S. cryophilus* (Table S6) and used MEME (Multiple Em for Motif Elicitation) to perform *de novo* motif discovery in 1000-bp sequences upstream of the start codons of this set of genes in each

241 species (Bailey et al., 2015). Manual inspection of the MEME-discovered motifs revealed that
242 the FLEX motif is highly conserved in these four species (Figure 2-figure supplement 2A).
243 Combining the 196 genes from all four species as the input for MEME analysis resulted in a 11-
244 bp motif matching the GTAAACAAACA FLEX motif sequence (Figure 2-figure supplement 2A).
245 This MEME-identified 11-bp motif was submitted to the FIMO (Find Individual Motif
246 Occurrences) tool of the MEME Suite to perform motif scanning in the genomes of the four
247 species using the default *P* value cutoff of 1E-4. The resulting 33089 FIMO hits were classified
248 into unreliable hits (*P* value > 3E-6) and confident hits (*P* value <= 3E-6) based on a comparison
249 of the number of hits in the Mei4 target genes in *S. pombe* and the number of hits in other *S.*
250 *pombe* genes. A total of 2917 confident hits (476, 716, 827 and 898 in *S. pombe*, *S. octosporus*,
251 *S. osmophilus* and *S. cryophilus*, respectively) were found (Tables S7-S8).
252
253 As expected, among the *wtf* genes in the *S. pombe* reference genome, only the four genes that
254 can express the *wtf*^{poison} transcript (*wtf4*, *wtf13*, *wtf19* and *wtf23*) possess confident FIMO hits in
255 intron 1 (Figure 2-figure supplement 2B). Inspecting intron 1 of the candidate *wtf* genes in the
256 other three species showed that, 20 of the 48 intact *wtf* candidate genes in *S. octosporus*
257 possess confident FIMO hits in intron 1, one of the two intact *wtf* candidate genes in *S.*
258 *cryophilus* possesses confident FIMO hits in intron 1, whereas none of the 31 intact *wtf*
259 candidate genes in *S. osmophilus* possesses confident FIMO hits in intron 1 (Figure 2-figure
260 supplement 2B, Tables S2-S4). Thus, the presence of the FLEX motif in intron 1 appears to be
261 a feature conserved in a substantial fraction of candidate *wtf* genes in *S. octosporus*.
262
263 To assess whether the presence of the FLEX motif in intron 1 of *wtf* candidate genes in *S.*
264 *octosporus* is an indication of the ability to express the *wtf*^{poison} transcript, we analyzed our long-
265 read RNA sequencing data of meiotic *S. octosporus* cells. All 48 intact *S. octosporus wtf*
266 candidate genes have long transcripts initiating from upstream of exon 1, and 31 of them also

have detectable short transcripts initiating from within intron 1 (Figure 2B, Figure 2-figure supplement 3, Table S2). Out of 20 intact *S. octosporus wtf* candidate genes with confident FIMO hits in intron 1, 17 have detectable short transcripts initiating from within intron 1. Thus, the presence of the FLEX motif in intron 1 correlates with the expression of short transcripts that likely correspond to the *wtf*^{poison} transcripts (P = 0.016, Fisher's exact test). Furthermore, among the 31 genes with detectable short transcripts, those with higher levels of the short transcript are more likely to harbor the FLEX motif in intron 1, as 9 of the top 10 genes ranked by the expression level of the short transcript contain the FLEX motif, whereas only 2 of the bottom 10 genes contain the FLEX motif (P = 0.0055, Fisher's exact test). Because 14 *S. octosporus wtf* candidate genes without confident FIMO hits in intron 1 nonetheless do have detectable short transcripts initiating from within intron 1, the lack of a conserved FLEX motif in intron 1 does not appear to preclude the expression of the *wtf*^{poison} transcript in *S. octosporus*. It is thus possible that some of the candidate *wtf* genes in *S. osmophilus* may also be able to express the *wtf*^{poison} transcript despite the absence of a conserved FLEX motif in intron 1.

Interestingly, most of the intact *wtf* candidate genes have an in-frame alternate translational start site near the beginning of exon 2, similar to the *wtf* drivers of *S. pombe* (Eickbush et al., 2019; Hu et al., 2017; Nuckolls et al., 2017). The only exceptions are *S. osmophilus wtf16* and S. *cryophilus wtf2*, which appear analogous to the *S. pombe* suppressor *wtf* genes in that they lack an alternate translational start site near the beginning of exon 2 (Figure 1-figure supplement 1) (Bravo Núñez et al., 2018a; Eickbush et al., 2019). No *wtf* candidate genes appeared similar to the unknown class of *S. pombe wtf* genes (Table S5) (Bravo Núñez et al., 2020a; Eickbush et al., 2019). We note that *wtf* candidate genes in *S. octosporus*, *S. osmophilus* and *S. cryophilus* share more homology among themselves than they do with *wtf* genes in *S. pombe* (Table S5).

We conclude based on the amino acid conservation, the conserved gene structure, a conserved promoter feature, and conserved presence of an alternate transcriptional start site in intron 1 and an alternate translational start site near the beginning of exon 2, that candidate *wtf* genes we identified in *S. octosporus*, *S. cryophilus* and *S. osmophilus* are members of the *wtf* gene family. We, therefore, will henceforth refer to them as *wtf* genes.

wtf genes in _S. octosporus_, _S. osmophilus_ and _S. cryophilus_ are associated with dispersed 5S rDNA sequences.

The *S. pombe wtf* genes derive their names from their association with solo long terminal repeats (LTRs) of Tf transposons (with Tf) (Bowen et al., 2003; Wood et al., 2002). Most *S. pombe wtf* genes are flanked on at least one side by a solo LTR (Figure 3; Bowen et al., 2003). A Tf-related full-length transposon was previously discovered in *S. cryophilus* (designated Tcry1) and we found Tf-related full-length transposons in our *S. osmophilus* assembly (Rhind et al., 2011;Table S9). In *S. cryophilus*, none of the 10 solo LTRs are associated with *wtf* genes. In *S. osmophilus,* five out of 36 solo LTRs are associated with *wtf* genes (Figure 3). *S. octosporus* does not contain recognizable transposons (Rhind et al., 2011).

Instead of a close association with transposon sequences, we found that most the *wtf* genes outside of *S. pombe* are closely associated with dispersed 5S rDNA genes (Figure 3, Figure 3-figure supplement 1). In *S. octosporus*, *S. osmophilus* and *S. cryophilus* respectively, 87% (72/83), 79% (33/42) and 40% (2/5) of *wtf* genes are associated with 5S rDNA genes (Table S10). Conversely, 93% (106/114), 55% (59/107) and 3.4% (4/117) of the 5S rDNA genes in these three species respectively are associated with *wtf* genes (Table S10).

In *S. octosporus*, *S. osmophilus* and *S. cryophilus*, we found there is often a gene from an uncharacterized gene family situated between the *wtf* gene and an upstream 5S rDNA gene.

We named this new gene family *wag* for ***w****tf*-**a**ssociated **g**ene (Figure 3; Table S2-S4, S11). Overall, we found that the genomic context of *wtf* genes could be described by a limited number of patterns, including those first identified in *S. pombe* that are largely specific to that species (Figure 3) (Bowen et al., 2003). These patterns likely reflect a few genomic contexts that were duplicated multiple times during the expansion of the gene family as not only the genes, but also the intergenic sequences within a given type of *wtf*-5S rDNA unit or 5S rDNA-*wag*-*wtf* unit are highly similar within a species (Figure 3-figure supplements 2-6).

wtf genes were likely present in the common ancestor of *S. octosporus*, *S. osmophilus*, *S. cryophilus* and *S. pombe*

We next examined whether the *wtf* genes were present in the common ancestor of *S. octosporus*, *S. osmophilus*, *S. cryophilus* and *S. pombe*. The alternate hypothesis is that the *wtf* genes were transferred between the species by horizontal gene transfer or by introgression. Horizontal gene transfer does occur in fission yeast but the possibility of cross-species introgression is unclear (Dawe et al., 2018; Jeffares et al., 2017; Rhind et al., 2011; Seike et al., 2019, 2015; Sipiczki, 1979; Sipiczki et al., 1982).

At the genome level, synteny is limited between *S. pombe* and non-*pombe* fission yeast species (Rhind et al., 2011). However, if the *wtf* gene family was vertically inherited from a common ancestor, it is possible that we may find one or more *wtf* loci that exhibit synteny between *S. pombe* and at least one non-*pombe* species. We therefore inspected the genes flanking *S. pombe wtf* genes to look for situations where orthologous genes in another species also flanked a *wtf* gene (Table S12). We found that in *S. octosporus*, *S. osmophilus* and *S. pombe*, one or more *wtf* genes are present between the *clr4* and *met17* genes (Figure 4A). This shared synteny could reflect that the ancestor of these species contained a *wtf* gene between *clr4* and

met17, but it could also mean that the whole *clr4-met17* region has undergone horizontal gene transfer or introgression.

To distinguish these possibilities, we analyzed the divergence of the *clr4-met17* region between species. Superficially, the region appears quite divergent, with multiple genes gained and/or lost in different lineages. This observation supports a long period of divergence that would be expected if the region descended from the common ancestor of these species. We next analyzed the divergence more precisely. Given the extremely rapid evolution of the *wtf* genes (Eickbush et al., 2019), we thought that the flanking genes would prove most informative. If the region was recently transferred between lineages by horizontal gene transfer, it was possible there may be two copies of *clr4* and/or *met17* in the recipient genome. *met17* has an ancient paralog (*SPAC23A1.14c*) present in all fission yeast species, but we found no evidence of recent duplications of *met17* or *clr4.* We also reconstructed phylogenies of the fission yeast *clr4* and *met17* genes and found that the gene trees were consistent with the species trees (Figure 4B-C). If the genes had been transferred between species, for example from the lineage leading to *S. pombe* to the lineage leading to *S. octosporus* and *S. osmophilus*, the gene tree should reflect that pattern. In this example, the *S. octosporus* and *S. osmophilus clr4* and *met17* genes should group with the *S. pombe* genes on trees, rather than with the *S. cryophilus* genes as we observed. In addition, the percent amino acid divergence we observed in pairwise comparisons between the orthologs revealed divergences similar to the average percent divergences between the species, except for *met17* of *S. octosporus*, which may have gained an intron and diverged extensively (Table S14). Together, our analyses are consistent with vertical transmission of *clr4* and *met17* and the *wtf* genes between them. This suggests the ancestor of *S. octosporus*, *S. osmophilus*, *S. cryophilus* and *S. pombe* had a *wtf* gene between *clr4* and *met17* and that the *wtf* gene was lost in the lineage leading to *S. cryophilus* (Figure 4A). We found additional shared synteny between *S. pombe wtf6* and *S. cryophilus wtf4*. Again,

370 phylogenetic evidence is consistent with a *wtf* gene being present at that locus in the ancestor
371 of *S. octosporus*, *S. osmophilus*, *S. cryophilus* and *S. pombe* and being lost in the lineage
372 leading to *S. octosporus* and *S. osmophilus* (Figure 4-figure supplement 1, Table S13-S14).
373
374 There were additional cases where an *S. pombe wtf* gene was flanked on one side by a gene
375 whose ortholog was also flanked by a *wtf* in one of the other species (Table S12). We designate
376 this partial synteny. We found three *S. pombe wtf* loci (*wtf27*, the *wtf30*+*wtf31*+*wtf10* locus, and
377 *wtf33* all in the *S. kambucha* isolate) with partial synteny with *wtf* genes in *S. octosporus* (*wtf4*,
378 *wtf31* and *wtf13;* Table S12) (Eickbush et al., 2019). Amongst those three loci, two were also in
379 partial synteny with *wtf* genes in *S. osmophilus* (*wtf5* and *wtf15*). Altogether, our analyses
380 indicate that *S. pombe*, *S. octosporus*, *S. osmophilus* and *S. cryophilus* inherited *wtf* genes from
381 a common ancestor with multiple *wtf* genes.
382
383 **_wtf_ genes show evolutionary signatures consistent with a history of genetic conflict**
384 We next wanted to determine if the *wtf* genes are ancient meiotic driver genes or if the genes
385 more recently acquired the ability to drive in the lineage leading to *S. pombe*. To address this,
386 we first analyzed the evolutionary history of the gene family in more depth. Meiotic drivers are
387 predicted to be rapidly evolving and the *S. pombe wtf* genes support this prediction (Eickbush et
388 al., 2019; Hu et al., 2017). This rapid evolution is thought to be driven by the genetic conflict
389 predicted to exist between meiotic drivers and the rest of the genome. The conflict arises
390 because the best interest of the driving haplotype (i.e. drive) is at odds with the best interest of
391 the rest of the genome, which is Mendelian allele transmission (Crow, 1991). The driving
392 haplotype gains an evolutionary advantage by driving, but this is generally bad for the fitness of
393 the organism (Zanders and Unckless, 2019). The rest of the genome therefore gains an
394 evolutionary advantage by suppressing drive. This leads to rapid evolution due to an

395 evolutionary arms race between the drive locus and unlinked genomic suppressors where each
396 side must constantly innovate (McLaughlin and Malik, 2017).
397
398 In *S. pombe*, the evolutionary innovation of *wtf* genes stems from gene duplications, expansion
399 and contraction of tandem repeats within the coding sequences, and extensive non-allelic gene
400 conversion within the family (Eickbush et al., 2019; Hu et al., 2017). We looked for similar
401 evidence of rapid evolutionary innovation in the *wtf* genes outside of *S. pombe.* As a first step,
402 we built a maximum likelihood phylogeny of intact *wtf* genes from all four species. For *S.*
403 *pombe*, we used the genes from the FY29033 isolate as it contains more intact *wtf* genes than
404 the reference genome strain. We also excluded genes from the unknown functional class of *S.*
405 *pombe* (*wtf7*, *wtf11*, *wtf14* and *wtf15*) because these genes are widely diverged from each other
406 and all other *wtf* genes. We observed that the *S. pombe wtf* genes grouped together in a well-
407 supported clade (Figure 5).
408
409 For the other three species, *wtf* genes did not cluster into species-specific monophyletic clades
410 (Figure 5). The *S. cryophilus* genes were found distributed within clades of *S. osmophilus*
411 genes. 37 *S. octosporus* genes grouped together in a well-supported clade. The remaining 11
412 *S. octosporus* genes grouped together within a well-supported clade that includes 2 *S.*
413 *osmophilus* genes (Figure 5). Interestingly, this clade of 13 genes contains most (11/14) of the
414 *S. octosporus wag*-associated intact *wtf* genes and two *S. osmophilus* genes in the clade are
415 also *wag*-associated. Overall, these patterns are consistent with a history dominated by
416 species-specific duplications and/or species-specific homogenization mediated by non-allelic
417 gene conversion.
418
419 We next explored the variation of *wtf* gene numbers to address if the variation is due to
420 extensive overall gene loss since these genes diverged from a common ancestor, variable

levels of gene duplication between lineages, or a more complex combination of gene gains and losses. To explore these possibilities, we first returned to our analyses of synteny. If gene loss was the predominant driver of variation in *wtf* gene number, we would expect to find that the *wtf* genes were usually found at a site that also contains a *wtf* gene in one or more additional species. Novel *wtf* gene duplications are more likely to be lineage specific. As described above, all but five *wtf* loci found in *S. pombe* exhibit no synteny in other species (Table S12). Similarly, there are 31, 12, and 2, species-specific *wtf* loci in *S. octosporus*, *S. osmophilus* and *S. cryophilus*, respectively (Table S15). These observations are consistent with novel gene duplications occurring in the lineages leading to all four species. Independent expansions are additionally supported by the different genomic contexts of the *wtf* genes in *S. pombe* (Tf-association) and the other species (*wag* and/or 5S rDNA-association). Gene losses are also likely within all lineages, as mentioned above for the loss of ancestral *wtf* gene(s) between *met17* and *clr4* in *S. cryophilus* (Figure 4A).

We next looked for signatures of non-allelic gene conversion within the newly discovered *wtf* genes. We started with genes found in synteny with a *wtf* gene in another lineage. These genes should be orthologous and thus group together in a well-supported clade. Non-allelic gene conversion, however, can overwrite genes and thus cause them to be more similar to *wtf* genes at ectopic sites. We focused on *S. octosporus* and *S. osmophilus* as these two species are most closely related and there are a large number—26—*wtf* loci showing synteny between these two species (Table S16-S17). We found that none of the genes from syntenic loci group together in a well-supported clade that excludes genes from other loci (Figure 5, Figure 5-source data 1). This suggests gene conversion has frequently overwritten genes in one or both of these two lineages.

We next analyzed all the genes within *S. octosporus* and *S. osmophilus* for signatures of gene conversion using the GARD (Genetic Algorithm Recombination Detection) program (Kosakovsky Pond et al., 2006a). This program builds multiple phylogenetic trees using different segments of genes. If the entire gene shares the same evolutionary history, the trees constructed from different parts of the genes should be the same. Ectopic gene conversion, however, can shuffle variation within a gene family and lead to differences between trees constructed from different parts of the genes. Consistent with the patterns described above, GARD detected evidence of non-allelic gene conversion within both *S. octosporus* and *S. osmophilus* (Figure 5-figure supplement 1).

Finally, we looked for potential evolutionary innovation due to expansion and contraction of tandem repeats within the coding sequences of the newly identified *wtf* genes. Exon 6 of some *S. pombe wtf* genes encodes a 7 amino acid sequence that can be repeated in tandem multiple times (Eickbush et al., 2019). An *S. pombe wtf* gene can drive without this sequence, but the number of repeat units found can be important for conferring specificity between a Wtf^{poison} protein and the $Wtf^{antidote}$ protein that neutralizes it (Bravo Núñez et al., 2018a; Nuckolls et al., 2020). The sequence is thus important for functional innovation of drivers and suppressors. We looked for amino acid repeats in our candidate *wtf* homologs and found a 7 amino acid sequence that was repeated a variable number of times in tandem in exon 4 of genes from *S. octosporus* and *S. osmophilus*. We generated sequence logos to visualize both the nucleotide sequences and amino acid sequences of the repeat in each species (Figure 5-figure supplement 2, Table S18). We found that the repeat sequences were similar in all three species, consistent with shared ancestry (Figure 5-figure supplement 2). For example, the *S. pombe* and *S. osmophilus* repeats both have IGNXXXG as the most common amino acid sequence. The region containing this 7-amino-acid repeat exhibits similar length variability in three species (Figure 5-figure supplement 2). Hence, like the *S. pombe wtf* drivers, the *wtf*

drivers of *S. octosporus* and *S. osmophilus* show signatures of evolutionary innovation via expansion and contraction of a repetitive coding sequence. Together with previous analyses of *S. pombe*, our analyses demonstrate an extensive history of evolutionary innovation within the *wtf* genes. This is consistent with the hypothesis that these genes have a long history as meiotic drivers.

wtf genes duplicated to pre-existing 5S rDNA genes

Given their association with dispersed 5S rDNA genes, we hypothesized that the *wtf* genes in the lineages leading to *S. octosporus*, *S. osmophilus* and *S. cryophilus* may have duplicated to pre-existing 5S rDNA genes. We propose two recombination models by which this could happen, ectopic gene conversion and integration of extrachromosomal DNA circles (Figure 6-Figure supplement 1A and B) (Cohen et al., 2010, 1999, 2006; Cohen and Segal, 2009; Daugherty and Zanders, 2019; Navrátilová et al., 2008; Paulsen et al., 2018). Under both models, lineage-restricted *wtf* loci flanked with two 5S rDNA genes (e.g., Species A in Figure 6A) are predicted to have synteny with loci containing a single 5S rDNA gene and no *wtf* genes in other species (e.g., Species B in Figure 6A). To test this, we first looked at sites where the *S. octosporus* locus contains a 5S-rDNA-flanked *wtf* gene, but the syntenic loci in *S. cryophilus* and *S. osmophilus* do not. There are 6 such *wtf* loci. In 83% (5 out of 6) of those sites, the *S. cryophilus* and *S. osmophilus* loci contain a 5S rDNA gene (Table S17 and S19). This is consistent with *wtf* genes duplicating to pre-existing 5S rDNA genes.

We saw similar evidence of *wtf* gene duplication to pre-existing 5S rDNA genes when we considered other species comparisons (Figure 6B). For example, we found that in 11 out of 12 sites where *S. osmophilus* has a 5S-rDNA-flanked *wtf* gene but *S. cryophilus* has no *wtf* genes, there is a 5S rDNA gene in *S. cryophilus* (Figure 6B, Table S19). Overall, these analyses support the hypothesis that *wtf* genes spread to pre-existing 5S rDNA genes in the lineages

leading to *S. octosporus* and *S. osmophilus*. It is important to note, however, that lineage-specific loss of 5S rDNA-associated *wtf* genes could, and likely do, also contribute to the patterns described above.

wtf genes in *S. octosporus*, *S. osmophilus* and *S. cryophilus* encode poison and antidote proteins

We next examined whether there was functional conservation between the *wtf* genes. There are relatively few genetic tools available in fission yeasts outside of *S. pombe*. We therefore first tested the functions of the genes outside of their endogenous species. We previously demonstrated that the *S. pombe* Wtf4^{poison} and Wtf4^{antidote} proteins can act in the budding yeast, *Saccharomyces cerevisiae*. Specifically, expression of the Wtf4^{poison} protein kills vegetative *S. cerevisiae* cells, and co-expression of the Wtf4^{antidote} protein neutralizes the toxicity of the Wtf4^{poison} protein (Nuckolls et al., 2020). We used this system to test if the *wtf* genes from the other fission yeast species also encode poison and antidote proteins.

We cloned coding sequences of the putative poison (encoded by exons 2-5) and antidote (encoded by exons 1-5) proteins of *S. octosporus wtf25* and *wtf61*, *S. osmophilus wtf19 and wtf41* and *S. cryophilus wtf1* under the control of a β-estradiol-inducible promoter on separate plasmids. We then introduced the plasmids into *S. cerevisiae* and analyzed the phenotypes of the resulting strains. We found that induction of each of the putative Wtf^{poison} proteins, except *S. osmophilus wtf19*, inhibited cell proliferation in *S. cerevisiae* (Figure 7A-C, Figure 7-figure supplement 1). Moreover, the toxicity of each functional Wtf^{poison} protein was partially neutralized by co-expression of the cognate (i.e., encoded on the same gene) Wtf^{antidote} proteins (Figure 7A-C; Figure 7-figure supplement 1B).

In *S. pombe*, the Wtf^{antidote} protein encoded by one *wtf* gene generally cannot neutralize the Wtf^{poison} protein encoded by a different *wtf* gene (Bravo Núñez et al., 2020b, 2018a; Hu et al., 2017). Instead, a high level of sequence identity appears to be required for a Wtf^{antidote} protein to co-assemble with and neutralize a Wtf^{poison} protein (Nuckolls et al., 2020). We tested if this feature was shared with *wtf* genes outside of *S. pombe*. We tested proteins from five pairs of *wtf* genes. Excluding the antidote protein-specific residues encoded in exon 1, the proteins encoded by each pair share from 13-76% amino acid identity. Like our previous observations in *S. pombe*, we found that Wtf^{antidote} proteins did not neutralize non-cognate Wtf^{poison} proteins (Figure 7-figure supplement 2A-E).

We imaged tagged versions of the *S. octosporus* Wtf25 proteins to see if the localization of the proteins in *S. cerevisiae* was similar to what we previously observed for *S. pombe* Wtf4 proteins. *S. octosporus* Wtf25^{poison}-GFP and Wtf25^{antidote}-mCherry were both functional (Figure 7A). *S. octosporus* Wtf25^{poison}-GFP was distributed throughout the cytoplasm, with some potential endoplasmic reticulum localization, similar to what we previously observed for *S. pombe* Wtf4^{poison}-GFP (Figure 7D, Figure 7-figure supplement 3). The *S. octosporus* Wtf25^{antidote}-mCherry localization was more restricted. We observed large aggregates outside the vacuole and some signal inside the vacuole (Figure 7E, Figure 7-figure supplement 3). This is slightly different from our previous observations with *S. pombe* Wtf4^{antidote} as that protein mostly accumulated outside the vacuole in the insoluble protein deposit, with less Wtf4^{antidote} protein observed within the vacuole (Nuckolls et al., 2020)

When the *S. octosporus* Wtf25^{poison}-GFP and Wtf25^{antidote}-mCherry proteins were co-expressed, we observed some colocalization of the proteins (Figure 7F, Figure 7-figure supplement 3). The colocalized proteins appear to be trafficked to the vacuole. These localization patterns are similar to our previous observations of *S. pombe* Wtf4 proteins where the Wtf4^{antidote} co-

549 assembles with the Wtf4^{poison} and causes a change of localization of the Wtf4^{poison} protein. With
550 *S. pombe* Wtf4 proteins, however, the co-expressed poison and antidote proteins mostly
551 accumulate outside the vacuole at the insoluble protein deposit, with less protein entering the
552 vacuole (Nuckolls et al., 2020). Overall, our results are consistent with broad, but not absolute,
553 functional conservation of the Wtf proteins, despite extensive amino acid divergence.
554
555 **_Wtf_ genes can cause meiotic drive in _S. octosporus_**
556 We next formally tested if *wtf* genes could cause meiotic drive outside of *S. pombe* using *S.*
557 *octosporus*, which among *S. octosporus*, *S. osmophilus* and *S. cryophilus* is the only one with
558 available genetic tools (Seike and Niki, 2017). According to our long-read RNA-seq data, only a
559 small fraction of *wtf* genes in *S. octosporus* have substantial levels of the short transcript
560 isoform (poison isoform) initiated from within intron 1 (Figure 2-figure supplement 3). We
561 preferentially tested such genes as we reasoned that a sufficiently high expression level of the
562 poison is essential for drive.
563 We successfully deleted seven *wtf* genes (*wtf25*, *wtf68*, *wtf33*, *wtf60*, *wtf46*, *wtf21* and *wtf62*, in
564 the order of decreasing expression levels of the poison isoform) in heterothallic haploid strains
565 of both mating types. No growth phenotypes were observed for these deletion mutants. We then
566 analyzed whether any of the deletions affected viability of spores derived from homozygous and
567 heterozygous crosses using octad dissection analysis (*S. octosporus* generates eight spores
568 per meiosis due to a post-meiotic mitosis prior to spore packaging (Chiu, 1996)).
569
570 In homozygous crosses, none of the deletions significantly altered spore viability comparing to
571 the wild-type control (Figure 8, Table S20). Thus, like previous observations for *S. pombe wtf*
572 genes (Bravo Núñez et al., 2018a; Hu et al., 2017; Nuckolls et al., 2017), these seven *S.*
573 *octosporus wtf* genes are not required for mating, meiosis, or sporulation. In heterozygous
574 crosses, deletion of *wtf25, wtf68* or *wtf33* caused notable and significant spore viability reduction

575 (> 5% spore viability reduction and $P < 0.05$, Fisher's exact test) and also resulted in significant
576 allele transmission bias against the *wtf* deletion allele relative to the wild-type *wtf*+ allele ($p <$
577 0.05, exact binomial test; Table S21-23, Figure 8-9, Figure 9-Figure supplement 1-2). These
578 results indicate that *wtf25*, *wtf68*, and *wtf33* are active meiotic drivers.
579
580 To further explore the octad dissection data, we classified octads derived from heterozygous
581 crosses according to the number of viable spores with a *wtf* gene deletion ("R", antibiotic
582 resistant) and the number of viable spores without a *wtf* gene deletion ("S", antibiotic sensitive)
583 in an octad. For example, an octad with 7 viable spores can be classified as either the 4R3S
584 type or the 3R4S type. If spore viability is not affected by *wtf* gene deletion, the ratios of 4R3S to
585 3R4S, 4R2S to 2R4S, 4R1S to 1R4S, and 4R0S to 0R4S should be about 1:1. For *wtf25*, *wtf68*,
586 and *wtf33*, the three genes deemed as active meiotic drivers based on the analysis of overall
587 spore data, most of these octad type ratios significantly deviate from 1:1 ($P < 0.05$, exact
588 binomial test; Figure 9C, Figure 9-figure supplements 1-2). The 4R2S to 2R4S ratio of *wtf60* and
589 the 4R3S to 3R4S ratio of *wtf46* also significantly deviate from 1:1 (Figure 9-figure supplement
590 3-4, Tables S24-S25), suggesting that *wtf60* and *wtf46* may have weak meiotic driver activities.
591 *wtf21* and *wtf62* did not cause significant deviation of octad type ratios (Figure 9-figure
592 supplements 5-6, Table S26-S27), consistent with the low expression levels of the poison
593 isoforms of these two genes (Figure 2-figure supplement 3). In fact, the levels of allele
594 transmission bias favoring the *wtf*+ allele appear to be correlated with the expression levels of
595 the poison isoform (Figure 9D).
596
597 **_S. octosporus wtf25_ is a poison and antidote meiotic driver**
598 To determine whether an active *wtf* gene in *S. octosporus* can cause meiotic drive at an ectopic
599 genomic locus, we constructed an integrating plasmid carrying a 2.5-kb genomic region
600 containing *wtf25* together with its upstream and downstream flanking 5S rDNA genes and

integrated the plasmid at the *leu1* locus in the *wtf25* deletion background. Octad dissection analysis indicated that *wtf25* integrated at the *leu1* locus can act as a meiotic driver in a heterozygous cross (*leu1Δ::wtf25*/*leu1*) and the level of meiotic drive was comparable to that caused by the endogenous *wtf25* gene (Figure 10B). This result indicates that *S. octosporus wtf25* can act in a locus-independent manner like the *S. pombe wtf* drivers.

wtf25 can express a long transcript isoform and a short transcript isoform through alternative transcriptional initiation (Figure 2B). Based on what is known about the *S. pombe wtf* genes and our analyses of *S. octosporus wtf25* in *S. cerevisiae* (Figure 7), we hypothesized that the long and short isoforms encode antidote and poison proteins, respectively (Hu et al., 2017; Nuckolls et al., 2017). We introduced point mutations into the predicted start codons of the long and short isoforms of *wtf25* integrated at the *leu1* locus and analyzed the effects of the mutations on spore viability (Figure 10A). The mutant with the predicted start codon in the short transcript isoform mutated (ATG to GCG, methionine to alanine), referred to as $wtf25^{antidote\text{-}only}$, was unable to kill spores not inheriting it in a *wtf25* deletion background (Figure 10B, Table S28). This supports our hypothesis that the short transcript encodes a spore-killing poison.

Analogously, we mutated the predicted start codon in the long transcript isoform and generated the $wtf25^{poison\text{-}only}$ mutant allele (Figure 10A). We could not obtain transformants of the plasmid carrying this mutant allele in the *wtf25* deletion background, possibly due to self-killing. Instead, we integrated the plasmid at the *leu1* locus in the wild-type background and crossed the resulting strain to a *wtf25Δ* strain. As a control, we integrated an empty vector at the *leu1* locus in the wild-type background and crossed the resulting strain to a *wtf25Δ* strain. Compared to the control, *wtf25Δ* spores (spores not inheriting the wild-type *wtf25* at the endogenous locus) derived from diploids carrying the $wtf25^{poison\text{-}only}$ allele suffered markedly more severe viability loss (Figure 10C). Among them, the *wtf25Δ* spores that also inherited the $wtf25^{poison\text{-}only}$ mutant

627 allele at the *leu1* locus were all inviable. These results further support the model that the short
628 isoform encodes a poison protein that confers killing but not protection. In addition, they
629 demonstrate that the long isoform is required for protection against spore killing.
630
631 **Discussion**
632 ***wtf* genes are ancient meiotic drivers**
633 Our analyses indicate that *wtf* genes were present in the common ancestor of *S. octosporus*, *S.*
634 *osmophilus*, *S. cryophilus* and *S. pombe*. As these species are estimated to have diverged ~119
635 million years ago (Rhind et al., 2011), we propose that the *wtf* gene family is over 100 million
636 years old. Our results suggest that the gene family has contained active meiotic driver genes
637 throughout their history in fission yeasts. First, the gene family exhibits several signatures of the
638 rapid evolutionary innovation typified by genes involved in genetic conflicts (Figure 5, Figure 5-
639 figure supplements 1-2); (Burt and Trivers, 2006; McLaughlin and Malik, 2017) . Also, genes
640 from all four species encode both poison and antidote proteins, like the known drivers in *S.*
641 *pombe* (Figure 7). In addition, genes from at least three species contain the FLEX regulatory
642 motif upstream of the open reading frame that encodes a poison protein, suggesting the genes
643 are expressed in meiosis (Figure 2). Our RNA sequencing data confirms this hypothesis in *S.*
644 *octosporus* (Figure 2B, Figure 2-figure supplement 2). And finally, we demonstrate that the *S.*
645 *octosporus* genes cause meiotic drive when heterozygous (Figure 9).
646
647 We have been unable to trace the history of the *wtf* gene family farther back than the ancestor
648 of *S. octosporus*, *S. osmophilus*, *S. cryophilus* and *S. pombe*. It is possible that the genes were
649 born *de novo* within this lineage. Alternately, it is possible the genes may also have entered the
650 lineage via horizontal gene transfer. Distinguishing these possibilities will likely prove difficult.
651 The old age and rapid evolution of the gene family largely restrict our ability to reconstruct the
652 sequence of the ancestral gene(s) with confidence. In addition, given that the genes encode

poison and antidote proteins, it is possible that any potential extant homologs outside of fission yeast will have experienced a history of genetic conflict and could be quite diverged from their ancestral state as well.

Repeat facilitated expansion of the *wtf* gene family

Our results indicate that the ancestor of the four species contained at least 2 *wtf* genes, and the extant species carry between 5-83 *wtf* genes, including pseudogenes. Our analyses are consistent with novel gene duplications occurring in the lineages leading to all four species. The *wtf* genes are compact and can autonomously cause drive. These features likely facilitated their spread within genomes. In this study, we show that non-allelic recombination using repetitive 5S rDNA sequences has likely facilitated the expansion of the *wtf* gene family in *S. octosporus* and *S. osmophilus*. This recombination could be non-allelic gene conversion but could also be crossovers involving extrachromosomal circles as many *wtf* genes are flanked by direct repeats of 5S rDNA genes (Figure 3, Figure 6). The later pathway was recently implicated in the spread of Rsp-like meiotic drive associated sequences in *Drosophila* species (Sproul et al., 2020). The newly formed *wtf* gene duplicates could be maintained at a high rate by selection given their potential to cause drive or to suppress drive of other *wtf* genes with a similar sequence.

It may be relevant that both the Tf LTRs and the dispersed 5S rDNA genes cluster spatially in the nucleus. The Tf LTR transposons are bound by CENP-B family proteins and are clustered to a nuclear domain known as the Tf body in a process that requires the CENP-B family protein Cbp1, the histone lysine H3-K4 methyltransferase Set1 and the Ku protein Pku80 (Johansen and Cam, 2015). The 5S rDNA genes are transcribed by RNA polymerase III (pol III) and cluster with other pol III transcribed-genes within the nucleus (Daulny et al., 2016; Haeusler and Engelke, 2006). Such clustering may promote the duplication of *wtf* genes to novel repeat-associated sites in the genome. The clusters could potentially affect recombination outcomes.

679 Factors found in the clusters could limit crossover recombination events between non-allelic
680 sites that would generate costly chromosome rearrangements. The clusters could also facilitate
681 non-allelic gene conversion that helps enable the rapid evolution of the *wtf* genes (Eickbush et
682 al., 2019; Hu et al., 2017).
683
684 It is also interesting to note that *Rsp-like* sequences of *Drosophila* mentioned above also spread
685 to distributed repetitive sequences that cluster within nuclei (Herbette et al., 2021). Furthermore,
686 genes of the *Dox* gene family in *D. simulans* are associated with satellite DNA 359 which has
687 been proposed to have facilitated expansion of the family (Muirhead and Presgraves, 2021;
688 Vedanayagam et al., 2021). Experimental analyses of the effect of clustered repeats on
689 sequence duplication and ectopic recombination outcomes are required to test these ideas
690 (Muirhead and Presgraves, 2021; Vedanayagam et al., 2021).
691
692 **Model for the long-term persistence of *wtf* drivers**
693 Theoretical models of drive generally consider a single, stationary drive locus (Crow, 1991). The
694 reality of the long-term evolution of the *wtf* drivers is a great deal more complex. The *wtf* drivers
695 are part of a large, rapidly evolving gene family that also includes drive suppressors (Bravo
696 Núñez et al., 2018a). We propose that this complexity creates a cycle of driver death and rebirth
697 analogous to the mythological phoenix (Figure 11). *wtf* drivers are perpetually reborn anew via
698 gene duplication and rapid evolution of existing genes. This rebirth allows the genes to evade
699 extinction by short-circuiting the two main paths to extinction mentioned earlier: extinction
700 following suppression and extinction following fixation. We discuss both paths in more detail
701 below.
702
703 We propose that the number and diversity of the *wtf* drivers create a significant challenge for the
704 evolution of suppressors. The mapped genic suppressors of *wtf* drivers are other *wtf* genes.

Importantly, however, the *wtf*-mediated suppression in all known cases is highly specific in that the antidotes only neutralize poison proteins with amino acid sequences that are highly similar to the antidote. Because of this, even changing two amino acids is sufficient to disrupt the ability of a $Wtf^{antidote}$ to neutralize a Wtf^{poison} (Bravo Núñez et al., 2018a). In addition, the fitness benefits of suppressing one driver are minimized if there are still several active drivers (Bravo Núñez et al., 2020b). It is not yet clear if suppressors could act more broadly against multiple *wtf* drivers. Sequence-directed transcriptional silencing via installation of heterochromatin, similar to that used to control transposons, could be a potential route for widespread silencing of *wtf* drivers (Mizuguchi et al., 2017). However, silencing of *wtf* drivers would not be trivial to evolve for several reasons (Nuckolls et al., 2021). In particular, the regions linked to *wtf* genes benefit from their drive. Because of this, variants that resisted heterochromatin installation and *wtf* silencing would have an advantage over those that were permissive to heterochromatin installation.

Driver fixation is a second route to driver extinction. Population surveys of *wtf* gene diversity are currently available only in *S. pombe* where the entire complement of *wtf* genes has been assembled for 4 isolates (Eickbush et al., 2019; Hu et al., 2017). This limited analysis suggested that the rapid evolution of *wtf* genes makes fixation of any given sequence unlikely. Within this group, there is only one locus (*wtf4*) where all four isolates contain a driver. The sequence of the *wtf4* driver, however, is not fixed. Even the two most similar *wtf4* drivers, from the reference genome (*Sp*) and the *S. kambucha* isolate (*Sk*), are distinct drivers in that the antidote from *Sp wtf4* does not neutralize the poison from *Sk wtf4* and *vice versa* (Nuckolls et al., 2017). The non-allelic gene conversion of *wtf* genes and the expansion and contraction of repetitive coding sequence that largely drive the evolution of the *wtf* genes in *S. pombe* also occurred in the *S. octosporus* and *S. osmophilus* lineages. Those lineages also have many (>20) *wtf* genes, so fixation may also be unlikely within those species. *S. cryophilus*, however, has fewer *wtf* genes

and has lost the repetitive coding sequence shared by the other drivers, so fixation may be more likely in that species.

An alternate hypothesis to explain the long-term persistence of the *wtf* drivers is that the genes are not merely selfish parasites. It is possible that *wtf* drivers promote fitness in some way that we have yet to discover. This theoretical additional function of the *wtf* genes could have promoted their long-term maintenance in fission yeast genomes. It is important to note, however, that genes do not need to promote fitness to be maintained in genomes and there is currently no evidence supporting a role of *wtf* drivers in promoting fitness, except in cases where they suppress other *wtf* drivers.

Other ancient drivers?

Currently, the *wtf* drivers are unique amongst cloned meiotic drivers in both their abundance within genomes and their longevity over evolutionary time. This, however, may very well change. Currently, many meiotic drive loci are unmapped. Many more drivers that confer more subtle transmission biases also likely remain undetected (Wei et al., 2017). As more genomes are fully assembled and more drivers are mapped, it seems plausible that the *wtf* gene family will be joined by other equally abundant and persistent families of meiotic drivers.

Material and Methods

Nanopore sequencing and assembly of the *S. osmophilus* genome

To sequence the *S. osmophilus* genome, we extracted genomic DNA with the QIAGEN Genomic-tip kit. We then used a standard ligation sequencing prep and kit (SQK-LSK109; Oxford Nanopore Technologies), including DNA end repair using the NEB End Prep Enzyme, FFPE prep with the NEB FFPE DNA repair mix, and ligation using NEB Quick Ligase. We

sequenced using two Flongle Sequencers and performed base calling with guppy version 2.1.3. This generated approximately 521 megabases of sequence or approximately 40x coverage. We then performed de novo assembly pathway using canu v1.8 and the ovl overlapper with a predicted genome size of 13 mb and a corrected error rate of 0.12. We corrected our assembly using pilon with paired end illumina data generated with the same DNA. We assembled 11 nuclear contigs with a total length of 11.3 mb and one mitochondrial contig that was 68 kb in length. Assembly statistics were generated using an existing perl script (https://github.com/SchwarzEM/ems_perl/blob/master/fasta/count_fasta_residues.pl). The assembled genome scored at 89% complete with BUSCO v3.0.2 which is comparable to the score for the closely related species *S. octosporus* (Simão et al., 2015). Base called reads were deposited on the SRA under project accession code PRJNA839783.

RNA sequencing and Nanopore cDNA sequencing

Sample preparation

For RNA sequencing and ONT (Oxford Nanopore Technologies) cDNA sequencing of *S. octosporus* diploid cells undergoing azygotic meiosis, we crossed DY44617 and DY44598 on a SPASK plate (1% glucose, 7.3 mM KH2PO4, vitamins, 45 mg/L of leucine, adenine, uracil, histidine and lysine) for about 12 hours. Cells were spread on YES plates (0.5% yeast extract, 3% glucose, 200 mg/L of leucine, adenine, uracil and histidine) containing nourseothricin (NAT) and G418 (YES+NAT&G418) for diploid cell selection. After 3 days, colonies grown up on YES+NAT&G418 plates were collected and spread on YES plates. After 24 hours, cells were washed off from YES plates and spread on SPASK plates for azygotic meiosis induction. Approximately 5 OD_{600} units of cells were harvested and snap frozen using liquid nitrogen 19 hours after the start of meiosis induction.

RNA extraction

All collected cells were thawed on ice for about 5 minutes and then washed once with chilled DEPC water. The cell pellets were resuspended with TES buffer (10 mM Tris pH 7.5, 10 mM EDTA pH 8.0, 0.5% SDS), and mixed with acidic phenol-chloroform (1:1) immediately. The samples were incubated in a 65ºC heat block for 1 hour. Then the samples were centrifuged at 4ºC, and the aqueous phase was collected. The aqueous phase was then treated with phenol-chloroform (1:1) and chloroform:isoamyl alcohol (24:1) successively. 3 M NaAc (pH 5.2) and isopropanol were added to the aqueous phase and mixed thoroughly by inverting. The mixture was stored at -20ºC overnight and then centrifuged at 4ºC. After centrifuging, the supernatants were removed, and the RNA pellets were washed with 70% ethanol. RNA samples were dissolved in DEPC water after air-drying.

RNA sequencing

For RNA sequencing, we prepared total RNA from *S. octosporus* cells undergoing azygotic meiosis as described above. Sequencing library construction and Illumina 150-bp paired-end sequencing were performed by Annoroad Gene Technology (Beijing, China). The raw sequencing reads were processed using fastp (version:0.20.0), with default parameters. The cleaned reads were mapped to a high-quality *S. octosporus* reference genome (http://bifx-core.bio.ed.ac.uk/~ptong/genome_assembly/oct_genome.fa) using STAR (version: 2.6.0a) with the following settings: '--alignIntronMin 29 --alignIntronMax 819 --outFilterMultimapNmax 1 --outFilterMismatchNmax 0 --alignEndsType EndToEnd' (Dobin et al., 2016). Illumina sequencing data were deposited at NCBI SRA under the accession number SRR17543073.

Long-read cDNA sequencing

For long-read cDNA sequencing using the Oxford Nanopore Technologies (ONT) platform, we prepared total RNA as described above. Sequencing library construction and ONT cDNA sequencing were performed by Biomarker Technologies (Qingdao, China). Through processing

808 the reads using pychopper (version 2.3.1), we obtained 2,839,411 full-length reads. We
809 performed further data analysis using FLAIR (Full-Length Alternative Isoform analysis of RNA,
810 version 1.5) (Tang et al., 2020). FLAIR was designed to perform reads mapping, reads
811 correcting, and isoform clustering for noisy long reads generated by ONT cDNA sequencing,
812 and it can be run optionally with short-read RNA sequencing data to help increasing the
813 accuracy of splicing site identification in isoforms. We mapped full-length reads to the *S.*
814 *octosporus* reference genome mentioned above using 'flair.py align' submodule with default
815 parameters. The splicing junction information generated by short-read RNA sequencing was
816 firstly extracted using a FLAIR script called "junctions_from_sam.py" from the reads mapping
817 SAM file then submitted to "flair.py correct" submodule. Finally, we generated high-quality
818 transcript information by running "flair.py collapse" submodule with default parameters (Tang et
819 al., 2020). The ONT cDNA reads mapping results were visualized using the Integrative
820 Genomics Viewer (IGV) (Robinson et al., 2011) (an example is shown in Figure 2B) and the
821 transcripts generated by FLAIR were used for *wtf* and *wag* gene structure annotation polishing.
822 ONT cDNA sequencing data were deposited at NCBI SRA under the accession number
823 SRR17543072.
824
825 ***S. osmophilus* genome annotation.**
826 For *S. osmophilus,* we annotated all the coding sequences with the Augustus gene prediction
827 software webpage (Stanke et al., 2006) (http://bioinf.uni-greifswald.de/webaugustus/). First, we
828 trained Augustus software with *S. octosporus* genome from Tong et al., 2019, and we uploaded
829 the cDNA sequences of *S. octosporus* genes from (Rhind et al., 2011). This training set allowed
830 Augustus to construct a model to then predict *S. osmophilus* genes. Augustus annotated the
831 predicted exons and introns of all the genes in *S. osmophilus* genome found in Figure 1-source
832 data 2. To match *S. osmophilus* genes with orthologous gens within *S. octosporus*, *S.*
833 *cryophilus* and *S. pombe*, we extracted all the predict translations of *S. osmophilus* genes and

used OrthoVenn2 to find orthologs for each genes (Xu et al., 2019). The orthologs are reported in Figure 1-source data 3.

Calculating amino acid identity between *Schizosaccharomyces* species.

To calculate the percentage amino acid identity shared between proteins of the different *Schizosaccharomyces* species, we used BLASTp (default parameters) to compare each protein sequence to a protein database created for each genome (Altschul et al., 1990). For example, we compared all the genes of *S. osmophilus* with the *S. octosporus* database. We then compared all the genes of *S. octosporus* with the *S. osmophilus* database. The best hit was saved for each gene from the reciprocal BLASTp to calculate the percentage of identity between two orthologs (Figure 1-source data 3). We then calculate the mean of all the percentage identity (all the genes) between the two genomes. The percentage of identity for each paired comparisons between genomes can be found in Table S1. This percentage of identity was used to verify and construct the *Schizosaccharomyces* phylogeny in Figure 1B in concordance with previously published results (Brysch-Herzberg et al., 2019; Rhind et al., 2011).

Sequence homology search

To find *wtf* genes outside of *S. pombe,* we performed a PSI-BLAST search within the *Schizosaccharomyces* species with the *S. pombe wtf4* gene as a query (E-value threshold 0.05, Word size=3, matrix=BLOSUM62, gap existence=11, gap extension=1, PSI-BLAST threshold=0.005) (Altschul et al., 1997). We repeated the search until no new significant hits were found (E-value threshold <0.05). Then we perform a BLASTn search using novel *wtf* genes from *S. octosporus*, *S. osmophilus* and *S. cryophilus* as queries to find additional *wtf* genes and pseudogenes within each genome (E-value threshold <0.05) (Altschul et al., 1990).

To search for *S. japonicus wtf* genes, we used sequences of *S. octosporus*, *S cryophilus,* and *S. pombe wtf* genes as query for BLAST with *S. japonicus* (Altschul et al., 1990; Rhind et al., 2011). This yielded no hits. We also carried out a MEME motif search of all the available *wtf* genes sequences and then perform PSI-BLAST to find genes with *wtf* genes motifs in *S. japonicus* (parameters: expect threshold: 10, word size: 3, matrix: BLOSUM62, gap costs = existence: 11, extension: 1, PSI-BLAST threshold: 0.005) (Altschul et al., 1997). This also yielded no conclusive hits. Finally, we manually inspected *S. japonicus* genes defined as lineage-specific by OrthoVenn2 to search for multi-exon (5-6) candidate genes with a potential alternate translational start site in intron 1 or exon 2, similar to the *wtf* drivers (Xu et al., 2019). This search also yielded no promising hits.

wtf, wag, LTR and 5S rDNA gene annotation _S. octosporus wtf_ and _wag_ genes

To annotate *wtf* genes in *S. octosporus* we used two different approaches listed below. First, we aligned the short-read RNA-sequencing data described above to the *S. octosporus* genome using Geneious Prime® 2021.1.1 (https://www.geneious.com). For each *wtf* and *wag* gene identified, we manually viewed RNA-sequencing data and used it to annotate the exons and introns. For genes and pseudogenes with insufficient sequence coverage, we determined coding sequences using homology to other *wtf* or *wag* genes that we were able to annotate with RNA-sequencing data. Specifically, we first aligned the unannotated genes with annotated genes using MAFFT with parameters L-INS-I (200PAM scoring matrix/k=2; Gap open penalty of 2; offset of 0.123) (Katoh, 2002; Katoh and Standley, 2013). We then used the alignment to manually inspect genes to annotate splicing sites and predict coding sequences. Genes with incomplete coding sequences, including those determined to have lost splice sites, were considered pseudogenes.

884 Secondly, we further polished the annotation of *wtf* and *wag* genes according to the ONT cDNA
885 sequencing data. We generated high-quality transcript information using the FLAIR pipeline as
886 described above and we predicted the longest open reading frame of these transcripts using
887 TransDecoder (version: 5.5.0). We manually compared the coding frame of FLAIR transcripts
888 mapped at the *wtf* or *wag* loci with the gene annotation obtained in the first approach and
889 refined the gene structure annotation. Both pipeline of annotation gave similar results, we
890 resolved manually the discrepancies between the different annotations and reported the
891 annotation of *S. octosporus wtf* genes in Table S2.
892
893 **_S. osmophilus wtf_ and _wag_ genes annotations**
894 We first annotated *S. osmophilus wtf* and *wag* genes using Augustus prediction (trained with *S.*
895 *octosporus* data) (Stanke et al., 2008). We then manually inspected the annotations using
896 alignments of all the *S. osmophilus wtf* or *wag* genes generated by MAFFT (L-INS-I; 200PAM
897 scoring matrix/k=2; Gap open penalty of 2; offset of 0.123) (Katoh, 2002; Katoh and Standley,
898 2013). Genes with 4 exons were annotated as pseudogenes when a 5th exon was not predicted
899 by Augustus and was found to be absent after inspection of the alignment. In many of these
900 pseudogenes, the 5th exons were degenerate with accumulated stop codons.
901
902 **5S rDNA annotation**
903 To annotate 5S rDNA in the genomes of *S. octosporus* and *S. cryophilus* we used BLASTn
904 using annotated 5S rDNA sequences in each genome as a query (Altschul et al., 1990). For *S.*
905 *osmophilus*, we used an *S. octosporus* 5S rDNA gene as a query. In all genomes, hits with 70-
906 100% DNA sequences identity were considered 5S rDNA genes. All 5S rDNA can be found in
907 GTF files of annotated genomes (Figure 1-source data 4 to 6)
908
909 **LTR annotation**

To annotate Tf transposon LTRs in *S. osmophilus*, we used BLASTn to search for sequences similar to the already annotated LTRs found in *S. cryophilus* and *S. pombe* (Rhind et al., 2011). We found many hits in *S. osmophilus* (E-value less than 0.05). In addition, we also used the LTR_retriever program which identified additional LTRs in *S. osmophilus* (Ou and Jiang, 2018). All the LTR identified are reported in Table S9.

de novo discovery and genome-wide scanning of the FLEX motif (Mei4-binding motif)

To identify Mei4-binding motifs, we firstly compiled a list of 70 *S. pombe* Mei4 target genes. These genes were selected as Mei4 target genes based on the following criterion: (1) they were shown to contain Mei4 ChIP-seq peaks at two time points during meiosis (3 hour and 4 hour into meiosis) (Alves-Rodrigues et al., 2016); and (2) they are among the middle meiosis genes whose transcript levels were reduced in *mei4*Δ and increased when Mei4p was overexpressed (Mata et al., 2007). Among these 70 *S. pombe* Mei4 target genes, we further selected 49 genes that have single copy orthologs in other fission yeast species according to Rhind et al 2011 and the result of our orthovenn2 analysis (Table S6). We extracted the 1000-bp sequences upstream of the start codon of these 49 genes in each species and performed *de novo* motif discovery using MEME (http://meme-suite.org/index.html) (Bailey and Elkan, 1994). Manual inspection of all resulting motifs identified FLEX motifs in *S. pombe*, *S. octosporus*, *S. osmophilus* and *S. cryophilus* (Figure 2-figure supplementary 2A). We then combined all 196 genes in the four fission yeast species as input for *de novo* motif discovery and the resulting 11-bp FLEX motif was submitted to the FIMO tool (Grant et al., 2011) for genome-wide motif scanning. A total of 33089 FIMO hits were found in the four fission yeast species using the default *P* value cutoff of 1E-4. By comparing the number of hits in Mei4 target genes and the number of hits in other genes, we chose *P* value < 3E-6 as the criterion for deeming a FIMO hit confident. In the reference *S. pombe* genome, there are a total of 476 FIMO hits meeting this criterion. Among the 49 *S. pombe* genes used for motif discovery, 59.2% (29 out of 49 genes)

harbor at least one confident hit in the 1000-bp region upstream of the start codon, whereas for the other *S. pombe* genes, 6.5% (328 out of 5073 genes) harbor at least one confident hit in the 1000-bp region upstream of the start codon (P = 7.47E-22, Fisher's exact test). The statistics of FIMO hits is shown in Table S7, and all confident FIMO hits are listed in Table S8.

DNA Sequence alignments and phylogenic tree construction.

All DNA or amino acid sequence alignments were constructed using the MAFFT (Katoh, 2002; Katoh and Standley, 2013) plugin in Geneious Prime® 2021.1.1 with parameters L-INS-I (200PAM scoring matrix/k=2; Gap open penalty of 2; offset of 0.123). We generated trees using the PhyML 3.0 (Guindon et al., 2010) in the webpage http://www.atgc-montpellier.fr/phyml/. The substitution model used was selected by Smart model Selection which calculates an AIC (Akaike Information Criterion) for each substitution model and then selects the best model for the dataset (Akaike, 1998; Lefort et al., 2017). The starting tree for each phylogeny was generated by BIONJ, an improved version of neighbor-joining (Gascuel, 1997). The trees were then improved with NNI (nearest neighbor interchange) (Joseph Felsenstein, 2004). The branch support was calculated by Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-like aLRT) (Anisimova and Gascuel, 2006). Then the trees were rooted by midpoint using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree).

Analyses of repetitive regions within *wtf* genes

We aligned the full length of all *S. octosporus*, *S. osmophilus wtf genes* within each species using MAFFT with parameters L-INS-I (200PAM scoring matrix/k=2; Gap open penalty of 2; offset of 0.123) (Katoh, 2002; Katoh and Standley, 2013) using Geneious Prime® 2021.1.1 (https://www.geneious.com). We then manually identified the repeat region within the alignments and manually quantified the number of bases within the repeat.

To obtain sequence logos of *S. octosporus* and *S. osmophilus* repeats in exon 4, we extracted the first complete repeat for all *wtf* genes containing a repeat. We then separately aligned all the *S. octosporus* and *S. osmophilus* repeats to produce FASTA files (Figure 5-supplement 2 source data 1 and 4) which we uploaded to the Weblogo3 interface (http://weblogo.threeplusone.com/) (Crooks, 2004). The output generated the logos displayed in Figure 5-figure supplement 2.

GARD analyses of recombination within *wtf* gene family.

To study the recombination within *wtf* gene family within a species, we first produced an alignment of the coding sequence of *wtf* genes with Translation align in Geneious Prime® 2021.1.1 (https://www.geneious.com/) with MAFFT alignment L-INS-I (200PAM scoring matrix/k=2; Gap open penalty of 2; offset of 0.123) (Katoh, 2002; Katoh and Standley, 2013). We then used our alignments to find recombination events within the *wtf* gene family by using GARD (Kosakovsky Pond et al., 2006a) with general discrete model of site-to-site variation with three class rates executed within the Datamonkey website (https://www.datamonkey.org/) (Weaver et al., 2018).

Syntenic analysis

To find *wtf* loci shared by *Schizosaccharomyces* species (Figure 4 and Figure 4-figure 1) and to assay the relationship between *wtf* loci and ancestral 5S rDNA sites (Figure 6), we manually inspected synteny of loci in *S. octosporus, S. osmophilus, S. cryophilus,* and *S. pombe.* In order to study the synteny between different *wtf* loci, we used OrthoVenn2 file generated previously (see *S. osmophilus* genome annotation section of Material and Methods) and the Ensembl fungi database to identify the orthologous genes (Howe et al., 2019; Xu et al., 2019). For each *wtf* loci we identified the immediately upstream and downstream gene and then the corresponding orthologs in each species. If the gene immediately upstream and/or downstream of the *wtf* loci

did not correspond to any ortholog, we use the gene after and so on. An analogous approach was used with the analysis of 5S rDNA sites. All the data is reported in Table S12-S13, S16, and S17.

S. cerevisiae LExA-ER-AD β-estradiol inducible system

The LExA-ER-AD system (Ottoz et al., 2014) uses a heterologous transcription factor containing LexA DNA-binding protein, the human estrogen receptor (ER) and an activation domain (AD). β-estradiol binds the human estrogen receptor (ER) and tightly regulates the activity of the LexA-ER-AD transcription factor. The LexA DNA-binding domain recognizes *lexA* boxes in the target promoter.

Cloning *S. octosporus*, *S. osmophilus* and *S. cryophilus wtf*^{poison} and *wtf*^{antidote} alleles for expression in *S. cerevisiae*

All plasmids used in this study are listed in Table S31**.** All oligos used in this study are listed in S30.

Cloning *S. octosporus wtf61*^{poison} (SOCG_04114) under the control of *a β*-estradiol inducible promoter. We amplified the predicted coding sequence of the *S. octosporus wtf61*^{poison} from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 1432 and 1442. The *CYC1* terminator was digested from pSZB395 (Nuckolls et al. 2020) using SfiI and XhoI. We then cloned *S. octosporus wtf61*^{poison} CDS and the CYC1 terminator into XhoI and BamHI site of pSZB385 to generate SZB985. We then digested pSZB985 with XhoI and BamHI to extract *wtf61*^{poison} CDS with the CYC1 terminator. We next PCR amplified the LexA promoter (LexApr) using oligos 1195 and 1240 from FRP1642 (Addgene #58442, *Ottoz et al. 2014*). We then cloned both the promoter and the *wtf6*^{poison} CDS fragment into pRS316 (Sikorski and Hieter, 1989) digested with KpnI and BamHI to generate pSZB1040.

Cloning *S. octosporus wtf61*^{antidote} (SOCG_04114) under the control of *a β-estradiol* inducible promoter. We amplified the predicted *S. octosporus wtf61*^{antidote} from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 2011 and 2170. We PCR amplified LexApr using oligos 1195 and 1240 from FRP1642 (Addgene #58442, *Ottoz et al. 2014*). We PCR amplified CYC1 terminator from pSZB1040 using oligos 2194 and 2195. We then used overlap PCR to stitch together *S. octosporus wtf61*^{antidote} and *CYC1* terminator via PCR using oligos 2011 and 2195. We digested LexApr with KpnI and XhoI. We digested the fragment *S. octosporus wtf61*^{antidote}-*CYC1* with XhoI and BamHI. Finally we cloned LexApr and *S. octosporus wtf61*^{antidote}-*CYC1* fragments into pRS314 (Sikorski and Hieter, 1989) digested with KpnI and BamHI to generate pSZB1108.

Cloning *S. cryophilus wtf1*^{poison} (SPOG_03611) under the control of *a β-estradiol* inducible promoter. We amplified the predicted coding sequence of the *S. cryophilus wtf1*^{poison} (SPOG_03611) from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 2277 and 2278. We amplified CYC1 terminator from pSZB1040 using oligos 2279 and 2170. We used overlap PCR to stitch together *S. cryophilus wtf1*^{poison} with *CYC1* terminator using oligos 2277 and 2170. We digested that PCR product with XhoI and BamHI. We also amplified the LexApr from pSZB1040 using oligos 1195 and 1240 and then digested with KpnI and XhoI. We then cloned the *S. cryophilus wtf1*^{poison}-*CYC1* and LexApr cassettes into of pRS316 (Sikorski and Hieter, 1989) digested with KpnI and BamHI to generate pSZB1122.

Cloning *S. cryophilus wtf1*^{antidote} (SPOG_03611) under the control of *a β-estradiol* inducible promoter. We amplified the predicted coding sequence of *S. cryophilus wtf1*^{antidote} from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 2276 and 2278. We amplified *CYC1* terminator from pSZB1040 via PCR using oligos 2279 and 2170. We used overlap PCR to stitch

1040 together *S. cryophilus wtf1*^{antidote} and *CYC1* terminator using oligos 2276 and 2170. We then
1041 digested the resulting PCR product with XhoI and BamHI. The LexApr was amplified from
1042 pSZB1040 via PCR using oligos 1195 and 1240 and then digested with KpnI and XhoI. We then
1043 cloned both the promoter and *S. cryophilus wtf1*^{antidote}*-CYC1* fragments into pRS314 (Sikorski
1044 and Hieter, 1989) digested with KpnI and BamHI to generate pSZB1192.
1045
1046 Cloning *S. osmophilus wtf41*^{poison} under the control of *a β-estradiol* inducible promoter. We
1047 amplified the predicted coding sequence of *S. osmophilus wtf41*^{poison} from a gBlock synthetized
1048 by IDT (Coralville, IA) via PCR using oligos 2783 and 2780. We amplified the *CYC1* terminator
1049 from pSZB1040 via PCR using oligos 2781 and 2771. We amplified the LexApr from pSZB1040
1050 via PCR using oligos 1195 and 2778. We used overlap PCR to stitch together LexApr*, S.*
1051 *osmophilus wtf41*^{poison} and the *CYC1* terminator using oligos 1195 and 2771. We then cloned
1052 the resulting KpnI-digested product into pRS316 (Sikorski and Hieter, 1989) digested with KpnI
1053 to generate pSZB1327.
1054
1055 Cloning *S. osmophilus wtf41*^{antidote} under the control of *a β-estradiol* inducible promoter. We
1056 amplified the predicted coding sequence of *S. osmophilus wtf41*^{antidote} from a gBlock synthetized
1057 by IDT (Coralville, IA) via PCR using oligos 2779 and 2780. We amplified the *CYC1* terminator
1058 from pSZB1040 via PCR using oligos 2781 and 2771. We amplified LexApr from pSZB1040 via
1059 PCR using oligos 1195 and 2782. We use overlap PCR to stitch together the three fragments
1060 using oligos 1195 and 2771. We then cloned the KpnI-digested product pRS314 (Sikorski and
1061 Hieter, 1989) digested with KpnI to generate pSZB1325.
1062
1063 Cloning *S. octosporus wtf25*^{poison} (SOCG_04480)-GFP under the control of *a β-estradiol*
1064 inducible promoter. We amplified the predicted coding sequence of *S. octosporus wtf25*^{poison}
1065 from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 2669 and 2830. We

amplified LexApr from SZB1040 via PCR using oligos 1195 and 2668. We amplified GFP from pKT0127 (Sheff and Thorn, 2004) via PCR using oligos 2831 and 2832. We amplified the *CYC1* terminator from SZB1040 using oligos 2833 and 2771. We used overlap PCR to stitch together LexApr-*S. octosporus wtf25*^{poison}-GFP-*CYC1* terminator using oligos 1195 and 2771. We then cloned the KpnI-digested product into pRS316 (Sikorski and Hieter, 1989) digested with KpnI generate SZB1353.

Cloning *S. octosporus wtf25*^{antidote} (SOCG_04480) mCherry under the control of *a β-estradiol* inducible promoter. We amplified the predicted coding sequence of *S. octosporus wtf25*^{antidote} from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 2662 and 2663. We amplified LexApr from pSZB1040 via PCR using oligos 1195 and 2661. We amplified mCherry from pSZB457 via PCR using oligos 2664 and 2665. We amplified *CYC1* terminator from pSZB1040 via PCR using oligos 2666 and 2771. We used overlap PCR to stitch together the three products. We then cloned the resulting KpnI-digested PCR product into pRS314 (Sikorski and Hieter, 1989) digested with KpnI to generate pSZB1347.

Cloning *S. osmophilus wtf19*^{poison} under the control of *a β-estradiol* inducible promoter. We amplified the predicted coding sequence of *S. osmophilus wtf19*^{poison} from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 2777 and 2774. We amplified LexApr from pSZB1040 via PCR using oligos 1195 and 2776. We amplified *CYC1* terminator from pSZB1040 via PCR using oligos 2775 and 2771. We use overlap PCR to stitch together LexApr-*S. osmophilus wtf19*^{poison}-mCherry-*CYC1* terminator using oligos 1195 and 2771. We cloned LexApr-*S. osmophilus wtf19*^{poison} -*CYC1* terminator into pRS316 (Sikorski and Hieter, 1989) digested with KpnI to generate pSZB1324.

Cloning *S. osmophilus wtf19*^{poison} under the control of *a β-estradiol* inducible promoter.

We amplified the predicted coding sequence of *S. osmophilus wtf19*^{antidote} from a gBlock synthetized by IDT (Coralville, IA) via PCR using oligos 2773 and 2774. We amplified LexApr from pSZB1040 via PCR using oligos 1195+2772. We amplified *CYC1* terminator from pSZB1040 via PCR using oligos 2775 and 2771. We used overlap PCR to stitch together LexApr-*S. osmophilus wtf19*^{antidote}-*CYC1* terminator using oligos 1195 and 2771. We cloned LexApr-*S. osmophilus wtf19*^{poison}- *CYC1* terminator into pRS314 (Sikorski and Hieter, 1989) digested with KpnI to generate pSZB1322.

Plasmid transformation in *S. cerevisiae*

All yeast strains used in this study are listed in Table S29 with detailed genotype and citation information. Plasmids used in this study are listed in Table S31**.** We transformed plasmids into *S. cerevisiae* SZY1637 (Nuckolls et al., 2020) using a protocol modified from (Elble, 1992). Specifically, we incubated a yeast colony in a mix of 240 µL 50% PEG3500, 36 µL 1 M lithium acetate, 50 µL boiled salmon sperm DNA (10 mg/ml), and 10 µL plasmid for 4-6 hours at 30°C before selecting transformants. We selected transformants on Synthetic Complete (SC) media (6.7 g/L yeast nitrogen base without amino acids and with ammonium sulfate, 2% agar, 1X amino acid mix, 2% glucose) lacking histidine, uracil, and tryptophane (SC -His -Ura -Trp).

Spot assays in *S. cerevisiae*

We grew 5 mL overnight cultures in SC -His -Ura -Trp of each strain. We then diluted each culture to an OD_{600} of 1 and performed a serial dilution. We then plated 10 µL of each dilution on a solid SC -His -Ura -Trp petri plate with or without 500 nM β-estradiol.

Imaging Wtf proteins expressed in *S. cerevisiae*

For imaging of Wtf proteins expressed in *S. cerevisiae* (Figure 7D-F), we first grew 5 mL saturated overnight cultures in SC -His -Ura -Trp media. The next day, we diluted 1 mL of each

saturated culture into 4 mLs of fresh SC -His -Ura -Trp media. We then added β-estradiol to a final concentration of 500 nM to induce *wtf* expression and shook the cultures at 30°C for 4 hours prior to imaging.

Cells (5 µL concentrated culture) were then imaged on an LSM-780 (Zeiss) with a 40x LD C-Apochromat (NA = 1.1) objective. A physical zoom of 8 was used which yielded an XY pixel size of 0.052 µm. The fluorescence of GFP was excited with the 488 nm laser and filtered through a 491-553 nm bandpass filter before being collected onto a GaAsP detector running in photon counting mode. The fluorescence of mCherry was excited with the 561 nm laser and filtered through a 562-624 nm bandpass filter before being collected onto the same detector.

S. octosporus strains

The two wild-type heterothallic *S. octosporus* strains (DY44286=NIG10005 and DY44284=NIG10006) were a kind gift from Dr. Hironori Niki and all other *S. octosporus* strains were constructed based on these two heterothallic strains. *S. octosporus*-related genetic methods are performed according to or adapted from genetic methods for *S. pombe* (Forsburg and Rhind, 2006; Seike and Niki, 2017). The construction of *wtf* gene deletion strains was carried out by PCR-based gene targeting using an SV40-EM7 (SVEM) promoter-containing G418-resistance marker referred to here as *kanSVEM* (Erler et al., 2006). As sequences between the *wtf*-flanking 5S rDNA genes share high similarity among different *wtf* gene loci, to ensure the specificity of gene deletion, we used homologous arm sequences outside of 5S rDNA genes and the length of at least one homologous arm was above 1 kb. All *wtf* gene deletion strains were verified using PCR. PCR primer sequences are listed in Table S30.

To analyze the spore killing activity of *wtf25* at an ectopic genomic locus, we constructed integrating plasmids based on the pDB4978 vector described below. A pDB4978-based plasmid

was linearized with NotI digestion and integrated at the *leu1* (*SOCG_02003*) locus. Transformants were selected by the resistance to clonNAT conferred by the natMX marker on pDB4978. Successful integration resulted in the deletion of the ORF sequence of the *leu1* (*SOCG_02003*) gene and leucine auxotrophic phenotype (Figure 10).

Integration plasmids for *S. octosporus*

All *S. octosporus* plasmids were generated by recombination cloning using the ClonExpressII One Step Cloning Kit (Vazyme, Nanjing, China). For the construction of the pDB4978 vector, the plasmid pAV0584 (Vještica et al., 2019) was firstly digested using NotI and HindIII, and the largest resulting fragment (about 4.5-kb) was purified and then digested using SpeI to obtain an approximately 3.7-kb fragment containing AmpR, ori, and the natMX marker. A sequence containing the f1ori and multiple cloning sites was PCR amplified from pAV0584 using primers oGS-177 and oGS-178 (oligo sequences are listed in Table S30). The sequences upstream and downstream of the *leu1(SOCG_02003)* ORF were amplified from *S. octosporus* genomic DNA using primers oGS-192 and oGS-193, and primers oGS-195 and oGS-197, respectively. Finally, all four fragments were combined by recombination cloning to generate the pDB4978 vector.

Spore viability analysis

Spore viability was assessed by octad dissection using a TDM50 tetrad dissection microscope (Micro Video Instruments, Avon, USA). The method of octad dissection was adapted from (Seike and Niki, 2017) and a detailed description of the experiment procedure follows. First, to maximize mating efficiency, before mating, all parental strains were streaked on YES plates for overnight growth. Then, parental strains were mixed at a one-to-one ratio and dropped on PMG plate (or PMG plates with the leucine supplement for leucine auxotrophic strains) and incubated at 30°C. After 2 days, about 1 OD_{600} unit of cells were resuspended in 200 µl of 1 mg/ml solution of snailase (Beijing Solarbio Science & Technology Co.). The mixture was incubated without

agitation at 25°C for 1 day and then the supernatant was aspirated. Snailase-treated cells were diluted in sterile water and then dropped on a YES plate for octad dissection. After dissection, plates were incubated at 30°C for about 5 days, and then plates were scanned, and the genotypes of colonies were determined by replica plating.

For data analysis, we excluded spores dissected from asci with fewer than 8 spores (asci with fewer than 8 spores are rare when sporulation was conducted on PMG plates) and octads containing >4 spores harboring one allele of a heterozygous locus (excluded octads represent < 2% of the octads analyzed). Numeric data of octad dissection analysis are in Figure 8-source data 1; Figure 9-supplement 1-6 source data 1 and the scanned plate photos are in the Figure 8-source data 2; Figure 9-source data 2 ; Figure 9-supplement 1-6 source data 2 and 3. For statistical analysis of the spore viability data, Fisher's exact test was performed using the web page https://www.langsrud.com/fisher.htm and exact binomial test were performed using an Excel spreadsheet downloaded from http://www.biostathandbook.com/exactgof.html (McDonald, 2009).

References

Abbott S, Fairbanks DJ. 2016. Experiments on Plant Hybrids by Gregor Mendel. doi:10.1534/genetics.116.195198

Abe H, Shimoda C. 2001. Autoregulated expression of Schizosaccharomyces pombe meiosis-specific transcription factor Mei4 and a genome-wide search for its target genes. *Genetics*.

Akaike H. 1998. Information Theory and an Extension of the Maximum Likelihood Principle. Springer, New York, NY. pp. 199–213. doi:10.1007/978-1-4612-1694-0_15

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* **215**:403–410. doi:10.1016/S0022-2836(05)80360-2

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped

1196 BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic*
1197 *Acids Res*. doi:10.1093/nar/25.17.3389
1198 Alves-Rodrigues I, Ferreira PG, Moldón A, Vivancos AP, Hidalgo E, Guigó R, Ayté J. 2016.
1199 Spatiotemporal Control of Forkhead Binding to DNA Regulates the Meiotic Gene
1200 Expression Program. *Cell Rep* **14**:885–895. doi:10.1016/j.celrep.2015.12.074
1201 Anisimova M, Gascuel O. 2006. Approximate Likelihood-Ratio Test for Branches: A Fast,
1202 Accurate, and Powerful Alternative. *Syst Biol* **55**:539–552.
1203 doi:10.1080/10635150600755453
1204 Bailey TL, Elkan C. 1994. Fitting a mixture model by expectation maximization to discover
1205 motifs in biopolymers. *Proceedings Int Conf Intell Syst Mol Biol* **2**:28–36.
1206 Bailey TL, Johnson J, Grant CE, Noble WS. 2015. The MEME Suite. *Nucleic Acids Res*
1207 **43**:W39–W49. doi:10.1093/nar/gkv416
1208 Bastide H, Cazemajor M, Ogereau D, Derome N, Hospital F, Montchamp-Moreau C. 2011.
1209 Rapid rise and fall of selfish sex-ratio X chromosomes in drosophila simulans:
1210 Spatiotemporal analysis of phenotypic and molecular data. *Mol Biol Evol* **28**:2461–2470.
1211 doi:10.1093/molbev/msr074
1212 Bowen NJ, Jordan IK, Epstein JA, Wood V, Levin HL. 2003. Retrotransposons and their
1213 recognition of pol II promoters: A comprehensive survey of the transposable elements from
1214 the complete genome sequence of Schizosaccharomyces pombe. *Genome Res* **13**:1984–
1215 1997. doi:10.1101/gr.1191603
1216 Bravo Núñez MA, Lange JJ, Zanders SE. 2018a. A suppressor of a wtf poison-antidote meiotic
1217 driver acts via mimicry of the driver's antidote. *PLOS Genet* **14**:e1007836.
1218 doi:10.1371/journal.pgen.1007836
1219 Bravo Núñez MA, Nuckolls NL, Zanders SE. 2018b. Genetic Villains: Killer Meiotic Drivers.
1220 *Trends Genet* **34**:424–433. doi:10.1016/j.tig.2018.02.003
1221 Bravo Núñez MA, Sabbarini IM, Eickbush MT, Liang Y, Lange JJ, Kent AM, Zanders SE, Nuñez

MAB, Sabbarini IM, Eickbush MT, Liang Y, Lange JJ, Kent AM, Zanders SE, Bravo Núñez MA, Sabbarini IM, Eickbush MT, Liang Y, Lange JJ, Kent AM, Zanders SE, María Angélica Bravo Núñez, Ibrahim M. Sabbarini MTE, Yue Liang, Jeffrey J. Lange, Aubrey M. Kent and SEZ, Nuñez MAB, Sabbarini IM, Eickbush MT, Liang Y, Lange JJ, Kent AM, Zanders SE. 2020a. Dramatically diverse Schizosaccharomyces pombe wtf meiotic drivers all display high gamete-killing efficiency. *PLoS Genet* **16**:e1008350. doi:10.1371/journal.pgen.1008350

Bravo Núñez MA, Sabbarini IM, Eide LE, Unckless RL, Zanders SE. 2020b. Atypical meiosis can be adaptive in outcrossed S. pombe due to wtf meiotic drivers. *Elife* **9**:2020.04.28.066035. doi:10.1101/2020.04.28.066035

Brysch-Herzberg M, Tobias A, Seidel M, Wittmann R, Wohlmann E, Fischer R, Dlauchy D, Peter G. 2019. Schizosaccharomyces osmophilus sp. nov., an osmophilic fission yeast occurring in bee bread of different solitary bee species. *FEMS Yeast Res*. doi:10.1093/femsyr/foz038

Burt A, Trivers R. 2006. Genes in Conflict: The Biology of Selfish Genetic Elements.

Carvalho AB, Vaz SC. 1999. Are Drosophila SR drive chromosomes always balanced? *Heredity (Edinb)* **83**:221–228. doi:10.1038/sj.hdy.6886100

Cazemajor M, Landre C, Montchampmoreau C, Landré C, Montchamp-Moreau C. 1997. The Sex-Ratio Trait in Drosophila simulans : Genetic Analysis of Distortion and Suppression. *Genet Soc Am* **147**:635–642.

Chen J, Ding J, Ouyang Y, Du H, Yang J, Cheng K, Zhao J, Qiu S, Zhang X, Yao J, Liu K, Wang L, Xu C, Li X, Xue Y, Xia M, Ji Q, Lu J, Xu M, Zhang Q. 2008. A triallelic system of S5 is a major regulator of the reproductive barrier and compatibility of indica-japonica hybrids in rice. *Proc Natl Acad Sci U S A* **105**:11436–11441. doi:10.1073/pnas.0804761105

Chiu S-W. 1996. Patterns in Fungal Development. *Cambridge Univ Press*. https://books.google.com/books?hl=fr&lr=&id=CHakwyrnPKIC&oi=fnd&pg=PP13&ots=H4rl

9LBeLJ&sig=EC8FyALrW3oYtGaNUrYiG5w5xv4#v=snippet&q=octosporus&f=false

Cohen S, Agmon N, Sobol O, Segal D. 2010. Open Access RESEARCH Extrachromosomal circles of satellite repeats and 5S ribosomal DNA in human cells, Mobile DNA. doi:10.1186/1759-8753-1-11

Cohen S, Menut S, Méchali M. 1999. Regulated Formation of Extrachromosomal Circular DNA Molecules during Development in Xenopus laevis. *Mol Cell Biol* **19**:6682–6689. doi:10.1128/mcb.19.10.6682

Cohen S, Segal D. 2009. Extrachromosomal circular DNA in eukaryotes: Possible involvement in the plasticity of tandem repeats. *Cytogenet Genome Res*. doi:10.1159/000218136

Cohen Z, Bacharach E, Lavi S. 2006. Mouse major satellite DNA is prone to eccDNA formation via DNA Ligase IV-dependent pathway. *Oncogene* **25**:4515–4524. doi:10.1038/sj.onc.1209485

Courret C, Chang CH, Wei KHC, Montchamp-Moreau C, Larracuente AM. 2019a. Meiotic drive mechanisms: Lessons from Drosophila. *Proc R Soc B Biol Sci* **286**:20191430. doi:10.1098/rspb.2019.1430

Courret C, Gérard PR, Ogereau D, Falque M, Moreau L, Montchamp-Moreau C. 2019b. X-chromosome meiotic drive in Drosophila simulans: a QTL approach reveals the complex polygenic determinism of Paris drive suppression. *Heredity (Edinb)* **122**:906–915. doi:10.1038/s41437-018-0163-1

Crooks GE. 2004. WebLogo: A Sequence Logo Generator. *Genome Res* **14**:1188–1190. doi:10.1101/gr.849004

Crow JF. 1991. Why Mendelian Segregation so exact? *BioEssays* **84**:487–492.

Daugherty MD, Zanders SE. 2019. Gene conversion generates evolutionary novelty that fuels genetic conflicts. *Curr Opin Genet Dev* **58**–**59**:49–54. doi:10.1016/j.gde.2019.07.011

Daulny A, Mejía-Ramírez E, Reina O, Rosado-Lugo J, Aguilar-Arnal L, Auer H, Zaratiegui M, Azorin F. 2016. The fission yeast CENP-B protein Abp1 prevents pervasive transcription of

repetitive DNA elements. *Biochim Biophys Acta - Gene Regul Mech* **1859**:1314–1321. doi:10.1016/j.bbagrm.2016.06.009

Dawe RK, Lowry EG, Gent JI, Stitzer MC, Swentowsky KW, Higgins DM, Ross-Ibarra J, Wallace JG, Kanizay LB, Alabady M, Qiu W, Tseng K-F, Wang N, Gao Z, Birchler JA, Harkess AE, Hodges AL, Hiatt EN. 2018. A Kinesin-14 Motor Activates Neocentromeres to Promote Meiotic Drive in Maize. *Cell* **173**:839-850.e18. doi:10.1016/j.cell.2018.03.009

Dyer KA, Charlesworth B, Jaenike J. 2007. Chromosome-wide linkage disequilibrium as a consequence of meiotic drive. *Proc Natl Acad Sci* **104**:1587–1592. doi:10.1073/pnas.0605578104

Eickbush MT, Young JM, Zanders SE. 2019. Killer Meiotic Drive and Dynamic Evolution of the wtf Gene Family. *Mol Biol Evol* **36**:1–44. doi:10.1101/461004

Elble R. 1992. A simple and efficient procedure for transformation of yeasts. *Biotechniques* **13**:18–20.

Erler A, Maresca M, Fu J, Stewart AF. 2006. Recombineering reagents for improved inducible expression and selection marker re-use inSchizosaccharomyces pombe. *Yeast* **23**:813–823. doi:10.1002/yea.1396

Finseth FR, Nelson TC, Fishman L. 2021. Selfish chromosomal drive shapes recent centromeric histone evolution in monkeyflowers. *PLoS Genet* **17**:1–20. doi:10.1371/journal.pgen.1009418

Forsburg SL, Rhind N. 2006. Basic methods for fission yeast. *Yeast* **23**:173–183. doi:10.1002/yea.1347

Gascuel O. 1997. BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Mol Biol Evol* **14**:685–695. doi:10.1093/oxfordjournals.molbev.a025808

Grant CE, Bailey TL, Noble WS. 2011. FIMO: scanning for occurrences of a given motif. *Bioinformatics* **27**:1017–1018. doi:10.1093/bioinformatics/btr064

Grognet P, Lalucque H, Malagnac F, Silar P, Heitman J. 2014. Genes That Bias Mendelian

Segregation. *PLoS Genet* **10**:e1004387. doi:10.1371/journal.pgen.1004387

Guindon S, Dufayard J-FF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst Biol* **59**:307–321. doi:10.1093/sysbio/syq010

Haeusler RA, Engelke DR. 2006. Spatial organization of transcription by RNA polymerase III. *Nucleic Acids Res* **34**:4826–4836. doi:10.1093/nar/gkl656

Hamilton WD. 1967. Extraordinary Sex Ratios. *Science (80-)* **156**:477–488. doi:10.1126/science.156.3774.477

Hatcher MJ. 2000. Persistence of selfish genetic elements: Population structure and conflict. *Trends Ecol Evol*. doi:10.1016/S0169-5347(00)01875-9

Helleu Q, Gérard PR, Dubruille R, Ogereau D, Prud'homme B, Loppin B, Montchamp-Moreau C. 2016. Rapid evolution of a Y-chromosome heterochromatin protein underlies sex chromosome meiotic drive. *Proc Natl Acad Sci* **113**:4110–4115. doi:10.1073/pnas.1519332113

Herbette M, Wei X, Chang C-HH, Larracuente AM, Loppin B, Dubruille RR. 2021. Distinct spermiogenic phenotypes underlie sperm elimination in the Segregation Distorter meiotic drive system, PLoS Genetics. doi:10.1371/journal.pgen.1009662

Hoff KJ, Stanke M. 2018. Predicting Genes in Single Genomes with AUGUSTUS. *Curr Protoc Bioinforma* e57. doi:10.1002/cpbi.57

Howe KL, Contreras-Moreira B, De Silva N, Maslen G, Akanni W, Allen J, Alvarez-Jarreta J, Barba M, Bolser DM, Cambell L, Carbajo M, Chakiachvili M, Christensen M, Cummins C, Cuzick A, Davis P, Fexova S, Gall A, George N, Gil L, Gupta P, Hammond-Kosack KE, Haskell E, Hunt SE, Jaiswal P, Janacek SH, Kersey PJ, Langridge N, Maheswari U, Maurel T, Mcdowall MD, Moore B, Muffato M, Naamati G, Naithani S, Olson A, Papatheodorou I, Patricio M, Paulini M, Pedro H, Perry E, Preece J, Rosello M, Russell M, Sitnik V, Staines DM, Stein J, Tello-Ruiz MK, Trevanion SJ, Urban M, Wei S, Ware D, Williams G, Yates

AD, Flicek P. 2019. Ensembl Genomes 2020-enabling non-vertebrate genomic research. *Nucleic Acids Res* **48**:689–695. doi:10.1093/nar/gkz890

Hu W, Jiang Z-D Di, Suo F, Zheng J-XX, He W-ZZ, Du L-LL. 2017. A large gene family in fission yeast encodes spore killers that subvert Mendel's law. *Elife* **6**:1–3. doi:10.7554/elife.26057

Huang X, Zhao Q, Han B. 2015. Comparative population genomics reveals strong divergence and infrequent introgression between Asian and African rice. *Mol Plant*. doi:10.1016/j.molp.2015.01.010

Jeffares DC, Jolly C, Hoti M, Speed D, Shaw L, Rallis C, Balloux F, Dessimoz C, Bähler J, Sedlazeck FJ. 2017. Transient structural variations have strong effects on quantitative traits and reproductive isolation in fission yeast. *Nat Commun* **8**. doi:10.1038/ncomms14061

Johansen P, Cam HP. 2015. Suppression of meiotic recombination by CENP-B homologs in Schizosaccharomyces pombe. *Genetics* **201**:897–904. doi:10.1534/genetics.115.179465

Joseph Felsenstein. 2004. Inferring Phylogenies. *Sinauer Assoc , Sanderland, Massachusetts*.

Kanizay LB, Pyhäjärvi T, Lowry EG, Hufford MB, Peterson DG, Ross-Ibarra J, Dawe RK. 2013. Diversity and abundance of the abnormal chromosome 10 meiotic drive complex in Zea mays. *Heredity (Edinb)* **110**:570–577. doi:10.1038/hdy.2013.2

Katoh K. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* **30**:3059–3066. doi:10.1093/nar/gkf436

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol Biol Evol* **30**:772–780. doi:10.1093/molbev/mst010

Koide Y, Ogino A, Yoshikawa T, Kitashima Y, Saito N, Kanaoka Y, Onishi K, Yoshitake Y, Tsukiyama T, Saito H, Teraishi M, Yamagata Y, Uemura A, Takagi H, Hayashi Y, Abe T, Fukuta Y, Okumoto Y, Kanazawa A. 2018. Lineage-specific gene acquisition or loss is involved in interspecific hybrid sterility in rice. *Proc Natl Acad Sci* **115**:E1955–E1962.

1352 doi:10.1073/pnas.1711656115

1353 Kosakovsky Pond SL, Posada D, Gravenor MB, Woelk CH, Frost SDW. 2006a. Automated

1354 Phylogenetic Detection of Recombination Using a Genetic Algorithm. *Mol Biol Evol*

1355 **23**:1891–1901. doi:10.1093/molbev/msl051

1356 Kosakovsky Pond SL, Posada D, Gravenor MB, Woelk CH, Frost SDWW, Kosakovsky SL,

1357 Posada D, Gravenor MB, Woelk CH, Frost SDWW. 2006b. GARD: a genetic algorithm for

1358 recombination detection. *Bioinformatics* **22**:3096–3098. doi:10.1093/bioinformatics/btl474

1359 Kumon T, Ma J, Akins RB, Stefanik D, Nordgren CE, Kim J, Levine MT, Lampson MA. 2021.

1360 Parallel pathways for recruiting effector proteins determine centromere drive and

1361 suppression. *Cell* **184**:4904-4918.e11. doi:10.1016/j.cell.2021.07.037

1362 Larracuente AM, Presgraves DC. 2012. The Selfish Segregation Distorter Gene Complex of

1363 Drosophila melanogaster. *Genetics* **192**:33–53. doi:10.1534/genetics.112.141390

1364 Lefort V, Longueville J-EE, Gascuel O. 2017. SMS: Smart Model Selection in PhyML **34**:2422–

1365 2424. doi:10.1093/molbev/msx149

1366 Lin CJ, Hu F, Dubruille R, Vedanayagam J, Wen J, Smibert P, Loppin B, Lai EC. 2018. The

1367 hpRNA/RNAi Pathway Is Essential to Resolve Intragenomic Conflict in the Drosophila Male

1368 Germline. *Dev Cell* **46**:316-326.e5. doi:10.1016/j.devcel.2018.07.004

1369 Lindholm AK, Dyer KA, Firman RC, Fishman L, Forstmeier W, Holman L, Johannesson H, Knief

1370 U, Kokko H, Larracuente AM, Manser A, Montchamp-Moreau C, Petrosyan VG,

1371 Pomiankowski A, Presgraves DC, Safronova LD, Sutter A, Unckless RL, Verspoor RL,

1372 Wedell N, Wilkinson GS, Price TAR, Anna K. Lindholm, 1,* Kelly A. Dyer, 2 Renée C.

1373 Firman, 3 Lila Fishman, 4 Wolfgang Forstmeier, 5 Luke Holman, 6 Hanna Johannesson, 7

1374 Ulrich Knief, 5 Hanna Kokko, 1 Amanda M. Larracuente, 8 Andri Manser, 1 Catherine

1375 Montchamp-Moreau, 9 Varos G. Petrosyan, 15 and Tom A.R. Price13, Lindholm AK, Dyer

1376 KA, Firman RC, Fishman L, Forstmeier W, Holman L, Johannesson H, Knief U, Kokko H,

1377 Larracuente AM, Manser A, Montchamp-Moreau C, Petrosyan VG, Pomiankowski A,

Presgraves DC, Safronova LD, Sutter A, Unckless RL, Verspoor RL, Wedell N, Wilkinson GS, Price TAR. 2016. The Ecology and Evolutionary Dynamics of Meiotic Drive. *Trends Ecol Evol* **31**:315–326. doi:10.1016/j.tree.2016.02.001

Long Y, Zhao L, Niu B, Su J, Wu HH, Chen Y, Zhang Q, Guo J, Zhuang C, Mei M, Xia J, Wang L, Wu HH, Liu Y-GG. 2008. Hybrid male sterility in rice controlled by interaction between divergent alleles of two adjacent genes. *Proc Natl Acad Sci U S A* **105**:18871–18876. doi:10.1073/pnas.0810108105

Lyon MF. 2003. Transmission Ratio Distortion in Mice. *Annu Rev Genet* **37**:393–408. doi:10.1146/annurev.genet.37.110801.143030

Mata J, Wilbrey A, Bähler J. 2007. Transcriptional regulatory network for sexual differentiation in fission yeast. *Genome Biol* **8**:R217. doi:10.1186/gb-2007-8-10-r217

McDonald JH. 2009. Handbook of Biological Statistics. *Sparky House Publ Balt MD* **Vol 2**:180–185.

McLaughlin RN, Malik HS. 2017. Genetic conflicts: The usual suspects and beyond. *J Exp Biol* **220**:6–17. doi:10.1242/jeb.148148

Meiklejohn CD, Landeen EL, Gordon KE, Rzatkiewicz T, Kingan SB, Geneva AJ, Vedanayagam JP, Muirhead CA, Garrigan D, Stern DL, Presgraves DC. 2018. Gene flow mediates the role of sex chromosome meiotic drive during complex speciation. *Elife* **7**:024711. doi:10.7554/eLife.35468

Mizuguchi T, Taneja N, Matsuda E, Belton J-M, FitzGerald P, Dekker J, Grewal SIS. 2017. Shelterin components mediate genome reorganization in response to replication stress. *Proc Natl Acad Sci* **114**:5479–5484. doi:10.1073/pnas.1705527114

Muirhead CA, Presgraves DC. 2021. Satellite DNA-mediated diversification of a sex-ratio meiotic drive gene family in Drosophila. *Nat Ecol Evol* 1–9. doi:10.1038/s41559-021-01543-8

Navrátilová A, Koblížková A, Macas J. 2008. Survey of extrachromosomal circular DNA derived

1404 from plant satellite repeats. *BMC Plant Biol* **8**:90. doi:10.1186/1471-2229-8-90

1405 Nuckolls NL, Mok AC, Lange JJ, Yi K, Kandola TS, Hunn AM, McCroskey S, Snyder JL, Bravo
1406 Núñez MA, McClain M, McKinney SA, Wood C, Halfmann R, Zanders SE. 2020. The wtf4
1407 meiotic driver utilizes controlled protein aggregation to generate selective cell death. *Elife*
1408 **9**:2020.02.05.935874. doi:10.7554/eLife.55694

1409 Nuckolls NL, Núñez MAB, Eickbush MT, Young JM, Lange JJ, Yu JS, Smith GR, Jaspersen SL,
1410 Malik HS, Zanders SE. 2017. Wtf Genes Are Prolific Dual Poison-Antidote Meiotic Drivers.
1411 *Elife* **6**:1–43. doi:10.7554/eLife.26033

1412 Nuckolls NL, Srinivasa AN, Mok AC, Bravo Núñez MA, Lange JJ, Gallagher TJ, Seidel CW,
1413 Zanders SE. 2021. S. pombe wtf genes use dual transcriptional regulation and selective
1414 protein exclusion from spores to cause meiotic drive. *bioRxiv* 2021.09.30.462505.
1415 doi:10.1101/2021.09.30.462505

1416 Ottoz DSM, Rudolf F, Stelling J. 2014. Inducible, tightly regulated and growth condition-
1417 independent transcription factor in Saccharomyces cerevisiae. *Nucleic Acids Res* **42**:130.
1418 doi:10.1093/nar/gku616

1419 Ou S, Jiang N. 2018. LTR_retriever: A Highly Accurate and Sensitive Program for Identification
1420 of Long Terminal Repeat Retrotransposons. *Plant Physiol* **176**:1410–1422.
1421 doi:10.1104/pp.17.01310

1422 Paulsen T, Kumar P, Koseoglu MM, Dutta A. 2018. Discoveries of Extrachromosomal Circles of
1423 DNA in Normal and Tumor Cells. *Trends Genet*. doi:10.1016/j.tig.2017.12.010

1424 Price TARR, Verspoor R, Wedell N. 2019. Ancient gene drives: an evolutionary paradox. *Proc R*
1425 *Soc B Biol Sci* **286**:20192267. doi:10.1098/rspb.2019.2267

1426 Rhind N, Chen Z, Yassour M, Thompson DA, Haas BJ, Habib N, Wapinski I, Roy S, Lin MF,
1427 Heiman DI, Young SK, Furuya K, Guo Y, Pidoux A, Chen HM, Robbertse B, Goldberg JM,
1428 Aoki K, Bayne EH, Berlin AM, Desjardins CA, Dobbs E, Dukaj L, Fan L, FitzGerald MG,
1429 French C, Gujja S, Hansen K, Keifenheim D, Levin JZ, Mosher RA, Muller CA, Pfiffner J,

1430 Priest M, Russ C, Smialowska A, Swoboda P, Sykes SM, Vaughn M, Vengrova S, Yoder
1431 R, Zeng Q, Allshire R, Baulcombe D, Birren BW, Brown W, Ekwall K, Kellis M,
1432 Leatherwood J, Levin H, Margalit H, Martienssen R, Nieduszynski CA, Spatafora JW,
1433 Friedman N, Dalgaard JZ, Baumann P, Niki H, Regev A, Nusbaum C, Müller CA, Pfiffner J,
1434 Priest M, Russ C, Smialowska A, Swoboda P, Sykes SM, Vaughn M, Vengrova S, Yoder
1435 R, Zeng Q, Allshire R, Baulcombe D, Birren BW, Brown W, Ekwall K, Kellis M,
1436 Leatherwood J, Levin H, Margalit H, Martienssen R, Nieduszynski CA, Spatafora JW,
1437 Friedman N, Dalgaard JZ, Baumann P, Niki H, Regev A, Nusbaum C, Muller CA, Pfiffner J,
1438 Priest M, Russ C, Smialowska A, Swoboda P, Sykes SM, Vaughn M, Vengrova S, Yoder
1439 R, Zeng Q, Allshire R, Baulcombe D, Birren BW, Brown W, Ekwall K, Kellis M,
1440 Leatherwood J, Levin H, Margalit H, Martienssen R, Nieduszynski CA, Spatafora JW,
1441 Friedman N, Dalgaard JZ, Baumann P, Niki H, Regev A, Nusbaum C, Müller CA, Pfiffner J,
1442 Priest M, Russ C, Smialowska A, Swoboda P, Sykes SM, Vaughn M, Vengrova S, Yoder
1443 R, Zeng Q, Allshire R, Baulcombe D, Birren BW, Brown W, Ekwall K, Kellis M,
1444 Leatherwood J, Levin H, Margalit H, Martienssen R, Nieduszynski CA, Spatafora JW,
1445 Friedman N, Dalgaard JZ, Baumann P, Niki H, Regev A, Nusbaum C. 2011. Comparative
1446 functional genomics of the fission yeasts. *Science (80-)* **332**:930–936.
1447 doi:10.1126/science.1203357

1448 Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP. 2011.
1449 Integrative genomics viewer. *Nat Biotechnol* **29**:24–26. doi:10.1038/nbt.1754

1450 Ross-Ibarra J, Tenaillon M, Gaut BS. 2009. Historical divergence and gene flow in the genus
1451 Zea. *Genetics* **181**:1399–1413. doi:10.1534/genetics.108.097238

1452 Sakata M, Takano-Kai N, Miyazaki Y, Kanamori H, Wu J, Matsumoto T, Doi K, Yasui H,
1453 Yoshimura A, Yamagata Y. 2021. Domain Unknown Function DUF1668-Containing Genes
1454 in Multiple Lineages Are Responsible for F1 Pollen Sterility in Rice. *Front Plant Sci*
1455 **11**:2314. doi:10.3389/fpls.2020.632420

1456 Sandler L, Novitski E. 1957. Meiotic Drive as an Evolutionary Force. *Am Nat* **91**:105–110.
1457 doi:10.1086/281969
1458 Seike T, Nakamura T, Shimoda C. 2015. Molecular coevolution of a sex pheromone and its
1459 receptor triggers reproductive isolation in Schizosaccharomyces pombe. *Proc Natl Acad*
1460 *Sci* **112**:4405–4410. doi:10.1073/pnas.1501661112
1461 Seike T, Niki H. 2017. Mating response and construction of heterothallic strains of the fission
1462 yeast Schizosaccharomyces octosporus. *FEMS Yeast Res* **17**:1–14.
1463 doi:10.1093/femsyr/fox045
1464 Seike T, Shimoda C, Niki H. 2019. Asymmetric diversification of mating pheromones in fission
1465 yeast. *PLOS Biol* **17**:e3000101. doi:10.1371/journal.pbio.3000101
1466 Sheff MA, Thorn KS. 2004. Optimized cassettes for fluorescent protein tagging
1467 inSaccharomyces cerevisiae. *Yeast* **21**:661–670. doi:10.1002/yea.1130
1468 Shen R, Wang L, Liu X, Wu J, Jin W, Zhao X, Xie X, Zhu Q, Tang H, Li Q, Chen L, Liu Y-G.
1469 2017. Genomic structural variation-mediated allelic suppression causes hybrid male
1470 sterility in rice. *Nat Commun* **8**:1310. doi:10.1038/s41467-017-01400-y
1471 Sikorski RS, Hieter P. 1989. A system of shuttle vectors and yeast host strains designed for
1472 efficient manipulation of DNA in Saccharomyces cerevisiae. *Genetics* **122**:19–27.
1473 Simão FA, Waterhouse RM, Ioannidis P, Kriventseva E V., Zdobnov EM. 2015. BUSCO:
1474 Assessing genome assembly and annotation completeness with single-copy orthologs.
1475 *Bioinformatics* **31**:3210–3212. doi:10.1093/bioinformatics/btv351
1476 Sipiczki M. 1979. Interspecific protoplast fusion in fission yeasts. *Curr Microbiol* **3**:37–40.
1477 doi:10.1007/BF02603131
1478 Sipiczki M, Kucsera J, Ulaszewski S, Zsolt J. 1982. Hybridization Studies by Crossing and
1479 Protoplast Fusion within the Genus Schizosaccharomyces Lindner. *Microbiology*
1480 **128**:1989–2000. doi:10.1099/00221287-128-9-1989
1481 Sproul JS, Khost DE, Eickbush DG, Negm S, Wei X, Wong I, Larracuente AM. 2020. Dynamic

1482 Evolution of Euchromatic Satellites on the X Chromosome in Drosophila melanogaster and
1483 the simulans Clade. *Mol Biol Evol* **37**:2241–2256. doi:10.1093/molbev/msaa078
1484 Stanke M, Diekhans M, Baertsch R, Haussler D. 2008. Using native and syntenically mapped
1485 cDNA alignments to improve de novo gene finding. *Bioinformatics* **24**:637–644.
1486 doi:10.1093/bioinformatics/btn013
1487 Stanke M, Schöffmann O, Morgenstern B, Waack S. 2006. Gene prediction in eukaryotes with a
1488 generalized hidden Markov model that uses hints from external sources. *BMC*
1489 *Bioinformatics* **7**. doi:10.1186/1471-2105-7-62
1490 Tamura K. 2003. Temporal Patterns of Fruit Fly (Drosophila) Evolution Revealed by Mutation
1491 Clocks. *Mol Biol Evol* **21**:36–44. doi:10.1093/molbev/msg236
1492 Tang AD, Soulette CM, van Baren MJ, Hart K, Hrabeta-Robinson E, Wu CJ, Brooks AN. 2020.
1493 Full-length transcript characterization of SF3B1 mutation in chronic lymphocytic leukemia
1494 reveals downregulation of retained introns. *Nat Commun* **11**:1438. doi:10.1038/s41467-
1495 020-15171-6
1496 Tao Y, Masly JP, Araripe L, Ke Y, Hartl DL. 2007. A sex-ratio meiotic drive system in Drosophila
1497 simulans. I: An autosomal suppressor. *PLoS Biol* **5**:2560–2575.
1498 doi:10.1371/journal.pbio.0050292
1499 Tong P, Pidoux AL, Toda NRTT, Ard R, Berger H, Shukla M, Torres-Garcia J, Mueller CA,
1500 Nieduszynski CA, Allshire RC, Müller CA, Nieduszynski CA, Allshire RC. 2019.
1501 Interspecies conservation of organisation and function between nonhomologous regional
1502 centromeres. *Nat Commun* **10**. doi:10.1038/s41467-019-09824-4
1503 Unckless RL, Larracuente AM, Clark AG. 2015. Sex-Ratio Meiotic Drive and Y-Linked
1504 Resistance in Drosophila affinis. *Genetics* **199**:831–840. doi:10.1534/genetics.114.173948
1505 Vedanayagam J, Lin C-J, Lai EC. 2021. Rapid evolutionary dynamics of an expanding family of
1506 meiotic drive factors and their hpRNA suppressors. *Nat Ecol Evol* **5**:1613–1623.
1507 doi:10.1038/s41559-021-01592-z

1508 Vještica A, Marek M, Nkosi PJ, Merlini L, Liu G, Bérard M, Billault-Chaumartin I, Martin SG.
1509 2019. A toolbox of Stable Integration Vectors (SIV) in the fission yeast
1510 Schizosaccharomyces pombe. *J Cell Sci*. doi:10.1242/jcs.240754
1511 Vogan AA, Ament-Velásquez SL, Bastiaans E, Wallerman O, Saupe SJ, Suh A, Johannesson
1512 H. 2021. The Enterprise , a massive transposon carrying Spok meiotic drive genes.
1513 *Genome Res* **31**:789–798. doi:10.1101/gr.267609.120
1514 Weaver S, Shank SD, Spielman SJ, Li M, Muse S V, Kosakovsky Pond SL. 2018. Datamonkey
1515 2.0: A modern web application for characterizing selective and other evolutionary
1516 processes. *Mol Biol Evol* **35**:773–777. doi:10.1093/molbev/msx335
1517 Wei KHC, Reddy HM, Rathnam C, Lee J, Lin D, Ji S, Mason JM, Clark AG, Barbash DA. 2017.
1518 A pooled sequencing approach identifies a candidate meiotic driver in Drosophila. *Genetics*
1519 **206**:451–465. doi:10.1534/genetics.116.197335
1520 Wood V, Gwilliam R, Rajandream M-A, Lyne M, Lyne R, Stewart A, Sgouros J, Peat N, Hayles
1521 J, Baker S, Basham D, Bowman S, Brooks K, Brown D, Brown S, Chillingworth T,
1522 Churcher C, Collins M, Connor R, Cronin A, Davis P, Feltwell T, Fraser A, Gentles S,
1523 Goble A, Hamlin N, Harris D, Hidalgo J, Hodgson G, Holroyd S, Hornsby T, Howarth S,
1524 Huckle EJ, Hunt S, Jagels K, James K, Jones L, Jones M, Leather S, McDonald S, McLean
1525 J, Mooney P, Moule S, Mungall K, Murphy L, Niblett D, Odell C, Oliver K, O'Neil S,
1526 Pearson D, Quail MA, Rabbinowitsch E, Rutherford K, Rutter S, Saunders D, Seeger K,
1527 Sharp S, Skelton J, Simmonds M, Squares R, Squares S, Stevens K, Taylor K, Taylor RG,
1528 Tivey A, Walsh S, Warren T, Whitehead S, Woodward J, Volckaert G, Aert R, Robben J,
1529 Grymonprez B, Weltjens I, Vanstreels E, Rieger M, Schäfer M, Müller-Auer S, Gabel C,
1530 Fuchs M, Fritzc C, Holzer E, Moestl D, Hilbert H, Borzym K, Langer I, Beck A, Lehrach H,
1531 Reinhardt R, Pohl TM, Eger P, Zimmermann W, Wedler H, Wambutt R, Purnelle B,
1532 Goffeau A, Cadieu E, Dréano S, Gloux S, Lelaure V, Mottier S, Galibert F, Aves SJ, Xiang
1533 Z, Hunt C, Moore K, Hurst SM, Lucas M, Rochet M, Gaillardin C, Tallada VA, Garzon A,

Thode G, Daga RR, Cruzado L, Jimenez J, Sánchez M, del Rey F, Benito J, Domínguez A, Revuelta JL, Moreno S, Armstrong J, Forsburg SL, Cerrutti L, Lowe T, McCombie WR, Paulsen I, Potashkin J, Shpakovski G V., Ussery D, Barrell BG, Nurse P. 2002. The genome sequence of Schizosaccharomyces pombe. *Nature* **415**:871–880. doi:10.1038/nature724

Xie Y, Tang J, Xie X, Li X, Huang J, Fei Y, Han J, Chen S, Tang H, Zhao X, Tao D, Xu P, Liu YG, Chen L. 2019. An asymmetric allelic interaction drives allele transmission bias in interspecific rice hybrids. *Nat Commun* **10**:1–10. doi:10.1038/s41467-019-10488-3

Xie Y, Xu P, Huang J, Ma S, Xie X, Tao D, Chen L, Liu Y-G. 2017. Interspecific Hybrid Sterility in Rice Is Mediated by OgTPR1 at the S1 Locus Encoding a Peptidase-like Protein. *Mol Plant* **10**:1137–1140. doi:10.1016/j.molp.2017.05.005

Xu L, Dong Z, Fang L, Luo Y, Wei Z, Guo H, Zhang G, Gu YQ, Coleman-Derr D, Xia Q, Wang Y. 2019. OrthoVenn2: a web server for whole-genome comparison and annotation of orthologous clusters across multiple species. *Nucleic Acids Res* **47**:W52–W58. doi:10.1093/nar/gkz333

Yang J, Zhao X, Cheng K, Du H, Ouyang Y, Chen J, Qiu S, Huang J, Jiang Y, Jiang L, Ding J, Wang J, Xu C, Li X, Zhang Q. 2012. A Killer-Protector System Regulates Both Hybrid Sterility and Segregation Distortion in Rice. *Science (80-)* **337**:1336–1340. doi:10.1126/science.1223702

Yu Y, Zhao Z, Shi Y, Tian H, Liu L, Bian X, Xu Y, Zheng X, Gan L, Shen Y, Wang Chaolong, Yu X, Wang Chunming, Zhang X, Guo X, Wang J, Ikehashi H, Jiang L, Wan J. 2016. Hybrid sterility in rice (Oryza sativa L.) involves the tetratricopeptide repeat domain containing protein. *Genetics* **203**:1439–1451. doi:10.1534/GENETICS.115.183848/-/DC1

Zanders S, Johannesson H. 2021. Molecular Mechanisms and Evolutionary Consequences of Spore Killers in Ascomycetes. *Microbiol Mol Biol Rev*. doi:10.1128/mmbr.00016-21

Zanders SE, Unckless RL. 2019. Fertility Costs of Meiotic Drivers. *Curr Biol*.

doi:10.1016/j.cub.2019.03.046

Zimmering S, Sandler L, Nicoletti B. 1970. Mechanisms of Meiotic Drive. *Annu Rev Genet* **4**:409–436. doi:10.1146/annurev.ge.04.120170.002205

Figure legends

Figure 1: *wtf* homologs are found outside of *S. pombe*.

(A) Model for meiotic drive of *wtf* genes in *S. pombe*, modified from (Nuckolls et al., 2017). All spores are exposed to the poison protein, but those that inherit the *wtf* driver are rescued by the antidote protein. **(B)** Phylogeny of *Schizosaccharomyces* species including the numbers of *wtf* homologs found by PSI-BLAST and BLASTn searches. MYA represents million years ago. *The *S. osmophilus* genome is not fully assembled, so the number represents the *wtf* homologs found within the assembled contigs. The phylogeny is based on published reports (Brysch-Herzberg et al., 2019; Rhind et al., 2011) and our own analyses with the added partial assembly of *S. osmophilus*. Annotations of all the identified genes can be found in Figure 1-source data 2.

Figure 1-source data 1: *S. osmophilus* genome assembly.

Fasta file containing the partial genome assembly of *S. osmophilus* from this study.

Figure 1-source data 2: Predicted *S. osmophilus* gene annotations.

We used the Augustus program (Stanke et al., 2006) to predict *S. osmophilus* gene annotations using a model based on *S. octosporus* genes. Augustus generated a GTF file with all the predicted genes.

Figure 1-source data 3: Orthologous genes in *Schizosaccharomyces*.

A list of orthologous gene sets within *Schizosaccharomyces* generated by Orthovenn 2 (Xu et al., 2019) and each comparison between orthologs was assessed by BLAST (Altschul et al., 1990). The columns display in order query species, query gene, subject species, subject gene, percentage of identity and length of the query.

Figure 1-source data 4: *S. octosporus* genome annotation.

GFF file of *S. octosporus* genome.

Figure 1-source data 5: *S. osmophilus* genome annotation.

GFF file of *S. osmophilus* genome.

Figure 1-source data 6: S. cryophilus genome annotation.

GFF file of *S. cryophilus* genome.

Figure 1-figure supplement 1: Maps of the *wtf* gene family members in *S. octosporus, S. osmophilus, S. cryophilus, and S. pombe*.

Genome maps of *wtf* genes from **(A)** *S. octosporus*, **(B)** *S. osmophilus*, **(C)** *S. cryophilus*, and **(D)** *S. pombe*. Genes on the forward strand are shown above each chromosome, whereas genes on the reverse strand are shown below chromosomes. Genes that we predict to be intact drivers because they contain an alternate translational start site near the beginning of exon 2 are shown in purple. Genes that we predict to be drive suppressors because they lack the potential alternate start site are shown in green. Predicted pseudogenes are indicated with an asterisk*. The four *S. pombe wtf* genes with unknown functions are shown in light blue. The *S. pombe* map is modified from (Eickbush et al., 2019). Annotations of the novel *wtf* genes can be found in Tables S2-S4.

Figure 2: *S. pombe wtf* genes share features with other *wtf* genes outside of *S. pombe*. **(A)** Schematic *wtf* loci of the *Schizosaccharomyces* species. Orange boxes correspond to exons (E1 indicates exon 1, etc.), the red boxes represent 5S rDNA genes, the blue box represents a pseudogenized *wag* gene and the yellow box is an LTR from a Tf transposon. The predicted translational start sites for the antidote (ATG in exon 1) and poison (ATG in exon 2) proteins are indicated, as is the FLEX transcriptional regulatory motif (Table S2-S4). **(B)** Long-read RNA sequencing of mRNAs from meiotic *S. octosporus* cells revealed two main transcript isoforms of the *wtf25* gene, presumably encoding an antidote and a poison protein, respectively. cDNA reads obtained using the Oxford Nanopore Technologies (ONT) platform are shown in pink. Blue lines indicate sequences missing in the reads due to splicing. The diagram at the top depicts the two main transcript isoforms. The 3' transcript ends shown in the diagram correspond to the major transcript end revealed by cDNA reads.

Figure 2-source data 1: ***S. octosporus* RNA-seq data**.
Long-read RNA sequence data (Oxford Nanopore) are available at the NCBI SRA under the accession number SRR17543072. Standard RNA sequence data (Illumina) are available at the NCBI SRA under the accession number SRR17543073.

Figure 2-figure supplement 1: Limited conservation of Wtf proteins.
The percent identity shared amongst all 113 Wtf predicted antidote proteins from *S. octosporus*, *S. osmophilus, S. cryophilus* and *S. pombe* (isolate FY29033) aligned with MAFFT (L-INS-I; BLOSSUM62 scoring matrix/k=2; Gap open penalty of 2; offset of 0.123) (Katoh, 2002; Katoh and Standley, 2013). The data are shown in 10 amino acid sliding windows. The alignment can be found in Figure 2-figure supplement 1-source data 1.

Figure 2-figure supplement 1-source data 1: **Multi-alignment of all 113 Wtf predicted antidote proteins of *S. octosporus*, *S. osmophilus*, *S. cryophilus*, and *S. pombe*.**

Alignment of 113 predicted antidotes Wtf proteins made using MAFFT (Katoh, 2002; Katoh and Standley, 2013). This alignment was used to generate Figure 2-figure supplement 1.

Figure 2-figure supplement 2: **Many *wtf* genes in *S. octosporus* harbor the FLEX motif in intron 1**.

(A) The FLEX motif identified by the *de novo* motif discovery tool MEME. 49 Mei4 target genes in *S. pombe* and their orthologs in *S. octosporus*, *S. osmophilus* and *S. cryophilus* were used as input for MEME. MEME analyses were conducted for each species separately and for all species combined. **(B)** *wtf* genes containing the FLEX motif in intron 1. The motif scanning tool FIMO was used to find the FLEX motif in the genomes of *S. pombe*, *S. octosporus*, *S. cryophilus* and *S. osmophilus*. The 11-bp FLEX motif identified by the MEME analysis using 146 genes as input was provided to FIMO for motif scanning. All *wtf* genes containing a FIMO hit in intron 1 are shown with the *P* value of the FIMO hit in intron 1 presented on a –log10 scale. We found the default *P* value cutoff of FIMO (1E-4) being too loose and applied a cutoff of 3E-6 to distinguish confident hits from unreliable hits.

Figure 2-figure supplement 3: **Transcription levels of predicted poison and antidote isoforms of intact *wtf* genes in *S. octosporus***.

Long-read (Oxford Nanopore) RNA sequencing was performed on mRNAs isolated from *S. octosporus* cells undergoing meiosis. All intact *wtf* genes are shown with the read count of the long transcript (encoding putative antidote) in grey and the read count of the short transcript (encoding putative poison) in black. The bold gene names indicate the genes with a confident FLEX motif hit in intron 1. The underlined gene names indicate the genes analyzed by deletion (Figures 8-9). The read counts of the two isoforms can be found in Table S2.

Figure 3: Genomic context of *wtf* genes.

The *wtf* genes are found in a limited number of genomic contexts. The *wtf* genes are represented as orange boxes, *wag* genes are in blue, and LTRs are in yellow. NA indicates not applicable as *wag* genes are absent from *S. pombe* and LTRs are absent from *S. octosporus.*

Figure 3-figure supplement 1: Distance between 5S rDNA and *wtf* genes.

The distance in base pairs between 5S rDNA and the coding sequence of a *wtf* gene in **(A)** *S. osmophilus* and **(B)** *S. octosporus***.** Only *wtf* genes with a flanking 5S rDNA were considered. The *wtf* gene is collapsed at 0 and the flanking sequences were considered in 100 base pair bins.

Figure 3-figure supplement 2 Homology between distinct 5S rDNA-*wtf* and *wag-wtf* units.

The regions containing *wtf* genes with the indicated genomic contexts were aligned with MAFFT to find the percent sequences identity (Katoh, 2002; Katoh and Standley, 2013). The percent identity is shown in 50 base pair sliding windows. **(A)** The percent identity shared amongst 37 *wtf*-5S rDNA units from *S. octosporus*. **(B)** The percent identity shared amongst 17 *wtf-wag* units from *S. octosporus*.

Figure 3-figure supplement 2-source data 1: **Multi-alignment of 37 *S. octosporus* 5S rDNA-*wtf* units.**

DNA MAFFT alignment of 37 5S rDNA-*wtf-5S rDNA* unit of *S. octosporus* used to make Figure 3-figure supplement 2A.

Figure 3-figure supplement 2-source data 2: **Multi-alignment of 17 *S. octosporus wtf-wag* units.**

Multi DNA alignment using MAFFT of 17 *wtf-wag* units from *S. octosporus* used to make Figure 3-figure supplement 2B.

Figure 3-figure supplement 3: *S. octosporus wtf* gene units supported by maximum likelihood phylogeny.

The regions flanking the *wtf* genes in *S. octosporus* were sorted into the color-coded groups shown based on maximum phylogenies shown in Figure 3-figure supplement 4 and Figure 3-figure supplement 5. Orange boxes correspond to *wtf* genes, the red boxes represent 5S rDNA genes, and the blue boxes represent *wag* genes. Genomic contexts without *wag* genes and with *wag* genes are shown separately in **(A)** and **(B)**.

Figure 3-figure supplement 4: Maximum likelihood phylogeny of the regions between *S. octosporus wtf* genes and a downstream flanking 5S rDNA gene.

The regions downstream of 67 *S. octosporus wtf* genes with a downstream 5S rDNA gene were aligned with MAFFT (Katoh, 2002) and a maximum likelihood phylogeny was built with PhyML(Guindon et al., 2010). Branch support values shown at the nodes (0-1) are SH-like aLRT values. The shaded clades and letter designations correspond to the colors and letters shown in Figure 3-figure supplement 3.

Figure 3-figure supplement 4-source data 1: **Multi-alignment of the regions downstream of 67 *S. octosporus wtf* with a downstream 5S rDNA.**

DNA alignment built using MAFFT of the regions downstream of 67 *S. octosporus wtf* with a downstream 5S rDNA.

Figure 3-figure supplement 4-source data 2: Phylogeny of the regions downstream of 67 *S. octosporus wtf* genes with a downstream 5S rDNA.

Phylogeny generated by PhyML of the downstream regions of 67 *S. octosporus wtf* genes with a downstream 5S rDNA in Newick format (Guindon et al., 2010). The labels are SH-like aLRT values for support of the nodes (0-1).

Figure 3-figure supplement 5: Maximum likelihood phylogeny of the regions between *S. octosporus wtf* genes and an upstream flanking 5S rDNA gene.

The regions upstream of 40 *S. octosporus wtf* genes with an upstream 5S rDNA gene were aligned with MAFFT and a maximum likelihood phylogeny was built with PhyML. Branch support values shown at the nodes (0-1) are SH-like aLRT values. The shaded clades and letter designations correspond to the colors and letters shown in Figure 3-figure supplement 3.

Figure 3-figure supplement 5-source data 1: **Multi-alignment of the regions upstream of 40 *S. octosporus wtf* with an upstream 5S rDNA.**

DNA alignment made using MAFFT of the regions upstream of 40 *S. octosporus wtf* genes with an upstream 5S rDNA.

Figure 3-figure supplement 5-source data 2: Phylogeny of the regions upstream of 40 *S. octosporus wtf* genes with an upstream 5S rDNA.

Phylogeny generated by PhyML of the upstream regions of 40 *S. octosporus wtf* genes with an upstream 5S rDNA in a Newick format. The labels are SH-like aLRT values for support of the nodes (0-1).

Figure 3-figure supplement 6: Maximum likelihood phylogeny of *S. octosporus wtf* genes.

The sequences of 83 *S. octosporus wtf* genes were aligned using MAFFT and a maximum likelihood phylogeny was constructed using PhyML. Branch support values shown at the nodes

(0-1) are SH-like aLRT values. The color-coded letter designations to the right of the gene names indicate the phylogenetic groupings of the sequences flanking the *wtf* genes from Figure 3-figure supplement 3.

Figure 3-figure supplement 6-source data 1: Multi-alignment of 83 *S. octosporus wtf* genes.

DNA multi-alignment using MAFFT of 83 *S. octosporus wtf* genes in PHYLIP format.

Figure 3-figure supplement 6-source data 2: Phylogeny of 83 *S. octosporus wtf* genes.

Phylogeny generated by PhyML of 83 *S. octosporus wtf* genes in Newick format. Support values are SH-like aLRT values (0-1).

Figure 4: Shared *wtf* locus in three fission yeast species.

(A) The syntenic region between *clr4* and *met17* in *S. octosporus*, *S. osmophilus*, *S. cryophilus,* and *S. pombe* is shown. The *S. pombe* locus shown is from the *S. kambucha* isolate. The orange boxes represent *wtf* genes, the blue boxes represent *wag* genes, the red arrows represent 5S rDNA, the green arrow represents *tRNA-his,* the grey boxes represent genes without a homolog in this region in the species shown and the black boxes represent genes that are syntenic between the species. The phylogenetic relationship between species is shown to the left of the DNA representation. The orthologs of *clr4* **(B)** and *met17* **(C)** were aligned and used to build neighbor-joining trees that were midpoint rooted. Branch support (0-100) was calculated using bootstrap.

Figure 4-source data 1: **Multi-alignment of *Schizosaccharomyces clr4* genes and neighbor-joining tree.**

DNA MAFFT alignment of *Schizosaccharomyces clr4* from *S. octosporus, S. osmophilus, S. cryophilus, S. pombe, and S. japonicus* in PHYLIP format.

Figure 4-source data 2: **Neighbor-joining tree of *Schizosaccharomyces clr4* genes.**
Phylogenetic tree of *Schizosaccharomyces clr4* from *S. octosporus, S. osmophilus, S. cryophilus, S. pombe, and S. japonicus* in Newick format. Bootstrap values are displayed from 0 to 100.

Figure 4-source data 3: **Multi-alignment of *Schizosaccharomyces met17* genes.**
DNA MAFFT alignment of *Schizosaccharomyces met17* from *S. octosporus, S. osmophilus, S. cryophilus, S. pombe, and S. japonicus* in PHYLIP format.

Figure 4-source data 4: **Neighbor-joining tree of *Schizosaccharomyces met17* genes.**
Neighbor-joining tree of *Schizosaccharomyces met17* from *S. octosporus, S. osmophilus, S. cryophilus, S. pombe, and S. japonicus* in Newick format. Bootstrap values are displayed from 0 to 100.

Figure 4-figure supplement 1: Synteny between *S. cryophilus wtf4* and *S. pombe wtf6*.
(**A**) The syntenic region containing *cyp9* and *ago1* is shown for all fission yeast species. An inversion in the *S. pombe* lineage separated *cyp9* and *ago1*. There is a *wtf* gene upstream of *ago1* in both *S. pombe* and *S. cryophilus*. The orange boxes represent the *wtf* genes. Five genes are numbered and shown in green to illustrate that the ancestor of *S. pombe* and *S. cryophilus* likely had a *wtf* gene between *cyp9* and *ago1*. The black boxes represent additional orthologous genes in synteny. The orthologs of *cyp9* **(B)** and *ago1* **(C)** were aligned and used to build neighbor-joining trees that were midpoint rooted. Branch support (0-100) was calculated using bootstrap.

Figure 4-figure supplement 1-source data 1: **Multi-alignment of *Schizosaccharomyces ago1* genes and neighbor-joining tree.**

DNA alignment built using MAFFT of *Schizosaccharomyces ago1* from *S. octosporus, S. osmophilus, S. cryophilus, S. pombe, and S. japonicus* in PHYLIP format.

Figure 4-figure supplement 1-source data 2: **Neighbor-joining tree of S*chizosaccharomyces ago1* genes.**

Neighbor-joining tree of *Schizosaccharomyces ago1* in Newick format. Bootstrap values are displayed from 0 to 100.

Figure 4-figure supplement 1-source data 3: **Multi-alignment of *Schizosaccharomyces cyp9* genes.**

DNA alignment built using MAFFT of *Schizosaccharomyces cyp9* from *S. octosporus, S. osmophilus, S. cryophilus, S. pombe, and S. japonicus* in PHYLIP format.

Figure 4-figure supplement 1-source data 4: **Neighbor-joining tree of *Schizosaccharomyces cyp9* genes.**

Phylogeny of *Schizosaccharomyces cyp9* from *S. octosporus, S. osmophilus, S. cryophilus, S. pombe, and S. japonicus* phylogenetic tree of *Schizosaccharomyces met17* in Newick format.

Figure 5: Gene duplication and non-allelic gene conversion within *wtf* gene family.

All the predicted intact Wtf antidote amino acid sequences were aligned using MAFFT from Figure 2-figure supplement 1-source data 1 and used to build a maximum likelihood tree using PhyML. The *S. pombe* sequences were from the FY29033 isolate as it has more *wtf* genes than the reference genome. The *S. pombe* genes are shown in black, *S. octosporus* genes are in

magenta, *S. osmophilus* genes are dark blue, and the *S. cryophilus* genes are cyan. The triangles represent multiple genes with the precise number indicated on the right. The branch support values (0-1) are SH-like aLRT values and are shown at each node.

Figure 5-source data 1: **Maximum Likelihood phylogeny of 113 *wtf* genes.**

Phylogenetic tree of 113 *wtf* genes from *S. octosporus, S. cryophilus, S. osmophilus,* and S. *pombe* in Newick format. Branch support values are SH-like aLRT values.

Figure 5-figure supplement 1: GARD analysis consistent with non-allelic gene conversion within *wtf* genes.

We used GARD (genetic algorithm for the detection of recombination) (Kosakovsky Pond et al., 2006b) analysis to look for evidence of gene conversion within the *wtf* genes of **(A)** *S. octosporus*, **(B)** *S. osmophilus* and **(C)** *S. pombe.* We considered only genes predicted to be meiotic drivers or suppressors. This analysis found that a hypothesis allowing multiple trees for different segments of the alignment is >100 times more likely than a hypothesis allowing only a single tree, supporting that non-allelic recombination has occurred within *wtf* genes. The analysis identified two likely breakpoints in each species. For *S. pombe* the analysis is from (Eickbush et al., 2019).

Figure 5-figure supplement 1-source data 1: GARD analysis of *S. octosporus wtf* genes.

GARD analysis of *S. octosporus wtf* predicted meiotic drivers and suppressors. This analysis found that a hypothesis allowing multiple trees for different segments of the alignment is >100 times more likely than a hypothesis allowing only a single tree, supporting that nonallelic recombination has occurred within the gene family. The analysis identified two likely breakpoints corresponding to positions 204 and 355 in the alignment, yielding three segments as depicted by the colored rectangles at the top of the figure. Both breakpoints have strong statistical

support (***; p<0.0004). The trees generated for each segment (below) are distinct. The yellow highlighting is to help illustrate the incongruence between the trees.

Figure 5-figure supplement 1 source data 2: GARD analysis of *S. osmophilus wtf* genes.

GARD analysis of *S. osmophilus wtf* predicted meiotic drivers and suppressors. This analysis found that a hypothesis allowing multiple trees for different segments of the alignment is >100 times more likely than a hypothesis allowing only a single tree, supporting that nonallelic recombination has occurred within the gene family. The analysis identified two likely breakpoints corresponding to positions 159 and 298 in the alignment, yielding three segments as depicted by the colored rectangles at the top of the figure. Both breakpoints have strong statistical support (***; p<0.0004). The trees generated for each segment (below) are distinct. The yellow highlighting is to help illustrate the incongruence between the trees.

Figure 5-figure supplement 2: Contraction and expansion of repeat sequences in *wtf* genes.

The *wtf* genes of *S. octosporus* **(A)** *S. osmophilus* **(C)**, and *S. pombe* **(E)** can contain the indicated repetitive sequences. The DNA (top) and amino acid (bottom) sequences logos representing the repeat regions are shown for each species. The size distribution of the repeat regions for all *S. octosporus* **(A)** *S. osmophilus* **(C)**, and *S. pombe* **(E)** *wtf* genes is shown. The sizes are presented in base pairs instead of repeat units because the terminal repeats are not always full length. The *S. pombe* data are from (Eickbush et al., 2019). The repeat count in exon 4 of *S. octosporus wtf* genes and the repeat count in exon 4 of *S. osmophilus wtf* genes is shown in supplementary Table S21.

Figure 6: *wtf* genes duplicated into pre-existing 5S rDNA.

Testing if lineage restricted *wtf* genes occur at sites where the ancestral species is inferred to have had a 5S rDNA gene. An example of this situation is illustrated in **(A)** where species A has a 5S-rDNA-flanked *wtf* gene and species B has a 5S rDNA gene at the syntenic locus. **(B)** Number of *wtf*+5S rDNA loci in species A (any of the gene layouts illustrated in (**A**)) with 5S rDNA at the syntenic locus in species B. This analysis only considers loci that contain 5S-rDNA-flanked *wtf* gene in species A but contain no *wtf* genes in species B. Table S16 and S17 were used to test this hypothesis.

Figure 6-figure supplement 1: *wtf* gene duplication models.

(A) Model of duplication via non-allelic gene conversion: 1) double strand of DNA with 5S rDNA depicted in red. 2) A double strand DNA break (DSB) within the 5S rDNA 3) 5' end resection. 4) Strand invasion of an ectopic locus with a *wtf* gene flanked by 5S rDNA genes. 5) The repair template containing the *wtf* gene is copied to the site of the initiating DSB. 6) Strand displacement and annealing of the broken DNA. 7) Synthesis of DNA with *wtf* gene in the other strand and ligation to finalize repair. 8) *wtf* gene duplicated in a new locus **(B)** 1) 5S rDNA-*wtf*-*5S rDNA* unit. 2) Crossing-over between 5S rDNA repeats flanking a *wtf* gene can generate an extrachromosomal circular DNA. 3) This circle can recombine with an ectopic locus containing a 5S rDNA. 4) Generation of a new *wtf* locus.

Figure 7: ***wtf* genes can encode for poison and antidote proteins.**

Spot assay of serial dilutions of *S. cerevisiae* cells on non-inducing (SC -His -Trp -Ura) and inducing (SC -His -Trp -Ura +500 nM β-estradiol) media. Each strain contains [*TRP1*] and [*URA3*] ARS CEN plasmids that are either empty (EV) or carry the indicated β-estradiol inducible *wtf* alleles. **(A)** *S. octosporus wtf25*^{poison}*-GFP* and *wtf25*^{antiddote}*-mCherry* (**B**) *S. osmophilus wtf41*^{poison} and *wtf41*^{antidote}, and **(C)** *S. cryophilus wtf1*^{poison} and *wtf1*^{antidote}. The dilution factor is 0.2 starting at OD=1. **(D)** A representative cell carrying a [*URA3*] plasmid with β-

estradiol inducible *S. octosporus wtf25^{poison}-GFP* (cyan). **(E)** A representative cell carrying a [*TRP1*] plasmid with β-estradiol inducible *S. octosporus wtf25^{antidote}-mCherry* (magenta). **(F)** A representative *S. cerevisiae* cell carrying a [*URA3*] plasmid with β-estradiol inducible *S. octosporus wtf25^{poison}-GFP* (cyan) and [*TRP1*] plasmid with β-estradiol inducible *S. octosporus wtf25^{antidote}-mCherry* (magenta). In all the experiments, the cells were imaged approximately 4 hours after induction with 500 nM β-estradiol. TL= transmitted light. Scale bar represents 2 μm.

Figure 7-figure supplement 1: Some *wtf* genes outside of *S. pombe* encode for poison and antidote proteins.

Spot assay of serial dilutions of *S. cerevisiae* cells on non-inducing (SC -His -Trp -Ura) and inducing (SC -His -Trp -Ura +500 nM β-estradiol) media. Each strain contains [*TRP1*] and [*URA3*] ARS CEN plasmids that are either empty (EV) or carry the indicated β-estradiol inducible alleles. **(A)** *S. osmophilus wtf19^{poison}* and *wtf19^{antidote}* **(B)** *S. octosporus wtf61^{poison}* and *wtf61^{antidote}*. The dilution factor is 0.1 for **(A)** 0.2 for **(B)** with a starting OD=1 for both panels.

Figure 7-figure supplement 2: Non-cognate Wtf^{antidotes} fail to rescue cells from Wtf^{poisons}.

Spot assay of serial dilutions of *S. cerevisiae* cells on non-inducing (SC -His -Trp -Ura) and inducing (SC -His -Trp -Ura +500 nM β-estradiol) media. Each strain contains [*TRP1*] and [*URA3*] ARS CEN plasmids that are either empty (EV) or carry the indicated β-estradiol inducible *Wtf^{poison}* and *wtf^{antidote}* alleles. **(A)** *S. octosporus wtf61* and *S. osmophilus wtf41* **(B)** *S. cryophilus wtf1* and *S. osmophilus wtf41* **(C)** *S. octosporus wtf61* and *S. octosporus wtf25* **(D)** *S. cryophilus wtf1* and *S. octosporus wtf25* and **(E)** *S. pombe wtf4* and *S. octosporus wtf25*. In **C-E**, the Wtf25^{poison} protein was tagged with GFP and the Wtf25^{antidote} protein was tagged with mCherry. The percent identity between the coding sequences of the *wtf^{poison}* alleles and the percent amino acid identity shared by the Wtf^{poison} proteins is shown at the top of each panel. The dilution factor for all plates is 0.2 starting at OD=1.

Figure 7- figure supplement 3: The distribution of *S. octosporus* Wtf25 proteins is similar to *S. pombe* Wtf4 proteins.

(A) Images of cells carrying a [*URA3*] plasmid with β-estradiol inducible *S. octosporus wtf25^{poison}-GFP*. Wtf25^{poison}-GFP signal is distributed in the cytoplasm, with potential endoplasmic reticulum (ER) localization (yellow arrows). **(B)** Images of cells carrying a [*TRP1*] plasmid with β-estradiol inducible *S. octosporus wtf25^{antidote}-mCherry* (magenta). Wtf25^{antidote}-mCherry signal can be observed within vacuoles (white arrows) and as large aggregates (yellow arrows). **(C)** Images of cells carrying a [*URA3*] plasmid with β-estradiol inducible *S. octosporus wtf25^{poison}-GFP* (cyan) and [*TRP1*] plasmid with β-estradiol inducible *S. octosporus wtf25^{antidote}-mCherry* (magenta). There is colocalization of Wtf25^{poison}-GFP and Wtf25^{antidote}-mCherry signal within vacuoles (black arrows). In all the experiments, the cells were imaged approximately 4 hours after induction with 500 nM β-estradiol. TL= transmitted light. Scale bars represents 2 μm. All images were captured with the same settings. Images in panel A are not shown at the same brightness and contrast as panels B and C to better visualize the signal.

Figure 8: Three *S. octosporus wtf* genes, when individually deleted, caused spore viability loss in heterozygous crosses but not in homozygous crosses.

Deletion mutants of seven *S. octosporus wtf* genes were obtained, and crosses were performed. Heterozygous deletion cross but not homozygous deletion cross of *wtf25*, *wtf68* or *wtf33* resulted in significant spore viability loss. Spore viability was measured using octad dissection analysis (see methods). Representative octads are shown in Figure 9, Figure 9-supplements 1-6 and Figure 8 and 9-source data 2. Numerical data are provided in Table S21. *P* values (Fisher's exact test) for crosses with > 5% spore viability reduction compared to the wild-type control are shown and calculated in Figure 8-source data 1.

Figure 8-source data 1: **Octad analysis tables.**

Summary of spore viability is shown in table 1 and tables 2-8 test the significance of the difference of spore viability by Fisher's exact test.

Figure 8-source data 2: Octad dissection raw data.

Wild type cross raw data files are shown as a pdf file with each cross in the upper left of the images.

Figure 9: Some *S. octosporus wtf* genes cause meiotic drive.

(A) Representative octads dissected from asci produced from a *wtf25* heterozygous deletion cross. The labels A to H indicate the 8 spores dissected from each ascus and the labels 1 to 11 indicate the 11 asci analyzed. The genotypes of clones were determined by replica plating onto antibiotic-containing plates. Raw data of all octads can be found in Figure 9-source data 2. **(B)** The percentages of spores that were viable and with indicated genotypes produced by *wtf25*$^{+}$/*wtf25*Δ cross. The *P* value was calculated using exact binomial test and numerical data are provided in Figure 9-source data 1. **(C)** Classification of octads derived from *wtf25*$^{+}$/*wtf25*Δ cross according to the number of viable spores with and without a *wtf* gene deletion. The *P* values were calculated using the exact binomial test. The *P* values are only displayed if a pair of octad types have more than 5 octads in total, as *P* values cannot reach the significance threshold if the total number of octads ≤ 5. **(D)** Correlation between transmission distortion ratio and poison isoform expression level. The transmission distortion ratio represents the proportion of *wtf* containing spores in total viable spores produced by a *wtf*$^{+}$/*wtf*Δ heterozygote and the read counts are those shown in Figure 2-figure supplement 3. Numerical data of transmission distortion ratio of each *wtf* gene can be found in Table S21-S27.

Figure 9-source data 1: **Numerical data of the octad dissection analysis of *wtf25* heterozygous deletion cross.**

Octad analysis table with spore viability of *wtf25*$^{+}$/*wtf25*Δ heterozygous cross can be found in table 1.1. Corresponding octad genotypes are found in table 1.2. Spore viability data of homozygous diploid are in table 2. Exact binomial test was performed in table 3.1, and the Fisher's exact test is calculated in table 3.2.

Figure 9-source data 2: ***wtf25* heterozygous diploid octad dissection raw data.**

wtf25^{+}/*wtf25*Δ heterozygous diploid raw data files are shown as a pdf file with each cross in the upper left of the images.

Figure 9-source data 3: ***wtf25* homozygous diploid octad dissection raw data.**

*wtf25*Δ/*wtf25*Δ homozygous diploid raw data files are shown as a pdf file with each cross in the upper left of the images.

Figure 9-figure supplement 1: Octad dissection analysis of *wtf68* heterozygous deletion cross.

(A) Representative octads dissected from asci produced from a *wtf68* heterozygous deletion cross. The labels A to H indicate the 8 spores dissected from each ascus and the labels 1 to 11 indicate the 11 asci analyzed. The genotypes of clones were determined by replica plating. Raw data of all octads can be found in Figure 9-figure supplement 1-source data 2. **(B)** The percentages of spores that were viable and with indicated genotypes in *wtf68*$^{+}$/*wtf68*Δ cross. The *P* value was calculated using exact binomial test and numerical data are provided in Figure 9-figure supplement 1-source data 1. **(C)** Classification of octads derived from *wtf68*$^{+}$/*wtf68*Δ cross according to the number of viable spores with and without a *wtf* gene deletion. The *P* values were calculated using the exact binomial test. The *P* values are only

displayed if a pair of octad types have more than 5 octads in total, as *P* values cannot reach the significance threshold if the total number of octads ≤ 5.

Figure 9-figure supplement 1-source data 1: **Numerical data of the octad dissection analysis of *wtf68* heterozygous deletion cross.**

Raw data file with spore viability of *wtf68*$^{+}$/*wtf68*Δ heterozygous cross can be found in table 1.1. Corresponding octad genotypes are found in table 1.2. Spore viability data of homozygous diploid are in table 2. Exact binomial test was performed in table 3.1, and the Fisher's exact test is calculated in table 3.2.

Figure 9-figure supplement 1-source data 2: ***wtf68* heterozygous diploid octad dissection raw data.**

wtf68^{+}/*wtf68*Δ heterozygous diploid raw data files are shown as a pdf file with each cross in the upper left of the images.

Figure 9-figure supplement 1-source data 3: ***wtf68* homozygous diploid octad dissection raw data.**

*wtf68*Δ/*wtf68*Δ homozygous diploid raw data files are shown as a pdf file with each cross in the upper left of the images.

Figure 9-figure supplement 2: Octad dissection analysis of *wtf33* heterozygous deletion cross.

(A) Representative octads dissected from asci produced from a *wtf33* heterozygous deletion cross. The labels A to H indicate the 8 spores dissected from each ascus and the labels 1 to 11 indicate the 11 asci analyzed. The genotypes of clones were determined by replica plating. Raw data of all octads can be found in Figure 9-figure supplement 2-source data 2. **(B)** The

percentages of spores that were viable and with indicated genotypes in *wtf33*$^{+}$/*wtf33*Δ cross. The *P* value was calculated using exact binomial test and numerical data are provided in Figure 9-supplement 2-source data 1. **(C)** Classification of octads derived from *wtf33*$^{+}$/*wtf33*Δ cross according to the number of viable spores with and without a *wtf* gene deletion. The *P* values were calculated using the exact binomial test. The *P* values are only displayed if a pair of octad types have more than 5 octads in total, as *P* values cannot reach the significance threshold if the total number of octads ≤ 5.

Figure 9-figure supplement 2-source data 1: **Numerical data of the octad dissection analysis of *wtf33* heterozygous deletion cross.**

Raw data file with spore viability of *wtf33*$^{+}$/*wtf33*Δ heterozygous cross can be found in table 1.1. Corresponding octad genotypes are found in table 1.2. Spore viability data of homozygous diploid are in table 2. Exact binomial test was performed in table 3.1, and the Fisher's exact test is calculated in table 3.2.

Figure 9-figure supplement 2-source data 2: ***wtf33* heterozygous diploid octad dissection raw data.**

wtf33^{+}/*wtf33*Δ heterozygous diploid raw data files are shown as a pdf file with each cross in the upper left of the images.

Figure 9-figure supplement 2-source data 3: ***wtf33* homozygous diploid octad dissection raw data.**

*wtf33*Δ/*wtf33*Δ homozygous diploid raw data files are shown as a pdf file with each cross in the upper left of the images.

Figure 9-figure supplement 3: Octad dissection analysis of *wtf60* heterozygous deletion cross.

(A) Representative octads dissected from asci produced from a *wtf60* heterozygous deletion cross. The labels A to H indicate the 8 spores dissected from each ascus and the labels 1 to 11 indicate the 11 asci analyzed. The genotypes of clones were determined by replica plating. Raw data of all octads can be found in Figure 9-figure supplement 4-source data 2. **(B)** The percentage of spores that were viable and with indicated genotypes in *wtf60*$^{+}$/*wtf60*Δ cross. The *P* value was calculated using exact binomial test and numerical data are provided in Figure 9-figure supplement 4-source data 1. **(C)** Classification of octads derived from *wtf60*$^{+}$/*wtf60*Δ cross according to the number of viable spores with and without a *wtf* gene deletion. The *P* values were calculated using the exact binomial test. The *P* values are only displayed if a pair of octad types have more than 5 octads in total, as *P* values cannot reach the significance threshold if the total number of octads ≤ 5.

Figure 9-figure supplement 3-source data 1: Numerical data of the octad dissection analysis of *wtf60* heterozygous deletion cross.

Raw data file with spore viability of *wtf60*$^{+}$/*wtf60*Δ heterozygous cross can be found in table 1.1. Corresponding octad genotypes are found in table 1.2. Spore viability data of homozygous diploid are in table 2. Exact binomial test was performed in table 3.1, and the Fisher's exact test is calculated in table 3.2.

Figure 9-figure supplement 3-source data 2: ***wtf60* heterozygous diploid octad dissection raw data.**

wtf60^{+}/*wtf60*Δ heterozygous diploid raw data files are shown as a pdf file with each cross in the upper left of the images.

Figure 9-figure supplement 3-source data 3: ***wtf60* homozygous diploid octad dissection raw data.**

*wtf60*Δ/ *wtf60*Δ homozygous diploid raw data files are shown as a pdf file with each cross in the upper left of the images.

Figure 9-figure supplement 4: Octad dissection analysis of *wtf46* heterozygous deletion cross.

(A) Representative octads dissected from asci produced from a *wtf46* heterozygous deletion cross. The labels A to H indicate the 8 spores dissected from each ascus and the labels 1 to 11 indicate the 11 asci analyzed. The genotypes of clones were determined by replica plating. Raw data of all octads can be found in Figure 9-figure supplement 3-source data 2. **(B)** The percentage of spores that were viable and with indicated genotypes in *wtf46*$^{+}$/*wtf46*Δ cross. The *P* value was calculated using exact binomial test and numerical data are provided in Figure 9-figure supplement 3-source data 1. **(C)** Classification of octads derived from *wtf46*$^{+}$/*wtf46*Δ cross according to the number of viable spores with and without a *wtf* gene deletion. The *P* values were calculated using the exact binomial test. The *P* values are only displayed if a pair of octad types have more than 5 octads in total, as *P* values cannot reach the significance threshold if the total number of octads ≤ 5.

Figure 9-figure supplement 4-source data 1: **Numerical data of the octad dissection analysis of *wtf46* heterozygous deletion cross.**

Raw data file with spore viability of *wtf46*$^{+}$/*wtf46*Δ heterozygous cross can be found in table 1.1. Corresponding octad genotypes are found in table 1.2. Spore viability data of homozygous diploid are in table 2. Exact binomial test was performed in table 3.1, and the Fisher's exact test is calculated in table 3.2.

Figure 9-figure supplement 4-source data 2: ***wtf46*** **heterozygous diploid octad dissection raw data.**

wtf46^{+}/*wtf46*Δ heterozygous diploid raw data files are shown as a pdf file with each cross in the upper left of the images.

Figure 9-figure supplement 4-source data 3: ***wtf46*** **homozygous diploid octad dissection raw data.**

*wtf46*Δ/*wtf46*Δ homozygous diploid raw data files are shown as a pdf file with each cross in the upper left of the images.

Figure 9-figure supplement 5: Octad dissection analysis of *wtf21* heterozygous deletion cross.

(A) Representative octads dissected from asci produced from a *wtf21* heterozygous diploid. The coordinates A to H stands for 8 spores dissected from one ascus rows 1 to 11 represent 11 octad asci analyzed. The genotypes of clones were determined by replica plating. Raw data of all octads can be found in Figure 9-figure supplement 6-source data 1. **(B)** The percentage of spores that were viable and with indicated genotypes in *wtf21*$^{+}$/*wtf21*Δ cross. The *P* value was calculated using exact binomial test and numerical data are provided in Figure 9-figure supplement 6-source data 1. **(C)** Classification of octads derived from *wtf21*$^{+}$/*wtf21*Δ cross according to the number of viable spores with and without a *wtf* gene deletion. The *P* values were calculated using the exact binomial test. The *P* values are only displayed if a pair of octad types have more than 5 octads in total, as *P* values cannot reach the significance threshold if the total number of octads ≤ 5.

Figure 9-figure supplement 5-source data 1: **Numerical data of the octad dissection analysis of *wtf21* heterozygous deletion cross.**

Raw data file with spore viability of *wtf21*$^{+}$/*wtf21*Δ heterozygous cross can be found in table 1.1. Corresponding octad genotypes are found in table 1.2. Spore viability data of homozygous diploid are in table 2. Exact binomial test was performed in table 3.1, and the Fisher's exact test is calculated in table 3.2.

Figure 9-figure supplement 5-source data 2: ***wtf21*** **heterozygous diploid octad dissection raw data.**

wtf21^{+}*//wtf21*Δ heterozygous diploid raw data files are shown as a pdf file with each cross in the upper left of the images.

Figure 9-figure supplement 5-source data 3: ***wtf21*** **homozygous diploid octad dissection raw data.**

*wtf21*Δ/*wtf21*Δ homozygous diploid raw data files are shown as a pdf file with each cross in the upper left of the images.

Figure 9-figure supplement 6 Octad dissection analysis of *wtf62* heterozygous deletion cross.

(A) Representative octads dissected from asci produced from a *wtf62* heterozygous deletion cross. The labels A to H indicate the 8 spores dissected from each ascus and the labels 1 to 11 indicate the 11 asci analyzed. The genotypes of clones were determined by replica plating. Raw data of all octads can be found in Figure 9-figure supplement 6-source data 2. **(B)** The percentage of spores that were viable and with indicated genotypes in *wtf62*$^{+}$/*wtf62*Δ cross. The *P* value was calculated using exact binomial test and numerical data are provided in Figure 9-figure supplement 6-source data 1. **(C)** Classification of octads derived from *wtf62*$^{+}$/*wtf62*Δ cross according to the number of viable spores with and without a *wtf* gene deletion. The *P* values were calculated using the exact binomial test. The *P* values are only displayed if a pair of octad

types have more than 5 octads in total, as *P* values cannot reach the significance threshold if the total number of octads ≤ 5.

Figure 9-figure supplement 6-source data 1: **Numerical data of the octad dissection analysis of *wtf62* heterozygous deletion cross.**

Raw data file with spore viability of *wtf62*$^{+}$/*wtf62*Δ heterozygous cross can be found in table 1.1. Corresponding octad genotypes are found in table 1.2. Spore viability data of homozygous diploid are in table 2. Exact binomial test was performed in table 3.1, and the Fisher's exact test is calculated in table 3.2.

Figure 9-figure supplement 6-source data 2: ***wtf62* heterozygous diploid octad dissection raw data.**

wtf62^{+}/*wtf62*Δ heterozygous diploid raw data files are shown as a pdf file with each cross in the upper left of the images.

Figure 9-figure supplement 6-source data 3: ***wtf62* homozygous diploid octad dissection raw data.**

*wtf62*Δ/ *wtf62*Δ homozygous diploid raw data files are shown as a pdf file with each cross in the upper left of the images.

Figure 10: *S. octosporus wtf25* is a poison-and-antidote killer meiotic driver.

(A) Schematic of the *wtf25* alleles integrated at the *leu1* (*SOCG_02003*) locus. Black asterisks indicate start codon mutations. The start codon for the putative *wtf25*^{poison} coding sequence is mutated in the *wtf25*$^{antidote\text{-}only}$ allele and the start codon for the putative *wtf25*^{antidote} coding sequence is mutated in the *wtf25*$^{poison\text{-}only}$ allele. **(B)** The wild-type *wtf25* allele integrated at the *leu1* locus can act as a meiotic driver by killing spores not inheriting it in a heterozygous

cross while *wtf25*$^{antidote\text{-}only}$ mutant allele integrated at the same locus was unable to kill spores not inheriting it in a heterozygous cross. *P* value calculations using a binomial test of goodness-of-fit are shown in Figure 10-source data 1 and 2. **(C)** The *wtf25*$^{poison\text{-}only}$ allele integrated at *leu1* can cause self-killing in spores that do not inherit wild-type *wtf25* at the endogenous locus. The effects of the *wtf25*$^{poison\text{-}only}$ allele were compared to a control cross in which an empty vector was integrated at *leu1*. Numerical data are provided in Table S28 and the *P* value calculation is shown in Figure 10-source data 3.

Figure 10-source data 1: **Raw data of the octad dissection analysis of *wtf25* integrated at *leu1*.**

Table 1 shows the viability of heterozygous diploid spores. Exact binomial test of goodness-of-fit was calculated in Table 2, and the resulting *P* value is displayed in Figure 10B.

Figure 10-source data 2: Raw data of the octad dissection analysis of *wtf25*$^{antidote\text{-}only}$ integrated at *leu1*.

Table 1 shows the viability of heterozygous diploid spores. Exact binomial test of goodness-of-fit was calculated in Table 2, and the resulting *P* value is displayed in Figure 10B.

Figure 10-source data 3: Raw data of the octad dissection analysis of *wtf25*$^{poison\text{-}only}$ integrated at *leu1*.

Empty plasmid control strain spore viability can be found in Table 1.1. *wtf25*$^{poison\text{-}only}$ octad dissection spore viability results can be found in Table 1.2. The *P* value was calculated with a Fisher's exact test in Table 2.

Figure 11: Model for long-term persistence of *wtf* meiotic drivers.

The presence of dispersed repetitive elements like LTRs (yellow) or 5S rDNA (red) associated with *wtf* genes (orange) may facilitate duplication of the genes to novel sites in the genome by recombination mechanisms schematized in Figure 6. Non-allelic gene conversion and expansion/contraction of repeat elements can also fuel the birth and rejuvenation of *wtf* meiotic drivers allowing them to avoid fixation, suppression, mutational decay and, ultimately, extinction.

Table S1: Percent amino acid identity of all 1:1 orthologs in *Schizosaccharomyces.*

Orthologous gene sets between pairs of *Schizosaccharomyces* species were identified using a combination of Orthovenn2 and BLASTp (Xu et al., 2019). All proteins from a given species were aligned the proteins of the other species and the best hit for each was used to determine the amino acid identity. All the percent identity values between a pair of species were then used to calculate the average amino acid identity between the two species. The genome used for finding proteins sequences was generate by Rhind et al., 2011 for *S. octosporus*, *S. cryophilus*, *S. pombe*, and *S. japonicus*. The *S. osmophilus* genome was sequenced and annotated in this study (see methods). The orthologs list can be found in Figure 1-Source data 3.

Table S2: Location and features of *S. octosporus wtf* genes.

S. octosporus wtf genes names are found in column A. The gene locations are described from columns B to F. If the gene is associated with a *wag* gene, the *wag* gene name and orientation are indicated in columns G and H. Column K indicates whether the *wtf* gene is associated with a 5S rDNA gene (immediately adjacent to the *wtf* or outside a flanking *wag* gene). The strand location of 5S rDNA genes that may be found upstream of the *wtf* gene is described in column I, while the strand location for 5S rDNA genes that may be downstream of the *wtf* gene is described in column J. *wtf* genes and the associated 5S rDNA are considered to be in tandem when they are encoded in the same strand and in the same direction. The *wtf* and *wag* genes

are all in a divergent orientation in that they are on opposite strands and transcribed in opposite directions. Column L details if there is a 5S rDNA upstream, downstream or if there is a 5S rDNA gene both upstream and downstream the *wtf* gene. Column M describes our prediction if the *wtf* gene encodes a driver (intact poison start codon), an antidote-only gene (no start codon for poison) or is a pseudogene (premature stop codon). Columns N and O show the read counts of the two isoforms detected with long read RNA-seq, respectively, with the long isoform predicted to encode an antidote protein and the short isoform predicted to encode a poison protein. Column P indicates if a FIMO motif scanning hit of the FLEX motif was present in intron 1 of the *wtf* gene. Column Q provides the location of the FIMO hit in intron 1 (only the best scoring FIMO hit is shown if more than one hit was found). Column R shows the strand the FIMO hit is on. Columns S and T show the *P* value of the FIMO hit and the sequence of the FIMO hit, respectively.

Table S3: Location and features of *S. osmophilus wtf* genes.

S. osmophilus wtf genes names are found in column A. The gene locations are described from columns B to F. If the gene is associated with a *wag* gene, the *wag* gene name and orientation are indicated in columns G and H. Column K indicates whether the *wtf* gene is associated with a 5S rDNA gene (immediately adjacent to the *wtf* or outside a flanking *wag* gene). The strand location of 5S rDNA genes that may be found upstream of the *wtf* gene is described in column I, while the strand location for 5S rDNA genes that may be downstream of the *wtf* gene is described in column J. *wtf* genes and the associated 5S rDNA are in tandem when they are encoded in the same strand and in the same direction. Column L details if there is a 5S rDNA upstream, downstream or if there is a 5S rDNA gene both upstream and downstream the *wtf* gene. Column M describes our prediction if the *wtf* gene encodes a driver (intact poison start codon), an antidote-only gene (no start codon for poison) or is a pseudogene (premature stop codon). Columns N and O indicated the strand of the LTR and orientation relative to the *wtf*

gene. As above, tandem orientation means same orientation and same strand, convergent means the elements are on opposite strands but are transcribed toward each other. Divergent means that the elements are in different strands and are transcribed in opposite directions. Column P indicates if a FIMO motif scanning hit of the FLEX motif was present in intron 1 of the *wtf* gene. Column Q provides the location of the FIMO hit in intron 1 (only the best scoring FIMO hit is shown if more than one hit was found). Column R shows the strand the FIMO hit is on. Columns S and T show the *P* value of the FIMO hit and the sequence of the FIMO hit, respectively.

Table S4: Location and features of *S. cryophilus wtf* genes.

S. cryophilus wtf genes names are found in column A. The gene locations are described from columns B to F. If the gene is associated with a *wag* gene, the *wag* gene name and orientation are indicated in columns G and H. Column K indicates whether the *wtf* gene is associated with a 5S rDNA gene (immediately adjacent to the *wtf* or outside a flanking *wag* gene). The strand location of 5S rDNA genes that may be found upstream of the *wtf* gene is described in column I, while the strand location for 5S rDNA genes that may be downstream of the *wtf* gene is described in column J. *wtf* genes and the associated 5S rDNA are in tandem (column L) when they are encoded in the same strand and in the same direction. Column L details if there is a 5S rDNA upstream, downstream or if there is a 5S rDNA gene both upstream and downstream the *wtf* gene. Column M describes our prediction if the *wtf* gene encodes a driver (intact poison start codon), an antidote-only gene (no start codon for poison) or is a pseudogene (premature stop codon). Column N indicates if a FIMO motif scanning hit of the FLEX motif was present in intron 1 of the *wtf* gene. Column O provides the location of the FIMO hit in intron 1 (only the best scoring FIMO hit is shown if more than one hit was found). Column P shows the strand the FIMO hit is on. Columns Q and R show the *P* value of the FIMO hit and the sequence of the FIMO hit, respectively.

Table S5: Pairwise amino acid identity of intact *wtf* genes.

Using MAFFT with parameters L-INS-I (200PAM scoring matrix/k=2; Gap open penalty of 2; offset of 0.123), we aligned all the predicted coding sequences of the intact *wtf* genes from *S. octosporus, S. osmophilus*, *S. cryophilus* and *S. pombe*. The longest isoform (i.e. antidote) of each protein, when two isoforms are predicted, was used. The table shows the percent amino acid identity shared between all pairs of genes. The cells are color-coded such that pairs with higher similarity are shaded a darker red.

Table S6: Genes used for FLEX motif discovery.

This table lists the 49 *S. pombe* Mei4 target genes and their orthologs in three other fission yeast species used for FLEX motif discovery.

Table S7: Summary statistics of genome-wide FLEX motif scanning.

FIMO hits were classified into unreliable hits and confident hits using the *P* value cutoff of 3E-6. This table lists the numbers of total FIMO hits, unreliable hits and confident hits in each species.

Table S8: Confident hits of FLEX motif scanning.

This table lists the confident FIMO hits in the four fission yeast species.

Table S9: Locations of LTR sequences in *S. osmophilus.*

We used BLASTn with *S. cryophilus* LTR sequences as queries to identify *S. osmophilus* LTRs. In addition, we also used as LTR_retriever (see Methods). The table reports the location, length, and orientation of each LTR identified.

Table S10: Summary of association between 5S rDNA and *wtf* genes within *Schizosaccharomyces* genomes.

The table lists the number of 5S rDNA genes in each species and details how many of those 5S rDNA genes are associated with a locus that contains one or more *wtf* genes. Additional unannotated 5S rDNA genes were identified within the *S. octosporus* and *S. cryophilus* genomes using BLASTn. In *S. osmophilus*, all 5S rDNA genes were identified by BLASTn. A gene was considered a *bona fide* 5S rDNA gene if it shared more than 70% sequence identity with another 5S rDNA gene in that genome. A 5S rDNA was considered associated with a *wtf* locus if it was immediately adjacent to a *wtf* gene, or if it was adjacent to a *wag* gene flanking a *wtf* gene.

Table S11: *wag* gene transcripts in *S. octosporus.*

Annotation of *wag* genes of *S. octosporus* with the corresponding SOCG names, where applicable, in column B. Genes with early stop codons relative to consensus sequences are considered pseudogenes (column H).

Table S12: Synteny analysis of the regions containing *wtf* genes in *S. pombe* (i.e. Figure 4 and Figure 4-figure supplement 1).

For each *S. pombe wtf* locus (from the *S. kambucha* isolate; column A), we noted the genes directly upstream and downstream excluding *wag* genes (columns H and I). We next found the orthologs of those *wtf*-flanking genes in *S. osmophilus* (columns J and K), *S. octosporus* (columns L and M), and *S. cryophilus* (columns N and O). If the orthologs of the genes that flank a *wtf* in *S. pombe* also flank a single *wtf* locus in another species, the *wtf* genes were considered to share ‘complete’ synteny. If the orthologs both flank *wtf* genes, but not the same *wtf* gene in a different species, we dubbed this scenario ‘double partial synteny.’ If only one of the two orthologs flank a *wtf* gene in another species, we considered that ‘partial synteny.’ The

synteny analyses results for *S. cryophilus*, *S. octosporus* and *S. osmophilus* are reported in columns B-C, D-E, and F-G, respectively.

Table S13: *S. cryophilus wtf* genes in synteny with *S. octosporus*, *S. osmophilus*, and *S. pombe wtf* genes (Figure 4-supplement figure 1).

For each *S. cryophilus wtf* gene (column A), we noted the genes directly upstream and downstream, excluding *wag* genes (columns H and I). We next found the orthologs of those *wtf*-flanking genes in *S. octosporus* (columns J and K), *S. pombe* (columns L and M), and *S. osmophilus* (columns N and O). If the orthologs both flank *wtf* genes, but not the same *wtf* gene in a different species, we dubbed this scenario 'double partial synteny.' If only one of the two orthologs flank a *wtf* gene in another species, we considered that 'partial synteny.' The synteny analyses results for *S. octosporus*, *S. pombe*, and *S. osmophilus* are reported in columns B-C, D-E, and F-G, respectively.

Table S14: Percent amino acid identity of genes flanking *wtf* genes at syntenic loci (i.e. Figure 4 and Figure 4-figure supplement 1).

The amino acid sequences of genes flanking the *S. pombe wtf* loci shown in Figure 4 (*wtf34*) and Figure 4 -figure supplement 1 (*wtf6*) were aligned with their orthologs from all other *Schizosaccharomyces* species using MAFFT L-INS-I (200PAM scoring matrix/k=2; Gap open penalty of 2; offset of 0.123). The tables depict the pairwise percent amino acid identity between all ortholog pairs. Comparisons between the genes flanking *S. pombe wtf34* (*clr4* and *met17*) are shown at the top while the comparisons between the genes flanking *S. pombe wtf6* (*ago1* and *cyp9*) are shown below.

Table S15: Species-specific *wtf* genes.

Summary of the species-specific *wtf* loci and genes found in each species. The *S. kambucha* isolate of *S. pombe* was used for this table and the reference genomes were used for the other species. The gene names of the species-specific *wtf* genes are shown in the final column. Genes found at separate loci are separated by commas and genes found at a centromere are shown in bold.

Table S16: Analyzing if 5S rDNA genes are found at loci syntenic to 5S rDNA-adjacent *S. osmophilus wtf* genes in other species (i.e. Figure 6).

For each *S. osmophilus wtf* locus (column A), we noted the genes directly upstream and downstream (columns D and E) excluding any *wag* genes. We next found the orthologs of those *wtf*-flanking genes in *S. octosporus* (columns F and G), and *S. cryophilus* (columns H and I). The synteny analyses results comparing *S. osmophilus wtf* loci to *S. octosporus* are shown in columns B and C. If the orthologs of the genes that flank a *wtf* in *S. osmophilus* also flank a single *wtf* locus in the queried species, the *wtf* genes were considered to share 'complete' synteny. If the orthologs both flank a *wtf* locus, but not the same *wtf* locus in the queried species, we dubbed this scenario 'double partial synteny.' If only one of the two orthologs flank a *wtf* gene in the queried species, we considered that 'partial synteny.' For the analysis, we considered loci in complete synteny where there was a *wtf* gene flanked by a 5S rDNA gene in *S. osmophilus* (column J), but no *wtf* gene at the syntenic locus in the queried species (columns K and M, respectively). We evaluated if the *wtf*-lacking syntenic locus in *S. octosporus* or *S. cryophilus* contained a 5S rDNA gene (columns L and N, respectively). The loci that met our criteria and were considered in the analysis are listed in columns O and P for *S. octosporus* and *S. cryophilus*, respectively. In column Q we considered each locus to be a lineage specific locus meaning no synteny found in other species.

Table S17: Analyzing if 5S rDNA genes are found at loci syntenic to 5S rDNA-adjacent *S. octosporus wtf* genes in other species (i.e. Figure 6).

For each *S. octosporus wtf* locus (column A), we noted the genes directly upstream and downstream (columns D and E) excluding any *wag* genes. We next found the orthologs of those *wtf*-flanking genes in *S. osmophilus* (columns F and G), and *S. cryophilus* (columns H and I). The synteny analyses results comparing *S. octosporus wtf* loci to *S. osmophilus* are shown in columns B and C. If the orthologs of the genes that flank a *wtf* in *S. octosporus* also flank a single *wtf* locus in the queried species, the *wtf* genes were considered to share 'complete' synteny. If the orthologs both flank a *wtf* locus, but not the same *wtf* locus in the queried species, we dubbed this scenario 'double partial synteny.' If only one of the two orthologs flank a *wtf* gene in the queried species, we considered that 'partial synteny.' For the analysis, we considered loci in complete synteny where there was a *wtf* gene flanked by a 5S rDNA gene in *S. octosporus* (column J), but no *wtf* gene at the syntenic locus in the queried species (columns K and M, respectively). We evaluated if the *wtf*-lacking syntenic locus in *S. osmophilus* or *S. cryophilus* contained a 5S rDNA gene (columns L and N, respectively). The loci that met our criteria and were considered in the analysis are listed in columns O and P for *S. osmophilus* and *S. cryophilus*, respectively. In column Q we considered each locus to be a lineage specific locus meaning no synteny found in other species.

Table S18: Repeat count within exon 4 in *S. octosporus* and *S. osmophilus wtf* genes (i.e. Figure 5-supplement figure 2).

This tab contains 4 tables. From left to right, the first table displays the size, in base pairs of the repeat region found in each intact *S. octosporus wtf* genes. These sizes were determined manually in each gene. The next table summarizes how many *S. octosporus wtf* genes were

found with repeat regions of the indicated ranges. The following two tables repeat the analyses with the *S. osmophilus wtf* genes.

Table S19: Expanded analysis of *wtf*+5S rDNA loci in species A with 5S rDNA at the locus in species B (i.e. Figure 6).

Expanded table of data presented in Figure 6. The analysis considers *wtf*+5S rDNA loci that are present in species A that are not found in species B. The total number of such sites, in addition to how many of the sites have a 5S rDNA gene at the syntenic site in species B is reported. The *wtf* genes considered are shown in the last column. Those with a 5S rDNA gene at the syntenic site in species B are shown in bold. Genes found at separate loci are separated by commas.

Table S20: Total viability numerical data summary.

Table S21: *wtf25*(SOCG_04480) deletion related numerical data of the octad dissection analysis.

Table S22: *wtf68*(SOCG_01240) deletion related numerical data of the octad dissection analysis.

Table S23: *wtf33* deletion related numerical data of the octad dissection analysis.

Table S24: *wtf46*(SOCG_00084) deletion related numerical data of the octad dissection analysis.

Table S25: *wtf60*(SOCG_04742) deletion related numerical data of the octad dissection analysis.

Table S26: *wtf62*(SOCG_04077) deletion related numerical data of the octad dissection analysis.

Table S27: *wtf21*(SOCG_02322) deletion related numerical data of the octad dissection analysis.

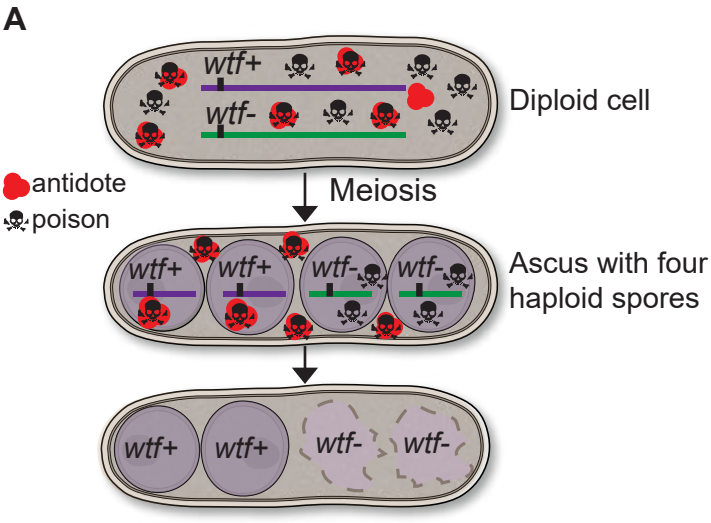
Table S28: octo-pSIV-leu1-1D plasmid related numerical data of the octad spore dissection analysis.

Table S29: Yeast strain summary.

Table S30: Oligos summary.

Table S31: Plasmids summary.

Figure 1: *wtf* homologs are found outside of *S. pombe*.



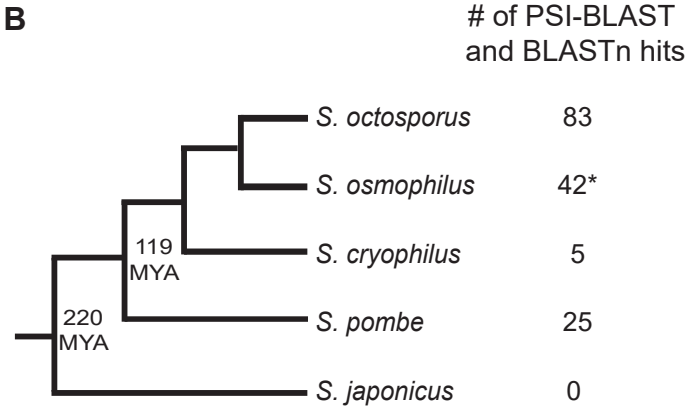


Figure 1-figure supplement 1: Maps of the *wtf* gene family members in *S. octosporus*, *S. osmophilus*, *S. cryophilus* and *S. pombe*.

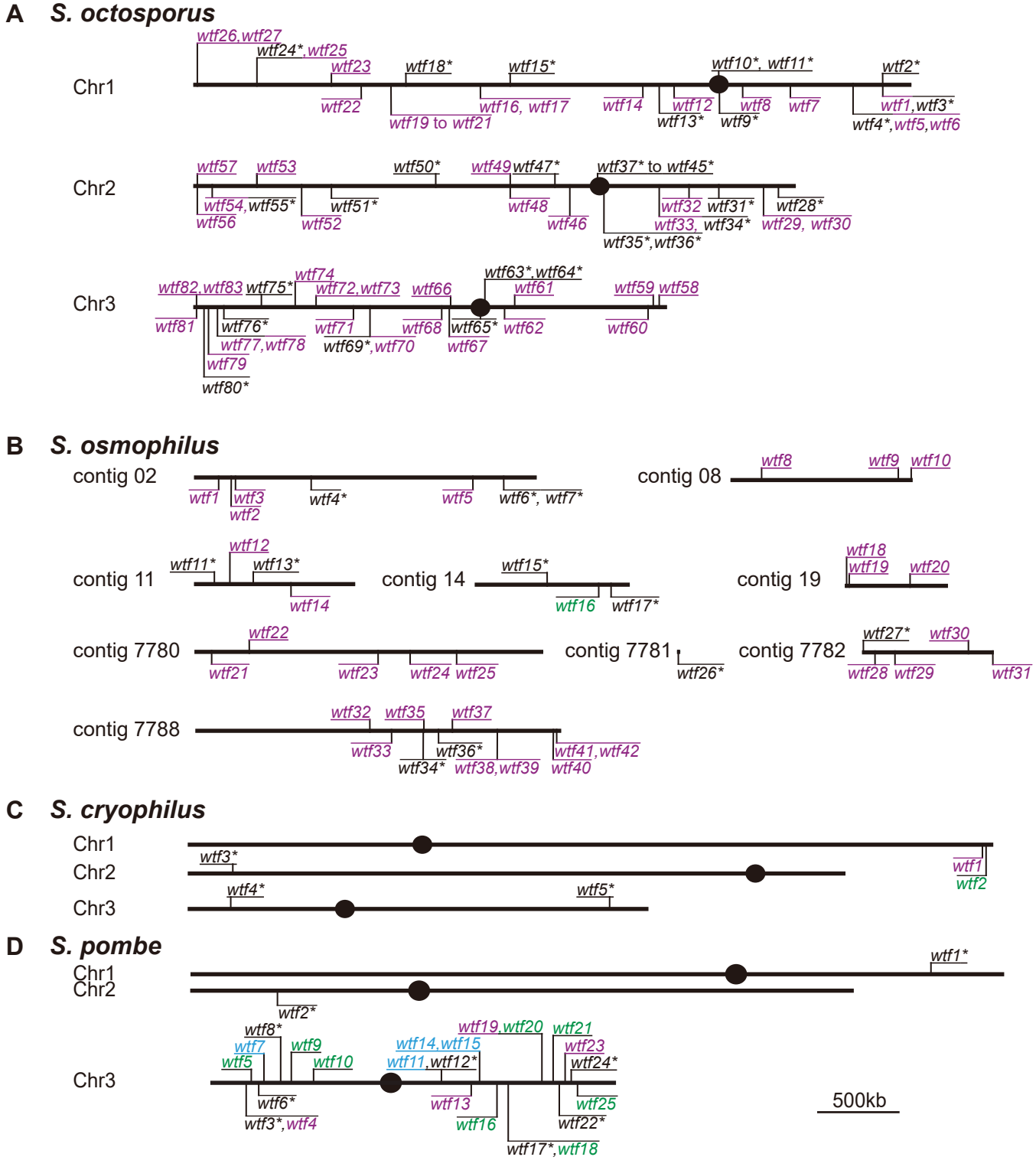


Figure 2: *S. pombe wtf* genes share features with other *wtf* genes outside of *S.pombe*.

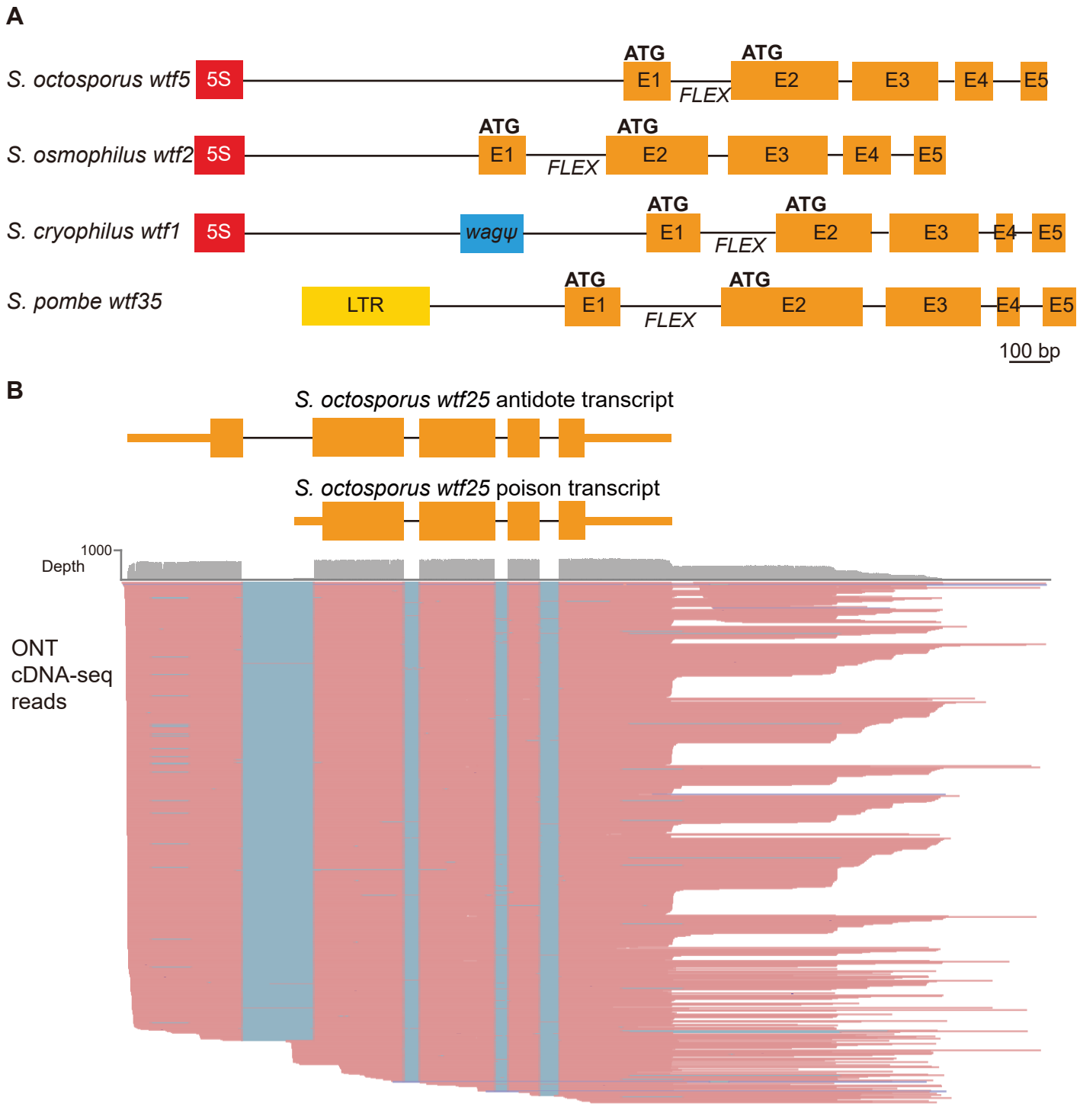


Figure 2-figure supplement 1: Limited conservation of Wtf proteins.

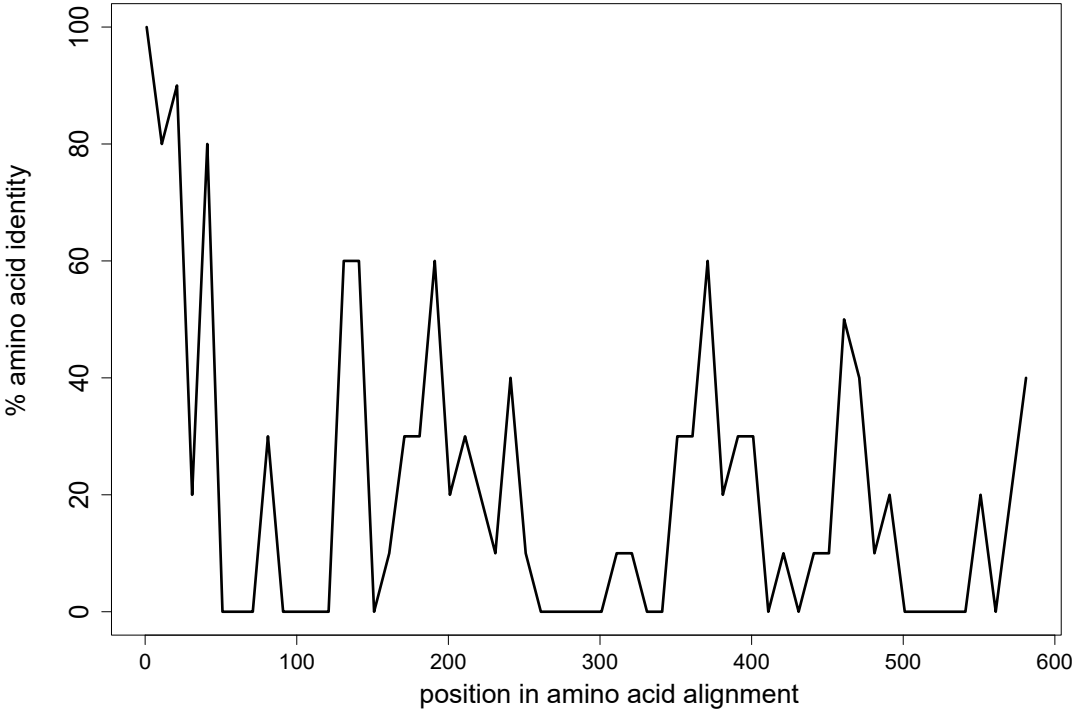


Figure 2-figure supplement 2: Many *wtf* genes in *S. octosporus* harbor the FLEX motif in intron 1

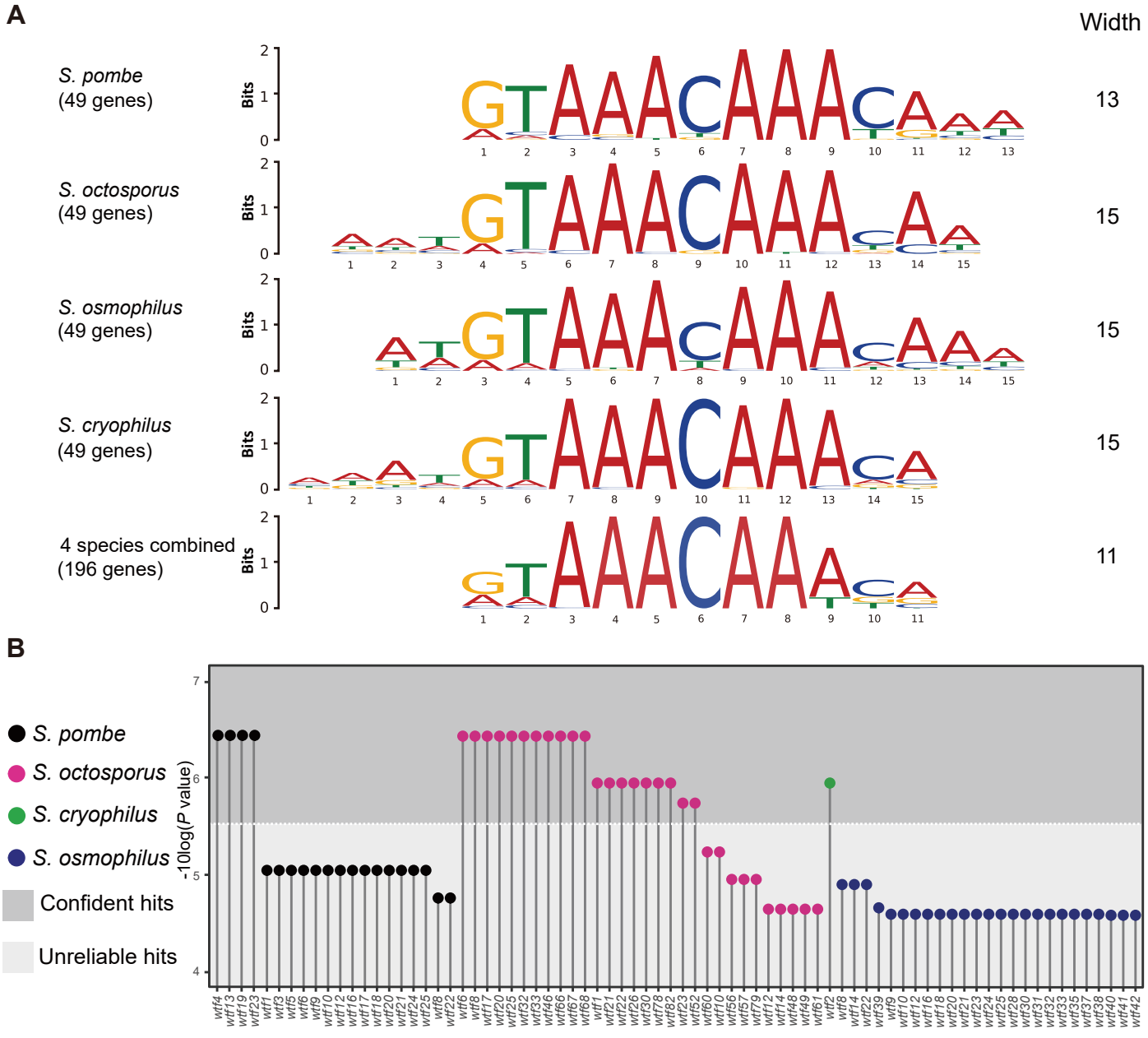


Figure 2-figure supplement 3: Transcription levels of predicted poison and antidote isoforms of intact *wtf* genes in *S. octosporus*.

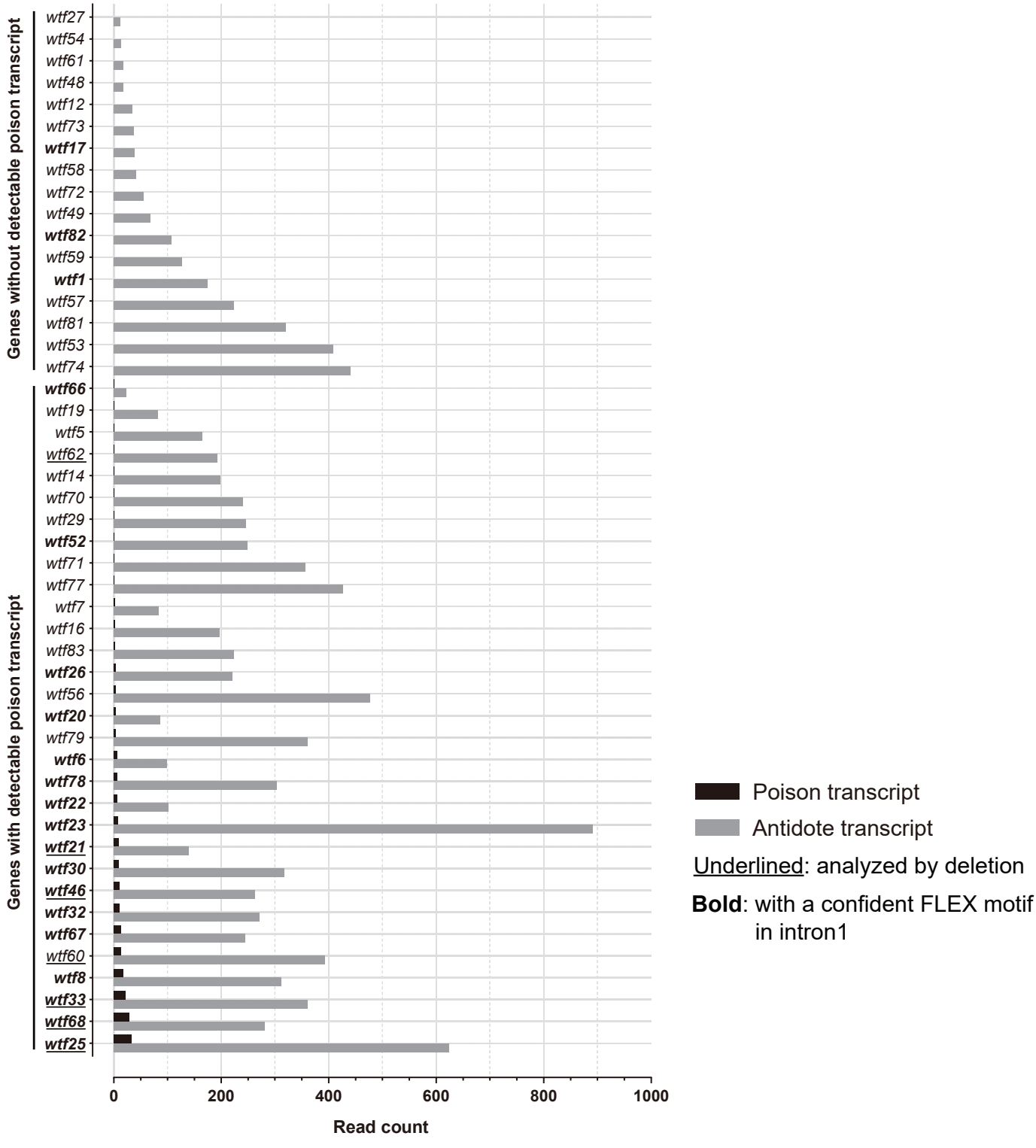


Figure 3: Genomic context of *wtf* genes.

Figure 3-figure supplement 1: Distance between 5S rDNA and *wtf* genes

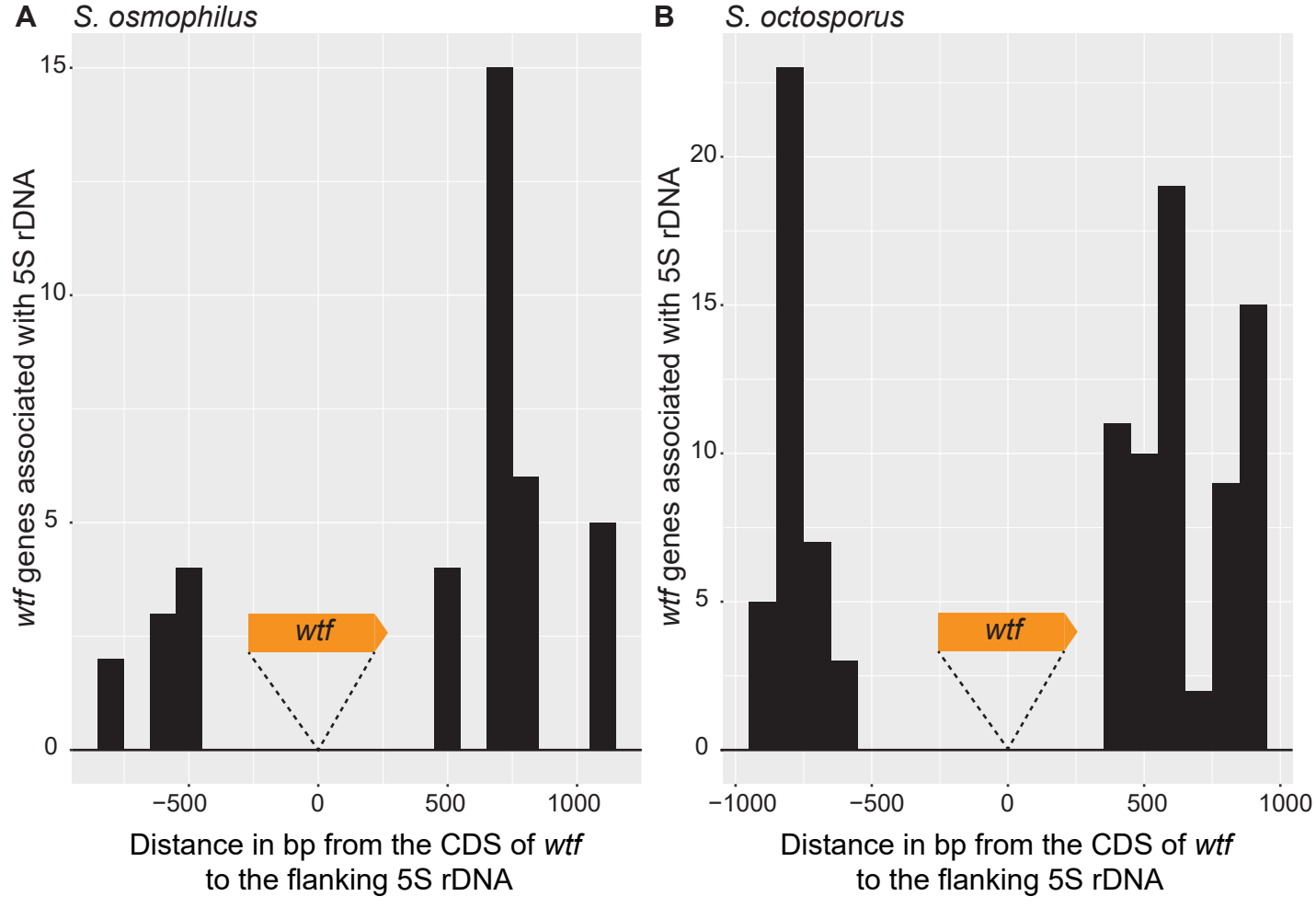


Figure 3-figure supplement 2: Homology between distinct 5S rDNA-*wtf* and *wag-wtf* units

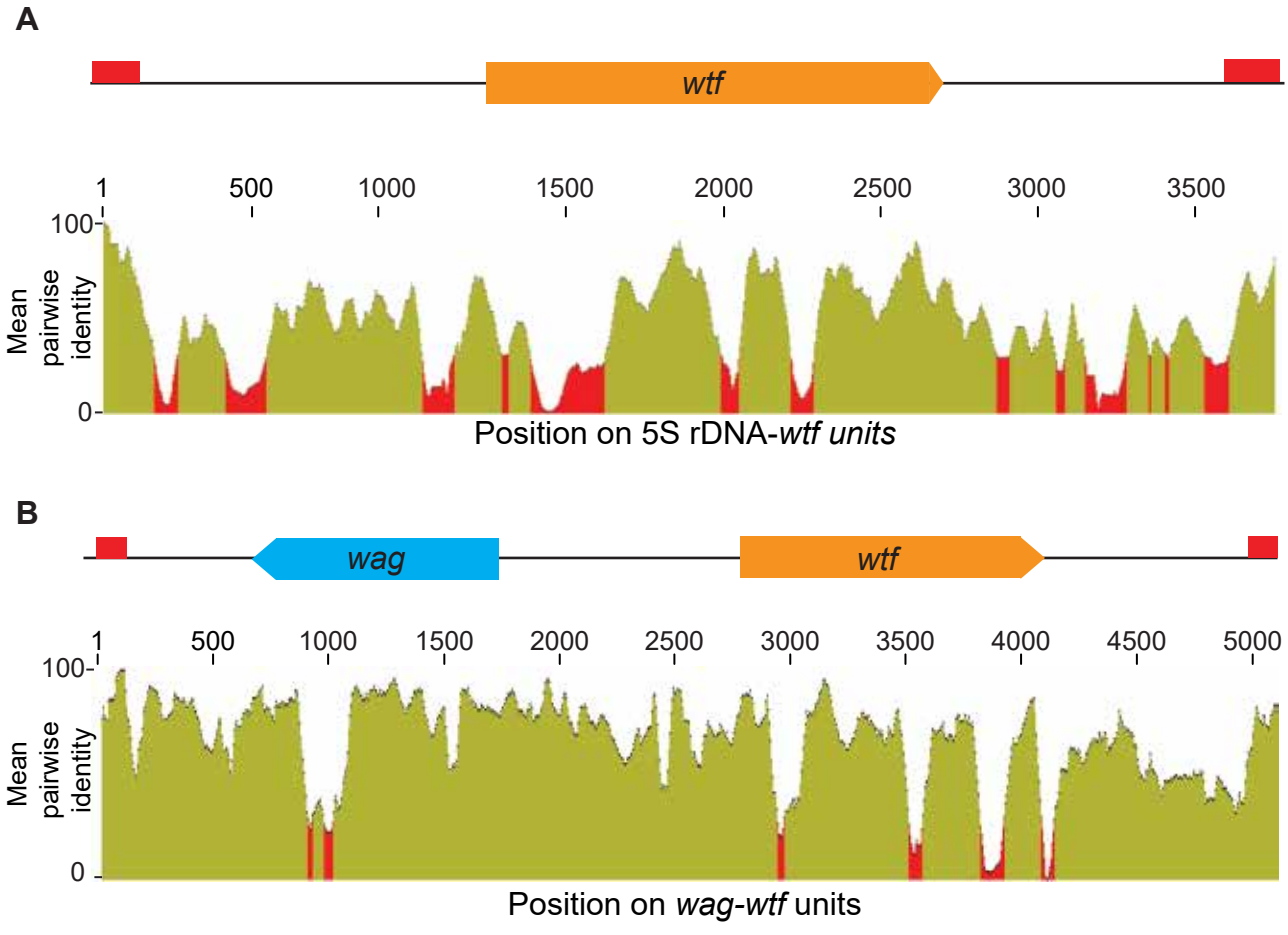


Figure 3-figure supplement 3: *S. octosporus wtf* gene units supported by maximum likelihood phylogeny.

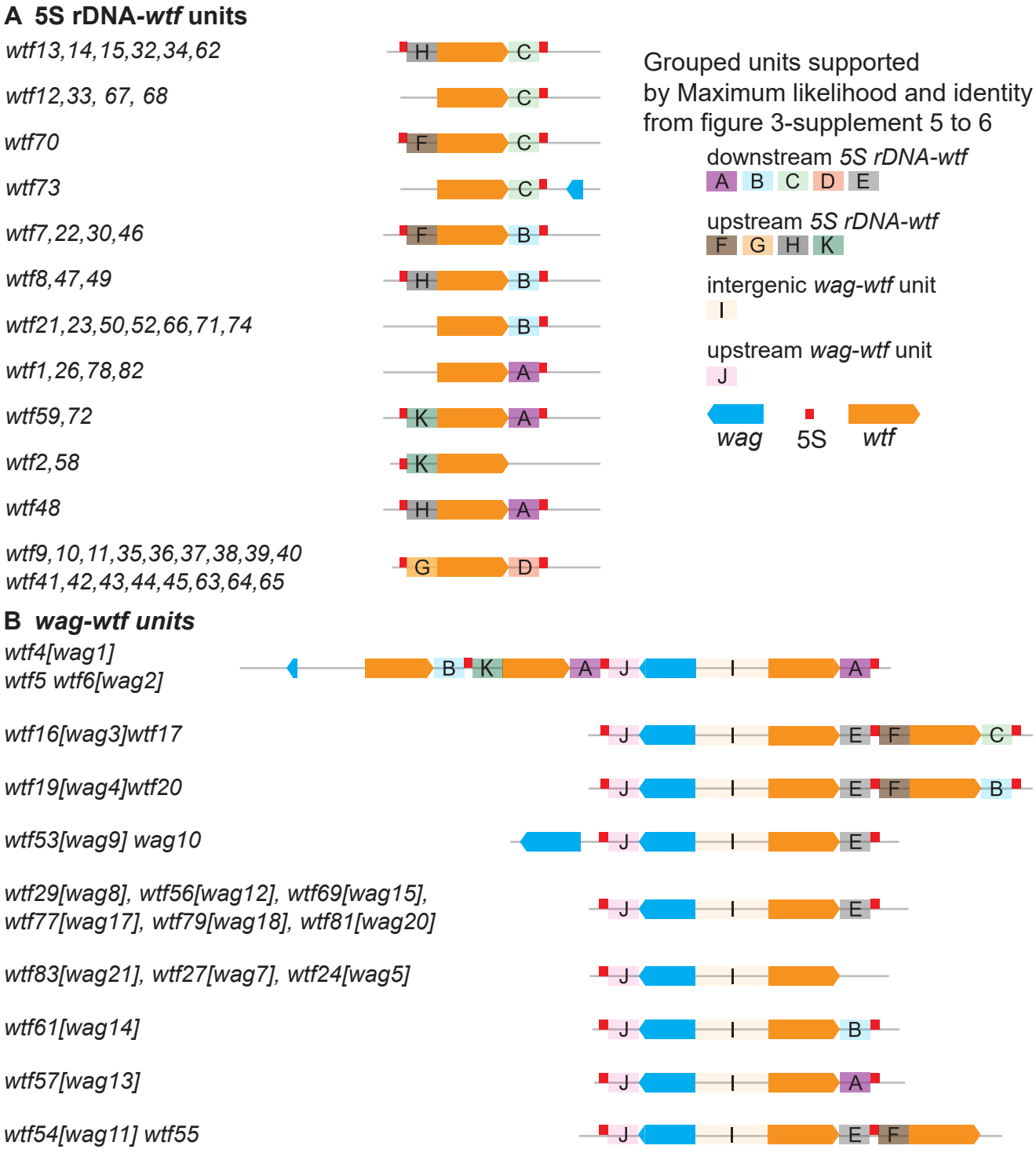


Figure 3-figure supplement 4: Maximum likelihood phylogeny of the regions between *S. octosporus wtf* genes and a downstream flanking 5S rDNA gene.

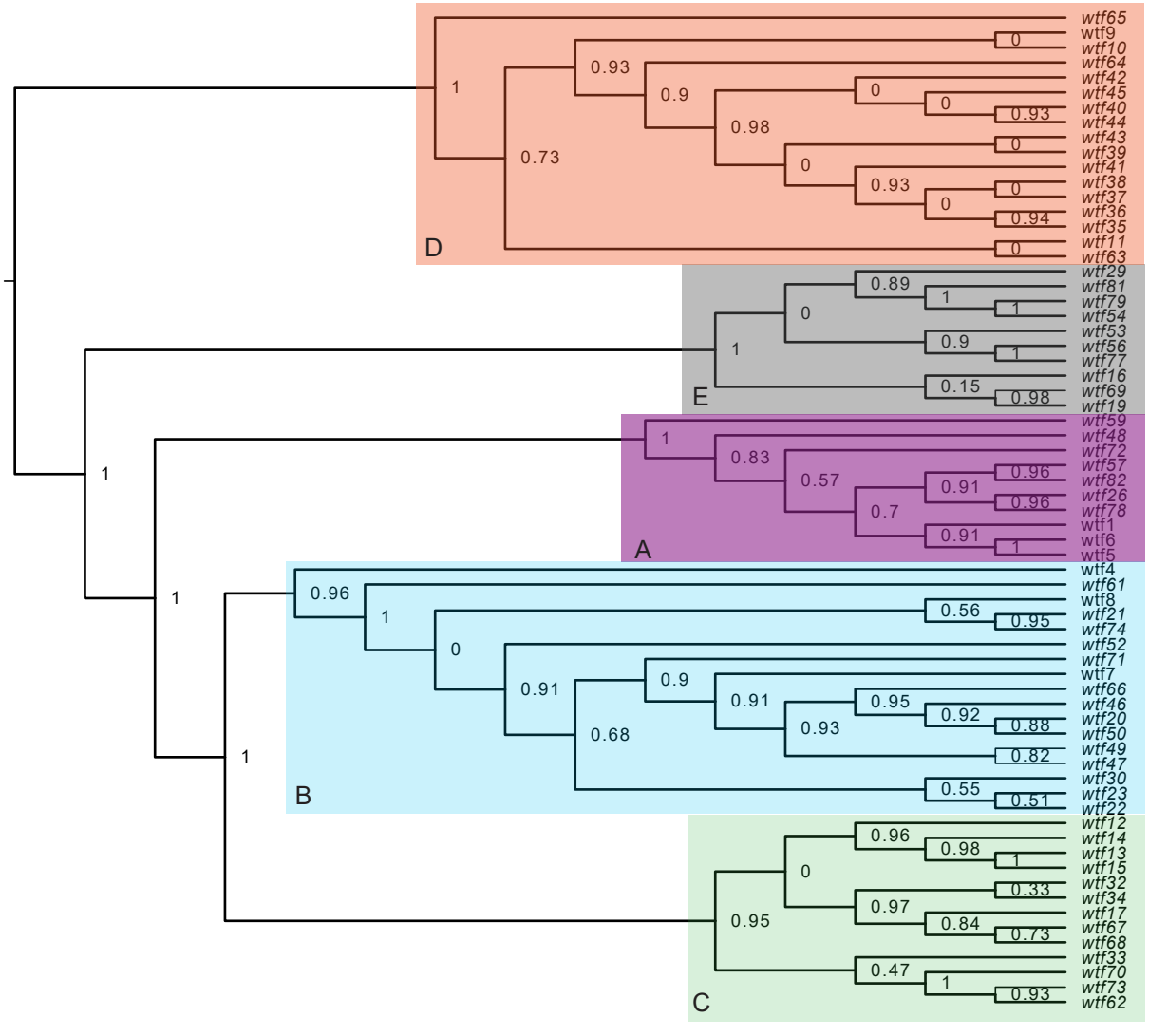


Figure 3-figure supplement 5: Maximum likelihood phylogeny of the regions between *S. octosporus wtf* genes and a upstream flanking 5S rDNA gene.

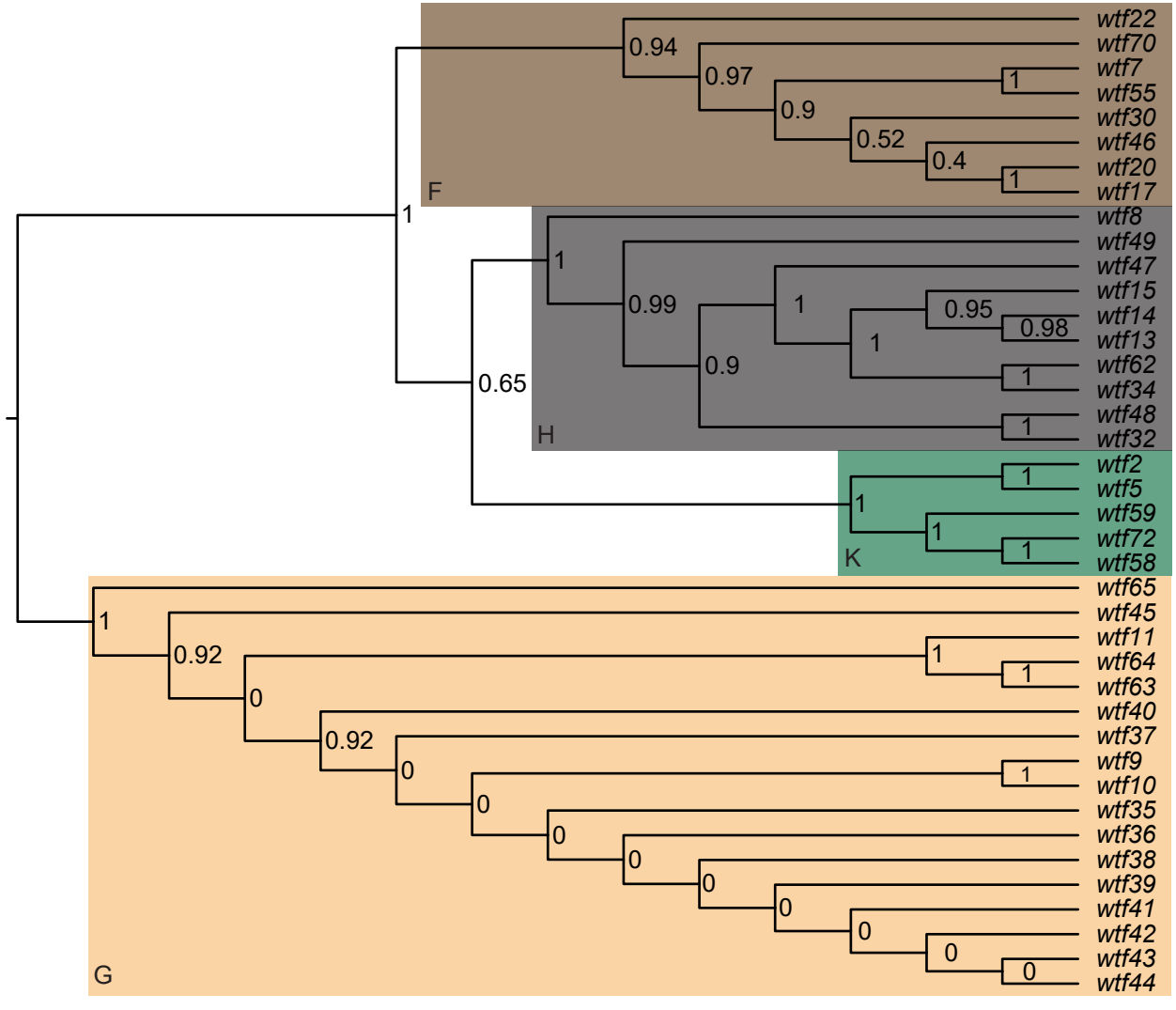


Figure 3-figure supplement 6: Maximum Likelihood phylogeny of *S. octosporus wtf* genes

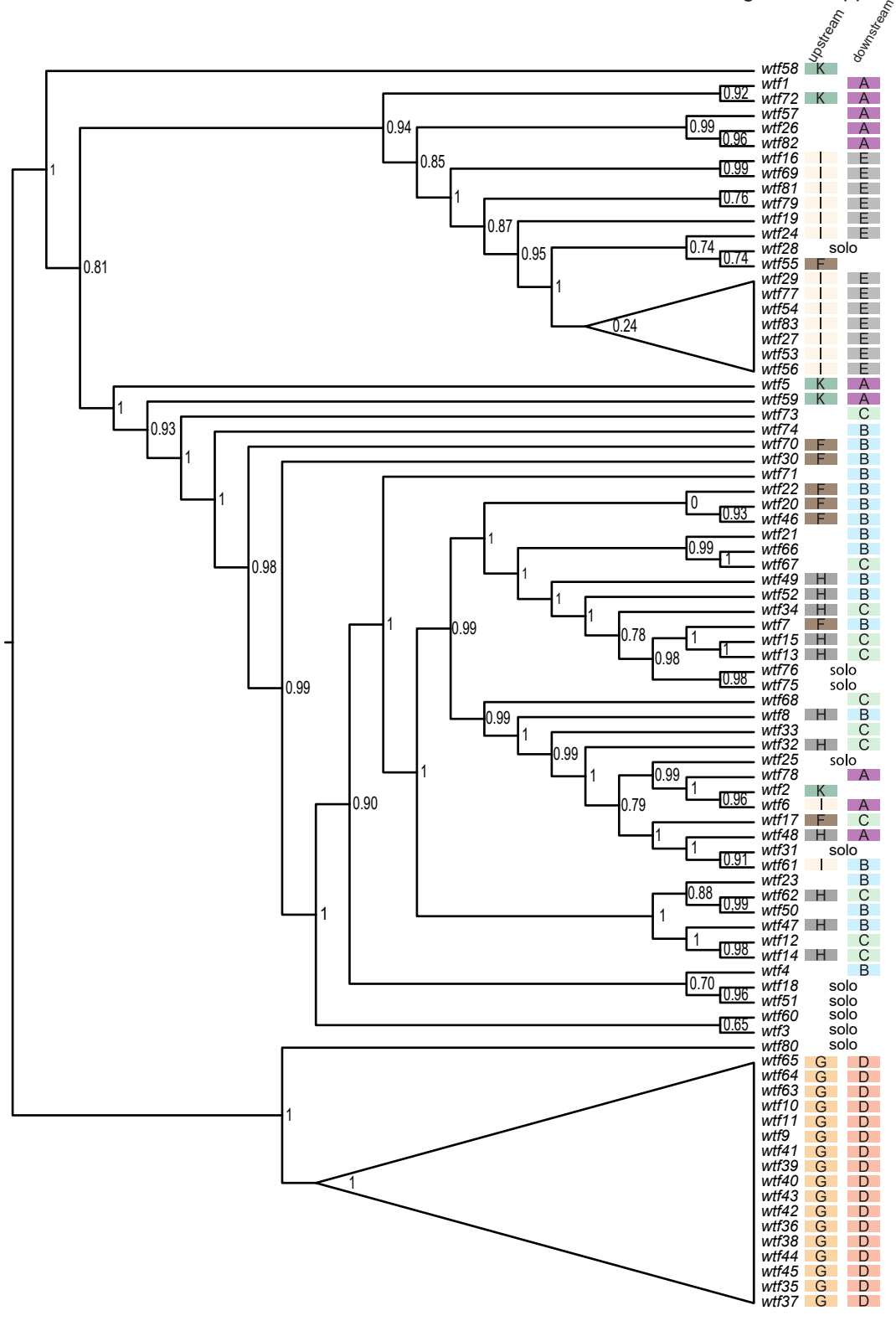


Figure 4: *Shared wtf* locus in three fission yeast species.

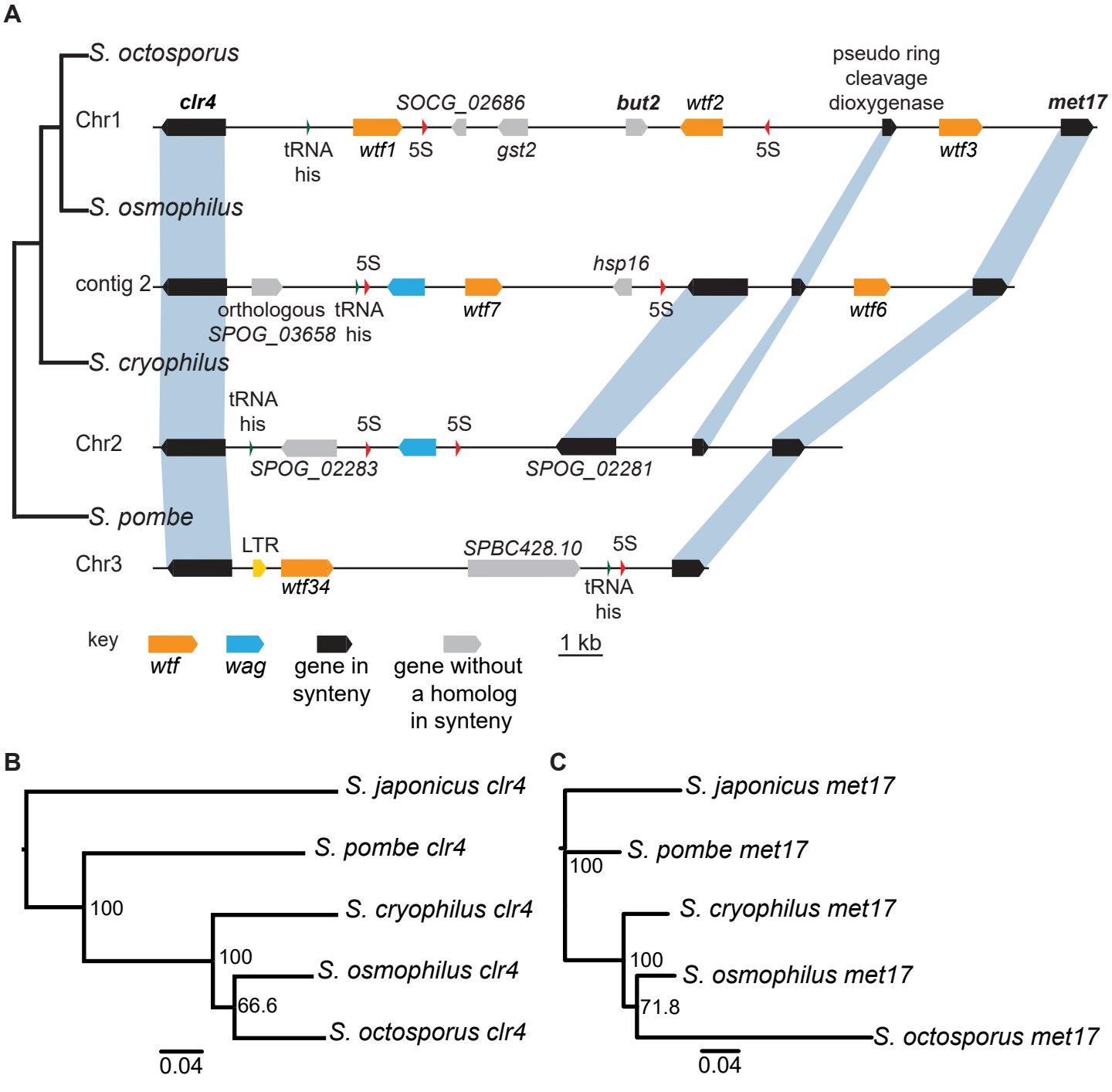


Figure 4-figure supplement 1: Synteny between *S. cryophilus wtf4* and *S. pombe wtf6*.

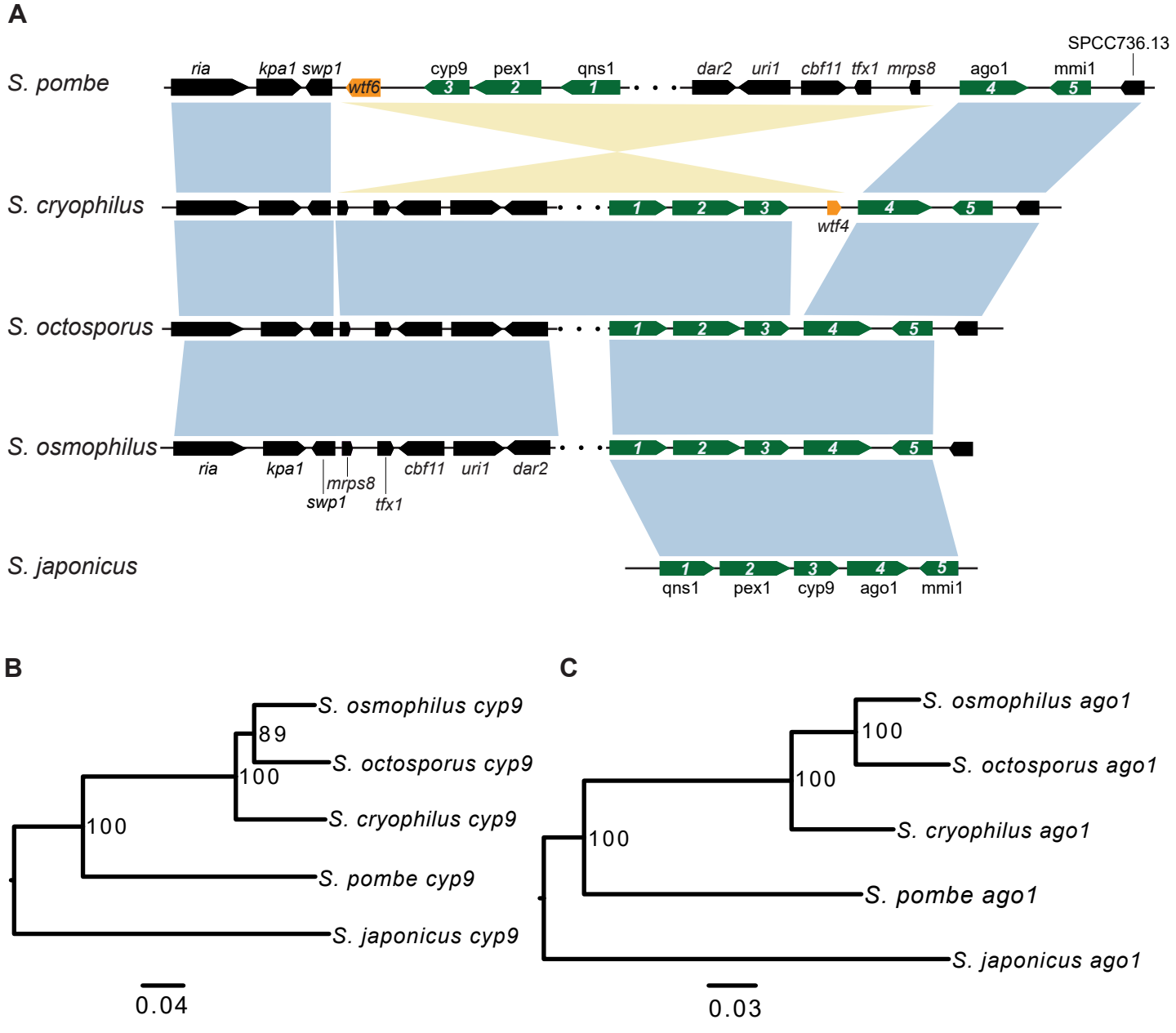


Figure 5: Gene duplication and non-allelic gene conversion within *wtf* gene family.

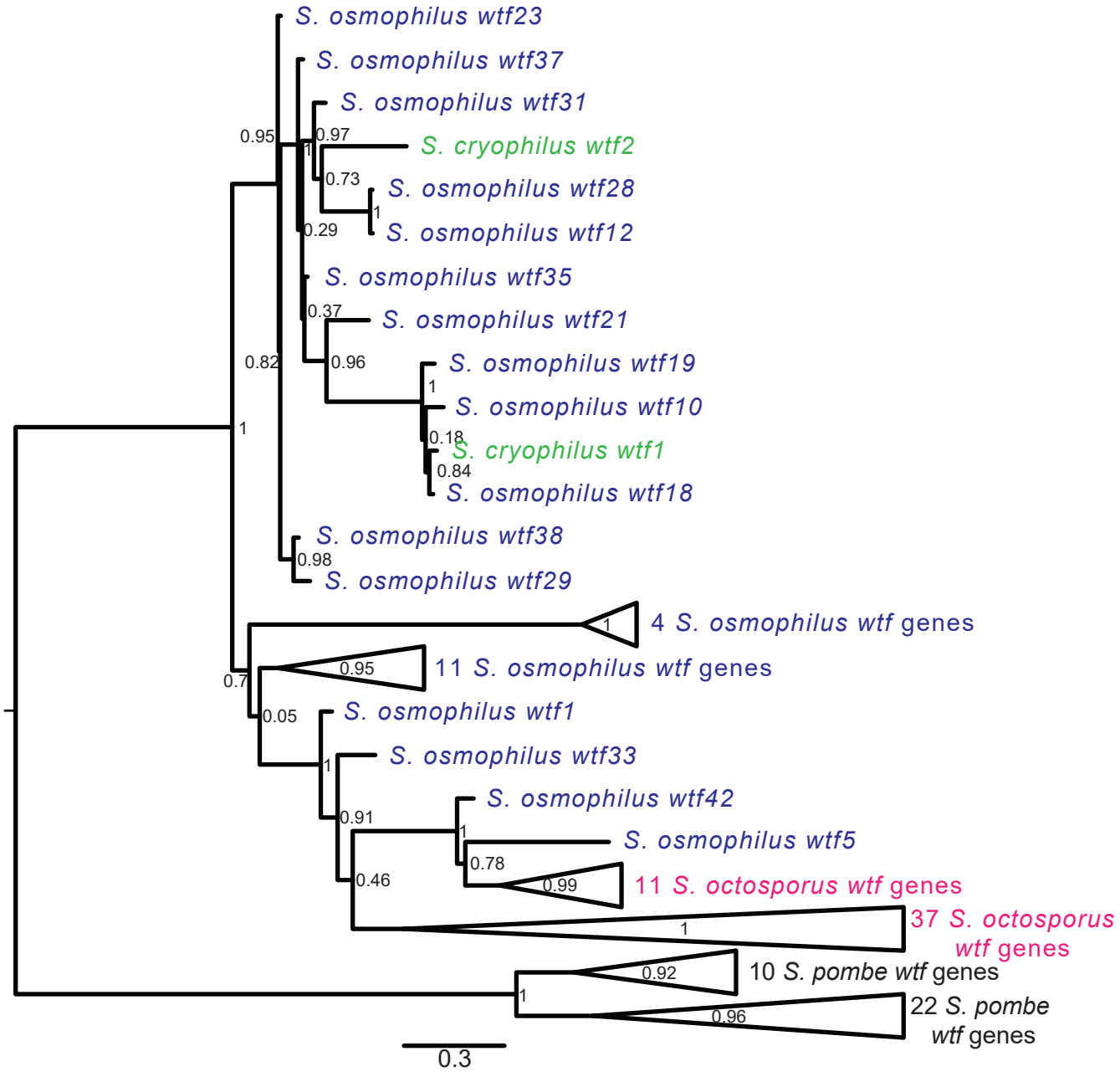


Figure 5-figure supplement 1: GARD analysis consistent with non-allelic gene conversion within of *wtf* genes.

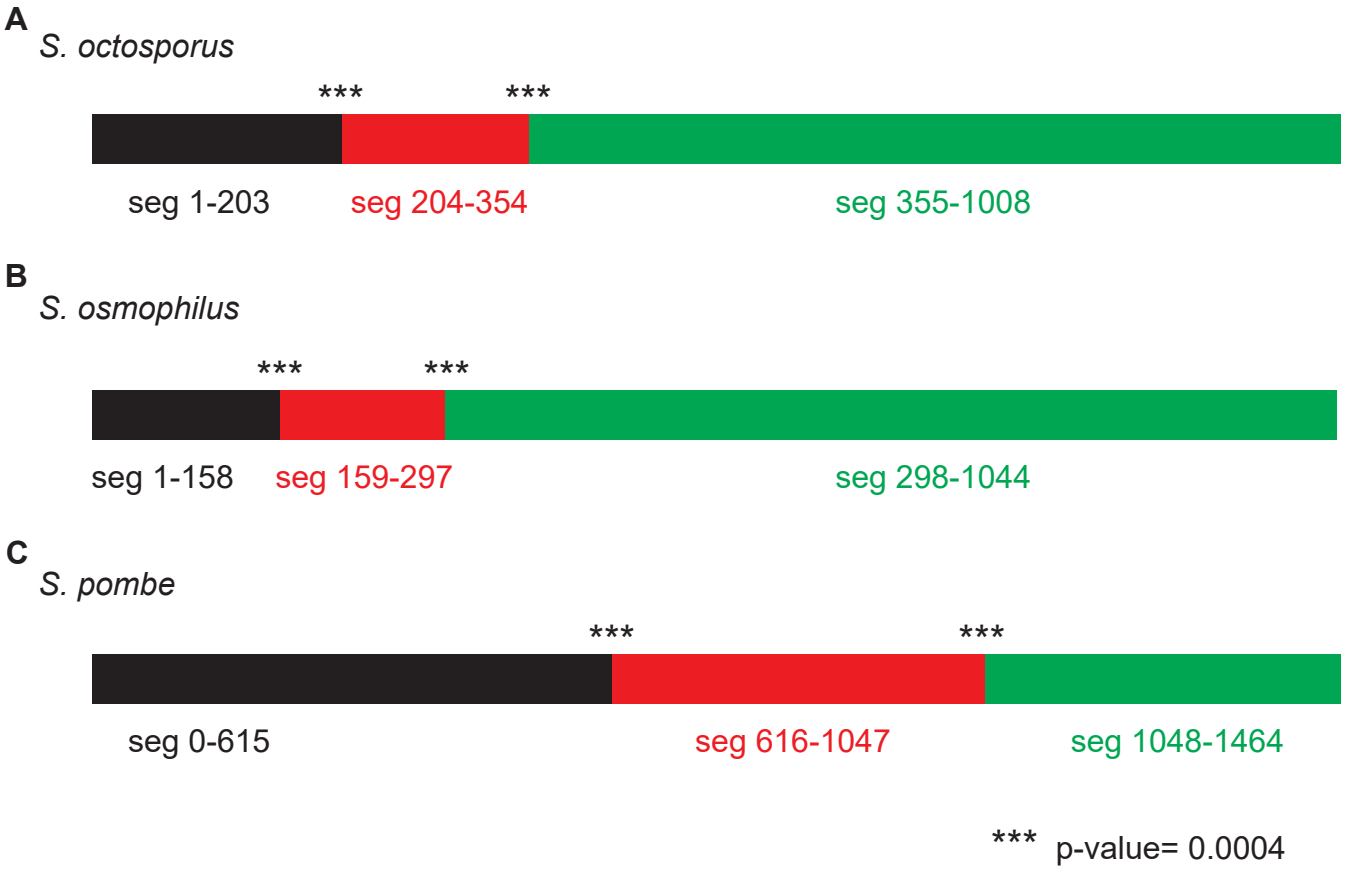


Figure 5-figure supplement 2: Contraction and expansion of repeat sequences in *wtf* genes

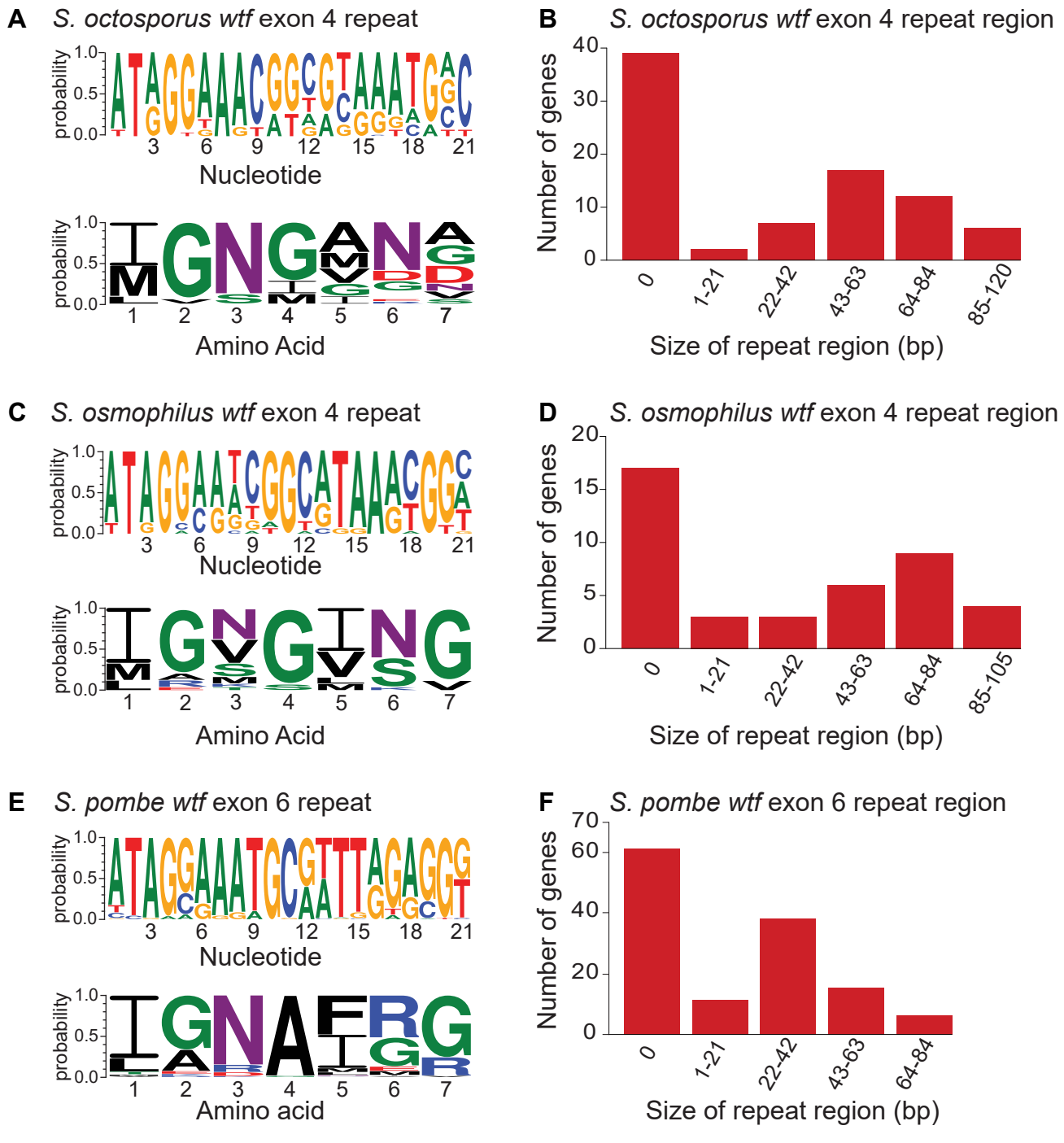
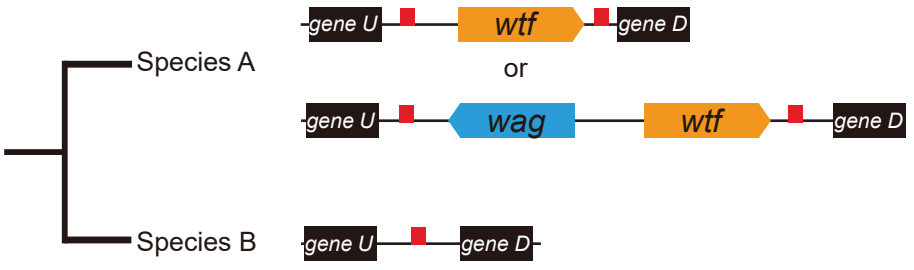


Figure 6: *wtf* genes duplicated into pre-existing 5S rDNA

A



B

Species A	**Species B**	*wtf*+5S rDNA locus in species A with a lone 5S rDNA at the locus in species B*
S. osmophilus	*S. octosporus*	2 out 4
	S. cryophilus	11 out 12
	S. cryophilus and S. octosporus	1 out 1
S. octosporus	*S. cryophilus*	10 out 11
	S. osmophilus	7 out 9
	S. cryophilus and S. osmophilus	5 out 6

*considers only *wtf* where no *wtf* is found in species B

Figure 6-figure supplement 1: *wtf* gene duplication models

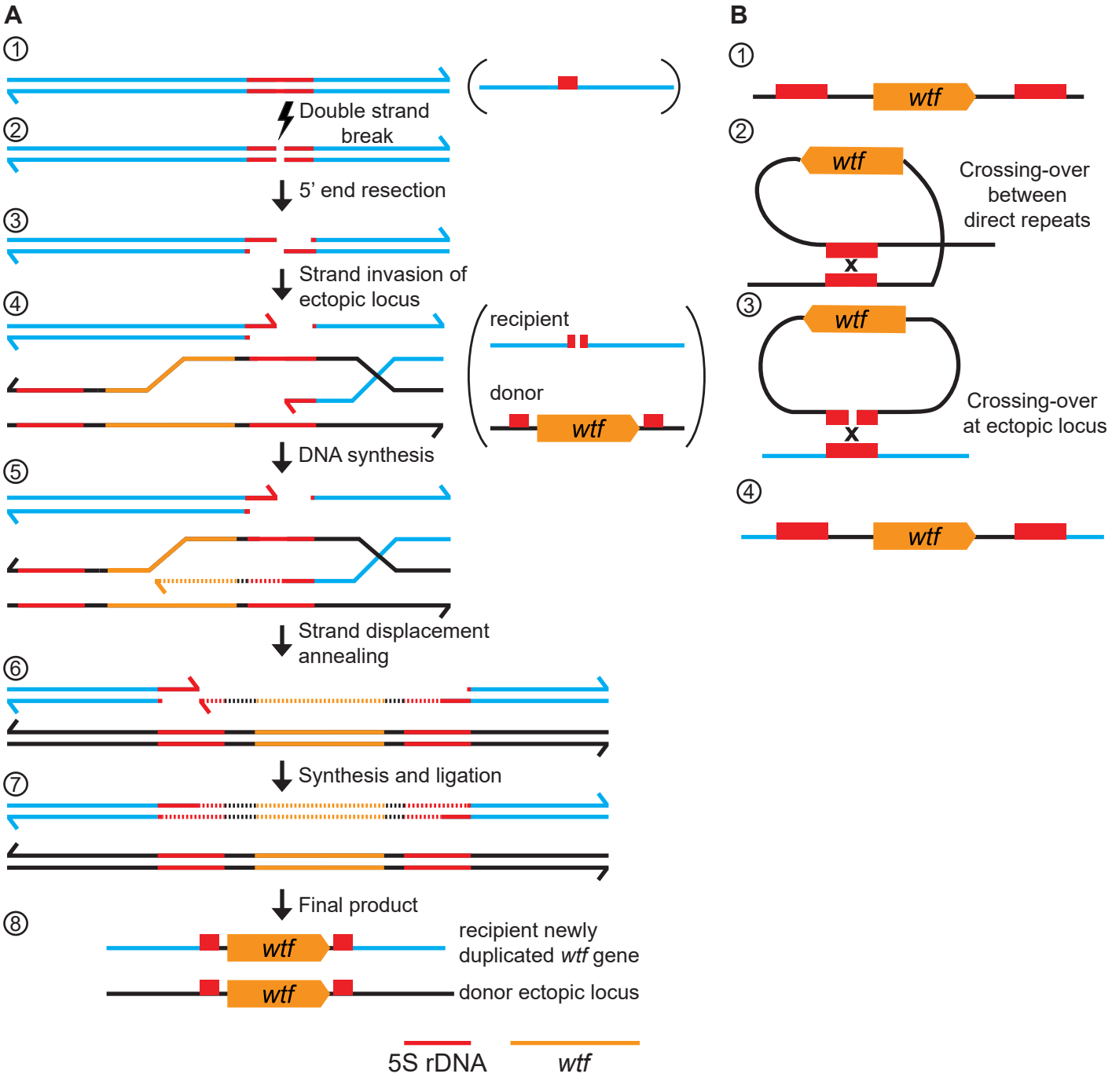
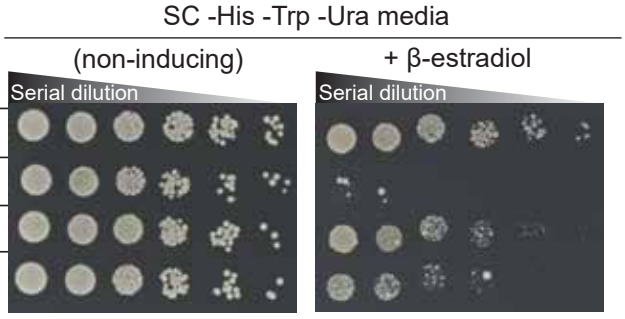


Figure 7: *wtf* genes can encode for poison and antidote proteins.

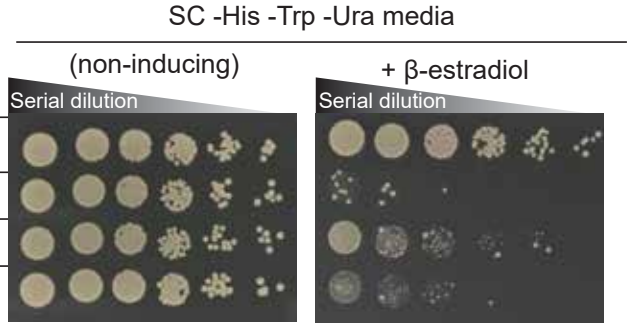
A *S. octosporus wtf25*

plasmids		
[*TRP1*]		[*URA3*]
[EV]	+	[EV]
[EV]	+	[*wtf25*^{poison}-*GFP*]
[*wtf25*^{antidote}-*mCh*]	+	[EV]
[*wtf25*^{antidote}-*mCh*]	+	[*wtf25*^{poison}-*GFP*]



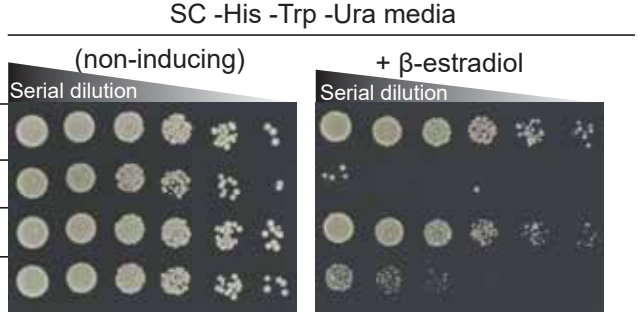
B *S. osmophilus wtf41*

plasmids		
[*TRP1*]		[*URA3*]
[EV]	+	[EV]
[EV]	+	[*wtf41*^{poison}]
[*wtf41*^{antidote}]	+	[EV]
[*wtf41*^{antidote}]	+	[*wtf41*^{poison}]

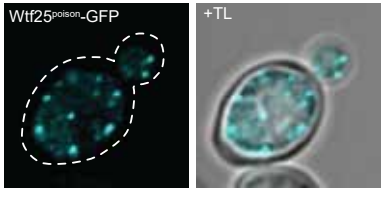


C *S. cryophilus wtf1*

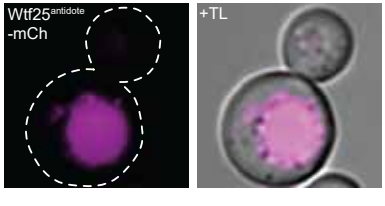
plasmids		
[*TRP1*]		[*URA3*]
[EV]	+	[EV]
[EV]	+	[*wtf1*^{poison}]
[*wtf1*^{antidote}]	+	[EV]
[*wtf1*^{antidote}]	+	[*wtf1*^{poison}]



D [*wtf25*^{poison}-*GFP*]+[EV] cell



E [*wtf25*^{antidote}-*mCh*]+[EV] cell



F [*wtf25*^{poison}-*GFP*]+[*wtf25*^{antidote}-*mCh*] cell

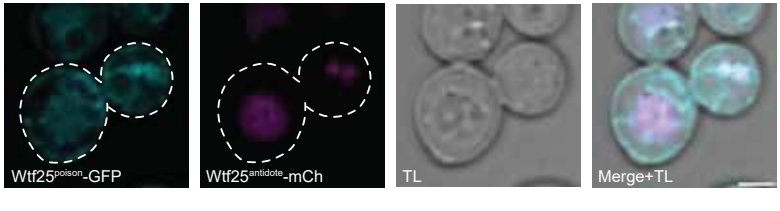


Figure 7-figure supplement 1: Some *wtf* genes outside of *S. pombe* encode for poison and antidote proteins

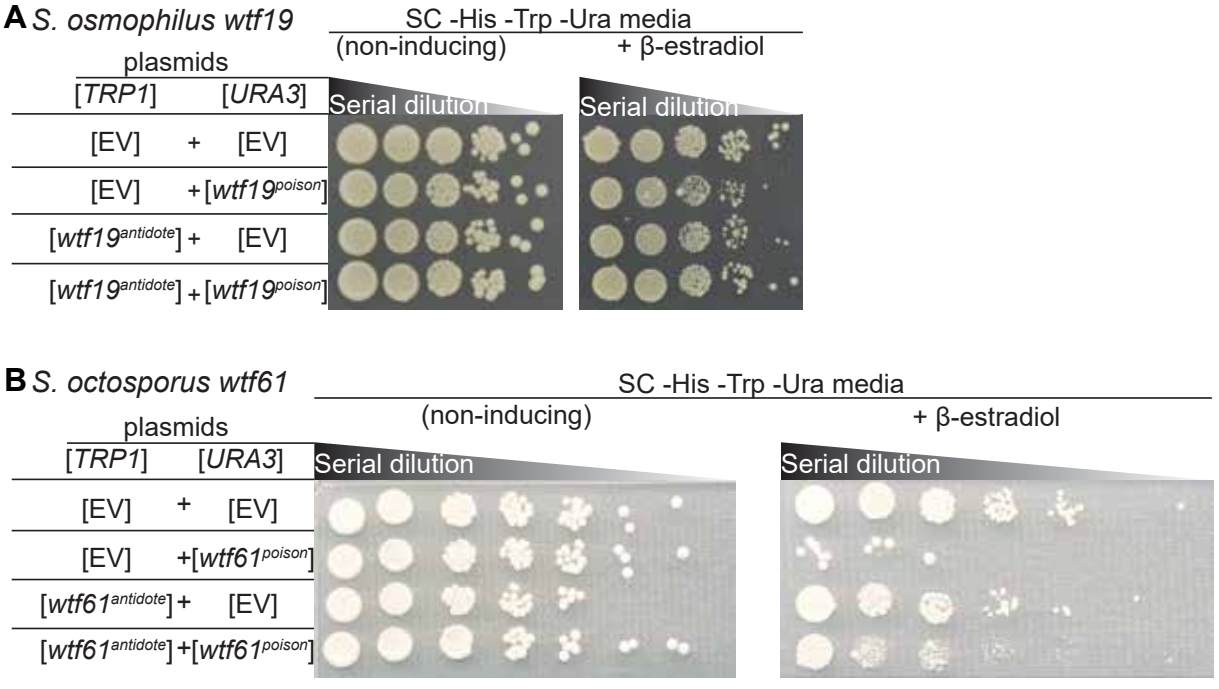
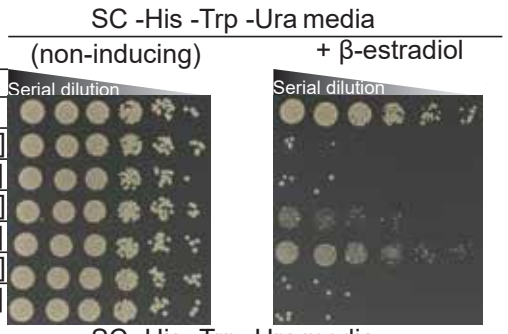


Figure 7-figure supplement 2: Non-cognate Wtf^{antidotes} fail to rescue cells from Wtf^{poisons}.

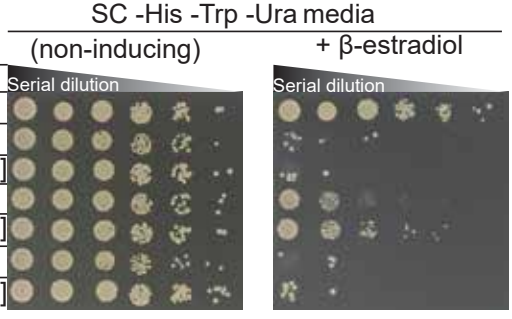
A 66.8% CDS identity
48.7% aa identity

plasmids		
[*TRP1*]		[*URA3*]
[EV]	+	[EV]
[EV]	+	[*S. osmophilus wtf41*^{poison}]
[EV]	+	[*S. octosporus wtf61*^{poison}]
[*S. osmophilus wtf41*^{antidote}]	+	[*S. osmophilus wtf41*^{poison}]
[*S. octosporus wtf61*^{antidote}]	+	[*S. octosporus wtf61*^{poison}]
[*S. octosporus wtf61*^{antidote}]	+	[*S. osmophilus wtf41*^{poison}]
[*S. osmophilus wtf41*^{antidote}]	+	[*S. octosporus wtf61*^{poison}]



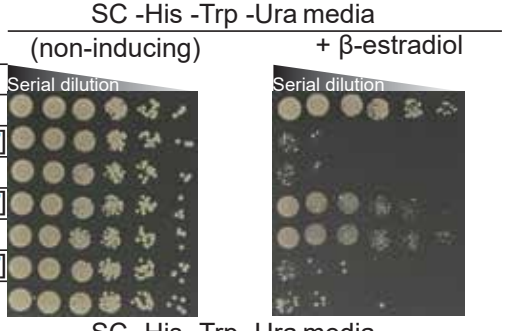
B 45.3% CDS identity
29.1% aa identity

plasmids		
[*TRP1*]		[*URA3*]
[EV]	+	[EV]
[EV]	+	[*S. cryophilus wtf1*^{poison}]
[EV]	+	[*S. osmophilus wtf41*^{poison}]
[*S. cryophilus wtf1*^{antidote}]	+	[*S. cryophilus wtf1*^{poison}]
[*S. osmophilus wtf41*^{antidote}]	+	[*S. osmophilus wtf41*^{poison}]
[*S. osmophilus wtf41*^{antidote}]	+	[*S. cryophilus wtf1*^{poison}]
[*S. cryophilus wtf1*^{antidote}]	+	[*S. osmophilus wtf41*^{poison}]



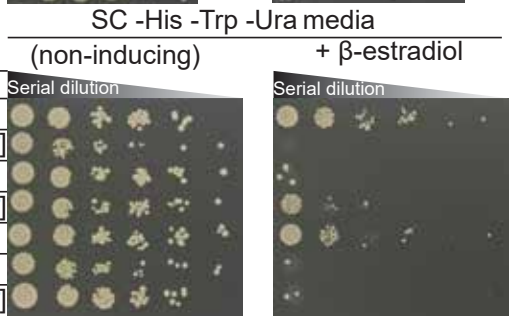
C 82.1% CDS identity
75.6% aa identity

plasmids		
[*TRP1*]		[*URA3*]
[EV]	+	[EV]
[EV]	+	[*S. octosporus wtf25*^{poison}-GFP]
[EV]	+	[*S. octosporus wtf61*^{poison}]
[*S. octosporus wtf25*^{antidote}-mCh]	+	[*S. octosporus wtf25*^{poison}-GFP]
[*S. octosporus wtf61*^{antidote}]	+	[*S. octosporus wtf61*^{poison}]
[*S. octosporus wtf61*^{antidote}]	+	[*S. octosporus wtf25*^{poison}-GFP]
[*S. octosporus wtf25*^{antidote}-mCh]	+	[*S. octosporus wtf61*^{poison}]



D 48.7% CDS identity
30.8% aa identity

plasmids		
[*TRP1*]		[*URA3*]
[EV]	+	[EV]
[EV]	+	[*S. octosporus wtf25*^{poison}-GFP]
[EV]	+	[*S. cryophilus wtf1*^{poison}]
[*S. octosporus wtf25*^{antidote}-mCh]	+	[*S. octosporus wtf25*^{poison}-GFP]
[*S. cryophilus wtf1*^{antidote}]	+	[*S. cryophilus wtf1*^{poison}]
[*S. octosporus wtf25*^{antidote}-mCh]	+	[*S. cryophilus wtf1*^{poison}]
[*S. cryophilus wtf1*^{antidote}]	+	[*S. octosporus wtf25*^{poison}-GFP]



E 29.7% CDS identity
12.6% aa identity

plasmids		
[*TRP1*]		[*URA3*]
[EV]	+	[EV]
[EV]	+	[*S. pombe wtf4*^{poison}-GFP]
[EV]	+	[*S. octosporus wtf25*^{poison}-GFP]
[*S. pombe wtf4*^{antidote}]	+	[*S. pombe wtf4*^{poison}-GFP]
[*S. octosporus wtf25*^{antidote}-mCh]	+	[*S. octosporus wtf25*^{poison}-GFP]
[*S. octosporus wtf25*^{antidote}-mCh]	+	[*S. pombe wtf4*^{poison}-GFP]
[*S. pombe wtf4*^{antidote}]	+	[*S. octosporus wtf25*^{poison}-GFP]

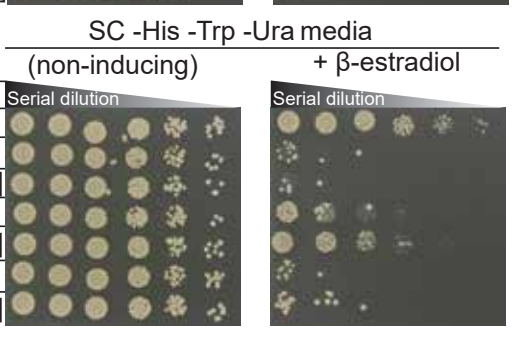


Figure 7-figure supplement 3: The distribution of *S. octosporus* Wtf25 proteins is similar to *S. pombe* Wtf4 proteins.

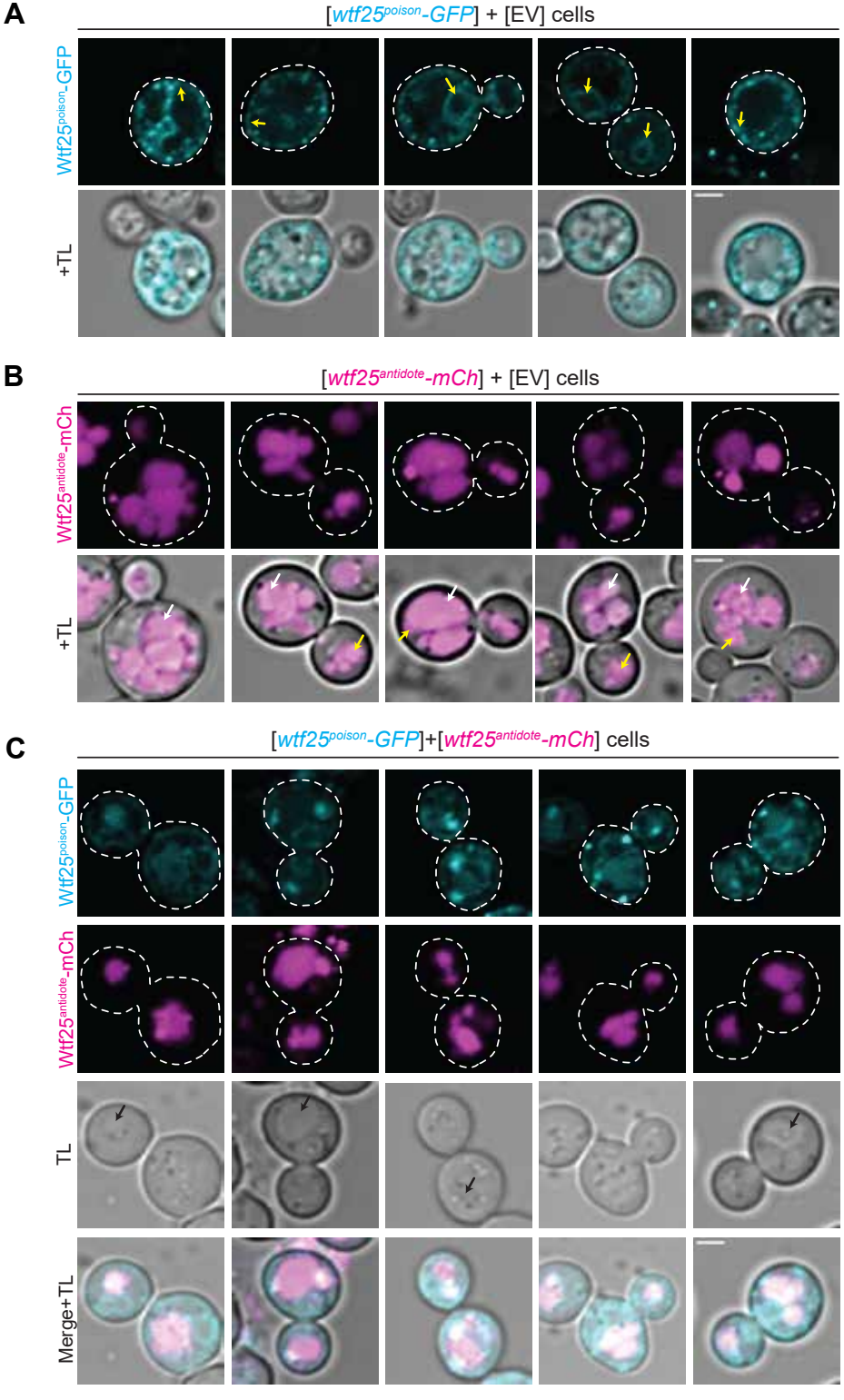


Figure 8: Three *S. octosporus wtf* genes, when individually deleted, caused spore viability loss in heterozygous crosses but not in homozygous crosses.

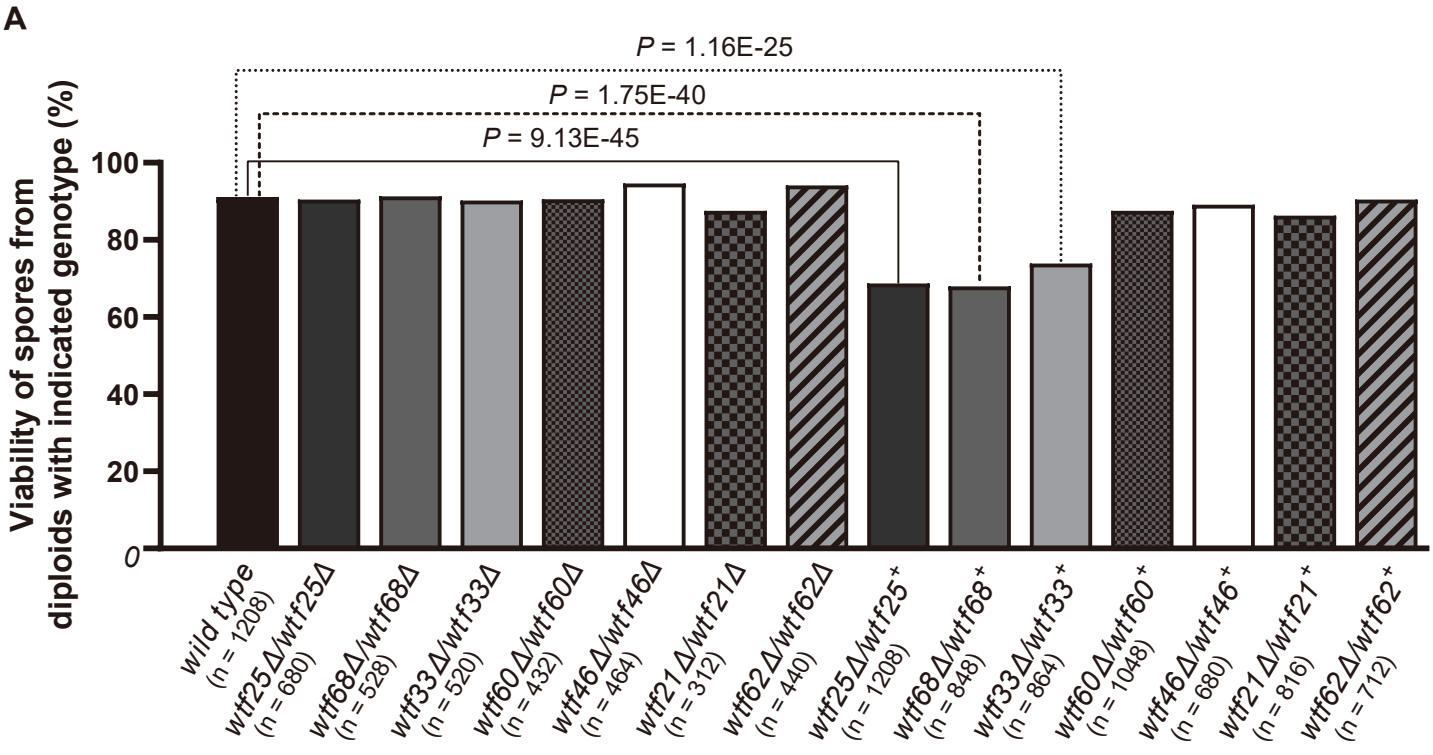
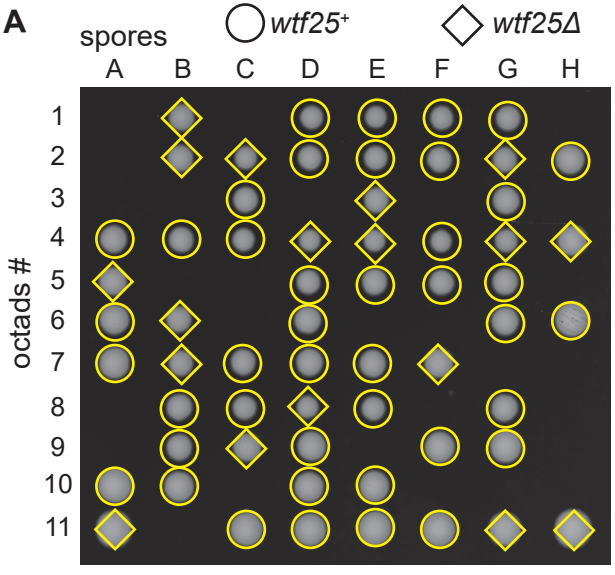
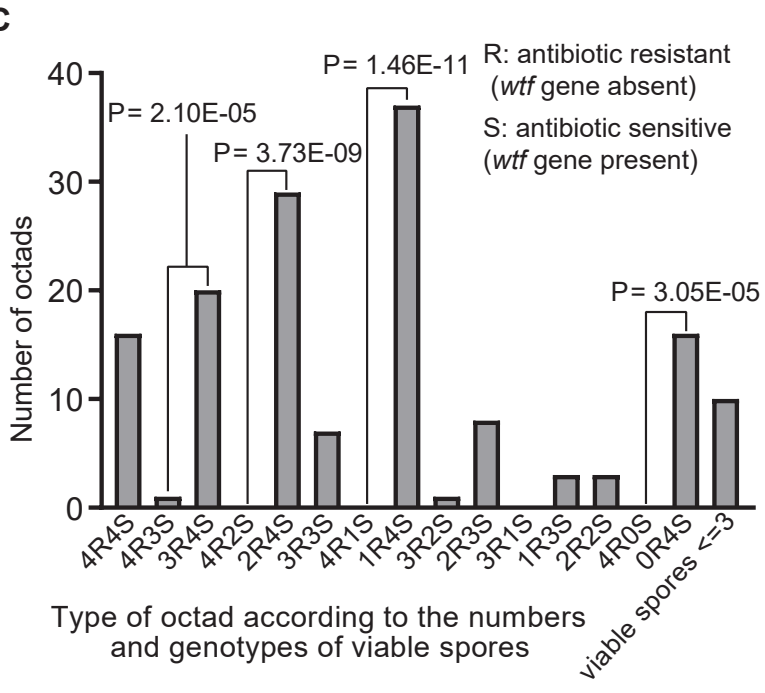


Figure 9: Some *S. octosporus wtf* genes cause meiotic drive.





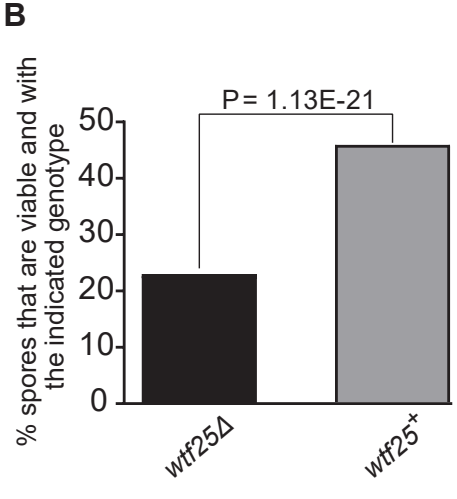


Figure 9-figure supplement 1: Octad dissection analysis of *wtf68* heterozygous deletion cross.

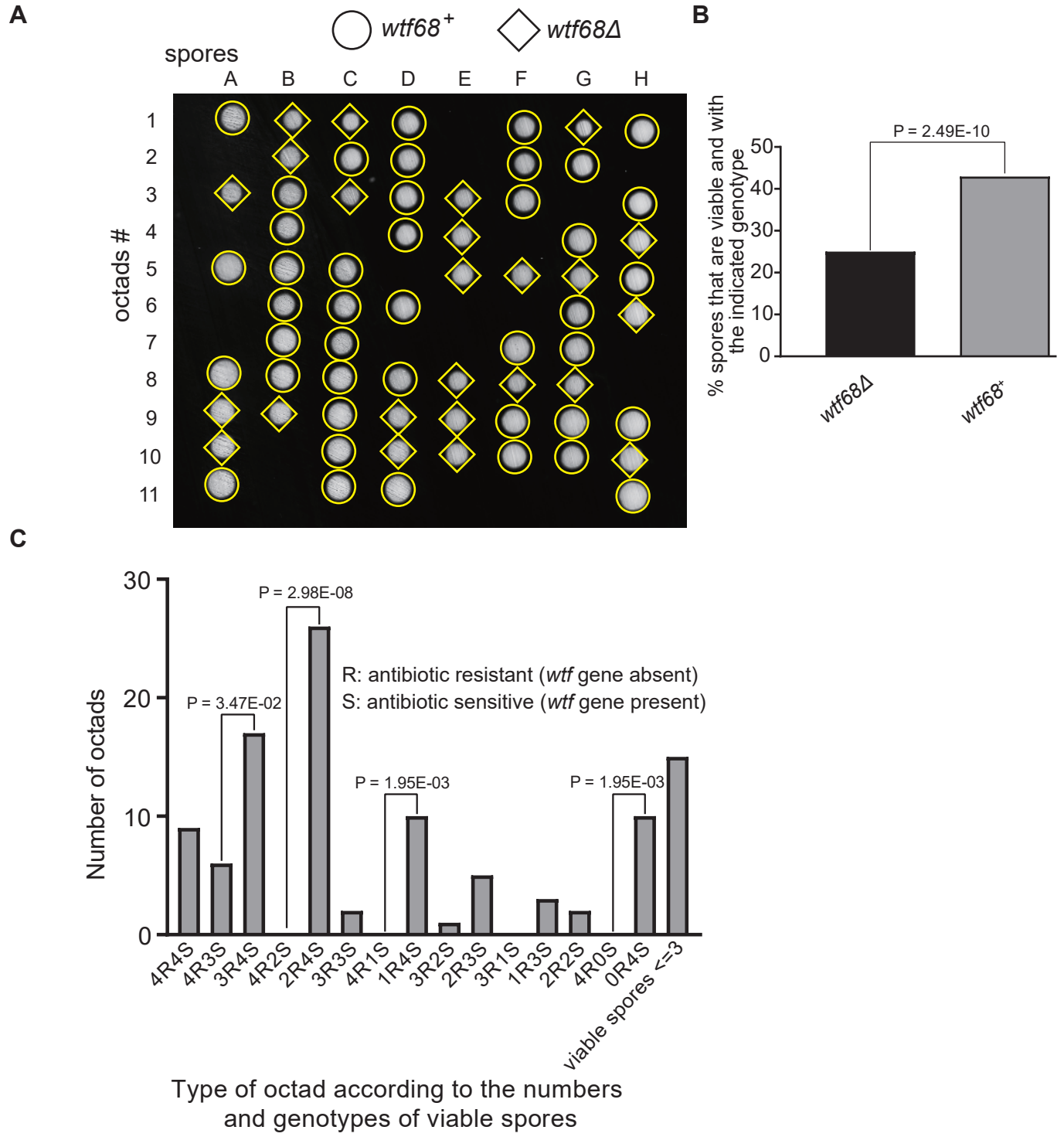


Figure 9-figure supplement 2: Octad dissection analysis of *wtf33* heterozygous deletion cross.

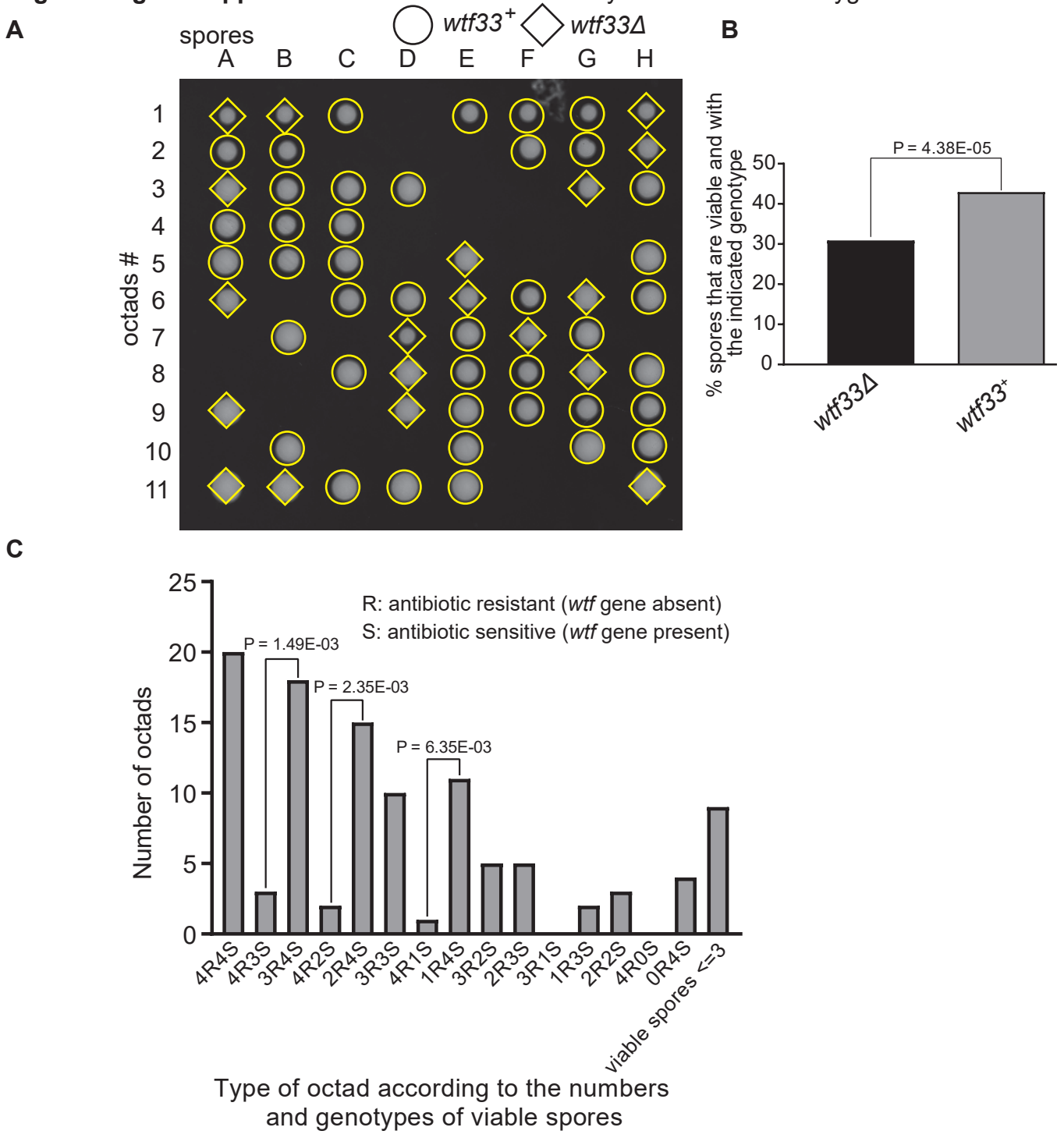


Figure 9-figure supplement 3: Octad dissection analysis of *wtf60* heterozygous deletion cross.

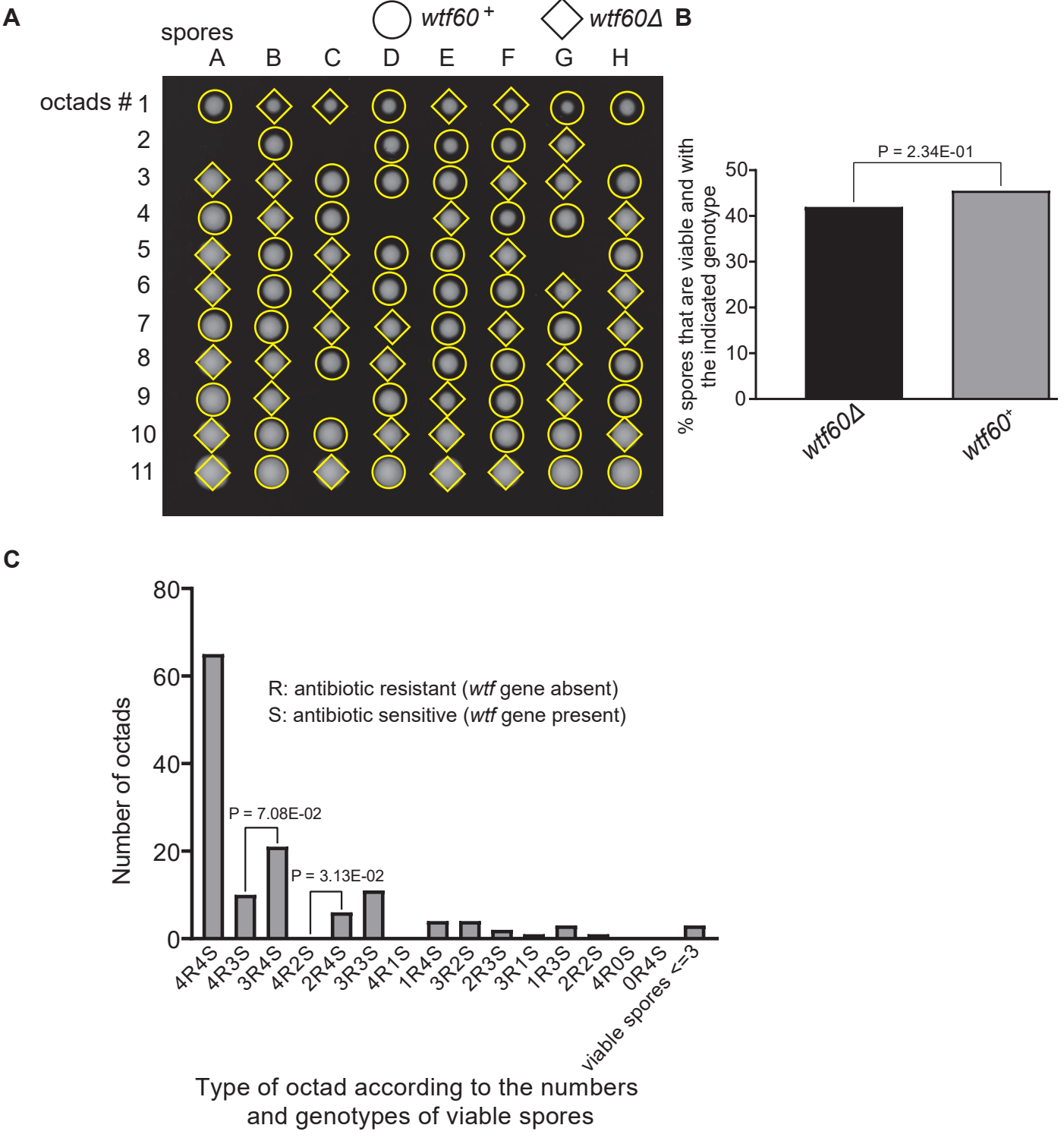


Figure 9-figure supplement 4: Octad dissection analysis of *wtf46* heterozygous deletion cross.

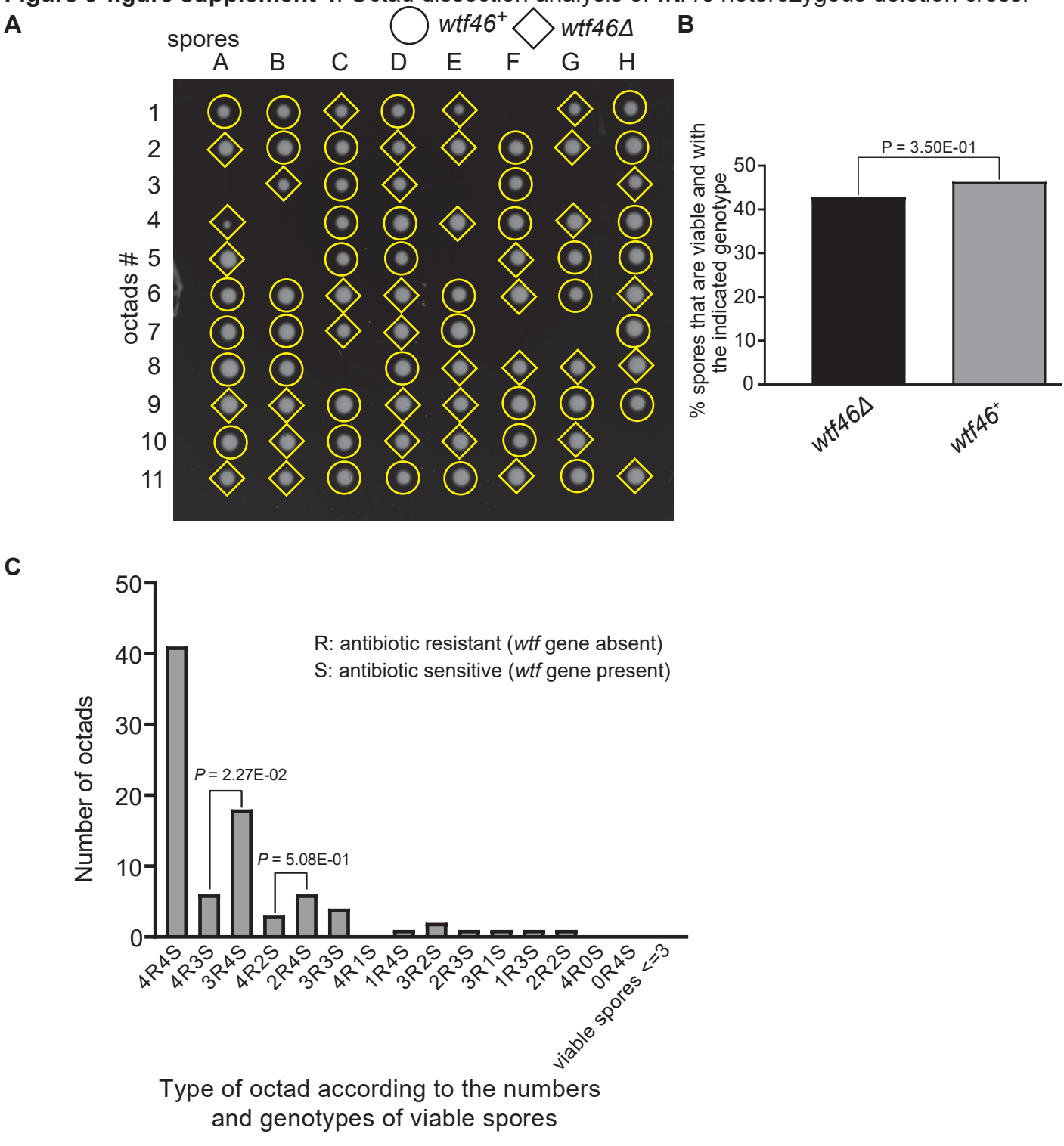


Figure 9-figure supplement 5: Octad dissection analysis of *wtf21* heterozygous deletion cross.

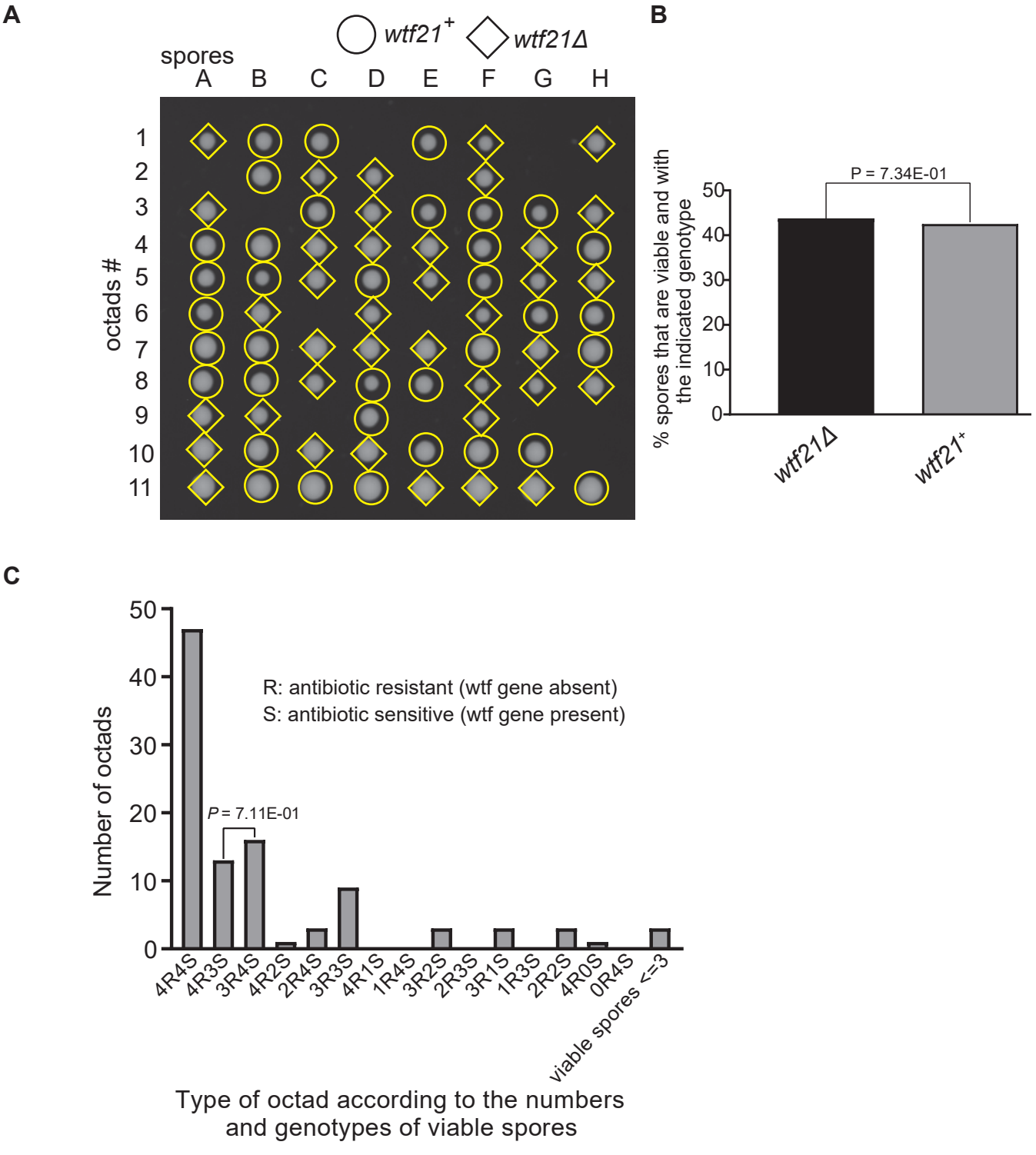


Figure 9-figure supplement 6: Octad dissection analysis of *wtf62* heterozygous deletion cross.

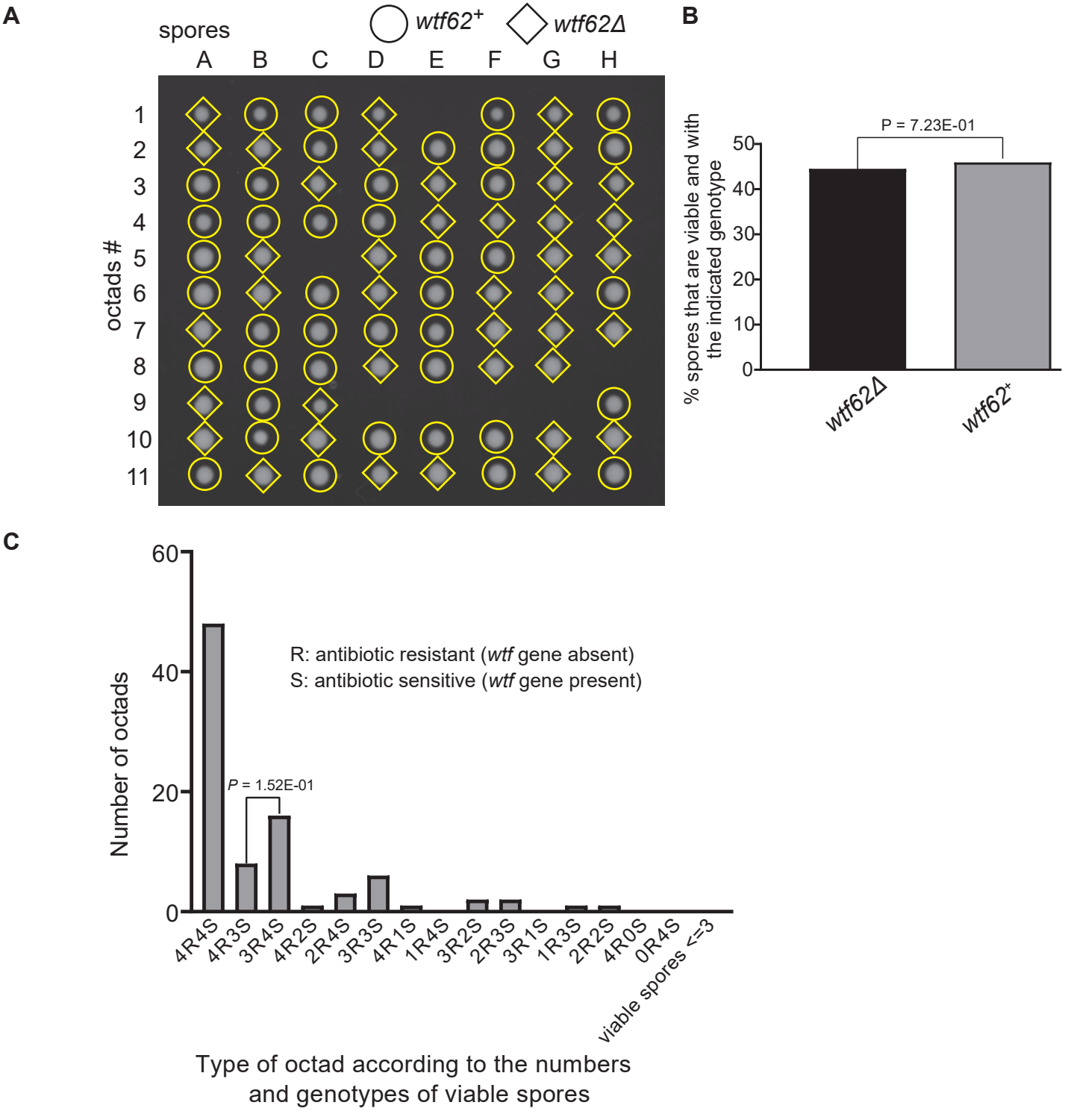


Figure 10: *S. octosporus wtf25* is a poison-and-antidote killer meiotic driver

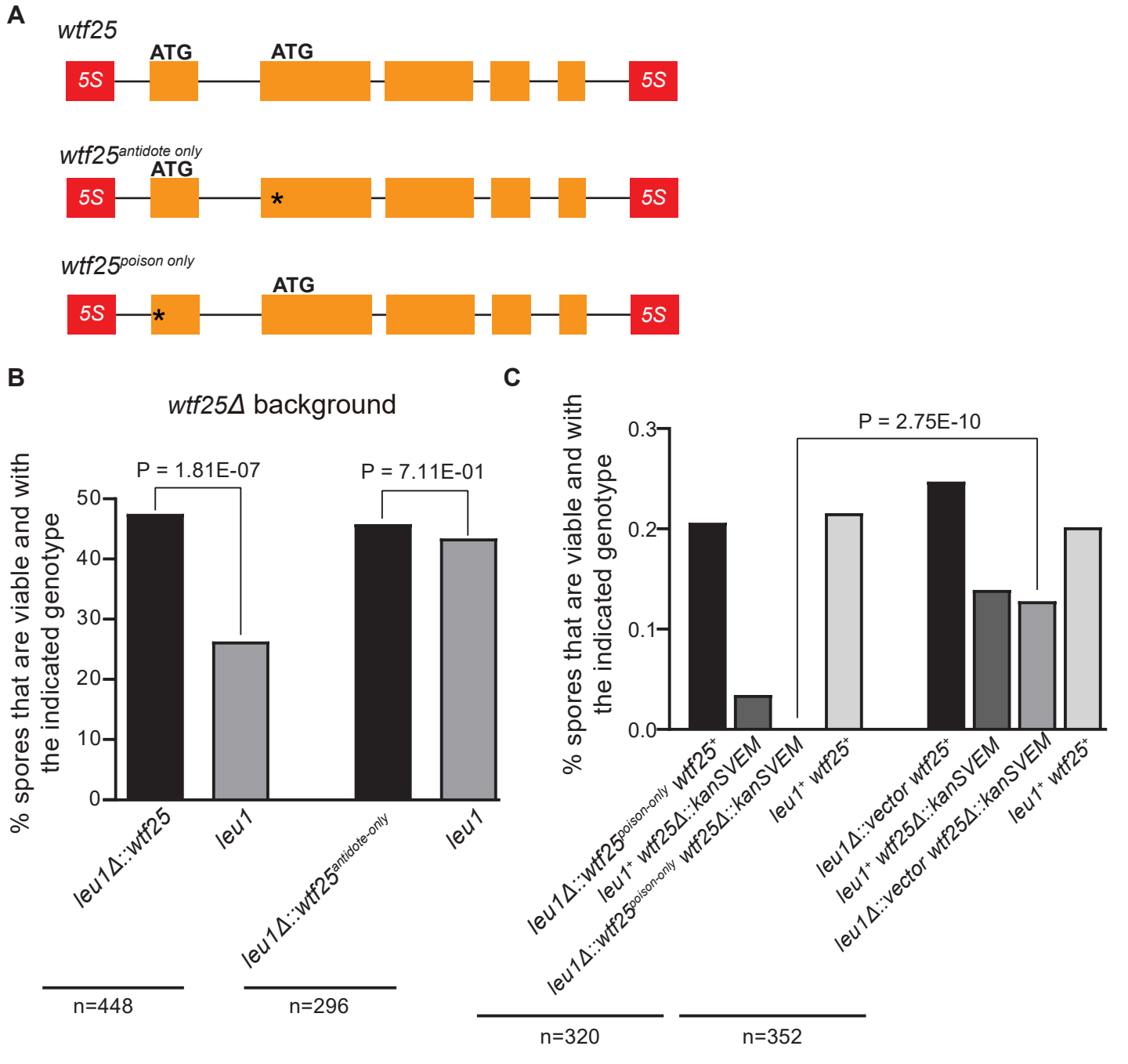


Figure 11: Model for long-term persistence of *wtf* meiotic drivers