

RESEARCH ARTICLE

Dramatically diverse *Schizosaccharomyces pombe wtf* meiotic drivers all display high gamete-killing efficiency

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Abstract

Meiotic drivers are selfish alleles that can force their transmission into more than 50% of the viable gametes made by heterozygotes. Meiotic drivers are known to cause infertility in a diverse range of eukaryotes and are predicted to affect the evolution of genome structure and meiosis. The *wtf* gene family of *Schizosaccharomyces pombe* includes both meiotic drivers and drive suppressors and thus offers a tractable model organism to study drive systems. Currently, only a handful of *wtf* genes have been functionally characterized and those genes only partially reflect the diversity of the *wtf* gene family. In this work, we functionally test 22 additional *wtf* genes for meiotic drive phenotypes. We identify eight new drivers that share between 30–90% amino acid identity with previously characterized drivers. Despite the vast divergence between these genes, they generally drive into >85% of gametes when heterozygous. We also identify three *wtf* genes that suppress other *wtf* drivers, including two that also act as autonomous drivers. Additionally, we find that *wtf* genes do not underlie a weak (64% allele transmission) meiotic driver on chromosome 1. Finally, we find that some Wtf proteins have expression or localization patterns that are distinct from the poison and antidote proteins encoded by drivers and suppressors, suggesting some *wtf* genes may have non-meiotic drive functions. Overall, this work expands our understanding of the *wtf* gene family and the burden selfish driver genes impose on *S. pombe*.

Author summary

During gametogenesis, the two gene copies at a given locus, known as alleles, are each transmitted to 50% of the gametes (e.g. sperm). However, some alleles cheat so that they are found in more than the expected 50% of gametes, often at the expense of fertility. This selfish behavior is known as meiotic drive. Some members of the *wtf* gene family in the fission yeast *Schizosaccharomyces pombe* kill the gametes (spores) that do not inherit them, resulting in meiotic drive favoring the *wtf* allele. Other *wtf* genes act as suppressors of drive. However, the *wtf* gene family is diverse and only a small subset of the genes has

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Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: S.E.Z. and M.A.B.N. are inventors on a patent application based on *wtf* killers (patent application serial 62/491,107). All other authors declare that no competing interests exist.

been characterized. Here we analyze the functions of other members of this gene family and found eight new drivers as well as three new suppressors of drive. Surprisingly, we find that drive is relatively insensitive to changes in *wtf* gene sequence as highly diverged *wtf* genes execute gamete killing with similar efficiency. Finally, we also find that the expression and localization of some Wtf proteins are distinct from those of known drivers and suppressors, suggesting that these proteins may have non-meiotic drive functions.

Introduction

During meiosis, diploid cells divide to produce haploid gametes (e.g. sperm). This process is generally fair in that each parental allele of a gene is represented equally in the gametes [1]. However, many eukaryotic genomes contain 'selfish' elements that bias their own transmission such that they are overrepresented in the viable gametes [2–4]. These loci are known as meiotic drivers and they can both directly and indirectly reduce fitness through a number of mechanisms (reviewed in [5, 6]).

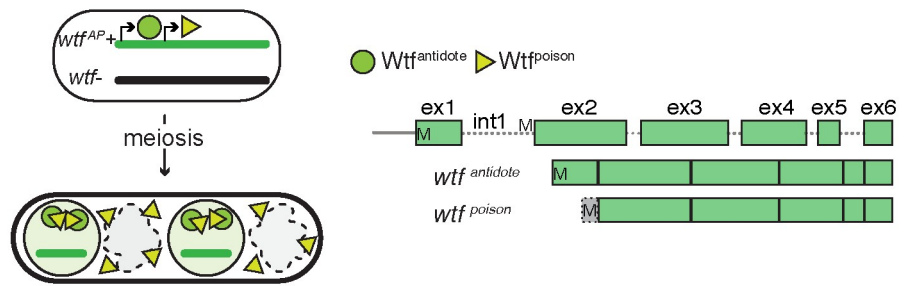
Meiotic drivers can be in conflict with unlinked genes that do not gain a transmission advantage but must bear the fitness burdens often imposed by these selfish elements [7–10]. This genetic conflict is thought to favor the emergence of unlinked allele variants that can suppress the effects of drivers. In turn, variants of drivers that can evade this suppression will have a selective advantage [4, 8, 11]. The conflict between drivers and suppressors is predicted to lead to the rapid evolution of both sets of genes. This is analogous to the genetic arms race observed between viruses and host immune systems [12, 13]. Arms races caused by meiotic drivers are predicted to affect the evolution of gametogenesis and genome structure [14, 15].

Empirical analyses of meiotic drive/suppressor systems have traditionally been limited by the complexity of most known drive systems. However, the *wtf* (*with tf*) gene family of the fission yeast *Schizosaccharomyces pombe* offers an opportunity to study meiotic drive in a highly tractable model system. Some *wtf* genes are meiotic drivers and others act as suppressors of drive [16–18]. Characterized *wtf* drivers kill the meiotic products (spores) that do not inherit them from a *wtf*⁺/*wtf*⁻ heterozygous diploid by producing two proteins, Wtf^{poison} and Wtf^{antidote}. These proteins are encoded on largely overlapping transcripts, but the antidote message includes a 5' exon (exon 1) not found in the poison message (Fig 1A). The poison protein localizes throughout the spore sac (ascus) and acts on every spore in an indiscriminate fashion. The antidote protein, however, is highly enriched within the *wtf*⁺ spores, rescuing them from destruction by the poison [16, 17]. Similarly, the known drive-suppressing *wtf* genes produce a protein that mimics the antidote of a driver, rescuing the spores that inherit the suppressor from the driver's poison [18].

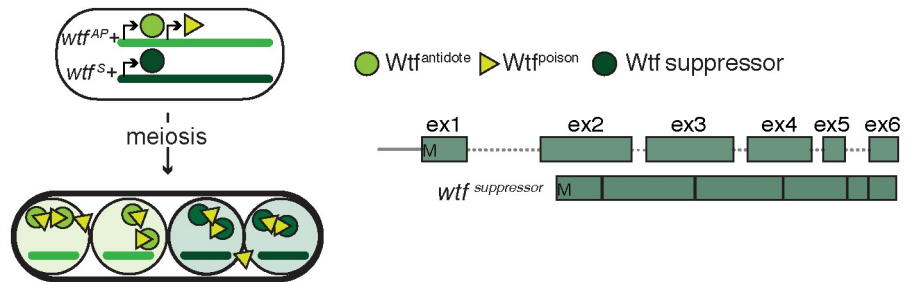
The *wtf* family contains 25 genes (including pseudogenes) in the *S. pombe* reference strain, L972 (referred to here as 'Sp') [19, 20]. This gene family is rapidly evolving and other *S. pombe* isolates in which the *wtf* genes have been assembled contain up to 38 members [17, 21]. Currently, only a handful of these genes have been functionally characterized to be either drivers or suppressors [16–18]. Although the characterized genes have provided mechanistic insight, they do not represent the extensive diversity within the *wtf* gene family. It is currently unclear if the sequence diversity of *wtf* genes corresponds with phenotypic diversity or if some *wtf* genes could have a function outside of drive.

To aid in the functional characterization of the *wtf* gene family, we previously sorted them into three classes. **Class 1** genes are predicted to be meiotic drivers (Fig 1A). These genes contain two potential transcriptional and translational start sites that could be used to encode

A Class 1: predicted poison-antidote meiotic driver



B Class 2: predicted meiotic drive suppressor



C Class 3: no predicted function

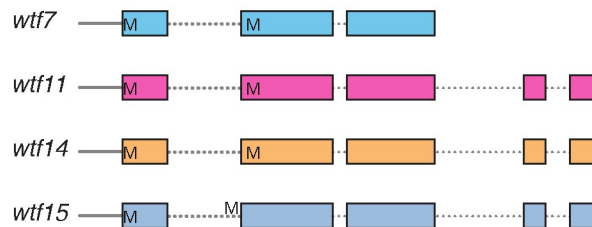


Fig 1. Classification of the *wtf* gene family. (A) Model of how known *wtf* meiotic drivers kill the spores that do not inherit them from a *wtf*⁺/*wtf*⁻ diploid. *wtf* meiotic drivers (shown as *wtf*^{AP}) make a trans-acting poison and a spore-enriched antidote that is specifically found in the spores that inherit the *wtf*⁺ allele. Class 1 contains both 6- and 5-exon *wtf* genes. (B) Model for how antidote-only (shown as *wtf*^S) *wtf* genes can work as suppressors of *wtf* meiotic drivers. The spores that inherit the *wtf* antidote-only gene are protected from a specific Wtf^{poison}. Class 2 contains the predicted antidote-only *wtf* genes. (C) Gene structure of class 3 genes. These genes have no predicted function. M's (methionine) represent potential translational start sites.

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distinct poison and antidote proteins. **Class 2** genes are predicted drive suppressors that appear to encode only Wtf^{antidote} proteins (Fig 1B). The class 1 and class 2 genes are rapidly evolving, largely due to extensive ectopic gene conversion that has shuffled sequence variation and muddled orthology within the family [17, 21]. **Class 3** includes four diverse genes (*wtf7*, *wtf11*, *wtf14*, and *wtf15*) that have no predicted function, but are grouped together only because they are unlike all other *wtf* genes (Fig 1C). These genes also have two potential translational start sites, but unlike class 1 genes, they produce only one major transcript type [22]. Finally, the class 3 *wtf* genes have not undergone ectopic gene conversion, so distinct *S. pombe* isolates have clear orthologs of each class 3 gene [21].

More extensive characterization of *wtf* genes is required to understand the functional breadth of these genes and their potential evolutionary impact on *S. pombe*. Here we use a combination of genetics and cell biology approaches to functionally analyze 22 previously

untested *wtf* genes. We find that a tremendous breadth of Wtf protein sequences can build highly efficient poison-antidote drivers and drive suppressors. Moreover, we discover that some *wtf* genes can simultaneously act as drivers and as suppressors of other drive loci. Surprisingly, we also find that *wtf* genes do not underlie a previously identified but unmapped locus on chromosome 1 that causes an allele transmission bias into viable spores. Finally, we discover that the potential cellular roles of *wtf* family members extend beyond gametogenesis. This work demonstrates that the landscape of drive systems in *S. pombe* is even richer than previously anticipated and underscores the potential of this simple model system to reveal unexpected insights about meiotic drive.

Results

Class 1 contains autonomous meiotic drivers

To test the ability of class 1 and class 2 *wtf* genes to drive autonomously, we cloned untested genes representing unique subclasses from four isolates that largely reflect the range of *S. pombe* diversity: *Sp*, *S. kambucha* (*Sk*), FY29033, and CBS5557 (S1 Fig) [23, 24]. We then assayed the ability of these *wtf* genes to drive in one or more ectopic strain backgrounds (e.g. testing *Sk wtf* genes in *Sp*). We used ectopic strain backgrounds because drive occurs in heterozygotes and it is simpler to insert genes into *S. pombe* than it is to delete the repetitive *wtf* genes at their endogenous loci. Additionally, drivers may be less likely to be suppressed in an ectopic background [25].

To do these analyses, we first linked each *wtf* gene under the control of its endogenous promoter to a selectable drug resistance marker (*kanMX4* or *hphMX6*) and integrated them into the *ade6* locus (of either *Sp* and/or *Sk*). We then crossed each haploid carrying a *wtf* gene of interest to a wild-type strain from the same background to generate hemizygous (*wtf::drug^R/ade6+*) diploid strains. We induced the hemizygous diploids to undergo meiosis and then assayed the presence of each *wtf* gene of interest in the viable spore population using the linked selectable markers. In the absence of suppressors, genes capable of autonomous drive are expected to be overrepresented in the viable progeny (>50%).

Most genes within class 1 have six exons, including all previously described *wtf* drivers. Class 1 also includes two 5-exon *wtf* genes (S1 Fig) [21]. We tested ten 6-exon and two 5-exon *wtf* genes from class 1 (Fig 2A, diploids 1–16). We found that seven of the 6-exon genes and one 5-exon gene exhibited significant drive in at least one strain background (Fig 2A). These genes are: *Sk wtf9*, *Sk wtf19*, *Sk wtf30*, *Sk wtf33*, *Sp wtf19*, FY29033 *wtf36*, FY29033 *wtf18*, and FY29033 *wtf35*. Interestingly, these genes are incredibly diverse and share as little as 30% pairwise amino acid identity (S2A Fig). Moreover, all of the drivers except one (*Sp wtf19*) drive into >90% of the progeny when tested under the same conditions [16, 18]. These results demonstrate that a remarkably wide range of proteins can execute spore-killing similarly well.

Surprisingly, the 5-exon FY29033 *wtf35* meiotic driver lacks sequences homologous to what is exon 4 in the other known 6-exon drivers. In addition, the gene lacks the 7-amino acid repeat that is found in between 1 and 4 copies in the last exon of all other drivers [21]. Despite these differences, the localization of the FY29033 Wtf35 proteins was indistinguishable from that observed in other drivers (S3 Fig, S1 Table, diploid 39). These observations indicate that the aforementioned sequences can be dispensable for Wtf protein localization and drive.

The four class 1 genes that did not exhibit drive in our tests were *Sk wtf27*, *Sk wtf29*, *Sp wtf23*, and CBS5557 *wtf23* (Fig 2A, diploids 12–16). CBS5557 *wtf23* completely lacks a specific 11-amino acid repeat within exon three that can be found in 2–7 copies in all characterized drivers except FY29033 *wtf35*, which contains only four amino acids of the repeat [21]. We found no coding sequence features in *Sk wtf27*, *Sk wtf29*, or *Sp wtf23* that distinguish these

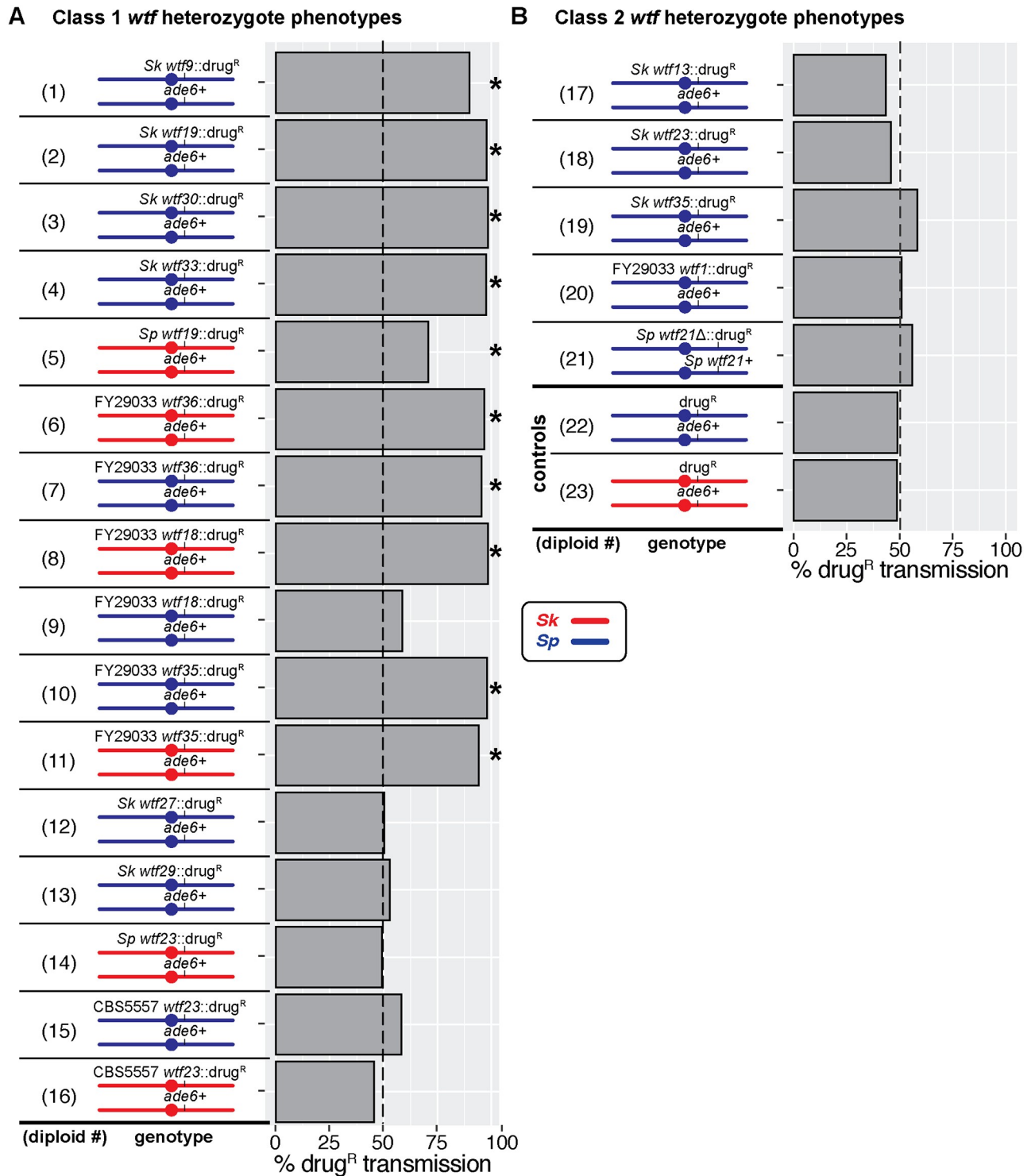


Fig 2. Phenotypes of diploids carrying a hemizygous or heterozygous *wtf* allele. Allele transmission of (A) class 1 *wtf* gene and (B) class 2 *wtf* gene heterozygotes. We used the *kanMX4* or *hphMX6* drug resistance markers (*drug^R*) linked to the *wtf* allele of interest and the *ade6* marker to follow allele transmission. The presence of the *kanMX4* marker conferred resistance to G418 (*G418^R*), while presence of the *hphMX6* marker conferred resistance to Hygromycin B (*HYG^R*). For diploids 1–20 and control diploids 22 and 23, we excluded the spores that had inherited both *ade6+* and drug resistance markers as these are likely aneuploid or diploid. For diploid 21, allele transmission was assayed using only the drug marker. Diploids 1–4,7,9,10,12,13,15, and 17–20 were compared to control diploid 22; diploids 5,6,8,11,14, and 16 were compared to the control diploid 23. *Sp* chromosomes are depicted in blue and *Sk* chromosomes are shown in red. * indicates a p-value < 0.05 (G-test). We genotyped at least 200 haploid offspring for each diploid. We tested some of the *wtf* transgenes using multiple independent strains (i.e. one in which the transgene was marked with *kanMX4* and one marked with *hphMX6*) and we present the combined data. The complete raw data are presented in [S1 Table](#).

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genes from the confirmed drivers. However, it is possible that these four genes are not capable of driving or they are not transcribed. It is also possible that these genes are suppressed in the background in which we tested them. We previously saw background-specific suppression of the *Sp wtf13* driver, and similar suppression explains why we observed drive of FY29033 *wtf18* in the *Sk*, but not in the *Sp* background (see below) [18]. Overall, our analyses are consistent with class 1 containing a wide diversity of autonomous meiotic drive genes.

The class 2 *wtf* genes are either known or predicted antidote-only drive suppressors (Fig 1B, S1 Fig). Like the class 1 genes, class 2 contains both 5-exon and 6-exon genes, although only 6-exon genes have been previously tested [16–18]. Interestingly, class 2 also contains *Sp wtf21*, which was previously reported to be an essential gene because a heterozygous mutant (*Sp wtf21/wtf21Δ*) transmitted only the wild-type *Sp wtf21* allele to viable gametes [26].

We first tested four class 2 genes (*Sk wtf13*, *Sk wtf23*, *Sk wtf35*, and FY29033 *wtf1*) using the same approach described above for class 1 genes. As expected for genes predicted to produce only antidotes, we found that none of the genes tested exhibited drive in an ectopic strain background (Fig 2B, diploids 17–20). We also revisited the idea that *Sp wtf21* is an essential gene [26]. We found that we could generate a deletion of *Sp wtf21* in a haploid strain, indicating the gene is not essential in that strain background. Moreover, we did not observe drive in an *Sp wtf21/wtf21Δ* heterozygote (Fig 2B, diploid 21).

Finally, we tested if we could observe autonomous meiotic drive by the class 3 *wtf* genes (*wtf7*, *wtf11*, *wtf14*, and *wtf15*) in *Sp*. Unlike the other *wtf* genes (class 1 and class 2), each natural isolate has a clear ortholog of each class 3 *wtf* gene [21]. We therefore had to use a different strategy to test if these genes could drive. Instead of introducing the genes into an ectopic strain background, we assayed whether these genes could drive when heterozygous at their endogenous loci. We deleted *wtf7* and *wtf11* individually, and *wtf14* and *wtf15* together as they are adjacent to each other. In diploids heterozygous for any of these *wtf* gene deletions, the wild-type alleles were transmitted to ~50% of the viable spores (S4A Fig). This indicates that these genes cannot drive or that they are suppressed in the *Sp* background. We also assayed a homozygous deletion strain lacking all of the class 3 genes. We observed no fertility defects, indicating these genes are not required for sexual reproduction (S4B Fig). We also observed no growth defects in haploids lacking some or all of the class 3 genes (S4C Fig).

***wtf* genes in both class 1 and class 2 can act as suppressors of drive**

Previous work identified alleles of *wtf18* in *Sp* and *Sk* as suppressors of meiotic drive, but we wanted to test if other *wtf* genes could also act as drive suppressors [18]. Within drivers and their known suppressors, the Wtf^{antidote} proteins share high levels of amino acid identity with the poisons they neutralize. This similarity may be particularly important within the C-terminus [18]. We used this knowledge to guide our search for other drive suppressors. We found that the DNA sequence in the last three exons of the class 2 gene FY29033 *wtf1* shares >99% identity to the sequence in the FY29033 *wtf35* driver (Fig 3A). We therefore predicted that FY29033 *wtf1* could be a suppressor of the FY29033 *wtf35* driver. To test this, we made FY29033 *wtf35*/FY29033 *wtf1* diploids in an *Sp* background and assayed transmission of both genes into progeny. FY29033 *wtf35* was no longer able to drive in the presence of FY29033 *wtf1* (Fig 3C, compare diploid 10 to 24). Additionally, FY29033 *wtf1* was able to rescue the fertility defect caused by FY29033 *wtf35* (Fig 3C). These results demonstrate that FY29033 *wtf1* is a suppressor of FY29033 *wtf35*.

To broaden our search for suppressors, we examined why FY29033 *wtf18* showed drive in *Sk*, but not in *Sp*. Specifically, we looked for *wtf* genes in *Sp* that could work as a suppressor of FY29033 *wtf18*. We noticed that the last 776 base pairs of FY29033 *wtf18* were identical to the

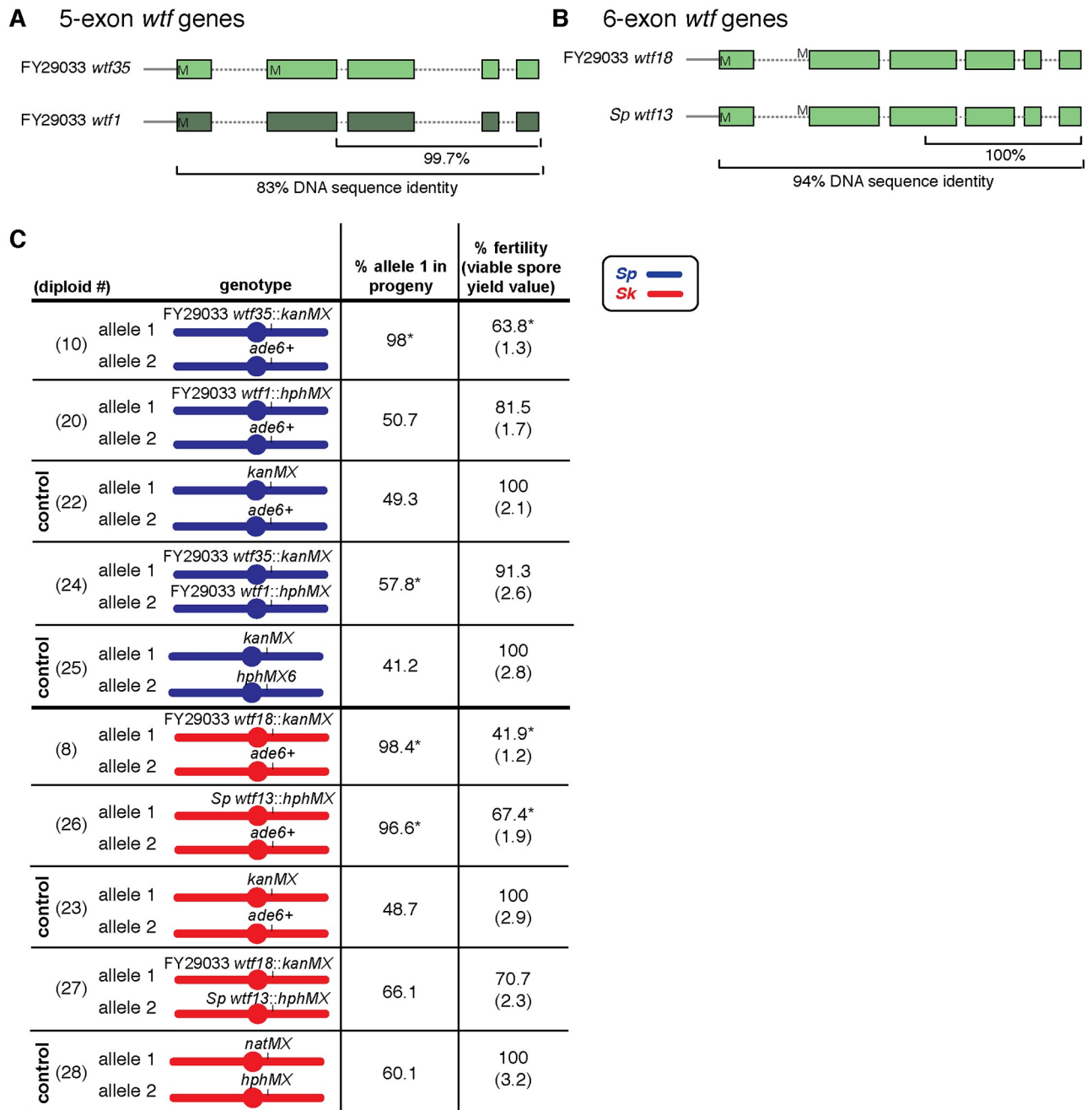


Fig 3. *wtf* genes can be both drivers and suppressors of drive. Cartoons illustrating the similarity between (A) FY29033 *wtf35* and FY29033 *wtf1* and (B) FY29033 *wtf18* and *Sp wtf13*. M's (methionine) represent the translational start sites. (C) Allele transmission and fertility phenotypes of *Sp* (blue) and *Sk* (red) diploids with the indicated transgenes integrated at *ade6*. Allele transmission was determined by following the genetic markers (*ade6* or drug resistance) linked to each allele. Diploids 10 and 20 were compared to control diploid 22, diploid 24 was compared to control diploid 25, diploids 8 and 26 were compared to control diploid 23, and diploid 27 was compared to control diploid 28. The data for diploid 26 were previously published in [18]. We normalized the fertility values to the control diploids and reported both the percentage and the viable spore yield value. * indicates p-value of < 0.05 (G-test [allele transmission] and Wilcoxon test [fertility]). We genotyped more than 200 haploid spores for each diploid. Spores that inherited both markers at *ade6* (*Ade+* G418^R, *Ade+* HYG^R, or G418^R HYG^R) were excluded from the analyses as they are likely aneuploid or diploid. We present the complete raw data in S1 and S2 Tables.

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Sp wtf13 meiotic driver (Fig 3B). This suggested that the *Sp Wtf13*^{antidote} could potentially neutralize the FY29033 *Wtf18*^{poison} and *vice versa*. To test this, we generated FY29033 *wtf18::kanMX4/Sp wtf13::hphMX6* heterozygotes in an *Sk* strain background and assayed their phenotypes. We observed that drive of both genes was suppressed in the heterozygote relative to hemizygotes containing only one of the drivers (Fig 3C, compare diploids 8 and 26 to diploid 27). These results show that *wtf* drivers can also function as suppressors of each other.

***wtf* genes are not responsible for meiotic drive of *Sk* chromosome 1**

We previously detected a weak allele transmission bias that favored *Sk* chromosome 1 in the viable progeny of *Sp/Sk* hybrid diploids [27]. We reexamined this observation here with hybrid diploids that are heterozygous for *Sp* and *Sk* copies of chromosome 1, but are homozygous for *Sk* chromosomes 2 and 3. These hybrids were also unable to initiate meiotic recombination due to deletion of *rec12* (*SPO11* homolog), which is required for programmed meiotic double-stranded break formation. The *rec12* deletion ensured chromosome-wide linkage on chromosome 1, allowing us to monitor transmission of the unmapped locus into the spore progeny. Consistent with our previous observations, we observed 64.3% of the viable spores generated by the hybrids inherited *Sk* chromosome 1 (S5A Fig).

The observed transmission bias is consistent with the presence of a driver on *Sk* chromosome 1. However, it is also possible that Dobzhansky-Muller incompatibilities between *Sp* chromosome 1 and *Sk* chromosomes 2 and/or 3 explain the transmission bias [28]. To distinguish these possibilities, we tested a different *rec12Δ* hybrid diploid that was heterozygous for *Sp* and *Sk* chromosome 1, but homozygous for *Sp* chromosomes 2 and 3. We observed that 70.5% of the viable spores generated by this hybrid inherited *Sk* chromosome 1 (S5B Fig). This result cannot be explained by incompatibilities because a fully compatible genotype (*Sp* chromosomes 1–3) is underrepresented. Together, our results indicate that meiotic drive, not genetic incompatibilities underlie this transmission bias.

We postulated that a *wtf* gene could underlie this drive phenotype. There is only one *wtf* locus, *wtf1*, on both *Sp* and *Sk* chromosome 1. Surprisingly, the coding sequences for the *Sp* and *Sk* alleles are identical and both are predicted to be pseudogenes due to an early in-frame stop codon. This suggested that *wtf* genes do not underlie the drive of *Sk* chromosome 1. To directly test this idea, we deleted *Sk wtf1* from the first hybrid described above. We found that the transmission of *Sk* chromosome 1 was not significantly different after deleting *Sk wtf1* (S5A Fig), indicating that *Sk* chromosome 1 encodes a non-*wtf* meiotic driver.

Class 3 *wtf* genes are not required for drive of *Sk wtf4*

We previously analyzed the sequences of the class 3 *wtf* genes across 57 isolates of *S. pombe* and found that *wtf7*, *wtf11*, and *wtf15* show signatures of positive selection [21]. This suggests that selection is favoring novelty and led us to speculate that these genes could be involved in meiotic drive. Given that we failed to observe drive of these class 3 genes (S4A Fig), we thought they could perhaps be modifying drive of other *wtf* genes. All class 3 *wtf* genes are linked to drive loci so we predicted that they could facilitate drive, as they would also gain a transmission advantage from a linked driver [8].

We tested this idea by generating diploids hemizygous for the *Sk wtf4* meiotic driver (*Sk wtf4::kanMX4/ade6+*) and lacking all four of the class 3 *wtf* genes. *Sk wtf4* was found in nearly 100% of the viable spores generated by this quadruple deletion mutant (S4D Fig, compare diploid 32 to 33). These results show that *wtf7*, *wtf11*, *wtf14*, and *wtf15* are not required to facilitate drive of the *Sk wtf4* driver.

Localization of class 3 *Sk* Wtf proteins in *Sp*

We next decided to take a more agnostic approach to look for possible functions of Wtf7, Wtf11, Wtf14, and Wtf15 proteins by investigating their expression and localization in cells. We analyzed a published data set from a proteomics meiotic time course study [29] and found that peptides from Wtf11, Wtf14, and Wtf15, but not Wtf7, were all detected during meiosis in at least two replicate experiments (S6 Fig). Wtf11 and Wtf15 were detected in one or more time points taken after the first meiotic division. Interestingly, the levels of Wtf14 remained steady throughout meiosis (S6 Fig). These patterns are both unlike those of class 1 and class 2 Wtf proteins which increase in abundance as meiosis progresses [18, 29].

To assay the localization of the proteins within cells, we tagged each of the class 3 *wtf* genes (cloned from *Sk*) with GFP at the C-terminus and integrated them at the *ade6* locus of *Sp*. The tagged genes were under the control of their endogenous promoters. We then separately imaged haploid and diploid cells grown in rich media during logarithmic cell growth. We also imaged the haploid and diploid cells again in stationary phase. Finally, we imaged diploid cells undergoing meiosis and the products of meiosis (tetrads).

We were not able to detect any GFP fluorescence under any conditions in cells containing the *wtf11-GFP* allele (S7 Fig) [29]. The reasons for this are unclear, given the proteomics data indicates the protein is expressed in meiosis. We also did not detect Wtf7-GFP or Wtf15-GFP in vegetatively growing haploids or diploids (S8 Fig). However, we did observe GFP fluorescence in tetrads produced by diploids heterozygous for *wtf7-GFP* and tetrads produced by *wtf15-GFP* heterozygotes (Fig 4 and S9 Fig). This late expression of Wtf15-GFP is consistent with the proteomics data set (Wtf7 was not detected by proteomics) [29]. In tetrads generated by diploids with one GFP-tagged copy of Wtf7 or Wtf15, we observed that the signal was greatly enriched in two of the four spores (Fig 4). We speculate that the two spores with bright GFP signal are those that inherited the tagged allele. This would suggest that *wtf7-GFP* and *wtf15-GFP* are both expressed after spore individualization, similar to the antidotes of *wtf* drivers and suppressors [16, 18]. Wtf15-GFP exhibited a diffuse localization pattern that largely filled the spores (Fig 4B and S9B Fig). The localization pattern of Wtf7-GFP varied in different spores. In some instances, Wtf7-GFP made a ring-like structure next to Nsp1-mCherry (nucleoporin marker) (Fig 4A and S9A Fig). However, we also observed other cases where Wtf7-GFP seemed to be clustered within the spore (Fig 4A).

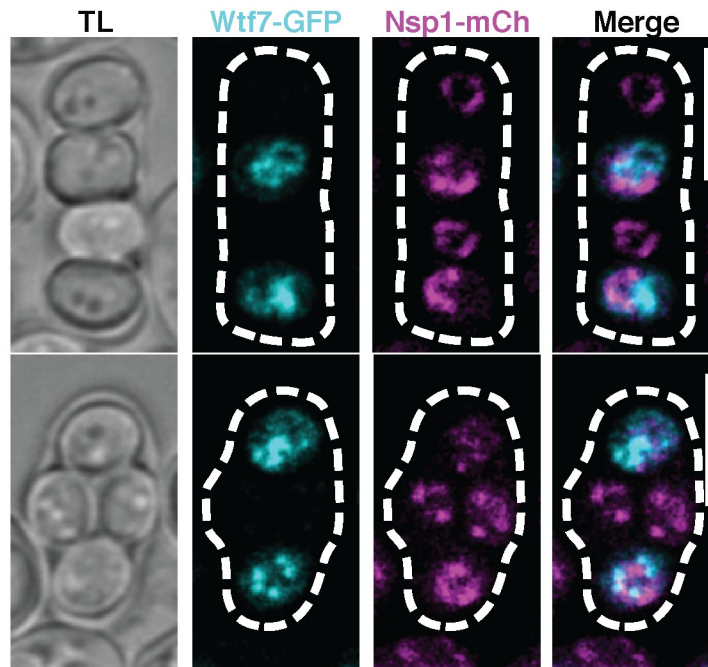
Unlike all other Wtf proteins, we saw expression of Wtf14-GFP in vegetative cells. We saw that Wtf14-GFP colocalized with the endoplasmic reticulum (ER) marker *pbip1::mCherry-AHDL* in a punctate pattern in vegetative haploid and diploid cells (Fig 5A and 5B, S10 Fig) [30]. This localization became more pronounced in cells during stationary phase (i.e. after ~16 hours growth in rich media). These observations are consistent with previous work showing that *Sp* Wtf14-YFP expressed from a repressible promoter localizes to the ER [31] (Fig 5A and 5B, S10 Fig). We also saw ER localization of Wtf14-GFP in two of the four spores generated by diploids heterozygous for *wtf14-GFP* (Fig 5C). Similar to what has been observed in *Saccharomyces cerevisiae*, ER proteins seem to be generated *de novo* in spores [32]. This localization pattern likely reflects expression of *wtf14-GFP* in the two spores that inherited the tagged *wtf14* allele.

Discussion

S. pombe genomes harbor a surprising number of active drive genes

Overall, we identified eight new *wtf* genes capable of meiotic drive. The *S. pombe* isolates with assembled *wtf* sequences contain between 4–14 predicted drivers [17, 21]. Our current work

A tetrads from *ade6-::Sk wtf7-GFP/ade6+*, *nsp1+/nsp1-mCherry*



B tetrad from *ade6-::Sk wtf15-GFP/ade6+*

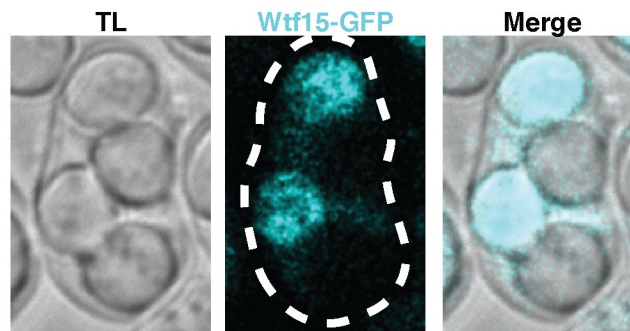


Fig 4. *Sk wtf7-GFP* and *Sk wtf15-GFP* are expressed in spores. (A) Representative images of *Sk Wtf7-GFP* localization in spores generated by diploids heterozygous for both *Sk wtf7-GFP* (green) and *nsp1-mCherry* (magenta; nucleoporin marker). (B) Tetrad generated from a diploid heterozygous for *Sk wtf15-GFP*. We linearly unmixed these images (see [Materials and methods, S9 Fig](#)). We adjusted the brightness and contrast differently for each image and smoothed them using Gaussian blur. Scale bar represents 5 μ m. TL, transmitted light.

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shows that many of these predicted drive genes are able to drive in the right genetic context. This work expands the rapidly growing number of *bona fide* drive genes identified in recent years [4, 16–18, 33–43].

Moreover, our results highlight that one species can house a surprising number of functional meiotic drive genes. This is not unique to *S. pombe* as several species are known to harbor multiple gamete-killing drivers. These species include plants, fungi, insects, and animals [16–18, 27, 34–38, 41, 42, 44–47]. For example, in *Drosophila simulans*, there are three genetically distinct X-linked male drive systems. Two of these systems are paralogous, whereas the third arose independently [46, 48–50]. Additionally, species can also house multiple copies of ‘true meiotic drive loci’ that bias chromosome segregation as well. Maize, for example,

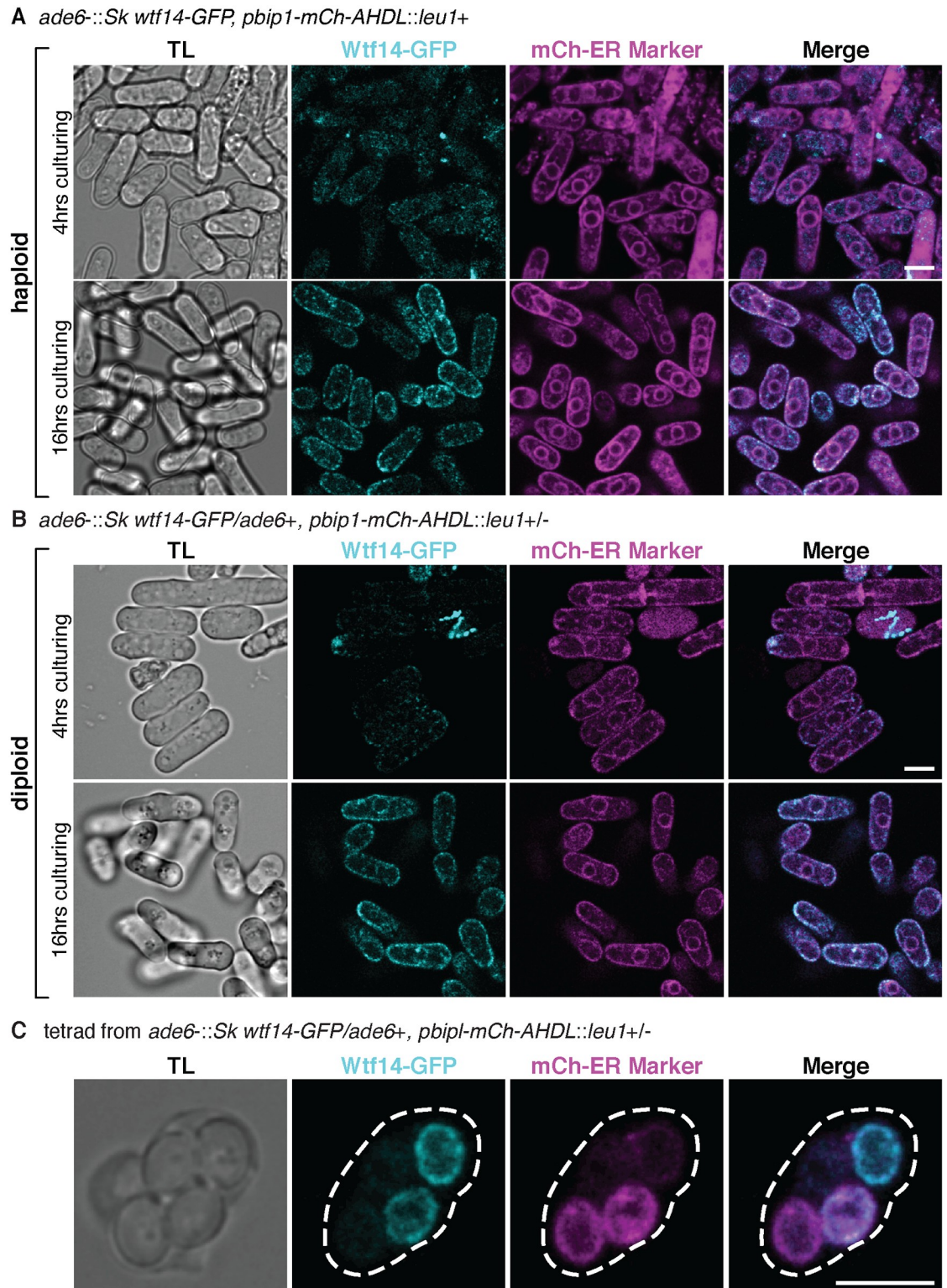


Fig 5. *Sk wtf14-GFP* is broadly expressed and localizes to the perinuclear endoplasmic reticulum (ER). Representative images of *Sk Wtf14-GFP* (cyan) and ER marker mCherry-AHDL (magenta) in (A) haploids and (B) diploids after 4 hours and 16 hours of culturing. The imaged diploids were heterozygous for both *Sk wtf14-GFP* and *pbip1-mCh-AHDL*. (C) Tetrad generated by a diploid heterozygous for *Sk wtf14-GFP* and *pbip1-mCh-AHDL*. We linearly unmixed the images in A and B (see [Materials and methods, S10 Fig](#)). We adjusted the brightness and contrast differently for each image and smoothed them using Gaussian blur. The scale bar represents 5 μ m. TL, transmitted light.

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contains different numbers and sizes of heterochromatic regions (knobs) that have preferential segregation during female meiosis [51–53]. Similarly, drive of more than one centromere has been observed in species of *Mus* [54, 55].

In *S. pombe*, one is compelled to ask how a genome can contain so many genes that act to destroy gametes. The costs of the selfish *wtf* genes could be at least partially offset because each genome also harbors multiple suppressors of drive. It is possible that we failed to observe drive of *Sp wtf23*, *Sk wtf27*, and *Sk wtf29* in this study due to the presence of suppressors. Consistent with this idea, we identified three new *wtf* genes in this work that act to suppress drive (FY29033 *wtf1*, *Sp wtf13*, and FY29033 *wtf18*). We also found that two of these *wtf* genes (*Sp wtf13* and FY29033 *wtf18*) can simultaneously promote their own drive while suppressing the drive of other *wtf* genes.

An alternate hypothesis is that the cost of *wtf* drivers could be mitigated if the genes are rarely in a heterozygous state (in which they drive and decrease fertility). A population genetic study of linkage disequilibrium between polymorphisms concluded that *S. pombe* rarely outcrosses with genetically diverged partners [56]. If so, why would so many meiotic drivers flourish in a species that rarely outcrosses? The extraordinary rate of *wtf* evolution may offer a solution to that paradox. Changes in class 1 and class 2 *wtf* gene sequences accumulate very rapidly due to extensive ectopic gene conversion within the family and due to the expansion and contraction of two sets of repeated sequences within the coding sequences of the genes [17, 21]. Because of this, crosses between otherwise isogenic isolates can generate diploids that are heterozygous at *wtf* loci [18]. The rate of such changes may be slow enough that few *wtf* drivers are likely to be heterozygous when closely related isolates mate. This could limit the fitness burden of carrying multiple active drive genes, while also maintaining the selfish selective advantage enjoyed by active drivers (or their suppressors) [18].

Finally, our new findings are consistent with the idea that *Wtf* antidotes must be highly similar to the poisons they neutralize [18]. Similarity between drivers and suppressors has also been observed in other systems and this similarity could potentially serve as a general guide to identify suppressors [36, 37, 50].

In many ways, the landscape of meiotic drive genes in *S. pombe* is similar to that in *Podospora*. This filamentous fungus also contains multiple meiotic drive loci, including the *het-s* drive system in *P. anserina*, the best understood driver in any system at the molecular level [45, 57]. In addition, *Podospora* contains its own multi-gene family of single-gene poison-antidote meiotic drivers, the *Spok* spore killers [36, 37]. Like the *wtf* genes, the *Spok* genes are variable in number and location between isolates, leading to varying degrees of spore-killing phenotypes in crosses. Different isolates of *Podospora* contain various *Spok* genes (*Spok1*, *Spok2*, *Spok3*, and *Spok4*). Like *wtf* drivers, some *Spok* genes can both drive and suppress other drivers [36, 37]. Similar to FY29033 *wtf18* and *Sp wtf13* in which suppression is bidirectional, *Spok1* and *Spok4* confer mutual resistance to each other [37]. However, this case is not general among the *Spok* drivers. For example, the *Spok1* gene is resistant to the *Spok2* driver, but not *vice versa* [36].

The *wtf* genes also show some marked differences from the *Spok* gene family. Firstly, the same sequence appears to encode both the poison and antidote functions for a given *Spok* gene. In addition, the driving *wtf* genes are diverse in amino acid identity (30–90%), whereas the *Spok* genes are more conserved (>94% DNA sequence identity) within *P. anserina*. This could be due to the predicted enzymatic (nuclease and kinase) functions of SPOK proteins, which may constrain their divergence [36, 37].

Interestingly, although both *wtf* and *Spok* spore killers evolved independently and use different genes to enact drive, the parallels suggest that there could be recurrent themes shared by different drive systems. These characteristics could perhaps be exploited to aid in the discovery and characterization of novel natural drive systems, especially in organisms with low genetic

tractability that show evidence of meiotic drive. Additionally, these shared themes could help us understand how artificial gene drive systems may evolve in populations.

The potential functions of class 3 *wtf* genes remain largely unknown

Our final goal in this work was to test the potential functions of the class 3 *wtf* genes: *wtf7*, *wtf11*, *wtf14*, and *wtf15*. We observed no context in which we could detect Wtf11-GFP, but proteomics data indicate it is expressed late in meiosis [29]. We found that none of the class 3 *wtf* genes are required for vegetative growth. Additionally, our results showed that *wtf7*, *wtf11*, *wtf14*, and *wtf15* cannot drive at their endogenous loci, nor are they required to enable the drive of a known *wtf* driver. However, we did find that *wtf7*, *wtf14*, and *wtf15* are expressed in a spore-specific manner, similar to the antidotes encoded by *wtf* drivers and suppressors. The functional significance of the class 3 *wtf* genes is unclear as none of these genes are required for viability or fertility. Finally, we observed that Wtf14-GFP is localized to the ER during vegetative growth and in spores. This is the first observed expression of a Wtf protein during vegetative growth, demonstrating the potential for this gene to function outside of gametogenesis.

Building a *wtf* meiotic driver

Our studies have elucidated that the ‘rules’ underlying the construction of *wtf* meiotic drivers are curiously lax. Firstly, a surprisingly wide breadth of sequences are equally capable of enacting drive. The sequence encoded in exon 1, found in antidote messages, is the most conserved (68–100% amino acid identity amongst *bona fide* antidotes), suggesting this region may have specific interacting partners. The rest of the Wtf protein is strikingly diverse. For example, the *Sk* Wtf9 and FY29033 Wtf35 proteins share <30% amino acid identity (S2B Fig). However, both genes can drive into >90% of the functional gametes generated by a heterozygote.

In addition, our new analysis of the FY29033 *wtf35* driver revealed that the 7-amino acid repeat sequences encoded in the last exon of the characterized 6-exon *wtf* drivers are dispensable for drive. This is surprising because we previously found that matching numbers of repeats in the Wtf^{antidote} and Wtf^{poison} proteins was important for poison-antidote specificity (for the *Sp wtf13* driver and the *Sp wtf18-2* suppressor) [18]. The fact that FY29033 *wtf35* drives without these repeats suggests that they may function as spacers between more functionally important flanking domains. The important feature could be that having the same distance between the flanking domains helps to create compatible poison and antidote protein pairs.

It is curious that such diverse proteins act as equally effective Wtf poisons (or Wtf antidotes). It seems unlikely that the toxicity of the Wtf^{poison} proteins could be due to an enzymatic function, as it is unclear how an enzyme could maintain equal functionality while diverging so dramatically. In addition, it also seems unlikely that the toxicity relies on specific protein-protein interactions between the poison and some other factor, given the differences between the different poison proteins. We favor a model in which some other shared feature of the Wtf proteins, such as the multiple predicted transmembrane domains, may be important for their function. Future studies of the mechanisms used by the Wtf proteins will be guided by and hopefully reveal the molecular basis of these puzzling observations.

Materials and methods

Viable spore yield assay and allele transmission

To assay fertility and allele transmission, we began by generating heterozygous diploids as described in [27]. We grew the haploid parental strains in YEL (0.5% yeast extract, 3% dextrose, and 250 mg/L adenine, histidine, leucine, lysine, and uracil) to saturation at 32°C. Using

the saturated cultures, we added 300 μ l of each strain to an Eppendorf tube and vortexed it to mix the cells. We spun these cells down and resuspended the cell pellet in sterile ddH₂O. We then plated 100 μ l of the cell mixture onto SPA (1% dextrose, 7.3 mM KH₂PO₄, vitamins, and agar) or SPAS (SPA + 45 mg/L adenine, histidine, leucine, lysine, and uracil) plates and incubated the plates at 25°C for ~16 hours. This allowed the haploids to mate and form diploids. Because some of the strains we used are homothallic (meaning they can switch mating type), we selected for heterozygous diploids. Most of our haploid parental strains had complementary auxotrophic markers which allowed us to select for heterozygous diploids by streaking the mated cell mix on minimal media. We then restreaked colonies that grew on minimal media to further isolate single colonies on minimal media. However, some of the heterozygous diploids were adenine auxotrophs. To select those diploids, we did the same procedure using minimal media supplemented with 45 mg/L of adenine.

Next, we cultured the heterozygous diploids in YEL overnight to saturation in a 32°C shaker. We then plated 50 μ l of our saturated culture onto SPA to allow the diploids to sporulate (for three days at 25°C). We also diluted the cultures and subsequently plated the cells onto YEA (YEL + agar), and let them grow for three days at 32°C. These plates were then replica-plated to minimal media (+ adenine for adenine auxotrophs) as well as any other selective media to further confirm we isolated heterozygous diploids. From the YEA plates, we also calculated the colony-forming units (CFU) to determine the concentration of viable diploids in the YEL culture. We next scraped the cells off of the three day old SPA plates into 500 μ l of sterile ddH₂O and treated them with 5 μ l of glucuslase (Sigma-Aldrich) for four hours at 32°C to shed the ascus membrane and wall to release the spores [58]. We then killed any remaining vegetative cells by adding 500 μ l of 60% ethanol for 10 minutes at room temperature. Next, we washed the spores with ddH₂O and resuspended them in 500 μ l of sterile ddH₂O. We diluted the spores and plated them onto YEA and let them grow into colonies at 32°C for three to five days to determine the CFU. Additionally, we picked colonies from the YEA plate onto a YEA master plate and grew the plate at 32°C for ~24 hours. We then replica-plated the master plate to yeast nitrogen-base plates with a specific dropout of either adenine, histidine, uracil, lysine, or leucine, and plates containing various drugs (G418, Hygromycin B, and Nourseothricin) to determine the genotype of each spore and thus assay allele transmission. Resistance to G418, Hygromycin B, or Nourseothricin is conferred by the *kanMX4*, *hphMX6*, or *natMX4* cassettes, respectively. For each genotype reported, we generated at least two independent diploids via crossing for each genotype tested.

Viable spore yield calculations

To determine the fertility of selected stable heterozygous diploids, we calculated the viable spore yield (number of viable spores recovered from SPA/ the number of viable diploid cells plated on SPA). This is a slight variation of the method describe by Smith, as we frequently start with diploid cells rather than haploids [58]. The number of viable cells used in these calculations was determined using the CFU counts described above. For each genotype tested, we generated at least two independent diploids via crossing.

To determine the viable spore yield of homothallic haploids (i.e. SZY2254 and SZY3529), we first grew the strains in YEL cultures overnight at 32°C with shaking. We then spread 100 μ l of each saturated culture onto SPA plates and left the plates at 25°C for three days. This step allowed the haploid cells to mate and subsequently sporulate. Using the same culture, we also performed serial dilutions and plated them onto YEA to quantify the number of haploids in the original culture. After three days, we scraped the spores off of the SPA plates and treated them as described above. Next, we performed a series of dilutions and plated the spores onto

YEA plates to quantify the number of viable spores. We then incubated these plates at 32°C for five days. To determine the fertility, we calculated the viable spore yield (number of viable spores recovered from SPA/ number of haploids plated on SPA).

Strain construction: *ade6*-integrating vectors

All strain names and genotypes are presented in [S4 Table](#). To assay allele transmission of different *wtf* genes, we used *ade6*-integrating vectors containing *kanMX4* or *hphMX6* drug resistance markers [59, 60]. pSZB188 (empty vector with *kanMX4* resistance) was published in [16]. pSZB386 (empty vector with *hphMX6* resistance) was published in [18]. pSZB387 is identical to pSZB386. We also generated a *ura4*-integrating vector linked to *kanMX4*. We ordered a gBlock from IDT (Coralville, IA) with a mutant *ura4* allele. This mutant allele contained 5' and 3' deletions. We also deleted 50 bp from the middle of the gene which we replaced with a KpnI site. We then blunt-cloned this gBlock into the PvuII site of pFA6 to generate pSZB331. All of the vectors described above have a KpnI site within the mutant *ade6*-targeting cassette or the *ura4*-targeting cassette that we cut to linearize the plasmid.

Additionally, we made an *ade6*-integrating vector linked to *natMX4*. Similar to how pSZB188 and pSZB386 were constructed, we amplified the 5' and 3' side of the *ade6* gene with oligos 588+589 and 590+591, respectively. We then stitched the two fragments together using overlap PCR. One of the oligos introduced a KpnI site in the middle of the gene. We then digested the fragment with BamHI and XhoI and ligated it into the BamHI and SalI sites of pAG25, to generate pSZB722. Due to the presence of KpnI sites within the *natMX4* cassette, we mutated the KpnI site at *ade6* to a StuI site. To do this, we used the QuickChange II XL Site-Directed Mutagenesis Kit from Agilent Technologies with pSZB722 as a template and oligos 2165 and 2166 to make pSZB849. We used StuI to linearize the plasmid. We then introduced plasmids into yeast using a variation of the standard lithium acetate protocol [61]. The variation to the protocol is that we do not sonicate the cells. Proper integration of these vectors at *ade6* yields an Ade⁻ phenotype (red colonies). To make the *ade6*-integrating vectors with the *wtf* transgenes, we cloned the *wtf* alleles using the oligos, DNA templates, restriction enzymes, and target sites described in [S5 Table](#). For each gene, we cloned at least 500 bp upstream and 300 bp downstream of each gene in an effort to include regulatory sequences. The promoter regions of the class 1 and class 2 *wtf* genes are highly similar and 288 bp of upstream and 325 bp of downstream sequences were sufficient to ensure expression of previously tested drivers and suppressors [16–18]. We verified each plasmid by Sanger sequencing the entire gene and the associated flanking sequences.

We could not directly amplify FY29033 *wtf1* alone from the genome, due to the repetitive nature of the *wtf* genes. Instead, we first amplified both FY29033 *wtf1* + *wtf36* tandem genes together from the FY29033 strain using oligos 1346 and 1348. From that PCR product, we amplified the FY29033 *wtf1* allele using oligos 1352 and 1592. We then digested this fragment with SacI and subsequently ligated this allele into the SacI site of pSZB387 to make pSZB879. We verified the clone by sequencing the gene and flanking sequences. The plasmids are described in [S6 Table](#).

Deleting *Sk wtf1*, *Sp wtf7*, *Sp wtf11*, and *Sp wtf14* + *wtf15*

To generate an *Sk wtf1* deletion cassette, we first amplified the regions (~750 bp) upstream and downstream of the *wtf1* locus using oligo pairs 645+656 and 2158+646, respectively. The upstream region includes the Tf1 transposon flanking the left side of the *Sk wtf1* locus. For these PCR reactions, we used SZY661 as a template. We then amplified the *hphMX6* cassette from pAG32 with oligos 657 and 2159 [60]. We stitched all three fragments together using

overlap PCR and transformed this deletion cassette into SZY298 to generate SZY3829. We confirmed the deletion using a series of PCR reactions: two oligo pairs with one oligo external to and one oligo within the deletion cassette (660+AO638 and AO1112+661) and a pair of oligos with one oligo outside of the deletion cassette and one oligo internal to the *Sk wtf1* locus (660+2287).

To generate an *Sp wtf7* deletion cassette, we first began by amplifying the regions (~1 kb) upstream and downstream of the gene with oligos 1565 + 1566 and oligos 1569 + 1570, respectively. We then amplified the drug cassettes, either *hphMX6* or *natMX4*, with oligos 1567 and 1568 using pAG32 or pAG25 as templates, respectively [60]. These oligos contained tails with homology to the upstream and downstream sequence of *Sp wtf7*. We then stitched these separate fragments together using overlap PCR and subsequently transformed it into yeast using the standard lithium acetate protocol [61]. We deleted *Sp wtf7* in the SZY44 strain to generate SZY2309, and deleted *Sp wtf7* in SZY643 to generate strains SZY2310 and SZY2336. We confirmed these deletions using a series of PCR reactions: oligo pairs with one oligo outside of the deletion cassette and one oligo internal to the gene (1571+1586 and 1572+1585), and 2 oligo pairs in which one oligo was external to and one oligo was within the deletion cassette (1571+AO638 and 1572+AO1112). Additionally, we further confirmed deletion of the gene using oligos internal to *Sp wtf7* (2152 and 2153).

To generate an *Sp wtf11* deletion cassette, we began by amplifying the regions (~500 bp) upstream and downstream of the gene using oligos 1667+1669 and oligos 1670+1672, respectively. Next, we amplified the *hphMX6* drug cassette with oligos 1668 and 1671 using pAG32 as a template [60]. We put these fragments together using overlap PCR and transformed it into SZY2336 to generate strains SZY2854 and SZY2855. This deletion was confirmed with two pairs of oligos with one oligo outside of the deletion cassette and one oligo internal to *wtf11* (1705+1707 and 1706+1707) and 2 pairs of oligos in which one oligo was external to the deletion cassette and one oligo was internal to the *hphMX6* cassette (1705+AO638 and 1706+1842). We further confirmed the deletion of the gene with a PCR reaction using oligos internal to *Sp wtf11* (2154 and 2155).

To delete the tandem *Sp wtf14* and *wtf15* genes, we first made a deletion cassette via PCR. To do this, we amplified the upstream region (~600 bp) of *Sp wtf14* using oligos 1649 and 1651, and the downstream region (~1 kb) of *Sp wtf15* with oligos 1655 and 1657. We also amplified the *natMX4* drug cassette from pAG25 with oligos 1650 and 1656 and then stitched the three fragments together using overlap PCR [60]. We then transformed this construct into SZY643 to generate strains SZY2856 and SZY2857. We confirmed the deletion of *Sp wtf14* and *Sp wtf15* via PCR using two oligo pairs in which one oligo was external to the deletion cassette and one oligo was within the *wtf14* and *wtf15* locus (1709+1710 and 1658+1711). We also did a PCR with in which one oligo was external to the deletion cassette and one was internal to the *natMX4* cassette (1709+AO638 and 1658+1843). Additionally, we did a PCR with oligos within the *Sp wtf14* + *wtf15* locus (2156 and 2157) to confirm the absence of the genes.

To assay the effect of the class 3 *wtf* genes, we generated strains lacking *wtf7*, *wtf11*, *wtf14*, and *wtf15* genes in *Sp*. First, we exchanged the *natMX4* gene marking the *wtf14* + *wtf15* deletion with either *kanMX4* or *CaURA3MX* cassette as described in [62]. Briefly, we amplified the *kanMX4* (from pFA6) or *CaURA3MX* (from pFA6-mTurq2-URA3MX) cassettes using oligos PR78 and PR79 [59, 63]. We then transformed the *kanMX4* fragment into SZY2856 to generate the yeast strain SZY3444, and the *CaURA3MX* fragment into SZY2856 to generate SZY3448. We then generated quadruple deletion mutant strains via crosses.

Spot assay

To determine if a mutant strain lacking class 3 *wtf* genes had growth defects, we first cultured the strains in 5 ml of YEL at 32°C with shaking overnight. We then diluted the cultures to an OD₆₀₀ of 1. We carried out serial dilutions and spotted 5 μl onto YEA plates and incubated them at 32°C for 2 days, after which the plates were imaged.

Deleting *Sp wtf21* using CRISPR

We used the *S. pombe* CRISPR-Cas9 genome editing system from [64] to generate the *Sp wtf21Δ::kanMX4* mutation in SZY890. This system uses two plasmids. The first one expresses a guide RNA to the target sequence and the second plasmid expresses Cas9 (pMZ222). To generate the plasmid carrying a guide RNA targeting *Sp wtf21* (pSZB197), we annealed oligos 623 and 624 to each other and ligated them into the CspCI site of pMZ283. Next, we transformed pMZ222 and pSZB197 into *Sp* (SZY643) along with a *wtf21Δ::kanMX4* repair cassette (see below). We initially selected for yeast that contained both plasmids (Leu⁺ Ura⁺) and subsequently selected for G418-resistant colonies. We then screened for the desired mutants by PCR using oligos 631 and 632 that flank *wtf21*, but are outside of the region amplified in the repair cassette. We generated the repair cassette using PCR to build a fragment containing the regions upstream and downstream of *wtf21* flanking the *kanMX4* gene [59]. We amplified the upstream and downstream regions with oligos 625+626 and 629+630, respectively, using *Sp* genomic DNA as a template. We amplified the *kanMX4* gene with oligos 627+628. We then stitched the three fragments together using overlap PCR to generate the repair cassette.

We confirmed the *Sp wtf21Δ::kanMX4* deletion via PCR. We used two oligo pairs in which one oligo was external to the deletion cassette and one oligo was within the *wtf21* locus (631+1154 and 1557+632). We also did two sets of PCRs where one oligo was external to the deletion cassette and one was internal to the *kanMX4* cassette (631+AO638 and AO1112+632). In addition, we further confirmed the deletion via Sanger sequencing. Finally, we did a PCR with oligos within the *Sp wtf21* locus (1635+1636 and 1635+1637) to confirm the absence of the gene.

C-terminally GFP-tagged *wtf* alleles

We generated *Sk wtf7*, *wtf11*, and *wtf15* GFP-tagged alleles using the following strategy. We first amplified the *Sk wtf* alleles (*wtf7*, *wtf11*, and *wtf15*) with their endogenous promoters using genomic DNA from SZY661 using oligo pairs 1359+1360 for *wtf7*, 1362+2210 for *wtf11*, and 991+1368 for *wtf15*. We then amplified yEGFP using pKT127 [65] as a template with oligos 1361+634 for *wtf7*, 2211+634 for *wtf11*, and 1369+634 for *wtf15*. We then stitched the two respective fragments together using overlap PCR and digested the fragments with SacI. We then cloned these fragments into the SacI site of pSZB188 to generate pSZB691 (*wtf7-GFP*), pSZB1087 (*wtf11-GFP*), and pSZB698 (*wtf15-GFP*).

To generate *Sk wtf14-GFP*, we amplified *wtf14* from pSZB378 using oligos 1365 and 1366. To amplify yEGFP, we used oligos 1367 and 634 and used pKT127 as a template [65]. We then used overlap PCR to stitch the two fragments together and digested the fragment with SacI. We then cloned it into the SacI site of pSZB188 to generate pSZB696.

To generate FY29033 *wtf35-GFP*, we amplified *wtf35* (from the upstream region to exon 5) from pSZB788 using oligos 1036 and 1349. We then digested this fragment with PvuII. We also ordered a gBlock from IDT (Coralville, IA) that contained 182 bp upstream of the stop codon of *wtf35*, a 24 bp linker (encoding GDGAGLIN), and the first 44 bp of yEGFP. To amplify yEGFP, we used oligos 2371 and 634 and used pKT127 as a template [65]. We then used overlap PCR to stitch the three fragments together and digested this new fragment with SacI. We then ligated it into the SacI site of pSZB188 to generate pSZB1176.

Imaging GFP-tagged Wtf proteins

To determine the localization of Wtf7-GFP, Wtf11-GFP, Wtf14-GFP, and Wtf15-GFP during haploid and diploid vegetative growth, we imaged cells during logarithmic and stationary phase. To image the cells during stationary phase, we first made a 5 ml YEL culture of each haploid and diploid strain and grew them for 16 hours in a 32°C shaker. We then imaged these cells as our stationary phase samples. To obtain cells in logarithmic phase, we used the YEL cultures described above and diluted them 1:10 in 5 ml cultures and incubated the tubes with shaking at 32°C for four hours. To image the cells, we spotted 3 µl of culture onto a glass slide pre-coated with lectin and covered with a glass coverslip to keep the cells in place for imaging.

To image the spore sacs, we used diploids that had sporulated on SPA plates at 25°C for two days. To prepare cells for imaging, we first scraped the cells off of SPA plates and mixed them with 3 µl of lectin. We then plated them on glass slides and covered with a glass coverslip.

For most experiments, we imaged the cells on an LSM-780 (Zeiss) AxioObserver confocal microscope with a 40X C-Apochromat water-immersion objective (Zeiss, NA = 1.2) or a 40X LD C-Apochromat water-immersion objective (Zeiss, NA = 1.1). We acquired images of every field of cells in two ways. We acquired a channel mode image to obtain a transmitted light image. For the images in Fig 5C, we acquired the fluorescence images by exciting GFP at 488 nm and collecting its emission between a 500–553 bandpass filter, while we excited mCherry at 561 nm and collected its emission between a 562–615 nm bandpass filter. For all other images, we acquired images in lambda mode over the entire visible range, with GFP and mCherry excitation at 488 and 561 nm, respectively, to obtain the true fluorescence. To eliminate cross talk, we collected the GFP and mCherry lambda images separately. To distinguish true GFP and mCherry signal from autofluorescence, we linearly unmixed the lambda mode data for GFP and mCherry using reference GFP and mCherry images and an in-house plug-in in ImageJ (<https://imagej.nih.gov/ij/>). When YFP was employed, we acquired the fluorescence by exciting YFP at 488 nm. We then collected the fluorescence spectrally as with GFP and mCherry above.

For S3A Fig, we used an LSM-700 (Zeiss) AxioObserver.Z1 confocal microscope with a 63x Plan Apochromat oil-immersion objective (Zeiss, NA = 1.4). The fluorescence was excited at 488 and 555 nm for GFP and mCherry, respectively. The GFP fluorescence was collected through a 400–577 nm bandpass filter while the mCherry was collected through a 577–700 nm bandpass filter in multitrack mode. The continuously variable secondary dichroic was set at 578 nm. The transmitted light was collected using the 488 nm excitation.

Analysis of meiotic proteomic data

We used the data set collected by Krapp et al and determined the relative protein levels following the method described in [29]. We considered all Wtf proteins that were detected in at least one timepoint in at least two of the three replicate experiments. We plotted the mean of the quantified Wtf protein for each timepoint that had at least two replicates, regardless of the number of replicates at that specific timepoint.

Alignments

To determine the DNA and amino acid sequence identity shared by *wtf* genes and proteins, we aligned the sequences using Geneious Prime (<https://www.geneious.com>). We used the Geneious aligner and performed a global alignment with free end gap using the default parameters. For DNA sequence alignments, the parameters we used were: cost matrix = 65% similarity, gap open penalty = 12, and gap extension penalty = 3. For protein sequence alignments we used Blosum62 as the matrix, gap open penalty = 12, gap extension penalty = 3, and refinement iterations = 2.

Supporting information

S1 Fig. Classification of the *wtf* gene family based on their sequence and structure (21).

Class 1 is predicted to contain poison-antidote meiotic drivers. Class 2 *wtf* genes are predicted to be antidote-only genes and suppressors of drive. *wtf7*, *wtf11*, *wtf14*, and *wtf15* are all grouped in class 3 solely because they do not look like any other *wtf* gene in this family, or each other. The *wtf* genes that have been shown in this study or were previously characterized as meiotic drivers or suppressors are shown in bold [16–18]. The *wtf* genes that were tested in this study but did not show a meiotic drive or drive suppressor phenotype are underlined. *wtf* genes with in-frame stop codons are depicted with a “*” next to the gene name and ‘M’ highlights the start codons and the in frame ATG codons near the start of exon 2.

(TIF)

S2 Fig. *wtf* meiotic drivers are extremely diverse. (A) The amino acid identity shared by pairs of Wtf^{antidote} proteins encoded by *bona fide* meiotic drivers [16–18]. The range of colors corresponds with the ranges of percent amino acid identity between the two compared Wtf^{antidote} proteins. Higher identities are depicted with lighter colors, while lower identities are depicted by darker colors. (B) Manual alignment of the antidotes of *Sk* Wtf9 and FY29033 Wtf35. Using this method, the percent amino acid identity between these two proteins is 24%.

(TIF)

S3 Fig. Localization of *Sk* Wtf4-GFP, *Sp* Wtf13-YFP, and FY29033 Wtf35-GFP. Tetrads showing the localization of (A) *Sk* Wtf4-GFP, (B) *Sp* Wtf13-YFP, and (C) FY29033 Wtf35-GFP, and transmitted light (TL) using hemizygous transgenes integrated at *ade6* [16, 18]. Brightness and contrast were adjusted differently for each image and the images were smoothed using Gaussian blur. The scale bar represents 3 μ m.

(TIF)

S4 Fig. Class 3 *wtf* genes do not exhibit meiotic drive phenotypes. (A) Allele transmission into spores from diploids heterozygous for the class 3 *wtf* genes. No significant differences in the allele transmission were found using a G-test when compared to the control *ura4* locus. (B) Fertility of wild-type and mutant backgrounds (normalized to wild-type). The fertility values were not significantly different (Wilcoxon test). The complete raw data are presented in S2 Table. (C) Serial dilutions of strains with the denoted genotypes were spotted onto YEA plates and grown for 2 days at 32°C. (D) Allele transmission of the *Sk wtf4* meiotic driver in a wild-type and mutant background. We found no significant difference between diploid 32 and diploid 33 using a G-test. For (A) and (D), we genotyped more than 200 spore progeny for each diploid and the complete raw data are presented in S1 Table.

(TIF)

S5 Fig. The allele transmission bias favoring *Sk* chromosome 1 is not caused by *wtf* genes.

(A) Allele transmission of *Sk* chromosome 1 (K1) in *rec12*- diploids. Diploid 35 represents combined data from crosses of three sets of haploid parents. (B) Allele transmission of chromosome 1 from *Sp* or *Sk* (P1 or K1, respectively) in *rec12*- diploids. For A and B, heterozygous markers at the *lys1* and/or *rec12* loci were used to genotype chromosome 1. At least 400 spore progeny were genotyped for each diploid. * indicates a p-value < 0.05 (G-test). The complete raw data are presented in S3 Table.

(TIF)

S6 Fig. Class 3 Wtf proteins are present during meiosis. Analysis of Wtf protein levels during meiosis from the data of Krapp et al [29]. In short, Krapp et al prepared a spike-in control by pooling heavy-labeled proteins collected from 0, 3, 5, and 8 hours after meiotic induction.

They then compared the constant spike-in control to light-labeled experimental samples collected at the indicated time points [29]. We have plotted the average relative abundance (light-labeled protein to heavy-labeled protein) of the indicated Wtf proteins from three replicate experiments. Peptides for all genes shown were detected in at least two replicate experiments, although not always in each time point. Time points where the protein was detected in less than three experiments are indicated with open circles. Due to the high sequence similarity between *Sp* Wtf4 and Wtf13 proteins, and *Sp* Wtf19 and Wtf23 proteins, the data points for these two pairs of proteins were merged.

(TIF)

S7 Fig. *Sk wtf11-GFP* expression was not observed during vegetative growth or sporulation.

Representative images of cells containing *Sk* Wtf11-GFP (green) in (A) haploids, (B) heterozygous (*wtf11-GFP/ade+*) diploids, and (C) a tetrad generated by heterozygous diploids. The GFP signal was not greater than background GFP. We adjusted the brightness and contrast to observe the background. The adjustments were different for each image. We smoothed the images using Gaussian blur. We verified the green autofluorescence via spectral imaging. TL, transmitted light. Scale bar represents 5 μ m.

(TIF)

S8 Fig. *Sk wtf7-GFP* and *Sk wtf15-GFP* expression was not observed in vegetative haploids or diploids.

(A) Representative images of Wtf7-GFP (in green) in haploids (left) and heterozygous diploids (right) during logarithmic phase and saturation. (B) Representative images of Wtf15-GFP (in green) in haploids (left) and heterozygous diploids (right) during logarithmic phase and saturation. The GFP signal was not greater than background GFP. We linearly unmixed these images, adjusted the brightness and contrast differently for each image, and smoothed each image using Gaussian blur. The brightness and contrast were adjusted to observe the background. We verified the green autofluorescence via spectral imaging. The scale bar represents 5 μ m. TL represents transmitted light.

(TIF)

S9 Fig. Linear unmixing of *Sk* Wtf7-GFP and *Sk* Wtf15-GFP localization in spores.

(A) Linear unmixing of the representative images shown in Fig 4A of *Sk* Wtf7-GFP (cyan) and Nsp1-mCherry (magenta). We normalized the intensities of the green and red autofluorescence to the intensities of the GFP and mCherry signals, respectively. (B) Linear unmixing of the representative image shown in Fig 4B of *Sk* Wtf15-GFP (cyan) localization. We normalized the intensity of the green autofluorescence to the intensity of the GFP channel. We adjusted the brightness and contrast differently for each image and smoothed them using Gaussian blur. The scale bar represents 5 μ m and TL represents transmitted light.

(TIF)

S10 Fig. Linear unmixing of *Sk* Wtf14-GFP in haploid and diploid cells.

Linear unmixing of (A) haploids and (B) diploids containing *Sk* Wtf14-GFP (cyan) and mCherry-AHDL (magenta) from the representative images shown in Fig 5A and 5B [30]. We adjusted the intensities of the autofluorescence images to their respective channels. We adjusted brightness and contrast differently for each image and smoothed them using Gaussian blur. TL represents transmitted light. Scale bar represents 5 μ m.

(TIF)

S1 Table. Raw data of allele transmission from Figs 2 and 3, and S4 Fig. Each of the horizontal lines represents the relevant genotype and allele transmission from the indicated diploid into spores. The first column (C1) represents the diploid number, which matches the numbers

in Figs 2 and 3, and S4 Fig. In columns C2-C5, the strain number (SZY) and relevant genotype of the haploid parent strains used to determine the allele transmission at the drive locus (*ade6* or *wtf* locus) are shown. *Sp*, *Sk*, CBS5557, and FY29033 alleles are labeled in blue, red, yellow, and green, respectively. Columns C6-C8 indicate which phenotypes were followed at the control locus (*ura4*) and the number of progeny that showed the indicated phenotypes. Columns C9 and C10 indicate the phenotypes that were followed at the drive loci (*ade6* or *wtf* locus) and the number of haploid progeny that exhibited the indicated phenotypes. Some of the progeny inherited both markers from the parent strains at the *ade6* locus. DRUG^R represents inheritance of either the *kanMX4* or *hphMX6* marker. G418^R signifies inheritance of the *kanMX4* marker, HYG^R denotes inheritance of the *hphMX6* marker, and NAT^R represents inheritance of the *natMX4* marker. Inheritance of these markers confers resistance to G418, Hygromycin B, or Nourseothricin, respectively. The number of the progeny that inherited both markers is presented in column C11 and the percentage of the progeny with this phenotype is shown in column C12. These progeny were excluded from the data presented in Figs 2 and 3, and S3 Fig. Column C13 shows the fraction of the haploid progeny that inherited the genotype of allele 1. Column C14 shows the fraction of the haploid progeny that inherited the genotype of allele 2. Column C15 shows the total progeny assayed excluding the progeny that inherited both genetic markers at *ade6*. Column C16 shows the total progeny. Column C17 shows the total number of independent diploids assayed for each cross. The last column (C18) shows the p-value calculated by comparing diploids 1–4,7,9,10,12,13,15,17–20 to control diploid 22; diploid 24 to control diploid 25; diploids 5,6,8,11,14,16 and 26 to control diploid 23; diploid 27 to control diploid 28; diploids 21, 29, 30, and 31 to the control *ura4* locus; and diploid 33 to diploid 32. We previously published the allele transmission data for diploid 26 [18]. (PDF)

S2 Table. Raw data for fertility from Fig 3 and S4 Fig. Each column represents the diploid assayed, which matches the diploid number in Fig 3 and S4 Fig. The first row underneath the diploid number shows the SZY numbers of the haploid parent strains. We present all the viable spore yield values from independent assays. For relative fertility, we normalized diploids 10 and 20 to control diploid 22; diploid 24 to control diploid 25; diploids 8 and 26 to control diploid 23; and diploid 27 to control diploid 28. We previously published the viable spore yield data for diploid 26 [18]. We normalized the average viable spore yield value from the h⁹⁰ mating of strain SZY3529 (*Sp wtf7Δ*, *wtf11Δ*, *wtf14+wtf15Δ*) to the average viable spore yield value of the wild-type control strain SZY2254. We calculated the p-value comparison using the Wilcoxon test. (PDF)

S3 Table. Raw data of allele transmission from S5 Fig. Each of the horizontal lines represents the relevant genotype and allele transmission of the indicated diploid. The first column represents the diploid number, which matches the numbers in S5 Fig. In columns 2–5, the strain number (SZY) and relevant genotype of the haploid parent strains used to determine the allele transmission of chromosome 1 of *Sp* and *Sk*. Columns 6 and 7 indicate the number of progeny that exhibited the indicated phenotypes and were followed in chromosome 1 (*lys1* or *rec12*). Column 8 shows the total progeny assayed. Column 9 indicates the percent allele transmission of the *Sk* chromosome 1. Control diploid 34 (*Sk/Sk*), diploid 36 (*Sp/Sk* hybrid *wtf1Δ*), diploid 37 (*Sp/Sp*), and diploid 38 (*Sp/Sk* hybrid) are represented by one pair of haploid parents. Diploid 35 (*Sp/Sk* hybrid) shows the data from three different pairs of haploid parents. In two of the crosses, we followed chromosome 1 using the *lys1* markers. In the third cross, we followed chromosome 1 using the *rec12* markers. Column 10 shows the p-value (G-test) calculated by comparing diploid 35 and 36 to control diploid 34, and diploid 38 to control diploid 37.

Column 11 shows the total number of diploids assayed for each cross.
(PDF)

S4 Table. Yeast strains used in this study. The strain SZY643 contains the *wtf18-2* allele [18].
(PDF)

S5 Table. Table summary of plasmid constructions. Column 1 lists the *wtf* gene cloned into each vector. Column 2 denotes the isolate origin of each *wtf* gene in column 1. The DNA templates and oligos used in the PCR reactions to amplify the *wtf* alleles are shown in columns 3 and 4, respectively. We digested each of the amplified fragments with the enzymes reported in column 5 and then integrated into the target site listed in column 6. The strain number of each of the plasmids that we generated is reported in column 7. The description of each plasmid can be found in [S6 Table](#).
(PDF)

S6 Table. Plasmids used in this study.
(PDF)

S7 Table. Oligos used in this study.
(PDF)

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References

- Abbott S, Fairbanks DJ. Experiments on plant hybrids by Gregor Mendel. *Genetics*. 2016; 204(2):407–22. <https://doi.org/10.1534/genetics.116.195198> PMID: 27729492
- Sandler L, Novitski E. Meiotic drive as an evolutionary force. *The American Naturalist*. 1957; 91(857):105–10.
- Zimmering S, Sandler L, Nicoletti B. Mechanisms of meiotic drive. *Annu Rev Genet*. 1970; 4:409–36. <https://doi.org/10.1146/annurev.ge.04.120170.002205> PMID: 4950062
- Burt A, Trivers R. *Genes in conflict: the biology of selfish genetic elements*. Cambridge, Mass.: Belknap Press of Harvard University Press; 2006. viii, 602 p., 8 p. of plates p.
- Lindholm AK, Dyer KA, Firman RC, Fishman L, Forstmeier W, Holman L, et al. The ecology and evolutionary dynamics of meiotic drive. *Trends Ecol Evol*. 2016; 31(4):315–26. <https://doi.org/10.1016/j.tree.2016.02.001> PMID: 26920473
- Zanders SE, Unckless RL. Fertility costs of meiotic drivers. *Current Biology*. 2019; 29(11):R512–R20. <https://doi.org/10.1016/j.cub.2019.03.046> PMID: 31163165
- Dyer KA, Charlesworth B, Jaenike J. Chromosome-wide linkage disequilibrium as a consequence of meiotic drive. *Proc Natl Acad Sci U S A*. 2007; 104(5):1587–92. <https://doi.org/10.1073/pnas.0605578104> PMID: 17242362
- Crow JF. Why is Mendelian segregation so exact? *Bioessays*. 1991; 13(6):305–12. <https://doi.org/10.1002/bies.950130609> PMID: 1909864
- Price TAR, Wedell N. Selfish genetic elements and sexual selection: their impact on male fertility. *Genetica*. 2007; 132(3):295. <https://doi.org/10.1007/s10709-007-9173-2> PMID: 17647082
- Sutter A, Lindholm AK. Detrimental effects of an autosomal selfish genetic element on sperm competitiveness in house mice. *Proceedings of the Royal Society B: Biological Sciences*. 2015; 282(1811).
- Hartl DL. Modifier theory and meiotic drive. *Theoretical Population Biology*. 1975; 7(2):168–74. [https://doi.org/10.1016/0040-5809\(75\)90012-x](https://doi.org/10.1016/0040-5809(75)90012-x) PMID: 1145501
- McLaughlin RN Jr., Malik HS. Genetic conflicts: the usual suspects and beyond. *J Exp Biol*. 2017; 220(Pt 1):6–17. <https://doi.org/10.1242/jeb.148148> PMID: 28057823
- Daugherty MD, Malik HS. Rules of engagement: molecular insights from host-virus arms races. *Annu Rev Genet*. 2012; 46:677–700. <https://doi.org/10.1146/annurev-genet-110711-155522> PMID: 23145935
- Henikoff S, Ahmad K, Malik HS. The centromere paradox: stable inheritance with rapidly evolving DNA. *Science*. 2001; 293(5532):1098–102. <https://doi.org/10.1126/science.1062939> PMID: 11498581
- Pardo-Manuel de Villena F, Sapienza C. Female meiosis drives karyotypic evolution in mammals. *Genetics*. 2001; 159(3):1179–89. PMID: 11729161
- Nuckolls NL, Bravo Núñez MA, Eickbush MT, Young JM, Lange JJ, Yu JS, et al. *wtf* genes are prolific dual poison-antidote meiotic drivers. *eLIFE*. 2017; 6:e26033. <https://doi.org/10.7554/eLife.26033> PMID: 28631612
- Hu W, Jiang Z, Suo F, Zheng J, He W, Du L. A large gene family in fission yeast encodes spore killers that subvert Mendel's law. *eLIFE*. 2017; 6:e26057. <https://doi.org/10.7554/eLife.26057> PMID: 28631610
- Bravo Núñez MA, Lange JJ, Zanders SE. A suppressor of a *wtf* poison-antidote meiotic driver acts via mimicry of the driver's antidote. *PLOS Genetics*. 2018; 14(11):e1007836. <https://doi.org/10.1371/journal.pgen.1007836> PMID: 30475921
- Wood V, Gwilliam R, Rajandream MA, Lyne M, Lyne R, Stewart A, et al. The genome sequence of *Schizosaccharomyces pombe*. *Nature*. 2002; 415(6874):871–80. <https://doi.org/10.1038/nature724> PMID: 11859360
- Lock A, Rutherford K, Harris MA, Hayles J, Oliver SG, Bahler J, et al. PomBase 2018: user-driven reimplementation of the fission yeast database provides rapid and intuitive access to diverse, interconnected information. *Nucleic acids research*. 2019; 47(D1):D821–d7. <https://doi.org/10.1093/nar/gky961> PMID: 30321395

21. Eickbush MT, Young JM, Zanders SE. Killer meiotic drive and dynamic evolution of the *wtf* gene family. *Molecular Biology and Evolution*. 2019; 36(6):1201–14. <https://doi.org/10.1093/molbev/msz052> PMID: 30991417
22. Kuang Z, Boeke JD, Canzar S. The dynamic landscape of fission yeast meiosis alternative-splice isoforms. *Genome Res*. 2016.
23. Jeffares DC, Rallis C, Rieux A, Speed D, Převorovský M, Mourier T, et al. The genomic and phenotypic diversity of *Schizosaccharomyces pombe*. *Nature Genetics*. 2015; 47:235. <https://doi.org/10.1038/ng.3215> PMID: 25665008
24. Tusso S, Nieuwenhuis BPS, Sedlazeck FJ, Davey JW, Jeffares DC, Wolf JBW. Ancestral admixture is the main determinant of global biodiversity in fission yeast. *Molecular biology and evolution*. 2019; 36(9):1975–89. <https://doi.org/10.1093/molbev/msz126> PMID: 31225876
25. Orr HA, Irving S. Segregation distortion in hybrids between the Bogota and USA subspecies of *Drosophila pseudoobscura*. *Genetics*. 2005; 169(2):671–82. <https://doi.org/10.1534/genetics.104.033274> PMID: 15654115
26. Kim D-U, Hayles J, Kim D, Wood V, Park H-O, Won M, et al. Analysis of a genome-wide set of gene deletions in the fission yeast *Schizosaccharomyces pombe*. *Nature Biotechnology*. 2010; 28:617. <https://doi.org/10.1038/nbt.1628> PMID: 20473289
27. Zanders SE, Eickbush MT, Yu JS, Kang JW, Fowler KR, Smith GR, et al. Genome rearrangements and pervasive meiotic drive cause hybrid infertility in fission yeast. *eLIFE*. 2014; 3:e02630. <https://doi.org/10.7554/eLife.02630> PMID: 24963140
28. Coyne JA, Orr HA. *Speciation*. Sunderland, Mass.: Sinauer Associates; 2004. xiii, 545, 2 p. of plates p.
29. Krapp A, Hamelin R, Armand F, Chiappe D, Krapp L, Cano E, et al. Analysis of the *S. pombe* meiotic proteome reveals a switch from anabolic to catabolic processes and extensive post-transcriptional regulation. *Cell Reports*. 2019; 26(4):1044–58. e5. <https://doi.org/10.1016/j.celrep.2018.12.075> PMID: 30673600
30. Zhang D, Vjestica A, Oliferenko S. The cortical ER network limits the permissive zone for actomyosin ring assembly. *Current Biology*. 2010; 20(11):1029–34. PMID: 20434336
31. Matsuyama A, Arai R, Yashiroda Y, Shirai A, Kamata A, Sekido S, et al. ORFeome cloning and global analysis of protein localization in the fission yeast *Schizosaccharomyces pombe*. *Nat Biotechnol*. 2006; 24(7):841–7. <https://doi.org/10.1038/nbt1222> PMID: 16823372
32. Suda Y, Nakanishi H, Mathieson EM, Neiman AM. Alternative modes of organellar segregation during sporulation in *Saccharomyces cerevisiae*. *Eukaryotic Cell*. 2007; 6(11):2009–17. <https://doi.org/10.1128/EC.00238-07> PMID: 17905927
33. Didion JP, Morgan AP, Clayshulte AM, McMullan RC, Yadgary L, Petkov PM, et al. A multi-megabase copy number gain causes maternal transmission ratio distortion on mouse chromosome 2. *PLOS Genetics*. 2015; 11(2):e1004850. <https://doi.org/10.1371/journal.pgen.1004850> PMID: 25679959
34. Long Y, Zhao L, Niu B, Su J, Wu H, Chen Y, et al. Hybrid male sterility in rice controlled by interaction between divergent alleles of two adjacent genes. *Proc Natl Acad Sci U S A*. 2008; 105(48):18871–6. <https://doi.org/10.1073/pnas.0810108105> PMID: 19033192
35. Hammond TM, Rehard DG, Xiao H, Shiu PK. Molecular dissection of *Neurospora* spore killer meiotic drive elements. *Proc Natl Acad Sci U S A*. 2012; 109(30):12093–8. <https://doi.org/10.1073/pnas.1203267109> PMID: 22753473
36. Grognet P, Lalucque H, Malagnac F, Silar P. Genes that bias Mendelian segregation. *PLOS Genetics*. 2014; 10(5):e1004387. <https://doi.org/10.1371/journal.pgen.1004387> PMID: 24830502
37. Vogan AA, Ament-Velásquez SL, Granger-Farbos A, Svedberg J, Bastiaans E, Debets AJM, et al. Combinations of Spok genes create multiple meiotic drivers in *Podospora*. *eLIFE*. 2019; 8:e46454. <https://doi.org/10.7554/eLife.46454> PMID: 31347500
38. Rhoades NA, Harvey AM, Samarajeewa DA, Svedberg J, Yusifov A, Abusharekh A, et al. Identification of *rfk-1*, a meiotic driver undergoing RNA editing in *Neurospora*. *Genetics*. 2019; 212(1):93–110. <https://doi.org/10.1534/genetics.119.302122> PMID: 30918007
39. Pieper KE, Unckless RL, Dyer KA. A fast-evolving X-linked duplicate of importin- α 2 is overexpressed in sex-ratio drive in *Drosophila neotestacea*. *Molecular Ecology*. 2018; 27(24):5165–79. <https://doi.org/10.1111/mec.14928> PMID: 30411843
40. Xie Y, Tang J, Xie X, Li X, Huang J, Fei Y, et al. An asymmetric allelic interaction drives allele transmission bias in interspecific rice hybrids. *Nature Communications*. 2019; 10(1):2501. <https://doi.org/10.1038/s41467-019-10488-3> PMID: 31175302
41. Yu X, Zhao Z, Zheng X, Zhou J, Kong W, Wang P, et al. A selfish genetic element confers non-Mendelian inheritance in rice. *Science*. 2018; 360(6393):1130–2. <https://doi.org/10.1126/science.aar4279> PMID: 29880691

42. Yang J, Zhao X, Cheng K, Du H, Ouyang Y, Chen J, et al. A killer-protector system regulates both hybrid sterility and segregation distortion in rice. *Science*. 2012; 337(6100):1336–40. <https://doi.org/10.1126/science.1223702> PMID: 22984070
43. Bauer H, Schindler S, Charron Y, Willert J, Kusecek B, Herrmann BG. The nucleoside diphosphate kinase gene *Nme3* acts as quantitative trait locus promoting non-Mendelian inheritance. *PLOS Genetics*. 2012; 8(3):e1002567. <https://doi.org/10.1371/journal.pgen.1002567> PMID: 22438820
44. Chen J, Ding J, Ouyang Y, Du H, Yang J, Cheng K, et al. 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*. 2008; 105(32):11436–41. <https://doi.org/10.1073/pnas.0804761105> PMID: 18678896
45. Dalstra HJ, van der Zee R, Swart K, Hoekstra RF, Saupe SJ, Debets AJ. Non-mendelian inheritance of the HET-s prion or HET-s prion domains determines the *het-S* spore killing system in *Podospora anserina*. *Fungal genetics and biology: FG & B*. 2005; 42(10):836–47.
46. Tao Y, Hartl DL, Laurie CC. Sex-ratio segregation distortion associated with reproductive isolation in *Drosophila*. *Proc Natl Acad Sci U S A*. 2001; 98(23):13183–8. <https://doi.org/10.1073/pnas.231478798> PMID: 11687638
47. Tao Y, Araripe L, Kingan SB, Ke Y, Xiao H, Hartl DL. A sex-ratio meiotic drive system in *Drosophila simulans*. II: An X-linked distorter. *PLOS Biology*. 2007; 5(11):e293. <https://doi.org/10.1371/journal.pbio.0050293> PMID: 17988173
48. Merçot H, Atlan A, Jacques M, Montchamp-Moreau C. Sex-ratio distortion in *Drosophila simulans*: co-occurrence of a meiotic drive and a suppressor of drive. *Journal of Evolutionary Biology*. 1995; 8(3):283–300.
49. Dermitzakis ET, Masly JP, Waldrip HM, Clark AG. Non-Mendelian segregation of sex chromosomes in heterospecific *Drosophila* males. *Genetics*. 2000; 154(2):687–94. PMID: 10655222
50. Lin C-J, Hu F, Dubruille R, Vedanayagam J, Wen J, Smibert P, et al. The hpRNA/RNAi pathway is essential to resolve intragenomic conflict in the *Drosophila* male germline. *Developmental Cell*. 2018; 46(3):316–26.e5. <https://doi.org/10.1016/j.devcel.2018.07.004> PMID: 30086302
51. Rhoades MM. Preferential segregation in maize. *Genetics*. 1942; 27(4):395–407. PMID: 17247049
52. Kato Yamakake TA. Cytological studies of maize [*Zea mays* L.] and teosinte [*Zea mexicana* Schrader Kuntze] in relation to their origin and evolution. Amherst, Mass.: Massachusetts Agricultural Experiment Station; 1976. ca. 200 p. p.
53. Dawe RK, Lowry EG, Gent JI, Stitzer MC, Swentowsky KW, Higgins DM, et al. A kinesin-14 motor activates neocentromeres to promote meiotic drive in maize. *Cell*. 2018; 173(4):839–50.e18. <https://doi.org/10.1016/j.cell.2018.03.009> PMID: 29628142
54. Akera T, Chmátal L, Trimm E, Yang K, Aonbangkhen C, Chenoweth DM, et al. Spindle asymmetry drives non-Mendelian chromosome segregation. *Science*. 2017; 358(6363):668–72. <https://doi.org/10.1126/science.aan0092> PMID: 29097549
55. Akera T, Trimm E, Lampson MA. Molecular strategies of meiotic cheating by selfish centromeres. *Cell*. 2019; 178(5):1132–44.e10. <https://doi.org/10.1016/j.cell.2019.07.001> PMID: 31402175
56. Farlow A, Long H, Arnoux S, Sung W, Doak TG, Nordborg M, et al. The spontaneous mutation rate in the fission yeast *Schizosaccharomyces pombe*. *Genetics*. 2015; 201(2):737–44. <https://doi.org/10.1534/genetics.115.177329> PMID: 26265703
57. Dalstra HJ, Swart K, Debets AJ, Saupe SJ, Hoekstra RF. Sexual transmission of the [Het-S] prion leads to meiotic drive in *Podospora anserina*. *Proc Natl Acad Sci U S A*. 2003; 100(11):6616–21. <https://doi.org/10.1073/pnas.1030058100> PMID: 12719532
58. Smith GR. Genetic analysis of meiotic recombination in *Schizosaccharomyces pombe*. *Methods Mol Biol*. 2009; 557:65–76. https://doi.org/10.1007/978-1-59745-527-5_6 PMID: 19799177
59. Wach A, Brachat A, Pohlmann R, Philippsen P. New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast*. 1994; 10(13):1793–808. <https://doi.org/10.1002/yea.320101310> PMID: 7747518
60. Goldstein AL, McCusker JH. Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. *Yeast*. 1999; 15(14):1541–53. [https://doi.org/10.1002/\(SICI\)1097-0061\(199910\)15:14<1541::AID-YEA476>3.0.CO;2-K](https://doi.org/10.1002/(SICI)1097-0061(199910)15:14<1541::AID-YEA476>3.0.CO;2-K) PMID: 10514571
61. Schiestl RH, Gietz RD. High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. *Current Genetics*. 1989; 16(5):339–46.
62. Gardner JM, Jaspersen SL. Manipulating the yeast genome: deletion, mutation, and tagging by PCR. In: Smith JS, Burke DJ, editors. *Yeast Genetics: Methods and Protocols*. New York, NY: Springer New York; 2014. p. 45–78.

63. Chen J, Smoyer CJ, Slaughter BD, Unruh JR, Jaspersen SL. The SUN protein Mps3 controls Ndc1 distribution and function on the nuclear membrane. *The Journal of Cell Biology*. 2014; 204(4):523–39. <https://doi.org/10.1083/jcb.201307043> PMID: 24515347
64. Jacobs JZ, Ciccaglione KM, Tournier V, Zaratiegui M. Implementation of the CRISPR-Cas9 system in fission yeast. *Nature Communications*. 2014; 5:5344. <https://doi.org/10.1038/ncomms6344> PMID: 25352017
65. Sheff MA, Thorn KS. Optimized cassettes for fluorescent protein tagging in *Saccharomyces cerevisiae*. *Yeast*. 2004; 21(8):661–70. <https://doi.org/10.1002/yea.1130> PMID: 15197731