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RESEARCH ARTICLE

Figures and figure supplements

wtf genes are prolific dual poison-antidote meiotic drivers

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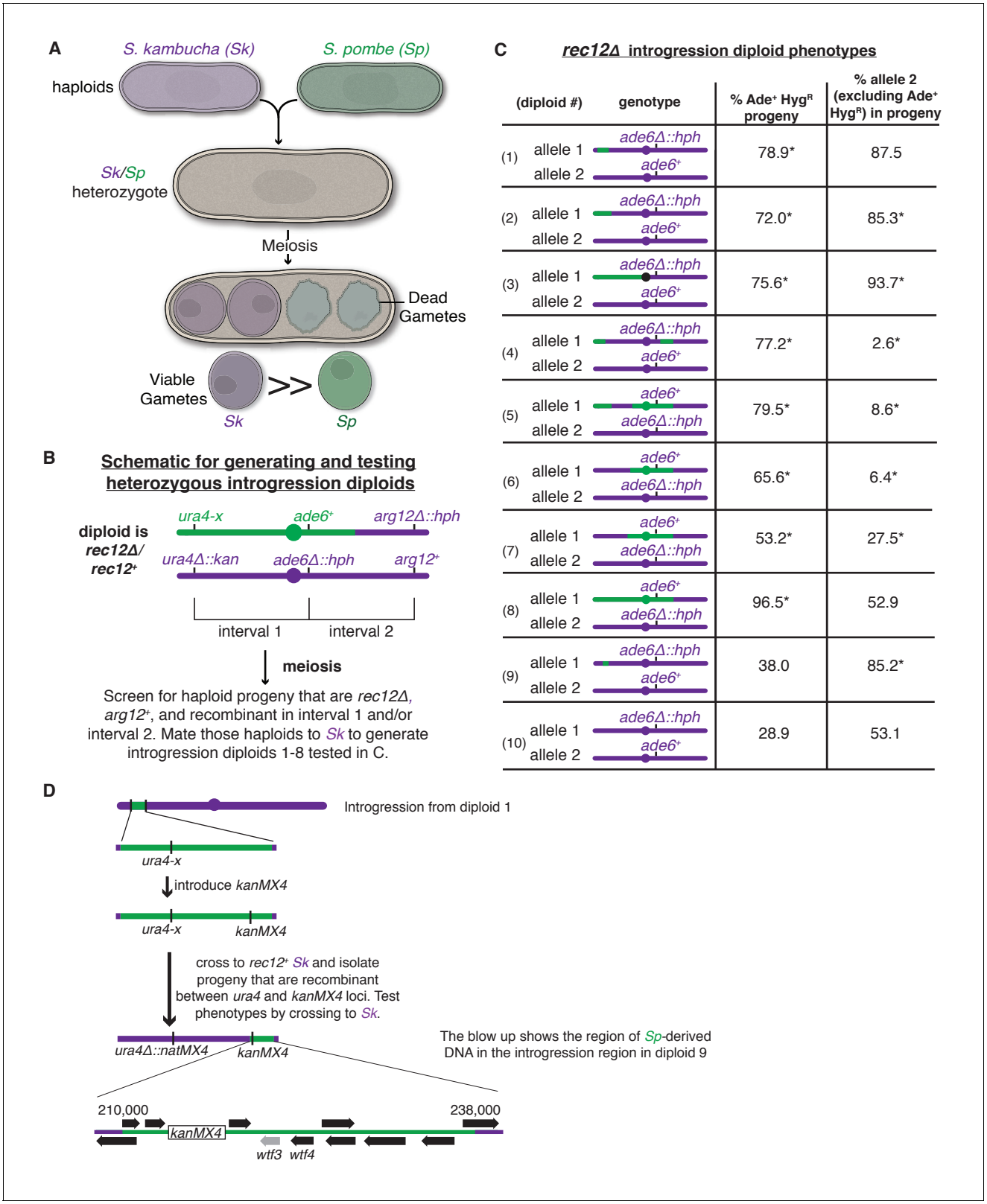

Figure 1. A complex meiotic drive landscape on *Sk* and *Sp* chromosome 3 is revealed by recombination mapping. (**A**) A cross between *Sk* and *Sp* generates a heterozygote that has low fertility and preferentially transmits *Sk* alleles on all three chromosomes into viable gametes (***Zanders et al., 2014***). (**B**) Generation of chromosome 3 introgression diploids 1–8. *Sk*-derived DNA is shown in purple while *Sp*-derived DNA is shown in green. The origin of the *Sp/Sk* mosaic chromosome is depicted in ***Figure 1—figure supplement 1***. (**C**) Phenotypes of *rec12Δ/rec12Δ* introgression/*Sk* diploids. See

Figure 1 continued on next page

Figure 1 continued

Figure 1—source data 1 for breakpoints between *Sk*-derived DNA (purple) and *Sp*-derived DNA (green). Chromosome transmission was followed using the heterozygous markers at the *ade6* locus: *hph* is short for the *hphMX4* marker gene which confers resistance to hygromycin (Hyg^R). The percentage of gametes that inherit both markers (heterozygous disomes, likely aneuploids and diploids) and (after excluding the heterozygous disomes) the percent of gametes that inherit the marker from the pure *Sk* chromosome are shown. Over 100 viable gametes were tested for each diploid; raw data can be found in ***Figure 1—source data 2***. * indicates p-value<0.01 (G-test) compared to *rec12Δ/rec12Δ Sk* control (from ***Zanders et al. (2014)***). (**D**) Fine-scale mapping of the drive locus starting with the introgression from diploid 1. Strains that were recombinant between the *ura4* locus and an introduced *kanMX4* marker gene were selected and their phenotypes were tested in crosses to *Sk*. The recombinant strain with the smallest amount of *Sp* DNA that retained the phenotype (sensitivity to drive by an *Sk* chromosome) is shown in detail. This introgression strain was mated to *Sk* to generate diploid 9. These analyses identified a ~30 kb candidate region (see blow up) containing a drive locus. In *Sp*, this region contains *wtf4* and the *wtf3* pseudogene. The syntenic region in *Sk* contains only one *wtf* gene, *wtf4*.

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The following source data is available for figure 1:

Source data 1. Breakpoints between *Sp* and *Sk*-derived DNA sequences.

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Source data 2. Raw data underlying ***Figure 1C***.

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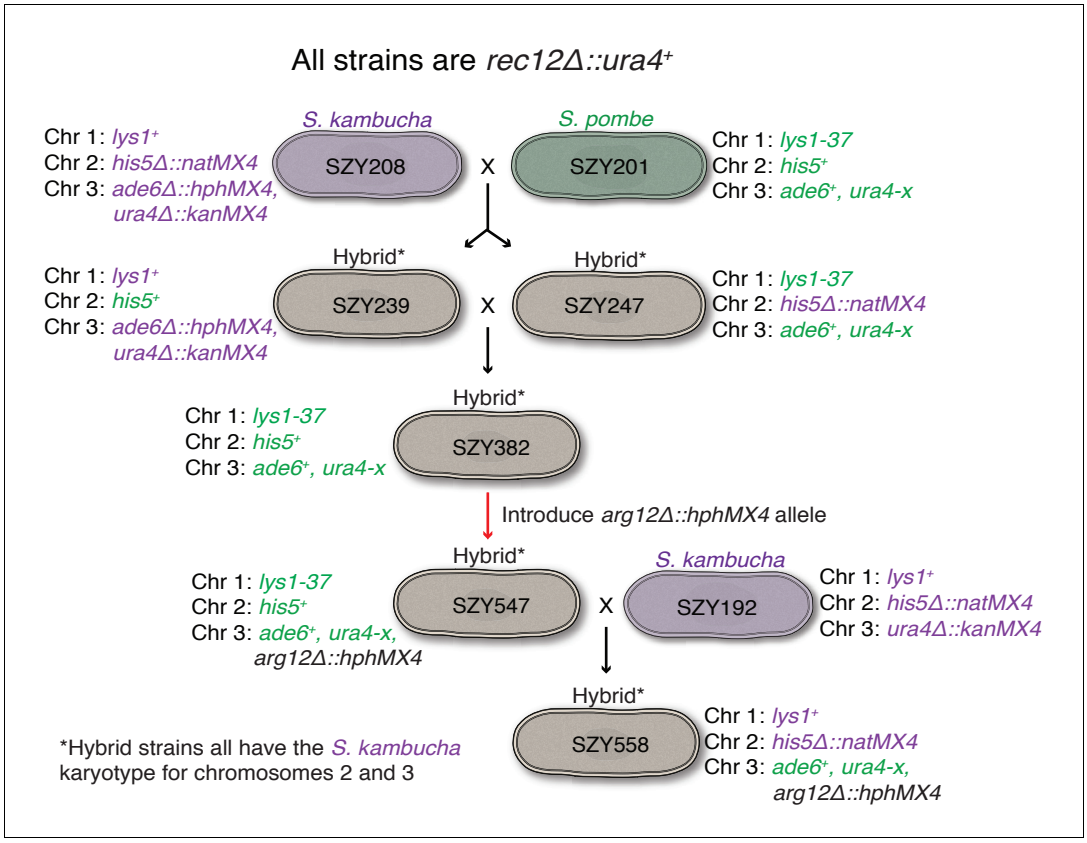

Figure 1—figure supplement 1. Generation of mosaic chromosome 3 used in ***Figure 1B***. The goal of these crosses was to generate a strain containing mostly *Sp*-derived DNA on chromosome 3 in an otherwise *Sk* background. This effort was complicated by the different karyotypes of *Sp* and *Sk* chromosomes 2 and 3 (***Zanders et al., 2014***). We used *rec12*Δ strains to limit recombination, but rare recombinants (e.g. SZY239 and SZY247) can still be obtained via selection. Markers derived from the *Sk* parent are shown in purple, while *Sp*-derived markers are green. We first isolated hybrids in which *Sk* and *Sp* markers on chromosomes 2 and 3 were uncoupled, suggesting rare recombination events had occurred between *Sk* and *Sp* chromosomes 2 and 3. Such events have the potential to generate chromosome 3 variants with mostly *Sp* DNA, but with an *Sk* karyotype, as occurred in SZY247. We then performed the illustrated crosses to move that chromosome into a different strain background with pure *Sk* chromosomes 1 and 2. We finally sequenced SZY558 and verified the strain has *Sk* chromosomes 1 and 2 and *Sp* DNA on chromosome 3 until between SNPs at positions 1,804,477 and 1,810,659.
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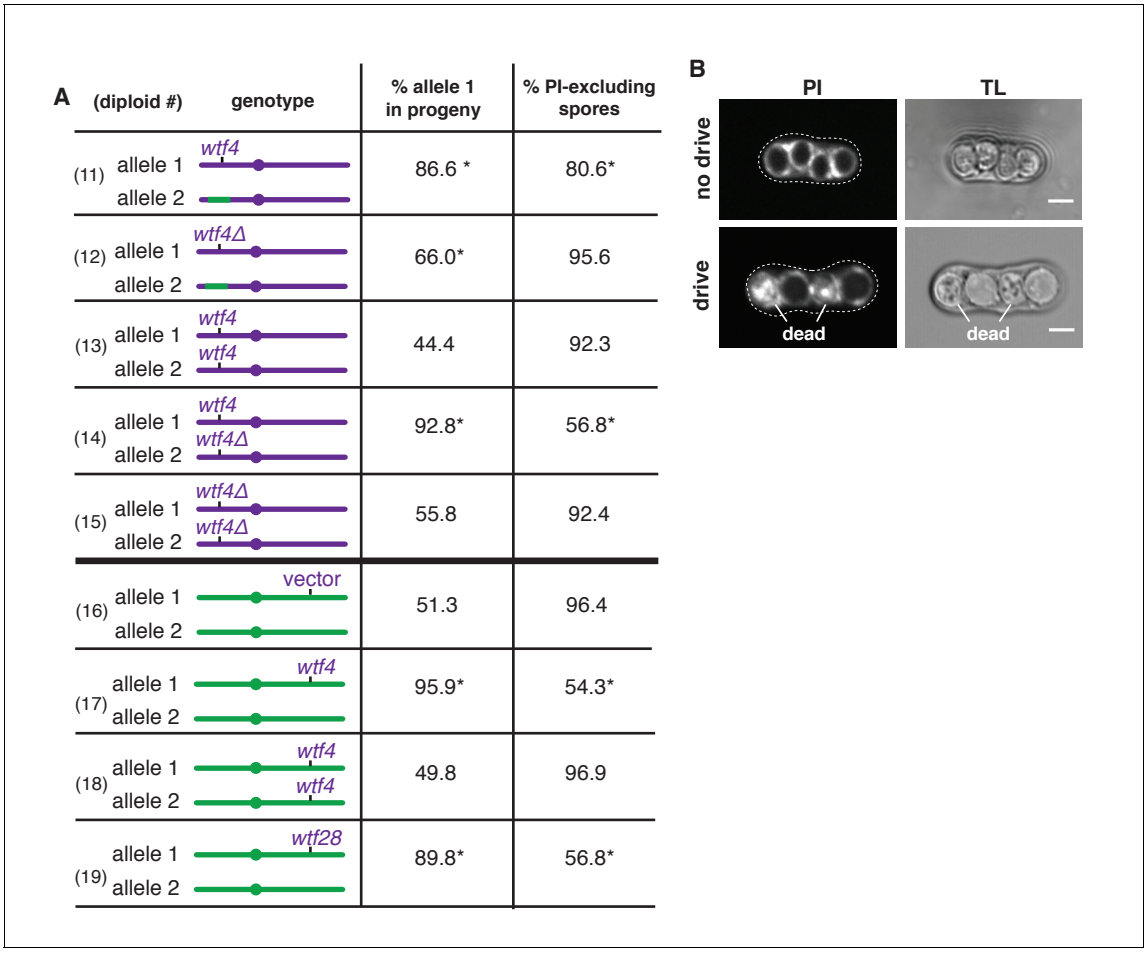

Figure 2. *Sk wtf4* is a self-sufficient meiotic driver that kills gametes that do not inherit the gene. (**A**) Allele transmission and propidium iodide (PI) staining phenotypes of diploids 11–19. *Sk*-derived DNA is purple, *Sp*-derived DNA is green. The cartoons depict chromosome 3. Chromosomes 1 and 2 are derived from *Sk* in diploids 11–15, but are from *Sp* in diploids 16–19. For diploids 11–15, allele transmission was monitored by following heterozygous markers at the *ura4* locus, which is tightly linked to *wtf4* (estimated 7–17 cM based on physical distance [***Young et al., 2002***]). PI dye is excluded from living spores, but not dead spores that have lost membrane integrity, such as those destroyed by drive. The percent of spores that exclude PI is shown as a proxy of fertility (***Figure 2—source data 1***). The PI phenotypes and *ura4* locus allele transmission for diploids 11, 12, 14 and 15 were compared to those of the wild-type *Sk* control (diploid 13). * indicates p-value<0.01 (G-test). For diploids 16–19, allele transmission was followed using markers at the *ade6* locus, which is where the empty vector or *wtf* gene constructs are integrated. The integrations introduced a dominant drug resistance gene and mutated $ade6^{+}$. Because these diploids all had codominant alleles at *ade6*, we could detect progeny that inherited both *ade6* alleles (less than 10% of the total population). These progeny are excluded from the data presented above, but all the raw data are presented in ***Supplementary file 1***. The PI phenotypes and allele transmission for diploids 17–19 were compared to the empty vector control (diploid 16) and * indicates p-value<0.01 (G-test). See ***Supplementary file 1*** for the markers used for each diploid and the raw data for allele transmission and ***Supplementary file 2*** for the PI staining raw data. Over 200 viable gametes were scored for allele transmission and over 200 spores (>50 4-spore asci) were assayed for PI staining. (**B**) Images of PI staining and transmitted light (TL) in an ascus with no drive containing all alive spores (top) and in an ascus with drive where two of the four spores are dead (bottom). Scale bar represents three microns.

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The following source data is available for figure 2:

Source data 1. PI staining correlates with viable spore yield as a measure of fertility in wild-type and *wtf* heterozygous crosses.

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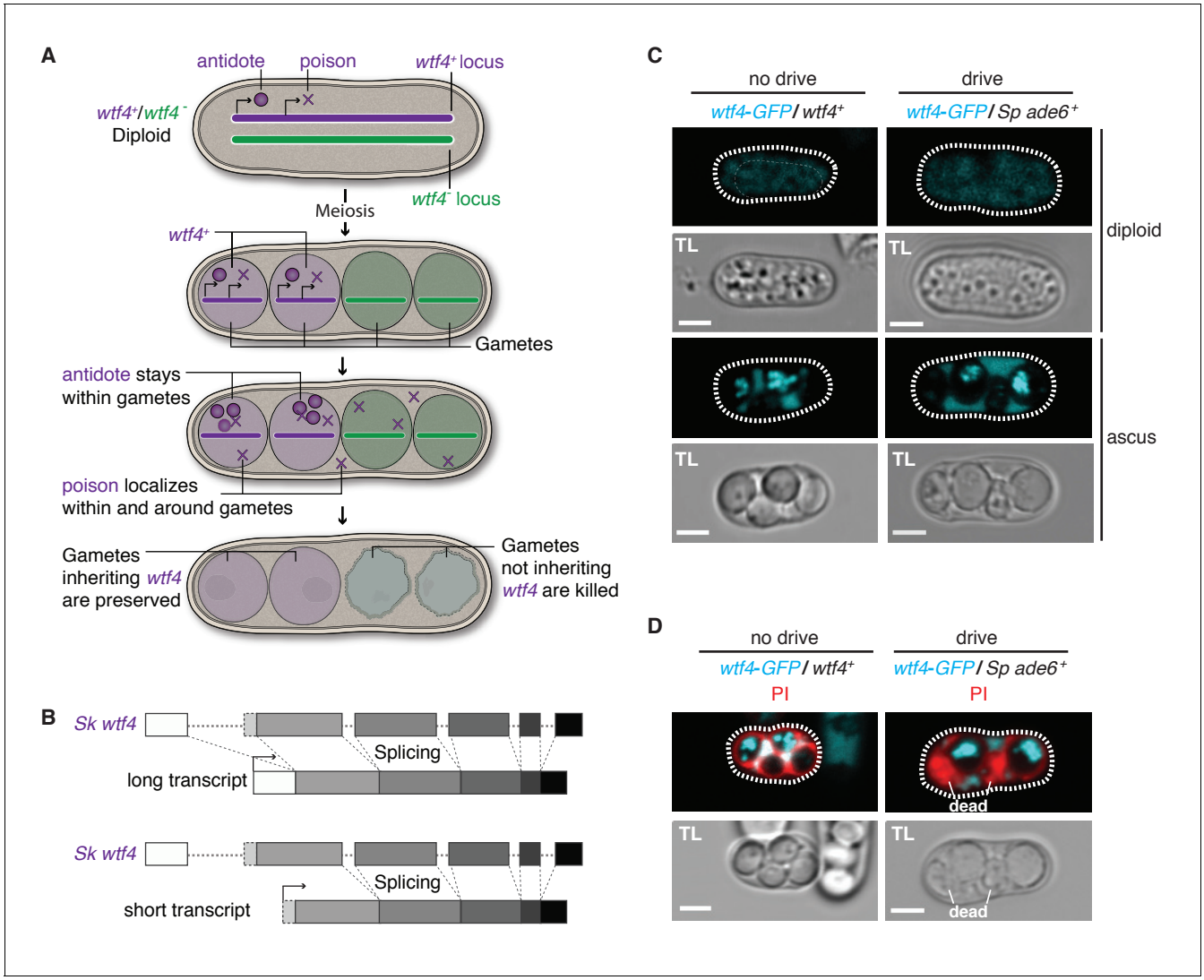

Figure 3. *Sk wtf4* has the capacity to make two proteins and Wtf4-GFP shows a dual localization pattern. (**A**) Model for meiotic drive of *Sk wtf4* via a poison-antidote mechanism. (**B**) *wtf4* creates a long and an alternative short transcript. See ***Figure 3—figure supplement 1*** for a depiction of the long-read RNA sequencing data on which this model is based (***Kuang et al., 2017***). (**C**) *Sk* Wtf4-GFP localization in diploids where drive does [right] or does not occur [left]. Cells were imaged prior to the first meiotic division [top] and as mature asci [bottom]. (**D**) Asci generated by diploids of the same genotypes as in (**C**) stained with PI to label dead cells (those lacking *wtf4*).

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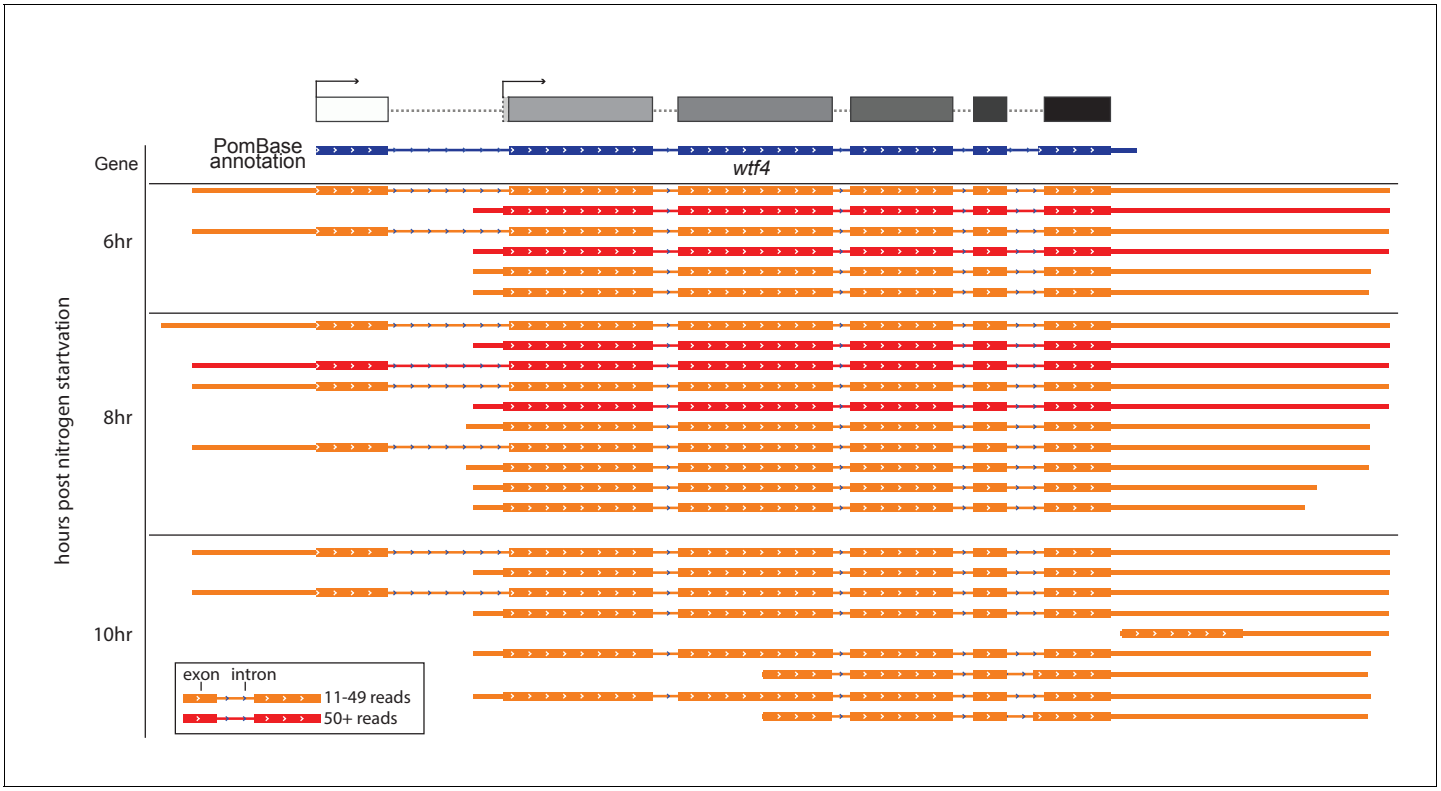

Figure 3—figure supplement 1. *Sp wtf4* has alternate transcriptional start sites. Our annotation of the *wtf4* gene with alternate start sites predicted is shown at the top in the same format as ***Figures 3–5***. The PomBase annotation for *Sp wtf4* is shown below that in blue. The transcript locations from one replicate of the meiotic transcript time courses sequenced by ***Kuang et al. (2017)*** are shown below in red and orange. The IsoSeq consensus reads shown should represent full-length transcripts, and each represents a number of individual sequencing reads. Only transcripts represented by 11 or more reads are displayed. Many of the transcripts vary by only a few nucleotides at the 5′ or 3′ ends and appear identical in the image. The time the samples were taken after meiotic induction are shown on the left. No transcripts with 11 or more reads were observed at earlier time points. Introns are represented by thin lines with blue arrows and the coding sequences are represented by the thick boxes. There are two major transcriptional start sites and the splice sites of intron 5 are different from those in the PomBase annotation. We did not verify two possible additional transcript types observed only at 10 hr, or explore their possible functional relevance. The data were visualized using IGV (***Thorvaldsdottir et al., 2013***).

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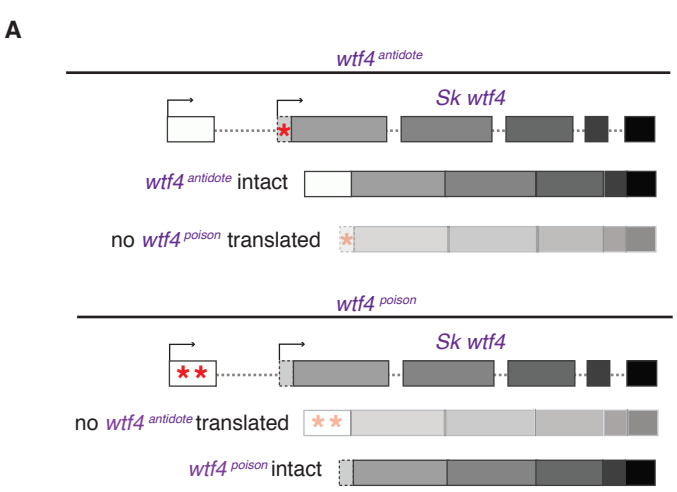

B

(diploid #)	genotype	% allele 1 in progeny	% PI-excluding spores
(20)	allele 1: wtf4 antidote; allele 2:	46.1	97.8
(21)	allele 1: wtf4; allele 2: wtf4 antidote	53.7	94.1
(22)	allele 1: wtf4 poison; allele 2:	37.7 *	14.1 *
(23)	allele 1: wtf4; allele 2: wtf4 poison	96.5 *	56.9 *
(24)	allele 1: wtf4 antidote; allele 2: wtf4 poison	88.0 *	44.6 *

Figure 4. *Sk wtf4* creates two proteins using alternate transcripts: an antidote and a gamete-killing poison. (**A**) Separation of function *wtf4* alleles. The red stars indicate start codon mutations. (**B**) Allele transmission and PI staining phenotypes of *Sp* diploids with the indicated *Sk wtf4* alleles integrated at *ade6* on chromosome 3, as in diploids 16–19 in ***Figure 2A***. Spores that inherited both alleles at *ade6* are eliminated from the data presented above, but the complete data are found in ***Supplementary file 1***. * indicates p-value<0.01 (G-test) compared to empty vector (or wild-type control) for allele transmission and fertility as assayed by PI staining. See ***Supplementary file 1*** for raw data and the markers used to monitor allele transmission for each diploid and ***Supplementary file 2*** for the PI staining raw data. Over 200 viable gametes were scored for allele transmission for all diploids except diploid 24, from which we genotyped 50. Over 200 spores (>50 4-spore asci) were assayed for PI staining of each diploid.

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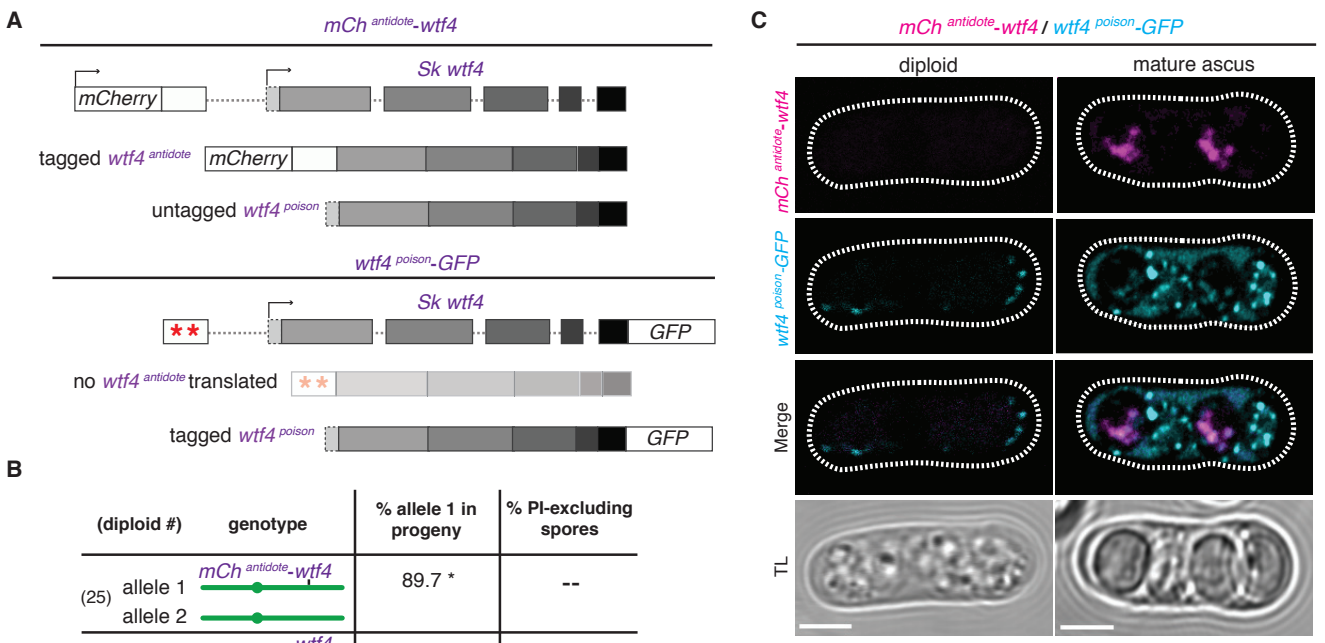

B

(diploid #)	genotype	% allele 1 in progeny	% PI-excluding spores
(25)	allele 1: *mCh antidote-wtf4* allele 2:	89.7 *	--
(26)	allele 1: *wtf4* allele 2: *mCh antidote-wtf4*	48.4	--
(27)	allele 1: *wtf4 poison-GFP* allele 2:	56.0	49.6 *
(28)	allele 1: *wtf4* allele 2: *wtf4 poison-GFP*	91.5 *	55.0 *

Figure 5. Wtf4 antidote is spore-specific and Wtf4 poison spreads throughout the ascus. (**A**) Constructs tagging either the Wtf4 antidote (top) or poison (bottom) proteins. The red stars indicate start codon mutations. (**B**) Allele transmission and PI staining phenotypes for tagged alleles, as in ***Figure 4B***. See ***Supplementary file 1*** for raw data and the markers used to monitor allele transmission for each diploid and ***Supplementary file 2*** for the PI staining raw data. We could not reliably use PI to assay fertility of mCherry-tagged strains because of color similarity, but in viable spore yield assays the *mCherry*^{antidote}-*wtf4* allele gave a similar phenotype to *wtf4*. * indicates p-value<0.01 (G-test) compared to empty vector (or wild-type control). Over 200 viable gametes were scored for allele transmission and over 200 spores (>50 4-spore asci) were assayed for PI staining. (**C**) Wtf4 poison (cyan) and antidote (magenta) protein localization prior to the first meiotic division (left) and in a mature ascus (right). Scale bar represents three microns. TL, transmitted light.

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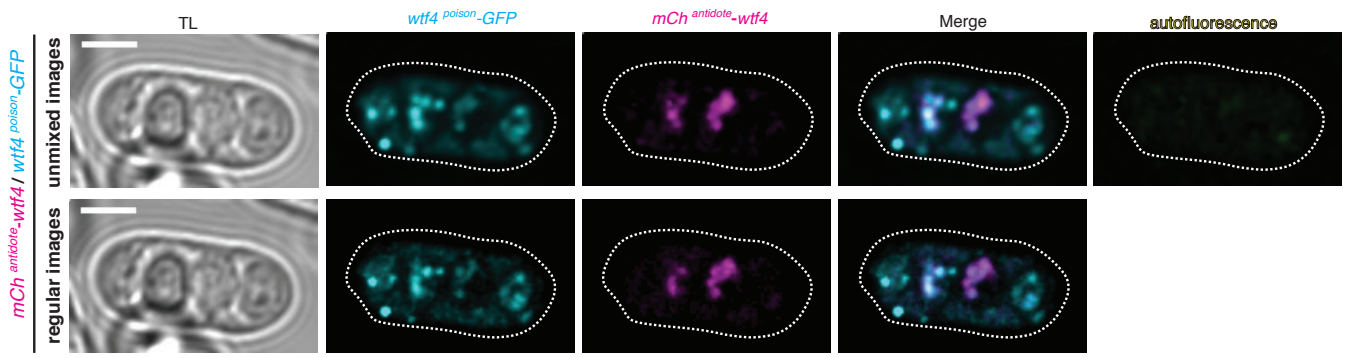

Figure 5—figure supplement 1. Spectral unmixing verifies true signal. Wtf4 poison (cyan) and antidote (magenta) protein localization in a mature ascus processed using linear unmixing [top] and unprocessed [bottom]. Scale bar represents three microns.

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