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Figures and figure supplements

Genome rearrangements and pervasive meiotic drive cause hybrid infertility in fission yeast

Sarah E Zanders, et al.

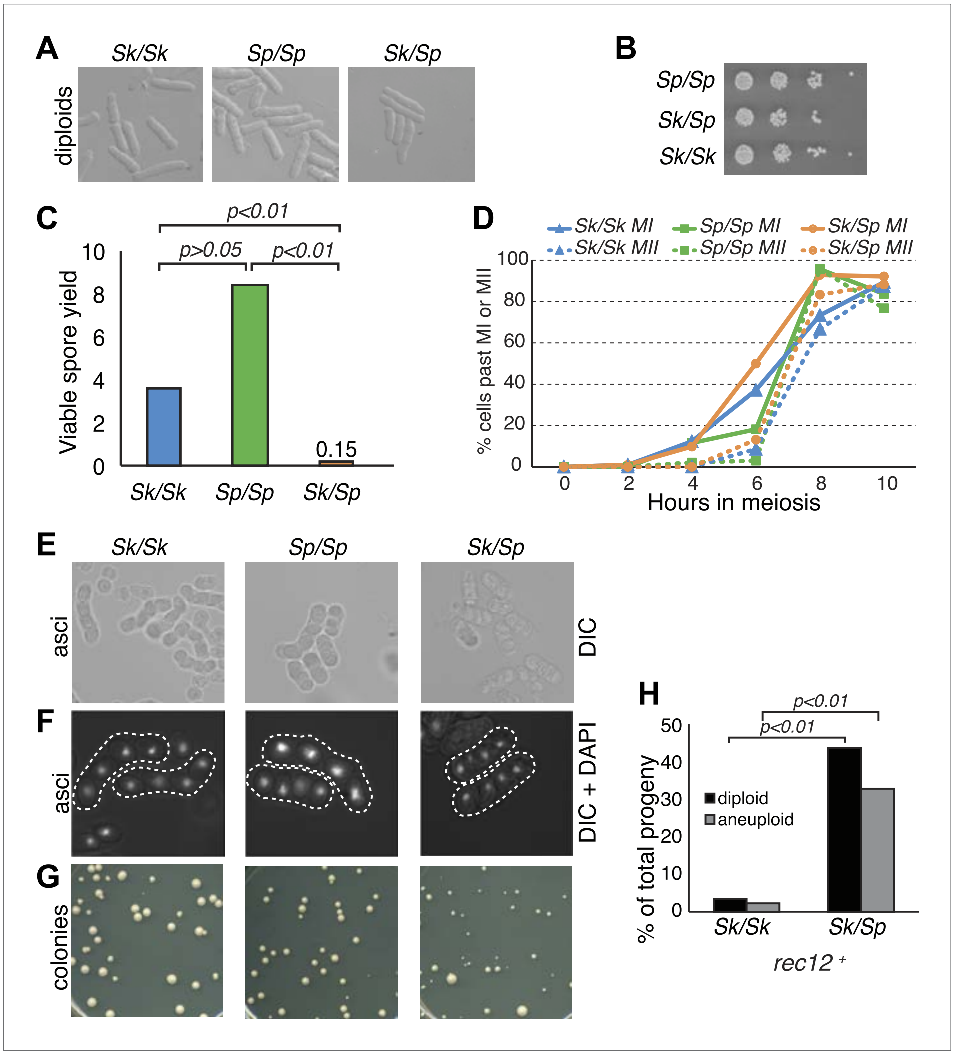

Figure 1. *Sk/Sp* hybrids are healthy but exhibit low fertility. (**A**) *Sk/Sp* hybrid diploids are morphologically similar to pure species diploids. (**B**) *Sk/Sp* hybrid diploids show no gross growth defects relative to pure species controls. (**C**) Viable spore yield tests show that *Sk/Sp* fertility is low relative to pure species controls (averages of n ≥ 5 experiments, p-values obtained using *t* test). This assay does not directly measure viable spores per meiosis, so values can exceed 4. (**D**) *Sk/Sp* hybrids complete both meiotic divisions with timing similar to that of pure species controls (representative experiment of 3, n ≥200 cells for each data point). (**E** and **F**) The asci produced by *Sk/Sp* hybrids contain spores that are more irregular and transparent than pure species asci. (**G**) The viable spores produced by *Sk/Sp* hybrids often grow into small irregularly sized and shaped colonies. (**H**) The majority of the viable spores produced by *Sk/Sp* hybrids are aneuploid or diploid (p-values obtained using G-test, n >200 for each). These data are also shown in ***Figure 5A***.

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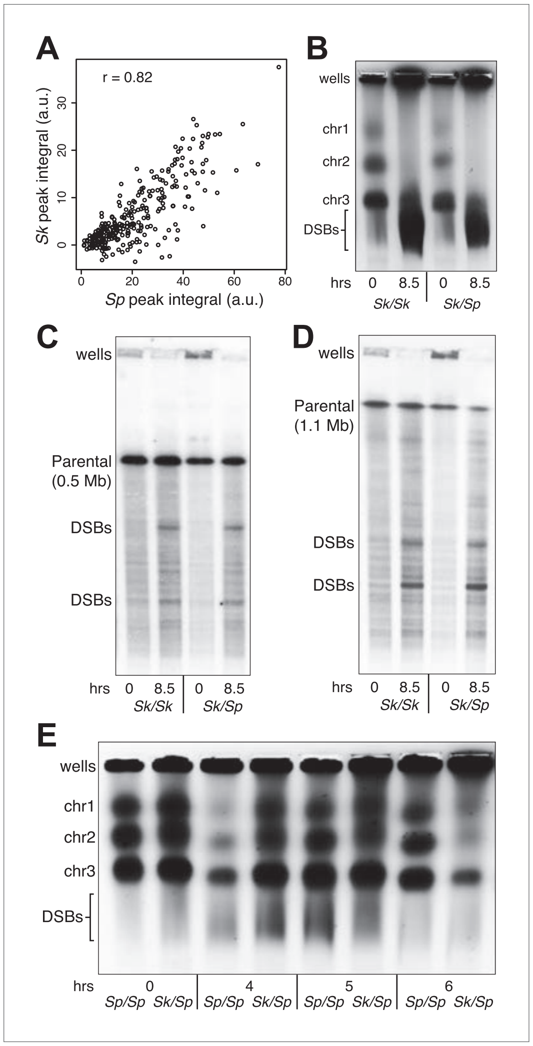

Figure 2. DSB hotspot divergence and repair in *Sk/Sp* hybrids. (**A**) We used ChIP–chip of Rec12-FLAG from *rad50S Sk* meiotic cultures to assay DSB hotspots and compared the profile to the published DSB hotspot maps of *Sp* (***Fowler et al., 2013***). We then compared the Rec12-enrichment in *Sk* at 286 defined *Sp* hotspots and found a strong correlation between Rec12 enrichments between the two species at these sites. (**B**) *Sk/Sp* cells are proficient at inducing DSBs. Ethidium bromide stained pulsed-field gel of diploids at 0 and 8.5 hr after inducing meiosis in liquid cultures. These diploids are *rad50S* mutants, so DSBs form normally but are not repaired. As DSBs are formed, the three full-sized chromosome bands disappear and the DNA runs as smaller broken fragments on the gel. (**C** and **D**) We find that DSBs are formed at similar locations and similar frequencies in *Sk/Sp* and *Sk/Sk*. Southern blots of pulsed-field gels to obtain a closer view of DSB formation in *rad50S* diploids probed to visualize two NotI restriction fragments known as NotI J [shown in (**C**)] and NotI D [shown in (**D**)]. Prior to DSB formation, most of the DNA runs as a single large band. After all break formation (8.5 hr) smaller cut fragments become apparent at the same
Figure 2. Continued on next page

Figure 2. Continued

sites in *Sk/Sp* and *Sk/Sk*. (**E**) DSBs are efficiently repaired in *Sk/Sp*. Ethidium bromide stained pulsed-field gel of *rad50*[+] diploids at the given times after the induction of meiosis show that DSBs do not accumulate more in *Sk/Sp* than the *Sp/Sp* control during meiotic prophase. Together with those in (**B**) these data demonstrate that *Sk/Sp* cells form and efficiently repair DSBs.

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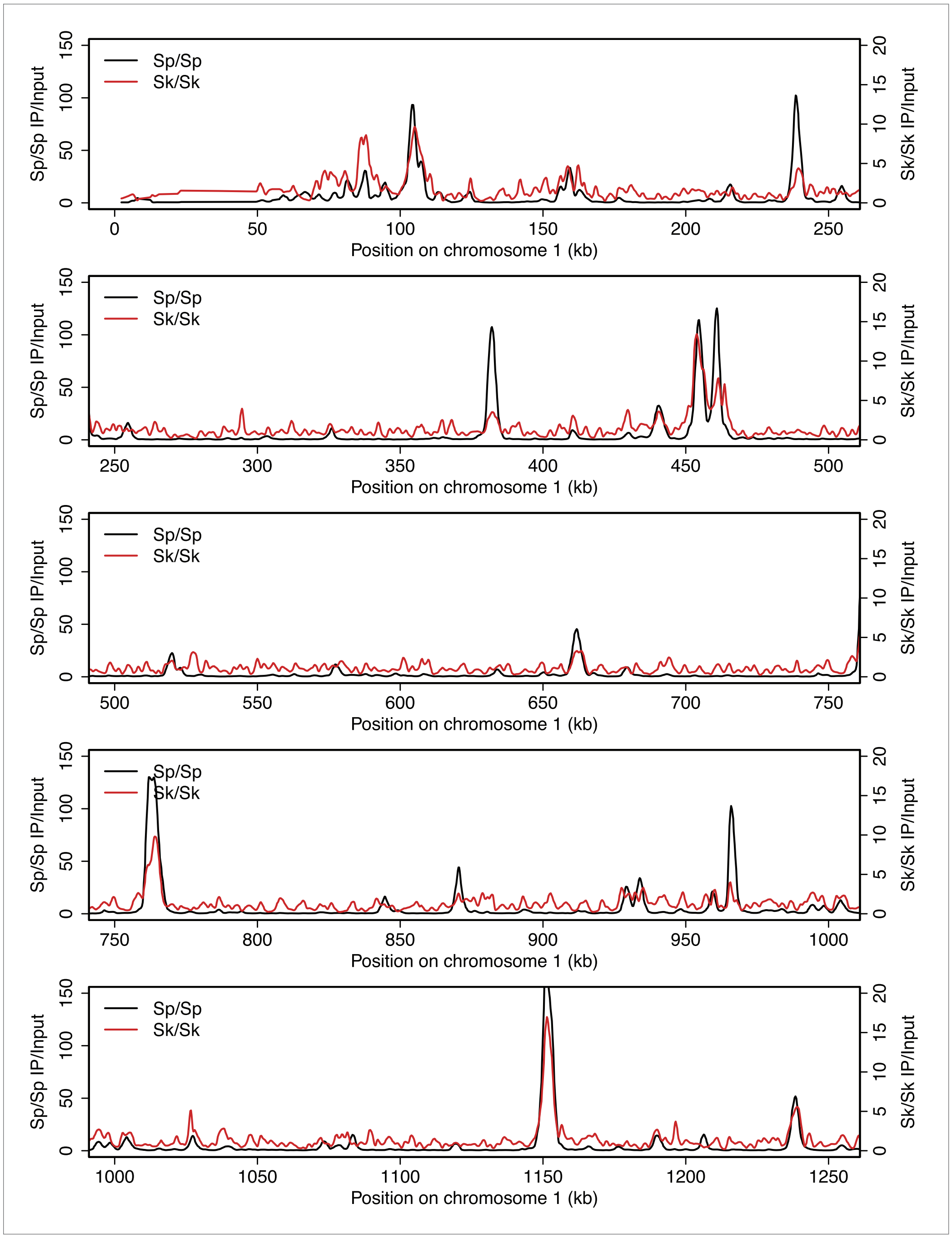

Figure 2—figure supplement 1. DSB hotspots in *Sk* and *Sp*.

DOI: 10.7554/eLife.02630.005

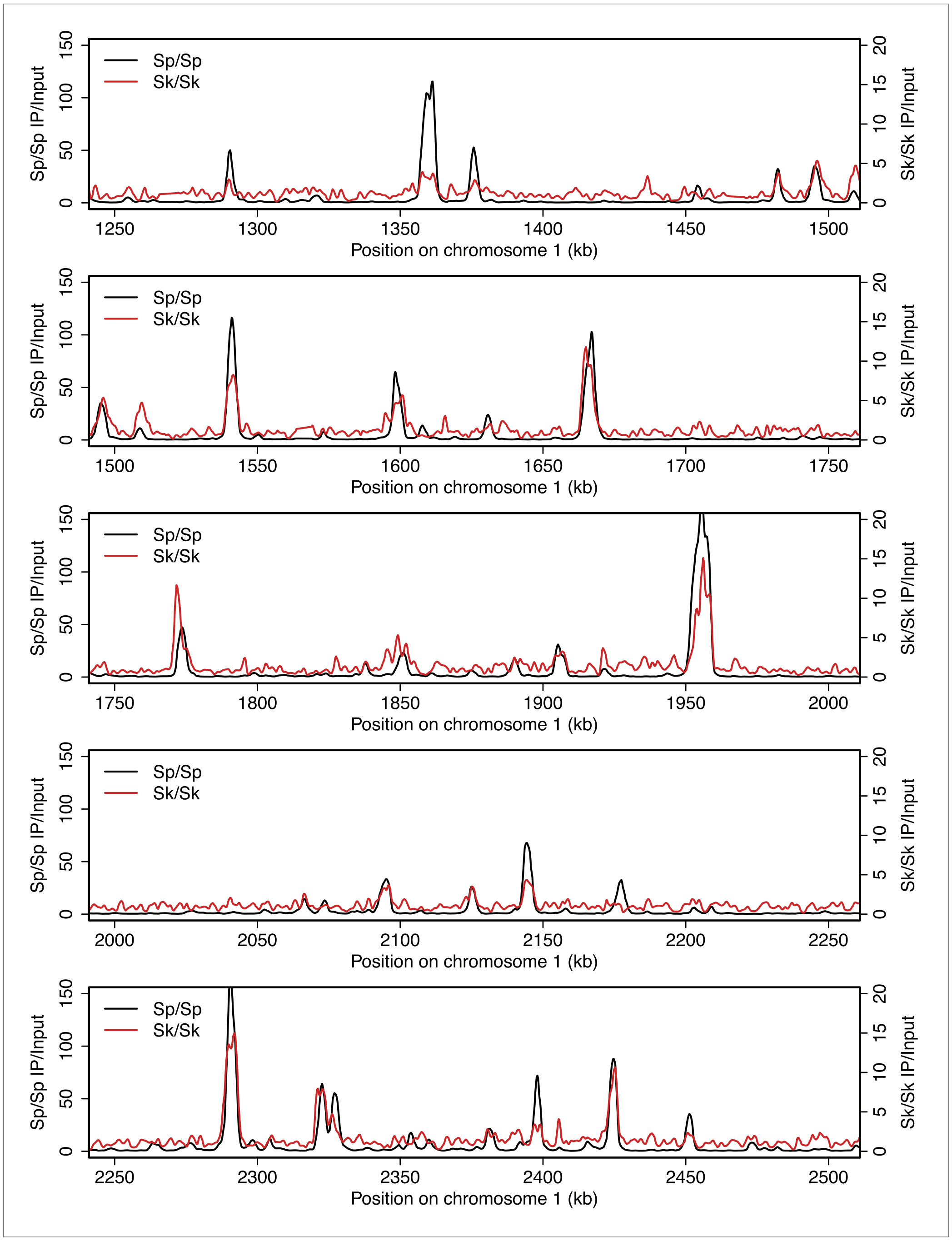

Figure 2—figure supplement 2. DSB hotspots in *Sk* and *Sp*.

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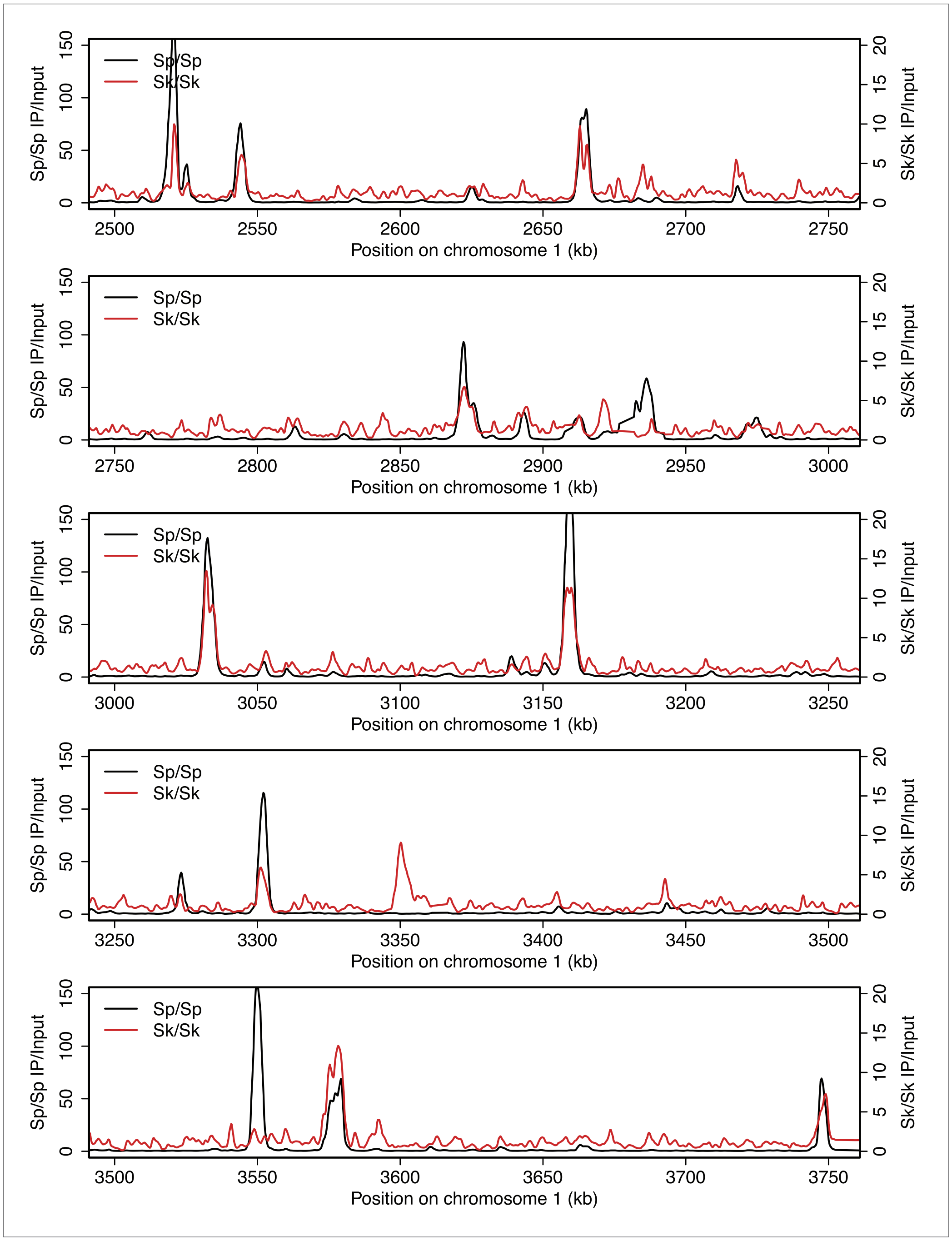

Figure 2—figure supplement 3. DSB hotspots in *Sk* and *Sp*.

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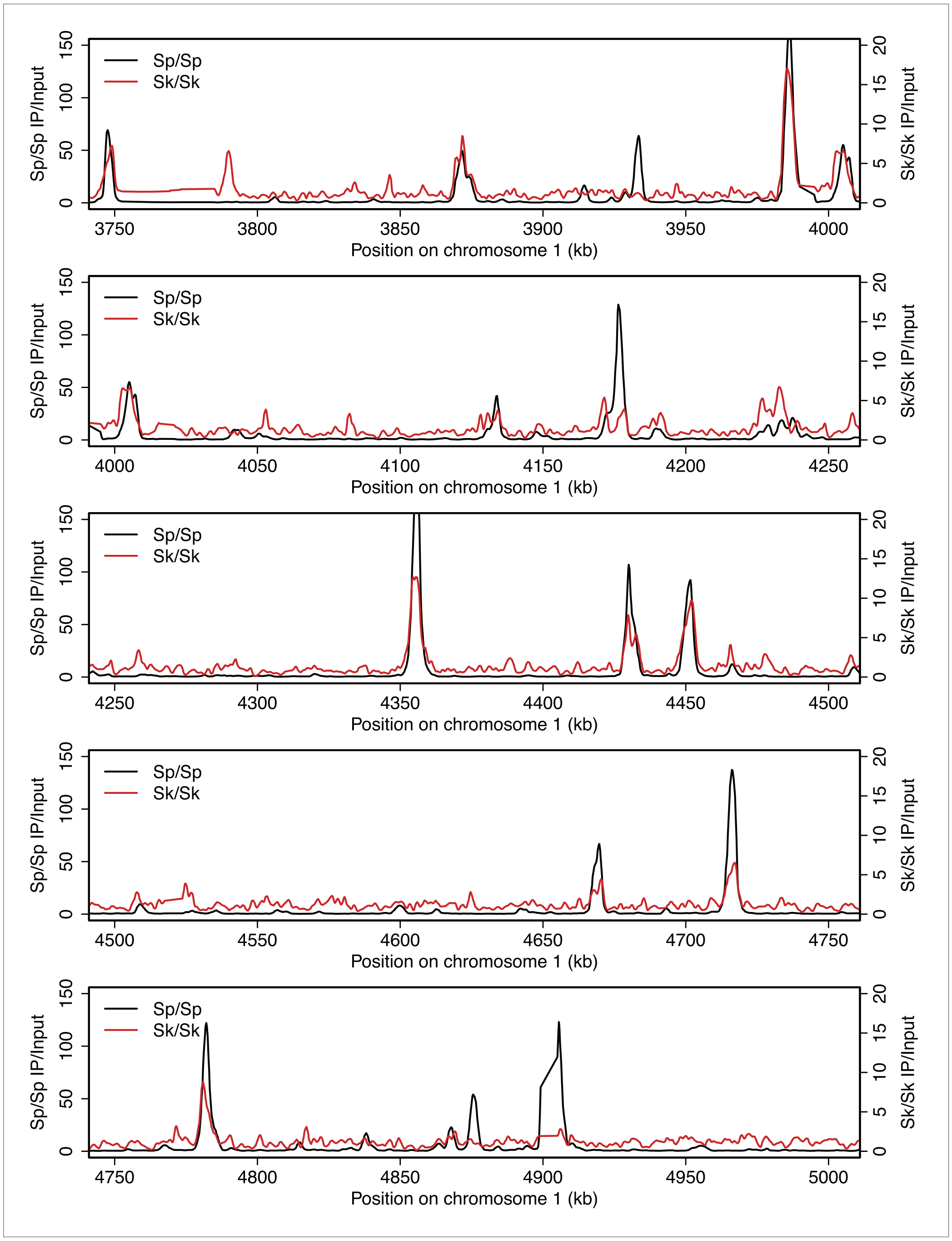

Figure 2—figure supplement 4. DSB hotspots in *Sk* and *Sp*.
DOI: 10.7554/eLife.02630.008

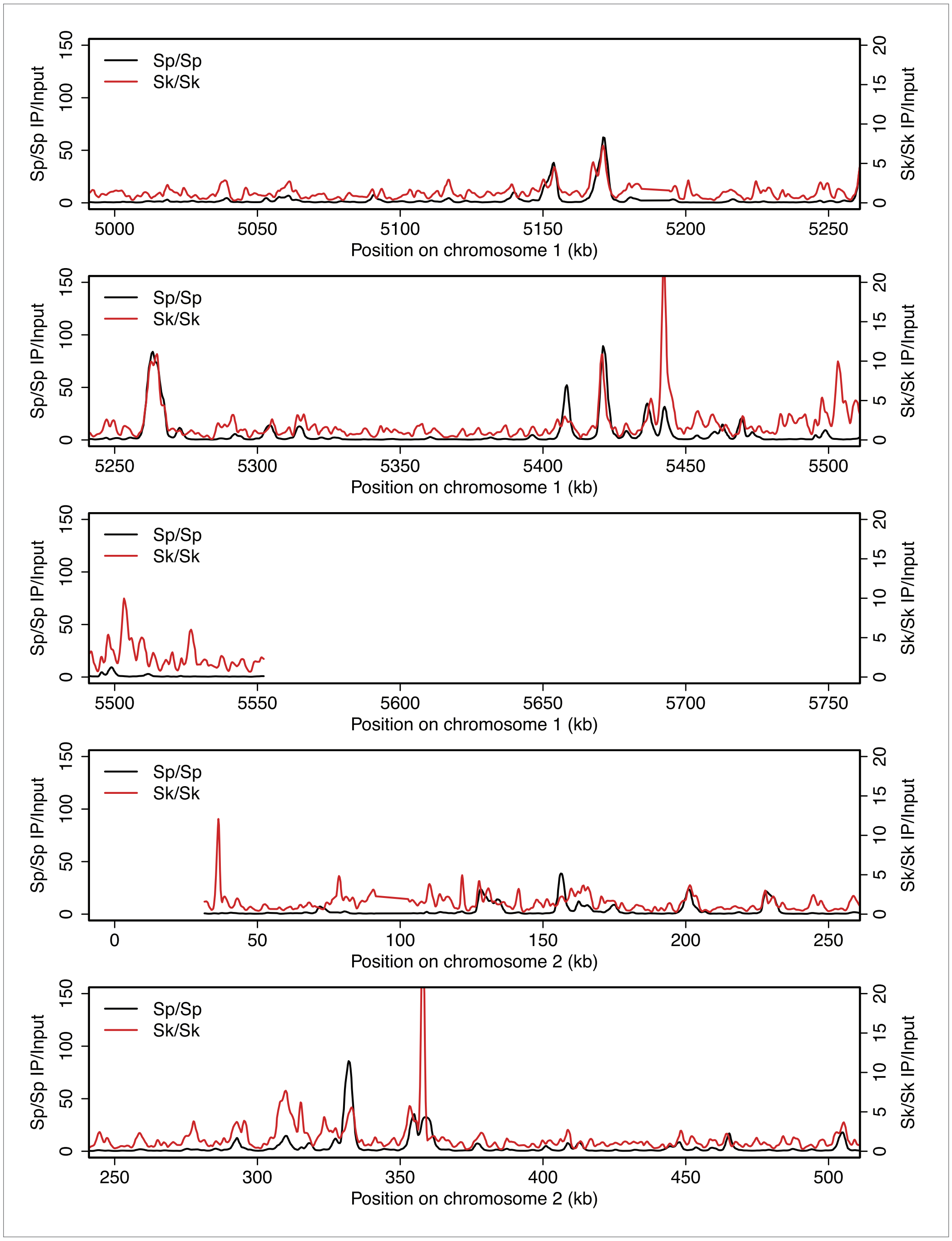

Figure 2—figure supplement 5. DSB hotspots in *Sk* and *Sp*.

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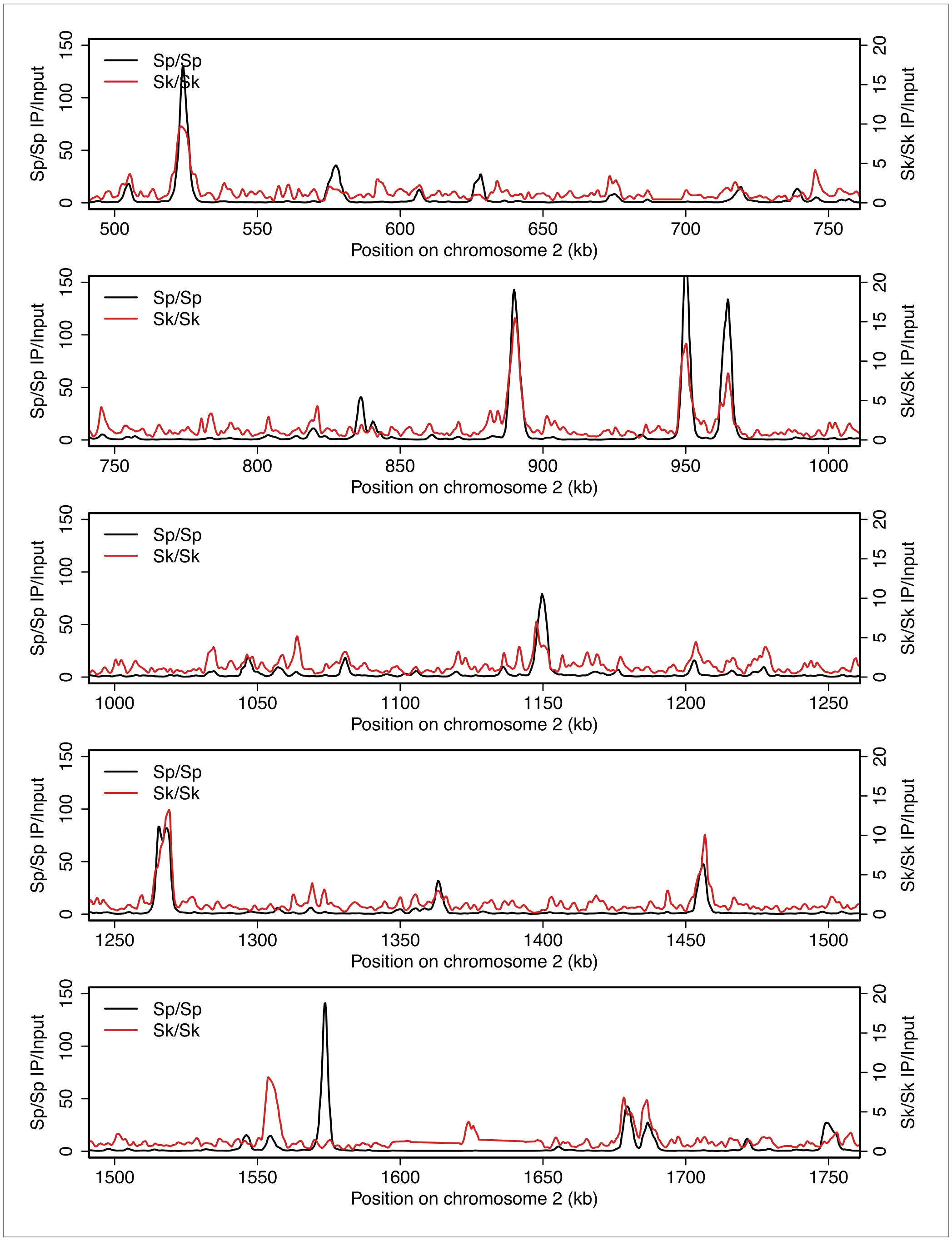

Figure 2—figure supplement 6. DSB hotspots in *Sk* and *Sp*.

DOI: 10.7554/eLife.02630.010

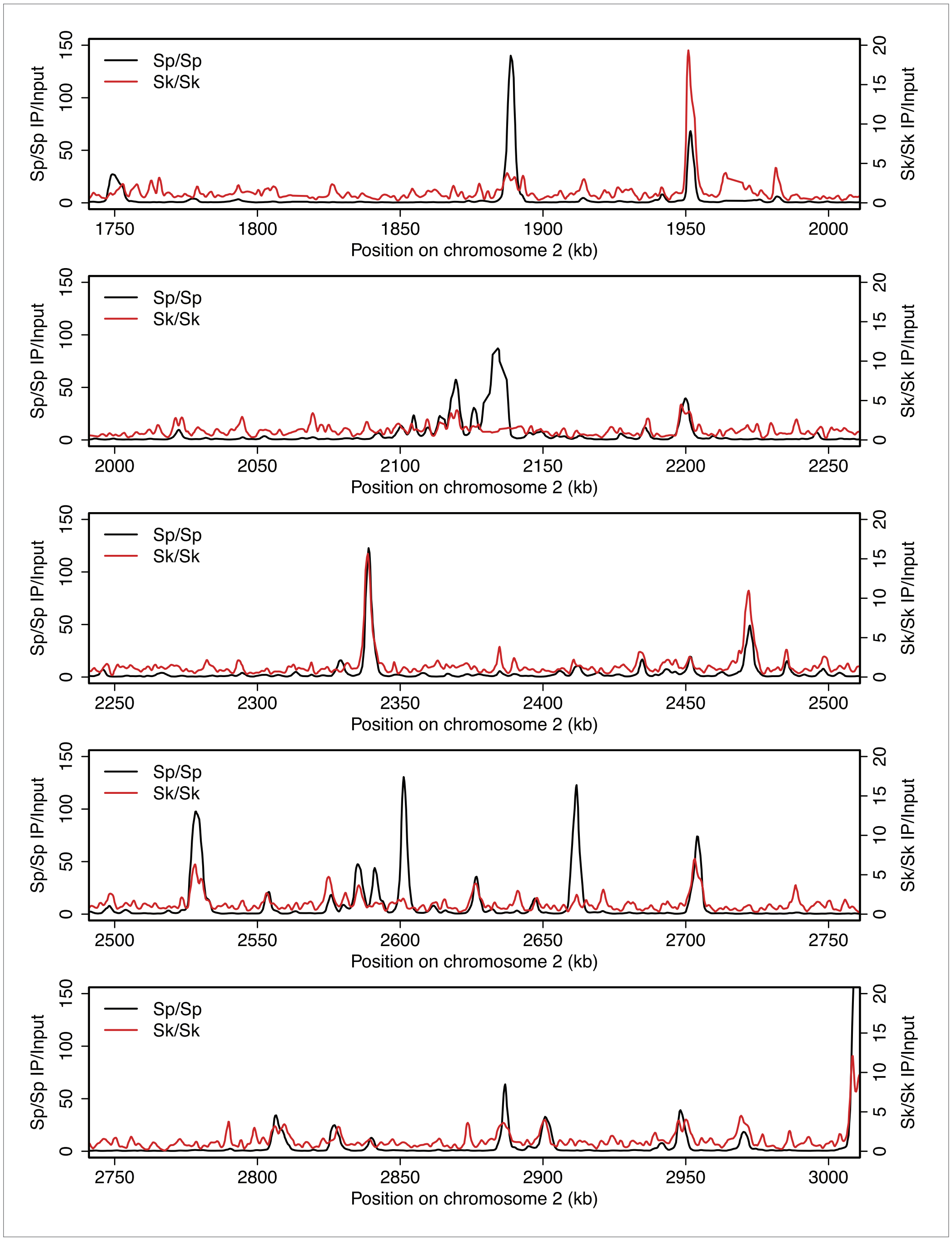

Figure 2—figure supplement 7. DSB hotspots in *Sk* and *Sp*.
DOI: 10.7554/eLife.02630.011

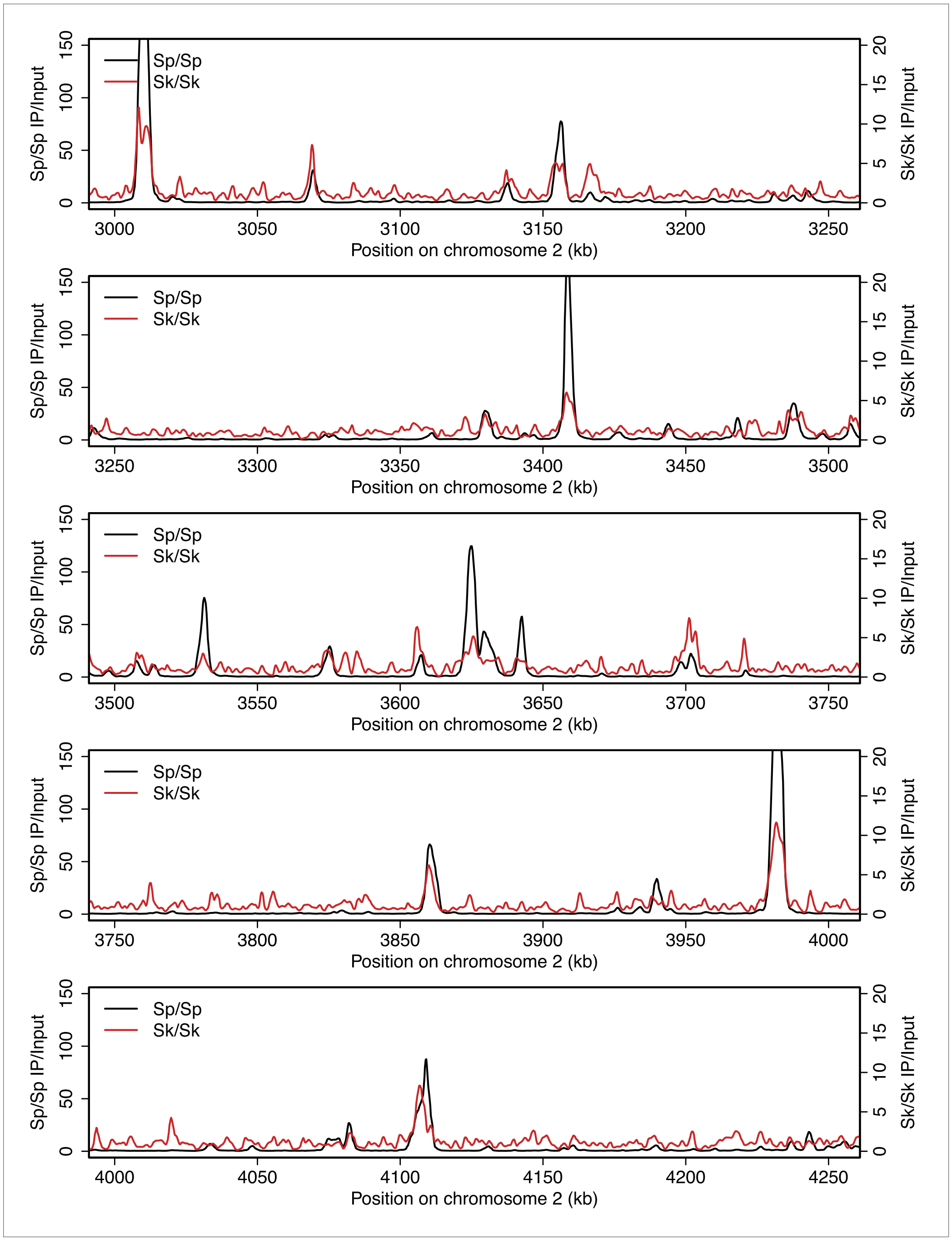

Figure 2—figure supplement 8. DSB hotspots in *Sk* and *Sp*.
DOI: 10.7554/eLife.02630.012

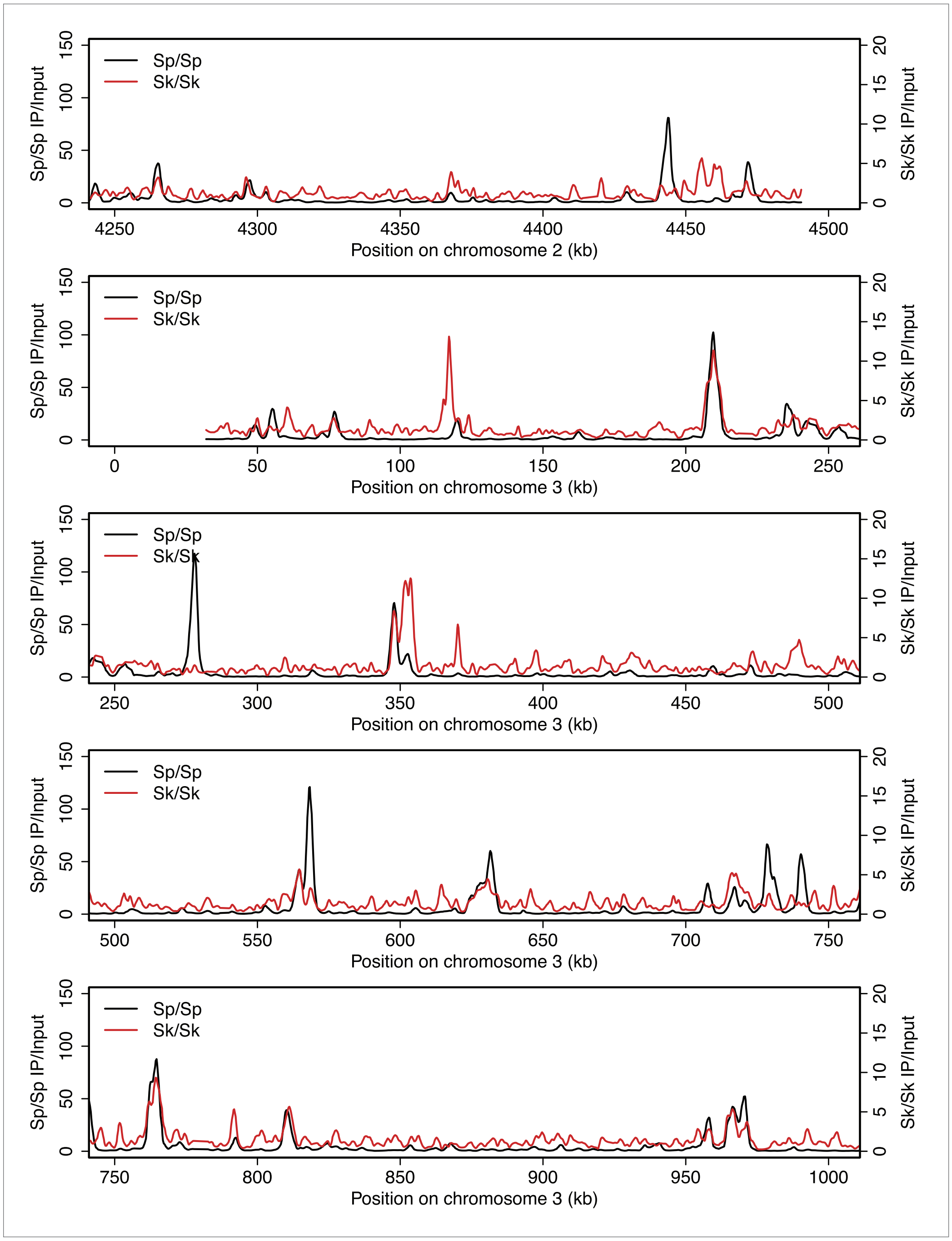

Figure 2—figure supplement 9. DSB hotspots in *Sk* and *Sp*.

DOI: 10.7554/eLife.02630.013

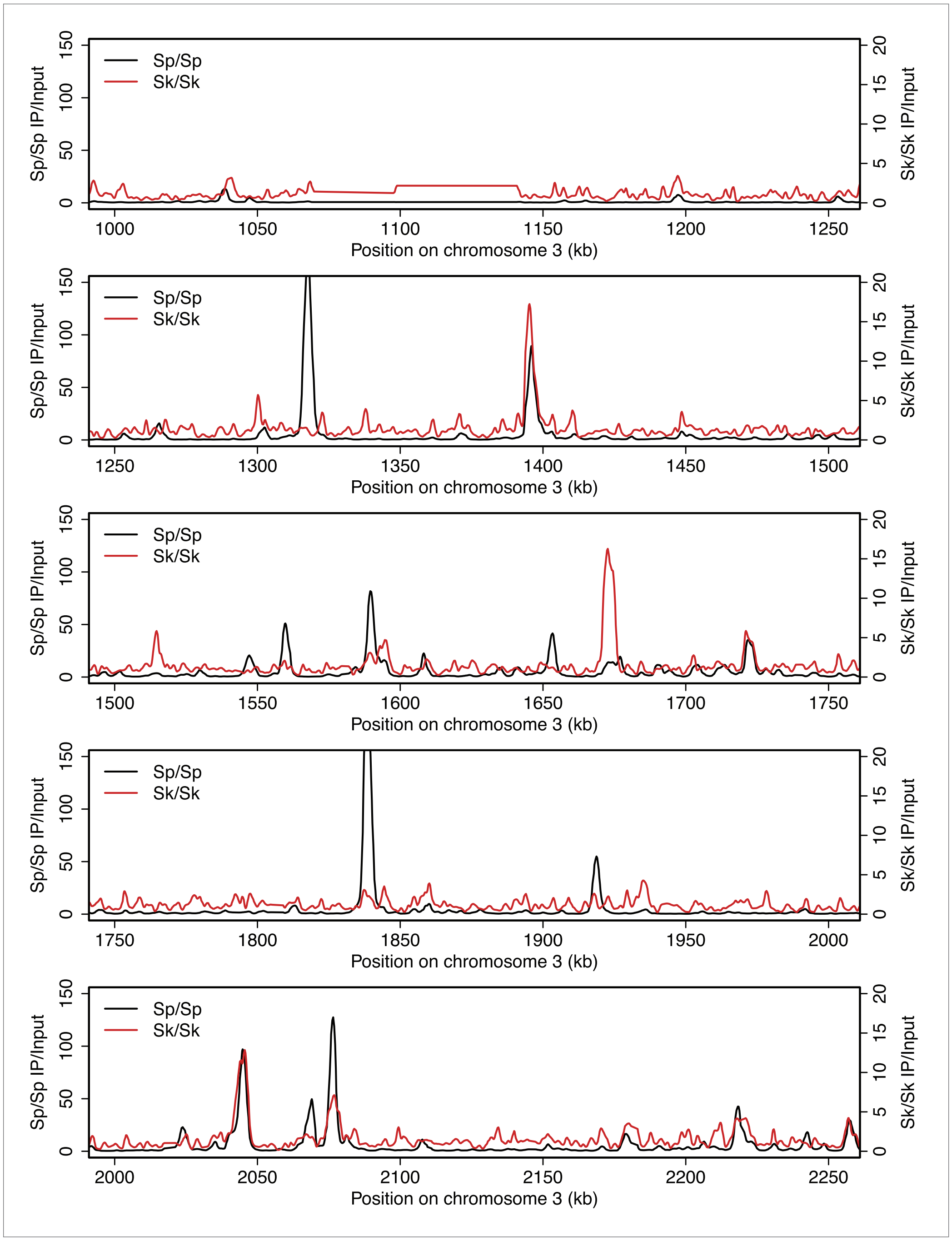

Figure 2—figure supplement 10. DSB hotspots in *Sk* and *Sp*.
DOI: 10.7554/eLife.02630.014

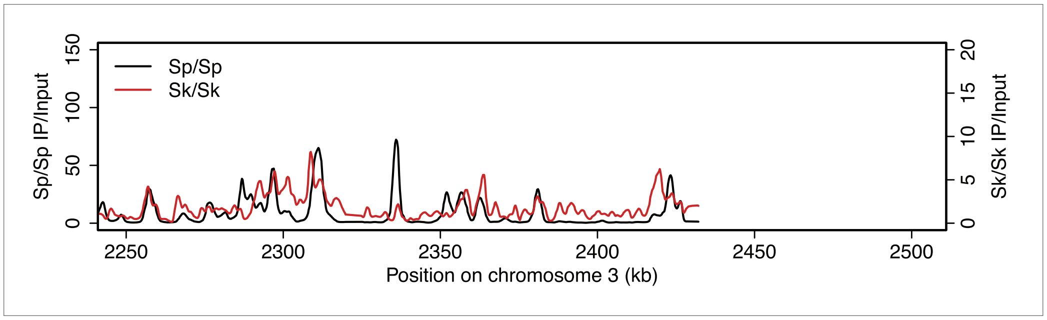

Figure 2—figure supplement 11. DSB hotspots in *Sk* and *Sp*.
DOI: 10.7554/eLife.02630.015

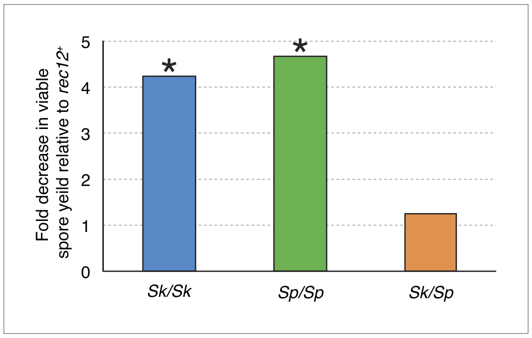

Figure 3. Recombination does not alter *Sk/Sp* hybrid fertility. The average *rec12⁺* viable spore yield of each diploid was divided by that of the corresponding *rec12Δ* mutant. For the pure species diploids, the viable spore yield was significantly lower in the absence of Rec12 (n ≥ 5 experiments for each genotype; * *t* test p<0.05). The viable spore yield of *Sk/Sp* hybrids, on the other hand, was not significantly different between *rec12⁺* and *rec12Δ Sk/Sp* hybrids (p=0.42). This indicates that recombination likely hurts fertility just as much as it promotes fertility in *Sk/Sp* hybrid meiosis. These data are shown in a different format in ***Figure 5A***.
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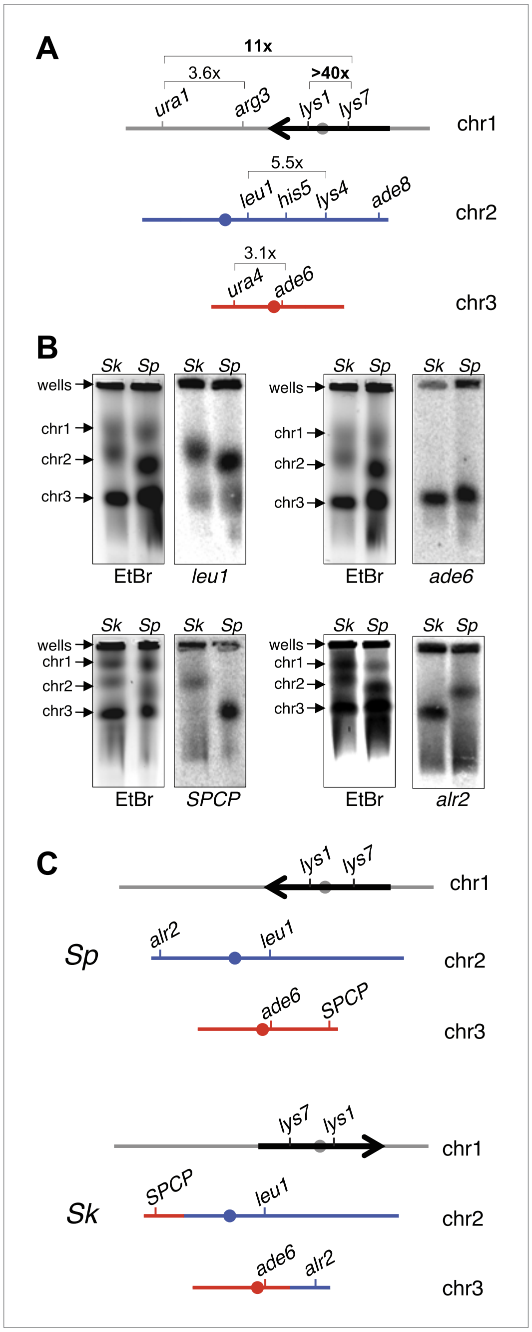

Figure 4. Chromosome rearrangements limit the recovery of recombinant progeny in *Sk/Sp* hybrids. (**A**) A cartoon illustrating the fold decrease in recombinant frequencies in *Sk/Sp* spores compared to that in *Sp/Sp* spores. The detailed recombination data are in ***Figure 4—figure supplements 2 and 3***. The *Sp* karyotype is depicted with a grey/black chromosome 1, blue chromosome 2, and red chromosome 3. The backwards black arrow indicates an inversion in *Sp* relative to the ancestral karyotype. (**B**) Pulsed-field gels separating *Sp* and *Sk* chromosomes and Southern blots of the gels probed with DNA from the indicated loci revealed a reciprocal translocation that includes several

Figure 4. Continued on next page

Figure 4. Continued

essential genes including *alr2* and *SPCP1E11.08* (abbreviated *SPCP*). The EtBr-stained gels are on the left and the Southern blots are on the right in each pair. (**C**) A cartoon summary of the karyotype differences between *Sp* and *Sk*. The arrow indicates the location of the inversion in *Sp*. A few landmark loci are shown.

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Chromosome	Interval	Recombinants	Total	*Sk*/*Sk* distance (cM)	*Sp*/*Sp* distance (cM)	Fold Change
2	*lys4 – his4*	3	129	2.3	12	5.2
2	*his4 – ade8*	42	132	50.6	94	1.9
3	*ade6 – ura4*	72	157	unlinked	190	NA

Figure 4—figure supplement 1. Recombination frequencies in *Sk*.

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Chr	Interval	Recombinants	Total	*Sk*/*Sp* distance (cM)	*Sp*/*Sp* distance (cM)	Fold decrease
1	*ura1 – arg3*	95	257	67	240	3.6
1	*ura1 – lys7*	32	95	56	600	11
1	*ura1 – lys1*	53	257	54	480	8.9
1	*arg3 – lys1*	33	257	27	230	8.5
1	*arg3 – lys7*	52	212	34	360	11
1	*lys1 – lys7*	0	95	0	120	>40
2	*leu1 – his5*	12	201	6.1	46	7.5
2	*his5 – lys4*	57	201	42	200	4.8
2	*leu1 – lys4*	59	201	44	240	5.5
2	*lys4 – his4*	17	307	5.9	12	2
2	*his4 – ade8*	43	307	16	94	5.9
2	*lys4 – ade8*	48	307	19	100	5.3
3	*ade6 – ura4*	51	144	62	190	3.1
2 and 3	*leu1 – ade6*	20	101	25	NA	NA

Figure 4—figure supplement 2. Recombination frequencies in *Sk*/Sp hybrids are low relative to *Sp*.

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strains crossed	Chr.	Parental *Sp*		Parental *Sk*		Recombinant 1		Recombinant 2		Hybrid distance (cM)	*Sp* Distance (cM)
		Genotype	#	Genotype	#	Genotype	#	Genotype	#		
SZY142x210	1	*arg3- lys7-*	51	*arg3+ lys7+*	109	*arg3- lys7+*	36	*arg3+ lys7-*	16	34	360
SZY142x180	1	*ura1- arg3-*	64	*ura1+ arg3+*	98	*ura1- arg3+*	52	*ura1+ arg3-*	43	67	240
SZY142x180*	1	*ura1- lys7-*	63	*ura1+ lys7+*	0	*ura1- lys7+*	0	*ura1+ lys7-*	32	56	600
SZY142x180*	1	*lys1+ lys7-*	95	*lys1- lys7+*	NA	*lys1+ lys7+*	0	*lys1- lys7-*	NA	0	120
SZY142x180	1	*ura1- lys1+*	63	*ura1+ lys1-*	109	*ura1- lys1-*	53	*ura1+ lys1+*	32	54	480
SZY142x180	1	*arg3- lys1+*	74	*arg3+ lys1-*	129	*arg3- lys1-*	33	*arg3+ lys1+*	21	27	230
SZY128x71	2	*leu1- his5-*	12	*leu1+ his5+*	177	*leu1- his5+*	1	*leu1+ his5-*	11	6.1	46
SZY128x71	2	*his5- lys4+*	15	*his5+ lys4-*	129	*his5- lys4-*	8	*his5+ lys4+*	49	42	200
SZY128x71	2	*leu1- lys4+*	9	*leu1+ lys4-*	133	*leu1- lys4-*	4	*leu1+ lys4+*	55	44	240
SZY127x94	2	*lys4+ his4+*	104	*lys4- his4-*	186	*lys4+ his4-*	8	*lys4- his4+*	9	5.9	12
SZY127x94	2	*his4+ ade8-*	91	*his4- ade8+*	173	*his4+ ade8+*	22	*his4- ade8-*	21	16	94
SZY127x94	2	*lys4+ ade8-*	88	*lys4- ade8+*	171	*lys4+ ade8+*	24	*lys4- ade8-*	24	19	110
SZY297x480	3	*ade6+ ura4+*	22	*ade6- ura4-*	71	*ade6+ ura4-*	4	*ade6- ura4+*	47	62	190

Figure 4—figure supplement 3. *Sk* alleles are underrepresented in the progeny of *Sk/Sp* hybrids.
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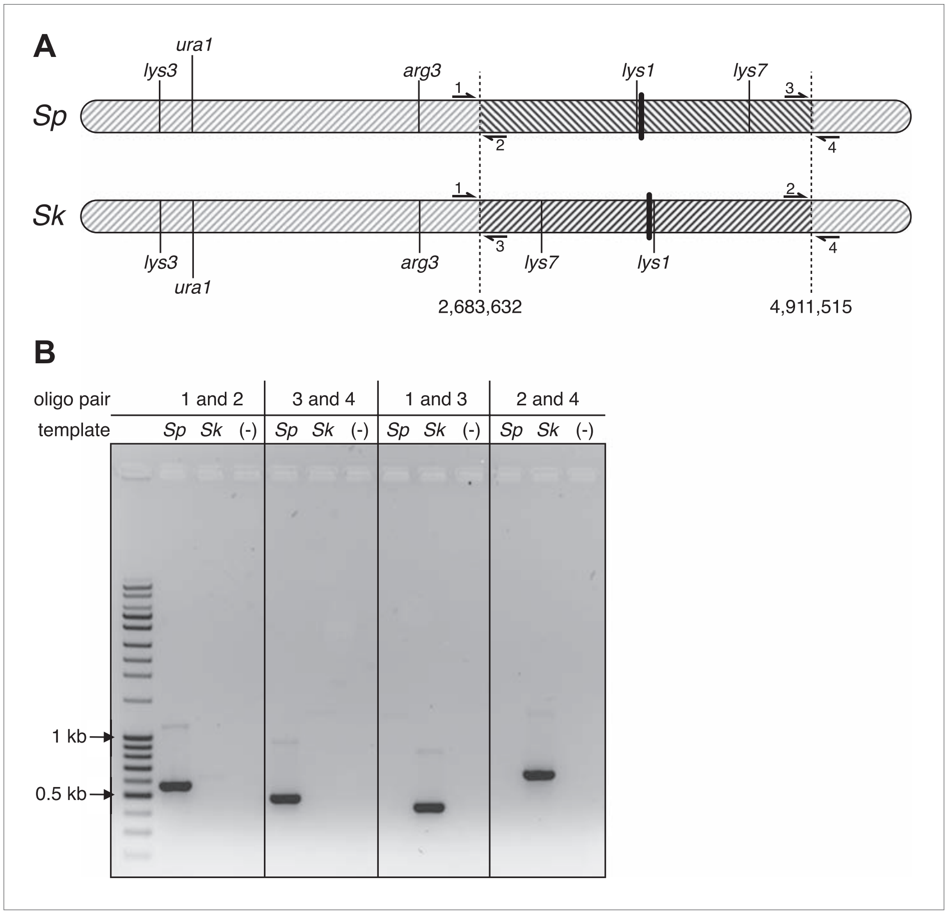

Figure 4—figure supplement 4. *Sp* has an inversion on chromosome 1 relative to *Sk*.
DOI: 10.7554/eLife.02630.021

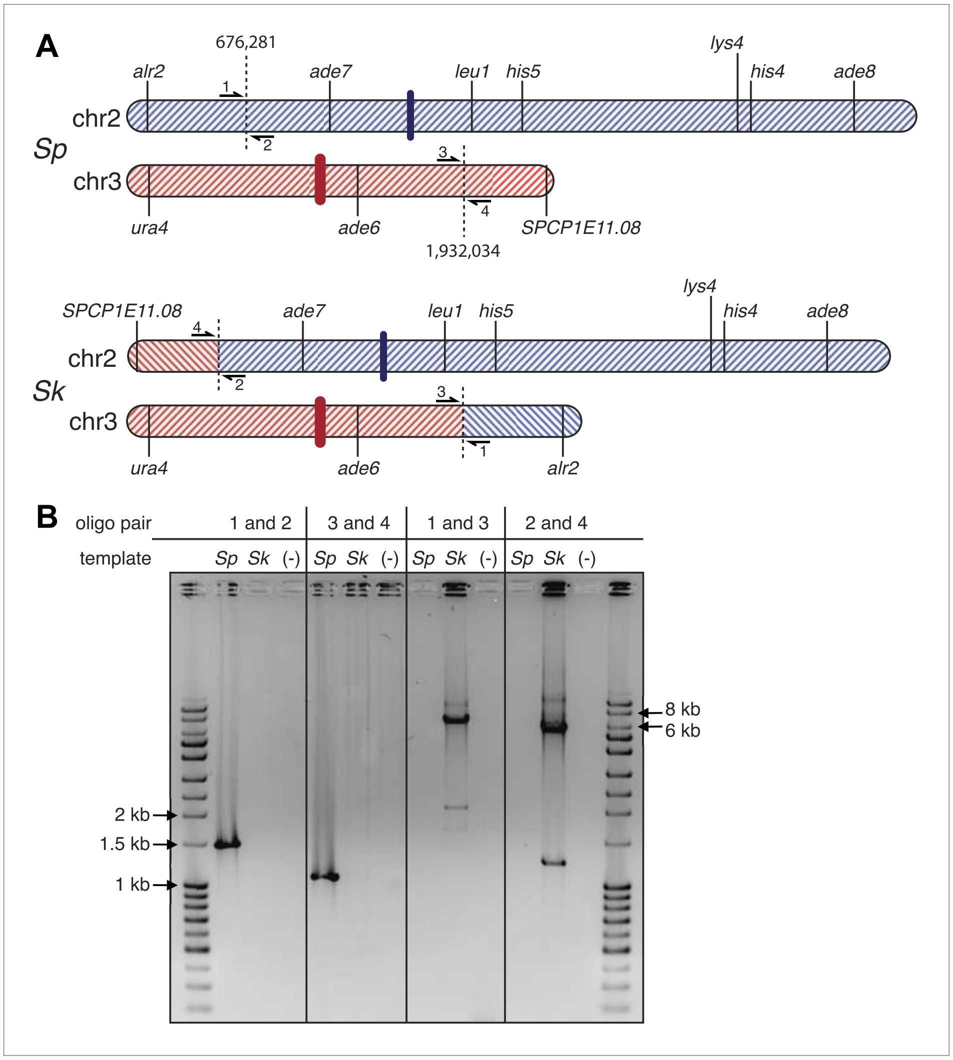

Figure 4—figure supplement 5. *Sk* has a reciprocal translocation between chromosomes 2 and 3.
DOI: 10.7554/eLife.02630.022

A

Diploid	viable spores/cell	% aneuploid	% diploid	aneuploids/cell	diploids/cell
Sp/Sp	8.4	not done	not done	not done	not done
Sp/Sp rec12Δ	1.8	5.2	4.1	0.094	0.074
Sk/Sk	3.6	2.2	3.5	0.079	0.13
Sk/Sk rec12Δ	0.85	26	3.2	0.22	0.027
Sk/Sp	0.15	33	44	0.050	0.066
Sk/Sp rec12Δ	0.13	77	8.8	0.092	0.011

B

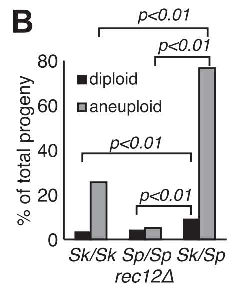

Figure 5. Increased aneuploidy amongst viable *Sk/Sp* gametes is recombination-independent. (**A**) We calculated both viable spore yield (viable spores/cell) as well as the fraction of viable spores that are aneuploid or diploid ('Materials and methods'). In the absence of Rec12, the relative frequencies of aneuploids and diploids are elevated in all cases. However, there is significantly more aneuploidy and diploidy of viable spores produced by *rec12Δ Sk/Sp* hybrids than by *rec12Δ* pure species controls. This shows the phenotype is not caused solely by recombination defects. In addition, *Sk/Sp* diploids do not generate more aneuploids or diploids relative to the number of cells induced to undergo meiosis compared to pure-species controls. Some of these data are presented in a different format in ***Figures 1H*** and ***Figure 3***. (**B**) A bar graph illustrating the fraction of the viable spores produced by the indicated *rec12Δ* diploids that are aneuploid or diploid (G-test, n >300 for each).
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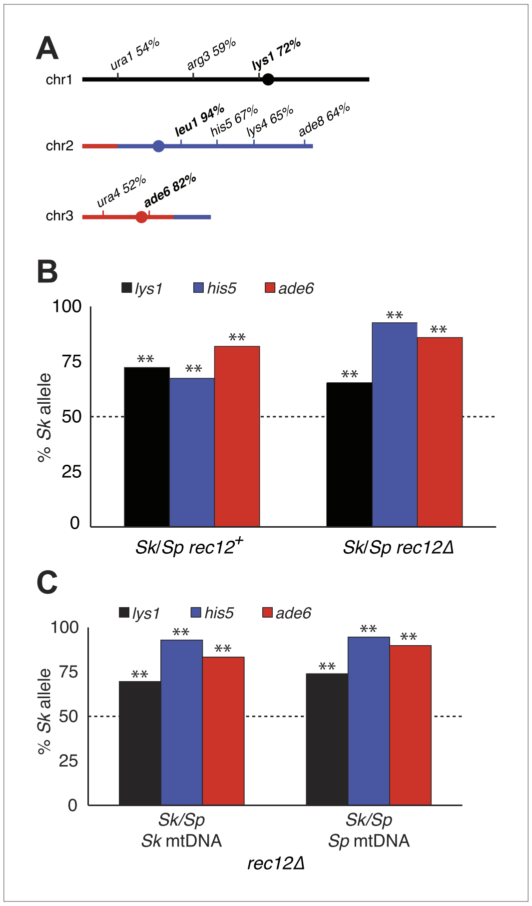

Figure 6. Alleles on all three *Sk* chromosomes show drive (independent of mitochondrial DNA type). (**A**) *Sk* alleles were inherited by significantly more than 50% of the viable spores produced by *Sk/Sp* hybrids, except *ura1* and *ura4* (G-test p<0.01; n >100 for each). The markers nearest to the meiotic drive loci (i.e., those showing the greatest bias towards *Sk* inheritance) are shown in boldface. The color scheme is the same as that in ***Figure 4***. The data underlying these numbers are shown in ***Figure 4—figure supplement 3***, and ***Figure 6—figure supplement 1***. (**B**) The *Sk* alleles of *lys1*, *his5* and *ade6* show significant drive both in the presence and absence of recombination (**p<0.01, n >300 for *lys1* and *his5*, n >80 for *ade6*). The amount of *his5* drive is greater in the absence to Rec12 due to enhanced linkage with the driving locus. The data underlying this graph are shown in ***Figure 6—figure supplement 1***. (**C**) Incompatibilities between the *Sk* mitochondrial DNA and *Sp* nuclear genes are not responsible for the drive phenotype because we observed the same drive in *rec12Δ Sk/Sp* hybrids with either *Sk* or *Sp*-derived mitochondrial DNA (**p<0.01, n >200 for *lys1* and *his5*, n >50 for *ade6*). The data underlying this graph are shown in ***Figure 6—figure supplement 4***.

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	Sp/Sp rec12⁺ (n=328)		*Sp/Sp rec12Δ* (n=368)		*Sk/Sk rec12*⁺ (n=227)		*Sk/Sk rec12Δ* (n=342)		*Sk/Sp rec12*⁺ (n=621)		*Sk/Sp rec12Δ* (n=589)	
locus	observed	%	observed	%	observed	%	observed	%	observed	%	observed	%
his5⁺	149	45.4	174	49.9	113	51.6	158	47.7	236	67.4	40	7.4
his5⁻	179	54.6	175	51.1	106	48.4	173	52.3	114	32.6	497	92.6
lys1⁺	159	48.5	168	48.1	111	50.7	173	52.3	97	27.7	350	65.2
lys1⁻	169	51.5	181	51.9	108	49.3	158	47.7	253	72.3	187	34.8
ade6⁺	178	54.3	153	46.4	94	43.9	129	53.1	26	18.1	12	14.1
ade6⁻	150	45.7	177	53.6	120	56.1	114	46.9	118	81.9	73	85.9
diploid		ND	19	5.2	8	3.5	11	3.2	271	43.6	52	8.8
aneuploid		ND	19	5.2	5	2.2	88	25.7	204	32.9	452	76.7
VSY	8.4		1.8		3.6		0.85		0.15		0.13	

Figure 6—figure supplement 1. Summary of *Sk/Sp* hybrid and pure species diploid meiotic phenotypes and distribution of alleles in their progeny.
DOI: 10.7554/eLife.02630.025

	aneuploids		haploids	
	Sk/Sp rec12⁺ (n=204)		*Sk/Sp rec12*⁺ (n=146)	
locus	observed	%	observed	%
his5⁺	134	65.7	102	69.9
his5⁻	70	34.3	44	30.1
lys1⁺	59	28.9	38	26.0
lys1⁻	145	71.1	108	74.0

Figure 6—figure supplement 2. Biased transmission favoring *Sk* alleles on chromosomes 1 and 2 is observed in aneuploid and haploid spores.
DOI: 10.7554/eLife.02630.026

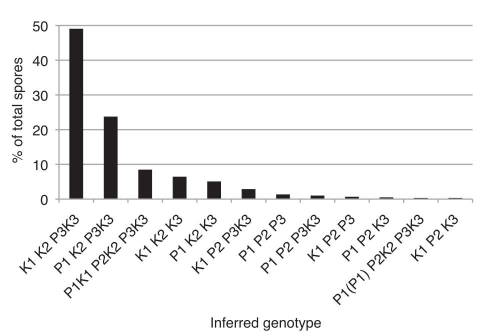

Figure 6—figure supplement 3. Distribution of progeny from *rec12Δ Sk/Sp* hybrid meiosis.
DOI: 10.7554/eLife.02630.027

locus	*Sk/Sp rec12Δ* (n=339) *Sk* mtDNA observed	%	*Sk/Sp rec12Δ* (n=341) *Sp* mtDNA observed	%
his5$^+$	21	7.1	16	5.4
his5$^-$	275	92.9	283	94.6
lys1$^+$	206	69.6	221	73.9
lys1$^-$	90	30.4	78	26.1
ade6$^+$	9	16.7	6	10.2
ade6$^-$	45	83.3	53	89.8

Figure 6—figure supplement 4. Meiotic drive in *Sk/Sp* hybrids is independent of mitochondrial DNA.
DOI: 10.7554/eLife.02630.028

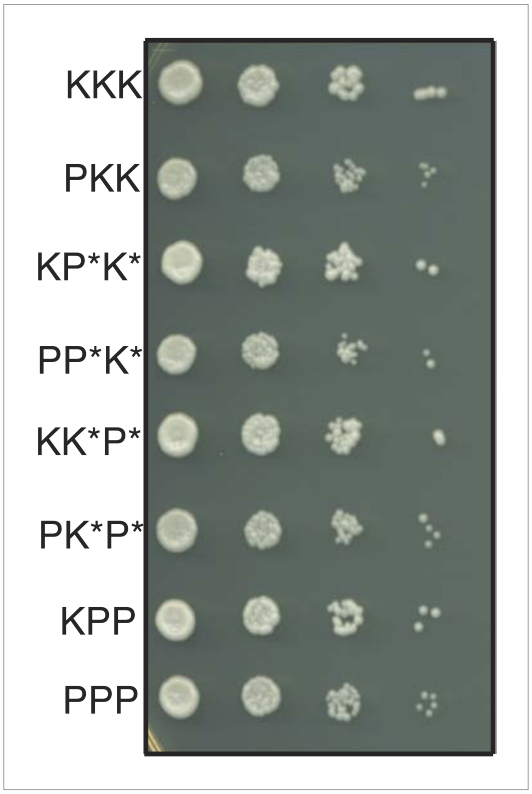

Figure 7. The haploid progeny of *Sk/Sp* hybrids have similar growth rates. The progeny of *rec12Δ Sk/Sp* hybrids with the indicated chromosomes were diluted and grown on rich YEA medium. KKK indicates the *Sk* parental genotype, whereas PPP indicates the *Sp* parental genotype. The strains were genotyped using *lys1*, *his5* and *ade6* alleles on chromosomes 1, 2, and 3, respectively. Strains that inherit intact chromosomes 2 and 3 from different species are non-viable because they lack many essential genes. However, we do rarely recover viable recombinant strains that have alleles from *Sk* chromosome 2 and *Sp* chromosome 3 (and vice versa). Potentially recombinant chromosomes are denoted with an *. All haploid strains recovered have growth rates similar to that of the parental species, suggesting mitotic growth defects do not underlie differential recovery of the genotypes.

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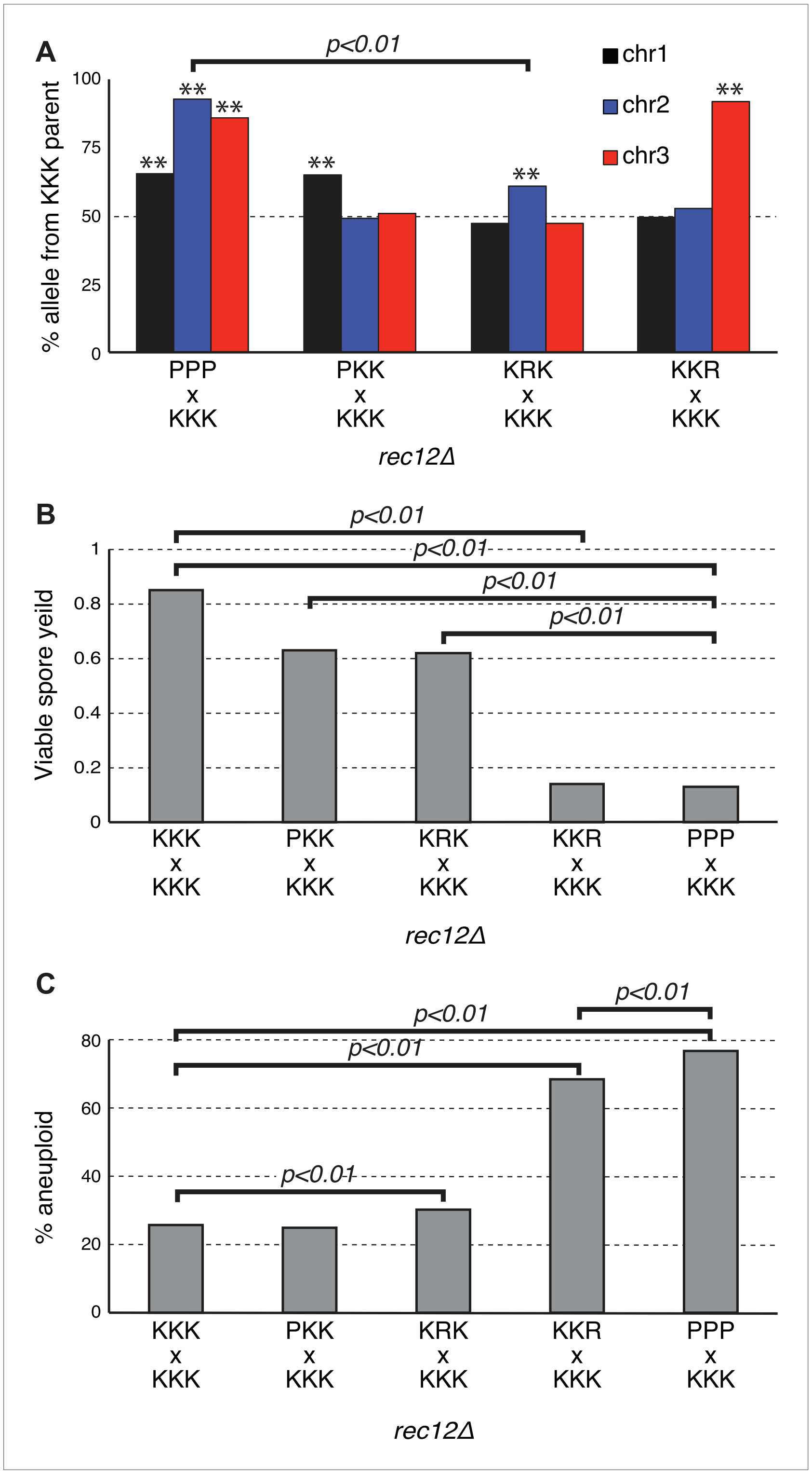

Figure 8. *Sk* drive alleles are autonomous and contribute to hybrid infertility. Aneuploidy is largely caused by heterozygosity of *Sk* and *Sp* DNA on chromosome 3. (**A**) Comparison of meiotic drive phenotypes between *rec12Δ* diploids generated by mating *Sk* to *Sp* or to haploid strains obtained from *Sk/Sp* hybrids. 'R' indicates a recombinant chromosome (***Figure 8—figure supplement 1***), which is compatible with all *Sk* chromosomes but does not contain a meiotic drive allele. All *Sk* chromosomes can drive autonomously (** indicates drive; G-test p<0.01). However, the drive of *Sk* chr2 is lower in the KRK/KKK diploid than in pure PPP/KKK hybrids (G-test; n >500 for chromosomes 1 and 2, n >80 for chromosome 3 in each cross). The PPP/KKK data are also shown in ***Figure 6B***.
Figure 8. Continued on next page

Figure 8. Continued

(**B**) Fertility defects of hybrids parallel the amount of drive observed amongst the viable spores (see A, p-values obtained from t-tests, averages of at least five experiments are shown). This is consistent with drive causing spore death. (**C**) The high aneuploidy amongst the viable progeny of *Sk/Sp* hybrids is largely due to heterozygosity of one or more loci on chromosome 3 (G-test, n >500 for each cross). The PPP/KKK and KKK/KKK viable spore yield and aneuploid data are also shown in ***Figure 5***. The data underlying these graphs are summarized in ***Figure 8—figure supplement 2***.

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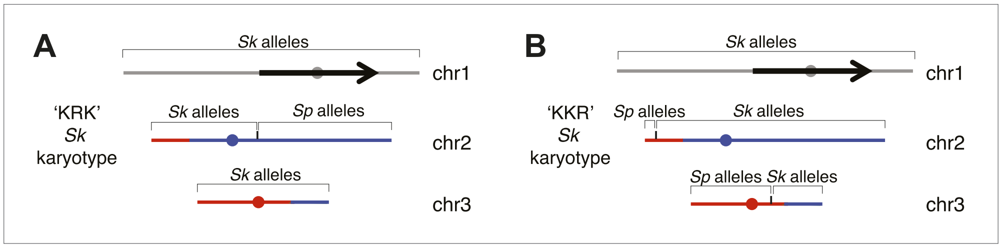

Figure 8—figure supplement 1. Genotype of recombinant strains used in ***Figure 8***.

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	PPP x KKK		PKK x KKK		KRK x KKK		KKR x KKK	
	rec12Δ n=589		*rec12Δ* n=684		*rec12Δ* n=708		*rec12Δ* n=801	
locus	**observed**	**%**	**observed**	**%**	**observed**	**%**	**observed**	**%**
lys1⁺	**350**	**65.2**	**411**	**65.1**	364	52.7	384	50.5
lys1⁻	187	34.8	220	34.9	**326**	**47.3**	**377**	**49.5**
his5⁺	40	7.4	321	50.9	270	39.2	**400**	**52.6**
his5⁻	**497**	**92.6**	**310**	**49.1**	**420**	**60.8**	361	47.4
ade6⁺	12	14.1	226	49.0	**224**	**47.4**	19	8.9
ade6⁻	**73**	**85.9**	**235**	**51.0**	252	52.6	**194**	**91.1**
diploid	**52**	8.8	26	3.8	12	1.7	33	4.1
aneuploid	**452**	76.7	170	24.9	214	30.2	548	68.4
VSY	**0.13**		**0.63**		**0.62**		**0.14**	

Figure 8—figure supplement 2. Summary of meiotic phenotypes for *Sk/Sp* hybrids and diploids with one heterozygous chromosome and the distribution of alleles in their viable progeny.

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