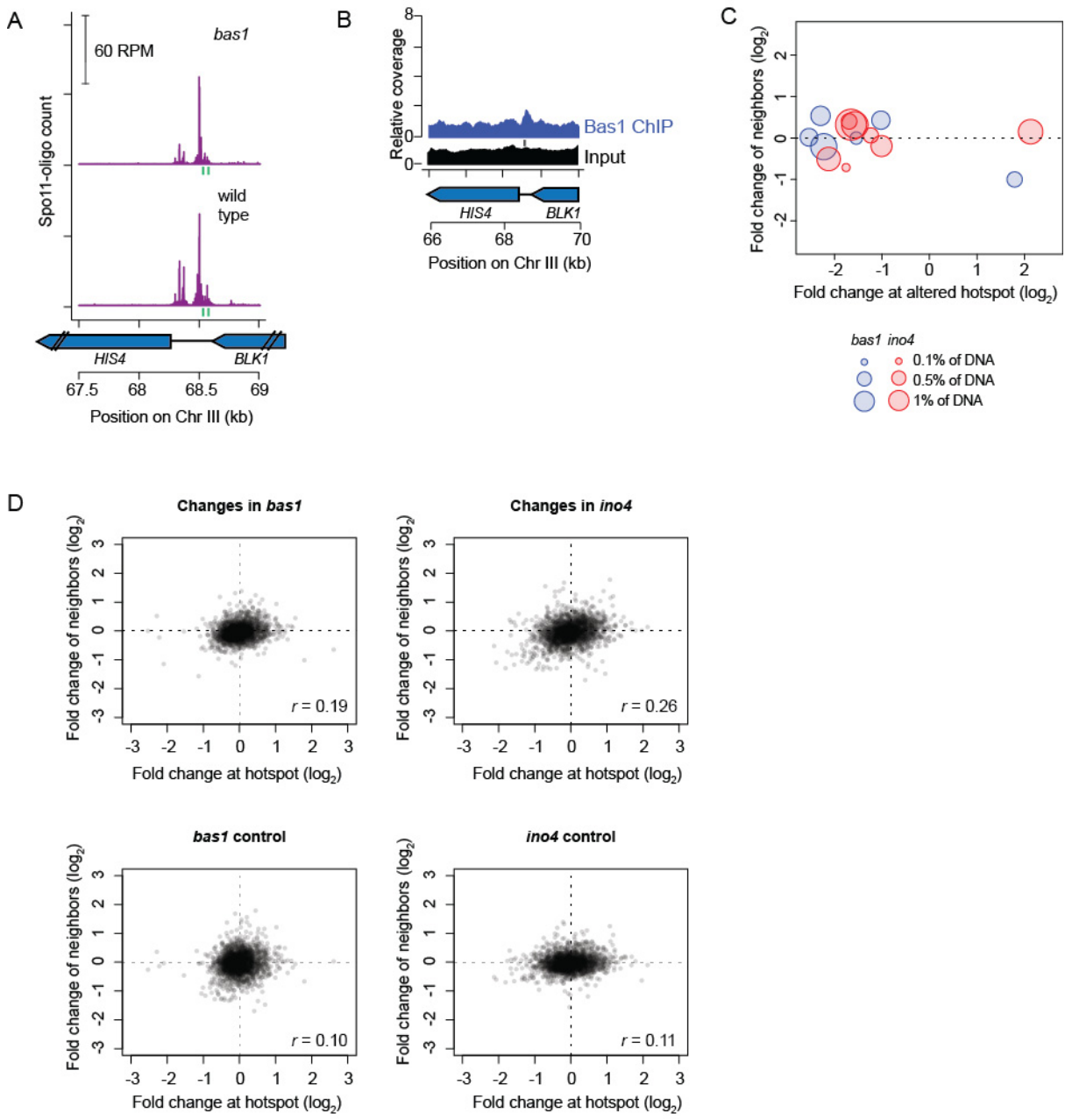

Figure S1 (A,B) Spo11 oligos (A) and Bas1 ChIP-seq signals (B) at the hotspot in the *HIS4* promoter. Green vertical ticks indicate matches to the Bas1 motif. *HIS4* is a modest hotspot in wild-type SK1 (870 RPM on average, equivalent to <2% of DNA broken; **Table S3**) and Spo11-oligo counts decrease to 64% of wild type in the *bas1* mutant (**Table S3**). A weak Bas1 ChIP-seq signal was discernible in the *HIS4* promoter, but was not sufficiently strong to pass our threshold for calling a Bas1 binding peak. (C,D) Hotspot competition is not a major contributor to altered DSB distributions in TF mutants. It is known that a very strong artificial hotspot can inhibit DSB formation at natural DSB hotspots nearby (see Main Text). Such behavior predicts that a hotspot whose intrinsic activity is altered by a TF mutation should show compensatory changes in the opposite direction for its neighbors: hotspots that decrease activity should show increased activity among the neighbors, and vice versa. The bubble plot in panel C compares the log fold change in Spo11-oligo counts at specific hotspots in *bas1* (blue) or *ino4* (red) mutants with the total log fold change of Spo11-oligo counts within the neighbors of those hotspots (within 5 kb on either side). Only hotspots that overlap ChIP-seq peaks of the respective TF, have ≥100 RPM average in at least one genotype, and show ≥2-fold change of

Spo11-oligo count within the hotspot are shown. The area of each point is proportional to the Spo11-oligo count (i.e., DSB strength) in either wild type or the TF mutant, whichever was higher. There was no significant correlation between the change in these hotspots and the change in their neighbors (p = 0.313). Panel D is similar, but for all hotspots with ≥100 RPM average in at least one genotype. The top panels show fold changes in the *bas1* (left) or *ino4* (right) mutant. As controls for non-specific correlations, the bottom panels compare the fold change within each hotspot in one TF mutant with the fold change within the hotspot's neighbors in the other TF mutant. Only very weak positive correlations are seen, which is opposite the direction predicted for a hotspot competition effect. As discussed in the Main Text, it is likely that the lack of a signature of hotspot competition is because the hotspots whose activity changes have relatively low DSB frequencies, so their effects on their neighbors are too modest to be apparent when assayed in a cell population.

Table S1 Yeast strains

Strain number	Genotype
SKY3821	*MATa/MATα; ho::LYS2/"; lys2/"; ura3/"; leu2/"; arg4-Bgl/"; nuc1Δ::LEU2/"; SPO11-His6-flag3-loxP-kanMX-loxP/"*
SKY3860	*MATa/MATα; ho::LYS2/"; lys2/"; ura3/"; leu2/"; arg4-Bgl/"; nuc1Δ::LEU2/"; SPO11-His6-flag3-loxP-kanMX-loxP/"; bas1Δ::hphMX/"; sae2Δ::NatMX/"*
SKY3880	*MATa/MATα; ho::LYS2/"; lys2/"; ura3/"; leu2/"; arg4-Bgl/"; nuc1Δ::LEU2/"; SPO11-His6-flag3-loxP-kanMX-loxP/"; sae2Δ::NatMX/"*
SKY4019	*MATa/MATα; ho::LYS2/"; lys2/"; ura3/"; leu2/"; arg4-Bgl/"; nuc1Δ::LEU2/"; SPO11-His6-flag3-loxP-kanMX-loxP/"; bas1Δ::hphMX/"*
SKY4603	*MATa/MATα; ho::LYS2/"; lys2/"; ura3/"; leu2/"; arg4-Nsp/"; nuc1Δ::LEU2/"; BAS1-myc13-KanMX/"*
SKY4680	*MATa/MATα; ho::LYS2/"; lys2/"; ura3/"; leu2/"; arg4-Bgl/"; nuc1Δ::LEU2/"; SPO11-His6-flag3-loxP-kanMX-loxP/"; ino4Δ::hphMX/"*
SKY4696	*MATa/MATα; ho::LYS2/"; lys2/"; ura3/"; leu2/"; arg4/"; nuc1Δ::LEU2/"; INO4-myc13-KanMX/"*
SKY4846	*MATa/MATα; ho::LYS2/"; lys2/"; ura3/"; leu2/"; arg4/"; nuc1Δ::LEU2/"; SPO11-His6-flag3-loxP-kanMX-loxP/"; ino4Δ::hphMX/"; sae2Δ::NatMX/"*

Table S2 Spo11-oligo sequencing statistics

Sample	Total sequenced	Total mapped[a]	Total filtered[b]	Uniquely mapped[c]
wild type sample 1	4,361,438	4,302,910 (98.6%)	3,762,134 (86.2%)	3,633,091 (83.3%)
wild type sample 2	2,783,885	2,700,065 (97.0%)	2,360,467 (84.8%)	2,280,452 (81.9%)
bas1 sample 1	5,615,623	5,545,624 (98.8%)	4,823,794 (85.9%)	4,665,416 (83.1%)
bas1 sample 2	5,219,925	3,906,560 (74.8%)	3,212,796 (61.5%)	2,983,690 (57.2%)
bas1 sample 3	5,410,693	5,349,120 (98.9%)	4,794,677 (88.6%)	4,542,315 (84.0%)
bas1 sample 4	5,401,936	5,211,575 (96.5%)	4,540,169 (84.0%)	4,413,658 (81.7%)
ino4 sample 1	3,241,998	3,203,647 (98.8%)	2,867,655 (88.4%)	2,664,527 (82.2%)
ino4 sample 2	3,631,197	3,614,320 (99.5%)	3,188,652 (87.8%)	3,117,705 (85.9%)
ino4 sample 3	4,160,807	4,127,194 (99.2%)	3,616,144 (86.9%)	3,480,102 (83.6%)

[a] The fraction of sequences that could not be mapped likely reflect sequencing errors, adaptor dimers, PCR dimers and *bona fide* Spo11-oligos derived from genomic regions that are unique to SK1 (i.e., not found in the S288C reference strain).
[b] Total number of reads that filtered to get rid of reads with poor alignment and/or adaptor clipping.
[c] Total number of reads that mapped to only one position in the genome.

Tables S3-S7

Available for download as Excel files at www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.178293/-/DC1

Table S3 **Spo11-oligo hotspots called from a combined map merging the wild-type, *bas1* and *ino4* average maps**

Table S4 **Bas1 ChIP-seq peak positions and signals**

Table S5 **Ino4 ChIP-seq peak positions and signals**

Table S6 **RNA-seq differential gene expression in *bas1* mutants**

Table S7 **RNA-seq differential gene expression in *ino4* mutants**