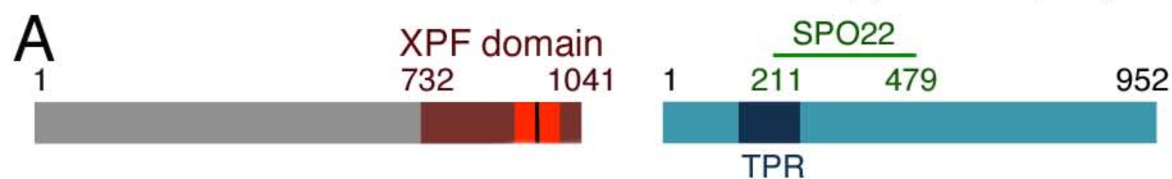
Dubois Supplementary Fig. 1



B yeast two-hybrid

Zip2N Zip2C

Zip4N Zip4C

BT AD - Zip2 Zip2N Zip2C

-

Zip4

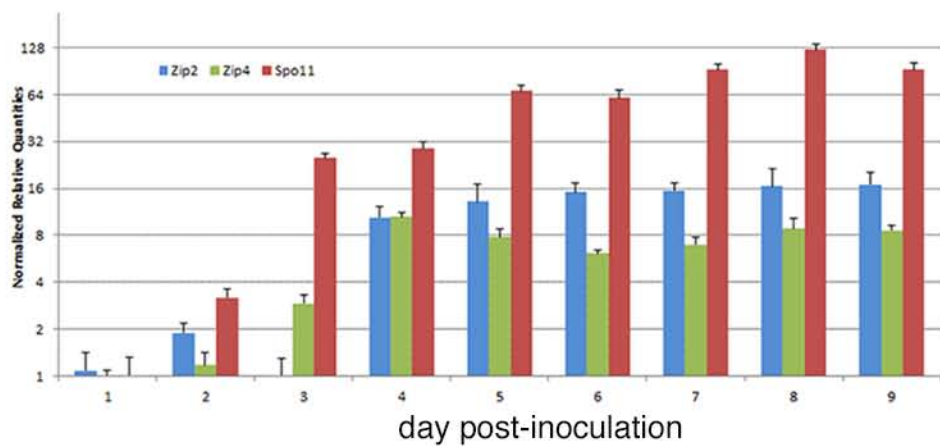
Zip4N

Zip4C

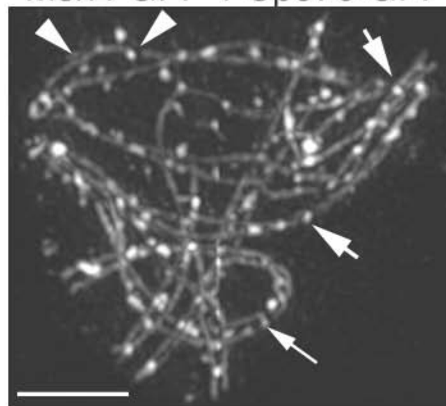
Gal4 AD	Gal4 BT
-	-
Zip2	-
Zip2N	-
Zip2C	-
Zip4	-
-	Zip4
Zip2	Zip4
Zip2N	Zip4
Zip2C	Zip4
Zip4	Zip4
-	Zip4N
Zip2	Zip4N
-	Zip4C
Zip2	Zip4C

–Leu/–Trp

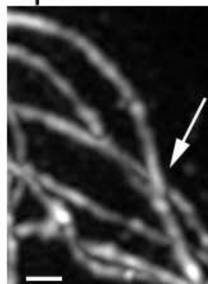
–Leu/–Trp/–Ade/–His



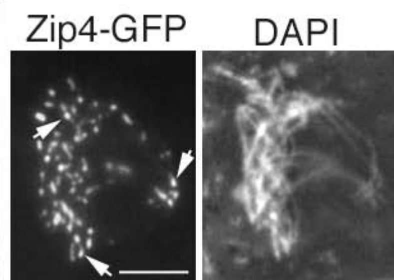
A zygotene nucleus
Msh4-GFP + Spo76-GFP



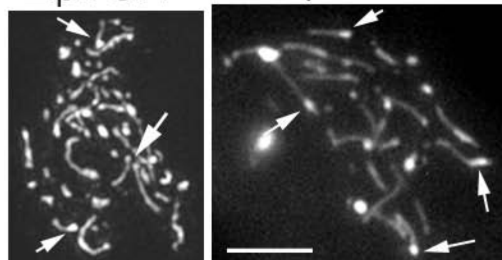
B
Spo76-GFP



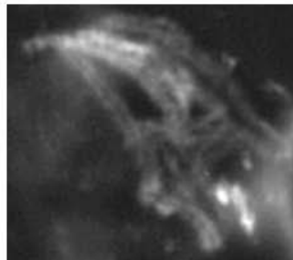
C
Zip4-GFP DAPI



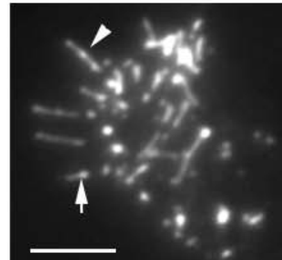
D
Zip2-GFP Zip4-GFP



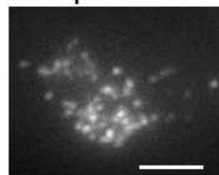
DAPI



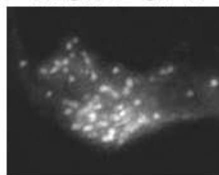
Zip2-GFP



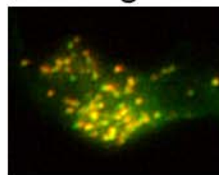
E
Zip2-mC



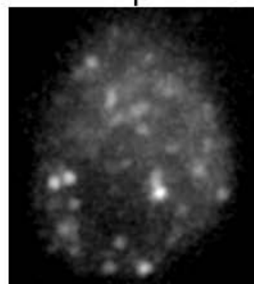
Msh4-GFP



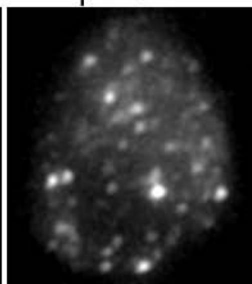
merge



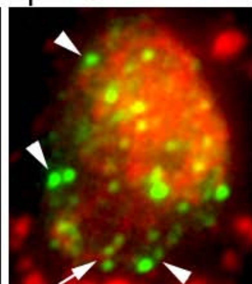
F *mer3*
Zip4-mC



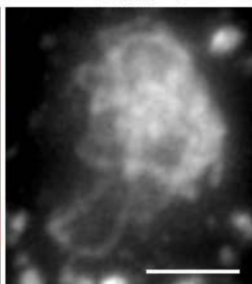
Zip2-GFP

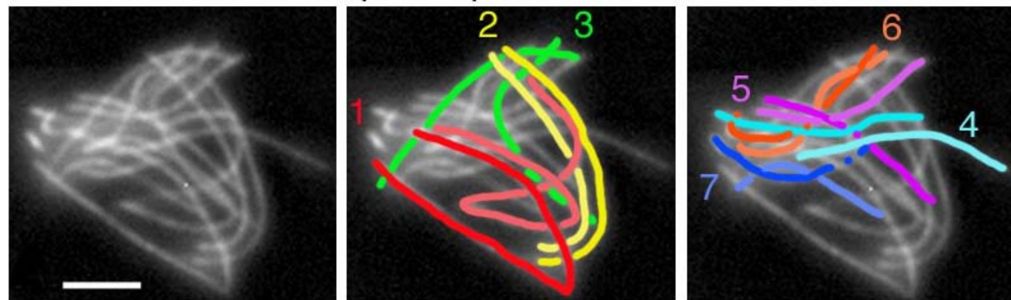


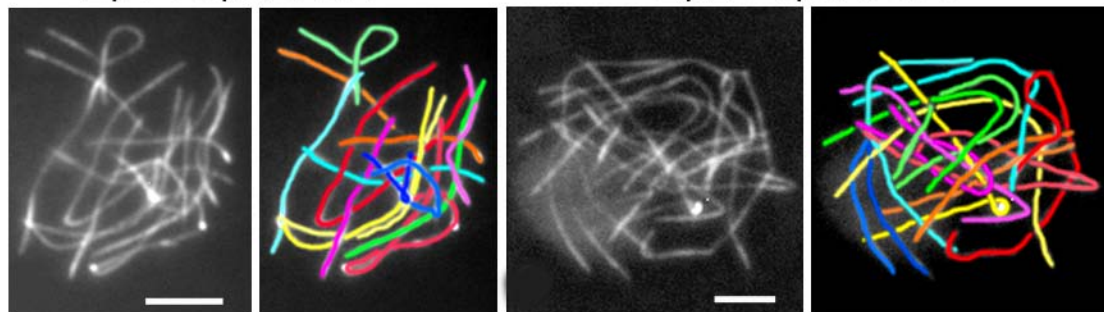
Zip2-GFP+DAPI

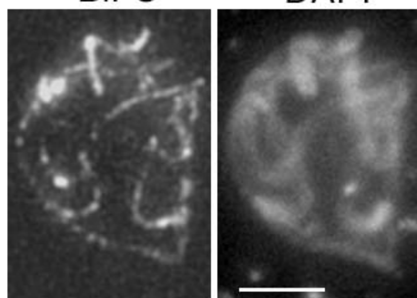


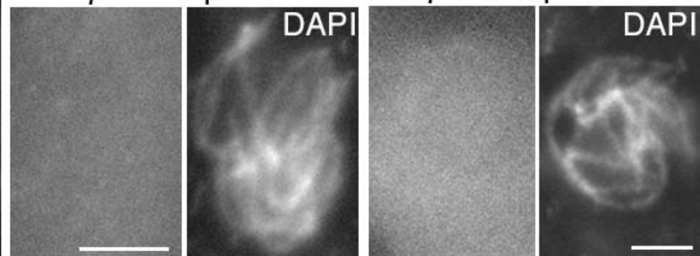
DAPI




A
zip4 + Spo76-GFP
1
2
3
4
5
6
7


zip4 + Spo76-GFP
zip2 + Spo76-GFP

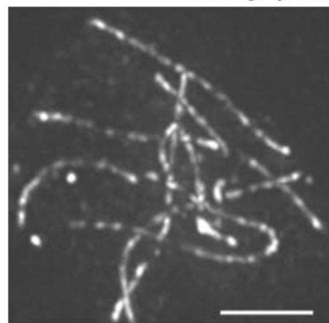

B
BiFC
DAPI

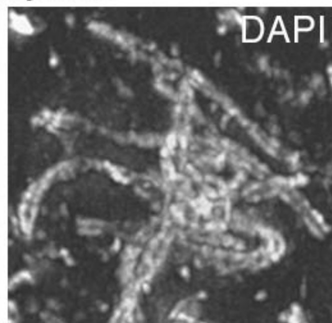

C
zip2 + Zip4-GFP
DAPI
zip4 + Zip2-GFP
DAPI

Dubois Supplementary Fig. 4

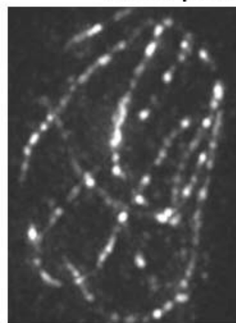
A Zip4-GFP

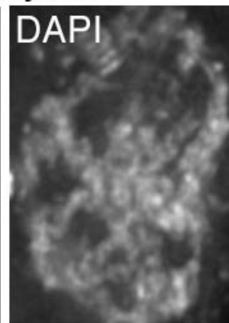
early pachytene



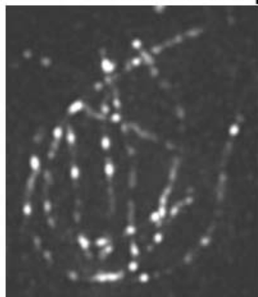


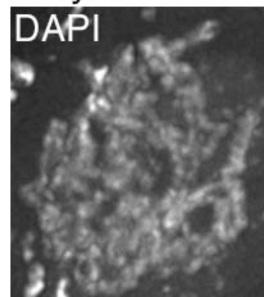
mid-pachytene



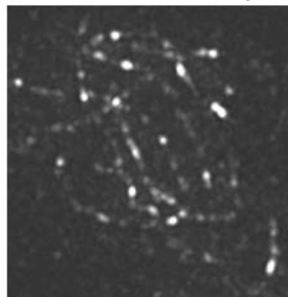


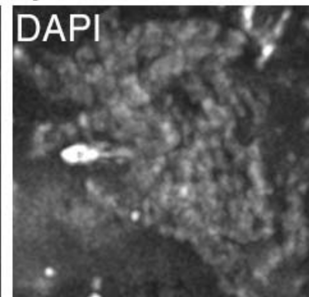
mid-late pachytene



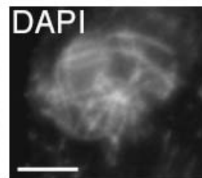


late pachytene

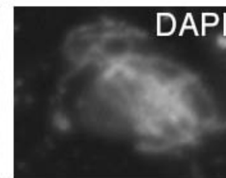




B *zip4* + Hei10-GFP



zip2 + Hei10-GFP



SI Appendix Figure Legends

Fig. S1. Zip2 and Zip4 Interaction and transcripts.

(*A*) Schematic domain organization of the predicted 1041 amino-acid Sordaria Zip2 protein (left) and the predicted 952 amino-acid Sordaria Zip4 protein (right). Indicated are: the conserved motifs of Zip2 (brown, XPF domain; red, DNA-binding domain) and Zip4 (dark cyan, predicted TPR and green line, SPO22 motif). Application of TPRpred software shows that Sordaria Zip4 contains three tetra-tricopeptide repeats (TPR), which are supposed to mediate protein-protein interactions.

(*B*) Yeast two-hybrid analysis confirms that Sordaria Zip2 and Zip4 interact with one another. Upper panel, left: cartoon summarizing the interacting domains: Zip2N (1-731), Zip2C (732-1041), Zip4N (1-479) and Zip4C (480-952). Right: interactions were detected between the two full-length proteins; also, the N-terminus of Zip2 is sufficient for interaction with Zip4 and both the N- and the C- terminus of Zip4 interact with Zip2, indication that the TPR domain is not essential for the interaction. Lower panel indicates the details and controls of the interactions between the recombinant proteins fused with the GAL4 activation domain (AD) and recombinant proteins fused with the GAL4 DNA-binding domain (BT). Growth on SD–Leu/–Trp is the positive control of the diploid strains. Growth on SD–Leu/–Trp/–Ade/–His medium indicates an interaction. Rapidly growing cultures were serially diluted 10-fold at each step and were spotted in rows. Left to right: from no dilution to 10^{-5} dilution.

(*C*) Comparison of the *ZIP2* (blue), *ZIP4* (green) and *SPO11* (brown) transcripts during Sordaria vegetative and sexual cycles by RT-qPCR. Histograms reflect the heterogeneity of the meiotic stages in the fruiting bodies. As asci/meiocytes are formed in successive waves (first ones between day 2 and 3), fruiting bodies from days 4 to 6 contain essentially meiosis I and meiosis II asci but also fewer asci than fruiting bodies from days 7 to 9, which now contain asci at all stages between prophase I to ascospore formation. Normalized Relative Quantities (NRQ) are scaled to minimal (lowest relative quantity = 1) in 2-log scale. All NRQ above 2 have p-value < 0.01. Error bars indicate the standard deviation calculated with the NRQ of four biological replicates for each day. Reference genes (*PDF2*, *TIP*, *UBC* and *CIT1)* were selected with geNorm. The geometric average of the expression of the four reference genes was used for the normalization of the expression of *ZIP2, ZIP4* and *SPO11*.

Fig. S2. Coalignment and SC formation.

(*A*) Mid-zygotene nucleus: 3D-SIM with Spo76-GFP and Msh4-GFP co-staining. The different steps, from "hanging" foci (arrowheads) to bridges (thin arrow) and synapsis (large arrows) are observable in the nucleus, indicating that bridge formation is a rather transient step.

(*B*) Late leptotene nucleus with axes (Spo76-GFP) coaligned at ~ 400nm. Arrow points to an interlocking between two homologs.

(*C*) At early zygotene, Zip4-GFP occurs mostly as foci and as few lines extending in one direction from foci (arrows).

(*D*) At mid-zygotene, more lines form by asymmetric nucleation (arrows) from a Zip2 focus (left) or a Zip4 focus (middle), or by fusion of early lines (right, arrowhead).

(*E*) Msh4 colocalizes with Zip2 at early zygotene.

(*F*) In the absence of Mer3, few colocalizing Zip2 and Zip4 foci are localized on chromosomes (arrow points to two of them), but the majority of foci is localized in the nucleoplasm (arrowheads).

Bars = 2 microns, except for Fig. 2*B* = 400nm.

Fig. S3. Coalignment defects, BiFC and Zip2-Zip4 interdependency.

(*A*) Examples of almost complete coalignment (upper panel) and partial coalignment (lower panel) in *zip4* and *zip2.* The 7 bivalents are distinguishable by their lengths and color (e.g. 2 longest: red and yellow, smallest: blue).

(*B*) Early pachytene nucleus by BiFC reconstitution of the Zip2-Zip4-GFP signal.

(*C*) Zip4 and Zip2 are dependent upon one another for their nuclear localization.

Bars = 2 microns.

Fig. S4. Zip2-Zip4 focus morphologies throughout pachytene and Hei10 localization.

(*A*) Early to late pachytene nuclei stained by Zip4-GFP. Uniformly-sized foci at early pachytene (upper panel, left); at mid pachytene some foci increase in size and brightness (upper panel, right); mixture of bright and dim foci at mid-late pachytene (lower panel, left) and finally even more dim foci and fewer bright foci at late pachytene (lower panel, right). Pachytene sub-stages can be defined with accuracy by chromatin compactness: thin and sharp at early pachytene, which allows to see the two homologs (upper panel, left) to more and more compact and diffuse (lower

panel), thus permitting detailed temporal analyzes.

(*B*) Hei10-GFP localization in absence of Zip4 (left) or Zip2 (right). Hei10-GFP appears as a dense perinuclear halo in both mutants as in wild type (34), but no foci (left) or only one Hei10 focus (arrow, right) is visible in the mutants.

Bars = 2 microns.