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Identification of chromosome sequence motifs that mediate meiotic pairing and synapsis in *C. elegans*

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Caenorhabditis elegans **chromosomes contain specialized regions called pairing centres, which mediate homologous pairing and synapsis during meiosis. Four related proteins, ZIM-1, 2, 3 and HIM-8, associate with these sites and are required for their essential functions. Here we show that short sequence elements enriched in the corresponding chromosome regions selectively recruit these proteins *in vivo*. *In vitro* analysis using SELEX indicates that the binding specificity of each protein arises from a combination of two zinc fingers and an adjacent domain. Insertion of a cluster of recruiting motifs into a chromosome lacking its endogenous pairing centre is sufficient to restore homologous pairing, synapsis, crossover recombination and segregation. These findings help to illuminate how chromosome sites mediate essential aspects of meiotic chromosome dynamics.**

Studies of genome rearrangements in the nematode *C. elegans* have shown that particular regions near one end of each chromosome are required in *cis* for homologous recombination and segregation during meiosis. Translocations or deletions of these regions suppress genetic exchange across large chromosome regions[1–5]. These 'homologue recognition regions', or 'pairing centres', stabilize pairing and promote assembly of the synaptonemal complex between homologous chromosomes[6,7].

A family of four paralogous proteins, each containing two atypical C2H2 zinc fingers, is required for pairing centre function[8,9]. Each protein localizes to the pairing centres of one or two pairs of chromosomes during early meiotic prophase: ZIM-1 on chromosomes II and III, ZIM-2 on chromosome V, ZIM-3 on chromosome I and IV, and HIM-8 on the X chromosome. Loss of any of these proteins results in defects in pairing, synapsis, recombination and segregation of the corresponding chromosomes.

Cis-acting elements that underlie pairing centre function have not yet been described. Here we identify sequence motifs enriched on each chromosome that specifically recruit the cognate zinc finger protein required for that chromosome to undergo faithful meiotic segregation. This *in vivo* analysis is corroborated by *in vitro* binding experiments that show the basis for their sequence specificity. Integration of these sequences onto a chromosome deficient in pairing centre activity is sufficient to restore meiotic chromosome pairing and synapsis. Moreover, we demonstrate that these recruitment motifs do not require a specific chromosome position, and that one zinc finger protein can substitute for another to promote meiotic interactions of a particular chromosome.

RESULTS

Identification of X chromosome pairing centre sequences

The X chromosome pairing centre has been previously mapped to the region distal to (or left of, by *C. elegans* convention) the *dpy-3* locus, 2.15 Mb from the left telomere[3,10]. X chromosomes lacking this region usually fail to synapse or undergo exchange and consequently missegregate, resulting in an elevated frequency of XO (male) progeny[3], known as the high incidence of males, or Him, phenotype[11].

To delimit the region containing the X chromosome pairing centre more precisely, chromosome deficiencies were mapped using single nucleotide polymorphisms (SNPs)[12,13]. We analysed three deficiencies that eliminate both pairing centre function and cytologically detectable HIM-8 localization (Fig. 1b–d)[3]. Each of these three deficiencies lacked all markers tested between 50 kb and 1.46 Mb from the left end (see Methods; Fig. 1a), but did not delete a marker at 2.07 Mb. By contrast, *yDf19*, an X chromosome deficiency that retains HIM-8 staining (Fig. 1e) and undergoes normal meiotic segregation[14] lacked the leftmost markers scored, but its right breakpoint was found to lie between 1.06 and 1.17 Mb from the left end (Fig. 1a). These data indicate that elements sufficient to recruit HIM-8 and confer pairing centre activity are contained within sequences between 1.06 and 2.07 Mb from the left end of the X chromosome.

Candidate sequences within this 1-Mb region were injected into wild-type *C. elegans* to test for HIM-8 binding. The resulting transgenic animals carried high-copy extrachromosomal arrays, which typically contain megabases of the injected DNA and are transmitted through mitosis and

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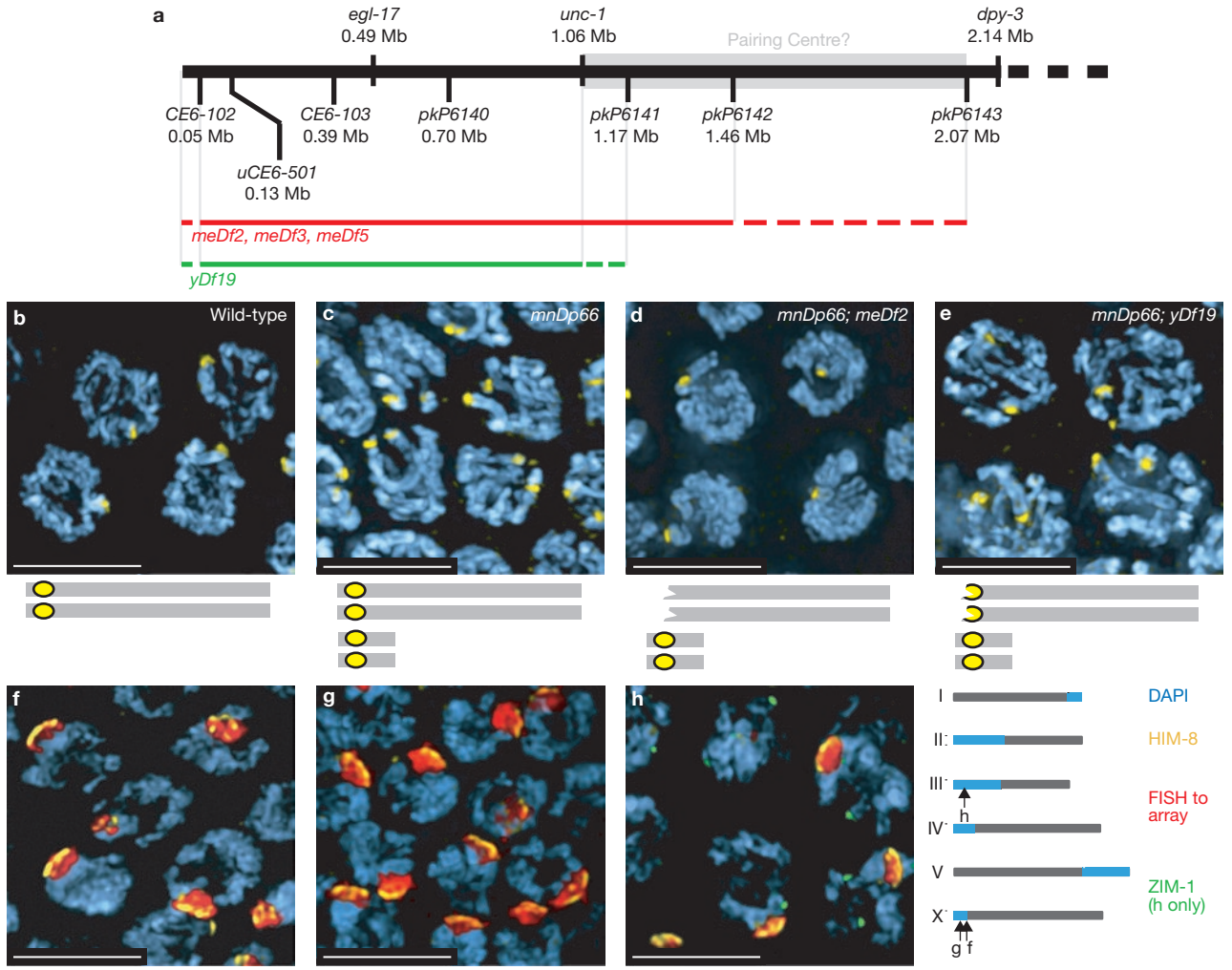

Figure 1 The X chromosome pairing centre region. (**a**) Left two megabases of the X chromosome. Genetic and physical markers used for mapping are indicated. Three deficiencies that remove the pairing centre (*meDf2*, *meDf3*, and *meDf5*) and one that does not (*yDf19*) were mapped. All pairing centre deficiencies remove *pk6142* but not *pk6143*, indicating breakpoints between 1.46 and 2.07 Mb from the left end. *yDf19* removes *unc-1* but not *pk6141*, indicating a breakpoint between 1.06 and 1.17 Mb from the left end. (**b**–**e**) HIM-8 immunofluorescence (yellow) in meiotic nuclei from hermaphrodites of the indicated genotypes. Diagrams of the X chromosomes and the *mnDp66* duplication, which is required for viability in deficiency homozygotes, are shown. (**f**–**h**) HIM-8 immunofluorescence (yellow) was combined with FISH (red) to test for recruitment of HIM-8 to extrachromosomal arrays. The diagram on right indicates the genomic location of the sequences tested in each panel: 539 bp amplicon from cosmid K06A9 on XL (**f**); cluster of TTGGTCAGTGCA repeats from *X*L (**g**); cluster of 4 HIM-8 recruitment motifs from IIIL recruits HIM-8 (yellow) but not ZIM-1 (green) (**h**). All images are maximum-intensity projections of deconvolved 3D stacks. Scale bars, 5 μm.

meiosis (see Methods)[15]. Combining FISH with immunofluorescence, we tested whether candidate arrays recruited HIM-8 in germline nuclei. Although this approach is unbiased with respect to candidate sequences, it does require that HIM-8 recognize a sequence motif or other element within the chromatin context of an extrachromosomal array, which undergoes transcriptional silencing and enriched H3K9 dimethylation in germline nuclei[16]. Although we did not know *a priori* whether HIM-8 would bind to arrays, we were encouraged by the success of an analogous approach to identify sequence elements that recruit *C. elegans* dosage compensation complex proteins in somatic nuclei[17,18].

From an initial pool of cosmids that recruited HIM-8, we narrowed the recruitment activity to smaller fragments, ultimately to a 539-bp amplicon (Fig. 1f; Supplementary Information, Table S1). Centred within this short sequence are five and a half copies of a 21-bp repeat, and no other repetitive element, coding sequence or other feature of obvious interest.

Computational analysis revealed that a 12-bp motif, (TTGGTCAGTGCT) contained within the larger repeat is enriched on the X chromosome, relative to the autosomes, and in the pairing centre region relative to the entire X chromosome (Supplementary Information, Fig. S1a). When degeneracy was allowed, we found that some closely related sequences were also enriched in the pairing centre region and that TTGGTCAGTGCA, which differs at the 3′ nucleotide, is even more abundant than the original motif (Fig. 2; Supplementary Information, Fig. S1). Interestingly, a version of this sequence lacking the 5′ base was previously identified computationally as the most overrepresented oligonucleotide on the X chromosome and named CeRep50 (ref. 19).

To test whether TTGGTCAGTGCA could also recruit HIM-8, we amplified two short regions from the left end of the X chromosome, each containing several copies of this motif with different flanking sequences. Both amplicons recruited HIM-8 (Fig. 1g; Supplementary Information, Table S1), as did a synthetic oligonucleotide containing

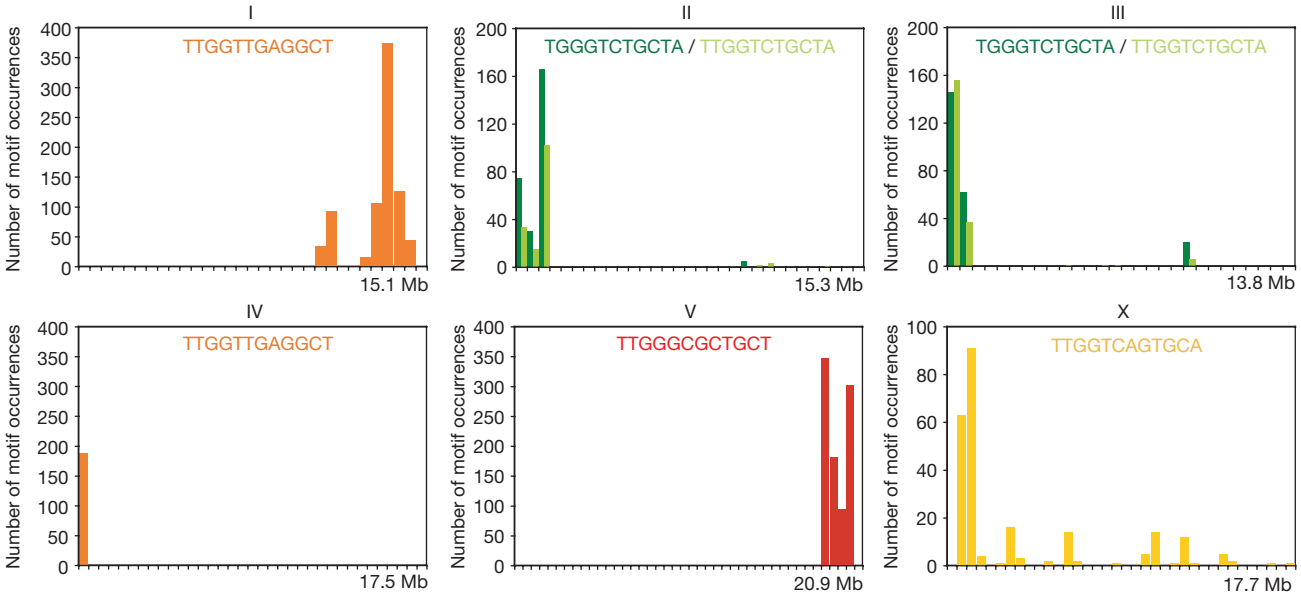

Figure 2 ZIM/HIM-8 recruitment motifs. Distribution of the most abundant ZIM-1 (green), ZIM-2 (red), ZIM-3 (orange) and HIM-8 (yellow) recruitment motif on the six *C. elegans* chromosomes. Each bin along the *x* axis represents a 500-kb genomic segment. Note that *y* axes show different scales for each chromosome.

four tandem copies of this motif interspersed with flanking spacers. TTGGTCAGTGCA is therefore sufficient to recruit HIM-8.

Both of these related HIM-8 recruiting motifs are highly enriched near the left end of the X chromosome and are most often found in short, tandemly oriented clusters with a predominant 21-base periodicity (Supplementary Information, Table S2)[19]. Many copies lie within introns of known or predicted genes. Small clusters or isolated occurrences of these motifs can be found elsewhere on the X chromosome and occasionally on autosomes. *yDf19*, the deficiency chromosome with a functional pairing centre, retains about half of the motifs normally found in the left 2 Mb of the X chromosome, whereas *meDf2*, *meDf3* and *meDf5* remove between 94–100% of the motifs found in this dense cluster.

Detection of HIM-8 in germline nuclei by immunofluorescence microscopy revealed a single primary association site in the genome, corresponding to the region of the X chromosome removed by *meDf2*, *meDf3*, and *meDf5* (Fig. 1b–d)[9]. However, granular staining of HIM-8 was detected elsewhere on the chromatin, which may reflect binding to related motifs elsewhere in the genome. HIM-8 might weakly promote X chromosome segregation even in the absence of the pairing centre, as loss of *him-8* function results in a more severe meiotic phenotype than X chromosome pairing centre deficiencies[9]. To assay the recruitment potential of clusters outside the pairing centre, we amplified clusters of motifs from the centre and right end of the X chromosome, and also from chromosome III. Each of these amplicons recruited HIM-8 in the high-copy extrachromosomal array assay (Fig. 1h; Supplementary Information, Table S1). Although HIM-8 recruitment motifs elsewhere on the X may contribute to segregation, they lack key pairing centre functions; in particular, they do not measurably stabilize homologue pairing in the absence of synapsis[7]. This suggests that pairing centres require a minimal density of binding sites, or perhaps other *cis*-acting components, for full function.

Identification of autosomal pairing centre sequence motifs

Our evidence indicates that HIM-8 is recruited *in vivo* by a sequence previously identified *in silico* as the most overrepresented short oligonucleotide on the X chromosome. Earlier computational analysis also identified overrepresented sequences on each autosome, relative to the other five chromosomes. These motifs, designated as CeRep45-49, were found to be asymmetrically enriched towards one end of each chromosome[19]. These regions of enrichment roughly correspond with pairing centres, which have been mapped to varying precision on different chromosomes. However, these observations were based entirely on computational analysis and no functions have previously been demonstrated for these abundant motifs.

We revamped the earlier analysis, incorporating the knowledge that pairing centres on chromosomes I and IV share a common zinc finger protein (ZIM-3), as do II and III (ZIM-1)[8]. We found that CeRep45, the most overrepresented sequence on chromosome I[19], is also highly enriched in a 120-kb window within the pairing centre region on chromosome IV (Fig. 2; Supplementary Information, Fig. S2). Interestingly, although most repeats on chromosome I are clustered in an alternating orientation (or 'inverted' clusters, Supplementary Information, Table S2), with a total period of 68 bp, the copies on chromosome IV are mostly in tandemly oriented clusters with a 19-bp periodicity (Supplementary Information, Table S2). Amplicons spanning clusters of this motif from either chromosome I or IV strongly recruited ZIM-3 to extrachromosomal arrays (Supplementary Information, Fig. S5a, S5c, Table S1).

Chromosomes II and III both require ZIM-1 for meiotic pairing and synapsis[8]. Although neither CeRep45 nor CeRep46, the sequences most overrepresented on chromosomes II and III, respectively, is highly abundant on the other chromosome, we found that both repeats are associated with a distinct motif, $T^{G}/_{T}GGTCTGCTA$, which is enriched on both chromosomes (Fig. 2; Supplementary Information, Fig. S3). Amplicons containing clusters of these elements showed specific recruitment of ZIM-1 (Supplementary Information, Fig. S5b, Table S1). Although this motif is predominantly in inverted clusters on chromosomes II and III, the predominant spacing of these elements is different on the two chromosomes (Supplementary Information, Table S2).

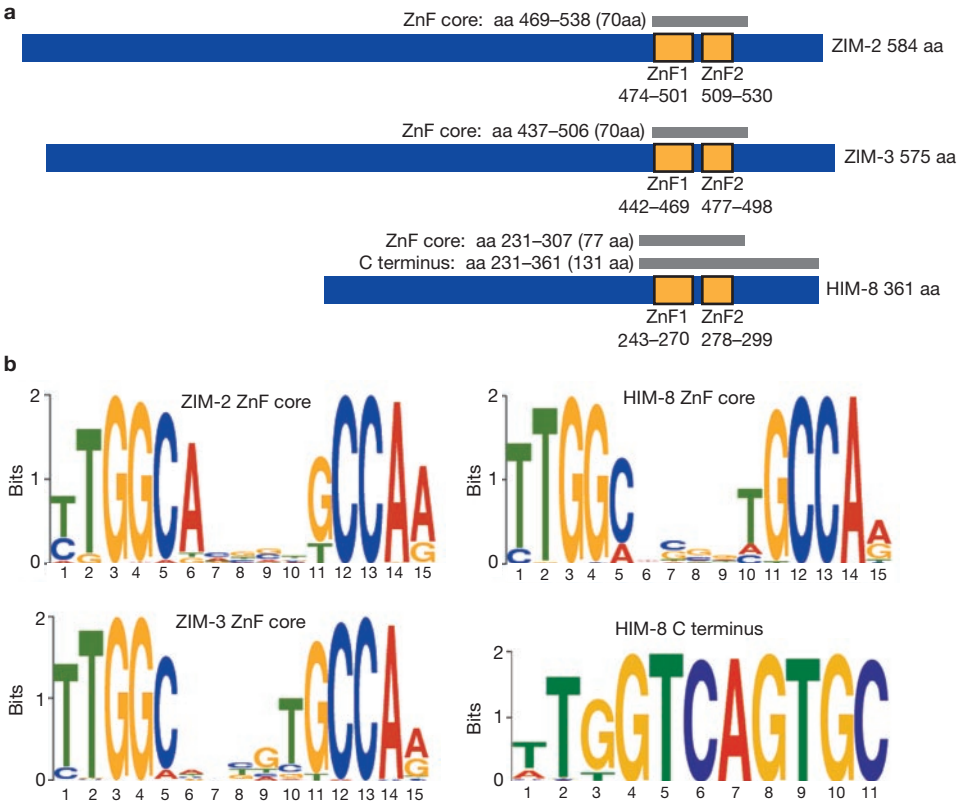

Figure 3 Sequence-specific binding by ZIM-2, ZIM-3, and HIM-8 protein fragments. (**a**) Diagram of ZIM-2, ZIM-3, and HIM-8 showing the full-length proteins (blue), the position of the zinc fingers (ZnF, gold boxes), and the protein fragments expressed *in vitro* and subjected to SELEX analysis (grey bars). (**b**) Consensus motifs derived using MEME from the unique sequences identified following four iterations of SELEX (Supplementary Information, Table S3). Protein fragments containing the core ZnF domains of each protein show specific binding to the DNA sequence TTGGC. The randomized region in the library used for SELEX is 21–26 bp, which is significantly larger than the stretch that a 2-finger module can specify. For this reason, oligonucleotides containing two binding sites are more likely to be co-precipitated with tagged proteins than sequences containing a single site (E. Rebar, personal communication). A C-terminal fragment of HIM-8, including the zinc finger domains, specifically binds to the same sequence identified by recruitment of HIM-8 to extrachromosomal arrays.

Recruitment of pairing centre proteins to chromosome V was more enigmatic. The CeRep49 sequence ([T]TGGGCGCTGCT)[19] seemed an excellent candidate for ZIM-2 recruitment, as it is highly enriched on chromosome V and also because its base composition and length are similar to those of motifs that recruit the other proteins (Fig. 2; Supplementary Information, Fig. S4). Indeed, we did find that one cluster of this repeat specifically recruited ZIM-2, although less robustly than other ZIM-recruiting arrays (Supplementary Information, Fig. S5d, Table S1). However, a different cluster from chromosome V containing both this motif and the motif TTGGTCGCTGCT, which differs at the underlined base, strongly recruited both ZIM-2 and HIM-8 (Supplementary Information, Fig. S5e, f, Table S1). As shown previously[19], the organization of CeRep49 repeats on chromosome V is bimodal with respect to spacing, with prominent 18- and 32-base periodicities. Clusters with 32-bp spacing contain CeRep49 alone, whereas clusters with 18-bp spacing always include this G–>T variant motif (Supplementary Information, Table S2). It is unclear whether co-recruitment of HIM-8 to arrays containing the second class of cluster is due to the variant sequence, or to the distinct spacing, but it is suggestive that the T variant is more similar to the X chromosome repeat, TTGGTCAGTGCA. No obvious recruitment of HIM-8 is detected cytologically on chromosome V, and genetic evidence indicates that only ZIM-2 is required for efficient pairing and synapsis of this chromosome[8]. Nevertheless, indirect evidence suggests that HIM-8 might contribute to pairing of chromosome V in the absence of ZIM-2, as appreciable crossing-over is detected cytologically in a *zim-2* mutant[8].

In vitro analysis of pairing centre sequences

HIM-8 and the ZIM proteins each contain two short domains resembling C2H2 zinc fingers[20], the most common DNA binding motif in metazoa[21]. Data presented here and in previous work[8,9] indicate that defined chromosomal sequences are able to recruit HIM-8 and the ZIM proteins *in vivo,* and that missense mutations in the zinc finger domains of HIM-8 disrupt chromosome association[9]. However, the spacing between the Zn-coordinating Cys and His residues in the first finger of the ZIM/HIM-8 family is distinct from the canonical C2H2 spacing seen in proteins such as Zif268 (ref. 22) or Sp1(ref. 23). Furthermore, a single canonical C2H2 zinc finger usually specifies only a 3 or 4-bp subsite[22], raising the question of how these unusual proteins with two zinc fingers might recognize the non-palindromic sequences of about 12 bp that we identified.

To determine whether the C2H2 zinc fingers in the ZIM/HIM-8 proteins can bind DNA sequence-specifically, we used a SELEX assay[24]. Briefly, protein fragments expressed *in vitro* were incubated with a

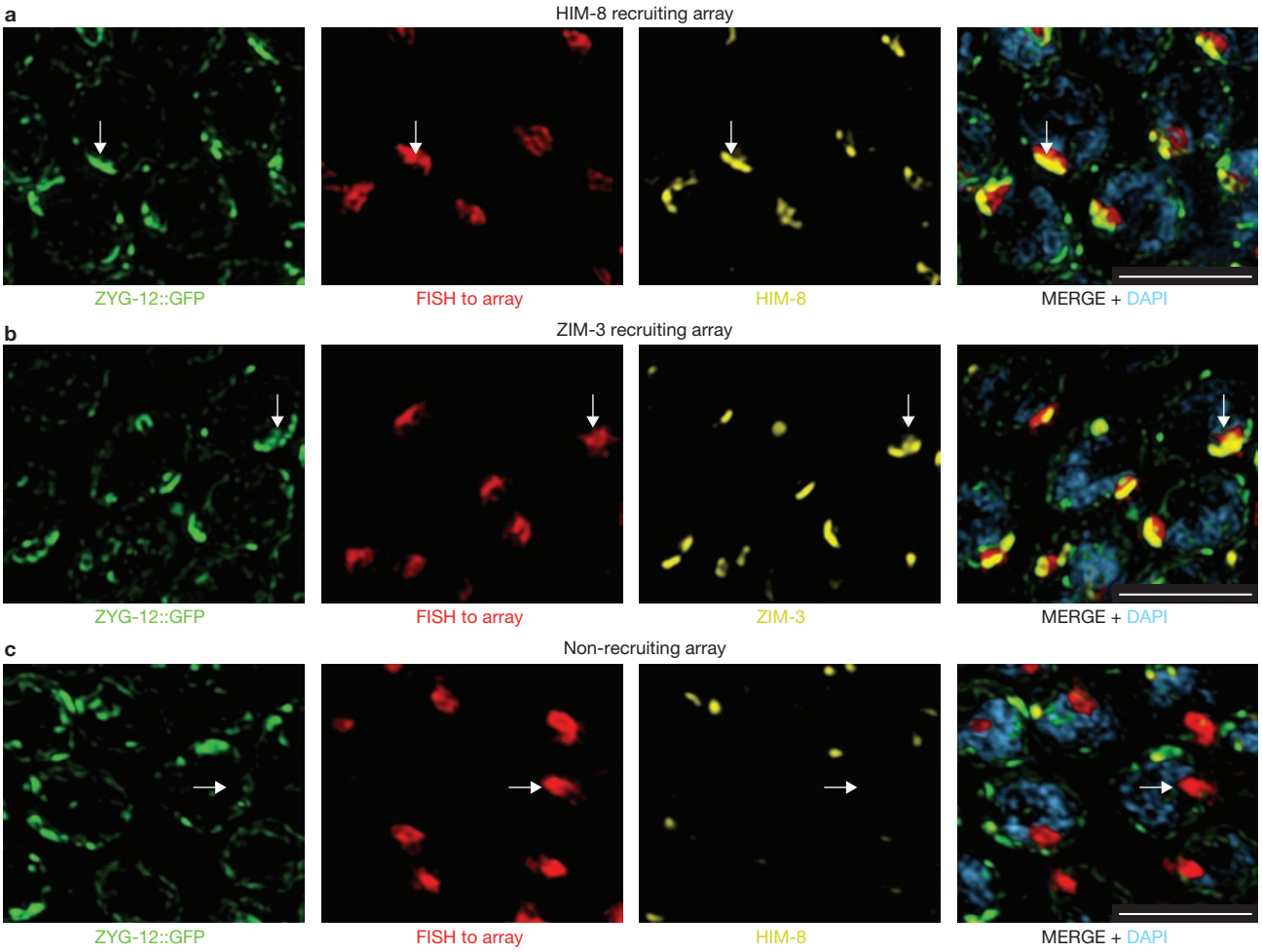

Figure 4 ZIM/HIM-8 recruiting arrays associate with nuclear envelope components. (**a**–**c**) Combined FISH detection of extrachromosomal arrays (red) and immunolocalization of HIM-8 or ZIM-3 (yellow) and ZYG-12:GFP (green). HIM-8 (**a**) and ZIM-3 (**b**) recruiting arrays interact with a large patch of ZYG-12 at the nuclear envelope. The array shown in **c** does not recruit HIM-8 or any of the ZIM proteins, and does not colocalize with ZYG-12. Arrows indicate clear examples of colocalization (or lack thereof, **c**). All images are maximum-intensity projections of deconvolved 3D stacks. Scale bars, 5 μm.

library of double-stranded DNA fragments carrying a randomized 21-bp stretch of DNA. The protein-bound DNA was isolated and amplified, and the cycle was reiterated three times more, after which the bound DNA fragments were sequenced and analysed to derive a consensus. The assay produced very similar results when this analysis was performed with the core zinc finger domains of HIM-8, ZIM-2 and ZIM-3. In all three cases, the pentamer motif TTGGC clearly emerged as the preferred binding site (Fig. 3; Supplementary Information, Table S3). Interestingly, this sequence is similar but not identical to one end of all of the binding motifs we identified *in vivo*. We next expressed a longer fragment of HIM-8, including the region from the zinc finger domain to the carboxy terminus of the protein. This fragment bound specifically to oligonucleotides containing the consensus $^{T}/_{A}$TGGTCAGTGC, identical to the full length HIM-8 recruiting repeat we identified *in vivo* (Fig. 3b; Supplementary Information, Table S3).

Taken together, the SELEX results indicate that HIM-8 uses a composite protein–DNA interaction domain to recognize its full target site. We infer that the distinct recruitment motifs of the ZIM proteins are probably recognized by the combined specificities of their zinc finger domain and a short adjacent C-terminal domain in each protein, and that the zinc fingers probably specify one end of each binding site containing TTGG. These findings suggest a direct correspondence between each zinc finger protein and the sequences we identified *in vivo*, suggesting that other co-factors are unlikely to be required to recruit these proteins to their cognate chromosome sequences. Future work may elucidate how these composite DNA-binding domains interact with their cognate binding sites.

ZIM/HIM-8 recruiting arrays interact with the nuclear envelope

Chromosomal pairing centres are physically associated with the nuclear envelope during early meiotic prophase[8,9]. We observed that when ZIM/HIM-8 proteins are recruited to the extrachromosomal arrays, they also concentrate at the interface between the array and the nuclear envelope (see Fig. 1f–h). This suggests that the zinc finger proteins recruit their binding sites to the periphery of the array and the nucleus. In work detailed elsewhere (Sato *et al.* submitted), we have found that sites of contact between endogenous pairing centres and the nuclear envelope are enriched for several proteins that contribute to chromosome segregation. These include the inner and outer nuclear membrane proteins SUN-1 and ZYG-12, which concentrate at discrete patches along the

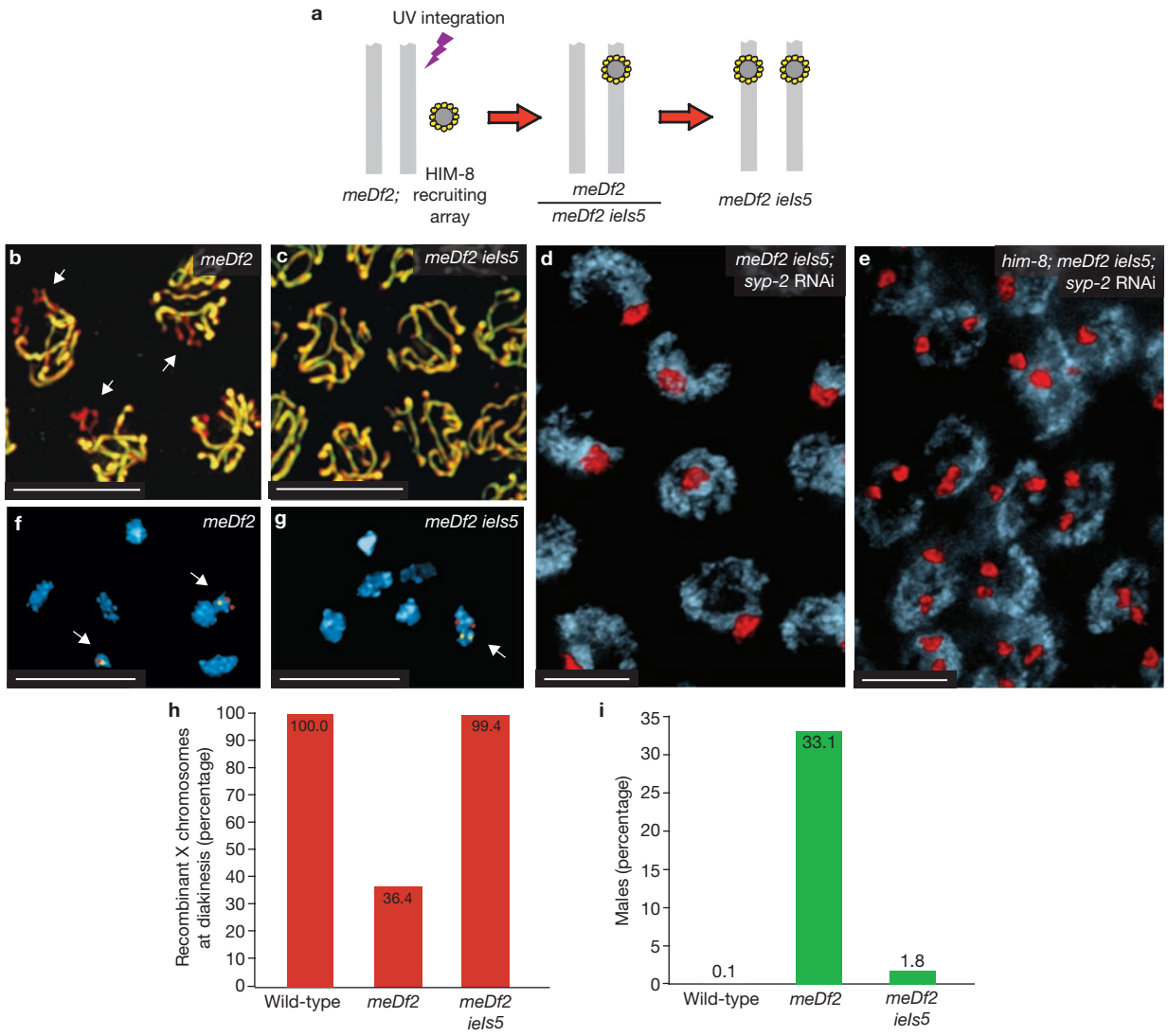

Figure 5 HIM-8 recruitment motifs are sufficient for pairing centre function. (**a**) Integration of a HIM-8 recruiting array (see Fig. 1f) onto *meDf2*, an X chromosome deficient in pairing centres. (**b**) Hermaphrodite homozygous for *meDf2* has unsynapsed X chromosomes in most pachytene nuclei, visualized as axial elements marked by HTP-3 (red) lacking the central region protein SYP-1 (green). Arrows indicate examples of unsynapsed chromosomes. (**c**) Most meiotic nuclei in *meDf2 ieIs5* hermaphrodites are fully synapsed. (**d**, **e**) Stabilization of pairing in the absence of synapsis (*syp-2* RNAi) was examined by performing FISH to the integrated array (red). In *meDf2 ieIs5* oocytes (**d**) the arrays are paired, as indicated by only a single region of FISH staining. In *him-8; meDf2 ieIs5* oocytes (**e**) the arrays are unpaired, indicating that pairing between integrated arrays is *him-8* dependent, as seen between endogenous pairing centres[9]. (**f**, **g**) Oocytes at diakinesis in *meDf2* and *meDf2 ieIs5* hermaphrodites. FISH probes to the centre (yellow) and right end (red) identify the X chromosomes. Arrows indicate non-recombinant (univalent) and recombinant (bivalent) X chromosomes in *meDf2* and *meDf2 ieIs5* hermaphrodites, respectively. (**h**) Quantification of recombinant X chromosomes. (**i**) Quantification of males produced by self-fertilizing hermaphrodites of the indicated genotypes. Scale bars, 5 μm.

nuclear surface during early prophase[25,26]. We therefore tested whether arrays that recruit ZIM/HIM-8 proteins also associate with these nuclear envelope components. We compared the behaviour of three extrachromosomal arrays, two that recruit different zinc finger proteins, and a control, non-recruiting array (Fig. 1g; Supplementary Information, Fig. S5c, Table S1). The ZIM/HIM-8 recruiting arrays clearly associated with patches of SUN-1 and ZYG-12 at the nuclear envelope, in contrast to the non-recruiting array (Fig. 4 and data not shown). Thus, ZIM/HIM-8 recruitment motifs are sufficient to link the arrays to a protein complex that probably tethers these sequences to the nuclear envelope and mediates interactions with cytoplasmic dynein and microtubules (Sato *et al.* submitted).

HIM-8 recruiting sequences are sufficient to restore pairing centre activity to the X chromosome

Pairing centres contribute to meiotic chromosomal segregation by stabilizing homologue pairing and promoting synapsis[7]. To test whether a dense cluster of HIM-8 recruitment motifs is sufficient to restore these pairing centre functions to an X chromosome lacking its endogenous pairing centre, a HIM-8-recruiting extrachromosomal array was integrated onto the *meDf2* chromosome, which is deficient in 'pairing centre activity' (*meDf2 ieIs5*; see Methods; Fig. 5a). Whereas the X chromosomes in *meDf2* homozygotes usually fail to synapse (Fig. 5b)[7], in *meDf2 ieIs5* nearly all nuclei showed complete homologous synapsis (Fig. 5c), demonstrating that HIM-8 recruitment by the

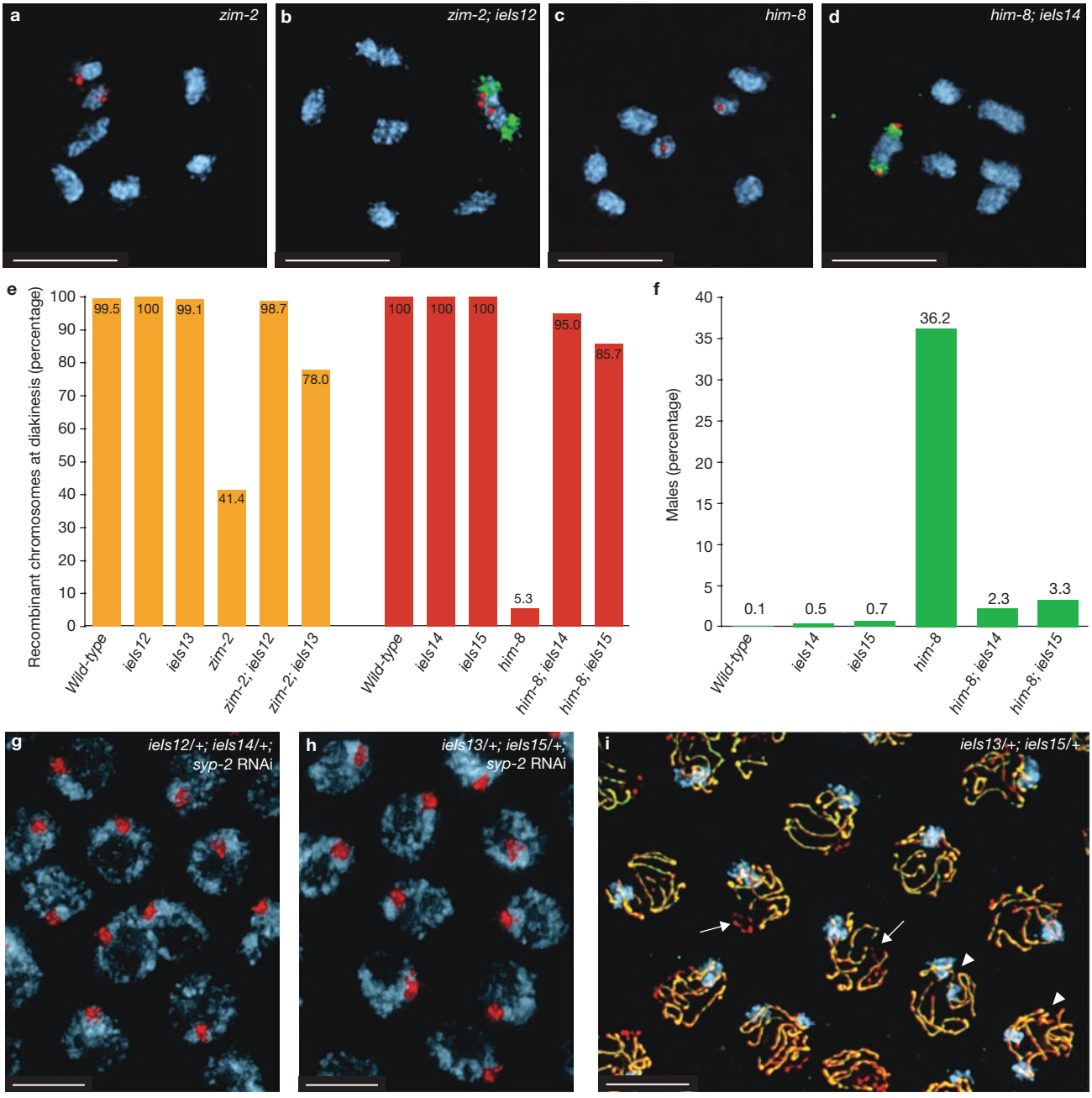

Figure 6 ZIM/HIM-8 proteins can interchangeably support pairing centre function. (**a**–**d**) Oocytes at diakinesis from *zim-2*, *zim-2;ielsl2, him-8* and *him-8;ielsl4* hermaphrodites. FISH probes to the 5S rDNA (red in **a**, **b**) and an *X*-chromosome repeat (red in **c**, **d**) were used to identify chromosomes *V* and *X*, respectively. In animals carrying chromosomal insertions of ZIM-2/HIM-8 binding sites *ielsl2* (**b**) and *ielsl4* (**d**), the chromosome-specific probes localize to a single bivalent, which is also marked by a FISH probe to λ DNA (green), indicating that the insertion of binding sites restored crossover recombination on chromosome V in *zim-2* animals and the X chromosome in *him-8* animals. (**e**) Quantification of bivalent X (red) and V (orange) chromosomes. (**f**) Quantification of males produced by self-fertilizing hermaphrodites of the indicated genotypes. (**g**, **h**) Arrays of binding sites (red) inserted into two different chromosomes *(ielsl2/+; ielsl4/+* and *ielsl3/+; ielsl5/+)* were assayed for their ability to promote stable pairing between non-homologous chromosomes in the absence of synapsis (*syp-2* RNAi). (**i**) Synapsis was analysed in animals heterozygous for two different insertions (*ielsl3/+; ielsl5/+*) by immunostaining of synaptonemal complex components. Nuclei containing unsynapsed chromosomes, visualized as segments positive for the axial element protein HTP-3, (red) but lacking transverse filament proteins including SYP-1 (green), usually contain integrated arrays (blue) that are paired and synapsed with each other, indicating non-homologous synapsis between chromosomes V and X (arrows). In contrast, nuclei with fully synapsed chromosomes often contained unpaired arrays (blue), indicating that all chromosomes are likely synapsed with their appropriate homologues (arrowheads). Scale bars, 5 μm.

inserted array can mediate proper synapsis of the X chromosomes. We also analysed pairing of the X chromosomes in the absence of synapsis by knocking down expression of *syp-2*, an essential synaptonemal complex component[27], by RNA interference (RNAi). Throughout the early meiotic region of the gonad, hybridization to the integrated array showed only a single region of staining (Fig. 5d), indicating that these inserted sequences can stabilize pairing between homologous X chromosomes in the absence of synapsis. By introducing the *him-8(tm611)*

null allele[9], we found that both the pairing and synapsis mediated by the integrated array require HIM-8 function (Fig. 5e and data not shown), much as these events do when mediated by the endogenous X chromosome PC.

Pairing and synapsis enable chromosomes to complete crossover recombination, which is required for chiasma formation and homologue segregation at the first meiotic division. To determine whether *meDf2 ieIs5* chromosomes undergo crossing-over, we scored the frequency of bivalents at diakinesis (Fig. 5f–h). As shown previously[3], most oocytes (65.6%) in *meDf2* hermaphrodites had univalent X chromosomes. By contrast, 99.4% of oocyte nuclei in *meDf2 ieIs5* homozygotes had bivalent (recombinant) X chromosomes. The fidelity of X chromosome meiotic segregation can be quantified in *C. elegans* by the frequency of male self-progeny produced by hermaphrodites. *meDf2 ieIs5* hermaphrodites produced 1.8% males, which is markedly fewer than *meDf2* homozygotes (33.1%; Fig. 5i). We conclude that integration of many copies of a 539-bp segment that recruits HIM-8 rescues pairing, synapsis, crossing-over and segregation defects arising from deletion of the endogenous pairing centre.

Pairing centre function does not require a specific chromosome position or zinc finger protein

In our initial rescue experiment, HIM-8 motifs were fortuitously integrated towards the left end of the *meDf2* chromosome, near their natural location. We carried out additional experiments to determine whether the position on the chromosome is essential for pairing centre activity, and also whether a particular chromosome requires a specific member of the ZIM/HIM-8 family. Identification of sequences that recruit both HIM-8 and ZIM-2 to arrays allowed us to address these questions about pairing centre plasticity. We UV-irradiated animals carrying such an array and screened for integration events onto either chromosome V or X (Supplementary Information, Fig. S5e, f). Two independent integrations into the left (non-pairing centre) arm of chromosome V were recovered (*ieIs12* and *ieIs13*), as were two integrations on the right (non-pairing centre) arm of *X* (*ieIs14* and *ieIs15*). The integrated arrays on chromosome V were homozygosed and crossed with *zim-2(tm574),* which eliminates ZIM-2 activity[8], whereas the X chromosome integrants were crossed with *him-8(mn253)* to abrogate HIM-8 function. In all cases the presence of the array on chromosome V or X resulted in restoration of bivalent formation relative to *zim-2* or *him-8* mutants, respectively (Fig. 6a–e). Disjunction of the X chromosome (as measured by the frequency of male self-progeny) was also rescued in the absence of HIM-8, presumably by ZIM-2 association with the X-linked integrated array (Fig. 6f). These results indicate that ZIM-2 can promote crossing-over on the X chromosome, that HIM-8 can promote crossing-over on V, and that the major ZIM/HIM-8 binding cluster does not require a specific chromosome position to function.

Integration of the same extrachromosomal array onto two different chromosomes also provided an opportunity to address whether such 'artificial pairing centres' can promote pairing and synapsis between non-homologous chromosomes. We generated trans-heterozygotes by crossing worms with the ZIM-2/HIM-8 recruiting array integrated on V (*ieIs12* or *ieIs13*) to animals with the same array integrated on the X chromosomes (*ieIs14* or *ieIs15*). We looked at whether such 'matching' pairing centres could stabilize pairing between non-homologous chromosomes in the absence of synapsis, and/or promote non-homologous synapsis. To look at pairing in the absence of synapsis, we visualized the integrated arrays in worms subjected to *syp-2* RNAi. In all four *trans*-heterozygous combinations, the ZIM-2/HIM-8 recruiting arrays on X and V were consistently paired (99% of nuclei) throughout the normal leptotene/zygotene and pachytene regions of the gonad (Fig. 6g, h; Supplementary Information, Table S4), indicating that non-homologous chromosomes did indeed undergo robust, stable pairing.

When synapsis was allowed to proceed in animals carrying the matching integrated arrays on non-homologous chromosomes, the meiotic configuration of individual nuclei was more variable, probably because of competition between the endogenous pairing centres and the integrated arrays (Fig. 6i; Supplementary Information, Table S4). In 71% of pachytene-stage nuclei, the integrated arrays were together and the associated non-homologous chromosomes were synapsed, whereas the true homologous partners of the two array-bearing chromosomes remained unsynapsed. 10% of nuclei showed complete homologous synapsis with the integrated arrays apart, indicating that the arrays occasionally failed to induce non-homologous synapsis even if they had initially paired. An additional 6% of nuclei showed complete synapsis, yet the integrated arrays were closely associated. This may reflect physical association between two pairs of homologously synapsed chromosomes; alternatively the normal copies of V and X may have synapsed with each other, or aberrantly loaded synaptonemal complex without pairing.

Together, these data suggest that the artificial pairing centres created by integrated zinc finger-binding arrays have potent pairing activity and that they can sometimes, but not always, mediate synapsis between non-homologous chromosomes even in the presence of homologous partners carrying intact pairing centres. These results are consistent with the idea that the integrated arrays and endogenous pairing centres compete to initiate synapsis. We emphasize, however, that the integrated arrays have important differences from endogenous pairing centres. Not only are the repeats probably present at higher density and much higher copy number within the artificial pairing centres, but within natural pairing centres the zinc finger-recruiting motifs are distributed among hundreds of kilobases of chromosome-specific sequences. We consider it likely that these interspersed sequences have an important role in specifying partner choice, perhaps by stabilizing and/or destabilizing pairing. This would explain why pairing between ZIM-1- or ZIM-3-binding pairing centres on different chromosomes is not detected in wild-type animals[8].

ZIM/HIM-8 recruiting arrays induce meiotic defects

Consistent with the aberrant interactions observed between arrays on non-homologous chromosomes, animals carrying free HIM-8 and ZIM recruiting arrays showed meiotic defects, including unsynapsed chromosomes, univalents, and male and inviable progeny (Supplementary Information, Fig. S6). These defects may reflect aberrant pairing between arrays and endogenous pairing centres dependent on the same zinc finger protein or titration of the ZIM/HIM-8 proteins off the endogenous pairing centre onto the array. However, these are unlikely to be the only explanations, as other chromosomes also showed segregation defects; for example, ZIM-binding arrays induced a weak Him phenotype, indicating missegregation of the X chromosome. This suggests that the arrays may titrate limiting components, such as nuclear envelope proteins, away from endogenous pairing centres that do not share the same zinc finger dependence.

DISCUSSION

These findings help to illuminate the mechanisms underlying homologue pairing, synapsis and segregation during meiosis. They demonstrate that pairing centre functions are defined by the binding of ZIM/HIM-8 proteins,

through a composite DNA binding domain, to a concentrated locus on each chromosome, and that consequent association with a complex of proteins at the nuclear envelope is both necessary and sufficient to facilitate specific pairing and synapsis between homologous chromosomes.

Although evidence presented here shows that a high-copy, high-density cluster of HIM-8 or ZIM protein binding sites can promote chromosome pairing and synapsis, the question of how chromosomes recognize their appropriate partners remains unclear. Previous work has demonstrated that the identity of the HIM-8/ZIM protein cannot specify partner choice during meiosis; indeed, we suspect that all pairing centres are likely to have derived from a common motif recognized by a single ancestral binding protein[8]. This would be more analogous to the situation in many other organisms, where shared telomeric repeats mediate association with the nuclear envelope to promote meiotic chromosome interactions. In light of this, it is interesting that the pentameric motif recognized by the HIM-8 and ZIM zinc finger cores (TTGGC) is closely related to the telomeric repeat in *C. elegans* (TTAGGC). It seems most likely that homology is assessed at unique sequences interspersed with and/or adjacent to the major clusters of binding sites on each chromosome. Future work may reveal how this assessment is accomplished, and how recruitment of HIM-8 and the ZIM proteins contributes to this fundamental mechanism underlying sexual reproduction. □

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturecellbiology/

Note: Supplementary Information is available on the Nature Cell Biology website.

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AUTHOR CONTRIBUTIONS

C.M.P. and A.F.D. designed most experiments and wrote the manuscript. J.H.C. provided cDNA clones for the SELEX assays, which were executed by X.M. and L.Z. with guidance from F.D.U. All other experiments were performed by C.M.P.

COMPETING INTERESTS

The authors declare competing financial interests. X.M., L.Z. and F.D.U. are full-time employees of Sangamo BioSciences, Inc.

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METHODS

SNP mapping. The boundaries of the deficiencies *meDf2*, *meDf3, meDf5,* and *yDf19* were mapped using 'snip-SNPs', polymorphisms that alter a restriction site between N2 Bristol and a Hawaiian isolate of *C. elegans*[12,13]. A *unc-1 dpy-3* strain with Hawaiian-derived SNPs to the left of *dpy-3* was generated by extensive outcrossing to the Hawaiian strain CB4856. *mnDp66; Df* males were then crossed to these Hawaiian *unc-1 dpy-3* hermaphrodites. Cross progeny were allowed to self-fertilize, and Unc nonDpy F2s (lacking *mnDp66,* which carries N2 SNP alleles) were lysed and their DNA was genotyped for seven SNPs ranging from 0.05–2.07 Mb from the left end of the *X* chromosome. Detection of only the Hawaiian digest pattern indicated that a SNP lies within the deletion, whereas both N2 and Hawaiian alleles were expected for SNPs outside the deletion.

Progeny analysis. Hermaphrodites were picked to individual plates as L4s and moved daily to fresh plates until they no longer laid eggs. All of their self-progeny were counted to determine the proportion of unhatched (dead) eggs and adult males and hermaphrodites. The following strains were analysed: *ieEx69* (ZIM-3 recruiting array derived from the pairing centre region of chromosome I; n = 1370), *ieEx29* (ZIM-1 recruiting array derived from an amplicon in the pairing centre region of chromosome II; n = 1,754), *ieEx41* (HIM-8 recruiting array generated from pairing centre region of X chromosome; n = 3,170), wild-type (n = 1,954), *mnDp66;meDf2* (n = 2,217), *mnDp66;unc-119(ed3);meDf2 ieIs5* (n = 3,637), *ieIs14* (n = 2,370), *ieIs15* (n = 1,663), *him-8(mn253)* (n = 1,315), *him-8(mn253); ieIs14* (n = 1,526), and *him-8(mn253); ieIs15* (n = 1,111).

Extrachromosomal arrays and integration. Extrachromosomal arrays were generated by injecting DNA mixtures including a plasmid carrying a phenotypic marker (*rol6(su1006), unc-119*(+), *or myo-2*::GFP) at 10–100 μg ml^{-1}, phage λ DNA (*Hind*III digested; New England Biolabs) at 50–100 μg ml^{-1}, and candidate DNA sequences on cosmids, plasmids, or PCR products, at 10–50 μg ml^{-1}. PCR primers are listed in Supplementary Information, Table S1.

A synthetic 84-bp oligonucleotide consisted of four tandem copies of the sequence AATTTGTGTTGGTCAGTGCAA. Both DNA strands were synthesized, annealed and co-injected with plasmids carrying phenotypic markers. Similar results were obtained when the same sequence was cloned into a TOPO vector (Invitrogen).

To integrate a HIM-8 recruiting array containing a 539-bp amplicon from cosmid K06A9 (*ieEx22*), array-bearing animals were crossed to the X chromosome pairing centre deficiency strain *mnDp66 (X;I);unc-119(ed3) III; meDf2 X*. *ieEx22* contains an *unc-119* rescuing construct, so the resulting array-bearing, *meDf2* F2 animals were nonUnc and Him. The worms were washed four times in M9 and placed on a 10-cm unseeded NGM plate. Once the liquid had absorbed into the agar, the plate was placed uncovered into a Stratalinker, and irradiated with 350 J m^{-2} of 254-nm light. The animals were allowed to recover for 4 h on food. Four L4s or young adults were then transferred to each of 40 plates. When their progeny reached the L4 larval stage, 500 of these F1s were picked to individual plates. From each F1, three F2 animals were picked to individual plates and their progeny (F3s) were screened for stable transmission of the nonUnc phenotype. A single integrant that mapped to the X chromosome was recovered. This array-containing X chromosome was outcrossed three times to *meDf2* animals to generate the *mnDp66 (X;I); unc-119(ed3) III; meDf2 ieIs5* X strain used for analysis.

Because a duplication of the X chromosome pairing centre region, *mnDp66*, was present in the original irradiated animals, we checked to make sure that the improvement in segregation was not a result of this duplication recombining back onto the X chromosome. The *unc-1* locus is present on *mnDp66* and absent from *meDf2*. The *dpy-3* locus is present on *meDf2* and absent on *mnDp66.* We therefore crossed *mnDp66; meDf2 ieIs5* hermaphrodites to N2 males, and mated the resulting male cross progeny, *mnDp66/+; meDf2 ieIs5*, to *unc-1 dpy-3* hermaphrodites. Unc nonDpy progeny (*unc-1 dpy-3/meDf2 ieIs5*) were recovered at the expected frequency of 50%, indicating that the *unc-1* region is still missing from the *meDf2 ieIs5* X chromosome.

Chromosomal integration of a ZIM-2/HIM-8 recruiting array was performed similarly to the integration of the HIM-8 recruiting array above, except that the extrachromosomal array was derived from a region of sequence motifs from chromosome V with 18-bp spacing and carried a dominant *rol-6(su1006)* marker and except for the presence of the array (*ieEx75*), the parental animals carried a normal karyotype. F1s (230) were individually plated after UV irradiation. Ten lines were identified that produced 100% Rol progeny, of which two mapped to the left arm of chromosome V (*ieIs12* and *ieIs13)* and two mapped to the right arm of the X chromosome *(ieIs14* and *ieIs15)*.

Immunofluorescence and FISH. Cytological methods were performed as described previously[9]. A FISH probe specific for the extrachromosomal and integrated arrays was synthesized from λ-phage DNA as previously described[8]. Chromosome-specific probes recognizing the middle and right ends of the *X* chromosome and to the 5S rDNA on chromosome V have been described previously[9,28].

To quantify the frequency of recombinant chromosomes, hermaphrodites were picked as L4s and maintained at 15 °C for three days. Adults were dissected, fixed, and hybridized with appropriate fluorescent probes to allow unambiguous identification of the relevant chromosomes. Three-dimensional images of oocyte nuclei at diakinesis were recorded and chromosomes were scored as bivalent (both homologues connected) or univalent (separate). The number of nuclei scored for each experiment were as follows: X chromosomes in wild-type (n = 162), *mnDp66;meDf2* (n = 121), *mnDp66;unc-119(ed3);meDf2 ieIs5* (n = 174), *ieIs14* (n = 157), *ieIs15* (n = 110), *him-8(mn253)* (n = 150), *him-8(mn253); ieIs14* (n = 238), and *him-8(mn253); ieIs15* (n = 300); chromosome V in the following strains: wild-type (n = 194), *ieIs12* (n = 105), *ieIs13* (n = 115), *zim-2(tm574)* (n = 140), *zim-2(tm574);ieIs12* (n = 155), and *zim-2(tm574);ieIs13* (n = 127). For all experiments involving integrated arrays, an array-specific probe was included to confirm that the chromosome being analysed (that is, X or V) contained an array.

Feeding RNAi. To examine chromosome pairing in the absence of synapsis, expression of *syp-2*, which encodes essential component of the central region of synaptonemal complex, was eliminated by feeding RNAi in *mnDp66; unc-119(ed3); meDf2 ieIs5* and *mnDp66; unc-119(ed3); him-8(tm611); meDf2 ieIs5* animals, and also in the *trans*-heterozygous progeny of [*ieIs12* or *ieIs13]* and [*ieIs14* or *ieIs15].* L4 larvae were placed on plates containing IPTG with lawns of bacteria containing RNAi clone sjj_C24G6.1 (ref. 29). Their progeny were dissected and stained 20–24 h post-L4. To examine cross-progeny of animals carrying arrays on different chromosomes, matings were carried out on *syp-2* RNAi lawns and the resulting transheterozygotes were maintained on *syp-2* RNAi plates until dissection, 20–24 h post-L4. Absence of synapsis was verified by SYP-1 immunofluorescence in all analysed animals.

Computational identification of motifs. To determine whether sequences related to TTGGTCAGTGCT might also be enriched on the X chromosome, a Perl script was written to search the genome, allowing variation at one nucleotide within the sequence at a time. The script was run iteratively to analyse any related sequences that were enriched at least 5-fold on the X chromosome relative to the autosomes. All such derived, enriched X chromosome motifs are presented in Supplementary Information, Fig. S1a. The same method was used to identify sequences related to the ZIM recruitment motifs that are enriched on the autosomes (Supplementary Information, Figs S2a, S3a, Fig. S4a).

SELEX. *In vitro* site selection by SELEX was performed exactly as described previously[24]. Fragments of *ZIM/HIM-8* cDNA constructs were amplified by PCR, in a two-step scheme, to yield linear products carrying HA-tags at the carboxy terminus. A library of DNA fragments carrying a 21-bp randomized sequence flanked by a constant region was used for experiments on ZIM-2 and ZIM-3, whereas two libraries, one with a 21-bp and the other a 26-bp randomized portion, was used for experiments on HIM-8 (data presented are collate findings from both). The MEME server[30] was used to identify motifs and generate the logograms presented.

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a

	I	*II*	*III*	*IV*	*V*	*X*
TTGGTCAGTGCA	3	8	23	4	13	239
TTGGTCAGTGCT	7	5	8	1	3	83
TTTGTCAGTGCA	5	4	12	4	6	82
TTGGTCAGTGCG	2	3	1	2	1	50
CTGGTCAGTGCA	1	2	1	2	1	40
GTGGTCAGTGCA	1	2	4	0	5	36
TTGGTCAGTGCC	0	2	2	6	3	30
ATGGTCAGTGCA	2	4	1	0	2	26
TCGGTCAGTGCA	0	2	1	0	3	20

b

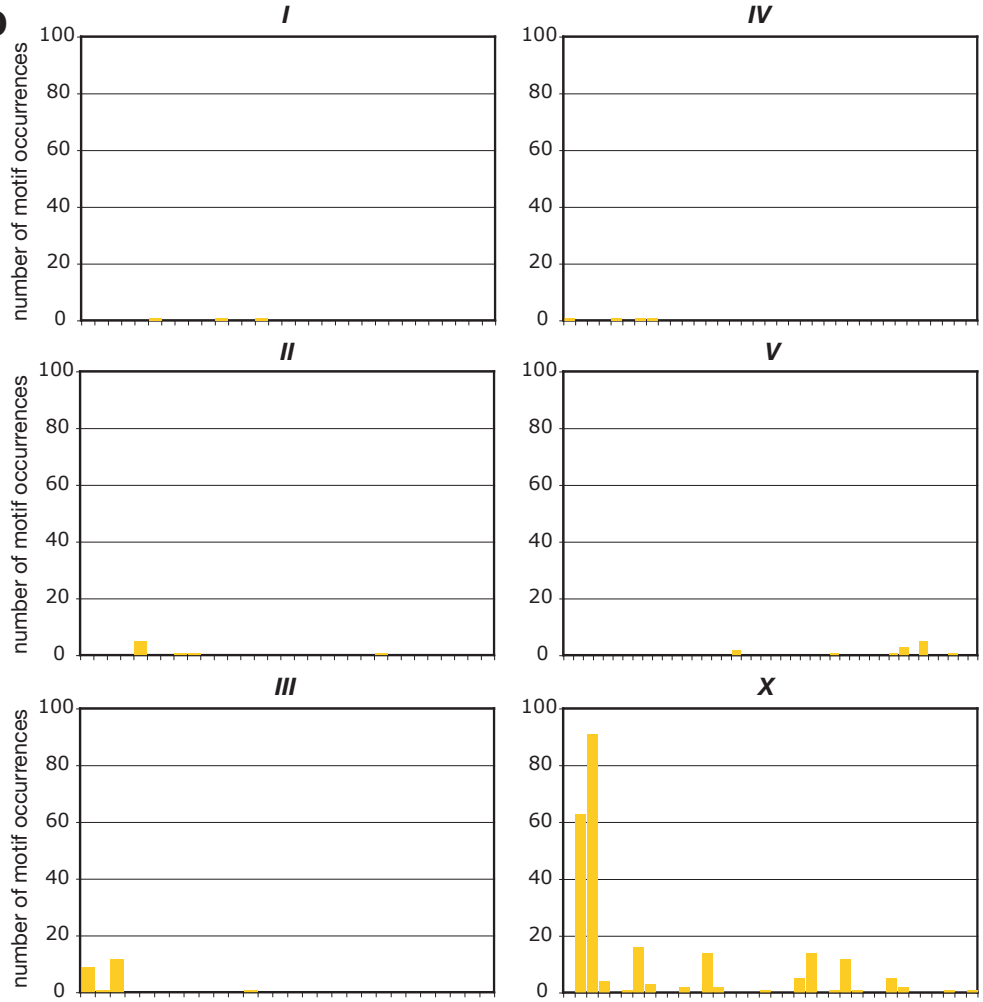


Figure S1 HIM-8 recruitment motifs. (**a**) A family of related motifs is enriched on the *X* chromosome relative to the autosomes. The two most abundant motifs both recruit HIM-8 in our extrachromosomal array assay. (**b**) Distribution of the most abundant HIM-8 recruitment motif (yellow in (**a**)). The number of occurrences in each 500-kb region is plotted over the length of each chromosome. The majority of HIM-8 recruitment motifs on the *X* chromosome lie between 0.5 and 1.5 Mb from the left end.

a

	I	*II*	*III*	*IV*	*V*	*X*
TTGGTTGAGGCT	797	1	10	190	1	2
TTGAGTGAGGCT	91	1	1	126	4	4
TTGATTGAGGCT	26	1	2	21	2	4
TTAAGTGAGGCT	8	0	0	20	0	1
TTCGTTGAGGCT	20	0	1	6	0	1
TTGGTTGAGACT	11	0	0	12	2	0

b

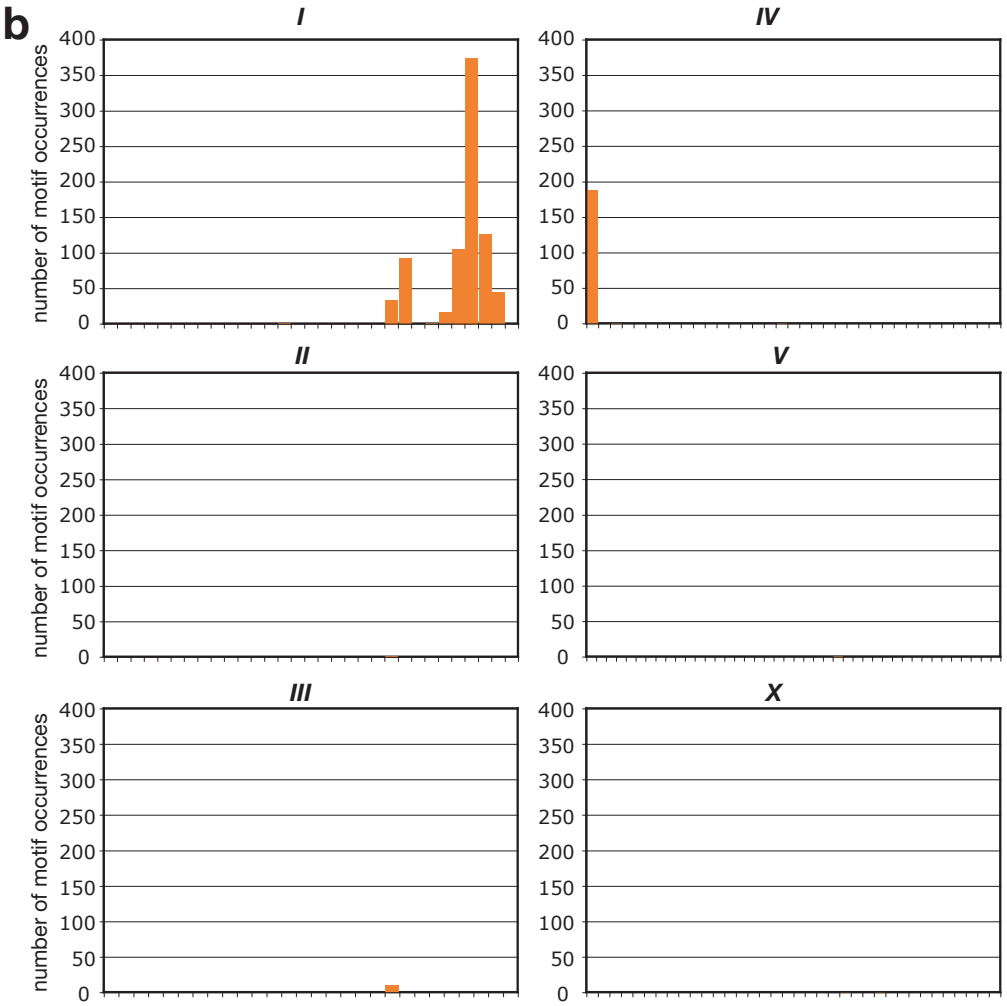


Figure S2 ZIM-3 recruitment motifs. (**a**) A family of related motifs is enriched on chromosomes *I* and *IV* relative to the other chromosomes. (**b**) Distribution of the most abundant ZIM-3 recruitment motif (orange in (**a**)). The number of occurrences in each 500-kb region is plotted over the length of each chromosome.

a

	I	*II*	*III*	*IV*	*V*	*X*
TGGGTCTGCTA	1	277	228	2	61	3
TTGGTCTGCTA	1	157	202	1	28	7
TGGGTATGCTA	2	7	133	1	0	2
ATGGTCTGCTA	3	25	75	2	24	6

b

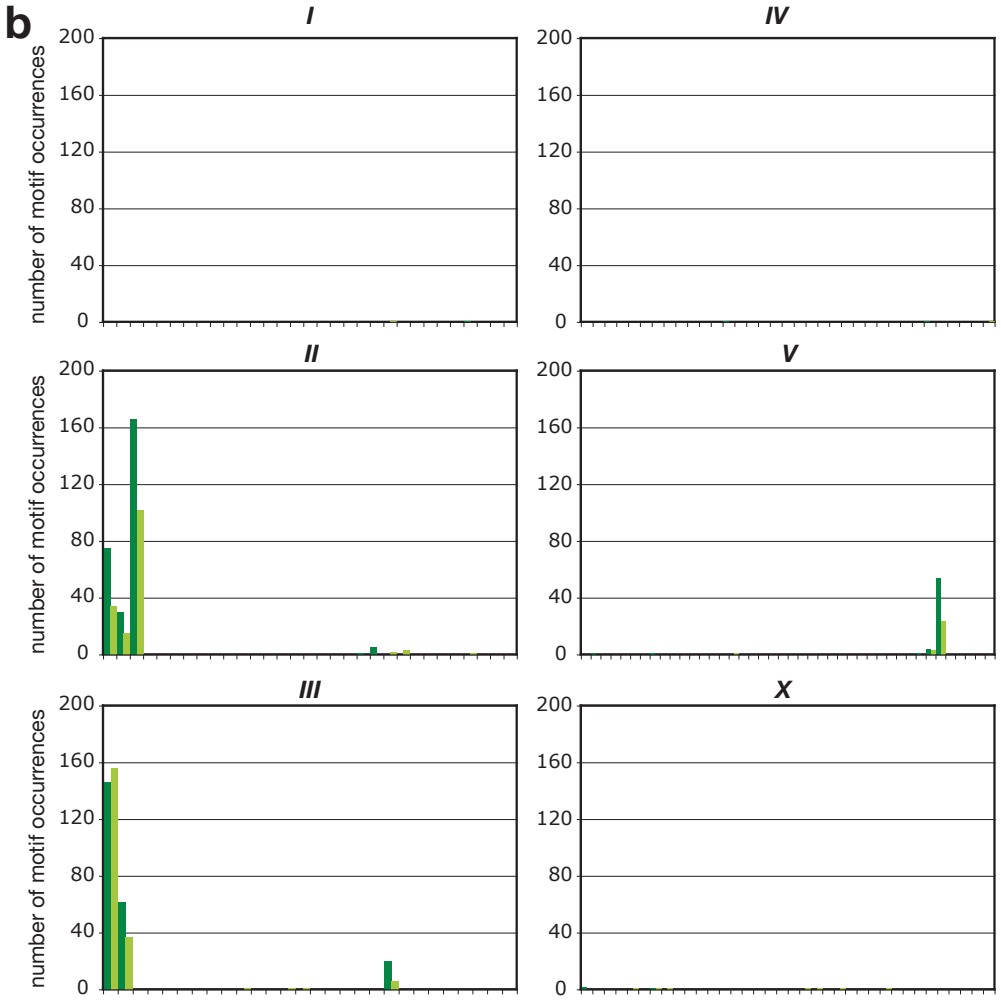


Figure S3 ZIM-1 recruitment motifs. (**a**) A family of related motifs is enriched on chromosomes *II* and *III* relative to the other chromosomes. (**b**) Distribution of the two most abundant ZIM-1 recruitment motifs (dark and light green in (**a**)). The number of occurrences in each 500-kb region is plotted over the length of each chromosome.

a

	I	*II*	*III*	*IV*	*V*	*X*
TTGGGCGCTGCT	0	1	0	0	926	90
TTGGTCGCTGCT	3	0	2	1	105	9
TTAGGCGCTGCT	0	0	0	1	44	3
TTGAGCGCTGCT	1	2	1	0	17	1
TTGGGCTCTGCT	2	1	1	0	11	1

b

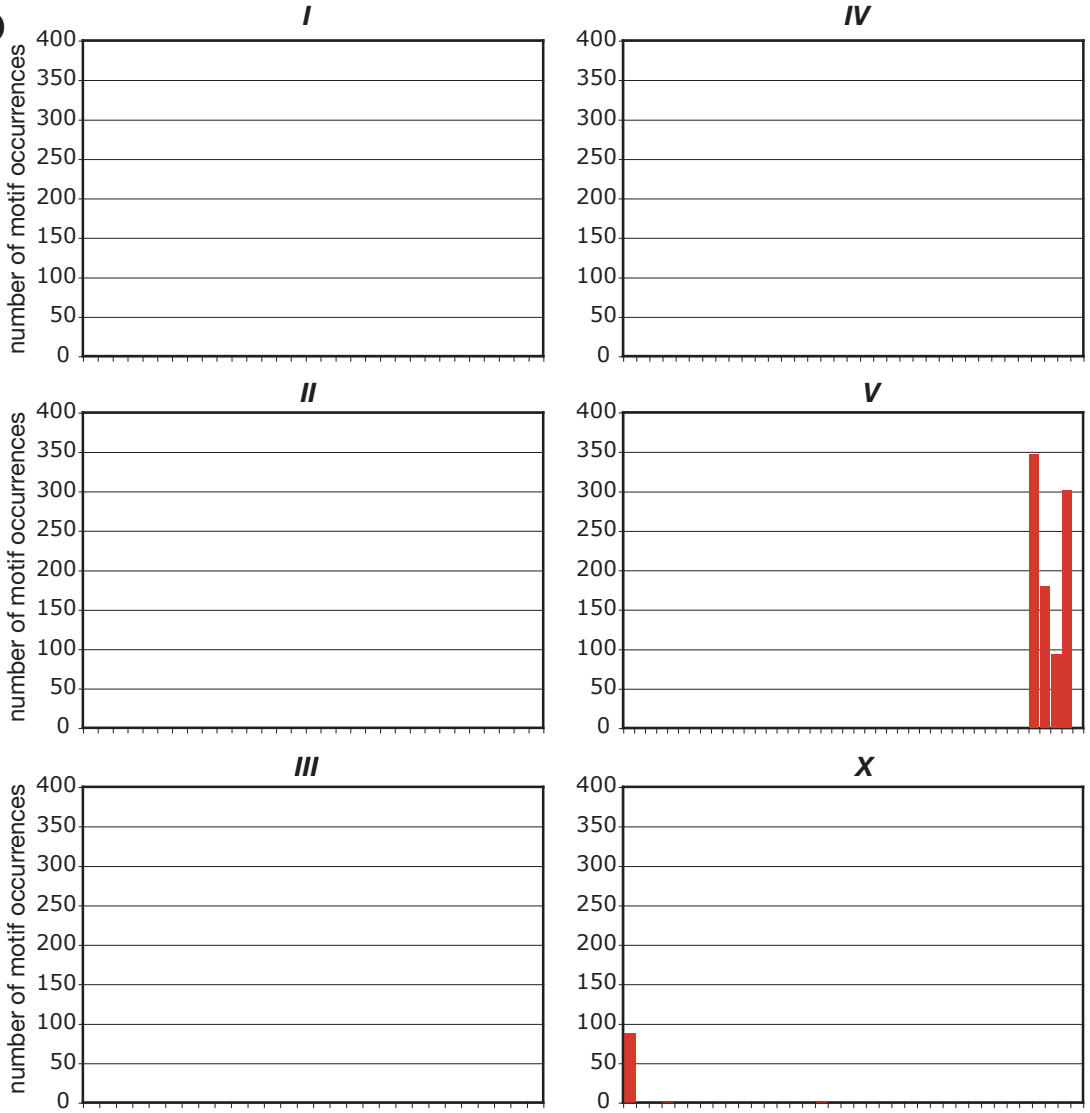


Figure S4 ZIM-2 recruitment motifs. (**a**) A family of related motifs is enriched on chromosome *V* relative to the other chromosomes. (**b**) Distribution of the most abundant ZIM-2 recruitment motif (red in (**a**)). The number of occurrences in each 500-kb region is plotted over the length of each chromosome.

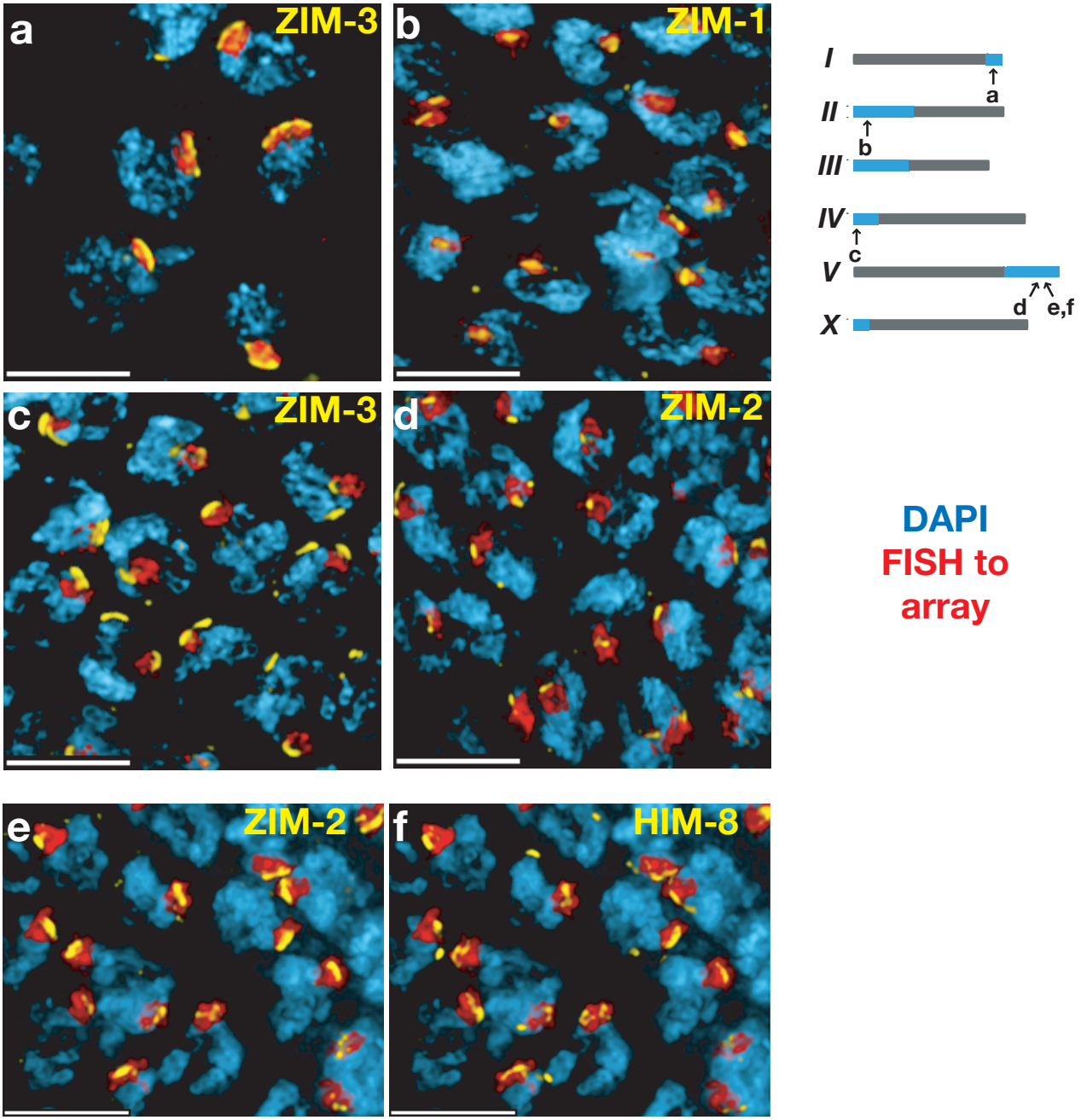
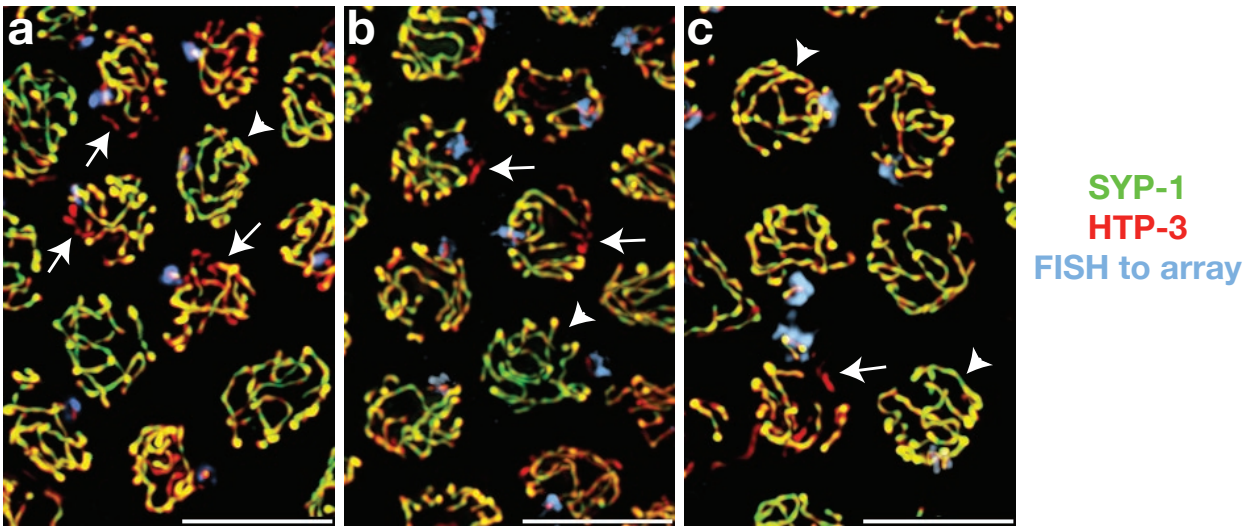


Figure S5 Recruitment of ZIMs to autosomal motifs. (**a-f**) Immunostaining of the indicated ZIM protein (yellow) was combined with FISH to the extrachromosomal array (red). Diagram to the right indicates location in the genome from which each PCR product in (**a-f**) was generated. See Materials and Methods and table S1 for details. Recruitment of ZIM-2 by an amplicon from chromosome *V* (**d**) was less robust than the other examples. An extrachromosomal array generated from a segment on chromosome *V* recruits both ZIM-2 (**e**) and HIM-8 (**f**). The proteins show similar but not precisely overlapping localization to the array. Note that in (**f**), a HIM-8 focus can be seen that localizes to the endogenous *X* chromosome PC, in addition to the array. All images are maximum-intensity projections of deconvolved 3D stacks. Scale bars represent 5μm.



d

	% males (# of adults scored)	range of % males	% viable embryos (# of embryos scored)	range of % viable embryos
I	0.7 (1046)	0.0 - 2.5	76.4 (1370)	61.8 - 86.7
II	0.7 (1243)	0.0 - 5.1	70.9 (1754)	21.7 - 98.9
X	4.3 (2991)	2.1 - 12.3	94.4 (3170)	85.6 - 100.0

Figure S6 ZIM/HIM-8 recruiting arrays produce meiotic defects. (**a-c**) Unsynapsed chromosomes are visualized as regions of HTP-3 staining (red) that lack SYP-1 (green). Extrachromosomal arrays are detected by FISH to λ-phage DNA (blue). Nuclei with unsynapsed (arrows) and fully synapsed chromosomes (arrowheads) are observed. Some nuclei lack the array, due to irregular mitotic segregation and resulting mosaicism. All images are maximum intensity projections. Scale bars represent 5 μm. (**a**) ZIM-3 recruiting array derived from the chromosome *I* PC region. (**b**) ZIM-1 recruiting array generated from the chromosome *II* PC region. (**c**) HIM-8 recruiting array generated from the *X* chromosome PC region. (**d**) Frequency of males and viable embryos observed among whole broods of hermaphrodites carrying extrachromosomal arrays generated from the PC region of chromosome *I* (*ieEx69*), *II* (*ieEx29*), or *X* (*ieEx41*). The ranges of observed segregation defects for individual hermaphrodites carrying the same array are indicated. Individual arrays preferentially disrupted segregation of the particular chromosomes that depend on the ZnF protein they recruit. For example, many of the HIM-8 recruiting lines produced abundant male self-progeny but few dead embryos, indicating a preferential effect on meiotic segregation of the *X* chromosomes. In contrast, several ZIM-1 and ZIM-3 recruiting lines produced many dead eggs with a moderate number of males, more reminiscent of the *zim* mutant phenotypes (C. M. Phillips, A. F. Dernburg, *Dev Cell* **11**, 817 (Dec, 2006)).

Chromosome	Name	Description	Figure	Type	Start location	End location	Length	Forward primer	Reverse primer	Recruitment	Co-transformation Marker
X	*ieEx2*	T08D2		cosmid	159,154	199,130	39,977			none	*unc-119+*
	ieEx4, ieEx5, ieEx8	cosmid pool A		pool			98,652			none	*rol-6*
		(ZK380)		cosmid	1,628,595	1,656,984	28,390				
		(R09H3)		cosmid	1,657,483	1,668,169	10,687				
		(F07G6)		cosmid	1,702,425	1,741,393	38,969				
		(F31A9)		cosmid	1,777,293	1,797,898	20,606				
	not available	cosmid pool B		pool			168,964			HIM-8	*myo-2::GFP*
		(K06A9)		cosmid	1,520,293	1,559,865	39,573				
		(F07G6)		cosmid	1,702,425	1,741,393	38,969				
		(B0294)		cosmid	1,892,089	1,902,865	10,777				
		(F52D2)		cosmid	1,948,255	1,989,639	41,385				
		(F49H12)		cosmid	2,056,571	2,094,830	38,260				
	not available	B0294		cosmid	1,892,089	1,902,865	10,777			none	*myo-2::GFP*
	not available	K06A9		cosmid	1,520,293	1,559,865	39,573			HIM-8	*myo-2::GFP*
	ieEx9	K06A9-left		pool	1,525,558	1,540,404	14,847			none	*myo-2::GFP*
				PCR product	1,525,558	1,530,937	5,380	gtgcattgatttgggagagg	cagaaacggttctcaagtgg		
				PCR product	1,530,918	1,536,182	5,265	ccacttgagaaccgtttctg	ttgggatggagtgcttttgg		
				PCR product	1,536,161	1,540,404	4,244	atccaaaagcactccatccc	cctaccaatctgaagtccag		
	not available	K06A9_5kb		PCR product	1,540,385	1,545,886	5,502	ctggacttcagattggtagg	gagggacatacacctttgtc	HIM-8	*unc-119+, myo-2::GFP*
	ieEx10, ieEx11	K06A9_1kb_1		PCR product	1,540,385	1,541,396	1,012	ctggacttcagattggtagg	ggctcaaaaagtgaacaggg	none	*myo-2::GFP*
	ieEx12	K06A9_1kb_2		PCR product	1,541,260	1,542,366	1,107	acgccccgttacttttttcc	ctaatcagttttggcaagcttc	none	*myo-2::GFP*
	ieEx13	K06A9_1kb_3		PCR product	1,542,115	1,543,479	1,365	acataccgacatcatagcag	gaggtgtgaaatgttgcagg	none	*myo-2::GFP*
	ieEx14	K06A9_1kb_5		PCR product	1,544,055	1,545,118	1,064	gtcgtgacaatcacatgacac	tattccacaagtctcggagg	HIM-8	*myo-2::GFP*
	ieEx15	K06A9_1kb_6		PCR product	1,544,910	1,545,886	977	ttagttctccggcttatccc	gagggacatacacctttgtc	none	*myo-2::GFP*
	ieEx17	K06A9_546bp		PCR product	1,544,055	1,544,600	546	gtcgtgacaatcacatgacac	cacaatttatgagtggtagatc	none	*unc-119+, myo-2::GFP*
	ieEx18-ieEx26	K06A9_539bp	1f	PCR product	1,544,477	1,545,015	539	tgataagaaacttttgtaaatggc	gaattgatatgcaaaagttgatc	HIM-8	*unc-119+, myo-2::GFP*
	ieEx39-ieEx42	left_end_1	1g, 4a, S6c	PCR product	1,292,750	1,293,641	892	actatgtgaccattgcacccc	gacccagttggaagcattcc	HIM-8	*unc-119+, myo-2::GFP*
	ieEx44, ieEx47	left_end_2		PCR product	920,701	921,111	411	cactgacgacaattaccacg	aattggtagagggaaatggc	HIM-8	*unc-119+, myo-2::GFP*
	ieEx73, ieEx74	middle		PCR product	6,286,941	6,288,814	1,874	tccagaagtatttgtccgcc	atggactaaagacggaccac	HIM-8	*rol-6*
	ieEx71, ieEx72	right_end		PCR product	12,126,536	12,127,269	734	agacaacattcacagcctgc	tcccattcagagtttccctg	HIM-8	*rol-6*
	ieEx56, ieEx60	84 bp oligo		oligonucleotide			84			HIM-8	*unc-119+, myo-2::GFP*
I	*ieEx69, ieEx70*	*I*	S5a, S6a	PCR product	13,883,701	13,885,547	1,847	gacacttactgcacagcagg	tcatttgtgtccagtagggg	ZIM-3	*unc-119+, myo-2::GFP*
II	*ieEx29, ieEx30*	*II*	S6b, S6b	PCR product	1,303,119	1,304,835	1,717	tagtaggaacgagccaattcc	attggagaaatttgggcctgc	ZIM-1	*unc-119+, myo-2::GFP*
III	*ieEx31, ieEx32*	*III*		PCR product	78,566	79,885	1,320	cgatttgtcacgtgtagaagtg	atttagcagacccagctatgg	ZIM-1 (weak)	*unc-119+, myo-2::GFP*
	ieEx78	*III*		PCR product	340,603	341,464	862	ttttgttcgggcgtcattcg	aatgcgaagaaggcagaagc	ZIM-1 (weak)	*rol-6*
	ieEx77	*X* PC on *III*	1h	PCR product	1,243,666	1,244,174	509	ggataggagttttttgccgc	tgaattttgccataaattgccc	HIM-8	*rol-6*
IV	*ieEx33, ieEx34*	*IV*	4b, S5c	PCR product	122,506	123,738	1,233	gatgccagcttgtcgaagag	gaggatgatgttccgttgag	ZIM-3	*unc-119+, myo-2::GFP*
	ieEx37, ieEx38	40dH08	4c	fosmid	2,441,775	2,477,542	35,768			none	*unc-119+, myo-2::GFP*
V	*ieEx35, ieEx36*	*V* (32bp)	S5d	PCR product	18,877,099	18,878,680	1,582	tcccaaactcggcaaacctg	tttgtagcgttagcagcacc	ZIM-2 (weak)	*unc-119+, myo-2::GFP*
	ieEx75, ieEx76	*V* (18bp)	S5e, S5f	PCR product	19,110,852	19,112,083	1,232	cacgttgttttggttggagg	tctttggagctgttgaagcg	ZIM-2/HIM-8	*rol-6*

Table S1 Transgenic lines generated for ZIM/HIM-8 recruitment assays. The genomic location, size of clone or amplicon, primers (if applicable), and co-transformation markers are indicated for the transgenic lines generated in this work. Also indicated are the results of the recruitment assay and the figure (if any) in which cytological data are presented.

Chromosome	ZIM	Recruitment motif	Orientation	Spacing	
I	ZIM-3	TTGGTTGAGGCT	inverted	35-40 bp / 28 bp	total interval of 68 bp
II	ZIM-1	TGGGTCTGCTA TTGGTCTGCTA	inverted	28 bp / 35 bp	total interval of 63 bp
III	ZIM-1	TGGGTCTGCTA TTGGTCTGCTA	inverted	14 bp / 17 bp	total interval of 31 bp
IV	ZIM-3	TTGGTTGAGGCT	tandem	19 bp	
V	ZIM-2	TTGGGCGCTGCT	tandem	32 bp	
V	ZIM-2	TTGGGCGCTGCT TTGGTCGCTGCT	tandem	18 bp	
X	HIM-8	TTGGTCAGTGCA	tandem	21 bp	

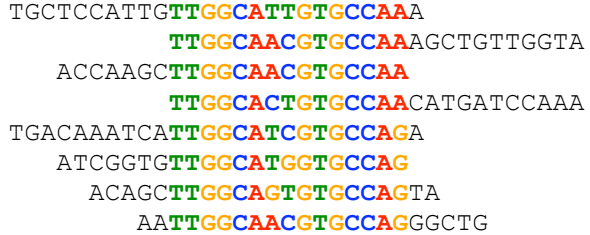
Table S2 ZIM/HIM-8 recruitment motifs vary in spacing and relative orientation. Relative orientation and spacing of recruitment motifs within clusters are indicated. Both isolated, inverted pairs and larger inverted clusters of ZIM-3 recruitment motifs are common in the PC region of chromosome *I*. By contrast, chromosome *IV* contains this motif primarily in closely spaced tandem clusters. On both chromosomes *II* and *III*, ZIM-1 binding clusters contain alternating copies of the two most common motifs in inverted orientation. However, the predominant spacing between clustered copies is different on the two chromosomes. Chromosome *V* has two subtypes of binding site clusters, both containing motifs in tandem orientation. One type contains only the TTGGGCGCTGCT with a predominant spacing of approximately 32 bp. The second class contains a mixture of TTGGGCGCTGCT and TTGGTCGCTGCT, with a tighter spacing.

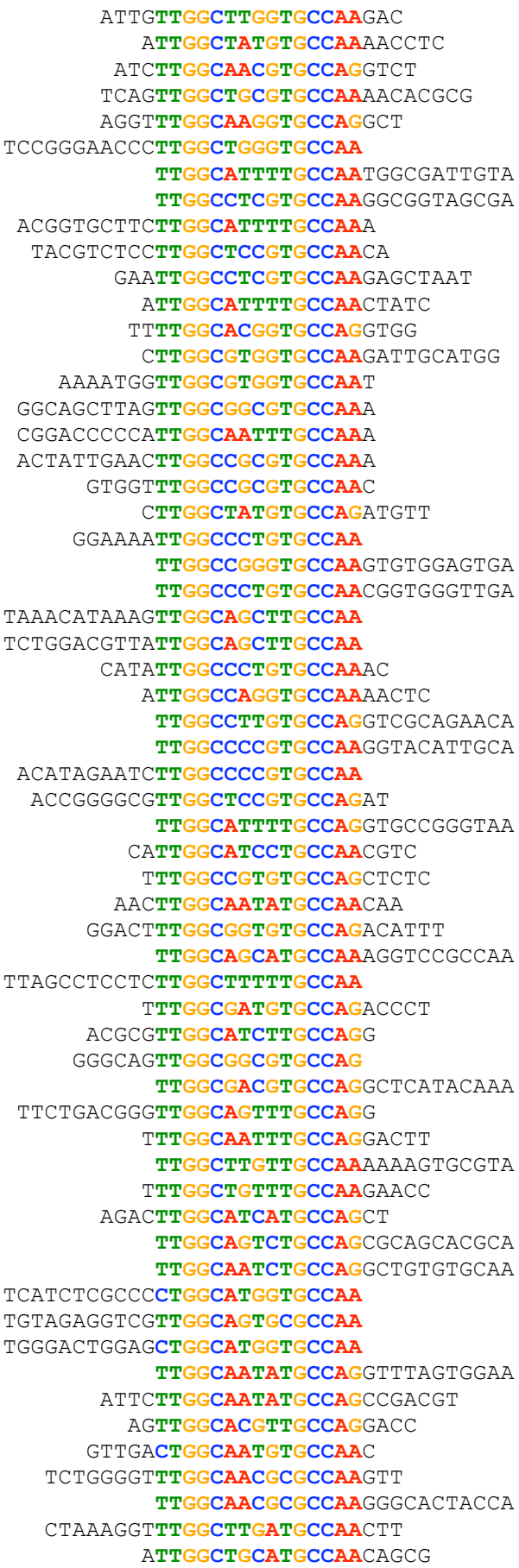
Table S3. Oligonucleotide sequences from SELEX assays. Unique oligonucleotides bound by the ZIM-2, ZIM-3, and HIM-8 protein fragments following four cycles of the SELEX assay. The sequences have been aligned and the consensus motifs derived by MEME analysis are highlighted.

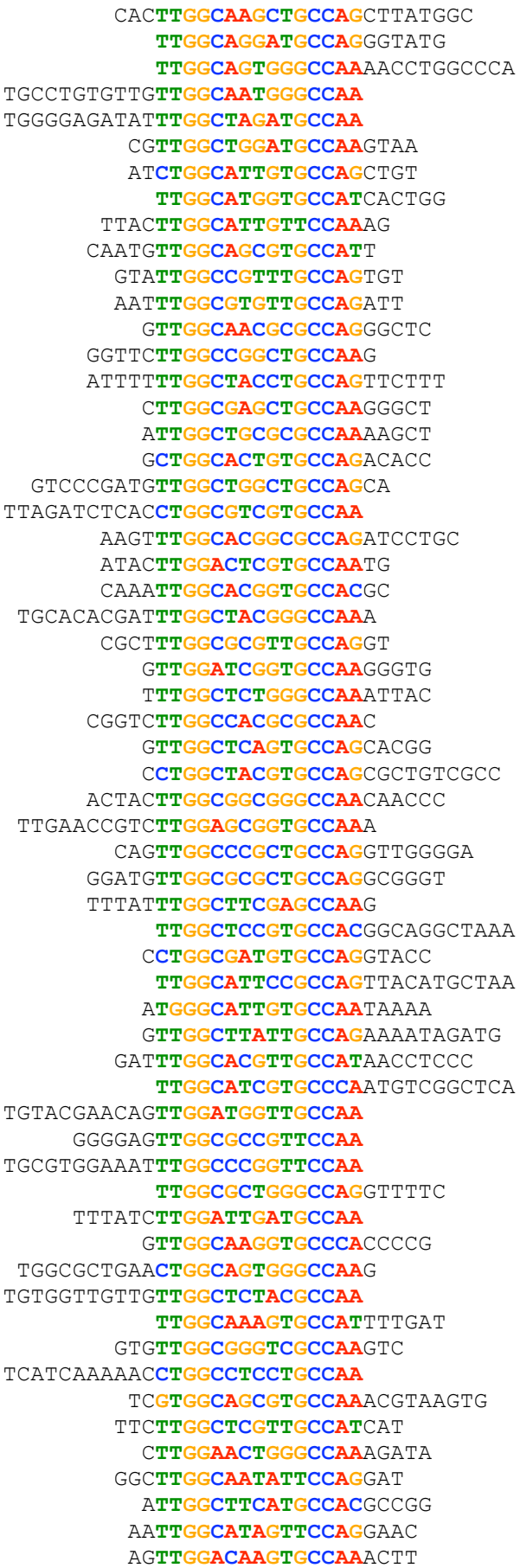
(a) Oligonucleotides bound to ZIM-2 ZnF core fragment
(101 unique sequences, 21 bp each)

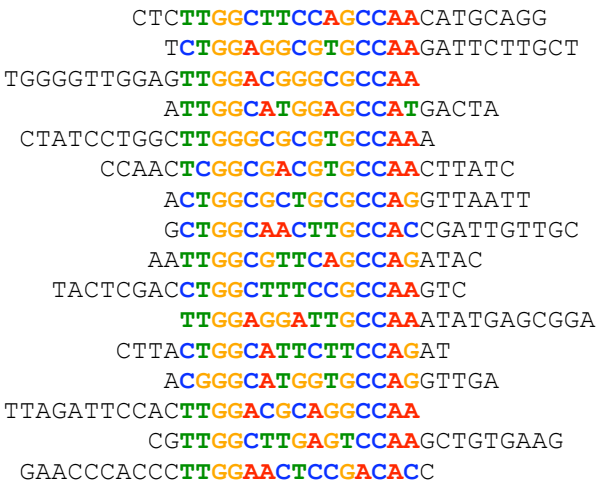
```
   CGTGTTGGCAAGGTGCCAAAG
    CTGTTGGCACGCTGCCAAGTG
   TGAATTGGCACCCTGCCAAAT
      CTTGGCACGCGGCCAAGGCCT
    CACTTGGCAATCTGCCAAGAA
     ACCTGGCAAGGTGCCAACCTC
 CAATCTCTGGCACGCTGCCAA
   TCACCTGGCAAGGTGCCAAGT
  GACTCTTGGCAACGGGCCAAA
   CATGTTGGCAATGCGCCAATA
   CATGTTGGCAATGCGCCAATA
      CCTGGCACCCTGCCAACCCAG
   GACCCTGGCAATCTGCCAATA
  TGACGCTGGCACGATGCCAAG
   GGCTTTGGCACGCTTCCAATTA
       CTGGCACGATGCCAAGGTTGG
    TTTTTGGCAAGGTTCCAATAT
     ATTTGGCATTGGGCCAAAGGA
       TTGGCACCCAGCCAAGGGCCA
  TACCGCTGGCACCCGGCCAAT
    ATGTTGGCAGGGCGCCAAGTG
  ATGGTTTGGCAATGTTCCAAA
  GCTATTTGGCACGAAGCCAAT
  TCAACCTGGCACCATGCCAAC
     TGCTGGCAGGGTGCCAATTAG
      CTTGGCAACCAGCCAAATAAC
    ACCTCTGGCAATGCGCCAAAC
  CCAGAATTGGCACTCTGCCAGC
      ACTTGGCATTGCGCCAAAGGG
     CATCTGGCACCCCGCCAATCT
       CCTGGCACCCCGCCAACTGCT
       TCTGGCACGAGGCCAATTGGT
    ATCACTGGCATTCTGCCAACC
   GCACCTTGGCAACCTGCCAGA
   ACCGCTTGGCACACGGCCAAT
      ACCTGGCACGGTGCCAGTGGA
       CTTGGCAACCTGCCAGGGCGC
   TCCAGTTGGCAGTCCGCCAA
      GGTTGGCAACGGTCCAACAGC
        TTGGCACCGCTCCAACACAGA
      GATTGGCATGACGCCAAGACG
        TTGGCATCGAGCCAACAATCA
  AGAGTGTTGGCAAGTCGCCAA
    CTTACTGGCAAGTTGCCAAAT
      AGTTGGCAATATGCCAGGATT
        ATGGCACGGTGCCAAACTTTAC
  CCGAGGTTGGCACGTAGCCAA
  AGGTGCTGGCACAGGGCCAAG
      CTTGGCAGGGGTCCAATTAGT
 TTGGTCTTGGCAACGATCCAA
  AAATTCTGGCAAAGGGCCAAATT
  GTAAATTGGCACTACGCCAGC
       CTGGCACTTGGCCAACTGTGG
```

(b) Oligonucleotides bound to ZIM-3 ZnF core fragment
(144 unique sequences, 21 and 26 bp)

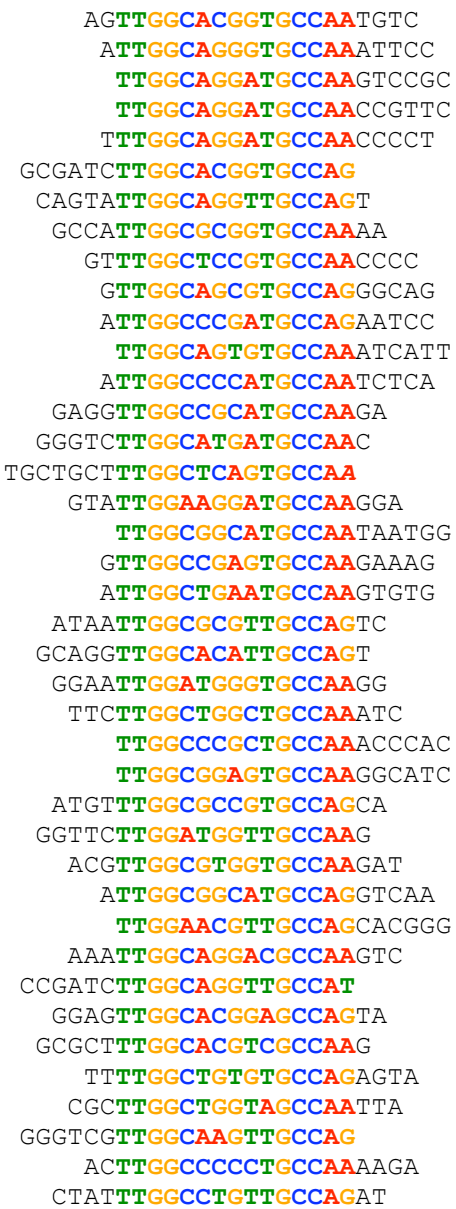


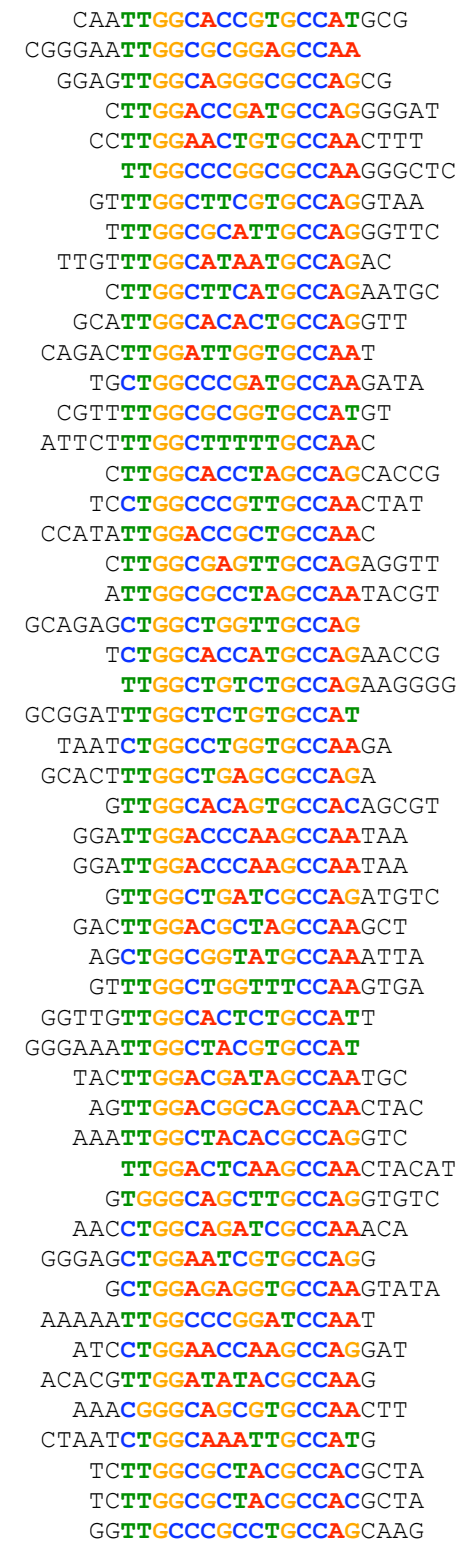




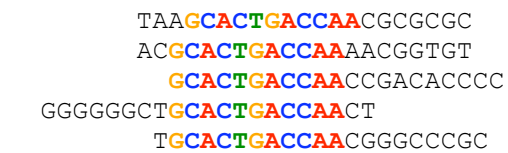


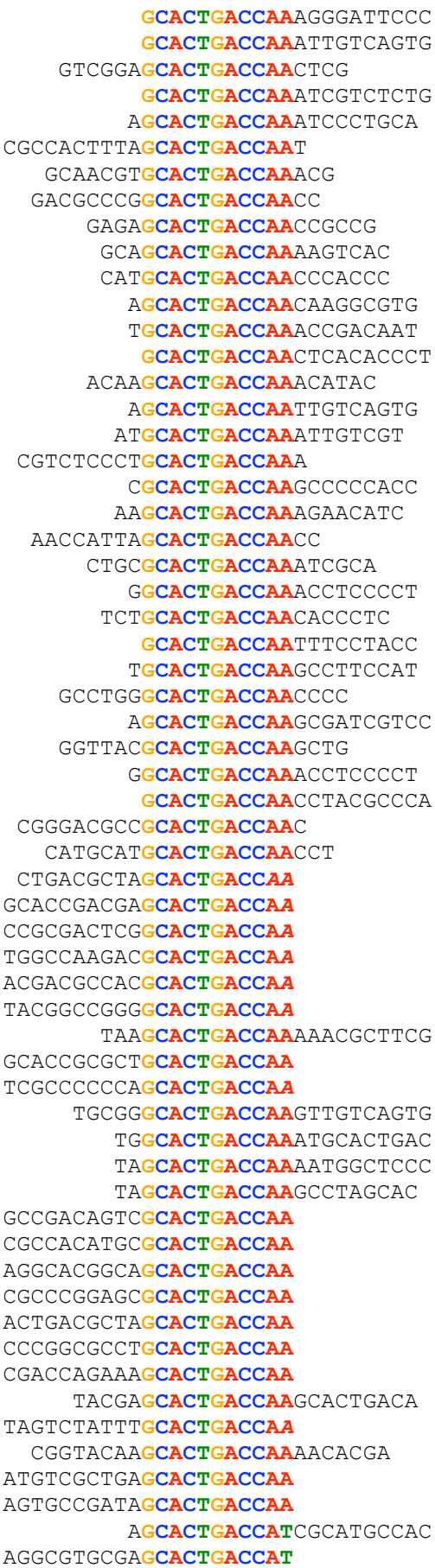
(c) Oligonucleotides bound to HIM-8 ZnF core fragment
(91 unique sequences, 21 bp each)

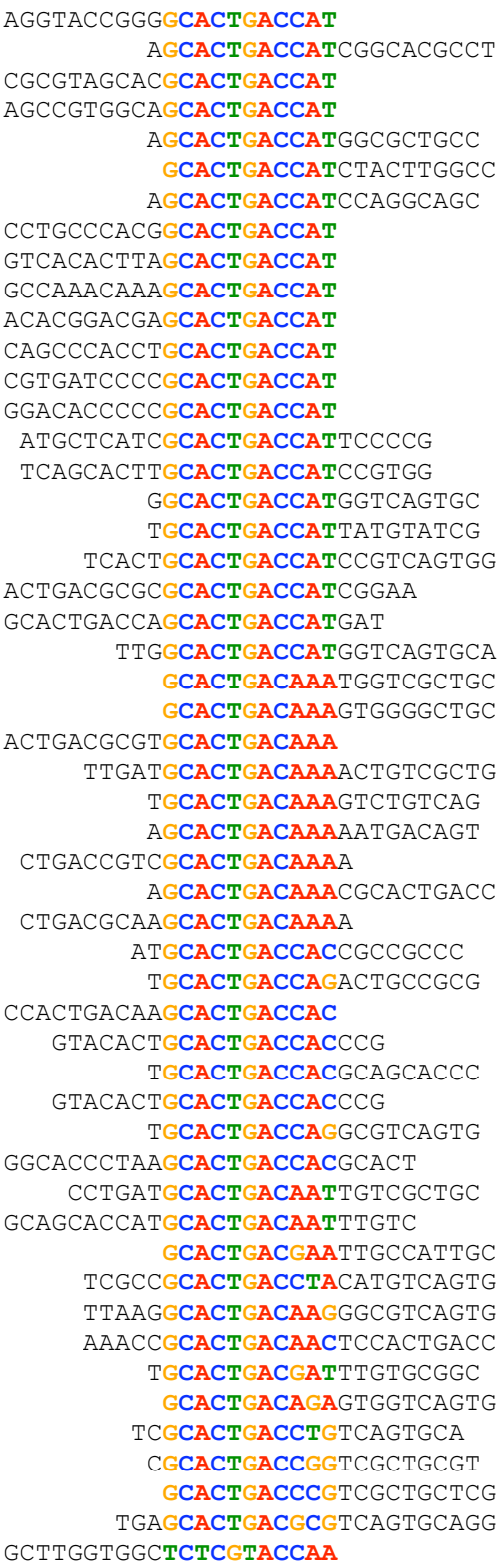




(d) Oligonucleotides bound to HIM-8 C-terminal fragment
(117 unique sequences, 21 and 26 bp)







(a) ***ieIs13/+; ieIs15/+; syp-2*** **RNAi**

	paired arrays	unpaired arrays
1	35	0
2	45	1
total	80	1
percent	**98.8**	**1.2**

(b) ***ieIs13/+; ieIs15/+***

	incomplete synapsis; paired arrays	complete synapsis; unpaired arrays	complete synapsis; paired arrays	incomplete synapsis; unpaired arrays
1	34	8	3	9
2	41	2	6	0
3	55	9	2	13
total	130	19	11	22
percent	**71.4**	**10.4**	**6.0**	**12.1**

Table S4 Artifical PCs can promote pairing and synapsis between nonhomologous chromosomes. (**a**) The number of nuclei with paired ZIM/HIM-8 recruiting arrays in *ieIs13/+; ieIs15/+; syp-2* RNAi was scored in two gonads from different animals. (**b**) Nuclei in three individual gonads of the genotype *ieIs13/+; ieIs15/+* were scored for complete or incomplete synapsis and pairing of the integrated ZIM/HIM-8 recruiting arrays.