The inferred HIM-5 amino acid sequence

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MSRIRSNNDN IIILTDEQRK TVGRIAGRSQ NRNTSKKIAD GPYFLPRYRI
RDNAERSVGA RFKSLPQKEQ DEVVNEAFSN LREYLKKREP FYAKLRKANS
KYSSKPKERE KSVDSNDEAD RRNKGNKKTQ KNASKNCQIE KSSNNSGILK
KSGSGISVAS KPKKSVAFAP GVYEDLSTDD DLEFLNSVIV NSDRPTSQCD
NPARRMCGRP PTKHRDTEQS QEITGSKKQK IFPTPHEKPA WWSFRIPKKR
AQ
```

Results from using LALIGN to compare him-5 with hsp83 from *Drosophila melanogaster*.

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Waterman-Eggert score: 72;  21.6 bits; E(1) <  0.054
24.0% identity (53.8% similar) in 104 aa overlap (48-151:158-255)
        50        60        70        80        90       100       110       120
him-5  YRIRDNAERSVGARFKSLPQKEQDEVVNEAFSNLREYLKKREPFYAKLRKANSKYSSKPKEREKSVDSNDEADRRNKGNK
       . .: .  . .:   : .   ..:..     :...: ..:.  : .   :   .     ::::: : :.:::: ..: .
HSP83  FTVRADNSEPLGRGTKIVLYIKEDQTDYLEESKIKEIVNKHSQFIGYPIKLLVE-----KEREKEV-SDDEADDEKKEGD
       160       170       180       190       200       210             220       230

       130       140       150
him-5  KTQKNASKNCQIEKSSNNSGILKK
       . ..  . . .::  ...    ::
HSP83  EKKEMETDEPKIEDVGEDEDADKK
             240       250
```

Figure S1 The inferred amino acid sequence of HIM-5 is highly basic and novel.The region of optimal alignment with HSP83 from Drosophila melanogaster is highlighted in red and the alignment is shown at the bottom. The E value is 0.05, as shown. The sequence KEREKxVxSxxDEAD is not identical in the HSP83 proteins from other species so its function is not known.

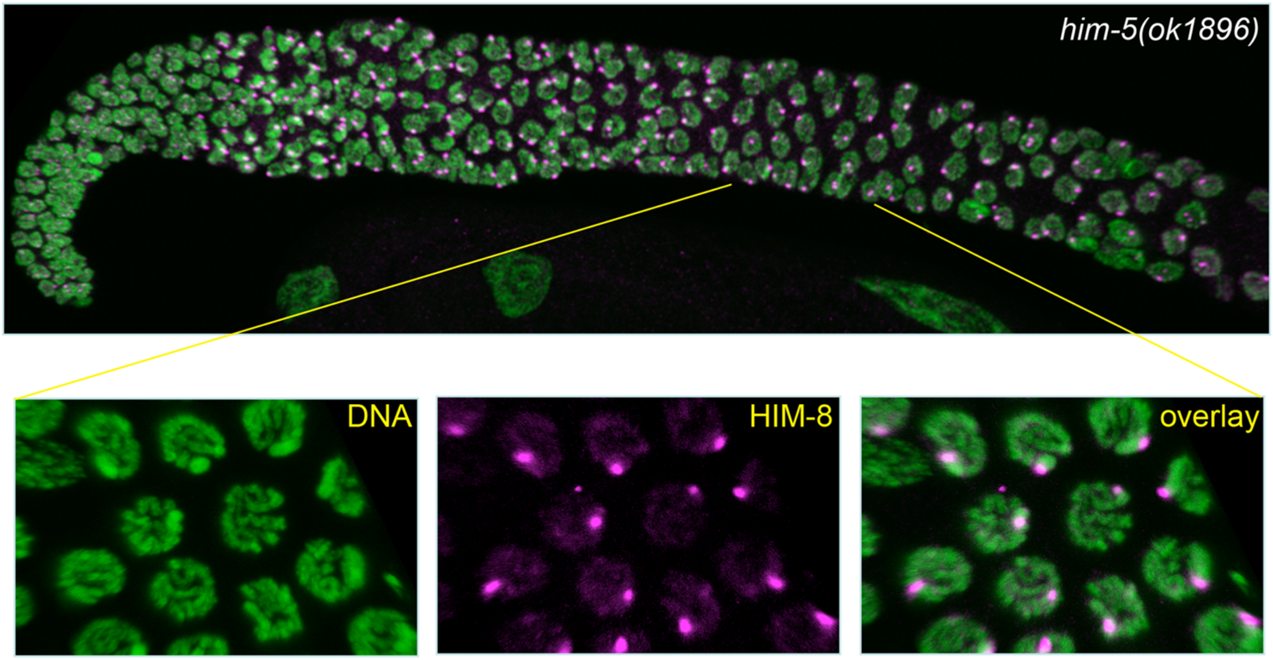

Figure S2 Pairing is normal in him-5 mutants. Shown is a him-5(ok1896) germline (top) and a zoomed in region of mid-pachytene (below). Anti-HIM-8 (magenta) staining indicates that full pairing is achieved between X chromosomes.

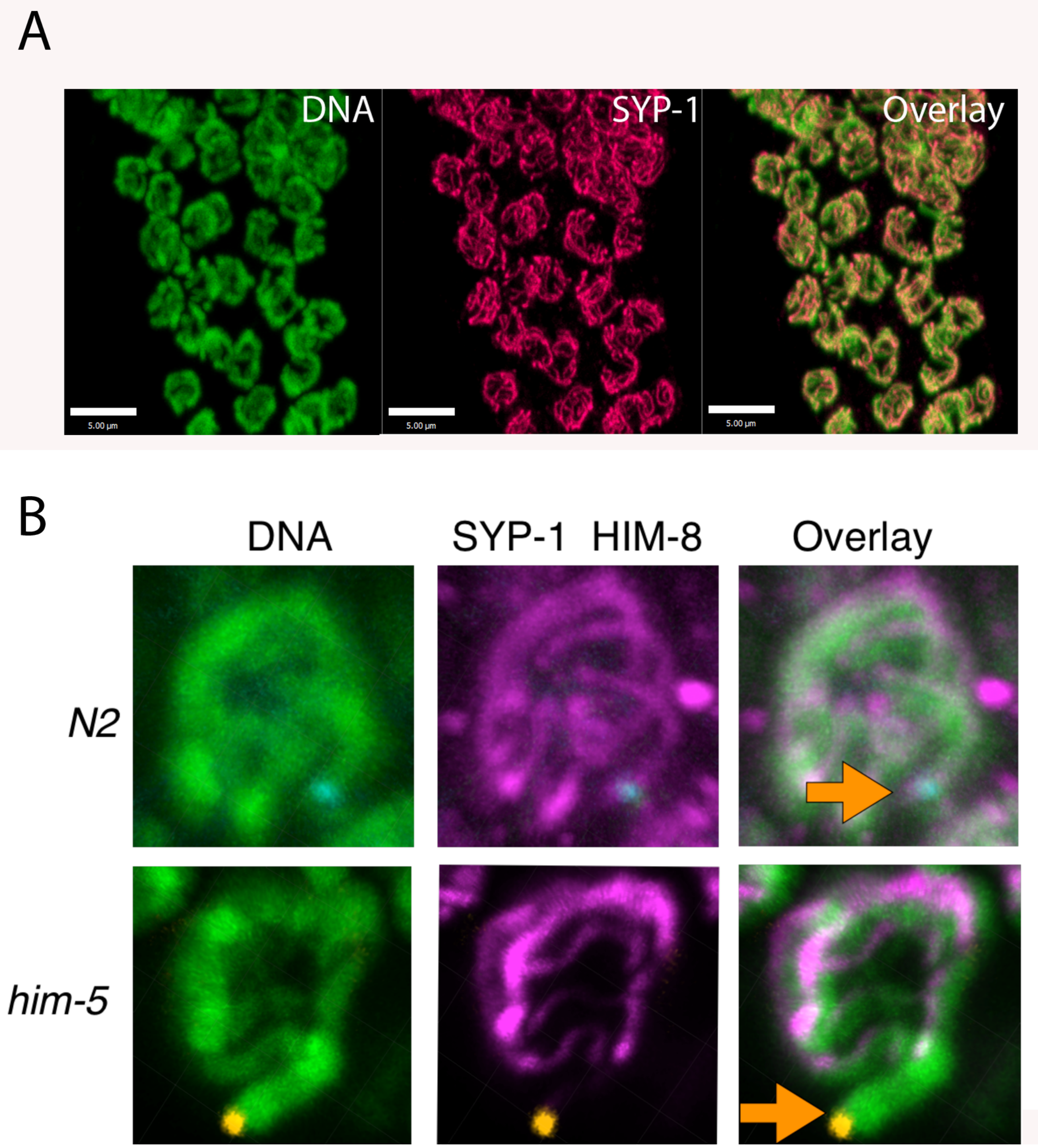

Figure S3 him-5 mutants have a desynapsed X chromosome at pachytene. A. The SC is fully established in early pachytene as shown by the complete coincidence of SYP staining (magenta) with DNA (green). B. A mid-to-late pachytene nucleus stained for DNA (green), SYP-1 (magenta), and HIM-8 (cyan in wild type; yellow in him-5) is shown. In wild type, all chromosomes are fully synapsed. As seen in the overlay on the bottom row, one chromosome is desynapsed and lacks SYP-1 staining in him-5(ok1896) mutants. The desynapsed chromosome stains with an antibody against the X chromosome pairing center binding protein, HIM-8 indicating that this is the X chromosome. Note that a single HIM-8 focus is seen indicating that the X chromosomes remain paired after desynapsis.

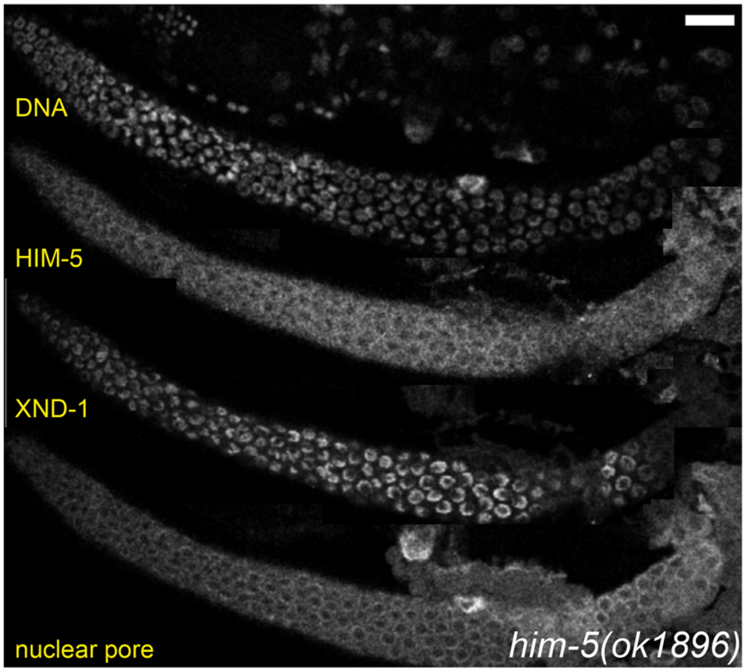

Figure S4 A *him-5* deletion lacks HIM-5 but retains localized XND-1. Germlines from *him-5(ok1896)* were stained for DNA (top), HIM-5, XND-1, and the nuclear pore, as indicated. The *him-5* mutants lack HIM-5 staining, as expected for the deletion. Conversely, XND-1 stains normally, indication that the wild type activity of *him-5* is not needed for XND-1 localization.

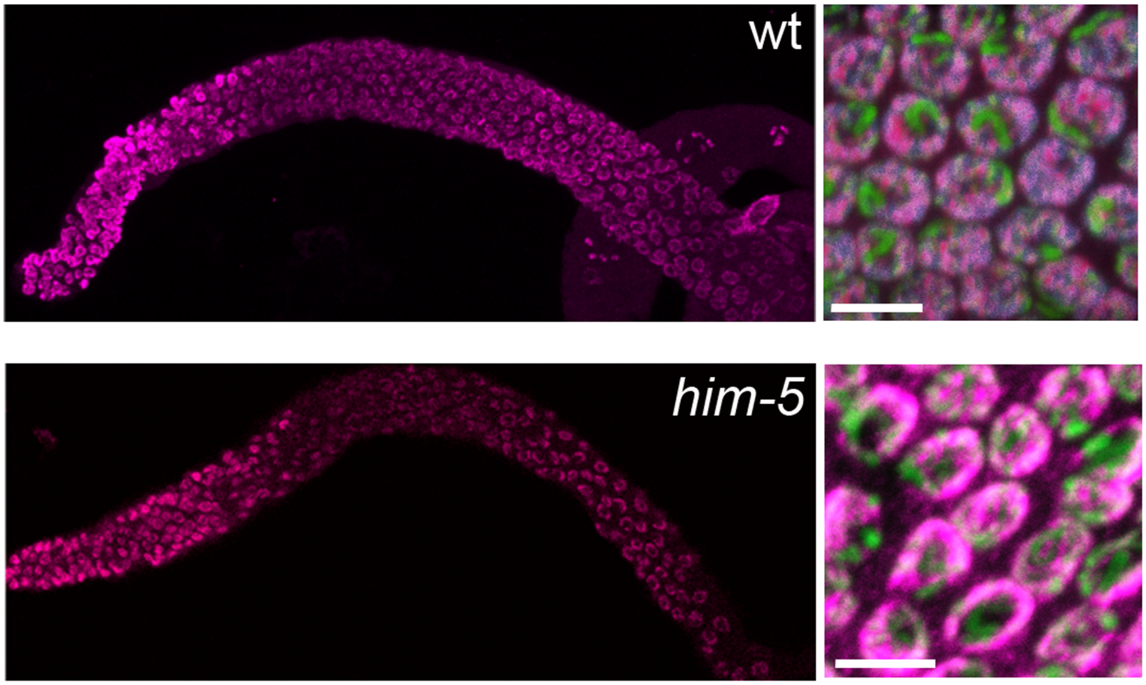

Figure S5 him-5 does not affect H2AK5 acetylation. H2AK5Ac is indicated by magenta. A germline (left) and mid-pachytene nuclei (right) are shown from wild type and him-5 (ok1896). No consistent differences between wild type and him-5 are observed. These data reveal consistent lack of H2AK5Ac on a single chromosome, which we infer from DAPI intensity to be the X chromosome.

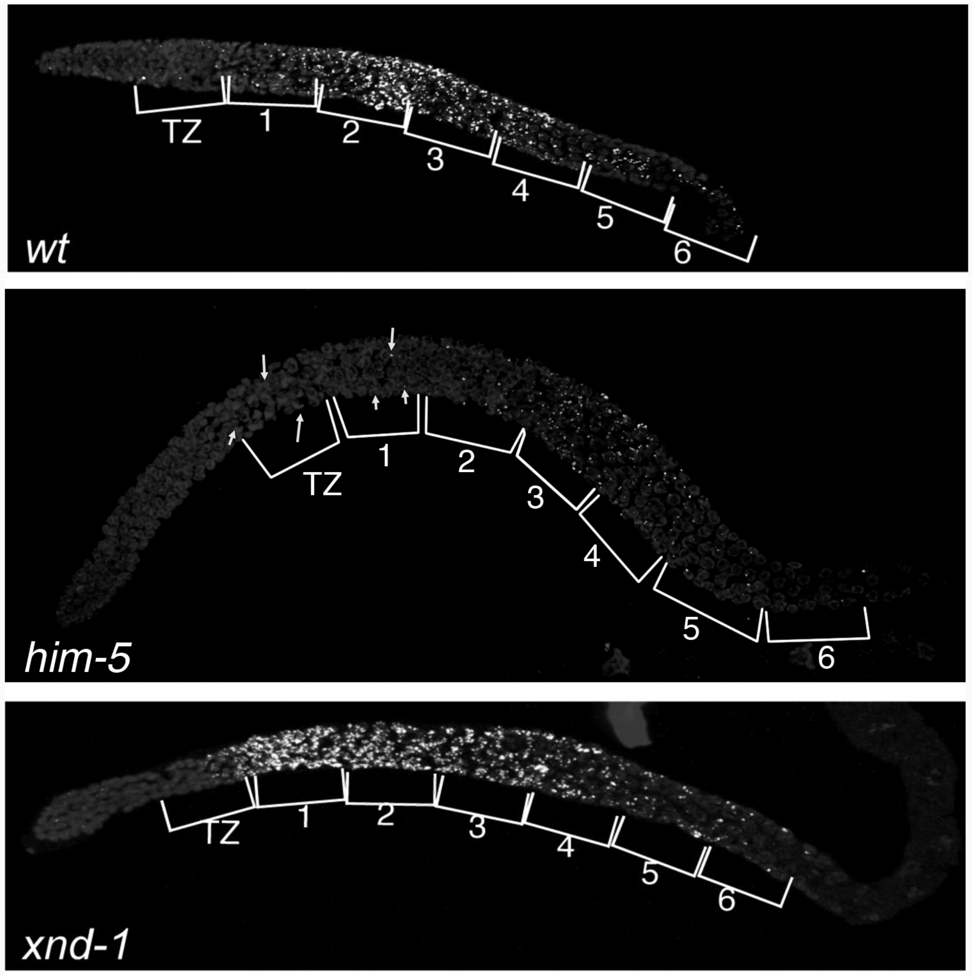

Figure S6 RAD-51 dynamics differs in him-5 and xnd-1. DNA (grey) and RAD-51 (white foci) are shown as maximum projections from confocal stacks through wild type (top), him-5(ok1896) (middle), and xnd-1(ok709) (bottom) germlines. The germlines were divided into seven equal sized regions from the transition zone (zygotene) to the pachytene- diplotene border and number of RAD-51 foci/ nucleus was quantified (see Figure 7B). The white foci in wild type in regions 5 and 6 are due to background staining with the anti-RAD-51 antibody and can be discerned in the rachis in 3D projections (not shown). In him-5, arrows point to small RAD-51 foci in the distal region. Breaks in this region can readily be observed in this region in Figure 7A.

Table S1 RNA interference of D1086.4 gives male progeny

dsRNA[a]	# P0 ines with males[b]	Frequency	# F1 lines with males	Frequency
D1086.4 5’	2/16[&]	2-5%	1/40	~5%
D1086.4 3’	4/22[#]	2-10%	3/40[c]	5-20%
D1086.5	0/18	N.A.	0/20	N.A.

[a] dsRNAs were injected into one day old adult wild type (N2) worms and allowed to lay on fresh plates each day for 3 days.

[b] Males were only observed on plates from the 48-72 time period post-injection.

[c] Frequency of males were 5% and 20%, the latter having a parent that gave 10% males.

Table S2 Crossover distribution on chromosome I from oocytes

Interval (Mb)	**0.17 -1.91**	**1.91-4.59**	**4.59-10.72**	**10.72-12.05**	**12.05-14.68**	***N***
wt [a]	8.9 (**21**)	10.6 (**25**)	0.0 (**0**)	5.5 (**13**)	26.3 (**62**)	**236**
him-5	5.4* (**17**)	11.1 (**35**)	16.2** (**51**)	7.3 (**23**)	10.5** (**33**)	**320**

Values are map units for each interval (number of COs per interval)

The change in crossover distribution between *N2* and *him-5* is statistically significant: χ^2(4,N=122)=1471, *p*< .0001

*, **Significant difference in map size of the interval between *wild type* and *him-5* (*p<0.1; **p<0.005)

[a] Data is the same as in WAGNER *et al.* 2010.

Table S3 Crossover distribution on chromosome I from sperm

Interval (Mb)	**0.17 -1.91**	**1.91-4.59**	**4.59-10.72**	**10.72-12.05**	**12.05-14.68**	***N***
***wt*[a]**	14.0 (**46**)	12.8 (**42**)	6.1 (**20**)	4.3 (**14**)	13.4 (**44**)	**328**
him-5	7.9* (**22**)	13.0 (**36**)	17.3** (**48**)	3.6 (**10**)	6.1** (**17**)	**282**

Values are map units for each interval (number of COs per interval)

The change in crossover distribution between *N2* and *him-5* is statistically significant: c^2 (4, N=133)=79, *p*< .0001

*, **Significant difference in map size of the interval between *wild type* and *him-5* (*p<0.05; **p<0.005)

[a] Data is the same as in WAGNER *et al.* 2010.

Table S4 Percentage of males post-irradiation

	0-12hr	N	12-24	N	24-36	N	36-48	N
N2 control	0	**268**	0	**656**	0	**591**	0	**333**
N2 IR	0	**609**	0	**1375**	0	**1074**	0	**662**
him-5 control	35	**172**	30.8	**466**	33.4	**416**	35.3	**222**
him-5 IR	45	**496**	8.4	**1279**	11.0	**1065**	16.1	**430**

Data represents the compilation of two independent experiments.

Values represent the percentage of males in the viable progeny and were calculated a (total number of males)/ (total wild type hermaphrodites + males) for each time point after exposure to 20Gy radiation. Since XXX Dpy progeny are sub-viable, they were excluded from these analyses.

Table S5 Hatching rates post-irradiation

	0-12hr	N	12-24hr	N	24-36hr	N	36-48hr	N
N2 control	100	**268**	100	**656**	100	**591**	100	**334**
N2 IR	88	**692**	100	**1375**	99.0	**1074**	98.2	**674**
him-5 control	72	**239**	75.7*	**616**	78.6	**448**	69.6	**316**
him-5 IR	77	**631**	92.8	**1411**	88.1	**1142**	78.4	**548**

Data represents the compilation of two independent experiments.

Values represent the percentage of the total viable progeny/total # eggs laid (N) for each time point after exposure to 20Gy radiation.

* The change in hatching rates between *him-5* and *him-5* post-IR is statistically significant: χ^2(1,N=616)= 17.280, $p<$ .0001

Table S6 Apoptosis analysis with acridine orange

# Apoptotic **Nuclei/Gonad**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Average**	**N**	**mean**	**St. Dev**	**SE Mean**
N2	0	4	3	8	4	1	1	0	2.90	**21**	2.905	1.3381	0.292
him-5(e1490)	2	4	7	5	1	4	0	1	2.78	**25**	2.76	1.7861	0.357

Student t-test DF:43 T-value 0.3143 P-value=0.7548

Table S7 Total number of meiotic breaks analyzed by RAD-51 foci after *rad-54(RNAi)*

Breaks per Nucleus	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**Avg**	**N**	**Mean**	**St. Dev**	**SE Mean**
N2	0	0	0	0	0	2	4	3	10	12	5	14.1	**36**	14.1	1.4	0.23
him-5 (ok1896)	1	2	8	16	11	5	10	2	1	0	0	9.9	**56**	9.9	1.7	0.23

Student t-test DF: 84 T-value 13.0838 P-value = <0.00001