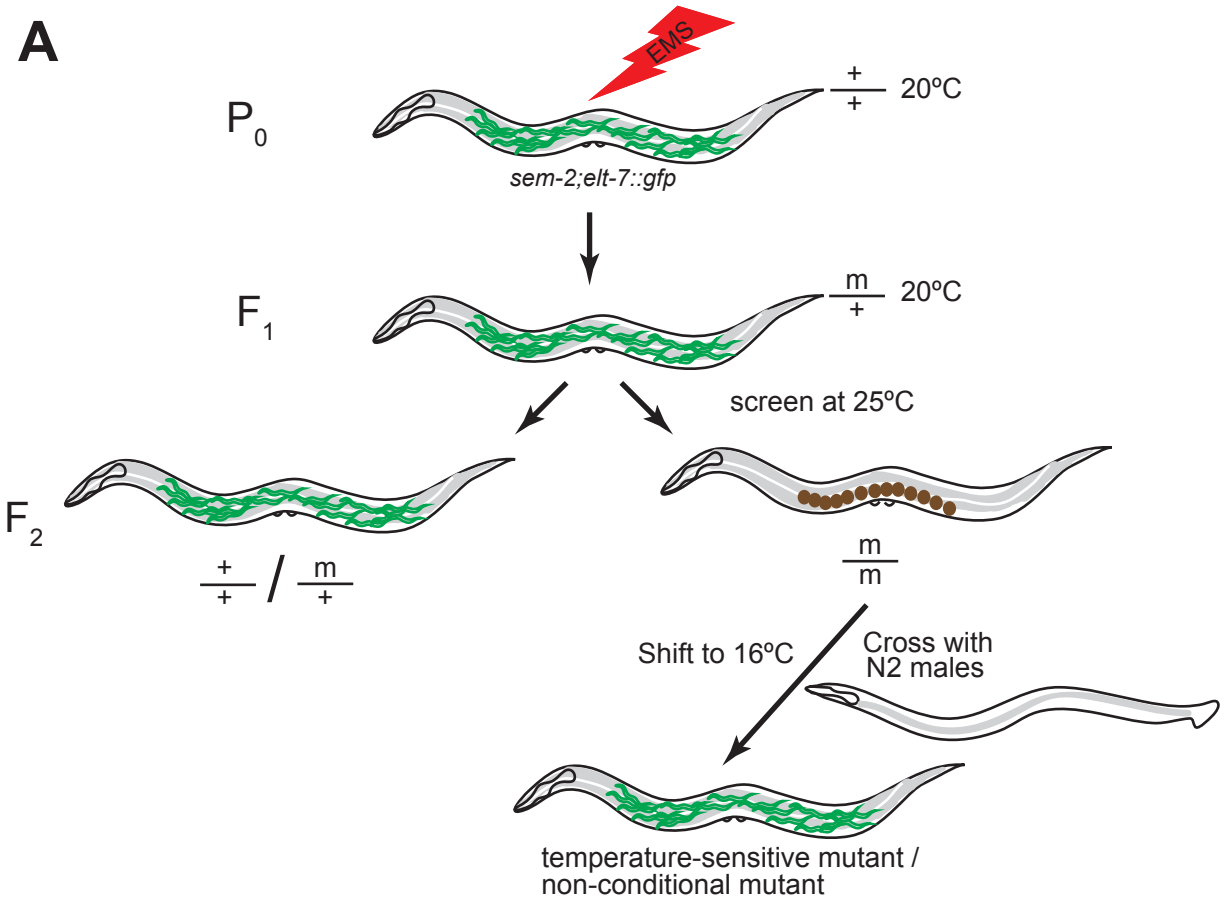

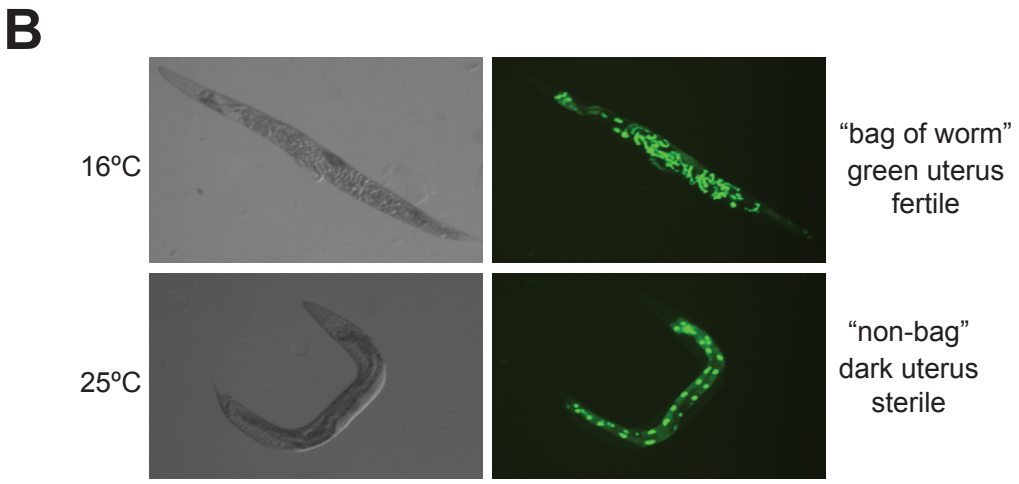

Figure S1. Strategy to isolate *spe-38(as38)* shown in Figure 1
(A) Schematic diagram of the genetic screen.
The L4 larvae of the genotype *sem-2;elt-7::gfp* were mutagenized using EMS (P0); sterile mutation afflicting the germ cells will lead to the production of heterozygous mutant in F1 generation; one fourth of the resultant generation (F2) will be homozygous for a sterile mutation, which can be identified by the lack of larvae in their uterus at 25°C. Candidate mutants were shifted to 16°C and wild-type males were added to recover each Spe mutant.
(B) Representative example of a temperature-sensitive, Spe mutant in *sem-2;elt-7::gfp* genetic background grown at permissive (16°C) or restrictive (25°C) temperatures. The *elt-7::gfp* drives GFP expression in the intestinal nuclei of all worms. The green embryos and larvae are observed only in the uteri of fertile hermaphrodites.

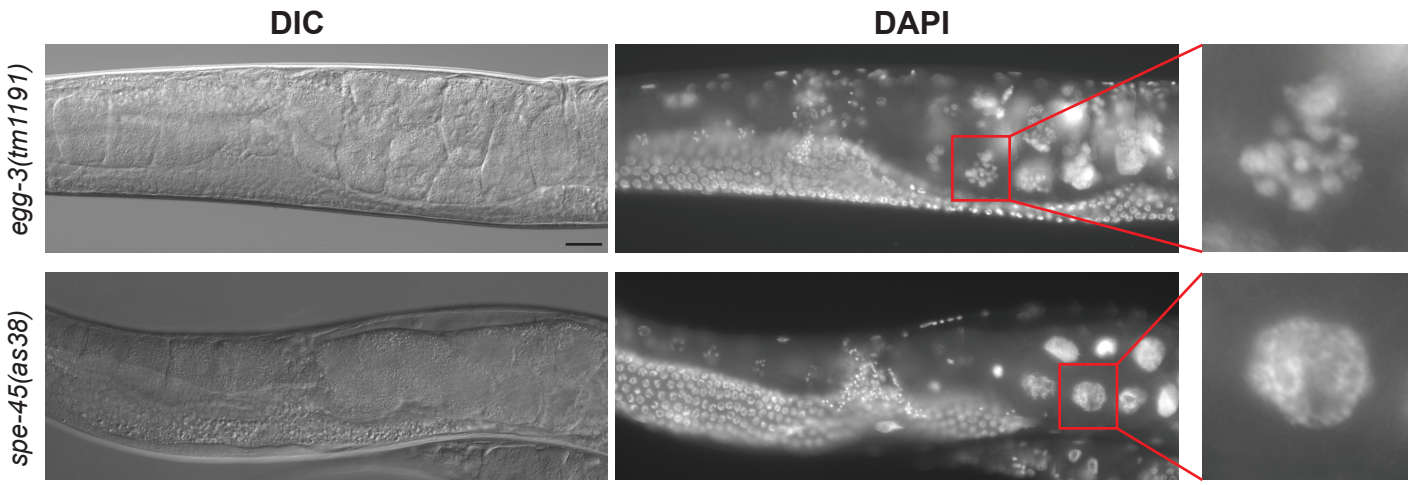

Figure S2. *spe-45(as38)* sperm fail to enter oocyte (related to Figure 2)
DAPI staining of the *egg-3(tm1191)* and *spe-45(as38)* hermaphrodites.
The sperm entry contributes centrosomes in *egg-3* mutants, resulting in the segregation of replicated chromosomal mass. Failure of sperm entry in *spe-45* mutant, and the failure to inherit sperm-derived centrosome thereof, results in endomitotic replication (Emo) phenotype, wherein chromosomal replication is unaccompanied by its segregation.The right most panels show the magnified areas indicated by the red box. Scale bar: 20μm

MQALLYFTACLTFVDSKRFVLLDPDDPNLEKSASNITDNDVRSRSIKMAKFRKWLKYDVDCQFI

SRSELAVKISQGRCPHESATEIKPTTTTTKKPKSFFFKRKRKPLKKKDNSLFSNENKESVVRAVVG

as38 (W → Stop)

NNKMHEIDWTPLSFPDLELVSGTMVTFKCDEMNRKKKRKKHEEIEFVE**W**FVNGKRINPSWFDW

RVSVSIDGHLGIWPIGEGDGGHFECLSNGQLIASVTVTVVPISKVLVNGLFNYLFVCAIFAVATIPIG

CLLGNRNQEKKEIEVDRMEEFLAENVFKTDQMAKEKVAGIIEKQGVVDERQLIESKAKGNRSTIMI

LLQKPNTMKEKEKEDQKVSNNPAPAASASTEGATTVTEGTTAAETTVATETTATGTGTTEAAATTV

TETNEEADNEEDEEEDEDDDDDGSVDGTTAGSTESKGPGTTSKSTDKGKKKKKTKKGGKKKKS

GAGKGKGKKKSKVSKEKKGGKKVQKKKPASKPTKKKK

Figure S3. Amino acid sequences of SPE-45/OIG-7 (related to Figure 3)

The immunoglobulin domain is underlined and Trp178 is in bold. The transmembrane domain is in blue.

Table S1 (related to Figure 1)

Pre-fertilization events [S1] are normal in *spe-45(as38)* mutants.

Events	**Wild type**	***spe-45(as38)***
Gonad morphology	+	+
Increase in oocyte size	+	+
Disappearance of nucleolus	+	+
Nuclear migration	+	+
Nuclear envelope breakdown	+	+
Cortical rearrangement	+	+
Ovulation	+	+

Table S2. *spe-45(as38)* was mapped to Chromosome IV (related to Figure 3)

Marker	Chromosome	Sterile F2s	Fertile F2s
dpy-5(e61) unc-13(e51)	I	11	53
unc-4(e120) rol-1(e91)	II	5	17
dpy-17(e164) unc-32(e189)	III	8	55
unc-17(e113) dpy-13(e184)	IV	0	66
unc-46(e177) dpy-11(e224)	V	6	18

The males harboring indicated marker mutations were crossed with *spe-45(as38)* hermaphrodites. The total numbers of sterile and fertile worms were counted from the F2 generations that exhibit marker mutants' phenotypes.

Supplemental Experimental Procedures

Strains

The following strains were used in this study: N2 Bristol, Hawaiian (CB4856), *sem-2(n1343);Is[Pelt-7::gfp; rol-6(su1006)], spe-45(as38), spe-45(tm3715), sDf1, sDf27, him-5(e1490), fem-1(hc17ts), lon-1(e185) sup-5(e1464), dpy-5(e61)*. The strains were maintained as described previously [S2, S3]. The *spe-45(as38)* and the *spe-45(tm3715)* mutants were out-crossed with N2 males at least 10 times prior to analyses. The *spe-45(as38)* mutants were maintained by incubating at 16°C; the *spe-45(tm3715)* mutants were maintained by crossing with males, and selecting F2 generation for Spe phenotype.

Genetic screen to isolate sterile mutants

For an overview of our screening strategy see Figure S1. We prepared a synchronized L1 population of *sem-2(n1343);Is[Pelt-7::gfp; rol-6(su1006)]* worms by hypochlorite treatment. We placed approximately 10,000 L1 larvae on NGM plates seeded with *E. coli* OP50 and allowed them to mature until L4 larvae, at which point we washed the plates with M9 buffer to harvest synchronized population of L4 larvae, and placed into 50 ml conical tube. We then added 5ml of 50mM EMS to the L4 larvae and incubated them at room temperature for five hours (Figure S1). Next, we removed the supernatant and washed the mutagenized worms thrice with M9 buffer, and placed them onto NGM plate seeded with "egg food", which was prepared by mixing 6 g of dried egg powder with 4ml of the bacterial slurry (*E. coli* HB101). The worms were allowed to grow until most of the F2 generation became young adults at 25°C. The plates were screened for the absence of any GFP signal inside the uterus of the F2 worms to select for the putative sterile mutants. Candidates were picked and placed singly onto NGM plates and five N2 males were added and incubated at 16°C for 2-3 days. We looked for the plate that had progeny and selected them for further characterization.

Positional cloning of *spe-45(as38)*

The two-factor, three-factor and SNP mapping and deficiency mapping were done as described previously [S4, S5]. Complementation tests against all known sperm mutants in the *spe-45* region were done as described previously [S6].

Whole genome sequencing

The N2 and *spe-45(as38)* strains were grown at 16° C on 150 mm plates seeded with *E. coli* OP50. The worms were harvested and the genomic DNA was extracted using the blood and cell culture DNA mini kit (Qiagen, CA). Sequencing libraries were constructed using the TruSeq DNA prep kit (Illumina, CA) per the manufacturer's protocol. Single-end 50bp sequencing on a HiSeq 2000 instrument (Illumina, CA) yielded a minimum of 44 million reads (~22X genome coverage) per sample. Variants were identified using a pipeline of BFAST [S7] for alignment, SAMtools [S8] for variant calling, and ANNOVAR [S9] for annotation, with *C. elegans* genome version WS220 as the reference. Variants identified in N2 were considered background alleles and filtered from the list of *spe-45(as38)* candidates.

Brood size

Brood sizes of the hermaphrodites were determined as described previously [S10]. Briefly, we selected L4 hermaphrodites based on the crescent-shaped vulvae, and placed them singly on to plates seeded with *Escherichia coli* OP50. The P0 worms were sequentially transferred onto new plates every day for the next three days. The total number of F1 worms in each of those plates was calculated after three days from the day the P0 were transferred. Unfertilized oocytes laid on the plates are visually distinguishable from the fertilized eggs. Total number of unfertilized oocytes and eggs were calculated by serially transferring the worm to a new plate every day for the period of three days from L4.

DAPI staining

DAPI staining was done as described previously [S11]. Young adults were transferred from a culture plate into the watch glass containing 500 μl of M9 buffer to get rid of the bacterial film coating the worms. The worms are transferred into a watch glass containing 500μl of ice-cold methanol to fix the worms for 5 min, and then transferred into M9 buffer. Finally, the worms were placed on a 2% agarose pad; 7 μl of the Vectashield mounting medium containing DAPI (Vector Laboratories Inc., CA) was placed on the worm and kept in the dark for 5 min before placing the cover slip. At least ten worms were analyzed. The gonadal region was photographed at 461 nm wavelength.

Sperm activation

Spermatids and spermatozoa of males were observed as described previously [S12, S13]. The L4 males were selected and kept on a NGM plate overnight to prevent the loss of sperm through mating. Twenty to thirty virgin males were placed into 10μl of sperm media kept on the slide. The worms were cut using a 15 gauge needle (BD Sciences, CA) to release the spermatids; coverslips were placed and the spermatids were observed under Nomarski optics. The same procedure was followed to observe spermatozoa, except that the worms were dissected in sperm media containing 200 μg/ml of Pronase E [S14]. At least ten fields were examined for each genotype. The sperm are considered fully active if pseudopods are found in greater than 90% of the cells in the field.

Male fertility

The *fem-1(hc17ts)* females were crossed with *him-5* males or *spe-45;him-5* males at 25°C for one day. The males were removed from the mating plate and the brood sizes of the resulting crosses were measured.

Sperm migration

The *fem-1(hc17)* hermaphrodites were maintained and allowed to lay eggs at 15°C. The adult worms were removed from the plates, and the plates were shifted to 25°C until most of the worms reached L4 stage. The L4 larvae were selected and transferred onto a NGM plate seeded with *E. coli* OP50. The plates were incubated at 25°C for a day to let the L4 stage become young adult.

The *fem-1(hc17ts)* young adult was transferred singly onto a mating plate and either left unmated or mated with single male of the genotypes *him-5* or *spe-45(as38);him-5* for 3 hours at 25°C. Then, the *fem-1(hc17ts)* females were transferred from the mating plate to a fresh NGM plate seeded with OP50 and incubated it at 25°C for one hour to allow the sperm to migrate from the vulva to the spermatheca, after which the *fem-1(hc17ts)* females were stained with DAPI and viewed under the microscope.

Transgenic rescue

Extrachromosomal arrays carrying a mixture of different genomic DNAs were generated by microinjection of fosmids or cosmids into N2 worms. These fosmids (Source Bioscience, UK)

and cosmids (gift from Sanger Institute, UK) carry defined regions of the *C. elegans* genome as indicated in Figure 2A. The microinjection of the worms was done as described previously [S14, S14]. The transgenic worms were then crossed into *spe-45(as38)* to mobilize the transgene into the *as38* genetic background. The *spe-45* genomic DNA only partially rescued the *spe-45(as38)* phenotype, which could be because expression of a transgene in germ cells is often silenced [S16].

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