

CGTTCGTCAATGTATTTTCAGAAGGCCTT -2,106  
GGATTTTTTCCGTTTTTCATCTctgaaattcaggaaaaatcgataaaatttcaagatttgagaatttttcttcaaaaaaaaggg -2,023  
taaatttgtgattttttaaacaattttttatgtaaaattgcagaaaaatcaataaaattcccgatatttgtgcaactgtttt -1,940  
ttcaaaaaaggttaatttcaagatttttgaaacatttttttgaaaaatttgagaaaaatcaattaaatttcaggttttgataa -1,857  
ttgttttcttcaaaaaaaaggggtgaattttcgattttttgaaatttttttgtaaaattgcagaaaaatcgataaaattt -1,774  
caagatttgagcatttttcttcaaaaaatgtaactttctgatttttcaaatatttttcttgtaaaatttgagaaaaatcga -1,691  
taaaatttcacgtacctattttcttcaactattttcttcaaaaaatttcttaaaactcttaaaaaagcttttttttttagtt -1,608  
tttttagctaaatttttgaaattttcgagctttttgaaattttaccagataaaatttttcggtcgaaataccagattttga -1,525  
gcactttttgtcttcaaaaaatcttcaaaaaatccagtaaaatcgtaaaacttcaagcttttttgaaattttcaataaatt -1,442  
ctgacattttccagaaaaacaagcgatttttgtgaaaaatagtcataaaatttggtgatttttcgtagcttttgacaatttgcag -1,359  
tcatttttcagatagaaaaatcaatatataaagggtgaatttccactttttgataaaaaatgcactaatttttgagagttttt -1,276  
ttgaaaaatagtcgaaatatgctgactttcggagttttggacaatttccagtcatttttgcgagactttgcaaaagaaaacca -1,193  
gagttttgtgaaaaatgtgcaatttttgcatacttaacacaaaaaacatgaaaaatttgatgattttccagtcatttttcagc -1,110  
aaaaaaaaatcatttttctaactgttttaccgaaaaaaacaattatttttcaggtacgcacTGGTTTCCTTCAGGATTTTCAGA -1,027  
CTTTCCTCTTTCCTTTCATCGTAAACTCGTGTTCGGCGACTTCTCTCAGTAGCTTTAGCTTATAAGCGTCCGGCGAAGTGGC -944  
CAACTCGTGTGATTTTTCCCTGTTTAACGATGTAATACGGGTTTGAGCGAGAAAAATCCGGCGGATCCATGAGATTTACCACct -861  
gaaattgaggaaaaatcgataaaatctcgaggttttccacagctggaatacCTCGGCACGTGGCACCATTTTATTATCAATGTA -778  
ATATTTGATCCTTTTTCTTTCCAACCTTGACGAACAATCTTCACTTCTGAATTTTCAAACGCCATTAGACGCTTTTCCGAGTTGT -695  
CAAAGGTGATTTTCGACACGAGCGTGGGCGACTTTCGGTCCGGTACTCTCGTGAAGCAGACCAAGACGTTGTCTTCTTTCAAA -612  
TGAGCATATTCATCGAAAGAACAATGAAATCGctgaaaaattgacatttttcagctttttccgcagaaattcatgcggtaac -529  
CATGAAAAAATTTGGATTTTCCCGATCCATTTTCCTCCGACGACGCTTCGAGCGTGGACTGAAGCCCGACACATTTGGTGTG -446  
TCTTTGTAGCTTCGAAAGCCGGTGATTTCTCACctgaaaaatagggaaaaatcgatgaaaaacttgggaaaaactcacTTCTTTGA -363  
TCTTCATcggtttattgcatcaagtgatgggatgttgaCTgaaacatcgataatatagatgagaaaaatcgagaatttaagtc -280  
aaaaaaaaacttttgaatgaggaaaaatcgatggaaatcgcaaaaaaacactgaaaaatcagcaaaaaataaatttcaaattcaaag -197  
cgaagaattcgcgggcgcgacccgggactagaaaatccccactcggccaccctgtgtgtactcctctcggacaaggatatttcg -114  
aaaaatgagggtttttccaaataaaaaactgtttatttctcggaaaaataactagaaaaccacggaaaaaacgtgtctaaaaacaaaa -31

eb34 ATG M to ATA I

M N G V A G E S P S Y 11  
aactaaagcagaataacaaaaaattatttatttcaattatttctcacaAAAATG AACGGAGTAGCTGGCGAATCGCCGAGCTA 53

eb41 GGC G to GAC D

D E Y L T Y F T P G D R I L I S E N H P N H A Q R L N 38  
CGACGAGTACCTGACGTATTTACACCCGGGCGATCGAATTTCTGATATCGGAAAAATCATCCAAATCATGCACAACGCCTCAATT 136  
S Y N N V A W T K T H L A 51

CGTACAATAATGTTGCGTGGACGAAAGACACATTTGGCTgtgagtgccagaaaaagaaaaaatgctgaaatctgaaattttttg 219  
L L G H I G Y I E S I N E H 65

ctgagaatctcttcaaaaacaggcaaatcttcaacattttcagCTTCTCGGTTCACATTTGGCTATATCGAGAGCATCAACGAGCA 302  
R H T A N V R V Y Y A V P Q N P E L 83

CCGGCACACTGCAAAATGTTCCGGTTCCTACTACGCAGTTCCGCAAAAATCCAGAGCTTgtaagctgattttttgaagattttgtgt 385  
aaaatgtgtgatttttccgaaatctagcgatctttttgtactttttccattgaaaaatcgcaaaaaaaagtttaaattgtct 468

ggatactaccgtaaatctttaaaggcgcacacattttactgatcgcgaccaggagaagtattgcgaaagtggcgtgctgcttt 551  
aaaaggctactgtaatttttgattttcaatatcgaatttttaaatgaaaattggttgataacggtacagaaaactgaaatcttc 634

attgaagatacttaaaaacttttctgaatttttttgatttcaagctttcagaggtaaacactgaaaaaaagagcctttttccgaa 717  
F K L S T E W P L D A L E F P Q C 100

tttttcatcccaattttaaattttaaattttcagTTCAAGCTCTCTACCGAATGGCCGCTGGACGCGTTGGAAATTCCTTCAATG 800  
I D H S K G D L V A I T R G D P E K T T I G I V A T K 127

TATAGATCATTTCAAAGGTGATCTCGTTGCAATAACCCGCGGAGATCCGGAAAAACGACAATCGGAATAGTGGCAACGAAGG 883  
E P L N L G G I P K H L V Q L K S A S N P T I T R E I Q 155

AACCATTGAATTTAGGTGGAAATCCGAAGCATTTGGTTCAACTGAAAAGCGCCTCGAATCCGACGATTAACCCGGGAGATTCAG 966  
V A V N A N T G W T D E R R D I F L C P V Y S G P R L S 183

GTGGCGTTAATGCGAATACGGGATGGACTGATGAGAGACGGGATATCTTTTTGTGTCCGGTTTATTCGGGTCCCCGATTTGTC 1,049  
M I A R D N N E P S V H T Y K gk936708 g to a splice donor mutation 198

AATGATTGCTAGAGATAATAATGAGCCGTCGGTTCATACTTATAAGgtagtaacttcaggcatgtctgctctcaaaactgcc 1,132  
tgcttacacgcttatctgattatgtgctacctagttgctactatcgaatagcctgtcaaatggtgtgctgatgctgaaaa 1,215

tgctgaaaaatgctgccataaaagcttgtctgctacatatttgctgattgttcataggttaagcttacaacctacatacc 1,298

tacatgcctacttgcttacacgcctgcctccgcgactacctgcttgttttttctgccttctacatgtttgctgaaaaatg 1,381  
cgtctgcataaaagcctgtctgctacatatttgctgctggtccataggtcagcttacatgcctacatgcctacctgcctatc 1,464

H T I H G Y T P D T L M 210  
 tgcctacctacctacatgcctacctgaaagataaatcgattttttcagCACACAATCCACGGCTACACCCCGACACCTTAAT 1,547  
 eb36 GCG A to ACG T  
 Q V I A N W G E D A L S Y A L L V E S I R S D P Q Q V 237  
 GCAAGTGATCGCGAATTGGGGTGAAGACCGCTGAGCTATGCGCTTCTCGTGAATCGATCCGATCCGGATCCGCAACAGTTC 1,630  
 hc54 CTT L to TTT F  
 R T L F D G Q L P L F R A V A D D L R N V V V M L V A L 265  
 GGACCCTTTTCGACGGTCAACTGCCACTTTTCCGTGCTGTCGCCGATGATCTACGGAATGTTGTTGTGATGCTTGTCCGCTTC 1,713  
 gk587388 GGT G to AAT S  
 G A D R T A R D S E N R T I I H V A A E R 286  
 GGTGCAGATAGAACCCGACAGAGATTCCGAGAATCGAACTATCATTCATGTGGCTGCGGAGAGgtgggaaatcgtaaaaaaatc 1,796  
 eb35 ATG M to ATA I, eb42 CTG L to CAG Q  
 G L D K M L D T V M L L L P 300  
 gatttaaaaaaaattaaaaaaatcaataattcttgcagAGGTCTTGACAAAAATGCTGGATACTGTAATGCTTCTTCTCCCAA 1,879  
 K D I N S Q A A N G L T P L H L A A R H A H A A C I D R 328  
 AAGACATCAATTCACAAGCGGCCAACGGTCTAACACCGCTTTCATTTGGCCGCCGTCACGCCACGCAGCTTGTATCGATCGG 1,962  
 L L G T S T C I P C V P N N F G D T L L H E V C R L P E 356  
 CTTCTCGGTACGAGTACTTGTATTCCGTGTGTTCCGAACAATTTTGGCGACACACTTTTGCATGAGGTTGTCCGGCTGCCAGA 2,045  
 S S N K K A 362  
 GTCTCGAATAAGgtttgtgttattgatttttccgaaaactgaccagaaaatcgataatcgctaaaaacaattttcagAAAGCGG 2,128  
 A I S R I L T N T R A N I H H V N N S N M T P I Q I A I 390  
 CAATAAGTAGAATTCAGCAAATACTCGTGCGAACATTCATCACGTGAACAATAGCAATATGACACCCATCCAAATGCAATT 2,211  
 M S G H V S T V E Q L L L 403  
 ATGAGTGGTCATGTCAGgtaaaaacctctattagaagtgcaccacccccctccaacattttcagCACCGTCGAGCAGCTTCTCCT 2,294  
 L R A S Y R N T T S K T G M S A L H F A A A S G H A N 430  
 GTTGCCTGCCTCTACCCTAATACCACCTCGAAAATGGAATGAGCGCATTGCATTTTGGCCGTGCCTCTGGCCATGCGAATG 2,377  
 eb37 g to a splice donor mutation; a downstream gt splice donor frameshifts the  
 coding sequence, adding 9 novel amino acids followed by a premature stop codon \*  
 V V N K L I S I 437  
 TTGTCAATAAACTTATTTCCgtaggtgaaaaaaattagtagtgttggtgcccaggtttatgcaaaaactcgccacgtagtt 2,460  
 ttttaggatttttttgccgaaaaataatgaatttaagatcaaaaatttttctcgaaaaatttttaatttttgcgcaaaaattttttt 2,543  
 A W P R S P P S \*  
 L G L E V H R R D K F G 449  
 ccagaaatttgaattttccgacaacttttttaattattttcttgcagCTTGCCCTAGAAGTCCACCGTGCAGCAAGTTCCGTT 2,626  
 R G V L H Y A L E K W T G E A E K D C N R L A A I Q A L 477  
 CGCGGAGTACTCCACTACGCCCTCGAAAAATGGACAGGAGAACCGGAAAAAGACTGTAATCGGCTTGACGCAATTCAGGCTCT 2,709  
 V K A G A P S N I I D L N G Q T P V F Q L I R E M L S 504  
 CGTTAAAGCCGGCGCTCCATCGAATATATATCGATTTGAAATGGACAGACACCCGGTTTTCCAGTTGATTCGTGAAATGTTGAGCA 2,792  
 N S E Q Y P A S L V P V C A Q L V R T N L N L D E M A S 532  
 ATTCGGAGCAATATCCGGCGTGTGTTGTTCCGTTTGTGCTCAACTTGTTCGTACGAACCTGAATCTTGATGAGATGGCAAGC 2,875  
 R L R P M W Q L A T I C F L V A N G A D L N V K 556  
 CGACTTCGTCCGATGTGGCAACTTGCCACTATTTGCTTTTTTGGTTGCTAATGGAGCGGATTTGAATGTCAAgttgggtctattt 2,958  
 tttttaaaagaaaatcgataatttttctctttttctgggaaaaatcgatattcttcaagaaagtcaatatttgcgataaaaaatcg 3,041  
 K I N N V  
 atatttaaaaaaaatcgattttctctagaaaaatggtttttcgacaaaaatcgatagttttcagaaaaatcaataatgtcc 3,124  
 eb39 g to a splice acceptor mutation; an upstream ag splice donor causes a novel  
 in-frame 8 amino acid insertion in an otherwise wild type MIB-1 protein  
 P F K D R R G M T V M D L C E E S S F R S I I V H I A Q 581  
 ctttcagAGATCGCAGAGGGATGACCGTGATGGATTTGTGCGAAGAAATCATCATTTAGATCGATTATCGTTTCATATTGCACAG 3,207  
 T K Q R 585  
 ACTAAGCAGAGgttgggatttcagtgatggccgcccgaatgagaaaaactcgccaccaattgatttttgcattttttttttga 3,290  
 aaagtttctgaaattgatgcgtaaggtactatcaaatatagagaatcaacaaacaaaaaaatgggtggccgagtttccgaa 3,373  
 Q V M P M M M A M S E D K F D S 601  
 aaaattcggccatgtcgcacaaaactcaatttcagACAGGTGATGCCGATGATGATGGCGATGAGTGAGGATAAATTCGACTCGA 3,456  
 T E V T M C T F S C L N S V A T V K L D P C G H R V A C 629  
 CAGAAGTAACAATGTGCACATTTTCATGTCTCAATTCGGTTGCCACTGTCAAAATTTGGACCCGTGCGGTCATCGGGTCCGTTGT 3,539  
 V D C T E K V A I R R C P V C R Q F I N E A H D Q 654  
 GTCGATTGTACGGAGAAAGTGGCAATCCGCCGATGCCAGTGTGTCGTCATTTATCAACGAGGCTCATGATCAAGgtattttg 3,622

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cgaggatttacggggtgtggcctagaaataccttttttagagttttctaggccacgttggcaatttgatcaattttgttttcgt 3,705
                                                                 D G K P V Q I G 662
ggaaaatcgattatcgatgtatcgatttttctctgtataactaaatcgatgtttaaacagACGGAAAGCCAGTTCAAATCGG 3,788
S R C H E P S D G D R Q Q V S A E V R K Q I A D D A A 689
CTCCCCGTGCCATGAACCATCCGATGGCGATCGTCAACAAGTTTCGGCAGAAGTTTCGCAAACAAATCGCCGACGATGCGGGCGA 3,871
R E A K I E V E R E K Q N E L N Q L R K R L E Q L E L E 717
GAGAGGCGAAAAATCGAAGTGGAAACGTGAAAAGCAGAAATGAGTTGAATCAACTGAGAAAACGTTTGGAGCAGCTCGAGTTGGAG 3,954
eb33 TGC C to TAC Y
T N C A I C M D L K I A 729
ACGAACTGCGCGATTTGTATGGATTTGAAGATTGCGgtgagtatggctttaacaaaaaatcaataaattatcgatttttga 4,037
                                                                 V V F N C G H T A C V D C A D K L K K Q C H 751
aaaattaaatattttcagGTTGTATTTAATTGTGGACATACGGCTTGTGTGGATTGTGCCGATAAACTGAAGAAGCAATGTCA 4,120
I C R K T I E T M Q P I Y S 765
CATCTGCAGAAAGACTATFCGAGACGATGCAGCCGATCTACTCGtgaatatcaatttattgcttttgattgtgtataactccatt 4,203
tctgtgtaaataaataagattttattgagaaataaaggggttttaactg 4,252

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**Supplemental Figure 1:** The *mib-1* gene, protein and various sequence features:

*mib-1* (Y47D3A.22) exons are shaded in red and include the empirically determined *mib-1* transcription start (SMART II kit, Clontech), defined here as #1 in the sequence numbering scheme. Amino acids are numbered in italics and show the wild type protein sequence.

Introns are in lower case and have no shading

*smc-3* (Y47D3A.26a) exons are shaded in magenta but coding is on the opposite strand from *mib-1*. Only part of the *smc-3* gene is shown, including the entire region used for the 5'UTR/promoter fusion described in Figure 8.

Translation start codons are ATG text at 22 for *mib-1* and -356 for *smc-3*

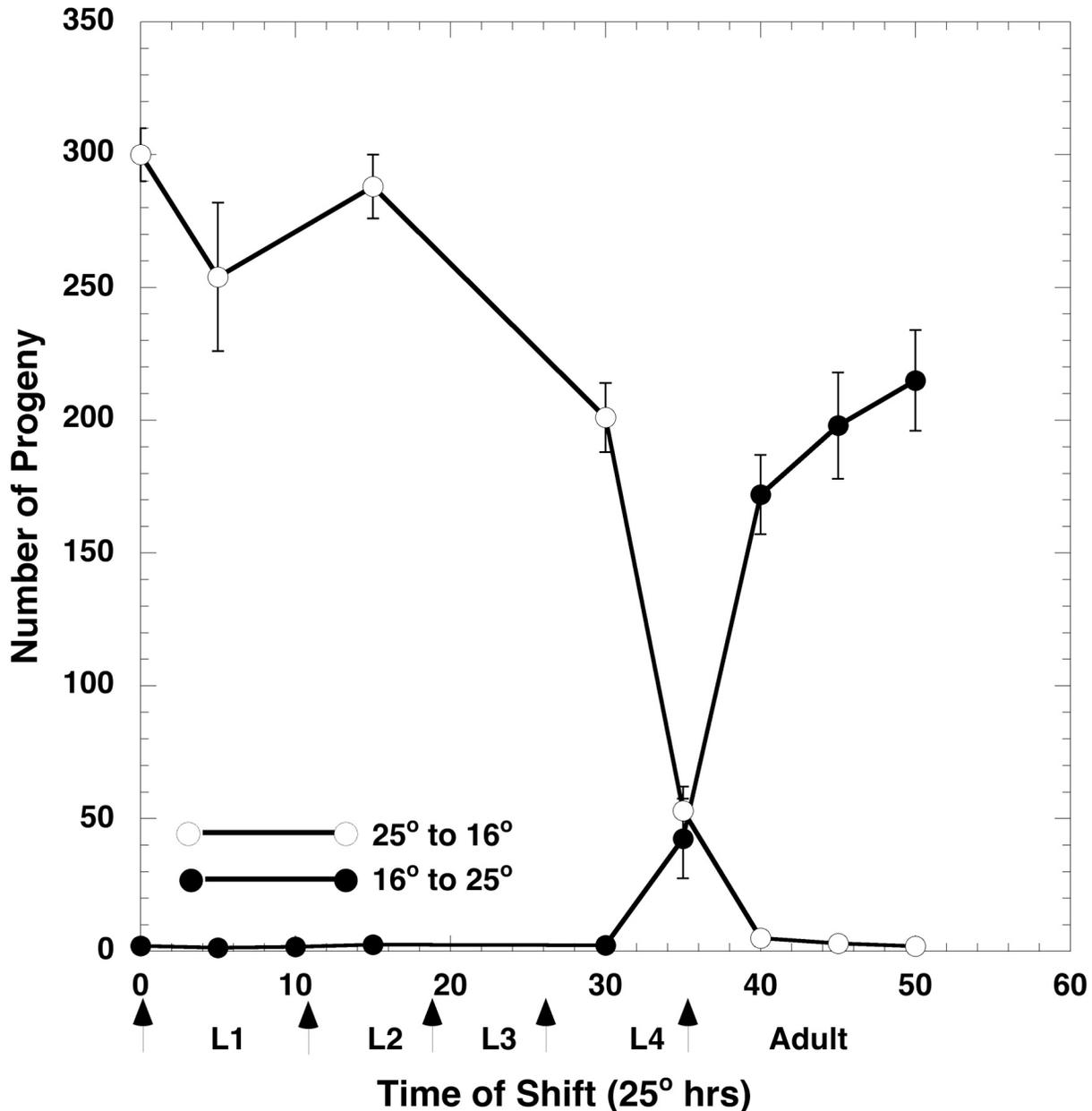
UTR sequence as defined by cDNA's described in Wormbase appears in gray text. At the 5' end, the longest cDNA is PE\_SS\_GG2740#FAF3E5. This is considerably longer than the empirically determined 5' transcription start for unknown reasons.

ct antisense to *smc-3* outtron acceptor for SL1

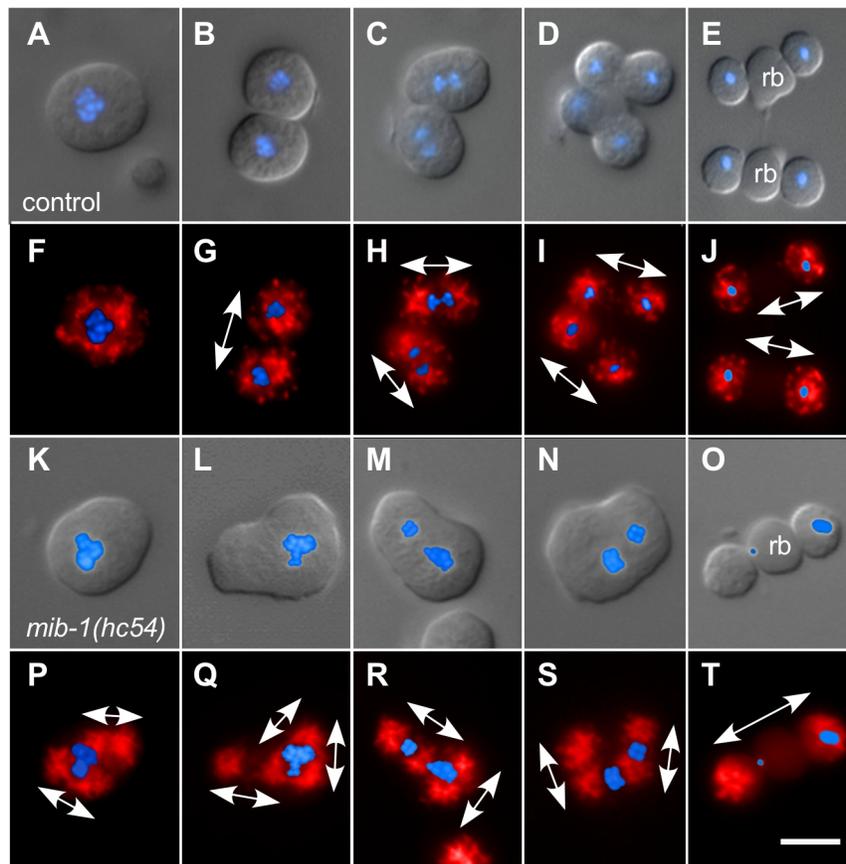
g (at -145 ) and T (at 968), respectively, marks the 5' and 3' ends of the 1,113 bp region deleted in the *mib-1*(*eb154*) mutation.

Point mutations are indicated in aqua shading

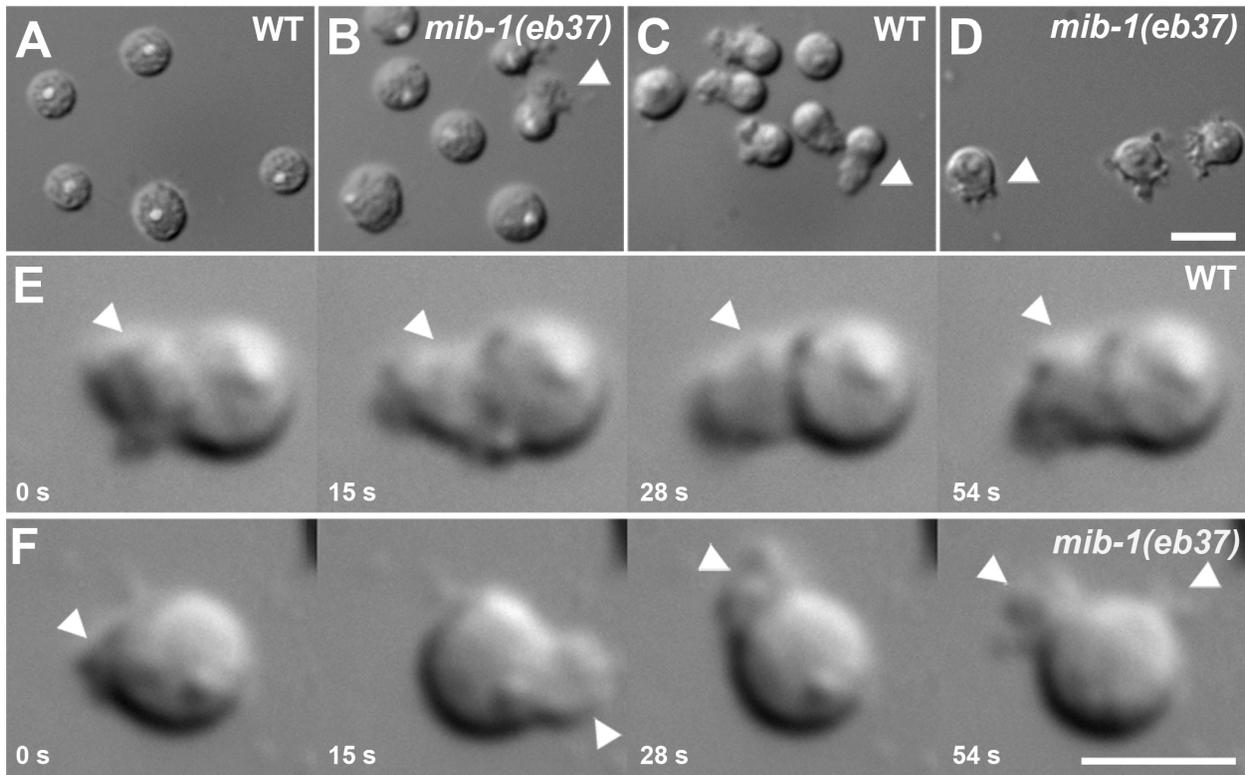
The effects of the *mib-1*(*eb37*) and *mib-1*(*eb39*) splicing mutations on the protein sequence are indicated in yellow shading; these highlighted residues are not included in amino acid numbering.



**Supplemental Figure 2:** TSP curves for *mib-1(eb154)*. *mib-1(eb154)* hermaphrodites were shifted from permissive (16°) to restrictive (25°) temperature, or vice versa, at intervals throughout development to determine when the MIB-1 protein is required for fertility (the temperature-sensitive period, or TSP). The mean number of progeny produced by 9-14 *mib-1(eb154)* hermaphrodites is plotted against their age at temperature shift, in 25° hours since hatch. Error bars are standard error of the mean. Data from upshifts (16° to 25°) and downshifts (25° to 16°) are plotted separately. Progeny counts plotted at 50 hrs are control values from animals that were not shifted. The approximate positions of life cycle stages are indicated below the X axis.



**Figure S3: Spermatogenesis in live cells.** Live *him-5* (wild type control; A-J) or *mib-1(hc54); him-5* (K-T), where DNA appears in blue and mitochondria are shown in red. Double-headed arrows indicate the presumptive cell division planes. (A, F) a primary spermatocyte with centrally located chromosomes surrounded by mitochondria; (B, G) secondary spermatocytes showing a well-ordered division plane and mitochondria concentrated around the chromosomes; (C, H) dividing secondary spermatocytes showing mitochondria positioned between the spindle poles and the plasma membrane; (D, I) dividing secondary spermatocytes at a later stage than (C, H) showing that mitochondria surround the nucleus; (E, J) as spermatids become distinct from the residual body (rb), the mitochondria concentrate around the condensed nucleus; (K, P) a *mib-1* spermatocyte with two division planes has both sets of chromosomes in one location; (L, Q) a *mib-1* spermatocyte that has three division planes but all of its chromosomes in one location; (M, R) a *mib-1* spermatocyte that has two division planes 90° to each other with chromosomes centrally located (upper left) or off-center (lower right) relative to the mitochondria; (N, S) a *mib-1* spermatocyte with two division planes, one with centrally locate chromosomes (right) and one with improperly positioned chromosomes (left; O, T) two spermatids, one (right) with and one (left) without a nucleus, separating from the residual body (rb) with a small DNA mass near the nucleus-free spermatid.



**Figure S4: Wild type and *mib-1(eb37)* male-derived sperm.** (A) Control (*him-5*) spermatids are round and have a nucleus (white dot) that is usually located centrally; (B) *mib-1(eb37)*; *him-5* spermatids have nuclei (white dot) that are frequently off-center. One spontaneously activated spermatozoon with a pseudopod (arrowhead) is present. (C and D) DIC micrographs of dissected male sperm activated *in vitro* with Pronase. (C) Control (*him-5*) spermatozoa each extend a single pseudopod (arrowhead) that is approximately equal in length to the cell body; (D) *mib-1(eb37)*; *him-5* spermatozoa extend short, abnormal pseudopods. (E and F) Frame-grabbed images from movies of immobilized male-derived sperm that have been activated with Pronase. The time (in seconds, s) is indicated in the left corner of each frame. (E) A control (*him-5*) spermatozoon has a single pseudopod (arrowheads) that maintains a stable position relative to the cell body (frame grabbed from supplemental movie S1); (F) A *mib-1(eb37)*; *him-5* spermatozoon extends a short, unstable pseudopod (arrowheads) that rapidly changes position. In the last frame, two regions extend a pseudopod (frame grabbed from supplemental movie S3). The scale bars are 5 μm and the one in D applies to A-D while the one in F applies to E and F.

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      (eb34)I                (eb41)D
C. elegans  MNGVAGESPSYDEYLYTFTPGDRILISENHPNHAQRNLSYNNVAVTKTHLALLGHIGYIESINEHRHTANVRVYAVPQNPELFLKSTEW 90
C. briggsae MDQEDENSVDSTQSEINFRLGDRVLISEIGPNHAQRNLYNNISWTKTHMALLGHVGFYISLNEARRTAEVRFYTLPPCTDLLQILTEW 90
C. remanei  MELNNTVQSPQSSETFIEGDRVLISEISPNYAQRNLYNNVNWTKTHMALLGHVGYIYSIDEEKRTALVRVYALPPSTDLLQNLTEW 90
C. brenneri MESADESSSENTQSSDFRAGDRVLISEISPNFAQRNLYNNVIWTKTHMALLGHVGYIFSIINEKKSALVRIFYTLPPSAELLQNLTEW 90
C. tropicalis MDSNDEITEESARIGNIFHQGDRVLISEISPSHAQRNLSYNSLIWTKTHMALLGHVGYINSLNEERRTASVCIFYSLPPSTELLQNLTEW 90
      *:      .      .      *      **:*      *      .      *      *      *      *      *      *      *      *      *      *      *      *
C. elegans  PLDALEFPQCIDHSKGLDVAITRGDPEKTTIGIVATKEPLNLGGIPKHLVQLKSASNPTITREIQVAVNANTGWTDERRIDFLCPVYSGP 180
C. briggsae PLEALEYPRMVEHSKGDVVAITRGDPKITSIGVIAANEIVSFEFVGPVYVSLKNAENPTVMRDIQVTVDVNPGWTDGRRDLFLCPVYSGP 180
C. remanei  PLEALEIPKRVESKGDVVAITRGDPLKTTIGVIAAKETVFEFVGPVYVSLKNAENPTVMRDIQVTVDVNPGWTDGRRDLFLCPVYSGP 180
C. brenneri PLDALEVPQVVEHSKGDIVAITRGDPSKTTIGVIAAKETVFEFVGPVYVSLKNAENPTVMRDIQVTVDVNPGWTDGRRDLFLCPVYSGP 180
C. tropicalis PLEALEIPKRVESKGDIVAITRGDPLKTTIGVIAASSETVIEFVGPVYVSLKHAENSALTRDIQVTVDVNPGWTDGRRDLFLCPVYSGP 180
      **:*      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      (gk936708)X                (eb36)T                (hc54)F                (gk587388)S
C. elegans  RLSMIARDNNEPSVHTYKHTIHGYTPDTLMQVIANWGEDALSAYLLVESIRSDPQQVRTLFDGQLPLFRAVADDLRNVVVMLVALGADRT 270
C. briggsae RLSMITRDRNEHSIHTYKHTIHGYTTENLMIINWNTEDSGSYAKLVESIRANPQQVRSLFDGQLPLFRAVSDDLWNVVVMLISLGADKT 270
C. remanei  RLSMITRDRNEHSIHTYKHTIHGYTTDLNMIINWNTEDSSSYAKLVESIRANPQQVRFDFDGLPFRFRAVSDDLWNVVVMLISLGAEN 270
C. brenneri RLSMITRDRNEHSIHTYKHTIHGFNTDLNMIINWNTEDSGSYAKLVESIRANPQQVRTLFDGQLPLFRAVSDDLWNVVVMLISVGADKN 270
C. tropicalis RLSMITRDRNEHSIHTYKHTIHGYTTDLNMIINWNTEDSSSYAKLVESVRANPQHVRTLFDGQLPLFRAVSDDLWNVVVMLISVGADKN 270
      *****:*. *      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      (eb35, eb42)IQ
C. elegans  ARDSERNITVHVAERGLDKMLDTVMLLLPKDINSQAANGLTPLHLAARHAHAACIDRLLGTSTCIPCVPNFGDTLLHEVCRLEPSSNK 360
C. briggsae VRDGSERTIVHVAERGLDKMLEGVLLLLPKEVNSQSANGDTPHLAARHAHAACIDRLLGTSACLPCVANNGDTPLHEVCKLPESGNK 360
C. remanei  AKDSQNTIVHVAERGLDKMLEGVLLLLPKEVNSQSITGDTPLHLASRHAHAACIDRLLGTSACLPCVANNGDTPHEVCKLPESGNK 360
C. brenneri ARDANEKTIHVAERGLDKMLEGVLLLLPKEVNSQKNGDTPHLAARHAHAACIDRLLGTSACLPCVSNIGDTPLHEVCKLPESGNK 360
C. tropicalis ARDGNDRITVHVAEKGLDKLLEGVLLLLPKEVNSQSRNGDTPHLASRHAHAACIDRLLGSSSCLPCVANNGDTPLHEVCKLPESGNK 360
      :. *      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      (eb37)X
C. elegans  KAAISRILTNTRANIHVVNSNMTPIQIAIMSGHVSTVEQLLLLRASYRNTTSKTMGSALHFAAASGHANVVNKLISLGLVHRRDKFGR 450
C. briggsae KAAISRILTNTRANIHQMNSSMTPLQISIAEGHVSTVDQLLLLRSSHRNTTTKNGMNALHYAAGCGHNVVVKLISLGLVHRRDKFGR 450
C. remanei  KAAISRIMTNTRANIHQMNSANMTPLQISIFGGHVNTVEQLLLLRSSYRNTTTKNGMNALHYAAGCGHNVVVKLIMLGLVHRRDRFGR 450
C. brenneri KAAISRILTNTRANIHQMNSNMTPLQISISGGHVSTVEQLLLLRSSYRNTTTKNGMNALHYAAGCGHNVVVKLIALGLVHRRDKFGR 450
C. tropicalis KAAISRILTNTRANIHQMNSANLTPQISIHGGHVSTVEQLLLLRSSYRNTTSKDMNALHYAAGCGHNVVVKLISLGLVHRRDRLGR 450
      *****:*****:*. *      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
C. elegans  GVLHYALEKWTGEAEKDCNRLAAIQALVKAGAPSNIIDLNGQTPVFQQLIREMNSSEQYPASLVPVCAQLVTRTNLNLDEMASRLRPMWQL 540
C. briggsae GVLHYALEKWTGEADKDCGRLATIQALVRAGAPSNLVDLNGQTPVFQQLIREIMKTNEQYPTLIPVCAQLIRTNLNLDEMASRLRPMWQL 540
C. remanei  GVLHYALEKWTGEAEKDCGRLASIQALVKAGAPPVVDLNGQTPIFQLVKDMLKQNEQYAVSLVPMCAQLVTRTNLNLDEMASRLRPMWQL 540
C. brenneri GVLHYALEKWTGEAEKDCGRLATIQALVKAGAPPVVDLNGQTPVFQQLVLDILNKQYAVSLVPMCAQLVTRTNLNLDEMASRLRPMWQL 540
C. tropicalis GVLHYALEKWTGEAEKDCGRLATIQALVKAGAPPVVDLNGQTPVFQQLVLDILKNNEQFPSTLVPVCAQLVTRTNLNLDEMASRLRPMWQL 540
      *****:*****:*. *      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      (eb39)X
C. elegans  ATICFLVANGADLNKDRKGMVMDLCEESSFRSIIIVHIAQTQKQVMPMMAMSEDKFDSTEVMTCTFSCLSNVATVKLDPCGHRVACV 630
C. briggsae ATICFMVSNAGADLNKDRKGVSAVDLCTGSSLRPIIVHISQTKQRTVMPM-MSMALDKFDAAEVMCTFSCLDVATVKLNPCGHRVACV 629
C. remanei  ATICFMVSNAGADLNKDRKGVSAVDLCEDSLRLPIIVHISQTKQRTVMPM-LAMSQDKFDAAEVMCTFSCFDSVATVKLNPCGHRVACV 629
C. brenneri ATICFMVSNAGADLNKDRKGVSAVDLCEDSLRLPIIVHISQTKQRTVMPM-MALSQDNFDAAEVMCTFSCLDVATVKLNPCGHRVACV 629
C. tropicalis ATICFMVSNAGADLNKDRKGLTIADLCEDSLRLPIIVHISQTKQRTVMPM-LAMSQDRFDAAEVMCTFSCLDVATVKLNPCGHRVACV 629
      *****:*****:*. *      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
C. elegans  DCTEKVAIRRCPCVCRQFINEAHDQDGKPVQIGSRCHEPSDGRQ--QVSAEVRKQIADDAAREAKIEVEREKQNELNQLRKRKLEQLELET 718
C. briggsae DCAEKTAIRRCPCVCYQFIAEAHDQDGPVQIGFRSGEPSDFDRKTPQLSAEYCKKIAEDAAREAKIEVEREKQNELNLLRKKLEQLELET 719
C. remanei  DCAEKTAIRRCPCVCYQFIAGAHHDGTVQVIGSRSGESTDFGRQHSQLSAEICKKIAEDAAREAKIEFEREKQNELNLLRKKLEQLELET 719
C. brenneri DCAEKTIRRCPCVCYQFIAGAYDQDGIQVIGSRSSPSDLERQNPQLSAEICKKIAEDAAREAKIEVEREKQNELNLLRKKLEQLELET 719
C. tropicalis DCAERTSIRRCPCVCYQFIAGASDQDGIQVIGSRSSSETLELERQTPQLSAEIRKKAEDAAREAKIEVEREKQNELNLLRKKLEQLELET 719
      **:*      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      (eb33)Y
C. elegans  NCAICMDLKIIVFVFCGHTACVDCADKLLKQCHI CRKTIETMQPIYS 765
C. briggsae SCAICMDSKIEIVFVFCGHTACVECADKLNKQCHI CRKTIETMQPIYS 766
C. remanei  SCAICMDSKIEIVFVFCGHTACVECTDKLLKQCHI CRKPIETMQPIYS 766
C. brenneri SCAICMDSKIAIVFVFCGHTACVECADKLLKQCHI CRKTIETMQPIYS 766
C. tropicalis SCAICMDSKIAIVFVFCGHTACVDCADKLNKQCHI CRKTIETMQPIYS 766
      .***** *      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *

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**Supplemental Figure 5:** Clustal Omega (Sievers et al., 2011) alignment of MIB-1 proteins from five *Caenorhabditis* species. Except for *C. elegans*, the others are derived from genomic sequences and, in the case of *C. remanei*, *C. brenneri* and *C. tropicalis*, exon calling errors were corrected by visual inspection,

which was possible because there is high MIB-1 homology among these species. Identical amino acids that are indicated by an “\*”, closely similar amino acids are indicated by a “:” and somewhat similar amino acids are indicated by a “.”. The original accession numbers are: NP\_499452 for *C. elegans* (Abbott et al., 1998), CAP32032.2 for *C. briggsae* (Stein et al., 2003), XP\_003104188.1 for *C. remanei* (unpublished, Washington University Genome Sequencing Center), EGT34718.1 for *C. brenneri* (unpublished, Washington University Genome Sequencing Center), Csp11.Scaffold628.g7302.t1 for *C. tropicalis* (from: <http://blast.caenorhabditis.org/#result>). The positions of nine mutations created in this study plus two from the Million Mutation Project that were partially evaluated are indicated (Thompson et al., 2013). X indicates the position of mutations that affect splice sites and alter or truncate the coding sequence (see supplemental Figure 1).

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aaatcgatgaaaaacttgggaaaactcacTTCTTTGATCTTCATcggtttattgcatcaagtgatgggatgttga<sup>ct</sup>gaaaca  
 tcgataatatagatgagaaaaatcgagaatttaagtcaaaaaaaactttttgaaatgaggaaaaatcgatggaaatcgaaaaaa  
 cactgaaaaatcagcaaaaaataaatttcaaattcaaagcgaagaattcgcgggcgcgaccgggactagaaaatccccactcggc  
 antisense to HL45 primer  
 Cacc<sup>g</sup>gtgtgtactccctctcggacaaggatatttcgaaaaatgaggggtttttccaaataaaaaacgtgttatttctcggaaaaa -68  
 tactagaaaaccacggaaaaacgtgtctaaaaacaaaaaaactaaagcagaataacaaaaaattattta<sup>tttcaattattttctca</sup> 16  
 M S K G E E L F T G V V P I L V E L D G D V N G H K  
 caaaaATGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCAATTTCTTGAATTAGATGGTGATGTTAATGGGCACAAA 21/78  
 F S V S G E G E G D A T Y G K L T L K F I C T T G K L P  
 TTTTCTGTCTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACCTTACCCTTAAATTTTATTTGCACTACTGGAAAACCTACC 161  
 V P W P T L V T T F  
 TGTTCATGGgtaaagtttaaacatataataactaactaacctgattattttaaattttcagCCAACACTTGTCACTACTTTTCT 244  
 C Y G V Q C F S R Y P D H M K Q H D F F K S A M P E G Y  
 GTTATGGTGTTCATGCTTcTCGAGATACCCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCGAAGGTTAT 327  
 V Q E R T I F F K D D G N Y K T  
 GTACAGGAAAGAACTATATTTTTCAAGATGACGGGAACACAAGACAGgtaaagtttaaacagttcggtaactaactaaccata 410  
 R A E V K F E G D T L V N R I E L K G I D F  
 catattttaaattttcagGTGCTGAAGTCAAGTTTGAAGGTGATAACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTA 493  
 K E D G N I L G H K L E Y N Y N S H N V Y I M A D K Q K  
 AAGAAGATGGAAACATTTCTTGGACACAAAATTGGAATCAACTATAACTCACACAATGTATACATCATGGCAGACAAAACAAA 576  
 N G I K V N F K I R H  
 AATGGAATCAAAGTTgtaaagtttaaacatgattttactaactaactaatctgattttaaattttcagAACTTCAAATTTAGACA 659  
 N I E D G S V Q L A D H Y Q Q N T P I G D G P V L L P  
 CAACATTTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTTGGCGATGGCCCTGTCTTTTACCAG 742  
 D N H Y L S T Q S A L S K D P N E K R D H M V L L E F V  
 ACAACATTTACCTGTCCACACAATCTGCCCTTTTCGAAAGATCCAACGAAAAGAGAGACCACATGGTCTTCTTTGAGTTTGTA 825  
 T A A G I T H G M D E L Y K G G G M N G V A G E S P S Y  
 ACAGCTGCTGGGATTACACATGGCATGGAcGAACTATACAAAggagggcgaATGAACGGAGTAGCTGGCGAATCGCCGAGCTA 876/53  
 D E Y L T Y F T P G D R I L I S E N H P N H A Q R L N  
 CGACGAGTACCTGACGTATTTACACCCGGGCGATCGAATTTCTGATATCGGAAAAATCATCCAAATCATGCACAACGCCCTCAATT 136  
 S Y N N V A W T K T H L A  
 CGTACAATAATGTTGCGTGGACGAAGACACATTTGGCTgtgagtgccagaaaaagaaaaaaatgctgaaatctgaaattttttg 219  
 L L G H I G Y I E S I N E H  
 ctgagaatctcttcaaaacaggcaaatcttcaacattttcagCTTCTCGGTACATTTGGCTATATCGAGAGCATCAACGAGCA 302  
 R H T A N V R V Y Y A V P Q N P E L  
 CCGGCACACTGCAAAATGTTCCGGTTTACTACGCAGTTCCGCAAAATCCAGAGCTTgtaaagctgattttttgaaagattttgtgt 385  
 Aaaatgtgtgatttttcggaatctagcgcgactttttttgactttttttccattgaaaaatcggaaaaaaaagtttaaattgtct 468  
 Ggatactaccgtaatcgtttaaaggcgcacacatttttactgatcgcgccaggagaagtattgcaaaagtgccgtgctgcttt 551  
 Aaaaggctactgtaatttttgattttcaaatcgaatttttaaatgaaaattggttgataacggtacagaaactgaaatcttc 634  
 Attgaaagatacttaaaacttttctgaatttttttgatttcaagctttcagaggtaacactgaaaaaagagcctttttccgaa 717  
 F K L S T E W P L D A L E F P Q C  
 tttttcatcccaaattttaaattttaaattttcagTTCAAGCTCTCTACCGAATGGCCGCTGGACCGGTTGGAATTTCCCTCAATG 800  
 I D H S K G D L V A I T R G D P E K T T I G I V A T K  
 TATAGATCATTTCAAAAAGGTGATCTCGTTGCAATAACCCGCGGAGATCCGGAAAAAACGACAATCGGAATAGTGGCAACGAAGC 883  
 E P L N L G G I P K H L V Q L K S A S N P T I T R E I Q  
 AACCATTTGAATTTAGGTGGAATTCGAAGCATTTGGTTCAACTGAAAAGCGCCTCGAATCCGACGATTACCCGGGAGATTCAG 966  
 V A V N A N T G W T D E R R D I F L C P V Y S G P R L S  
 GTGGCGTTAATGCGAATACGGGATGGACTGATGAGAGACGGGATATCTTTTTGTGTCCGGTTTATTCGGGTCCCCGATTGTC 1,049

**Supplemental Figure 6:** A *gfp::mib-1* gene fusion created by CRISPR genome engineering techniques.

Sequence features include:

*gfp* exon sequences are shaded in green and derived from pPD95.81 (Fire Lab Vector Kit, 1995). Three glycine codons link the 3' end of the *gfp* gene sequence to the start of *mib-1* coding sequence.

**G** (at -145) and **T** (at 968), respectively, marks the 5' and 3' ends of the 1,113 bp region deleted in the *mib-1(eb154)* mutation.

*mib-1* (Y47D3A.22) exons, shaded in red, include the empirically determined *mib-1* transcription start. Only part of the *mib-1* gene sequence is shown.

*smc-3* (Y47D3A.26a) exons are shaded in magenta and coding is on the opposite strand from *mib-1*. Only part of the *smc-3* gene is shown.

Translation start codons are in white text

Introns are in lower case and have no shading

Wild type *mib-1* had a **C** (at -150) was mutated to a **G** and a **G** (at 972) was mutated to a **T** (this silent mutation, like wild type, encodes alanine). These point mutations were introduced so that Cas9 would not cut the template during CRISPR-mediated insertion of *gfp* sequence into *mib-1(eb154)*.

**ct** antisense to *smc-3* outtron acceptor for SL1

**Supplemental Table 1**  
***mib-1* mutations**

<b>Allele</b>	<b>Mutagen</b>	<b>Screen</b>	<b>Tester<sup>1</sup></b>	<b>Mutation<sup>2</sup></b>	<b>Mutation type</b>
<i>mib-1(hc54)</i>	50 mM EMS	F2	NA	C 1657 T	L247F missense
<i>mib-1(eb33)</i>	50 mM EMS	F1	<i>hc54</i>	G 3962 A	C720Y missense
<i>mib-1(eb34)</i>	50 mM EMS	F1	<i>hc54</i>	G 24 A	M1I missense
<i>mib-1(eb35)</i>	50 mM EMS	F1	<i>tDf7</i>	G 1851 A	M291I missense
<i>mib-1(eb36)</i>	50 mM EMS	F1	<i>hc54</i>	G 1588 A	A224T missense
<i>mib-1(eb37)</i>	50 mM EMS	F1	<i>hc54</i>	G 2398 A	Cryptic splice donor used; encodes 437 of SPE-16 plus 9 novel amino acids followed by a premature nonsense codon
<i>mib-1(eb39)</i>	50 mM EMS	F1	<i>hc54</i>	G 3131 A	Cryptic splice acceptor used; novel 8 amino acid insertion between amino acids 556 and 557
<i>mib-1(eb41)</i>	4.25 mM ENU	F1	<i>tDf7</i>	G 83 A	G21D missense
<i>mib-1(eb42)</i>	4.25 mM ENU	F1	<i>tDf7</i>	T 1853 A	L292Q missense
<i>mib-1(eb154)</i>	CRISPR Cas9	F2	NA	deletes from -145 to 968	1,113 bp deletion; removes coding sequence for first 156 amino acids
<i>mib-1(eb1s26)</i>	CRISPR Cas9	F2	NA	GFP insertion at 21	876 bp insertion of <i>gfp</i> plus three glycine codons before <i>mib-1</i> coding sequence

<sup>1</sup>Tester refers to the *mib-1* affecting mutation used to select noncomplementing new alleles. <sup>2</sup>Nucleotide number is from the *mib-1* transcription start

**Supplemental Table 2  
Primers Used for PCR and CRISPR Genome Engineering**

<b>Primer</b>	<b>5' to 3' sequence</b>	<b>Position in <i>mib-1</i></b>
Fspe16C1Sal1	GTTCGACATGAACGGAGTAGCTGGCGAATCG	22 to 45 sense primer; <i>Sal</i> I restriction site
Rspe16C1BglII	AGATCTTCACGAGTAGATCGGCTGCATCGTC	4,166 to 4,141 antisense primer; <i>Bgl</i> II restriction site
Rspe16NRFBglII	AGATCTCGATCTTTGACATTCAAATCCGCTCC	3,137 to 3,132 and 2,947 to 2,928 antisense primer; <i>Bgl</i> II restriction site
MR83	GCTTGTATCGATCGGCTTCTCGG	1,948 to 1,970 sense primer
MR84	CGCCATCATCATCGGCATCACCTG	3,431 to 3,407 antisense primer
MR100	CGTTCGTCAATGTATTTT CAGAAGGCC	-2133 to -2105 sense primer
MR101	AGTCGACCTGCAGGCATGCAAGCTC GATTCCGCCAGCTACTCCGTTTCAT	45 to 22 antisense primer; <i>pPD95.75 antisense primer</i> <sup>1</sup>
MR102	AGCTTGCATGCCTGCAGGTCG	NA; <i>pPD95.75 sense primer</i> <sup>1</sup>
MR103	AAGGGCCCCGTACGGCCGACTA	NA; <i>pPD95.75 antisense primer</i> <sup>1</sup>
TK287	CGTCTACGCTGCTCTCATCCTTCA	NA; <i>rla-1 sense primer</i>
TK288	ATTCCTCCTTTGGCTCCTCCTTCTTCTT	NA; <i>rla-1 antisense primer</i>
HL45	GAGAGGAGTACACACCGGGGTT TTAGAGCTAGAAATAGCAAGT	-130 to -148 antisense guide sequence and forward Q5 for sgRNA <sup>2</sup>
HL46	ACCCGGGAGATTCAGGTGGGTT TTAGAGCTAGAAATAGCAAGT	952 to 970 guide sequence and forward Q5 for sgRNA <sup>2</sup>
HL56	ATTCGCATTAACCGCCGGGGTT TTAGAGCTAGAAATAGCAAGT	984 to 968, -146 to -147 <i>mib-1(eb154)</i> antisense guide sequence and forward Q5 for sgRNA <sup>2</sup>
HL57	TCCCCACTCGGCCACCCGGGTT TTAGAGCTAGAAATAGCAAGT	-161 to -143 <i>mib-1(eb154)</i> sense guide sequence and forward Q5 for sgRNA <sup>2</sup>

Some primers are bipartite, being complementary to *C. elegans* genomic DNA in one region and the insert within a plasmid or a restriction site in another region; *italic* text is used to differentiate these two template sources.

<sup>1</sup>Fire lab 1995 vector kit

<sup>2</sup> Dickinson, D. J., Ward, J. D., Reiner, D. J. and Goldstein, B. (2013). Engineering the *Caenorhabditis elegans* genome using Cas9-triggered homologous recombination. *Nature Methods* 10, 1028-1034.