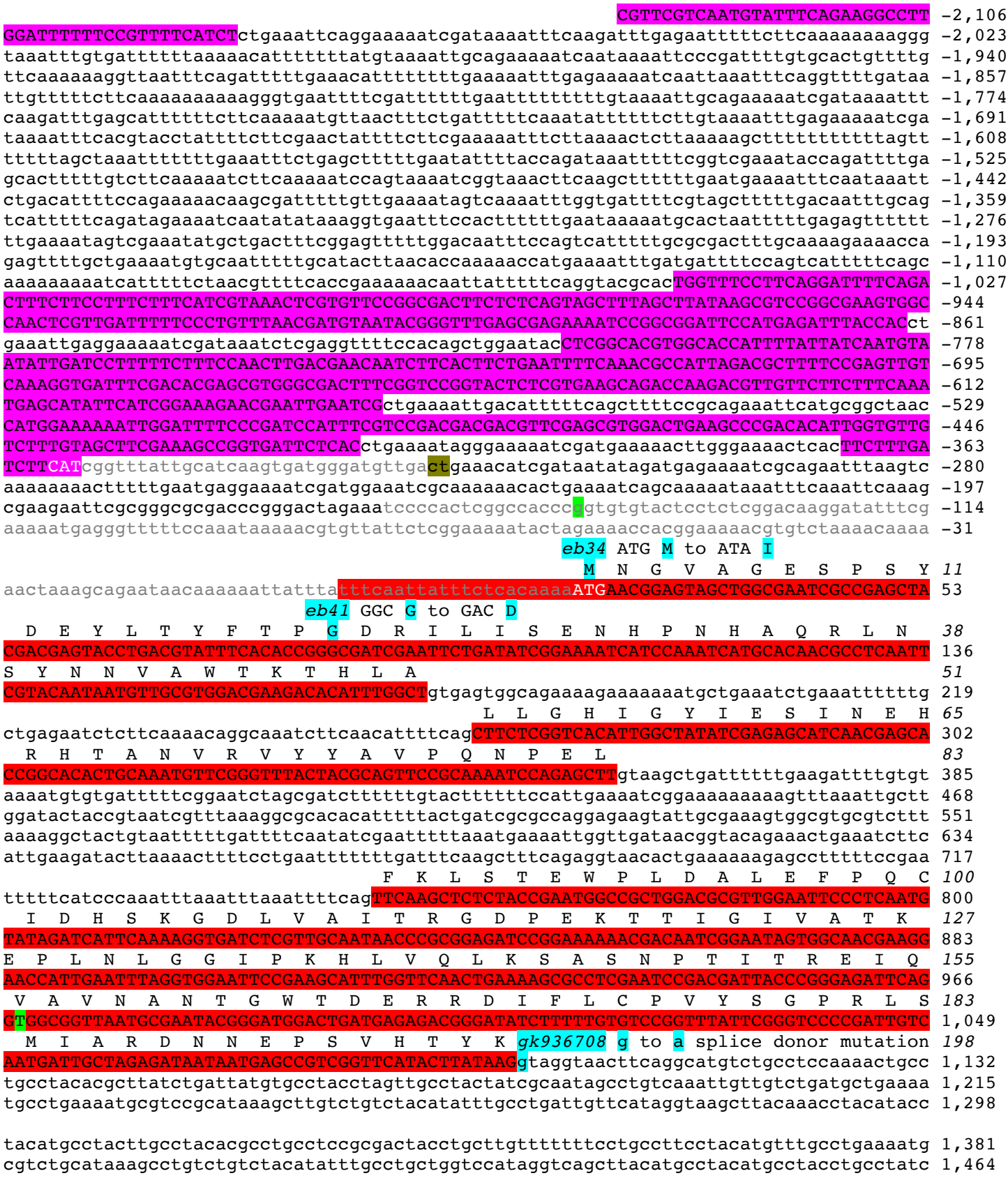
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aaaaatgagggttttccaaataaaaacgtgttattctcggaaaaatactagaaaaccacggaaaaacgtgtctaaaacaaaa -31
eb34 ATG M to ATA I
M N G V A G E S P S Y 11
aactaaagcagaataacaaaaaattatttatttcaattatttctcacaaaaATGAACGGAGTAGCTGGCGAATCGCCGAGCTA 53
eb41 GGC G to GAC D
D E Y L T Y F T P G D R I L I S E N H P N H A Q R L N 38
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S Y N N V A W T K T H L A 51
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L L G H I G Y I E S I N E H 65
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R H T A N V R V Y Y A V P Q N P E L 83
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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
AACCATTGAATTTAGGTGGAATTCCGAAGCATTTGGTTCAACTGAAAAGCGCCTCGAATCCGACGATTACCCGGGAGATTCAG 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
GTGGCGGTTAATGCGAATACGGGATGGACTGATGAGAGACGGGATATCTTTTTGTGTCCGGTTTATTCGGGTCCCCGATTGTC 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
AATGATTGCTAGAGATAATAATGAGCCGTCGGTTCATACTTATAAGgtaggtaacttcaggcatgtctgcctccaaaactgcc 1,132
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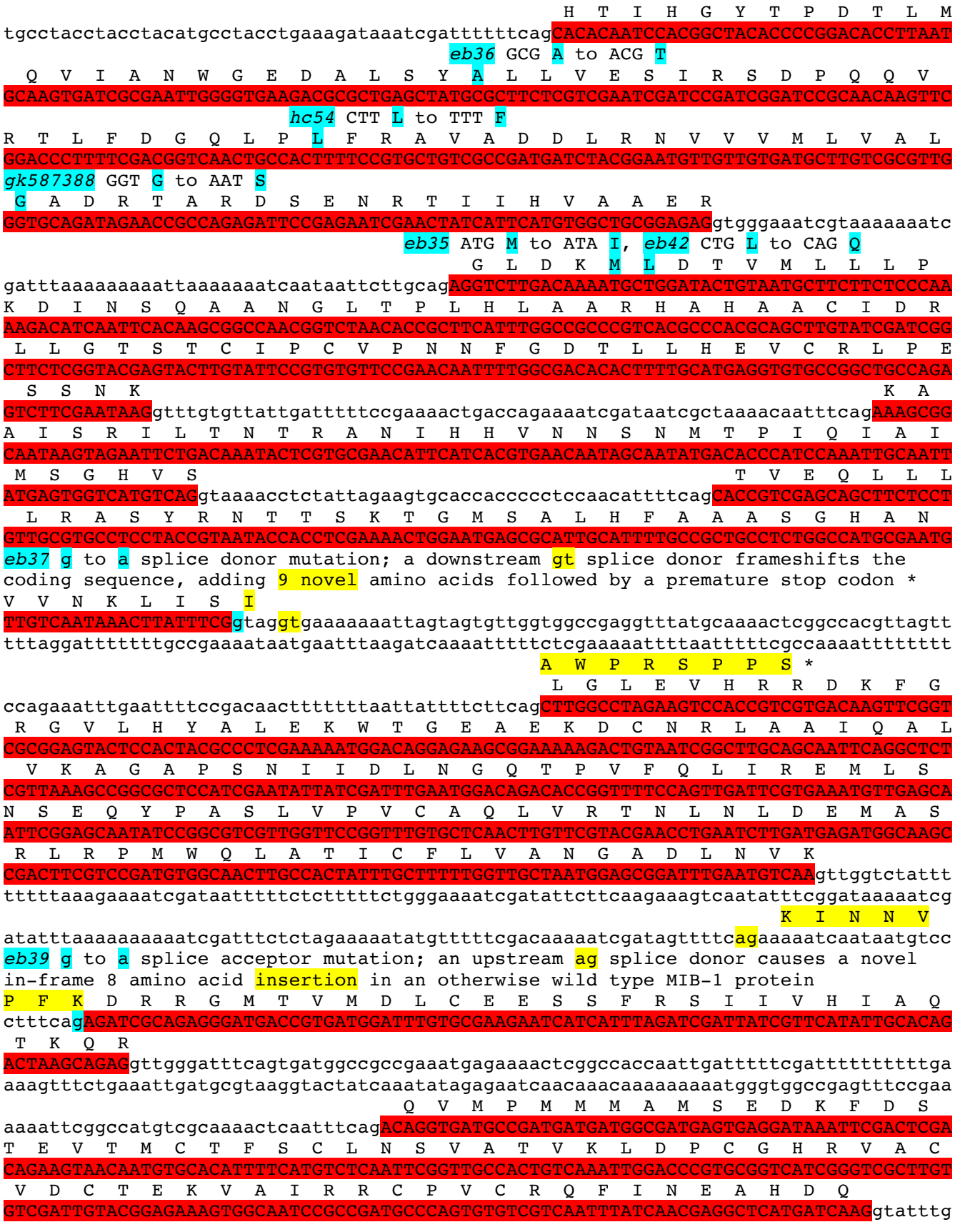
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eb36 GCG A to ACG T
Q V I A N W G E D A L S Y A L L V E S I R S D P Q Q V 237
GCAAGTGATCGCGAATTGGGGTGAAGACGCGCTGAGCTATGCGCTTCTCGTCGAATCGATCCGATCGGATCCGCAACAAGTTC 1,630
hc54 CTT L to TTT F
R T L F D G Q L P L F R A V A D D L R N V V V M L V A L 265
GGACCCTTTTCGACGGTCAACTGCCACTTTTCCGTGCTGTCGCCGATGATCTACGGAATGTTGTTGTGATGCTTGTCGCGTTG 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
GGTGCAGATAGAACCGCCAGAGATTCCGAGAATCGAACTATCATTCATGTGGCTGCGGAGAGgtgggaaatcgtaaaaaaatc 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
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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
AAGACATCAATTCACAAGCGGCCAACGGTCTAACACCGCTTCATTTGGCCGCCCGTCACGCCCACGCAGCTTGTATCGATCGG 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
CTTCTCGGTACGAGTACTTGTATTCCGTGTGTTCCGAACAATTTTGGCGACACACTTTTGCATGAGGTGTGCCGGCTGCCAGA 2,045
S S N K K A 362
GTCTTCGAATAAGgtttgtgttattgatttttccgaaaactgaccagaaaatcgataatcgctaaaacaatttcagAAAGCGG 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
CAATAAGTAGAATTCTGACAAATACTCGTGCGAACATTCATCACGTGAACAATAGCAATATGACACCCATCCAAATTGCAATT 2,211
M S G H V S T V E Q L L L 403
ATGAGTGGTCATGTCAGgtaaaacctctattagaagtgcaccacccctccaacattttcagCACCGTCGAGCAGCTTCTCCT 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
GTTGCGTGCCTCCTACCGTAATACCACCTCGAAAACTGGAATGAGCGCATTGCATTTTGCCGCTGCCTCTGGCCATGCGAATG 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
TTGTCAATAAACTTATTTCGgtaggtgaaaaaaattagtagtgttggtggccgaggtttatgcaaaactcggccacgttagtt 2,460
tttaggatttttttgccgaaaataatgaatttaagatcaaaatttttctcgaaaaatttaatttttcgccaaaatttttttt 2,543
A W P R S P P S *
L G L E V H R R D K F G 449
ccagaaatttgaattttccgacaacttttttttaattatttttcttcagCTTGGCCTAGAAGTCCACCGTCGTGACAAGTTCGGT 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
CGCGGAGTACTCCACTACGCCCTCGAAAAATGGACAGGAGAAGCGGAAAAAGACTGTAATCGGCTTGCAGCAATTCAGGCTCT 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
CGTTAAAGCCGGCGCTCCATCGAATATTATCGATTTGAATGGACAGACACCGGTTTTCCAGTTGATTCGTGAAATGTTGAGCA 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
ATTCGGAGCAATATCCGGCGTCGTTGGTTCCGGTTTGTGCTCAACTTGTTCGTACGAACCTGAATCTTGATGAGATGGCAAGC 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
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tttttaaagaaaatcgataatttttctctttttctgggaaaatcgatattcttcaagaaagtcaatatttcggataaaaatcg 3,041
K I N N V
atatttaaaaaaaaaatcgatttctctagaaaaatatgtttttcgacaaaaatcgatagtttcagaaaaatcaataatgtcc 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
ctttcagAGATCGCAGAGGGATGACCGTGATGGATTTGTGCGAAGAATCATCATTTAGATCGATTATCGTTCATATTGCACAG 3,207
T K Q R 585
ACTAAGCAGAGgttgggatttcagtgatggccgccgaaatgagaaaactcggccaccaattgatttttcgatttttttttttga 3,290
aaagtttctgaaattgatgcgtaaggtactatcaaatatagagaatcaacaaacaaaaaaaaaatgggtggccgagtttccgaa 3,373
Q V M P M M M A M S E D K F D S 601
aaaattcggccatgtcgcaaaactcaatttcagACAGGTGATGCCGATGATGATGGCGATGAGTGAGGATAAATTCGACTCGA 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
CAGAAGTAACAATGTGCACATTTTCATGTCTCAATTCGGTTGCCACTGTCAAATTGGACCCGTGCGGTCATCGGGTCGCTTGT 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
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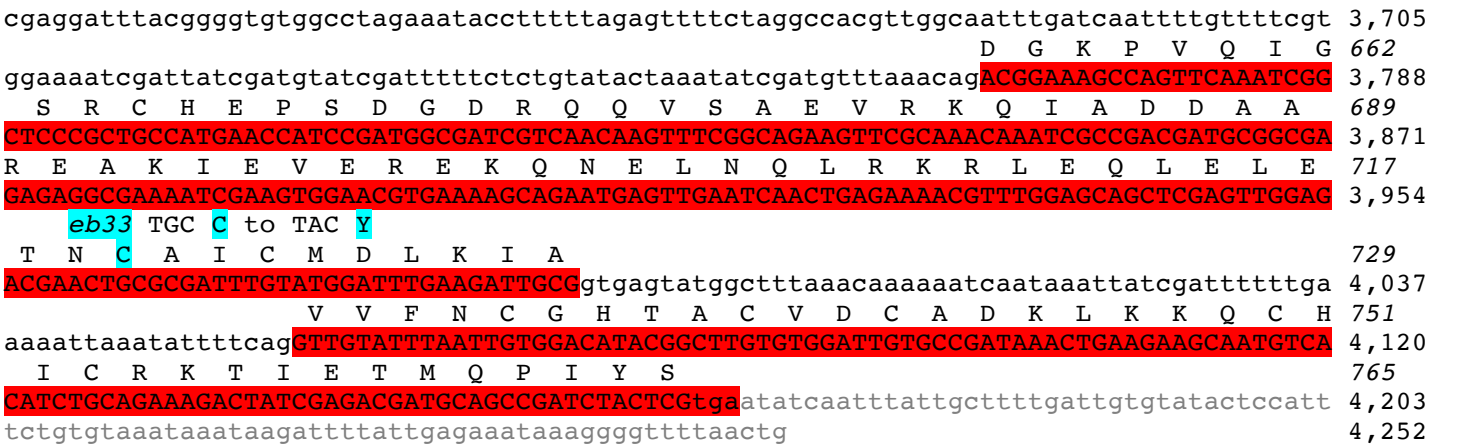


Supplemental Figure 1: The *mib-1* gene, protein and various sequence features:

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*

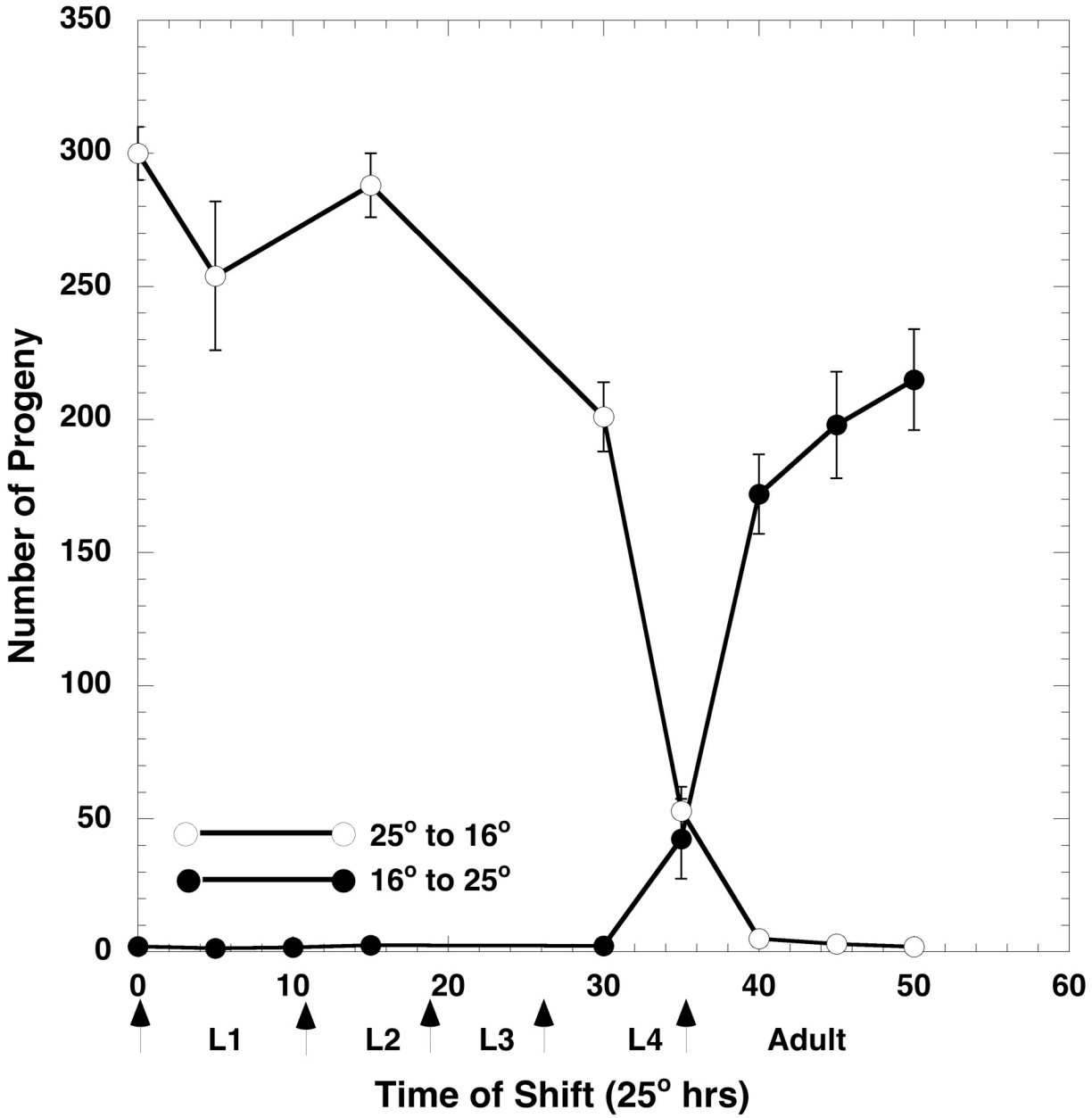
UTR sequence as defined by cDNA's described in Wormbase appears in gray text. At the 5' end, the longest cDNA is PE_SS_GG2740#FAF3E5. This is considerably longer than the empirically determined 5'transcription start for unknown reasons.

ct antisense to *smc-3* outron 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 (16º) to restrictive (25º) 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 25º hours since hatch. Error bars are standard error of the mean. Data from upshifts (16º to 25º) and downshifts (25º to 16º) 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.

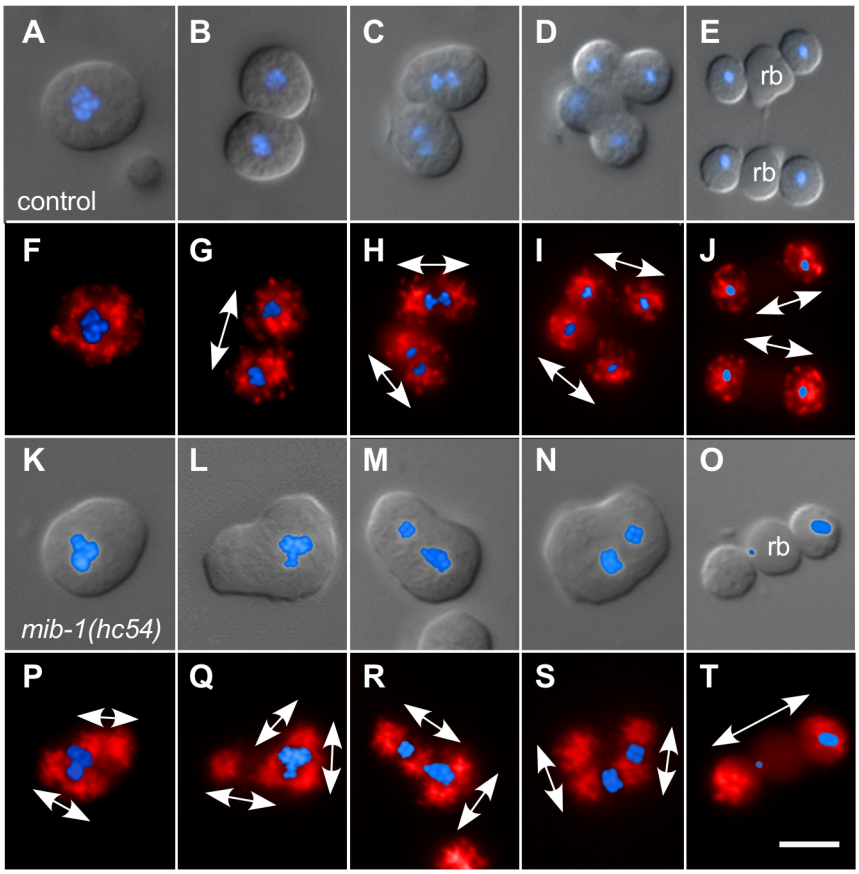


Figure S3: Spermatogenesis in live cells. Live *him-5* (wild type control; A-J) or *mib-1(hc54); him-5* (K-T), where DNA appears in blue and mitochondria are shown in red. Double-headed arrows indicate the presumptive cell division planes. (A, F) a primary spermatocyte with centrally located chromosomes 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^{o} 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.

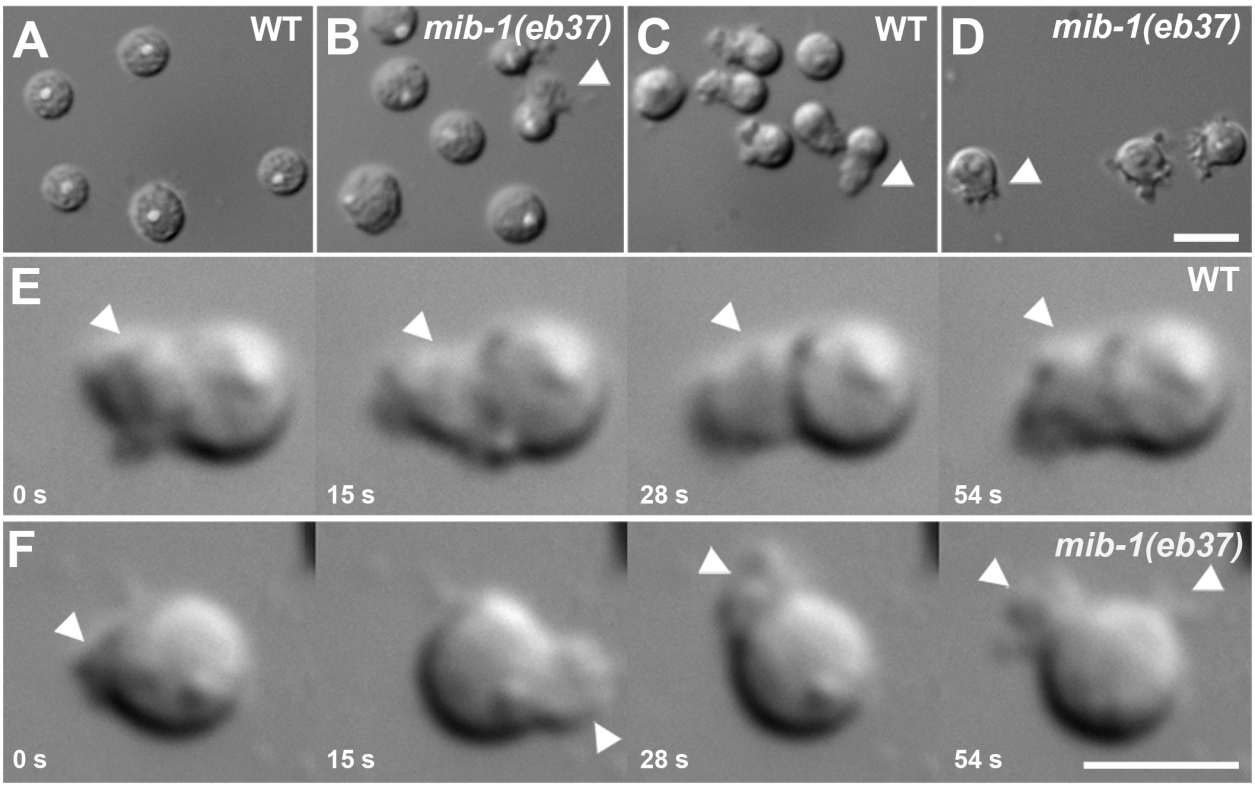
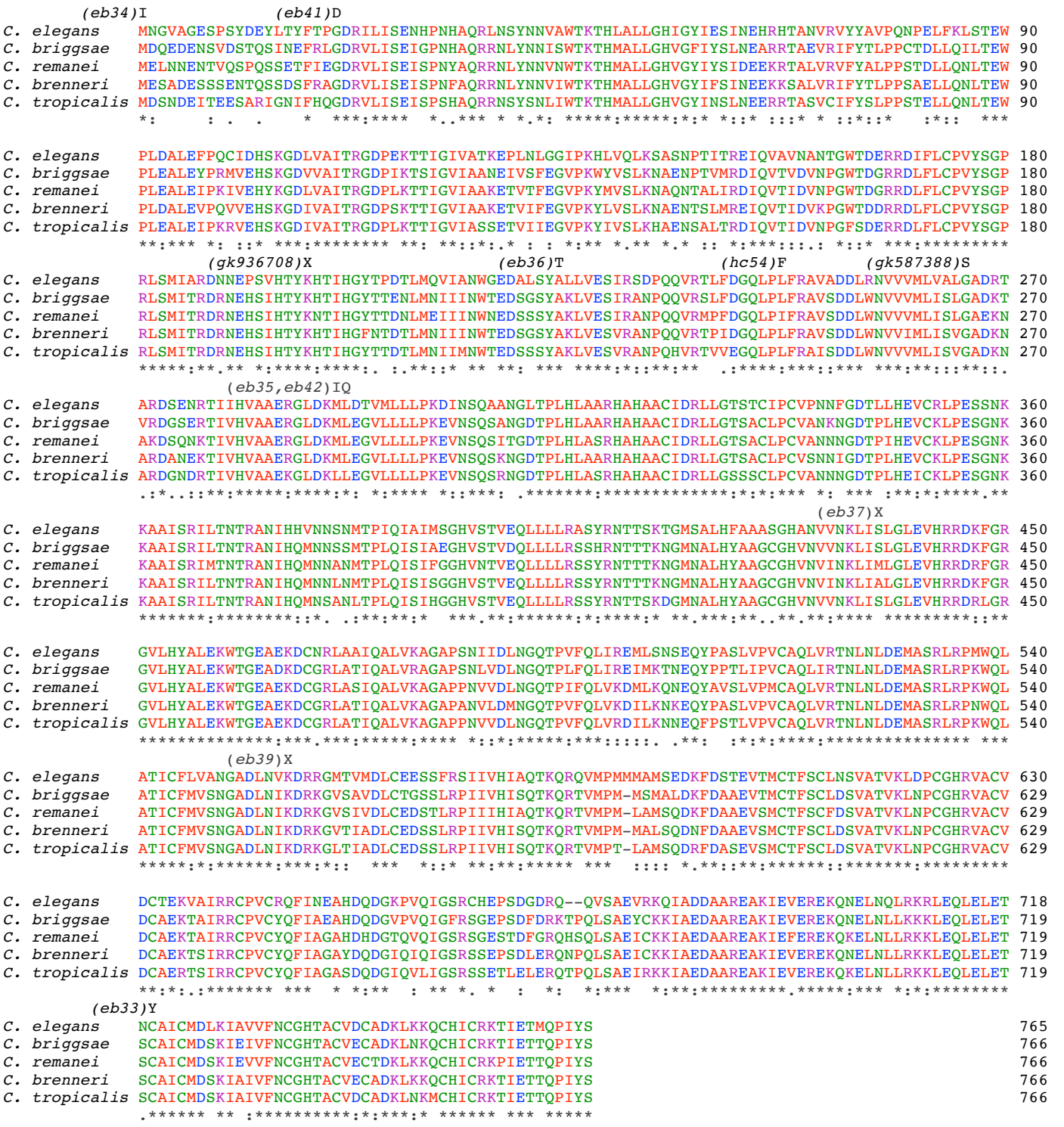


Figure S4: Wild type and *mib-1(eb37)* male-derived sperm. (A) Control (*him-5*) spermatids are round and have a nucleus (white dot) that is usually located centrally; (B) *mib-1(eb37); him-5* spermatids have nuclei (white dot) that are frequently off-center. One spontaneously activated spermatozoon 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.



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).

SUPPLEMENTAL REFERENCES

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Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Soding, J., et al. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Sys. Biol.* **7**, 539.

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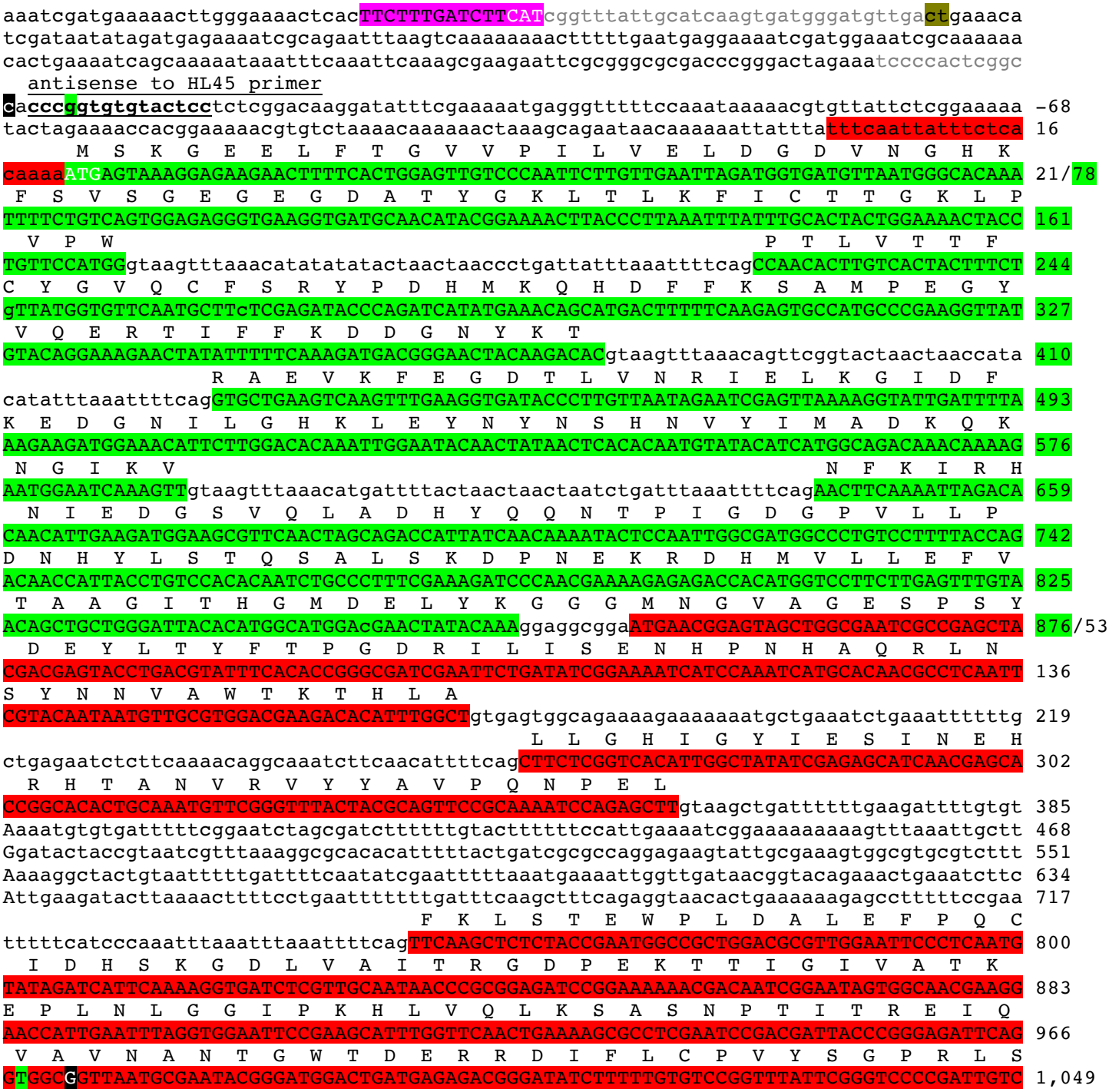

Supplemental Figure 6: A *gfp::mib-1* gene fusion created by CRISPR genome engineering techniques. Sequence features include:

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* outron acceptor for SL1

Supplemental Table 1
mib-1 mutations

Allele	Mutagen	Screen	Tester[1]	Mutation[2]	Mutation type
mib-1(hc54)	50 mM EMS	F2	NA	C 1657 T	L247F missense
mib-1(eb33)	50 mM EMS	F1	*hc54*	G 3962 A	C720Y missense
mib-1(eb34)	50 mM EMS	F1	*hc54*	G 24 A	M1I missense
mib-1(eb35)	50 mM EMS	F1	*tDf7*	G 1851 A	M291I missense
mib-1(eb36)	50 mM EMS	F1	*hc54*	G 1588 A	A224T missense
mib-1(eb37)	50 mM EMS	F1	*hc54*	G 2398 A	Cryptic splice donor used; encodes 437 of SPE-16 plus 9 novel amino acids followed by a premature nonsense codon
mib-1(eb39)	50 mM EMS	F1	*hc54*	G 3131 A	Cryptic splice acceptor used; novel 8 amino acid insertion between amino acids 556 and 557
mib-1(eb41)	4.25 mM ENU	F1	*tDf7*	G 83 A	G21D missense
mib-1(eb42)	4.25 mM ENU	F1	*tDf7*	T 1853 A	L292Q missense
mib-1(eb154)	CRISPR Cas9	F2	NA	deletes from -145 to 968	1,113 bp deletion; removes coding sequence for first 156 amino acids
mib-1(ebIs26)	CRISPR Cas9	F2	NA	GFP insertion at 21	876 bp insertion of *gfp* plus three glycine codons before *mib-1* coding sequence

[1]Tester refers to the *mib-1* affecting mutation used to select noncomplementing new alleles. [2]Nucleotide number is from the *mib-1* transcription start

Supplemental Table 2
Primers Used for PCR and CRISPR Genome Engineering

Primer	5' to 3' sequence	Position in *mib-1*
Fspe16C1Sal1	*GTCGAC*ATGAACGGAGTAGCTGGCGAATCG	22 to 45 sense primer; *Sal*I restriction site
Rspe16C1BglII	*AGATCT*TCACGAGTAGATCGGCTGCATCGTC	4,166 to 4,141 antisense primer; *Bgl*II restriction site
Rspe16NRFBglII	*AGATCT*CGATCTTTGACATTCAAATCCGCTCC	3,137 to 3,132 and 2,947 to 2,928 antisense primer; *Bgl*II restriction site
MR83	GCTTGTATCGATCGGCTTCTCGG	1,948 to 1,970 sense primer
MR84	CGCCATCATCATCGGCATCACCTG	3,431 to 3,407 antisense primer
MR100	CGTTCGTCAATGTATTTCAGAAGGCC	-2133 to -2105 sense primer
MR101	*AGTCGACCTGCAGGCATGCAAGCTC* GATTCGCCAGCTACTCCGTTCAT	45 to 22 antisense primer; *pPD95.75 antisense primer*[1]
MR102	AGCTTGCATGCCTGCAGGTCG	NA; pPD95.75 sense primer[1]
MR103	AAGGGCCCGTACGGCCGACTA	NA; pPD95.75 antisense primer[1]
TK287	CGTCTACGCTGCTCTCATCCTTCA	NA; *rla-1* sense primer
TK288	ATTCCTCCTTTGGCTCCTCCTTCTTCTT	NA; *rla-1* antisense primer
HL45	*GAGAGGAGTACACACCGGG*GTT TTAGAGCTAGAAATAGCAAGT	*-130 to -148 antisense guide sequence* and forward Q5 for sgRNA[2]
HL46	*ACCCGGGAGATTCAGGTGG*GTT TTAGAGCTAGAAATAGCAAGT	*952 to 970 guide sequence* and forward Q5 for sgRNA[2]
HL56	*ATTCGCATTAACCGCCGGG*GTT TTAGAGCTAGAAATAGCAAGT	*984 to 968, -146 to -147 mib-1(eb154) antisense guide sequence* and forward Q5 for sgRNA[2]
HL57	*TCCCCACTCGGCCACCCGG*GTT TTAGAGCTAGAAATAGCAAGT	*-161 to -143 mib-1(eb154) sense guide sequence* and forward Q5 for sgRNA[2]

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.

[1]Fire lab 1995 vector kit

[2] 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.