Oligonucleotides used in this study

For the following CRISPR targeting RNAs (crRNAs), target-specific sequences have a yellow background. For repair templates, altered nucleotides are in red, altered restriction endonucleases are underlined, and crRNA target sequences have a yellow background. For dsRNA template primers, target-specific sequences are underlined.

C04A11.4 (WY1208; *fd130*)

crRNA: 5'-CAGGAGUUCUGUUACGAAGGGUUUUAGAGCUAUGCU-3'
Alt-RTM CRISPR-Cas9 tracrRNA: IDT (CA#1072534) was used for all CRISPR injections.
Locus amplification: 5'-CCACCGAACAAACCCAATTG-3' and 5'-CGGAGCATTTAAGCCACCAT-3'
Locus sequencing: 5'-ATATTCGGTCATTGTGGCCC-3' and 5'-CTGGGTTTCACACTGCAACA-3'

F56D12.6 (WY1209; *fd131*)

crRNA: 5'-GCUCGAUCGGAAGCCGGCGCGUUUUAGAGCUAUGCU-3'
Locus amplification: 5'-GACTATCGCCTCAACTCAGA-3' and 5'-CTTCTCTATTTCCGCAGCTC-3'
Locus sequencing: 5'-GGAAGCGTGGATTCTGTGAT-3' and 5'-CAGCGAGCACCTTTTTACGA-3'
dsRNA primers: 5'-TAATACGACTCACTATAGGGAGAGGACGATTGGCGGAACAAAA-3' and
5'-TAATACGACTCACTATAGGGAGAGTGTCCAGGTTCGAATTTCC-3'

B0302.1 (WY1217; *fd139*)

crRNA: 5'-TGAGCCGATTCTCTCGTCTGGUUUUAGAGCUAUGCU-3'
Locus amplification: 5'-GCAACACGCCTATCACAGTT-3' and 5'-TCCGGAGAATCTGTTCTCTG-3'
Locus sequencing: 5'-TCAAATGTCGGACGAGGAGA-3' and 5'-CATTTAACTGAGGGTACCCG-3'
dsRNA primers: 5'-TAATACGACTCACTATAGGGAGACCATTCGAATATGCCCGCAA-3' and
5'-TAATACGACTCACTATAGGGAGATGAGTCACTGGTTTTGCCTG-3'

F48E8.5 (WY1211; *fd133*)

crRNA: 5'-GCAAAGAGTTTGAAGCGAATGUUUUAGAGCUAUGCU-3'
Locus amplification: 5'-GTTCTCAATCCGCGAGGCA-3' and 5'-TCGATTACAAGCCGAGTGCT-3'
Locus sequencing: 5'-GGAGCAGATTCTGAAGGAGA-3' and 5'-ACAGTTCTTGGCTTCCTCTG-3'
Repair template:
5'-CTTGTCGAGGATGACGTGCCGAATGTCAGATTCAACGCCGCAAAGAGTTTAAAGCGAA
TTGAAAAGAACTTGACCCCAAGGTGACAGGAAAATCTTTTTCACTATCCCAATTT-3'
(Creates DraI site)

W07E11.1

crRNA: 5'-TCAAACTTTGCACGGAGAACGUUUUAGAGCUAUGCU-3'
Locus amplification: 5'-CTCAGAAGCTGGAGTTGGAA-3' and 5'-TCTCTCCAGCAACTCCTCTA-3'
Locus sequencing: 5'-GTGCTCACGATGAACAACCT-3' and 5'-AATGGATCCGAACTCGAGCA-3'
Repair template:
GATGAGAAAATGTCACTTGAACACCTGCCCGGTTGGAGTCGCTACTCAAGATCTTGTTCTCCGTGCAAAGTTTGA
TGGAAAGCCAGAACACGTTGTCAACTATATG
(Creates BglII site)
dsRNA primers:

5’-TAATACGACTCACTATAGGGAGAACTTAAAGTGCGCTAACCCG-3’ and
5’-TAATACGACTCACTATAGGGAGACCACCAGAGAGACATTTTCC-3’

T09B9.4
crRNA: 5’-GCTTGTTGGAATGTGCAATCGUUUUAGAGCUAUGCU-3’
Locus amplification: 5’-ATCAGGAGGAATTGATGCGC-3’ and 5’-CGGCTTCGATACGAATAGCT-3’
Repair template:
ATCGTCAGCAACTCAACGAAACTGCCCATTTGCACATTGAAAACAAAAAGCTAGTTGGAATATGCAATCAAGAGA
GACGTCGATCGCAAGCTACAGAGGCTGAAGTCAAAAAATgttagtga
(Ablates HindIII site)
dsRNA primers:
5’-TAATACGACTCACTATAGGGAGAACACCGCTGCCTGATAATCA-3’ and
5’-TAATACGACTCACTATAGGGAGATGACTTCAGCCTCTGTAGCT-3’

F53A9.7
crRNA: 5’-GCTTGTTGGAATGTGCAATCTCAGTGTGAACAACAGTTGG-3’
Locus amplification: 5’-GATCGGGCGTCAAAAATGGG-3’ and 5’-ACTACACACCACTTTGAAGAAACA-3’
Repair template:
CATACGGAGGACACGGAGGATATGCTCCACCACCAGTCCATGGAGCTCCAGAATACATGCCACCGACGGTCGTT
CACACTGACGGAGGACATCACGGACACGTTGACACCCATCACCATGA
(Creates Hpy188III site)

T05A10.1
crRNA: 5’-GCTTGTTGGAATGTGCAATCATGCATGTCCGCTCCACCTG-3’
Locus amplification: 5’-ATAGCGATGGAACGACACCA-3’ and 5’-GGATACCTGAGAGTTGGAAGG-3’
Repair template:
AACGATCACTATTGGCCTTCGCCGGATCGTCGATCATGCATGTCCGCTCCATCTGTAGCAATGCAACGTGACTTTG
ATGACCGAGGTGTGTGAAACTCGGATCTGGAT
(Creates SfcI site)

R04E5.10
crRNA: 5’-GCTTGTTGGAATGTGCAATCATTGAAATCTCCGAATTTAA-3’
Locus amplification: 5’-AGCACAAAGATGAATCAATCGTCA-3’ and 5- TGACTGTACGATACGTGTGCAT-3’
Repair template:
ATATATTAAAAACAATTACAGATTCATTCATTGAAATCTCCGAATTTAATAGATGATATCGTTGCTACTCAAATCTA
CAGCTTCGATTTCTCCAAAAACACAAAGACAGTT
(Creates EcoRV site)

WGS, variant detection, and sibling subtraction method (SSM)

Our sequencing effort was designed to provide 20× average coverage of the *C. elegans* genome (105 Mb) to provide adequate depth to detect causative single-nucleotide polymorphisms (SNPs) in our experimental datasets. 2×125 paired-end library construction (150-bp insert) and sequencing were performed using the Tru-Seq DNA PCR-free Library Preparation Kit, and all

samples were sequenced in one lane on an Illumina HiSeq2000. We sequenced genomes from the non-mutant comparator for each mutant and also each of the five mutants described in this paper. All raw Illumina data are deposited in the NCBI Sequence Read Archive (http://trace.ncbi.nlm.nih.gov/Traces/sra) (BioProject ID PRJNA415825). Quality assessment was carried out with FastQC (*http://www.bioinformatics.babraham.ac.uk/projects/fastqc/*). Residual adapters and low-quality and short reads were removed via Trimmomatic v0.35 (BOLGER *et al.* 2014).

Following quality control, we performed the remaining analyses with Galaxy (AFGAN *et al.* 2016) following a modified version of the CloudMap protocol (MINEVICH *et al.* 2012). Protocols described below (*SSM Variant Detection* and *SSM Variant Subtraction*) can be accessed through the shared workflows on UseGalaxy.org. Individual tools used in Galaxy are noted in italics below (LI AND DURBIN 2009; LI *et al.* 2009; MCKENNA *et al.* 2010; CINGOLANI *et al.* 2012a; CINGOLANI *et al.* 2012b).

Tool	Function	Parameters
Map with BWA for Illumina **[Galaxy version 1.2.3]**	Aligns reads to *the C. elegans* WS220 (ce10)] genome	• The parameters used for alignment were as follows: Library mate-paired = Paired-end, Maximum edit distance (aln -n) = 0 • Fraction of missing alignments given 2% uniform base error rate (aln -n) = 0.04 • Maximum number of gap opens (aln -o) = 1, Maximum number of gap extensions (aln -e) = -1 • Disallow long deletion within [value] bp towards the 3'-end (aln -d) = 16 • Disallow insertion/deletion within [value] bp towards the end (aln -i) = 5 • Number of first subsequences to take as seed (aln -l) = -1, Maximum edit distance in the seed (aln -k) = 2 • Mismatch penalty (aln -M) =3 • Gap open penalty (aln -O) = 11 • Gap extension penalty (aln -E) = 4 • Proceed with suboptimal alignments if there are no more than INT equally best hits (aln -R) = Null • Disable iterative search (aln -N) = No • Maximum number of alignments to output in the XA tag for reads paired properly (samse/sampe -n) = 3 • Maximum number of alignments to output in the XA tag for disconcordant read pairs (excluding singletons) (sampe -N) = 10, Maximum insert size for a read pair to be considered as being mapped properly (sampe -a) = 500 • Maximum occurrences of a read for pairing (sampe -o) = 100000 • Specify the read group for this file? (samse/sampe -r) = No • Suppress the header in the output SAM file = No • Job Resource Parameters = Use default job resource parameters

Filter SAM **[Galaxy version 1.0.0]**	Removes Orphaned and misaligned pairs	• 1: Flag Read is paired = Yes • 2: Flag Read is mapped in proper pair = Yes
Add or Replace Groups **[Galaxy version 1.56.0]**	Alters BAM file read groups	• Read group ID (ID tag) = 1 • Read group sample name (SM tag) = rgSM, • Read group library (LB tag) = rgLB • Read group platform (PL tag) = rgPU • Specify additional (optional) arguments = Use pre-set defaults • Output bam instead of sam = Yes.
Realigner Target Creator **[Galaxy version 0.0.4]**	Identifies Intervals for local realignment	**[(1)]** Basic GATK options: • How strict should we be in validating the pedigree information = STRICT • Interval set rule = UNION • Type of reads downsampling to employ at a given locus = NONE • Type of BAQ calculation to apply in the engine = OFF, • BAQ gap open penalty (Phred Scaled) = 40 • Use the original base quality scores from the OQ tag = No • Value to be used for all base quality scores • when some are missing = -1 • How strict should we be with validation = STRICT Interval merging rule = ALL • Disable experimental low-memory sharing functionality = No Makes the GATK behave non-deterministically that is, the random numbers generated will be different in every run = No. **[(2)]** Basic Analysis options: • Window size for calculating entropy or SNP clusters (windowSize) = 10 • Fraction of base qualities needing to mismatch for a position to have high entropy (mismatchFraction) = 0.15 • Minimum reads at a locus to enable using the entropy calculation (minReadsAtLocus) = 4 • Maximum interval size = 500
Indel Realigner **[Galaxy version 0.0.6]**	Performs Local alignment based on sequence intervals identified by *Realigner Target Creator*[Galaxy version 0.0.4]	Basic GATK and Basic Analysis options: • LOD threshold above which the realigner will proceed to realign = 5.0 • Use only known indels provided as RODs = No **[(1)]** Basic GATK options: • How strict should we be in validating the pedigree information = STRICT, Interval set rule = UNION • Type of reads downsampling to employ at a given locus = NONE • Type of BAQ calculation to apply in the engine = OFF • BAQ gap open penalty (Phred Scaled) = 40.0

		• Use the original base quality scores from the OQ tag = No • Value to be used for all base quality scores, when some are missing = -1 • How strict should we be with validation = STRICT • Interval merging rule = ALL • Disable experimental low-memory sharing functionality = No Makes the GATK behave non-deterministically that is, the random numbers generated will be different in every run = No. **[(2)]** Basic Analysis options: • Percentage of mismatching base quality scores at a position to be considered having high entropy = 0.15 • Simplify BAM = No • Consensus Determination Model = USE_READS • Maximum insert size of read pairs that we attempt to realign = 3000 • Maximum positional move in basepairs that a read can be adjusted during realignment = 200 • Max alternate consensuses to try = 30 • Max reads (chosen randomly) used for finding the potential alternate consensus = 120 • Max reads allowed at an interval for realignment = 20000 • Don't output the original cigar or alignment start tags for each realigned read in the output bam = No
Mark Duplicate Reads **[Galaxy version 1.56.0]**	Removes Duplicate reads	• Remove duplicates from output file = Yes • Assume reads are already ordered = Yes • Regular expression that can be used to parse read names in the incoming SAM file = [a-zA-Z0-9]+:[0-9]:([0-9]+):([0-9]+):([0-9]+).* • The maximum offset between two duplicate clusters in order to consider them optical duplicates = 100
Generate pileup from BAM dataset **[Galaxy version 1.1.2]**	Generates pileup file	• Whether or not to print the mapping quality as the last column = Do not print the mapping quality as the last column • Whether or not to print only output pileup lines containing indels = print all lines • Where to cap mapping quality = 30 • Call consensus according to MAQ model? = No
Varscan **[Galaxy version 0.1]**	Allows for filtering variants across a range of allele frequency thresholds with SNPs and INDELs called separately	• Select your desired range of allele frequency thresholds = 100% - FA1, FA3; 90% -FA2, FA4 (Sup); 0% - FA1, FA3; 10% - FA2, FA4 (Non-Sup) • Minimum read depth = 8 (Sup); 3 (Non-Sup) • Minimum supporting reads =1 • Minimum base quality at a position to count a read =24 • Minimum frequency to call homozygote = FA1, FA3 - 100%; FA2, FA4 - 90% • p-value threshold for calling variants = 0.99

VCFCombine **[Galaxy Version 1.0.0_rc1.0]**	Combines SNP and INDEL VCF	• Select both SNP and INDEL files generated by the tool *Varscan*.
VCF-VCF intersect [Galaxy version 1.0.0_rc1.0	Performs Subtraction	• Mutant variant file was selected as the second VCF dataset and the NMSC variant file as the first VCF dataset. Then the following options were chosen to perform the subtraction: • Union or intersection = Intersect • Invert selection? = Yes • compare records up to this many bp away (window size) = 30 • output whole loci when one alternate allele matches = Yes • Advanced controls = Don't use advanced options.
SnpEff **[Galaxy version 1.0]**	Annotates Variants	• Input format = VCF • Output format = Tabular • Genome = *Caenorhabditis elegans*: WS220.64 • Upstream/Downstream length = 10000 bases • Filter homozygous/heterozygous changes = No Filter • Filter homozygous/heterozygous changes = None • Chromosomal position = Use default (based on input type).

Results from the above analysis can be downloaded from Galaxy and further subsetted according to individual need (*e.g.,* variants within coding regions or that occur on a particular chromosome). In addition, we recommend confirming candidate variants intended for experimental validation further by examining the individual non-mutant comparator and mutant alignments within a genome browser such as IGV (Robinson *et al.* 2011; Thorvaldsdóttir *et al.* 2013). This is to remove false positive candidates that are the result of a low number of reads in the Non-suppressed sibling strains. These are not called as variants in the Non-suppressed siblings and thus are not subtracted.

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