Supplementary Figure 1

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C. elegans   TTCACCAAAGCTCTATCCGAACGAGTACGAATCACACATGCC---------
C. remanei   TTTCCCACAGTTCTACTAGAATG---TCACATGTCATTCGCC---------
C. briggsae  ===================================================
C. brenneri  -----------TCTATTCAAACGGGCTCCCTTCCCCCAGACC---------


C. elegans   ------TCCCCCACAAATTATTG--ATCTACATCCCCATTTGGTATAGTGT
C. remanei   ------CCGCCCACTTCCCATAGTTAAC----------------ATAGTGA
C. briggsae  ============================================ATAGTGA
C. brenneri  ------CCGCCCCCTACACACAA--AAC----------------ATAGTGG


C. elegans   CTTACAGGACTCTCAATATGTCAGCTGCATGATAAATGGTCCCTATATAAT
C. remanei   CGTGCGGAACCCGGGAGATGTCGGTTACTTCCCAAGACGTCCCTATATAAG
C. briggsae  CGCGCGAAACCCGGGAGATGTCGGCTGCTATCTAGGGGGTCCCTATATAAA
C. brenneri  AGGGCGGAACCCGTGAGATGTCGGCTGCTGTGTAGGTACCCCCTATATAAG


C. elegans   TGAATTGCAAATCTA-AATGTTTGTTCTTCCGAGAACATATACTAAAATTG
C. remanei   AAACTTTCTTCTGAATAGATATTGTTCTTCCGAGAACATATACTAAAATTG
C. briggsae  CAGATGACCTATAGA-AGATTTCGTTCTTCCGAGAACATCAACTAAAATTG
C. brenneri  GGGGTGCCCTTTCCAAGCATCTCGTTCTTCCGAGAACATATACTAAAATTG


C. elegans   GAACAATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCAAA
C. remanei   GAACAATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCAAA
C. briggsae  GAACAATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCAAA
C. brenneri  GAACAATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCAAA


C. elegans   TTCGTGAAGCGTTCCAAATTTTTTCAATAATATTCTA-----GATATCCCT
C. remanei   TTCGTGAAGCGTTCCAAATTTTTT--GTCTAATTTTG==============
C. briggsae  TTCGTGAAGCGTTCCAAATTTTTT--GTCCGATTTTA-----TTTGATGAC
C. brenneri  TTCGTGAAGCGTTCCAAATTTTTT------------------GGTATCAAC


C. elegans   TTTTGAAACTAA-AAT--------GAAATACTCTAAATTTTT--GA-----
C. remanei   ===================================================
C. briggsae  CTG----ATAAGAAAT--------AATGT-------AAATTTTGGAACTAT
C. brenneri  CTTTACCATTAG-AAT--------AATTTGTTCGAAGGATTT---------
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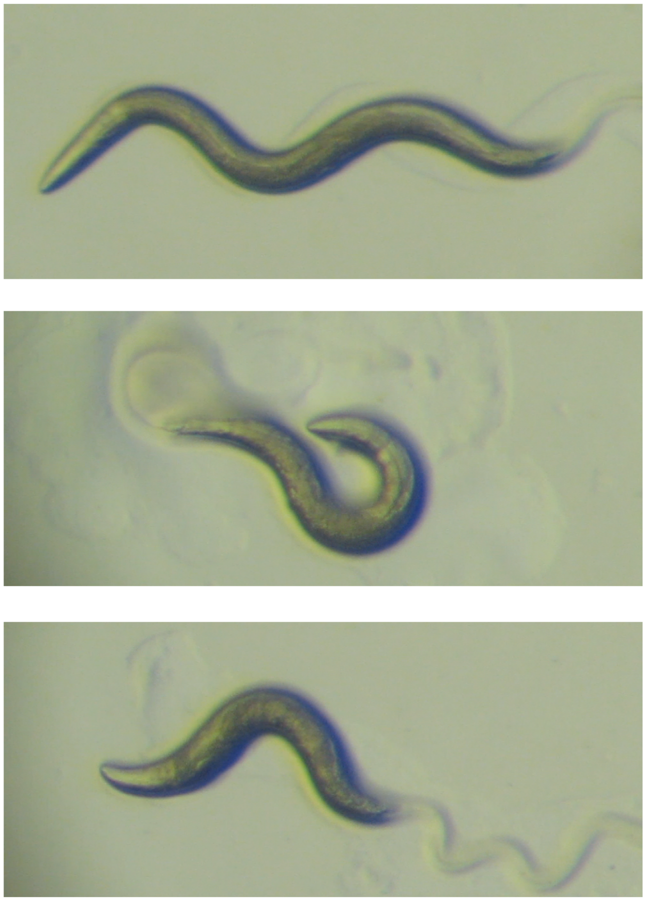
Supplementary Fig. 1: A putative U6 snRNA locus found on *C. elegans* Chromosome IV and aligned with similar sequences from three other Caenorhabditis species. In green is the conserved snRNA sequence; our promoter conservatively includes 500 bases of upstream sequence and 237 bases of downstream sequence that flank it.

Supplementary Figure 2



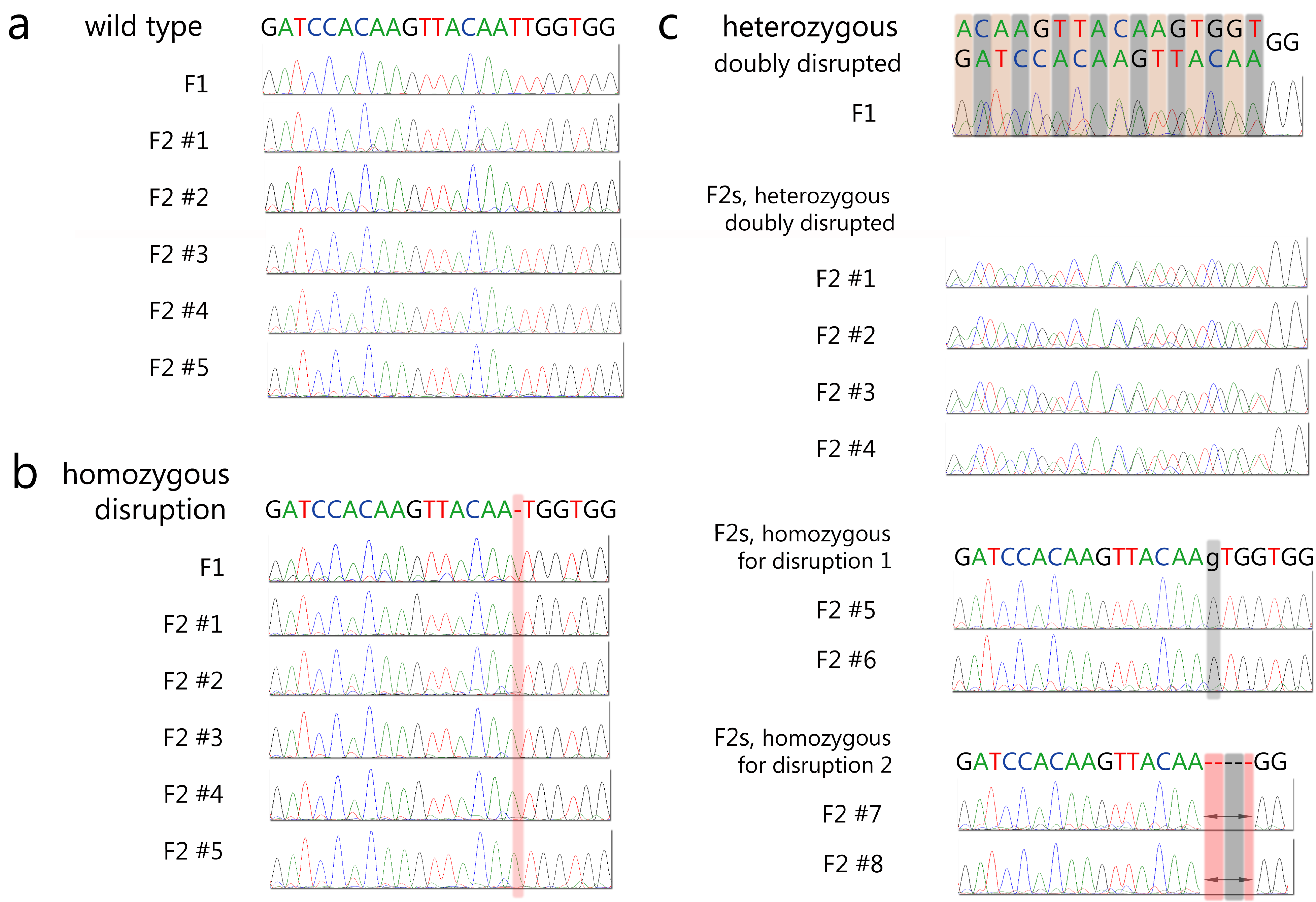
wild type

disrupted *unc-119*

disrupted *dpy-13*

Supplementary Fig. 2: Images of worms from our wild-type background line, a disrupted *unc-119* line, and a disrupted *dpy-13* line.

Supplementary Figure 3



Supplementary Fig. 3: Sequencing results of F_1 and F_2 progeny from animals injected with cas9 and sgRNAs specific for the *klp-12* locus. (A) The sequence and traces of a wild-type F_1 and five of its F_2 progeny. (B) The sequence and traces of a disrupted F_1, carrying two copies of the same allele, and five of its F_2 progeny, all of which were also homozygous for the same disruption. (C) The sequences and traces of a heterozygous, doubly disrupted F_1 (each deciphered mutant sequence is listed above the sequencing trace) and eight of its F_2 progeny. Four of these eight F_2 progeny are similarly heterozygous, while two are homozygous for one disrupted allele and the last two are homozygous for the other disrupted allele. In all cases, the final three nucleotides (TGG) represent the PAM sequence.

Supplementary Figure 4

Target *unc-119* sequence:	GAATTTTCTG AAATTAAAGA CGG
Off-Target sequence 1:	GAGGTTTGAG AAATTAAAGA CGG
N2:	GAGGTTTGAG AAATTAAAGA CGG
unc-119 mutant 1:	GAGGTTTGAG AAATTAAAGA CGG
unc-119 mutant 2:	GAGGTTTGAG AAATTAAAGA CGG

Target *unc-119* sequence:	GAATTTTCTG AAATTAAAGA CGG
Off-Target sequence 2:	ATTCTGTTTG AAATTAAAGA TGG
N2:	ATTCTGTTTG AAATTAAAGA TGG
unc-119 mutant 1:	ATTCTGTTTG AAATTAAAGA TGG
unc-119 mutant 2:	ATTCTGTTTG AAATTAAAGA TGG

Target *unc-119* sequence:	GAATTTTCTG AAATTAAAGA CGG
Off-Target sequence 3:	TAATTTTAGG AAATTAAAGA AGG
N2:	TAATTTTAGG AAATTAAAGA AGG
unc-119 mutant 1:	TAATTTTAGG AAATTAAAGA AGG
unc-119 mutant 2:	TAATTTTAGG AAATTAAAGA AGG

Target *dpy-13* sequence:	GGACATTGAC ACTAAAATCA AGG
Off-Target sequence 1:	TTCAATTGCC ACTAAAATCA TGG
N2:	TTCAATTGCC ACTAAAATCA TGG
dpy-13 mutant 1:	TTCAATTGCC ACTAAAATCA TGG

Target *dpy-13* sequence:	GGACATTGAC ACTAAAATCA AGG
Off-Target sequence 2:	GCAAAATTCC ACTAAAATCA AGG
N2:	GCAAAATTCC ACTAAAATCA AGG
dpy-13 mutant 1:	GCAAAATTCC ACTAAAATCA AGG

Target *klp-12* sequence:	GATCCACAAG TTACAATTGG TGG
Off-Target sequence 1:	CCACTTGAAG TTACAATTGG TGG
N2:	CCACTTGAAG TTACAATTGG TGG
klp-12 mutant 1:	CCACTTGAAG TTACAATTGG TGG
klp-12 mutant 2:	CCACTTGAAG TTACAATTGG TGG

Target *Y61A9LA.1* sequence:	GGATGGATGT GTAGTCAATT CGG
Off-Target sequence 1:	CCTCCTCACT GTAGTCAATT TGG
N2:	CCTCCTCACT GTAGTCAATT TGG
Y61A9LA.1 mutant 1:	CCTCCTCACT GTAGTCAATT TGG
Y61A9LA.1 mutant 2:	CCTCCTCACT GTAGTCAATT TGG

Target *Y61A9LA.1* sequence:	GGATGGATGT GTAGTCAATT CGG
Off-Target sequence 2:	CACTTCGATT GTAGTCAATT TGG
N2:	CACTTCGATT GTAGTCAATT TGG
Y61A9LA.1 mutant 1:	CACTTCGATT GTAGTCAATT TGG
Y61A9LA.1 mutant 2:	CACTTCGATT GTAGTCAATT TGG

Supplementary Fig. 4: Genotyping results at off-target loci. Mismatches between targeting sgRNAs and off-target loci are highlighted in red. In all examined cases, no indels were found at the off-target loci.

Supplementary Table 1

Experiment	F1s	Disruptions
Cas9 only	150	0
sgRNA only	150	0
Cas9 + gRNA, F1s mCherry(-)	214	0
Total	514	0

Supplementary Table 1: Summary of results from control microinjection experiments. No mutants were recovered.

Synthesized codon-optimized Cas9-intron-SV40 NLS sequence

ATGGACAAGAAGTACTCCATCGGACTCGACATCGGAACCAACTCCGTCGGA
TGGGCCGTCATCACCGACGAGTACAAGGTCCCATCCAAGAAGTTCAAGGTC
CTCGGAAACACCGACCGTCACTCCATCAAGAAGAACCTCATCGGAGCCCTCC
TCTTCGACTCCGGAGAGACCGCCGAGGCCACCCGTCTCAAGCGTACCGCCCG
TCGTCGTTACACCCGTCGTAAGAACCGTATCTGCTACCTCCAGGAGATCTTCT
CCAACGAGATGGCCAAGGTCGACGACTCCTTCTTCCACCGTCTCGAGGAGTC
CTTCCTCGTCGAGGAGGACAAGAAGCACGAGCGTCACCCAATCTTCGGAAA
CATCGTCGACGAGGTCGCCTACCACGAGAAGTACCCAACCATCTACCACCTC
CGTAAGAAGCTCGTCGACTCCACCGACAAGGCCGACCTCCGTCTCATCTACC
TCGCCCTCGCCCACATGATCAAGTTCCGTGGACACTTCCTCATCGAGGGAG
ACCTCAACCCAGACAACTCCGACGTCGACAAGCTCTTCATCCAGCTCGTCCAG
ACCTACAACCAGCTCTTCGAGGAGAACCCAATCAACGCCTCCGGAGTCGACG
CCAAGGCCATCCTCTCCGCCCGTCTCTCCAAGTCCCGTCGTCTCGAGAACCTC
ATCGCCCAGCTCCCAGGAGAGAAGAAGAACGGACTCTTCGGAAACCTCATC
GCCCTCTCCCTCGGACTCACCCCAAACTTCAAGTCCAACTTCGACCTCGCCGA
GGACGCCAAGCTCCAGCTCTCCAAGGACACCTACGACGACGACCTCGACAA
CCTCCTCGCCCAGATCGGAGACCAGTACGCCGACCTCTTCCTCGCCGCCAAG
AACCTCTCCGACGCCATCCTCCTCTCCGACATCCTCCGTGTCAACACCGAGAT
CACCAAGGCCCCACTCTCCGCCTCCATGATCAAGCGTTACGACGAGCACCAC
CAGGACCTCACCCTCCTCAAGGCCCTCGTCCGTCAGCAGCTCCCAGAGAAGT
ACAAGGAGATCTTCTTCGACCAGTCCAAGAACGGATACGCCGGATACATCG
ACGGAGGAGCCTCCCAGGAGGAGTTCTACAAGTTCATCAAGCCAATCCTCG
AGAAGATGGACGGAACCGAGGAGCTCCTCGTCAAGCTCAACCGTGAGGAC
CTCCTCCGTAAGCAGCGTACCTTCGACAACGGATCCATCCCACACCAGATCC
ACCTCGGAGAGCTCCACGCCATCCTCCGTCGTCAGGAGGACTTCTACCCATT
CCTCAAGGACAACCGTGAGAAGATCGAGAAGATCCTCACCTTCCGTATCCC
ATACTACGTCGGACCACTCGCCCGTGGAAACTCCCGTTTCGCCTGGATGAC
CCGTAAGTCCGAGGAGACCATCACCCCATGGAACTTCGAGGAGGTCGTCG
ACAAGGGAGCCTCCGCCCAGTCCTTCATCGAGCGTATGACCAACTTCGACA
AGAACCTCCCAAACGAGAAGGTCCTCCCAAAGCACTCCCTCCTCTACGAGTA
CTTCACCGTCTACAACGAGCTCACCAAGGTCAAGTACGTCACCGAGGGAAT
GCGTAAGCCAGCCTTCCTCTCCGGAGAGCAGAAGAAGGCCATCGTCGACC
TCCTCTTCAAGACCAACCGTAAGGTCACCGTCAAGCAGCTCAAGGAGGACT
ACTTCAAGAAGATCGAGTGCTTCGACTCCGTCGAGATCTCCGGAGTCGAG
GACCGTTTCAACGCCTCCCTCGGAACCTACCACGACCTCCTCAAGATCATCA
AGGACAAGGACTTCCTCGACAACGAGGAGAACGAGGACATCCTCGAGGA
CATCGTCCTCACCCTCACCCTCTTCGAGGACCGTGAGATGATCGAGGAGCG
TCTCAAGACCTACGCCCACCTCTTCGACGACAAGGTCATGAAGCAGCTCAA
GCGTCGTCGTTACACCGGATGGGGACGTCTCTCCCGTAAGCTCATCAACGG
AATCCGTGACAAGCAGTCCGGAAAGACCATCCTCGACTTCCTCAAGTCCGA
CGGATTCGCCAACCGTAACGTAAGTTTAAACATGATTTTACTAACTAACTA

ATCTGATTTAAATTTTCAGTTCATGCAGCTCATCCACGACGACTCCCTCACC
TTCAAGGAGGACATCCAGAAGGCCCAGGTCTCCGGACAGGGAGACTCCC
TCCACGAGCACATCGCCAACCTCGCCGGATCCCCAGCCATCAAGAAGGGA
ATCCTCCAGACCGTCAAGGTCGTCGACGAGCTCGTCAAGGTCATGGGAC
GTCACAAGCCAGAGAACATCGTCATCGAGATGGCCCGTGAGAACCAGA
CCACCCAGAAGGGACAGAAGAACTCCCGTGAGCGTATGAAGCGTATCG
AGGAGGGAATCAAGGAGCTCGGATCCCAGATCCTCAAGGAGCACCCAG
TCGAGAACACCCAGCTCCAGAACGAGAAGCTCTACCTCTACTACCTCCAG
AACGGACGTGACATGTACGTCGACCAGGAGCTCGACATCAACCGTCTCT
CCGACTACGACGTCGACCACATCGTCCCACAGTCCTTCCTCAAGGACGAC
TCCATCGACAACAAGGTCCTCACCCGTTCCGACAAGAACCGTGGAAAGT
CCGACAACGTCCCATCCGAGGAGGTCGTCAAGAAGATGAAGAACTACT
GGCGTCAGCTCCTCAACGCCAAGCTCATCACCCAGCGTAAGTTCGACAA
CCTCACCAAGGCCGAGCGTGGAGGACTCTCCGAGCTCGACAAGGCCGG
ATTCATCAAGCGTCAGCTCGTCGAGACCCGTCAGATCACCAAGCACGTC
GCCCAGATCCTCGACTCCCGTATGAACACCAAGTACGACGAGAACGACA
AGCTCATCCGTGAGGTCAAGGTCATCACCCTCAAGTCCAAGCTCGTCTCCG
ACTTCCGTAAGGACTTCCAGTTCTACAAGGTCCGTGAGATCAACAACTACC
ACCACGCCCACGACGCCTACCTCAACGCCGTCGTCGGAACCGCCCTCATCAA
GAAGTACCCAAAGCTCGAGTCCGAGTTCGTCTACGGAGACTACAAGGTCT
ACGACGTCCGTAAGATGATCGCCAAGTCCGAGCAGGAGATCGGAAAGG
CCACCGCCAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCGA
GATCACCCTCGCCAACGGAGAGATCCGTAAGCGTCCACTCATCGAGACCA
ACGGAGAGACCGGAGAGATCGTCTGGGACAAGGGACGTGACTTCGCCA
CCGTCCGTAAGGTCCTCTCCATGCCACAGGTCAACATCGTCAAGAAGACC
GAGGTCCAGACCGGAGGATTCTCCAAGGAGTCCATCCTCCCAAAGCGTA
ACTCCGACAAGCTCATCGCCCGTAAGAAGGACTGGGACCCAAAGAAGTA
CGGAGGATTCGACTCCCCAACCGTCGCCTACTCCGTCCTCGTCGTCGCCA
AGGTCGAGAAGGGAAAGTCCAAGAAGCTCAAGTCCGTCAAGGAGCTC
CTCGGAATCACCATCATGGAGCGTTCCTCCTTCGAGAAGAACCCAATCG
ACTTCCTCGAGGCCAAGGGATACAAGGAGGTCAAGAAGGACCTCATCA
TCAAGCTCCCAAAGTACTCCCTCTTCGAGCTCGAGAACGGACGTAAGCG
TATGCTCGCCTCCGCCGGAGAGCTCCAGAAGGGAAACGAGCTCGCCCT
CCCATCCAAGTACGTCAACTTCCTCTACCTCGCCTCCCACTACGAGAAGC
TCAAGGGATCCCCAGAGGACAACGAGCAGAAGCAGCTCTTCGTCGAG
CAGCACAAGCACTACCTCGACGAGATCATCGAGCAGATCTCCGAGTTC
TCCAAGCGTGTCATCCTCGCCGACGCCAACCTCGACAAGGTCCTCTCCG
CCTACAACAAGCACCGTGACAAGCCAATCCGTGAGCAGGCCGAGAACA
TCATCCACCTCTTCACCCTCACCAACCTCGGAGCCCCAGCCGCCTTCAAG
TACTTCGACACCACCATCGACCGTAAGCGTTACACCTCCACCAAGGAGG
TCCTCGACGCCACCCTCATCCACCAGTCCATCACCGGACTCTACGAGACC
CGTATCGACCTCTCCCAGCTCGGAGGAGACTCCCGTGCCGACCCAAAG
AAGAAGCGTAAGGTCTGA

Peft-3::Cas9-SV40 NLS::tbb-2 3'UTR vector primers

pUC57 EcoRI Peft-3 F
CGACGTTGTAAAACGACGGCCAGTGAATTCGCACCTTTGGTCTTTTATTGTCAACT
Peft-3 cas9 start R
GTCGAGTCCGATGGAGTACTTCTTGTCCATTAAGCCTGCTTTTTTGTACAAACTTGTGAG
cas9 start F
ATGGACAAGAAGTACTCCATCGGACTCGAC
cas9 tbb-2 UTR R
GGGAATGCTTGAAAGGATTTTGCATTTATCTCAGACCTTACGCTTCTTCTTTGG
tbb-2 UTR F
GATAAATGCAAAATCCTTTCAAGCATTCC
tbb-2 UTR pUC57 R
AACAGCTATGACCATGATTACGCCAAGCTTTGAGACTTTTTTCTTGGCGGCACA

U6 pol III promoter, sgRNA, and downstream sequence

CTCCAAGAACTCGTACAAAAATGCTCTGAAGTAGGTCTCGAGATCAA
TACTGGGAAGACGAAAGTCTTGCGAAACCGATTCGCTGACCCCAGTA
AAGTCTACTTCGGTAGCCCTTCCCCCACCACCCAGCTCGACGACGTCGA
CGAGTACATCTACCTCGGTCGTCAAATCAACGCCCAAAACAACTTGAT
GCCGGAAATCCACCGAAGACGTCGAGCAGCCTGGGCTGCATTCAATG
GAATCAAGAATGCCACCGACTCCATCACCGACAAGAAGATTCGTGCG
AATCTGTTCGACTCAATTGTCCTTCCAGCGCTCACCTACGTTTCAGAAG
CCTGGACATTCACCAAAGCTCTATCCGAACGAGTACGAATCACACATG
CCTCCCCCACAAATTATTGATCTACATCCCCATTTGGTATAGTGTCTTA
CAGGACTCTCAATATGTCAGCTGCATGATAAATGGTCCCTATATAATT
GAATTGCAAATCTAAATGTTT**GAATTTTCTGAAATTAAAGAGTTTTA**
GAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG
AAAAAGTGGCACCGAGTCGGTGCTTTTTTTCAATAATATTCTAGATA
TCCCTTTTTGAAACTAAAATGAAATACTCTAAATTTTTGAGTTCAATG
TTTCAACTAATCTTGGTTAAAGGTAAATTCCCGGAGGGACGATTTTA
AAGTTGCCAGAGTTGATTAAATTGGTCCAAAAAAATTTTATTAGGAA
ACTCGAAAAAAAATTGATTTTTTTTGACAATATTCCAAAGAAAATTA
CAAAATCTCGACGCCAAACATAGTCGGCTGTG
(unc-119 target sequence = bold and underlined; sgRNA scaffold sequence = bold

Target genomic sequences

unc-119	GAATTTTCTGAAATTAAAGACGG
dpy-13	GGACATTGACACTAAAATCAAGG
klp-12	GATCCACAAGTTACAATTGGTGG

Y61A9LA.1	GGATGGATGTGTAGTCAATTCGG

U6 pol III promoter expression vector and sgRNA primers

U6prom EcoRI F
CGGGAATTCCTCCAAGAACTCGTACAAAAATGCTCT
U6prom HindIII R
CGGAAGCTTCACAGCCGACTATGTTTGGCGT
dpy-13 gRNA F
GGACATTGACACTAAAATCAGTTTTAGAGCTAGAAATAGCAAGTTA
dpy-13 gRNA R
TGATTTTAGTGTCAATGTCCAAACATTTAGATTTGCAATTCAATTATATAG
klp-12 gRNA F
GATCCACAAGTTACAATTGGGTTTTAGAGCTAGAAATAGCAAGTTA
klp-12 gRNA R
CCAATTGTAACTTGTGGATCAAACATTTAGATTTGCAATTCAATTATATAG
Y61A9LA.1 gRNA F
GGATGGATGTGTAGTCAATTGTTTTAGAGCTAGAAATAGCAAGTTA
Y61A9LA.1 gRNA R
AATTGACTACACATCCATCCAAACATTTAGATTTGCAATTCAATTATATAG

RT-PCR primers

cas9 RT-PCR F	CAAGCGTCGTCGTTACACCGGA
cas9 RT-PCR R	CTGGAGGATTCCCTTCTTGATGG
unc-119 gRNA RT-PCR F	GAATTTTCTGAAATTAAAGAGTTTTAGAGC
unc-119 gRNA RT-PCR R	GACTCGGTGCCACTTTTTCAAGTT
act-4 F	GTTTCCGTTGCCCAGAGGCTCT
act-4 R	CTCGTCGTACTCTTGCTTGGAGAT

Genotyping primers

dpy-13 gt F	CAACCTTGTCAACGGACGAAAGC
dpy-13 gt R	CATAGTTGTAGACGATTGGAAGAGTAAT
unc-119 NHEJ gt F	CACGGAACACGTGCTCTTCGAAA
unc-119 NHEJ gt R	CGATCCTTGAAAAAGTGCCGTTCAA
klp-12 gt F	CCATCGAATAATCCATCCACAAGTT
klp-12 gt R	GTTTCGCTTGGGGTGTCATGTT
Y61A9LA.1 gt F	CTCTCACTAGAAAATACACTTTTCAGC
Y61A9LA.1 gt R	AGTGCCATGAAGAACTCCTGCAT

Off-target genotyping primers

unc-119_off_target_1_F	CCT TGT GGA TAT GAA GTT TTG

unc-119_off_target_1_R	GCC ACT TTT TGA CAG TAA GT
unc-119_off_target_2_F	TGT GGA TTC GAT AGG TTG AA
unc-119_off_target_2_R	CTC CTT GTG TTT CTC GTA AAT
unc-119_off_target_3_F	TCG TTG GAT ACT ATT CTG AAT GGG A
unc-119_off_target_3_R	GCT GTC GAA TAA ATC TTG CTA TGG A
dpy-13_off_target_1_F	CAA AAA GTA ACC TAC AGT TCT GTC C
dpy-13_off_target_1_R	GGA ACA AAT GGC AAC AAA GAT AC
dpy-13_off_target_2_F	CTT CTC CGG AAA AAG CTC GAA ATT
dpy-13_off_target_2_R	GCA TTT AAT TTG TTT TCC ATG CAA C
klp-12_off_target_1_F	GAC GCA GAC TTC TCA ACT GAT TT
klp-12_off_target_1_R	GAC AAC ACA TTT GGC TCA GTT TTG
klp-12_off_target_2_F	CCT GAT AAA CGG GTC TCC AAG A
klp-12_off_target_2_R	CGG TTG TAA AAC TAA CTC TTC CCA T
Y61A9LA.1_off_target_1	CAA CAC TCA AGG AAA AAA GGC GAA
Y61A9LA.1_off_target_1	CAT ATC TGT CCT CAG ATG GAT TAT G
Y61A9LA.1_off_target_2	CTA GTC ATA CTG GTC CAT TAT GC
Y61A9LA.1_off_target_2	CAT CAA TTT CGA CGC ATA TTA TCT C

Supplementary Table 2: List of all oligonucleotides and relevant sequences used in this study.