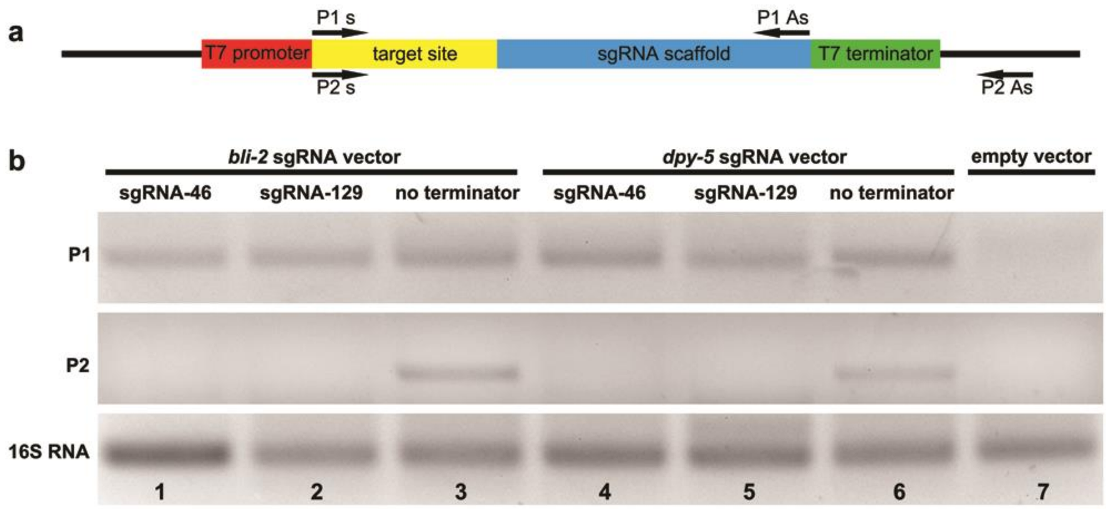

Figure S1A. The ts-gRNA encoding plasmids, used for the CRISPR-Cas9 feeding system and, are transcribed normally in bacteria.

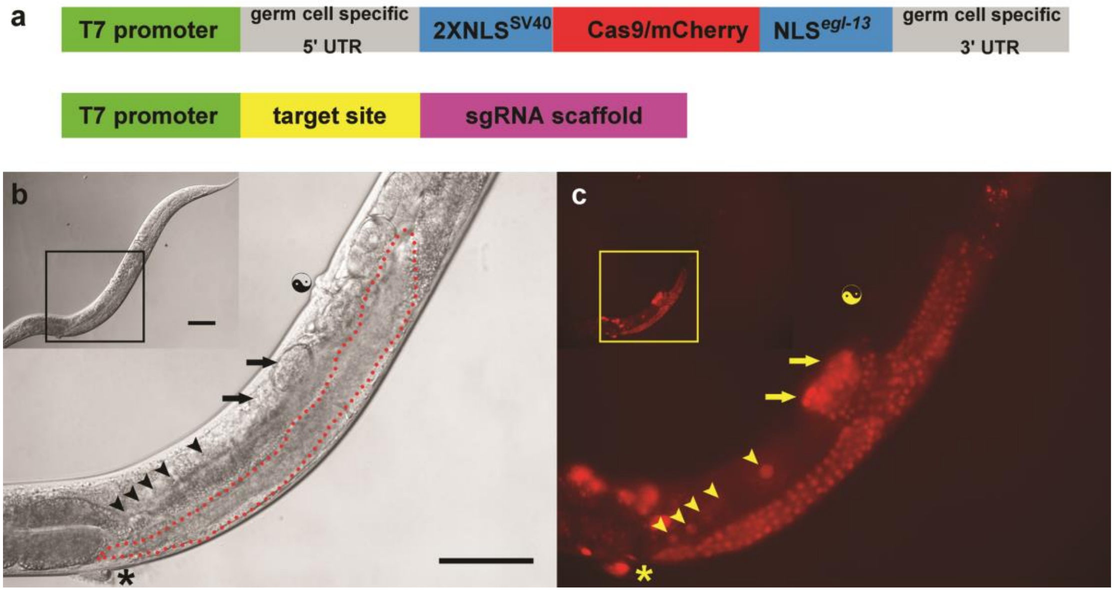

Figure S1B. Tracing the injected mCherry mRNA in a gonad arm of a hermaphrodite.

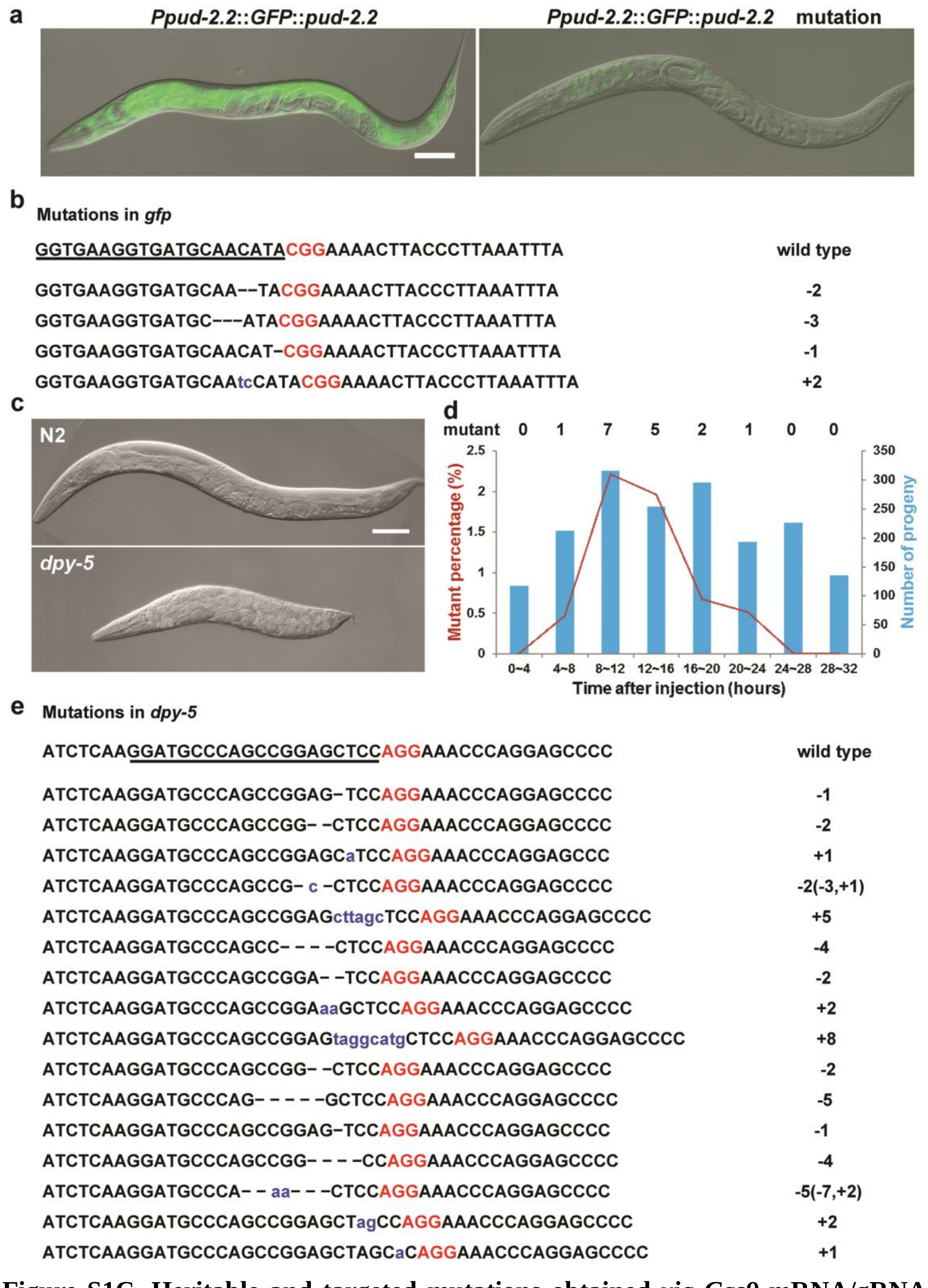

Figure S1C. Heritable and targeted mutations obtained *via Cas9* mRNA/gRNA RNA gonad injection.

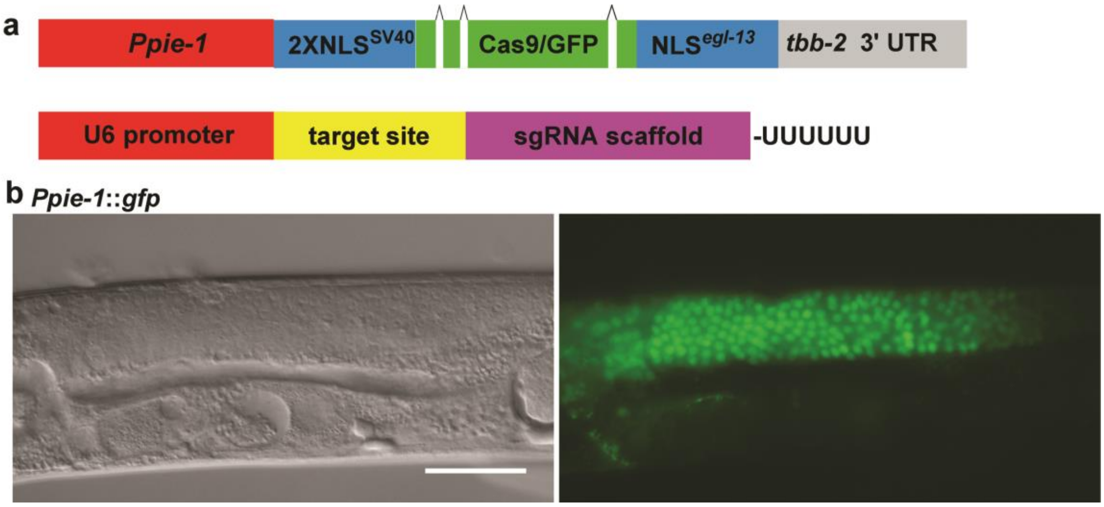

Figure S1D. Direct gonad injection of Cas9 and gRNA encoding plasmids.

Mutations in *dpy-5*

TCCATCTCAAGGATGCCCAGCCGGAGCTCCAGGAAACCCAGGAGCCCC	wild type
TCCATCTCAAGGATGCCCAGCCGGAGC−CCAGGAAACCCAGGAGCCCC	-1
TCCATCTCAAGGATGCCCAGCCGGAG−−CCAGGAAACCCAGGAGCCCC	-2
TCCATCTCAAGGATGCCCAGCCGGAGC−−CAGGAAACCCAGGAGCCCC	-2
TCCATCTCAAGGATGCCCAGCCGGAG−TCCAGGAAACCCAGGAGCCCC	-1
TCCATCTCAAGGATGCCCAGCCG−−−− TCCAGGAAACCCAGGAGCCCC	-4
TCCATCTCAAGGATGCCCAGCCGGAGCaaTCCAGGAAACCCAGGAGCCCC	+2
TCCATCTCAAGGATGCCCAGCCGGA−CTCCAGGAAACCCAGGAGCCCC	-1
TCCATCTCAAGGATGCCCAGCCGGAGC−CCAGGAAACCCAGGAGCCCC	-1
TCCATCTCAAGGATGCCCAGCCGGAGCg−CAGGAAACCCAGGAGCCCC	-1(-2,+1)

Figure S1E. Sequences of nine indels of *dpy-5* mutants *via* direct gonad DNA injection.

Figure S1F. The *dpy-5* and *bli-2* coding sequences and target sites selected for genome editing.

dpy-5 (target site + NGG motif)

aaaATGGTAAAGGCCGTCGTCGGATTCGGCGCTGCATGCGGAATCTCTGCTATCGTTGCTTGCCTTTGGG
CTGCACTTGTCATCACAAATGACATCAATGACATGTATGATGATGTGATGGGAGAGCTCGGAGGATTCA
GAGATATCTCTGATGACACTTGGGGAACCCTTCTCGACGTTCGTCACGGAGCCGGAGAGTCTGCTGAG
CAATACGTTCGTGGAATCTTCGGACGTCACAAGCGTTCCAACAGCCAATGCTCTTGCGGACTTCCATCT
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CCAGGAAACAACGGAAATCCAGGAGAGGAAGGACAAACTGGAGCCCAGGGACCAACTGGACAGCC
AGGAAAGGACGGATTCAACGGAAACGACGGAACTCCAGGACAAGCTGGACCACAAGGAGCCGTTGG
AGCCGATGCCGAATACTGCCCATGCCCAGAGAGAAAGCGCAGACGCGTCTAAactgttttcgtattcaaaaataaatatt
tatgtattcaa

bli-2(target site + NGG motif)

gaattATGGACGAGAAGGAACTGAATCATGAAGCTTCTATGCTTCGAAAGGTTGCATTTCTCGGGATTTGC
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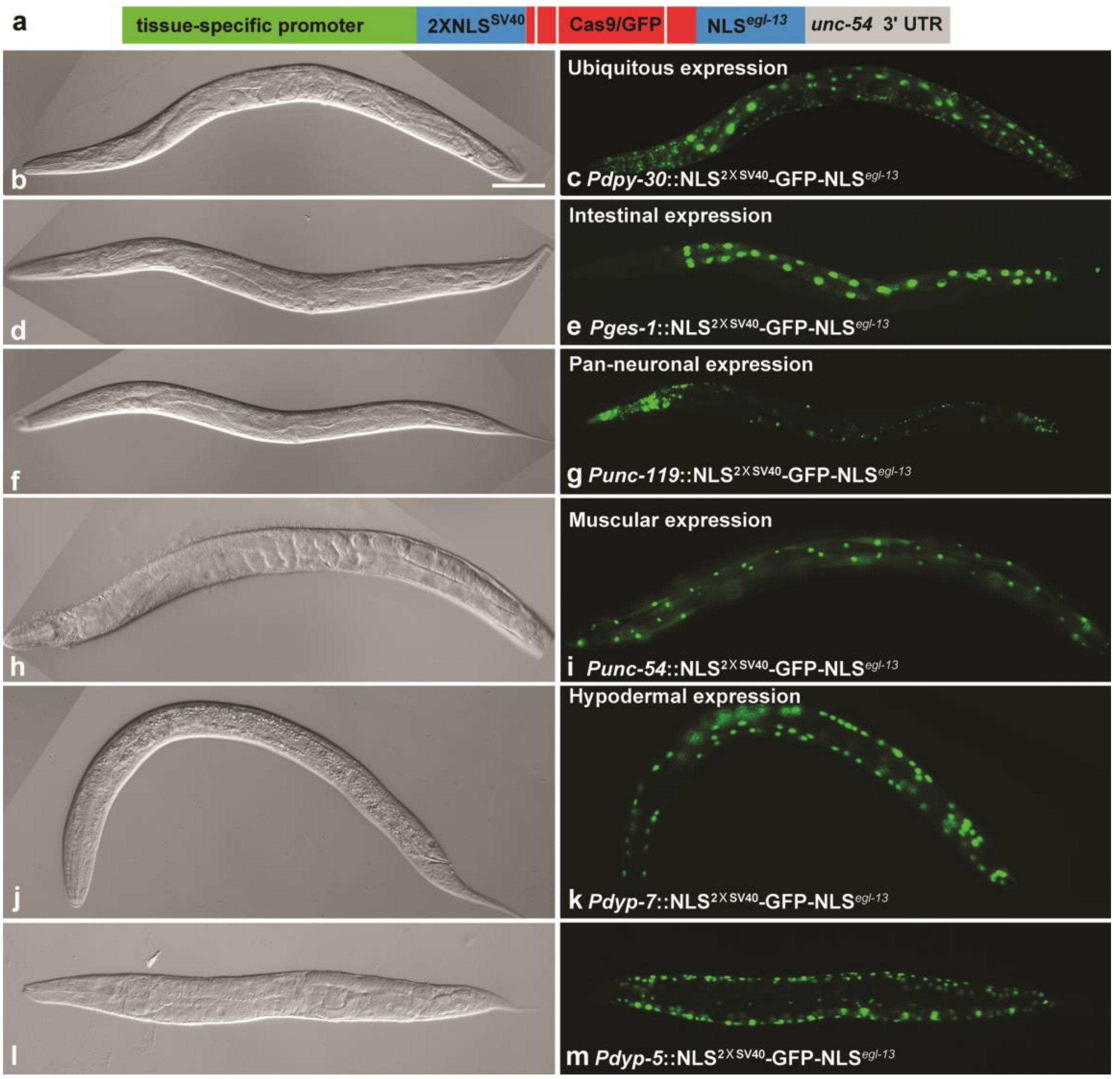

Figure S1G. The tissue-specific GFP is mainly found in the nuclei of transgenic worm lines.

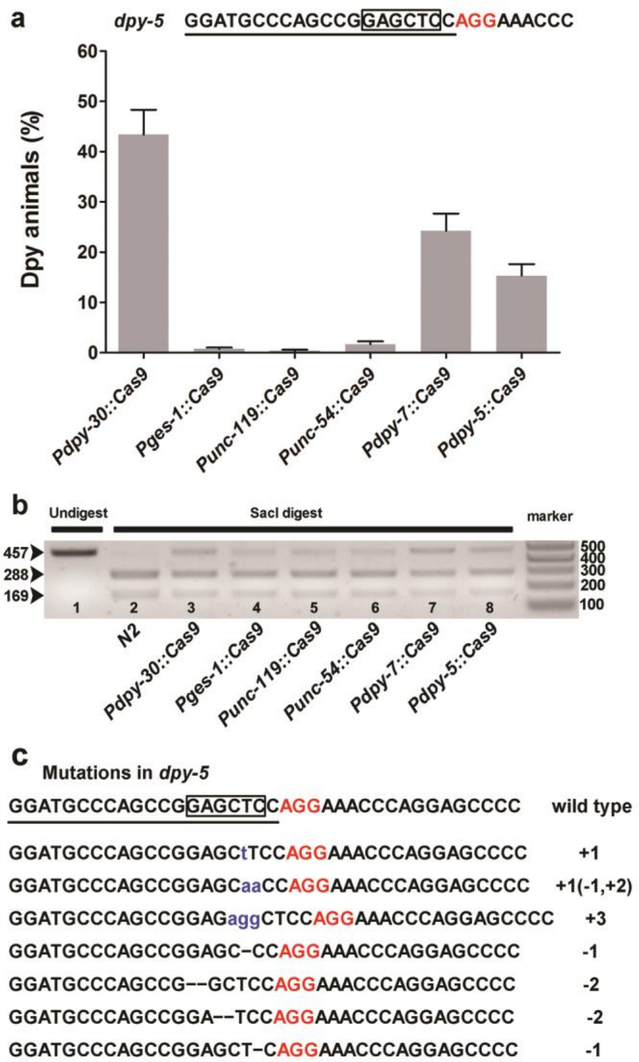

Figure S1H. Tissue-specific genome editing is achieved by the CRISPR-Cas9 feeding system.

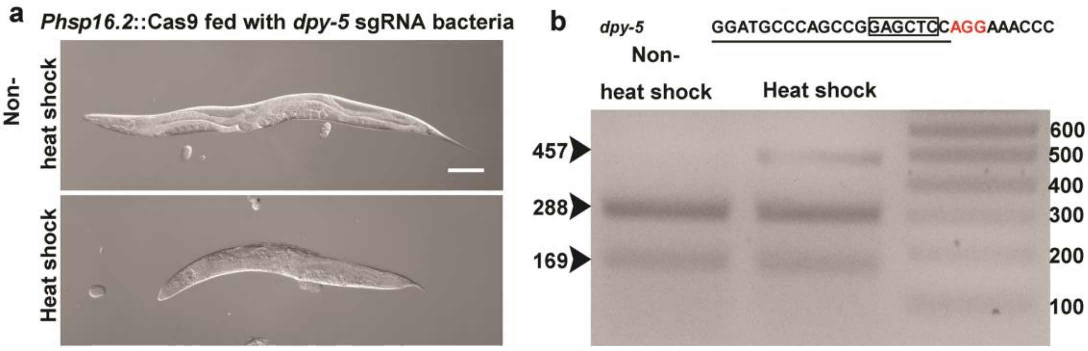

Figure S1I. Inducible knockout of *dpy-5* is achieved through the CRISPR-Cas9 feeding system.

Figure S1J. Sequences of Cas9 and gRNA encoding plasmid used in our study

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ATATTGTGCTTATCGCCAATCATCCGAATACTGCCAGCAAAATGCTCCAAGAACTCGTACAAAAATG
CTCTGAAGTAGGTCTCGAGATCAATACTGGGAAGACGAAAGTCTTGCGAAACCGATTCGCTGACCCC
AGTAAAGTCTACTTCGGTAGCCCTTCCCCCACCACCCAGCTCGACGACGTCGACGAGTACATCTACCT
CGGTCGTCAAATCAACGCCCAAAACAACTTGATGCCGGAAATCCACCGAAGACGTCGAGCAGCCTG
GGCTGCATTCAATGGAATCAAGAATGCCACCGACTCCATCACCGACAAGAAGATTCGTGCGAATCTG
TTCGACTCAATTGTCCTTCCAGCGCTCACCTACGTTTCAGAAGCCTGGACATTCACCAAAGCTCTATC
CGAACGAGTACGAATCACACATGCCTCCCCCACAAATTATTGATCTACATCCCCATTTGGTATAGTGT
CTTACAGGACTCTCAATATGTCAGCTGCATGATAAATGGTCCCTATATAATTGAATTGCAAATCTAAA
TGTTTGGGTCTTCGAGAAGACCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTT
ATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT

129-bp T7 RNA polymerase terminator:

CTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACC
CCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGAT

46-bp T7 RNA polymerase terminator:

TTGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTT

Supplementary information, Figure S1A. The ts-gRNA encoding plasmids, used for the CRISPR-Cas9 feeding system and, are transcribed normally in bacteria.

(**a**) Schematic map of the ts-gRNA, with transcription initiated by T7 RNA polymerase and terminated by the 46-bp/129-bp T7 RNA polymerase terminator. PCR primer pairs, P1s/P1As and P2s/P2As, are used to monitor ts-gRNA transcription states. (**b**) Confirmation of transcription of the (*dpy-5*/*bli-2*)ts-gRNA-46 (lane 1 and 4) and (*dpy-5* or *bli-2*)ts-gRNA-129 (lane 2 and 5) in the bacteria by RT-PCR using P1s/P1As and P2s/P2As primers. 16S RNA is an internal control. Vectors without any T7 RNA polymerase terminator (lane 3 and 6) and empty vector (lane 7) serve as positive and negative controls, respectively.

Supplementary information, Figure S1B. Tracing the injected mCherry mRNA in a gonad arm of a hermaphrodite.

(**a**) Schematic diagrams of DNA cassettes used for *in vitro* synthesis of optimized Cas9/mCherry mRNA and ts-gRNA. The Cas9 or mCherry coding region is flanked with two SV40 and one *egl-13* nuclear localization signals (NLSs). Germ cell specific 5' and 3' UTRs are attached to each end. Gene-specific target sequence is cloned into the gRNA vector, respectively. (**b**) DIC image of one arm of the injected hermaphrodite germline is shown. Dotted red line highlights the germ cells in the gonad. The inlet depicts the entire worm while boxed part indicates the enlarged portion. (**c**) The dark field of (**b**) shows where the mCherry fluorescence is. The boxed part in (**c**) is the corresponding fluorescent image of the boxed part in (**b**). The arrows represent the developing embryos in the reproductive duct, while the arrowheads represent the developing oocytes. Taiji (☯) indicates vulva. Asterisk marks the injection site. Scale bar, 100 µm.

Supplementary information, Figure S1C. Heritable and targeted mutations obtained *via Cas9* mRNA/gRNA RNA gonad injection.

(**a**) GFP fluorescence of a transgenic worm, P*pud-2.2::GFP::pud-2.2,* that carries a single-copy integrated *GFP* (left). The animal on the right carries a disrupted *GFP*, in which the residual signal is the gut auto-fluorescence. (**b**) Sequences of four indels obtained by direct gonad injecting *Cas9* mRNA and *gfp*ts-gRNA. The 20bp *gfp* target site is underlined, and the PAM is outlined in red. Mutations are shown relative to the wild type sequences. Deletions are denoted by dashes, and insertions are depicted in blue lowercases. The nature of the mutations is indicated as the number of base pairs inserted (+) or deleted (-). (**c**) Control worm (upper) and a *dpy-5* mutant (bottom) obtained by direct gonad injection of *Cas9* mRNA and *dpy-5*ts-gRNA. (**d**) A time course of the numbers of *dpy-5* mutants obtained after *Cas9* mRNA/*dpy-5*ts-gRNA injection. The left y axis values are percentages of identified mutants per period, and the red curve is plotted as the relative frequency of the entire time period. The x axis represents all tested time periods. The right y axis values are total progeny collected per period (blue histograms). (**e**) Sequences of 16 indels obtained by direct gonad injecting *Cas9* mRNA and *dpy-5ts-gRNA*. The 20bp *dpy-5* target sequence is underlined and the PAM is outlined in red. Mutations are shown relative to the wild type sequences. Deletions are denoted by dashes, and insertions are depicted in blue lowercases. The nature of the mutations is indicated as the number of base pairs inserted (+) or deleted (-). In the case of mutant alleles caused by both deletions and insertions, the deleted and inserted numbers are explained in parentheses after the total changed number. Scale bars, 100 μm.

Supplementary information, Figure S1D. Direct gonad injection of Cas9 and gRNA encoding plasmids.
(**a**) Diagram of germline-specific *Cas9* and gRNA expression vectors used to generate transgenic lines. Three artificial introns are engineered into Cas9 coding sequence and the resulted cassette or GFP coding sequence is under control of a germline specific *pie-1* promoter and flanked with two SV40 and an *egl-13* NLSs. The gRNA scaffold is cloned into a modified pMD18-T vector and its expression is under control of the U6 promoter and terminated by a stretch of 6 U residues. (**b**) Germline expression and nuclear localization of GFP. Scale bar is 100 μm.

Supplementary information, Figure S1E. Sequences of nine indels of *dpy-5* mutants *via* direct gonad DNA injection. Mutations are obtained by injecting P*pie-1*::*cas9* and *dpy-5ts-gRNA* encoding plasmids. *dpy-5* target sequence is underlined and the PAM is outlined in red. Mutations are shown relative to the wild type sequences. Deletions are denoted by dashes, and insertions are depicted in blue lowercases. The nature of the mutations is indicated as the number of base pairs inserted (+) or deleted (-).In the case of mutant alleles caused by both deletions and insertions, the deleted and inserted numbers are explained in parentheses after the total changed number.

Supplementary information, Figure S1F. The *dpy-5* and *bli-2* coding sequences and target sites selected for genome editing.

Supplementary information, Figure S1G. The tissue-specific GFP is mainly found in the nuclei of transgenic worm lines.
(**a**) Diagram of tissue-specific *Cas9* vector used to generate transgenic lines. Three artificial introns are engineered into Cas9 coding sequence and the resulted cassette or the enhanced-GFP coding sequence is under control of a tissue-specific promoter and flanked with SV40 and *egl-13* NLSs. (**b, d, f, h, j, l**) DIC images of the P*tissue-specific*::NLS$^{\mathbf{2\times SV40}}$-GFP-NLS$^{egl\text{-}13}$ transgenic animals. (**c, e, g, i, k, m**) Dark field views of the worms (GFP fluorescence) corresponding to those in left panel. Tissue-specific promoters used in this study are *dpy-30* promoter (**c**, for the ubiquitous expression), *ges-1* promoter (**e**, for the intestinal expression), *unc-119* promoter (**g**, for the pan-neuronal expression), *unc-54* promoter (**i**, for the muscle expression) and *dpy-7* promoter (**k**, for hypodermal expression). GFP is observed in nuclei of hypodermal cells in P*dpy-5*::NLS$^{\mathbf{2\times SV40}}$-GFP-NLS$^{egl\text{-}13}$ transgenic animals (**m**). Scale bar, 100μm.

Supplementary information, Figure S1H. Tissue-specific genome editing is achieved by the CRISPR-Cas9 feeding system.

(**a**) The *dpy-5* specific sequence (underlined) used to construct *dpy-5ts-gRNA* is shown in the top panel. The PAM motif (in red) is immediately adjacent to the underlined portion, and the boxed region is a SacI restriction site. The bottom panel shows the penetrance of Dpy phenotype observed in the progeny from each tissue-specific *Cas9* transgenic line fed on *dpy-5ts-gRNA*-46 expressing HT115 bacteria. (**b**) Tissue-specific somatic *dpy-5* mutations are checked by PCR and SacI restriction assays. Lanes 1 and 2: undigested and digested PCR products from N2 animals, respectively. Lane 3-8: restriction patterns of PCR products from each tissue-specific *Cas9* transgenic worms fed on *dpy-5ts-gRNA-46* expressing HT115 bacteria. (**c**) Sequences of seven indels obtained by the CRISPR-Cas9 feeding system. The underlined sequence is target specific (*dpy-5*). The short lines represent Cas9/gRNA-led deletions and blue lowercase letters are the insertions. Red capital letters represent the PAM motif. The nature of the mutations is indicated as the number of base pairs inserted (+) or deleted (-). If both deletion and insertion are observed, the fact is described in parentheses.

Supplementary information, Figure S1I. Inducible knockout of *dpy-5* is achieved through the CRISPR-Cas9 feeding system.

(**a**) Transgenic worms, P*hsp-12.6*::*Cas9*, exert Dpy phenotype if heat shocked (bottom) or appear normal without any heat treatment (upper) after feeding on *dpy-5ts-gRNA* containing bacteria starting at the L1 stage. (**b**) The *dpy-5* specific sequence (underlined) used to construct *dpy-5ts-gRNA* is shown in the top panel. The PAM motif (in red) is immediately adjacent to the underlined portion, and the boxed region is a SacI restriction site. The bottom panel shows the PCR and SacI restriction assay results. The left lane: digested PCR product of without heat treatment. The middle lane: a SacI-resistant PCR product appears after P*hsp-16.2*::*Cas9* worm is heat shock. The right panel: molecular marker. Scale bar is100 µm.

Supplementary information, Figure S1J. Sequences of Cas9 and gRNA encoding plasmid used in our study