Supplementary information, Data S1 Materials and Methods

Culture conditions and worm strains. The wild-type *C. elegans* strains N2 (Bristol), NL2099 *rrf-3*(*pk1426*) II, and MQD397 hqIs92 [pDYH59(P*unc-119*::unc-119+P*pud-2.2*::GFP::pud-2.2)] II (from Dr. Mengqiu Dong at the NIBS, Beijing)[1] were maintained under standard culture conditions[2]. Worms are normally cultured at 20°C and all experiments were performed at 20°C, or otherwise specified. Strains used in our study are listed in **Supplementary table 1H.**

ts-gRNA. Using the known sequence requirements of CRISPR-Cas9 mediated cleavage[3-5], we searched for targeting sequences in *C. elegans* genome based on the following criteria: (i) a protospacer adjacent motif (PAM) sequence, NGG, must be adjacent to the 3' end of the target sequence; and (ii) the first two nucleotides at 5'end of the target sequence had to be GG or GA, to allow efficient ts-gRNA transcription mediated by the T7 promoter *in vitro*. A consensus sequence, G-(G/A)-(N18)-NGG, was used to construct ts-gRNA encoding cassettes. We aimed to target the protein-coding genes to create frame-shift mutations in their ORFs. To quick detect the mutations, we also preferred the presence of a restriction site in the target specific sequence, upstream of the PAM. We used the USCS genome browser to search for a string of sequences that met the above criteria for each gene of interest.

The target sequences were made by annealing two gene specific complementary oligonucleotides with two recognition sites of BbsI, which generates 3' extruding 4 nucleotides overhang outside the recognition site, on each end (in opposite orientation). 50μM of each oligonucleotide was mixed and heated at 95 °C for 5min, incubated at 37 °C for10min, and restriction sites are arranged between the U6 or T7 promoter and gRNA scaffold, juxtaposed to each other in the gRNA scaffold encoding vectors. To quickly create different ts-gRNA cassettes, the BbsI restricted vector and annealed oligonucleotides were ligated according to their compatibility of their 3'overhangs. All cloned inserts were verified by sequencing.

Cas9 plasmid construction for RNA injection. 5' Capped *Cas9* or *mCherry* mRNA with Poly(A) tail was synthesized using the mMessage Machine (Ambion,#AM1340) and PolyA tailing Kits (Ambion, #AM1350), and purified by the RNeasyMini Kit (QIAGEN, Cat. 74104). To create the template for *Cas9* mRNA *in vitro* transcription, *Cas9* coding sequence (without introns) was first PCR amplified from plasmid 46168[6] using a forward primer containing an AgeI site and a reverse primer containing a NheI restriction site. The PCR product was cloned into L4440 restricted by AgeI and NheI. We amplified the *glp-1* 5′ UTR from N2 genomic DNA, fused it to two NLS^{SV40} through a two-step fusion PCR process, and added the SacII and AgeI recognition sites to the 5' and 3′ ends of the resulted PCR fragment, respectively. The restricted PCR fragment was then cloned to the upstream of *Cas9* coding sequence in L4440, which was digested beforehand with SacII and AgeI. The *glp-1* 3′ UTR was amplified from N2 genomic DNA and ligated to an NLS^{egl-13} and the resulted DNA fragment was cloned downstream of *Cas9* coding sequence in L4440. A T7 promoter was placed upstream of *glp-1* 5' UTR in L4440 to generate 5' UTR^{glp-1}-$NLS^{2\times SV40}$-Cas9-NLS^{egl-13}-3'UTR^{glp-1} cassette, which was used for *in vitro* synthesis of *Cas9* mRNA.5'UTR-$NLS^{2\times SV40}$-mCherry-NLS^{egl-13}-3'UTR cassette was created similarly, with the *Cas9* coding sequence replaced by mCherry coding sequence that was amplified from pcDNA3.1. The full sequence of T7 promoter-5'$UTR^{\text{germ cell-specific}}$-$NLS^{2\times SV40}$-Cas9-$NLS^{egl-13}$-3'$UTR^{\text{germ cell-specific}}$ is shown in **Supplementary Fig. 1J**.

Cas9 plasmid construction for DNA injection. To generate plasmid encoding *Cas9* used for germline or tissue specific expression, three artificial introns were introduced into *Cas9* coding sequence by fusion-PCR to generate $NLS^{2\times SV40}$-Cas9-NLS^{egl-13} segment, which was then cloned into pPD95.75 at the XmaI and EcoRI sites. Germline or tissue specific promoters (P*pie-1*, P*dpy-30*, P*ges-1*, P*unc-119*, P*unc-54* and P*dpy-7*) were PCR amplified from N2 genomic DNA, and placed upstream of the *Cas9* coding cassette. To generate transgenic lines (P*pie-1*::*Cas9*), the construct

(NLS$^{2\times SV40}$-Cas9-NLS^{egl-13} in pPD95.75) was modified by replacing *unc-54* 3' UTR with *tbb-2* 3'UTR and placing *pie-1* promoter upstream of the *Cas9* coding sequence. To test the efficiency of this plasmid, *GFP* coding sequence was used to replace *Cas9* sequence. The full sequence of P*pie-1*::NLS^{2xSV40}-Cas9-NLS^{egl-13}-*tbb-2* 3'UTR is shown in **Supplementary Fig. 1J**.

Microinjection and constructs used to generate transgenic worms. Gonad microinjection was performed according to standard *C. elegans* procedures in young adult hermaphrodites[7]. The microinjector was adjusted such that the pressure from the N2 gas tank was 300 kpa. The P_{inject} and $P_{balance}$ measurements were adjusted to 80 kpa and 20 kpa, respectively. The mix for RNA injection included 900 ng/ul *Cas9* mRNA or mCherry mRNA and 120ng/ul ts-gRNA. The normal plasmid DNA mix consisted of 25ng/μl linearized Cas9 coding plasmid, 100ng/μl ts-gRNA encoding plasmid, and 20ng/ul pRF-4[rol-6-(su1006)] that serves as a co-injection marker. A high concentration of injection DNA mix contains 500ng/μl P*pie-1*::*Cas9*, 200 ng/μl ts-gRNA and 20 ng/μl co-injection marker P*col-10*::*mCherry*. The low concentration of injection mix included 5 ng/μl linearized Cas9 or GFP coding sequence, 20ng/μl ts-gRNA, 5ng/μl *Pcol-10*::*mCherry*, 5 ng/μl *pRF-4*[rol-6-(su1006)], and 150 ng/μl fragmented *E.coli* genomic DNA (sonicated or PvuII digested) that served to prevent germline silencing effect[8, 9]. To generate tissue-specific and heat-inducible Cas9 transgenic lines, 20 ng/μl P*tissue-specific*::*Cas9* or P*hsp-16.2*::*Cas9* and 20 ng/μl P*col-10*::*mCherry* (a co-injection transgene marker) were injected into each gonad. At least three independent lines, stably carrying plasmid DNA as extra chromosomal arrays, were obtained per construct. The transgenes used in our study are listed in **Supplementary Table 1H**.

The ts-gRNA encoding plasmid construction and feeding assays. We fused a fragment amplified from T7 promoter-2×BbsI enzyme restriction site-gRNA scaffold cassette by overlapping-PCR[4], and the 46-bp/129-bp T7 terminator by annealing

complementary oligonucleotides. The resulted DNA fragment, T7 promoter-2xBbsI enzyme sites-gRNA scaffold-T7 terminator cassette was cloned into pMD18-T vector to become the empty vector. The annealed 18~20bp double stranded target DNA was inserted into the empty vector at the BbsI restriction sites to become (*dpy-5* or *bli-2*)ts-gRNA-46, or (*dpy-5* or *bli-2*)ts-gRNA-129 plasmid. The plasmid or empty vector was transformed into HT115 bacteria, of which a bacterial colony was inoculated and cultured in 6ml LB [ampicillin (100μg/ml) and tetracycline (50μg/ml)] at 37℃ overnight. The cultured bacteria were collected in 600μl LB and 1/6 of liquid bacteria were spread to the center of a LB agar plate (1mM IPTG, 100μg/ml ampicillin and 50μg/ml tetracycline). Let inoculated plates dry (wrapped in the aluminum foil to induce overnight at room temperature). To perform the germline-mediated mutagenesis, 10 L4 larval P*germ cell*::*Cas9* worms (P0) were picked up and fed with HT115 bacteria transformed with ts-gRNA-46-containing plasmid DNA. Each transgenic progeny of P0 (F1) with mCherry fluoresce was picked up and seeded onto a culture dish with normal HT115 bacteria. To perform the soma-specific mutagenesis, 10 young-adult-stage P*somatic cell*::*Cas9* worms (P0) are fed HT115 bacteria transformed with ts-gRNA-46-containing plasmid DNA. Individual transgenic progeny of P0 (F1) with mCherry fluoresce was picked up and fed continuously with HT115 bacteria containing ts-gRNA-46. Worms observed in F1 (P*somatic cell*::*Cas9*) or F2 (P*germ cell*::*Cas9*) with desired phenotypes were subjected to genotyping or PCR/restriction confirmation.

Heat shock treatment. Worms with synchronized developmental stage were collected by allowing 50–100 adult animals laying eggs for 1 h on a normal NGM plate first, removing the adults, and raising the embryos at 20°C before heat treatment. At desired developmental stage(s), the worms were treated on the agar plates floating in a 35°C water bath for one hour, and recovered at 20°C. The Dpy phenotype was then observed and scored.

Identification of mutants with obvious phenotypes or genotyping.

MQD470(hqIs92[pDYH59(P*unc-119*::unc-119+PF15E11.14::GFP::F15E11.14)]II;hqIs106[pDYH71(P*unc-119*::unc-119+PF15E11.13::cherry::F15E11.13)] IV) (a gift from Dong Mengqiu) worm gonads were injected with *Cas9* mRNA/ *gfpts-gRNA*, and the F1 progeny were examined by GFP fluorescence. The potential mutants were also subjected for sequencing confirmation. Mutants with visible phenotypes such as Dpy and Blistered were first singled out and transferred into a new NGM plate, followed by single worm PCR using NEB Phusion high fidelity polymerase. The PCR products were then sequenced or digested with specific restriction enzymes. For *dpy-5* somatic knockout assay, we PCR-amplified a 457-bp genomic DNA fragment containing the target site from transgenic animals fed on HT115 harboring *dpy-5*ts-gRNA-46 and digested the amplified DNA with SacI. After electrophoresis, we recovered the amplified DNA band and sequenced them.

RNA isolation and RT-PCR of bacteria. The overnight culture of transformed HT115 bacteria was collected by centrifuging at 1,2000rpm for 1min, and the supernatant was discarded. Total bacteria RNA was extracted using Trizol reagent (Invitrogen) and cleaned with RNeasy mini column (Qiagen, Cat. 74104). 2.5μg of total RNA was reverse transcribed using SuperScriptII reverse-transcriptase (Invitrogen) and random hexamers. The first-strand cDNA was subjected to RT-PCR to determine the length of ts-gRNA transcripts.

Primers. All primers used in this study are listed in **Supplementary Table 1I**.

Reference for Materials and Methods

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