Gene activation in *Caenorhabditis elegans* using the *Campylobacter jejuni* CRISPR-Cas9 feeding system

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Abstract

Clustered regularly interspaced palindromic repeats-based activation system, a powerful genetic manipulation technology, can modulate endogenous gene transcription in various organisms through fusing nuclease-deficient Cas9 to transcriptional regulatory domains. At present, this clustered regularly interspaced palindromic repeats-based activation system has been applied to activate gene expression by microinjection manner in *Caenorhabditis elegans*. However, this complicated and time-consuming injection manner is not suitable for efficient and high-throughput gene regulation with clustered regularly interspaced palindromic repeats-Cas9 system. Here, we engineered a *Campylobacter jejuni* clustered regularly interspaced palindromic repeats-Cas9-based gene activation system through bacteria feeding technique to delivering gene-specific sgRNA in *C. elegans*. It enables to activate various endogenous genes efficiently, as well as induce the corresponding phenotypes with a more efficient and labor-saving manner. Collectively, our results demonstrated that our novel d*Cj*Cas9-based activation feeding system holds great promise and potential in *C. elegans*.

Keywords: *Campylobacter jejuni* Cas9; gene activation; feeding; *Caenorhabditis elegans*

Introduction

The clustered regularly interspaced palindromic repeats (CRISPR)-CRISPR-associated protein (CRISPR-Cas) system is most often based on the Streptococcus pyogenes Cas9 (SpCas9) nuclease, which is an RNA-guided genome editing tool for genetic manipulation in various organisms (Bevacqua et al. 2021; Farbiak et al. 2021; Yin et al. 2021). In addition to SpCas9, various Cas9 orthologs from different bacteria have been characterized and developed, such as Neisseria meningitidis Cas9 (NmCas9), Staphylococcus aureus Cas9 (SaCas9), Francisella novicida Cas9 (FnCas9), and *Campylobacter jejuni* Cas9 (CjCas9). To date, the CRISPR-Cas9 system has been widely used for introducing indels and generating knockouts in vitro and in vivo (Bevacqua et al. 2021, Farbiak et al. 2021, Yin et al. 2021). Moreover, when a missense substitution of the catalytic site was introduced to eliminate Cas9 nuclease activity that did not compromise its DNA binding activity, nuclease-deficient Cas9 (dCas9) was also able to upregulate RNA-guided transcription by fusing with trans-activators (Mali et al. 2013). Among these various Cas9 orthologs, CjCas9, a newer CRISPR endonuclease, exhibits several unique features, including its smallest size (984 amino acid residues) for easier delivery, distinct target recognition of the 5’-NNNNACA-3’ or 5’-NNNNRYAC-3’ sequence, unique triple-helix tracrRNA structure, and potential for contact with the nucleotide sequences in both DNA strands of the target (Zhang et al. 2021).

In *Caenorhabditis elegans*, the CRISPR-Cas9 system, especially SpCas9, has been widely used for gene editing and transcriptional regulation, either alone or in combination with transcriptional regulatory domains (Long et al. 2015; Wei et al. 2019). Very recently, we also developed a robust CjCas9-based transcription activation system, miniCAFE, which combines a nuclease-deficient CjCas9 (dCjCas9) and the tripartite transcriptional activator VP64-p65-Rta (herpes simplex virus-derived VP64 activator, the human NF-KB p65 activator domain, and the Epstein-Barr-virus-derived R trans-activator, VPR), and then microinjected it into *C. elegans* to activate the expression of various genes (Zhang et al. 2021). Microinjection in *C. elegans* is a reliable, versatile, and frequently used method for delivering genetic constructs. However, microinjection requires a specialized micromanipulator and a skilled microinjection operator (Berkowitz et al. 2008), so it is not suitable for efficient and high-throughput gene disruption or regulation in the CRISPR-Cas9 system. In *C. elegans*, feeding-based RNAi is the most convenient and powerful method for silencing gene function by feeding animals with bacteria expressing dsRNA (Timmons et al. 2001). This “feeding” method has also been used for delivering guide-RNA to achieve CRISPR-Cas9-based gene disruptions (Liu et al. 2014). However, the CRISPR-based feeding system for gene activation has not been reported. Therefore, we engineered a CRISPR-Cas9-based gene activation system using a bacteria feeding technique to deliver gene-specific sgRNA to determine whether genes could be activated using a bacteria feeding-dependent CRISPR-Cas9 system.
Materials and methods

Caenorhabditis elegans strains and maintenance

Wild-type N2 (Bristol) was used as the wild-type strain [from the Caenorhabditis Genetic Center (CGC), University of Minnesota, USA]. Worms were grown and maintained on solid nematode growth medium (NGM) with the OP50 or HT115 Escherichia coli strains as food at 20°C.

Plasmid construction

The plasmid pCAG-VPR-L1-Cas9 D8A H559A was constructed by Rong Lab. To implement VPR-dCas9 or Cas9 fusion protein, microinjection was performed according to a standard protocol as previously described (Berkowitz et al. 2008). In brief, the relevant plasmids were injected into the gonads of young adult hermaphrodites. The DNA mixture for injection included 50 ng/μl Pdpy-30::VPR-L1-Cas9 D8A H559A or Pdpy-30::VP64-L1-Cas9 D8A H559A, 5 ng/μl Pmyo-2::GFP: H2B and 3 ng/μl Pmyo-3::mCherry (Pmyo-2::GFP: H2B and Pmyo-3::mCherry were used as the additional pharyngeal and body-wall fluorescence-bearing transgenic reporter plasmids, respectively). The VPR-dCas9 and VP64-dCas9 transgenic strains bearing the VPR-dCas9 or VP64-dCas9 expression plasmid and fluorescence reporter were obtained by microinjection as additional extrachromosomal arrays. To further generate stable transgenic worms, the extrachromosomal arrays were integrated into the chromosome by X-ray irradiation. The transgenic worms were back-crossed 4 times with the wild-type N2 strain before use.

The sgRNA bacteria feeding assay

The empty vector or gene-specific sgRNA plasmids were transformed into HT115(DE3) chemically competent cells using CaCl2 transformation protocols. The gene-specific sgRNA sequences were verified by Sanger sequencing. All sgRNA bacteria feeding experiments were performed at 20°C using an RNAi-like feeding protocol (Wan et al. 2021). Briefly, in IPTG-supplemented plates, the VPR-dCas9 or VP64-dCas9 transgenic worms were fed HT115 bacteria carrying empty vector or gene-specific sgRNA plasmids starting at the L1 larval stage until the young adult stage. The sgRNA-fed worms were used for subsequent experiments.

Microinjection and transgenic strains

To generate transgenic worms expressing the VPR-dCas9 or VP64-dCas9 fusion protein, microinjection was performed according to a standard protocol as previously described (Berkowitz et al. 2008). In brief, the relevant plasmids were injected into the gonads of young adult hermaphrodites. The DNA mixture for injection included 50 ng/μl Pdpy-30::VPR-L1-Cas9 D8A H559A or Pdpy-30::VP64-L1-Cas9 D8A H559A, 5 ng/μl Pmyo-2::GFP: H2B and 3 ng/μl Pmyo-3::mCherry (Pmyo-2::GFP: H2B and Pmyo-3::mCherry were used as the additional pharyngeal and body-wall fluorescence-bearing transgenic reporter plasmids, respectively). The VPR-dCas9 and VP64-dCas9 transgenic strains bearing the VPR-dCas9 or VP64-dCas9 expression plasmid and fluorescence reporter were obtained by microinjection as additional extrachromosomal arrays. To further generate stable transgenic worms, the extrachromosomal arrays were integrated into the chromosome by X-ray irradiation. The transgenic worms were back-crossed 4 times with the wild-type N2 strain before use.

Table 1. The sgRNAs and primers in this study.

| Gene   | Target genomic sequences (5'–3') | Primer sequences (5'–3') |
|--------|----------------------------------|-------------------------|
| myo-2  | ATAAAGATAGCAGAAAATGCGCAGG       | Forward ATAAAGATAGCAGAAAATGCGCAGG |
|        | AAAGGACCAC                      | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| lpl-4  | GATTGGACCTTCACATACACAC           | Forward GATTGGACCTTCACATACACAC |
|        | ACACACAC                        | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| lpl-5  | AATAATGGCTTGGCGCCTGTGAGGGTAC    | Forward AATAATGGCTTGGCGCCTGTGAGGGTAC |
|        | CAACGACC                        | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| oak-2  | TCTTGTGGAGAGCATATTAAAGTG         | Forward TCTTGTGGAGAGCATATTAAAGTG |
|        | TCTTACATAC                      | Reverse CAACGACC |
| pha-4  | ATGGAAGGTGAGTATGGGAGAG           | Forward ATGGAAGGTGAGTATGGGAGAG |
|        | GAGGATAC                        | Reverse CAACGACC |
| spr-4  | AACGCAAAGAGAGGTGTTGAG           | Forward AACGCAAAGAGGTGTTGAG |
|        | AGAGATAC                        | Reverse CAACGACC |
| pie-1  | GCAATATCGTCCTTATGCGGTGA         | Forward GCAATATCGTCCTTATGCGGTGA |
| (sgRNA1)|                                 | Reverse CAACGACC |
|        | GAAAGATAC                       | Forward CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| pie-1  | AAAATAATGGCTTGGATGATCCCA         | Forward AAAATAATGGCTTGGATGATCCCA |
| (sgRNA2)|                                 | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| pie-1  | TTTTCTCCAGTATGCTGACT          | Forward TTTTCTCCAGTATGCTGACT |
| (sgRNA3)|                                 | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| ges-1  | TTAACATCTTGTTGGAGTCATTACA       | Forward TTAACATCTTGTTGGAGTCATTACA |
| (sgRNA1)|                                 | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| ges-1  | AAAATCTTACGAGGTGCGACAAA         | Forward AAAATCTTACGAGGTGCGACAAA |
| (sgRNA2)|                                 | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| ges-1  | TTTTCTCGGTTCGGCGACAAA          | Forward TTTTCTCGGTTCGGCGACAAA |
| (sgRNA3)|                                 | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| rab-3  | TGTAGGACCTTCTTGTTAGAG           | Forward TGTAGGACCTTCTTGTTAGAG |
| (sgRNA1)|                                 | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| rab-3  | GAGCAATAC                       | Forward TGTAGGACCTTCTTGTTAGAG |
| (sgRNA2)|                                 | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| chr IV | AAAATCGGTGAAAACACACACAC         | Forward AAAATCGGTGAAAACACACACAC |
| (NC)   | AAACACAC                        | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |

Table 2. The sgRNA scaffold and T7 terminator cassette.

| Scaffold-T7 terminator | Primer sequences (5'–3') |
|------------------------|-------------------------|
| Forward ATGGAACGAGATGCTGCTGCTCA | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA | Forward ATGGAACGAGATGCTGCTGCTCA |
| Forward ATGGAACGAGATGCTGCTGCTCA | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA | Forward ATGGAACGAGATGCTGCTGCTCA |
| Forward ATGGAACGAGATGCTGCTGCTCA | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA | Forward ATGGAACGAGATGCTGCTGCTCA |
| Forward ATGGAACGAGATGCTGCTGCTCA | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA | Forward ATGGAACGAGATGCTGCTGCTCA |
| Forward ATGGAACGAGATGCTGCTGCTCA | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA | Forward ATGGAACGAGATGCTGCTGCTCA |
| Forward ATGGAACGAGATGCTGCTGCTCA | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA | Forward ATGGAACGAGATGCTGCTGCTCA |
| Forward ATGGAACGAGATGCTGCTGCTCA | Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA |
| Reverse CTTGCGCAATTTCGCTTCTGTAGCTGCTCA | Forward ATGGAACGAGATGCTGCTGCTCA |
RNA extraction and quantitative RT-PCR

Worms were fed HT115 bacteria carrying empty vector or gene-specific sgRNA plasmids until age-synchronized young adults and were then collected with M9 buffer and washed several times. Worm pellets were resuspended using AG RNA™ PRO reagent (Accurate Biology, Changsha, China). Total RNA was isolated by chloroform extraction and isopropanol precipitation. Afterward, 500 ng of total RNA was used for reverse transcription with a high-capacity cDNA transcription kit (RK20400, ABclonal, Wuhan, China). Quantitative real-time PCR was performed using SYBR Green Select Master Mix (RK21203, ABclonal, Wuhan, China). Quantitative real-time PCR was performed using a CFX96 real-time system (Bio Rad, CA, USA), and each experiment was repeated at least 3 times. Quantification of transcripts was normalized to the act-2 gene, and results were computed using the 2−ΔΔCT method. P-values were calculated using the 2-tailed Student’s t-test. The primers used in this study are shown in Table 3.

Fluorescence microscopy and image analyses

To assess the fluorescence intensity of myo-2::GFP, we fed VPR-dCjCas9 and VP64-dCjCas9 transgenic worms HT115 bacteria carrying an empty vector or sgRNA targeting the myo-2 promoter. We mounted the worms on 2% agar pads after they were anesthetized using M9 buffer containing NaCl (50 mM) and then observed fluorescence using a Nikon Ti2-U (Zhang et al. 2021). The GFP fluorescence intensity of each worm was analyzed using ImageJ as previously described. Each experiment used at least 30 animals. P values were calculated using the 2-tailed Student’s t-test.

Table 2. The full sequence of sgRNA cassette.

| T7 promoter-lac operator-target sequence- sgRNA (F + S) scaffold-T7 terminator cassette sequence (5′−3′) |
|-------------------------------------------------|
| ttatacgactctataagggaattgtggcggtaaaaccttcgaaccggttgtgcca-gaggttttttttcatggcgtttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt...
Fig. 1. Transcriptional activation of multiple targeted genes and corresponding phenotype induction in *C. elegans* using the *C. jejuni* CRISPR–Cas9 activation feeding system. a) Schematic of the VRP-dCjCas9 activation feeding system. VPR-dCjCas9 transgenic worms were generated by injection of the P*dpy-30*::VPR-dCjCas9 plasmid. By delivering the engineered sgRNA plasmid containing the gene-specific gRNA sequence through bacterial feeding, the VRP-dCjCas9 activator can activate gene expression in *C. elegans*. b) *P*myo-2::GFP fluorescence in the pharyngeal region of the VPR-dCjCas9 and 4|G3, 2022, Vol. 12, No. 6
were not affected by sgRNA-bacteria, which was verified using other internal reference genes (act-1 and csg-1; Hoogewijs et al. 2008, data not shown). In addition, to quantify transcripts, we used both empty vector L4440 and an sgRNA targeting an intergenic sequence as the negative control (Fig. 1f).

During injection of plasmid Pdpy-30-VPR-dCas9, Pmyo-2-GFP::H2B was used as a coinjection maker to ensure successful microinjection. To test the capacity of the VPR-dCas9 feeding system, we first examined whether our VPR-dCas9 activator could up-regulate the pharyngeal GFP fluorescence expression by sgRNA targeting the myo-2 promoter. We fed the VPR-dCas9 transgenic worms HT115 bacteria carrying with myo-2-specific sgRNA for promoter. We observed a significant increase in the fluorescence intensity of pharyngeal-GFP (Fig. 1b) and mRNA expression level when compared with the VPR-dCas9-only control worms (Fig. 1f), suggesting that the VPR-dCas9 feeding system could be used to activate the target gene in C. elegans.

Given the ability of the VPR-dCas9 system to upregulate reporter gene expression, we speculated that it might also induce endogenous gene transcription in C. elegans. Therefore, we used the VPR-dCas9 feeding system to activate a series of endogenous genes and conducted phenotype analyses.

First, we chose aak-2 and pha-4 to examine the ability of the VPR-dCas9 system to induce endogenous gene transcription. Both the AMPK α-catalytic subunit AAK-2 and the human FoxA transcription factor ortholog PHA-4 play important roles in lifespan regulation (Greer et al. 2007, Panowski et al. 2007). By delivering specific sgRNA targeting the aak-2 or pha-4 promoter using bacterial feeding, we observed a significant extension in mean lifespan (Fig. 1c) and greatly increased mRNA levels of the target (Fig. 1f), compared with control. Furthermore, we used lipid metabolism-regulating genes (lipl-4 and lipl-5) to assess our VPR-dCas9 system. The lysosomal acid lipases LIPL-4 and LIPL-5 have been reported to regulate lipid storage and longevity in C. elegans (Folick et al. 2015, Buis et al. 2019). Consistent with previous findings, using ORO staining, we found that the fat storage levels of C. elegans were significantly decreased when the VPR-dCas9 feeding system was used to target the lipl-4 or lipl-5 promoter, respectively (Fig. 1d). Moreover, up-regulation of the mRNA expression levels of lipl-4 or lipl-5 was also detected (Fig. 1f). Similar to aak-2 and pha-4, we also observed a significant lifespan extension when targeting lipl-4 promoter (Fig. 1c).

Recently, researchers have used engineered dCas9:VP64 by microinjection manner, to successfully induce the mammalian REST orthologue spr-4 transcriptional activation and lifespan extension in worms (Zullo et al. 2019). Consistently, using our VPR-dCas9 feeding system with spr-4-specific sgRNA, we also observed upregulation of the mRNA level of spr-4 with corresponding lifespan extension (Fig. 1, e and f). Altogether, these results suggest that the feeding-based VPR-dCas9 activation system enables efficient activation of endogenous genes in C. elegans.

Compared with the traditional microinjection activation system, our VPR-dCas9 feeding system, although not as efficient as microinjection systems, is more efficient and less labor-intensive than injection systems (Zhang et al. 2021). Another gene activation system using herpes simplex virus-derived VP64 as an activator has been reported in C. elegans (Long et al. 2015, Zullo et al. 2019). To compare the activation efficiency of VP64 and the VPR activator, we generated VP64-dCas9 transgenic worms and then assessed the ability of VP64 and the VPR activator to induce gene expression using the same sgRNA targeting the myo-2 promoter by feeding manner. Compared with the control worms, we observed a significant increase in the fluorescence intensity of pharyngeal-GFP both in VPR-dCas9 and VP64-dCas9 worms, but those of VP64-dCas9 worms were weaker, suggesting that the activation efficiency of the VPR activator was obviously stronger than that of VP64 (Fig. 1b).

Furthermore, to determine the activation efficiency of our VPR-dCas9 feeding system in different tissues, we attempted to activate tissue-specific genes including pie-1 (germline), ges-1 (intestine), and rab-3 (neuron), by delivering specific-sgRNAs of targets through bacteria feeding. We found significant up-regulation of the mRNA levels of ges-1 and rab-3, but not pie-1, by feeding the gene-specific sgRNA bacteria (Fig. 1h). These results suggest that our feeding-based VPR-dCas9 system exhibits different efficiencies in different tissues, with a high activation efficiency in the intestine, pharynx (confirmed by myo-2) and neuron, but poor efficiency (even nonfunctional) in the germline.

As so far, a modular and flexible platform for gene activation in vivo has been built using the dCas9 protein combined with trans-activator domains (Böhm et al. 2020, Chiarella et al. 2020). In this study, we developed a CRISPR-Cas9-based system to efficiently activate transcription through bacteria feeding to deliver gene-specific sgRNA in C. elegans, which is cost-effective and efficient. However, it is worth noting that targeting either the different genes or the same gene in different sites using our VPR-dCas9 feeding system exhibited markedly different activation efficiencies (Table 4). Therefore, a preliminary screening to obtain suitable and efficient sgRNAs is required before corresponding studies using the VPR-dCas9 feeding system. To better select the functional sgRNAs, we analyzed the distribution of all designed sgRNAs in the promoter region of the different target genes and found that the functional sgRNAs were primarily distributed between 400 and 700 bp upstream of the 5’UTR of target genes (Fig. 1g). Moreover, a previous study indicated that overexpression SID-1 and SID-2 transgenic worms could be selected as a powerful genetic background to increase gRNA uptake (Liu et al. 2014). In further study, we will attempt to use the SID-1
and SID-2 over-expression transgenic background to optimize our VPR-dCj Cas9 feeding system. Furthermore, the expression of most genes could be regulated by transcription factors or epigenetic modifiers. Therefore, combining dCjCas9 with other functional domains, such as an epigenetic modifier, would greatly expand the applicability of our CRISPR-Cas9 feeding system in C. elegans. Overall, this novel dCjCas9-based feeding system may hold great promise for genome editing, transcriptome modulation, and other applications in C. elegans.

**Data availability**

The C. elegans strains and plasmids are available upon request. **Table 1** contains the sgRNAs and primers sequence. **Table 2** contains the sgRNA screening of multiple targeted genes. **Table 3** contains the lifespan data and statistics. **Table 4** contains the sgRNA sequences and fold change values.
contains the full sequence of feeding sgRNA cassette. Table 3 contains the primers used in qRT-PCR. Table 4 contains the sgRNA screening data of all candidate genes. Table 5 contains the statistics data of all lifespan assays. The authors affirmed that all data necessary for confirming the conclusions of the article are present within the article.

QZ, Q-LW, and ZL designed the study, ZL, WD, CW, and QY conducted the experiments. Q-LW, ZL, and CW analyzed the data. ZL and WD wrote the manuscript. QZ and Q-LW reviewed and edited the manuscript.

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Conflicts of interest

None declared.

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