

**Supplementary information Table S1A. Mutagenizing *dpy-5* or *bli-2* by the CRISPR-Cas9 feeding system using two types of T7 RNA polymerase terminator**

| Experiment     | Gene         | Mutants/Total F1 progenies | Frequency (%) |
|----------------|--------------|----------------------------|---------------|
| 1 <sup>a</sup> | <i>bli-2</i> | 3/213                      | 1.41          |
| 2 <sup>a</sup> | <i>bli-2</i> | 5/492                      | 1.02          |
| 3 <sup>a</sup> | <i>bli-2</i> | 4/227                      | 1.76          |
| 1 <sup>b</sup> | <i>bli-2</i> | 0/533                      | 0             |
| 2 <sup>b</sup> | <i>bli-2</i> | 0/438                      | 0             |
| 1 <sup>a</sup> | <i>dpy-5</i> | 4/487                      | 0.82          |
| 2 <sup>a</sup> | <i>dpy-5</i> | 4/330                      | 1.21          |
| 3 <sup>a</sup> | <i>dpy-5</i> | 4/542                      | 0.74          |
| 1 <sup>b</sup> | <i>dpy-5</i> | 0/146                      | 0             |
| 2 <sup>b</sup> | <i>dpy-5</i> | 0/254                      | 0             |
| 3 <sup>b</sup> | <i>dpy-5</i> | 0/562                      | 0             |

**a:** Experiments employing ts-gRNA-46.

**b:** Experiments using ts-gRNA-129.

**Table S1B. The time window of *dpy-5* mutants obtaining by direct *Cas9* mRNA and *dpy-5ts-gRNA* gonad injection**

| Time course | Number of mutants | Number of total progenies | Mutant percentage (%) |
|-------------|-------------------|---------------------------|-----------------------|
| 0~4 hour    | 0                 | 117                       | 0                     |
| 4~8 hour    | 1                 | 213                       | 0.47                  |
| 8~12 hour   | 7                 | 316                       | 2.22                  |
| 12~16 hour  | 5                 | 254                       | 1.97                  |
| 16~20 hour  | 2                 | 296                       | 0.68                  |
| 20~24 hour  | 1                 | 193                       | 0.52                  |
| 24~28 hour  | 0                 | 227                       | 0                     |
| 28~32 hour  | 0                 | 136                       | 0                     |
| Total       | 16                | 1752                      | 0.91 (average)        |

**Table S1C. Summary of direct gonad DNA injection experiments to generate *dpy-5* mutants**

| Experiment           | Gene         | Injected worms (P0) | Disrupted F1 worms/F1 progenies | Frequency % |
|----------------------|--------------|---------------------|---------------------------------|-------------|
| 1 <sup>a</sup>       | <i>dpy-5</i> | ND                  | 3/37                            | 8.11        |
| 1 <sup>a</sup>       | <i>dpy-5</i> | 48                  | 3/43                            | 6.98        |
| Average <sup>a</sup> |              |                     |                                 | <b>7.54</b> |
| 1 <sup>b</sup>       | <i>dpy-5</i> | 30                  | 2/91                            | 2.19        |
| 2 <sup>b</sup>       | <i>dpy-5</i> | 16                  | 1/42                            | 2.38        |
| Average <sup>b</sup> |              |                     |                                 | <b>2.29</b> |

ND, not determined

**a** 500 ng/μl *Ppie-1::Cas9*+200 ng/μl U6::*dpy-5ts*-gRNA;

**b** 5 ng/μl *Ppie-1::Cas9*+20 ng/μl U6::*dpy-5ts*-gRNA;

**Table S1D. New heritable alleles of *dpy-5* and *bli-2* generated in this study**

| <i>dpy-5</i> mutagenesis   | Strain Name | Genotype              | Molecular Information                   | Mutation Type |            |
|--|-------------|-----------------------|---|---------------|------------|
|  | N2          | ---                   | ATCTCAAGGATGCCCGAGCTCCAGGAAACCCA        | Wild Type     |            |
| Cas9 mRNA/<br><i>dpy-5</i> sgRNA<br>gonad injection                                      | PLD5301     | <i>dpy-5(mfd5301)</i> | ATCTCAAGGATGCCCGAG-TCCAGGAAACCCA        | -1            | Frameshift |
|  | PLD5302     | <i>dpy-5(mfd5302)</i> | ATCTCAAGGATGCCCGAG--CTCCAGGAAACCCA      | -2            | Frameshift |
|  | PLD5303     | <i>dpy-5(mfd5303)</i> | ATCTCAAGGATGCCCGAGCaTCCAGGAAACCCA       | +1            | Frameshift |
|  | PLD5304     | <i>dpy-5(mfd5304)</i> | ATCTCAAGGATGCCCGAG- c -CTCCAGGAAACCCA   | -2(-3,+1)     | Frameshift |
|  | PLD5305     | <i>dpy-5(mfd5305)</i> | ATCTCAAGGATGCCCGAGGcttagCTCCAGGAAACCCA  | +5            | Frameshift |
|  | PLD5306     | <i>dpy-5(mfd5306)</i> | ATCTCAAGGATGCCCGAGC---CTCCAGGAAACCCA    | -4            | Frameshift |
|  | PLD5307     | <i>dpy-5(mfd5307)</i> | ATCTCAAGGATGCCCGAG--TCCAGGAAACCCA       | -2            | Frameshift |
|  | PLD5308     | <i>dpy-5(mfd5308)</i> | ATCTCAAGGATGCCCGAGAaaGCTCCAGGAAACCCA    | +2            | Frameshift |
|  | PLD5309     | <i>dpy-5(mfd5309)</i> | ATCTCAAGGATGCCCGAGtagcatGCTCCAGGAAACCCA | +8            | Frameshift |
|  | PLD5310     | <i>dpy-5(mfd5310)</i> | ATCTCAAGGATGCCCGAG--CTCCAGGAAACCCA      | -2            | Frameshift |
|  | PLD5311     | <i>dpy-5(mfd5311)</i> | ATCTCAAGGATGCCCGAG---GCTCCAGGAAACCCA    | -5            | Frameshift |
|  | PLD5312     | <i>dpy-5(mfd5312)</i> | ATCTCAAGGATGCCCGAG-TCCAGGAAACCCA        | -1            | Frameshift |
|  | PLD5313     | <i>dpy-5(mfd5313)</i> | ATCTCAAGGATGCCCGAG---CCAGGAAACCCA       | -4            | Frameshift |
|  | PLD5314     | <i>dpy-5(mfd5314)</i> | ATCTCAAGGATGCCCACTCC--aa--AGGAAACCCA    | -5(-7,+2)     | Frameshift |
|  | PLD5315     | <i>dpy-5(mfd5315)</i> | ATCTCAAGGATGCCCGAGCTagCCAGGAAACCCA      | +2            | Frameshift |
|  | PLD5316     | <i>dpy-5(mfd5316)</i> | ATCTCAAGGATGCCCGAGCTAGCaCAGGAAACCCA     | +1            | Frameshift |
| 500 ng/μl<br><i>Ppie-1::Cas9+</i><br>200 ng/μl<br><i>U6::dpy-5</i><br>sgRNA<br>transgene | PLD5317     | <i>dpy-5(mfd5317)</i> | ATCTCAAGGATGCCCGAGC-CCAGGAAACCCA        | -1            | Frameshift |
|  | PLD5318     | <i>dpy-5(mfd5318)</i> | ATCTCAAGGATGCCCGAG--CCAGGAAACCCA        | -2            | Frameshift |
|  | PLD5319     | <i>dpy-5(mfd5319)</i> | ATCTCAAGGATGCCCGAGC--CAGGAAACCCA        | -2            | Frameshift |
|  | PLD5320     | <i>dpy-5(mfd5320)</i> | ATCTCAAGGATGCCCGAG-TCCAGGAAACCCA        | -1            | Frameshift |
|  | PLD5321     | <i>dpy-5(mfd5321)</i> | ATCTCAAGGATGCCCGAG---TCCAGGAAACCCA      | -4            | Frameshift |
|  | PLD5322     | <i>dpy-5(mfd5322)</i> | ATCTCAAGGATGCCCGAGCaaTCCAGGAAACCCA      | +2            | Frameshift |
| 5 ng/μl<br><i>Ppie-1::Cas9+</i><br>20 ng/μl<br><i>U6::dpy-5</i><br>sgRNA<br>transgene    | PLD5323     | <i>dpy-5(mfd5323)</i> | ATCTCAAGGATGCCCGAG-CTCCAGGAAACCCA       | -1            | Frameshift |
|  | PLD5324     | <i>dpy-5(mfd5324)</i> | ATCTCAAGGATGCCCGAGC-CCAGGAAACCCA        | -1            | Frameshift |
|  | PLD5325     | <i>dpy-5(mfd5325)</i> | ATCTCAAGGATGCCCGAGCg-CAGGAAACCCA        | -1(-2,+1)     | Frameshift |
| CRISPR-Cas9<br>feeding system  | PLD5326     | <i>dpy-5(mfd5326)</i> | ATCTCAAGGATGCCCGAG--CCAGGAAACCCA        | -2            | Frameshift |
|  | PLD5327     | <i>dpy-5(mfd5327)</i> | ATCTCAAGGATGCCCGAG-TCCAGGAAACCCA        | -1            | Frameshift |
|  | PLD5328     | <i>dpy-5(mfd5328)</i> | ATCTCAAGGATGCCCGAGCagTCCAGGAAACCCA      | +2            | Frameshift |
|  | PLD5329     | <i>dpy-5(mfd5329)</i> | ATCTCAAGGATGCCCGAGC---GGAAACCCA         | -4            | Frameshift |
|  | PLD5330     | <i>dpy-5(mfd5330)</i> | ATCTCAAGGATGCCCGAGCT-CAGGAAACCCA        | -1            | Frameshift |

|                            |         |                       |   |    |            |
|----------------------------|---------|-----------------------|---|----|------------|
| CRISPR-Cas9 feeding system | PLD5331 | <i>dpy-5(mfd5331)</i> | ATCTCAAGGATGCCCGAGCCGGAGCTcgggTCCAGGAAACCC<br>A | +4 | Frameshift |
|                            | PLD5332 | <i>dpy-5(mfd5332)</i> | ATCTCAAGGATGCCCGAGGACTCCAGGAAACCCA              | +1 | Frameshift |
|                            | PLD5333 | <i>dpy-5(mfd5333)</i> | ATCTCAAGGATGCCCGAGG-TCCAGGAAACCCA               | -1 | Frameshift |
|                            | PLD5334 | <i>dpy-5(mfd5334)</i> | ATCTCAAGGATGCCCGACC-----CAGGAAACCCA             | -8 | Frameshift |
|                            | PLD5335 | <i>dpy-5(mfd5335)</i> | ATCTCAAGGATGCCCGAGG--CCAGGAAACCCA               | -2 | Frameshift |
|                            | PLD5336 | <i>dpy-5(mfd5336)</i> | ATCTCAAGGATGCCCGAGGCTC-AGGAAACCCA               | -1 | Frameshift |
|                            | PLD5337 | <i>dpy-5(mfd5337)</i> | ATCTCAAGGATGCCCGACC- ---TCCAGGAAACCCA           | -5 | Frameshift |

| <i>bli-2</i> mutagenesis   | Strain Name | Genotype              | Molecular Information                            | Mutation Type |            |
|----------------------------|-------------|-----------------------|--|---------------|------------|
| CRISPR-Cas9 feeding system | N2          | - - -                 | CGGATTC <u>AGTAGCAGCAAATCCTCTTTGGCTCCAGG</u>     | Wild Type     |            |
|                            | PLD2301     | <i>bli-2(mfd2301)</i> | CGGATTCAGTAGCAGCAAATCCT- <u>TTTGGCTCCAGG</u>     | -1            | Frameshift |
|                            | PLD2302     | <i>bli-2(mfd2302)</i> | CGGATTCAGTAGCAGCAA- <u>CCTCTTTGGCTCCAGG</u>      | -2            | Frameshift |
|                            | PLD2303     | <i>bli-2(mfd2303)</i> | CGGATTCAGTAGCAGCAAATCCTga <u>CTTTGGCTCCAGG</u>   | +2            | Frameshift |
|                            | PLD2304     | <i>bli-2(mfd2304)</i> | CGGATTCAGTAGCAGGac- <u>TCCTCTTTGGCTCCAGG</u>     | -2(-4, +2)    | Frameshift |
|                            | PLD2305     | <i>bli-2(mfd2305)</i> | CGGATTCAGTAGCAGCAAATCCT- <u>TTGGCTCCAGG</u>      | -2            | Frameshift |
|                            | PLD2306     | <i>bli-2(mfd2306)</i> | CGGATTCAGTAGCAGCAAATC- <u>TTGGCTCCAGG</u>        | -5            | Frameshift |
|                            | PLD2307     | <i>bli-2(mfd2307)</i> | CGGATTCAGTAGCAG- <u>TTTGGCTCCAGG</u>             | -7(-9, +2)    | Frameshift |
|                            | PLD2308     | <i>bli-2(mfd2308)</i> | CGGATTCAGTAGCAGCAAATCCTC- <u>TTGGCTCCAGG</u>     | -1            | Frameshift |
|                            | PLD2309     | <i>bli-2(mfd2309)</i> | CGGATTCAGTAGCAGCAAAT- <u>TTGGCTCCAGG</u>         | -5            | Frameshift |
|                            | PLD2310     | <i>bli-2(mfd2310)</i> | CGGATTCAGTAGCAGCAAATCCTCa <u>TTTGGCTCCAGG</u>    | +1            | Frameshift |
|                            | PLD2311     | <i>bli-2(mfd2311)</i> | CGGATTCAGTAGCAGCAAA- <u>CTTTGGCTCCAGG</u>        | -4            | Frameshift |
|                            | PLD2312     | <i>bli-2(mfd2121)</i> | CGGATTCAGTAGCAGCAAATCgaatcCT <u>TTTGGCTCCAGG</u> | +5            | Frameshift |

Note: A total of 37 *dpy-5* mutants obtained by direct Cas9 mRNA/*dpy-5*ts-gRNA gonad injection; *Ppie-1::Cas9/U6::dpy-5*ts-gRNA coding plasmid, or CRISPR-Cas9 feeding system, respectively, are shown. 12 *bli-2* mutants were obtained by CRISPR-Cas9 feeding system. The target sequences are underlined and the PAM is in red. The nature of the mutations is indicated as the number of base pairs inserted (+) or deleted (-). In case of mutant alleles caused by deletion and insertion, the deleted and inserted numbers are explained in parentheses after the total changed number. Sequencing results indicate that the lesion type of sequenced *Dpy* or *Bli* worms was frame-shift mutation.

**Table S1E. Summary of genome editing results using the CRISPR-Cas9 feeding system**

| Genotype  | Dpy worms | Number of total F1 worms | Dpy percentage (%) | <i>p</i> value  |
|---|-----------|--------------------------|--------------------|---|
| N2 fed on OP50  | 0         | 1860                     | 0                  | ----  |
| N2 fed on HT115 containing empty vector;  | 0         | 2215                     | 0                  | ----  |
| N2 fed on HT115 containing <i>dpy-5ts-gRNA-46</i> ;   | 1         | 2872                     | 0.035              | ----  |
| <i>pRF4</i> fed on OP50   | 0         | 287                      | 0                  | ----  |
| <i>pRF4</i> fed on HT115 containing empty vector;   | 0         | 375                      | 0                  | ----  |
| <i>pRF4</i> fed on HT115 containing <i>dpy-5ts-gRNA-46</i> ;                                      | 0         | 421                      | 0                  | ----  |
| <i>Pdpy-30::Cas9</i> ; <i>pRF4</i> fed on HT115 containing empty vector                           | 7         | 478                      | 1.46               | <i>p</i> <0.0001 with <i>Pdpy-30::Cas9</i> ; <i>pRF4</i> Student <i>t</i> -test |
| <i>Pdpy-30::Cas9</i> ; <i>pRF4</i> fed on HT115 containing <i>dpy-5ts-gRNA-46</i>                 | 225       | 461                      | 48.81              |   |
|   | 137       | 459                      | 38.16              |   |
|   | 238       | 598                      | 39.80              |   |
|   | 161       | 491                      | 32.79              |   |
| <i>Pges-1::Cas9</i> ; <i>pRF4</i> fed on HT115 containing empty vector                            | 0         | 250                      | 0                  | <i>p</i> =0.68 with <i>Pges-1::Cas9</i> ; <i>pRF4</i> Student <i>t</i> -test    |
| <i>Pges-1::Cas9</i> ; <i>pRF4</i> fed on HT115 containing <i>dpy-5ts-gRNA-46</i>                  | 3         | 336                      | 0.89               |   |
|   | 4         | 384                      | 1.04               |   |
|   | 7         | 532                      | 1.32               |   |
| <i>rrf-3</i> ; <i>Punc-119::Cas9</i> ; <i>pRF4</i> fed on HT115 containing empty vector           | 0         | 320                      | 0                  | <i>p</i> =0.77 with <i>Punc-119::Cas9</i> ; <i>pRF4</i> Student <i>t</i> -test  |
| <i>rrf-3</i> ; <i>Punc-119::Cas9</i> ; <i>pRF4</i> fed on HT115 containing <i>dpy-5ts-gRNA-46</i> | 2         | 562                      | 0.35               |   |
|   | 1         | 352                      | 0.28               |   |
|   | 1         | 383                      | 0.26               |   |
|   | 0         | 179                      | 0                  |   |
| <i>Punc-54::Cas9</i> ; <i>pRF4</i> fed on HT115 containing empty vector                           | 1         | 350                      | 0.28               | <i>p</i> =0.58 with <i>Punc-54::Cas9</i> ; <i>pRF4</i> Student <i>t</i> -test   |
| <i>Punc-54::Cas9</i> ; <i>pRF4</i> fed on HT115 containing <i>dpy-5ts-gRNA-46</i>                 | 4         | 248                      | 1.62               |   |
|   | 7         | 408                      | 2.21               |   |
|   | 9         | 379                      | 185                |   |
|   | 6         | 223                      | 2.69               |   |
| <i>Pdpy-7::Cas9</i> ; <i>pRF4</i> fed on HT115 containing empty vector                            | 5         | 385                      | 1.30               | <i>p</i> <0.0001 with <i>Pdpy-7::Cas9</i> ; <i>pRF4</i>                         |
| <i>Pdpy-7::Cas9</i> ; <i>pRF4</i> fed on  | 74        | 362                      | 20.44              |   |
|   | 90        | 413                      | 21.79              |   |

|  |     |     |       |  |
|--|-----|-----|-------|--|
| HT115 containing<br><i>dpy-5ts-gRNA-46</i>   | 132 | 491 | 26.88 | Student <i>t</i> -test   |
|  | 52  | 298 | 17.44 |  |
| <i>Pdpy-5::Cas9</i> ; <i>pRF4</i> fed on<br>HT115 containing empty vector              | 6   | 525 | 1.14  | <i>p</i> <0.0001 with<br><i>Pdpy-5::Cas9</i> ; <i>pRF4</i><br>Student <i>t</i> -test |
| <i>Pdpy-5::Cas9</i> ; <i>pRF4</i> fed on<br>HT115 containing<br><i>dpy-5ts-gRNA-46</i> | 138 | 769 | 17.94 |  |
|  | 83  | 474 | 17.51 |  |
|  | 79  | 573 | 13.79 |  |

**Table S1F. Dpy-like worms observed in 3 experiments using an inducible (*Phsp-16.2::Cas9*) fed on *dpy-5ts-gRNA* expressing bacteria or on empty vector bacteria after heat shock treatment**

| Experiment/Genotype                                 |  | Dpy worms | Total F1 progeny | Frequency (%) |
|---|--|-----------|------------------|---------------|
| <i>Phsp-16.2::Cas9</i> transgenic worms fed on OP50 |  | 0         | 126              | 0             |
| <b>1</b>  | <i>Phsp-16.2::Cas9</i> transgenic worms fed on HT115 containing empty vector           | 6         | 153              | 3.922         |
|   | <i>Phsp-16.2::Cas9</i> transgenic worms fed on HT115 containing <i>dpy-5ts-gRNA-46</i> | 56        | 182              | 30.80         |
| <b>2</b>  | <i>Phsp-16.2::Cas9</i> transgenic worms fed on HT115 containing empty vector           | 4         | 135              | 2.96          |
|   | <i>Phsp-16.2::Cas9</i> transgenic worms fed on HT115 containing <i>dpy-5ts-gRNA-46</i> | 39        | 143              | 27.3          |
| <b>3</b>  | <i>Phsp-16.2::Cas9</i> transgenic worms fed on HT115 containing empty vector           | 7         | 162              | 4.32          |
|   | <i>Phsp-16.2::Cas9</i> transgenic worms fed on HT115 containing <i>dpy-5ts-gRNA-46</i> | 48        | 179              | 26.8          |
| <b>Total</b>  | <i>Phsp-16.2::Cas9</i> transgenic worms fed on HT115 containing empty vector           | ---       | ---              | <b>3.73</b>   |
|   | <i>Phsp-16.2::Cas9</i> transgenic worms fed on HT115 containing <i>dpy-5ts-gRNA-46</i> | ---       | ---              | <b>28.3</b>   |



**Table S1G. The off-target effect using the CRISPR-Cas9 feeding system**

|                                  |                            |                              |            |            |
|----------------------------------|----------------------------|------------------------------|------------|------------|
| <i>gfp</i>                       | Target <i>gfp</i> sequence | GGTGAAGGTG                   | ATGCAACATA | <b>CGG</b> |
|                                  | Off-target sequence 1      | GAAAGTTTTG                   | ATGCAACATA | <b>TGG</b> |
|                                  | N2                         | GAAAGTTTTG                   | ATGCAACATA | <b>TGG</b> |
|                                  | <i>gfp</i> mutant 1        | GAAAGTTTTG                   | ATGCAACATA | <b>TGG</b> |
|                                  | <i>gfp</i> mutant 2        | GAAAGTTTTG                   | ATGCAACATA | <b>TGG</b> |
|                                  | <i>gfp</i> mutant 3        | GAAAGTTTTG                   | ATGCAACATA | <b>TGG</b> |
|                                  | <i>gfp</i> mutant 4        | GAAAGTTTTG                   | ATGCAACATA | <b>TGG</b> |
|                                  | Off-target sequence 2      | GGCTGTACCG                   | ATGCAACATA | <b>TGG</b> |
|                                  | N2                         | GGCTGTACCG                   | ATGCAACATA | <b>TGG</b> |
|                                  | <i>gfp</i> mutant 1        | GGCTGTACCG                   | ATGCAACATA | <b>TGG</b> |
|                                  | <i>gfp</i> mutant 2        | GGCTGTACCG                   | ATGCAACATA | <b>TGG</b> |
|                                  | <i>gfp</i> mutant 3        | GGCTGTACCG                   | ATGCAACATA | <b>TGG</b> |
|                                  | <i>gfp</i> mutant 4        | GGCTGTACCG                   | ATGCAACATA | <b>TGG</b> |
|                                  | <i>dpy-5</i>               | Target <i>dpy-5</i> sequence | GGATGCCAG  | CCGGAGCTCC |
| Off-target sequence 1            |                            | GATGGACAAG                   | CCGGAGCTCC | <b>AGG</b> |
| N2                               |                            | GATGGACAAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5301[ <i>dpy-5(mfd5301)</i> ] |                            | GATGGACAAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5306[ <i>dpy-5(mfd5306)</i> ] |                            | GATGGACAAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5315[ <i>dpy-5(mfd5315)</i> ] |                            | GATGGACAAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5318[ <i>dpy-5(mfd5318)</i> ] |                            | GATGGACAAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5325[ <i>dpy-5(mfd5325)</i> ] |                            | GATGGACAAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5327[ <i>dpy-5(mfd5327)</i> ] |                            | GATGGACAAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5333[ <i>dpy-5(mfd5333)</i> ] |                            | GATGGACAAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5335[ <i>dpy-5(mfd5335)</i> ] |                            | GATGGACAAG                   | CCGGAGCTCC | <b>AGG</b> |
|                                  |                            |                              |            |            |
| Target <i>dpy-5</i> sequence     |                            | GGATGCCAG                    | CCGGAGCTCC | <b>AGG</b> |
| Off-target sequence 2            |                            | GCTGGACCAG                   | CCGGAGCTCC | <b>AGG</b> |
| N2                               |                            | GCTGGACCAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5301[ <i>dpy-5(mfd5301)</i> ] |                            | GCTGGACCAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5306[ <i>dpy-5(mfd5306)</i> ] |                            | GCTGGACCAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5315[ <i>dpy-5(mfd5315)</i> ] |                            | GCTGGACCAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5318[ <i>dpy-5(mfd5318)</i> ] |                            | GCTGGACCAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5325[ <i>dpy-5(mfd5325)</i> ] |                            | GCTGGACCAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5327[ <i>dpy-5(mfd5327)</i> ] |                            | GCTGGACCAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5333[ <i>dpy-5(mfd5333)</i> ] |                            | GCTGGACCAG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5335[ <i>dpy-5(mfd5335)</i> ] |                            | GCTGGACCAG                   | CCGGAGCTCC | <b>AGG</b> |
|                                  |                            |                              |            |            |
| Target <i>dpy-5</i> sequence     |                            | GGATGCCAG                    | CCGGAGCTCC | <b>AGG</b> |
| Off-target sequence 3            |                            | AACGGAAACG                   | CCGGAGCTCC | <b>AGG</b> |
| N2                               |                            | AACGGAAACG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5301[ <i>dpy-5(mfd5301)</i> ] |                            | AACGGAAACG                   | CCGGAGCTCC | <b>AGG</b> |
| PLD5306[ <i>dpy-5(mfd5306)</i> ] | AACGGAAACG                 | CCGGAGCTCC                   | <b>AGG</b> |            |

|                                  |                                  |            |            |            |
|----------------------------------|----------------------------------|------------|------------|------------|
|                                  | PLD5315[ <i>dpy-5(mfd5315)</i> ] | AACGGAAACG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5318[ <i>dpy-5(mfd5318)</i> ] | AACGGAAACG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5325[ <i>dpy-5(mfd5325)</i> ] | AACGGAAACG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5327[ <i>dpy-5(mfd5327)</i> ] | AACGGAAACG | CCGGAGCTCC | <b>AGG</b> |
| <i>dpy-5</i>                     | PLD5333[ <i>dpy-5(mfd5333)</i> ] | AACGGAAACG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5335[ <i>dpy-5(mfd5335)</i> ] | AACGGAAACG | CCGGAGCTCC | <b>AGG</b> |
|                                  |                                  |            |            |            |
|                                  | Target <i>dpy-5</i> sequence     | GGATGCCAG  | CCGGAGCTCC | <b>AGG</b> |
|                                  | Off-target sequence 4            | CCAGGACCAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | N2                               | CCAGGACCAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5301[ <i>dpy-5(mfd5301)</i> ] | CCAGGACCAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5306[ <i>dpy-5(mfd5306)</i> ] | CCAGGACCAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5315[ <i>dpy-5(mfd5315)</i> ] | CCAGGACCAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5318[ <i>dpy-5(mfd5318)</i> ] | CCAGGACCAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5325[ <i>dpy-5(mfd5325)</i> ] | CCAGGACCAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5327[ <i>dpy-5(mfd5327)</i> ] | CCAGGACCAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5333[ <i>dpy-5(mfd5333)</i> ] | CCAGGACCAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5335[ <i>dpy-5(mfd5335)</i> ] | CCAGGACCAG | CCGGAGCTCC | <b>AGG</b> |
|                                  |                                  |            |            |            |
|                                  | Target <i>dpy-5</i> sequence     | GGATGCCAG  | CCGGAGCTCC | <b>AGG</b> |
|                                  | Off-target sequence 5            | GACGGAGAAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | N2                               | GACGGAGAAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5301[ <i>dpy-5(mfd5301)</i> ] | GACGGAGAAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5306[ <i>dpy-5(mfd5306)</i> ] | GACGGAGAAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5315[ <i>dpy-5(mfd5315)</i> ] | GACGGAGAAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5318[ <i>dpy-5(mfd5318)</i> ] | GACGGAGAAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5325[ <i>dpy-5(mfd5325)</i> ] | GACGGAGAAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5327[ <i>dpy-5(mfd5327)</i> ] | GACGGAGAAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5333[ <i>dpy-5(mfd5333)</i> ] | GACGGAGAAG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5335[ <i>dpy-5(mfd5335)</i> ] | GACGGAGAAG | CCGGAGCTCC | <b>AGG</b> |
|                                  |                                  |            |            |            |
|                                  | Target <i>dpy-5</i> sequence     | GGATGCCAG  | CCGGAGCTCC | <b>AGG</b> |
|                                  | Off-target sequence 6            | CCAGGAAACG | CCGGAGCTCC | <b>AGG</b> |
|                                  | N2                               | CCAGGAAACG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5301[ <i>dpy-5(mfd5301)</i> ] | CCAGGAAACG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5306[ <i>dpy-5(mfd5306)</i> ] | CCAGGAAACG | CCGGAGCTCC | <b>AGG</b> |
|                                  | PLD5315[ <i>dpy-5(mfd5315)</i> ] | CCAGGAAACG | CCGGAGCTCC | <b>AGG</b> |
| PLD5318[ <i>dpy-5(mfd5318)</i> ] | CCAGGAAACG                       | CCGGAGCTCC | <b>AGG</b> |            |
| PLD5325[ <i>dpy-5(mfd5325)</i> ] | CCAGGAAACG                       | CCGGAGCTCC | <b>AGG</b> |            |
| PLD5327[ <i>dpy-5(mfd5327)</i> ] | CCAGGAAACG                       | CCGGAGCTCC | <b>AGG</b> |            |
| PLD5333[ <i>dpy-5(mfd5333)</i> ] | CCAGGAAACG                       | CCGGAGCTCC | <b>AGG</b> |            |
| PLD5335[ <i>dpy-5(mfd5335)</i> ] | CCAGGAAACG                       | CCGGAGCTCC | <b>AGG</b> |            |
|                                  |                                  |            |            |            |

|              |                                  |                                  |             |            |            |
|--------------|----------------------------------|----------------------------------|-------------|------------|------------|
|              | Target <i>dpy-5</i> sequence     | GGATGCCCCAG                      | CCGGAGCTCC  | <b>AGG</b> |            |
|              | Off-target sequence 7            | CCTGGAAACG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | N2                               | CCTGGAAACG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5301[ <i>dpy-5(mfd5301)</i> ] | CCTGGAAACG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5306[ <i>dpy-5(mfd5306)</i> ] | CCTGGAAACG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5315[ <i>dpy-5(mfd5315)</i> ] | CCTGGAAACG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5318[ <i>dpy-5(mfd5318)</i> ] | CCTGGAAACG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5325[ <i>dpy-5(mfd5325)</i> ] | CCTGGAAACG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5327[ <i>dpy-5(mfd5327)</i> ] | CCTGGAAACG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5333[ <i>dpy-5(mfd5333)</i> ] | CCTGGAAACG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5335[ <i>dpy-5(mfd5335)</i> ] | CCTGGAAACG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              |                                  |                                  |             |            |            |
|              | Target <i>dpy-5</i> sequence     | GGATGCCCCAG                      | CCGGAGCTCC  | <b>AGG</b> |            |
|              | Off-target sequence 8            | CCAGGACAAG                       | CCGGAGCTCC  | <b>AGG</b> |            |
| <i>dpy-5</i> | N2                               | CCAGGACAAG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5301[ <i>dpy-5(mfd5301)</i> ] | CCAGGACAAG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5306[ <i>dpy-5(mfd5306)</i> ] | CCAGGACAAG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5315[ <i>dpy-5(mfd5315)</i> ] | CCAGGACAAG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5318[ <i>dpy-5(mfd5318)</i> ] | CCAGGACAAG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5325[ <i>dpy-5(mfd5325)</i> ] | CCAGGACAAG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5327[ <i>dpy-5(mfd5327)</i> ] | CCAGGACAAG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5333[ <i>dpy-5(mfd5333)</i> ] | CCAGGACAAG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              | PLD5335[ <i>dpy-5(mfd5335)</i> ] | CCAGGACAAG                       | CCGGAGCTCC  | <b>AGG</b> |            |
|              |                                  |                                  |             |            |            |
|              |                                  | Target <i>dpy-5</i> sequence     | GGATGCCCCAG | CCGGAGCTCC | <b>AGG</b> |
|              |                                  | Off-target sequence 9            | GCTGGAAGAG  | CCGGAGCTCC | <b>AGG</b> |
|              |                                  | N2                               | GCTGGAAGAG  | CCGGAGCTCC | <b>AGG</b> |
|              |                                  | PLD5301[ <i>dpy-5(mfd5301)</i> ] | GCTGGAAGAG  | CCGGAGCTCC | <b>AGG</b> |
|              |                                  | PLD5306[ <i>dpy-5(mfd5306)</i> ] | GCTGGAAGAG  | CCGGAGCTCC | <b>AGG</b> |
|              |                                  | PLD5315[ <i>dpy-5(mfd5315)</i> ] | GCTGGAAGAG  | CCGGAGCTCC | <b>AGG</b> |
|              |                                  | PLD5318[ <i>dpy-5(mfd5318)</i> ] | GCTGGAAGAG  | CCGGAGCTCC | <b>AGG</b> |
|              |                                  | PLD5325[ <i>dpy-5(mfd5325)</i> ] | GCTGGAAGAG  | CCGGAGCTCC | <b>AGG</b> |
|              |                                  | PLD5327[ <i>dpy-5(mfd5327)</i> ] | GCTGGAAGAG  | CCGGAGCTCC | <b>AGG</b> |
|              |                                  | PLD5333[ <i>dpy-5(mfd5333)</i> ] | GCTGGAAGAG  | CCGGAGCTCC | <b>AGG</b> |
|              |                                  | PLD5335[ <i>dpy-5(mfd5335)</i> ] | GCTGGAAGAG  | CCGGAGCTCC | <b>AGG</b> |
|              |                                  |                                  |             |            |            |
|              |                                  | Target <i>dpy-5</i> sequence     | GGATGCCCCAG | CCGGAGCTCC | <b>AGG</b> |
|              |                                  | Off-target sequence 10           | CCAGGACCAG  | CCGGAGCTCC | <b>TGG</b> |
|              |                                  | N2                               | CCAGGACCAG  | CCGGAGCTCC | <b>TGG</b> |
|              |                                  | PLD5301[ <i>dpy-5(mfd5301)</i> ] | CCAGGACCAG  | CCGGAGCTCC | <b>TGG</b> |
|              | PLD5306[ <i>dpy-5(mfd5306)</i> ] | CCAGGACCAG                       | CCGGAGCTCC  | <b>TGG</b> |            |
|              | PLD5315[ <i>dpy-5(mfd5315)</i> ] | CCAGGACCAG                       | CCGGAGCTCC  | <b>TGG</b> |            |
|              | PLD5318[ <i>dpy-5(mfd5318)</i> ] | CCAGGACCAG                       | CCGGAGCTCC  | <b>TGG</b> |            |

|                     |                                  |                                  |            |            |            |
|---------------------|----------------------------------|----------------------------------|------------|------------|------------|
|                     | PLD5325[ <i>dpy-5(mfd5325)</i> ] | CCAGGACCAG                       | CCGGAGCTCC | <b>TGG</b> |            |
|                     | PLD5327[ <i>dpy-5(mfd5327)</i> ] | CCAGGACCAG                       | CCGGAGCTCC | <b>TGG</b> |            |
|                     | PLD5333[ <i>dpy-5(mfd5333)</i> ] | CCAGGACCAG                       | CCGGAGCTCC | <b>TGG</b> |            |
|                     | PLD5335[ <i>dpy-5(mfd5335)</i> ] | CCAGGACCAG                       | CCGGAGCTCC | <b>TGG</b> |            |
| <b><i>bli-2</i></b> | Target <i>bli-2</i> sequence     | AGTAGCAGC                        | AAATCCTCTT | <b>TGG</b> |            |
|                     | Off-target sequence 1            | AAAAACTCC                        | AAATCCTCTT | <b>TGG</b> |            |
|                     | N2                               | AAAAACTCC                        | AAATCCTCTT | <b>TGG</b> |            |
|                     | PLD2301[ <i>bli-2(mfd2301)</i> ] | AAAAACTCC                        | AAATCCTCTT | <b>TGG</b> |            |
|                     | PLD2302[ <i>bli-2(mfd2302)</i> ] | AAAAACTCC                        | AAATCCTCTT | <b>TGG</b> |            |
|                     | PLD2303[ <i>bli-2(mfd2303)</i> ] | AAAAACTCC                        | AAATCCTCTT | <b>TGG</b> |            |
|                     | PLD2304[ <i>bli-2(mfd2304)</i> ] | AAAAACTCC                        | AAATCCTCTT | <b>TGG</b> |            |
|                     | PLD2305[ <i>bli-2(mfd2305)</i> ] | AAAAACTCC                        | AAATCCTCTT | <b>TGG</b> |            |
|                     | PLD2306[ <i>bli-2(mfd2306)</i> ] | AAAAACTCC                        | AAATCCTCTT | <b>TGG</b> |            |
|                     | PLD2307[ <i>bli-2(mfd2307)</i> ] | AAAAACTCC                        | AAATCCTCTT | <b>TGG</b> |            |
|                     | PLD2308[ <i>bli-2(mfd2308)</i> ] | AAAAACTCC                        | AAATCCTCTT | <b>TGG</b> |            |
|                     | PLD2309[ <i>bli-2(mfd2309)</i> ] | AAAAACTCC                        | AAATCCTCTT | <b>TGG</b> |            |
|                     | PLD2310[ <i>bli-2(mfd2310)</i> ] | AAAAACTCC                        | AAATCCTCTT | <b>TGG</b> |            |
|                     | PLD2311[ <i>bli-2(mfd2311)</i> ] | AAAAACTCC                        | AAATCCTCTT | <b>TGG</b> |            |
|                     | PLD2312[ <i>bli-2(mfd2312)</i> ] | AAAAACTCC                        | AAATCCTCTT | <b>TGG</b> |            |
|                     |                                  |                                  |            |            |            |
|                     |                                  | Target <i>bli-2</i> sequence     | AGTAGCAGC  | AAATCCTCTT | <b>TGG</b> |
|                     |                                  | Off-target sequence 2            | ACTAGTTATC | AAATCCTCTT | <b>AGG</b> |
|                     |                                  | N2                               | ACTAGTTATC | AAATCCTCTT | <b>AGG</b> |
|                     |                                  | PLD2301[ <i>bli-2(mfd2301)</i> ] | ACTAGTTATC | AAATCCTCTT | <b>AGG</b> |
|                     |                                  | PLD2302[ <i>bli-2(mfd2302)</i> ] | ACTAGTTATC | AAATCCTCTT | <b>AGG</b> |
|                     |                                  | PLD2303[ <i>bli-2(mfd2303)</i> ] | ACTAGTTATC | AAATCCTCTT | <b>AGG</b> |
|                     |                                  | PLD2304[ <i>bli-2(mfd2304)</i> ] | ACTAGTTATC | AAATCCTCTT | <b>AGG</b> |
|                     |                                  | PLD2305[ <i>bli-2(mfd2305)</i> ] | ACTAGTTATC | AAATCCTCTT | <b>AGG</b> |
|                     |                                  | PLD2306[ <i>bli-2(mfd2306)</i> ] | ACTAGTTATC | AAATCCTCTT | <b>AGG</b> |
|                     |                                  | PLD2307[ <i>bli-2(mfd2307)</i> ] | ACTAGTTATC | AAATCCTCTT | <b>AGG</b> |
|                     |                                  | PLD2308[ <i>bli-2(mfd2308)</i> ] | ACTAGTTATC | AAATCCTCTT | <b>AGG</b> |
|                     |                                  | PLD2309[ <i>bli-2(mfd2309)</i> ] | ACTAGTTATC | AAATCCTCTT | <b>AGG</b> |
|                     | PLD2310[ <i>bli-2(mfd2310)</i> ] | ACTAGTTATC                       | AAATCCTCTT | <b>AGG</b> |            |
|                     | PLD2311[ <i>bli-2(mfd2311)</i> ] | ACTAGTTATC                       | AAATCCTCTT | <b>AGG</b> |            |
|                     | PLD2312[ <i>bli-2(mfd2312)</i> ] | ACTAGTTATC                       | AAATCCTCTT | <b>AGG</b> |            |

The mismatched bases of the potential off-target sequences are in red, compared to the target sequences.

**Table S1H. The *C. elegans* Strains used in this study**

| Strain name | Genotype   | Method                         | Resource     |
|-------------|--|--------------------------------|--------------|
| N2          | ---  | ---                            | CGC          |
| MQD397      | hqIs92[pDYH59( <i>Punc-119::unc-119+Ppud-2.2::GFP::pud-2.2</i> )]  | Microinjection and integration | Mengqiu Dong |
| PLD2001     | mfdEx2001[ <i>Ppie-1::NLS<sup>2×SV40</sup>-GFP-NLS<sup>egl-13</sup>-tbb-2 3'UTR</i> ;<br><i>Pcol-10::mCherry</i> ; <i>pRF-4 (+)</i> ]        | Microinjection                 | This study   |
| PLD2002     | mfdEx2002[ <i>Ppie-1::NLS<sup>2×SV40</sup>-Cas9-NLS<sup>egl-13</sup>-tbb-2 3'UTR</i> ;<br><i>U6::dpy-5 sgRNA</i> ; <i>pRF-4 (+)</i> ]        | Microinjection                 | This study   |
| PLD2003     | mfdEx2003[ <i>Ppie-1::NLS<sup>2×SV40</sup>-Cas9-NLS<sup>egl-13</sup>-tbb-2 3'UTR</i> ;<br><i>U6::dpy-5 sgRNA</i> ; <i>Pcol-10::mCherry</i> ] | Microinjection                 | This study   |
| PLD2004     | mfdEx2004[ <i>Ppie-1::NLS<sup>2×SV40</sup>-Cas9-NLS<sup>egl-13</sup>-tbb-2 3'UTR</i> ;<br><i>Pcol-10::mCherry</i> ]                          | Microinjection                 | This study   |
| PLD2005     | mfdEx2005[ <i>Pdpy-30::NLS<sup>2×SV40</sup>-GFP-NLS<sup>egl-13</sup>-unc-54 3'UTR</i> ;<br><i>Pcol-10::mCherry</i> ]                         | Microinjection                 | This study   |
| PLD2006     | mfdEx2006[ <i>Pges-1::NLS<sup>2×SV40</sup>-GFP-NLS<sup>egl-13</sup>-unc-54 3'UTR</i> ;<br><i>Pcol-10::mCherry</i> ]                          | Microinjection                 | This study   |
| PLD2007     | mfdEx2007[ <i>Punc-119::NLS<sup>2×SV40</sup>-GFP-NLS<sup>egl-13</sup>-unc-54 3'UTR</i> ;<br><i>Pcol-10::mCherry</i> ]                        | Microinjection                 | This study   |
| PLD2008     | mfdEx2008[ <i>Punc-54::NLS<sup>2×SV40</sup>-GFP-NLS<sup>egl-13</sup>-unc-54 3'UTR</i> ;<br><i>Pcol-10::mCherry</i> ]                         | Microinjection                 | This study   |
| PLD2009     | mfdEx2009[ <i>Pdpy-7::NLS<sup>2×SV40</sup>-GFP-NLS<sup>egl-13</sup>-unc-54 3'UTR</i> ;<br><i>Pcol-10::mCherry</i> ]                          | Microinjection                 | This study   |
| PLD2010     | mfdEx2010[ <i>Pdpy-5::NLS<sup>2×SV40</sup>-GFP-NLS<sup>egl-13</sup>-unc-54 3'UTR</i> ;<br><i>Pcol-10::mCherry</i> ]                          | Microinjection                 | This study   |
| PLD2011     | mfdEx2011[ <i>Pdpy-30::NLS<sup>2×SV40</sup>-Cas9-NLS<sup>egl-13</sup>-unc-54 3'UTR</i> ;<br><i>Pcol-10::mCherry</i> ]                        | Microinjection                 | This study   |
| PLD2012     | mfdEx2012[ <i>Pges-1::NLS<sup>2×SV40</sup>-Cas9-NLS<sup>egl-13</sup>-unc-54 3'UTR</i> ;<br><i>Pcol-10::mCherry</i> ]                         | Microinjection                 | This study   |
| PLD2013     | mfdEx2013[ <i>Punc-119::NLS<sup>2×SV40</sup>-Cas9-NLS<sup>egl-13</sup>-unc-54 3'UTR</i> ;<br><i>Pcol-10::mCherry</i> ]                       | Microinjection                 | This study   |
| PLD2014     | mfdEx2014[ <i>Punc-54::NLS<sup>2×SV40</sup>-Cas9-NLS<sup>egl-13</sup>-unc-54 3'UTR</i> ;<br><i>Pcol-10::mCherry</i> ]                        | Microinjection                 | This study   |
| PLD2015     | mfdEx2015[ <i>Pdpy-7::NLS<sup>2×SV40</sup>-Cas9-NLS<sup>egl-13</sup>-unc-54 3'UTR</i> ;<br><i>Pcol-10::mCherry</i> ]                         | Microinjection                 | This study   |
| PLD2016     | mfdEx2016[ <i>Pdpy-5::NLS<sup>2×SV40</sup>-Cas9-NLS<sup>egl-13</sup>-unc-54 3'UTR</i> ;<br><i>Pcol-10::mCherry</i> ]                         | Microinjection                 | This study   |
| PLD2017     | mfdEx2017[ <i>Phsp-16.2::NLS<sup>2×SV40</sup>-Cas9-NLS<sup>egl-13</sup>-unc-54 3'UTR</i> ;<br><i>Pcol-10::mCherry</i> ]                      | Microinjection                 | This study   |

**Table S11. Primers used in our study (5'→3')**

|  |                       |
|--|-----------------------|
| <i>pie-1</i> promoter <i>s</i>                     | AAAAGTCTATTCTGGGTTC   |
| <i>pie-1</i> promoter <i>As</i>                    | CTGGAAAAGAAAATTTGATTT |
| <i>dpy-30</i> promoter <i>s</i>                    | TTTATCTGCGATGGTGC     |
| <i>dpy-30</i> promoter <i>As</i>                   | TGAGATATCAAAGGCTTCTG  |
| <i>unc-119</i> promoter <i>s</i>                   | AAACCACGGGCGAC        |
| <i>unc-119</i> promoter <i>As</i>                  | ATATATGCTGTTGTAGCTGA  |
| <i>unc-54</i> promoter <i>s</i>                    | GTGCCAATGTAAGTCCG     |
| <i>unc-54</i> promoter <i>As</i>                   | TTTCTCTACCTCAAACTAATG |
| <i>ges-1</i> promoter <i>s</i>                     | CAACATCACGGACCCAAT    |
| <i>ges-1</i> promoter <i>As</i>                    | CTGAAACTGTAAGACGCAGAC |
| <i>dpy-7</i> promoter <i>s</i>                     | GACGAGGATGTTTCTATG    |
| <i>dpy-7</i> promoter <i>As</i>                    | TATCTGGAACAAAATGTAAG  |
| <i>dpy-5</i> promoter <i>s</i>                     | TTACTTATTTGTGCTTCGTC  |
| <i>dpy-5</i> promoter <i>As</i>                    | AAAGTTAGCAAGTTCAAAAG  |
| <i>hsp-16.2</i> promoter <i>s</i>                  | AGCGTTTCAAGTATCC      |
| <i>hsp-16.2</i> promoter <i>As</i>                 | GATTATAGTTTGAAGATTTC  |
| <i>dpy-5</i> PCR for <i>sacI</i> cutting <i>s</i>  | ATGATGATGTGATGGGAGA   |
| <i>dpy-5</i> PCR for <i>sacI</i> cutting <i>As</i> | TCCTGGTTGTCCGTCTT     |
| <i>tbb-2</i> 3'UTR <i>s</i>                        | ATGCAAGATCCTTTCAAGC   |
| <i>tbb-2</i> 3'UTR <i>As</i>                       | TTGGCGGCACAATAAAG     |
| P1 <i>bli-2 s</i>                                  | AGTAGCAGCAAATCCTCTT   |
| P1 <i>dpy-5s</i>                                   | GGATGCCAGCCGGAGCTCC   |
| P1 <i>As</i>                                       | AGCACCGACTCGGTGCCAC   |
| P2 <i>bli-2 s</i>                                  | AGTAGCAGCAAATCCTCTT   |
| P2 <i>dpy-5s</i>                                   | GGATGCCAGCCGGAGCTCC   |
| P2 <i>As</i>                                       | CCCCAGGCTTTACACTTTATG |
| 16S rRNA <i>s</i>                                  | CTCCTACGGGAGGCAGCAG   |
| 16S rRNA <i>As</i>                                 | GTATTACCGCGGCTGCTG    |