## Supplementary materials

## Supplementary tables

#### **Table S1: Genetic lesions**

|  |  |  |
| --- | --- | --- |
| ***Cbr-lin-4*** https://wormbase.org/species/c\_briggsae/gene/WBGene00255721#g--10 | | ***Allele type*** |
| WT | GCCTGTTCCCTGAGACCTCAAGTGTGAGCGTTCTGAACAT | wt |
| *ae54* | GCCTGTT------GACCTCAAGTGTGAGCGTTCTGAACAT | 0 |
| *ae55* | GCCTGTT--------**T**CTCAAGTGTGAGCGTTCTGAACAT | 0 |
| *ae71* | GCCTGT-------GACCTCAAGTGTGAGCGTTCTGAACAT | 0 |
| *ae72* | GCCTG-------AGACCTCAAGTGTGAGCGTTCTGAACAT | 0 |
| *ae79* | GCC------CTGAGACCTCAAGTGTGAGCGTTCTGAACAT | 0 |
| ***Cel-lin-4*** https://wormbase.org/species/c\_elegans/gene/WBGene00002993#g--10 | |  |
| WT | GCCTGTTCCCTGAGACCTCAAGTGTGAGTGTACTATTGAT | wt |
| *ae53* | GCCTGT-------GACCTCAAGTGTGAGTGTACTATTGAT | 0 |
| ***Cbr-lin-14*** https://wormbase.org/species/c\_briggsae/gene/WBGene00036988#g--10 | |  |
| WT | E V H D L R T A V N  GAGGTTCACGATCTACGGACGGCAGTAAAT | wt |
| *ae62* | E V H G R Q \*  GAGGTTCACG-------GACGGCAGTAAAT | 0 |
| *ae63* | E V H Y G R Q \*  GAGGTTCAC----TACGGACGGCAGTAAAT | 0 |
| *ae51* | E V H D H D G R Q \*  GAGGTTCACGATC**ACG**ACGGACGGCAGTAAAT | 0 |
| *ae58* | ATTCCAAAAAAAAATTCGCCCT< 1381 bp del.>CCTCGGAA | gf |
| *ae59* | ATTCCAAAAAAAAATTCGCCCTTTCTATCCCATCC< 1368 bp del.>CCTCGGAA | gf |
| ***Cbr-lin-28*** https://wormbase.org/species/c\_briggsae/gene/WBGene00033626#g--10 | |  |
| WT | D D N T G E D L F V H Q S N L N M Q G F R  GATGATAACACCGGGGAAGATCTTTTTGTGCATCAATCCAATTTGAATATGCAAGGATTTAGAA | wt |
| *ae25* | D D N R G R S F C A S I Q F E Y A R I \*  GATGATA--ACCGGGGAAGATCTTTTTGTGCATCAATCCAATTTGAATATGCAAGGATTTAGAA | (0) |
| *ae35* | D D M E R V K V M Q H T E K I F L C I N P I \*  GATGATA**TGGAGAGGGTGAAGGTGATGCAACATACGGAA**AAGATCTTTTTGTGCATCAATCCAATTTGAAT | (0) |
| *ae39* | D R G R S F C A S I Q F E Y A R I \*  GAT--------CG**T**GGAAGATCTTTTTGTGCATCAATCCAATTTGAATATGCAAGGATTTAGAA | (0) |
| *ae80* | D D N <160 aa del.> K A G K \*  GATGATAAC< 1025 bp del.>AAGGCTGGAAAATAGgcgtcgagaccatggagaac | 0 |
| ***Cbr-lin-29*** https://wormbase.org/species/c\_briggsae/gene/WBGene00025748#g--10 | |  |
| WT | Y L S Q H M R I H L G I K P F G P C N Y C G K  TACCTCTCCCAACACATGCGAATCCATTTGGGAATCAAACCGTTTGGGCCATGCAATTATTGTGGAAAG  K F T Q L S H L Q Q H I R T H T G E K P Y K  AAGTTCACACAGCTCTCACATCTTCAACAACACATTCGGACGCACACGGGAGAGAAACCGTATAAA | wt |
| *ae75* | Y L S H A N P F G N Q T V W A M Q L L W K  TACCTCTC-----ACATGCGAATCCATTTGGGAATCAAACCGTTTGGGCCATGCAATTATTGTGGAAAG  E V H T A L T S S T T H S D A H G R E T V \*  AAGTTCACACAGCTCTCACATCTTCAACAACACATTCGGACGCACACGGGAGAGAAACCGTATAAA | 0 |
| ***Cbr-lin-41*** https://wormbase.org/species/c\_briggsae/gene/WBGene00040670#g--10 | |  |
| WT | M T T T T S T A T L T L E T T D G G E Q H  ATGACCACCACCACGAGTACGGCAACGCTGACACTGGAAACCACCGACGGCGGTGAGCAGCAC | wt |
| *ae76* | M T T K P P P R V R Q R \*  ATGACCACCA**AACCACCA**CCACGAGTACGGCAACGCTGACACTGGAAACCACCGACGGCGGTGAGCAGCAC | lf |

#### **Table S1: Genetic lesions (continued)**

|  |  |  |
| --- | --- | --- |
| *ae77* | M T T M P K D P A K P P A K A Q V V G W P P V R  ATGACCACCA**TGCCTAAAGATCCAGCCAAACCTCCGGCCAAGGCACAAGTTGTGGGATGGCCACCGGTGAGA**  S Y R K N V M V S C Q K S S G G P E A A A F V K  **TCATACCGGAAGAACGTGATGGTTTCCTGCCAAAAATCAAGCGGTGGCCCGGAGGCGGCGGCGTTCGTGAAG**  T T S T A T L R R R K E D D H H A \*  **A**CCACGAGTACGGCAACGCTGA**GAAGACGTAAAGAGGATGACCACCATGCCTAAAGATCCAGCCAAACCTCC**  **GGCCAAGGCACAAGTTGTGGGATGGCCACCGGTGAGATCATACCGGAAGAACGTGATGGTTTCCTGCCAAAA**  **ATCAAGCGGTGGCCCGGAGGCGGCGGCGTCGA**CACTGGAAACCACCGACGGCGGTGAGCAGCAC | 0 |
| ***Cbr-lin-46*** https://wormbase.org/species/c\_briggsae/gene/WBGene00031058#g--10 | |  |
| WT | R Y P P Q S K V V E  CGATATCCACCTCAATCCAAAGTTGTAGAA | wt |
| *ae38* | R Y P P W Y H P S Y P N Q T D L P S L I R G L G  CGATATCCACC**ATGGTATCACCCATCTTATCCGAATCAGACTGATCTTCCCAGCCTTATCCGCGGTCTCGGT**  L R Y S S C Q K R K N C I \*  **CTCAGATACAGTTCTTGCCAAAAGCGCAAAAACTGTATCTGACGCATATCGACGCAGAAGTGGAAGGCGAAG**  TCCAAAGTTGTAGAA | 0 |
| WT | M S S V G F  TAAACATTTACATAA*TTACAG*GTAAAACCAAGAATTGTATCAGTGGGAGTCAATCCAATGAGTTCTGTAGGATTTC | wt |
| *ae43* | TAAACATTTACATAA*TTACAG*GTAAAACCAAGAATTGT------GGGAGTCAATCCAATGAGTTCTGTAGGATTTC | gf |
| *ae44* | TAAACATTTACATAATTAC-----------------------------------------AGTTCTGTAGGATTTC | lf |
| ***Cbr-let-7*** https://www.mirbase.org/cgi-bin/mirna\_entry.pl?acc=Cbr-let-7 | |  |
| WT | ATTTTTCAGGGGATTGCAGGATGATGGCTCTACACTGGGGTACGGTGAGGTAGTAGGTTGTATAGTTTAG | wt |
| *ae47* | ATTTTTCAGGGGATTGCAGGATGATGGCTCTACACTGGGGTGAGGT**GGTTGTATAGTTTAGAATATTACTCTCGGT**  **AGTTTAGAATATTACTCTCGG**AGTAGGTTGTATAGTTTAG | 0 |
| *ae48* | ATTTTTCAGGG-------------------------------------------------TATAGTTTAG | 0 |
| *ae50* | ATTTTTCAGGGGATTGCAGGATG--------------------------------------TATAGTTTA | 0 |
| ***Cbr-mir-48*** https://www.mirbase.org/cgi-bin/mirna\_entry.pl?acc=Cbr-mir-48 | |  |
| WT | CATTCGGGATGTTGAGGTAGGCTCAGTAGATGCGAGGAGATCCACCATTCCTCACATCGTCTGTCCTAACTCG | wt |
| *ae65* | GTTTTTCGATATCTCACATAGAAATAGAG< 2180 bp del. >ATTCCTCACATCGTCTGTCCTAACTCG | 0 |
| ***Cbr-mir-84*** https://www.mirbase.org/cgi-bin/mirna\_entry.pl?acc=Cbr-mir-84 | |  |
| WT | TTTTGAACAGCCGAGGAAGTTGGAATGATGTTGACTTTTCAGTTACGTCGACAGCATTGCAAACTACCTCAGA | wt |
| *ae68* | TTTTGAACAGCCGAG----TTGGAATGATGTTGACTTTTCAGTTACGTCGACAGCATTGCAAACTACCTCAGA | 0 |
| *ae69* | TTTTGAACAGCCGA---AGTTGGAATGATGTTGACTTTTCAGTTACGTCGACAGCATTGCAAACTACCTCAGA | 0 |
| *ae70* | TTTTGAACAGCCGAGGAA-------TGATGTTGACTTTTCAGTTACGTCGACAGCATTGCAAACTACCTCAGA | 0 |
| ***Cbr-mir-241*** https://www.mirbase.org/cgi-bin/mirna\_entry.pl?acc=Cbr-mir-241 | |  |
| WT | TCTTTTGTACCTCTTCCGCAAGACGGTGTCAAAGCTGAGGTAGGTGTGAGAAATGACGAAAGGCTCTTAATCG | wt |
| *ae64* | TCTTTTGTACCTCTTCCGCA**TCT**-----------------T**CC**GTGTGAGAAATGACGAAAGGCTCTTAATCG | 0 |
| ***Cbr-mir-241 Cbr-mir-48*** | |  |
| *ae73* | AAATGCACGTATAGGATGGGCTTCT< 12,897 bp del.>CGGGTTGGGACACAAACAACTCTTT | 0 |
| ***Cbr-lin-28::AID*** | |  |
| *aeIs13* | E T E T A D K A G K M P K D P A K P P A K A Q V V  GAGACTGAAACTGC**C**GA**T**AA**A**GCTGGAAAA**ATGCCTAAAGATCCAGCCAAACCTCCGGCCAAGGCACAAGTTGT**  G W P P V R S Y R K N V M V S C Q K S S G G P E  **GGGATGGCCACCGGTGAGATCATACCGGAAGAACGTGATGGTTTCCTGCCAAAAATCAAGCGGTGGCCCGGAGG**  A A A F V K \*  **CGGCGGCGTTCGTGAAGTAG**acaaggctggaaaaTAG | AID |
| ***Cbr-hbl-1::AID*** | |  |
| *aeIs12* | A L H M Y Q A K H Q M P K D P A K P P A K A Q V V  GCTCTCCACATGTA**T**CA**G**GC**T**AA**G**CATCAG**ATGCCTAAAGATCCAGCCAAACCTCCGGCCAAGGCACAAGTTGT**  G W P P V R S Y R K N V M V S C Q K S S G G P E  **GGGATGGCCACCGGTGAGATCATACCGGAAGAACGTGATGGTTTCCTGCCAAAAATCAAGCGGTGGCCCGGAGG**  A A A F V K \*  **CGGCGGCGTTCGTGAAGTAG**taccaagcgaaacatcagTGA | AID |

#### **Table S1: Genetic lesions (continued)**

|  |  |  |
| --- | --- | --- |
| ***Cbr-lin-41::AID*** | |  |
| *aeIs14* | M T T M P K D P A K P P A K A Q V V  aaaaaagaagacgtaaagaggATGACCACC**ATGCCTAAAGATCCAGCCAAACCTCCGGCCAAGGCACAAGTTGT**  G W P P V R S Y R K N V M V S C Q K S S G G P E  **GGGATGGCCACCGGTGAGATCATACCGGAAGAACGTGATGGTTTCCTGCCAAAAATCAAGCGGTGGCCCGGAGG**  A A A F V K T T S T A T  **CGGCGGCGTTCGTGAAG**ACCACGAGTACGGCAACG | AID |

Dashes, deleted sequence. Bold, insertion/substitution. Underline, mature miRNA. Italics, trans splice site. Angle brackets, large deletion. Lowercase, duplicated sequence at AID insertion site. Allele type: 0, null; (0), likely null but possibly strong loss-of-function; lf, loss-of-function (not null); gf, gain-of-function; wt, wildtype.

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#### **Table S2: adult alae in *Cbr-lin-4(0)* and *Cbr-lin-14(gf)* strains.**

|  |  |  |  |
| --- | --- | --- | --- |
| Alae completeness | *Cbr-lin-4(ae55)*  young adults  n = 12 | *Cbr-lin-4(ae55)*  egg-producing adults  n = 18 | *Cbr-lin-14(ae58) 3'UTR Δ*  egg-producing adults  n = 21 |
| Full | 8% | 17% |  |
| Gaps | 8% | 78% |  |
| Half | 17% | 6% |  |
| Patches | 50% |  | 48% |
| No alae | 17% |  | 52% |

Alae gaps mean that more than 50%, but less than 100% of seam cells generated adult alae, alae patches - more than 0% but less than 50%, half means that approximately 50% of seam cells generated adult alae.

#### **Table S3: Intestinal nuclei number in strains expressing *TIR1(F79G)*.**

|  |  |
| --- | --- |
| Genotype | Average number of intestinal nuclei at L3 and older |
| Wild type | 33.1+/-1.8 (n = 60) |
| *TIR1(F79G) II* without auxin | 25.7+/-3.3 (n = 17) |
| *TIR1(F79G) II*; *Cbr-hbl-1::AID* on 5-Ph-IAA | 22.6+/-2.5 (n = 10) |
| *TIR1(F79G) II*; 3let-7s, *Cbr-hbl-1::AID* on 5-Ph-IAA | 25.7+/-2.7 (n = 15) |

## Supplementary figures and legends

#### **Fig. S1. Seam cell lineages of *C. briggsae* are identical to those of *C. elegans*.** (A) DIC microphotograph of an L1 worm less than 3 hours after hatching. Ten seam cells are outlined with the dotted line. (B) Cell lineage diagram summarizing the divisions of V1-V4 and V6 seam cells during the four larval stages. Vertical lines indicate the passage of time, and horizontal lines indicate a cell division, with those that stop indicating terminal differentiation, either by joining the hypodermal syncytium, or by differentiation into adult seam cells that produce alae (gray square with horizontal lines). The red square highlights symmetric cell divisions that each produce two daughters that remain at the seam and continue dividing. This cell lineage is identical for *C. elegans* and *C. briggsae*.

#### **Fig. S2. Intestinal nuclei divide after the L1 stage in *C. elegans* and *C. briggsae*.** Division in *C. briggsae* occurs after the L2 stage, but not in *C. elegans*. “Early L1” refers to animals before the L1 seam cell divisions. “L1 – early L2” refers to animals during and after the L1 seam cells divisions but before the L2 (symmetric) seam cell divisions. “L2” refers to animals both during and after the L2 seam cell divisions. Statistical analysis is described in Materials and Methods.

#### **Fig. S3. *Cbr-lin-4(0)* mutants have a reiterative phenotype.** DIC micrographs showing (A) the vulval region in a *Cbr-lin-4(ae54)* L4 animal compared to a wild-type L4 animal. The vulval precursor cells failed to divide. Asterisk indicates the vulva area, and above the asterisk is the lysed anchor cell. (B) *Cbr-lin-4(ae54)* adult where the vulva failed to form resulting in the accumulation of embryos in the uterus. Arrowhead points at the vulva area. (C) Gapped alae in an adult *Cbr-lin-4(ae54)* animal; arrow points at the alae gap.

#### **Fig. S4. *Cbr-lin-4(0)* mutants reiterate L2 seam cell fates**. DIC microphotograph of *Cbr-lin-4(ae54)* L3 larva with an increased number of seam cells (elongated cells located at the midline).

#### **Fig. S5. *Cbr-lin-4(0)* mutants can become dauers but incompletely.** DIC microphotograph of the central body of a *Cbr-lin-4(ae54)* dauer larva. Part of its body is swollen and lacks the dauer cuticle.

#### **Fig. S6. Genomic locations of genetic lesions listed in Table S1**. Black rectangles, coding exons. White rectangles, non-coding regions. Arrows indicate lesions listed in Table S1. The bars for *Cbr-lin-14* alleles *ae58* and *ae59* show the extent of the deletions. Lesions and modifications not shown were at either the 5’ or 3’ end of the coding regions, as indicated in Table S1 and described in the text.

#### **Fig. S7. The overall morphology of *Cbr-lin-14(0)* mutants is almost identical to that of *Cel-lin-14(lf)* mutants.** DIC microphotographs of *Cbr-lin-14(ae62)* mutant and *Cel-lin-14::AID* mutants on 5-Ph-IAA (20°C).

#### **Fig. S8. The number of seam cells in *Cbr-lin-14(0)* mutants is slightly reduced compared to the wild type.** Seam cells were counted in L4 animals for two *Cbr-lin-14(0)* alleles, *ae62* and *ae63*. A small reduction in the seam cell number might be a result of skipping of the L2 divisions by some seam cells. Statistical analysis is described in Materials and Methods.

#### **Fig. S9. Gonad disorganization manifests with age and is slightly suppressed by *Cbr-lin-46(0)* in *Cbr-lin-28(0)* mutants*.***(A) Gonad disorganization in *Cbr-lin-28(0)* mutants is partially suppressed by the dauer pathway, although *p-value* was > 0.05 between post-dauers and normal animals at 20°C. *Cbr-lin-46(0)* mutation partly suppressed *Cbr-lin-28(0)* gonad disorganization, *p* < 0.005 when the data from all temperatures was combined in one data set in each strain. (B) Gonad disorganization manifests with age. In both *Cbr-lin-28(0)* and *Cbr-lin-28(0)*; *Cbr-lin-46(0)* double mutants, gonad disorganization was observed in few animals within 24 hours after they reached the adult or arrested L4 stage, 2 days later a higher fraction of animals had disorganized gonads (20°C). Animals’ age after hatching is indicated below the chart. Sample sizes are specified in parenthesis above the bars. Statistical analysis is described in Materials and Methods.

#### **Fig. S10. *Cbr-lin-28::GFP* transgenes.** Top: An approximately 5.5 kb genomic sequence containing the *Cbr-lin-28* coding region and 5’ and 3’ regulatory regions was cloned into a plasmid. A nematode-optimized GFP was fused in-frame upstream of the stop codon (green). Arrows show locations of mutations in CSD and CCHC domains that inactivate the protein. Gray box above the 3’UTR shows the extent of the deletion that removes the miRNA sites. Middle: A schematic of the CBR-LIN-28 protein showing the locations of inactivating mutations in the CSD domain and CCHC motifs. Sequences of wild type and mutant transgenes are shown. Bottom: A schematic of the *Cbr-lin-28* 3’UTR showing the location of the recognition sites for let-7 and lin-4 miRNAs (miR). Grey bar shows the extent of the deletion removing the miRNA sites. The sequences flanking the deletion are shown.

#### **Fig. S11 *Cbr-lin-46(0)* mutants have slight vulval developmental defects.** A DIC microphotograph of a *Cbr-lin-46(ae44)* animal with vulval protrusions (20°C) compared to vulva of a wild-type animal.

#### **Fig. S12. *Cbr-lin-28(0); Cbr-lin-46(0)* sometimes arrest at L4.** DIC microphotographs showing (A) a successfully molted *Cbr-lin-28(ae39); Cbr-lin-46(ae38)* animal with adult vulva and eggs; (B) a *Cbr-lin-28(ae39); Cbr-lin-46(ae38)* animal with an arrested L4 phenotype. Arrowhead points at the L4-shaped vulva and asterisks indicate embryos.

#### **Fig. S13. A deletion in *Cbr-lin-46*’s5'UTR causes a precocious phenotype.** (A) The 5’UTR of *C. elegans* and *C. briggsae lin-46* and the *Cbr-lin-46(ae43)* allele. The gray box highlights the conserved 36-nt 5’UTR, with a splice acceptor for a trans-spliced leader to the left and the start of the ORF to the right. (B) DIC microphotograph of *Cbr-lin-46* 5'UTRdeletion mutants, showing protruding vulvae and accumulated eggs. (C) DIC microphotograph of a cuticle of *Cbr-lin-46(ae43)* L4 larva with precocious alae (outlined by a dotted line). (D) *lin-46* 5'UTRdeletion mutants do not skip L2 stages (20°C).

#### **Fig. S14. *Cbr-mir-241(0)*, *Cbr-mir-84(0)*, single and double mutants do not have reiterations of L2 stages** at (A) 20°C or (B) 15°C. There was no statistically significant difference between WT and any other group at both temperatures (*p-value* > 0.05). Statistical analysis is described in Materials and Methods.

**Fig. S15. *Cbr-mir-241(0)*; *Cbr-mir-84(0)* double mutants occasionally develop egg-laying defects.** DIC microphotographs showing (A) a *Cbr-mir-241(ae64); Cbr-mir-84(ae70)* double mutant with accumulated eggs; other *Cbr-mir-241(0), Cbr-mir-84(0)* single and double mutants had similar occasional phenotypes; (B) slightly abnormal vulvae shapes of the *Cbr-mir-241(ae64);Cbr-mir-84(ae70)* double mutants; other *Cbr-mir-241(0), Cbr-mir-84(0)* single and double mutants occasionally had similar abnormal vulvae. Compare to Fig. S11.

**Fig. S16. Simultaneous depletion of *Cbr-lin-28* and *Cbr-hbl-1* leads to gonad migration defects.** DIC microphotographs showing (A) gonad arms in a *Cbr-hbl-1::AID; Cbr-lin-28::AID* late L3 worm on 5-Ph-IAA; gonad arms are extended towards the ends instead of reflexing compared to (B) wild-type early L4 animal with normal gonad reflexion at anterior end. Also, compare to Fig. 2A. Animals are oriented anterior end left, dorsal side up.

#### **Fig. S17. *Cbr-let-7(0)* mutants have egg-laying defects.** DIC micrographs of (A) a *Cbr-let-7(ae48)* mutant with accumulated eggs. (B) Vulva of *Cbr-let-7(ae47)*. Compare to Fig. S11. Mutant vulvae protrude more. Animals are oriented anterior end left, dorsal side up.

#### **Fig. S18. *Cbr-let-7(0)* mutants have thinner alae than the wild type.** DIC micrographs of (left) Wild type *C. briggsae* adult alae, and (right) *Cbr-let-7(ae47)* adult alae in animals of approximately the same age. In *Cbr-let-7(0)* mutants adult alae appear to be thinner. Animals are oriented anterior end left, dorsal side up.

#### **Fig. S19. *Cbr-let-7(0)* mutants have extra molts.** DIC micrograph of an adult *Cbr-let-7(ae47)* worm with cuticle coming off at the anterior end, indicated by the arrowhead.

#### **Fig. S20. Seam cell number in *Cbr-let-7(0)* mutants is similar to wild type.** Although there is an increase in the seam cell number in some animals at lower temperatures, we cannot conclude that this is due to reiteration of L2 cell fates as opposed to other reasons. Statistical analysis is described in Materials and Methods.

#### **Fig. S21. *Cbr-lin-28(0); Cbr-let-7(0)* double mutants have extra molts.** DIC micrograph showing an *Cbr-lin-28(ae39); Cbr-let-7(ae47)* adult with a partly shed cuticle. Incomplete extra molts were common for this strain.

#### **Fig. S22.** **DIC micrograph of a typical *Cbr-lin-29(ae75)* adult.** The abnormal vulva is typical for this mutant, but the tail is not.

#### **Fig. S23. DIC micrograph showing a typical vulva of adult *Cbr-lin-28(ae39); Cbr-lin-29(ae75)* animals.** The defect is similar to *Cbr-lin-28(ae39)* single mutant. Anterior end is to the left.

#### **Fig. S24. The heterochronic pathway of *C. elegans*.** The scheme shows main components of the heterochronic pathway of *C. elegans* and their regulatory relationships. Bars indicate documented inhibitory activities. Blue boxes indicate the three regulatory modules. Arrows indicate the larval stages that the modules promote.