**Supplementary File 1**

**Table S1**

**Viability and fertility of representative transgenic worm strains**

|  |  |  |  |
| --- | --- | --- | --- |
| Strains | Eggs laid (±SD)  (6 Hermaphrodites) | Egg viability (±SD) (%) | Male progeny (±SD) (%) |
| WT | 279.83 (±35.61) | 107.62 (±5.47) | 0.16 (±0.17) |
| *zhp-1::AID::3xFLAG; GFP::cosa-1; Psun-1::TIR1* | 250.5 (±30.43) | 113.80 (±3.58) | 0.12 (±0.30) |
| *zhp-2::AID::3xFLAG; GFP::cosa-1; Psun-1::TIR1* | 270.00 (±18.25) | 110.58 (±3.27) | 0.11 (±0.17) |
| *zhp-3::AID::3xFLAG; GFP::cosa-1; Psun-1::TIR1* | 263.83 (±20.62) | 108.52 (±2.92) | 0.06 (±0.14) |
| *GFP::cosa-1; Psun-1::TIR1; zhp-4::AID::3xFLAG* | 260.33 (±16.84) | 109.12 (±2.69) | 0.34 (±0.35) |
| *zhp-2::AID::3xFLAG; GFP::cosa-1; Psun-1::TIR1, spo-11::AID::3xFLAG* | 277.67 (±21.90) | 110.47 (±3.21) | 0.11 (±0.17) |
| *plk-2::HA, zhp-2::AID::3xFLAG, zhp-3::V5; dsb-2::AID::3xFLAG, GFP::cosa-1; Psun-1::TIR1* | 270.17 (±21.10) | 108.33 (±5.31) | 0.29 (±0.35) |
| *plk-2::AID::3xFLAG, zhp-2::HA; GFP::cosa-1; Psun-1::TIR1* | 255.00 (±12.13) | 111.28 (±2.10) | 0.30 (±0.36) |

Note: viability of >100% is a consequence of failing to count some embryos.

**Table S2**

**Alleles generated in this study**

|  |  |  |
| --- | --- | --- |
| Allele | Genotype | Information about mutagenesis |
| *ie43* | *zhp-1(ie43)/hT2*; stop codon + frameshift + Xho1 after aa6; sequence inserted is 5’-TAAGCTCGAG-3’ | generated using *dpy-10* Co-CRISPR, balanced with *hT2 [bli-4(e937) let-?(q782) qIs48] (I; III),* #2 |
| *ie44* | *zhp-1(ie44)/hT2*; stop codon + frameshift + Xho1 after aa6; sequence inserted is 5’-TAAGCTCGAG-3’ | generated using *dpy-10* Co-CRISPR, balanced with *hT2 [bli-4(e937) let-?(q782) qIs48] (I; III)*, #4 |
| *ie45* | *zhp-2(ie45)/hT2*; stop codon + frameshift after aa7; nucleotide c22 was replaced by 5’-TAATAATTAATTAG-3’ | generated using *dpy-10* Co-CRISPR, balanced with *hT2 [bli-4(e937) let-?(q782) qIs48] (I; III)*, #18 |
| *ie46* | *zhp-1(ie46)*; stop codon + frameshift + Xho1 after aa6; sequence inserted is 5’-TAAGCTCGAG-3’; in Hawaii CB4586 background | generated using *dpy-10* Co-CRISPR in Hawaii CB4586. #1 |
| *ie51* | *htp-3(ie51)* mutant | generated using *dpy-10* Co-CRISPR in *zhp-1(ie62[zhp-1::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie52* | *htp-3(ie52)* mutant | generated using *dpy-10* Co-CRISPR in *zhp-2(ie67[zhp-2::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie58* | *dsb-2(ie58[dsb-2::AID::3xFLAG])* | generated using *dpy-10* Co-CRISPR in *zhp-2(ie67[zhp-2::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie59* | *spo-11(ie59[spo-11::AID::3xFLAG])* | generated using *dpy-10* Co-CRISPR in *zhp-1(ie62[zhp-1::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie60* | *spo-11(ie60[spo-11::AID::3xFLAG])* | generated using *dpy-10* Co-CRISPR in *zhp-2(ie67[zhp-2::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie61* | *zhp-1(ie61[zhp-1::AID::3xFLAG])* | generated using *dpy-10* Co-CRISPR in *ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie62* | *zhp-1(ie62[zhp-1::AID::3xFLAG])* | generated using *dpy-10* Co-CRISPR in *meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie63* | *zhp-2(ie63[zhp-2::HA])* | generated using *dpy-10* Co-CRISPR in *zhp-1(ie61[zhp-1::AID::3xFLAG]) I; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie64* | *zhp-3(ie64[zhp-3::V5])* | generated using *dpy-10* Co-CRISPR in *zhp-1(ie61[zhp-1::AID::3xFLAG]) I; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie65* | *zhp-4(ie65[zhp-4::HA])* | generated using *dpy-10* Co-CRISPR in *zhp-1(ie61[zhp-1::AID::3xFLAG]) I; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie66* | *zhp-2(ie66[zhp-2::AID::3xFLAG])* | generated using *dpy-10* Co-CRISPR in *ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie67* | *zhp-2(ie67[zhp-2::AID::3xFLAG])* | generated using *dpy-10* Co-CRISPR in *meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie68* | *zhp-1(ie68[zhp-1::V5])* | generated using *dpy-10* Co-CRISPR in *zhp-2(ie66[zhp-2::AID::3xFLAG]) I; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie69* | *zhp-3(ie69[zhp-3::V5])* | generated using *dpy-10* Co-CRISPR in *zhp-2(ie66[zhp-2::AID::3xFLAG]) I; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie70* | *zhp-4(ie70[zhp-4::HA])* | generated using *dpy-10* Co-CRISPR in *zhp-2(ie66[zhp-2::AID::3xFLAG]) I; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie72* | *zhp-2(ie72[zhp-2::HA])* | generated using Cas9 in *syp-1(me17) V/nT1[unc-?(n754) let-? qIs50] (IV;V)* |
| *ie73* | *zhp-2(ie73[zhp-2::HA])* | generated using *dpy-10* Co-CRISPR in *spo-11(me44)/ieT1* |
| *ie74* | *zhp-2(ie74[zhp-2::HA])* | generated using CRISPR/Cas9 in *msh-5(me23) IV/nT1[unc-?(n754) let-?] (IV;V)* |
| *ie75* | *zhp-2(ie75[zhp-2::HA])* | generated using CRISPR/Cas9 in *cosa-1(we12) III/qC1 III* |
| *ie76* | *zhp-3(ie76[zhp-3::AID::3xFLAG])* | generated using *dpy-10* Co-CRISPR in *ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie77* | *zhp-3(ie77[zhp-3::AID::3xFLAG])* | generated using *dpy-10* Co-CRISPR in *meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie78* | *zhp-1(ie78[zhp-1::V5])* | generated using *dpy-10* Co-CRISPR in *zhp-3(ie76[zhp-3::AID::3xFLAG]) I; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie79* | *zhp-2(ie79[zhp-2::HA])* | generated using *dpy-10* Co-CRISPR in *zhp-3(ie76[zhp-3::AID::3xFLAG]) I; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie80* | *zhp-3(ie80[zhp-3::AID::GFP])* | generated using *dpy-10* Co-CRISPR in *ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie81* | *zhp-4(ie81[zhp-4::HA])* | generated using *dpy-10* Co-CRISPR in *zhp-3(ie80[zhp-3::AID::GFP]) I; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie82* | *zhp-4(ie82[zhp-4::AID::3xFLAG])* | generated using *dpy-10* Co-CRISPR in *ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie83* | *zhp-1(ie83[zhp-1::V5])* | generated using *dpy-10* Co-CRISPR in *ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV; zhp-4(ie82[zhp-4::AID::3xFLAG]) V* |
| *ie84* | *zhp-2(ie84[zhp-2::HA])* | generated using *dpy-10* Co-CRISPR in *ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV; zhp-4(ie82[zhp-4::AID::3xFLAG]) V* |
| *ie85* | *zhp-3(ie85[zhp-3::V5])* | generated using *dpy-10* Co-CRISPR in *ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV; zhp-4(ie82[zhp-4::AID::3xFLAG]) V* |
| *ie86* | *zhp-4(ie86[zhp-4::HA])* | generated using *dpy-10* Co-CRISPR in *zhp-3(jf61)/hT2 [bli-4(e937) let-?(q782) qIs48] (I; III)* |
| *ie87* | *zhp-4(ie87[zhp-4::HA])* | generated using CRISPR/Cas9 in *syp-1(me17) V/nT1[unc-?(n754) let-? qIs50] (IV;V)* |
| *ie88* | *zhp-4(ie88[zhp-4::HA])* | generated using *dpy-10* Co-CRISPR in *spo-11(me44)/ieT1* |
| *ie89* | *zhp-4(ie89[zhp-4::HA])* | generated using CRISPR/Cas9 in *msh-5(me23) IV/nT1[unc-?(n754) let-?] (IV;V)* |
| *ie90* | *zhp-4(ie90[zhp-4::HA])* | generated using CRISPR/Cas9 in *cosa-1(we12) III/qC1 III* |
| *ie92* | *zhp-3(ie92[zhp-3::V5])* | generated using *dpy-10* Co-CRISPR |
| *ie103* | *zhp-3(ie103[zhp-3::V5])* | generated using *dpy-10* Co-CRISPR in *dsb-2(ie58[dsb-2::AID::3xFLAG]), meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie104* | *zhp-3(ie104[zhp-3::V5])* | generated using *dpy-10* Co-CRISPR in *zhp-2(ie67[zhp-2::AID::3xFLAG]) I; dsb-2(ie58[dsb-2::AID::3xFLAG]), meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie105* | *plk-2(ie105[plk-2::HA])* | generated using *dpy-10* Co-CRISPR in *zhp-3(ie103[zhp-3::V5]) I; dsb-2(ie58[dsb-2::AID::3xFLAG]), meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie106* | *plk-2(ie106[plk-2::HA])* | generated using *dpy-10* Co-CRISPR in *zhp-2(ie67[zhp-2::AID::3xFLAG]), zhp-3(ie104[zhp-3::V5]) I; dsb-2(ie58[dsb-2::AID::3xFLAG]), meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie107* | *zhp-2(ie107[zhp-2::HA])* | generated using *dpy-10* Co-CRISPR in *meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV*. (*ie107* and *ie108* were made from a single injection) |
| *ie108* | *plk-2(ie108[plk-2::AID::3xFLAG])* | generated using *dpy-10* Co-CRISPR in *meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV*. (*ie107* and *ie108* were made from a single injection) |
| *ie109* | *plk-2(ie109[plk-2::HA])* | generated using *dpy-10* Co-CRISPR in *zhp-1(ie62[zhp-1::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |
| *ie110* | *plk-2(ie110[plk-2::HA])* | generated using *dpy-10* Co-CRISPR in *zhp-2(ie67[zhp-2::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* |

**Table S3**

**crRNAs, repair templates and genotyping primers for transgenes generated in this study**

|  |  |  |
| --- | --- | --- |
| Transgenes | crRNAs and repair templates | Genotyping primers and fragment sizes |
| *zhp-1* null mutant in *ie43* or *ie44* | 5’- ttcatcgtttgcaatggatg -3’; 5’-gttgttcggcatgtttcacagaagacgtgggagcaggcggtgataaaaaattgtcttttacttggcgagcatccacatccattctcgagcttagcaaacgatgaactccatagtgcctacagttctgaaataaatgcaaattgttatttttgaaccatgtttggaacg -3’ | F: 5’- gaaaattatcgaaaaactgtcacaaac -3’; R: 5’- gttttccattttcaattgaattgacttc -3’;  WT, 563bp; Mutant, 573bp=297bp+276bp (XhoI digestion) |
| *zhp-2* null mutant in *ie45* | 5’- tggatccaatgcaatcattg -3’; 5’-ctttcgtacagttttgacagaaaacgtgaccgcatgctgttaggtacagtttcgtttgatttggtttaatgccacaatgattgcctcgagttagcattggatccaatccatacgtgtttcgagtcttaaaatacgattaaataaagctcaaagacttctgctagataacaag -3’ | Nucleotide c22 was replaced by taataattaattag.  F: 5’ gactcgaaacacgtatggattg-3’;  R: 5’-ggtacagtttcgtttgatttg-3’;  WT, 72bp; Mutant, 85bp |
| *zhp-1* null mutant in *ie46* | 5’- ttcatcgtttgcaatggatg -3’; 5’- gttgttcggcatgtttcacagaagacgtgggagcaggcggtgataaaaaattgtcttttacttggcgagcatccacatccattctcgagcttagcaaacgatgaactccatagtgcctacagttctgaaataaatgcaaattgttatttttgaaccatgtttggaacg -3’ | F: 5’- gaaaattatcgaaaaactgtcacaaac -3’; R: 5’- gttttccattttcaattgaattgacttc -3’;  WT, 563bp; Mutant, 573bp=297bp+276bp (XhoI digestion) |
| *htp-3* null mutant in *ie51* or *ie52* | 5’- agtgccgaaatcggatacgg -3’ and 5’- tgttcatcgacttcaactgg -3’; 5’- gtcactaaacacgaatcccgtggaaacgtttgtctggacctttgtctatgactcaaccacatctgcaagtgccgaaatctgatgaagtcgatgaacagccagaagaacaaaatgatgatgatgctcagaattctcttcaaattgactctgatgctcagaattctcttcaaattgactctg -3’ | N/A |
| *dsb-2::AID::3xFLAG* in *ie58* | 5’- gaatcgtgttgctcaagctg -3’; 5’- gaagatgtgagaatatatttgtctaaaaacaatggaaagaatgtaattttccaggtcaccgaaccaccaataaacgatatattctttgatcgttttcatcaaccacaactcgaacagcacgattctgttccagaagatggagctggatcacctaaagatccagccaaacctccggccaaggcacaagttgtgggatggccaccggtgagatcataccggaagaacgtgatggtttcctgccaaaaatcaagcggtggcccggaggcggcggcgttcgtgaagggagccggatctgattataaagaccatgatggagactataaggatcacgatattgattacaaagacgatgatgataaatgattatttggaaaaattctaaaaatttaaacttgtatcatttgaatctgtaaattttttttcaagtatatttaag -3’ | F: 5’- ctaagcgttgaagtagccgacaaatg -3’; R: 5’- cgagctaaactggtccgcgaagag -3’;  WT, 480bp; inserted, 702bp |
| *spo-11::AID::3xFLAG* in *ie59* or *ie60* | 5’- tactcgtttataatcgtctc -3’; 5’- gacaggagtatatggctcgattcttgattgctccgagagtcatgtcgatagaaaaagaaattccgattcaaccagaaaccatcatcaacgaatacggatcacctaaagatccagccaaacctccggccaaggcacaagttgtgggatggccaccggtgagatcataccggaagaacgtgatggtttcctgccaaaaatcaagcggtggcccggaggcggcggcgttcgtgaagggagccggttctgattataaagaccatgatggagactataaggatcacgatattgattacaaagacgatgatgataaaggtagttcgacagatgatgatcgggaagcaaaagacgatgactatatagattcggatgctgaagaaaagttccagaatatgatagataatgatagtg -3’ | F: 5’- gtgacacgagaattagactggatg -3’; R: 5’- attgaggaggagcactctg -3’;  WT, 532bp; inserted, 730bp |
| *zhp-1::AID::3xFLAG* in *ie61* or *ie62* | 5’- tcgtctcaatcgaatcgtgg -3’; 5’-cgttgctggttcagatgtagataatgacgaattgctcgattatgtatgctgatatcattgatttttctgttcctaaagttgctttttcaggatcttctaggattgaggaatcgatctgattcatcatcatccaactgctcgtctcaatctaacagaggaggatctctgtttggagctggatcacctaaagatccagccaaacctccggccaaggcacaagttgtgggatggccaccggtgagatcataccggaagaacgtgatggtttcctgccaaaaatcaagcggtggcccggaggcggcggcgttcgtgaagggagccggatctgattataaagaccatgatggagactataaggatcacgatattgattacaaagacgatgatgataaataacataatctgtaatatagatgaccatagttttatttttcagctataaacataaaaatgcttgatttatcttcttcccacatatccagagcaaaacgtgacac -3’ | F: 5’- cttcggaatttgtatgtattccctgatcg -3’; R: 5’- cgtgtcgcttgatttatctagtcctag -3’;  WT, 562bp; inserted, 784bp |
| *zhp-2::HA* in *ie63, ie72, ie73, ie74, ie75, ie79, ie84* or *ie107* | 5’- gagttttattgacgaagaat -3’; 5’- gatcataggaatagattgtttggcgcaggacttgagcatccgtcgccaattcttcgtcaaggagccggatcttacccctacgatgtcccagattatgcttaaaactccctgtatgtcacctccgtatttttatcctttttccacaatttttatttc -3’ | F: 5’- gcaattgctgtgattgattaagtg -3’; R: 5’- ggagatattacgggggcaataaac -3’;  WT, 211bp; inserted, 250bp |
| *zhp-3::V5* in *ie64, ie69, ie85, ie92, ie103* or *ie104* | 5’- gagattaaaacattaatcgg -3’; 5’- gttttcaggatacctcgcacaaaggaaaccgatcaatggtcggagcttcattggacccgccgatggagccggatctggaaagccaattccaaacccacttcttggactcgactccacctaatgttttaatctcgtttttttctgaattcgttctttatttgttgatatatacatc -3’ | F: 5’- cgaccctctcatcaactaacac -3’; R: 5’- tttcagcactcttcgggtc -3’;  WT, 434bp; inserted, 488bp |
| *zhp-4::HA* in *ie65, ie70, ie81, ie86, ie87, ie88, ie89* or *ie90* | 5’- gagaaaagcacaaggagcat -3’; 5’- ctctgcaatattttacatgataaaatgcgacacatatattaaaaaaaatcgattcaagcataatctgggacatcgtaggggtatgatccagctccttgactattacgatgctccttgtgcttttctctttgagcttcatgttttttcaagtacgggaac -3’ | F: 5’- ggaaatttatcgattttttcggaaattgattg -3’; R: 5’- catgataaaatgcgacacatatattaaa -3’;  WT, 236bp; inserted, 275bp |
| *zhp-2::AID::3xFLAG* in *ie66* or *ie67* | 5’- gagttttattgacgaagaat -3’; 5’- gcaattgctgtgattgattaagtgtaattttttccagatcgattatagtcgccgtgatcataggaatagattgtttggcgcaggacttgagcatccgtcgccaattcttcgtcaaggagctggatcacctaaagatccagccaaacctccggccaaggcacaagttgtgggatggccaccggtgagatcataccggaagaacgtgatggtttcctgccaaaaatcaagcggtggcccggaggcggcggcgttcgtgaagggagccggatctgattataaagaccatgatggagactataaggatcacgatattgattacaaagacgatgatgataaataaaactccctgtatgtcacctccgtatttttatcctttttccacaatttttatttctattcaaaatcaaaggtttattgcccccgtaatatctcc -3’ | F: 5’- ctccaatgttagttttatctcctgttttc -3’; R: 5’- cgtcgcgttttgtaccataaaacaatc -3’;  WT, 476bp; inserted, 698bp |
| *zhp-1::V5* in *ie68, ie78* or *ie83* | 5’- tcgtctcaatcgaatcgtgg -3’; 5’- catttttatgtttatagctgaaaaataaaactatggtcatctatattacagattatgttaggtggagtcgagtccaagaagtgggtttggaattggctttcctgatccagctccaaacagagatcctcctctgttagattgagacgagcagttggatgatgatgaatcagatcgattcctcaatcctagaagatcc -3’ | F: 5’- cgttgctggttcagatgtagataatg -3’; R: 5’- gtgtcacgttttgctctggatatgtg -3’;  WT, 275bp; inserted, 329bp |
| *zhp-3::AID::3xFLAG* in *ie76* or *ie77* | 5’- gagattaaaacattaatcgg -3’; 5’- gtgtactctgacgttttcaggatacctcgcacaaaggaaaccgatcaatggtcggagcttcattggacccgccgatggagctggatcacctaaagatccagccaaacctccggccaaggcacaagttgtgggatggccaccggtgagatcataccggaagaacgtgatggtttcctgccaaaaatcaagcggtggcccggaggcggcggcgttcgtgaagggagccggatctgattataaagaccatgatggagactataaggatcacgatattgattacaaagacgatgatgataaataatgttttaatctcgtttttttctgaattcgttctttatttgttgatatatacatcatgtacaattcaactttaatg -3’ | F: 5’- cgaccctctcatcaactaacac -3’; R: 5’- tttcagcactcttcgggtc -3’;  WT, 434bp; inserted, 656bp |
| *zhp-3::AID::GFP* in *ie80* | 5’- gagattaaaacattaatcgg-3’; 5’- aactaacacacttacacacttttttcgtctctcactctctcattccgggcgctttttgcgcctttccgctccctctatattccaattctttcgatttttgccacgtgtactctgacgttttcaggatacctcgcacaaaggaaaccgatcaatggtcggagcttcattggacccgccgatggaggatctggaggcggttctggcggaggttctggtcctaaagatccagccaaacctccggccaaggcacaagttgtgggatggccaccggtgagatcataccggaagaacgtgatggtttcctgccaaaaatcaagcggtggcccggaggcggcggcgttcgtgaaggtttctaaaggagaagaacttttcacaggagttgttccaatacttgttgaacttgatggagatgtaaatggacataagttctctgtttctggcgaaggagaaggagatgctacttatggaaaacttactctcaagttcatttgcactactggaaaacttcccgttccatggccaactcttgttactactttgacttatggagttcaatgtttcgctcgttatccagaccatatgaaacaacacgatttcttcaaatctgctatgccagaaggatatgttcaagaaagaactattttcttcaaagatgatggaaactacaaaactcgtgcggaagtaaagtttgagggagatactcttgtaaatagaatagaactcaaaggaatagatttcaaagaagatggaaatatacttggacataaacttgaatataactacaatagtcataaagtttatattactgctgataaacaaaaaaatggaataaaagtaaacttcaaaactcgtcataatatagaagatggaagtgttcaacttgctgaccattaccaacaaaatactccaataggagatggcccagttcttctcccagataatcattatctttctactcaatctgctctttctaaagacccaaatgaaaaaagagaccatatggttcttttagagttcgttactgcggcgggaataactcttggaatggatgaactttacaaataatgttttaatctcgtttttttctgaattcgttctttatttgttgatatatacatcatgtacaattcaactttaatgtttatttcataaaaattgggttaaaaattccaaatttctttttctctacctagttcacatctgatttaaattttatttgaatatttcg -3’ | F: 5’- cgaccctctcatcaactaacac -3’; R: 5’- cacgttcttccggtatgatctc -3’;  WT, no product; inserted, 307bp.  or  F: 5’- cgaccctctcatcaactaacac -3’; R2: 5’- tttcagcactcttcgggtc -3’  WT, 434bp; inserted, 1316bp |
| *zhp-4::AID::3xFLAG* in *ie82* | 5’- gagaaaagcacaaggagcat -3’; 5’- gttcccgtacttgaaaaaacatgaagctcaaagagaaaagcacaaggagcatcgtaatagtcaaggagctggatcacctaaagatccagccaaacctccggccaaggcacaagttgtgggatggccaccggtgagatcataccggaagaacgtgatggtttcctgccaaaaatcaagcggtggcccggaggcggcggcgttcgtgaagggagccggatctgattataaagaccatgatggagactataaggatcacgatattgattacaaagacgatgatgataaatgaatcgattttttttaatatatgtgtcgcattttatcatg -3’ | F: 5’- ggaaatttatcgattttttcggaaattgattg -3’; R: 5’- ctaaaactgtactttcaggtcataaatgtatc -3’;  WT, 382bp; inserted, 604bp |
| *plk-2::HA* in *ie105, ie106, ie109* or *ie110* | 5’- tcgattttcttagcgacgcg -3’; 5’-caacgtccgtttggaatctgcagcagatatccagccggcttatccatcatcctcgcgtcgcggagccggatcttacccctacgatgtcccagattatgcttaagaaaatcgatctgcaacaaattgagctcatttccccttaccggttttg -3’ | F: 5’- ggagaagttcctccatcgaattc -3’; R: 5’- ggataaaattgattacaaacacggac -3’;  WT, 294bp; inserted, 333bp |
| *plk-2::AID::3xFLAG* in *ie108* | 5’- tcgattttcttagcgacgcg -3’; 5’-gaaagaggagacgaaacacaatgcaccggcggccaatgcagtacgccttccatcgacttccagcaacgtccgtttggaatctgcagcagatatccagccggcttatccatcatcctcgcgtcgcggagctggatcacctaaagatccagccaaacctccggccaaggcacaagttgtgggatggccaccggtgagatcataccggaagaacgtgatggtttcctgccaaaaatcaagcggtggcccggaggcggcggcgttcgtgaagggagccggatctgattataaagaccatgatggagactataaggatcacgatattgattacaaagacgatgatgataaataagaaaatcgatctgcaacaaattgagctcatttccccttaccggttttgatatttctctgatcaatacacttttatgtccgtgtttgtaatcaattttatcc -3’ | F: 5’- ggagaagttcctccatcgaattc -3’; R: 5’- gagcatgatgacacccgaatgtttg -3’;  WT, 451bp; inserted, 673bp |

**Table S4**

**Strains used in this study**

|  |  |  |
| --- | --- | --- |
| Strains | Source | Identifier |
| *C. elegans:* Hawaiian wild-type isolate | Caenorhabditis Genetics Center | CB4856 |
| *C. elegans: syp-1 (me17) V/nT1[unc-?(n754) let-? qIs50] (IV;V)* | MacQueen et al., 2002; Caenorhabditis Genetics Center | AV307/CA30 |
| *C. elegans: spo-11(me44) IV/ieT1* | Chan et al., 2004; Hayashi et al., 2007; this paper | AV157 |
| *C. elegans: dsb-2(tm6047) II* | This paper; 421 bp deletion  null allele mutant; [Japanese KO consortium](http://shigen.nig.ac.jp/c.elegans/mutants/DetailsSearch?lang=english&seq=6047" \t "_blank) | CA1153 |
| *C. elegans: zhp-3(jf61) I/hT2 [bli-4(e937) let-?(q782) qIs48] (I; III)* | Jantsch et al., 2004; Bhalla et al., 2008 | UV1/CA685 |
| *C. elegans: msh-5(me23) IV/nT1 [unc-?(n754) let-?] (IV;V)* | Kelly et al., 2000; Caenorhabditis Genetics Center | AV115 |
| *C. elegans: meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II* | Yokoo et al., 2012; Caenorhabditis Genetics Center | AV630 |
| *C. elegans: ieSi38[sun-1p::TIR1::mRuby::sun-1 3’ UTR, Cbr-unc-119 (+)] IV* | Zhang et al., 2015; Caenorhabditis Genetics Center | CA1199 |
| *C. elegans: zhp-1(ie43) I/ hT2 [bli-4(e937) let-?(q782) qIs48] (I; III)* | This paper | CA1356 |
| *C. elegans: zhp-1(ie44) I/ hT2 [bli-4(e937) let-?(q782) qIs48] (I; III)* | This paper | CA1357 |
| *C. elegans: zhp-2(ie45) I/ hT2 [bli-4(e937) let-?(q782) qIs48] (I; III)* | This paper | CA1358 |
| *C. elegans: zhp-1(ie46) I in Hawaii background* | This paper | CA1359 |
| *C. elegans: meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1364 |
| *C. elegans: zhp-1(ie61[zhp-1::AID::3xFLAG]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1366 |
| *C. elegans: zhp-1(ie62[zhp-1::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1367 |
| *C. elegans: htp-3(ie51), zhp-1(ie62[zhp-1::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1368 |
| *C. elegans: zhp-1(ie62[zhp-1::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)], spo-11(ie59[spo-11::AID::3xFLAG]) IV* | This paper | CA1369 |
| *C. elegans: zhp-1(ie61[zhp-1::AID::3xFLAG]), zhp-2(ie63[zhp-2::HA]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV;* | This paper | CA1370 |
| *C. elegans: zhp-1(ie61[zhp-1::AID::3xFLAG]), zhp-3(ie64[zhp-3::V5]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV;* | This paper | CA1371 |
| *C. elegans: zhp-1(ie61[zhp-1::AID::3xFLAG]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV; zhp-4(ie65[zhp-4::HA]) V* | This paper | CA1372 |
| *C. elegans: zhp-2(ie66[zhp-2::AID::3xFLAG]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1374 |
| *C. elegans: zhp-2(ie67[zhp-2::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1375 |
| *C. elegans: htp-3(ie52), zhp-2(ie66[zhp-2::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1376 |
| *C. elegans: zhp-2(ie66[zhp-2::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)], spo-11(ie60[spo-11::AID::3xFLAG]) IV* | This paper | CA1377 |
| *C. elegans: zhp-1(ie68[zhp-1::V5]), zhp-2(ie66[zhp-2::AID::3xFLAG]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1378 |
| *C. elegans: zhp-2(ie66[zhp-2::AID::3xFLAG]), zhp-3(ie69[zhp-3::V5]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1379 |
| *C. elegans: zhp-2(ie66[zhp-2::AID::3xFLAG]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV; zhp-4(ie70[zhp-4::HA]) V* | This paper | CA1380 |
| *C. elegans: zhp-2(ie72[zhp-2::HA]) I; syp-1(me17) V/nT1[unc-?(n754) let-? qIs50] (IV;V)* | This paper | CA1383 |
| *C. elegans: zhp-2(ie73[zhp-2::HA]) I; spo-11(me44) IV/ ieT1* | This paper | CA1384 |
| *C. elegans: zhp-2(ie74[zhp-2::HA]) I; msh-5(me23) IV/nT1[unc-?(n754) let-?] (IV;V)* | This paper | CA1385 |
| *C. elegans: zhp-2(ie75[zhp-2::HA]) I; cosa-1(we12) III/qC1* | This paper | CA1386 |
| *C. elegans: zhp-3(ie76[zhp-3::AID::3xFLAG]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1387 |
| *C. elegans: zhp-3(ie77[zhp-3::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1388 |
| *C. elegans: zhp-1(ie78[zhp-1::V5]), zhp-3(ie76[zhp-3::AID::3xFLAG]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1389 |
| *C. elegans: zhp-2(ie79[zhp-2::HA]), zhp-3(ie76[zhp-3::AID::3xFLAG]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1390 |
| *C. elegans: zhp-3(ie80[zhp-3::AID::GFP]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1391 |
| *C. elegans: zhp-3(ie80[zhp-3::AID::GFP]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV; zhp-4(ie81[zhp-4::HA]) V* | This paper | CA1392 |
| *C. elegans: unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV; zhp-4(ie82[zhp-4::AID::3xFLAG]) V* | This paper | CA1393 |
| *C. elegans: meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV; zhp-4(ie82[zhp-4::AID::3xFLAG]) V* | This paper | CA1394 |
| *C. elegans: zhp-1(ie83[zhp-1::V5]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV; zhp-4(ie82[zhp-4::AID::3xFLAG]) V* | This paper | CA1395 |
| *C. elegans: zhp-2(ie84[zhp-2::HA]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV; zhp-4(ie82[zhp-4::AID::3xFLAG]) V* | This paper | CA1396 |
| *C. elegans: zhp-3(ie85[zhp-3::V5]) I; unc-119(ed3) III; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV; zhp-4(ie82[zhp-4::AID::3xFLAG]) V* | This paper | CA1397 |
| *C. elegans: zhp-3(jf61) I/ hT2 [bli-4(e937) let-?(q782) qIs48] (I; III); zhp-4(ie86[zhp-4::HA]) V* | This paper | CA1398 |
| *C. elegans: syp-1(me17) V/nT1[unc-?(n754) let-? qIs50] (IV;V); zhp-4(ie87[zhp-4::HA]) V* | This paper | CA1399 |
| *C. elegans: spo-11(me44) IV/ieT1; zhp-4(ie88[zhp-4::HA]) V* | This paper | CA1400 |
| *C. elegans: msh-5(me23) IV/nT1[unc-?(n754) let-?] (IV;V); zhp-4(ie89[zhp-4::HA]) V* | This paper | CA1401 |
| *C. elegans: cosa-1(we12) III/qC1; zhp-4(ie90[zhp-4::HA]) V* | This paper | CA1402 |
| *C. elegans: zhp-3(ie92[zhp-3::V5]) I* | This paper | CA1404 |
| *C. elegans: zhp-3(ie92[zhp-3::V5]) I; dsb-2(tm6047) II* | This paper | CA1405 |
| *C. elegans: zhp-1(ie78[zhp-1::V5]), zhp-3(ie76[zhp-3::AID::3xFLAG]) I; syp-1 (me17) V/nT1[unc-?(n754) let-? qIs50] (IV;V)* | This paper | CA1410 |
| *C. elegans: dsb-2(ie58[dsb-2::AID::3xFLAG]), meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1421 |
| *C. elegans: zhp-2(ie67[zhp-2::AID::3xFLAG]) I; dsb-2(ie58[dsb-2::AID::3xFLAG]), meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1422 |
| *C. elegans: meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)], spo-11(ie59[spo-11::AID::3xFLAG]), IV* | This paper | CA1423 |
| *C. elegans: zhp-1(ie62[zhp-1::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV; zhp-4(ie82[zhp-4::AID::3xFALG]) V* | This paper | CA1424 |
| *C. elegans: zhp-3(ie103[zhp-3::V5]) I; dsb-2(ie58[dsb-2::AID::3xFLAG]), meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1425 |
| *C. elegans: zhp-2(ie67[zhp-2::AID::3xFLAG]), zhp-3(ie104[zhp-3::V5]) I; dsb-2(ie58[dsb-2::AID::3xFLAG]), meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1426 |
| *C. elegans: plk-2(ie105[plk-2::HA]), zhp-3(ie103[zhp-3::V5]) I; dsb-2(ie58[dsb-2::AID::3xFLAG]), meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1427 |
| *C. elegans: plk-2(ie106[plk-2::HA]), zhp-2(ie67[zhp-2::AID::3xFLAG]), zhp-3(ie104[zhp-3::V5]) I; dsb-2(ie58[dsb-2::AID::3xFLAG]), meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1428 |
| *C. elegans: plk-2(ie108[plk-2::AID::3xFLAG]), zhp-2(ie107[zhp-2::HA]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1429 |
| *C. elegans: plk-2(ie109[plk-2::HA]), zhp-1(ie62[zhp-1::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1430 |
| *C. elegans: plk-2(ie110[plk-2::HA]), zhp-2(ie67[zhp-1::AID::3xFLAG]) I; meIs8[pie-1p::GFP::cosa-1, unc-119(+)] II; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR, Cbr-unc-119(+)] IV* | This paper | CA1431 |