Supplementary Materials and Methods

Constructs and generation of transgenic lines

Constructs used in this study are listed in supplementary material Table S4. Except where noted below, constructs were generated using Gibson assembly (Gibson et al., 2009), and verified by sequencing of new junction regions. All TIR1 expression constructs were derivatives of MosSCI vector pCFJ151 (Frokjaer-Jensen et al., 2008), except for P*sun-1*::*TIR1*::*mRuby*::*sun-1* 3'UTR, which was derived from pCFJ178. A codon-optimized TIR1 gene with two introns was assembled from multiple gBlocks (IDT) (supplementary material Fig. S1). This coding sequence was used to replace the *sun-1* coding sequence in pOR131 (Rog and Dernburg, 2015), a derivative of pCFJ178 containing the *sun-1* promoter and coding sequence inserted upstream of a codon-optimized mRuby gene, followed by the *sun-1* 3'UTR. In the resulting construct, TIR1 and mRuby are separated by a 3x(Gly-Gly-Ser-Gly) linker.

A plasmid encoding P*eft-3*::*degron*::*GFP*::*unc-54* 3'UTR was also derived from pCFJ151. The degron-GFP cassette was generated by fusion of a minimal degron domain derived from IAA17 (supplementary material Fig. S1) to the N-terminus of codon-optimized EmGFP (Rog and Dernburg, 2015) using PCR fusion. The *eft-3* promoter was amplified from pDD122 (Dickinson et al., 2013). The *myo-2* promoter and *unc-54* 3'UTR were amplified from pCFJ90 (Frokjaer-Jensen et al., 2008). The *ges-1* promoter, including 1999 bp upstream of the start codon, was amplified from genomic DNA (Staab et al., 2013).

MosSCI transformation was performed using protocols described in WormBuilder (Frokjaer-Jensen et al., 2008). The MosSCI insertion strains EG6700, EG8079, EG8080 or EG8081 were used for injection.

The P*smu-2*::*degron*::*smu-2*::*smu-2* 3'UTR construct was generated by insertion of the degron immediately downstream of the start codon of *smu-2* gene in pAS17 (Spartz et al., 2004). The pRF4 plasmid expressing *rol-6* (su1006), which produces a dominant Roller phenotype, was used as co-transformation marker. Extrachromosomal arrays were obtained by injecting plasmid DNA into the germ line (Mello et al., 1991).

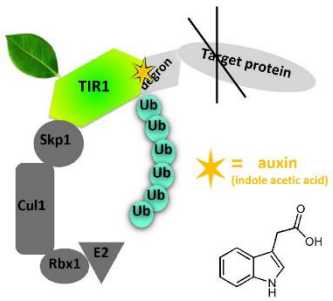
Plasmids for tagging the *dhc-1* locus by CRISPR/Cas9-mediated editing were constructed as follows: P*eft-3*::*Cas9*::*htp-3* 3'UTR was generated by removal of sgRNA expression elements

in pDD122 (Dickinson et al., 2013) and by replacement of the *tbb-2* 3'UTR with the *htp-3* 3'UTR. The *htp-3* 3'UTR was intended to restrict the expression of Cas9 to the meiotic germline (MacQueen et al., 2005; Merritt et al., 2008). P_{U6}*::sgRNA (F+E)* targeting *dhc-1* was generated by inserting the guide RNA sequence (5'-GTTCTACCAACGAGGAGTTGCAT-3') or (5'-GCGAGTGGTGAAATCAGTTGA-3') into the modified pMB70 plasmid (Waaijers et al., 2013). The sgRNA in pMB70 was optimized with an A-U flip and hairpin extension, which was shown to avoid premature termination of U6 Pol-III transcription and to promote sgRNA-Cas9 assembly (Chen et al., 2013), thereby enhancing the efficiency of genomic editing (Ward, 2015). The guide RNA sequence for tagging *dhc-1* was chosen using MIT CRISPR DESIGN tool at website http://crispr.mit.edu/. The homologous repair plasmid (pLZ50) was constructed using Gibson assembly using the following PCR amplified fragments: 2452 bp upstream of the *dhc-1* stop codon (including point mutations in each guide RNA cut site), the linker-degron-GFP cassette, 2459 bp downstream of the stop codon of *dhc-1*, and the pCFJ151 backbone. All constructs were verified by sequencing. Editing of *dhc-1* was accomplished using protocols described previously (Dickinson et al., 2013; Waaijers et al., 2013).

Editing of the *nhr-23* and *nhr-25* loci was performed using a previously described *pha-1(ts)* co-conversion protocol and validated sgRNA sequences (Ward, 2015). For *nhr-25* knock-ins, a degron-TEV-3xFLAG cassette was synthesized as a gBlock (IDT) with 62/35 bp homology arms and TOPO cloned to produce pJW1354, which was PCR amplified to produce a linear repair template with extended 85/80 bp homology arms (with respect to mutations to disrupt the PAM and the insertion site, respectively). *pha-1(ts)* animals grown on *cku-80* RNAi were injected with 50 ng/µl pJW1259 (Cas9 plasmid), 10 ng/µl of oligo 2221 (a *pha-1(ts)* repair oligo that introduces a silent *Mlu*I site for genotyping), 25 ng/µl of the degron-TEV-3xFLAG PCR product, 50 ng/µl of pJW1363 (P_{U6}*::nhr-25* sgRNA), 10 ng/µl pJW1328 (P_{U6}*::pha-1* sgRNA; AddGene plasmid #69488). For *nhr-23* knock-in experiments, a degron-TEV-3xFLAG cassette with 39/40 bp homology arms was TOPO cloned to produce pJW1355, which was PCR amplified to produce a linear repair template with 79/80 bp homology arms (with respect to mutations to disrupt the PAM and the insertion site, respectively). *pha-1(ts)* animals grown on *cku-80* RNAi were injected with 50 ng/µl of pJW1254 (Cas9 plasmid with P_{U6}*::nhr-23 sgRNA*), 50 ng/µl oligo 2221, 37.5 ng/µl of the degron-3xFLAG PCR product, and 50 ng/µl pJW1328.

Following injection of *pha-1(ts)* mutants, *pha-1(+)* progeny with the desired knock-ins were identified by PCR followed by diagnostic BamHI digestion, as in Ward, 2015.

A



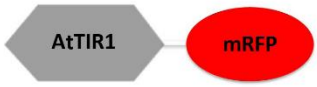
B TIR1 gene sequence

ATGCAAAAGAGAATCGCCTTGTCGTTTCCAGAAGAAGTACTCGAGCACGTCTTCTCCTTCATCCAACTCGACAAGGACCGTAACTCCGTCTCCCTCG
TCTGCAAGTCCTGGTACGAGATCGAGCGTTGGTGCCGTCGTAAGGTCTTCATCGGAAACTGCTACGCCGTCTCCCCAGCCACCGTCATCCGTCGTTT
CCCAAAGGTCCGTTCCGTCGAGCTCAAGGGAAAGCCACACTTCGCCGACTTCAACCTCGTCCCAGACGGATGGGGAGGATACGTCTACCCATGGATC
GAGGCCATGTCCTCCTCCTACACCTGGCTCGAGGAGATCCGTCTCAAGCGTATGGTCGTCACCGACGACTGCCTCGAGCTCATCGCCAAGTCCTTCA
AGAACTTCAAGGTCCTCGTCCTCTCCTCCTGCGAGGGATTCTCCACCGACGGACTCGCCGCCATCGCCGCCACCTGCCGTAACCTCAAGgtaagttt
aaacatatatatactaactaaccctgattatttaaattttcagGAGCTCGACCTCCGTGAGTCCGACGTCGACGAGGTCTCCGGACACTGGCTCTCC
CACTTCCCAGACACCTACACCTCCCTCGTCTCCCTCAACATCTCCTGCCTCGCCTCCGAGGTCTCCTTCTCCGCCCTCGAGCGTCTCGTCACCCGTT
GCCCAAACCTCAAGTCCCTCAAGCTCAACCGTGCCGTCCCACTCGAGAAGCTCGCCACCCTCCTCCAACGTGCCCCACAACTCGAGGAGCTCGGAAC
CGGAGGATACACCGCCGAGGTCCGTCCAGACGTCTACTCCGGACTCTCCGTCGCCCTCTCCGGATGCAAGgtaagtttaaacagttcggtactaact
aaccatacatatttaaatttttcagGAGCTCCGTTGCCTCTCCGGATTCTGGGACGCCGTCCCAGCCTACCTCCCAGCCGTCTACTCCGTCTGCTCCC
GTCTCACCACCCTCAACCTCTCCTACGCCACCGTCCAATCCTACGACCTCGTCAAGCTCCTCTGCCAATGCCCAAAGCTCCAACGTCTCTGGGTCCT
CGACTACATCGAGGACGCCGGACTCGAGGTCCTCGCCTCCACCTGCAAGGACCTCCGTGAGCTCCGTGTCTTCCCATCCGAGCCATTCGTCATGGAG
CCAAACGTCGCCCTCACCGAGCAAGGACTCGTCTCCGTCTCCATGGGATGCCCAAAGCTCGAGTCCGTCCTCTACTTCTGCCGTCAAATGACCAACG
CCGCCCTCATCACCATCGCCCGTAACCGTCCAAACATGACCCGTTTCCGTCTCTGCATCATCGAGCCAAAGGCCCCAGACTACCTCACCCTCGAGCC
ACTCGACATCGGATTCGGAGCCATCGTCGAGCACTGCAAGGACCTCCGTCGTCTCTCCCTCTCCGGACTCCTCACCGACAAGGTCTTCGAGTACATC
GGAACCTACGCCAAGAAGATGGAGATGCTCTCCGTCGCCTTCGCCGGAGACTCCGACCTCGGACTCCACCACGTCCTCTCCGGATGCGACTCCCTCC
GTAAGCTCGAGATCCGTGACTGCCCATTCGGAGACAAGGCCCTCCTCGCCAACGCCTCCAAGCTCGAGACCATGCGTTCCCTCTGGATGTCCTCCTG
CTCCGTCTCCTTCGGAGCCTGCAAGCTCCTCGGACAAAAGATGCCAAAGCTCAACGTCGAGGTCATCGACGAGCGTGGAGCCCCAGACTCCGTCCA
GAGTCCTGCCCAGTCGAGCGTGTCTTCATCTACCGTACCGTCGCCGGACCACGTTTCGACATGCCAGGATTCGTCTGGAACATGGACCAAGACTCCA
CCATGCGTTTCTCCCGTCAAATCATCACCACCAACGGACTC

C TIR1 protein sequence

MQKRIALSFPEEVLEHVFSFIQLDKDRNSVSLVCKSWYEIERWCRRKVFIGNCYAVSPATVIRRFPKVRSVELKGKPHFADFNLVPDGWGGYVYPWI
EAMSSSYTWLEEIRLKRMVVTDDCLELIAKSFKNFKVLVLSSCEGFSTDGLAAIAATCRNLKELDLRESDVDDVSGHWLSHFPDTYTSLVSLNISCL
ASEVSFSALERLVTRCPNLKSLKLNRAVPLEKLATLLQRAPQLEELGTGGYTAEVRPDVYSGLSVALSGCKELRCLSGFWDAVPAYLPAVYSVCSRL
TTLNLSYATVQSYDLVKLLCQCPKLQRLWVLDYIEDAGLEVLASTCKDLRELRVFPSEPFVMEPNVALTEQGLVSVSMGCPKLESVLYFCRQMTNAA
LITIARNRPNMTRFRLCIIEPKAPDYLTLEPLDIGFGAIVEHCKDLRRLSLSGLLTDKVFEYIGTYAKKMEMLSVAFAGDSDLGHHVLSGCDSLRK
LEIRDCPFGDKALLANASKLETMRSLWMSSCSVSFGACKLLGQKMPKLNVEVIDERGAPDSRPESCPVERVFIYRTVAGPRFDMPGFVWNMDQDSTM
RFSRQIITTNGL

D



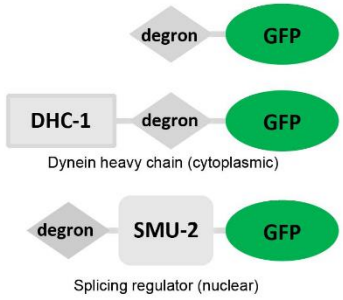
TIR1 constructs inserted by MosSCI:

Ubiquitous somatic expression: $P_{eft\text{-}3}$::*TIR1*::*unc-54* 3' UTR

Tissue-specific: $P_{ges\text{-}1}$::*TIR1*::*unc-54* 3' UTR, $P_{myo\text{-}2}$, etc.

Germline and early embryo: $P_{sun\text{-}1}$::*TIR1*::*sun-1* 3' UTR

G



Degron-tagged proteins expressed from arrays or engineered by insertion

E Degron DNA sequence

ATGCCTAAAGATCCAGCCAAACCTCCGGCCAAGGCACAAGTTGTGGGATGGCCACCGGTGAGATCATACCGGAAGAACGTGATGGTTTCCTGCCAAA
AATCAAGCGGTGGCCCGGAGGCGGCGGCGTTCGTGAAG

F Degron peptide sequence

MPKDPAKPPAKAQVVGWPPVRSYRKNVMVSCQKSSGGPEAAAFVK

Figure S1. Schematic of the AID system, TIR1 and degron sequences, and transgenes.

(A) Schematic of the AID system. Heterologously expressed *Arabidopsis thaliana* TIR1 (AtTIR1) F-box protein interacts with endogenous components to form a functional E3 ubiquitin ligase. In the presence of auxin, AtTIR1 binds the degron-tagged protein of interest. Degron-tagged proteins are polyubiquitinated and degraded by the proteasome. (B) The coding sequence of TIR1. *AtTIR1* sequence was codon optimized for *C. elegans*. Two point mutations (D170E and M473L) were incorporated to increase the affinity of *At*TIR1 for its substrates. Two introns (gray bases in lowercase) were inserted into *AtTIR1* to enhance expression. The start codon is shown in blue. (C) Corresponding amino acid sequence of TIR1. The two point mutations (D170E and M473L) are highlighted in red. (D) Design of TIR1 trangenes. The mRuby coding sequence was fused to TIR1 for visualization. A number of different drivers were generated for ubiquitous and tissue-specific TIR1 expression in *C. elegans*. All TIR1 fusion constructs were inserted as single-copy transgenes by MosSCI. (E) Coding sequence of the degron. The minimal degron domain from *Arabidopsis thaliana* IAA17 was chosen for expression in *C. elegans*. The start codon (blue) was included for expression at the N-terminus of target proteins. (F) Corresponding amino acid sequence of the degron. (G) Target constructs. The degron was placed at either end or in the middle of fusion proteins. GFP was fused to targets for visualization of expression and degradation. The *degron-GFP* transgene was inserted into the genome by MosSCI. A *degron-GFP* tag was inserted at the 3' end of the *dhc-1* coding sequence using CRISPR/Cas9-mediated editing. The *degron::smu-2::GFP* transgene was expressed from extrachromosomal arrays.

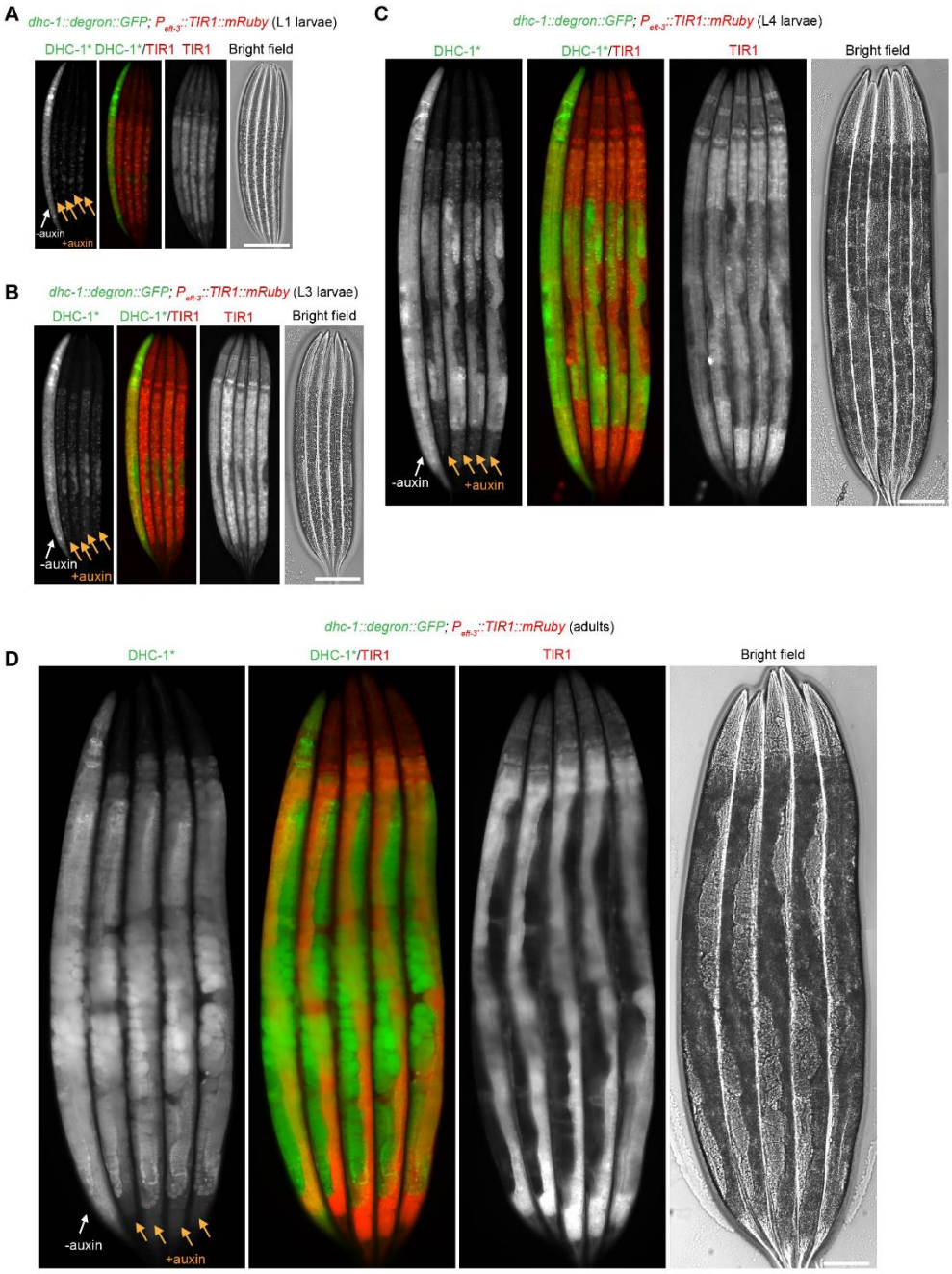


Figure S2. The AID system rapidly degrades degron-tagged proteins at various developmental stages.

(A) L1 larvae, (B) L3 larvae, (C) L4 larvae and (D) adults expressing *dhc-1-degron-GFP* and *P*$_{eft-3}$*::TIR1::mRuby::unc-54* 3'UTR MosSCI transgene were treated with (+) or without (-) 1mM auxin for two hours. Scale bar, 50 μm.

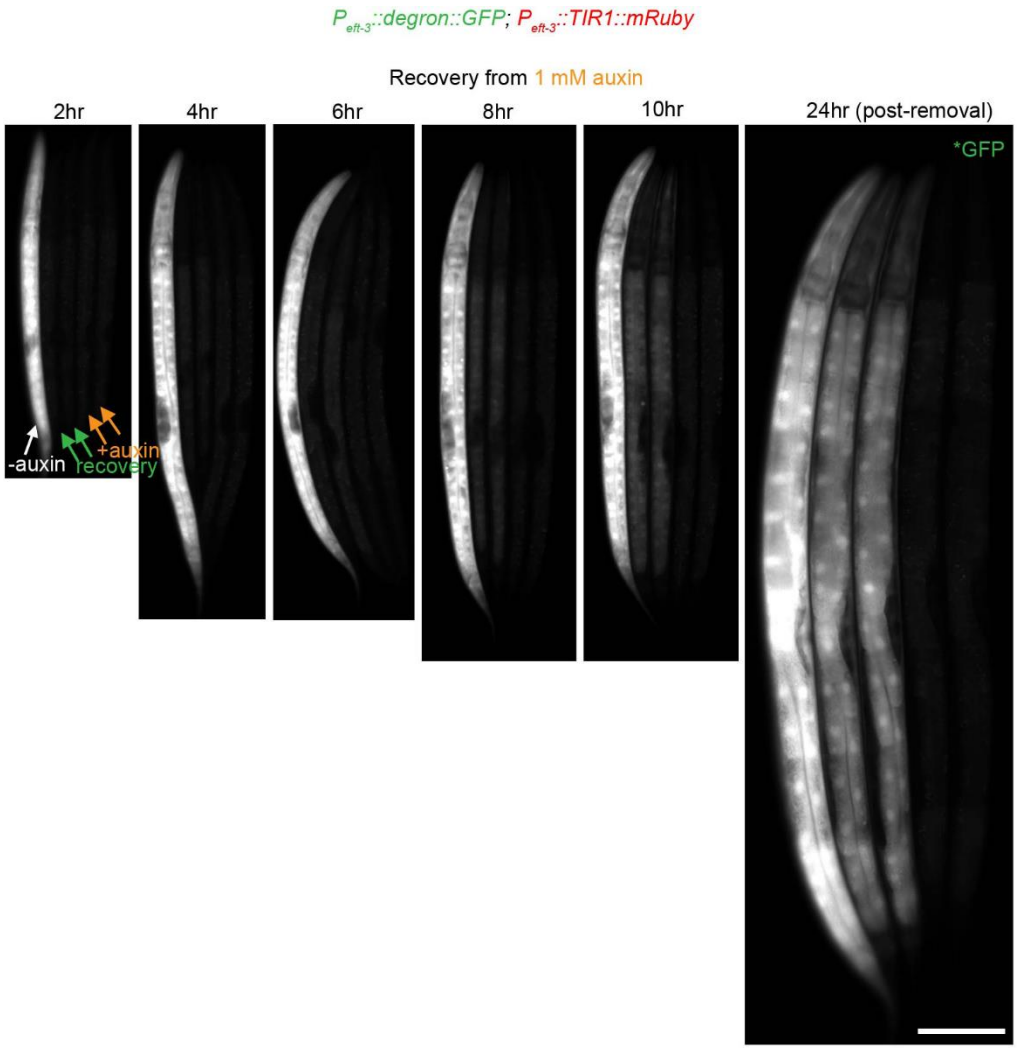


Figure S3. The recovery rate depends on the auxin concentration used for degradation.

L1 larvae treated with 1 mM auxin for two hours as in Fig. 2C were transferred to fresh NGM plates. Recovery of degron-tagged GFP was examined at the indicated time points. Worms without auxin treatment and those always on auxin plates were included as controls. Scale bar, 50 μm.

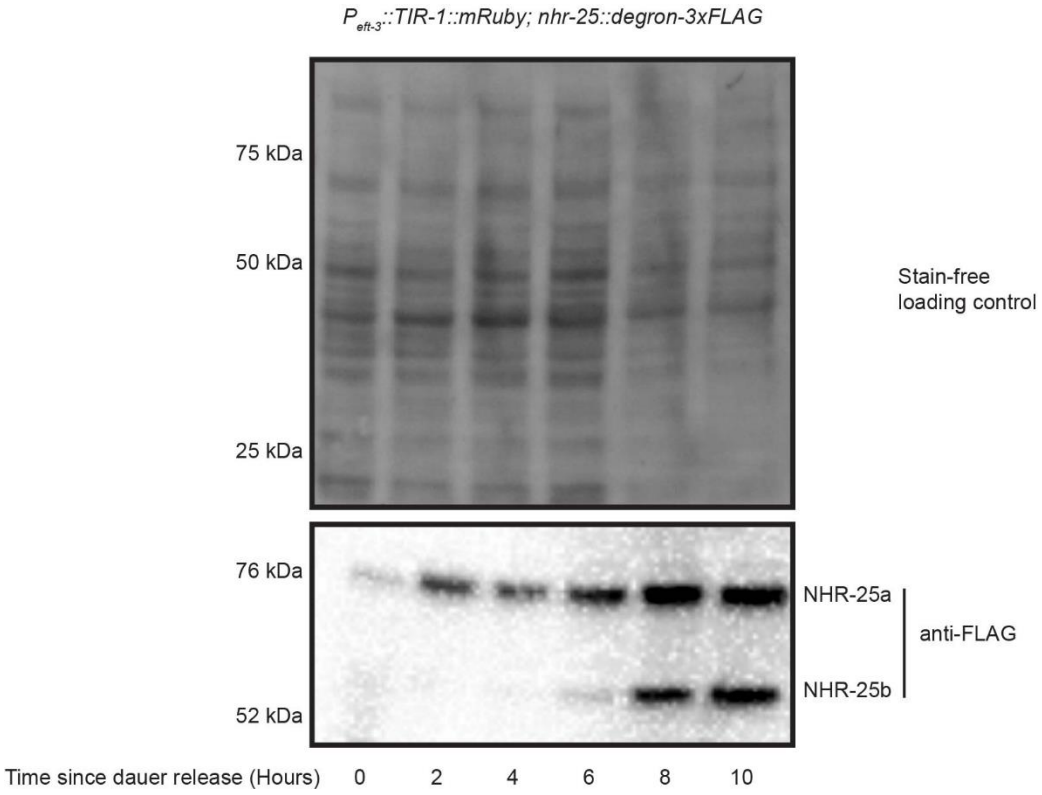


Figure S4. Time course of NHR-25-degron-3xFLAG expression following dauer release. Larvae were grown in liquid culture and analyzed as for the immunoblot shown in Figure 3B. The initial time point sampled before feeding to release animals from dauer arrest. Samples were then taken every two hours, to ten hours. Lysates were made by boiling worms in SDS sample buffer, resolved by SDS-PAGE, and probed with anti-FLAG-HRP (1:2000) to detect NHR-25 levels. Stain-free (Bio-Rad) analysis of total protein on each blot is provided as a loading control. Marker size (in kilodaltons) is provided.

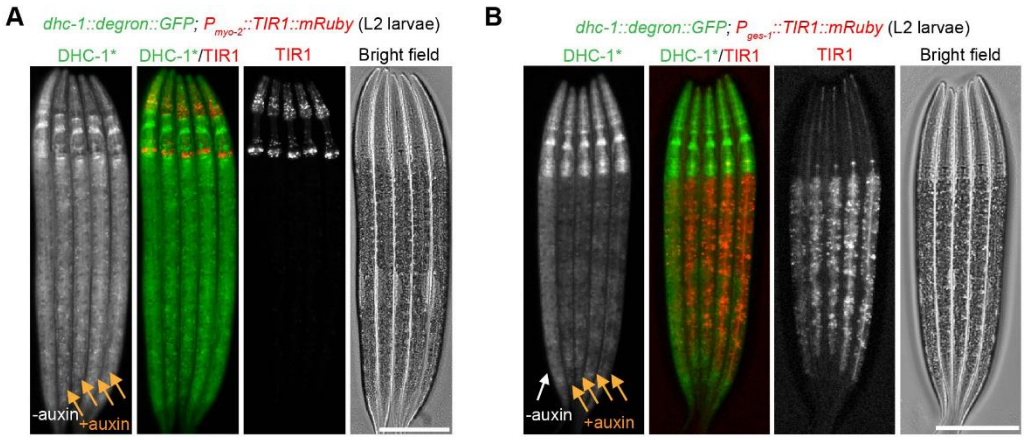


Figure S5. The AID system enables tissue-specific degradation in *C. elegans*.

(A) The *myo-2* promoter was used to drive TIR1 expression in pharyngeal muscle. Animals expressing this transgene and degron-tagged DHC-1 were treated with (+) or without (–) 1 mM auxin for three hours. Scale bar, 50 µm. L2 larvae are shown for clarity since they have small germlines; similar results were obtained for all larval and adult stages. (B) TIR1 was expressed in the intestine from the *ges-1* promoter, and combined with ubiquitously-expressed DHC-1::degron::GFP. Worms were treated with (+) or without (–) 1 mM auxin for two hours. Scale bar, 50 µm.

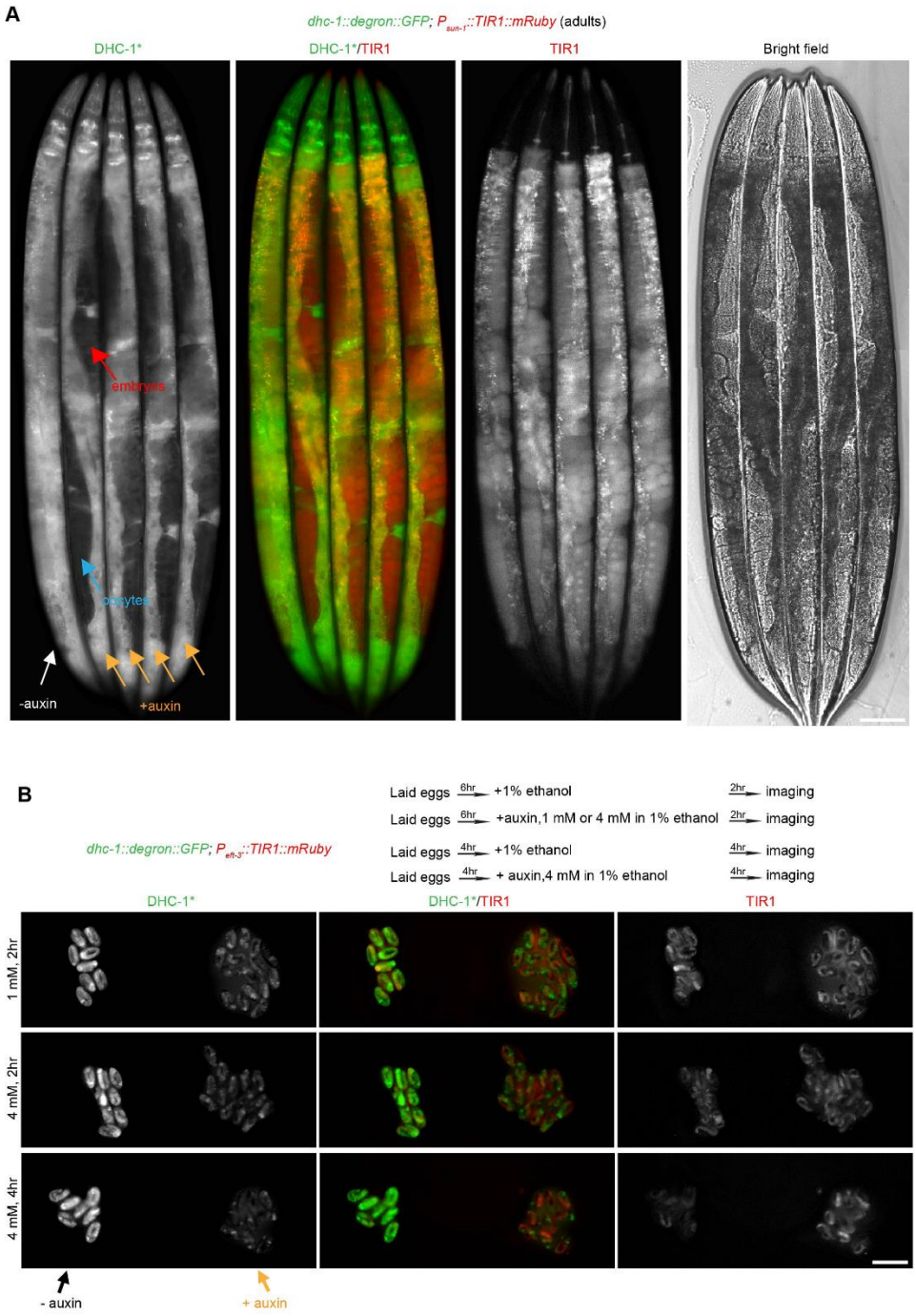


Figure S6. Auxin inducible degradation in maturing oocytes, early embryos and laid eggs.

(A) Young adults expressing *dhc-1::degron::GFP* and $P_{sun\text{-}1}$*::TIR1::mRuby* trangenes were treated with (+) or without (–) 1 mM auxin for four hours. Scale bar, 50 µm. (B) Inducible degradation by auxin treatment of laid eggs. Eggs laid by *dhc-1::degron::GFP*; $P_{eft\text{-}3}$*::TIR1::mRuby::unc-54* 3'UTR hermaphrodites were treated with 1 mM or 4 mM auxin (+) or without (–) auxin in S basal buffer for indicated times. Scale bar, 50 µm.

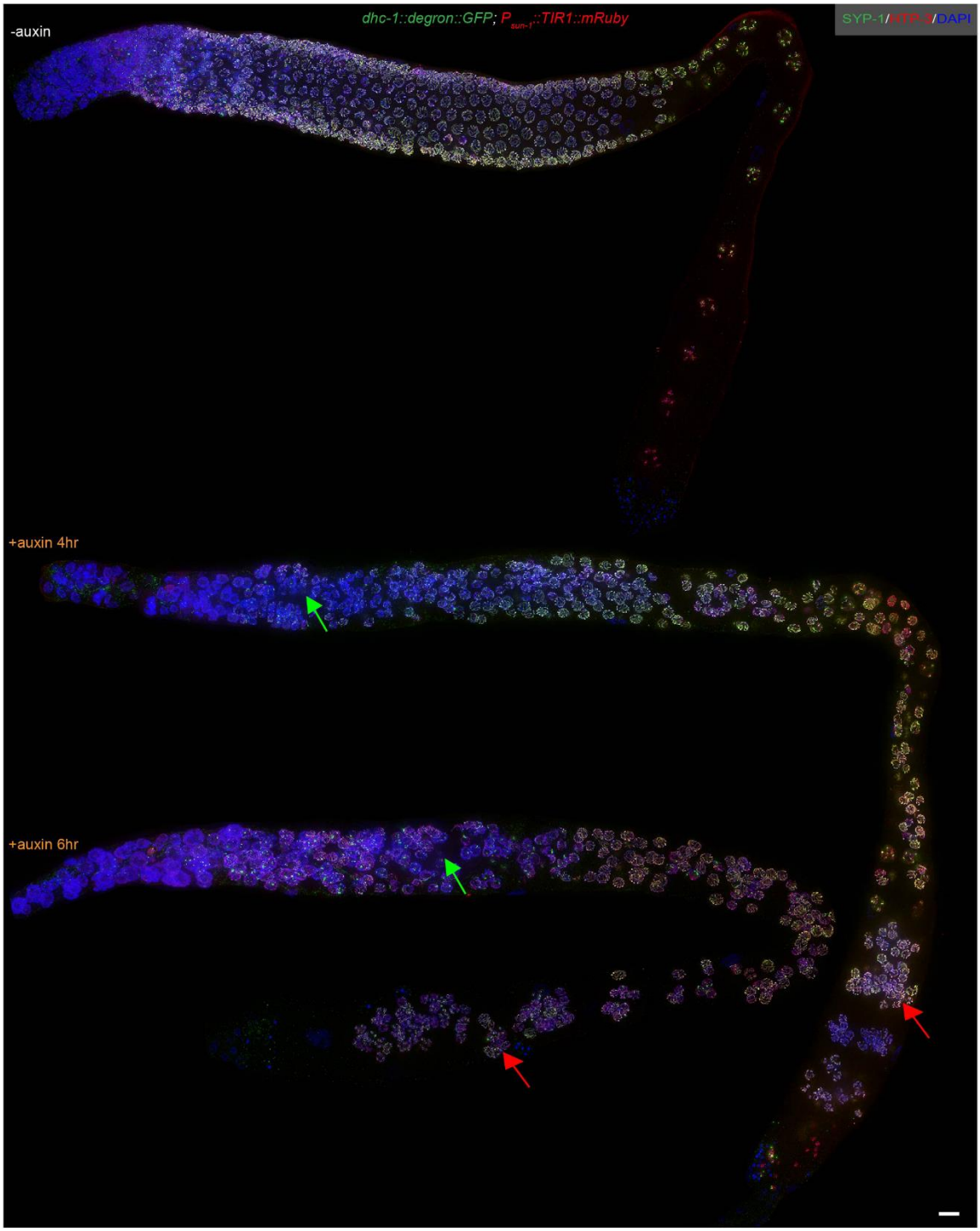


Figure S7. Conditional degradation of DHC-1 in the germline reveals its essential functions in meiotic progression and oocyte maturation during *C. elegans* meiosis.

Low–magnification views of the gonads shown at higher magnification in Figure 5B. Green arrows indicate abnormal nuclear positioning of cells in the distal gonad (early meiotic prophase), and red arrows indicate the proximal region of the germline. In auxin-treated animals, the proximal regions contain large numbers of nuclei with pachytene-like morphology, rather than the single-file organization of nuclei at diakinesis observed during normal oocyte maturation, as in the control image at the top. Scale bar, 5 μm.

Table S1. 1 mM auxin or AID related transgenes do not affect fertility or chromosomal segregation in meiosis at 20ºC

Strain	Treatment	Average progeny (5-6 hermaphrodites)	% Males (Males/Total progeny)
WT	Control	273.8	0.22 (3/1369)
WT	1 mM auxin	276.4	0 (0/1382)
dhc-1::degron::GFP	Control	285.2	0.63 (9/1426)
dhc-1::degron::GFP	1 mM auxin	253.2	0 (0/1266)
Peft-3::TIR1::mRuby	Control	271.2	0.25 (4/1627)
Peft-3::TIR1::mRuby	1 mM auxin	278.2	0.06 (1/1669)
dhc-1::degron::GFP; Peft-3::TIR1::mRuby	Control	275.0	0.18 (3/1650)
Pmyo-2::TIR1::mRuby	Control	314.5	0.05 (1/1887)
Pges-1::TIR1::mRuby	Control	316.7	0.05 (1/1900)
Psun-1::TIR1::mRuby	Control	310.4	0.13 (2/1552)
Psun-1::TIR1::mRuby	1 mM auxin	284	0.14 (2/1420)
dhc-1::degron::GFP; Psun-1::TIR1::mRuby	Control	249.2	0.27 (4/1495)

Table S2. 1 mM auxin treatment or AID related transgenes expression do not affect developmental rate at 20ºC

Strain	Treatment	0 hr (n L1)	16 hr (n early L4)	22 hr (n young adult)
WT	Control	100	100	100
WT	1 mM auxin	100	100	99
Peft-3::TIR1:mRuby	Control	100	98	100
Peft-3::TIR1:mRuby	1 mM auxin	100	100	100
Psun-1::TIR1:mRuby	Control	100	96	98
Psun-1::TIR1:mRuby	1 mM auxin	100	97	99
dhc-1::degron::GFP	Control	100	100	100
dhc-1::degron::GFP	1 mM auxin	100	99	99
dhc-1::degron::GFP; Peft-3::TIR1:mRuby	Control	100	100	100
dhc-1::degron::GFP; Psun-1::TIR1:mRuby	1 mM auxin	100	96	98

0 hr is the time at which 100 synchronized L1s were plated. The number of animals at the expected developmental stage at 16 hours and 22 hours post-plating is provided for each strain or condition.

Table S3. 4 mM auxin causes a reduction in brood size at 25ºC

Strain	Treatment	Brood size (± s.d.)	% Embryonic lethality
Peft-3::TIR1::mRuby	Control[1]	194±35	0
Peft-3::TIR1::mRuby	4 mM auxin	120±17	0.1
Peft-3::TIR1::mRuby; nhr-25::degron-TEV-3xFLAG	Control[1]	251±30	0
Peft-3::TIR1::mRuby; nhr-25::degron-TEV-3xFLAG	4 mM auxin	146±37	3.7
Peft-3::TIR1::mRuby; nhr-23::degron-TEV-3xFLAG	Control[1]	173±92	0.1
Peft-3::TIR1::mRuby; nhr-23::degron-TEV-3xFLAG	4 mM auxin	155±35	4.7

[1]Control data same as in Table 1, as brood sizes were performed together

Table S4. Constructs used in this study

Construct	Information
pLZ8	P*eft-3::Cas9::htp-3* 3'UTR, generated from pDD122
pLZ29	P*eft-3::degron::GFP::unc-54* 3'UTR, pCFJ151
pLZ31	P*eft-3::TIR1::mRuby::unc-54* 3'UTR, pCFJ151
pLZ37	P*ges-1::TIR1::mRuby::unc-54* 3'UTR, pCFJ151
pLZ50	*dhc-1 homology arm #1::degron::GFP::dhc-1 homology arm #2*, pCFJ151 backbone
pLZ51	P*U6::sgRNA(F+E)* targeting *dhc-1* #1, pMB70 modified
pLZ52	P*U6::sgRNA(F+E)* targeting *dhc-1* #2, pMB70 modified
pLZ68	P*myo-2::TIR1::mRuby::unc-54* 3'UTR, pCFJ151
pZC3	P*sun-1::TIR1::mRuby::sun-1* 3'UTR, pCFJ178
pZC4	P*smu-2::degron::smu-2::GFP::smu-2* 3'UTR, pAS17
pJW1328	P*U6::pha-(ts)* targeting sgRNA
pJW1354	Gly-Ser-4xGly linker-degron-TEV-3xFLAG with 62/35 bp *nhr-25* homology arms
pJW1355	Gly-Ser-4xGly linker-degron-TEV-3xFLAG with 39/40 bp *nhr-23* homology arms
pJW1363	P*U6::nhr-25* targeting sgRNA
pJW1254	Cas9 plasmid with P*U6::nhr-23* targeting sgRNA

Table S5. *C. elegans* strains used in this study

Strain	Genotype	Method	Resource
N2	WT	NA	CGC
GE24	*pha-1(e2123) III*	NA	CGC
EG6700	*unc-119(ed3); oxTi10882(+) IV*	NA	CGC
EG8079	*unc-119(ed3); oxTi179(+) II*	NA	CGC
EG8080	*unc-119(ed3); oxTi444(+) III*	NA	CGC
EG8081	*unc-119(ed3); oxTi177(+) IV*	NA	CGC
CA1199	*unc-119(ed3); ieSi38 [Psun-1::TIR1::mRuby::sun-1 3'UTR, cb-unc-119(+)] IV*	Microinjection	This study
CA1200	*unc-119(ed3); ieSi57 [Peft-3::TIR1::mRuby::unc-54 3'UTR, cb-unc-119(+)] II*	Microinjection	This study
CA1201	*iesi38; ieEx21 [Psmu-2::degron::smu-2::GFP::smu-2 3'UTR, pRF4]*	CA1199 cross with CA1203	This study
CA1202	*ieSi57; ieSi58 [Peft-3::degron::GFP::unc-54 3'UTR, cb-unc-119(+)] IV*	CA1200 cross with CA1204	This study
CA1203	*ieEx21*	Microinjection	This study
CA1204	*unc-119(ed3); ieSi58*	Microinjection	This study
CA1205	*unc-119(ed3); ieSi59 [Peft-3::degron::GFP::unc-54 3'UTR, cb-unc-119(+)] III*	Microinjection	This study
CA1206	*ieSi57; ieSi59*	CA1200 cross with CA1205	This study
CA1207	*dhc-1(ie28[dhc-1::degron::GFP])*	Microinjection	This study
CA1208	*unc-119(ed3); ieSi60 [Pmyo-2::TIR1::mRuby::unc-54 3'UTR, cb-unc-119(+)] II*	Microinjection	This study
CA1209	*unc-119(ed3); ieSi61 [Pges-1::TIR1::mRuby::unc-54 3'UTR, cb-unc-119(+)] II*	Microinjection	This study
CA1210	*ie28; ieSi57*	CA1207 cross with CA1200	This study
CA1211	*ieEx21; ieSi57*	CA1203 cross with CA1200	This study
CA1212	*ie28; ieSi60*	CA1207 cross with CA1208	This study
CA1213	*ie28; ieSi61*	CA1207 cross with CA1209	This study
CA1214	*ieEx21; ieSi61*	CA1203 cross with CA1209	This study
CA1215	*ie28; ieSi38*	CA1207 cross with CA1199	This study
KRY84	*nhr-25(kry59(nhr-25::degron-TEV-3xFLAG))*X	Microinjection	This study
KRY85	*iSi57; nhr-25(kry59)*	CA1200 cross with KRY84	This study
KRY87	*nhr-23(kry61(nhr-23::degron-TEV-3xFLAG))I*	Microinjection	This study
KRY88	*nhr-23(kry61); iSi57*	CA1200 cross with KRY87	This study

Supplementary reference

Chen, B., Gilbert, L. A., Cimini, B. A., Schnitzbauer, J., Zhang, W., Li, G. W., Park, J., Blackburn, E. H., Weissman, J. S., Qi, L. S., et al. (2013). Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system. *Cell* **155**, 1479-1491.

Dickinson, D. J., Ward, J. D., Reiner, D. J. and Goldstein, B. (2013). Engineering the *Caenorhabditis elegans* genome using Cas9-triggered homologous recombination. *Nat. Methods* **10**, 1028-1034.

Frokjaer-Jensen, C., Davis, M. W., Hopkins, C. E., Newman, B. J., Thummel, J. M., Olesen, S. P., Grunnet, M. and Jorgensen, E. M. (2008). Single-copy insertion of transgenes in *Caenorhabditis elegans*. *Nat. Genet.* **40**, 1375-1383.

Gibson, D. G., Young, L., Chuang, R. Y., Venter, J. C., Hutchison, C. A., 3rd and Smith, H. O. (2009). Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat. Methods* **6**, 343-345.

MacQueen, A. J., Phillips, C. M., Bhalla, N., Weiser, P., Villeneuve, A. M. and Dernburg, A. F. (2005). Chromosome sites play dual roles to establish homologous synapsis during meiosis in *C. elegans*. *Cell* **123**, 1037-1050.

Mello, C. C., Kramer, J. M., Stinchcomb, D. and Ambros, V. (1991). Efficient gene transfer in *C.elegans*: extrachromosomal maintenance and integration of transforming sequences. *EMBO J.* **10**, 3959-3970.

Merritt, C., Rasoloson, D., Ko, D. and Seydoux, G. (2008). 3' UTRs are the primary regulators of gene expression in the *C. elegans* germline. *Curr. Biol.* **18**, 1476-1482.

Rog, O. and Dernburg, A. F. (2015). Direct Visualization Reveals Kinetics of Meiotic Chromosome Synapsis. *Cell Rep.* **10**, 1639-1645.

Spartz, A. K., Herman, R. K. and Shaw, J. E. (2004). SMU-2 and SMU-1, *Caenorhabditis elegans* homologs of mammalian spliceosome-associated proteins RED and fSAP57, work together to affect splice site choice. *Mol. Cell Biol.* **24**, 6811-6823.

Staab, T. A., Griffen, T. C., Corcoran, C., Evgrafov, O., Knowles, J. A. and Sieburth, D. (2013). The conserved SKN-1/Nrf2 stress response pathway regulates synaptic function in *Caenorhabditis elegans*. *PLoS Genet.* **9**, e1003354.

Waaijers, S., Portegijs, V., Kerver, J., Lemmens, B. B., Tijsterman, M., van den Heuvel, S. and Boxem, M. (2013). CRISPR/Cas9-targeted mutagenesis in *Caenorhabditis elegans*. *Genetics* **195**, 1187-1191.

Ward, J. D. (2015). Rapid and precise engineering of the *Caenorhabditis elegans* genome with lethal mutation co-conversion and inactivation of NHEJ repair. *Genetics* **199**, 363-377.