The auxin-inducible degradation (AID) system enables versatile conditional protein depletion in *C. elegans*

Liangyu Zhang\textsuperscript{1,2,3,4}, Jordan D. Ward\textsuperscript{5}, Ze Cheng\textsuperscript{1}, and Abby F. Dernburg\textsuperscript{1,2,3,4,*}

\textsuperscript{1}Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA 94720-3220, USA

\textsuperscript{2}Howard Hughes Medical Institute, 4000 Jones Bridge Road, Chevy Chase, MD 20815, USA

\textsuperscript{3}Life Sciences Division, Department of Genome Dynamics, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

\textsuperscript{4}California Institute for Quantitative Biosciences, Berkeley, CA 94720, USA

\textsuperscript{5}Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA 94158, USA
*Author for correspondence (afderburg@lbl.gov).
ABSTRACT

Experimental manipulation of protein abundance in living cells or organisms is an essential strategy for investigation of biological regulatory mechanisms. While powerful techniques for protein expression have been developed in *C. elegans*, existing tools for conditional disruption of protein function are far more limited. To address this, we have adapted the auxin-inducible degradation (AID) system discovered in plants to enable conditional protein depletion in *C. elegans*. We report that expression of a modified *Arabidopsis* TIR1 F-box protein mediates robust auxin-independent depletion of degron-tagged targets. We document the effectiveness of this system for depletion of nuclear and cytoplasmic proteins in diverse somatic and germline tissues throughout development. Target proteins were depleted in as little as 20-30 minutes, and their expression could be reestablished upon auxin removal. We have engineered strains expressing TIR1 under the control of various promoter and 3’ UTR sequences to drive tissue-specific or temporally regulated expression. The degron tag can be efficiently introduced by CRISPR/Cas9-based genome editing.
We have harnessed this system to explore the roles of dynamically expressed nuclear hormone receptors in molting, and to analyze meiosis-specific roles for proteins required for germline proliferation. Together, our results demonstrate that the AID system provides a powerful new tool for spatiotemporal regulation and analysis of protein function in a metazoan model organism.

KEYWORDS: *C. elegans*, genetic tool, degron, auxin, auxin-inducible degradation, tissue-specific depletion
INTRODUCTION

Techniques for precise temporal and spatial control of protein expression enable detailed analysis of developmental mechanisms. In *C. elegans*, a variety of tools for stage- or tissue-specific expression have been developed, including the hsf-1 system (Bacaj and Shaham, 2007), drug-induced protein stabilization (Cho et al., 2013), FLP-mediated excision of FRT-flanked transcriptional terminators (Davis et al., 2008), and the Q-system (Wei et al., 2012). However, available methods for conditional protein depletion are far more limited. Depletion of gene products in specific stages and tissues has been achieved through RNAi (Qadota et al., 2007), or by gene disruption via tissue-specific expression of sequence-specific nucleases (Cheng et al., 2013; Shen et al., 2014). However, these approaches are indirect and irreversible, since they rely on inactivation of a gene or on mRNA degradation. Additionally, there is often a substantial lag between induction and protein depletion, the duration of which depends on mRNA and/or protein stability (Elbashir et al., 2001; Fire et al., 1998).
Degrons, amino acid sequences that direct proteasomal destruction of tagged proteins, have become extremely powerful experimental tools, particularly in yeast. A recent report repurposed an endogenous, developmentally regulated degradation pathway in *C. elegans* (Armenti et al., 2014) for experimental manipulation of proteins in this system. In cells or tissues engineered to express ZIF-1, an E3 ubiquitin ligase substrate-recognition subunit, proteins fused to a 36 amino acid ZF1 degron can be quickly degraded. This system holds great promise, but also has some limitations. It cannot be used in the germline, since the native role of this pathway is to degrade germline-expressed proteins upon fertilization, and ectopic ZIF-1 expression would therefore disrupt essential germline functions. Conditional depletion using this system also relies on *zif-1* induction by heat shock, which can interfere with some processes and requires some lag time.

The auxin-inducible degradation (AID) system from plants has enabled rapid, conditional protein depletion in yeast and cultured vertebrate cells (Holland et al., 2012; Nishimura et al., 2009). This system relies on expression of a plant-specific F-
box protein, TIR1, which regulates diverse aspects of plant growth and morphogenesis in response to the phytohormone auxin (Gray et al., 1999; Ruegger et al., 1998). TIR1 is the substrate recognition component of a Skp1–Cullin–F-box (SCF) E3 ubiquitin ligase complex, which recognizes substrates only in the presence of auxin (indole-3-acetic acid, or IAA) and targets them for degradation by the proteasome (Dharmasiri et al., 2005; Kepinski and Leyser, 2005; Tan et al., 2007). When expressed in heterologous systems, TIR1 can interact with endogenous Skp1 and Cullin proteins to form a functional, auxin-dependent ubiquitin E3 ligase (Holland et al., 2012; Kanke et al., 2011; Kreidenweiss et al., 2013; Nishimura et al., 2009; Philip and Waters, 2015). However, to our knowledge, this approach has not been previously used in any intact metazoan system.

We have now adapted the AID system for small-molecule inducible protein degradation in *C. elegans* (supplementary material Fig. S1A). We report that expression of TIR enables rapid, reversible, auxin-dependent degradation of nuclear and cytoplasmic targets in all tissues and developmental stages tested. We have
applied this system to analyze nuclear hormone receptor control of molting, and meiosis-specific roles for proteins required for germline proliferation, demonstrating the versatility of this system for dissecting protein function in a widely-used model organism.
RESULTS

Design strategy for the auxin-inducible degradation (AID) system in *C. elegans*

TIR1-dependent protein degradation has been most extensively characterized in the model plant *Arabidopsis thaliana*. The TIR1 gene from rice (*Oryza sativa*) was found to yield more robust degradation when expressed in budding yeast and vertebrate cells than the orthologs from *Arabidopsis* and *Gossypium hirsutum* (cotton; (Nishimura et al., 2009). However, the standard laboratory culture temperature for *C. elegans* (20˚C) is closer to the preferred range for *Arabidopsis* (23-25˚C), so we chose to express the *Arabidopsis* TIR1 protein sequence in *C. elegans*, in part to take advantage of prior molecular studies of this protein. We incorporated two point mutations (D170E and M473L) shown to increase the affinity of AtTIR1 for its substrates and to increase auxin sensitivity without causing auxin-independent activity (Yu et al., 2013) (supplementary material Fig. S1B,C). We constructed a synthetic TIR1 gene that was codon optimized for *C. elegans* and contained two introns (supplementary material Fig. S1B). This gene was fused to a
codon-optimized red fluorescent protein (mRuby) gene (Rog and Dernburg, 2015) to permit visualization of TIR1 expression, and placed under the control of several different germline and somatic regulatory elements (supplementary material Fig. S1D). Throughout this study, the *unc-54* 3’ UTR was used for all somatic TIR1 drivers, and the *sun-1* 3’ UTR was used for germline expression. Worm strains with integrated copies of these transgenes were created by transposon-mediated single-copy insertion (MosSCI; Frokjaer-Jensen et al., 2008).

We fused a 44-amino acid minimal degron sequence (Morawska and Ulrich, 2013) (supplementary material Fig. S1E,F) derived from the *Arabidopsis thaliana* IAA17 protein to two broadly-expressed *C. elegans* genes, *smu-2* and *dhc-1*, together with a synthetic GFP gene (Rog and Dernburg, 2015) to enable visualization and monitoring of the target proteins (supplementary material Fig. S1G). The splicing regulator SMU-2 was selected as a candidate nuclear target because it is one of two known genes that can be readily expressed from extrachromosomomal arrays in both the soma and the germline (Spartz et al., 2004), facilitating strain construction. We
engineered a degron::smu-2::GFP transgene and confirmed robust protein expression from extrachromosomal arrays. The dynein heavy chain (DHC-1) was chosen as a cytoplasmic target because it is ubiquitously expressed and essential, and because of our interest in the roles of dynein during meiotic prophase (Sato et al., 2009; Wynne et al., 2012). A degron-GFP tag was inserted at the 3’ end of the endogenous dhc-1 coding sequence using CRISPR/Cas9-mediated editing (Dickinson et al., 2013). We also constructed strains expressing degron-tagged GFP from a stably inserted transgene, which served as a functionally inert reporter (supplementary material Fig. S1G).

The AID system degrades cytoplasmic and nuclear proteins at all developmental stages

We first introduced a construct designed to express TIR1-mRuby from the strong, ubiquitous eft-3 promoter and the 3’ UTR from unc-54, which is broadly permissive for somatic expression. Visualization of red fluorescence confirmed that
TIR1 was expressed in most or all somatic tissues throughout development, but not in the germline, as expected. We crossed this transgene to a strain in which the endogenous \textit{dhc-1} gene was C-terminally tagged with a degron-GFP cassette using CRISPR/Cas9-mediated editing. In the presence of 1 mM auxin, DHC-1 was depleted within two hours in somatic tissues at various developmental stages, from L1 to adult (Fig.1 A,B; supplementary material Fig. S2). Degradation was quantified in L2 larvae by determining green fluorescence intensity in whole worms, which showed a 91.6% reduction in GFP signal in auxin treated animals compared to control animals. The residual fluorescence is likely due to undegraded DHC-1 in the germline, where TIR1 was not expressed, since the somatic fluorescence was no higher than in wild-type animals lacking GFP-tagged \textit{dhc-1}.

We also crossed extrachromosomal arrays encoding a degron- and GFP-tagged SMU-2 protein into our pan-somatic TIR1 strain to test whether this nuclear protein could be targeted by the TIR1 ubiquitin ligase complex. When these animals were exposed to 1 mM auxin, fluorescent SMU-2 disappeared throughout the soma.
within two hours in L2 larvae and three hours in adult worms (Fig.1 C-E). Thus, the AID system permits protein depletion throughout larval and adult stages.

To analyze the kinetics of AID-mediated protein degradation in adult animals, we used a strain in which TIR1-mRuby and degron-tagged GFP were co-expressed using the same regulatory sequences (the eft-3 promoter and unc-54 3’ UTR). The abundance of degron-tagged GFP was measured over time in adult worms exposed to a range of auxin concentrations. We found that the degradation rate depended on the concentration of auxin: in the presence of ≥0.5 mM auxin, degron-GFP was reduced to 50% of its initial level within 20 minutes, and was undetectable within 45 minutes (Fig. 2 A,B), while lower auxin concentrations resulted in slower depletion of degron-GFP.

**AID-mediated degradation is reversible**

We next investigated whether AID-mediated protein degradation is reversible. We treated L1 larvae with various concentrations of auxin, and monitored the
depletion and recovery of degron-tagged GFP using fluorescence imaging. We found that treatment with 25 μM auxin was sufficient to eliminate degron-GFP within two hours (Fig. 2C). The recovery rate depended strongly on the concentration of auxin used for depletion (Fig. 2C-E; supplementary material Fig. S3): after removal from 25 μM auxin, visible GFP fluorescence was observed within two hours, and reached half the level seen in untreated animals within seven hours (Fig. 2D,E). When degradation was induced with 1 mM auxin, recovery of GFP expression required substantially longer (Fig. 2E; supplementary material Fig. S3). Thus, auxin concentrations should be tested and optimized for specific targets and tissues, particularly for experiments in which reversibility is desired.

We note that rates of AID-mediate protein degradation and recovery also depended on the developmental stage of the treated animals (data not shown): degradation occurred more quickly in young larvae than in adults. This may reflect differences in the rates of auxin uptake or diffusion through tissues, as well as potential differences in the abundance of expressed TIR1 and other endogenous SCF.
components at various developmental stages. The recovery rate of specific proteins will clearly depend on transcription and translation rates, which are also likely to vary during development.

The AID system enables functional analysis of nuclear hormone receptors during development

Due to our interest in nuclear hormone control of developmental gene regulatory networks (Ward et al., 2013; Ward et al., 2014), as test cases, we targeted two essential nuclear hormone receptors, NHR-23 and NHR-25. A degron::3xFLAG cassette was PCR amplified with 40 bp homology arms (Paix et al., 2014) and inserted into the 3’ ends of the endogenous nhr-23 and nhr-25 coding sequences using pha-1 co-conversion (Ward, 2015). This approach enabled homozygous knock-in animals to be obtained within nine days of injection. These alleles were then crossed into the pan-somatic P_{ept-3::TIR1::mRuby} strain. In the absence of auxin, the resulting strains showed normal brood sizes and viability, demonstrating that the
tags did not interfere with NHR-23 or NHR-25 function (Fig. 3A). However, following treatment with 1 mM auxin, the *nhr-25* degron line displayed a strong reduction in brood size (Fig. 3A), and a spectrum of other defects consistent with inactivation of NHR-25 (Asahina et al., 2006; Chen et al., 2004): gonad abnormalities (Fig. 3A,B), molting defects (Fig. 3A,B), and complete sterility among the F1 progeny of treated animals. Auxin treatment of the degron-tagged *nhr-23* animals did not affect the number of their F1 progeny (Fig. 3A), but 100% of these progeny arrested as L1 larvae, which were also Dumpy (Fig. 3A,B). In a previous study, when *nhr-23* was inactivated by RNAi at the same stage (L4) at which we initiated auxin treatment, only 2% of progeny arrested at the L1 stage, with additional progeny arresting at L2 and L3 (Kostrouchova et al., 2001). These data indicate that the AID system can produce more penetrant phenotypes than RNAi depletion.

We next wished to assess the kinetics of degradation of these nuclear hormone receptors. A time course of NHR-25-degron-3xFLAG expression following dauer release revealed that expression increased dramatically over the next 6-8
hours (supplementary material Fig. S4), so we focused on this time point. We released animals from dauer arrest in liquid culture by feeding with HB101 bacteria for six hours. Animals were then treated with either 0.25% ethanol (control) or 1 mM auxin, and NHR-25-degron-3xFLAG levels were monitored by anti-FLAG immunoblotting. While NHR-25-degron-3xFLAG levels were unchanged in the control, the target was largely depleted within 20 min after auxin addition, and almost undetectable after 40 minutes (Fig. 3C). Thus, this approach enables precise time-resolved analysis of proteins that are dynamically expressed during development. This versatility should also allow modulation of protein levels in large cultures, permitting new types of biochemical experiments.

The AID system allows tissue-specific degradation

We next tested whether degradation could be spatially restricted to specific somatic tissues. We generated a strain expressing a TIR1-mRuby transgene under control of a myo-2 promoter. After confirming that red fluorescence was specifically
detected in pharyngeal muscle, we crossed it to the ubiquitously expressed dhc-1-degron-GFP transgene. After exposure to 1 mM auxin, we observed an obvious decline in green fluorescence within the pharynx, while DHC-1-degron-GFP in other tissues remained unchanged relative to untreated controls (supplementary material Fig. S5A). Restriction of TIR1 expression to the intestine by driving it from the ges-1 promoter resulted in loss of DHC-1-degron-GFP only in the gut (supplementary material Fig. S5B). Similarly, we observed auxin-dependent depletion of the degron-tagged nuclear protein SMU-2 only in tissues where TIR1 was expressed (Fig. 4A-C). To quantify the tissue-specific degradation, we dissected worms specifically expressing TIR1 in intestine and determined the degradation of degron-SMU-2-GFP in intestine by measuring the green fluorescence in intestinal nuclei. We detected 98.3% of SMU-2 was degraded in intestine following auxin treatment (Fig. 4B).

One of our key goals in developing the AID system was to enable conditional protein depletion in the germline. To express TIR1 throughout this tissue, we used the promoter and 3’UTR from the sun-1 gene, which is expressed in both the mitotic
and meiotic region of the germline as well as in mitotic cells of the early embryo (Malone et al., 2003; Minn et al., 2009). When this transgene was crossed into worms expressing degron-tagged DHC-1-GFP, we observed disappearance of DHC-1 fluorescence throughout the germline, including in maturing oocytes, where expression was initially strongest (supplementary material Fig. S6A). We confirmed that tagged DHC-1 was undetectable throughout the germline by immunofluorescence microscopy following auxin exposure (Fig. 4D). Notably, DHC-1 was undetectable in the early embryos inside the mothers following auxin treatment (supplementary material Fig. S6A). Thus, the \( P_{sun-1::TIR1::mRuby} \) trangene is an effective tool for depleting target proteins in early embryos as well as throughout the mitotic and meiotic germline.

Because \textit{C. elegans} embryos are surrounded by an eggshell and vitelline membrane that prevent entry of many molecules (Carvalho et al., 2011), we considered it unlikely that the AID system would be useful in embryos after they were laid. Nevertheless, we tested the system by treating embryos expressing TIR1
and degron-tagged DHC-1-GFP with auxin. To avoid weakening the eggshell by treatment with bleach, we allowed adult hermaphrodites to lay embryos on auxin-free plates, then washed these embryos into S basal medium containing 1 mM or 4 mM auxin. To our surprise, we observed a striking reduction in GFP fluorescence following auxin treatment. Although degradation of the target was incomplete (supplementary material Fig. S6B), high magnification revealed that the residual green fluorescence was spatially restricted to cells in which little or no TIR1-mRuby was expressed from our $P_{eft-3/unc-54\,3'}$ UTR transgene (Fig. 4E). We therefore conclude that auxin can penetrate the eggshell and induce effective target degradation in embryos. Development of other drivers for TIR1 would likely enable auxin-mediated degradation in specific embryonic tissues.

We conclude that the AID system provides a robust tool for conditional depletion of proteins of interest in many, and likely all, somatic and germline tissues.
Conditional degradation of DHC-1 in the germline reveals multiple roles in meiotic progression

To validate the utility of the AID system to interrogate germline functions, we analyzed the effects of depleting the dynein heavy chain protein (DHC-1). Meiosis is a specialized cell division process characterized by homologous chromosome pairing, synapsis, and segregation into daughter cells. Using RNAi and temperature sensitive mutants, dynein activity was previously demonstrated to be essential for formation of the synaptonemal complex during *C. elegans* meiosis (Sato et al., 2009), which in turn is required for stable lengthwise association between homologous chromosomes. These earlier experiments were complicated by the essential role of dynein in mitotic chromosome segregation, which is required for germline proliferation. We first exposed animals expressing GFP/degron-tagged DHC-1 and our pan-germline TIR1 (*Psun-1::TIR1::mRuby*) to 1 mM auxin to monitor the degradation kinetics of DHC-1 by fluorescence. We found that DHC-1 became
undetectable throughout the germline within 45 minutes of auxin treatment, and verified this by immunofluorescence (Fig. 5A).

To determine the effects of DHC-1 depletion on meiotic prophase, we dissected animals after exposure to auxin for several hours, so that a pool of nuclei entered and progressed through early prophase in the absence of DHC-1. Early meiotic nuclei in these germlines showed obvious defects in chromosome synapsis, as indicated by aberrant localization of SYP-1 (Fig. 5B,C), a synaptonemal complex protein (MacQueen et al., 2002), consistent with our previous findings (Sato et al., 2009). We also observed effects that had not been apparent when dynein was depleted by RNAi or temperature-sensitive mutations. These included global disorganization of germline nuclei (supplementary material Fig. S7), consistent with a previously-demonstrated role of dynein in maintaining nuclear position in germline cells (Zhou et al., 2009), as well as a novel defect in oocyte maturation (supplementary material Fig. S7). These effects likely reflect more complete
abrogation of dynein function than we were able to achieve by RNAi and/or temperature shifts.

Viability, fertility, and development are unaffected by TIR1 expression and auxin treatment

The utility of a technique in addressing developmental mechanisms relies on its minimal side effects. So far, we have observed no obvious side effects of either long-term auxin exposure within the useful concentration range or from TIR1 expression. We found that expression of TIR1 from strong drivers in the soma or germline had no effect on brood size or embryonic viability at either 25ºC (Fig. 3A) or 20ºC (supplementary material Table S1). Because we were concerned that expression of TIR1 might sequester other SCF complex components, we specifically looked for phenotypes associated with inactivation of the germline-expressed Skp1-related proteins (SKR-1/SKR-2) (Nayak et al., 2002) in the germlines of animals expressing TIR1 from the strong sun-1 promoter, but observed no such
abnormalities. Moreover, neither exposure to 1 mM auxin or TIR1 expression affected developmental rate (supplementary material Table S2).

We did observe a modest reduction in brood size when animals were exposed to 4 mM auxin for extended periods at 25°C (supplementary material Table S3). This may be an indirect effect, since bacterial growth was somewhat inhibited at this concentration. Since the maximal rate of target degradation is obtained at lower concentrations (Figure 2), auxin should be used at 1 mM or lower concentrations, conditions under which we observed no apparent side effects.

DISCUSSION

Rapid, conditional and reversible protein degradation is an invaluable tool for probing protein function in cellular or developmental processes. A variety of methods have been developed to conditionally stabilize or destabilize proteins (Armenti et al., 2014; Banaszynski et al., 2006; Bonger et al., 2011; Caussinus et al., 2012; Cho et al., 2013; Dohmen et al., 1994; Raina and Crews, 2010; Renicke et al,
2013; Taxis et al., 2009; Zhou et al., 2000), but many of these approaches are unsuitable for use in *C. elegans* or have significant limitations. Here, we demonstrate that the AID system allows efficient, rapid degradation of nuclear and cytoplasmic proteins at all developmental stages. Protein expression recovered upon auxin removal, with lower auxin doses facilitating faster recovery. By spatially restricting TIR1 expression through various promoter and 3’UTR sequences, we achieved tissue-specific depletion of degron-tagged target proteins, with auxin exposure providing temporal control. Auxin concentration and the stage of the animal/embryo influence the degradation and recovery rates of target proteins. Accordingly, the relevant kinetic parameters may need to be tested and optimized for specific experiments. The developmental stage likely influences the rate of auxin uptake and diffusion or transport through tissues, the abundance of endogenous Skp1/Cul1 or other TIR1 partners, and the expression levels of both TIR1 and the target protein. For experiments in which recovery of expression is desired, it will also be important to consider gene-specific transcription and translation rates.
A number of features make the AID system particularly attractive and simple to implement. Auxin is inexpensive and is moderately water-soluble, making it easy to administer in plates or liquid growth media. Liquid culture is particularly well suited for experiments that require large amounts of starting material (e.g. IP-mass spec, ChIP-seq). While many drugs show poor efficacy in *C. elegans* due to limited permeability of the egg or cuticle, efficient export, and other toxin-resistance mechanisms (Broeks et al., 1995; Lindblom et al., 2001), we have found that exogenous treatment with auxin can induce degradation at all developmental stages, even during embryogenesis. Auxin-mediated degradation is also efficient in the absence of food (data not shown) making it useful for analysis of processes induced by starvation, such as autophagy, L1 arrest, or dauer formation. This feature should also allow production of large populations of synchronized animals depleted for a protein of interest. The small size of the degron enables efficient knock-in by co-conversion (Arribere et al., 2014; Kim et al., 2014; Ward, 2015), or selection-based CRISPR editing (Dickinson et al., 2015; Dickinson et al., 2013; Norris et al., 2015). The degron can be fused to the N- or C-terminus of target proteins, and can even be
inserted internally. By fusing an epitope tag or fluorescent protein to the target along with the degron, the same engineered protein can be localized, purified, and inducibly degraded, providing a multifunctional tool for experimental biology. Crucially, exposure to auxin over the effective concentration range had no detectable effects on worm viability, morphology, or fertility, nor did expression of TIR1 under strong drivers in the soma or germline (Fig. 3A; supplementary material Table S1-S3). These attributes should make the system applicable to a wide range of questions in cell and developmental biology.

Although the auxin-inducible degradation system we describe here is robust and specific, future directed optimization of the AID system may enhance its utility in *C. elegans*. We have characterized the system using a gain-of-function allele of the TIR1 gene from *Arabidopsis*, but it may be useful to compare the performance of this protein to orthologs or paralogs from other plants, with and without the corresponding mutations. Ongoing efforts to develop more potent auxin agonists may also provide other small molecule tools, although the high water solubility,
small size, low toxicity, and nominal cost of IAA may prove difficult to improve upon.

It may also be possible to develop smaller and/or higher-activity degron sequences
or to enhance TIR1 activity through further evolutionary or mutational analysis.

However, in its present incarnation, the AID system represents a highly versatile tool
for rapid, conditional, robust tissue-specific and stage-specific protein degradation in

*C. elegans.*

Provided that a model organism has a set of tissue-specific regulatory
elements and that auxin can penetrate a tissue of interest, our adaptation of the AID
system for *C. elegans* should provide a road map for importing this technology into
other metazoan model organisms.
MATERIALS AND METHODS

Constructs and generation of transgenic lines

Constructs used in this study are listed in supplementary material Table S4. More information about constructs and transgenic lines are provided in supplementary Materials and Methods.

The constructs and transgenic worm lines used in this study will be made available through AddGene and CGC, respectively.

Strains

All strains were maintained on NGM plates at 20°C except where otherwise noted. Strains used in this study are listed in supplementary material Table S5.

To obtain highly synchronized larvae without bleaching, adults of the indicated genotypes were transferred onto seeded NGM plates and allowed to lay eggs for an hour. Adults were then removed and the embryos were cultured for appropriate times to allow them to reach the indicated developmental stages.
Synchronized adults were obtained by picking L4 larvae and maintaining them for 20-24 hours at 20°C.

To obtain the synchronized L1 larvae used to generate the data in supplementary material Table S2, two plates of each strain were suspended using M9 buffer. After a wash with M9 buffer, worms were bleached for 4 minutes. Eggs were then washed twice with M9 buffer, and starved in M9 buffer overnight to synchronize to L1 stage.

**Auxin treatment**

Unless otherwise indicated, auxin treatment was performed by transferring worms to bacteria-seeded plates containing auxin. The natural auxin indole-3-acetic acid (IAA) was purchased from Alfa Aesar (Cat. # A10556). A 400 mM stock solution in ethanol was prepared and was stored at 4°C for up to one month. Auxin was added into the NGM agar, cooled to about 50°C, before pouring plates. Because we found that high concentrations of auxin (e.g., 4 mM) inhibited bacterial growth, a
fresh OP50 culture was highly concentrated before spreading plates. Plates were left at room temperature for 1-2 days to allow bacterial lawn growth.

For auxin treatment in liquid culture, S basal buffer was supplemented with 3% (v/v) pelleted OP50 and the indicated concentration of auxin. For all auxin treatments, 0.25% ethanol was used as control.

**Viability and fertility**

To score total progeny (brood size) and male self-progeny, L4 hermaphrodites were picked onto individual plates with or without auxin, and transferred to new plates daily over 4 days. The eggs laid on each plate were counted after removing the parent. Viable progeny and male progeny were quantified when the F1 reached L4 or adult stages (2-3 days post egg laying).

**Microscopy and image acquisition**

To permit direct comparisons of worms of different genotypes or experimental conditions, animals were lined up side-by-side on agarose pads immediately prior to imaging. Briefly, 2-3 µl of buffer containing 100 mM sodium
azide was spotted on a freshly-made 2% agarose pad, and 4-6 worms were then transferred into the liquid spot using a pick. As the liquid absorbed into the pad, worms were quickly manipulated to lie side-by-side, and overlaid with a coverslip or not. Fluorescence images were acquired immediately to avoid dehydration of the animals. Wide-field optical sections at 1-µm z-spacing were acquired with a DeltaVision Elite microscope (Applied Precision) using a 10X N.A. 0.40 air objective, and pseudocolored using the SoftWoRx package or Adobe Photoshop. Images were not deconvolved. For each data stack, a single optical section near the middle of the animals with the highest GFP signal was selected for presentation. For the images in Fig. 3B, animals were picked into 2-3 microliters of buffer containing 100 mM sodium azide on a freshly-made 2% agarose pad, and overlaid with a coverslip. Images were acquired using DIC optics and a 63x objective on an Axioplan 2 (Zeiss) microscope running Micromanager.

To quantify the degradation in Fig. 1B and 1D, all treatments and image collection were performed in parallel. Images were acquired as described above
using a constant exposure for GFP (DHC-1-degron-GFP or degron-SMU-2-GFP), which was set to maximize signal/noise while avoiding camera saturation. Fluorescence quantification was performed on a single, unprocessed optical section from the middle of each data stack. Worms were outlined using the selection tool in ImageJ (NIH), and the average green fluorescence intensity for each animal was measured using a plugin (‘Analyze’-‘Measure RGB’) in ImageJ. Background intensity values, measured in wild type worms treated in parallel with auxin, were subtracted from each measurement. The fluorescence intensity in treated worms was normalized by dividing the value for each worm by the measured intensity in an untreated worm in the same microscope field. An analogous approach was used to measure the rate of recovery of protein expression after auxin removal in Fig. 2E, with background intensities measured in worms that remained on auxin plates during the recovery period.

To quantify intestine-specific degradation of SMU-2, as reported in Fig. 4B, worms were dissected to extrude their intestines in 1X Egg Buffer (25 mM HEPES, pH
7.4, 118 mM NaCl, 48 mM KCl, 2 mM EDTA, 0.5 mM EGTA) without detergents, then fixed with 1% formaldehyde for 2 min, washed with PBST, stained with DAPI, washed again, and mounted in glycerol-NPG mounting medium. All images were collected as stacks of 16 optical sections at intervals of 0.5 μm using a DeltaVision Elite microscope (Applied Precision) with a 20X N.A. 0.75 air objective. A maximum-intensity projection through the data stack was calculated. Individual intestinal nuclei in these images were manually segmented in ImageJ based on the DAPI signal, and their average green fluorescence intensity was measured as described above. Background fluorescence was measured in nuclei from wild-type worms treated in parallel, and this value was subtracted from the mean nuclear intensity value for each worm. These background-corrected values were expressed as a percent of the mean nuclear fluorescence intensity measured in control (non-auxin-treated) worms. Data are reported as mean ± S.D. for all worms in 3 independent experiments.

Immunofluorescence experiments were performed according to published protocols (Phillips et al., 2009). Briefly, young adult hermaphrodites (20-24 hours
post-L4) were dissected in Egg Buffer containing 15 mM sodium azide and 0.1% Tween 20, followed by fixation with 1% formaldehyde in the same buffer on a coverslip for 1 min. The coverslip with worms was then picked up using a Histobond slide (VWR), blotted to remove any excess fixative, and frozen on dry ice. After removal of the coverslip, slides with adhered worms were transferred to -20°C methanol for 1 min. Samples were then washed in PBST (PBS containing 0.1% Tween 20) and blocked with Blocking Reagent (Roche) in PBST. Primary antibody incubations were performed overnight at 4°C. After washing with PBST, secondary antibody incubations, and DAPI staining were conducted sequentially at room temperature. Primary antibodies used were as follows: HTP-3 (Guinea pig, 1:500), SYP-1 (Rabbit, 1:500). Secondary antibodies labeled with Alexa 488, Cy3 or Cy5 were purchased from Jackson Immunoresearch. All images were acquired as z-stacks through 8-μm depth at intervals of 0.2 μm using a DeltaVision Elite microscope (Applied Precision) with a 100X N.A. 1.4 oil-immersion objective. Image deconvolution, projection, and colorization were performed using the SoftWoRx package and Photoshop CC 2014 (Adobe).
Western blotting

For anti-GFP western blots (Fig. 2A), 20-30 adult worms of the indicated genotypes were picked into SDS sample buffer and lysed by boiling for 30 min, with occasional vortexing. Whole-worm lysates were separated on 4-12% polyacrylamide gradient gels and blotted onto nitrocellulose membranes. Antibodies against GFP (Roche) and α-tubulin (Sigma-Aldrich) were used at 1:1000 and 1:5000 respectively. HRP-conjugated secondary antibodies (Jackson Laboratory) and ECL reagents (Amersham) were used for detection.

To quantify western blots, TIF images were recorded for each blot using a Chemidoc system (BioRad), converted to 8-bit grayscale using Adobe Photoshop, and the integrated intensity of each GFP and α-tubulin band was calculated using ImageJ (NIH). The GFP band intensity was normalized by dividing by the corresponding α-tubulin band intensity. Each normalized GFP band intensity was expressed as a percentage of the intensity at t=0.
For anti-FLAG western blots (Fig. 3C), a synchronized dauer culture was generated as previously described (Wang and Kim, 2003). Animals were released from dauer by feeding with HB101 and cultured for six hours at 25ºC with 150 rpm shaking. A “0 minute” sample (1500 animals) was taken, and then either 0.25% ethanol or 1 mM auxin was added. At the indicated time points, 1500 animals were harvested, washed, and resuspended in 100 µl of M9+gelatin. Thirty µl of 4 x SDS sample buffer was added, and lysates were made by boiling for 10 minutes, freezing on dry ice for 20 minutes, and boiling for 10 minutes. Proteins were resolved, probed, and imaged as described previously (Ward, 2015). Anti-FLAG conjugated to horseradish peroxidase (1:2000) (Sigma no. 8592) was used, and the blot was developed using SuperSignal West Femto ECL substrate (Thermo Scientific no. 34095).
Acknowledgements

Some strains used in this study were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The *smu-2::GFP* plasmid pAS17 was kindly provided by Todd Starich, Caroline Spike, and Jocelyn Shaw (University of Minnesota, USA). We thank members of the Dernburg lab for helpful suggestions during the course of this work and for critical reading of the manuscript.

Competing interests

The authors declare no competing financial interests.

Author contributions

All authors designed the experiments. L.Z., J.D.W. and Z.C. performed the experiments. L.Z. and A.F.D performed data analysis. L.Z., J.D.W. and A.F.D wrote the paper.

Funding

This work was supported by funding to the Dernburg Lab from the National Institutes of Health (GM065591) and the Howard Hughes Medical Institute. J.D.W. was supported by the National Institute of General Medical Sciences of the NIH under award no. K99GM107345. Additional support was from NIH (CA20535) and U.S. National Science Foundation (MCB 1157767) awards to K. Yamamoto.
Fig. 1. The auxin-inducible degradation (AID) system enables degradation of cytoplasmic and nuclear proteins in larval and adult *C. elegans*. (A) Inducible
degradation of the cytoplasmic dynein heavy chain, DHC-1, in the soma. Animals with a degron-GFP cassette inserted at the 3' end of the endogenous dhc-1 gene in a $P_{eft-3}::TIR1::mRuby::unc-54$ 3'UTR genetic background were treated with (+) or without (-) 1 mM auxin for two hours. Worms were then immobilized and imaged. Wild type (WT) worms treated with 1 mM auxin for two hours were included to measure background fluorescence. L2 larvae are shown for clarity because their germline has not yet proliferated extensively, facilitating observation of somatic degradation; other developmental stages are shown in supplementary material Fig. S2. Scale bar, 50 μm. (B) Quantification of DHC-1-degron-GFP degradation in A as described in “Materials and Methods“. Data are presented as the mean ± S.D. from three independent experiments ($n = 18$ worms). (C) Inducible degradation of the nuclear SMU-2 protein in the soma. L2 larvae expressing degron-SMU-2-GFP from an extrachromosomal array and mRuby-tagged TIR1 from an integrated transgene were treated, immobilized and imaged as described in A. Scale bar, 50 μm. (D) Quantification of degron-SMU-2-GFP degradation in C. Data are presented as the mean ± S.D. from three independent experiments ($n = 17$ worms). (E) Inducible
degradation in the adult soma. Young adult worms expressing degron-SMU-2-GFP and TIR1-mRuby were treated with (+) or without (-) 1 mM auxin for three hours and then immobilized and imaged as described in A. Scale bar, 50 μm.
Fig. 2. AID-mediated degradation is rapid and reversible. (A) Young adult worms expressing degron-tagged GFP and TIR1-mRuby from the same somatic driver ($P_{eft-3}$::unc-54 3’UTR) were treated with auxin in S basal buffer containing OP50. Worms
were then lysed at various time points, and Western blots were performed using antibodies against GFP and tubulin. (B) Degradation rates were determined using the blots shown in A as described in “Materials and Methods”. Data are presented as mean ± S.D. from three independent experiments. (C) Low concentrations of auxin permit efficient degradation in larvae. L1 larvae expressing degron-GFP and TIR1-mRuby were treated with 25 μM or 1 mM auxin (+) or without (-) auxin for two hours. Worms were then immobilized and imaged as mentioned in Fig. 1. Scale bar, 50 μm. (D) Conditional degradation is reversible following removal of auxin. L1 larvae treated with 25 μM auxin for two hours in C were transferred onto fresh NGM plates. Recovery of degron-tagged GFP was examined at indicated time points. Worms without auxin treatment and those left on auxin plates were included as controls. Scale bar, 50 μm. (E) Quantification of the relative recovery rates in D (recovery from 25 μM auxin) and in supplementary material Fig. S3 (recovery from 1 mM auxin) as described in “Materials and Methods”. Data are presented as means ± S.D. from three independent experiments.
Fig. 3. The AID system enables functional analysis of nuclear hormone receptors during development. (A) Brood sizes (± S.D.) and embryonic lethality in progeny of control or 1 mM auxin treated animals of the indicated genotype. Synchronized
animals (n≥173) of the indicated genotype were obtained by a timed, 4-hour egglaying period on control or auxin-containing plates. These animals were scored for developmental rate (% animals that were L4 or adults following 48 hours at 25°C), larval arrest prior to L4, molting defects, and gonadal defects. (B) Representative images of animals from the timed egg lay on control or 1 mM auxin plates following 60 hours at 25°C. In the absence of auxin, no defects were seen in any genotype, however in the presence of auxin, worms expressing degron-tagged NHR-25 and pan-somatic TIR1 showed molting defects (arrow indicated unshed cuticle) and gonadal defects such as tumorous germlines (note lack of eggs and abnormal germline). Animals expressing degron-tagged NHR-23 and pan-somatic TIR1 uniformly arrested as L1 larvae. Scale bar, 50 µm. (C) Temporal analysis of inducible protein degradation. Worms of the indicated genotypes were grown for six hours at 25°C following dauer release before 0.25% ethanol (control) or 1 mM auxin were added and samples were harvested every 20 minutes. Lysates were resolved by SDS-PAGE and immunoblotted with the indicated antibodies. Stain-free (Bio-Rad) analysis of total protein on each blot is provided as a loading control. Marker size
(in kilodaltons) is provided. Two isoforms of NHR-25 (a and b) are detected, similar to that described by Ward, 2015.
Fig. 4. The AID system permits tissue-specific degradation in *C. elegans*. (A) The *ges-1* promoter was used to drive TIR1 expression in the intestine. L3 larvae carrying
this transgene and degron-tagged SMU-2 from arrays were treated with (+) or without (-) 1 mM auxin for three hours. Worms were then dissected and intestines were extruded to monitor residual SMU-2-GFP in this tissue. DNA was stained with 4',6-diamidino-2-phenylindole (DAPI) to indicate the nuclei. Insets show higher-magnification views of the outlined regions. Scale bar, 50 µm. (B) Quantification of degron-SMU-2-GFP degradation in the intestine. Data are presented as the mean ± S.D. from three independent experiments (n = 144 nuclei, 15 worms). (C) Tissue-specific degradation in adults. Young adult worms expressing degron-SMU-2-GFP from arrays and TIR1 in the intestine were treated with (+) or without (-) 1 mM auxin for three hours. Wild type worms (WT) treated with auxin were included as background control. Scale bar, 50 µm. (D) Inducible degradation in the germline. Young adults expressing TIR1 driven by the sun-1 promoter and 3'UTR along with degron-tagged DHC-1 were treated with (+) or without (-) 1 mM auxin for two hours. Worms were then dissected, fixed, and imaged. Scale bar, 50 µm. (E) Inducible degradation in embryos. Eggs laid by hermaphrodites expressing dhc-1::degron::GFP
and \( P_{eft-3}::TIR1::mRuby::unc-54 \) 3'UTR were treated with 1 mM or 4 mM auxin (+) or without (-) auxin in S basal buffer for indicated times. Scale bar, 5 μm.
Fig. 5. Conditional depletion of DHC-1 in the germline reveals its essential function in meiosis. (A) Rapid degradation of DHC-1-degron-GFP in the germline. 

dhc-1::degron::GFP, Psun-1::TIR1::mRuby young adult animals were treated with 1mM auxin (+) or without (-) auxin for the indicated time. Worms were then dissected, fixed, stained, and imaged. Four times enlarged images were shown for indicating
efficient degradation. Scale bar, 50 μm. (B) Low–magnification views of germlines stained for SYP-1 (green) and HTP-3 (red) to monitor synapsis. SYP-1 is a synaptonemal complex protein, while HTP-3 is a component of the chromosome axes (MacQueen et al., 2002; MacQueen et al., 2005). Synapsis defect was indicated by mislocalization of SYP-1. dhc-1::degron::GFP, psun-1::TIR1::mRuby adults were treated with (+) or without (-) 1 mM auxin for the indicated times. Scale bar, 5 μm. (C) Higher magnification views from the corresponding regions in B. Scale bar, 5 μm.
References

Armenti, S. T., Lohmer, L. L., Sherwood, D. R. and Nance, J. (2014). Repurposing an endogenous degradation system for rapid and targeted depletion of C. elegans proteins. Development 141, 4640-4647.

Arribere, J. A., Bell, R. T., Fu, B. X., Artiles, K. L., Hartman, P. S. and Fire, A. Z. (2014). Efficient marker-free recovery of custom genetic modifications with CRISPR/Cas9 in Caenorhabditis elegans. Genetics 198, 837-846.

Asahina, M., Valenta, T., Silhankova, M., Korinek, V. and Jindra, M. (2006). Crosstalk between a nuclear receptor and beta-catenin signaling decides cell fates in the C. elegans somatic gonad. Dev. Cell 11, 203-211.

Bacaj, T. and Shaham, S. (2007). Temporal control of cell-specific transgene expression in Caenorhabditis elegans. Genetics 176, 2651-2655.

Banaszynski, L. A., Chen, L. C., Maynard-Smith, L. A., Ooi, A. G. and Wandless, T. J. (2006). A rapid, reversible, and tunable method to regulate protein function in living cells using synthetic small molecules. Cell 126, 995-1004.

Bonger, K. M., Chen, L. C., Liu, C. W. and Wandless, T. J. (2011). Small-molecule displacement of a cryptic degron causes conditional protein degradation. Nat. Chem. Biol. 7, 531-537.

Broeks, A., Janssen, H. W., Calafat, J. and Plasterk, R. H. (1995). A P-glycoprotein protects Caenorhabditis elegans against natural toxins. EMBO J. 14, 1858-1866.

Carvalho, A., Olson, S. K., Gutierrez, E., Zhang, K., Noble, L. B., Zanin, E., Desai, A., Groisman, A. and Oegema, K. (2011). Acute drug treatment in the early C. elegans embryo. PLoS One 6, e24656.

Caustrinus, E., Kanca, O. and Affolter, M. (2012). Fluorescent fusion protein knockout mediated by anti-GFP nanobody. Nat. Struct. Mol. Biol. 19, 117-121.

Chen, Z., Eastburn, D. J. and Han, M. (2004). The Caenorhabditis elegans nuclear receptor gene nhr-25 regulates epidermal cell development. Mol. Cell Biol. 24, 7345-7358.

Cheng, Z., Yi, P., Wang, X., Chai, Y., Feng, G., Yang, Y., Liang, X., Zhu, Z., Li, W. and Ou, G. (2013). Conditional targeted genome editing using somatically expressed TALENs in C. elegans. Nat. Biotechnol. 31, 934-937.

Cho, U., Zimmerman, S. M., Chen, L. C., Owen, E., Kim, J. V., Kim, S. K. and Wandless, T. J. (2013). Rapid and tunable control of protein stability in Caenorhabditis elegans using a small molecule. PLoS One 8, e72393.

Davis, M. W., Morton, J. J., Carroll, D. and Jorgensen, E. M. (2008). Gene activation using FLP recombinase in C. elegans. PLoS Genet. 4, e1000028.

Dharmasiri, N., Dharmasiri, S. and Estelle, M. (2005). The F-box protein TIR1 is an auxin receptor. Nature 435, 441-445.

Dickinson, D. J., Pani, A. M., Heppert, J. K., Higgins, C. D. and Goldstein, B. (2015). Streamlined Genome Engineering with a Self-Excising Drug Selection Cassette. Genetics. 200, 1035-1049.

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.

Dohmen, R. J., Wu, P. and Varshavsky, A. (1994). Heat-inducible degron: a method for constructing temperature-sensitive mutants. Science 263, 1273-1276.

Elbashir, S. M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K. and Tuschl, T. (2001). Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411, 494-498.

Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E. and Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391, 806-811.
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.

Gray, W. M., del Pozo, J. C., Walker, L., Hobbie, L., Risseeuw, E., Banks, T., Crosby, W. L., Yang, M., Ma, H. and Estelle, M. (1999). Identification of an SCF ubiquitin-ligase complex required for auxin response in *Arabidopsis thaliana*. *Genes Dev.* **13**, 1678-1691.

Holland, A. J., Fachinetti, D., Han, J. S. and Cleveland, D. W. (2012). Inducible, reversible system for the rapid and complete degradation of proteins in mammalian cells. *Proc. Natl. Acad. Sci. U S A* **109**, E3350-3357.

Kanke, M., Nishimura, K., Kanemaki, M., Kakimoto, T., Takahashi, T. S., Nakagawa, T. and Masukata, H. (2011). Auxin-inducible protein depletion system in fission yeast. *BMC Cell Biol.* **12**, 8.

Kepinski, S. and Leyser, O. (2005). The *Arabidopsis* F-box protein TIR1 is an auxin receptor. *Nature* **435**, 446-451.

Kim, H., Ishidate, T., Ghanta, K. S., Seth, M., Conte, D., Jr., Shirayama, M. and Mello, C. C. (2014). A co-CRISPR strategy for efficient genome editing in *Caenorhabditis elegans*. *Genetics* **197**, 1069-1080.

Kostrouchova, M., Krause, M., Kostrouch, Z. and Rall, J. E. (2001). Nuclear hormone receptor CHR3 is a critical regulator of all four larval molts of the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U S A* **98**, 7360-7365.

Kostrouchova, M., Krause, M., Kostrouch, Z. and Rall, J. E. (2001). Nuclear hormone receptor CHR3 is a critical regulator of all four larval molts of the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U S A* **98**, 7360-7365.

Kreidenweiss, A., Hopkins, A. V. and Mordmuller, B. (2013). 2A and the auxin-based degron system facilitate control of protein levels in *Plasmodium falciparum*. *PLoS One* **8**, e78661.

Kreidenweiss, A., Hopkins, A. V. and Mordmuller, B. (2013). 2A and the auxin-based degron system facilitate control of protein levels in *Plasmodium falciparum*. *PLoS One* **8**, e78661.

Lindblom, T. H., Pierce, G. J. and Sluder, A. E. (2001). A *C. elegans* orphan nuclear receptor contributes to xenobiotic resistance. *Curr. Biol.* **11**, 864-868.

MacQueen, A. J., Colaiacco, M. P., McDonald, K. and Villeneuve, A. M. (2002). Synapsis-dependent and -independent mechanisms stabilize homolog pairing during meiotic prophase in *C. elegans*. *Genes Dev.* **16**, 2428-2442.

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.

Malone, C. J., Misner, L., Le Bot, N., Tsai, M. C., Campbell, J. M., Ahringer, J. and White, J. G. (2003). The *C. elegans* hook protein, ZYG-12, mediates the essential attachment between the centrosome and nucleus. *Cell* **115**, 825-836.

Minn, I. L., Rolls, M. M., Hanna-Rose, W. and Malone, C. J. (2009). SUN-1 and ZYG-12, mediators of centrosome-nucleus attachment, are a functional SUN/KASH pair in *Caenorhabditis elegans*. *Mol. Biol. Cell* **20**, 4586-4595.

Morawska, M. and Ulrich, H. D. (2013). An expanded tool kit for the auxin-inducible degron system in budding yeast. *Yeast* **30**, 341-351.

Nayak, S., Santiago, F. E., Jin, H., Lin, D., Schedl, T. and Kipreos, E. T. (2002). The *Caenorhabditis elegans* Skp1-related gene family: diverse functions in cell proliferation, morphogenesis, and meiosis. *Curr Biol* **12**, 277-287.

Nishimura, K., Fukagawa, T., Takisawa, H., Kakimoto, T. and Kanemaki, M. (2009). An auxin-based degron system for the rapid depletion of proteins in nonplant cells. *Nat. Methods* **6**, 917-922.

Norris, A. D., Kim, H. M., Colaiacovo, M. P. and Calarco, J. A. (2015). Efficient Genome Editing in *Caenorhabditis elegans* with a Toolkit of Dual Marker Selection Cassettes. *Genetics* **201**, 449-458.

Paix, A., Wang, Y., Smith, H. E., Lee, C. Y., Calidas, D., Lu, T., Smith, J., Schmidt, H., Krause, M. W. and Seydoux, G. (2014). Scalable and versatile genome editing using linear DNAs with microhomology to Cas9 Sites in *Caenorhabditis elegans*. *Genetics* **198**, 1347-1356.

Philip, N. and Waters, A. P. (2015). Conditional Degradation of *Plasmodium* Calcineurin Reveals Functions in Parasite Colonization of both Host and Vector. *Cell Host Microbe* **18**, 122-131.
Phillips, C. M., McDonald, K. L. and Dernburg, A. F. (2009). Cytological analysis of meiosis in *Caenorhabditis elegans*. *Methods Mol. Biol.* **558**, 171-195.

Qadota, H., Inoue, M., Hikita, T., Koppen, M., Hardin, J. D., Amano, M., Moerman, D. G. and Kaibuchi, K. (2007). Establishment of a tissue-specific RNAi system in *C. elegans*. *Gene* **400**, 166-173.

Raina, K. and Crews, C. M. (2010). Chemical inducers of targeted protein degradation. *J. Biol. Chem.* **285**, 11057-11060.

Renicke, C., Schuster, D., Usherenko, S., Essen, L. O. and Taxis, C. (2013). A LOV2 domain-based optogenetic tool to control protein degradation and cellular function. *Chem. Biol.* **20**, 619-626.

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

Ruegger, M., Dewey, E., Gray, W. M., Hobbie, L., Turner, J. and Estelle, M. (1998). The TIR1 protein of *Arabidopsis* functions in auxin response and is related to human SKP2 and yeast grr1p. *Genes Dev.* **12**, 198-207.

Sato, A., Isaac, B., Phillips, C. M., Rillo, R., Carlton, P. M., Wynne, D. J., Kasad, R. A. and Dernburg, A. F. (2009). Cytoskeletal forces span the nuclear envelope to coordinate meiotic chromosome pairing and synapsis. *Cell* **139**, 907-919.

Shen, Z., Zhang, X., Chai, Y., Zhu, Z., Yi, P., Feng, G., Li, W. and Ou, G. (2014). Conditional knockouts generated by engineered CRISPR-Cas9 endonuclease reveal the roles of coronin in *C. elegans* neural development. *Dev. Cell* **30**, 625-636.

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.

Tan, X., Calderon-Villalobos, L. I., Sharon, M., Zheng, C., Robinson, C. V., Estelle, M. and Zheng, N. (2007). Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature* **446**, 640-645.

Taxis, C., Stier, G., Spadaccini, R. and Knop, M. (2009). Efficient protein depletion by genetically controlled deprotection of a dormant N-degron. *Mol. Syst. Biol.* **5**, 267.

Wang, J. and Kim, S. K. (2003). Global analysis of dauer gene expression in *Caenorhabditis elegans*. *Development* **130**, 1621-1634.

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.

Ward, J. D., Bojanala, N., Bernal, T., Ashrafi, K., Asahina, M. and Yamamoto, K. R. (2013). Sumoylated NHR-25/NRSA regulates cell fate during *C. elegans* vulval development. *PLoS Genet.* **9**, e1003992.

Wang, J. D., Mullaney, B., Ashrafi, K., et al. (2014). Defects in the *C. elegans* acyl-CoA synthase, *acs-3*, and nuclear hormone receptor, *nhr-25*, cause sensitivity to distinct, but overlapping stresses. *PLoS One* **9**, e92552.

Wei, X., Potter, C. J., Luo, L. and Shen, K. (2012). Controlling gene expression with the Q repressible binary expression system in *Caenorhabditis elegans*. *Nat. Methods* **9**, 391-395.

Wynne, D. J., Rog, O., Carlton, P. M. and Dernburg, A. F. (2012). Dynein-dependent processive chromosome motions promote homologous pairing in *C. elegans* meiosis. *J. Cell Biol.* **196**, 47-64.

Yu, H., Moss, B. L., Jang, S. S., Prigge, M., Klavins, E., Nemhauser, J. L. and Estelle, M. (2013). Mutations in the TIR1 auxin receptor that increase affinity for auxin/indole-3-acetic acid proteins result in auxin hypersensitivity. *Plant Physiol.* **162**, 295-303.

Zhou, K., Rolls, M. M., Hall, D. H., Malone, C. J. and Hanna-Rose, W. (2009). A ZYG-12-dynein interaction at the nuclear envelope defines cytoskeletal architecture in the *C. elegans* gonad. *J Cell Biol* **186**, 229-241.