Identification of a nuclear receptor/coactivator developmental signaling pathway in the nematode parasite Strongyloides stercoralis

Mi Cheong Cheonga, Zhu Wangb, Tegegn G. Jaletab, Xinshe Lib, James B. Lokb, Steven A. Kliewera,c,1, and David J. Mangelsdorfa,d,1

DAF-12 is nematode-specific nuclear receptor that has been proposed to govern development of the infectious stage of parasitic species, including Strongyloides stercoralis. Here, we identified a parasite-specific coactivator, called DAF-12 interacting protein-1 (DIP-1), that is required for DAF-12 ligand-dependent transcriptional activity. DIP-1 is found only in Strongyloides spp. and selectively interacts with DAF-12 through an atypical receptor binding motif. Using CRISPR/Cas9-directed mutagenesis, we demonstrate that DAF-12 is required for the requisite developmental arrest and the ligand-dependent reactivation of infectious S. stercoralis infective third-stage larvae, and that these effects require the DIP-1 coactivator. These studies reveal the existence of a distinct nuclear receptor/coactivator signaling pathway that governs parasite development.

DAF-12 | nuclear receptor | coactivator | nematode parasite | Strongyloides stercoralis

Parasitic nematodes are an enormous medical and economic burden worldwide (1). Despite the large number of diseases caused by these nematodes, the number of effective anthelmintic drugs is limited and resistance is an emerging problem (2, 3). To develop new drugs that target parasitic nematodes, a better understanding of the mechanisms that control the parasitic nematode life cycle is needed. Many parasitic nematodes undergo a complicated life cycle that requires several different host species to propagate, and so suitable models to study them are lacking. To circumvent this problem, Caenorhabditis elegans has served as a model system for parasitic nematode research because many aspects of development and gene function are conserved between C. elegans and parasitic species. Nevertheless, a complete understanding of the biology of parasitic nematodes and the mechanisms that are common to and differ from C. elegans are necessary to identify new therapeutic targets in these important pathogens.

DAF-12 is a nuclear hormone receptor initially identified in C. elegans (4) and is an important regulator of worm metabolism and development (4, 5). The transcriptional activity of DAF-12 is similar to that of other endocrine steroid receptors and depends on the binding of nematode-specific steroid hormones called dafachronic acids (DA) (6, 7). This endocrine signaling pathway governs a key checkpoint in the life cycle C. elegans, by determining whether the worm arrests its development and enters a dormant larval stage, called dauer diapause, or continuously develops to mature reproductive adults. During unfavorable conditions, the DA hormone is not produced and DAF-12 functions as a transcriptional corepressor that results in dauer diapause, or continuously develops to mature reproductive adults. Under favorable conditions, DA is synthesized, resulting in DAF-12-dependent expression of genes involved in promoting reproductive development and preventing dauer arrest.

DAF-12 is one of the most well-conserved signaling pathways between C. elegans and parasitic worms (8). The dauer stage resembles the infective third stage (L3) of parasitic nematodes, which is specialized to seek out and infect host organisms. Orthologs of DAF-12 have been identified in all parasitic species surveyed, including hookworms, threadworms, and filarial parasitic nematodes (9–14). One such parasite is Strongyloides stercoralis, which infects 200 to 300 million persons worldwide and under certain conditions can cause a disseminated hyperinfection that is frequently fatal if untreated (15, 16). The DAF-12 homolog of S. stercoralis has 42% sequence similarity with C. elegans DAF-12 and a similar affinity for binding DA (10). Previous studies showed that exogenous DA treatment in postfree-living S. stercoralis prevents the formation of the L3 stage (10, 11). Moreover, the most active ligand of DAF-12, Δ7-DA, significantly reduces parasite burdens in mice with an S. stercoralis hyperinfection (17). Taken together, these studies suggest that DAF-12 has potential as a therapeutic target for treating S. stercoralis parasitism and perhaps parasitism by other nematodes. Thus, characterizing the DAF-12 signaling pathway in S. stercoralis is essential to explore this potential.

**Significance**
Understanding the molecular mechanisms controlling nematode parasite infection is important to developing new therapeutic strategies. The DAF-12 nuclear receptor signaling pathway in free-living nematodes regulates a process known as the dauer diapause. In nematode parasites, this signaling pathway is believed to govern the infectious stage. To investigate this hypothesis, we characterized the orthologous components of DAF-12 and a parasite-specific transcriptional coactivator in the potentially lethal parasite Strongyloides stercoralis. Using a CRISPR method developed specifically for S. stercoralis, we demonstrate that DAF-12 and its coactivator are required for both the entry into and exit from the infectious stage. These studies further highlight the potential of therapeutically targeting this pathways in S. stercoralis and other nematode parasites.

Author contributions: M.C.C., J.B.L., S.A.K., and D.J.M. designed research; M.C.C. and Z.W. performed research; J.B.L., S.A.K., and D.J.M. supervised the project; T.G.J. and X.L. contributed new reagents/analytic tools; M.C.C., Z.W., J.B.L., S.A.K., and D.J.M. analyzed data; and M.C.C., J.B.L., S.A.K., and D.J.M. wrote the paper.

Reviewers: A.A., Max Planck Institute for Biology of Ageing; and D.D.M., Baylor College of Medicine.

The authors declare no competing interest.

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1To whom correspondence may be addressed. Email: Steven.Kliewer@UTSouthwestern.edu or davo.mango@utsouthwestern.edu.

This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2021864118/-/DCSupplemental.

Published February 18, 2021.
As transcription factors, the activities of nuclear hormone receptors like DAF-12 are tuned by coregulators (corepressors and coactivators). Corepressors generally interact with unliganded receptors to repress target gene expression, whereas coactivators typically interact with ligand-bound receptors to stimulate gene expression. In mammals, several dozen nuclear hormone coregulators have been identified and found to be associated with numerous disease states and metabolic disorders ([18]). In *C. elegans*, the ligand-dependent activity of DAF-12 is governed by one known coregulator, the corepressor DIN-1 ([19]).

The study of this coregulator has added to our understanding of daf-12 phenotypic complexity and the endocrine signaling network that it regulates. However, interestingly, DIN-1 is not present in *S. stercoralis*, leaving open the question of how the transactivation of DAF-12 is regulated in the parasite.

In this study, we identified a *Strongyloides* parasite-specific DAF-12 coactivator called DAF-12 interacting protein-1 (DIP-1) and investigated the requirement of both the DAF-12 receptor and the coactivator for their roles in regulating the parasite’s lifecycle. We find that *S. stercoralis* (Ss)-DAF-12 and Ss-DIP-1 are key factors for *S. stercoralis* to progress to the infectious iL3 stage in its life cycle, and thus represent potential new drug targets to combat *S. stercoralis* infection.

**Results**

**Ss-DAF-12 Is Essential for Development of Infectious-Stage Parasites.**

We previously showed that Δ7-DA binds and activates the *S. stercoralis* daf-12 gene homolog, Ss-daf-12, and that premature transactivation of Ss-DAF-12 blocks iL3 development ([10], [11]). However, the lack of an appropriate experimental system has prevented the study of the direct involvement of Ss-DAF-12 in governing the parasite’s biology. Although RNAi has not been effective in *S. stercoralis*, CRISPR-based approaches have been successful ([20]). To that end, we used CRISPR editing to disrupt the ligand-dependent activation function-2 (AF-2) domain of Ss-daf-12 with the insertion of a homology-directed repair template that contains a GFP reporter gene under the control of the *Strongyloides ratti* (Sr) eef-1 promoter (Fig. 1A). The Sr-eef-1 promoter drives a high level of reporter expression in *S. stercoralis* that permits selection of CRISPR-targeted *F1* progeny expressing GFP from injected postparasitic free-living females. Single-worm PCR was performed with primers shown in *SI Appendix* comparing a wild-type iL3 worm (9Δ) and is consistent with that observed in Δ7-DA–treated worms (9Δ) and with the conclusion that at the infectious stage of the lifecycle, Ss-DAF-12 is essential for the development of infective larvae.

To further address the timing and requirement of Ss-daf-12 for iL3 development, we repeated the experiment and collected worms beginning at 3 d after injection when the postfree-living L2 worms are just beginning to transition to the iL3 stage (Fig. 1C). At this time point, viable GFP+ worms were present, but all had defects in body morphology and molting, or had developed to precocious L4/adult-stage worms that had bypassed the arrested iL3 stage (Fig. 1C and D). This latter phenotype is similar to that observed in Δ7-DA–treated worms (9Δ) and is consistent with the conclusion that at the infectious stage of the lifecycle, Ss-DAF-12 is required for developmental arrest and iL3 formation. Accordingly, some Ss-daf-12 mutant larvae developed to morphologically fully formed second-generation free-living females (Fig. 1D). In *C. elegans*, a similar DAF-12 AF-2 mutation is called dauer-defective; these worms are not able to arrest as third-stage dauer larvae and instead exhibit the mutant phenotypes ([4]) similar to those observed in the Ss-daf-12 AF-2 mutation.

**Ss-DIP-1 Is a Parasite-Specific DAF-12 Coactivator.**

Given that *S. stercoralis* lacks any of the known nuclear receptor coregulators, we performed a yeast-two hybrid screen to identify the coactivator that is responsible for Ss-DAF-12 transactivation. We used the Ss-DAF-12 ligand-binding domain (LBD) as bait and screened against proteins from a cDNA library of genes expressed in the postfree-living L1, L2, and iL3 stages. We first confirmed that the Ss-DAF-12 LBD behaves similarly to other nuclear receptors by demonstrating that it can interact with the *C. elegans* cofactor, DIN-1S, in a DA-specific manner (*SI Appendix*, Fig. S2). We then

![Fig. 1.](https://doi.org/10.1073/pnas.2021864118)

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screened for *S. stercoralis*-specific proteins that interact with Ss-DAF-12. We identified one protein that interacts with Ss-DAF-12 in the presence of Δ7-DA (Fig. 2A), which we named Ss-DIP-1, as a candidate coactivator. Interestingly, the screen did not reveal any candidate corepressors. Ss-DIP-1 is a 525-amino acid protein with no readily identifiable functional domains (Fig. 2B). Moreover, no genes with significant similarity to Ss-dip-1 are present in the *C. elegans* (Ce) genome. The only organisms that we found with genes homologous to Ss-DIP-1 were in the parasitic nematode family Strongyloidae (SI Appendix, Fig. S3), suggesting that this coactivator might have evolved after this family diverged from other nematodes. Consistent with this phylogenetic specificity, Ce-DAF-12, which shares 42% identity in its LBD with Ss-DAF-12, interacted only weakly with Ss-DIP-1 (Fig. 2A).

To confirm the interaction between Ss-DAF-12 and Ss-DIP-1, we performed a mammalian two-hybrid assay. To overcome the poor expression of Ss-DAF-12 in mammalian cells, we used a chimeric receptor construct in which the Ce-DAF-12 DNA binding domain (DBD) was fused to the LBD of Ss-DAF-12 (10). The resulting construct (CeSs-DAF-12) was fused to the VP16 transactivation domain and assayed for its interaction with Gal4-Ss-DIP-1 in COS-7 cells. We used SRC-1 (steroid receptor coactivator-1) as a positive control because it is an established mammalian coactivator that interacts with Ce-DAF-12 and Ss-DAF-12 (6, 10). Despite being expressed in mammalian cells (SI Appendix, Fig. S4A), Gal4-Ss-DIP-1 increased expression of the reporter gene over 30-fold in the presence of VP16-CeSs-DAF-12 and its ligand, Δ7-DA (SI Appendix, Fig. S4B). Notably, when the Ss-DIP-1 sequence was codon-optimized for increased expression in mammalian cells (SI Appendix, Fig. S4A), the ligand-dependent interaction with DAF-12 increased to a level (>200-fold) that was similar to that observed with the SRC-1 control (Fig. 2C and SI Appendix, Fig. S4B). Numerous mammalian coactivators have been shown to interact with nuclear receptors (22, 23). SRC-1, for example, interacts with a broad range of nuclear receptors, including mammalian liver X nuclear receptor (LXR) and retinoid X receptor (RXR), as well as Ce-DAF-12 and Ss-DAF-12 (Fig. 2C). In contrast, Ss-DIP-1 coactivated only the DAF-12 receptor from *Strongyloides* spp. In addition to coactivating DAF-12 from *S. stercoralis* (Fig. 2C), Ss-DIP-1 from the related species, *Strongyloides* nattii, also markedly enhanced the ligand-dependent activation of DAF-12 (SI Appendix, Fig. S5), further demonstrating its unique receptor and species specificity.

We next tested transactivation of Ss-DAF-12 on a luciferase reporter containing the *lit-1* kinase promoter, which is an established DAF-12 target gene with identified DAF-12 binding sites. In the presence of Ss-DIP-1, Ss-DAF-12 transactivated the *lit-1* promoter and Δ7-DA markedly enhanced this activation (Fig. 2D), demonstrating that DIP-1 is a functional coactivator of Ss-DAF-12.

**Ss-DIP-1/Ss-DAF-12 Interaction Domains.** Nuclear receptor coactivators often have a conserved LXXLL motif, which mediates their interactions with nuclear receptors (24). However, Ss-DIP-1 does not have this motif. To identify the region of Ss-DIP-1 that interacts with Ss-DAF-12, we performed mammalian two-hybrid assays with truncation mutants of Ss-DIP-1 (amino acids 1 to 100, 1 to 67, and 1 to 34) and confirmed their expression in mammalian cells (SI Appendix, Fig. S6). Amino acids 1 to 100 and 1 to 67 interacted with Ss-DAF-12, while amino acids 1 to 34 did not, suggesting that residues 34 to 67 are important for interaction with Ss-DIP-1 (Fig. 2E). Inspection of this region revealed a single motif (FPTLL) that is similar to the canonical LXXLL motif. To test the importance of this motif, we individually mutated each of these residues to alanine. F56A, P57A, T58A, and L60A substitutions in Ss-DIP-1 caused modest decreases in its interaction with Ss-DAF-12, while the L59A mutant and F56A/P57A double-mutant caused severe impairment of this interaction (Fig. 3B). To further demonstrate the importance of this motif, we substituted the phenylalanine at amino acid 56 with a leucine to convert it from FXXLL to a canonical LXXLL motif. This F56L substitution in Ss-DIP-1 markedly decreased its interaction with Ss-DAF-12 (Fig. 3C). These results suggest that in contrast to other coregulators that preferentially mediate their interactions with receptors through LXXLL motifs, the FXXLL motif in Ss-DIP-1 is a specific adaptation for mediating its interaction with Ss-DAF-12.

To further characterize the molecular basis of Ss-DIP-1 coactivation of DAF-12, we investigated the structural features of Ss-DAF-12 responsible for the ligand-dependent interaction. Nuclear receptor LBDs have a conserved tripartite structure composed of a globular domain of α-helices (numbered H1 to H12) arranged in a three-layer “sandwich.” The X-ray crystal structure of Ss-DAF-12 has a similar structure and previous structural studies identified several amino acid residues required for ligand binding, including tryptophan-611 (10). Mutation of this residue (W611R) in Ss-DAF-12 abolished receptor transactivation as expected and disrupted the interactions with both Ss-DIP-1 and SRC-1 (Fig. 3D). In other nuclear receptors, the AF-2 domain in helix 12 is required for ligand-dependent interaction with coactivators like SRC-1 (25). In Ss-DAF-12 the glutamate (E747) in the AF-2 domain is conserved and, like in other nuclear receptors, forms hydrogen bonds with LXXLL coactivator sequences to form a charge clamp that stabilizes the receptor/coactivator interaction (26). Mutation of this residue (E747Q) in Ss-DAF-12 disrupted the ligand-dependent interaction between Ss-DAF-12 and Ss-DIP-1 (Fig. 3D). This result indicates that the AF-2 domain of Ss-DAF-12 mediates binding to Ss-DIP-1 similar to other coactivator–nuclear receptor interactions.

**Ss-dip-1 Expression.** To investigate *Ss-dip-1* expression in *S. stercoralis*, we generated a GFP reporter worm using a *Ss-dip-1p::GFP* expression construct. This construct showed GFP expression in head neurons, the pharynx, and the hypodermis (Fig. 4D). Using a *Ss-daf-12p::GFP* construct, we found that *Ss-daf-12* has a similar expression pattern, further supporting the coordinate role of these proteins and their biologically relevant interaction (Fig. 4B). We found that expression levels of both *Ss-dip-1* and *Ss-daf-12* reporters increased in the hypodermis at the iL3 stage (Fig. 4C).

**Ss-DIP-1 Controls iL3 Recovery by Ss-DAF-12.** Taken together, these findings support a model in which the entry and exit into the infectious iL3 stage is governed by a DAF-12–dependent gene-expression program similar to what has been observed in *C. elegans* entry and exit from dauer (5). To further test the role of Ss-DIP-1 in governing iL3 reactivation in vivo, we disrupted *DIP-1* expression in the *Sr-eef-1p::GFP* construct as a positive control because it is an established DAF-12 target gene with identified DAF-12 binding sites. In the presence of Ss-DIP-1, Ss-DAF-12 transactivated the *lit-1* promoter and Δ7-DA markedly enhanced this activation (Fig. 2D), demonstrating that DIP-1 is a functional coactivator of Ss-DAF-12.
as has been shown previously (27). Notably, disruption of Ss-dip-1 resulted in a similar phenotype and decreased parasite mortality (Fig. 5D), consistent with the notion that Ss-DIP-1 plays an important role in Δ7-DA–induced iL3 activation.

Finally, we examined genes that are known to be regulated by DAF-12 using qPCR. Previous work showed that Ss-acs-1, Ss-acbp-3, and Ss-F28H7.3 are regulated by Δ7-DA (5). Coactivators generally interact with ligand-bound nuclear receptors to stimulate gene expression. Therefore, we hypothesized that Ss-DIP-1 also regulates Ss-DAF-12 target gene expression. As expected, increased expression of Ss-acs-1, Ss-acbp-3, and Ss-F28H7.3 genes was abrogated in Ss-dip-1 CRISPR worms (Fig. 5E). These results support the notion that Ss-DIP-1 controls iL3 recovery through the transactivation of Ss-DAF-12. These findings confirm that Ss-DAF-12 is a key switch for parasitic development and that Ss-DIP-1 controls iL3 recovery as a Ss-DAF-12 coactivator.

**Discussion**

DAF-12 is a hormone-dependent nuclear receptor that is found uniquely in nematodes. In *C. elegans* it is a master transcriptional regulator of the metabolic and developmental program that controls dauer diapause and reproductive maturity (4, 5, 19). In parasitic species, the role of DAF-12 has not been thoroughly investigated but it has been proposed to govern the iL3 stage (8), which is similar to the L3 dauer stage found in free-living species (28). In this study, using newly developed transgenic techniques in the human parasite, *S. stercoralis*, we show that Ss-DAF-12 is a key factor in governing iL3-stage biology, and that this role requires an atypical transcriptional cofactor, Ss-DIP-1.

The discovery of Ss-DIP-1 as a DAF-12–specific coactivator provides an unprecedented understanding of a key molecular mechanism that governs parasite infection. Intriguingly, while Ss-DIP-1 shares features similar to the known canonical nuclear

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**Fig. 2.** Ss-DIP-1 is a Ss-DAF-12 coactivator. (A) Yeast two-hybrid interaction of Ss-DIP-1 with the ligand binding domain (L) of Ss-DAF-12 or Ce-DAF-12 in the presence of Δ7-DA (1 μM). Serial dilutions of yeast cells were spotted on both permissive (DDO and DDO/X) and selective (QDO/X/A) media and incubated for 3 d at 30 °C. DDO indicate SD/-Leu/-Trp and QDO indicate SD/-Ade/-His/-Leu/-Trp. “X” is X-α-gal and A indicates Aureobasidin A. (B) Ss-dip-1 (SSTP_0000616600) cDNA sequence and predicted protein product. Underline and red lettering indicate position of the FXXLL motif. (C) Mammalian two-hybrid interaction of Ss-DIP-1 with various nuclear receptors in COS-7 cells. Agonists used were 1 μM T0901317 (LXR), 10 μM 9-cis retinoic acid (RXR), 1 μM Δ7-DA (DAF-12). ***P < 0.0001, and *P < 0.05 by two-way ANOVA test comparing agonist to vehicle-treated cells (n = 3 ± SD). (D) Ss-DIP-1 increases ligand-dependent transactivation of DAF-12 on a *lit-1* reporter gene in cotransfected SL2 cells. ***P < 0.0001 by two-way ANOVA test comparing cells with Ss-DIP-1 to those without Ss-DIP-1 (n = 3 ± SD).
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formation of iL3 larvae, 2) postfree-living worms that lack DAF-12 activity fail to respond to Δ7-DA and never enter the infectious stage, and 3) postfree-living worms that lack Ss-DIP-1 also fail to respond to Δ7-DA.

An interesting nuance of DAF-12 function is that it is both a transcriptional repressor (in the absence of ligand) and a transcriptional activator (in the presence of ligand). Thus, the disruption of DAF-12 function in S. stercoralis results in a phenotype similar to the dauer-defective phenotype observed in C. elegans in which the loss of the transrepressor function prevents the worms from developmental arrest as iL3 larvae. Instead, many of these larvae progress to adult worms. A similar, albeit less penetrant, phenotype has been observed recently in the related rodent parasite, S. ratti, using an RNAi knockdown approach, further validating the evolutionary conservation of this pathway (12). The same phenotype can be achieved by inducing DAF-12 activity in postfree-living wild-type larvae with exogenous Δ7-DA. Both cases result in precocious adult worms that are morphologically abnormal and die within a few days. Notably, in S. stercoralis these DA-treated, postfree-living worms are no longer able to establish host infection. These results suggest that S. stercoralis needs other host factors in addition to DAF-12 activation to fully resume their developmental program. Alternatively, it is possible that either Ss-DAF-12 or Δ7-DA may have additional roles in other stages of the life cycle. These results raise several questions, including the identity of the endogenous DAF-12 ligand from parasitic nematodes and when and how it is synthesized. It may be that like C. elegans, Ss-DAF-12 is important for hormone-mediated developmental timing regulation by heterochronic genes (e.g., let-7-family miRNAs) in S. stercoralis (32, 33).

In summary, the discovery of DIP-1 as a parasite-specific, DAF-12-specific coactivator provides a unique understanding of a key molecular mechanism that governs parasite infection. Our work also advances an understanding of the mechanisms that allow nuclear receptors to fine-tune their transcriptional responses.

**Materials and Methods**

**Yeast Two-Hybrid Screening.** We performed yeast two-hybrid screening using the MATCHMAKER Gold yeast two-hybrid system (Clontech), following protocols provided by the manufacturer. Briefly, bait vectors were transformed into yeast strain Y2HGold (MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, α). The baits were transformed into yeast strain Y2HGold (MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, α).

**Fig. 5.** Ss-DIP-1 controls iL3 recovery. (A) CRISPR target site and strategy for homology-directed disruption of Ss-dip-1. Red line indicates position of the insert repair template and guide sequence and PAM site (underlined). Position of forward (F) and reverse (R) primers are shown. (B) Representative image of single-worm genotyping. Nested primers in A were used to amplify successful integration of Sr-eef-1::GFP into the target site. PCR product size for primary PCR with F1 and R1 primers is 884 bp. PCR product size for the nested PCR with F2 and R2 primers is 688 bp (arrowhead). (C) Frequency of iL3 feeding in Ss-dip-1 knockout (KO) versus wild-type (WT) and Ss-unc-22 KO control worms. Worms were induced to start feeding by placing in DMEM media. ***P < 0.0001 by two-way ANOVA test comparing Δ7-DA-treated WT to Ss-dip-1 mutant worms (n = 5 ± SD). See SI Appendix, Table S3 for number of animals tested. (D) Ss-dip-1 mutant worms show a decreased response to 1 μM Δ7-DA as assayed by iL3 mortality. ***P < 0.001 by two-way ANOVA test comparing Δ7-DA-treated WT to Ss-dip-1 mutant worms (n = 5 ± SD). See SI Appendix, Table S3 for number of animals tested. (E) Ss-daf-12 target gene expression determined by qPCR. S. stercoralis iL3 were treated with or without 400 nM Δ7-DA. **P < 0.001, ***P < 0.0001 by two-way ANOVA test comparing Δ7-DA to vehicle treated worms (n = 3 ± SD).
gal4a, gal80A, LSY2::GAL1UAS-GalI1TATA-His3, GAL2UAS-GalI1TATA-Ade2, URA3::MEL1UAS-MelI1ATA, AUR1-C MEL1). Variable length Ce-daF-12 and Ss-daF-12 cDNA fragments were cloned into pGBK7 vector. Full-length (FL) indicates amino acids 1 to 750; hinge and ligand binding domains (L) indicates amino acids 240 to 754 (Ce-DAF-12) or 184 to 754 (Ss-DAF-12); and ligand binding domain (L) indicates amino acids 502 to 754 (Ce-DAF-12) or 513 to 754 (Ss-DAF-12). For the preys, we used the “Mate & Plate” Library system and followed the manufacturer’s protocol (Takara Bio USA, Inc., Mountain View, CA). S. stercoralis IL3 cDNA library was cloned into the pGADT7 vector and transformed into the yeast strain Y187 (MATα used. SD/-Leu/-Trp selects diploids containing pGBKT7 and pGADT7. X-MEL1). For selection, SD/-Leu/-Trp/X-MEL1 colonies were screened; three colonies were identified as not false positives, one of which was found to be DIP-1. Selected colonies were verified by growth on SD/-Ade/-His/-Leu/Trp-X-gal/Aba (Aureobasidin A) plates were used. SD/-Leu-Trp selects diploids containing pGBK7 and pGADT7. X-gal/AbA was used to select for protein-protein interaction. Approximately 8 million colonies were screened; three colonies were identified as not false positives, one of which was found to be DIP-1. Selected colonies were verified by growth on SD/-Ade/-His/-Leu/Trp-X-gal/Aba minimal plates.

**Cell Culture and Reporter Assay.** For the mammalian two-hybrid assay, COS-7 cells were maintained in DMEM containing 10% fetal bovine serum, 2 mM glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin in 5% CO2 at 37 °C. COS-7 cells were transfected in 96-well plates using 50 ng of luciferase reporter, 10 ng CMV-β-galactosidase luciferase reporter, 15-ng nuclear receptor expression plasmids (VP16-Ce-DAF-12, VP16-CeS-Cs-DAF-12, VP16-hXRR and VP16-hLXRR), and 15-ng empty vector or cofactor expression plasmids (GAL4-Ss-DIP-1, GAL4-SRC), as previously described (10). For DIP-1 truncation constructs, the ss-dip-1 cDNA encoding amino acids 1 to 525, 1 to 100, 1 to 67, and 1 to 34 were cloned into the CMX-GAL4 expression vector for use in the mammalian two-hybrid assay. Ethanol (vehicle) or indicated compounds were added to each well 6 h posttransfection, incubated for 16 h, and cells were then harvested for dual luciferase assay. Ss-DIP-1 (SRAE_000054200) was identified with TBLASTN to search the S. ratti genome in WormBase ParaSite using Ss-DIP-1. Ss-DIP-1 and Ss-DIP-1 cavodons were codon-optimized and purchased from Integrated DNA Technologies (https://www.idtdna.com/pages/tools/codon-optimization-tool?returnurl=%2FCodonOpt). Schneider’s Drosophila medium was used for cDNA synthesis. cDNA was preamplified (TaqMan preAmp Master Mix, Thermo Fisher) before being analyzed by qPCR. Relative mRNA levels were normalized to 18S ribosomal RNA levels. Data are presented as fold-changes of relative mRNA levels in Δ7-DA versus vehicle-treated worms.

**Transgene Construction.** To generate the ss-dip-1::GFP construct, 1.8 kb of the ss-dip-1 promoter upstream of the predicted ss-dip-1 gene was amplified and cloned with GFP::era-1 3′UTR from pPV529 vector. Primer sequences are listed in **Table S1**. For Ss-daF-12::GFP, 5.5 kb of the ss-daF-12 promoter upstream of the gene amplified and cloned with GFP::era-1 3′UTR from pPV529. Sequences were obtained from the Wormbase ParaSite website (https://parasite.wormbase.org/index.html). To generate transgenic progenies, these plasmids (50 ng/μL) were microinjected with RFP (pAJ50) injection markers (50 ng/μL).

**Statistical Analysis.** For all experiments, two-way ANOVA analysis was used for multigroup analyses and plots were created with Prism v8 (GraphPad Software). Statistical probabilities P < 0.05 were considered significant.

**Data Availability.** All study data are included in the article and supporting information.

**ACKNOWLEDGMENTS.** We thank members of the J.B.L. and D.J.M.–S.A.K. laboratories for comments and technical assistance and Dr. Elissa A. Hallem for critical reading of the manuscript. We also wish to thank members of the J.B.L. and D.J.M. laboratories for comments and technical assistance and Dr. Elissa A. Hallem for critical reading of the manuscript. This work was supported by NIH Grants R33 AI05856 (to J.B.L., S.A.K., and D.J.M.) and R01 AI056688 (to J.B.L.); Robert A. Welch Foundation Grants I-1558 (to S.A.K.) and I-1275 (to D.J.M.); and the Howard Hughes Medical Institute (D.J.M.).

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