Neuronal IL-17 controls *C. elegans* developmental diapause through CEP-1/p53

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Abstract. How metazoan development is coordinated with nutrient availability remains unclear. Here, we show that the activity of CEP-1, the C. elegans ortholog of the tumor suppressor p53, switches organismal development between reproductive growth and dormancy by responding to nutrient availability signaled by neuronal cytokine, ILC-17.1. Specifically, ILC-17.1 is released from amphid neurons upon food availability and promotes glucose utilization and represses CEP-1/p53: CEP-1/p53 repression promotes growth. In the absence of ILC-17.1, CEP-1/p53 is activated, accumulates in post-embryonically dividing progenitor blast cells, upregulates cell-cycle inhibitors, decreases cytochrome C levels, and causes larvae to arrest as stress-resistant, quiescent dauers. We propose a model whereby ILC-17.1 signaling links nutrient availability and energy metabolism to cell cycle progression and cell fate decisions during development through CEP-1/p53. These findings also suggest that the developmental function of the p53-gene family could have shaped their evolution and are relevant to our understanding of neuroimmune mechanisms in cancer.
During metazoan development, nutrient availability is coordinated with the division, growth and metabolic activity of individual cells through cell-cell communication. This is also the case in the invertebrate *C. elegans*, a free-living bacterivore, which displays a dramatic developmental plasticity to ensure that its growth and reproduction match available resources\textsuperscript{1-10}. When *C. elegans* larvae hatch under optimal conditions (e.g., in the laboratory at 20°C, at low population densities, on abundant food provided by lawns of the *E. coli* strain, OP50) they continue development into reproducing adults. However, if they hatch under suboptimal conditions, such as in the paucity of food, at high population densities, or high ambient temperatures, larvae implement an alternative developmental program and arrest as quiescent, stress-resistant larvae called ‘dauer’ larvae. Dauer larvae display metabolic and organismal phenotypes specialized for dispersal and survival, and can remain for months in an arrested state to resume development into reproductive adults when conditions become favorable again\textsuperscript{1-10}. Studies from several labs have identified molecular pathways that mediate the dauer decision, showing that growth promoting molecules, the best-characterized of which are the insulins, the transforming β growth factor (TGFβ/DAF-7) and lipid based dafachronic acid hormones, are released by sensory neurons and other cells to license continued growth and development; adverse environments inhibit these growth promoting signals and trigger dauer arrest\textsuperscript{1-11}. In addition, a number of quantitative trait loci (QTL) also modulate dauer arrest\textsuperscript{12}. Yet, how the dauer entry decision results in a coordinated change in cell fates across different tissues and is linked with the systemic shut-down of anabolic pathways still remains poorly understood.

An important group of proteins that mediate cell-cell communication and metabolism in metazoa are secreted proteins called cytokines\textsuperscript{13,14}. The IL-17 cytokines are one such evolutionarily conserved family of proinflammatory cytokines that are released by specialized immune cells at the interface between an organism and the outside world to activate immune surveillance, barrier
function and wound healing, but in humans, also promote cancers and autoimmune disease such as psoriasis. More recently IL-17s have been shown to play key immunometabolic roles in modulating mammalian physiology and disease. Here, we show that the *C. elegans* IL-17 ortholog, ILC-17.1, is released from amphid neurons in response to food, and coordinates cell division and cell fate decisions with metabolism by controlling the activity of the *C. elegans* tumor suppressor p53 ortholog, CEP-1. Specifically, ILC-17.1 release that occurs in the presence of food suppresses CEP-1/p53. CEP-1/p53 is activated upon the loss of ILC-17.1, and remarkably, this switches whole organism development from continuous growth to dormancy. The p53-like tumor suppressor genes are found in all multicellular animals where they prevent the transmission of damaged DNA by activating a multifaceted program that controls cell cycle checkpoints, mediates reversible growth arrest or apoptosis, and controls metabolic flux in cells. Our studies show that these functions of CEP-1/p53 also act to control developmental quiescence of a whole organism, *C. elegans*, suggesting that the developmental function of the p53-gene family could have shaped their evolution.

Results

ILC-17.1 is released by *C. elegans* amphid neurons in response to food and prevents dauer arrest.

*C. elegans* express three IL-17 orthologs. Of these, ILC-17.1, which has been shown to act as a neuromodulator, is expressed by a subset of specialized neurons with epithelial properties called amphid neurons that are exposed to the outside and sense information from the environment. We discovered that a deletion of *ilc-17.1*, *syb5296*, that removes almost all the coding sequence (2173bp out of 2980 bp; Supplementary Fig. S1a) and abolishes mRNA expression (Supplementary Fig. S1b) caused larvae to constitutively enter the dauer state even under optimal growth conditions (Supplementary Fig. S1c-d). Dauer larvae can be identified by their growth arrest, distinct morphology that includes specialized cuticular structures called alae,
a constricted pharynx, and arrested germline, as well as decreased pharyngeal pumping rates and detergent (1% SDS) resistance\(^3\)\(^-\)\(^9\). *ilc-17.1*(syb5296)X dauer larvae displayed all these characteristic morphological and physiological features (Supplementary Fig. S1e-j). Under optimal growth conditions at 20°C, *ilc-17.1* deleted larvae entered the dauer state transiently: approximately 30% (31.2 ±5%) were SDS-resistant 48 hours post-hatching on OP50 bacteria, and most larvae exited dauer and become reproductive adults by 72 hrs (Supplementary Fig. S1c, d). Under the same conditions, none of the wildtype larvae were detergent resistant, nor did any larvae arrest as dauers (Supplementary Fig. S1c, d). However, as with other mutations that promote dauer entry\(^3\)\(^,\)\(^4\)\(^,\)\(^28\), the dauer arrest of *ilc-17.1* deletion mutants was dramatically accentuated at the slightly more stressful ambient temperature of 25°C, which still supported the growth of all wildtype larvae into reproductive adults, but caused practically 100% of larvae lacking *ilc-17.1* to enter and persist in the arrested dauer state (Fig.1a-d).

Because the molecular basis for dauer regulation has been the target of extensive genetic screens that are considered to be saturated\(^3\)\(^,\)\(^5\)\(^,\)\(^29\)\(^-\)\(^31\), and IL-17.1 had not been previously implicated in the regulation of dauer, we confirmed the role of *ilc-17.1* in dauer entry by (i) examining the developmental phenotype of another independently generated CRISPR deletion in *ilc-17.1*, syb5297 (loss of 2188 bp of 2980 bp; Supplementary Fig. S1a), (ii) by backcrossing *ilc-17.1*, syb5297, and (iii) by downregulating *ilc-17.1* and its receptor\(^25\)\(^,\)\(^26\) in wildtype (N2) larvae using RNA interference (RNAi). Like *ilc-17.1* (syb5296), *ilc-17.1* (syb5297) created using a different guide RNA (see Materials and Methods) also displayed a completely penetrant dauer arrest at 25°C (Fig. 1a,b). In *C. elegans* there have been no off-target effects observed using CRISPR even after whole genome sequencing by several labs\(^32\)\(^-\)\(^34\). Nevertheless, due to reviewer’s skepticism, we considered whether a similarly large CRISPR-mediated deletion of any gene could, in itself, predispose larvae to enter dauer, and examined whether of the deletion of a related gene *F25D1.3*, syb7367(loss of 1475 bp of 1642 bp) also triggered dauer arrest. This did not
(Supplementary Fig. S1k). *ilc-17.1* (syb5296) larvae, however, continued to arrest as dauer even after they were backcrossed 2X time into a wildtype background (Supplementary Fig. S1k). In addition, RNAi-mediated downregulation of *ilc-17.1* in wildtype (N2) animals, but not control RNAi treatment, also caused a small but significant number of larvae to arrest as SDS-resistant dauer larvae at 25°C, (Supplementary Fig. S1l). ILC-17.1 signals though cytokine receptors which in *C. elegans* consists of ILCR-1/ILCR-2. The ILCR-2 subunit is widely expressed, as determined using a bicistronic SL2 cassette to tag the *ilcr-2* receptor at its endogenous locus with GFP (Supplementary Fig. S2a). Downregulation of *ilcr-2* in wildtype (N2) animals through RNAi caused over one-third, 39.6% ± 4%, of the wildtype larvae to arrest as dauer and did not alter the dauer entry of *ilc-17.1* deleted larvae (Fig. 1e). RNAi, being a partial downregulation of mRNA levels, is generally insufficient to induce dauer arrest with even known dauer pathway genes. Therefore, the RNAi-induced dauer entry of larvae exposed to *ilc-17.1* or *ilcr-2* RNAi for less than 48 hrs. post-hatching, (see Materials and Methods) was not only statistically significant, but also provided additional support for the role of *ilc-17.1* in dauer entry. Thus, together, these observations indicated that during normal development, ILC-17.1 signaling, likely through the ubiquitously expressed ILCR-2 receptors, was required for growth of larvae to reproducing adults; in the absence of ILC-17.1, or the ILCR-2 receptor, larvae arrested growth and entered the dauer state, transiently, or for prolonged durations.

The dauer phenotype of *ilc-17.1* deletion mutants could be rescued by re-expressing *ilc-17.1* under the control of its own promoter and 3' UTR regions (Fig. 1b; see Supplementary Fig. S2b for expression levels of rescue construct in comparison to the endogenous gene). The extent of rescue varied based on the animal’s diet (ILC-17.1 re-expression caused an approximately 32.8% rescue on OP50 bacteria, Fig 1b, but 63.6% rescue on HT115 bacteria, Supplementary Fig. S2c; Chi-squared = 121, df = 1, p-value <2e-16), suggesting that the role of ILC-17.1 signaling in dauer might be related to food, a prominent modulator of the *C. elegans* dauer decision. The systemic
effects of the \textit{ilc-17.1} deletion and rescue were surprising because previous studies using transgenic \textit{ilc-17.1} reporter constructs had shown that \textit{ilc-17.1} mRNA expression is restricted to a small subset of neurons, a pair of amphid sensory neurons (ASE) and two pairs of interneurons (AUA and RMG)\textsuperscript{25}. The expression of the endogenous \textit{ilc-17.1} gene was also restricted to a few neurons: mCherry expressed as a bicistronic SL2 cassette along with \textit{ilc-17.1} mRNA from the endogenous \textit{ilc-17.1} locus was also only visible in a few dye-filling amphid neurons (Supplementary Fig. S2d). Therefore, to understand how ILC-17.1 acts systemically, we re-expressed \textit{ilc-17.1} ectopically in \textit{ilc-17.1} deleted larvae, remote from its normal site of expression, using the muscle-specific \textit{unc-54} promoter (Supplementary Fig. S2e), and asked whether ILC-17.1 could act in a cell non-autonomous manner to promote development, or whether its only functioned cell autonomously in amphid neurons. Our expectation was that if ILC-17.1 controlled developmental pathways by acting in a cell-autonomous manner in the amphid neurons, ectopic expression would not rescue the dauer arrest, whereas a cell non-autonomous or endocrine mode of activity could result in a rescue. Consistent with a cell non-autonomous mode of action, and as was the case for the previously reported effects of ILC-17.1 on behavior\textsuperscript{25}, ILC-17.1 expressed ectopically from the \textit{unc-54} promoter rescued dauer arrest of \textit{ilc-17.1} deleted larvae (Fig.1b and Supplementary Fig. S2c). The rescue from this ectopic expression did not vary with bacterial diet (Fig 1b vs Supplementary Fig. S2c), perhaps because of its non-neuronal expression and/or higher expression levels when compared to that from the endogenous promoter (Supplementary Fig. S2b, f). RNAi mediated downregulation of \textit{ilcr-2} reduced the percentage of \textit{ilc-17.1} larvae that were rescued from dauer arrest by re-expressing ILC-17.1 either under its own promoter, or from the muscle promoter (Fig. 1e). This effect was significant, but only \textasciitilde 10\%, reflecting our limited understanding of the stoichiometry of ILC-17.1 ligand-receptor interactions as they relate to the timing of dauer arrest that occurs in the absence of ILC-17.1. Nevertheless, these studies indicated that ILC-17.1 signaling was required for continuous growth and suggested that ILC-17.1 might normally be secreted to act cell non-autonomously to promote development.
Therefore, to more directly examine whether ILC-17.1 is normally secreted from amphid neurons, we immunolocalized ILC-17.1 protein in *C. elegans* larvae using strains expressing ILC-17.1 tagged at its endogenous locus using a HA-tag. Suspecting that food played a role in ILC-17.1 signaling, we conducted these studies on larvae that were hatched in the absence or presence of food (OP50; Fig. 1f). While *ilc-17.1* mRNA localization was not altered by food availability, and remained restricted to the amphid neurons both in the presence and absence of food as detected by mCherry expressed from the endogenous *ilc-17.1* locus (Fig. 1f; left), ILC-17.1 protein was present outside the amphid neurons, concentrated in the pharynx when larvae were exposed to food (OP50), but not when larvae hatched in the absence of food (Fig.1f; right, quantification in Supplementary Fig. S2g). This suggested that ILC-17.1 protein was secreted and either retained, stabilized, or taken up by the pharynx in response to food signals [we could not reliably detect ILC-17.1 protein in distal tissues such as the intestine or coelomocytes]. The secretion of ILC-17.1 protein was also supported by immunolocalization studies on the muscle-expressed ILC-17.1 protein which, although expressed under a well-characterized muscle-specific promoter (i.e. *unc-54*; Supplementary Fig. S2e), could be constitutively detected outside muscle cells, throughout the animal (Supplementary Fig.S2h). Thus, the presence of ILC-17.1 protein in regions outside its cellular sites of mRNA synthesis provided strong support that ILC-17.1 is secreted, and in the case of the endogenous ILC-17.1 protein, secretion occurs in response to food availability.

Sequencing total RNA (RNA-seq) extracted from bleach synchronized larvae grown for 32-36 hours at 25°C when *ilc-17.1* mutant larvae were *en route* to dauer arrest, and wildtype larvae were in their late L2/early L3 stage *en route* to developing into reproducing adults, showed that *ilc-17.1* mutant larvae expressed the molecular signatures of dauer larvae that were distinct from wildtype larvae and from *ilc-17.1* deletion mutants that re-expressed ILC-17.1 (Supplementary...
Figure S3a; Supplementary Table 1-3). Thus, anabolic processes such as DNA replication and ribosome biogenesis were downregulated (Fig. 1g), and catabolic processes and stress responses such as autophagy, fatty acid metabolism, glutathione metabolism, and xenobiotic defense pathways were upregulated (Fig. 1h). In addition, ilc-17.1 deleted larvae expressed dauer-specific collagen genes that differ from the collagens of larvae undergoing continuous development (Fig. 1i; Supplementary Table 4). To evaluate the extent to which the ilc-17.1 deleted dauers were similar to previously described C. elegans dauers that accumulate in the population upon starvation or crowding, we compared the global gene expression changes of ilc-17.1 mutant larvae with previously published gene expression changes in larvae entering dauer in response to starvation or pheromone treatment (Supplementary Fig. S3 b,c; Supplementary Tables 5, 6). Consistent with a role for ILC-17.1 in signaling nutrient availability, the expression profile of ilc-17.1 deleted larvae was more closely correlated to dauer larvae generated by starvation (Supplementary Fig. S3b), compared to ascaroside induced dauers (Supplementary Fig. S3c). These differences were surprising and suggest that the trigger and route of dauer entry determines the gene expression profile of C. elegans dauer larvae.

ILC-17.1 loss activates CEP-1/p53 to trigger dauer entry through the activation of DAF-16/FOXO, DAF-3/SMAD-DAF-5/Ski complex and steroid hormone pathways. To understand how the loss of ILC-17.1 was triggering dauer entry we first considered the possibility that, through its role as a neuromodulator, the secretion of ILC-17.1 modulated the animals’ sensory perception of food or feeding behavior, and the lack of ilc-17.1 prevented larvae from finding or ingesting food. However, this did not seem to be the case (Supplementary Fig. S4a): ilc-17.1 deleted animals did not exhibit decreased chemotaxis towards lawns of OP50, or towards organic molecules like lysine or diacetyl thought to mimic the presence of bacteria. Animals lacking ILC-17.1 were also able to feed like wildtype animals, and the rate of pharyngeal pumping, a measure that is typically correlated with food uptake, was the same in wildtype animals
and animals lacking ILC-17.1, both in the presence and absence of food (Supplementary Fig. S4b). Also, prior to their dauer entry, *ilc-17.1* deletion mutants accumulated slightly, but not significantly fewer numbers of red latex beads in their intestinal lumen that served as a proxy for rates of bacterial ingestion (Supplementary Fig. S4c). These data suggested that *ilc-17.1* deficient larvae arrested development and entered a dauer diapause state despite being able to find and ingest nutrients.

Therefore, to answer how the loss of ILC-17.1 triggered dauer entry we next adopted a genetic approach, leveraging the wealth of studies on *C. elegans* dauer formation. In *C. elegans*, insulins released in the presence of optimal food activate or inhibit the sole insulin receptor ortholog, DAF-2, to modulate the function of the Forkhead transcription factor DAF-16. DAF-2 signaling supports continuous growth programs: loss of DAF-2 signaling leads to the activation of DAF-16 and promotes dauer entry. Likewise, TGFβ/DAF-7 ligands released under optimal growth conditions antagonize the activity of the transcription factor complex, DAF-3/SMAD-DAF-5/Ski, to permit continuous growth: inhibition of DAF-7 activates DAF-3/SMAD-DAF-5/Ski and leads to dauer formation. Both insulins and TGF-β result in the production of the sterol based dafachronic acid hormones that act through DAF-12, a nuclear hormone receptor, to support the development of adult reproductive physiology. The absence of dafachronic acid pathway components also triggers dauer arrest, and providing exogenous dafachronic acids to mutant animals deficient in insulin or TGFβ signaling, or decreasing DAF-12 activity, can bypass their dauer arrest. Finally, a mutation in a transmembrane guanylyl cyclase *daf-11*, that causes chemosensory defects also triggers dauer arrest. *ilc-17.1* deletion mutants lacked obvious chemosensory defects (Supplementary Fig. 4a-c), and continued to arrest as dauers on 5mM 8-Bromo-cGMP, which is known to rescue *daf-11*-associated dauer formation (Supplementary Fig. 4d). Therefore, to identify signal transduction pathways through which the loss of ILC-17.1...
might act to control the dauer decision, we examined whether the other dauer effectors, daf-16, daf-5, or dafachronic acid signaling, modulated dauer formation of ilc-17.1 mutant larvae.

A daf-16 loss of function mutation, mu86⁴⁴, completely suppressed dauer formation in animals lacking ILC-17.1, indicating that activation of DAF-16 was one mechanism responsible for their dauer arrest (Fig. 2a). The activation of DAF-16 in animals lacking ILC-17.1 could be quantified by an increase in the proportion of larvae with DAF-16::GFP⁴⁵ positive intestinal nuclei even at 20°C (Fig. 2b), and the upregulation of DAF-16 target genes⁴⁶,⁴⁷ both at 20°C (Supplementary Fig. S4e) and 25°C (Fig. 2c). DAF-16 activation could be suppressed by re-expressing ILC-17.1 in ilc-17.1 deletion mutants under its endogenous promoter, or ectopically, under the muscle-specific promoter (Fig. 2c). Similarly, a loss of function mutation in daf-5, e1386⁴⁸ also completely suppressed the dauer arrest of ilc-17.1 mutant larvae (Fig. 2d). Finally, and consistent with the role of the DAF-12 pathway downstream of DAF-16 and DAF-5/DAF-3, downregulating daf-12 by RNAi significantly decreased the number of larvae that arrested as dauers (Supplementary Fig. S5a), and exposing ilc-17.1 deficient larvae to exogenous Δ7-dafachronic acid at concentrations of 50 nM⁴², partially but significantly suppressed their dauer arrest and only ~65% instead of ~100% of the larvae were dauers (Fig 2e). The activation of DAF-16/FOXO and DAF-3/SMAD-DAF-5/Ski by the loss of ILC-17.1 was not simply because ilc-17.1 deleted larvae were deficient in the expression of insulin signaling and TGFβ/ DAF-7 signaling pathway components. The expression levels of daf-2 mRNA and the 28 insulin ligands⁴⁹,⁵⁰ were not decreased in ilc-17.1 deletion mutants, (Supplementary Fig. S5b daf-2 boxed; Supplementary Table 7), and the expression of ins-4 was even slightly higher (Supplementary Fig. S5c). The expression of insulin peptides INS-4 and DAF-28⁵⁰ were also not altered in larvae deficient in ilc-17.1, as seen using GFP translational reporters of these insulin ligands (Supplementary Fig. S5d, e). In addition, the dauer arrest of a mutation in the insulin receptor, daf-2 (e1370)⁵¹, could not be rescued by ILC-17.1 overexpression, suggesting that ILC-17.1 did not act genetically downstream of daf-2.
Similarly, the mRNA expression levels of the main TGFβ components were mostly unaltered in ilc-17.1, and daf-5 levels were even downregulated (Supplementary Fig. S5g; Supplementary Table 8). Thus, these data together indicated that ILC-17.1 acted upstream of DAF-16/FOXO, DAF-3/SMAD-DAF-5/Ski, and the DAF-9/DAF-12 endocrine pathways to modulate dauer entry.

In wildtype animals, DAF-16/FOXO and DAF-3/SMAD-DAF-5/Ski complex are thought to act independently, and in parallel to modulate the dauer decision. Therefore, to identify mechanism(s) that led to the activation of both these pathways in ilc-17.1 deleted larvae, we used RNAi to downregulate genes known to interact with these dauer effectors (Supplementary Fig. S5a). Intriguingly, amongst the possible candidates, we found that the downregulation of cep-1, the C. elegans ortholog of p53 (Supplementary Fig. S5a), or a deletion in cep-1, gk138, completely rescued the dauer arrest of ilc-17.1 deletion mutants and nearly all (98%) of cep-1;ilc-17.1 double mutant larvae grew into reproductive adults (Fig 2f-h).

Although p53 is best studied as a tumor suppressor, p53-like proteins modulate vertebrate development through as yet poorly understood mechanisms. The C. elegans p53 ortholog, cep-1 has not been previously implicated in C. elegans development. Nevertheless, as could be predicted by the dauer rescue of ilc-17.1 larvae upon cep-1 deletion, the overexpression of CEP-1 alone was sufficient to arrest growth and promote an almost completely penetrant dauer phenotype in larvae that developed at 25°C (Fig. 3a, b; see Supplementary Fig. S6a for mRNA levels). CEP-1 overexpression was achieved by expressing a functional, fluorescently tagged CEP-1 under its own promoter, CEP-1::GFP, that was able to complement a cep-1 deletion, cep-1(lg12501), as a genomically integrated multicopy array in a wildtype background. CEP-1::GFP did not induce dauer arrest in the background of the cep-1 deletion, cep-1(lg12501), (Figure 3c), indicating that it was the higher expression levels of cep-1 (Supplementary Fig. S6a),
and not the GFP tag on CEP-1/p53, that was responsible for the dauer arrest of CEP-1::GFP overexpressing larvae. CEP-1/p53 overexpression not only caused dauer arrest at 25°C, but also prompted larvae to transiently enter dauer at 20°C, as seen by their SDS-resistance (Supplementary Fig. S6b), phenocopying the ilc-17.1 deletion. RNA-seq analysis showed that genes differentially expressed in CEP-1/p53 overexpressing larvae en route to their dauer arrest significantly overlapped with those expressed in larvae lacking ILC-17.1 (65% of the 15,443 differentially expressed genes overlapped, padj.< 0.05; Supplementary Fig. S6c; Supplementary Tables 9, 10). CEP-1/p53 overexpressing larvae also upregulated dauer-specific collagens (Supplementary Fig. S6d; Supplementary Table 4), downregulated genes involved in anabolism (Supplementary Fig. S6e), and upregulated genes involved in catabolism (Supplementary Fig. S6f). In addition, between 15 hours to 32 hours post-hatching at 25°C, CEP-1/p53 overexpressing larvae and ilc-17.1 deletion mutants showed a remarkably constant level of gene expression, and only 340 and 34 genes respectively changed expression (p<0.05), compared to the > 10,000 genes that were either upregulated or downregulated in wildtype larvae and cep-1;ilc-17.1 double mutant larvae that were in the process of developing into reproductive adults (PCA analysis; Supplementary Fig. S6g; Supplementary Table 11), suggesting that the dauer decision was implemented by 15 hrs post-hatching.

Like in the ILC-17.1 deficient animals, the completely penetrant 25°C-dauer arrest of larvae overexpressing CEP-1 was dependent on DAF-16, DAF-5 and the steroid hormone pathway. Downregulating daf-16 expression by RNAi (Fig. 3d; we could not generate CEP-1/p53 overexpressing and daf-16 double mutant larvae, perhaps because of the site of integration of CEP-1::GFP transgene into the genome) or crossing CEP-1/p53 overexpressing larvae into daf-5, e1386, suppressed their dauer arrest (Fig. 3e). Treatment with 50nM exogenous Δ7-dafachronic acid also allowed a significant proportion of CEP-1/p53 overexpressing larvae to bypass dauer arrest (Fig. 3e). In accordance, DAF-16 target genes were also upregulated in
larvae overexpressing CEP-1/p53 at both 25°C (Fig 3f) and 20°C (Supplementary Fig. S7a). In addition, and most convincing, the upregulation of *daf-16* target genes in *ilc-17.1* deletion mutant larvae was *cep-1* dependent at both 25°C and 20°C (Fig 3f; Supplementary Fig. S7a). As we showed for ILC-17.1, CEP-1/p53 induced dauer entry appeared to be somewhat specific to reduced ILC-17.1 signaling, as the 25°C-dauer arrest of *daf-2*(e1370) III larvae that occurred due to reduced insulin signaling did not depend on *cep-1* (Supplementary Fig. S7b). Moreover, in agreement with previous reports, *cep-1* was not required for dauer induction at high temperatures as *cep-1* (gk138) I larvae could enter dauer at 27°C just like wildtype animals (Supplementary Fig. S7c).

To confirm that CEP-1/p53 was indeed activated in the absence of ILC-17.1, we tested whether known targets of CEP-1/p53, the BH3-only proteins *egl-1* and *ced-13*, were upregulated in *ilc-17.1* deleted larvae in a CEP-1/p53 dependent manner. As could be expected, *egl-1* and *ced-13* mRNA levels were upregulated upon CEP-1/p53 overexpression in larvae both at 25°C (Fig 3g) and 20°C (Supplementary Fig. S7d). *egl-1* and *ced-13* were also upregulated in a CEP-1/p53-dependent manner in *ilc-17.1* deletion mutants at both 25°C and 20°C, and could be suppressed by rescuing *ilc-17.1* deletion mutants with ILC-17.1 re-expressed under its endogenous-, or muscle-specific promoters (Fig 3g; Supplementary Fig. S7d). In addition, Chromatin Immunoprecipitation followed by quantitative PCR (ChIP-qPCR) using animals carrying an endogenous CRISPR/Cas9 FLAG tagged *cep-1* showed that more CEP-1/p53 was bound to the promoter regions of *egl-1* and *ced-13* in larvae lacking *ilc-17.1* when compared to wildtype larvae, and the increased occupancy was abrogated when *ilc-17.1* deletion mutants were rescued from dauer arrest by re-expressing ILC-17.1 (Fig. 3h).

In mammalian cells, activation of p53 in response to stress increases p53 protein levels due to its escape from constitutive degradation mediated by the E3 ubiquitin ligase MDM2. *C. elegans*
the genome lacks MDM2 orthologs, but CEP-1/p53 is controlled through translational and posttranslational modifications\textsuperscript{62,71}, although the mechanisms of activation remain incompletely understood. Western analysis of FLAG-tagged endogenous CEP-1/p53 in \textit{C. elegans} showed that CEP-1::FLAG expression was increased in larvae lacking \textit{ilc-17.1} (Fig 3i). As a positive control we used gamma irradiation at doses known to activate CEP-1/p53 in the germline, and this also increased CEP-1::FLAG levels (Fig 3i)\textsuperscript{67,69}. These results together showed that CEP-1/p53 was activated upon loss of ILC-17.1 signaling and triggered the dauer arrest of \textit{ilc-17.1} deletion mutants through the DAF-16/FOXO and DAF-3/SMAD-DAF-5/Ski (and likely DAF-12) pathways. Thus, it appeared that ILC-17.1-CEP-1/p53 could act as a node to integrate and coordinate the diverse cellular, physiological and metabolic effects of the DAF-2/Insulin signaling- and TGFβ/DAF-7-growth hormone signaling pathways that normally act in parallel to control dauer arrest. However, as we showed for DAF-2, these transcription complexes also likely receive independent information through the insulin receptor, DAF-2, and the TGFβ/DAF-7 receptors.

**ILC-17.1 modulates CEP-1/p53 activity in ventral blast cells (P cells) of larvae and directly or indirectly controls cell cycle progression and cell fate programs.**

During \textit{C. elegans} development, most cell divisions are completed during embryogenesis, but a subset of somatic and germline cells divide post-embryonically during and beyond the L1 stage, to generate adult tissues\textsuperscript{72-77}. Previous studies have shown that upon dauer entry these cells maintain or re-establish multipotency to be able to continue development when conditions become favorable again\textsuperscript{78,79}, and this is mediated by the known dauer signaling pathways that act to different extents, in different post-embryonically developing tissues, to modulate their cell cycle G1/S transition, natural re-programming, and/or differentiation\textsuperscript{80-87}. Because of its conserved functions in promoting reversible cell cycle arrest and stem cell differentiation\textsuperscript{19-21,88}, we speculated that CEP-1/p53 activated by the loss of ILC-17.1, was also restraining cell cycle progression and suppressing adult fates in post-embryonically developing cells when nutrients
were scarce. To test this possibility, we (i) examined the localization of CEP-1/p53 in developing *ilc-17.1* deleted larvae and *cep-1* overexpressing larvae during their dauer entry, (ii) examined whether CEP-1/p53 activity in *ilc-17.1* deletion mutants (and CEP-1/p53 overexpressing larvae) altered cell fate or increased the expression of cell cycle inhibitors, and (iii) examined whether decreasing the expression of the cell cycle inhibitors that may be upregulated could systemically rescue dauer arrest.

Previous studies have shown that CEP-1/p53 is expressed in the adult germline, and in a subset of larval pharyngeal muscle and neuronal cells. This has been visualized by other groups using the same functional, fluorescently tagged CEP-1, CEP-1::GFP which complements the *cep-1* deletion, *cep-1(lg12501)*, where endogenous CEP-1 protein is not expressed. We found that in addition to these cells, CEP-1/p53 was also expressed in the ventral somatic progenitor blast cells (P cells) early during larval development (15-20 hours post-hatching; Fig. 4a). The ventral P blast cells are multipotent progenitor cells that divide post-embryonically during larval stage 1 (L1) to generate neuroblasts, hypodermal blast cells and vulval precursor cells which later form adult tissue. These cells express the Paired-box gene *pax-3*, the sole *C. elegans* PAX-3 transcription factor homolog, during these early stages of development. CEP-1::GFP colocalized with approximately 50% of *pax-3*-driven mCherry that can be used to mark P cells, 15 hours post-hatching at 20°C, and accumulated in more *pax-3* expressing cells under dauer inducing conditions of 25°C (Fig. 4a, b). In addition, CEP-1::GFP expression levels in P blast cells increased at 25°C in larvae overexpressing CEP-1/p53 or lacking ILC-17.1, when compared to larvae at 20°C, or when compared to CEP-1::GFP expression in *cep-1* (lg12501) larvae that do not arrest as dauers (Fig. 4c). Notably, CEP-1::GFP expression in blast cells of *cep-1* (lg12501) larvae did not vary between 20°C and 25°C (Fig. 4c), indicating that increased CEP-1/p53 levels in blast cells of larvae overexpressing CEP-1/p53, or lacking ILC-17.1, was likely related to their dauer entry and not due to changes in temperature *per se*. Thus, during dauer arrest of *ilc-17*...
deletion mutants, or CEP-1/p53 overexpressing larvae, CEP-1/p53 appeared to be activated in
the ventral P blast cells that would arrest lineage progression as dauers.

Larvae overexpressing CEP-1/p53 and ilc-17.1 deleted larvae also upregulated cell cycle
inhibitors that are known to be direct or indirect targets of CEP-1/p53: one of the two p21
homologs, cki-1, and phg-1, the C. elegans homologue of Growth arrest-specific 1 or Gas1 were
upregulated (Fig. 4d). phg-1, but not cki-1, was a direct target of CEP-1/p53, as ilc-17.1
deletion mutants displayed increased occupancy of CEP-1/p53 at the promoter of the phg-1
gene (Fig. 4e), but not the cki-1 gene (Supplementary Fig. S7e). The increased expression of
these genes and occupancy at phg-1 promoter was restored to wildtype levels upon re-
expressing ILC-17.1 (Fig. 4d, e). For technical reasons we could not localize phg-1 or cki-1
mRNA in larvae during these early stages of development and therefore do not know whether
the increased expression occurred in the P blast cells. Nevertheless, the increased expression
of phg-1 and cki-1 played a causal role in dauer arrest: downregulation by RNAi was sufficient
to significantly, but modestly rescue dauer arrest in ilc-17.1 deletion mutants and CEP-1/p53
overexpressing larvae, and promote their development into adults (Supplementary Fig. S7f).
Crossing ilc-17.1 deletion mutants into phg-1 deletion mutants, ok2741, resulted in a greater
rescue and ~40% escaped dauer arrest and grew into reproductive adults, but surprisingly, over
60% of the ilc-17.1;phg-1 double mutants still remained arrested as dauers (Fig. 4f;
Supplementary Fig. S7g), suggesting that CEP-1/p53 activity might be acting in cooperation
with other cell cycle or dauer pathway genes. In addition, a proportion of ilc-17.1 deletion
mutant and CEP-1/p53 overexpressing larvae that appeared morphologically dauer, displayed
modest but significant non-dauer physiological traits on phg-1 and cki-1 RNAi such as increased
rates of sporadic pumping (Supplementary Fig. S7h; wildtype larvae at larval stage 2, L2, were
used as controls), suggesting there there may also occur a wider, albeit incomplete, dauer
rescue in some tissues. The dauer arrest of daf-2 mutant larvae which did not depend on cep-
was also not rescued upon downregulating phg-1 or cki-1, nor were the pumping rates of daf-
2 dauers altered, and these remained even lower than in ilc-17.1 deleted or CEP-1/p53
overexpressing dauers (Supplementary Fig. S7f, h). These data together showed that cell cycle
inhibitors that were CEP-1/p53-targets were upregulated upon loss of ILC-17.1 signaling or
overexpressing CEP-1/p53 and downregulating these inhibitors was sufficient, at least in part, to
cause a systemic rescue from dauer arrest.

Given these observations, we asked whether overexpressing CEP-1/p53 in P blast cells alone,
under the pax-3 promoter, might drive larvae to arrest as dauers. This expectation turned out to
be too simplistic, and was not supported, as the majority (85.7 ± 5%; n=6 experiments, 122 larvae
total) of pax-3p::CEP-1 expressing larvae grew into adults at 25°C, although 14.3 ± 4.5% did not
complete development and arrested at various early larval (<L4) stages. However, CEP-1/p53
expression in the P blast cells caused anteroposterior patterning defects in larvae and 11.8 ±2.9%
of larvae displayed dorsalization, vulvaless and dumpy phenotypes (representative example; Fig.
4g) suggesting that CEP-1/p53 expression could alter the cell fates of the P blast cell lineages76,93-
95. Consistent with these observations, in larvae lacking ILC-17.1 or overexpressing CEP-1/p53,
HOM-C homeobox genes required for anterio-posterior body patterning76,93-95 were differentially
expressed compared to wildtype or ilc-17.1; cep-1 double mutant larvae (Fig 4h; p< 0.05). This
included lin-39 (Scr/Dfd/Hox3–5) which maintains P cells competent to respond to later inductive
signals and prevents aberrant cell fusions, and mab-5 (Antp/Hox6–8) and egl-5 (Abd-A/Abd-
B/Hox9–13), involved in mid- and posterior body patterning, which were maintained at modestly,
but significantly higher levels when compared to larvae continuously developing into adults
(compare expression levels between 15 hr and 32 hr larvae in Supplementary Table 12). Thus,
while further studies are required to better understand how CEP-1/p53 activity in ilc-17.1 deleted
larvae triggers dauer arrest, these data together indicate that CEP-1/p53-induced increase in cell
cycle inhibitors such as phg-1 (in P cells or other cells), and its ability to directly or indirectly
modulate cell fate in the ventral P cell lineage during development, where CEP-1/p53 accumulates during dauer arrest, may collaborate to pause growth programs of the animal.

ILC-17.1 links glucose metabolism to CEP-1/p53 activity in progenitor blast cells (P cells) of larvae.

The release of ILC-17.1 from amphid neurons in response to food suggested the intriguing possibility that ILC-17.1 signaling linked CEP-1/p53-mediated cell fate decisions to energy metabolism, since limited nutrient availability, glucose starvation or energy deprivation are all known to activate p53 in mammalian cells. Indeed, the dauer arrest of ilc-17.1 mutant larvae appeared related to an impairment in their ability to utilize glucose normally available through their diets: decreasing glucose availability by downregulating fgt-1, the main Glucose transporter (GLUT) responsible for glucose absorption in C. elegans, decreased the extent of dauer-rescue conferred by ILC-17.1 re-expression in ilc-17.1 mutant larvae (Fig. 5a). Similarly, fgt-1 RNAi increased the numbers of ilc-17.1 deletion mutant larvae that arrested as dauers under optimal conditions at 20°C (Fig. 5b). Promoting glucose utilization and/or metabolism by providing extra glucose rescued the dauer arrest of ilc-17.1 deficient larvae (Fig. 5c; Supplementary Fig. S8a). This effect of glucose supplementation also depended on glucose utilization and metabolism. Impairing glucose import through fgt-1 RNAi decreased the percentage of larvae that were rescued by the extra glucose (Supplementary Fig. S8b), and no rescue was observed on the non-hydrolyzable glucose analog 2-Deoxy-d-glucose (2-DOG; Supplementary Fig. S8c) which cannot undergo glycolysis and does not enter the metabolic pathway. We ruled out the involvement of mitochondrial reactive oxygen species, ROS, known to be increased under glucose-rich diets by using mitochondria-targeted antioxidant, Mito-Tempo, that scavenges ROS (Supplementary Fig. S8c). The rescue also did not appear to be due to the non-specific effect of nutritional supplementation (e.g. L-glutamine, another important carbon source for metabolism, or skim milk powder that can be ingested by C. elegans; Supplementary Fig. S8d). Downregulating fgt-1 in
wildtype larvae, while not sufficient to trigger dauer arrest, modestly increased the expression of CEP-1/p53 target egl-1 (but not ced-13; Supplementary Fig. S8e). Thus, these data supported the possibility that the activation of CEP-1/p53 upon the loss of ILC-17.1 could, at least in part, occur due an impairment in the ilc-17.1 deleted larvae’s ability to utilize glucose.

If glucose impairment was responsible for CEP-1/p53 activation, glucose supplementation should also rescue the dauer arrest of larvae overexpressing CEP-1/p53. This was the case (Fig 5c; Supplementary Fig. S8a), also arguing that decreased glucose intake in the absence of ILC-17.1 was not a consequence of CEP-1/p53 activation, but instead acted logically upstream of CEP-1/p53 activation. In agreement, providing extra glucose decreased the levels of CEP-1::GFP that accumulated in the ventral P blast cells (Fig. 5d), suppressed the increased expression of phg-1, the direct target of CEP-1/p53, which was in part responsible for dauer arrest of ilc-17.1 deleted animals (Fig. 5e), and decreased DAF-16/FOXO activation (Fig.5e). Surprisingly, not only did CEP-1/p53 appear to be activated by the inability of ilc-17.1 to utilize glucose normally, but CEP-1/p53 activity, in turn decreased the expression levels of key metabolic enzymes involved in glucose metabolism: phosphofructokinase-1.2 (pfk-1.2), the rate limiting enzyme in glycolysis, and cytochrome c (cyc-2.2), the subunit of complex IV of the electron transport chain responsible for the final transfer of reducing equivalents to O₂. This was visible in the RNA-seq data (Supplementary Figure S9 a-f; Supplementary Table 13) and confirmed by qRT-PCR (Supplementary Figure S9g, Fig. 5f). In mammalian cells, p53 activity is known to decrease the expression of enzymes required for glycolysis and indeed, the decrease in mRNA expression of pfk-1.2 was a consequence of CEP-1/p53 activation in the ilc-17.1 deleted larvae: the deletion of cep-1 rescued the low mRNA levels (Supplementary Figure S9g). Surprisingly, the decrease in cyc-2.2 expression was also dependent on CEP-1/p53, but also on DAF-16/FOXO, as the deletion of cep-1 or daf-16 rescued the low cyc-2.2 mRNA levels in ilc-17.1 deleted larvae (Fig. 5f). Moreover, although the downregulation of pfk-1.2 or cyc-2.2 alone was not sufficient to induce...
dauer in wildtype animals, RNAi induced downregulation of cyc-2.2 in ilc-17.1 deleted larvae increased the percentage of larvae that arrested as dauers under optimal conditions at temperatures of 20°C, suggesting that CEP-1/p53-dependent decrease of cyc-2.2 could feedback onto ilc-17.1 loss to increase dauer propensity (Supplementary Figure S9h). These data, together, strongly suggested that the loss of ILC-17.1 resulted in an impairment in glucose utilization and thus activated CEP-1/p53, which, upon activation, could further limit glucose utilization by downregulating genes important for glycolysis and OXPHOS. Thus, ILC-17 secretion from amphid neurons that occurred in response to food availability appeared to link cell fate decisions with energy metabolism through CEP-1/p53.

ILC-17.1 suppresses CEP-1/p53 in C. elegans and human epithelial cells.

Since our data pointed towards a model whereby ILC-17.1 signaling that occurred constitutively, under favorable conditions in the presence of food, suppressed CEP-1/p53 to promote continuous development, we directly examined whether IL-17 suppressed CEP-1/p53 activity. As was shown in B lymphoma cells\textsuperscript{101}, stimulation of untransformed human epithelial cells with human recombinant IL-17A also induced a modest but significant downregulation of p53 expression levels (Fig. 6a). This was similar to the decrease in CEP-1/p53 levels seen in C. elegans upon overexpression of ILC-17.1 (Fig. 3i). For the experiments in mammalian cells, IL-17A stimulation of epithelial cells was confirmed by measurement of secreted CXCL5, a key cytokine downstream of IL-17A signaling\textsuperscript{102} (Supplementary Fig. S10a).

In C. elegans too, increased ILC-17.1 levels suppressed CEP-1/p53 activation: overexpressing ILC-17.1 in animals also overexpressing CEP-1/p53 inhibited their dauer arrest (Fig. 6b), inhibited the increase in mRNA levels of CEP-1/p53 target genes, egl-1, ced-13 in CEP-1/p53 overexpressing animals (Fig 3g; Supplementary Fig S7d), cki-1 and phg-1 (Fig. 4d), and partially restored the decreased cyc-2.2 mRNA levels that resulted from CEP-1/p53
overexpression (Fig 5f). In *C. elegans* the activity of CEP-1/p53 has been best studied in the adult germline where CEP-1/p53 controls germline apoptosis in response to genotoxic insults\(^{60,62,67,68}\). Therefore, we used gamma irradiation to cause DNA damage in day-one adult animals, and measured the number of germline apoptotic corpses that result 24 hr. later, to determine whether increased ILC-17.1 levels could also inhibit this canonical activity of CEP-1/p53 (Fig 6c). As has been previously shown\(^{68}\), and consistent with the role of p53 in controlling DNA-damage induced-, but not physiological apoptosis, *cep-1 (gk138)* adults lacking functional CEP-1/p53 displayed only a mild decrease in the rates of physiological apoptosis when compared to wildtype animals (Fig. 6c). However, as also shown previously\(^{68}\), in these animals gamma irradiation did not increase apoptosis (Fig. 6c). CEP-1/p53 overexpression, surprisingly, caused a significant increase in physiological apoptosis, and a marked increase in irradiation-induced apoptosis, both of which were decreased upon also overexpressing ILC-17.1 in these animals (Fig 6c). Overexpressing *ilc-17.1* alone also decreased both physiological or irradiation-induced apoptosis compared to that in wildtype animals, but this effect was mild and did not reach significance (average nos. of cells undergoing physiological apoptosis in N2 v ILC-17.1 overexpressing animals were 3.56 ± 1.3 and 2.53 ± 1.1 cells, and upon irradiation, these increased to 7.1 ± 1.8 in N2, and 5.72 ± 2.2 in IL-17 overexpressing animals). Also, for reasons we do not understand, *ilc-17.1* deletion mutants did not display changes in apoptosis when compared to wildtype animals, suggesting perhaps that the functions of ILC-17.1 in adult tissue, and/or germline cells may be more nuanced when compared to its developmental function. Nevertheless, these data confirmed that, consistent with our working model (Fig. 6d), ILC-17.1 could inhibit CEP-1/p53. In addition, they suggested the possibility that the roles of *ilc-17.1* signaling and CEP-1/p53 activity might diverge post-development.

**Discussion**
Here we show, for the first time, a role for the cytokine IL-17, and p53 in the development of an invertebrate species, *C. elegans*. Specifically, we show that CEP-1/p53 repression during early larval development ensures the continuous growth of the animal into reproductive adults, and this repression is mediated by ILC-17.1 released by amphid neurons upon food availability. Reduced ILC-17.1 signaling, expected to occur when food is scarce, activates CEP-1/p53, which then triggers dauer arrest through the well characterized dauer activating pathways, DAF-16/FOXO, DAF-3/SMAD-DAF-5/Ski and also, perhaps also through steroid hormone signaling. In its role in promoting dauer arrest CEP-1/p53 accumulates in multipotent P blast cells that differentiate postembryonically into adult tissues, restrains cell cycle progression, and alters cell fate decisions. In addition, while promoting dauer arrest, CEP-1/p53 alters metabolic flux by directly or indirectly controlling the levels of phosphofructokinase (*pfk-1*) and cytochrome C (*cyc-2.2*). Notably, these are functions of p53 in its role as a tumor suppressor. We propose a working model whereby ILC-17.1 secretion from amphid neurons that occurs in response to food availability links cell fate decisions with energy metabolism through the conserved, multifaceted program of CEP-1/p53 (Fig. 6d). Our data also support the possibility that this ancient function of p53, conserved in all multicellular animals and shown here to modulate an invertebrate developmental program, could have been the driving force in the evolution of the p53 gene-family.

It is intriguing that during normal *C. elegans* development CEP-1/p53 appears to modulate the fate of multipotent P blast cells. In mammals too, normal p53 activity is essential for maintaining stem cell quiescence in the hematopoietic system and other organs during steady state tissue homeostasis, and unrestrained p53 activity is associated with a developmental syndrome resembling CHARGE (coloboma, heart defects, atresia choanae, growth retardation, genital abnormalities, and ear abnormalities), through its effects on cell cycle progression, apoptosis and the migration of neural crest cells. Cytokines such as IL-17 modulate glucose metabolism...
in mammalian models, and glucose deprivation can, in turn, activate p53. Thus, as seen in *C. elegans*, the ability of p53-proteins to link metabolic control with cell cycle progression and stem cell fate could be a conserved mechanism leveraged by several signaling pathways similar to IL-17s to coordinate metazoan development with nutrient availability.

The specific mechanisms by which ILC-17.1/CEP-1 axis promotes dauer entry remain to be better explored. In addition, we are yet to dissect the temporal requirements, sites of action, and mechanisms and consequences of ILC-17.1 signaling required to control CEP-1/p53 activation, as well as its effects on adult traits such as longevity and stress resistance--traits linked to other dauer-activating pathways. While the discovery of new *C. elegans* dauer pathway genes is surprising given the extensive genetic screens that have been conducted, genes that require a complete deletion to confer a dauer phenotype, QTLs that interact in complex ways to limit the discovery of dauer phenotypes, or genes that show functional redundancy and tolerance to quantitative variation such as seen with CEP-1/p53 overexpression, could have been missed. Indeed, a previously characterized *ilc-17.1* mutation *ilc-17.1*(tm5218), generated through EMS mutagenesis and in the background of a linked mutation in *npr-1*, itself known to be a modifier of the dauer phenotype, does not trigger dauer arrest under the same conditions (Supplementary Figure S10b). Intriguingly, our RNA-seq data show that gene expression changes between 15 hr. post-hatching and 32 hr. post-hatching are negligible in *ilc-17.1* deletion mutants and CEP-1/p53 overexpressing larvae, suggesting that this pathway is one of the very early checkpoints in the larval decision between continuous growth and dauer arrest. Thus, it is plausible that ILC-17.1 and CEP-1/p53 could even act as early as in the maternal germline, or during embryogenesis, to modulate developmental decisions.
It is interesting that ILC-17.1 is secreted by *C. elegans* sensory neurons to control CEP-1/p53 activity. Neurons control dauer entry also though TGFβ, insulin and steroid hormone signaling. In addition, other stress-responsive transcriptional programs such as the cellular response to protein misfolding that are cell autonomous in individual cells are controlled through neuronal signaling in a metazoan such as *C. elegans*, allowing the nervous system to coordinate tissue-specific transcriptional and epigenetic responses with organismal physiology and behavior\textsuperscript{105-108}. Nutrient scarcity has shaped much of evolution, and the ability of cells and organisms to sense nutrient availability and prepare in anticipation to pause cell cycle progression and growth and maintain a quiescent state until resources are optimal, has unquestionable selective advantages. For *C. elegans*, like for most organisms, the availability of food is not guaranteed. Neuronal ILC-17.1 signaling appears to be one mechanism which allows the animal to match the cell fate programs in the developing larvae and metabolic requirements with resource availability. In mammals, resident neurons and immune molecules in peripheral tissue profoundly influence cell fate decisions to remodel tissue architecture during development, organogenesis, inflammatory responses, and in diseases such as cancer\textsuperscript{109,110}. It is therefore not far-fetched to imagine that mammalian neurons control p53 activity, cell quiescence and stem cell fate in response to nutrient availability through IL-17s, as seen in *C. elegans*, although, perhaps in mammals this occurs locally through peripheral innervation and immune cells. Such a control mechanism could open new avenues for therapeutic intervention in cancer and other IL-17-mediated diseases.
**Materials and Methods**

**C. elegans strains**

*C. elegans* strains used in this study are listed in Table 1. Strains were procured from Caenorhabditis Genetics Center (CGC, Twin Cities, MN), generated in the laboratory or generated by Suny Biotech (Suzhou, Jiangsu, China 215028). All strains will be available upon request, after publication.

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<td>Prahlad Lab/ SunyBiotech</td>
<td><em>ilc-17.1</em> deletion, 2188bp deletion, and the 9bp and 118bp sequences were left in the 5' and 3' deletion end, respectively of the 2315 bp <em>ilc-17.1</em> gene</td>
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<tr>
<td>RW10645</td>
<td>stIs10645 [pax-3p::HIS-24::mCherry + <em>unc-119</em>(+); <em>unc-119</em> (ed3)]</td>
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<tr>
<td>VEP062</td>
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<td>Prahlad Lab</td>
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<tr>
<td>VEP059</td>
<td>stIs10645 [pax-3p::HIS-24::mCherry + <em>unc-119</em>(+); <em>unc-119</em> (ed3); gtIs1[cep-1::GFP + <em>unc-119</em>(+)]</td>
<td>Prahlad Lab</td>
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<tr>
<td>VEP063</td>
<td>praEx026(pax-3p::CEP-1A cDNA::3xFLAG::<em>unc-54</em> UTR; <em>pmyo-2</em>::mCherry::<em>unc-54</em> UTR)</td>
<td>Prahlad Lab</td>
<td></td>
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<tr>
<td>AX5690</td>
<td>npr-1(ad609)X; <em>ilc-17.1</em>(tm5218)X</td>
<td>Mario de Bono’s lab/ C. elegans National Bioresource Project, Japan</td>
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CRISPR/Cas9 was used to create following *C. elegans* strains:

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<tr>
<th>Background Strain</th>
<th>Type of editing</th>
<th>Description/position of editing</th>
<th>Gene</th>
<th>Resulting strain</th>
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<tr>
<td>N2, <em>C. elegans</em> var Bristol</td>
<td>Deletion (syb5296)</td>
<td>2173bp deletion, and the 15bp and 127bp sequences were left in the 5' and 3' deletion end, respectively of the 2135 bp <em>ilc-17.1</em> gene. sgRNA used - CCAAAATCACCCACACAGGACA AA</td>
<td>*(ilc-17.1)*X</td>
<td>VEP032</td>
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<td>N2, <em>C. elegans</em> var Bristol</td>
<td>Deletion (syb5297)</td>
<td>2188bp deletion, and the 9bp and 118bp sequences were left in the 5' and 3' deletion end, respectively of the 2135 bp <em>ilc-17.1</em> gene. sgRNA used - tagGCAAATGCGAATGCGGATG G</td>
<td>*(ilc-17.1)*X</td>
<td>VEP032</td>
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<td>N2, <em>C. elegans</em> var Bristol</td>
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<td>VEP043</td>
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<td>VEP044</td>
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<td>Insertion; <em>SL2::GFP</em> C'-terminus</td>
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<td>*(ilcr-2)*II</td>
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N2, *C. elegans* var Bristol

<table>
<thead>
<tr>
<th>Insertion; 3X FLAG</th>
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<tbody>
<tr>
<td>(cep-1)I</td>
</tr>
<tr>
<td>VEP040</td>
</tr>
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</table>

N2, *C. elegans* var Bristol

Deletion (1475bp of 1692 bp)

Pralhad Lab/ SunyBiotech

F25D1.3 (syb7367) V

PHX7367

Generation of transgenic strains

i) Backcrossing of *ilc-17.1* (syb5296) X

The *ilc-17.1* (syb5296) X strain was crossed to wildtype (N2) males, and the the male F1 progeny which harbored the *ilc-17.1* (syb5296) allele on their X chromosome were backcrossed to the *ilc-17.1* (syb5296) X strain and homozygous F2s were selected by PCR. This procedure was conducted 2X times to generate the 2X backcrossed *ilc-17.1* (syb5296) X line.

ii) Generation of *cep-1* overexpression strain

TG12 (*cep-1*(lg12501) I; *unc-119* (ed4) III; [CEP-1::GFP + *unc-119* (+)]) is a strain that expresses a functional CEP-1 tagged with GFP integrated into its genome, as determined by the rescue of *cep-1*(lg12501) phenotype. To overexpress CEP-1 we backcrossed the TG12 strain with wild-type (N2) worms and selected F2 progeny that were homozygous for the wild-type *cep-1* gene, lacked the *cep-1*(lg12501) I mutation (confirmed by PCR), and were homozygous for the CEP-1::GFP transgene (confirmed by 100% GFP expression amongst the F3 and F4 progeny). Overexpression of cep-1 mRNA was verified by qPCR (Supplementary Figure S6d). The CEP-1::GFP construct was PCR amplified from the final transgenic VEP037 *C. elegans* strain, and sequence verified.

iii) Generation of ILC-17.1 overexpression strain under its own promoter and 3'UTR

To generate the strain VEP024 – praEx021 (*ilc-17.1p::* *ilc-17.1* (cDNA)::3xFLAG::*ilc-17.1* 3'UTR); pmyo-2::*mCherry::*unc-54 3'UTR], which overexpressed *ilc-17.1* under the endogenous *ilc-17.1* promoter and *ilc-17.1* 3'UTR, we first amplified 3 kb of genomic sequence upstream of...
the translational start site (ilc-17.1 promotor) and 1 kb of genomic sequence downstream from
the translational stop codon (ilc-17.1 3' UTR). These regions were cloned using Gateway
technology into pDONR221. ilc-17.1 cDNA fused to C-terminal 3xFLAG sequence was
synthesized as a gBlock (Integrated DNA technologies). These three fragments were then
submitted for gene synthesis service through GenScript to generate the expression vector
pUC57(ilc-17.1p:: ilc-17.1 (cDNA)::3xFLAG:: ilc-17.1 3'UTR). All plasmids were sequence
verified. The ilc-17.1 expression vector was then injected at 97.5 ng/ul along with the co-
injection marker pCFJ90 (pmyo-2::mCherry::unc-54 3'UTR) at 2.5 ng/ul by InVivo Biosystems
injection express service. Animals expressing mCherry were singled, lines transmitting the
extrachromosomal array were established, and mCherry positive progeny were PCR verified to
ensure they were transmitting ilc-17.1p:: ilc-17.1 (cDNA)::3xFLAG:: ilc-17.1 3'UTR. These lines
were then harvested for Western blot to verify expression of protein.

iv) Generation of ILC-17.1 overexpression strain under the unc-54 muscle promoter
and tbb-2 3'UTR

To generate VEP031, we overexpressed ILC-17.1 under muscle promoter by fusing - [(unc-
54p:: ilc-17.1 (cDNA)::3xFLAG] which was synthesized as a gBlock (Integrated DNA
technologies) and tbb-2 3' UTR which was amplified from genomic DNA. The two fragments
were cloned into pUC19 plasmid as the backbone using Gibson assembly111 to create the
overexpression plasmid [(unc-54p:: ilc-17.1 (cDNA)::3xFLAG::tbb-2 3'UTR); pdat-1::ssmito] and
sequence verified. The construct was injected at 100 ng/ul along with the co-injection marker
that expressed GFP in the dopaminergic neurons under a dat-1 promoter [ pdat-1], at 10 ng/ul
into wildtype (N2) worms. Animals expressing GFP in their dopaminergic neurons were singled,
lines were established, and GFP-positive progeny were PCR verified to ensure that they were
also transmitting harbored the ilc-17.1 transgene. These lines were then harvested for Western
blot to verify expression of protein.
v) Generation of CEP-1 overexpression strain under the pax-3 P-cell promoter and unc-54 3'UTR

To generate the strain VEP063 – praEx026(pax-3p::CEP-1A cDNA::3xFLAG::unc-54 UTR; pmyo-2::mCherry::unc-54 UTR)], which overexpressed CEP-1 under the P-cell promoter pax-3 and unc-54 3'UTR, we first amplified 2655 bp of genomic sequence upstream of the translational start site (pax-3 promoter), 1932 bp of CEP-1A full length cDNA sequence without a stop codon from N2 cDNA, and 851 bp of sequence was amplified from vector pDONRP2r-P3(3xFLAG::unc-54 3'UTR). These three sequences were then cloned using a BP reaction into the appropriate DONR vectors for the Multisite Gateway Pro cloning kit (Invitrogen) according to manufacturer's guidelines. An expression vector was created by performing the multisite LR reaction using pDEST R1-R2 with the three entry clones generated above to generate pDEST R1-R2 (pax-3p::CEP-1A::3xFLAG::unc-54 3' UTR) according to manufacturer’s instructions. All plasmids were sequence verified. The CEP-1 expression vector was then injected at 120 ng/ul along with the co-injection marker pCFJ90 (pmyo-2::mCherry::unc-54 3'UTR) at 5 ng/ul into N2 wildtype animals. Animals expressing mCherry were singled, lines transmitting the extrachromosomal array were established, and mCherry positive progeny were then harvested for Western blot to verify expression of protein.

vi) Generation of crosses.

Crosses between strains were generated according to standard procedures. The primers used to genotype strains are included in the table below.

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<thead>
<tr>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
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<td>AGTTTTGAGATTGAGGGCAAA</td>
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<td>AAGCTGCTGCTTCACTCTC</td>
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<td>ATGGGACTCGAGTTGAGTG</td>
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</table>


<table>
<thead>
<tr>
<th></th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>TJ1</td>
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<td>CB1386</td>
<td>CGG CTT GAT CGG ATA GTG AT</td>
</tr>
<tr>
<td></td>
<td>Wild-type PCR product was digested by BstNI</td>
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</tr>
</tbody>
</table>

**Antibodies used:** Growth conditions for *C. elegans* strains

All strains were grown and maintained at 20°C unless otherwise mentioned. Animals were grown in 20°C incubators (humidity controlled) on 60mm nematode growth media (NGM) plates by passaging 8-15 L4s (depending on the strain) onto a fresh plates. Extra care was taken to ensure equal worm densities across all strains. Animals were fed *Escherichia coli* OP50 obtained from Caenorhabditis Genetics Center (CGC) that were seeded (OD$_{600}$=1.5 and this was strictly maintained throughout the experiments) onto culture plates 2 days before use. The NGM plate thickness was controlled by pouring 8.9ml of autoclaved liquid NGM per 60mm plate. Laboratory temperature was maintained at 20°C and monitored throughout. For all experiments, age-
matched day-one hermaphrodites, or larvae timed to reach specific developmental stages as mentioned in the figure legend, were used.

For all experiments with larvae, the timings were adjusted to account for the differences in growth rates at different temperatures. Thus, at 25°C, larvae were harvested 30-36 hrs. post bleach hatch or egg lay, and at 20°C, larvae were harvested 40 hrs. post bleach hatch or egg lay.

**Obtaining synchronized embryos by ‘bleach-hatching’**

'Bleach-hatching' was performed as previously described\(^{105}\). Populations of 250-300 gravid adults were generated by passaging L4s, as described above. These plates were used for obtaining synchronized embryos by bleach-induced solubilization of the adults. Specifically, animals were washed off the plates with 1X PBS and pelleted by centrifuging at 2665Xg for 30s. The PBS was removed carefully, and worms were gently vortexed in the presence of bleaching solution [250µl 1N NaOH, 200µl standard (regular) bleach and 550µl sterile water] until all the worm bodies had dissolved (approximately 5-6 minutes), and only eggs were viable. The eggs were pelleted by centrifugation (2665Xg for 45s), bleaching solution was carefully removed and then embryos were washed with sterile water 3-4 times and counted under the microscope. The desired number of embryos were seeded on fresh OP50 plates and allowed to grow at 20°C or 25°C for specific time periods depending on the experimental need. If >5% eggs remained unhatched, these plates were discarded.

**Dauer Assay**

Embryos were allowed to hatch and grow on OP50 plates at 20°C or 25°C for this assay. Embryos were generated according to one of the following two methods: (i) Day-one gravid adults that had grown under normal culture conditions (on OP50 at 20°C) were bleach dissolved as described above, and ~50-100 embryos obtained from these gravid adults were seeded on fresh OP50 plates or (ii) day-one gravid adults were allowed to lay eggs on fresh OP50 (or RNAi) plates for
2-4 hours at 20°C, the adults were removed, and then embryos were allowed to develop at 20°C or 25°C under humified condition for 48-72 hrs. The former, bleach-synchronization method was used for RNA-seq method and experiments requiring large numbers of age-matched larvae. The latter, timed-egg lay method was used for all other experiments. For each of the strains and experiments, when we had to use bleach synchronization, we first ensured that bleaching alone did not alter the percentage of dauers or gene expression profiles by conducting a pilot experiment using both methods side-by-side. This was the case for all experiments, except experiments where glucose rescued the dauer phenotype. Here, although the trends were the same, and glucose resulted in a significant rescue of ilc-17.1 deletion mutants and cep-1 OE larvae that were generated either by bleach-synchronization or timed-egg lay, bleach synchronization caused a lower percentage to be rescued from dauer arrest. To assess dauer entry, the number of larvae that arrested as dauers (determined by phenotype and/or resistance to 1%SDS) and those that developed into L4s or adults were counted and percentage of dauers was calculated. The total number of embryos in each plate was ~50-100. Each experiment was performed in triplicate or more.

For the *ilc-17.1* deletion mutants, in order to ascertain that the dauer phenotype was not simply due to the presence of a background mutation, we also conducted the dauer assay after backcrossing the *ilc-17.1* (syb5296) X animals with wild-type Bristol N2 animals, 2X times. The backcrossed strains also arrested as dauers under that same conditions and 100 ± 0% larvae (n=3 independent repeats, 212 larvae scored), arrested as dauers at 25°C as determined by SDS resistance compared to 0% of wildtype larvae.

**SDS treatment**

Larvae were washed off the dauer assay plates, washed 1X with PBS to remove bacteria, treated with 1% sodium dodecyl sulfate (SDS) for 30 minutes, washed again with M9, and then transferred
onto a fresh plate. Larvae could be scored easily as dead/dissolved or as live dauer larvae (based on their phenotype, and movement).

**RNAi mediated downregulation of genes**

All RNAi clones used were verified by sequencing, and plates were seeded with RNAi bacteria for a maximum of two days before being used. Day-one gravid adults grown on OP50 were allowed to lay eggs on RNAi-bacteria seeded plates for 2-4 hours at 20°C. A second generation of RNAi knockdown was performed where necessary by transferring L4’s on existing RNAi plates to fresh plates for knockdown in their progeny. The adults were allowed lay eggs and then removed, and the embryos were transferred to 25°C (or kept at 20°C) under humified condition for 48-72 hrs. The number of larvae that arrested as dauers (determined by phenotype and/or resistance to 1%SDS) and those that developed into L4s or adults were counted and percentage of dauers was calculated.

**Exposure to food [OP50] availability**

Gravid day-one ilc-17.1(pra03 [ilc-17.1::SL2::mCherry]) X adults or ilc-17.1(pra04 [ilc-17.1::3xHA]) X adults were bleach dissolved and embryos were placed either on empty plates with no food or NGM plates seeded with OP50 and allowed to grow for 24-36 hours at 25°C. To observe mRNA expression of ilc-17.1, ilc-17.1(pra03 [ilc-17.1::3xHA]) X larvae were anesthetized with 1mM levamisole, mounted on 1% agarose pads, and imaged using a Confocal SPE microscope using optimal settings. mCherry expression served to mark cells that expressed ilc-17.1 mRNA. To identify where ILC-17.1 protein was expressed prior to food exposure, and after food exposure, 24-36 hour ilc-17.1(pra04 [ilc-17.1::3xHA]) X larvae were fixed and immunostained using Bouin’s Tube Fixation method (see below).

**Immunofluorescence**
Nematodes were fixed and immunostained using a modification of the Bouin’s Tube Fixation method. Worms were fixed for 30 min. at room temperature (RT) in 400 μl Bouin’s fix (Sigma Aldrich) + 600 μl methanol and 10 μl β-mercaptoethanol by tumbling, freeze cracked three times in liquid nitrogen and again tumbled for 30 mins at RT. For permeabilization, the fixative was removed and exchanged for borate-Triton-β-mercaptoethanol (BTB: 1xBorate Buffer, 0.5% Triton and 2% β-mercaptoethanol) solution. Worms were tumbled 3 times for 1 hour each in fresh BTB solution at RT. Worms were washed with PBS-0.05% Tween and incubated in block-solution (5% BSA). Staining with primary antibody was performed overnight at 4 °C, incubation with secondary antibody (Donkey anti-Mouse Alexa Fluor 488, 1:2000) for 2-4 hours at RT. All antibody dilutions were performed using antibody buffer containing 5% BSA. Samples were mounted onto glass slides using VECTASHIELD antifade mounting medium (Vector Laboratories, Burlingame, CA, USA). Primary antibodies used: (i) Monoclonal ANTI-FLAG M2, Sigma Aldrich, 1:500, (ii) Mouse anti-HA antibody, Thermofisher; 2-2.2.14, at 1:1000, (iii) anti β-Actin, Cell Signaling Technology, #4967, 1:1000.

Chemotaxis

Chemotaxis assay was performed on 9 cm petri dishes containing NGM. Two marks were made on the back of the plate at opposite sides of the plate about 0.5 cm from the edge of the agar. About 5 μl of attractant diluted in water was placed on the agar over one mark, and 5 μl of water was placed as the control over the opposite mark. Attractants and concentrations used are listed in drugs and metabolites section. 5 ul sodium azide with the concentration of 1M was also placed at both the attractant source and the control source. This drug could anesthetize animals within about a 0.5-cm radius of the attractant. Age synchronized day-one adult worms were transferred to the middle of the NGM plates at a point equidistant from the middle of each odorant. After 1 hr., the assay was quantified by counting the number of worms that had left the...
center origin a chemotaxis index was calculated \([\#\text{Odor} - \#\text{Control}] / [\#\text{Odor} + \#\text{Control}]\). Each repeat consisted of 50 worms, and experiments were repeated a minimum of three times.

Measuring Pharyngeal pumping

Synchronized day-one adults were singled onto NGM plates seeded with OP50, and onto plates without OP50. Pharyngeal pumping rates were determined by recording the pharyngeal region of animals by video using a Leica S9i digital stereo microscope at 5X magnification and slowing down the video to manually count the number of ‘pumps’ in 10 seconds, three times per animal. The mean of these pumps was determined and the number of pumps/minute calculated. One complete cycle of synchronous contraction and relaxation of the corpus and the terminal bulb was counted as a pump.

Feeding of fluorescent latex beads

Overnight 5ml OP50 culture LB was pelleted and resuspended in fresh 0.5ml LB to concentrate bacteria. 1 µl of fluorescent beads of 0.5µm mean particle size that mimic size of *E. coli* (Sigma L3280, red fluorescence) was added to 1 ml of concentrated OP50 [1:1000 ratio (v/v)], and 100ul of the mixture was seeded onto NGM plates. Synchronized day-one adults were allowed to lay eggs on the bead containing plates for 2 hours at 20°C and then removed. After 40 hours, larvae were picked onto 1% agarose pads on glass slides and anesthetized using 10mM levamisole. Z-stack images of the larvae were taken on Leica TCS SPE confocal microscope and number of beads within a set area near the tail region per larvae was quantified.

Drug and metabolite treatment

The following drugs or metabolites were used:

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<th>Drug/metabolite</th>
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<th>Final concentration</th>
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</tr>
<tr>
<td>Glucose</td>
<td>Research products International</td>
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<tr>
<td>8-Bromo-cGMP</td>
<td>Cayman Chemical</td>
<td>5mM</td>
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</table>

The drug solution (0.5 ml) was spread onto NGM agar plates containing an OP50 lawn and left for 1 hr. to dry or used for chemotaxis assays as described. Age synchronized day-one adult worms were transferred to the middle of the NGM plates for 4 hrs. and dauer assay were performed as described. Sterile water was used as control.

8-Bromo-cGMP solution was first added to standard NGM plates and allowed to dry for 1 hour. 1.5 OD op50 was added on top and allowed to grow for 1 day before starting the dauer assay as described.

Plates containing D-glucose were prepared as described in published methods, except previously except that we seeded live OP50 onto Glucose plates. Stock solutions of 0.1 and 1M Sterile D-glucose was prepared and added into NGM agar to obtain final concentration of 5mM and 50mM, respectively. For control plate, equivalent amount of sterile water was added to NGM agar. The plates were left to dry for 2 days at RT before seeding 1.5 OD OP50 onto them.

After drying at RT for another 1 day, dauer assays were performed as described.

Fluorescence Image analysis
ImageJ (Fiji) v1.53i was used for measuring fluorescence intensity as follows: single planes [DAF-16::GFP, CEP-1::GFP, TG12] or projections of z-planes [DAF-28, INS-4, fluorescent latex beads, pax-3p::mCherry] were used for measuring fluorescence intensity. The region of interest was circled using the circle selection tool. The mean fluorescence intensity of each circled area was recorded using the measurement tool. When appropriate, the background fluorescence intensity was measured and subtracted. Quantification of measurements was done in Microsoft Excel/GraphPad.

**RNA-sequencing and Data analysis**

**a) RNA isolation, library preparation and sequencing**

For the Total RNA-seq samples: Day-one adult worms were bleach-hatched and ~3200 eggs/genotype (~800 eggs/plate and 4 plates/genotype) were seeded on fresh OP50 plates and allowed to grow for 30-34 hrs. at 25°C. Worms were washed with sterile water and total RNA was extracted from biological triplicates using the Direct-zol RNA Miniprep Kits (catalog no. R2050, Zymo Research). Libraries were prepared using the Illumina Stranded Total RNA Prep with Ribo-Zero Plus, for rRNA depletion (catalog no. 20040525, Illumina). Samples were sequenced in one lane of the Illumina NovaSeq 6000, generating 2x150bp paired-end reads.

For the mRNA-seq samples: Day-one adult worms were bleach-hatched and ~3200 eggs/genotype (~800 eggs/plate and 4 plates/genotype) were seeded on fresh OP50 plates and allowed to grow for either 15 hours or 32 hours at 25°C. Worms were washed with sterile water and total RNA was extracted from biological triplicates using the Direct-zol RNA Miniprep. Libraries were prepared using Illumina TruSeq Stranded mRNA kit.
Samples were sequenced in one lane of the Illumina NovaSeq 6000, generating 2x100bp paired-end reads.

b) RNA-seq analysis

The quality of the RNA sequences was assessed with FastQC. Adapters and sequence reads with a quality lower than Q25 were trimmed by using Trimgalore \(^1\) (v0.67). Ribosomal RNA (rRNA) contamination was filtered with sortmeRNA\(^2\) (version 4.3.4). Sequencing reads were aligned to the *C. elegans* genome (WBcel235\(^3\)) using the Star aligner\(^4\) (v2.7.9a) and then the aligned reads were quantified with FeatureCounts v2.0.1 from the R package Rsubread\(^5\). Differential expression analysis was performed using DESeq2\(^6\) and genes with FDR corrected p-value of < 0.05 were considered significant. Pairwise distance analysis (sample-to-sample) was performed by using normalized counts coupled with the variance stabilization transformation (VST) on the complete set of genes and calculating the Euclidean distance between the replicates. Principal Component Analysis (PCA) was done using the plotPCA function in DESeq2\(^6\) with the VST transformed data. The mRNA-seq was analyzed following a similar protocol as described above, excluding the SortmeRNA step.

The microarray data for the *C. elegans* dauer stage was obtained from the Supplementary Table 1 of the study by Wang, J. & Kim (2003)\(^7\). The expression values of the dauer stage were defined as the dauers expression (fold-change) at 0hr relative to the mixed staged reference RNA. Sequences IDs from the array were converted to standard gene nomenclature using Simplemine in Wormbase\(^8\). The correlation between the expression at the dauer stage and the fold change in expression in the *ilc-17.1* deletion mutant relative to the wild type, was determined by performing a Spearman Rank Test.

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\(^1\) TrimGalore
\(^2\) SortmeRNA
\(^3\) WBcel235
\(^4\) Star
\(^5\) Rsubread
\(^6\) DESeq2
\(^7\) Wang, J. & Kim (2003)
\(^8\) Wormbase
The expression of C. elegans larvae (L1) treated with ascaroside cocktail was obtained from Cohen, et al. The correlation between the Fold-Change in expression in the larvae treated with ascaroside relative to N2 larvae and the fold change in expression in the *ilc-17.1* deletion mutant relative to the wild type, was determined by performing a Spearman Rank Test. The differentially expressed genes (DEGs) between N2 and daf-1(m40) was obtained from Hu, et al. and were presented as Log2(Fold-Change) values. The overlap between differentially expressed genes (DEGs) in the cep-1 overexpression mutant and the *ilc-17.1* deletion mutant was tested using Fisher exact test from the Gene Overlap package. Venn diagram was done using the R package VennDiagram.

c) Functional analysis

Gene ontology analysis (GO) and KEGG enrichment analysis was performed on the differentially expressed genes by using ClusterProfiler 4.0. Terms with an FDR corrected p-value <0.05 were considered significant. The annotations were obtained from R package org.Ce.eg.db (version 3.14) and the KEGG database. The annotations of genes as part of the metabolic pathways were obtained from the database Wormpaths and heatmaps were done using the R packages Complexheatmap and pheatmap.

Progenitor blast cell (P cells) imaging and quantification.

Synchronized day-one adults were allowed to lay eggs on OP50 plates for 2 hours at 20°C and then removed. The plates were then placed at 20°C or 25°C. After 15 hours, about 10 L1 larvae were picked onto 1% agarose pads on glass slides and anesthetized using 10mM levamisole and imaged on a Leica SP8. Cells expressing pax-3p::mCherry and CEP-1::GFP were scored, and the percent pax-3p::mCherry cells that also expressed CEP-1::GFP was calculated for individual worms.
DiO dye filling assay to identify amphid neurons

A stock dye solution containing 2mg/ml DiO (Molecular Probes, catalog # D-275) in dimethyl formamide is made and maintained under dark conditions. Day-one adult worms were transferred into an eppendorf tube with 1 ml of M9, spun down at 2000-3000 rpm and supernatant removed. Worms were resuspended in 1 ml of M9 and 5 microliter DiO stock sol (1:200 dilution) was added and incubated on a slow shaker for 2 hours at room temperature. Worms were spun down and washed with M9 twice before transferring them onto agar pads with 1mM levamisole to visualize by fluorescence using a Leica TCS SPE confocal microscope.

Dauer pharyngeal pumping

Synchronized day-one adults were allowed to lay eggs on OP50 plates for 2 hours at 20°C and then removed. The plates were then placed at 25°C, and larvae were allowed to arrest as dauers or grow into L4 larvae (RNAi treatment) . On day 3, larvae that remained arrested as dauers were used to measure pharyngeal pumping rates using Zeiss Axio Observer.A1 at 40X magnification. WT (N2) L2 stage larvae were used to measure pumping rates as controls. Pumping rates were measured by counting the number of grinder movements in the terminal bulb per minute. The average of three biological replicates consisting of 10 dauer larvae each was quantified.

Scoring apoptotic nuclei with Acridine Orange

Acridine orange (AO) staining was performed as previously described (Hefel et al. 2021). AO staining to detect apoptotic germline cells was performed by picking 50 L4 animals to a fresh plate and allowing them to grow into day-one adults for 24 hours at 20°C. Day 1 adults were then either exposed to 75 Gy from a Cesium source or left unirradiated. 24 hours later, AO staining was performed.. For each trial, 10 mg/ml AO stain was freshly prepared and then diluted 1:400 with M9 buffer. Worms were then picked to a tube of the diluted AO stain, wrapped
tightly with foil, and rotated at room temperature for 1 hour. After mixing, worms were
transferred to a fresh OP50 plate and allowed to crawl away from residual AO stain for 1 hour
before being picked to a droplet of 10 mM levamisole on a 1% agarose pad. A cover slip was
added, and worms were imaged immediately using the Leica fluorescence microscope on 60x
magnification. It was important to visualize AO within 10 mins after mounting animals on the
pad. The number of AO stain positive cells found in 12 individual gonad arms per experiment
were recorded for each sample.

**Gamma irradiating C. elegans to activate CEP-1/p53**

100 L4 larvae were picked onto an OP50 seeded plate, allowed to mature into day-one adults
and exposed to 75 Gy gamma irradiation, and subsequently harvested for Western blot
analysis. To determine the number of AO positive apoptotic nuclei, at least 50 L4 larvae were
exposed to 75 Gy gamma irradiation. The number of AO positive cells were scored 24 hrs. later,
when animals had matured into day-one adults.

**Western blot analysis**

  **i. Western blot analysis of C. elegans**

Western blot analysis was performed with 30–100 adult day-one animals, or approximately 400
30-36 hr. larvae as indicated. Animals were harvested in 15 µl of 1X PBS (pH 7.4), and 4X
Laemmli sample buffer (catalog no. 1610737, Bio-Rad) supplemented with 10% β-
mercaptoethanol was added and samples were boiled for 30 min. Whole-worm lysates were
resolved on 12% SDS-PAGE gels and transferred onto nitrocellulose membrane (catalog no.
1620115, Bio-Rad). Membranes were blocked with Odyssey Blocking Buffer (part no. 927–50000,
LI-COR). Immunoblots were imaged using LI-COR Odyssey Infrared Imaging System (LI-COR
Biotechnology, Lincoln, NE). Mouse anti-FLAG M2 antibody (catalog no. F1804, RRID:AB_262044,_Sigma Aldrich) was used at 1:500 to detect CEP-1::FLAG. Mouse anti-HA
antibody (Thermofisher; 2-2.2.14), was used at 1:1000 to detect ILC-17.1::HA. Mouse anti-α-tubulin primary antibody (AA4.3, RRID:AB_579793), developed by C. Walsh, was obtained from the Developmental Studies Hybridoma Bank (DSHB), created by the National Institute of Child Health and Human Development (NICHD) of the National Institute of Health (NIH), and maintained at the Department of Biology, University of Iowa. The following secondary antibodies were used: Donkey anti-mouse IgG (H and L) Antibody IRDye 800CW Conjugated (Licor) and Alexa Fluor 680 goat anti-rabbit IgG (H+L) (Invitrogen). LI-COR Image Studio software (RRID:SCR_015795) was used to quantify protein levels in different samples, relative to α-tubulin levels. Fold change of protein levels was calculated relative to wildtype (N2)/ controls.

ii. Western blot analysis of epithelial cell lines

A549 epithelial cells were lysed in a modified M2 buffer containing 20mM tris[hydroxymethyl]aminomethane, 150mM NaCl at pH 7.4, 0.5% NP40, 3mM EDTA, 3mM EGTA, 4mM PMSF, and a Complete Mini, EDTA-free protease inhibitor tablet according to the manufacturer’s instructions (Roche, #11836170001). Epithelial cells were collected by mechanical dislodgment in lysis buffer at individual time points, incubated for 20 minutes on ice, and spun at 14,000 x g for 5 minutes to remove insoluble material. 25ug protein was resolved by NuPAGE™ 4-12% Bis-Tris gel (Invitrogen) and transferred to PVDF for immunoblotting. Anti-p53 (Cell Signaling Technology, #9282) and β-actin (DSHB, 224-236-1) primary antibodies were used with HRP-conjugated secondary antibodies to anti-rabbit IgG (Jackson ImmunoResearch, #111-035-003) and anti-mouse IgG (Invitrogen, #62-6520). Densitometry was analyzed with ImageJ software.

RNA extraction and real-time quantitative reverse-transcriptase PCR (RT-PCR)

Worms were bleach-hatched as described above and ~400-800 eggs/plate (2 plates/strain) were seeded on fresh OP50 plates and were allowed to grow (i) for 30-34 hrs. at 25°C or (ii) for 36 hrs.
at 20°C and then harvested for RNA extraction. RNA was extracted as described earlier. Briefly, plates were washed with sterile water and centrifuged. Water was carefully removed and 300 µl of Trizol (catalog no. 400753, Life Technologies) was added and snap-frozen immediately in liquid nitrogen. Samples were thawed on ice and then lysed using a Precellys 24 homogenizer (Bertin Corp.). RNA was then purified as detailed with appropriate volumes of reagents modified to 300 µl of Trizol. The RNA pellet was dissolved in 17 µl of RNase-free water. The purified RNA was then treated with deoxyribonuclease using the TURBO DNA-free kit (catalog no. AM1907, Life Technologies) as per the manufacturer’s protocol. cDNA was generated by using the iScript cDNA Synthesis Kit (catalog no. 170–8891, Bio-Rad). qRT-PCR was performed using PowerUp SYBR Green Master Mix (catalog no. A25742, Thermo Fisher Scientific) in QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific) at a 10 µl sample volume, in a 96-well plate (catalog no. 4346907, Thermo Fisher Scientific). The relative amounts of mRNA were determined using the ΔΔCt method for quantitation. We selected *pmp-3* as an appropriate internal control for gene expression analysis in *C. elegans*.

All relative changes of mRNA were normalized to either that of the wild-type control or the control for each genotype (specified in figure legends). Each experiment was repeated a minimum of three times. For qPCR reactions, the amplification of a single product with no primer dimers was confirmed by melt-curve analysis performed at the end of the reaction. Primers were designed using Primer3 software and generated by Integrated DNA Technologies. The primers used for the qRT-PCR analysis are listed below:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer (5’-3’)</th>
<th>Reverse primer (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>pfk-1.2</em></td>
<td>TTGCATCGAATCTGTGAAGC</td>
<td>TCGCTGCAGTCAAAGCTAGA</td>
</tr>
<tr>
<td><em>cyc-2.2</em></td>
<td>CGGTGGAGCTATTCCAGAAG</td>
<td>GCAACTTGTCCGGATTGTCT</td>
</tr>
<tr>
<td><em>egl-1</em></td>
<td>TCCAAGCTAGCAGCAATGTG</td>
<td>GCGAAAAAGTCCAGAAGACG</td>
</tr>
</tbody>
</table>
Chromatin immunoprecipitation (ChIP)

Chromatin immunoprecipitation (ChIP) was performed as described earlier (Das et al., 2020). Day-one adult worms were bleach-hatched as described above and ~1600 eggs were seeded (~800 eggs/plate and 2 plates/genotype) on fresh OP5O plates. Plates were kept at 25°C for 30-34 hrs. and larvae were washed with 1X PBS (pH 7.4) and cross-linked with freshly prepared 2% formaldehyde (catalog no. 252549, Sigma Aldrich) at room temperature for 10 min followed by addition of 250 mM Tris (pH 7.4) at room temperature for 10 min. Samples were then washed three times in ice-cold 1X PBS supplemented with protease inhibitor cocktail and snap-frozen in liquid nitrogen. The worm pellet was resuspended in FA buffer [50 mM HEPES (pH 7.4), 150 mM NaCl, 50 mM EDTA, 1% Triton-X-100, 0.5% SDS and 0.1% sodium deoxycholate], supplemented with 1 mM DTT and protease inhibitor cocktail. The suspended worm pellet was lysed using a Precellys 24 homogenizer (Bertin Corp.), and then sonicated in a Bioruptor Pico Sonication System (catalog no. B0106001, Diagenode) (15 cycles of 30 s on/off).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>ced-13</td>
<td>GCAACTCAACACCGTGAA</td>
<td>CAATGCTGCGATACGTCTTG</td>
</tr>
<tr>
<td>phg-1</td>
<td>CAGAGGAGCTGTGACCGACA</td>
<td>TCGTCCTGACGATGCTGT</td>
</tr>
<tr>
<td>sod-3</td>
<td>CACTGCTCAAAGTCTTCA</td>
<td>ATGGGAGATCTGGGAGAGTG</td>
</tr>
<tr>
<td>mtl-1</td>
<td>TGGATGTTAGGGAGACTGCAA</td>
<td>CATTCTTAAATGAGCCGCAGCA</td>
</tr>
<tr>
<td>lys-7</td>
<td>GCCGTCACAAGTGGCATCTT</td>
<td>GGGTTGTATGCACGAAGCGAA</td>
</tr>
<tr>
<td>pmp-3</td>
<td>TAGAGTCAAGGGTCTGAGT</td>
<td>ATCGGCACCAAGGAAGCTG</td>
</tr>
<tr>
<td>cep-1(a+b isoform)</td>
<td>GCTCACTCTGTGACTGCTGAGT</td>
<td>AACCCAAGGTATCTGGGAACCTTT</td>
</tr>
<tr>
<td>cep-1(a isoform)</td>
<td>GTTGTGCTCGACTCCAAAAG</td>
<td>GGCAACGCTTTCTCAATTACAGTT</td>
</tr>
</tbody>
</table>
Endogenous CEP-1 was immunoprecipitated with anti-FLAG M2 magnetic bead (catalog no. M-8823, Sigma-Aldrich). Beads were first pre-cleared with salmon sperm DNA (catalog no. 15632-011, Invitrogen). Worm lysate was incubated at 4°C overnight with the pre-cleared FLAG beads. Beads were washed with low salt, high salt and LiCl wash buffers and then eluted in buffer containing EDTA, SDS and sodium bicarbonate. The elute was then de-crosslinked overnight in presence of Proteinase K. The DNA was purified by ChIP DNA purification kit (catalog no. D5205, Zymo Research). qPCR analysis of DNA was performed as described above using primer sets specific for different target genes. For all ChIP experiments, 10% of total lysate was used as 'input' and chromatin immunoprecipitated by different antibodies were expressed as % input values. The primers used for ChIP experiments, and the expected amplicon sizes are as follows:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>Forward primer (5'-3')</th>
<th>Reverse primer (5'-3')</th>
<th>Amplicon size</th>
</tr>
</thead>
<tbody>
<tr>
<td>egl-1</td>
<td>TSS +4 to +123</td>
<td>CTCACCTTTGCCTCAACCTC</td>
<td>CGAGGAGAAGTCCTAGACG</td>
<td>120 bp</td>
</tr>
<tr>
<td>ced-13</td>
<td>Promoter (-428 to -310)</td>
<td>CATTCTTTGGCCGTGCTCAT</td>
<td>AGGCAATCTAGCATGCACCT</td>
<td>119 bp</td>
</tr>
<tr>
<td>phg-1</td>
<td>Promoter (-428 to -294)</td>
<td>GCCAAACCTTCCAGATTATACA</td>
<td>TTCCTAGATAAGGTTAGATGAGAGA</td>
<td>135 bp</td>
</tr>
</tbody>
</table>
| phg-1 | Intron 1 (+223 to +326) | AAGCTGAGCTCCGA 
AACAA | TTTCCCCTAAAACGAGACAT | 104 bp |
**Treatment of mouse epithelial cell lines with human IL-17 and Nutlin3A**

A549 epithelial cells were seeded in a 6-well plate overnight and stimulated with 50-100ng/mL recombinant human-IL-17 (Peprotech, SKU 200-17) or 10uM Nutlin3A (Tocris, 675576-98-4), and supernatants and cell lysates were harvested 18 hours later.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Each ‘experiment’ refers to a set of independent biological samples that undergo treatment together. No statistical methods were used to predetermine sample size, and experiments were not randomized, but some were blinded. For the assays where we scored dauers, for each experiment, independent biological samples of the different strains were allowed to lay eggs and the eggs were subjected to ‘treatment’ or ‘control’ conditions. The results of an experiment generated counts categorized as either (a) dauers, or (b) non-dauers. The counts of dauers/non-dauers for the different types of experiments were used to generate a contingency table, and a chi-squared test was performed to test the independence (whether the results are related or not) of the experiment. This Chi-square statistic, degrees of freedom (df) and p value for each experiment is reported. Yates' continuity correction was used to prevent overestimation of...
statistical significance for small data. For qPCR or other experiments, the data were analyzed by using Student’s t-test and/or one-way ANOVA with Tukey’s correction (GraphPad Prism software) as described in respective figure legends. P values are indicated as follows: *p<0.05; **p< 0.01; ***p<0.001, ns, not significant.

DATA AVAILABILITY

The following data sets were generated

Genes differentially expressed in the following C. elegans larvae bleach-hatched and allowed to grow for 30-36 hr. at 25°C: wild type (N2), ilc-17.1(syb5296) X and praEx022 (unc-54p::ilc-17.1 cDNA::3XFLAG::tbb-2 3'UTR; pdat-1::GFP::unc-54 3'UTR).

Genes deferentially expressed in the following C. elegans larvae bleach-hatched and allowed to grow for 15 hr. and 32 hr. at 25°C: wild type (N2), ilc-17.1(syb5296) X, cep-1 (gk138) I, ilc-17.1(syb5296) X; cep-1 (gk138) I, and VEP036 (unc-119 (ed4); gtIs1 [CEP-1::GFP + unc-119 (+)].

**Figure 1:** ILC-17.1 is released by larval amphid neurons in response to food availability and reduced ILC-17.1 signaling causes dauer arrest.

a. Schematic of experiment to assess dauer arrest. For each experiment, independent samples of 10 day-one adults of each strain laid eggs for two hours at 20°C on 60 mm NGM plates with OP50 lawns. Adults were removed, and embryos were transferred to 25°C. Approximately 48 hr. later, larval phenotype was scored and categorized as either dauers, or larvae that continued development into stage 4 larvae (L4) and/or young adults. If unsure, SDS-resistance was used to confirm dauer arrest. Extent of crowding on plates was comparable. [Representative average numbers of embryos/plates from experiments in b are 64, 68, 56, 46 and 65 for wildtype (N2), ilc-17.1(syb5296) X, ilc-17.1(syb5297) X, ilc-17.1(syb5296) rescued with ilc-17.1p::ilc-17.1, and ilc-17.1(syb5296) rescued with unc-54p::ilc-17.1 respectively]. Pearson's Chi-squared test with Yates' continuity correction was used to determine significance throughout the manuscript, unless otherwise stated. Chi-squared values were calculated for whole experiments, and for pair-wise comparisons. All legends show Chi-squared, df and p values for the whole experiment; p values for pair-wise comparisons are depicted on graphs using ***p < 0.001, **p < 0.01, *p < 0.05 and ns, not significant.

b. Percentage larvae that arrest as dauers. ***p<0.001. (n=3-12 experiments; Chi-squared = 2359.6, df = 4, p-value < 2.2e-16.)

c, d. Representative micrographs 48 hours post-hatching at 25°C. **Top (c):** Wildtype (N2) grew to L4s. **Bottom (d):** ilc-17.1(syb5296) larvae arrested as dauers. Scale bar, 0.5mm.

e. **Top:** Schematic of experiment. **Bottom:** Percentage of wildtype (N2) and ilc-17.1 larvae that arrest as dauers on control (L4440) and ilcr-2 RNAi. ***p < 0.001, ns, not significant (n=3-10 experiments. Chi-squared = 1815.1, df = 7, p-value < 2.2e-16.)
**f. Top:** Schematic of experiment to localize *ilc-17.1* mRNA and ILC-17.1 protein in the presence and absence of food (OP50). Embryos were hatched at 20°C and imaged after 24-36 hours (L1 stage larvae). **Left panels:** Representative micrographs of z-section showing mCherry co-expressed with *ilc-17.1* mRNA in amphid neurons. **Right panels:** projection of z-sections showing immunostaining with anti-HA antibody to visualize CRISPR tagged endogenous ILC-17.1::HA protein. Pharyngeal region of larvae (top panel) hatched in the absence of food, and (middle panel) in the presence of food (OP50). **Bottom panels:** Controls (N2) that do not express HA or mCherry used to assess specificity of the fluorescence signal. (n>3 experiments, 4-5 larvae each). Scale bar, 5µm.

**g.** KEGG enrichments associated with differentially downregulated genes (p.adjust <0.05) in *ilc-17.1* mutant larvae (RNA-seq on 32-36 hr. larvae).

**h.** KEGG enrichments associated with differentially upregulated genes (p.adjust <0.05) in *ilc-17.1* mutant larvae (RNA-seq on 32-36 hr. larvae).

**i.** Heatmap depicting expression levels (log2 normalized counts) of the major dauer-specific collagens in wildtype (N2) as L2-L3 larvae *en route* to development into reproductive adults, *ilc-17.1* deletion mutants *en route* to dauer entry and *ilc-17.1* deletion mutants rescued from dauer entry with *unc-54p::ilc-17.1*.

Bars show mean ± S.E.M. Individual data points in the bar graphs in b and e represent the % dauers/experiment, and the bar graph depicts mean of these percentages.
Figure 2: Reduced ILC-17.1 signaling acts through DAF-16/FOXO, DAF-3/SMAD-DAF-1114/5/Ski complex, steroid hormone signaling and CEP-1/p53 to control dauer arrest.

a. Percent dauers in \textit{ilc-17.1} deletion mutants and \textit{ilc-17.1;daf-16} double mutant larvae. 

\[ ***p < 0.001, (n=4-8 \text{ experiments, Chi-squared} = 497.65, \text{ df} = 1, \text{ p-value} < 2.2e-16). \]

b. \textbf{Left}: Percent larvae showing DAF-16::GFP nuclear localization in intestinal cells. Larvae were grown at 20°C for 36 hrs. \( n = 3 \) experiments with 10-15 larvae each. Each data point represents average no. of larvae with nuclear DAF-16::GFP/experiment. \[ ***p < 0.001, (\text{unpaired t-test}). \]

Note: experiments were conducted at 20°C, since DAF-16::GFP localized constitutively in intestinal nuclei even in wildtype larvae at 25°C. \textbf{Right}: representative micrographs showing intestinal DAF-16::GFP (boxed) in wildtype and \textit{ilc-17.1} deletion mutant larvae. Scale bar, 5µm.

c. Average sod-3, mtl-1, and lys-7 mRNA levels in larvae 32-36 hr. post-hatching at 25°C. mRNA levels were determined relative to \textit{pmp-3} and normalized to wildtype (N2) values. \( n = 4-10 \) experiments. \[ ***p < 0.001, **p < 0.01, \text{ns=not significant, (unpaired t-test)}. \]

d. Percent dauers in \textit{ilc-17.1} mutants and \textit{ilc-17.1;daf-5} double mutants. \[ ***p < 0.001, (n=3-5 \text{ experiments, Chi-squared} = 787.3, \text{ df} = 3, \text{ p-value} < 2.2e-16). \]

e. Percent dauers in \textit{ilc-17.1} mutants on control (H2O) and exogenous 50nM \( \Delta7 \)-dafachronic acid.

\[ ***p < 0.001, (n=3-5 \text{ experiments, Chi-squared} = 784.32, \text{ df} = 3, \text{ p-value} < 2.2e-16). \]

f-h. \textbf{f}, percent dauers in \textit{ilc-17.1} mutants and \textit{ilc-17.1;cep-1} double mutants. \[ ***p < 0.001, (n=3-5 \text{ experiments, Chi-squared} = 1029.2, \text{ df} = 3, \text{ p-value} < 2.2e-16). \]

Representative micrographs of \textbf{g}, \textit{ilc-17.1} deleted larvae arrested as dauers, and \textbf{h}, \textit{ilc-17.1;cep-1} double mutants that grew to L4s, 48 hr. post-hatching at 25°C. Scale bar, 0.5mm.
Bars show the mean ± S.E.M. Individual data points in bar graphs in a, d, e, f represent the average % dauers/experiment, Pearson's Chi-squared test with Yates' continuity correction.
Figure 3: CEP-1/p53 acts downstream of reduced ILC-17.1 signaling and activates DAF-16/FOXO, DAF-3/SMAD-DAF-5/Ski complex to control dauer entry.

a-c. a, Percent dauers in wildtype (N2), cep-1 (lg12501); CEP-1::GFP animals that do not overexpress CEP-1/p53, and CEP-1/p53 overexpressing larvae. ***p < 0.001, (n = 3-4 experiments, Chi-squared = 569.35, df = 2, p-value < 2.2e-16). For cep-1 mRNA expression levels see Supplementary Fig. S6a. Representative micrographs 48 hrs. post-hatching at 25°C, showing in b, CEP-1/p53 overexpressing larvae arrested as dauers and c, cep-1 (lg12501); CEP-1::GFP as L4s. Scale bar, 0.5mm.

d. Percent dauers in wildtype (N2), and CEP-1/p53 overexpressing larvae on control (Ctrl; L4440) and daf-16 RNAi. ***p < 0.001, **p < 0.01 (n = 3-4 experiments, Chi-squared = 494.22, df = 3, p-value < 2.2e-16).

e. Percent dauers in wildtype (N2), daf-5 (e1386), CEP-1/p53 overexpressing larvae, CEP-1/p53 overexpressing larvae crossed to daf-5 (e1386), and N2 and CEP-1/p53 overexpressing larvae treated with 50nM Δ7-dafachronic acid (DA). ***p < 0.001, **p < 0.01, ns, not significant, (n = 3-4 experiments, Chi-squared = 982.66, df = 5, p-value < 2.2e-16).

f. Average sod-3, mtl-1, and lys-7 mRNA levels in larvae 32-36 hr. post-hatching at 25°C. mRNA levels were determined relative to pmp-3 and normalized to wildtype (N2) values. n = 4-10 experiments. ***p < 0.001, **p < 0.01, ns = not significant, (unpaired t-test).

g. Average egl-1 and ced-13 mRNA levels in 32-36 hr. larvae grown at 25°C. mRNA levels were determined relative to pmp-3 and normalized to wildtype (N2) values. n = 6-7 experiments. ***p < 0.001, **p < 0.01, *p < 0.05, ns = not significant, (unpaired t-test).
h. CEP-1 occupancy (expressed as percent input) in 32-36 hr. larvae grown at 25°C, measured at the promoter proximal regions of egl-1 and ced-13 (Top, schematic). CEP-1 occupancy was assayed in animals where endogenous cep-1 was FLAG tagged at its C-terminus using CRISPR/Cas9, and immunoprecipitated with anti-FLAG antibody. Strains used: CEP-1::FLAG expressing animals in control wildtype background (Ctrl), in ilc-17.1 background, and in ilc-17.1 rescued from dauer arrest by unc-54p::ilc-17.1 (ilc-17.1;unc-54p::ilc-17.1 CDS). n=4 experiments. ***p < 0.001, **p < 0.01, *p < 0.05. (unpaired t-test).

i. Top: Representative Western blot showing CEP-1::FLAG and tubulin when crossed into unc-54p::ilc-17.1, and ilc-17.1 deletion backgrounds. cep-1 RNAi serves to show specificity. Samples constitute 32-36 hr. larvae grown at 25°C, except for positive control, irradiated sample (4; adults).

Bottom: CEP-1 levels were quantified relative to tubulin and normalized to values from control animals (Ctrl; CEP-1::FLAG in wildtype background). n=4 experiments. Numbers in data bars correspond to representative bands on western. ***p < 0.001, **p < 0.01 (unpaired t-test).

Bars show the mean ± S.E.M. Individual points in bar graphs in a, d, e represent the % dauers/experiment, Pearson’s Chi-squared test with Yates’ continuity correction.
Figure 4. CEP-1/p53 accumulates in multipotent progenitor cells (P cells) during dauer entry, upregulates cell cycle inhibitors, and modulates cell fate.

a. Representative micrographs of 15 hr. L1 larvae at 25°C, showing pax-3p::mCherry marking ventral postembryonic blast cells (P cells; top panel; see schematic above), CEP-1::GFP expression (middle panel), and overlap (bottom panel) to show co-localization. Co-localized cells are marked with *. CEP-1::GFP expressing cells that do not co-localize with pax-3p::mCherry marked with #. Insets: zoom-in on the P blast cells. Scale bar=5μm.

b. Percent co-localization between pax-3p::mCherry-expressing cells and CEP-1::GFP expressing cells at 20°C and 25°C. n=3 experiments, 10-15 larvae/experiment. **p < 0.001, (unpaired t-test). Each data point represents the percent of pax-3p::mCherry-expressing cells that colocalize with CEP-1::GFP in one animal.

c. Fluorescent intensity (arbitrary units; AU) measured in P cells of L1 larvae, 15 hr. post-hatching at 20°C and 25°C. Strains used: cep-1 (lg12501); CEP-1::GFP animals that do not overexpress CEP-1/p53, CEP-1/p53 overexpressing larvae, and ilc-17.1 deletion mutants expressing CEP-1::GFP. n=3 experiments, 5-6 larvae/experiment. **p < 0.001, ns, not significant (unpaired t-test). Each data point represents the average fluorescence intensity value across five P cells in one animal (see Material and Methods).

d. Average cki-1 and phg-1 mRNA levels in 32-36 hr. larvae grown at 25°C. mRNA levels were determined relative to pmp-3 and normalized to wildtype (N2) values. n=4-6 experiments. *p < 0.05. (unpaired t-test).

e. CEP-1 occupancy (expressed as percent input) in 32-36 hr. larvae grown at 25°C, measured at the promoter proximal region of phg-1 (Top, schematic). CEP-1 occupancy was assayed in
animals where endogenous cep-1 was FLAG tagged at its C-terminus using CRISPR/Cas9, and immunoprecipitated with anti-FLAG antibody. Stains used: CEP-1::FLAG expressing animals in control wildtype background (Ctrl), in ilc-17.1 background, and in ilc-17.1 rescued from dauer with ilc-17.1; unc-54p::ilc-17.1 CDS. n=4 experiments. *p < 0.05. (unpaired t-test).

f. Percent dauers in ilc-17.1 larvae and ilc-17.1;phg-1 double mutants. **p < 0.05, ***p<0.001 (n=6 experiments, Chi-squared = 826.95, df = 3, p-value< 2.2e-16). Individual points in the bar graph represent the % dauers/experiment, Pearson's Chi-squared test with Yates' continuity correction.

g. Top: Representative micrographs of wildtype (N2) animals and animals overexpressing CEP-1 driven by the pax-3 promoter or 25°C. Scale bar, 50µm. Bottom: Percent larvae that displayed body morphology defects. **p<0.01, (unpaired t-test)

h. Top: Schematic showing homeobox gene expression patterns during post-embryonic development. Bottom: Heatmap depicting expression levels (log2 normalized counts) of the homeobox genes on Chromosome III, 32 hrs post-hatching at 25°C, in ilc-17.1 deletion mutants and CEP-1 overexpressing larvae that enter dauer, compared to ilc-17.1; cep-1 double mutants and wildtype (N2) larvae that continue development. (Also see Supplementary Table 12).

Bars show the mean ± S.E.M.
Figure 5. Impaired glucose utilization in ILC-17.1 deficient larvae activates CEP-1/p53 and decreases the expression of Cytochrome C.

a. Left: Schematic of experimental design. Right: Percent dauers at 25°C amongst wildtype (N2), \( ilc-17 \) deletion mutants, and \( ilc-17.1 \) deletion mutants rescued by expressing \( unc-54p::ilc-17.1 \) on Control (L4440) and \( fgt-1 \) RNAi. \(*p < 0.001, **p < 0.01, \text{ns, not significant. (n= 5 experiments, Chi-squared} = 1817.1, df = 5, \text{p-value < 2.2e-16).}

b. Left: Schematic of experimental design. Right: Percent SDS (1%)-resistant dauers that accumulate amongst wildtype (N2) and \( ilc-17.1 \) deletion mutants growing on Control (L4440) and \( fgt-1 \) RNAi under optimal growth conditions of 20°C. \(**p < 0.001, **p < 0.01, \text{ns, not significant. (n= 3 experiments, Chi-squared} = 206.82, df = 7, \text{p-value < 2.2e-16).}

c. Left: Schematic of experimental design. Right: Percent dauers amongst wildtype (N2) larvae, \( ilc-17.1 \) deletion mutants and CEP-1/p53 overexpressing animals on Control (H2O) and 50mM glucose at 25°C. \( **p < 0.01, \text{ns, not significant. (n= 3-6 experiments, Chi-squared} = 2882.3, df = 5, \text{p-value < 2.2e-16).}

d. Fluorescent intensity (arbitrary units; AU) measured in P cells in L1 larvae on Control (H2O) and 50mM glucose, 15 hrs. post-hatching at 25°C. Strains: cep-1 (lg12501); CEP-1::GFP and CEP-1/p53 overexpressing larvae. n=2-3 experiments, 3-5 larvae/experiment. \(**p < 0.001, **p < 0.01, \text{ns, not significant (unpaired t-test). Each data point indicates the average fluorescence intensity value across five P cells in one animal.}

e. Average \( phg-1, sod-3 \) and \( mtl-1 \) mRNA levels in 32-36 hr. larvae that arrest as dauers (on H2O, control) and larvae that bypass dauer (50mM glucose) at 25°C. mRNA levels were determined
relative to *pmp-3* and normalized to wildtype (N2) values. n=3 experiments. ***p < 0.001, **p < 0.01, ns, not significant (unpaired t-test).

f. Average *cyc-2.2* mRNA levels in 32-36 hr. larvae grown at 25°C. mRNA levels were determined relative to *pmp-3* and normalized to the wildtype (N2) values. n=4-6 experiments. ***p < 0.001, **p < 0.01, *p < 0.05. (unpaired t-test).

Bars show the mean ± S.E.M. Individual points in the bar graphs in a, b, c represent the % dauers/experiment, Pearson’s Chi-squared test with Yates’ continuity correction.
Figure 6: ILC-17.1 suppresses CEP-1/p53 in *C. elegans* and mammalian epithelial cells.

**a. Top:** Representative Western blot showing p53 levels in A459 epithelial cells stimulated for 18hrs. with increasing doses of rIL-17. β-actin was used as a loading control and Nutlin was used as a positive control. **Bottom:** Quantification of p53 levels normalized to actin levels. n= 4 independent experiments. **p<0.01. (One-Way ANOVA with uncorrected Fisher’s LSD; df=2).**

**b.** Percent dauers upon overexpressing ILC-17.1 (expression under the *unc-54* promoter) in cep-1 overexpressing larvae at 25°C. ***p < 0.001. (n=4 experiments, Chi-squared = 1245.4, df = 2, p-value < 2.2e-16).**

**c. Top:** Schematic of germline apoptosis (apoptotic cells in red) scored with Acridine Orange. **Bottom:** Average numbers of apoptotic cells in day-two adult animals under control, non-irradiated conditions, and upon irradiation with 75 Gy (day-one adults were irradiated). n= 3 experiments, and 12 gonad arms/experiment. ***p<0.001, **p<0.01 (analysis of variance (ANOVA) with Tukey's correction, df=13). Note: p values between N2 vs *ilc-17.1* OE (*unc-54p*), non-irradiated and irradiated are p=0.0192 and p=0.0623, if compared by themselves (unpaired t-test), but do not rise to significance when corrected for the multiple comparisons.

**d.** Working model: ILC-17.1 secretion from larval amphid neurons that occurs in response to food availability links cell fate decisions with glucose metabolism and DAF-16, DAF-3/DAF-5 activation through CEP-1/p53.

Data in all graphs show mean ± S.E.M.
Supplementary Figure S1: ILC-17.1 modulates C. elegans developmental diapause.

a. Schematic of ilc-17.1 gene depicting the syb5296 and syb5297 deletions made by CRISPR/Cas9 editing using two distinct guide RNAs. ilc-17.1 (syb5296) X deletion mutants were used in all future experiments.

b. Average mRNA expression in ilc-17.1 deletion mutants relative to wildtype (N2). n=4-8 experiments. ***p < 0.001 (unpaired t-test).

c. Schematic of experiment leveraging the resistance of dauers to 1% SDS used to identify dauers developing under optimal growth conditions at 20°C.

d. Percent ilc-17.1 larvae that transiently enter the dauer state as seen by SDS-resistance 48 hrs and 72 hrs. post-hatching under optimal conditions at 20°C. ***p < 0.001, **p < 0.01 (n=4 experiments, Chi-squared = 166.53, df = 3, p-value < 2.2e-16).

e-i. Representative micrographs showing DIC images of e, whole ilc-17.1 deletion mutant dauer larvae (inset shows alae), f, pharyngeal region and h, arrested germline 72 hr. post-hatching at 25°C. Wildtype (N2) larval stage 2 (L2) animals used as comparisons: g, pharyngeal region and i, germline. Scale bar, a=50µm, f-i, 5 µm.

j. Average pharyngeal pumping rates/minute in ilc-17.1 deletion mutants relative to wildtype (N2) larval stage 2 (L2) animals. n=4-8 experiments. ***p < 0.001, (unpaired t-test).

k. Percent F25D1.3, syb7367, and 2X backcrossed ilc-17.1 larvae that arrest as dauers at 25°C. ***p < 0.001, (n=3 experiments; 50-100 larvae each).
**Top:** Schematic of experiment. **Bottom:** Percent wildtype (N2) larvae that arrest as dauers as determined by SDS-resistance, 48 hrs post-hatching on control (Ctrl; L4440) and *ilc-17.1* RNAi at 25°C. *p < 0.05. (n=5 experiments, Chi-squared = 5.1712, df = 1, p-value = 0.02296).

Bars show the mean ± S.E.M. Individual points in the bar graphs in d, k represent the % dauers/experiment, Pearson's Chi-squared test with Yates' continuity correction.
Supplementary Figure S2: Characterization of ILC-17.1 signaling pathway components in *C. elegans*.

a. **Top**: Schematic of CRISPR insertion of SL2::GFP into ilcr-2 locus to identify sites of ilcr-2 expression. **Bottom, Upper panels**: Representative micrographs showing GFP expression in cells of the pharynx (left), epidermis (middle; arrows) and gonad (right). **Bottom, lower panels**: Control, wildtype (N2) non-transgenic animals not expressing GFP confirm specificity of GFP expression. Scale bar=5µm.

b. Average expression in *ilc-17.1* mRNA in 32-26 hr. larvae of wildtype (N2), *ilc-17.1* deletion mutants rescued with *ilc-17.1p::ilc-17.1* and *unc-54p::ilc-17.1*. Values normalized to *pmp-3* and shown relative to wildtype (N2) values. *p<0.05 (unpaired t-test).

c. Percentage of wildtype (N2) and *ilc-17.1* larvae that arrest as dauers on control HT115 (L4440) bacteria. ***p < 0.001 (n=10-20 experiments, Chi-squared = 3206, df = 3, p-value < 2.2e-16). Representative average numbers of embryos/plates of wildtype (N2), *ilc-17.1*, *ilc-17.1* rescued with *ilc-17.1p::ilc-17.1*, and *ilc-17.1* rescued with *unc-54p::ilc-17.1* on HT115 (L4440) bacteria are 59, 66, 66, 46 respectively. Individual points in the bar graphs represent the % dauers/experiment, Pearson's Chi-squared test with Yates' continuity correction.

d. **Top**: Schematic of CRISPR insertion of SL2::mCherry into *ilc-17.1* locus. **Bottom, upper panel**: Representative z-section micrographs showing mCherry expression in amphid neurons of *C. elegans* expressing mCherry as a bicistronic SL2 cassette along with the endogenous *ilc-17.1* gene to report on sites of *ilc-17.1* mRNA expression. Amphid neurons were identified by DiO dye filling (green); mCherry overlapped with a subset of DiO filled neurons. **Bottom, lower panel**: Control, wildtype (N2) animals not expressing mCherry confirm specificity of mCherry expression. Scale bar=5µm.
e. Top: Schematic of *ilc-17.1::flag* driven by the *unc-54* promoter [*ilc-17.1 OE (unc-54p)*] that was used to rescue *ilc-17.1* deletion mutants to assess ILC-17.1 protein localization. **Bottom:** Representative micrographs showing a z-section of these animals immunostained with α-FLAG antibody, and α-actin (to show expression in body wall muscle cells). Scale bar=5µm

f. Top: Representative Western blots showing (upper panel) expression of ILC-17.1::FLAG rescue constructs under the *ilc-17.1* promoter (1) and under the *unc-54* promoter (2) in *ilc-17.1* deletion mutants. Wildtype (N2) were used to show specificity of α-FLAG antibody. (lower panel), tubulin. **Bottom:** Quantification of ILC-17.1::FLAG levels in *ilc-17.1* deletion mutant animals relative to tubulin, and normalized to FLAG levels in (1). n=3 experiments. ***p < 0.001 (unpaired t-test).

g. Quantification of fluorescent intensity in pharynx versus background (arbitrary units; AU), in larvae harboring CRISPR tagged endogenous ILC-17.1::HA, immunostained with anti-HA (Figure 1f). n=3 experiments. 5-6 larvae/experiment. ***p < 0.001, (unpaired t-test).

Representative micrographs showing z-sections through animals overexpressing ILC-17.1::FLAG in their body wall muscle cells as in e. Note: ILC-17.1::FLAG is detected at the amphid commissures near the pharynx (top panel; arrow) and in the epidermis (bottom panel; arrows) in addition to the body wall muscle cells (e), Scale bar=5µm

Bars show the mean ± S.E.M.
Supplementary Figure S3: RNA-seq analysis of ILC-17.1 deficient larvae.

a. Pair-wise distance matrix of RNA-seq samples shows the expected clustering of total RNA of the biological triplicates of each strain [Strains used: wildtype (N2), ilc-17.1, and ilc-17.1 rescued with unc-54p::ilc-17.1 CDS]

b. Spearman correlation between all genes in a microarray analysis of dauer larvae that were induced by starvation and ilc-17.1 deleted larvae en route to dauer arrest grown for 32-36 hrs. at 25°C.

c. Spearman correlation between all genes in L1 larvae exposed to ascarosides and ilc-17.1 deleted larvae en route to dauer arrest grown for 32-36 hrs. at 25°C.
Supplementary Figure S4: ILC-17.1 loss does not affect the animal’s chemotaxis or pumping and is associated with changes in daf-16 target gene expression.

a. Top: Schematic of experimental design. Bottom: Average chemotaxis index towards OP50, 0.01% v/v diacetyl, and 1M lysine for ilc-17.1 deletion mutants and wildtype (N2) day-one adults at 20°C. n= 5-8 experiments. ns, not significant (unpaired t-test). Data points: average chemotaxis/experiment.

b. Number of pharyngeal pumps/min in ilc-17.1 deletion mutants and wildtype (N2) day-one adults at 20°C, on OP50 lawns, and on plates without food. n=3 experiments, with at least 5 animals/experiment scored. Data points: pumping rates of individual animals. ns, not significant (unpaired t-test).

c. Average numbers of fluorescent latex beads in similar areas of distal gut lumen of 40-hour ilc-17.1 deletion mutants and wildtype (N2) larvae at 20°C. n=3 experiments, with at least 10 animals/experiment. Data points: the numbers of beads in individual animals. ns, not significant (unpaired t-test).

d. Percent dauers amongst wildtype (N2) and ilc-17.1 deletion mutants raised on Control (H2O) and 5mM 8-bromo-cGMP at 25°C. ***p < 0.001, Pearson’s Chi-squared test with Yates correction, (n=3 experiments. Chi squared = 798, df = 3, p-value <2e-16.). Individual points in the bar graphs represent the % dauers/experiment.

e. Average sod-3, mtl-1, and lys-7 mRNA levels in 40 hr. old larvae grown at 20°C. mRNA levels were determined relative to pmp-3 and normalized to wildtype (N2) values. n=3 experiments. ***p < 0.001, **p < 0.01, ns, not significant (unpaired t-test).
Data in all graphs show mean ± S.E.M.
Supplementary Figure S5: ILC-17.1 deficiency does not alter the expression levels of Insulin signaling- or TGFβ signaling-pathway components.

a. Percent dauers in *ilc-17.1* deletion mutant larvae following RNAi-mediated downregulation of several genes known to interact with DAF-16 and DAF3/DAF-5. *daf-16* RNAi was used as a positive control. ***p < 0.001, ns, not significant. (*n*=3-5 experiments, Chi-squared = 156.66, df = 3, p-value < 2.2e-16).

b. Heatmap depicting expression levels (log2 normalized counts) of *daf-2* (boxed) and insulin ligands in the *ilc-17.1* deletion mutants, *ilc-17.1* deletion mutants rescued by unc-54p::ilc-17.1 and wildtype (N2). Arrows highlight lack of changes in *ins-1* and *ins-18* mRNA. RNA-seq data from larvae grown for 32-36 hrs. at 25°C.

c. Average mRNA levels (insulins) in 32-36 hr. larvae grown at 25°C. mRNA levels were determined relative to *pmp-3* and normalized to wildtype (N2) values. *n*=3 experiments. **p < 0.001, ns, not significant (unpaired t-test).

d. **Left:** Representative micrographs showing DAF-28::GFP expression in the last intestinal cell of 32-36hr. larvae at 25°C of *ilc-17.1* deletion mutants and wildtype (N2). Images are projections of confocal z-sections. Scale bar: 5µm. **Right:** Quantification of fluorescent intensity (arbitrary units, AU) of DAF-28::GFP. *n*=3 experiments. Data points: the fluorescence intensity of DAF-28::GFP in individual animals. ns, not significant (unpaired t-test).

e. **Top:** Representative micrographs showing INS-4::GFP expression in the pharynx and neurons of 32-36 hr. larvae at 25°C in *ilc-17.1* deletion mutants and wildtype (N2). Images are projections of confocal z-sections. Scale bar: 5µm. **Bottom:** Bars represent percent larvae that
express GFP in the different tissues where INS-4 is known to be expressed. n=3 experiments of 5-10 larvae of each strain. ns, not significant (unpaired t-test).

f. Overexpressing ILC-17.1 under unc-54p::ilc-17.1 CDS [ilc-17.1 OE (unc-54p)] in daf-2 (e1370) III strains does not rescue their dauer arrest. ***p < 0.001, ns, not significant (n=3 experiments, Chi-squared = 521, df = 3, p-value < 2.2e-16).

g. Heatmap depicting expression levels (log2 normalized counts) of TGFβ signaling pathway components in the ilc-17.1 deletion mutants, ilc-17.1 deletion mutants rescued by unc-54p::ilc-17.1, and wildtype (N2).

Data in all graphs show mean ± S.E.M. Individual points in the bar graphs in e, g, represent the % dauers/experiment, Pearson's Chi-squared test with Yates' continuity correction.
Supplementary Figure S6: CEP-1/p53 overexpression induces dauer arrest.

a. Top: Schematic of cep-1 mRNA, isoform b. Bottom: Average cep-1 isoform b mRNA levels in 32-36 hr. larvae grown at 25°C. mRNA levels were determined relative to pmp-3 and normalized to wildtype (N2) values. n=3 experiments. **p < 0.001, *p < 0.05 (unpaired t-test).

b. Top: Schematic of SDS treatment to assess dauer formation at 20°C. Bottom: Percent of wildtype (N2) and cep-1 overexpressing larvae (cep-1 OE) that enter a dauer state during development, 48 hrs. and 72 hrs. post-hatching at 20°C. ***p < 0.001, (n=3 experiments, Chi-squared = 414.65, df = 3, p-value < 2.2e-16). Individual points in the bar graphs represent the % dauers/experiment, Pearson's Chi-squared test with Yates' continuity correction.

c. Venn diagram depicting overlap between significantly differentially expressed genes (p<0.05) in ilc-17.1 deletion mutants and CEP-1/p53 overexpressing larvae, 32-36 hr. post-hatching at 25°C en route to dauer entry. p=0.0; hypergeometric test (Also see Supplementary Tables 9, 10)

d. Heatmap comparing expression levels (log2 normalized counts) of the major dauer-specific collagens in the ilc-17.1 deletion mutants, CEP-1/p53 overexpressing larvae, ilc-17.1; cep-1 double mutants, ilc-17.1 deletion mutants rescued by unc-54p::ilc-17.1, and wildtype (N2).

e. KEGG enrichments associated with differentially downregulated genes (p.adjust <0.05) in CEP-1/p53 overexpressing larvae [RNA-seq: 32-36 hr. larvae grown at 25°C; Supplementary Tables 9].
f. KEGG enrichments associated with differentially upregulated genes (padjust <0.05) in CEP-1/p53 overexpressing larvae [RNA-seq: 32-36 hr. larvae grown at 25°C; Supplementary Tables 9].

g. Principle Component Analysis (PCA) of the observed variance in the triplicate RNA-seq samples collected during development, 15 hrs. and 32 hrs. post-hatching at 25°C, of ilc-17.1 deletion mutants, CEP-1/p53 overexpressing larvae, ilc-17.1; cep-1 double mutants and wildtype (N2) larvae (Supplementary Tables 9).

Data in all graphs show mean ± S.E.M.
Supplementary Figure S7: CEP-1/p53 overexpression upregulates DAF-16/FOXO targets and cell cycle inhibitors to induce dauer arrest.

Average sod-3, mtl-1, and lys-7 mRNA levels in 40 hr. larvae grown at 20°C. mRNA levels were determined relative to pmp-3 and normalized to wildtype (N2) values. Strains used are on X-axis. n=6-10 experiments. ***p < 0.001, **p < 0.01, ns, not significant. (unpaired t-test).

b. Percent dauers in daf-2 and cep-1;daf-2 double mutants. Note: no rescue. n=3 experiments. ns, not significant. (Chi-squared = Not appropriate, df = 1, p-value = no significant)

c. Percent high temperature (27.5°C; HID phenotype) dauers in wildtype (N2) and cep-1 deletion mutants. ns, not significant. (n=4 experiments, Chi-squared = 0.67295, df = 1, p-value = 0.412).

d. Average egl-1 and ced-13 mRNA levels in 40 hr. larvae grown at 20°C. mRNA levels were determined relative to pmp-3 and normalized to wildtype (N2) values. n=6-7 experiments. ***p < 0.001, **p < 0.01, ns, not significant (unpaired t-test).

e. CEP-1 occupancy (expressed as percent input) in 32-36 hr. larvae grown at 25°C, measured at three promoter proximal regions of cki-1 (Schematic on top). Strains used: CEP-1::FLAG expressing animals in wildtype background (Ctrl), in ilc-17.1 deletion background. n=4 experiments. ns, not significant. (unpaired t-test).

f. Percent dauers in ilc-17.1 larvae, CEP-1/p53 overexpressing larvae and daf-2 larvae upon RNAi induced downregulation of cki-1 and phg-1. ***p < 0.001, **p < 0.01, ns, not significant (n=6 experiments, Chi-squared = 7443.7, df = 14, p-value < 2.2e-16).
g. Representative micrograph of *ilc-17.1; phg-1* double mutants showing the dauer rescue of some individuals but not others (dauers marked with arrow).

h. Average pumps/minute in *ilc-17.1, cep-1 and daf-2* dauers when subjected to control (L4440; Ctrl), *cki-1* and *phg-1* RNAi. n= 4 experiments, and 10 dauers were scored per experiment. *p < 0.05, ns, not significant (unpaired t-test). Pumping rates in wildtype (N2), L2 larvae are shown for comparison.

Data in all graphs show mean ± S.E.M. Individual points in the bar graphs in b, c, f, represent the % dauers/experiment, Pearson's Chi-squared test with Yates' continuity correction.
Supplementary Figure S8: CEP-1/p53 activity can be repressed by glucose supplementation.

a. Percent dauers amongst *ilc-17.1* deletion mutants, CEP-1/p53 overexpressing larvae and wildtype (N2) larvae on 5 mM, 10mM and 50 mM glucose at 25°C. ***p < 0.001, **p < 0.01, ns, not significant. (n= 5-9 experiments, Chi-squared = 5263.6, df = 11, p-value < 2.2e-16)

b. Percent dauers amongst *ilc-17.1* deletion mutants and wildtype (N2) larvae on 50mM glucose on Control (Ctrl; L4440) and *fgt-1* RNAi. **p < 0.01, *p < 0.05, ns, not significant. (n=6 experiments, Chi-squared = 299.36, df = 3, p-value < 2.2e-16).

c. Percent dauers amongst *ilc-17.1* deletion mutants and wildtype (N2) larvae upon exposure to 50mM DOG, 50mM glucose, and 0.1mM MitoTEMPO at 25°C (see X-axis for different conditions). ***p < 0.001, ns, not significant, (n=3- experiments, Chi-squared = 1251.7, df = 9, p-value < 2.2e-16).

d. Percent dauers amongst *ilc-17.1* deletion mutants and wildtype (N2) larvae on 20mM Skim Milk powder and 0.26 mM L-Glutamine at 25°C. ns, not significant, (n=3 experiments, Chi-squared = 648.89, df = 5, p-value < 2.2e-16).

e. Average mRNA levels of *egl-1* and *ced-13* in wildtype (N2) larvae subjected to Control (Ctrl; L4440) and *fgt-1* RNAi. mRNA levels were determined relative to *pmp-3* and normalized to wildtype (N2) values. n=3 experiments, *p < 0.05, ns, not significant (unpaired t-test).

Data in all graphs show mean ± S.E.M. Individual points in the bar graphs in a-d represent the % dauers/experiment, Pearson's Chi-squared test with Yates' continuity correction.
Supplementary Figure S9: ILC-17.1 deficiency and CEP-1/p53 overexpression modulate key glucose metabolic enzymes.

a-f. Heatmap depicting expression levels (log2 normalized counts) of glycolysis and mitochondrial OXPHOS genes in the ilc-17.1 deletion mutants, CEP-1/p53 overexpressing animals, ilc-17.1; cep-1 double mutants, ilc-17.1 rescued with unc-54p::ilc-17.1, and wildtype (N2). Arrows: highlight decreases in pfk-1.2 and cyc-2.2 mRNA levels in ilc-17.1 deleted and CEP-1/p53 overexpressing larvae (Boxed). RNA-seq data was collected as previously described.

g. Average pfk-1.2 mRNA levels in 32-36 hr. old larvae grown at 25°C. mRNA levels were determined relative to pmp-3 and normalized to wildtype (N2) values. Strain names on X-axis. n=4-6 experiments. ***p < 0.001, **p < 0.01, *p < 0.05. (unpaired t-test).

h. **Top:** Schematic of SDS treatment to assess dauer formation at 20°C. **Bottom:** Percent of wildtype (N2) and ilc-17.1 deletion mutant larvae that enter a dauer state at 20°C, 48 hr. post-hatching, when subjected to control (Ctrl; L4440), cyc-2.2 and pfk-1.2 RNAi. **p < 0.01, ns, not significant (n=3-4 experiments, Chi-squared = 213.38, df = 5, p-value < 2.2e-16 ). Individual points in the bar graphs represent the % dauers/experiment, Pearson's Chi-squared test with Yates' continuity correction.

Data in all graphs show mean ± S.E.M.
Supplementary Figure S10: rIL-17 induces the expected cytokine response in human epithelial cells.

a. Soluble CXCL5 levels in the supernatant of A459 epithelial cells stimulated for 18hrs. with rIL-17 measured by ELISA. Data is a summary of 2 independent experiments, performed in triplicates. *p<0.05, (unpaired t-test). Data show mean ± S.E.M.

b. Percent wild-type (N2) and npr-1(ad609); ilc-17.1(tm5218) double mutant larvae that arrest as dauers at 25°C. ns, not significant (n=3 experiments; 50-100 larvae each).


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Author Contributions

All authors designed the study, performed experiments, analyzed data, and drafted the manuscript. P.D.I coordinated the mammalian experiments; V.P. coordinated *C. elegans* experiments and the project. All authors approved the final version of the manuscript.

Declaration of Interests

The authors declare no competing interests
Figure 1

**a**  
Egg lay on OP50, 20°C, 2 hrs  
Place embryos at 25°C  
Score dauers 48-72 hrs. later

**b**  
![Bar graph](Wild-type (N2))

**c**  
![Image](Wild-type (N2))

**d**  
![Image](ilc-17.1(syb5296))

**e**  
Egg lay at 20°C, 2 hrs on RNAi  
Place embryos at 25°C  
Score dauers 48-72 hrs later

**f**  
24-36 hr larvae hatched without food or on OP50 lawn, 25°C

**g**  
KEGG Enrichment  
*ilc-17.1* vs N2 downregulated

**h**  
KEGG Enrichment  
*ilc-17.1* vs N2 upregulated

**i**  
Expression

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Metabolism of xenobiotics by cytochrome P450
Drug metabolism - cytochrome P450
Fatty acid elongation
Pentose and glucuronate interconversions
Tyrosine metabolism
Retinol metabolism
Glutathione metabolism
Biosynthesis of unsaturated fatty acids
Longevity regulating pathway - worm
Drug metabolism - other enzymes
Tryptophan metabolism
Fatty acid degradation
Lysosome
Fatty acid metabolism
Peroxisome
Autophagy - animal
Oxidative phosphorylation
Biosynthesis of cofactors

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Figure 2

(a) 25°C, 48 hrs. post-egg lay

![Graph showing % dauer](image)

(b) 20°C, 40 hrs post-egg lay

![Images of DAF-16::GFP](image)

(c) Relative fold change (mRNA, 25°C)

![Bar graph showing fold change](image)

(d) 25°C, 48 hrs. post-egg lay

![Graph showing % dauer](image)

(e) 25°C, 48 hrs. post-egg lay

![Graph showing % dauer](image)

(f) 25°C, 48 hrs. post-egg lay

![Graph showing % dauer](image)

(g) 0.5mm

![Imagery](image)

(h) 0.5mm

![Imagery](image)
Figure 4

(a) 25°C, 15 hrs. post-egg lay

(b) 25°C, 48 hrs. post-egg lay

(c) 25°C, 48 hrs. post-egg lay

(d) Relative fold change (mRNA, 25°C)

(e) Arbitrary Fluoresence Units

(f) 25°C, 48 hrs. post-egg lay

(g) 25°C, 3-4 days post-egg lay

(h) Expression

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Figure 5

(a) Egg lay at 20°C, 2 hrs on Ctrl or fgt-1 RNAi

Place embryos at 25°C

Score dauers

(b) Egg lay at 20°C, 2 hrs on Ctrl or fgt-1 RNAi

Place embryos at 20°C

SDS resistance; Score dauers

(c) Egg lay -/+ glucose at 20°C, 2 hrs

Place embryos at 25°C

Score dauers

(d) 25°C, 15 hrs. post-egg lay

(e) phg-1 sod-3 mtl-1

(f) Relative fold change (mRNA, 25°C)

Relative fold change (cyc-2 mRNA, 25°C)
Figure 6

(a) Western blot analysis of p53 and Actin proteins under different conditions: Unstimulated, IL-17 50ng/mL, IL-17 100ng/mL, and Nutlin 10ug/mL. The blot shows 55 kDa and 43 kDa bands.

(b) Bar graph showing fold change in p53/Actin ratio under Unstimulated, IL-17 50ng/mL, and IL-17 100ng/mL conditions. A significant increase is observed in the IL-17 100ng/mL condition compared to the other groups.

(c) Diagram showing apoptosis stained with Acridine Orange at 20°C. The graph below shows the number of apoptotic nuclei stained in non-irradiated and irradiated samples. A significant increase in apoptosis is observed in the irradiated samples.

(d) Conceptual diagram illustrating the relationship between food, ILC-17.1, glucose, CEP-1/p53, DAF-16/FOXO, DAF-3/DAF-5, cell cycle, and cytochrome C. The diagram highlights the developmental progression and glucose utilization pathways.
Figure S1

(a) ilc-17.1 (syb5296) deletion (2173 bp)

(b) Relative fold change (ilc-17.1 mRNA, 20°C)

(c) Egg lay on OP50 plate at 20°C, 2 hrs. → Place embryos at 20°C → SDS resistance after 48 and 72 hrs.

(d) 20°C, 48 and 72 hrs. post-egg lay

(e) 50µm

(f) 5µm

(g) 5µm

(h) 5µm

(i) Egg lay on RNAi at 20°C, 2 hrs. → Place embryos at 25°C → SDS resistance after 48 hrs.

(j) Average pumps/min

(k) % dauers

(l) % SDS resistance

Relative fold change (ilc-17.1 mRNA, 20°C) for N2 and ilc-17.1 (syb5296) indicated by a bar graph with asterisks.

Egg lay on RNAi at 20°C, 2 hrs. followed by SDS resistance after 48 hrs. shown with a bar graph indicating a significant increase in % SDS resistance for ilc-17.1 RNAi compared to Ctrl RNAi.
**Figure S2**

(a) **ilc-2**: SL2::GFP

(b) Relative fold change (ilc-17.1 mRNA, 25 °C)

(c) Dauer formation and rescue on HT115 bacteria, 25 °C

(d) **ilc-17.1**: SL2::mCherry

(e) **unc-54p** ATG ilc-17.1 CDS 3X Flag TAA

(f) N2 1 2

25kDA 50kDA

ILC-17.1 Tubulin

(g) Pharynx Background

(h) **ilc-17.1 OE (unc-54p)** FLAG-tagged

**ilc-17.1 HA?**
Figure S3

(a) Heatmap showing expression levels of different genotypes.

(b) Scatter plot comparing Log2 fold change for N2 Starved Dauer vs Control and ilc-17.1 vs N2.

(c) Scatter plot comparing Log2 fold change for N2 L1 larvae on Ascaroside vs Control and ilc-17.1 vs N2.

Spearman Correlation:
- For N2 Starved Dauer vs Control and ilc-17.1 vs N2, $\rho = 0.21$, $p < 2.2e^{-16}$
- For N2 L1 larvae on Ascaroside vs Control and ilc-17.1 vs N2, $\rho = 0.031$, $p = 1e^{-05}$
Day-one adults placed at center of plate and control (H₂O), OP50, or odor placed at opposite end. Chemotaxis index, after 1 hour.

**Figure S4**

(a) Day-one adults placed at center of plate and control (H₂O), OP50, or odor placed at opposite end. Chemotaxis index, after 1 hour.

(b) Pharyngeal pumps/min.

(c) Mean latex beads/area in intestine.

(d) % dauers.

(e) Relative fold change (mRNA, 20°C).

### Notes

- **NS** indicates no significant difference.
- **Ctrl** and 8-bromo-cGMP are used to control for experimental conditions.
- The graphs show the relative fold change in mRNA levels for different conditions.

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Figure S5

(a) Bar graph showing the percentage of dauers in N2 and ilc-17.1 worms after RNAi treatment. The graph includes data for genes such as daf-16, cep-1, pha-4, jnk-1, aak-1, par-4, strd-1, hsf-1, ncl-1, pmk-1, sta-2, and daf-2.


(c) Relative fold change in mRNA expression for genes such as ins-3, ins-4, ins-17, ins-19, ins-32, and ins-37 in N2 and ilc-17.1 worms.

(d) Images showing GFP expression in N2 and ilc-17.1 worms.

(e) Graph showing the percentage of animals expressing ins-4p::GFP in neurons, muscle, epithelium, and pharynx.

(f) Bar graph showing the percentage of dauers in N2, daf-2, daf-28, and ilc-17.1 OE worms.

(g) Expression levels of various genes including bra-1, daf-1, daf-14, daf-3, daf-4, daf-5, daf-7, and daf-8.
Figure S6

a) Egg lay on OP50 plate at 20°C, 2 hrs. Place embryos at 20°C SDS resistance after 48 and 72hrs.

b) Overlap significant DEGs p < 0.0

KEGG Enrichment

c) Condition

f) KEGG Enrichment

c) ** Figure S6 **

- Egg lay on OP50 plate at 20°C, 2 hrs.
- Place embryos at 20°C SDS resistance after 48 and 72hrs.

- Overlap significant DEGs p < 0.0

- KEGG Enrichment

- Condition

- Figure S6
Figure S7

(a) Relative fold change (mRNA, 20°C) for various strains and treatments.

(b) % dauers at 25°C, 48 hrs post-egg lay.

(c) % SDS resistance at 27°C, 48 hrs post-egg lay.

(d) Relative fold change (mRNA, 20°C) for gll-1, ced-13.

(e) Expression levels of cki-1 in different promoter regions.

(f) % dauers at 25°C, 48 hrs post-egg lay.

(g) Average pumps per minute for various treatments.

(h) Average pumps per minute for various treatments.

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Figure S8

25°C, 48 hrs post-egg lay

(a) 

- Ctrl
- 5mM glucose
- 10mM glucose
- 50mM glucose

% dauers

(b) 

- Ctrl RNAi
- fgt-1 RNAi

% dauers

(c) 

- N2
- ilc-17.1

% dauers

(d) 

- Ctrl
- Skim milk powder
- L-glutamine

% dauers

(e) 

- Relative fold change (mRNA, 25°C)

- egl-1
- ced-13

- Ctrl RNAi
- fgt-1 RNAi

- Ctrl RNAi
- fgt-1 RNAi

- N2

- 0.0
- 0.5
- 1.0
- 1.5
- 2.0
- 2.5

- ns

- 0
- 20
- 40
- 60
- 80
- 100

- % Dauer

- N2
- ilc-17.1Δ

- 25°C, 48 hrs post-egg lay

- Ctrl
- 20mM SKM
- 0.26mM L-glutamine

- % Dauer

- 0
- 20
- 40
- 60
- 80
- 100

- Relative fold change (mRNA, 25°C)

- 0
- 0.5
- 1.0
- 1.5
- 2.0
- 2.5

- ns
Figure S9

a) Glycolysis

b) Complex I

c) Complex II

d) Complex III

e) Complex IV

f) Complex V

g) Relative fold change (pfk-1.2 mRNA, 25°C)

h) Egg lay on OP50 plate at 20°C, 2 hrs. → Place embryos at 20°C → SDS resistance after 48 hrs.
Figure S10

(a) CXCL5 (pg/ml)

Unstimulated
IL-17 (100ng/ml)

*  

(b) % dauers

N2  npr-1; ilc-17.1 (tm5218)

ns