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Article

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A cell non-autonomous FOXO/DAF-16-mediated germline quality assurance program that responds to somatic DNA damage

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Abstract

Germline integrity is critical for progeny fitness. Organisms deploy the DNA damage response (DDR) signalling to protect germline from genotoxic stress, facilitating cell-cycle arrest of germ cells and DNA repair or their apoptosis. Cell-autonomous regulation of germline quality is well-studied; however, how quality is enforced cell non-autonomously on sensing somatic DNA damage is less known. Using *Caenorhabditis elegans*, we show that DDR disruption, only in the uterus, when insulin-IGF-1 signalling (IIS) is low, arrests germline development and induces sterility in a FOXO/DAF-16 transcription factor (TF)-dependent manner. Without FOXO/DAF-16, germ cells of the IIS mutant escape arrest to produce poor quality oocytes, showing that the TF imposes strict quality control during low IIS. In response to low IIS in neurons, FOXO/DAF-16 works cell autonomously as well as non-autonomously to facilitate the arrest. Activated FOXO/DAF-16 promotes transcription of checkpoint and DDR genes, protecting germline integrity. However, on reducing DDR during low IIS, the TF decreases ERK/MPK-1 signaling below a threshold, and transcriptionally downregulates genes involved in spermatogenesis-to-oogenesis switch as well as *cdk-1*/Cyclin B to promote germline arrest. Altogether, our study reveals how cell non-autonomous function of FOXO/DAF-16 promotes germline quality and progeny fitness in response to somatic DNA damage.

Introduction

The propagation of a species depends on a healthy and productive germline. The stability of the genome is constantly under threat from extrinsic as well as cell-intrinsic genotoxic agents. Thus, all organisms invest heavily on protecting the germline against DNA damage. Generally, in response to DNA damage, an organism deploys an array of countermeasures. Depending on the type of DNA damage, organisms employ lesion-specific DNA repair pathways that can restore damage inflicted by ultra-violet rays (UV), ionizing radiation (IR) or reactive oxygen (ROS) and nitrogen species (RNS). Apart from these highly specialized DNA repair mechanisms, organisms also depend on DNA damage response (DDR) signaling to activate damage-responsive checkpoints, leading to cell cycle arrest to repair the damage or apoptosis, when damage is beyond repair. Perturbation of the DDR, in turn, leads to unrepaired DNA damage, genomic instability and are the basis of many human diseases like cancer, neurodegeneration as well as aging [1]. Unrepaired DNA lesions in the germline can lead to infertility, reduced progeny fitness and birth defects. The critical decision of reproductive commitment and germline proliferation is largely influenced by environmental conditions via soma to germline communication [2,3]. For example, irradiation (genotoxic stress) of somatic tissues has been shown to cause hormonal imbalance leading to increased incidences of infertility in female cancer patients[4]. However, it is less known whether or how an organism perceives intrinsic DNA damage signals in somatic tissues and regulates germline development to preserve progeny genome integrity

Research in *C. elegans* has elucidated the role of the conserved FOXO TF DAF-16 in somatic and germline quality assurance. Mutations in the neuroendocrine IIS pathway activate FOXO/DAF-16 to arrest development at dauer diapause [5,6]. The TF mediates arrest at the L1 larval stage when food is depleted [7]. Further, activated FOXO/DAF-16 delays aging, enhances resistance to stresses and increases life span under conditions of lowered IIS [8,9,10]. These IIS mutant animals maintain their germline stem cell pool even at an advanced age, and so, have delayed reproductive aging [11]. They produce better quality oocytes [12] with low chromosomal abnormalities as compared to wild-type (WT), but the mechanism is less understood [13]. Interestingly, the IIS receptor DAF-2 functions cell non-autonomously in the neuron whereas DAF-16 works in the intestine to regulate longevity [14,15]. The long reproductive span or higher oocyte quality of the *daf-2* mutant is dependent on muscle or intestinal DAF-16 activity [13]. However, it is not known whether activated FOXO/DAF-16 can sense DNA damage in somatic tissues and modulate germline development cell non-autonomously.

Here, we show that in *C. elegans*, a uterine tissue-specific perturbation of DDR in the IIS pathway mutants prevents germ cells from exiting the pachytene stage of meiosis and inhibits oogenesis. For

disruption of DDR and inducing DNA damage, we knocked-down (KD) *cdk-12* that is required for the transcription of DDR genes [16,17]. This sterility is reversed in the absence of DAF-16, leading to the production of poor-quality oocytes and developmentally retarded progeny. We elucidate the cell autonomous as well as non-autonomous requirements of the IIS pathway and FOXO/DAF-16 in orchestrating the arrest. We show that this is achieved by downregulating signaling of ERK-MPK-1 pathway along with the transcriptional downregulation of important genes required for germline development. Thus, our study elucidates a new cell non-autonomous role of the IIS pathway and FOXO/DAF-16 in ensuring germline quality in response to somatic perturbation of DDR and associated chance of genome instability in the progeny.

Results

The cyclin-dependent kinase gene *cdk-12* genetically interacts with the IIS pathway

We were interested in identifying genes that when knocked down induce chronic stress signaling, thereby enhancing dauer formation of the IIS receptor mutant *daf-2(e1370)* (referred to as *daf-2*) strain. Knocking down *cdk-12* using RNAi led to a significant increase in dauer formation **(Figure 1A)**. In line with its possible role in inducing stress, *cdk-12* RNAi, initiated at L4, reduced life span of wild-type (WT), *daf-2*, *daf-16(mgdf50)* (referred to as *daf-16*) and *daf-16;daf-2* to an equal extent **(Figure S1A-D, Supplementary table 1)**. Thus, CDK-12 depletion may cause chronic stress to the worms, thereby increasing dauer of *daf-2* and reducing life span in general.

Cdk-12 depletion during low IIS leads to DAF-16A isoform-dependent pachytene arrest of germline and sterility

Considering *cdk-12* knockdown may potentially induce stress, we asked whether this would affect progeny production. Interestingly, we found that the *daf-2* worms became sterile when they were grown on *cdk-12* RNAi from L1 onwards **(Figure 1B, C)**. The sterility is DAF-16-dependent as fertility was restored in *daf-16;daf-2*, signifying that DAF-16 regulates the germline arrest in *daf-2* **(Figure 1B, C)**. Importantly, this was not due to differential RNAi efficiency in the strains **(Figure S1E).**

The *C. elegans* hermaphrodite gonad has two U-shaped arms carrying germline stem cell (GSC) pool near the distal end, which divide mitotically and then enter meiotic prophase as they move away from the distal tip. Germ cells in meiosis produce sperms during larval 4 (L4) stage, and after the spermatogenesis to oogenesis switch [18,19], generate oocytes or undergo programmed cell death. The proximal gonad contains a stack of oocytes, followed by sperms residing in the spermatheca. Both arms have a common uterus, where fertilized eggs are stored until hatching [20] **(Figure 1D).**

To visualize the germline, we dissected the gonads of Day 1 adult animals and stained them with DAPI. Confocal imaging showed that in *cdk-12* RNAi-fed *daf-2* worms, sperms were formed but oogenesis halted due to arrest of germ cells in the pachytene stage of meiosis. In *daf-16;daf-2*, the arrest was reversed and oocyte formation ensued **(Figure 1E)**. Notably, upon *cdk-12* KD, the number of oocytes is reduced independent of DAF-16 **(Figure 1F)**. The number of germ cells of *daf-2* in the pachytene stage of meiosis was drastically reduced upon *cdk-12* KD **(Figure 1G, H)**; however, in *daf-16;daf-2* worms the reduction was largely abrogated **(Figure 1I, J)**, leading to oocyte formation. The number of mitotic and

transition zone nuclei remained unchanged in both cases **(Figure 1G-J)**. We also found that the canonical IIS signalling pathway components are involved as *cdk-12* KD in *age-1(hx546)* (mutant in mammalian PI3K ortholog) [21] and *pdk-1(sa680)* (mutant in mammalian PDK ortholog) [22].also arrested germline at the pachytene stage of meiosis **(Figure S1F, G)**.

DAF-16 has multiple isoforms with distinct and overlapping functions [10,15,23]. We knocked down *cdk-12* in *daf-16;daf-2;daf-16a(+)* (DAF-16a rescued), *daf-16;daf-2;daf-16b(+)* (DAF-16b rescued) and *daf-16;daf-2;daf-16d/f(+)* (DAF-16d/f rescued) to find that the effect is mainly driven by DAF-16a **(Figure 1K, L)**. Previously, DAF-16a isoform has been shown to play a major role in regulating lifespan, stress resistance and dauer formation [9,15,24,25]. Here, we show a predominant role of DAF-16a in preventing the pachytene exit of germ cells in *daf-2* when *cdk-12* is depleted.

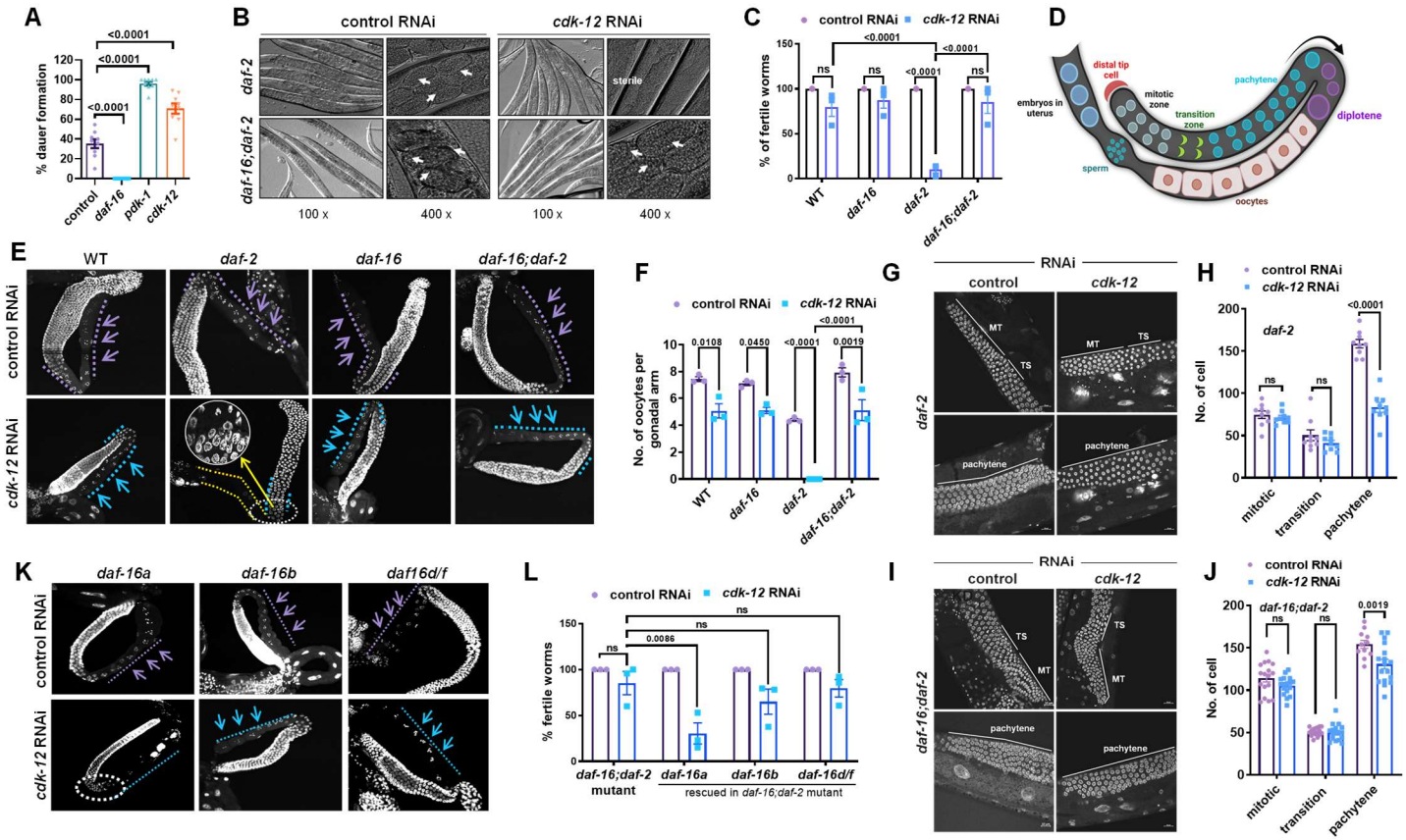

Figure 1. CDK-12 KD arrests germline of IIS mutant in a FOXO/DAF-16-dependent manner

(A) Percentage of dauer formation in *daf-2(e1370)* when *daf-16*, *pdk-1* or *cdk-12* is knocked down (KD) using RNAi. *Cdk-12* KD increased dauer formation of *daf-2(e1370).* Average of nine biological replicates (n≥40 for each replicate). One way ANOVA. Each point represents mean percentage of dauer formation for one biological replicate. Experiments performed at 22.5°C

(B) Representative images showing that *cdk-12* RNAi results in sterility in *daf-2(e1370)* worms that is rescued in *daf-2(e1370)*;*daf-16(mgdf50)*. Arrows show eggs. Image were captured at 100X and 400X magnification for each condition.

(C) Percentage of fertile worms in wild-type (WT), *daf-16(mgdf50), daf-2(e1370)* and *daf-16(mgdf50);daf-2(e1370)* on *cdk-12* RNAi. Most of the *daf-2(e1370)* worms are sterile on *cdk-12* KD that is rescued in *daf-16(mgdf50);daf-2(e1370)*. Average of three biological replicates (n≥25 for each experiment). Two-way ANOVA-Sidak multiple comparisons test.

(D) A diagrammatic representation of one of the two arms of the *C. elegans* gonad.

(E) Representative fluorescence images of dissected gonadal arms that were stained with DAPI. The germline arrests at the pachytene stage of meiosis 1 in *daf-2(e1370)* worms upon *cdk-12* KD; this was rescued in *daf-16(mgdf50);daf-2(e1370).* Image were captured at 400X magnification.

(F) Oocyte counts in WT, *daf-16(mgdf50), daf-2(e1370)* and *daf-16(mgdf50);daf-2(e1370)* on *cdk-12* RNAi. Average of three biological replicates (n≥15 for each experiment). Two-way ANOVA-Sidak multiple comparisons test.

(G-J) Representative fluorescence images of DAPI-stained dissected gonads of *daf-2(e1370)* and *daf-16(mgdf50);daf-2(e1370)* on control or *cdk-12* RNAi, showing germ cells in mitotic, transition and pachytene zones (G, I) and their quantification (H, J). n=9 (*daf-2*), n=17 (*daf-16;daf-2*) gonads for each condition used in quantification. One way ANOVA. Each point represents the number of mitotic (MT), transition (TS) or pachytene zones cell.

(K) Representative fluorescence images of DAPI-stained dissected gonads of *daf-16(mgdf50);daf-2(e1370)* worms, which have been transgenically rescued with different *daf-16* isoforms *(daf-16a, daf-16b* or *daf-16d/f)*, when grown on control or *cdk-12* RNAi. Arrows showing the oocytes. Image were captured at 400X magnification.

(L) Percentage of fertile worms in *daf-16(mgdf50);daf-2(e1370)* that are rescued with *daf-16* isoforms *(daf-16a, daf-16b* or *daf-16d/f)* on control or *cdk-12* RNAi. Average of three biological replicates (n≥40 for each replicate). Two-way ANOVA-Sidak multiple comparisons test.

Error bars are SEM. ns, non-significant. Unless otherwise mentioned, all experiments were performed at 20 ^{o}C. Source data is provided as a source data file.

FOXO/DAF-16 and CDK-12 promotes a germline quality assurance program

Since the *daf-16;daf-2* worms produce oocytes when *cdk-12* is knocked down, in contrast to *daf-2*, we determined the quality of oocytes. As previously reported, we also found the oocytes produced on day 3 of adulthood by *daf-2* to be of superior quality in comparison to the wild-type worms [13]. However, in *daf-16;daf-2,* the quality deteriorates significantly **(Figure S2A-C)** indicating the noted role of DAF-16 in the maintenance of better oocyte quality in *daf-2*. The quality of oocytes after *cdk-12* KD decreased in a DAF-16-independent manner **(Figure 2A, B)**. It may also be noted that *cdk-12* KD decreases the number of hatched progenies in all strains; however, no brood is generated in *daf-2*. The brood size is partially rescued in *daf-16;daf-2* **(Figure 2C)**.

Although most of the eggs that are laid by the *daf-16:daf-2* on *cdk-12* RNAi worms hatched **(Figure 2D)**, they failed to reach the L4 stage **(Figure 2E, S2D)**, indicating sub-optimal oocyte quality. Also, endomitotic oocytes (emo) that often develop due to defective fertilization [26], were more frequent in the proximal gonad of wild-type and *daf-16;daf-2* worms that were fed with *cdk-12* RNAi, compared to the *daf-2* worms **(Figure 2F)**. Thus, we conclude that *cdk-12* plays an important role in maintaining oocyte quality and activated DAF-16, under conditions of lowered IIS, enforces a germline quality assurance program that prevents the production of inferior quality progeny.

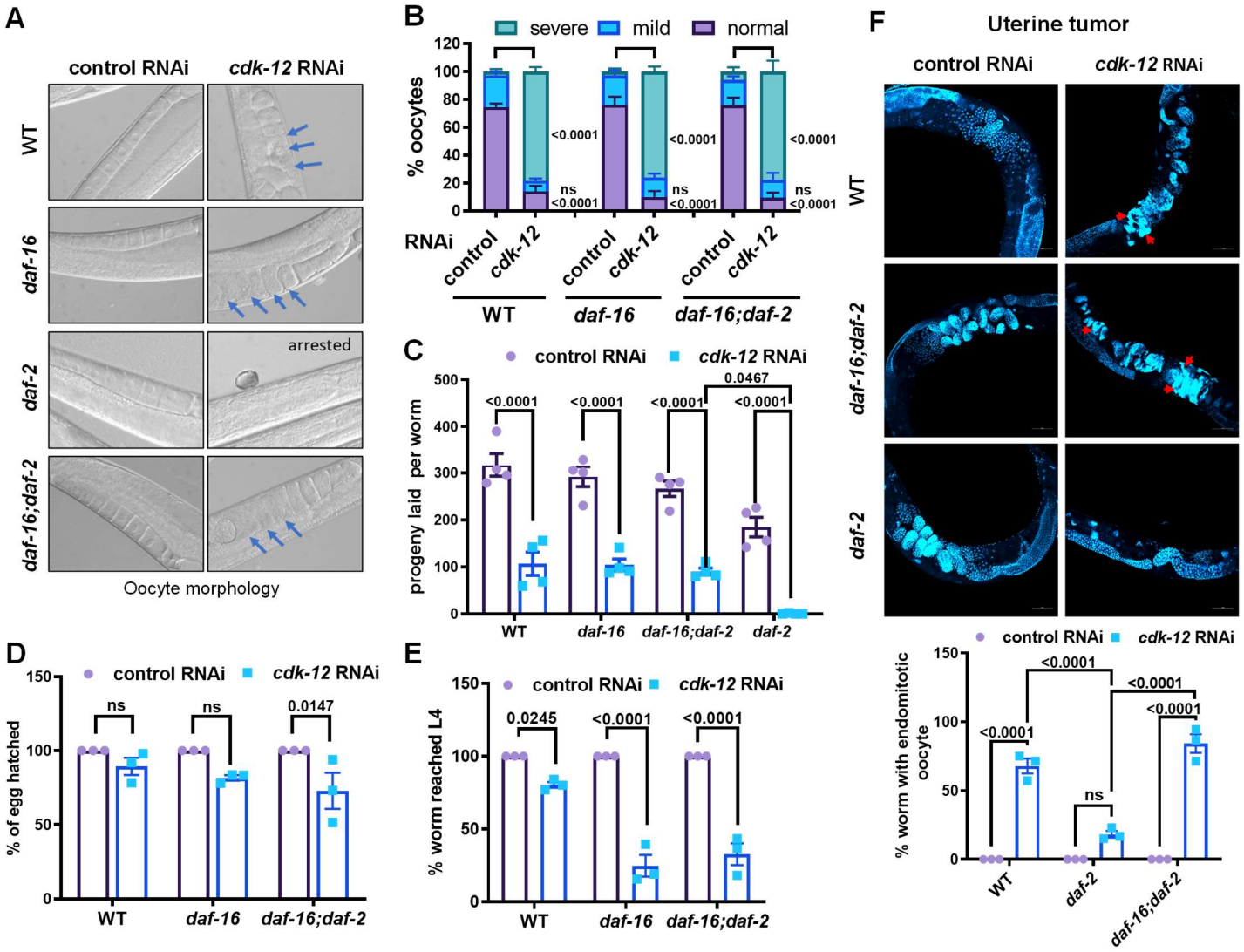

Figure 2. IIS pathway/*daf-16* and *cdk-12* regulate brood size, egg quality, oocyte quality and progeny health

(A) Representative DIC images of oocyte morphology when *cdk-12* was knocked down in WT, *daf-16(mgdf50), daf-2(e1370)* and *daf-16(mgdf50);daf-2(e1370)*. Blue arrows indicate morphologically disorganised oocyte. Image were captured at 400X magnification.

(B) Quantification of oocyte quality on day 1 of adulthood in WT, *daf-16(mgdf50), daf-2(e1370)* and *daf-16(mgdf50);daf-2(e1370)* grown on control or *cdk-12* RNAi. The quality was categorized as normal, or with mild or severe defects according to images represented in Figure S2A. Average of four biological replicates (n≥25 for each replicate). One way ANOVA.

(C) Lowered progeny count was observed in WT, *daf-16(mgdf50),* and *daf-16(mgdf50);daf-2(e1370)* on *cdk-12* RNAi, as compared to control RNAi. No progeny was observed in *daf-2(e1370)*. Average of four biological replicates (n≥14 for each replicate). Two-way ANOVA-Sidak multiple comparisons test.
(D) Percentage of eggs that hatched in WT, *daf-16(mgdf50),* and *daf-16(mgdf50);daf-2(e1370)* upon *cdk-12* RNAi, as compared to control RNAi. Average of three biological replicates (n≥15 for each replicate). One way ANOVA.
(E) The parental generation of different genetic background [WT, *daf-16(mgdf50)* or *daf-16(mgdf50);daf-2(e1370)*] was grown on *cdk-12* RNAi. The eggs were bleached and placed on control RNAi. Percentage of F1 that reached L4 or above after 72 hours is shown. Average of three biological replicates (n≥50 for each replicate). One way ANOVA.
(F) Representative confocal images of worms stained with DAPI showing more endomitotic oocyte in WT, and *daf-16(mgdf50);daf-2(e1370)* as compared to *daf-2(e1370)* on *cdk-12* RNAi. The quantification of data is presented below**.** Average of three biological replicates (n≥10 for each replicate). Two-way ANOVA-Sidak multiple comparisons test. Image were captured at 240X magnification.
Error bars are SEM. ns, non-significant. Experiments were performed at 20 ºC. Source data is provided as a source data file.

FOXO/DAF-16 and CDK-12 have shared transcriptional targets including DNA damage repair (DDR) genes

To understand the connection between the IIS pathway and CDK-12, we performed transcriptomics analysis at late L4 stage of WT, *daf-2* and *daf-16;daf-2* worm grown on control or *cdk-12* RNAi from L1 onwards. We found a large transcriptional response in *daf-2* when *cdk-12* is knocked down but not to that extent in WT (data not shown). Importantly, genes downregulated in *daf-2* on *cdk-12* RNAi are enriched for cell cycle, oogenesis, early embryonic development and hatching as well as DNA replication, repair processes **(Figure 3A)**. When we compared the expression of the germline genes between *daf-2* and *daf-16;daf-2*, we found two distinct clusters, with one dependent on and the other independent of DAF-16 **(Figure 3B)**. The fact that many important germline genes are downregulated in *daf-16;daf-2* supports our earlier observation that the quality of oocytes of the double mutant is poor. Out of the 4126 DAF-16-dependent genes upregulated in *daf-2*, 987 are also regulated by *cdk-12* **(Supplementary table 2)**, Similarly, out of the 1478 DAF-16-dependent genes downregulated in *daf-2*, 329 are *cdk-12* target, showing that DAF-16 and CDK-12 have shared transcriptional targets.

In mammalian cells, CDK12 specifically regulates genes involved in DNA damage response [17,27]. We also found the DDR gene expression in *daf-2* to be considerably down-regulated upon *cdk-12* KD. In addition, these genes were also dependent on DAF-16 **(Figure 3C)**. We validated this by quantitative real-time PCR (RT-PCR) **(Figure S3A)**. We also found many of these genes to be down-regulated in WT on *cdk-12* RNAi **(Figure S3B)**. The downregulation in *daf-2* was not due to differences in the sizes of the gonads upon *cdk-12* KD **(Figure S3C)**. Importantly, ChIP-seq data analysis in *daf-2* and *daf-16;daf-2* showed that many DNA damage checkpoint genes like *mrt-2*, *rad-51*, *rad-50* and *pch-2* and DNA damage repair and cell cycle genes have DAF-16 binding peaks in their promoter-proximal regions **(Figure 3D, S3D)**. suggesting that they may be direct targets of DAF-16. Together, these data show that genes involved in sensing and repairing DNA damage are common transcriptional targets of DAF-16 and CDK-12.

CDK-12 is required for efficient DNA damage repair

Mammalian CDK12 is known to regulate DDR genes and promote homologous recombination (HR)-mediated DNA repair [17,28]. Above, we also found CDK-12 to transcriptionally regulate the DDR genes in *C. elegans*. To determine whether CDK-12 KD leads to germline DNA damage, we utilized a chromosome fragmentation assay. In unirradiated worms, six highly condensed bivalent bodies can be

seen in the oocyte; however, unrepaired DNA strand breaks in irradiated worms lead to chromosome fragmentation/fusions [29]. We observed increased chromosome fragmentation and fusions in IR-treated wild-type worms upon *cdk-12* KD that suggests increased DNA damage **(Figure 3E)**. Next, we exposed the L4 or YA worms to different concentrations of DNA damaging agent camptothecin (CPT) **(Figure S3E)** or varying doses of Ionizing Radiation (IR) **(Figure 3F)** and found that *cdk-12* KD resulted in a lesser number of hatched eggs, highlighting their higher sensitivity, possibly due to compromised DNA damage repair. We also observed increased developmental arrest on IR treatment at L1 stage when *cdk-12* is KD, in a DAF-16-independent manner **(Figure 3G)**.

In agreement to the fact that *cdk-12* KD may lead to endogenous DNA damage, we observed higher apoptotic bodies per gonadal arm in *cdk-12* KD wild-type and *daf-2* worms **(Figure 3H)**. DNA damage in worm germline has been shown to evoke the innate immune response which in turn confers systemic resistance and enhances somatic stress endurance [30]. In our transcriptomic data, we find that KD of *cdk-12* up-regulates innate immune response genes independent of DAF-16 activation **(Figure S3F, G)**. Further, *cdk-12* depletion conferred increased heat stress resistance **(Figure S3H, I)** and *hsp-4::gfp* (Endoplasmic Reticulum Chaperon BiP ortholog) expression **(Figure S3J)**, as has been reported for DNA damage [30,31]. Thus, RNAi depletion of *cdk-12* may cause DNA damage in cells that may be sensed by DAF-16 in the *daf-2* mutant.

Further, we wanted to visualize the role of CDK-12 in somatic DNA damage. For this, we analysed the DAPI-stained adult intestinal cells. A total of 20 intestinal cells are present at hatching, a subset of which (8-12) divide, but do not undergo cytokinesis, thereby generating 28-32 binucleate intestinal cells by the end of the L1 stage [32]. Like mutations in some DDR genes, *atm-1* and *dog-1* [33], we also found elongated cells with chromosomal bridges upon *cdk-12* KD **(Figure 3I)**, much similar to L4 worms exposed to IR **(Figure S3K),** indicating the occurrence of DNA damage in the somatic cells [29,33].

Together, CDK-12 plays a pivotal role in the repair of damaged DNA, both in the *C. elegans* germline and somatic tissues to maintain genomic integrity. Therefore, knocking down *cdk-12* may lead to genomic instability that is sensed by activated DAF-16 in the *daf-2* mutant, leading to the germline arrest at pachytene stage of meiosis. The DNA damage on *cdk-12* KD also accelerates aging independent of DAF-16.

FOXO/DAF-16 confers increased DNA damage repair efficiency

To test the functional role of DAF-16 in DDR and its heightened engagement in *daf-2* to protect against DNA damage, we again utilized the chromosome fragmentation assay. Worms were treated with IR at L4 to induce DNA double-strand breaks, stained with DAPI after 48 hours post-radiation and imaged. We found *daf-2* worms to be highly resistant to IR, such that at 90 Gy most of the wild-type chromosomes were fragmented, but *daf-2* worms retained intact chromosomes. This IR resistance was conferred by DAF-16, as in the *daf-16;daf-2* worms, the chromosomes were fragmented to a similar extent as in wild-type with IR treatment **(Figure 3J)**.

A high dose of gamma radiation during early larval stages in *C. elegans* can result in sterility and developmental arrest if the damage is not repaired [34]. Upon treatment of *daf-2* and *daf-16;daf-2* worms with 140 Gy IR dose at the L1 stage, we found that *daf-16;daf-2* worms become sterile **(Figure 3K)**. However, remarkably, *daf-2* worms were mostly fertile. Similarly, resistance to somatic developmental arrest on IR treatment was observed in *daf-2*, in a *daf-16*-dependent manner **(Figure 3G)**. Together, our findings support a role of DAF-16 in regulating DNA damage repair during lowered IIS, thereby promoting resistance to DNA damage, supporting growth and reproduction.

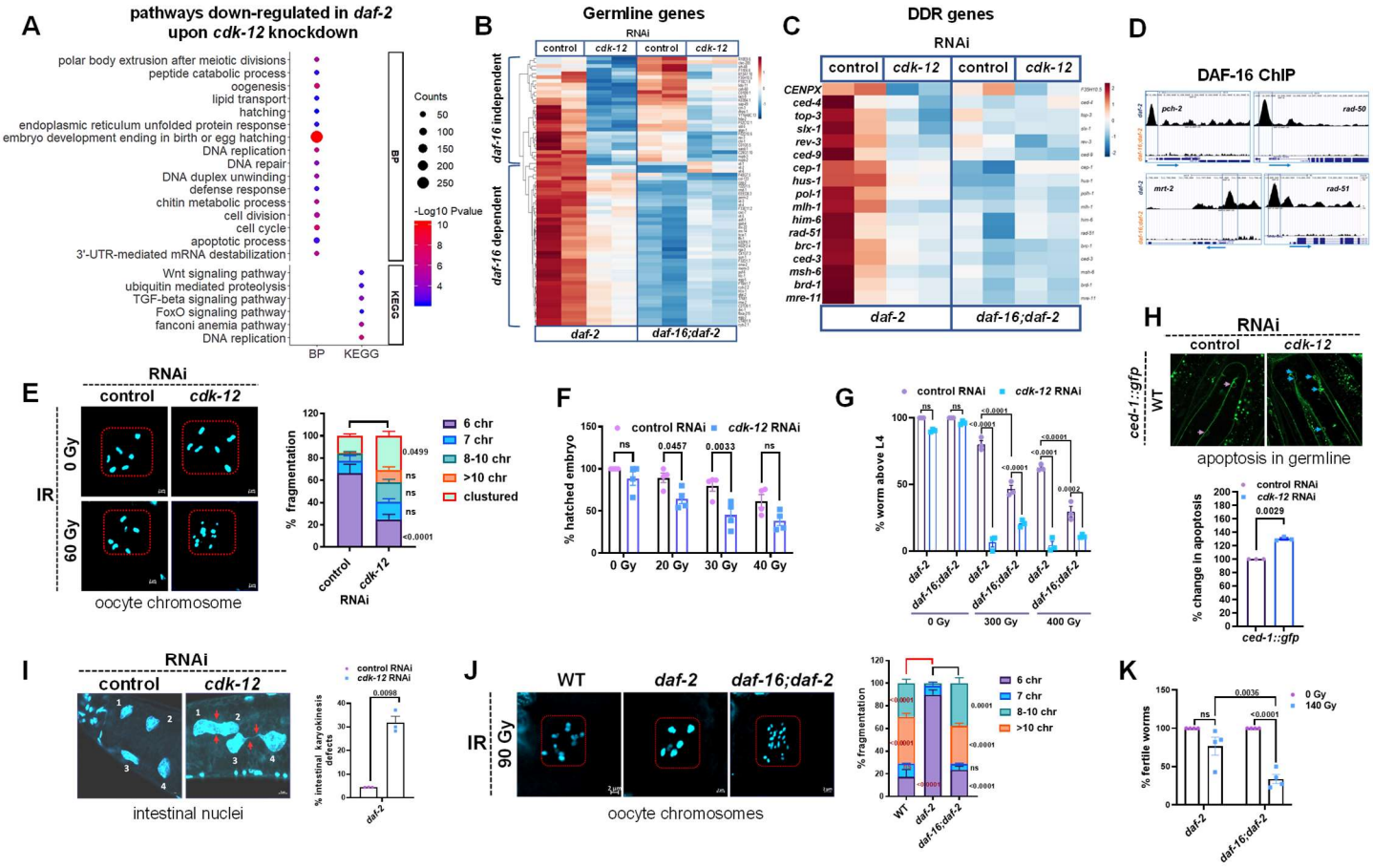

Figure 3. DAF-16 and CDK-12 regulate DDR gene expression for efficient DNA damage repair

(A) Gene Ontology (GO) Biological Processes (BP) term and KEGG pathway enrichment analysis of genes down regulated in *daf-2(e1370)* upon *cdk-12* KD using DAVID, as compared to control RNAi.

(B) A heat map showing differential changes in the expression pattern of genes involved in germline development in *daf-2(e1370)* and *daf-16(mgdf50);daf-2(e1370)* upon control or *cdk-12* RNAi.

(C) A heat map showing that DNA damage response (DDR) genes in *daf-2(e1370)* are down-regulated in a DAF-16-dependent manner. The DDR genes are also down-regulated in *daf-2(e1370)* upon cdk-12 RNAi, as compared to control RNAi.

(D) UCSC browser view of FOXO/DAF-16 peaks on *pch-2, rad-50, mrt-2* and *rad-51* promoters as analysed by ChIP-seq analysis of *daf-2(e1370)* and *daf-16(mgdf50);daf-2(e1370)* strains. Blue boxes represent the promoter regions of *pch-2, rad-50, mrt-2* and *rad-51* having DAF-16 binding peaks in *daf-2(e1370)*.

(E) Representative fluorescence images of DAPI-stained gonads showing oocytes with increased chromosome fragmentation upon γ-irradiation (60 Gy) in WT on *cdk-12* KD (left) and their quantification (right). Averages of four biological replicates (n≥59 oocyte for each replicate) are shown. One way ANOVA.

(F) Decrease in the percentage of hatched embryo in WT grown on *cdk-12* RNAi upon γ-irradiation, as compared to control RNAi. Average of four biological replicates are shown (n≥20 for each replicate). One way ANOVA.

(G) Worms of indicated strains were irradiated with different doses of γ-rays (0, 300, 400 Gy) at L1 larval stage and grown on control or *cdk-12* RNAi. After 96 hours, the percentage of worms that reached L4 or above was determined. Averages of 3 biological replicates (n≥100 for each replicate) are shown. Two-way ANOVA-Sidak multiple comparisons test.

(H) Representative images showing apoptotic cell (arrow) in the gonadal arm of *ced-1::gfp* upon *cdk-12* KD and their quantification**.** Average of three biological replicates are shown (n≥17 for each replicate). Unpaired t test with Welch's correction, Two-tailed.

(I) Representative fluorescence images of DAPI-stained worms showing incomplete separation of intestinal cell nucleus upon *cdk-12* KD (left) and its quantification (right). Average of three biological replicates (n≥70 intestinal cell for each replicate). Unpaired t test with Welch's correction, two-tailed.

(J) Representative DAPI-stained fluorescence images of oocytes showing chromosome fragmentation in WT, *daf-2(e1370)* and *daf-16(mgdf50);daf-2(e1370)* upon treatment with γ-irradiation (90 Gy) (left) and their quantification (right). Average of two biological replicates (n≥27 for each replicate) is shown. One way ANOVA.

(K) The *daf-2(e1370)* and *daf-16(mgdf50);daf-2(e1370)* worms were exposed to γ-irradiation (140 Gy) at L1 larval stage. Quantification showing percentage fertile worms. Arrows indicate sterile worms. Average of three biological replicates (n≥20 for each replicate) are shown. Two-way ANOVA-Sidak multiple comparisons test.

Error bars are SEM. ns, non-significant. Experiments were performed at 20 °C. Source data is provided as a source data file.

Regulation of pachytene arrest in *daf-2* upon DDR perturbation

IIS pathway couples nutrient sensing to meiosis progression and oocyte development to enable reproduction only when conditions are favourable for survival [2]. The well conserved LET-60 (RAS)-MEK-2 (ERK kinase)-MPK-1 (ERK1/2) pathway has several roles in the germline development and its maturation [35]. The RAS-ERK pathway works downstream of the IIS receptor *daf-2*. In response to nutrient availability, IIS activates MPK-1 (ERK) to promote meiotic progression. Thus, in the absence of nutrients or low food conditions, MPK-1 inhibition results in stalling of meiosis. In the *daf-2* germline stained with pMPK-1 antibody, the level of ERK activation is significantly lower than WT [36] **(Figure 4A, B)**. This potentially explains why *daf-2* have reduced brood size and oocyte numbers **(Figure 2C, S4A)**. This level is rescued to WT levels in *daf-16;daf-2* worms, showing that DAF-16 may negatively regulate pMPK-1 levels **(Figure 4A, B)**. When *cdk-12* is knocked down in WT, the levels of pMPK-1 is significantly reduced. However, the reduction is much more dramatic in *daf-2*, possibly below a threshold level **(Figure 4A, B)**. This may explain the complete arrest of the germline at pachytene stage **(Figure 1E)**. Importantly, in the *daf-16;daf-2*, the levels are restored **(Figure 4A, B)**, in line with the release of pachytene arrest in the double mutant **(Figure 1E)**.

It appears that downstream of *daf-2*, the ERK signalling and the canonical PI3K signalling co-ordinately regulate germline pachytene arrest. When *daf-2* is mutated, the pMPK-1 levels are lowered because of less signalling through the RAS pathway as well as due to the negative regulation of activated DAF-16 through the PI3K pathway. We have shown above that knocking out *daf-16* rescues the lower pMPK-1 in *daf-2* **(Figure 4A, B)**. So, we asked whether activating the ERK signalling can bypass the pachytene arrest in *daf-2* on *cdk-12* KD. We used an activated *ras* allele with constitutively high pMPK-1 phosphorylation [37]. In the *daf-2;let-60(gf)*, the pMPK-1 levels were upregulated **(Figure 4A, B)** and pachytene arrest was partially reversed **(Figure 4C-E)**. Although many eggs hatched to release L1 worms **(Figure S4B),** only about half of them were able to reach adulthood **(Figure 4F),** possibly pointing at their poor quality. Overall, we conclude that the ERK and the PI3 kinase pathways co-ordinately regulate meiosis arrest on sensing somatic DDR perturbations in *daf-2*.

Defective sperm to oogenesis switch and transcriptional downregulation of key cell cycle genes in *daf-2* on DDR perturbation

We have shown above that the sterility of *daf-2* on *cdk-12* RNAi may be due to inactive RAS-ERK signaling. RAS-ERK activation is critical for sperm-oocyte fate switch by regulating the timing the event

in *C. elegans* hermaphrodite[38]. We observed a two-folds increase in the number of sperms but no oocyte in *daf-2* upon *cdk-12* KD **(Figure 4G, H)**. So, we tested the mRNA levels of key sperm-oocyte switch genes and found their levels to be significantly reduced in *daf-2* **(Figure 4I**, but not in *daf-16;daf-2* **(Figure S4C)**. This decrease in expression of genes is due to the *cdk-12* KD *per se*, and not because of a reduction in germline size as at late-L4 (when RNA was collected), the germline size is comparable between control RNAi and *cdk-12* RNAi fed worms **(Figure S3C).**

Next, we asked if the sperm to oogenesis switch defect was accompanied by an underlying defect in other critical players of meiotic progression, namely, *cdk-1*, *cyb-1* and *cyb-3* [39]. To assess this, we determined the mRNA levels of these genes and found levels of all three to be significantly down-regulated in *daf-2* worms with *cdk-12* KD **(Figure 4J)**, whereas the gene levels were largely unchanged in *daf-16;daf-2 cdk-12* RNAi worms **(Figure S4D)**. Additionally, knocking down these genes individually led to sterility in *daf-2* worms **(Figure S4E, F)**, phenocopying the sterility upon *cdk-12* KD.

We further checked if a similar defect in sperm to oogenesis switch and downregulation of key cell cycle genes underlies the sterility upon DNA damage on IR exposure. We treated *daf-2* worms with 160 Gy IR at L1 and DAPI stained Day 1 adults. Surprisingly, we found that the sperm count increased around two-fold with a concomitant reduction in sperm to oocyte switch genes and *cdk-1*, *cyb-1*, and *cyb-3* RNA levels **(Figure S4G-I)**. Therefore, using CDK-12 knockdown and IR exposure to phenocopy DNA damage, we show that germline arrest on DDR perturbation in *daf-2* is brought about by defective sperm to oogenesis switching and reduction in the transcription of genes essential for meiotic progression. This, along with reduction of ERK/MPK-1 signaling, may be strategies employed by the *daf-2* hermaphrodite worms to prevent the production of poor-quality progeny when the DNA damage is beyond repair.

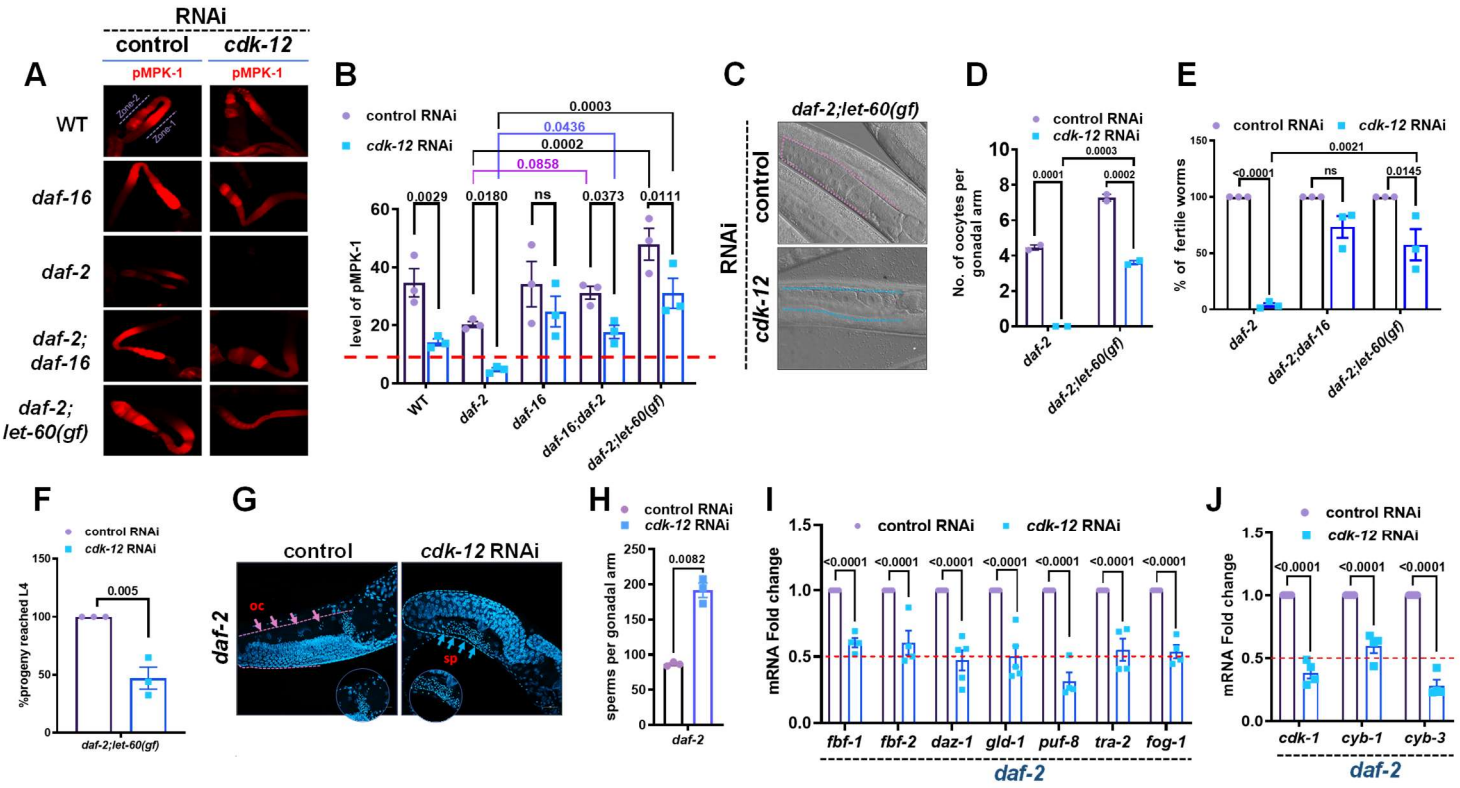

Figure 4. CDK-12 KD disturbs the balance of ERK-MPK and IIS signaling that regulates germline development

(A-B) Representative images of dissected gonads of WT, *daf-2(e1370), daf-16(mgdf50);daf-2(e1370)*, *daf-16(mgdf50)* and *daf-2(e1370);let-60(ga89)*, probed with anti-dpERK (red) (A) and its quantification (B) upon control or *cdk-12* RNAi. Average of three biological replicates (n≥10 for each replicate). Two-way ANOVA-Uncorrected Fisher's LSD multiple comparisons test. Zone-1 and zone-2 are the proximal and distal parts of the gonad, respectively. Red line in (B) is a presumptive threshold of pMPK-1 below which germline arrests.

(C) Representative DIC images of worms showing oocytes of *daf-2(e1370);let-60(ga89)* upon *cdk-12* KD.

(D) Quantification of oocyte number per gonadal arm of *daf-2(e1370)* and *daf-2(e1370);let-60(ga89)* upon *cdk-12* KD. Averages of two biological replicates (n≥20 for each replicate). Two-way ANOVA-Sidak multiple comparisons test.

(E) Percentage of fertile worms in *daf-2(e1370)*, *daf-16(mgdf50);daf-2(e1370)* and *daf-2(e1370);let-60(ga89)* on control or *cdk-12* RNAi. Average of three biological replicates (n≥25 for each replicateTwo-way ANOVA-Sidak multiple comparisons test. The concentration of IPTG used in this experiment is 0.4mM.

(F) The *daf-2(e1370);let-60(ga89)* were grown on control or *cdk-12* RNAi. The worms were bleached and their eggs grown on control RNAi. Percentage of hatched progeny that reached L4 larval stage is

plotted. Average of three biological replicates (n≥40 for each replicate). Unpaired t test with Welch's correction, Two-tailed.

(G, H) DAPI stained worms and quantification of the sperm count in *daf-2(e1370)* on control and *cdk-12* RNAi. Average of three biological replicates (n≈20 for each replicate). Unpaired t test with Welch's correction, Two-tailed.

(I) Quantitative RT-PCR analysis of sperm-to-oocyte switch genes in *daf-2(e1370)* on control or *cdk-12* RNAi. Expression levels were normalized to *actin*. Average of four biological replicates are shown. One way ANOVA

(J) Quantitative RT-PCR analysis of cell cycle regulator *cdk-1* and its binding partner *cyb-1* and *cyb-3* (mammalian Cyclin B orthologs) in *daf-2(e1370)* upon *cdk-12* KD, compared to control RNAi. Expression levels were normalized to *actin*. Average of four biological replicates are shown. One way ANOVA.

Error bars are SEM. ns, non-significant. Experiments were performed at 20 ºC. Source data is provided as a source data file.

Uterine tissue-specific DDR perturbation arrests germline in *daf-2*

Since *cdk-12* KD leads to impaired DDR and resulting DNA damage, we asked whether tissue-restricted DNA repair perturbations will lead to germline arrest in *daf-2*. We first used a germline-specific RNAi system to test if tissue autonomous depletion was sufficient for the arrest in *daf-2*. We used a *rde-1(-)* transgenic strain where *sun-1* promoter drives the expression of *rde-1* only in the germline of *daf-2* (germline-specific RNAi) [40]. We validated the strain by knocking down a germline-specific gene *glp-1* [41] which led to sterility **(Figure S5A)**, showing a functional germline RNAi machinery. A systemic KD of a soma-specific GATA transcription factor, *elt-2* [42] leads to developmental arrest in wild-type; however, the *daf-2* germline-specific RNAi worms were resistant to *elt-2* KD **(Figure S5A)**, showing the lack of RNAi in the somatic tissues. Surprisingly, we found KD of *cdk-12* only in germline does not lead to sterility **(Figure 5A, B)**, indicating that a soma-specific DDR malfunction may cause the germline arrest. However, depletion of *cdk-12* in the germline alone results in progenies that are developmentally arrested and sterile **(Figure 5C)**, showing that its function is required in the germline to maintain progeny quality. Importantly, it appears that activated DAF-16 only promotes germline arrest if the damage signal emanates from somatic tissues.

Next, we specifically knocked down *cdk-12* in different somatic tissues [43-46]. We found that knocking down *cdk-12* only in the uterine tissues was sufficient to arrest the germline in the *daf-2* worms at the pachytene stage of meiosis **(Figure 5D, S5B)**; no arrest was seen when the gene was knocked down in hypodermis, muscle, intestine or neurons **(Figure 5D)** and they produced healthy fertile progeny **(Figure S5C)**. This implies that KD of *cdk-12* in *daf-2* germ cells may lead to DNA damage resulting in poor progeny production. However, knocking it down in the somatic uterine tissue may activate DAF-16-dependent quality checkpoints that lead to cell-nonautonomous germline arrest.

Next, to determine the tissues where the IIS receptor functions, we used transgenic lines where the wild-type copy of *daf-2* is rescued only in the neurons (using either *unc-119* or *unc-14* promoters), muscles or intestine of the *daf-2* mutants [47] and then knocked down *cdk-12* using RNAi. We found that neuron-specific rescue of the *daf-2* gene led to a significant rescue of fertility, while little effect was seen in the case of muscle and intestine-specific rescue **(Figure 5E)**. We also determined where DAF-16 is required to sense and mediate the germline arrest in the *daf-2* mutant upon *cdk-12* KD. We found maximum arrest when *daf-16* is rescued in the muscle, neuron or uterine tissues of the *daf-16;daf-2* mutant worms **(Figure 5F)**, but not in the intestine. Together, these observations support a model where low neuronal IIS sensitizes uterine tissues to perturbations in DDR, leading to the arrest of germline at

the pachytene stage of meiosis. The DAF-16a isoform works in the somatic uterine tissues, apart from muscle and neurons, to implement the arrest **(Figure 5G)**.

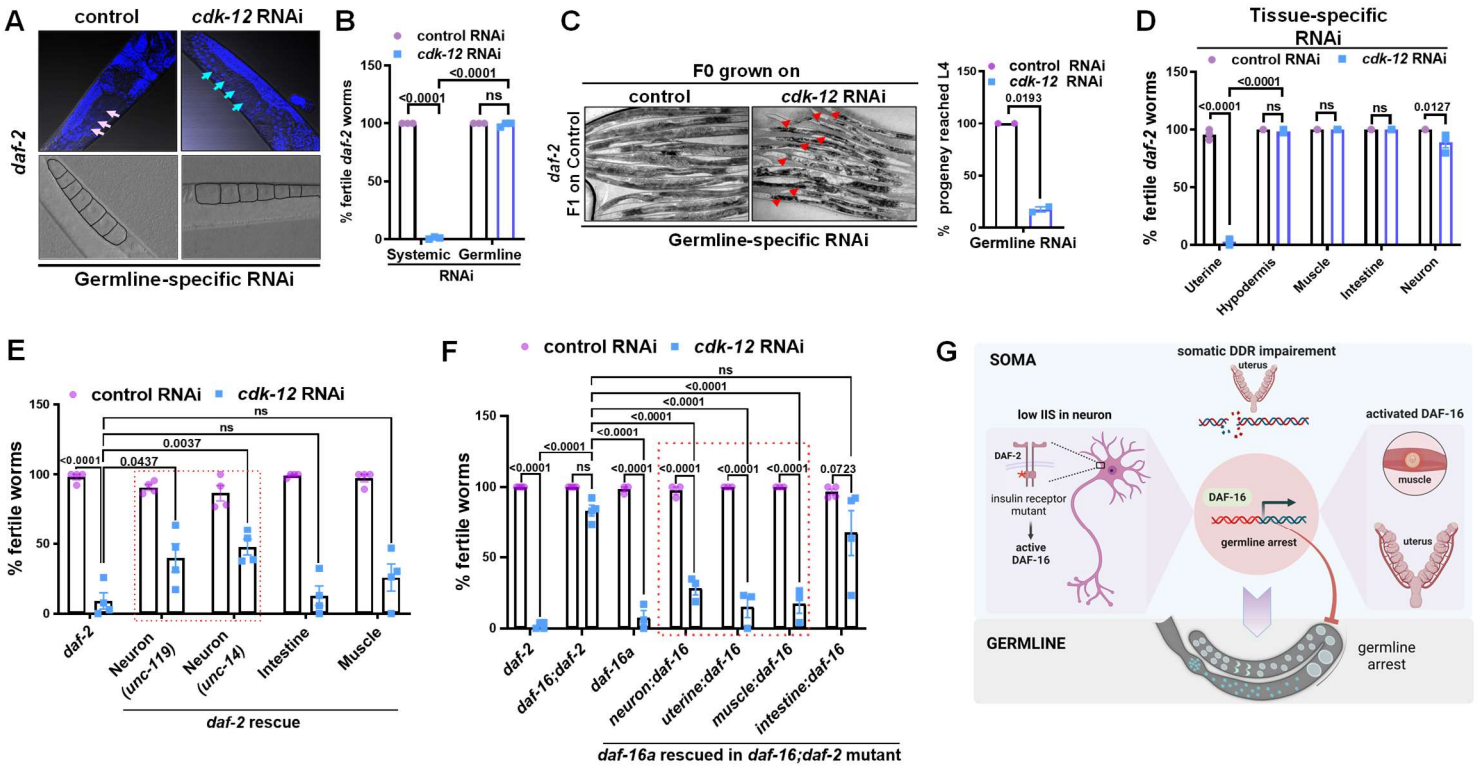

Figure 5. Cell non-autonomous signals from soma determines germline fate

(A) The *cdk-12* knock down by RNAi specifically in the germline of *daf-2(e1370);mkcSi13 II; rde-1(mkc36) V [mkcSi13 [sun-1p::rde-1::sun-1 3'UTR + unc-119(+)] II]* (germline-specific RNAi) strain produced no arrest. Arrows showing oocyte nuclei. Upper panels are 400x DAPI images and lower panels are bright field.

(B) Percentage of fertile worms in *daf-2(e1370)* and *daf-2(e1370);mkcSi13 II; rde-1(mkc36) V [mkcSi13 [sun-1p::rde-1::sun-1 3'UTR + unc-119(+)] II]* (germline-specific RNAi) on control or *cdk-12* RNAi. Average of three biological replicates (n≥30 for each replicate). Two-way ANOVA-Sidak multiple comparisons test.

(C) *Cdk-12* was knocked down specifically in the germline of *daf-2(e1370)* using germline-specific RNAi strain *(daf-2(e1370);mkcSi13 II; rde-1(mkc36) V [mkcSi13 [sun-1p::rde-1::sun-1 3'UTR + unc-119(+)] II])*. The eggs produced by these worms or the ones grown on control RNAi were transferred to fresh control RNAi plates. Representative brightfield images of these F1 progeny shown along with quantification. Average of two biological replicates (n≥30 for each replicate). Unpaired t test with Welch's correction, Two-tailed.

(D) Percentage of fertile worms when *cdk-12* is knocked down in different tissues of *daf-2(e1370).* Only uterine-specific knockdown [using *daf-2(e1370);rrf-3(pk1426) II; unc-119(ed4) III; rde-1(ne219) V;*

qyIs102] of *cdk-12* results in sterility. Average of three biological replicates (n≥19 for each replicate). Error bars are SEM. One way ANOVA.

(E) Percentage of fertile worms on *cdk-12* KD in strains where the *daf-2* gene is rescued either in the neurons, intestine or muscles of the *daf-2(e1370)* mutant. Average of four biological replicates (n≥30 for each replicate). Two-way ANOVA-Sidak multiple comparisons test.

(F) Percentage of fertile worms on *cdk-12* KD in strains where the *daf-16* gene is rescued either in the neurons, intestine, muscles or uterine tissues of the *daf-2(mu86)* mutant. Average of four biological replicates (n≥15 for each replicate). Two-way ANOVA-Sidak multiple comparisons test.

(G) A tentative model showing inter-tissue crosstalk of low IIS in the neuron and activated DAF-16/FOXO in the neuron or muscle or uterine tissue (somatic gonad) that is required to mediate the germline arrest in response to somatic DNA damage or DDR perturbation by *cdk-12* depletion.

Error bars are SEM. ns, non-significant. Experiments were performed at 20 ºC. Source data is provided as a source data file.

Discussion

In this study, we have shown that activated FOXO/DAF-16 senses the intrinsic somatic DNA damage and functions cell non-autonomously to regulate reproductive decision in order to safeguard the germline genomic integrity and progeny fitness.

CDK-12 is a well-studied protein that is involved in DDR and genome integrity in mammalian cells. We also show that in *C. elegans*, *cdk-12* regulates the expression of DDR genes and maintains genome integrity [48,49]. The depletion of *cdk-12* makes the worms susceptible to DNA damaging agents and induce spontaneous DNA damage both in the soma and the germline, implying a suboptimal repair pathway. We show *cdk-12* ablation reduces gamete quality, leading to increased infertility and decreased progeny fitness. It also led to retarded growth, and premature aging which are hallmarks of genomic instability. Thus, CDK-12 is an evolutionary well-conserved custodian of the genome that helps maintain DNA integrity, which we have used in our study as a genetic tool to analyse the effects of tissue-restrictive DDR perturbation and DNA damage.

To maintain genomic integrity, organisms have evolved an efficient DDR pathways, that senses and repairs DNA damage [50]. Defects in DDR is associated with reduced fitness, infertility and offspring with inherited diseases [51,52]. We identified that active FOXO/DAF-16 maintains genomic integrity by upregulating DNA repair genes, which could explain the longer lifespan and better oocyte quality of the low IIS mutants [53]. Apart from maintaining genomic stability, our study shows that activated FOXO/DAF-16 can sense DDR perturbation or DNA damage, stop reproduction by arresting the germline, and protect the genomic integrity of germ cell. In the absence of DAF-16, worms fail to arrest germline development and produce oocytes of poor quality that hatch into unhealthy progenies. Thus, activated FOXO/DAF-16 critically regulates reproductive decision by sensing the intrinsic threat of genomic instability. Previous studies has shown that DAF-16 acts as a nutrient sensor and mediates developmental arrest on starvation, as a protective mechanism [7]. Together, these data suggests that FOXO/DAF-16 acts as a master regulator of diverse cellular processes in maintaining genomic integrity, tissue homeostasis and reproduction.

We found that upon DDR perturbation and ensuing DNA damage, FOXO/DAF-16 enforces germline arrest by inactivating RAS-ERK signalling which is essential for germline proliferation and quality. We also observed reduced expression of cyclin-dependent kinase-1 gene (*cdk-1*) and its binding partner cyclin, *cyb-1,* and *cyb-3* genes, which may be due to dampening of the RAS-ERK signaling. In many cancers, RAS-ERK negatively regulates FOXO activity and promotes rapid proliferation[54]. Similarly,

we observed that constitutively activated RAS-ERK in the low IIS mutant (where FOXO/DAF-16 is activated) over-rides the germline arrest upon DDR perturbation, leading to the production of unhealthy progenies. Therefore, RAS-ERK and FOXO/DAF-16 regulate each other's activity and a fine balance is important for various biological process, including reproductive development.

Cell non-autonomous inter-tissue crosstalk helps an organism to perceive and respond to changing environment. Multiple studies in *C. elegans* have revealed cell non-autonomous crosstalk in stress response and longevity [55]. DAF-2 in the neuron and DAF-16 in the intestine is known to regulate longevity cell non-autonomously [14,15]. Muscle or intestinal FOXO/DAF-16 activity promotes long reproductive span or better oocyte quality of the *daf-2* mutant [13]. DAF-16 has also been shown to function in the uterine tissue to prevent decline in the germline progenitor cells with age [11]. However, it is not clear how DAF-16 cell non-autonomously regulates germline health. We show that perturbation of the DDR pathway only in the somatic uterine tissue of low IIS worm is sufficient to cause cell cycle arrest in the germline; perturbation in the germline itself does not lead to arrest but produces unhealthy progenies. This suggests that the somatic tissue, not the germline, senses stress signal of genome instability and shunt their energy and resources towards somatic maintenance rather than reproductive commitment. This is supported by the observations of heightened stress response pathways and retarded germline growth upon DDR perturbation.

Finally, we find that lowering of IIS is required in the neurons to activate FOXO/DAF-16 cell-autonomously in the neurons as well as non-autonomous in the muscle and uterine tissues to mediate cell cycle arrest. This soma to germline communication is most likely mediated by the DAF-12/dafachronic acid steroid signalling. It has been previously shown that DAF-12/dafachronic acid steroid signalling is required for germline to soma signalling for DAF-16-dependent longevity in germline mutants [56,57] Previous studies have also shown that neurons can sense cell intrinsic unfolded protein stress and mount a protective response in distal tissues [58]. Thus, from an evolutionary perspective, such a complex network of non-autonomous inter-tissue crosstalk likely helps an organism sense intrinsic or extrinsic stresses more efficiently and accurately. This may ensure the optimal survival as well as fitness across generations.

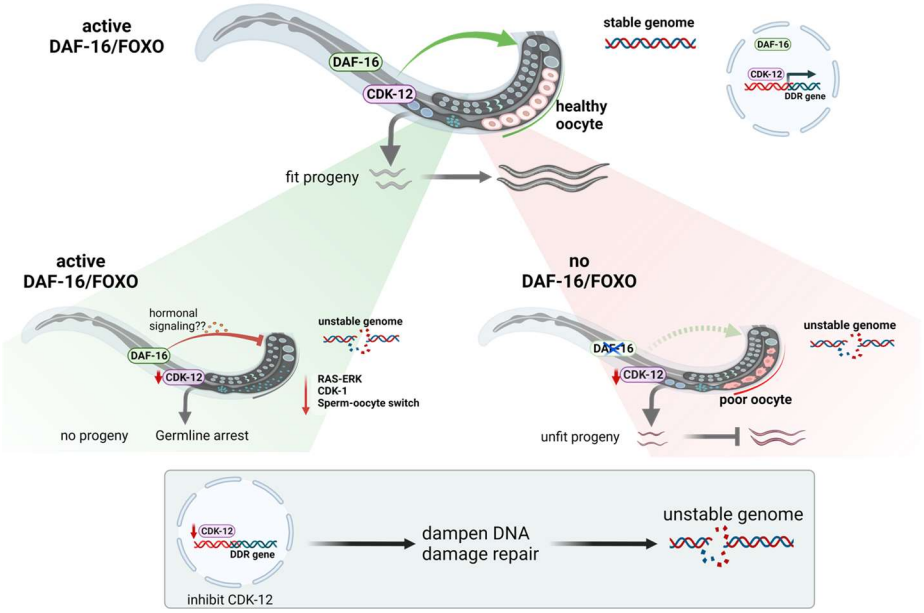

Figure 6. A model showing how activated DAF-16/FOXO may act as a quality control checkpoint from the somatic tissue, regulating reproductive decision, gamete quality and progeny health, likely via soma-germline hormonal signaling. Somatic DAF-16/FOXO may sense the DDR inactivation and send signals to germline to halt reproduction (by germline arrest) in order to protect the genome integrity of the germ cells/oocytes and maintain progeny fitness. In the absence of active DAF-16/FOXO, organisms fail to arrest reproduction and produces compromised oocyte and unhealthy progeny. DDR perturbation only in the somatic tissue (uterus) is sufficient to arrest the germline. DAF-16/FOXO arrests the germ cell development by inactivating the RAS-ERK signaling which is essential for germline proliferation and its maturation.

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Conflict of interest statement

The authors declare no conflict of interest.

Data availability statement

All data used in the study are presented as a Source data file. RNA-seq data is available with the ArrayExpress accession number E-MTAB-11189.

Experimental details

The experimental details are provided as a supplementary materials document.

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