Innate immunity mediated longevity and longevity induced by germ cell removal converge on the C-type lectin domain protein IRG-7

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Abstract

In *C. elegans*, removal of the germline triggers molecular events in the neighboring intestine, which sends an anti-aging signal to the rest of the animal. In this study, we identified an innate immunity related gene, named *irg-7*, as a novel mediator of longevity in germ-lineless animals. We consider *irg-7* to be an integral downstream component of the germline longevity pathway because its expression increases upon germ cell removal and its depletion interferes with the activation of the longevity-promoting transcription factors DAF-16 and DAF-12 in germ-lineless animals. Furthermore, *irg-7* activation by itself sensitizes the animals’ innate immune response and extends the lifespan of animals exposed to live bacteria. This lifespan-extending pathogen resistance relies on the somatic gonad as well as on many genes previously associated with the reproductive longevity pathway. This suggests that these genes are also relevant in animals with an intact gonad, and can affect their resistance to pathogens. Altogether, this study demonstrates the tight association between germline homeostasis and the immune response of animals, and raises the possibility that the reproductive system can act as a signaling center to divert resources towards defending against putative pathogen attacks.

Author summary

Increased sensitivity to pathogens is one of the hallmarks of aging. Thus, pathways that slow down the aging process should provide a remedy to this challenge. In this study, we have used the model organism *C. elegans* to identify a new longevity gene whose activation improves the resistance of the animals to pathogenic food and extends the lifespan of the animals. This improved resistance is the result of activation of the innate immune response and requires many genes known to promote longevity in animals whose germ-line has been removed. Nevertheless, this improved survival in the presence of pathogenic food is achieved without depleting the germline of the animals. This suggests that these longevity-promoting genes, many of which are conserved between worms and humans,
can also protect animals with an intact gonad by diverting resources towards defending against putative pathogen attacks.

Introduction

One of the most significant findings in the aging research field is the realization that lifespan is determined by the aging rate and by deleterious events that limit lifespan. Over the last few decades, a wealth of genes and signaling molecules that determine lifespan and longevity were identified. Genome-wide screens in the model organism *C. elegans* revealed that genes that influence aging fall into four conserved longevity pathways: the dietary restriction pathway, the insulin/IGF-1 pathway, the mitochondrial pathway and the reproductive pathway [1, 2]. In general, one common theme of these longevity regulatory pathways is their ability to shift resources towards enhanced maintenance and stress resistance of the soma [3].

In *C. elegans* and in *Drosophila*, germ cell removal can increase lifespan and increase resistance to oxidative stress, proteostasis stress and pathogens [4–9]. These beneficial effects of germ cell depletion require the presence of an intact somatic gonad [4, 5]. In addition, nuclear hormone signaling [5], autophagy [10–12], fat metabolism [10, 12], proteasome components [9, 13], microRNA-regulators [14] and a variety of transcription factors [5, 9–12, 15–18] are required for lifespan extension upon germ cell removal. Some of these genes (for example *daf-16*) are common mediators of several longevity pathways, whereas others are uniquely associated with the longevity of germlineless animals. Notably, many of the genes implicated in the longevity of germlineless animals act specifically in the intestine [19], which comprises the animal’s adipose tissue and liver, and is also the major site of pathogen colonization in *C. elegans*.

Although *C. elegans* has evolved a dedicated innate immune response to protect the animals from infecting pathogens [20, 21], one of the factors that limit *C. elegans* lifespan is its slightly pathogenic microbial diet, which colonizes and infects the intestine of aging animals [22–24]. Accordingly, mutants deficient in the central innate immunity PMK-1/ATF-7 pathway exhibit a shortened lifespan even on *E. coli* OP50, a weakened bacterial strain that is only slightly pathogenic to *C. elegans* [25]. Conversely, killing or limiting the proliferation of the bacterial food source extends *C. elegans* lifespan [22, 26]. Furthermore, many mutations that prolong lifespan also enhance the resistance of animals to pathogenic bacteria [6, 27–30]. This enhanced resistance to pathogens is thought to be the consequence of constant expression of a variety of innate immunity-related genes [17, 30–32], which act in parallel to the PMK-1/ATF-7 innate immunity pathway [6, 27–30]. Together, these imply that pathogen infection (even by the non-pathogenic OP50 *E. coli* strain) and the host immune response are important determinants of *C. elegans* lifespan. Here we identified the innate immunity related gene *irg-7* as a novel integral component of the reproductive longevity pathway, which links between genes implicated in this longevity pathway and pathogen resistance even in animals with an intact reproductive system.

Results

*irg-7(zc6)* mutants are long-lived

One of the characteristics of the aging process is a decline in cells’ ability to mount cellular stress responses under conditions of perturbed protein homeostasis in the cytosol, in the ER and in the mitochondria [34–36]. The failure to mount these stress responses abrogates the induction of the corresponding chaperones, whose deficiency further perturbs protein
homeostasis. We hypothesized that constitutive expression of chaperones may be beneficial for maintaining proteostasis in aged animals, and thus may contribute to their health-span and their longevity. To test this, we assessed the lifespan of \textit{irg-7(zc6)} mutants, in which the \textit{hsp-4} ER resident chaperone is constitutively expressed in the animals’ intestine due to a partially mapped background mutation ([37] and Fig 1A). This strain, which was originally named \textit{upr-1(zc6)}, was generated as part of a seminal study by David Ron’s group, to facilitate the identification of mutations in genes required for the induction of this ER resident chaperone [37]. We note that in the absence of information about the \textit{zc6} mutation, it was unclear whether these animals induce ER chaperone expression due to deregulation of gene expression or whether the induction is a reflection of ER stress and activation of the unfolded protein response (UPR). Either way, lifespan analysis of the \textit{irg-7(zc6)} mutants revealed that they lived up to 30% longer than control animals (Fig 1B and S1 Table). With the exception

Fig 1. \textit{irg-7(zc6)} mutation extends lifespan independently of UPR genes. (A) Representative fluorescence micrographs (100-fold magnification) of day-1 adults harboring an integrated \textit{Phsp-4::gfp} transgene. \textit{irg-7(zc6)} mutants expressed higher levels of the \textit{Phsp-4::gfp} than did wild type animals, predominantly in the posterior end of the intestine. Asterisks mark Student’s T-test values of $P<0.001$ compared to wild-type fluorescence. 50 animals were analyzed per genotype. Error bars represent SE of 3 independent biological replicates. (B) The \textit{irg-7(zc6)} mutation significantly extended the lifespan of otherwise wild-type, \textit{pek-1(ok275)}, \textit{atf-6(ok551)} or \textit{ire-1(RNAi)} animals. Mantel Cox $P$-value for the wild type vs. \textit{irg-7 (zc6)} single mutant comparison is in green. Mantel Cox $P$-value for the mutant vs. the mutant; \textit{irg-7(zc6)} double mutant comparison is in black. Mean lifespan and $P$-values are indicated within each graph. See S1 Table for additional lifespan data.

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of a developmental delay of 4 hours, the physiology of irg-7(zc6) mutants appeared to be similar to that of wild-type animals in terms of pumping rate, fecundity and progeny profile (S1 Fig).

If indeed the longevity of irg-7(zc6) mutants stems from the constitutive expression of ER-resident chaperones, whose expression is regulated by the activation of the unfolded protein response (UPR), then interference with UPR induction should curtail their lifespan. To test this, we introduced mutations in major UPR genes into the irg-7(zc6) strain and followed their lifespan. First, we introduced the irg-7(zc6) mutation into pek-1(ok275) and atf-6(ok551) mutants (harboring deletion mutations in the worm homologs of the PERK and ATF6 genes respectively). We found that the irg-7(zc6) mutation extended the lifespan of pek-1(ok275) and atf-6(ok551) mutants to a similar extent as it did in animals with an intact UPR (P > 0.2 in Cox Regression analysis of 3 independent experiments for each genotype) (Fig 1B and S1 Table). This implies that neither of these genes is required for the longevity of irg-7(zc6) mutants. Surprisingly, we could not introduce the irg-7(zc6) mutation into ire-1(ok799) and xbp-1(tm2457) mutants (harboring deletion mutations in the worm homologs of the IRE1 and XBP1 genes respectively). Specifically, we could not detect any homozygous xbp-1(-/-); irg-7(zc6) or ire-1(-/-); irg-7(zc6) double mutants among the viable progeny of heterozygous xbp-1(+/-) or ire-1(+/-) irg-7(zc6) mutants. This suggests that some activity of the ire-1/xbp-1 pathway is critical for the survival of irg-7(zc6) mutants. To circumvent this, we examined the longevity of irg-7(zc6) mutants treated with xbp-1 or ire-1 RNAi, which reduce the levels of their target genes, rather than completely eliminating them. Treatment with xbp-1 or ire-1 RNAi attenuated the expression of the Phsp4::gfp reporter in irg-7(zc6) mutants and was compatible with the survival of the irg-7(zc6) strain. Nevertheless, the irg-7(zc6) mutation still extended the lifespan of ire-1 or xbp-1 RNAi-treated animals to a similar extent as it did in animals with an intact UPR (P > 0.3 in Cox Regression analysis of 3 independent experiments) (Fig 1B and S1 Table). These findings suggest that the longevity of irg-7(zc6) mutants does not rely on high expression levels of ER resident chaperone.

zc6 is a gain of function mutation in the infection response gene irg-7

If not due to increased chaperone levels, why are upr-1/irg-7(zc6) mutants long-lived? An answer to this enigma may lie in the identity of the irg-7 gene. To associate the irg-7(zc6) mutation with its molecular and genetic identity, we completed the mapping of the irg-7(zc6) mutation. This mutation was previously characterized as a semi-dominant mutation located on chromosome X [37]. Using one-step whole-genome-sequencing and a SNP mapping strategy [38] we identified several candidate mutations within a mapping interval on chromosome X that may account for the Irg-7 phenotypes. We used RNAi to knockdown these candidate genes and searched for genes whose inactivation phenocopied or suppressed the phenotypes of the irg-7(zc6) mutants. We found that inactivation of f40f4.6 by RNAi suppressed the longevity of irg-7(zc6) mutants without affecting the lifespan of wild-type animals (Fig 2A and S2 Table). Inactivation of f40f4.6 by RNAi also suppressed the expression of the ER stress reporter in irg-7(zc6) mutants (Fig 2B), suggesting that the mutation in f40f4.6 is triggering the UPR in these animals. Since f40f4.6 RNAi suppresses rather than phenocopies the phenotypes associated with the irg-7(zc6) mutation, these experiments suggest that the irg-7(zc6) mutation is a gain of function (gof) mutation in the f40f4.6 gene.

In some cases gain of function mutations result in hyperactivation of the normal function of the encoded protein, whereas in other cases they may confer new activities. Likewise, overexpression of a protein can enhance its activity as well. Thus, we examined whether overexpression of F40F4.6 would extend lifespan similarly to the irg-7(zc6) gof mutation. To this
end, we determined the lifespan of animals carrying a fosmid that includes the f40f4.6 gene. We found that these animals lived longer than wild-type animals. In contrast, animals carrying a partially overlapping fosmid that does not include f40f4.6 had a normal lifespan (Fig 2C and S2 Table). These experiments suggest that the irg-7(zc6) mutation is a gain of function mutation that enhances the normal activity of the protein.
The \textit{zc6} mutation removes an inhibitory domain from the infection response gene \textit{irg-7}.

Not much is known about the \textit{irg-7} gene. Microarray studies demonstrated that the expression of \textit{irg-7/f40f4.6} is induced upon exposure to some pathogens [39] as well as under dietary restriction (in this study \textit{f40f4.6} has been referred to as \textit{drd-2} [40]). In support of these reports, we confirmed that infection by \textit{Photorhabdus luminescens} subsp Hb and \textit{Enterococcus faecalis} bacteria (henceforth referred to as HB and EF bacteria for brevity), induces the transcription of a reporter driven by the \textit{irg-7} promoter in the animals intestine (Fig 2D).

\textit{irg-7} encodes a protein of 2214 amino acids with a modular structure. It includes three EGF domains (that can be used for association with other regulatory proteins), a Von Willebrand factor type A domain (usually involved in adhesion via metal ion-dependent adhesion sites) and a C-type lectin domain (CTLD) (usually involved in binding to a wide variety of molecules such as sugars, proteins, lipids and inorganic compounds and implicated in pathogen recognition and clearance [41]) (Fig 2E). In addition, IRG-7 contains a putative signal sequence, but no putative transmembrane domain, and thus is likely to be secreted. Although each of these domains is individually evolutionarily conserved, we failed to identify any human homolog containing this exact combination of domains.

Sequencing of the \textit{irg-7} gene in animals carrying the \textit{zc6} mutation revealed a deletion of 901 base pairs between nucleotides 3241528–3242428 on chromosome X as well as an insertion of 2 nucleotides (S2 Fig). This deletion, which includes exon and intron regions, ultimately removes 221 amino acids from the protein product while preserving its open reading frame. The existence of a transcript encompassing the \textit{zc6} mutation was confirmed by RT-PCR and sequencing (S2 Fig). The region included in the deletion mutation encompasses one of the EGF-like domains of the protein (Fig 2E). Since the \textit{zc6} mutation is a \textit{gof} mutation, this indicates that the deleted EGF domain is an inhibitory domain.

\textit{irg-7} affects pathogen resistance

One of the factors that limit \textit{C. elegans} lifespan is its pathogenic food [22, 26]. CTLD-proteins have the capacity to bind carbohydrates that coat pathogens, and are induced upon contact with a variety of fungal and bacterial pathogens [41]. Thus, proteins harboring C-type lectin domains could potentially play a role in pathogen recognition and clearance [41]. Since IRG-7 harbors a CTLD domain, and since its expression is induced upon infection, we wondered whether the lifespan extension of \textit{irg-7 gof} mutants was associated with an improved ability to deal with pathogens. Hence, we examined whether the extended lifespan of \textit{irg-7 gof} mutants fed with live \textit{OP50} bacteria would persist if the animals were fed with killed bacteria. As previously reported [22, 26], we confirmed that the mere feeding of wild-type animals with dead bacteria extends their lifespan (Fig 3A). In contrast, a diet of heat-killed or UV-killed bacteria did not extend the lifespan of \textit{irg-7 gof} mutants (Fig 3A and S3 Table). This implies that unlike wild-type animals, the lifespan of \textit{irg-7 gof} mutants is not limited by the viability of its pathogenic food.

\textit{OP50 E. Coli} is only slightly pathogenic to \textit{C. elegans}. Thus, we next compared the survival of \textit{irg-7 gof} mutants and wild-type animals exposed to bacteria that is more pathogenic to \textit{C. elegans}. We focused on HB and EF bacteria, as these pathogenic bacteria induce \textit{irg-7} expression ([41] and Fig 2D). We found that the \textit{irg-7 gof} mutation extended the survival of the animals exposed to HB or EF bacteria (Fig 3B and S3 Table). Furthermore, treatment with \textit{irg-7} RNAi during larval development significantly compromised the survival of animals exposed to HB bacteria during adulthood (Fig 3C and S3 Table). This implies that the \textit{irg-7} gene normally promotes the survival of wild-type animals exposed to these pathogenic bacteria. Nevertheless, treatment with \textit{irg-7} RNAi during larval development did not affect the survival of animals fed...
with OP50 during adulthood (Fig 3D and S3 Table). The lack of lifespan shortening by \( \text{irg-7} \) RNAi in animals fed with OP50 bacteria, as opposed to the curtailed survival of the \( \text{irg-7} \) RNAi-treated animals on the HB bacteria, supports the conclusion that the diminished survival is a result of pathogen sensitivity rather than an aging-related phenotype. These results, together with a consideration of its structural molecular domains, implicate \( \text{irg-7} \) in pathogen resistance.

**\( \text{irg-7} \) gof activates the immune response**

In principle, the IRG-7 protein could enhance pathogen resistance by directly neutralizing pathogenic bacteria (for example via its specialized CTLD domain which can directly bind to
sugars encoating bacteria and perforate their cell membrane [42] or cause their spatial segregation of microbiota and the host intestine [43]). Alternatively, it may do so indirectly, by activating the animals’ immune response. One of the central signaling cascades of the innate immune response in *C. elegans* is the PMK-1/ATF-7 pathway [20, 21]. Thus, we examined whether the irg-7 gof mutation recruited this innate immunity pathway to the defense and well-being of the organism. To this end, we followed the expression of a GFP reporter driven by the promoter of *T24B8.5*, an ATF-7 target gene [20]. We found that the levels of the ATF-7 reporter increased in irg-7 gof mutants compared to animals with wild-type irg-7 (Mann-Whitney, *P* < 0.0001, Fig 4A). The level of the ATF-7 reporter in irg-7 gof mutants were the same as in animals exposed to EF (Mann-Whitney, *P* = 0.98) and PA14 pathogens (Mann-Whitney, *P* = 1.00) (Fig 4A). Interestingly, exposure to HB bacteria did not increase the expression of this ATF-7 reporter (Mann-Whitney, *P* = 1.00) (Fig 4A). These findings are consistent with the interpretation that the PMK-1/ATF-7 innate immunity pathway is activated in irg-7 gof mutants.
Next, we examined whether the increased expression of the ATF-7 reporter simply correlated with the \textit{irg-7} (zc6) mutation or whether it is important for the longevity of \textit{irg-7} \textit{gof} mutants. In support of the latter, we found that the longevity of \textit{irg-7} \textit{gof} mutants on live OP50 was completely dependent upon the integrity of the innate immunity PMK-1/ATF-7 pathway (Mantel-Cox \textit{P}>0.05 in six independent experiments---\textit{Fig 4B, and S4 Table}). Similarly, the improved survival of \textit{irg-7} \textit{gof} mutants on the pathogenic HB bacteria was also dependent upon the PMK-1/ATF-7 innate immunity pathway (Mantel-Cox \textit{P}>0.05 in three independent experiments) (\textit{Fig 4C and S4 Table}). Altogether, these findings support the conclusion that \textit{irg-7} activation acts as an immune-modulator, which engages the nematode’s innate immune response and improves the ability of animals to cope with the pathogenic food.

The ER stress response is constitutively active in \textit{irg-7} \textit{gof} mutants because of their immune response

One consequence of activation of the innate immune response is an increased load on the ER, presumably due to massive production of secreted antibacterial proteins [44]. Since the ER stress response is constitutively activated in the intestine of \textit{irg-7} \textit{gof} mutants (\textit{Fig 1A}, [37]), we wondered whether this stress response may be due to the activity of the PMK-1/ATF-7 innate immunity pathway in these mutants. Consistent with this possibility, inactivation of \textit{pmk-1} or \textit{atf-7} reduced the levels of the \textit{Phsp-4::gfp} ER-stress reporter in the intestine of the \textit{irg-7} \textit{gof} animals (Student’s T-test \textit{P}<0.002, \textit{Fig 4D}). Thus, the PMK-1/ATF-7 innate immunity pathway appears to contribute to the induction of the UPR in \textit{irg-7} \textit{gof} mutants.

\textit{irg-7} is required for lifespan extension upon germline removal

Many longevity pathways confer increased pathogen resistance [6, 27–30]. Hence we wondered whether the \textit{irg-7} gene is an integral part of any of the known longevity pathways. To this end, we compared the lifespan of wild-type animals and various long-lived mutants upon treatment with control or \textit{irg-7} RNAi. Using this approach, we found that \textit{irg-7} RNAi suppressed the extended lifespan of \textit{glp-1} mutants which lack a germline (Mantel-Cox \textit{P}<0.0001 in 3 independent experiments, \textit{Fig 5 and S5 Table}), although it had no effect on the lifespan of wild-type animals (Mantel-Cox \textit{P}>0.2 in 4 independent experiments, \textit{Fig 5 and S5 Table}). \textit{irg-7} RNAi did not affect the lifespan of animals that are long-lived due to reduced insulin/IGF1 signaling (i.e. \textit{daf-2} mutants), due to reduced mitochondrial respiration (\textit{clk-1} mutants) or due to a mutation in the \textit{eat-2} gene (a mutation in an acetyl-choline receptor that causes reduced pharyngeal pumping and extended longevity) (Mantel-Cox \textit{P}>0.2 in 3 independent experiments for each long-lived strain, \textit{Fig 5 and S5 Table}). The observation that \textit{irg-7} inactivation had only mild effects on wild-type lifespan, but almost completely prevented germline loss from extending lifespan, suggests that \textit{irg-7} plays a regulatory role specifically in this pathway.

\textit{irg-7} transcription is increased upon germ cell removal

After implicating \textit{irg-7} in the germline longevity pathway, we wondered whether its expression is regulated by this pathway. To this end, we examined whether the expression levels or expression pattern of \textit{irg-7} changes upon germ cell removal. Specifically, we compared the expression of the \textit{irg-7} transcription reporter in animals with a normal reproductive system and in animals with no germ cells. In both cases, the reporter driven by the \textit{irg-7} promoter was expressed specifically in the posterior intestinal cells, regardless of the presence or absence of the germ cells. However, the fluorescence levels of the transcriptional reporter increased upon germ cell removal (Student’s T-test \textit{P}<0.005, \textit{Fig 6A}). This indicates that \textit{irg-7} is associated with the germline longevity pathway in at least two ways. First, it is required for the longevity of
germlineless animals. Second, its transcription is regulated by the reproductive tissues. Interestingly, a similar increase in the levels of the transcriptional reporter was also observed in irg-7(zc6) mutants, suggesting that irg-7 activation indirectly promotes its own transcription as part of an auto-regulatory loop (Student’s T-test P < 0.005, Fig 6A).

**irg-7 is required for activation of parts of the transcriptional program in germlineless animals**

Since the germline longevity pathway engages many transcription factors, and irg-7 is a new factor in the germline longevity pathway, we examined whether the transcriptional network that promotes longevity in germlineless animals is affected by irg-7 depletion. To this end, we compared the induction of a set of reporters known to be induced by different transcription factors upon germ cell removal. We found that the fat-6 and lgg-1 reporters, target genes of the NHR-80 and PHA-4 transcription factors respectively [10, 15], were induced upon germ cell removal regardless of treatment with irg-7 RNAi (Fig 6B). In contrast, irg-7 inactivation compromised the induction of the reporters of sod-3 (a target gene of DAF-16) [45] and cdr-6 (a target gene of DAF-16 and DAF-12 [46, 47]) upon germ cell removal (Fig 6B).

Since irg-7 inactivation interfered with the induction of two daf-16 target genes in germlineless animals we hypothesized that irg-7 might be required for the activation of this transcription factor upon germ cell removal. A critical step in the activation of DAF-16 upon germ cell

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**Fig 5. irg-7 is part of the germline longevity pathway.** F40F4.6 RNAi significantly shortened the lifespan of glp-1 germlineless animals, but did not shorten the long lifespan of insulin/IGF1 signaling mutants (daf-2), dietary restricted mutants (eat-2), mutants in mitochondrial respiration mutants (clk-1) or wild-type animals. Mean lifespans and Mantel-Cox P values are indicated within each graph. Mantel Cox P-values for control RNAi vs. irg-7 RNAi-treated wild-type animals are in gray. Mantel Cox P-values for control RNAi vs. irg-7 RNAI-treated long-lived mutants are in blue. See S5 Table for more lifespan data.

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removal is its nuclear translocation in the intestine cells [45]. Thus, we used animals expressing fluorescently tagged DAF-16 to explore whether irg-7 was required for this step. We could not detect translocation of DAF-16::GFP to the nuclei of the intestine cells upon germ cell removal in animals treated with irg-7 RNAi, (Fig 6C).

The translocation of DAF-16 from the cytoplasm to the nucleus can be triggered by a variety of signals [48]. Hence, we wondered whether irg-7 inactivation globally abrogates DAF-16’s ability to translocate from the cytoplasm to the nucleus, or is it only impaired in response to a subset of signals (as in the case of germline depletion). To this end, we followed the affect of irg-7 RNAi on DAF-16’s intra-cellular localization in animals with reduced insulin/IGF1 signaling. We confirmed that when insulin signaling is perturbed, DAF-16::GFP translocates to the nuclei of all cells. However, in contrast to its interference with DAF-16::GFP’s translocation to the nuclei in germlineless animals, irg-7 RNAi did not affect the accumulation of DAF-16::GFP in the nuclei of insulin/IGF-1 signaling mutants (Fig 6C). This indicates that irg-7 is specifically required for the nuclear translocation of DAF-16 upon germ cell removal.

Inactivation of irg-7 also suppressed the induction of the cdr-6 reporter (Fig 6B). The transcription of the cdr-6 reporter is independently and additively induced upon germ cell removal by either the DAF-16 or the DAF-12 transcription factors [47]. In order to uncouple between
the two, we examined the expression of the *cdr-6* reporter in *daf-16*-deficient animals, which can still induce *cdr-6* expression upon germ cell removal in a *daf-12*-dependent manner. We found that *irg-7* RNAi suppressed the induction of the *cdr-6* reporter upon germ cell removal in this background as well (Fig 6B). This indicates that *irg-7* is an important regulator of the germline longevity pathway, which alters the transcriptional outputs of the DAF-16 and DAF-12 transcription factors upon germ cell removal.

**The longevity of *irg-7* gof mutants and longevity induced by germ cell removal depend on many common factors**

After establishing that *irg-7* gof mutants are long-lived, we explored which known lifespan-related genes are required for their longevity. Specifically, we used RNAi or deletion mutants to inactivate the expression of known longevity genes and then examined the ability of the *irg-7* mutants to live long. We found that the increased lifespan of *irg-7* gof mutants required most of the genes previously associated with the reproductive longevity pathway (Fig 7A and S6 Table). Some of these genes are uniquely associated with the reproductive longevity pathway (*tcr-1* [49], *kri-1* [50], *daf-9, daf-12* [5]). Others are common to the reproductive longevity pathway and other longevity pathways (e.g. *skn-1* [17, 51, 52], *daf-16* [5, 45, 53, 54], *hsf-1* [2, 55], *aak-2* [56] and *pha-4* [10, 57] which are also a part of the dietary restriction and/or insulin/IGF1 signaling longevity pathways). Silencing of additional genes, required for insulin/IGF-1-induced longevity but not required for the longevity of germline-less animals (*phi-3, skr-1* [13]), did not shorten the longevity of *irg-7* mutants by more than 5% (Fig 7A and S6 Table). Likewise, silencing of genes required uniquely for the mitochondrial longevity pathway (*cdr-2*, *ubl-5* [58, 59]), and/or the caloric-restriction longevity pathway (*wwp-1* [60]), did not shorten the longevity of *irg-7* gof mutants by more than 5% (Fig 7A and S6 Table). Thus, *irg-7*-mediated longevity appears to be related to the longevity mediated by germ cell removal, as the two rely on many common genes.

Lifespan extension by germ cell removal requires the presence of the somatic gonad [5, 47]. Thus, we examined whether lifespan extension by the *irg-7* gof mutation requires the somatic gonad as well. We found that instead of extending lifespan, the *irg-7* gof mutation shortened lifespan in the absence of a somatic gonad (Fig 7B and S6 Table). Thus, *irg-7* activation, similarly to germ cell removal, relies on the somatic gonad to promote longevity.

Finally, we examined the effect of the *irg-7*(*gof*) mutation on the longevity of animals that lack germ cells but have a somatic gonad. We found that the *irg-7* gof mutation did not increase the lifespan of germlineless animals (Fig 7C and S6 Table). This further supports the notion that the *irg-7* activation and germ cell removal increase lifespan by a common mechanism, which relies on many common genes and on the somatic gonad.

**irg-7** gof partially phenocopies the transcriptional reprogramming associated with germ cell removal

Since many transcription factors are required for the longevity of germlineless animals as well as for the longevity of *irg-7*(zc6) gof mutants (Fig 7A), we asked whether the *irg-7* gof mutation activates these transcription factors. Specifically, we focused on the DAF-16 and DAF-12 transcription factors, as *irg-7* was required for their activity in germlineless animals (Fig 6B and 6C).

First, we followed the levels of a *cdr-6* transcriptional reporter, which is transcribed both by DAF-16 and by DAF-12. We found that the *irg-7* gof mutation increased the level of the reporter in animals with an intact reproductive system by 1.5 fold (Student’s T-test *P*<0.0001, Fig 8A). This increase was dependent on the *daf-12* transcription factor, as it was not observed.
in its absence (Student’s T-test P = 0.075, Fig 8B). Thus, irg-7 gof activates the longevity-associated transcription factor DAF-12.

Next, we followed the levels of a sod-3 transcriptional reporter, which is a direct target gene of the DAF-16 transcription factor. Surprisingly, we did not detect an increase in the level of the sod-3 reporter in irg-7 gof mutants (Student’s T-test P = 0.067, Fig 8C). Likewise, we did not observe accumulation of DAF-16::GFP in the nuclei of irg-7(zc6) animals (Fig 8D). This could indicate that irg-7 gof is not sufficient to promote the nuclear translocation of the DAF-16 transcription factor to the nucleus, nor is it sufficient to significantly increase DAF-16 activity. Nevertheless, DAF-16 was required for the lifespan extension in irg-7 gof mutants (Fig 7A). Furthermore, DAF-16 was required for the improved survival of irg-7 gof mutants exposed to pathogenic HB bacteria (Fig 3E and S3 Table). These suggest that the basal activity
of DAF-16, which is below the sensitivity of this assay, but is not completely absent, is critical for the beneficial phenotypes of irg-7(zc6) gof mutants. Alternatively, it could be that the transcriptional targets of DAF-16 in animals with activated irg-7 are distinct from those examined here.

Germline homeostasis is modulated by HB bacteria and contributes to pathogen resistance

We found that irg-7 is implicated in pathogen resistance on one hand, and is an integral component of the reproductive longevity pathway on the other hand. Hence, we wondered whether these two processes are linked. Therefore, we asked whether germline physiology is modulated in the presence of pathogens. To this end, we examined whether germline homeostasis is modulated by the pathogenic HB bacteria. We found that exposure to HB bacteria increased germ cell apoptosis (Mann-Whitney P<0.0001, Fig 9A) and decreased the number
of mitotic germ cells (Mann-Whitney P<0.0001, Fig 9B) in the gonads of wild-type animals. To see if these changes in germline homeostasis contributed to the resistance of the animals to the pathogen, we decided to block some of these changes and to see if this increases the sensitivity of the animals to the pathogen. To this end, we used egl-1 mutants, which block germline apoptosis induced by Salmonella typhimurium [61]. As in the case of Salmonella typhimurium-induced germline apoptosis [61], a mutation in the egl-1 gene blocked HB bacteria-induced germline apoptosis (Mann-Whitney P = 1.00, Fig 9A) and increased the sensitivity of the animals to pathogen exposure (Mantel-Cox P<0.02 in 3 independent experiments, Fig 9C and S7 Table). These findings raise the possibility that the germline may serve as a tissue-level pathogen sensor whose depletion can promote animals’ survival in the presence of pathogens.

**irg-7** activation does not affect germline homeostasis when animals are fed with OP50 bacteria

Exposure to pathogenic bacteria increases the expression of the irg-7 gene and alters germline homeostasis. Hence, we examined whether these events occur consecutively such that pathogen exposure leads to activation of irg-7, which in turn affects germline homeostasis. To this end, we examined whether activation of *irg-7* is sufficient to affect germline homeostasis. *irg-7* activation was achieved by introducing the *irg-7 gof* mutation or by over-expressing multiple copies of a fosmid that includes the wild-type *irg-7* gene in animals fed with OP50 bacteria. We

Fig 9. Sensitivity of germline homeostasis to pathogenic HB bacteria improves the animals’ survival.

(A) Treatment with pathogenic HB bacteria induced germline apoptosis in an egl-1 dependent manner. Asterisks mark Mann-Whitney P values of P<0.001 of HB-treated animals (gray) compared to OP50 treated animals (black) of the same genotype. 45–50 animals were analyzed per genotype. Error bars represent SD.

(B) Amount of mitotic germ cells was determined in wild-type animals and in animals with activated *irg-7* upon exposure to OP50 (black) or HB bacteria (gray). Asterisks mark Mann-Whitney values of P<0.001 of HB-treated animals (gray) compared to OP50 treated animals (black) of the same genotype. Additional Mann-Whitney P values are indicated in the graph. Note that treatment with pathogenic HB bacteria reduced the amount of mitotic germ cells in the gonad of wild-type animals but not in animals with activated *irg-7*. Also note that activation of *irg-7* did not reduce, and even increased, the amount of mitotic germ cells in the animals. 25–30 DAPI-stained gonads were analyzed per genotype. Error bars represent SE of 3 independent biological replicates. (C) In the absence of pathogen-induced germline apoptosis, animals are more sensitive to the pathogenic bacteria. Mean survival and Mantel-Cox P-values are indicated within the graph. See S7 Table for additional survival data.

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found that the levels of germ cell apoptosis and the amount of mitotic germline in these animals were similar to those of wild-type animals (Mann-Whitney P > 0.3, Fig 9A and 9B). This indicates that under these conditions, germline homeostasis is not regulated by irg-7 activation. This once again indicates that irg-7 does not mediate its beneficial effects on OP50-fed animals by acting upstream to the germline. Rather, since irg-7 expression is induced upon germ cell removal, it should be placed downstream to the perturbations in germline homeostasis.

**irg-7 activation protects germline homeostasis upon exposure to HB bacteria**

In addition to the beneficial lifespan affects conferred by irg-7 activation in animals fed with OP50 bacteria, irg-7 activation also improves the survival of animals on pathogenic bacteria. Hence, we decided to examine whether under these conditions (upon exposure to pathogenic bacteria) irg-7 activation leads to perturbations in germline homeostasis. We found that that even in the presence of HB bacteria, no increase in germline apoptosis or reduction in the amount of mitotic germ cells was observed in animals with activated irg-7. Unexpectedly, the mitotic germline of animals with activated irg-7 exposed to HB bacteria was even more expanded than prior to their exposure to pathogenic bacteria (Mann-Whitney P < 0.0001, Fig 9B). Likewise, barely any apoptotic germ cells were detected in the gonads of animals with activated irg-7 upon treatment with HB bacteria (Fig 9A). Whereas the reasons for the robustness of germline homeostasis in irg-7 mutants is not known, an appealing speculation is that irg-7 activation successfully recruited the innate immune response to protect the animals from the pathogenic food and its toxic interaction with the animals' germline.

**Discussion**

Previous studies have demonstrated that signals from reproductive tissues influence longevity, yet only a fraction of the underlying genetic network that controls this process has been elucidated. In this study, we identified irg-7, as an integral downstream component of the germline longevity pathway.

irg-7 encodes an intestinally-produced secreted protein, harboring a single CTLD domain (typically associated with proteins involved in innate immunity anti-microbial activity) and several EGF domains. Interestingly, removal of one of these EGF domains activates the protein, assigning it as an auto-inhibitory domain. This auto-inhibition of IRG-7 is reminiscent of toxic mammalian CTLD-harboring proteins, which contain intramolecular inhibitory domains that maintain them in an inert inactive state, which can be switched into an active state by proteolytic removal of the inhibitory segment [42, 62, 63].

Our initial interest in irg-7(zc6) mutants was because of the constitutively high expression of the hsp-4 ER resident chaperone in their intestine. At first, it was unclear whether these animals induce ER chaperone expression due to deregulation of gene expression or as a reflection of ER stress and activation of the UPR. Our findings indicate that the hyperactivation of the ER stress response in these animals is a reflection and a consequence of their activated innate immune response. The UPR-mediated coordination between the secretory capacity and secretory load is critical in animals with an activated immune response. In the absence of such coordination, activation of the innate immune response is detrimental [44]. Consistent with this, complete depletion of the UPR genes ire-1 and/or xbp-1 using deletion mutants was lethal for irg-7 gof mutants, whose innate immune response is activated. Nevertheless, irg-7 gof mutants survived ire-1 and/or xbp-1 RNAi treatment. Furthermore, these RNAi treatments did not compromise their longevity. This dichotomy may indicate that a low level of UPR activity is sufficient to provide the basic adaptation required by the over-loaded ER in animals with an
activated innate immune response. This residual UPR activity may reflect low levels of UPR activity throughout the animal. Alternatively, it may reflect UPR activity in specific tissues, most likely in neurons, which are relatively resistant to RNAi treatment. This mechanism is consistent with previous findings that neuronal signaling can modulate the innate immune response in the C. elegans intestine [64, 65].

We consider irg-7 as an integral downstream component of the germline longevity pathway because its expression is increased upon germ cell depletion and because it is essential for the longevity of germineless animals. In germineless animals, depletion of irg-7 interferes with the activation of the longevity-promoting transcription factors DAF-16 and DAF-12. At the same time, irg-7 deficiency does not affect germ cell regulated activity of the transcription factors NHR-80 and PHA-4. Thus, at least two independent signaling pathways control the transcriptional network that is set upon germ cell depletion; only one of which implicates irg-7, which acts upstream or in parallel to daf-16 and daf-12. These perturbations are enough to preclude lifespan extension by germ cell depletion.

Although inactivation of irg-7 does not shorten the lifespan of animals with an intact reproductive system, its' activation extends the lifespan of the animals. The lifespan extension induced by irg-7 activation requires the presence of the somatic gonad, similarly to the longevity conferred by germ cell removal. In addition, it shares many genetic determinants with the reproductive longevity pathway. Although there is an extensive overlap between genes required for the lifespan extension induced by irg-7 activation and those required for longevity induced by germ cell depletion, the lifespan extension induced by irg-7 activation does not perturb germ-line homeostasis under normal growth conditions. Thus, the lifespan extension by irg-7 activation is independent of germinele depletion, yet requires many of the same longevity genes as in germineless animals.

How does irg-7 activation extend lifespan? Many CTLD proteins, which are abundant in the nematode genome (~280 genes), show a pathogen-specific induction during infection and are thought to be important for enhancing the nematode’s ability to fight off pathogens [66, 67]. Furthermore, the expression of several CTLD proteins is regulated by innate immunity pathways [6, 33, 67, 68]. Our data implicates irg-7 in regulating the animals’ sensitivity to pathogens in several ways. First, although the lifespan of wild-type animals is limited by its pathogenic food, in irg-7(zc6) mutants, lifespan was unaltered whether the animals were fed with live or with dead OP50 bacteria. Second, irg-7 activation promotes the survival of animals on pathogenic HB bacteria, whereas its inactivation renders wild-type animals more sensitive to the same pathogen. Third, irg-7 activates and engages the pmk-1/atf-7 innate immune response pathway to the defense and well-being of the animals and requires this innate immunity pathway for lifespan extension. Altogether, these imply that irg-7-associated life extension is tightly associated with its enhanced ability to defend the animals from the pathogenic toxicity of its food.

Since irg-7 is induced in germineless animals, and since its induction can lead to activation of the PMK-1/ATF-7 innate immunity pathway, our findings suggest that the canonical PMK-1/ATF-7 pathway may also be activated in germineless animals. Consistent with this, an increase of 1.3 fold in the transcript levels of several atf-7 target genes has been observed between germineless animals and animals with an intact reproductive system [17]. However, the transcript levels of the same target genes were reported unaltered upon germ cell removal in a previous study [6]. This dichotomy may be due to different normalization approaches taken by the two studies. Whereas both studies normalized transcript levels to a major housekeeping gene, the first study also took into account the differential number of cells composing animals with an intact gonad and those that lack a germline, whereas the second study did not. Our finding of increased expression of the ATF-7 target gene T24B8.5, obtained by a transcriptional reporter (and thus insensitive to the presence/absence of the germline in terms of normalization) yielded
a result consistent with the analysis of Steinbaugh et. al. [17], and suggests that the PMK-1/ATF-7 pathway may also be activated in germlineless animals and may contribute to their immunity. Nevertheless, this pathway may be less critical for the survival of germlineless animals, which can enhance their immune response via alternative transcription factors [6].

In addition to the innate immunity genes *pmk-1* and *atf-7*, the lifespan extension of *irg-7* gof mutants fed with live OP50 depends on the presence of the somatic gonad as well as on a variety of genes required for longevity induced by germ cell removal. This implicates the same systemic endocrine pathways that increase *C. elegans* lifespan upon germ cell removal in promoting the resistance of animals to pathogenic challenges. This conclusion is consistent with the expression/activation of many of the genes implicated in the reproductive longevity pathway specifically in the intestine, the same site where the host usually encounters the pathogenic bacteria. Furthermore, in addition to their contribution to germ cell-regulated longevity, both the somatic gonad and the transcription factor DAF-16 have been implicated in the enhanced resistance of germlineless animals to pathogens [6, 69]. Our findings now implicate additional germline-regulated genes such as *tcer-1, kri-1, daf-12, skn-1 and pha-4* in this innate immune response pathway. Interestingly, a bile acid biosynthetic pathway has already been implicated in the systemic communication of cellular stress and activation of the MAP kinase innate immunity pathway by stressed germline [70].

Interestingly, one of the genes required for the longevity of germlineless animals did not affect the longevity of *irg-7* gof mutants (Fig 7A). This gene encodes *nhr-80*, an intestinal transcription factor that transcribes lipid homeostasis-related genes [15]. This differential requirement of *nhr-80* is intriguing and may suggest that *nhr-80* is specifically required for the well-being of germlineless animals rather than an integral part of the reproductive longevity pathway. Nevertheless, this differential requirement of *nhr-80* is important as it genetically rules out the possibility that the *irg-7* gene acts upstream to the germ cells themselves, promoting lifespan extension by limiting the amount of germ cells in the animals. This conclusion is also supported by the fact that in *irg-7* gof mutants, germline homeostasis and progeny profiles are similar between wild-type animals and *irg-7* gof mutants (S1 Fig and Fig 9).

With the exception of *nhr-80*, the implication of a significant amount of components of the reproductive longevity pathway in pathogen resistance and innate immunity is thought-provoking, as it raises the possibility that the reproductive system can be used as a signaling center to divert resources towards defending against putative pathogen attacks (See model in Fig 10). Accordingly, perturbations to germ cell homeostasis (executed via regulation of germline proliferation and/or germline apoptosis) may serve as a surveillance center, putatively disrupted by pathogens and their toxins. Such a mode of action is consistent with recent studies that indicate that pathogen surveillance can be achieved indirectly by monitoring internal physiological cues that may be altered by pathogens and their toxins [71–74]; effectively diluting the germline. Accordingly, stress-induced perturbation in germ cell homeostasis can trigger a somatic defense response, including an innate immune response [70, 75].

Consistent with this model, exposure to some pathogens (as previously demonstrated in the case of *Salmonella typhimurium* infection [61] and as shown here for HB luminescence infection) perturbs germ cell homeostasis. In turn, germ cell depletion can activate innate immunity pathways via the expression of the innate immunity-promoting gene *irg-7* (Fig 6A), which in turn can activate the PMK-1/ATF-7 innate immunity pathway (Fig 4A). Likewise, germ cell depletion can promote innate immunity pathways by other means (i.e activation of the transcription factors DAF-16 and SKN-1 in the animals intestine, which in turn induce the expression of a variety of innate immunity-related genes [6, 17].

Finally, in further support of this model, the ability to relay a signal of distress via perturbations in germline homeostasis appears to be important for mounting an effective immune
response, as animals are less likely to survive some infections when germline apoptosis is blocked [61 and Fig 9].

One of the hallmarks of aging both in nematodes and in humans, is a progressively increased sensitivity to external pathogens, reflecting a failure of the immune response in the old. At least in *C. elegans*, the decline in the ability to combat pathogens is detrimental to the animals and limits their lifespan [76, 77]. Accordingly, it is not surprising that the same manipulations in the reproductive system that promote longevity also affect the animals’ innate immune response and increase their resistance to pathogens [6, 29, 30]. However, it is unlikely that the lifespan extension induced by germ cell removal is only the reflection of improved innate immunity. This is because germ cell removal provides additional benefits for the soma and the organism in addition to improving the animals’ resistance to pathogens. For example, germ cell depletion maintains proteasome activity [9], prolongs the responsiveness of multiple stress response pathways with age [8, 35] and confers resistance to multiple cellular stresses [7, 78]. Accordingly, the increase in lifespan achieved by the *irg-7* gof mutation and by feeding the animals with dead bacteria are not as big as that produced by germ-cell loss. Furthermore, *irg-7* gof only partially phenocopies the transcriptional reprogramming that normally occurs upon germ cell removal. Thus, improved innate immunity is only one of several benefits of animals whose germ cells are depleted. Nevertheless, finding new ways to maintain the immune

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**Fig 10. The reproductive innate immunity pathway model.** Pathogens are one of the factors that limit lifespan in *C. elegans* and in higher organisms. Our data implies that exposure to pathogens and/or their toxins can directly or indirectly modulate germline proliferation and survival resulting in a reproductive system with less germ cells. Germline depletion induces the transcription of *irg-7*, an innate immunity-related secreted protein whose induction promotes the activation of a somatic defense response. On one hand, *irg-7* enables the activation of bona-fida longevity-associated transcription factors, which support the induction of a longevity-promoting transcriptome. At the same time, *irg-7* enables the activation of *atf-7*, a dedicated innate immunity-related transcription factor. In this way, exposure to pathogens can be sensed by perturbations in germline homeostasis. Consequently, the reproductive system can serve as a signaling center to divert key metabolic resources towards defending against the putative pathogen attack by activating the innate immune response.

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response in aging animals that undergo immunosensesnce is an important goal that may post-pone major lifespan limiting events. Although the immune response in mammals is primarily adaptive in its nature, a basal innate immune response, based on a variety of antiseptic proteins including CTLD proteins, similar to those of the nematode, contributes to the immunity of mammals as well. Thus, better understanding of the molecular events that mediate this basic immune response, in the young and in the old, is important and holds great promise for human health.

**Materials and methods**

**Progeny profile**

L4 stage N2 or irg-7(zc6) worms were incubated at 20˚C and transferred to fresh plates twice a day until they stopped producing progeny. Worms that crawled off the plates, bagged or ruptured were removed from the data set. All progeny plates were incubated at 20˚C for 2 additional days and the number of worms that developed was determined.

**Plasmids and transgenic animals**

The *irg-7* promoter sequence (690 bp) was amplified from genomic DNA and cloned into the HindIII and XbaI sites, replacing a *myo-3* promoter in the pCF191 plasmid (previously described in [79]). Germline transformations were performed by injection of 50ng/μl plasmid with 15 ng/μl of *Punc-54::gfp* as a co-transformation marker.

**Fluorescence microscopy and quantification**

To follow expression of fluorescent proteins, transgenic animals were anaesthetized on 2% agarose pads containing 2mM levamisol. Images were taken with a CCD digital camera using a Nikon 90i fluorescence microscope. For each trial, exposure time was calibrated to minimize the number of saturated pixels and was kept constant throughout the experiment. Nikon NIS element software was used to quantify mean fluorescence intensity in the selected area encompassing whole worms.

**Lifespans**

RNAi treatments were performed continuously from the time of hatching unless indicated otherwise. In general, eggs were placed on plates seeded with the RNAi bacteria of interest. Lifespan of 90 animals per strain were scored every 1–2 days. Related lifespans were performed concurrently to minimize variability. In all experiments, lifespan was scored as of the L4 stage which was set as t = 0. Animals that ruptured or crawled off the plates were included in the lifespan analysis as censored worms. For lifespan on dead bacteria, bacteria was killed by 30 minutes of boiling prior to seeding or by UV irradiation. The statistical program SPSS was used to determine the means and the P values calculated using the log-rank (Mantel-Cox) method.

**DAPI staining of dissected gonads**

For quantifying the amounts of mitotic germ cells, gonads of day-1 adults were dissected, fixed and stained with DAPI as previously described [80]. In short, day-1 worms were transferred to unseeded (without bacteria) plates and then placed in 20 μl PBSx1 with 0.2mM Levamisole for immobilization. Once the worms were paralyzed, animals were decapitated with a needle to allow the removal of the gonads. The dissected gonads were fixed with 10% formaldehyde for 30 minutes. The fixed gonads were then washed twice in M9 and stained with 1 μg/mL DAPI (4’,6-diamidino-2-phenylindole) solution for 20 minutes. Worms were washed two times with
PBSTx1 and observed under the fluorescent microscope. The boundary of the region of mitotic cells was defined by the most distal row of cells containing nuclei with crescent-shaped DAPI morphology. The amount of mitotic nuclei was scored in sequential focal planes through the width of the germline.

**SYTO12 staining**

The number of apoptotic cells in the gonads of day-2 animals was assessed by scoring the number of SYTO12 labeled cells in the gonad. SYTO12 (Molecular Probes) staining was performed as previously described [81]. In short, to obtain an estimate of the relative numbers of corpses in different genetic backgrounds, 2-day adult animals were stained with SYTO 12 (Molecular Probes, Eugene, OR), a vital dye that preferentially stains apoptotic germ cells. Animals were stained by incubating them in a 33 μM aqueous solution of SYTO 12 supplemented with OP50 for 4–5 hours at 25°C. Animals were transferred back to new seeded plates to allow stained bacteria to be purged from the gut. After 30–60 minutes, animals were mounted on agarose pads and inspected using a Nikon eclipse 90i, equipped with standard epifluorescence filters and Nomarski optics. Only animals that stained brightly were scored.

**Pathogen survival**

*E. faecalis* OG1RF strain (ATCC 47077) was grown at 37 degrees in brain heart infusion (BHI) medium supplemented with Gentamicin (25–50μg/ml). *Photorhabdus luminescens subsp. L* strain (ATCC 29999) was grown at 30 degrees for 48 hours in Nutrient Broth medium (BD cat 234000). *Pseudomonas aeruginosa* was the clinical isolate PA14 strain. Bacteria were seeded on nematode growth medium (NGM) plates seeded with bacteria and incubated overnight at room temperature, with the exception of the fast killing experiment by *E. faecalis* where the bacteria was seeded on brain heart infusion (BHI) agar plates. Survival assays were initiated with 80–90 nematodes in the late L4/early day 1 stage, grown from eggs on plates seeded with OP50 bacteria or RNAi bacteria of interest. Survival was scored daily thereafter. The SPSS program was used to determine the means and the P values. P values were calculated using the log-rank (Mantel-Cox) method.

**Statistical analysis**

Error bars represent the standard error of the mean (SEM) of independent biological replicates unless indicated otherwise.

For a simple comparison between two data sets, P values were determined using unpaired Student’s *T*-test, assuming unequal variances.

For multiple comparisons, between multiple data sets, samples were analyzed by the Kruskal-Wallis method, followed by a post hoc analysis using the Mann-Whitney method with Bonferroni correction for multiple comparisons. The analysis was performed in R.

To compare pairs of lifespan and pathogen survival analysis (i.e a total of two genotypes), the Kaplan-Meier method was used to estimate survival as a function of time, and survival differences were analyzed by the Mantel-Cox log-rank test using the SPSS program.

To compare survival differences between two pairs of genotypes (i.e. a total set of four genotypes), Cox regression analysis of several genotype factors was performed to compare hazard ratios between genotypes. Contrasts were used to determine whether the (log) hazard ratio was significantly different under different genotypes. All performed regressions were corrected using the Bonferroni correction for multiple tests. The analysis was performed in R. This analysis is limited to samples with a constant proportional hazard ratio over time.
Strains, transgenic lines and fosmids
Information provided in S8 Table.

Supporting information

S1 Fig. irg-7(zc6) mutants have an overall normal physiology. (A) Average progeny numbers of wild-type animals and irg-7(zc6) mutants were assessed twice a day from early adulthood. No significant difference in the progeny profile was observed (P = 0.11). Plot averages 5 independent experiments. (B) Development assay monitoring the developmental rate of wild-type animals (black bars) and irg-7(zc6) mutants (grey bars) on OP50 at 20 degrees. Eggs from each genotype were placed on plates. After 48 hours, and every 2 hours intervals thereafter, worms that have reached or passed the L4 stage were scored. Error bars reflect SE of 3 experiments. No less than 90 animals were scored per strain. On average, wild-type animals reached the L4 stage after 48.9 hours (SD = 1.6) whereas irg-7(zc6) mutants reached the L4 stage on average after 53.0 hours (SD = 2.5). (C) Average pumping rate of wild-type animals, irg-7(zc6) and eat-2 mutants (known to have a reduced pumping rate) assessed on day 2 of adulthood. No significant difference occurs in the pumping rate between wild-type animals and irg-7 mutants (Student’s t-test values of P = 0.22). 20 animals were analyzed per genotype. Similar results were observed in an additional independent experiment.

(TIF)

S2 Fig. Sequences of the irg-7(zc6) mutation. Genomic DNA and cDNA from wild-type animals and irg-7(zc6) mutants was used as a template for a PCR reaction that encompasses the putative deleted area. The PCR products were then sequenced. Nucleotides present in the wild-type sequence, but not in the mutated sequence are marked in red. Likewise, an insertion of two nucleotides present in the mutant sequence, but not in the wild-type sequence is marked in red. Nucleotides flanking the deletion site are marked in yellow.

(DOCX)

S1 Table. Lifespan analysis of irg-7(zc6) mutants upon inactivation of ER-stress associated genes.
(XLSX)

S2 Table. Lifespan effects of f40f4.6 inactivation or overexpression on lifespan.
(XLSX)

S3 Table. irg-7 contributes to lifespan extension by improving pathogen resistance.
(XLSX)

S4 Table. irg-7 improves pathogen resistance via the PMK-1/ATF-7 innate immunity pathway.
(XLSX)

S5 Table. Effect of irg-7 inactivation using f40f4.6 RNAi on the lifespan of longevity mutants.
(XLSX)

S6 Table. Requirement of known longevity genes and reproductive tissues for lifespan extension by the irg-7(zc6) mutation.
(XLSX)

S7 Table. Germline apoptosis promotes pathogen resistance.
(XLSX)
S8 Table. Strains, transgenic lines and fosmids.

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