Prostaglandin signals from adult germline stem cells delay somatic ageing of *Caenorhabditis elegans*

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A moderate reduction in body temperature can induce a remarkable lifespan extension. Here we examine the link between cold temperature, germline fitness and organismal longevity. We show that low temperature reduces age-associated exhaustion of germline stem cells (GSCs) in *Caenorhabditis elegans*, a process modulated by thermosensory neurons. Notably, robust self-renewal of adult GSCs delays reproductive ageing and is required for extended lifespan at cold temperatures (10 °C, 15 °C). These cells release prostaglandin E2 (PGE2) to induce *cbs-1* expression in the intestine, increasing the somatic production of hydrogen sulfide, a gaseous signalling molecule that prolongs lifespan. Loss of adult GSCs reduces intestinal *cbs-1* expression and cold-induced longevity, whereas application of exogenous PGE2 rescues these phenotypes. Importantly, tissue-specific intestinal overexpression of *cbs-1* mimics cold-temperature conditions and extends longevity even at warm temperatures (25 °C). Thus, our results indicate that GSCs communicate with somatic tissues to coordinate extended reproductive capacity with longevity.

The ageing process is modulated by environmental and genetic factors. Although extreme cold is detrimental, a moderate reduction in body temperature induces a remarkable lifespan extension. As such, lowering body temperature is one of the most effective interventions to extend organismal lifespan. This phenomenon was first observed in poikilotherms, including *Caenorhabditis elegans*. For instance, *C. elegans* has a shorter lifespan when shifted from the standard culturing temperature (20 °C) to a warmer temperature (25 °C), whereas lower temperatures (for example, 10 °C or 15 °C) extend the lifespan. In mouse models, a 0.5–0.6 °C reduction in core body temperature induces a 20% lifespan extension. Although the effects of lowering core body temperature on longevity were originally reported more than a century ago, little is known about the mechanisms that underlie this process. Since temperature essentially influences every chemical and biological process, longevity was conventionally considered to ensue from a passive thermodynamic process. According to this view, cold temperature reduces the rate of chemical reactions and metabolism, resulting in slower molecular entropy, energy expenditure and pace of living. However, recent work in *C. elegans* reported that the cold-sensitive TRPA-1 channel detects low temperature during adulthood, leading to a calcium ion influx that promotes lifespan extension. Conversely, loss of the TRPA-1 channel shortens lifespan at cold temperatures, but does not affect lifespan at warm temperatures. Further studies identified that TRPA-1 acts in IL1 neurons, inducing neuroendocrine signalling circuits to extend longevity at cold temperatures. In addition, the co-chaperone *daf-41/p23* is also necessary for lifespan extension at low temperatures. Thus, cold-induced longevity is a regulated process that cannot be simply explained by passive changes in chemical reactions.

As with temperature, fecundity is also negatively correlated with lifespan extension. Although extreme cold is detrimental, a moderate reduction in body temperature induces a remarkable lifespan extension. Since tissue homoeostasis and physiological integrity are constantly challenged by ever-changing metabolic, pathological and environmental conditions, evolutionary pressure has been theorized to force a reallocation of energetic resources to prevent and repair damage to the germline. By this compromised distribution, the organism will ensure its reproduction, generating healthy and fit progeny. Along these lines, extensive evidence in *C. elegans* indicates that the germline promotes somatic ageing under standard and stress conditions. As such, germline-lacking worms can live up to 60% longer than those with germlines and exhibit increased resistance to a variety of environmental stressors, including high temperatures. Under standard (20 °C) and warm temperatures, the germline is responsible for the generation of signals that promote progressive deterioration of the soma and organismal ageing. Conversely, removal of the germline activates pro-longevity transcriptional factors in somatic tissues, resulting in lifespan extension. Eventually, these transcription factors modulate downstream processes within somatic cells, such as increased autophagy or proteome stability, that contribute more directly to the longevity phenotype. Thus, these findings establish proliferating germ cells as active inhibitors of pro-longevity pathways in somatic tissues, reducing organismal lifespan.

While the impact of the germline on lifespan at standard and warm temperatures has been extensively studied, here we examine the link between low temperature, germline fitness and organismal longevity. Remarkably, we find that cold temperature does not extend the lifespan of germline-lacking worms. Since germline-lacking worms live significantly longer at lower temperatures than wild-type worms, our results indicate that the germline is required for cold-induced longevity. Prompted by these results, we assess whether low temperature affects germline ageing. Indeed, low...
temperature ameliorates exhaustion of the germline stem cell (GSC) pool during adulthood, resulting in a delay in reproductive ageing. This process is regulated by thermosensory neurons, which detect low temperatures to maintain GSC self-renewal with age. Notably, cold-induced longevity is blocked by chemical and genetic interventions that specifically diminish adult GSC proliferation. By contrast, these interventions do not affect lifespan at higher temperatures. Robust proliferation of adult GSCs induces the expression of CBS-1 in somatic tissues such as the intestine, increasing the production of hydrogen sulfide (H₂S), a gaseous signalling molecule that extends lifespan²⁻⁴. Importantly, overexpression of CBS-1 in the intestine extends lifespan even at warm temperatures. In addition, we find that low temperature increases the production and release of prostaglandin E2 (PGE2), a hormone that modulates somatic tissues and promotes cold-induced longevity, by GSCs. Whereas loss of adult GSCs reduces somatic CBS-1 expression and cold-induced longevity, application of exogenous PGE2 rescues these phenotypes. Taken together, our results indicate that adult GSCs communicate with somatic tissues via prostaglandin signals to extend longevity at cold temperatures. This process coordinates extended reproductive capacity and long lifespan, without compromising either the germline or the soma.

Results

The germline is essential for cold-induced longevity during adulthood. With the strong lifespan extension induced by lowering body temperature, we investigated what role, if any, germ cells have in this phenotype. For this purpose, we first examined glp-1(e2141) mutant worms, which develop into adults lacking a germline due to a failure in germ-cell proliferation during development at the restrictive temperature (25 °C)⁶. We therefore raised glp-1(e2141) mutant larvae at 25 °C to obtain germline-lacking adults and shifted them to distinct temperatures. As reported previously⁶⁻⁸, glp-1 germline-lacking worms were long-lived at 20 °C compared with wild-type animals under the same conditions (Fig. 1a). By contrast, we found that glp-1 mutants lived significantly shorter than wild-type worms at lower temperatures (that is, 15°C, 10°C) (Fig. 1a). Although our results indicate that the pro-longevity effects of low temperature are diminished in glp-1 germline-lacking mutants (Fig. 1b), the fact that these worms are long-lived at 20°C makes our results difficult to interpret. To further assess the effect of germline depletion in cold-induced longevity, we used glp-4(bm2) mutants, a distinct germline-lacking strain that has no lifespan increase relative to wild-type worms at 20°C (refs. ⁹,¹¹) (Fig. 1c). Notably, low temperature (15°C) did not extend the lifespan of glp-4 germline-lacking worms, as we observed in glp-1 mutants (Fig. 1b,c). To determine whether this is a consequence of sterility per se, we assessed fer-15(b26);fem-1(hc17) worms, which are also sterile but which still contain a proliferating germline when raised at the restrictive temperature (25°C) during development (Supplementary Fig. 1). We found that low temperature during adulthood increased the lifespan of the control sterile strain but did not extend the lifespan of the glp-1 germline-lacking mutants (Supplementary Fig. 2). As a result, sterile control worms lived longer than glp-1 mutants at low temperature (Supplementary Fig. 2). Similar to fer-15;fem-1 worms, low temperature also extended the lifespan of distinct sterile mutants that have spermatogenesis defects but that contain a proliferating germline and generate oocytes, such as fog-1, fog-2 and spe-26 mutant worms¹²⁻¹⁸ (Fig. 1d,e). Thus, our results indicate that loss of cold-induced longevity is not due to sterility, but is specific to depletion of the germline.

In all of the above experiments, we raised both mutant and wild-type worms at the same temperature until they reached adulthood and then shifted them to distinct temperatures to assess the adult lifespan. Thus, our results suggest that the germline is particularly important during adulthood for the cold-induced longevity phenotype. In support of this hypothesis, exposure of wild-type C. elegans to low temperature only during adulthood was sufficient to prolong lifespan after development at either 25°C or 20°C (ref. ¹⁹) (Fig. 1f,g).

Cold temperature delays exhaustion of GSCs and reproductive ageing. Prompted by our results in germline-lacking worms, we hypothesized that temperature-associated changes in the adult germline determine cold-induced longevity. In adult C. elegans, the germline is composed of a mitotic zone at the distal end followed by regions containing cells from early meiotic stages to maturing gametes, extending proximally⁰ (Fig. 2a,b). Whereas adult germ cells are continuously lost during gametogenesis and cell death events, proliferating GSCs of the mitotic region self-renew and provide new pools of cells that enter meiosis to replenish the germline and generate gametes⁰. To determine the impact of temperature and age in adult germline proliferation, we raised wild-type larvae at 20°C until adulthood and then shifted them to different temperatures. Notably, the total number of germ cells within the mitotic region did not change with age or temperature (Fig. 2c and Supplementary Fig. 3). By contrast, low temperature delayed the age-associated decline in the percentage of mitotic cells, which were labelled with 5-bromodeoxyuridine (BrdU) (Fig. 2c,d). In addition, we observed active proliferation of germ cells for an extended fraction of the

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**Fig. 1** The germline is essential for cold-induced longevity during adulthood. a. Germline-lacking worms (glp-1(e2141)) are long-lived compared with the wild-type (N2) strain at 20°C (N2 20°C mean ± s.e.m., in days: 2015 ± 0.40, glp-1 20°C: 2690 ± 0.58, P < 0.0001). By contrast, germline-lacking worms are short-lived in comparison with wild-type worms at cold temperatures (N2 15°C: 29.01 ± 0.58, glp-1 15°C: 24.39 ± 0.56, P < 0.0001; N2 10°C: 42.94 ± 1.05, glp-1 10°C: 33.95 ± 0.46, P < 0.0001). b. Temperature reduction (15°C) extends the lifespan of wild-type worms (N2 20°C mean ± s.e.m.: 16.40 ± 0.60, N2 15°C: 26.73 ± 0.77, P < 0.0001) but not glp-1 germline-lacking worms (glp-1 20°C: 21.59 ± 0.66, glp-1 15°C: 21.78 ± 0.60, P = 0.0396). Wild-type worms are long-lived compared with glp-1 mutant worms at cold temperature (N2 15°C versus glp-1 15°C, P < 0.0001). c. Temperature reduction extends the lifespan of wild-type worms (N2 20°C mean ± s.e.m.: 17.83 ± 0.58, N2 15°C: 26.81 ± 0.90, P < 0.0001) but not glp-4(bn2) germline-lacking worms (glp-4 20°C: 16.44 ± 0.49, glp-4 15°C: 16.71 ± 0.45, P = 0.0379). Wild-type worms are long-lived compared with glp-4 mutant worms at cold temperature (N2 15°C versus glp-4 15°C, P < 0.0001). d. Temperature reduction extends the lifespan of sterile (fer-15(b26);fem-1(hc17) and fog-1(q250) mutant worms with a proliferating germline (N2 20°C mean ± s.e.m.: 18.17 ± 0.53 versus N2 15°C: 27.20 ± 0.67, P < 0.0001; fer-15;fem-1 20°C: 21.42 ± 0.50 versus fer-15;fem-1 15°C: 28.46 ± 0.74, P < 0.0001; fog-1 20°C: 19.62 ± 0.49 versus fog-1 15°C: 27.22 ± 0.70, P < 0.0001). e. Temperature reduction (15°C) extends the lifespan of sterile fog-2(q77) and spe-26(it122) mutant worms with a proliferating germline (N2 20°C mean ± s.e.m.: 17.01 ± 0.48 versus N2 15°C: 27.79 ± 0.60, P < 0.0001; fog-2 20°C: 16.55 ± 0.46 versus fog-2 15°C: 27.94 ± 0.62, P < 0.0001; spe-26 20°C: 17.51 ± 0.54 versus spe-26 15°C: 29.96 ± 0.67, P < 0.0001). f. Wild-type larvae were raised at 25°C, and then adult worms were shifted to the indicated temperatures. Exposure to low temperature (15°C) during adulthood is sufficient to extend longevity (P < 0.0001). Development and adulthood at 25°C mean ± s.e.m.: 13.38 ± 0.43, shifted to 20°C after development: 19.28 ± 0.44, shifted to 15°C after development: 29.07 ± 0.72, 15°C (development and adulthood): 29.89 ± 0.76. g. After development at 20°C, adult wild-type worms were shifted to the indicated temperatures. Exposure to low temperature (15°C) during adulthood extends longevity (P < 0.0001). 20°C (development and adulthood) mean ± s.e.m., in days: 20.25 ± 0.44, shifted to 25°C after development: 13.66 ± 0.37, shifted to 15°C after development: 29.11 ± 0.53, 15°C (development and adulthood): 26.65 ± 0.70. P values were calculated by two-sided log-rank test, n = 96 worms per condition. See Supplementary Data 3 for statistical analysis and replicate data for the lifespan experiments.
animal's mean lifespan at cold temperatures (Fig. 2e). At 25 °C, the percentage of proliferating germ cells was strongly reduced (that is, <10% of cells were BrdU-positive) at day 5, whereas the organismal mean lifespan was 14 d (Fig. 2d,e). At 20 °C, worms had a mean lifespan of 19.5 d with a strong decline in the percentage of proliferating germ cells at day 10. However, in adult worms at 15 °C the acute decline in proliferating germ cells was delayed until day 23, closer to their mean lifespan of 28 d. Likewise, worms at 10 °C maintained a high percentage of proliferating germ cells until day 37, while their mean lifespan was 43 d (Fig. 2d,e). Thus, these results indicate that low temperatures extend the ability of GSCs to self-renew during ageing, sustaining the pool of proliferating cells within the mitotic region.

In addition to their ability to self-renew, GSCs also produce differentiating gametes. Since more than half of the developing oocytes undergo apoptosis while the remaining cells progress to terminal differentiation and fertilization, GSCs are critical to replenish the pool of oocytes\(^39,40\). During development, the first germ cells in wild-type glp-1(e2141) undergo apoptosis while the remaining cells progress to terminal differentiation and fertilization, GSCs are critical to replenish the pool of oocytes\(^39,40\). During development, the first germ cells in wild-type glp-1(e2141) undergo apoptosis while the remaining cells progress to terminal differentiation and fertilization, GSCs are critical to replenish the pool of oocytes\(^39,40\). During development, the first germ cells in wild-type glp-1(e2141) undergo apoptosis while the remaining cells progress to terminal differentiation and fertilization, GSCs are critical to replenish the pool of oocytes\(^39,40\). 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hermaphrodites differentiate into spermatocytes, resulting in a limited number of 240–320 sperm, whereas subsequent germ cells generate oocytes during adulthood, which can then be ovulated and self-fertilized (Fig. 2a). At standard temperature, C. elegans hermaphrodites reproduce during the first 4–5 d of adulthood and quickly exhaust their supply of self-sperm. Likewise, worms at 15°C also self-reproduced until day 5 (Supplementary Fig. 4a). Although worms extended their self-reproductive period until day 8 of adulthood at 10°C (Supplementary Fig. 4a), the total number of eggs laid by hermaphrodites did not increase at cold temperatures (Supplementary Fig. 4b). Moreover, most of the laid eggs hatched at all of the temperatures tested (Supplementary Fig. 4c). However, this period is not sufficient to assess reproductive capacity because self-progeny brood sizes are determined by the number of self-sperm. To circumvent this limitation, 8-day adult hermaphrodites at each distinct temperature were mated with young males for 48 h to replenish the pool of sperm. At 10°C and 15°C, worms were able to lay eggs during the following 9 and 5 d after mating, respectively (Fig. 2f). By contrast, worms at 20°C laid an average of 10 eggs, with an average of 9 laid within the first 2 d after mating (<1 egg per day laid on days 3 and 4 after mating), and we did not observe any eggs laid at the higher temperature (25°C). Notably, the total number of eggs laid at low temperatures was markedly higher than that laid at 20°C (Fig. 2g). In addition, most of these eggs hatched into viable larvae (Fig. 2h). Taken together, our results indicate that cold temperatures sustain the GSC pool during adulthood, resulting in a higher maintenance of the proliferating germline region and reproductive capacity with age.

To assess whether maintenance of GSCs correlates with the capacity to induce cold-associated longevity, we shifted worms from standard (20°C) or warm (25°C) temperatures to a lower temperature (15°C) at different adult stages. Remarkably, cold-induced longevity was dramatically reduced when worms at 20°C were shifted to lower temperature at day 10 of adulthood (Fig. 3a), correlating with an acute decline in germline proliferation at 20°C (that is, <10% of cells were BrdU-positive). Similarly, worms displayed a diminished lifespan extension at cold temperature once they reached day 5 of adulthood at 25°C (Fig. 3b), correlating with the demise of germline proliferation at this temperature (Fig. 2d). Despite the reduction in the duration of cold-induced longevity (at 15°C), worms with a low percentage of proliferating germ cells still exhibited a partial lifespan extension when shifted to cold temperature (Fig. 3a,b). Moreover, we found that shifting the worms to cold temperature after an acute decline in germline proliferation could also partially recover this phenotype (Fig. 3c).

Since somatic tissues such as thermosensory neurons detect changes in temperature, we investigated whether these cells modulate germline maintenance at cold temperature. The cold-sensitive channel TRPA-1 senses temperature decreases in the intestine and neurons to extend lifespan. Concomitantly, loss of function of trpa-1 reduced the length of cold-induced longevity (Fig. 3d). At early adulthood stages (day 5), lack of trpa-1 did not affect germ-line proliferation at cold temperature (Fig. 3e and Supplementary Fig. 5). However, germline proliferation markedly decreased in trpa-1-lacking mutants compared with wild-type worms at older age (day 9), whereas the total number of germ cells within the mitotic region remained similar (Fig. 3e and Supplementary Fig. 5). Cold-sensitive IL1 neurons express trpa-1 and are required for the long-lived phenotype induced by low temperature. Because transgenic expression of trpa-1 in IL1 neurons alone is sufficient to rescue the short lifespan of trpa-1-lacking mutants at lower temperatures, we examined whether another thermosensory neurons could also contribute to this phenotype. A pair of thermoreceptor neurons, called AFD, are required for temperature-related behaviours but do not express the TRPA-1 channel. To assess whether AFD neurons modulate cold-induced longevity, we used worms expressing reconstituted caspase-3 under an AFD-specific promoter to kill this pair of neurons. As in our previous experiments with trpa-1 mutants, we raised AFD-ablated worms at 20°C during development and then shifted them to distinct temperatures to assess the adult lifespan. Notably, we found that AFD-ablated transgenic animals had significantly shorter lifespans than wild-type worms at cold temperatures (10°C, 15°C) (Fig. 3f and Supplementary Fig. 6a). By contrast, loss of AFD neurons did not affect lifespan at 20°C and 25°C (Supplementary Fig. 6b,c). To further assess the role of these neurons in cold-induced longevity, we examined two distinct strains carrying mutations in the ttx-1 gene that specifically disrupt AFD structure and function. Both ttx-1 mutants were short-lived when cultured at 10°C or 15°C during adulthood (Fig. 3g,h and Supplementary Fig. 7a–h). By contrast, ttx-1 mutants had no significant lifespan change relative to wild-type controls.

Fig. 2 | Cold temperature delays exhaustion of GSCs and reproductive ageing. a, Schematic representation of one gonad of the young adult hermaphrodite germ cell. Germ cells are derived from proliferating GSCs located at the mitotic region. Proliferating cells enter meiosis and move proximally from the mitotic region to the meiotic zones (transition zone, pachytene). Germ cell apoptosis occurs in the germline loop region (cell death zone). GSCs generate sperm during larval stages, then switch to oocyte production during adulthood. b, Representative of the mitotic region. The red dashed line corresponds to the point at which multiple germ cells exhibit an early meiotic phenotype, considered the boundary between the mitotic region and the transition zone (row 20 of cells from the most distal cell). c, BrdU staining of proliferating germ cells. Wild-type C. elegans were shifted to the distinct temperatures during adulthood and the germlines were extruded after 2 h of BrdU treatment at the indicated day (D) of age. e, Cell nuclei were stained with DAPI. Scale bar, 20 μm. The images are representative of four independent experiments. d, The graph represents the percentage of BrdU-positive cells per total nuclei within the mitotic region (mean ± s.e.m., 10°C D3–37, 15°C D3–23; n = 20 germlines, 15°C D23, 20°C D3–15, 25°C D3–10 (n = 15); germ lines were scored from three independent experiments). We did not examine the germline after D15 at 25°C, D18 at 20°C, D25 at 15°C and D37 at 10°C because the percentage of BrdU-positive cells was below 10% at these ages or temperatures. NS, not significant (P = 0.3322). e, The graph represents the average ± s.e.m. of the mean lifespan at 10°C (n = 15 independent experiments), 15°C (n = 56), 20°C (n = 29) and 25°C (n = 22). The graph also indicates until which day of the respective mean lifespan germ cell proliferation is maintained at each temperature (>10% of cells BrdU-positive in the mitotic region). As inferred from the data presented in the previous panel. f, Adult worms were cultured at the indicated temperature during adulthood. After the self-reproductive period, worms were mated at day 8 of adulthood with young males raised at 20°C and kept at the respective temperatures. The graph represents the number of eggs laid per worm every 24 h after mating (mean ± s.e.m., n = 24 worms scored per condition from three independent experiments). g, Total number of eggs laid per worm at different temperatures after mating at day 8 of adulthood (mean ± s.e.m., n = 24 worms from three independent experiments). No eggs were laid by mated worms at 25°C. h, Percentage of eggs that hatched at different temperatures (mean ± s.e.m., n = 24 worms from three independent experiments). The percentages of viable eggs after mating are similar at 15°C and 20°C (NS, not significant [P = 0.0715]). Although egg hatching decreases at 10°C, most of the eggs (81%) were still viable. * indicates that no eggs were laid. Statistical comparisons were made by two-tailed Student’s t-test for unpaired samples. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
worms at either 20°C or 25°C (Supplementary Fig. 7c–f). In both AFD-ablated and ttx-1 mutant worms, we observed a decrease in germline proliferation at cold temperature, particularly at older age (Fig. 3i and Supplementary Fig. 8). Collectively, our results indicate that detection of low temperature by thermosensory circuits such as IL1 and AFD neurons delays GSC exhaustion.

**Robust proliferation of adult GSCs determines cold-induced longevity.** Our experiments with germline-lacking worms indicate that the germline is required for cold-induced longevity. In addition, we found that low temperature maintains robust GSC proliferation during adulthood. Intrigued by these results, we assessed whether adult GSCs have a direct and active role in cold-induced longevity. To address this hypothesis, we let worms develop at 20°C into adults with a normal germline and then inhibited adult GSC function by different means, such as DNA synthesis inhibitors or genetic intervention. In adult worms, the germ cells within the mitotic region constitute the only pool of proliferating cells in the organism. Thus, treatment with 5-fluoro-2′-deoxyuridine (FUdR), an inhibitor of DNA synthesis, could particularly affect these cells in adult worms. Indeed, FUdR treatment initiated during adulthood markedly reduced GSC proliferation at both 20°C and 15°C (Fig. 4a). Although FUdR treatment did not affect adult lifespan at 20°C, it blocked the lifespan extension induced by cold temperature (Fig. 4b).
To further assess the requirement for adult proliferating germ cells in cold-induced longevity, we silenced distinct regulators of GSC proliferation. First, we knocked down mog-1, mog-4 and mog-5 genes, which encode the DEAH-box RNA helicases that are required for robust proliferation of germ cells during development. When we initiated RNAi treatment in young adults (day 1) after normal development of the germline, we also observed reduced proliferation within the mitotic region (Fig. 4c,d and Supplementary Fig. 9,10). Notably, downregulation of mog genes during adulthood diminished the lifespan extension induced by low temperature (Fig. 4e). By contrast, loss of mog expression during adulthood did not decrease lifespan at 20 °C and 25 °C (Fig. 4f,g). Besides mog genes, other RNA-binding proteins are also involved in the maintenance of germline proliferation, such as PGL-1, PGL-3 and GLH-1, which are all components of P-granules, a germline-specific RNA/protein complex. However, the germline proliferation requirement for these specific P-granule components is sensitive to temperature. As such, their individual loss can diminish germ-cell proliferation at high temperature (25 °C), but not at lower temperatures. Interestingly, knockdown of pgl genes slightly extends the lifespan of wild-type worms at high temperature. In contrast to downregulation of mog genes, we found that knockdown of pgl-1 alone during adulthood did not reduce germline proliferation (Fig. 4c,d) and cold-induced longevity at 15 °C (Fig. 4h). Likewise, downregulation of glh genes did not alter the long lifespan phenotype induced by low temperature (Fig. 4h).

Although these results indicate a link between germline proliferation and cold-induced longevity during adulthood, mog genes are also expressed in somatic tissues. To circumvent potential direct effects on somatic tissues, we knocked down the orthologues of the translational initiation factor E1F5A (that is, iff-1 and iff-2), which is duplicated in C. elegans. Whereas iff-2 is expressed only in somatic tissues, iff-1 is specifically expressed in the germline and is particularly abundant in the distal gonad, where mitotic divisions occur. In fact, iff-1 is essential for germline proliferation at both larval and adult stages. As such, iff-1 RNAi initiated during adulthood was sufficient to decrease the proliferation of germ cells in the distal gonad (Fig. 4c,d and Supplementary Fig. 9). Notably, knockdown of iff-1 throughout adulthood diminished the lifespan extension induced by low temperatures (10 °C and 15 °C) (Fig. 4i), but did not shorten lifespan at higher temperatures (that is, 20 °C and 25 °C) (Fig. 4k,l). By contrast, loss of iff-2 during adulthood did not impair germline proliferation (Fig. 4c,d) and cold-induced longevity (Fig. 4i,j). Following knockdown of elf-2A, a different translational initiation factor, worms also exhibited a normal proliferating germline (Fig. 4c,d) and extended lifespan, similar to that of control worms at cold temperature (Fig. 4i,j). Altogether, our data suggest that maintenance of adult GSC proliferation determines cold-induced longevity.

Prompted by these results, we assessed how temperature affects iff-1 mutants, which develop into young adult worms with very few germ cells (~ 40 germ nuclei) compared with controls (~ 2,000 germ nuclei), resembling glp-1 mutants. As seen in the glp-1 worms (Fig. 1b), iff-1 germline-lacking mutants were also long-lived at 20 °C but had shorter lifespans than the wild-type strain at 15 °C (Supplementary Fig. 11a,b). Although ablation of the germ line from development in either iff-1 or glp-1 mutants cannot be directly equated to the impairment of GSC proliferation induced by iff-1 RNAi at the adult stage, these results further support a role for the germline in cold-induced longevity. Along these lines, iff-1 knockdown during adulthood did not further decrease the lifespan of distinct germline-lacking strains at cold temperature (Supplementary Fig. 12a,b). As a more formal test of the specific role of adult GSCs, we knocked down iff-1 in adult sterile worms with a proliferating germline and found a decrease in cold-induced longevity (Supplementary Fig. 12c). These results not only strengthened the link between adult GSCs and cold-induced longevity, but also indicate that sterility does not affect the long lifespan phenotype. Indeed, knockdown of iff-1 during adulthood reduced GSC proliferation and cold-induced longevity (Fig. 4d,i), but did not strongly affect the number of eggs laid by self-fertilizing hermaphrodites (Supplementary Fig. 13a,b). Because maintenance of germline proliferation is required for cold-induced longevity, we investigated whether an over-proliferative germline could further extend lifespan at low temperature. Mutations in gld-1, a tumour-suppressor factor, promote re-entry of germ cells into the mitotic cycle and result in overproliferation. Likewise, knockdown of gld-1 also induces over-proliferation of the germline. Eventually, these over-proliferating germ cells break out of the gonad and fill the animal’s body, killing the worms at early adulthood stages. Accordingly, these worms exhibited a short lifespan at 20 °C (Supplementary Fig. 14a). Similarly, both tumorous gld-1 mutations and gld-1 knockdown from either development or adulthood also killed C. elegans early in life at low temperature (Supplementary Fig. 14b).

Collectively, our data indicate that cold-induced longevity is determined by an extended maintenance of the physiological pool of...
proliferating GSCs, while abnormal over-proliferation of these cells is deleterious for the animal.

**Adult proliferating germ cells induce cbs-1 in the intestine to extend lifespan.** Since our results suggest a direct regulation of cold-induced longevity by adult GSCs, we assessed whether these cells regulate somatic tissues to prolong lifespan. Whereas low temperature slightly increased motility, we did not observe defects in germline-lacking mutant worms compared with either wild-type or fer-15/fem-1 control sterile worms (Supplementary Fig. 15a). Likewise, inhibition of adult germline proliferation following iff-1 or mog-5 RNAi treatment did not affect motility at cold temperature (Supplementary Fig. 15b). We also examined body size and found that neither germline ablation nor inhibition of adult germline

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**Graphs:**

- **a:** Percentage alive at different temperatures over time.
- **b:** Percentage alive at different temperatures over time.
- **c:** % BrdU-positive cells in mitotic region at D5 and D9.
- **d:** Percentage alive at 15 °C over time.
- **e:** % BrdU-positive cells in mitotic region at Day 5 and Day 9.
- **f:** Percentage alive at 15 °C over time.
- **g:** Percentage alive at 15 °C over time.
- **h:** Percentage alive at 15 °C over time.
- **i:** % BrdU-positive cells in mitotic region at Day 5 and Day 9.
proliferation had a notable effect on the animal's size (Supplementary Fig. 15c–f). In C. elegans, metabolic rates increase with temperature and are often negatively correlated with longevity\(^\text{64}\). As indicated by lower oxygen consumption, the long lifespan of wild-type worms was accompanied by reduced metabolic rates at 15°C (Supplementary Fig. 16a). However, metabolic rates increased in fer-15; fem-1 sterile worms at cold temperature (Supplementary Fig. 16a), despite the fact that they exhibited a cold-induced longevity phenotype (Fig. 1d). In glp-1 germ-line-mutating mutants, we did not find significant changes in metabolic rates when they were shifted to 15°C (Supplementary Fig. 16a). More importantly, the short lifespan of iff-1 RNAi-treated worms did not correlate with increased metabolic rates at 15°C (Supplementary Fig. 16b). In fact, iff-1 RNAi-treated worms had a decrease in metabolic rates compared with control worms, resembling iff-2 RNAi treatment, which does not affect cold-induced longevity (Supplementary Fig. 16b). Thus, inhibition of adult GSC proliferation did not have a strong effect on features such as motility, body size or metabolic rates, indicating that these cells regulate cold-induced longevity by distinct mechanisms.

To gain further insights into the somatic changes induced by GSCs at low temperature, we used a quantitative proteomics approach. We identified 225 genes that were upregulated and 233 proteins that were downregulated at 20°C compared with at 15°C (Supplementary Data 1). We hypothesized that inhibition of germ-cell proliferation during adulthood at 15°C triggers similar changes in lifespan regulators to those induced by temperature increase. Thus, we examined the proteome of iff-1 RNAI-treated worms at cold temperature (Supplementary Data 1). Besides IFF-1 levels, quantitative proteomics analysis revealed that another 152 proteins were significantly changed following iff-1 knockdown during adulthood at 15°C (Supplementary Data 1). Among them, 27 proteins were also upregulated at 20°C (Fig. 5a, Supplementary Fig. 17 and Supplementary Data 1). Likewise, 27 proteins were downregulated in both iff-1 RNAI and 20°C conditions (Fig. 5a, Supplementary Fig. 17 and Supplementary Data 1). To examine whether these proteins modulate longevity at cold temperatures, we performed an RNAi screen (Supplementary Table 1). Notably, knockdown of several genes reduced cold-induced longevity, as we further validated in independent experiments (Fig. 5b–d). In particular, these genes encoded proteins that are increased at cold temperature and downregulated by loss of iff-1 (Fig. 5a).

**Fig. 4** | Adult GSC proliferation determines cold-induced longevity. a, BrdU staining of germlines from wild-type worms treated with 100 µg ml\(^{-1}\) FUdR during adulthood at the indicated temperatures after development at 20°C. Worms were examined at day 5 of adulthood. Nuclei were stained with DAPI. Scale bar, 20 µm. The images are representative of three independent experiments. b, FUdR during adulthood does not affect lifespan at 20°C (20°C mean ± s.e.m.: 18.23 ± 0.49, 20°C + FUdR: 18.28 ± 0.40, P = 0.8617), but reduces cold-induced longevity (15°C: 29.08 ± 0.60, 15°C + FUdR: 17.49 ± 0.40, P < 0.0001). c, BrdU staining of germlines from wild-type worms examined at day 5 of adulthood. Scale bar, 20 µm. The images are representative of three independent experiments. d, Percentage of BrdU-positive cells per total nuclei (mean ± s.e.m., vector/mog-5/iff-1 RNAI (n = 36 germlines), pgl-1/iff-2/ eif-2A RNAI (n = 36 germlines), from three independent experiments). Analysis by two-tailed Student’s t-test for unpaired samples: ***P < 0.0001.

e, Knockdown of mog genes shortens cold-induced longevity (15°C) in wild-type worms. Vector RNAI mean ± s.e.m.: 26.60 ± 0.74, mog-5: 20.52 ± 0.63 (P < 0.0001); mog-4: 21.83 ± 0.69 (P < 0.0001); mog-1: 22.87 ± 0.76 (P = 0.0020). f, mog RNAI does not reduce the lifespan of wild-type worms at 20°C. Vector RNAI mean ± s.e.m.: 17.96 ± 0.46, mog-5: 17.13 ± 0.28 (P = 0.0653), mog-4: 17.68 ± 0.34 (P = 0.3608), mog-1: 17.58 ± 0.34 (P = 0.2567). g, mog RNAI does not shorten the lifespan of wild-type worms at 25°C. Vector RNAI mean ± s.e.m.: 13.50 ± 0.42, mog-5: 13.93 ± 0.27 (P = 0.7344), mog-1: 14.88 ± 0.27 (P = 0.0420), mog-4: 14.76 ± 0.36 (P = 0.0685). h, Knockdown of pgl and pgh genes does not suppress cold-induced longevity in wild-type worms. Vector RNAI mean ± s.e.m.: 30.35 ± 0.85, pgl-5: 24.92 ± 0.44 (P < 0.0001), pgh-1: 30.96 ± 0.65 (P = 0.6424), pgh-2: 32.89 ± 0.72 (P = 0.2590), pgh-1: 29.64 ± 0.94 (P = 0.9853). i, glh-4: 29.57 ± 0.81 (P = 0.7474). j, Loss of iff-1 shortens cold-induced longevity in wild-type worms at 10°C. Vector RNAI mean ± s.e.m.: 42.45 ± 0.84, iff-1: 35.52 ± 0.54 (P < 0.0001), iff-2: 43.00 ± 0.66 (P = 0.6186), eif-2A: 43.11 ± 0.68 (P = 0.8526). j, iff-1 RNAI reduces cold-induced longevity in wild-type worms at 15°C. Vector RNAI mean ± s.e.m.: 29.19 ± 0.86, iff-1: 21.66 ± 0.76 (P < 0.0001), iff-2: 29.24 ± 0.85 (P = 0.8227), eif-2A: 28.53 ± 0.74 (P = 0.3853). k, iff-1 RNAI does not reduce lifespan at 20°C. Vector RNAI mean ± s.e.m.: 19.33 ± 0.45, iff-1: 19.79 ± 0.51 (P = 0.1371), iff-2: 19.61 ± 0.50 (P = 0.2137), eif-2A: 18.65 ± 0.44 (P = 0.2061). l, iff-1 RNAI induces a moderate lifespan extension at 25°C. Vector RNAI mean ± s.e.m.: 13.23 ± 0.40, iff-1: 15.36 ± 0.39 (P < 0.0001), iff-2: 13.69 ± 0.42 (P = 0.4603), eif-2A: 13.19 ± 0.33 (P = 0.6935). For lifespan experiments, P values were calculated by two-sided log-rank test, n = 96 worms per condition. RNAI initiated at day 1 of adulthood in all experiments. Supplementary Data 3 contains replicate data for the lifespan experiments.
proliferation (Fig. 6a,b), indicating an important role in somatic tissues. Indeed, neuron-specific knockdown of hsp-1 markedly shortened longevity at cold temperature, whereas its loss in either the muscle or intestine did not significantly affect lifespan (Supplementary Fig. 23a–c). However, knockdown of hsp-1 in the germline alone also decreased cold-induced longevity (Supplementary Fig. 23d,e). Thus, although cct subunits and hsp-1 could act in somatic tissues to extend lifespan, their direct effects in germ-cell proliferation also modulate organismal longevity. For this reason, we focused on cbs-1, the only regulator of cold-induced longevity that did not affect the number of proliferating germ cells (Fig. 6a,b). Whereas specific knockdown of cbs-1 in neurons did not change lifespan, its downregulation in either the muscle or intestine significantly shortened cold-induced longevity (Fig. 6c–e). Notably, longevity was not further reduced when the germline also responded to RNAi treatment (Fig. 6f,g). In addition, we found that knockdown of cbs-1 in the germline alone did not impair cold-induced longevity (Fig. 6h).

Altogether, our results suggest that proliferating germ cells induce cbs-1 in specific somatic tissues to extend longevity at cold temperature. To assess this hypothesis, we generated a transcriptional reporter construct. At 20°C and 25°C, cbs-1 was mostly...
Fig. 5 | cbs-1 and other factors modulate the long lifespan induced by adult GSCs at low temperatures. a, Heatmap depicting the log$_2$-fold change of the differentially expressed proteins in both iff-1 RNAi-treated worms at 15 °C and empty vector (EV) RNAi-treated worms at 20 °C compared with EV RNAi-treated worms at 15 °C. The fer-15(b26);fem-1(hc17) control strain was raised at the restrictive temperature (25 °C) during development to obtain sterile worms with a proliferating germline, which were then shifted to the indicated temperatures and RNAi treatment until day 6 of adulthood. Statistical comparisons were made by two-tailed Student’s t-test ($n=3$, $P<0.05$ was considered significant). Lifespan experiments following knockdown of the indicated proteins are presented in the following panels. Supplementary Fig. 17 presents the heatmap with the full list of significantly changed proteins. 

b, CCT subunits are required for cold-induced longevity (15 °C) in wild-type worms (EV RNAi mean ± s.e.m.: 28.26 ± 0.69, cct-2 RNAi: 18.58 ± 0.71, $P<0.0001$; cct-5 RNAi: 17.83 ± 0.68, $P<0.0001$; cct-6 RNAi: 16.15 ± 0.66, $P<0.0001$; hint-1 RNAi: 27.15 ± 0.69, $P=0.1722$). c, The hsp-1 chaperone is necessary for cold-induced longevity (15 °C) (EV RNAi mean ± s.e.m.: 25.49 ± 0.62, hsp-1 RNAi: 19.84 ± 0.49, $P<0.0001$; fkb-6 RNAi: 24.10 ± 0.73, $P=0.2535$). d, The RNA polymerase II ama-1 regulates the longevity of wild-type worms at 15 °C (EV RNAi mean ± s.e.m.: 28.54 ± 0.68, ama-1 RNAi: 23.67 ± 0.42, $P<0.0001$; ttr-6 RNAi: 27.95 ± 0.65, $P=0.4734$). 

e, Loss of cbs-1 decreases the longevity of wild-type worms at 15 °C (EV RNAi mean ± s.e.m.: 28.26 ± 0.69, cbs-1 RNAi: 24.54 ± 0.69, $P=0.0005$; cal-5 RNAi: 28.09 ± 0.78, $P=0.8688$; nlp-24 RNAi: 2716 ± 0.78, $P=0.0664$). f, Knockdown of cct subunits, hsp-1, ama-1 or cbs-1 decreases the long lifespan phenotype induced by 10 °C in wild-type worms ($P<0.0001$). EV RNAi mean ± s.e.m.: 43.31 ± 1.39, cct-2 RNAI: 26.36 ± 0.92, cct-5 RNAI: 26.76 ± 0.95, hsp-1 RNAI: 30.17 ± 0.82, ama-1 RNAI: 35.37 ± 0.87, cbs-1 RNAI: 33.57 ± 0.93. g, Knockdown of cct subunits, hsp-1, ama-1 or cbs-1 does not shorten lifespan at 20 °C. EV RNAi mean ± s.e.m.: 16.34 ± 0.37, cct-2 RNAI: median = 15.97 ± 0.41, $P=0.5386$, cct-5 RNAI: 17.01 ± 0.48, $P=0.1066$; hsp-1 RNAI: 17:10 ± 0.39, $P=0.1571$; ama-1 RNAI: 17.02 ± 0.35, $P=0.4403$; cbs-1 RNAI: 16.26 ± 0.43, $P=0.9592$. h, Knockdown of cct subunits, hsp-1, ama-1 or cbs-1 does not shorten lifespan at 25 °C. RNAi was initiated at day 1 of adulthood. EV RNAi mean ± s.e.m.: 13.95 ± 0.37, cct-2 RNAI: 13.20 ± 0.31, $P=0.9695$; cct-5 RNAI: 13.43 ± 0.29, $P=0.0472$; hsp-1 RNAI: 13.83 ± 0.36, $P=0.7389$; ama-1 RNAI: 14.07 ± 0.36, $P=0.8221$; cbs-1 RNAI: 14.53 ± 0.44, $P=0.1066$. In all the lifespan experiments, RNAi was started at day 1 of adulthood ($P$ values were calculated by two-sided log-rank test, $n=96$ worms per condition). See Supplementary Data 3 for statistical analysis and replicate data for the lifespan experiments.
expressed in the posterior intestine (Fig. 7a). At cold temperatures, cbs-1 expression increased, particularly in the intestine and also in the body muscle (Fig. 7a,b). Notably, FudR treatment reduced the high expression of cbs-1 in somatic tissues induced by cold temperature (Fig. 7a,b). Likewise, knockdown of either iff-1 or mog-5 decreased somatic expression of cbs-1 at cold temperature (Fig. 7c,d). Importantly, we found that somatic expression of cbs-1 was also downregulated when we specifically knocked down these factors in the germline alone (Supplementary Fig. 24a,b). Thus, our results indicate that proliferating germ cells regulate cold-associated induction of cbs-1 in somatic tissues such as the intestine.

With the strong connection between germline, cold-induced longevity and upregulation of somatic expression of cbs-1, we assessed whether intestinal overexpression of cbs-1 can rescue the short lifespan of germline-lacking worms at cold temperature. Whereas tissue-specific overexpression of cbs-1 in the intestine did not increase cold-induced longevity in wild-type worms, it extended the short lifespan of gfp-1 germline-lacking mutants (Supplementary Fig. 25a).

In addition, intestinal overexpression of cbs-1 also partially rescued the short lifespan of worms with defects in adult germline proliferation induced by FudR treatment at low temperature (Supplementary Fig. 25b). Collectively, our data suggest that intestinal upregulation of cbs-1 modulated by GSC proliferation contributes to cold-induced longevity.

CBS is a key enzyme in the transsulfuration pathway, which mediates the interconversion of cysteine and homocysteine. CBS activity produces cystathionine and hydrogen sulfide (H,S)\(^27\), which acts as a gaseous signalling molecule that reduces blood pressure\(^6\) and prevents neurodegeneration in mammals.\(^6\) In addition, dietary restriction induces high levels of H,S that contribute to the lifespan extension phenotype in yeast, worms, fruit flies and rodents.\(^6\) Importantly, exogenous addition of H,S is sufficient to extend the lifespan of C. elegans.\(^6\) Because upregulated levels of cbs-1 contributed to cold-induced longevity, we examined H,S levels. Notably, low temperatures increased H,S gas levels (Fig. 7e). Knockdown of cbs-1 diminished the production of H,S at cold temperature, whereas its overexpression increased H,S levels (Fig. 7f). Moreover, inhibition of germline proliferation by knockdown of either iff-1 or mog-5 was sufficient to decrease H,S production at 15°C, providing a link between H,S levels and the cold-induced longevity modulated by adult GSCs (Fig. 7h).

As shown above, tissue-specific overexpression of cbs-1 in the intestine alone did not further extend the longevity of worms with high endogenous levels of this protein at cold temperature (Supplementary Figs. 25, 26). Likewise, ubiquitous somatic overexpression of cbs-1 did not significantly increase cold-induced longevity (Supplementary Fig. 26). Thus, we investigated how somatic overexpression of cbs-1 affects lifespan at higher temperatures, at which worms have lower endogenous levels of cbs-1. Ubiquitous somatic overexpression of cbs-1 extends lifespan at 20°C (ref. 27) (Fig. 7i). Notably, we found that ubiquitous somatic upregulation of cbs-1 induced longevity even at warm temperature (25°C) (Fig. 7j). Although tissue-specific overexpression in the muscle or neurons also extended longevity, we observed the strongest effects following intestinal overexpression of cbs-1 at both 20°C and 25°C (Fig. 7k,l). Thus, upregulation of cbs-1 in the intestine could mimic low-temperature conditions and prolong lifespan. Altogether, our results suggest that adult proliferating germ cells induce cbs-1 expression in somatic tissues such as the intestine in a process that contributes to cold-induced longevity.

### Release of PGE2 by GSCs promotes longevity at cold temperatures

To assess the mechanism by which adult germ cells induce cbs-1 expression in the intestine at cold temperature, we performed transcriptome analysis of extruded germlines from wild-type worms following iff-1 knockdown or temperature increase (Supplementary Data 2). Compared with the germline of nematodes cultured at 15°C, we found 248 upregulated transcripts and 433 downregulated transcripts in the germlines of both iff-1 RNAi-treated worms and worms cultured at 20°C (Supplementary Fig. 27a and Supplementary Data 2). Among these common downregulated transcripts, we did not observe decreased expression of signalling peptides (that is, nlp, flp or insulin-like peptides) or genes involved in the synthesis of sex steroid hormones (for example, pregnenolone) (Supplementary Data 2). However, the expression of pges-2, the worm orthologue of human PTGES2, was downregulated in the germline by either iff-1 knockdown or temperature increase (Supplementary Fig. 27b and Supplementary Data 2). PTGES2 encodes a membrane-associated prostaglandin E synthase that catalyses the conversion of prostaglandin H2 into the more stable PGE2.\(^2\) Notably, we found that low temperature increased the levels of PGE2 (Fig. 8a). By western blot, we confirmed that worms exhibit higher protein levels of PGE2 in cold temperature (Fig. 8b). Immunohistochemistry experiments indicated that PGE2-2 is highly expressed in the germline compared with the intestine (Fig. 8c). Strikingly, low temperature promoted the enrichment of PGES-2 in the plasma membrane of germ cells, whereas this enzyme was essentially concentrated in the nucleus at higher temperature (Fig. 8c). Prompted by these results, we examined a
potential link between \textit{pges-2} and cold-induced longevity. Notably, \textit{pges-2} mutant animals had significantly shorter lifespans than wild-type worms at cold temperatures (Fig. 8d and Supplementary Fig. 28). However, they did not exhibit a decreased lifespan at 20 °C or 25 °C (Fig. 8d and Supplementary Fig. 28). Moreover, we found that \textit{pges-2} mutant worms exhibited a significant decline in somatic expression of \textit{cbs-1} at cold temperature compared with wild-type worms (Fig. 8e,f). By contrast, \textit{pges-2} mutation did not further decrease the low \textit{cbs-1} expression of worms at 20 °C (Fig. 8e,f). To further assess whether the prostaglandin E synthase activity of \textit{pges-2} is necessary for cold-induced longevity, we added exogenous PGE2 hormone. Indeed, PGE2 rescued the low somatic expression of \textit{cbs-1} in \textit{pges-2} mutants and extended their short lifespan at cold temperature (Fig. 8g–i). Recently, a study reported that the co-chaperone \textit{daf-41/p23} shortens lifespans at warm temperature and is necessary for the long lifespans induced by cold temperature\cite{17}. Importantly, epistasis experiments showed that the co-chaperone activity of \textit{daf-41/p23} is involved in lifespan regulation at warm temperature, but not at cold temperature\cite{17}. Thus, \textit{daf-41/p23} may modulate cold-induced longevity through a different pathway, although the detailed mechanism is not entirely understood. In addition to its co-chaperone activity, p23 also exhibits PGE2 synthase activity in vitro\cite{64}. Although experiments in mouse models failed to support a function for
p23 in PGE2 biosynthesis\textsuperscript{65}, we investigated whether the potential PGE2 synthase activity of daf-41/p23 modulates the effects of the germline in cold-induced longevity. We found that ubiquitous and germ-line-specific knockdown of daf-41/p23 did not diminish the somatic upregulation of cbs-1 induced by cold temperature (Supplementary Fig. 29a–d). In addition, knockdown of daf-41/p23 in the germline alone did not shorten longevity at cold temperature (Supplementary Fig. 29e). Taken together, our results support an important role for pges-2 in the germline in the modulation of cold-induced longevity, whereas daf-41/p23 is dispensable in this tissue for the long lifespan phenotype.

Since pges-2 mutants had normal numbers of proliferating germ cells (Supplementary Fig. 30), our results indicate that pges-2 activity in the germline modulates cold-induced longevity by influencing somatic tissues rather than by directly affecting germ cell proliferation. The release of prostaglandin from cells is mediated by a specific transporter, ABCC4 (ATP binding cassette subfamily C member 4)\textsuperscript{44}. Knockdown of mrp-6, the worm orthologue of ABCC4, diminished cold-induced longevity (Fig. 8j). By contrast, loss of mrp-6 did not shorten the lifespan of worms at 20°C (Fig. 8j). Whereas exogenous PGE2 hormone did not further extend cold-induced longevity in control worms, it rescued the short lifespan phenotype of mrp-6 RNAi-treated worms at cold temperature (Fig. 8k). Tissue-specific knockdown of mrp-6 in either the intestine or neurons did not affect longevity at cold temperature (Fig. 8l,m). Importantly, we found a decline in cold-induced longevity when the RNAi was efficient in the germline (Fig. 8n and Supplementary Fig. 31). Thus, these results suggest that the release of PGE2 by the germline regulates cold-induced longevity. To test this hypothesis, we inhibited adult GSC proliferation using FUDR treatment and examined intestinal cbs-1 expression following application of exogenous PGE2 hormone. Remarkably, exogenous PGE2 was sufficient to rescue the low expression of intestinal cbs-1 induced by FUDR treatment (Fig. 9a,b). Likewise, PGE2 rescued the decline in cold-induced longevity induced by loss of GSC proliferation (Fig. 9c). In addition, exogenous PGE2 extended the short lifespan phenotype of glp-1 germ-line-lacking worms at cold temperature (Supplementary Fig. 32). Altogether, our data indicate that the germline communicates with somatic tissues via PGE2 signals to extend longevity at cold temperature (Fig. 9d).

Discussion

The ageing process is regulated by a complex network of interconnected cell non-autonomous events, by which distinct tissues can influence the ageing of distal organs\textsuperscript{45}. In this intricate system, the germline has a central role in the regulation of somatic ageing\textsuperscript{46}. Particularly, previous studies found that the germline can actively promote the deterioration of the soma during the ageing process\textsuperscript{47,48}. Since food sources can be largely unpredictable and insufficient in nature, and the integrity of tissues is constantly challenged by environmental or metabolic conditions, this system may facilitate a compromised distribution of limited resources to protect the germline, ensuring the fitness of the progeny in line with the dispensable soma theory of ageing. However, here we find an unexpected role for the germline in the long lifespan phenotype induced by cold temperature. Notably, low temperature does not extend lifespan in germline-lacking mutants, providing a first indication that this tissue is required for cold-induced longevity. Ablation of the germline from development could induce numerous changes, but our results indicate that this tissue is particularly required during adulthood for cold-induced longevity. Intrigued by these results, we examined whether temperature-associated changes determine the fitness of the adult germline and how these changes affect cold-induced longevity. In addition to its effects on lifespan, we find that low temperature also slows down reproductive ageing. This process ensues from a delay in the age-associated exhaustion of adult GSCs, resulting in extended reproductive capacity. Notably, maintenance of GSC function is determined by the TRA-1 channel, which acts in IL1 neurons to detect low temperature. In addition to IL1 neurons, we find that dysregulation of thermosensory AFD neurons also shortens lifespan and germline proliferation at cold temperatures, but it does not reduce lifespan at standard or warm temperatures. Since AFD neurons do not express the TRPA-1 channel\textsuperscript{11}, it will be fascinating to examine how these cells sense and process low temperature to communicate with the germline. We speculate that AFD neurons constitutively release a pro-longevity signal when they are inactive or in a low-activity state in cold temperature conditions. Conversely, AFD activation by temperature increase may prevent the release of this pro-longevity factor. In addition to their effects at cold temperature, AFD neurons also induce a heat shock response under heat stress conditions (>30°C)\textsuperscript{66}. Although we did not
not observe any effects of AFD neurons on lifespan at a moderately warm temperature (25 °C), other studies reported that ablation of these neurons can reduce survival at 25 °C \([11] \). In our experiments, worms were maintained and raised at 20 °C during development before being shifted to distinct temperatures to assess adult lifespan. By contrast, worms were cultured in the aforementioned study at a given experimental temperature for at least two generations before adult lifespan analysis at that specific temperature. Moreover, the authors performed lifespan experiments on FUdR to inhibit progeny, while we avoided this treatment because of its effects on germline proliferation. Although it is difficult to directly compare the studies because of the divergent experimental conditions, these differences raise the intriguing possibility that adaptation to temperature and other conditions can also influence how AFD neurons process temperature signals to modulate lifespan.

Although our data indicate that somatic tissues such as IL1 and AFD neurons detect and process temperature changes to delay GSC exhaustion, we found that these cells, in turn, promote the somatic
fitness of distal tissues and organisinal longevity at low temperatures. To assess the requirement for adult GSCs in cold-induced longevity, we first treated adult worms with FUDR, which is extensively used to inhibit progeny at standard temperatures because it does not affect the longevity of wild-type worms under these conditions. We found that FUDR inhibits GSC proliferation at both 20°C and 15°C, but this demise of adult GSCs only shortens lifespan at cold temperature. However, caution is needed to interpret these results. Besides its effects on germline proliferation, FUDR treatment also improves proteostasis. In addition, FUDR affects bacterial ribonucleotide metabolism and has different effects depending on the Escherichia coli strain used to feed the worms, modulating host–microbiome co-metabolism in C. elegans. For this reason, we validated our data using RNAi of modulators of GSC proliferation during adulthood. For instance, we knocked down iff-1, a regulator of germline proliferation that is only expressed in this tissue. Loss of iff-1 during adulthood blocked cold-induced longevity, without reducing lifespan at warmer temperatures. Importantly, knockdown of iff-1 did not affect fertility in early adult stages but had a strong effect on GSC proliferation at later stages, supporting a specific signalling role for adult GSCs in cold-induced longevity.

We then identified that robust proliferation of GSCs upregulates proteostasis components (for example, cct subunits and hsp-1) as well as pro-longevity factors such as H2S. This signalling gaseous molecule is produced by cbs-1, expression of which is induced in somatic tissues such as the intestine by adult GSCs at low temperatures. Notably, intestinal cbs-1 overexpression was sufficient to extend longevity even at warm temperatures, mimicking cold-temperature conditions. It is important to note that intestinal cbs-1 overexpression did not completely rescue the pronounced short-lived phenotype of worms with defects in adult germline proliferation. There are different possible explanations for this partial rescue following intestinal cbs-1 overexpression. Besides its effects in the intestine, cold temperature also triggered cbs-1 expression in the muscle. Conversely, muscle-specific knockdown of cbs-1 decreased cold-induced longevity. Therefore, upregulation of cbs-1 in the muscle could contribute to cold-induced longevity. In addition, our results indicate that cbs-1 is not the only factor involved in cold-induced longevity and that upregulation of other factors can also contribute to this phenotype. For instance, loss of cct subunits in the intestine and neurons as well as knockdown of hsp-1 in neurons diminished cold-induced longevity.

Interestingly, gfp-1 germline-lacking mutant worms have been reported to also exhibit increased levels of cct subunits and H2S production in somatic tissues at standard and warm temperatures, contributing to lifespan extension under these conditions. Thus, a fascinating possibility is that germ cells can either promote or inhibit the same downstream pathways in somatic tissues depending on the environmental and physiological conditions. In addition, depletion of the germline from development could induce additional physiological changes compared to inhibition of GSC proliferation after formation of the adult germline. Our experiments in germline-lacking models were a first indication of a potential role for the germline in cold-induced longevity, which we further validated in rescue experiments by overexpressing cbs-1 in the intestine of these worms. However, absence of the germline cannot be directly equated with reduced adult GSC proliferation. Along these lines, we observed that both iff-1 or gfp-1 germline-lacking mutants were long-lived at 20°C, whereas iff-1 RNAi in adult wild-type worms did not extend lifespan at this temperature.

Little is known about the specific signalling molecules by which the germline communicates with somatic tissues. At cold temperature, our findings establish a role for PGE2 release by adult GSCs in extended longevity. Thus, this hormone could be an important determinant of long lifespan under specific conditions such as low temperature. Prostaglandins are lipid compounds derived from arachidonic acid. Interestingly, administration of arachidonic acid increases resistance of C. elegans to starvation and extends lifespan in conditions of food abundance. It is important to note that alterations in the synthesis or release of PGE2 do not completely block cold-induced longevity. Therefore, other signalling pathways could also contribute to the modulation of somatic tissues by germ cells at cold temperature.

Taken together, our results suggest a cell non-autonomous mechanism (that is, neurons–germline–intestine axis) that coordinates extended reproductive capacity with long lifespan at cold temperature, without the need to sacrifice either the germline or the soma. Since the germline and fertility are generally considered to induce somatic ageing, this unexpected pro-longevity role for adult GSCs has been largely overlooked.

Fig. 8 | Release of PGE2 by GSCs promotes cold-induced longevity. a, PGE2 levels in fer-15(fem-1) control sterile worms at day 6 of adulthood (8.4 mg worms per ml, mean ± s.e.m. from three independent experiments). Two-tailed Student’s t-test for unpaired samples: *P < 0.05, ***P < 0.0001, NS, not significant (P = 0.0519). b, Western blot of day 6 adult control sterile worms with antibodies to PGE2-2 and α-tubulin loading control. The graph represents PGE2-2 relative percentage values (corrected for α-tubulin) at 15°C (mean ± s.e.m., four independent experiments). Two-tailed Student’s t-test for unpaired samples: **P < 0.001. c, Immunostaining with antibody to PGE2-2. White and red arrows indicate the intestine and germline of day 6 adult wild-type worms, respectively. On the right, higher magnification of germ cells. DIC, differential interference contrast. Scale bar, 20 μm. Images are representative of three independent experiments. d, pges-2(ok3316) are short-lived at cold temperature compared with wild-type animals (pges-2 15°C mean ± s.e.m.: 30.10 ± 0.52, wild-type 15°C: 26.09 ± 0.66, P < 0.0001), but do not have shorter lifespans at 20°C (pges-2 20°C: 18.13 ± 0.41, wild-type 20°C: 19.13 ± 0.37, P = 0.1303). e, cbs-1p::GFP in adult wild-type and pges-2(ok3316) mutant worms (day 5). DIC, differential interference contrast. Scale bar, 1000 μm. Images are representative of three independent experiments. f, Quantification of cbs-1p::GFP in day 5 adult animals (mean ± s.e.m. relative to 15°C wild-type worms, 30 worms per condition from three independent experiments). Student’s t-test for unpaired samples: **P < 0.001. g, cbs-1p::GFP in day 8 adult worms at 15°C. Exogenous PGE2 rescues low expression of cbs-1 in pges-2(ok3316) mutant strains, Scale bar, 200 μm. Images are representative of two independent experiments. h, Quantification of cbs-1p::GFP in day 8 adult worms at 15°C (mean ± s.e.m. relative to non-treated wild-type worms. Wild-type (n = 12) and pges-2 (n = 15) from two independent experiments. Two-tailed Student’s t-test for unpaired samples: **P < 0.0001, NS, not significant: wild-type versus wild-type + PGE2 (P = 0.6614), wild-type versus pges-2 + PGE2 (P = 0.4073). i, Exogenous PGE2 extends the short lifespan of pges-2(ok3316) mutants at 15°C (Pges-2 versus pges-2 + PGE2, P < 0.0001). Wild-type mean ± s.e.m.: 28.07 ± 0.59, wild-type + PGE2: 28.64 ± 0.58, pges-2: 22.43 ± 0.73, pges-2 + PGE2: 26.54 ± 0.95. j, mrp-6 RNAi decreases the lifespan of wild-type worms at 15°C (vector RNAi mean ± s.e.m.: 29.64 ± 0.59, mrp-6: 26.66 ± 0.61, P < 0.0001), but not at 20°C (vector: 21.23 ± 0.58, mrp-6: 21.58 ± 0.64, P = 0.3707). RNAi was initiated during adulthood. k, Exogenous PGE2 does not prolong cold-induced longevity of control worms (vector RNAi mean ± s.e.m.: 29.85 ± 0.61 versus vector + PGE2: 29.98 ± 0.59, P = 0.9106), but extends the short lifespan of mrp-6 RNAi-treated worms (mrp-6: 26.37 ± 0.82 versus mrp-6 + PGE2: 31.43 ± 0.60, P < 0.0001). l, Neuronal-specific mrp-6 RNAi (vector RNAi: 23.87 ± 0.56, mrp-6: 24.18 ± 0.61, P = 0.5199). m, Intestinal-specific mrp-6 RNAi (vector RNAi: 24.86 ± 0.51, mrp-6: 26.15 ± 0.57, P = 0.0531). n, When the germline is sensitive to RNAi, mrp-6 RNAi reduces cold-induced longevity (vector RNAi: 27.77 ± 1.03, mrp-6: 23.32 ± 0.78, P = 0.0007). Lifespan experiments were analysed by two-sided log-rank test, n = 96 worms per condition. Supplementary Data 3 contains replicate lifespan experiments.
important implications for the understanding of the ageing process. Indeed, our results raise the intriguing possibility that germ cells activate distinct signals to differentially modulate somatic tissues depending on the physiological and environmental conditions. This could be of particular interest for our ever-ageing society, because several studies indicate that women who have later menopause tend to live longer and have a reduced risk of cardiovascular disease and less loss of bone density. An intriguing question is why the germline promotes longevity at cold temperature. In contrast to high temperatures, low temperature reduces the rate of cell death in several cell types.

![Graph of PGE2 levels](image1.png)

![Micrographs of PGES-2 and tubulin](image2.png)

![Graph of cbs-1p::GFP fluorescence](image3.png)

![Graph of KD neurons survival](image4.png)

![Graph of KD intestine + germline survival](image5.png)
of chemical reactions and molecular entropy, a process that may decrease the damage and deterioration of distinct tissues triggered by cellular metabolism. This process could ameliorate the pressure to prevent and repair damage to tissues. We speculate that these conditions facilitate the distribution of resources, reducing the need to compromise either the soma or the germline. Cold temperatures are detected by thermosensory neurons that delay the exhaustion of GSCs, resulting in the ability to reproduce at older ages. Since this process may also require a healthy soma, we speculate that GSCs actively communicate with other tissues such as the intestine to ensure the fitness of the organism and the ability to reproduce for extended periods.
Application of exogenous PGE2 rescues cbs-1 expression and cold-induced longevity in GSC-impaired worms. A. Representative images of GFP expressed under the control of the cbs-1 promoter in day 8 adult worms at 15°C. Application of exogenous PGE2 rescues low expression of cbs-1 caused by FUDr treatment (100 μg ml⁻¹). PGE2 was added from day 5 of adulthood. DIC, differential interference contrast. Scale bar, 200 μm. Images are representative of three independent experiments. B. Quantification of cbs-1:GFP expression in day 8 adult animals at 15°C. Graph represents the mean ± s.e.m. relative to non-treated worms (non-treated, PGE2, FUDr (n = 50 worms), FUDr + PGE2 (n = 65 worms), from three independent experiments). Statistical comparisons were made by Student's t-test for unpaired samples: ***P < 0.0001, NS, not significant: non-treated versus FUDr + 40 μM PGE2 (P = 0.2436), non-treated versus FUDr + 160 μM PGE2 (P = 0.8706). C. FUDr treatment reduces the lifespan of wild-type worms at cold temperature (non-treated versus FUDr, P < 0.0001), a phenotype rescued by application of exogenous PGE2 (PGE2 + FUDr versus FUDr, P < 0.0001). Non-treated mean ± s.e.m.: 29.30 ± 0.70, FUDr: 22.98 ± 0.63, FUDr + PGE2: 28.38 ± 0.64. P values were calculated by two-sided log-rank test, n = 96 worms per condition. See Supplementary Data 3 for statistical analysis and replicate data for the lifespan experiments. D. Model of cell non-autonomous communication between somatic tissues and the germline at cold temperature. Somatic tissues such as L1 and AFD neurons detect low temperature and ameliorate adult GSC exhaustion, which in turns delays reproductive ageing. Adult GSCs release PGE2 hormone to induce cbs-1 expression in the intestine, resulting in higher somatic production of pro-longevity H2S gas. The rewiring of somatic tissues by GSCs extends organismal lifespan. This process coordinates extended reproductive capacity with long lifespan at cold temperature.

**Methods**

C. elegans strains and maintenance. C. elegans strains were grown and maintained on standard nematode growth medium (NGM) seeded with E. coli (OP50). Wild-type (N2), CB4307 (glp-1[e2141]; mtl-30, sth-1[+], erm-1[+], rec-2[+], cem-2[+], ntr-2[+]; rol-6(su1006); unc-119(ed3)[+]), a DNA plasmid mixture containing ZC373.1p::GFP, rol-6(su1006)[+], unc-119[+], and gcy-39[+]. To generate the DVG177 strain (glp-1[e2141]; mtl-30, sth-1[+], erm-1[+], rec-2[+], cem-2[+], ntr-2[+]; rol-6(su1006); unc-119[+], gcy-39[+]) a DNA plasmid mixture was done in F2 progeny by PCR using the following primers: TGAACGCTGAGCATCCATAG and TGTCTCAGCACGCTCGAGAA.

RNAi constructs. RNAi-treated worms were fed E. coli (HT115) containing an empty control vector (L4440) or expressing double-stranded RNAi pgl-1, pgl-3, glh-1, mog-4, mog-5, gff-1, glf-1, glf-2, rps-15, rks-1, cct-2, cct-6, ctk-1, lim-1, RAI-1, RAI-2, RAI-3, C. elegans strains and maintenance. In the figure legends n represents the total number of uncensored animals/total number (uncensored + censored) of animals observed in each experiment. Lifespan experiments were conducted at the temperatures indicated in the corresponding figure. For the non-integrated RNAi constructs obtained from the Vega TR RNAi library, glh-4, mog-1, elf-2A, cct-5, ana-1, cbs-1, nlp-24, dad-1 and mar-6 RNAi constructs were obtained from the Ahringer RNAi Library. All constructs were sequence verified. For each RNAi, we assessed the knockdown efficiency at the distinct temperatures at which the RNAi was used (Supplementary Fig. 33).

Lifespan studies. In most of the lifespan experiments, synchronized larvae were raised and fed OP50 E. coli at 20°C until day 1 of adulthood. If wild-type larvae were raised at 25°C, this was noted in the figure (for example, Fig. 11, g and Fig. 3b). In experiments containing glp-1, glp-4, fer-15, fem-1, fog-1, fog-2 or spe-26 mutants, larvae were raised at the restrictive temperature (25°C) to obtain germline-lacking and sterile worms. Once worms reached adulthood, they were shifted to a given temperature that is, 10°C, 15°C, 20°C or 25°C on plates with HT115 E. coli carrying empty vector or RNAi clones. Worms (n = 96 per condition) were scored every day or every other day. From the initial worm population, the worms that were lost or buried into the medium as well as those that exhibited protruding vulva or that underwent bagging were censored. In the figure legends n represents the total number of uncensored animals/total number (uncensored + censored) of animals observed in each experiment. Lifespan experiments were conducted at the temperatures indicated in the corresponding figure. For the non-integrated RNAi constructs obtained from the Vega TR RNAi library, glh-4, mog-1, elf-2A, cct-5, ana-1, cbs-1, nlp-24, dad-1 and mar-6 RNAi constructs were obtained from the Ahringer RNAi Library. All constructs were sequence verified. For each RNAi, we assessed the knockdown efficiency at the distinct temperatures at which the RNAi was used (Supplementary Fig. 33).

Construction of cbs-1 C. elegans expression plasmids. To construct the C. elegans plasmid (pDV163) for ubiquitous somatic overexpression of cbs-1, pPD95.77 from the Fire Lab kit (Addgene) was digested with SphI and XmaI to insert 3.6 kb of the sur-5 promoter. The resultant vector was then digested with KpnI and EcoRI to excise GFP and insert a multi-cloning site. ZC373.1a (cbs-1) was PCR amplified from cDNA to include 5′ Nhel and 3′ NotI restriction sites and then cloned into the aforementioned vector. To generate the neuronal-specific overexpression cbs-1 plasmid (pDV188), pMS3 plasmid (pgl-1[pgl-1:pgl-1:6:unc-54:3′UTR]) carrying an inserted multi-cloning site was digested with 5′ Nhel and 3′ NotI restriction sites. Then, cbs-1 was PCR amplified from cDNA to include 5′ Nhel and 3′ NotI restriction sites and cloned into pMS1. Following the same strategy, cbs-1 was cloned into pHS2 (pgl-1[pgl-1:pgl-1:6:unc-54:3′UTR]) to construct intestinal-specific expression plasmid (pDV189). To generate the muscle-specific expression plasmid (pDV190), pMS3 plasmid (pgl-3:pgl-3:6:unc-54:3′UTR) was digested with 5′ XbaI and 3′ Ascl restriction sites. All constructs were sequence verified. pMS1–pMS3 were a gift from A. Dillin (University of California, Berkeley).

Construction of cbs-1 transcriptional reporter construct. To construct pDV184, pPD95.77 from the Fire Lab Kit was digested with SalI and BamHI. The promoter region and part of exon 1 of ZC373.1a (cbs-1) was PCR amplified from N2 cDNA to include ~1248 to 1878 and then cloned into the aforementioned vector using the same enzymes.

cbs-1 transcriptional reporter experiments and imaging. DVG156 worms were grown at 20°C until day 1 of adulthood and then transferred onto plates with...
wells were lysed in protein lysis buffer (50 mM Tris-HCl at pH 7.8, 150 mM NaCl, 0.25% sodium deoxycholate, 1 mM EDTA and protease inhibitor) using a Precellys 24 homogenizer. Worm lysates were centrifuged at 10,000 rpm for 10 min at 4 °C and the supernatant was collected. The total H2S production was determined using a standard BCA protein assay (Thermo Scientific). 3 mg of total protein were used for measurement.

Oxygen consumption. Oxygen consumption rates were measured using an Oroboros oxygraph 2k (Oroboros Instruments GmbH). 30 adult worms were used for each measurement. Data were analysed using DatLab7 software (version 7.3.0.3).

Motility assay. At day 6 of adulthood, worms were transferred to a drop of M9 buffer, and after 30 s of adaptation the number of body bends was counted for 30 s. A body bend is defined as a change in the direction of the bend at the mid-body.

Sample preparation for label-free quantitative proteomics and analysis. fer-15; fem-1 control sterile worms were lysed in ubea buffer (8M urea, 2M Thiourea and 10mM HEPES at pH 7.6) by sonication and cleared using centrifugation (13,000 rpm for 10 min). Supernatants were reduced (1 mM DTT, 30 min), alkylated (5 mM Iodoacetamide, 30 min), diluted 1:10 in PBS and 0.1% Tween 20. The samples were then centrifuged at 1:100 w/w ratio after diluting the urea concentration to 2M. One day later, samples were cleared (16,000 g at 20 min) and the supernatant was acidified. Peptides were cleaned using stage tip extraction. The liquid chromatography tandem mass spectrometry (LC–MS/MS) equipment consisted of an Easy LC 1000 coupled to a LTQ Orbitrap XL instrument (Thermo Finnigan). Peptides were separated on an in-house packed 50 cm column (1.9 μm C18 beads, Dr. Maisch) using a binary buffer system: 0.1% formic acid (buffer A) and 0.1% formic acid in acetonitrile (buffer B). The content of buffer B was raised from 7% to 23% within 120 min, followed by an increase to 45% within 10 min. Then, within 5 min buffer B fraction was raised to 80% and held for 5 min after which it was decreased to 5% within 2 min and held there for further 3 min before the next sample was loaded on the column. Eluting peptides were ionized by an applied voltage of 2.2 kV. The capillary temperature was 275 °C and the S-lens RF level was set to 60. MS1 spectra were acquired using a resolution of 70,000 (at 200 m/z), an automatic gain control (AGC) target of 3×10^6 and a maximum injection time of 60 ms. All labelfree proteomics data sets were analysed with MaxQuant software (version 1.5.3.8). We used the LFQ mode and MaxQuant default settings for protein identification and LFQ quantification. All downstream analyses were carried out on LFQ values with Perseus (version 1.5.2.4).

RNA isolation and sequencing. RNA from the extruded germlines of day 6 adult N2 worms was extracted using RNAbees (Tel-Test). Libraries were generated using the TrueSeq stranded mRNA Library Prep Kit (Illumina). Library preparation started with 1 μg total RNA. After selection (using poly-T oligo–attached magnetic beads), mRNA was purified and fragmented using divalent cations under denaturing conditions. The RNA fragments underwent reverse transcription using random primers followed by second strand cDNA synthesis with DNA polymerase 1 and RNase H. After end repair and A-tailing, indexing adapters were ligated. The products were then purified and amplified (2 μl template, 14 PCR cycles) to create the final cDNA libraries. After library validation and quantification (Agilent 2100 Bioanalyzer), equimolar amounts of library (5%) were pooled. The pool was quantified using the Qubit KAPA Library Quantification Kit (VWR) and the Applied Biosystems 7900HT Sequence Detection System. The pool was sequenced on an Illumina HiSeq 4000 sequencer with a paired-end (2×250 bp) protocol.

RNA sequencing (RNA-seq) data were analysed using a QuickNGS pipeline. Total RNA from three biological replicates was pooled. The pooled RNA was sequenced on an Illumina HiSeq 2500 sequencer with paired-end reads (2×100 bp) using standard protocols. The raw reads were trimmed using cutadapt version 0.10.1. The trimmed reads were aligned to the reference genome of C. elegans (Version 10.0) and the genome was annotated with augmented Cufflinks2 (version 2.1.1.). The QuickNgs pipeline performed read count means, fold change and P values with DEseq2 (version 1.4.5) and gene expression for the individual samples with Cufflinks2 (version 2.1.1) as fragments per kilobase of transcript per million (FPKM), in both cases using genomic annotation from the Ensembl database, version 87. Quantitative RT–PCR. Total RNA was isolated from approximately 2,000 synchronized day 5 adult worms using RNAbees; cDNA was generated using a ScriptSeq Flex cDNA synthesis kit (Quantabio). SybrGreen real-time qPCR experiments were performed with a 1:20 dilution of cDNA using a CFX384 Real-Time System Bio-Rad). Data were analysed with the comparative 2ΔΔCT method using the geometric mean of cdc–42 and pmp–3 as housekeeping genes. See Supplemental Table 2 for details about the primers used for this assay.
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Author contributions
H. J. L., A. N. and D. V. performed most of the experiments, data analysis and interpretation. S. K. assessed knockdown levels and contributed to lifespan assays and other experiments. G. C. performed some of the BrdU assays and helped with other experiments. M. S. S. generated the plasmids for tissue-specific overexpression that were used to clone ceh-1. M. H. performed oxygen consumption experiments. A. T. contributed expertise on metabolic rates and provided critical advice on the project. The manuscript was written by D. V. All authors discussed the results and commented on the manuscript.

Competing interests
The authors declare no competing interests.

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

No software was used

Data analysis

We used GraphPad PRISM 6 and OASIS software for statistical analysis. To quantify GFP fluorescence and body size, worms were outlined and quantified using ImageJ software (version 1.51s). We also used ImageJ (1.51s) to quantify densitometry of immunoblots. Oxygen consumption data were analyzed using DatLab7 software (version 7.3.0.3). Proteomics data sets were analyzed with the MaxQuant software version 1.5.3.8). We employed the LFQ mode and used MaxQuant default settings for protein identification and LFQ quantification. All downstream analyses of proteomics data were carried out on LFQ values with Perseus (version 1.5.2.4). RNA-seq data were analysed using a QuickNGS pipeline (Wagle, P., Nikolic, M. & Frommolt, P. QuickNGS elevates Next-Generation Sequencing data analysis to a new level of automation. BMC Genomics 16, 487, (2015)). This workflow system provided basic read quality check using FastQC (version 0.10.1) and read statistics using SAMtools (version 0.1.19). The basic data processing of the QuickNGS pipeline consists of a splicing-aware alignment using Tophat2 (version 2.0.10) followed by reference-guided transcriptome reassembly with Cufflinks2 (version 2.1.1). The QuickNGS pipeline calculated read count means, fold change and P-values with DEseq2 (version 1.4.5) and gene expression for the individual samples with Cufflinks2 (version 2.1.1) as FPKMs, using in both cases genomic annotation from the Ensembl database version 87.

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The manuscript includes the following Data Availability Statement: “The authors declare that the main data supporting the findings of this study are available within the article and its supplementary files. Transcriptome data have been deposited in Gene Expression Omnibus (GEO) under the accession code GSE123054. All the other data are also available from the corresponding author upon request.”

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
Sample size determination was done according to standard C. elegans approaches. Exact sample sizes are indicated in the corresponding figure legends and Supplementary Information.

Data exclusions
No data were excluded from the analyses.

Replication
At least three independent experiments for each assay were performed to verify the reproducibility of the findings. All the attempts of replication gave a similar outcome. Lifespan assays were done at least 2 times with 96 individuals each per condition. Exact numbers and statistics are provided in the Supplementary Information.

Randomization
For lifespan experiments, reporter quantification, BrdU assays, body size, egg counting, motility assays, worms were synchronized by picking young hermaphrodites adults and let them lay eggs for 6 hours. These young hermaphrodites were randomly picked from our maintenance plates. After egg laying for 6 hours, larvae were raised until adulthood and adult worms were then randomly assigned to the different treatment conditions (e.g., temperature, RNAi treatment) for the indicated assays. The different conditions were assessed in random order. The replicate experiments were usually performed by different authors involved in the study.

Blinding
The samples and different conditions were not processed in a blinded manner by the researchers participating in this study. However, worms were randomly assigned to the different treatment conditions, the different conditions were assessed in random order and the replicate experiments were usually performed by different authors involved in the study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☐ Antibodies |
| ☒ Eukaryotic cell lines |
| ☒ Palaeontology |
| ☒ Animals and other organisms |
| ☒ Human research participants |
| ☐ Clinical data |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ ChIP-seq |
| ☒ Flow cytometry |
| ☒ MRI-based neuroimaging |
### Antibodies

| Antibodies used | For Bromodeoxyuridine (BrdU) proliferation assays, we used Rat anti-BrdU monoclonal antibody (Clone BU1 / 7S (ICR1), Abcam, ab6326, Lot# GR545121, RRID:AB_305426, dilution 1:250) and Anti-Rat IgG secondary polyclonal antibody (Life Technologies, A11081, Lot# 1744742, RRID: AB_141738, 1:500). For western blot analysis, we used Rabbit anti-PGES2 polyclonal antibody (Bioss, bs-2639R, Lot# AH01125763, RRID:AB_10860215, 1:500) and Mouse anti-alpha-tubulin monoclonal antibody (Clone DM1A, Sigma, T6199, Lot# 115M4796V, RRID:AB_477583, 1:500). For germline and gut immunostaining, we used anti-PGES2 antibody (Bioss, bs-2639R, Lot# AH01125763, RRID:AB_10860215, 1:100) and Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary polyclonal Antibody, Alexa Fluor 488 (Life Technologies, A11008, RRID: AB_143165, Lot# 1408839, 1:500). |
| Validation | Validations of primary and secondary antibodies were done by the stated manufacturer's and supported by the publications indicated in the manufacturer's websites (please see below) |

**BrdU proliferation assays:**
- **Rat anti-BrdU monoclonal antibody**
  - Information provided by manufacturer (Abcam) and RRID portal:
  - Specificity/Applications: This antibody reacts with BrdU (5-Bromo-2'-deoxyuridine) in single stranded DNA, BrdU attached to a protein carrier or free BrdU. Suitable for ICC/IF
  - References: PMID:17206614, PMID:18399538, PMID:18613121, PMID:18666124, PMID:19003874, PMID:19048639, PMID:20878783, PMID:21246550, PMID:21452233, PMID:21800305, (…)
  - By ICC/IF, we validated that the only BrdU-positive cells are the germ cells within the mitotic region, which contains the only pool of proliferating cells in adult C. elegans.

- **Anti-Rat IgG secondary polyclonal antibody**
  - Information provided by manufacturer (Life Technologies) and RRID portal:
  - Specificity/Applications: Goat anti-Rat IgG. Suitable for ICC/IF
  - References: PMID:25209291, PMID:28292425, PMID:28472659, PMID:28821643, PMID:29257951, PMID:29429943, PMID:29456135, PMID:30293839, PMID:30639056, PMID:3069350
  - By ICC/IF, we validated that the only BrdU-positive cells are the germ cells within the mitotic region, which contains the only pool of proliferating cells in adult C. elegans.

**Western blot and immunofluorescence:**
- **anti-PGES2 polyclonal antibody**
  - Information provided by manufacturer (Bioss) and RRID portal:
  - Specificity/Applications: Reactivity with PGES-2 in numerous species such as rat, mouse, bovine, porcine, human, mouse, rat, human, pig, dog, cow, chicken. Suitable for WB and ICC/IF
  - References: PMID: 25070591 (ICC/IF), PMID: 28469528 (WB), PMID: 28780305 (WB)
  - By western blot, we detected protein size as expected in C. elegans and observed that low temperature increases PGES-2 levels as we validated by proteomics. By ICC/IF, we validated that that PGES-2 is highly expressed in the germ line compared to the intestine and that low temperature promoted the activation of PGES-2 (enrichment of PGES-2 in the plasma membrane of germ cells) whereas PGES-2 was essentially Inactivated (concentrated in the nucleus) at higher temperature, as we further confirmed by lifespan and tissue specific mrp-6 RNAi experiments at different temperatures.

- **anti-alpha-tubulin monoclonal antibody**
  - Information provided by manufacturer (Sigma) and RRID portal:
  - Specificity/Applications: Reactivity with mouse, yeast, human, chicken, rat, amphibian, fungi, bovine, mouse, Xenopus/amphibian, chicken/bird. Suitable for WB.
  - References: PMID:24956542, PMID:25521582, PMID:25785851, PMID:26562364, PMID:26910308, PMID:27336722, PMID:27641956, PMID:2794199, PMID:2794179, PMID:28317021, PMID:28344080, PMID:28388406, PMID:28452069, PMID:28486131, PMID:28552353, PMID:28602352, PMID:28602822, PMID:28618271
  - By western blot, we detected protein size as expected. We previously used this antibody to detect alpha-tubulin in C. elegans samples (PMID: 27892468).

- **Goat anti-Rabbit IgG**
  - Information provided by manufacturer (Life Technologies) and RRID portal:
  - Specificity/Applications: Goat anti-Rabbit IgG. Suitable for ICC/IF
  - References: PMID:10089887, PMID:10216085, PMID:10367906, PMID:10399916, PMID:10417176, PMID:10428032, PMID:10459018, PMID:10465787, PMID:10480903, PMID:10490100, PMID:10490101, PMID:10499794, PMID:10572057, PMID:10574968, PMID:10588653, PMID:10608877, PMID:10629214, PMID:10629215, PMID:10629217, PMID:10662920, PMID:10644749, PMID:10646502, PMID:1066056 (…)

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals
The study involved Caenorhabditis elegans strains with the following genotypes:

- Wild-type (N2)
- CB4037 (glp-1(e2141)III)
- SS104 (glp-4(bn2)I)
- CF512 (fer-15(b26);fem-1(hc17)IV) (fer-15 is also named rrf-3)
- TQ233 (trpa-1(ok999)IV)
- GN112 (pgIs2[ gcy-8p::TU#813 + gcy-8p::mCherry + gcy-8p::GFP + ttx-3p::GFP]),
- JN215 (tfI-1(tm4833)III;ht2 [bli-4(e937) let-7(q782) dls48];[I;III])
- PR767 (ttx-1(p767)IV)
- PY1283 (ttx-1(oy29) oys117 [gcy-8p::GFP + lin-15(+)]IV)
- BA837 (spe-26(n112)IV)
- CB4108 (fog-2(q71)IV)
- JK560 (fog-1(q250)) and JK1466 (glp-1(e485)dpv-5(e61)unc-13(e51))
- CF2424 (PR767 [ttx-1(p767)IV] outcrossed 4 times to wild-type N2)
- AA1724 (pges-2(ok3311)IV), generated by outcrossing the RB2421 strain (pges-2(ok3311)IV) 4 times to wild-type N2)
- TG6899 (xud6170a[qnp-6p::trpa-1::yfp]; trpa-1(ok999)IV)
- WM118 (rde-1(ne300)IV; neIs9[myo-3p::HA::RDE-1 + rol-6::GFP])
- VP303 (rde-1(ne219)IV; kbis7[ myo-2::GFP + rol-6::GFP])
- AM1345 (jamSi2[ mex-5p::rde-1::GFP + rde-1::GFP];
- TU3401 (sid-1(pk3321)IV; uls69[pCFJ90(myo-2::mCherry + unc-119(p::sid-1)])
- DCL569 (mkcSi13[sun-1p::rde-1::GFP + unc-119(+); rde-1::GFP])
- DVG146 (N2, ochEx130[sur-5p::ZC373.1, myo-3p::GFP])
- DVG147 (N2, ochEx131[sur-5p::ZC373.1, myo-3p::GFP])
- DVG162 (N2, ochEx145[ rgef-1p::ZC373.1, myo-3p::GFP])
- DVG163 (N2, ochEx146[ rgef-1p::ZC373.1, myo-3p::GFP])
- DVG166 (N2, ochEx149[ gly-19p::ZC373.1, myo-3p::GFP])
- DVG167 (N2, ochEx150[ gly-19p::ZC373.1, myo-3p::GFP])
- DVG173 (N2, ochEx156[ myo-3p::ZC373.1, myo-3p::GFP])
- DVG174 (N2, ochEx157[ myo-3p::ZC373.1, myo-3p::GFP])
- DVG9 (N2, ochEx6[myo-3p::GFP])
- DVG186 (glp-1(e2141)III; ochEx150[ gly-19p::ZC373.1, myo-3p::GFP])
- DVG156 (N2, ochEx140[ sun-1p::rde-1::GFP + UNC-119(+); rde-1::GFP])
- DVG185 (mkcSi13[ sun-1p::rde-1::GFP + UNC-119(+); rde-1::GFP])
- DVG177 (ochEx160[ ds-1p::GFP, prf4(rol-6)])

For all the studies we used hermaphrodite worms (except SS104 abd CB4037 mutant strains which lack a germline and CFS12, BA837, JK560 and CB4108 which have spermatogenesis defects).

For all the experiments, we started treatment conditions (e.g., RNAi, temperature) at day 1 of adulthood. For lifespan experiments, worms were assessed from day 1 adulthood until they died. For transcriptional reporter experiments worms were analyzed at day 7 of adulthood (otherwise, stated in the corresponding figure legend). qPCR analysis was performed on day 5-adulthood worms. H2S analysis was performed in worms at day 6 of adulthood. Quantification of PGE2 levels and western blots were performed in worms at day 6 of adulthood. Transcriptomics and proteomics experiments were performed on worms at day 6 of adulthood.

For the studies involving wild animals, ARRIVE guidelines are recommended for reporting animal research. The study did not involve wild animals.

Field-collected samples
This study was not involved samples collected from the field.

Ethics oversight
In this research, we used invertebrate C. elegans as an organismal model and no ethical approval was required. According to the "Zentrale Kommission für die Biologische Sicherheit" (ZKBS), the responsible entity inside the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit to assess the risk of Genetically Modified Organisms (GMO), genetic work with C. elegans is classified as risk group 1 (biological safety level 1: S1). Accordingly, we performed work on C. elegans in a S1-laboratory. The use of GMO in Germany is regulated by the "Gentechnik-Gesetz", and we followed the guidelines applying to S1 work with GMO (i.e., documentation of the project and of the, exact description of the creation and maintenance of the genetic modification or correct waste treatment).

Note that full information on the approval of the study protocol must also be provided in the manuscript.