

Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*

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Chromatin modifiers regulate lifespan in several organisms, raising the question of whether changes in chromatin states in the parental generation could be incompletely reprogrammed in the next generation and thereby affect the lifespan of descendants. The histone H3 lysine 4 trimethylation (H3K4me3) complex, composed of ASH-2, WDR-5 and the histone methyltransferase SET-2, regulates *Caenorhabditis elegans* lifespan. Here we show that deficiencies in the H3K4me3 chromatin modifiers ASH-2, WDR-5 or SET-2 in the parental generation extend the lifespan of descendants up until the third generation. The transgenerational inheritance of lifespan extension by members of the ASH-2 complex is dependent on the H3K4me3 demethylase RBR-2, and requires the presence of a functioning germline in the descendants. Transgenerational inheritance of lifespan is specific for the H3K4me3 methylation complex and is associated with epigenetic changes in gene expression. Thus, manipulation of specific chromatin modifiers only in parents can induce an epigenetic memory of longevity in descendants.

Transgenerational epigenetic inheritance has been described for some traits, including flower symmetry and colour in plants^{1–3}, progeny production in worms⁴, heat stress response and eye colour in *Drosophila*^{5–7}, and coat colour in mammals^{8–10}. However, the transgenerational epigenetic inheritance of longevity, and more generally of complex traits, is largely undefined. Chromatin modifiers have been shown to regulate longevity in several species^{11–18}, raising the possibility that chromatin changes in parents might not be entirely reset between generations and thereby also regulate longevity in descendants. Deficiencies in the H3K4me3 regulatory complex composed of ASH-2, WDR-5 and SET-2 extend lifespan in *C. elegans*¹². We asked if perturbation of members of the H3K4me3 regulatory complex (ASH-2, WDR-5 and SET-2) only in the parental generation could regulate the lifespan of descendants in subsequent generations in *C. elegans*.

Transgenerational inheritance of longevity

We first focused on WDR-5, a conserved regulatory component of the ASH-2 complex¹⁹ whose depletion decreases H3K4me3 levels^{12,20–22} and extends lifespan in worms¹². To test whether longevity could be inherited in a transgenerational epigenetic manner, we crossed wild-type (+/+) males with *wdr-5(ok1417)* mutant (*wdr-5/wdr-5*) hermaphrodites to generate F1 heterozygous hermaphrodites (Fig. 1a). These F1 heterozygous hermaphrodites were genotyped and then self-crossed to generate F2 hermaphrodites (wild type, heterozygous and homozygous at the *wdr-5* locus), which were genotyped after they had laid F3 generation progeny. In parallel, we crossed a wild-type male with a wild-type hermaphrodite to generate pure wild-type descendants and control for any beneficial longevity effects that could come from crossing rather than self-mating (Fig. 1a). Longevity of genetically wild-type descendants from wild-type or *wdr-5* mutant ancestors was compared in the F3, F4 and F5 generations. Interestingly, genetically wild-type F3 descendants from P0 *wdr-5* parents (+/+ from P0 *wdr-5* parents) still showed a ~20% extension of lifespan ($P < 0.0001$) compared to descendants from

pure wild-type parents (+/+ from P0 WT parents) (Fig. 1b). This 20% lifespan extension was similar in magnitude to the lifespan extension of pure F3 *wdr-5(ok1417)* mutants (*wdr-5/wdr-5*) (Fig. 1b). The lifespan of genetically wild-type descendants from *wdr-5(ok1417)* mutant parents (+/+ from P0 *wdr-5* parents) was still extended in the F4 generation (Fig. 1c), but was no longer extended in the F5 generation (Fig. 1d). Thus, *wdr-5* deficiency only in the parental generation can extend the lifespan of subsequent generations. Because the lifespan of F5 generation wild-type descendants from *wdr-5* mutant parents is no longer extended, the lifespan extension observed in the F3 and F4 generations is unlikely to be due to extraneous mutations that might have been present in the parental *wdr-5* mutant strain. Instead, the transgenerational inheritance of longevity may be due to epigenetic changes in H3K4me3 itself or in another molecule that can only be inherited for a limited number of generations.

We next asked if a transgenerational epigenetic heritability of lifespan was also observed with SET-2, the H3K4me3 methyltransferase enzyme that functions together with ASH-2 and WDR-5 to regulate H3K4me3 levels^{12,20–22} and longevity in *C. elegans*¹² (Fig. 2). Similar to what we observed for *wdr-5*, genetically wild-type descendants from *set-2(ok952)* mutants still had a ~30% extension of lifespan ($P < 0.0001$) in the F3 and F4 generations (Fig. 2b, c), but not in the F5 generation (Fig. 2d). Genetically wild-type F3 descendants from the reverse cross—P0 *set-2(ok952)* males crossed with wild-type hermaphrodites—were also long-lived (Supplementary Table 1), indicating that transgenerational inheritance of longevity is not linked to a particular gender in the parental generation.

ASH-2 is important for the conversion of H3K4 dimethylation (H3K4me2) to H3K4me3 (ref. 23). Knockdown of *ash-2* by RNA interference (RNAi) in worms decreases global H3K4me3 levels at the L3 stage^{12,22} and extends longevity¹². We asked if *ash-2* knockdown only in the parental generation affected the lifespan of several generations of descendants. Wild-type parent worms (P0) were placed on plates with bacteria expressing RNAi to *ash-2* from birth to the larval stage L4, then switched every day for 3 days onto plates

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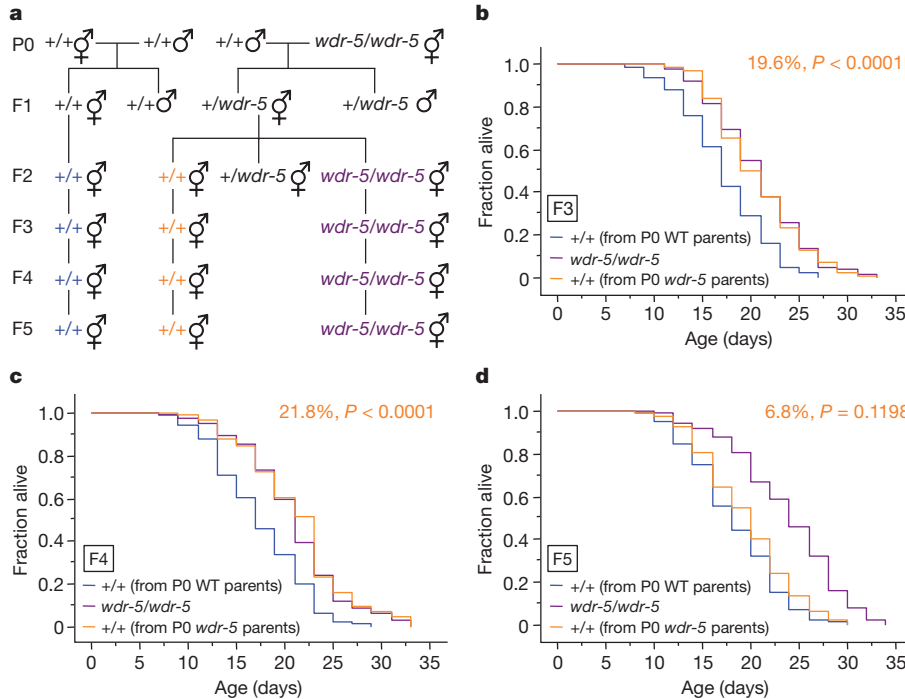


Figure 1 | Genetically wild-type descendants from *wdr-5* mutant parents have extended lifespan for several generations. **a**, Scheme for generating wild-type (+/+) descendants from *wdr-5(ok1417)* mutant worms (*wdr-5/wdr-5*). **b–d**, Lifespan of genetically wild-type F3 (**b**), F4 (**c**) and F5 (**d**) descendants

of *wdr-5(ok1417)* mutant worms (+/+ from P0 *wdr-5* parents) compared to descendants of wild-type worms (+/+ from P0 WT parents). Mean lifespan and statistics are presented in Supplementary Table 1.

containing OP50-1 bacteria and streptomycin to selectively prevent the growth of RNAi-expressing bacteria (Fig. 3a). Endogenous *ash-2* messenger RNA and ASH-2 protein levels were significantly decreased in the P0 generation, but returned to normal levels in subsequent generations (Fig. 3b, c), indicating that *ash-2* RNAi is not itself inherited. The lifespan of worms from the F1, F2 and F3 generations in which *ash-2* had been knocked down only in the P0

parental generation was still significantly extended (19–27%, $P < 0.0001$) compared to that of descendants of worms treated with empty vector control in the P0 parental generation (Fig. 3d–g). By contrast, F4 generation descendants no longer had extended lifespan (Fig. 3h). We obtained similar results after bleaching P0 worms to avoid potential carry over of RNAi-expressing bacteria (data not shown). Thus, alteration of the components of the H3K4me3

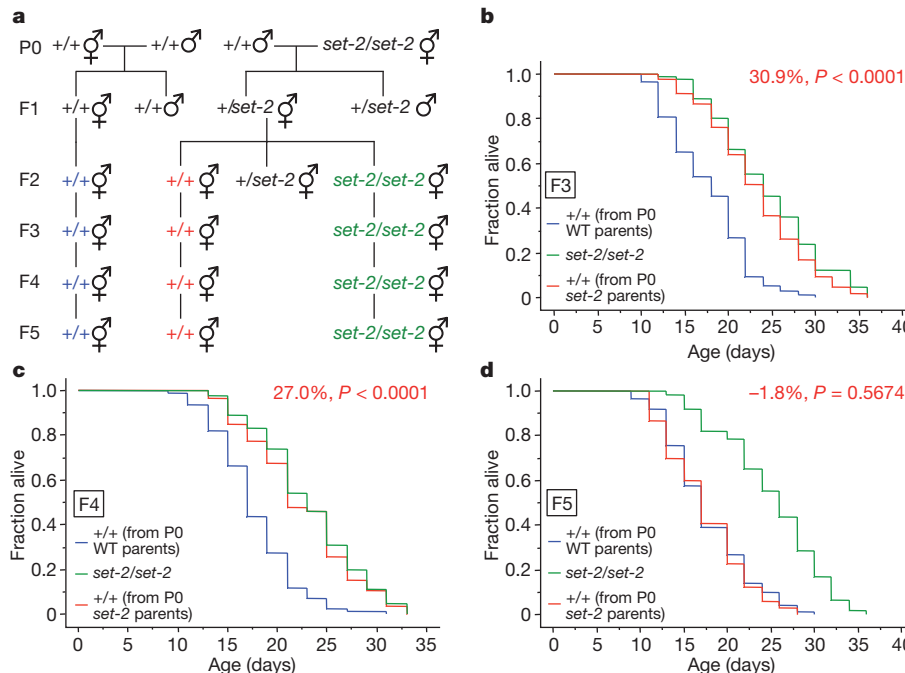


Figure 2 | Genetically wild-type descendants from *set-2* mutant parents have extended lifespan for several generations. **a**, Scheme for generating wild-type (+/+) descendants from *set-2(ok952)* mutant worms (*set-2/set-2*). **b–d**, Lifespan of genetically wild-type F3 (**b**), F4 (**c**) and F5 (**d**) descendants

from *set-2(ok952)* mutant worms (+/+ from P0 *set-2* parents) compared to descendants of wild-type worms (+/+ from P0 WT parents). Mean lifespan and statistics are presented in Supplementary Table 1.

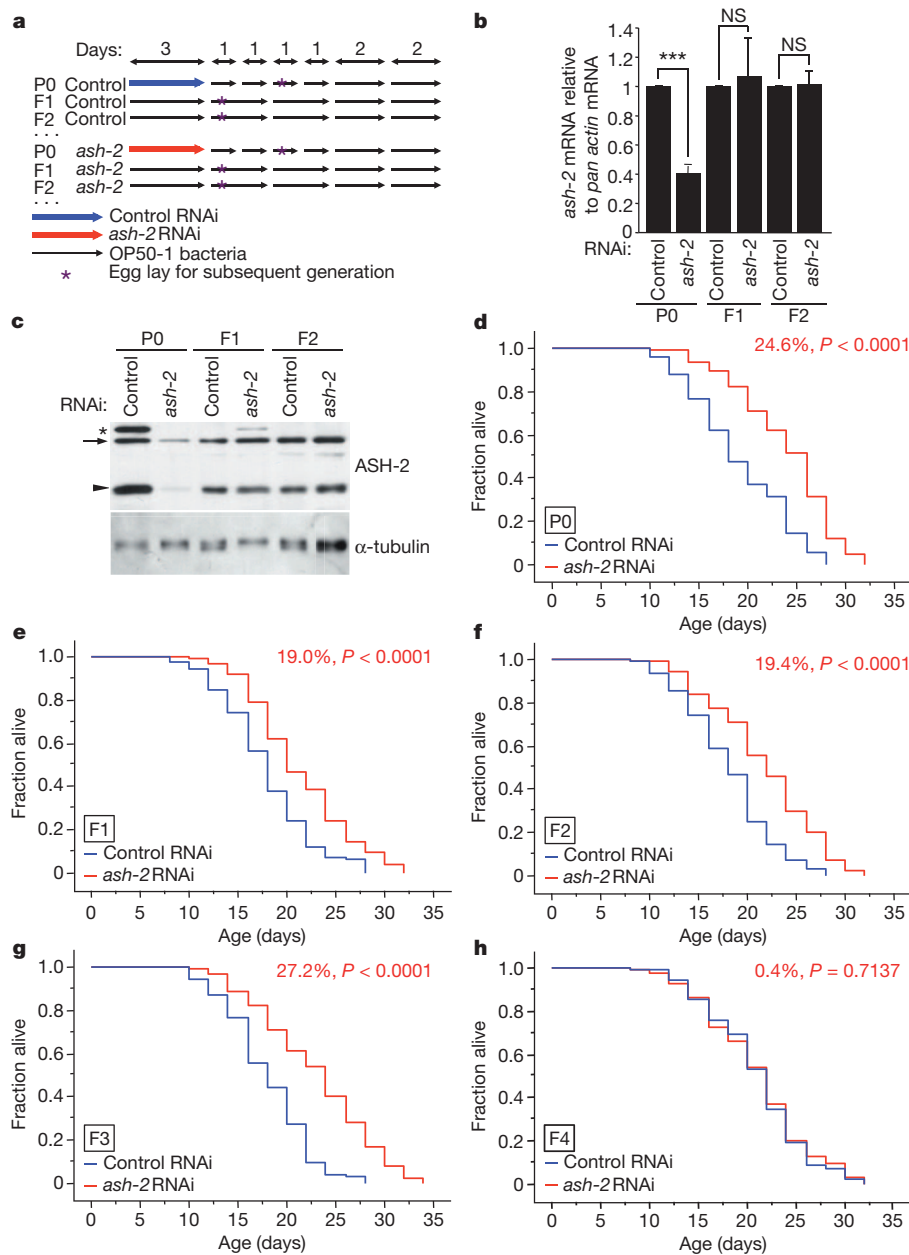


Figure 3 | Knockdown of *ash-2* only in the parental generation extends lifespan for several generations. **a**, Scheme for generating wild-type descendants from RNAi-treated parents. **b**, *ash-2* mRNA levels at day 7 in different generations of worms treated with *ash-2* RNAi or empty vector (control) only in the P0 generation. Mean \pm s.e.m. of three independent experiments. *** $P = 0.0002$ with paired *t*-test. **c**, ASH-2 protein levels at L3 stage in different generations of worms treated with *ash-2* RNAi or empty

vector (control) only in the P0 generation. Representative of two independent experiments. *, non-specific band; arrow, ASH-2; arrowhead, protein related to ASH-2, possibly a degradation product. **d-h**, Lifespan of P0 (**d**), F1 (**e**), F2 (**f**), F3 (**g**) and F4 (**h**) generations of worms with RNAi knockdown of *ash-2* or control RNAi (empty vector) in parents only. Mean lifespan and statistics are presented in Supplementary Table 2.

methyltransferase complex (ASH-2, WDR-5 and SET-2) in parents affects the lifespan of descendants, supporting the possibility that transgenerational inheritance of longevity is due to epigenetic changes that may only be inherited for a limited number of generations.

Importance of the H3K4me3 demethylase and germline

The H3K4me3 demethylase RBR-2 is necessary for the lifespan extension caused by deficiencies in members of the ASH-2 complex¹². We asked if the transgenerational extension of longevity induced by deficiencies in members of the ASH-2 complex is dependent on RBR-2. The lifespan of genetically wild-type F3 descendants from P0 *wdr-5* parents (+/+ from P0 *wdr-5* parents) was no longer extended in the presence of *rbr-2* RNAi (Fig. 4a, b). Similarly, F3 wild-type descendants

from *set-2;rbr-2* parents (+/+ from P0 *set-2;rbr-2* parents) were no longer long-lived (Supplementary Fig. 1). Together, these data indicate that the transgenerational inheritance of longevity due to deficiencies in H3K4 trimethylation complex members is dependent on the H3K4me3 demethylase RBR-2. The fact that the longevity of wild-type descendants of *wdr-5* and *set-2* mutants is reverted by deficiencies in *rbr-2* also indicates that this extended lifespan is unlikely to result from extraneous mutations in *wdr-5* or *set-2* strains. *rbr-2* mutation or knockdown did not lead to a shortening of lifespan in descendants (Supplementary Fig. 2), indicating that by itself, RBR-2 deficiency does not affect longevity in a transgenerational manner.

Longevity due to modulation of the ASH-2 complex is dependent on a functioning germline¹². To test if wild-type descendants of worms

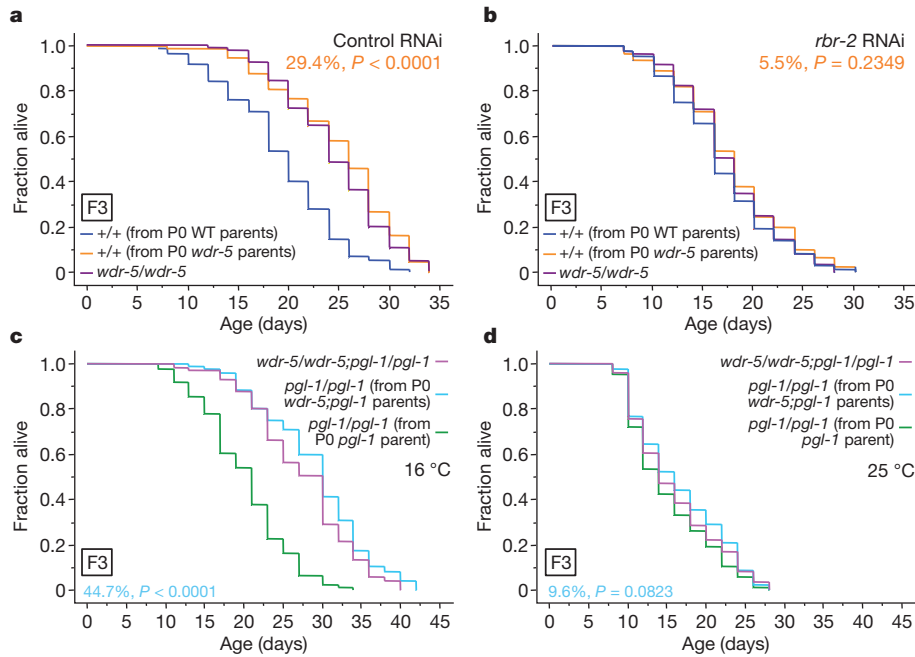


Figure 4 | Transgenerational inheritance of longevity by deficiencies in ASH-2 complex members is dependent on the presence of the H3K4me3 demethylase RBR-2 and an intact germline. **a, b,** Lifespan of genetically wild-type F3 descendants from *wdr-5(ok1417)* mutant worms (+/+ from P0 *wdr-5* parents) in the presence of empty vector (control RNAi) (**a**) or *rbr-2* RNAi

(**b**). **c, d,** Lifespan of *pgl-1* F3 descendants from *wdr-5(ok1417);pgl-1(bn101)* mutant worms (*pgl-1/pgl-1* from P0 *wdr-5;pgl-1* parents) compared with descendants from *pgl-1(bn101)* worms at the permissive temperature (16 °C) (**c**) and at the restrictive temperature (25 °C) (**d**). Mean lifespan and statistics are presented in Supplementary Tables 3 and 4.

with deficiencies in ASH-2 complex members also require the presence of a functioning germline for lifespan extension, we used temperature-sensitive feminized *fem-3(e2006)* mutant worms, which do not produce mature eggs at the restrictive temperature²⁴. Knockdown of *ash-2* and *wdr-5* only in parents extended the lifespan of the F1 generation in *fem-3(e2006)* mutant worms at the permissive temperature (16 °C), but not at the restrictive temperature (25 °C) (Supplementary Fig. 3). To independently examine if the germline is required for the longevity of

wild-type descendants of mutants of ASH-2 complex members, we used *pgl-1(bn101)* temperature-sensitive mutants that cannot form a functioning germline at the restrictive temperature²⁵ (Fig. 4c, d). F3 generation *pgl-1* descendants from *wdr-5;pgl-1* mutant parents no longer had an extended lifespan compared to *pgl-1* descendants from *pgl-1* parents at the restrictive temperature (25 °C) (Fig. 4c, d). Thus, a functioning adult germline is necessary for the long lifespan of wild-type descendants of parents with deficiencies in members of the ASH-2 complex.

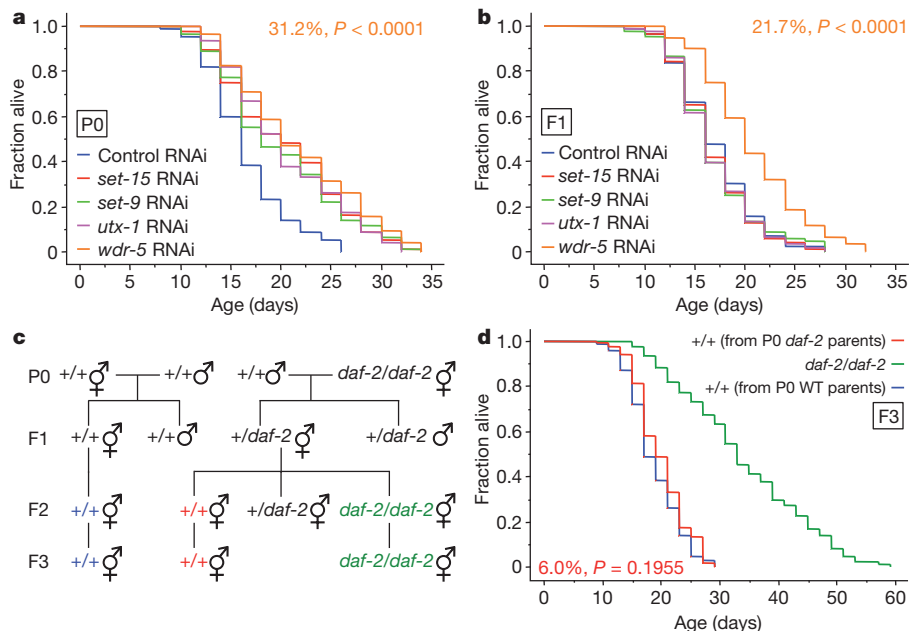


Figure 5 | Other longevity regulators do not have a transgenerational effect on lifespan. **a, b,** Lifespan of P0 (**a**) and F1 (**b**) generation descendants from worms treated with *set-9*, *set-15*, *utx-1* and *wdr-5* RNAi or control RNAi (empty vector) only in the P0 generation. **c,** Scheme for generating wild-type (+/+) F3 progeny from *daf-2(e1370)* mutant worms (*daf-2/daf-2*). **d,** Lifespan of genetically wild-type F3 descendants from *daf-2(e1370)* mutant worms (+/+ from P0 *daf-2* parents). Mean lifespan and statistics are presented in Supplementary Tables 2 and 4.

progeny from *daf-2(e1370)* mutant worms (*daf-2/daf-2*). **d,** Lifespan of genetically wild-type F3 descendants from *daf-2(e1370)* mutant worms (+/+ from P0 *daf-2* parents). Mean lifespan and statistics are presented in Supplementary Tables 2 and 4.

Specificity of epigenetic memory of lifespan

We then asked if the transgenerational inheritance of longevity is specific to H3K4me3 modifiers or if it is also observed with chromatin modifiers of other marks (*set-9*, *set-15* and *utx-1*), and more generally with genes in known longevity pathways: insulin signalling (*age-1* and *dod-23*), mitochondria (*cco-1* and *cyc-1*) and stress resistance (*asm-3*)^{12,17,18,26–32}. In contrast to what we observed for *ash-2* and *wdr-5*, knockdown of *set-9*, *set-15*, *utx-1*, *age-1*, *asm-3*, *cco-1*, *cyc-1* and *dod-23* only in parents did not extend the lifespan of the F1 generation (Fig. 5a, b and Supplementary Fig. 5). Similarly, genetically wild-type F3 descendants from long-lived *daf-2(e1370)*³³ mutant worms (+/+ from P0 *daf-2* parents) had no significant extension of lifespan (6% $P = 0.1955$) (Fig. 5c, d). Collectively, these findings indicate that transgenerational extension of longevity is relatively specific to H3K4me3 chromatin modifiers, and further indicate that the H3K4me3 mark may be important for epigenetic memory of lifespan between generations. As SET-9, SET-15 and UTX-1, unlike members of the ASH-2 complex, regulate lifespan in a manner that

is independent of the germline^{12,17,18}, it is also possible that transgenerational inheritance of longevity is specific to chromatin regulators that act in the germline.

Transgenerational inheritance of gene expression

We next determined if transgenerational inheritance of lifespan is associated with heritable changes in H3K4me3. Western blot and immunocytochemistry showed that global H3K4me3 levels were not decreased in F3 and F4 generation genetically wild-type descendants from *wdr-5* and *set-2* parents or in F1 and F2 generation descendants from *ash-2* or *wdr-5* knockdown only in parents (Fig. 6a, b and Supplementary Figs 6, 7). Thus, transgenerational inheritance of lifespan is unlikely to be mediated by a heritable global decrease in H3K4me3 levels. Transgenerational inheritance of lifespan might be associated with heritable local changes of H3K4me3 at certain loci, which could affect expression of certain genes involved in longevity. To test this idea, we compared gene expression genome-wide in wild-type descendants from *wdr-5* mutant and wild-type ancestors, and

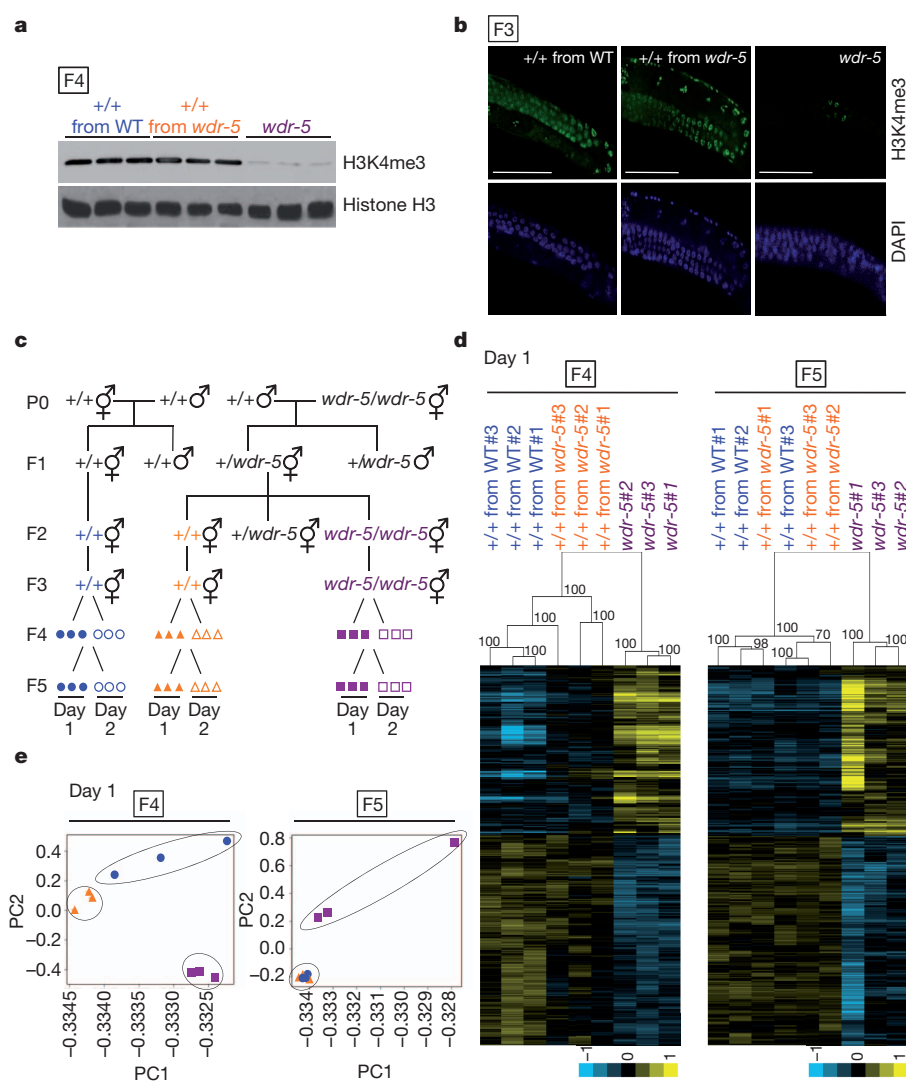


Figure 6 | Genetically wild-type descendants from *wdr-5* mutant parents exhibit differences in gene expression, but not in global H3K4me3 levels, compared to descendants from wild-type parents. **a**, **b**, Global H3K4me3 levels in the F4 generation by western blot (**a**) or in the F3 generation by immunocytochemistry (**b**) of L3 worms from genetically wild-type descendants from *wdr-5* parents (+/+ from *wdr-5*) or wild-type parents (+/+ from WT), and *wdr-5* mutants (*wdr-5*). Scale bars, 50 μ m. **c**, Scheme for generating wild-type descendants from a cross between *wdr-5(ok1417)* null mutant worms and wild-type worms. Symbols represent RNA samples from L3 worms from three

independent F2 ancestors on the first (closed symbols) or second (open symbols) day of egg-laying. **d**, Unbiased hierarchical clustering of WDR-5 regulated genes from the first day of egg-laying (Supplementary Table 9). Pvcust values are displayed on each node of the dendrogram. Values superior to 95 are considered significant. **e**, Principal component analysis (PCA) of the entire microarray data sets from the first day of egg-laying (Supplementary Table 5). PC, principal component. Symbols represent gene expression data from L3 worms collected on the first day of egg-laying (Fig. 6c).

pure *wdr-5* mutant descendants in the F4 and F5 generations (Fig. 6c). For each condition, we collected triplicates of L3 stage worms from the first or second day of egg-laying (Fig. 6c), with the first day of egg-laying corresponding to the samples used for lifespan assays. Statistical analysis of microarray (SAM) identified 759 genes that were differentially regulated in *wdr-5* pure mutants compared to wild-type worms, regardless of the generation (Supplementary Table 7) and egg-laying day (Supplementary Fig. 8a) ($P = 2.38 \times 10^{-116}$, hypergeometric probability). These WDR-5-regulated genes are enriched for longevity, development and growth gene ontology terms (Supplementary Fig. 8b), consistent with WDR-5's reported functions^{12,21,22}. As expected, WDR-5-regulated genes significantly overlap with ASH-2-regulated genes¹² ($P = 6.14 \times 10^{-12}$, hypergeometric probability, Supplementary Fig. 8c) and are enriched for H3K4me3 (refs 34, 35) ($P = 2.49 \times 10^{-34}$, hypergeometric probability, Supplementary Fig. 8d). These observations indicate that WDR-5 functions together with ASH-2 to regulate a subset of genes by modulating H3K4me3 at these loci.

We asked if the expression of some WDR-5-regulated genes might be transgenerationally inherited. Interestingly, a significant subset of WDR-5-regulated genes was still differentially regulated in wild-type descendants from *wdr-5* mutant worms in the F4 generation (Fig. 6d and Supplementary Fig. 10a), but not in the F5 generation (Fig. 6d and Supplementary Fig. 10a), consistent with the return to a normal lifespan in the F5 generation. Unbiased hierarchical clustering analysis showed that WDR-5-regulated genes in wild-type descendants from *wdr-5* mutant versus wild-type parents still clustered separately in the F4, but not the F5 generation (Fig. 6d and Supplementary Fig. 10a). Principal component analysis (PCA) confirmed that overall gene expression in wild-type descendants from *wdr-5* parents versus wild-type parents is easily distinguishable in the F4, but not the F5 generation (Fig. 6e and Supplementary Fig. 10b). Genes with transgenerational inheritance of expression were slightly more enriched for H3K4me3 than expected by chance ($P = 0.0123$ and $P = 0.0769$ for the first and second day of egg-laying, respectively, hypergeometric probability), and may represent the genes that are the most affected by the loss of the H3K4me3 mark. A number of these genes are known longevity regulators and are expressed in the germline (Supplementary Table 7). Gene ontology analysis of genes with transgenerational inheritance of expression shows enrichment for different types of metabolic pathways (Supplementary Figs 9, 10c), raising the possibility that changes in metabolism may play a role in the heritability of the phenotype. The genes with transgenerational inheritance of expression were different on the first versus second day of egg-laying, and were no longer identified when samples from different days of egg-laying were pooled (E.L.G. and A.B., data not shown). This could be because worms produced on the first day of egg-laying might be more susceptible to H3K4me3 depletion, because each collection day may represent a different snapshot in the rapidly changing L3 stage³⁶, or because of inherent stochasticity in the transgenerational inheritance of gene expression. Overall, these results indicate that ancestral H3K4me3 status influences the gene expression of descendants for several generations.

Discussion

Our study provides the first example of epigenetic inheritance of longevity. Histone methylation marks and DNA methylation are generally, but not always, erased between generations with epigenetic reprogramming^{37,38}. Our observations are consistent with the notion that H3K4me3 at specific loci may not be completely erased and replenished. Alternatively, the ASH-2/WDR-5/SET-2 complex could control the expression of the genes responsible for the erasure and replenishment of histone methylation marks between generations. Modulation of H3K4me3 modifiers in parents may also affect an unidentified protein or RNA that could in turn be inherited and cause lifespan changes. Interestingly, H3K4me3 regulators have been suggested to have a role in the inheritance of eye colour in *Drosophila*^{5,6} and of active transcriptional states in *Dictyostelium*³⁹. As the ASH-2

H3K4me3 regulatory complex is conserved from yeast to humans, manipulations of this complex in parents might have a heritable effect on longevity in mammals.

METHODS SUMMARY

Genetic crosses. For genetic crosses, wild-type or *pgl-1* male siblings were crossed to hermaphrodite siblings (to generate wild-type descendants) or to hermaphrodite mutants (to generate wild-type and mutant descendants). F1 hermaphrodites were allowed to lay progeny and were then genotyped by single-worm genotyping to ensure that they were heterozygous at the loci of the mutations of interest. Twenty F2 worms were placed on individual plates and allowed to lay ~40 eggs. F2 parents were then genotyped by single-worm genotyping and the lifespan of three independent lines was analysed. Progeny from each of these independent lines were collected after synchronized egg-laying for the F4 and F5 generations and analysed in lifespan assays. For microarray analysis, RNA samples were isolated at the F4 and F5 generations at the larval stage L3. Each sample was prepared from independent F2 clonal parents in triplicate with ~1,000 worms each.

Lifespan assays. Worm lifespan assays were performed at 20 °C, unless noted differently, without 5-fluoro-2'-deoxyuridine (FUdR), as described previously⁴⁰. For each lifespan assay, 90 worms per condition were used in three plates (30 worms per plate), unless noted differently. Worms that underwent 'matricide', that exhibited ruptured vulva, or crawled off the plates were censored. Statistical analyses of lifespan were performed on Kaplan–Meier survival curves in StatView 5.0.01 by log rank (Mantel–Cox) tests. The values from the Kaplan–Meier curves are included in the Supplementary Tables.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Information The raw unfiltered microarray results are deposited at the Gene Expression Omnibus (GEO) under the Subseries entry GSE31043. The raw unfiltered chromatin immunoprecipitation (ChIP)-chip data are deposited at GEO under the Subseries entry GSE30789. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to A.B. (anne.brunet@stanford.edu).

METHODS

Worm strains. *wdr-5(ok1417)* and *set-2(ok952)* strains were provided by the *Caenorhabditis* Genetics Center. Wild-type (N2), *wdr-5(ok1417)* and *set-2(ok952)* strains were genotyped for *mut-16(mg461)*, a mutation that affects RNAi efficiency and that was found as an extraneous mutation in several laboratory strains. These strains did not contain the *mut-16(mg461)* mutation. *wdr-5(ok1417)* mutant worms were backcrossed four to nine times by crossing wild-type males with *wdr-5(ok1417)* hermaphrodites. *set-2(ok952)* mutant worms were backcrossed four to six times by crossing wild-type males with *set-2(ok952)* hermaphrodites. The transgenerational inheritance of longevity was similar, both in terms of magnitude and number of generation, whether *wdr-5(ok1417)* and *set-2(ok952)* worms were backcrossed four to nine times or four to six times, respectively (see Supplementary Table 1), arguing against a simple backcrossing effect to explain the increased lifespan of wild-type descendants from *wdr-5* or *set-2* parents. *rbr-2(tm1231)* mutant worms were backcrossed seven times, *daf-2(e1370)* mutant worms were backcrossed an additional two times by our lab, *set-2(ok952)* and *rbr-2(tm1231)* were backcrossed two times and six times, respectively, before being crossed together to generate *set-2(ok952);rbr-2(tm1231)* and then crossed an additional time to wild-type worms; *wdr-5(ok1417)* and *pgl-1(bn101ts)* were backcrossed four times each before being crossed together to generate *wdr-5(ok1417);pgl-1(bn101ts)* and then crossed an additional time to *pgl-1(bn101ts)* worms. For crosses involving *set-2(ok952);rbr-2(tm1231)* mutant worms, six F3 progeny were genotyped for each independent line to ensure the genotype of the second mutant loci. Temperature-sensitive *fem-3(e2006)* mutant worms were either maintained at 16 °C or were switched to 25 °C at birth and maintained at this temperature for the entirety of their lifespan. Temperature-sensitive *pgl-1(bn101)* and *wdr-5(ok1417);pgl-1(bn101)* mutant worms were either maintained at 16 °C or were switched to 25 °C at the L4 stage in F2 parents. F3 progeny from these worms was maintained at 16 °C or 25 °C for the entirety of their lifespan.

RNA interference. Adult worms were placed on NGM plates containing ampicillin (100 mg ml⁻¹) and IPTG (0.4 mM) seeded with the respective bacteria and removed after 4–6 h to obtain synchronized populations of worms. HT115 (DE3) bacteria transformed with vectors expressing RNAi to the genes of interest were all obtained from the Ahringer library (a gift from M. W. Tan), except RNAi to *rbr-2* that was from the Open Biosystems library (a gift from K. Shen). At the L4 stage, P0 worms were moved to NGM plates containing streptomycin (300 µg ml⁻¹) seeded with OP50-1 bacteria, which are streptomycin-resistant, to eliminate any potentially remaining RNAi HT115 (DE3) bacteria, which are streptomycin-sensitive. P0 worms were switched to fresh OP50-1 seeded plates every day until day 6 of life (day 2 of adulthood). Day 6 P0 worms were allowed to lay eggs for 4–6 h and progeny from that stage were picked from these plates and their lifespan was examined. Subsequent generations were obtained by placing young adult F1, F2, F3, or F4 worms on fresh OP50-1 seeded plates for 4–6 h. To perform RNAi in *fem-3(e2006)* mutant worms, one set of P0 worms was maintained at 16 °C to allow them to lay eggs for the F1 generation, while a second set of P0 worms was analysed in lifespan assays at both 16 °C and 25 °C. RNAi to *rbr-2* was initiated at the eggs or L1 stage of F3 generation *wdr-5(ok1417)* mutant worms, wild-type descendants of *wdr-5(ok1417)* mutant worms, and wild-type descendants of wild-type parents.

Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). Two hundred worms were picked to NGM plates with OP50-1 bacteria overnight 2 days in a row. Worms were then picked to NGM plates without bacteria and washed three times with M9 buffer (22 mM KH₂PO₄, 34 mM K₂HPO₄, 86 mM NaCl, 1 mM MgSO₄). Worm pellets were resuspended in TRIzol (Invitrogen), followed by six freeze-thaw cycles in liquid N₂. One µg of total RNA was reverse-transcribed with oligo dT primers using Superscript II reverse transcriptase (Invitrogen) according to the manufacturer's protocol. Real time PCR was performed on a Bio-Rad iCycler or Roche LightCycler 480II using iQ SYBR green (Bio-Rad) or LightCycler480 SYBR green I master (Roche) with the following primers: pan-actin F: 5'-TCGGTATGGGACAGAAAGGAC-3', pan-actin R: 5'-CATCCCAGTTGGTGACGATA-3', *ash-2* F: 5'-CGATCGAAA CACGGAACGA-3', *ash-2* R: 5'-TGCCGGAATCTGCAGTTTTT-3', *wdr-5* F: 5'-CCCTGAAACAATACACTGGACACG-3', *wdr-5* R: 5'-AACTGGATGAC AATCGGAGGC-3'. The experiments were conducted in duplicate and the results were expressed as 2^{-(target gene number of cycles - pan-actin number of cycles)}.

Protein analysis by western blot. Worms were synchronously grown to the L3 stage and washed off plates with M9 buffer (22 mM KH₂PO₄, 34 mM K₂HPO₄, 86 mM NaCl, 1 mM MgSO₄). Worms were washed several times in M9 buffer and snap-frozen in liquid N₂. Sample buffer (2.36% SDS, 9.43% glycerol, 5% β-mercaptoethanol, 0.0945 M Tris HCl pH 6.8, 0.001% bromophenol blue) was added to worm pellets and they were repeatedly snap-frozen in liquid N₂. Worm extracts were sonicated three times for 30 s at ~15 W (VirSonic 600) and boiled for 2 min before being resolved on SDS-PAGE (10% or 14%) and transferred to nitrocellulose membranes. The membranes were incubated with primary

antibodies (H3K4me3 (Abcam ab8580, Millipore 07-473), 1:500; H3 (Abcam ab1791), 1:1,000; ASH-2 antibody⁴¹ (a gift from B. J. Meyer), 1:2,000, alpha-tubulin (Sigma T9026), 1:1,000), and the primary antibodies were visualized using horseradish-peroxidase-conjugated anti-rabbit secondary antibody (Calbiochem 401393) and ECL Plus (Amersham Biosciences).

Whole-mount immunocytochemistry. Worms were washed several times to remove bacteria and resuspended in fixing solution (160 mM KCl, 100 mM Tris-HCl pH 7.4, 40 mM NaCl, 20 mM Na₂EGTA, 1 mM EDTA, 10 mM spermidine HCl, 30 mM PIPES pH 7.4, 1% Triton X-100, 50% methanol, 2% formaldehyde) and subjected to two rounds of snap freezing in liquid N₂. The worms were fixed at 4 °C for 30 min and washed briefly in T buffer (100 mM Tris HCl pH 7.4, 1 mM EDTA, 1% Triton X-100) before a 1 h incubation in T buffer supplemented with 1% β-mercaptoethanol at 37 °C. The worms were washed with borate buffer (25 mM H₃BO₃, 12.5 mM NaOH pH 9.5) and then incubated in borate buffer containing 10 mM DTT for 15 min. Worms were blocked in PBST (PBS pH 7.4, 0.5% Triton X-100, 1 mM EDTA) containing 1% BSA for 30 min and incubated overnight with H3K4me3 antibody (Millipore 07-473; 1:100) and with Alexa Fluor 594 secondary antibody (Invitrogen; 1:25–1:100). DAPI (2 mg ml⁻¹) was added to visualize nuclei. The worms were mounted on a microscope slide and visualized using a Leica SP2 confocal system or a Zeiss Axioskop2 plus fluorescence microscope.

Single-worm genotyping. Single worms were placed in 5 µl of worm lysis buffer (50 mM KCl, 10 mM Tris pH 8.3, 2.5 mM MgCl₂, 0.45% NP40, 0.45% Tween-20, 0.01% gelatin (w/v) and 60 mg ml⁻¹ proteinase K), and incubated at -80 °C for 1 h, 60 °C for 1 h, and then 95 °C for 15 min. PCR reactions were performed using the following primers: *set-2* F: 5'-TGAAAGGATGATACTCGTGGGC-3', *set-2* R: 5'-CGATGAGAGAAAGGGGATTTTGTAAAC-3', *wdr-5* F: 5'-TTGTGTGT TCGCTGTGCATG-3', *wdr-5* R: 5'-GTATTTGCTCTCGGTGCATC-3', *mut-16* F: 5'-AATATTCGATCGGCAAGCAG-3', *mut-16* R: 5'-CCCAGGATACAG AAATAA-3', *rbr-2* F: 5'-CAAGTGTCTGTGTGCTGTGG-3', *rbr-2* R: 5'-TGGCGATTGGAACTCCGAG-3', *pgl-1* F: 5'-TGATGTGATTGCCGAG GAACAC-3', *pgl-1* R: 5'-GCTGAAGAAGACTGAAGACGCTAAG-3', *daf-2* F: 5'-ACCTGGATCGCTCAAGTTTG-3', *daf-2* R: 5'-TGCTTCGCTTTCAT CCGTGTGTC-3'.

PCR reactions were performed according to the manufacturer's protocol (Qiagen) and PCR reactions were resolved on agarose gels. *daf-2* PCR products were digested with BlnI to distinguish between wild-type and *daf-2(e1370)* genotypes. *pgl-1* PCR products were digested with MseI to distinguish between wild-type and *pgl-1(bn101)* genotypes.

Microarray analysis. Total RNA was isolated using an RNAqueous kit (Ambion). Microarray hybridization was performed at the Stanford Protein and Nucleic Acid facility with oligonucleotide arrays (Affymetrix, GeneChip *C. elegans* Genome Arrays). The raw unfiltered microarray results are deposited at the Gene Expression Omnibus (GEO) under the Subseries entry GSE31043. Background adjustment and normalization was performed with RMA (Robust Multiarray Analysis). Two-class unpaired analysis in significance analysis of microarrays (SAM)⁴² was performed with 100 permutations, a 10⁶ seed for the random number generator and a 5% false discovery rate (FDR) to compare gene expression in *wdr-5* mutants and wild-type descendants from wild-type parents. To obtain a 5% FDR, a 1.06 delta value was used for samples collected at the first day of egg-laying (day 1) and a 0.93 delta value for samples collected at the second day of egg-laying (day 2). Significantly changed probes from these two lists were then used to compare wild-type descendants from wild-type parents to wild-type descendants from *wdr-5* parents in each generation using a 5% FDR. To obtain a 5% FDR, a 0.66 delta value was used for the F4 generation at day 1, a 0.09 delta value was used for the F5 generation at day 1, a 0.92 delta value was used for the F4 generation at day 2, and a 0.61 delta value was used for the F5 generation at day 2. Similar results for wild-type descendants from *wdr-5* parents compared to wild-type descendants from wild-type parents were observed when SAM was performed with the entire normalized lists of genes.

Hierarchical clustering. A complete linkage hierarchical clustering on the subset of WDR-5 regulated genes for each day (Supplementary Tables 9 and 10) was performed using Gene Cluster 3.0. Clustering results were analysed further with Java TreeView. Further statistical analysis was performed using Pvcust⁴³. For the clustering analysis, genes and then arrays were centred using the mean. The R package Pvcust was used to apply complete linkage hierarchical clustering. As the data were centred, the uncentred Pearson correlation coefficient was used as a similarity measure, which was subsequently modified to dissimilarity by subtracting from 1. Experiments were conducted with 1,000 bootstrap replications.

Principal component analysis. Principal component analysis (PCA)⁴⁴ was conducted on the entire normalized lists of genes (Supplementary Tables 5 and 6). The data were centred to obtain unit variance before conducting the PCA analysis. The Pcomp function in the R package 'Stats' was used. The first and the second principal components (PC1 and PC2) were plotted.

H3K4me3 chromatin immunoprecipitation (ChIP)-chip data set from modENCODE and comparison between data sets. The H3K4me3 ChIP-chip data set was generated by the modENCODE consortium from worms at the L3 stage^{34,35}. The data, protocols, and antibody information can be accessed at the modENCODE Data Coordination Center (<http://intermine.modencode.org>), accession ID 3550. Use of this data set during the publication moratorium period was approved (S. Strome, personal communication). The raw unfiltered ChIP-chip data are deposited at GEO under the Subseries entry GSE30789. H3K4me3 ChIP intensity signals were divided by Input signals, log transformed, centred to mean zero, and scaled to standard deviation one. H3K4me3 enrichment peaks (4493) were called using the program ChIPOTle (ref. 45, <http://sourceforge.net/projects/chipotle-2/>) with a *P*-value cut-off of 10^{-20} , window size 500 bp, step size 100 bp, and the Bonferroni *P*-value correction. A list of gene coordinates (transcript start-end) was obtained from WormBase WS170 (<http://www.wormbase.org/>). Peaks were mapped to 5,062 genes by identifying the genes that had peaks

overlap with their 5' region (500 bp upstream and downstream from the transcript start site). For comparisons between different data sets, hypergeometric probabilities were calculated using <http://stattrek.com/Tables/Hypergeometric.aspx>.

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