

Additional file 1:Fig. S1

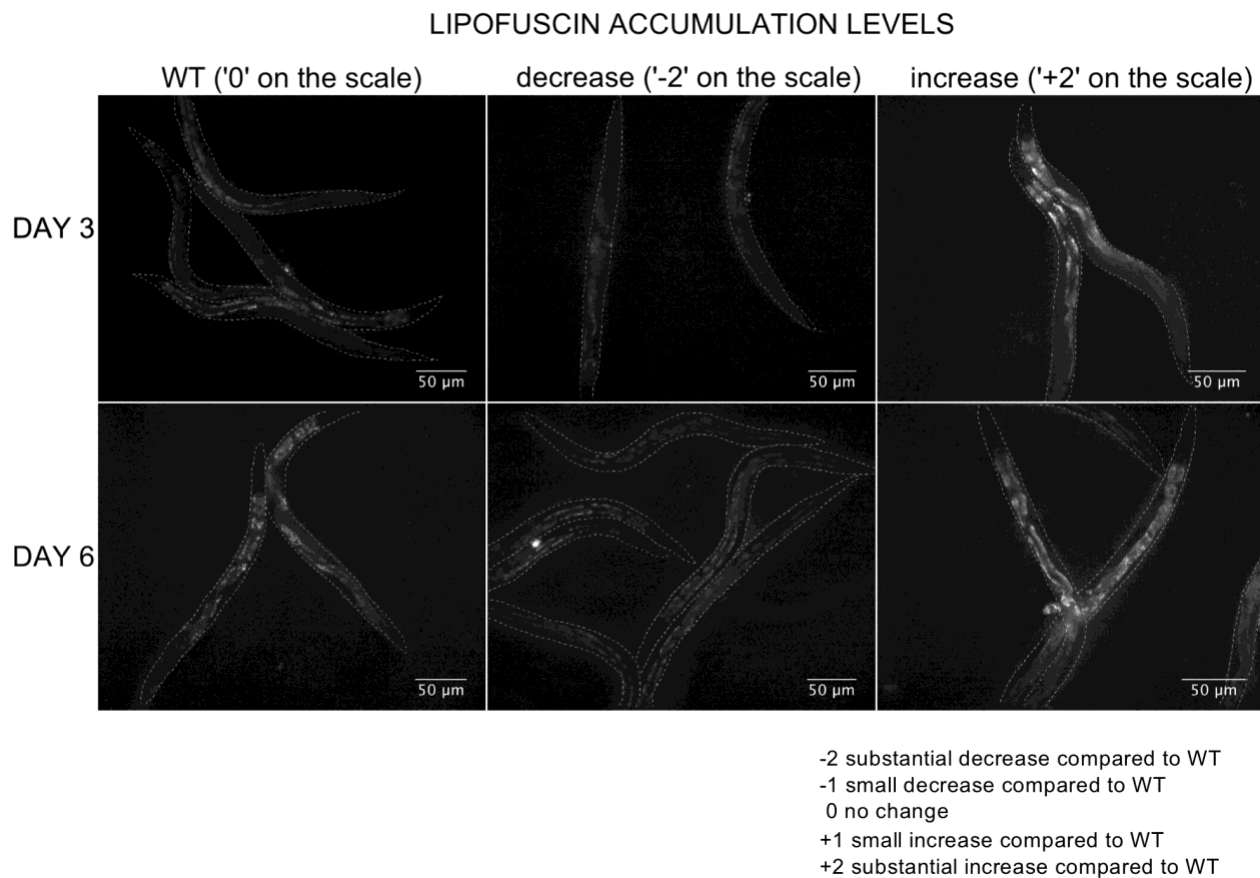


Figure S1. Examples of changes in lipofuscin accumulation after exposure to RNAi feeding clones

30 F1 progeny were picked at the L4 stage and assessed for lipofuscin accumulation at day 3 and day 6 of adulthood (15 worms/time point). Each set of worms analysed included the EV control to show the changes in lipofuscin levels at comparable ages. Accumulation of lipofuscin was assessed by eye in anaesthetised worms using a Zeiss AxioSKOP2 microscope, using the severity grading system detailed in the methods section.

Additional file 2:Table S1

| RNAi clone | median survival | maximum lifespan | Log Rank Test <i>p</i> value relative to EV control |
|----------------------------|-----------------|------------------|---|
| EV | 15 | 28 | |
| <i>mes-2</i> | 17 | 33 | 0.03 (*) |
| <i>jmjd-3.2</i> | 18 | 33 | 0.007 (**) |
| <i>cbp-1 (post larval)</i> | 18 | 27 | 0.008 (**) |
| <i>isw-1</i> | 18 | 34 | 0.003 (**) |
| EV | 15 | 25 | |
| <i>mes-3</i> | 20 | 35 | <0.0001 (****) |
| <i>mes-6</i> | 20 | 31 | <0.0001 (****) |
| EV | 18 | 28 | |
| <i>utx-1</i> | 20 | 32 | 0.003 (**) |
| EV | 13 | 25 | |
| <i>jmjd-3.1</i> | 13 | 21 | 0.43 (ns) |
| <i>jmjd-3.3</i> | 11 | 25 | 0.28 (ns) |

Table S1. Lifespan analysis of worms subjected to RNAi by feeding clones identified in and inspired by the primary lipofuscin screen

Lifespan was monitored following RNAi of the indicated gene. Average lifespan (in days) is shown, together with relevant *p* values compared with the control. In the case of *cbp-1*, where RNAi resulted in larval lethality of F1s, first generation P0 L4 worms were picked onto RNAi plates containing FUDR, and these were scored for lifespan extension. In the case of *mes-3* and *mes-6* RNAi, 2nd generation F2 worms were scored for lifespan extension. *****p*<0.0001, ****p*<0.001, ***p*<0.01, **p*<0.05, ns=not significant. EV= Empty Vector control (*i.e.* worms fed HT115 bacteria transformed with L4440 RNAi vector lacking a genomic insert). At least 60 animals were analysed for each condition.

Additional file 3:Table S2

| Fig ref | Strain / condition | no. of animals | mean lifespan | % lifespan change (vs control) | median lifespan | maximum lifespan | Log Rank Test <i>p</i> value relative to control |
|---------------|-------------------------|----------------|---------------|--------------------------------|-----------------|------------------|--|
| 1B | N2 control | 46 | 17.6 | | 18 | 26 | |
| | <i>mes-2(tm5007)</i> | 47 | 21.2 | 20% increase | 22 | 30 | 0.0003 (***) |
| | <i>mes-2(ok2480)</i> | 47 | 28.4 | 61% increase | 30 | 42 | <0.0001 (****) |
| 1B rep | N2 control | 95 | 20.5 | | 20 | 27 | |
| | <i>mes-2(tm5007)</i> | 95 | 22.5 | 10% increase | 22 | 33 | <0.0001 (****) |
| | N2 control | 51 | 15.6 | | 16 | 23 | |
| | <i>mes-2(ok2480)</i> | 50 | 21.7 | 36% increase | 21 | 33 | <0.0001 (****) |
| 1C | N2 control | 55 | 18.3 | | 18 | 30 | |
| | SP127 balancer control | 54 | 17.8 | | 18 | 28 | 0.4 (ns) |
| | <i>mes-2(bn11)</i> | 46 | 22.2 | 21% increase (vs N2) | 22 | 36 | <0.0001 (****) |
| | <i>mes-2(bn11)/+</i> | 59 | 22.6 | 23% increase (vs N2) | 22 | 38 | 0.0005 (***) compared with N2 |
| | | | | | | | |
| 1C rep | N2 control | 48 | 15.2 | | 15 | 27 | |
| | <i>mes-2(bn11)</i> | 59 | 19.6 | 30% increase (vs N2) | 19 | 35 | <0.0001 (****) |
| | N2 control | 95 | 20.5 | | 20 | 27 | |
| | <i>mes-2(bn11)/+</i> | 79 | 22.4 | 9% increase (vs N2) | 22 | 28 | <0.0001 (****) |
| 1E | N2 control | 46 | 17.6 | | 18 | 26 | |
| | <i>jmjd-3.2(tm3121)</i> | 45 | 20.5 | 16% increase | 20 | 32 | 0.005 (**) |
| 1E rep | N2 control | 55 | 18.3 | | 18 | 30 | |
| | <i>jmjd-3.2(tm3121)</i> | 57 | 20.8 | 14% increase | 20 | 34 | 0.012 (*) |
| 1F | N2 control | 48 | 14.1 | | 14 | 22 | |
| | <i>utx-1(tm3118)/+</i> | 42 | 19.4 | 38% increase | 20 | 34 | <0.0001 (****) |
| 1F rep | (see Fig 2C or 3F) | | | | | | |

Table S2. Statistical analysis of lifespan data relating to Figure 1

Full statistical analysis of lifespan data from Fig. 1 (**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns=not significant). Rep = repeat.

Additional file 4:Fig. S2

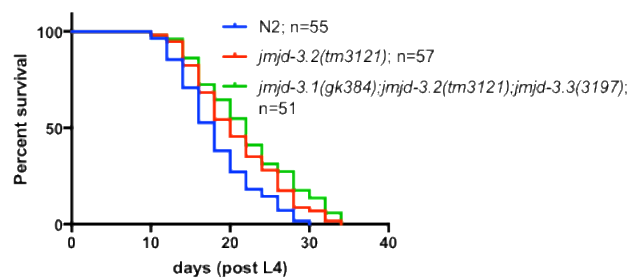


Figure S2. Lifespan analysis of *jmjd-3* mutants

The lifespan of *jmjd-3.2* animals was comparable to that of the *jmjd-3.1*; *jmjd-3.2*; *jmjd-3.3* triple mutants ($p=0.19$). *jmjd-3.2* animals are longer-lived compared to WT animals ($p=0.01$ (*)). See Additional file 5:Table S3 for the full statistical analysis of the lifespan data, including repeats.

Additional file 5:Table S3

| Fig ref | Strain / condition | no. of animals | mean lifespan | % lifespan change (vs control) | median lifespan | maximum lifespan | Log Rank Test <i>p</i> value relative to control |
|---------|--|----------------|---------------|--------------------------------|-----------------|------------------|--|
| S2 | N2 | 55 | 18.3 | | 18 | 30 | |
| | <i>jmjd-3.2(tm3121)</i> | 57 | 20.8 | 14% increase | 20 | 34 | 0.0117 (*) |
| | <i>jmjd-3.1(gk384); jmjd-3.2(tm3121); jmjd-3.3(tm3197)</i> | 51 | 22.2 | | 22 | 34 | 0.19 (ns) compared with <i>jmjd-3.2(tm3121)</i> |
| S2 rep | N2 | 79 | 14.7 | | 15 | 21 | |
| | <i>jmjd-3.2(tm3121)</i> | 76 | 16.1 | 10% increase | 16 | 25 | 0.003 (**) |
| | <i>jmjd-3.1(gk384); jmjd-3.2(tm3121); jmjd-3.3(tm3197)</i> | 75 | 15.5 | | 15 | 27 | 0.54 (ns) compared with <i>jmjd-3.2(tm3121)</i> |

Table S3. Statistical analysis of lifespan data relating to Figure S2

Full statistical analysis of lifespan data from Fig. S2 (*****p*<0.0001, ****p*<0.001, ***p*<0.01, **p*<0.05, ns=not significant). EV = empty vector control. Rep = repeat.

Additional file 6:Fig. S3

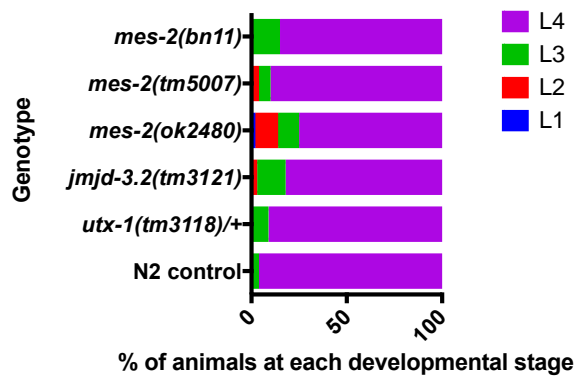


Figure S3. Developmental rates of *mes-2*, *jmjd-3.2* and *utx-1* mutant alleles

Gravid adults of each genotype were allowed to lay eggs for 1 hour before being removed from NGM plates seeded with OP50. The resulting progeny and their corresponding developmental stage were recorded after 72 hours at 20°C. n >60 animals for each strain.

Additional file 7:Table S4

| Fig ref | Strain / condition | no. of animals | mean lifespan | % lifespan change (vs control) | median lifespan | maximum lifespan | Log Rank Test <i>p</i> value relative to control |
|---------------|--|----------------|---------------|---|-----------------|------------------|---|
| 2A | N2 EV control | 50 | 17.2 | | 16 | 26 | |
| | <i>daf-16</i> RNAi | 56 | 13.6 | 21% decrease | 14 | 22 | <0.0001 (****) |
| | <i>mes-2(tm5007)</i> + EV control | 51 | 19.0 | 11% increase | 18 | 30 | 0.02 (*) |
| | <i>mes-2(tm5007)</i> + <i>daf-16</i> RNAi | 49 | 14.0 | 26% decrease (vs <i>mes-2</i> EV) | 14 | 18 | 0.94 (ns) compared with <i>daf-16</i> <0.0001 (****) compared with <i>mes-2</i> |
| 2A rep | N2 EV control | 57 | 14.3 | | 13 | 23 | |
| | <i>daf-16</i> RNAi | 56 | 12.5 | 13% decrease | 13 | 17 | 0.0008 (***) |
| | <i>mes-2(tm5007)</i> + EV control | 56 | 16.1 | 13% increase | 15 | 30 | 0.02 (*) |
| | <i>mes-2(tm5007)</i> + <i>daf-16</i> RNAi | 57 | 11.8 | 27% decrease (vs <i>mes-2</i> EV) | 13 | 15 | 0.08 (ns) compared with <i>daf-16</i> <0.0001 (****) compared with <i>mes-2</i> |
| 2B | N2 EV control | 50 | 17.2 | | 16 | 26 | |
| | <i>daf-16</i> RNAi | 57 | 13.8 | 20% decrease | 14 | 22 | <0.0001 (****) |
| | <i>jmjd-3.2(tm3121)</i> + EV control | 59 | 18.6 | 8% increase | 18 | 30 | 0.04 (*) |
| | <i>jmjd-3.2(tm3121)</i> + <i>daf-16</i> RNAi | 54 | 14.3 | 23% decrease (vs <i>jmjd-3.2</i> in EV) | 14 | 22 | 0.36 (ns) compared with <i>daf-16</i> <0.0001 (****) compared with <i>jmjd-3.2</i> |
| 2B rep | N2 EV control | 79 | 14.7 | | 15 | 21 | |
| | <i>daf-16</i> RNAi | 79 | 13 | 12% decrease | 13 | 19 | <0.0001 (****) |
| | <i>jmjd-3.2(tm3121)</i> + EV control | 76 | 16.1 | 10% increase | 16 | 25 | 0.003 (**) |

| | | | | | | | |
|-------------------|---|----|------|---|----|----|--|
| | <i>jmjd-3.2(tm3121)</i> + <i>daf-16</i> RNAi | 79 | 13.2 | 18% mdedcrease (vs <i>jmjd-3.2</i> in EV) | 13 | 20 | 0.68 (ns) compared with <i>daf-16</i> <0.0001 (****) compared with <i>jmjd-3.2</i> |
| 2C# | N2 EV control | 50 | 17.1 | | 16 | 26 | |
| | | 57 | 13.8 | 19% decrease | 14 | 22 | <0.0001 (****) |
| | <i>daf-16</i> RNAi | | | | | | |
| | <i>utx-1(tm3118)/+</i> + EV control | 52 | 21.3 | 25% increase | 20 | 34 | <0.0001 (****) |
| | <i>utx-1(tm3118)/+</i> + <i>daf-16</i> RNAi | 46 | 13.6 | 36% decrease (vs <i>tm3118</i> in EV) | 14 | 20 | 0.58 (ns) compared with <i>daf-16</i> <0.0001 (****) compared with <i>utx-1</i> |
| 2D | N2 EV control | 49 | 17.8 | | 18 | 28 | |
| | N2 + <i>utx-1</i> RNAi | 55 | 20.8 | 17% increase | 20 | 32 | 0.003 (**) |
| | <i>jmjd-3.2(tm3121)</i> + EV | 53 | 19.7 | 11% increase | 20 | 32 | 0.03 (*) |
| | <i>jmjd-3.2(tm3121)</i> + <i>utx-1</i> RNAi | 56 | 22.5 | 14% increase (vs <i>jmjd-3.2</i> in EV) | 22 | 36 | 0.007 (**) compared with <i>jmjd-3.2 EV</i> |
| | | | | | | | |
| 2D rep | N2 EV control | 79 | 14.7 | | 15 | 21 | |
| | N2 + <i>utx-1</i> RNAi | 79 | 20.8 | 41% increase | 19 | 34 | <0.0001 (****) |
| | <i>jmjd-3.2(tm3121)</i> + EV | 76 | 16.1 | 10% increase | 16 | 25 | 0.003 (**) |
| | <i>jmjd-3.2(tm3121)</i> + <i>utx-1</i> RNAi | 74 | 20.3 | 26% increase (vs <i>jmjd-3.2</i> in EV) | 20 | 37 | <0.0001 (****) compared with <i>jmjd-3.2 EV</i> |
| | | | | | | | |

Table S4. Statistical analysis of lifespan data relating to Fig 2

Full statistical analysis of lifespan data from Fig. 2 (****p<0.0001, ***p<0.001, **p<0.01, *p<0.05, ns=not significant). #consistent with data reported in [14,15]. EV = empty vector control. Rep = repeat.

Additional file 8:Fig. S4

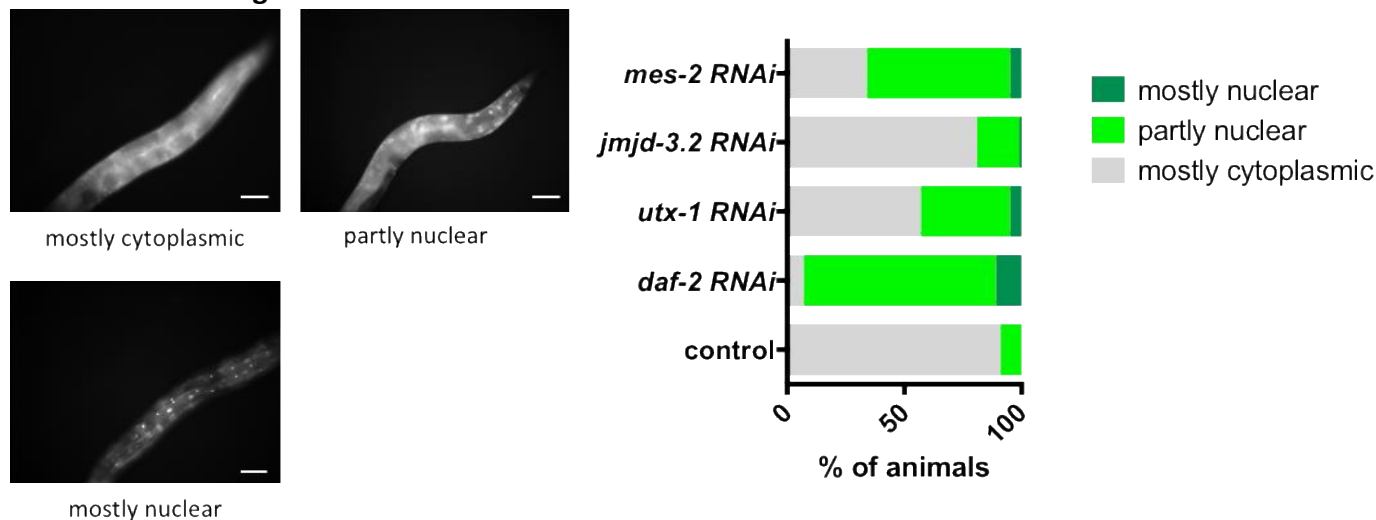


Figure S4. DAF-16::GFP translocation following *mes-2*, *jmjd-3.2* and *utx-1* RNAi

Nuclear translocation of *daf-16* using a translational GFP reporter (strain TJ356) was visualised in worms subjected to *mes-2*, *jmjd-3.2*, *utx-1* and *daf-2* RNAi. Worms were placed on RNAi plates at the L4 stage at 20°C and their progeny imaged using fluorescence microscopy when they had reached the third day of adulthood (n=at least 160 animals for each condition). Worms were divided into three categories, depending on the relative degree of DAF-16::GFP nuclear translocation: “mostly cytoplasmic” (where DAF-16::GFP is primarily localised in the cytoplasm), “partly nuclear” (where DAF-16::GFP is localised in both cytoplasm and nuclei) and “mostly nuclear” (where DAF-16::GFP is primarily localised in nuclei). A representative image for each class is shown in the left-hand panel, and the localisation data is shown in the right-hand panel. We used *daf-2* RNAi as a positive control, as this has previously been shown to cause extensive translocation of DAF-16 to the nucleus [14]. Scale Bar = 100µM.

Additional file 9:Table S5

| Fig ref | Strain / condition | no. of animals | mean lifespan | % lifespan change (vs control) | median lifespan | maximum lifespan | Log Rank Test <i>p</i> value relative to control |
|---------|---------------------------|----------------|---------------|------------------------------------|-----------------|------------------|--|
| 3C | N2 control | 59 | 16.5 | | 16 | 24 | |
| | N2 + <i>jmjd-3.2 OE</i> | 55 | 21.0 | 27% increase | 20 | 36 | <0.0001 (****) |
| | N2 + <i>jmjd-3.2DD OE</i> | 59 | 22.0 | 33% increase | 22 | 34 | <0.0001 (****) compared with <i>N2 control</i> 0.57 (ns) compared with <i>jmjd-3.2 OE</i> |
| 3C rep | N2 control | 53 | 13.9 | | 14 | 22 | |
| | N2 + <i>jmjd-3.2 OE</i> | 50 | 20.7 | 49% increase | 20 | 32 | <0.0001 (****) |
| | N2 + <i>jmjd-3.2DD OE</i> | 53 | 19.8 | 42% increase | 20 | 32 | <0.0001 (****) compared with <i>N2 control</i> 0.61 (ns) compared with <i>jmjd-3.2 OE</i> |
| 3D | N2 control | 55 | 18.3 | | 18 | 30 | |
| | N2 + <i>utx-1 OE</i> | 49 | 23.1 | 26% increase | 22 | 38 | <0.0001 (****) |
| | N2 + <i>utx-1DD OE</i> | 54 | 17 | 26% decrease (vs <i>utx-1 OE</i>) | 18 | 28 | 0.11 (ns) compared with <i>N2 control</i> <0.0001 (****) compared with <i>utx-1 OE</i> |
| 3D rep | N2 control | 52 | 17.5 | | 16 | 28 | |
| | N2 + <i>utx-1 OE</i> | 58 | 20.6 | 18% increase | 20 | 36 | 0.0013 (**) |

| | | | | | | | |
|----|--|----|------|--|----|----|---|
| | N2 + <i>utx-1DD OE</i> | 57 | 17.1 | 17% decrease (vs <i>utx-1 OE</i>) | 16 | 26 | 0.58 (ns) compared with <i>N2 control</i> 0.0002 (***) compared with <i>utx-1 OE</i> |
| 3E | N2 control | 99 | 15.6 | | 16 | 26 | |
| | <i>jmjd-3.2(tm3121)</i> | 45 | 20.2 | 29% increase | 20 | 32 | <0.0001 (****) |
| | <i>jmjd-3.2(tm3121)+ jmjd-3.2 OE</i> | 53 | 18.3 | 17% increase (vs N2) | 18 | 28 | 0.0007 (***) compared with <i>N2 control</i> 0.09 (ns) compared with <i>jmjd- 3.2(tm3121)</i> |
| | <i>jmjd-3.2(tm3121)+ jmjd-3.2DD OE</i> | 58 | 18.1 | 16% increase (vs N2) | 18 | 30 | 0.0008 (***) compared with <i>N2 control</i> 0.14 (ns) compared with <i>jmjd- 3.2(tm3121)</i> 0.81 (ns) compared with <i>jmjd- 3.2(tm3121) + jmjd-3.2 OE</i> |
| 3F | N2 control | 48 | 14.9 | | 14 | 22 | |
| | <i>utx-1(tm3118)/+</i> | 42 | 21.0 | 41% increase | 20 | 34 | <0.0001 (****) |
| | <i>utx-1(tm3118) + utx-1 OE</i> | 44 | 29.6 | 99% increase (vs N2) 41% increase (vs <i>utx- 1(tm3118/+)</i>) | 30 | 50 | <0.0001 (****) compared with <i>N2 control</i> <0.0001 (****) compared with <i>utx- 1(tm3118)/+</i> |
| | <i>utx-1(tm3118) + utx-1DD OE</i> | 44 | 20.1 | 35% increase (vs N2) | 19 | 34 | <0.0001 (****) |

| | | | | | | | |
|--------|-----------------|----|------|--|----|----|---|
| | | | | 32% decrease (vs utx-1(tm3118)+utx-1 OE) | | | compared with N2 control 0.49 (ns) compared with utx-1(tm3118)/+ <0.0001 (****) compared with utx-1(tm3118) + utx-1 OE |
| 3F rep | N2 control | 56 | 14.7 | | 14 | 24 | |
| | utx-1(tm3118)/+ | 46 | 16.9 | 15% increase | 16 | 30 | 0.0067 (**) |
| | utx-1(tm3118) | 46 | 21.3 | 45% increase (vs N2) 26% increase (vs utx-1(tm3118)/+) | 18 | 38 | <0.0001 (****) compared with N2 control <0.0001 (****) compared with utx-1(tm3118)/+ |
| | + utx-1 OE | | | | | | |
| | utx-1(tm3118) | 54 | 17.8 | 21% increase (vs N2) 16% decrease (vs utx-1(tm3118)+utx-1 OE) | 18 | 30 | 0.0001 (***) compared with N2 control 0.47 (ns) compared with utx-1(tm3118)/+ 0.0041 (**) compared with utx-1(tm3118) + utx-1 OE |

Table S5. Statistical analysis of lifespan data relating to Figure 3

Full statistical analysis of lifespan data from Fig. 3 (****p<0.0001, ***p<0.001, **p<0.01, *p<0.05, ns=not significant). Rep = repeat.

Additional file 10:Table S6

| Fig ref | Strain / condition | no. of animals | mean survival | % survival change (vs control) | median survival | maximum survival | Log Rank Test <i>p</i> value relative to control |
|---------|--|----------------|---------------|--------------------------------|-----------------|------------------|--|
| 4A | N2 control + 10mM paraquat | 47 | 49 hours | | 52 hours | 72 hours | |
| | <i>utx-1 OE</i> + 10mM paraquat | 49 | 61 hours | 25% increase | 59 hours | 95 hours | <0.0001 (****) |
| 4A rep | N2 control + 10mM paraquat | 50 | 49 hours | | 50 hours | 66 hours | |
| | <i>utx-1 OE</i> + 10mM paraquat | 49 | 57 hours | 16% increase | 61 hours | 89 hours | 0.0212 (*) |
| 4B | N2 control + UV (1000Jm ⁻²) | 40 | 4.6 | | 5 | 9 | |
| | <i>utx-1 OE</i> + UV (1000Jm ⁻²) | 45 | 6.5 | 41% increase | 7 | 11 | <0.0001 (****) |
| 4B rep | N2 control + UV (1000Jm ⁻²) | 55 | 3.9 | | 4 | 7 | |
| | <i>utx-1 OE</i> + UV (1000Jm ⁻²) | 54 | 5.4 | 38% increase | 6 | 10 | <0.0001 (****) |
| 4C | N2 control + 35°C acute heat stress | 40 | 6.9 | | 7.5 | 12 | |
| | <i>utx-1 OE</i> + 35°C acute heat stress | 45 | 18 | 161% increase | 20 | 30 | <0.0001 (****) |

Table S6. Statistical analysis of lifespan data relating to Figure 4

Full statistical analysis of survival data from Fig. 4 (*****p*<0.0001, ****p*<0.001, ***p*<0.01, **p*<0.05, ns=not significant). EV = empty vector control. Rep = repeat.

Additional file 11:Table S7

| Fig ref | Strain / condition | no. of animals | mean lifespan | % lifespan change (vs control) | median lifespan | maximum lifespan | Log Rank Test <i>p</i> value relative to control |
|-----------------|---|----------------|---------------|---|-----------------|------------------|---|
| 5A | N2 + EV control | 52 | 17.5 | | 16 | 28 | |
| | N2 + <i>daf-16</i> RNAi | 57 | 13.8 | 21% decrease | 14 | 22 | <0.0001 (****) compared with N2 control |
| | <i>utx-1(tm3118)</i> + <i>utx-1 OE</i> + EV control | 58 | 31.3 | 79% increase | 30 | 46 | <0.0001 (****) compared with N2 control |
| | <i>utx-1(tm3118)</i> + <i>utx-1 OE</i> + <i>daf-16</i> RNAi | 58 | 17.0 | 23% increase (vs N2 in <i>daf-16</i> RNAi) 46% decrease (vs <i>utx-1 OE</i> in <i>daf-16</i> RNAi) | 16 | 24 | <0.0001 (****) compared with <i>daf-16</i> RNAi <0.0001 (****) compared with <i>utx-1(tm3118)+utx-1 OE</i> |
| 5A rep | N2 + EV control | 54 | 16.3 | | 16 | 29 | |
| | N2 + <i>daf-16</i> RNAi | 56 | 13.3 | 18% decrease | 14 | 17 | <0.0001 (****) compared with N2 control |
| | <i>utx-1(tm3118)</i> + <i>utx-1 OE</i> + EV control | 57 | 30.0 | 84% increase | 31 | 47 | <0.0001 (****) compared with N2 control |
| | <i>utx-1(tm3118)</i> + <i>utx-1 OE</i> + <i>daf-16</i> RNAi | 53 | 14.2 | 7% increase (vs N2 in <i>daf-16</i> RNAi) 53% decrease (vs <i>utx-1 OE</i> in <i>daf-16</i> RNAi) | 14 | 19 | 0.0005 (***) compared with <i>daf-16</i> RNAi <0.0001 (****) compared with <i>utx-1(tm3118)+utx-1 OE</i> |
| 5B [#] | N2 + EV control | 56 | 14.5 | | 14 | 27 | |
| | N2 + <i>utx-1</i> RNAi | 56 | 19 | 31% increase | 19 | 28 | <0.0001 (****) |
| | <i>daf-2(e1370)</i> + EV control | 55 | 38.9 | 168% increase (vs N2) | 42 | 61 | <0.0001 (****) compared with N2 |

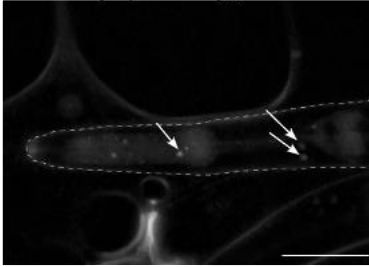
| | | | | | | | |
|-----------|---|----|------|---|----|----|--|
| | <i>daf-2(e1370) + utx-1 RNAi</i> | 58 | 39.1 | 170% increase (vs N2) | 42 | 56 | <0.0001 (****) compared with N2 0.45 (ns) compared with <i>daf-2(e1370)</i> |
| 5C | N2 | 58 | 17.2 | | 19 | 28 | |
| | <i>utx-1(tm3118) + utx-1 OE</i> | 56 | 27.9 | 62% increase | 32 | 42 | <0.0001 (****) compared with N2 |
| | <i>daf-2(e1370)</i> | 58 | 36.1 | 110% increase (vs N2) 29% increase (vs <i>utx-1(tm3118) + utx-1 OE</i>) | 39 | 53 | <0.0001 (****) compared with N2 <0.0001 (****) compared with <i>utx-1(tm3118) + utx-1 OE</i> |
| | <i>daf-2(e1370); utx-1(tm3118) + utx-1 OE</i> | 60 | 40.7 | 46% increase (vs <i>utx-1(tm3118) + utx-1 OE</i>) 13% increase (vs <i>daf-2(e1370)</i>) | 47 | 61 | <0.0001 (****) compared with <i>utx-1(tm3118) + utx-1 OE</i> 0.0023 (**) compared with <i>daf-2(e1370)</i> |
| 5C rep | N2 | 47 | 15.7 | | 17 | 23 | |
| | <i>utx-1(tm3118) + utx-1 OE</i> | 46 | 20.1 | 28% increase | 21 | 35 | <0.0001 (****) compared with N2 |
| | <i>daf-2(e1370)</i> | 59 | 33.0 | 110% increase (vs N2) 64% increase (vs <i>utx-1(tm3118) + utx-1 OE</i>) | 32 | 48 | <0.0001 (****) compared with N2 <0.0001 (****) compared with <i>utx-1(tm3118) + utx-1 OE</i> |
| | <i>daf-2(e1370); utx-1(tm3118) + utx-1 OE</i> | 52 | 42.0 | 109% increase (vs <i>utx-1(tm3118) + utx-1 OE</i>) 27% increase (vs <i>daf-2(e1370)</i>) | 42 | 62 | <0.0001 (****) compared with <i>utx-1(tm3118) + utx-1 OE</i> <0.0001 (****) compared with <i>daf-2(e1370)</i> |

Table S7. Statistical analysis of lifespan data relating to Figure 5

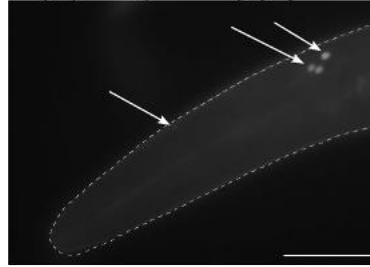
Full statistical analysis of lifespan data from Fig. 5 (****p<0.0001, ***p<0.001, **p<0.01, *p<0.05, ns=not significant). #consistent with data reported in [14]. EV = empty vector control. Rep = repeat.

Additional file 12:Fig. S5

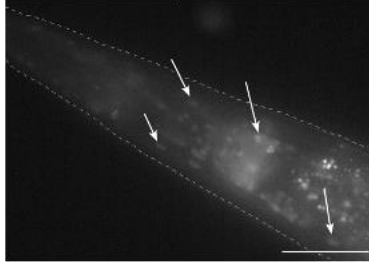
A *N2+jmjd-3.2::gfp*



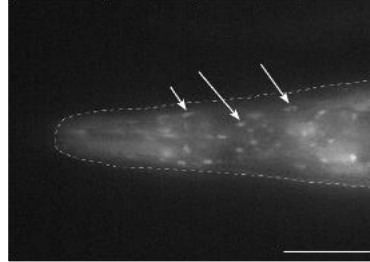
B *jmjd-3.2(tm3121)+jmjd-3.2::gfp*



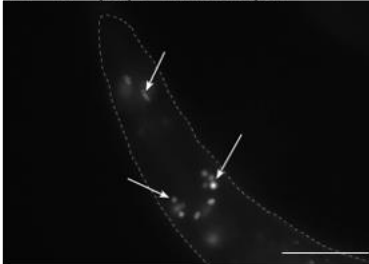
C *N2+utx-1::gfp*



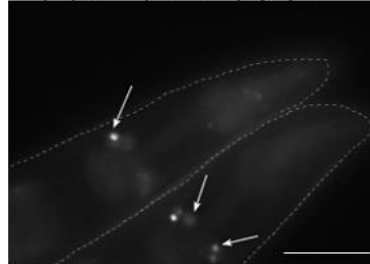
D *utx-1(tm3118)+utx-1::gfp*



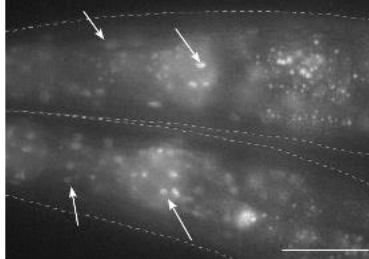
E *N2+jmjd-3.2DD::gfp*



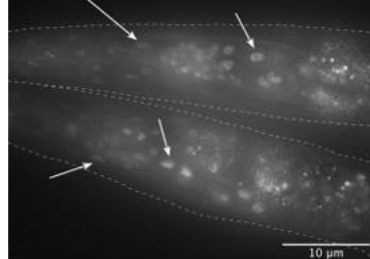
F *jmjd-3.2(tm3121)+jmjd-3.2DD::gfp*



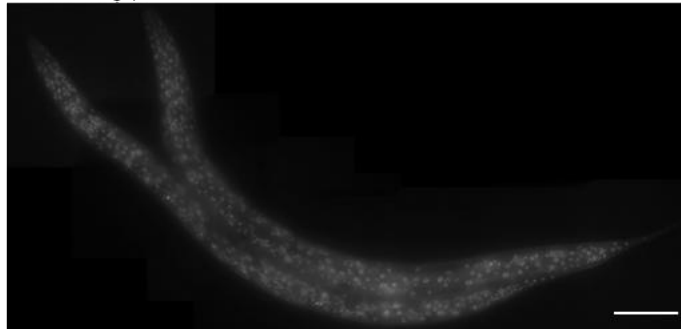
G *N2+utx-1DD::gfp*



H *utx-1(tm3118)+utx-1DD::gfp*



I *utx-1::gfp*



J *utx-1DD::gfp*

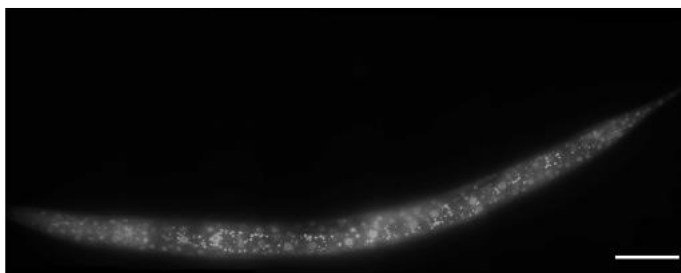


Figure S5. Spatial expression of *jmjd-3.2* and *utx-1*

A-B: Animals overexpressing a full length *jmjd-3.2::GFP* construct in wildtype (A) and *jmjd-3.2(tm3121)* mutants (B) were visualised by epifluorescence microscopy and showed spatial expression in a specific subset of neurons (white arrows). C-D: Animals overexpressing full length *utx-1::GFP* in wildtype (C) and *utx-1(tm3118)* mutant background (D) both showed ubiquitous expression (white arrows). E-H: The expression of demethylase dead *jmjd-3.2* (E-F) and *utx-1* (G-H) constructs showed a very similar pattern to the non-mutated constructs. I-J: Whole worm images of animals overexpressing *utx-1::GFP* (I) and *utx-1DD::GFP* (J) show ubiquitous expression of *utx-1*. Scale Bar (A-H) = 10µM; Scale Bar (I-J) = 20µM.

Additional file 13:Table S8

| Fig ref | Strain / condition | no. of animals | mean lifespan | % lifespan change (vs control) | median lifespan | maximum lifespan | Log Rank Test <i>p</i> value relative to control |
|-----------------|---------------------------------------|----------------|---------------|--------------------------------|-----------------|------------------|--|
| 6B | N2 control | 59 | 16.5 | | 16 | 24 | |
| | <i>myo-3p::utx-1::GFP</i> (muscle) | 53 | 15.1 | | 14 | 30 | 0.14 (ns) |
| | <i>dpy-7p::utx-1::GFP</i> (epidermis) | 42 | 17.1 | | 16 | 26 | 0.27 (ns) |
| | <i>rab-3p::utx-1::GFP</i> (neuron) | 55 | 22.8 | 38% increase | 22 | 34 | <0.0001 (****) |
| | <i>vha-6p::utx-1::GFP</i> (intestine) | 58 | 21.7 | 32% increase | 22 | 34 | <0.0001 (****) |
| 6B rep | see Fig S6A-B | | | | | | |
| 6C (mus) | NR350 + EV RNAi | 37 | 13.7 | | 14 | 20 | |
| | NR350 + <i>utx-1</i> RNAi | 43 | 13.7 | | 14 | 18 | 0.96 (ns) |
| 6C rep | NR350 + EV RNAi | 47 | 13.6 | | 14 | 22 | |
| | NR350 + <i>utx-1</i> RNAi | 48 | 13.3 | | 14 | 20 | 0.54 (ns) |
| 6D (epi) | NR222 + EV RNAi | 44 | 14.4 | | 14 | 24 | |
| | NR222 + <i>utx-1</i> RNAi | 43 | 19.4 | 35% increase | 20 | 28 | <0.0001 (****) |
| 6D rep | NR222 + EV RNAi | 55 | 15.8 | | 16 | 25 | |
| | NR222 + <i>utx-1</i> RNAi | 59 | 20.2 | 28% increase | 21 | 27 | <0.0001 (****) |
| 6E (neu) | TU3401 + EV RNAi | 48 | 15.1 | | 14 | 20 | |
| | TU3401 + <i>utx-1</i> RNAi | 48 | 21.8 | 44% increase | 22 | 29 | <0.0001 (****) |
| 6E rep | TU3401 + EV RNAi | 56 | 16.1 | | 16 | 21 | |
| | TU3401 + <i>utx-1</i> RNAi | 58 | 22.8 | 42% increase | 24 | 30 | <0.0001 (****) |
| 6F (int) | VP303 + EV RNAi | 51 | 14.7 | | 14 | 24 | |
| | VP303 + <i>utx-1</i> RNAi | 56 | 16.6 | 13% increase | 16 | 24 | 0.009 (**) |
| 6F rep | VP303 + EV RNAi | 42 | 13.7 | | 14 | 20 | |
| | VP303 + <i>utx-1</i> RNAi | 42 | 16.2 | 18% increase | 16 | 22 | 0.008 (**) |

Table S8. Statistical analysis of lifespan data relating to Figure 6

Full statistical analysis of lifespan data from Fig. 6 (*****p*<0.0001, ****p*<0.001, ***p*<0.01, **p*<0.05, ns=not significant). EV = empty vector control. Rep = repeat. Mus = muscle, epi = epidermal, neu = neuronal, int = intestinal.

Additional file 14:Fig. S6

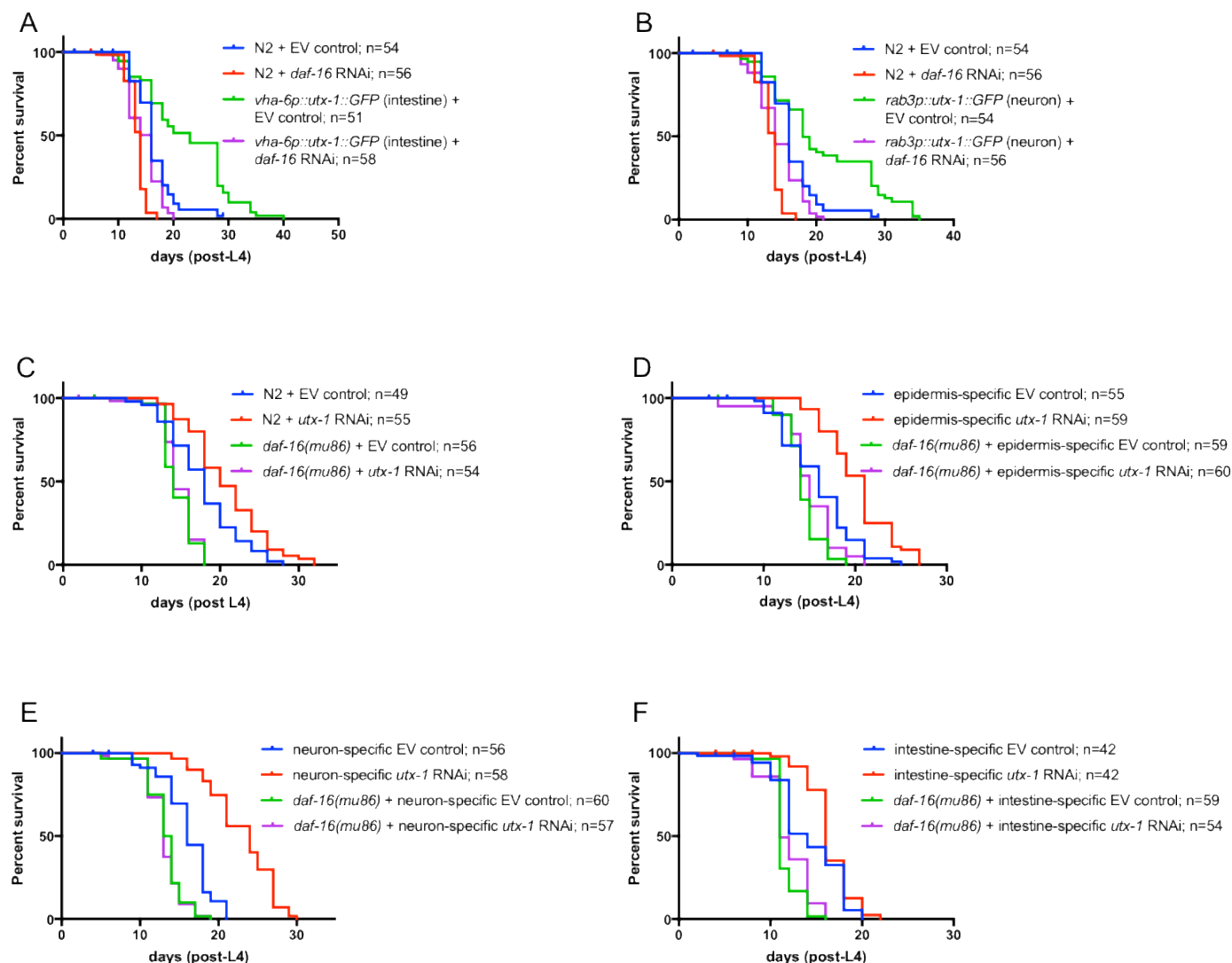


Figure S6. *daf-16* dependence of lifespan extension due to tissue-specific knockdown or overexpression of *utx-1*

A-B: Lifespan assays were performed on animals tissue-specifically overexpressing *utx-1* in the intestine (A) or neurons (B) subjected to *daf-16* RNAi. Overexpression of *utx-1* gave a moderate lifespan extension in both of these *utx-1* overexpressing strains subjected to *daf-16* RNAi ($p=0.0001$ (***) intestinal; $p=0.0002$ (***) neuronal), suggesting partial *daf-16* independence in each case. N2 and *daf-16* (RNAi) controls are shared between experiments A and B (as the experiments were performed as a large set) although the graphs are separated for clarity. C-F: Lifespan assays were performed on wild type and *daf-16(mu86)* animals subjected to global (C) as well as epidermis (D), neuron (E) and intestine-specific (F) knockdown of *utx-1*. Knockdown of *utx-1* in the wild type background in all cases caused lifespan extension, whereas this was largely abrogated in *daf-16(mu86)* mutants. EV= Empty Vector control (*i.e.* worms fed HT115 bacteria transformed with L4440 RNAi vector lacking a genomic insert). See Additional file 15: Table S9 for full statistical analysis of lifespan data, including repeats.

Additional file 15:Table S9

| Fig ref | Strain / condition | no. of animals | mean lifespan | median lifespan | maximum lifespan | Log Rank Test <i>p</i> value relative to control |
|----------------|--|----------------|---------------|-----------------|------------------|---|
| S6A | N2 EV control | 54 | 16.3 | 16 | 29 | |
| | N2 + <i>daf-16</i> RNAi | 56 | 13.3 | 14 | 17 | <0.0001 (****) compared with N2 control |
| | <i>vha-6p::utx-1::GFP</i> (intestine) + EV control | 51 | 22.4 | 23 | 40 | <0.0001 (****) compared with N2 control |
| | <i>vha-6p::utx-1::GFP</i> (intestine) + <i>daf-16</i> RNAi | 58 | 14.47 | 16 | 20 | 0.0001 (***) compared with <i>daf-16</i> RNAi <0.0001 (****) compared with <i>vha-6p::utx-1::GFP</i> |
| S6A rep | N2 EV control | 57 | 16.3 | 18 | 22 | |
| | N2 + <i>daf-16</i> RNAi | 59 | 14.9 | 13 | 18 | 0.002 (**) compared with N2 control |
| | <i>vha-6p::utx-1::GFP</i> (intestine) + EV control | 54 | 17.9 | 15 | 36 | 0.03 (*) compared with N2 control |
| | <i>vha-6p::utx-1::GFP</i> (intestine) + <i>daf-16</i> RNAi | 57 | 15.51 | 13 | 26 | 0.03 (*) compared with <i>daf-16</i> RNAi 0.008 (**) compared with <i>vha-6p::utx-1::GFP</i> |
| S6B | N2 EV control | 54 | 16.3 | 16 | 29 | |
| | N2 + <i>daf-16</i> RNAi | 56 | 13.3 | 14 | 17 | <0.0001 (****) compared with N2 control |
| | <i>rab-3p::utx-1::GFP</i> (neuron) + EV control | 54 | 20.7 | 30 | 46 | 0.0002 (***) compared with N2 control |
| | <i>rab-3p::utx-1::GFP</i> (neuron) + <i>daf-16</i> RNAi | 56 | 14.52 | 16 | 24 | 0.0002 (***) compared with <i>daf-16</i> RNAi |

| | | | | | | |
|--------------------|--|----|-------|----|----|--|
| | | | | | | <0.0001 (****) compared with <i>utx-1(tm3118)+ utx-1::GFP</i> |
| S6B rep | N2 EV control | 57 | 16.3 | 18 | 22 | |
| | N2 + <i>daf-16</i> RNAi | 59 | 14.9 | 13 | 18 | 0.002 (**) compared with <i>N2 control</i> |
| | <i>rab-3p::utx-1::GFP</i> (neuron) + EV control | 53 | 18.7 | 19 | 37 | 0.001 (**) compared with <i>N2 control</i> |
| | <i>rab-3p::utx-1::GFP</i> (neuron) + <i>daf-16</i> RNAi | 57 | 15.49 | 15 | 22 | 0.002 (**) compared with <i>daf-16 RNAi</i> 0.001 (**) compared with <i>utx-1(tm3118) +utx-1::GFP</i> |
| S6C | N2 + EV control | 49 | 17.8 | 18 | 28 | |
| | N2 + <i>utx-1</i> RNAi | 55 | 20.8 | 20 | 32 | 0.003 (**) (vs N2 + EV) |
| | <i>daf-16(mu86)</i> + EV control | 56 | 14.5 | 14 | 18 | <0.0001 (****) (vs N2 + EV) |
| | <i>daf-16(mu86)</i> + <i>utx-1</i> RNAi | 54 | 14.7 | 14 | 18 | 0.38 (ns) (vs <i>daf-16(mu86)+EV</i>) |
| S6C rep | N2 + EV control | 54 | 14.4 | 14 | 20 | |
| | N2 + <i>utx-1</i> RNAi | 54 | 16.3 | 16 | 22 | 0.005 (**) (vs N2 + EV) |
| | <i>daf-16(mu86)</i> + EV control | 56 | 14.3 | 14 | 17 | 0.002 (**) (vs N2 + EV) |
| | <i>daf-16(mu86)</i> + <i>utx-1</i> RNAi | 57 | 14.7 | 14 | 17 | 0.27 (ns) (vs <i>daf-16(mu86)+EV</i>) |
| S6D | epidermis-specific EV control (strain NR222) | 55 | 15.8 | 16 | 25 | |
| | epidermis-specific <i>utx-1</i> RNAi | 59 | 20.2 | 21 | 27 | <0.0001 (****) (vs epidermis- specific EV) |
| | <i>daf-16(mu86)</i> + epidermis-specific EV control (strain AW1708) | 59 | 14.3 | 16 | 22 | 0.0001 (***) (vs epidermis- specific EV) |

| | | | | | | |
|------------|--|----|------|------|----|---|
| S6D rep | <i>daf-16(mu86) +</i> epidermis-specific <i>utx-1</i> RNAi | 60 | 14.8 | 18 | 23 | 0.02 (*) (vs <i>daf-16(mu86)+epidermis-specific EV</i>) |
| | epidermis-specific EV control (strain NR222) | 44 | 14.4 | 14 | 24 | |
| | epidermis-specific <i>utx-1</i> RNAi | 43 | 19.4 | 20 | 28 | <0.0001 (****) (vs epidermis-specific EV) |
| | <i>daf-16(mu86) +</i> epidermis-specific EV control (strain AW1708) | 58 | 16.3 | 16 | 22 | 0.0001 (***) (vs epidermis-specific EV) |
| | <i>daf-16(mu86) +</i> epidermis-specific <i>utx-1</i> RNAi | 58 | 18.3 | 18 | 23 | 0.0001 (***) (vs <i>daf-16(mu86)+epidermis-specific EV</i>) |
| S6E | neuron-specific EV control (strain TU3401) | 56 | 16.1 | 16 | 21 | |
| | neuron-specific <i>utx-1</i> RNAi | 58 | 22.8 | 24 | 30 | <0.0001 (****) (vs neuron-specific EV) |
| | <i>daf-16(mu86) +</i> neuron-specific EV control (strain AW1709) | 60 | 13.3 | 13.5 | 19 | <0.0001 (****) (vs neuron-specific EV) |
| | <i>daf-16(mu86) +</i> neuron-specific <i>utx-1</i> RNAi | 57 | 13.0 | 13 | 19 | 0.57 (ns) (vs <i>daf-16(mu86)+neuron-specific EV</i>) |
| | | | | | | |
| S6E rep | neuron-specific EV control (strain TU3401) | 48 | 15.1 | 14 | 20 | |
| | neuron-specific <i>utx-1</i> RNAi | 48 | 21.8 | 22 | 29 | <0.0001 (****) (vs neuron-specific EV) |
| | <i>daf-16(mu86) +</i> neuron-specific EV control (strain AW1709) | 57 | 12.8 | 14 | 17 | <0.0001 (****) (vs neuron-specific EV) |
| | <i>daf-16(mu86) +</i> neuron-specific <i>utx-1</i> RNAi | 58 | 13.0 | 14 | 16 | 0.79 (ns) (vs <i>daf-16(mu86)+neuron-specific EV</i>) |
| | | | | | | |
| S6F | intestine-specific EV control (strain VP303) | 42 | 13.7 | 14 | 20 | |

| | | | | | | |
|------------|--|----|------|----|----|--|
| S6F rep | intestine-specific <i>utx-1</i> RNAi | 42 | 16.2 | 16 | 22 | 0.008 (**) (vs intestine- specific EV) |
| | <i>daf-16(mu86)</i> + intestine-specific EV control (strain AW1774) | 59 | 11.6 | 11 | 14 | <0.0001 (****) (vs intestine- specific-EV) |
| | <i>daf-16(mu86)</i> + intestine-specific <i>utx-1</i> RNAi | 54 | 11.9 | 11 | 14 | 0.07 (ns) (vs daf- 16(mu86)+intestin e-specific EV) |
| | intestine-specific EV control (strain VP303) | 51 | 14.7 | 14 | 24 | |
| | intestine-specific <i>utx-1</i> RNAi | 56 | 16.6 | 16 | 24 | 0.009 (**) (vs intestine- specific-EV) |
| | <i>daf-16(mu86)</i> + intestine-specific EV control (strain AW1774) | 56 | 12.5 | 12 | 16 | 0.0003 (***) (vs intestine- specific-EV) |
| | <i>daf-16(mu86)</i> + intestine-specific <i>utx-1</i> RNAi | 55 | 12.4 | 11 | 16 | 0.76 (ns) (vs daf- 16(mu86)+intestin e-specific EV) |
| | | | | | | |

Table S9. Statistical analysis of lifespan data relating to Figure S6

Full statistical analysis of lifespan data from Fig. S6 (****p<0.0001, ***p<0.001, **p<0.01, *p<0.05, ns=not significant). EV = empty vector control. Rep = repeat. Note some of the data corresponds to data in Figure 6 (Table S8) as these values were part of the same experiment, split off for clarity.

Additional file 16:Fig. S7

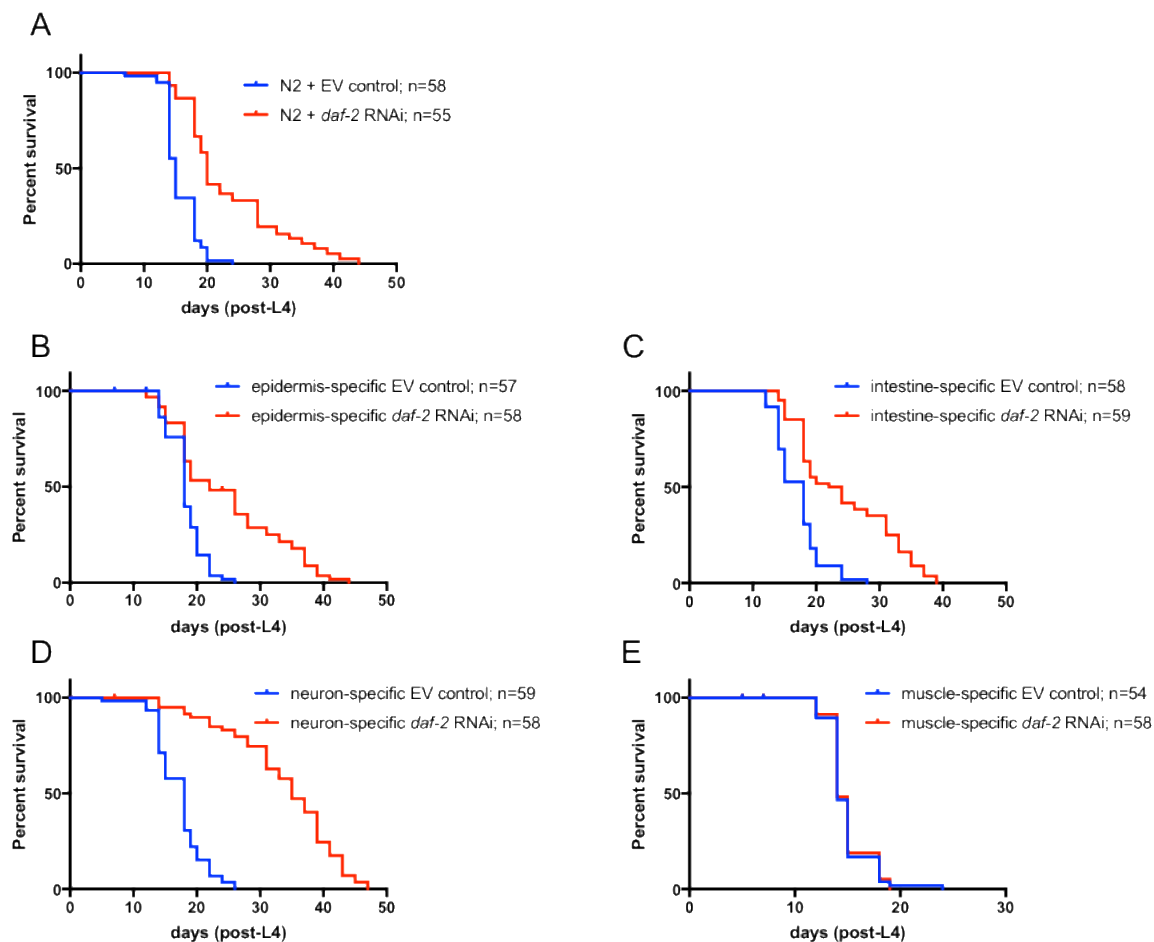


Figure S7. Knockdown of *daf-2* enhances longevity in a tissue-specific manner

A: Global depletion of *daf-2* was achieved through RNAi and promoted lifespan extension in wild-type animals compared to EV controls ($p < 0.0001$ (****)). B-E: Tissue-specific depletion of *daf-2* enhanced worm longevity when knocked down in the epidermis (B), intestine (C) and neurons (D) ($p < 0.0001$ (****) in all cases), but not in the muscle (E) ($p = 0.82$ (ns)), the same tissues implicated in improved longevity mediated by *utx-1* knockdown. EV= Empty Vector control (*i.e.* worms fed HT115 bacteria transformed with L4440 RNAi vector lacking a genomic insert). See Additional file 17: Table S10 for full statistical analysis of lifespan data.

Additional file 17:Table S10

| Fig ref | Strain / condition | no. of animals | mean lifespan | % lifespan change (vs control) | median lifespan | maximum lifespan | Log Rank Test <i>p</i> value relative to control |
|---------------------|---|----------------|---------------|--------------------------------|-----------------|------------------|--|
| S7A | N2 + EV RNAi | 58 | 15.7 | | 15 | 24 | |
| | N2 + <i>daf-2</i> RNAi | 55 | 22.3 | 42% increase | 20 | 44 | <0.0001 (****) |
| S7B (epi) | epidermis-specific EV control (strain: NR222) | 57 | 18.2 | | 18 | 26 | |
| | epidermis-specific <i>daf-2</i> RNAi | 58 | 24.4 | 34% increase | 22 | 44 | <0.0001 (****) |
| S7C (int) | intestine-specific EV control (strain: VP303) | 58 | 16.7 | | 18 | 28 | |
| | intestine-specific <i>daf-2</i> RNAi | 59 | 24.3 | 46% increase | 23 | 39 | <0.0001 (****) |
| S7D (neu) | neuron-specific EV control (strain: TU3401) | 59 | 17.2 | | 18 | 26 | |
| | neuron-specific <i>daf-2</i> RNAi | 58 | 33.7 | 96% increase | 35 | 47 | <0.0001 (****) |
| S7E (mus) | muscle-specific EV control (strain: NR350) | 54 | 14.9 | | 14 | 24 | |
| | muscle-specific <i>daf-2</i> RNAi | 58 | 14.9 | | 14 | 19 | 0.82 (ns) |

Table S10. Statistical analysis of lifespan data relating to Figure S7. Full statistical analysis of lifespan data from Fig. S7 (*****p*<0.0001, ****p*<0.001, ***p*<0.01, **p*<0.05, ns=not significant). EV = empty vector control. Epi = epidermal, int = intestinal neu = neuronal, mus = muscle.

Additional file 18:Table S11

| Strain name | Genotype | Strain name used for the purpose of this study |
|----------------|---|--|
| N2 | | wild type (WT) |
| | <i>mes-2(tm5007) II</i> | <i>mes-2(tm5007)</i> |
| SS186 | <i>mes-2(bn11) unc-4(e120)/mnC1 dpy-10(e128) unc-52(e444) II</i> | <i>mes-2(bn11)</i> |
| VC2409 | <i>mes-2(ok2480)/mT1 II; +/-mT1 [dpy-10(e128)] III</i> | <i>mes-2(ok2480)</i> |
| | <i>jmjd-3.2(tm3121) X</i> | <i>jmjd-3.2(tm3121)</i> |
| ZR332 | <i>jmjd-3.1(gk384) jmjd-3.2 (tm3121) jmjd-3.3(tm3197) X</i> | <i>jmjd-3</i> triple mutant |
| ZR252 | <i>utx-1(tm3118) X</i> | <i>utx-1(tm3118)</i> |
| ZR254 ZR255 | <i>utx-1(tm3118) X ; zrEx264[utx-1p::utx-1::GFP + rol-6⁺]</i> | <i>utx-1(tm3118)+ utx-1 OE</i> |
| ZR256 ZR257 | <i>utx-1(tm3118) X; zrEx295[utx-1p::utx-1DD::GFP + rol-6⁺]</i> | <i>utx-1(tm3118)+ utx-1DD OE</i> |
| ZR802 | <i>zrEx264[utx-1p::utx-1::GFP + rol-6⁺]</i> | <i>utx-1 OE</i> |
| ZK856 | <i>zrEx295[utx-1p::utx-1DD::GFP + rol-6⁺]</i> | <i>utx-1DD OE</i> |
| ZR693 ZR694 | <i>zrEx231[myo-3p::utx-1::GFP + rol-6⁺ + myo-2::rfp]</i> | <i>myo-3p::utx-1:: GFP</i> |
| ZR695 ZR696 | <i>zrEx233[dpy-7p::utx-1::GFP + rol-6⁺ + myo-2::rfp]</i> | <i>dpy-7p::utx-1:: GFP</i> |
| ZR697 ZR698 | <i>zrEx235[rab-3p::utx-1::GFP + rol-6⁺ + myo-2::rfp]</i> | <i>rab-3p::utx-1:: GFP</i> |
| ZR699 ZR700 | <i>zrEx237[vha-6p::utx-1::GFP + rol-6⁺ + myo-2::rfp]</i> | <i>vha-6p::utx-1:: GFP</i> |
| ZR701 ZR702 | <i>jmjd-3.2(tm3121) X zrEx239[jmjd-3.2p::jmjd-3.2::GFP + rol-6⁺ + myo-2::rfp]</i> | <i>jmjd-3.2(tm3121)+ jmjd-3.2 OE</i> |
| ZR703 ZR704 | <i>jmjd-3.2(tm3121) X zrEx241[jmjd-3.2p::jmjd3.2DD::GFP + rol-6⁺ + myo-2::rfp]</i> | <i>jmjd-3.2(tm3121) + jmjd-3.2DD OE</i> |
| ZR705 | <i>zrEx243[jmjd-3.2p::jmjd-3.2::GFP + rol-6⁺ +myo-2::rfp]</i> | <i>jmjd-3.2 OE</i> |
| ZR706 ZR707 | <i>zrEx245[jmjd-3.2p::jmjd-3.2DD::GFP + rol-6⁺ + myo-2::rfp]</i> | <i>jmjd-3.2DD OE</i> |
| VP303 | <i>rde-1(ne219) V; kbIs7 [nhx-2p::rde-1 + rol-6(su1006)]</i> | intestine specific RNAi |
| NR222 | <i>rde-1(ne219) V; kzIs9 [(pKK1260) lin-26p::NLS::GFP +</i> | epidermis specific RNAi |

| | | |
|--------|---|--|
| | <i>(pKK1253) lin-26p::rde-1 + rol-6(su1006)]</i> | |
| NR350 | <i>rde-1(ne219) V; kzl520 [hlh-1p::rde-1 + sur-5p::NLS::GFP]</i> | muscle specific RNAi |
| TU3401 | <i>sid-1(pk3321) V; uls69 [pCFJ90 (myo-2p::mCherry) + unc-119p::sid-1]</i> | neuronal specific RNAi |
| AW1708 | <i>daf-16(mu86) I; rde-1(ne219) V; kzl59 [(pKK1260) lin-26p::NLS::GFP + (pKK1253) lin-26p::rde-1 + rol-6(su1006)]</i> | <i>daf-16(mu86); epidermis-specific RNAi</i> |
| AW1709 | <i>daf-16(mu86) I; sid-1(pk3321) V; uls69 [pCFJ90 (myo-2p::mCherry) + unc-119p::sid-1]</i> | <i>daf-16(mu86); neuron-specific RNAi</i> |
| AW1774 | <i>daf-16(mu86) I; rde-1(ne219) V; kbls7 [nhx-2p::rde-1 + rol-6(su1006)]</i> | <i>daf-16(mu86); intestine-specific RNAi</i> |
| TJ356 | <i>zls356 [daf-16p::daf-16a/b::GFP + rol-6(su1006)]</i> | <i>daf-16::GFP translational reporter</i> |

Table S11. List of *C. elegans* strains used in this study. Strain names are indicated along with genotypes, plasmids and short names used in this report.