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Supplementary Materials for

N^6-methyldeoxyadenine and histone methylation mediate transgenerational survival advantages induced by hormetic heat stress

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The PDF file includes:

Figs. S1 to S10

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/7/1/eabc3026/DC1)

Tables S1 and S2

Fig. S1

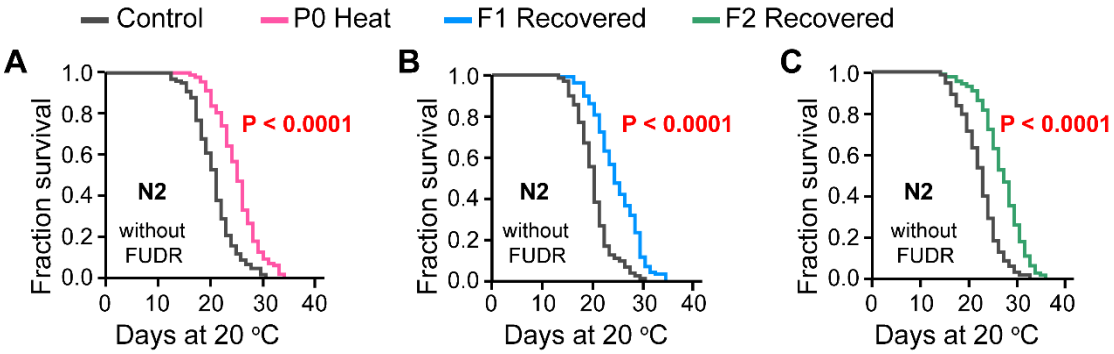


Fig. S1 Trans-generational inheritance of heat stress-induced survival benefits. Lifespan analyses of P0 **(A)**, F1 **(B)** and F2 **(C)** worms with or without treatment. Survival analyses were conducted in absence of FUDR. Lifespan was analyzed using the Kaplan-Meier test, P values were calculated using the log-rank test, and the lifespan values of the replicated tests are listed in Supporting File Table 1.

Fig. S2

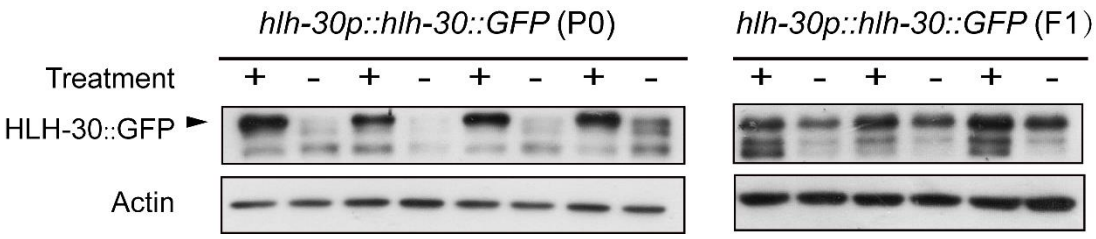


Fig. S2 HLH-30 expression. Protein level of HLH-30 in the P0 animals with or without treatment and their recovered progeny. For P0 (left panel), treatment meant day 1 adult worms exposed to 35 ℃ for 1 h; for F1 (right panel), treatment meant they were recovered progeny of heat shocked P0. Actin was shown as loading control.

Fig. S3

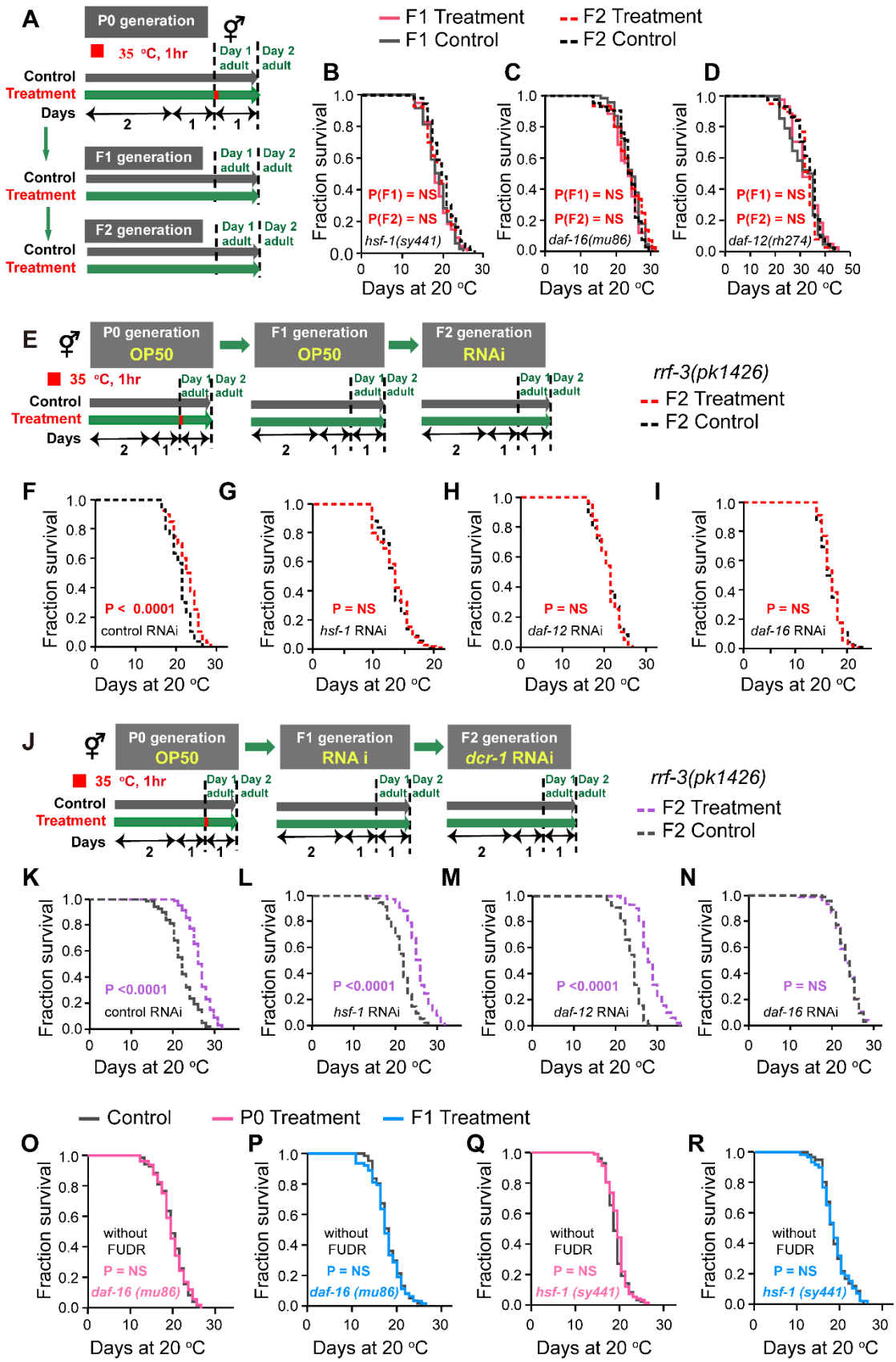

A
P0 generation
35 °C, 1hr
Day 1 adult
Day 2 adult
Control
Treatment
Days
2
1
1
F1 generation
F2 generation
F1 Treatment
F1 Control
F2 Treatment
F2 Control
B
C
D
Fraction survival
P(F1) = NS
P(F2) = NS
hsf-1(sy441)
daf-16(mu86)
daf-12(rh274)
Days at 20 °C
E
P0 generation OP50
F1 generation OP50
F2 generation RNAi
rrf-3(pk1426)
F2 Treatment
F2 Control
F
G
H
I
P < 0.0001
control RNAi
P = NS
hsf-1 RNAi
daf-12 RNAi
daf-16 RNAi
J
P0 generation OP50
F1 generation RNA i
F2 generation dcr-1 RNAi
K
L
M
N
P <0.0001
control RNAi
hsf-1 RNAi
daf-12 RNAi
daf-16 RNAi
Control
P0 Treatment
F1 Treatment
O
P
Q
R
without FUDR
P = NS
daf-16 (mu86)
hsf-1 (sy441)

Fig. S3 Intergenerational and trans-generational inheritance of heat stress-induced benefits requires DAF-16, DAF-12 and HSF-1. (A-D) Experimental scheme **(A)** and trans-generational inheritance tested in **(B)** *hsf-1* mutant (recovered F1 and F2), **(C)** *daf-16* mutant (recovered F1 and F2), and **(D)** *daf-12* mutant (recovered F1 and F2). **(E)** P0 and F1 worms were heat shocked without RNAi exposure, and F2 progeny were exposed to **(F)** control (HT115), **(G)** *hsf-1*, **(H)** *daf-12* and **(I)** *daf-16* RNAi to determine the requirement of *daf-16*, *daf-12* and *hsf-1* in the F2 generation (P value by log-rank test). **(J)** P0 parents were heat shocked without RNAi exposure, F1 progeny were exposed to **(K)** control (HT115), **(L)** *hsf-1*, **(M)** *daf-12* and **(N)** *daf-16* RNAi, F2 progeny were subjected to *dcr-1* RNAi to prevent the inheritance of RNAi, and then the lifespan of F2 progeny were analyzed. **(O-R)** Lifespan analyses of heat shocked P0 animals and their recovered F1 progeny, lifespan assays were conducted without FUDR treatment, and survival curve of **(O)** *daf-16* mutants (P0), **(P)** *daf-16* mutants (F1), **(Q)** *hsf-1* mutants (P0) and **(R)** *hsf-1* mutants (F1). Lifespan was analyzed using the Kaplan-Meier test, P values were calculated using the log-rank test. Detailed lifespan values are listed in Supporting File Table 1.

Fig. S4

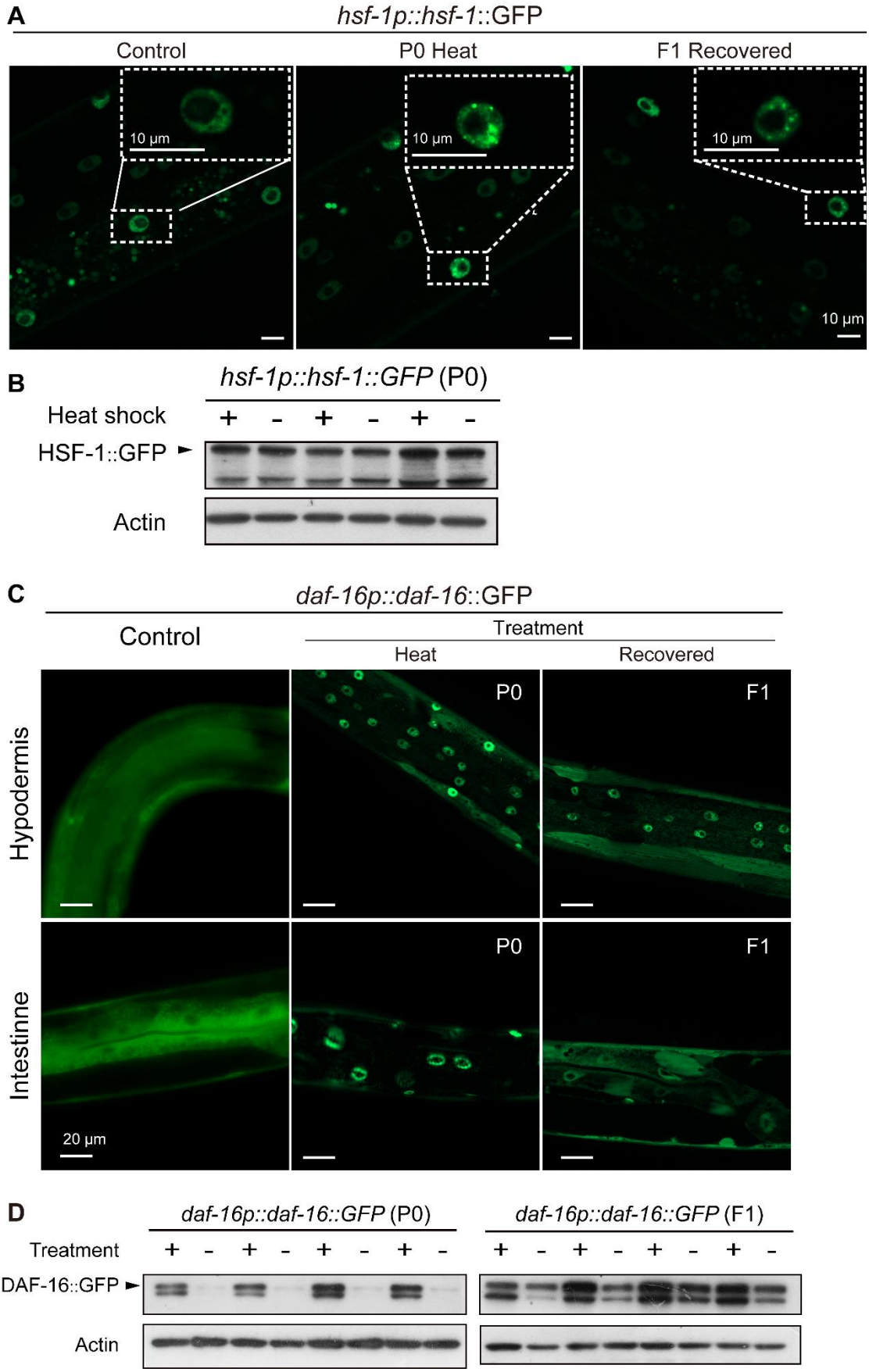

A
hsf-1p::hsf-1::GFP
Control
P0 Heat
F1 Recovered
10 μm
10 μm
10 μm
10 μm
B
hsf-1p::hsf-1::GFP (P0)
Heat shock
+ - + - + -
HSF-1::GFP
Actin
C
daf-16p::daf-16::GFP
Control
Treatment
Heat
Recovered
Hypodermis
Intestinne
P0
F1
P0
F1
20 μm
D
daf-16p::daf-16::GFP (P0)
daf-16p::daf-16::GFP (F1)
Treatment
+ - + - + - + -
+ - + - + - + -
DAF-16::GFP
Actin

Fig. S4 HSF-1::GFP and DAF-16::GFP nuclear translocation and expression. (A, C) Nuclear localization of HSF-1::GFP **(A)** and DAF-16::GFP **(C)** in the P0 transgenic *hsf-1::GFP* and *daf-16::GFP* worms with or without stress and their recovered progeny.**(B)** Protein level of HSF-1::GFP in the P0 transgenic *hsf-1::GFP* worms with or without heat stress. **(D)** Expression in the P0 *daf-16::GFP* transgenic worms with or without treatment and their recovered progeny. For P0 (middle panel in (C) and left panel in (D)), treatment meant day 1 adult worms exposed to 35 ℃ for 1 h; for F1 (right panel in (C) and right panel in (D)), treatment meant they were recovered progeny of heat shocked P0. Actin was shown as loading control.

Fig. S5

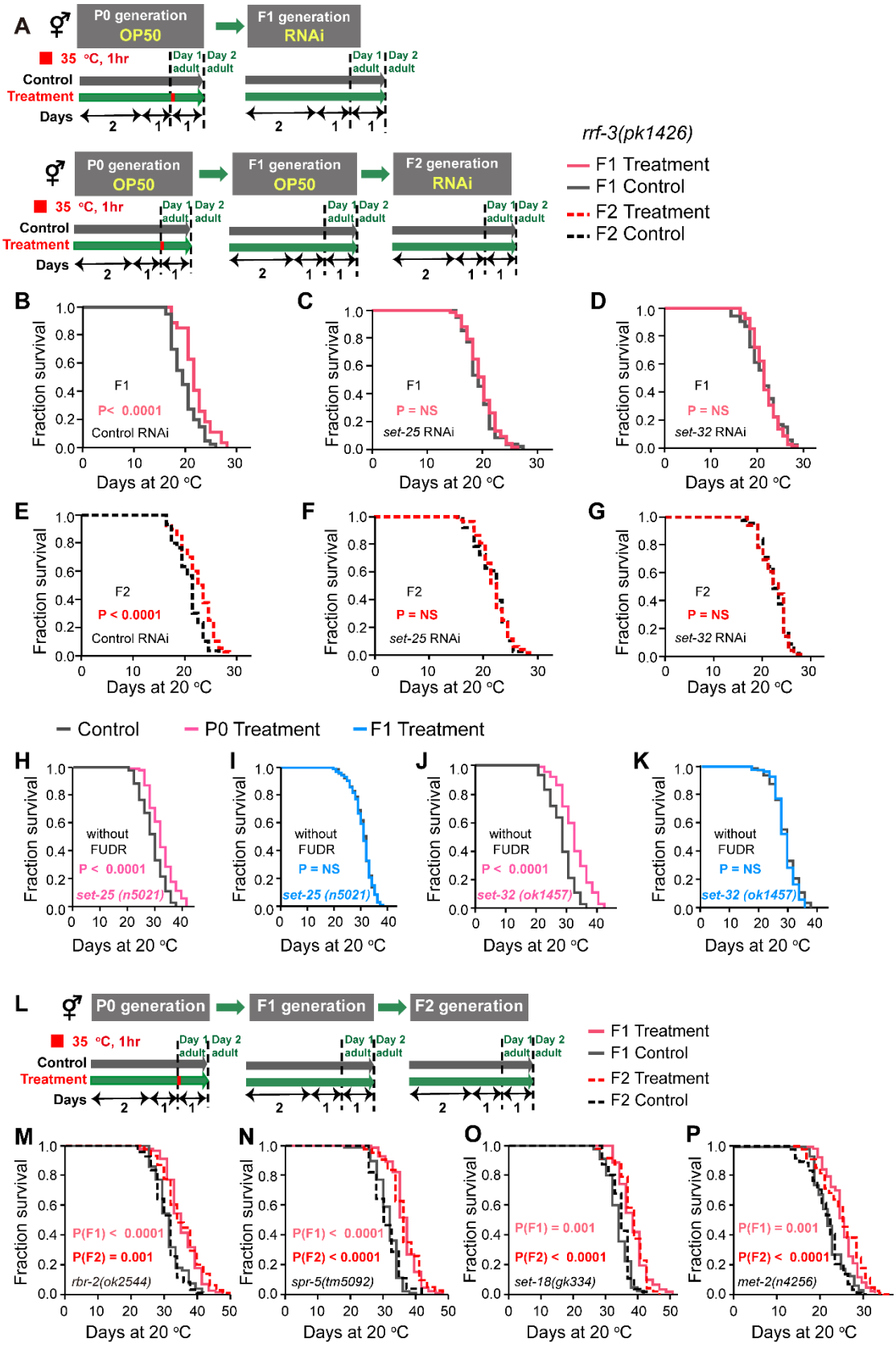


Fig. S5 Histone H3K9me3 modifiers, but not other modifiers, are specifically

required for TEI induced by heat stress. (A) Experimental scheme. **(B-D)** P0 worms were heat shocked without RNAi exposure, and then, lifespan analyses were conducted on F1 progeny were exposed to **(B)** control (HT115), **(C)** *set-25* RNAi, or **(D)** *set-32* RNAi. **(E-G)** Heat shocked P0 and recovered F1 worms were raised under normal condition, lifespan analyses were conducted on F2 progeny that were exposed to **(E)** control (HT115), **(F)** *set-25* RNAi, or **(G)** *set-32* RNAi. **(H-K)** Lifespan analyses of heat shocked P0 animals and their recovered progeny without FUDR. Survival curve of **(H)** *set-25* mutants (P0), **(I)** *set-25* mutants (recovered F1), **(J)** *set-32* mutants (P0) and **(K)** *set-32* mutants (recovered F1). Experimental scheme **(L)** and trans-generational inheritance tested in **(M)** *rbr-2* mutants (recovered F1 and F2), **(N)** *spr-5* mutants (recovered F1 and F2), **(O)** *set-18* mutants (recovered F1 and F2) and **(P)** *met-2* mutants (recovered F1 and F2). Lifespan was analyzed using the Kaplan-Meier test, and P values were calculated using the log-rank test, and the lifespan values of the replicated tests are listed in Supporting File Table 1.

Fig. S6

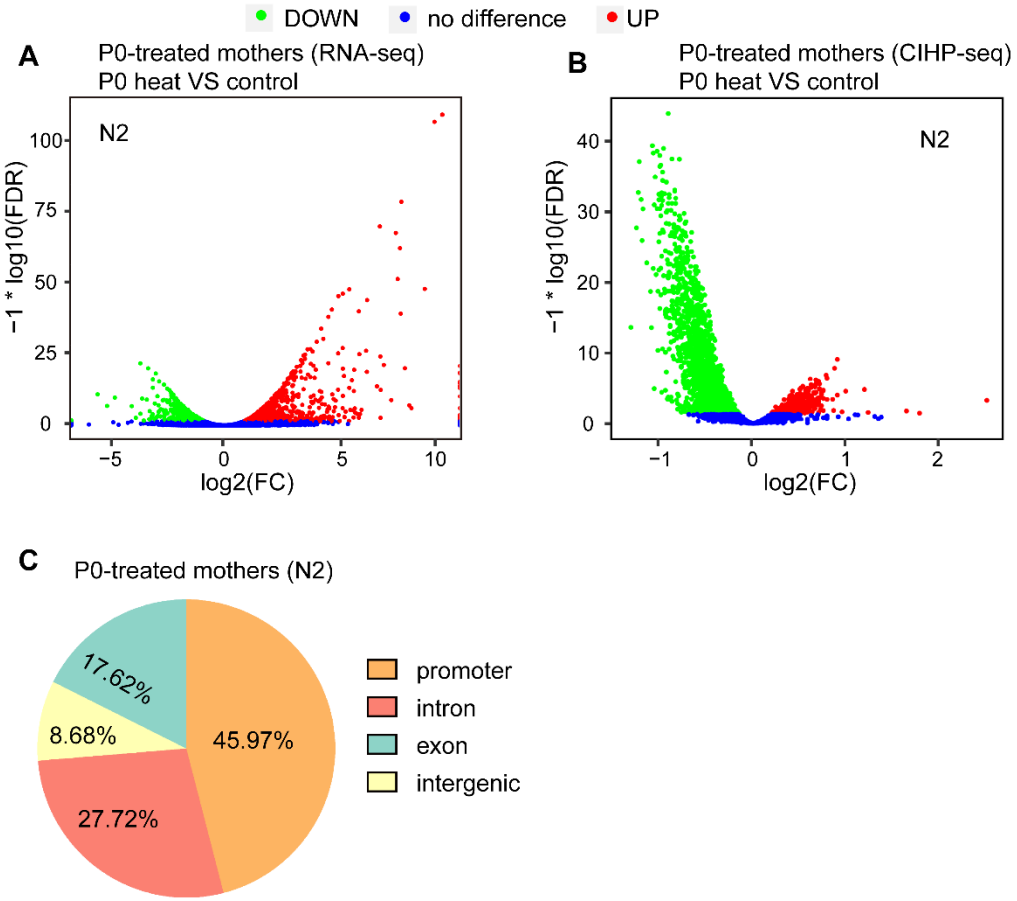


Fig. S6 Analyses of differentially expressed genes from P0 worms with or without heat shock in wild-type. (A) Volcano plot of the mRNAs differentially regulated in the P0 animals after heat shock. **(B)** Volcano plot of H3K9me3 differentially accumulated at genes in the P0 animals after heat shock. **(C)** Pie chart of the genomic distribution of H3K9me3 differentially accumulated peaks for P0 animals that were heat shocked versus that of the non-heat-shocked controls. The data shown refer to common genes as indicated by two independent assays.

Fig. S7

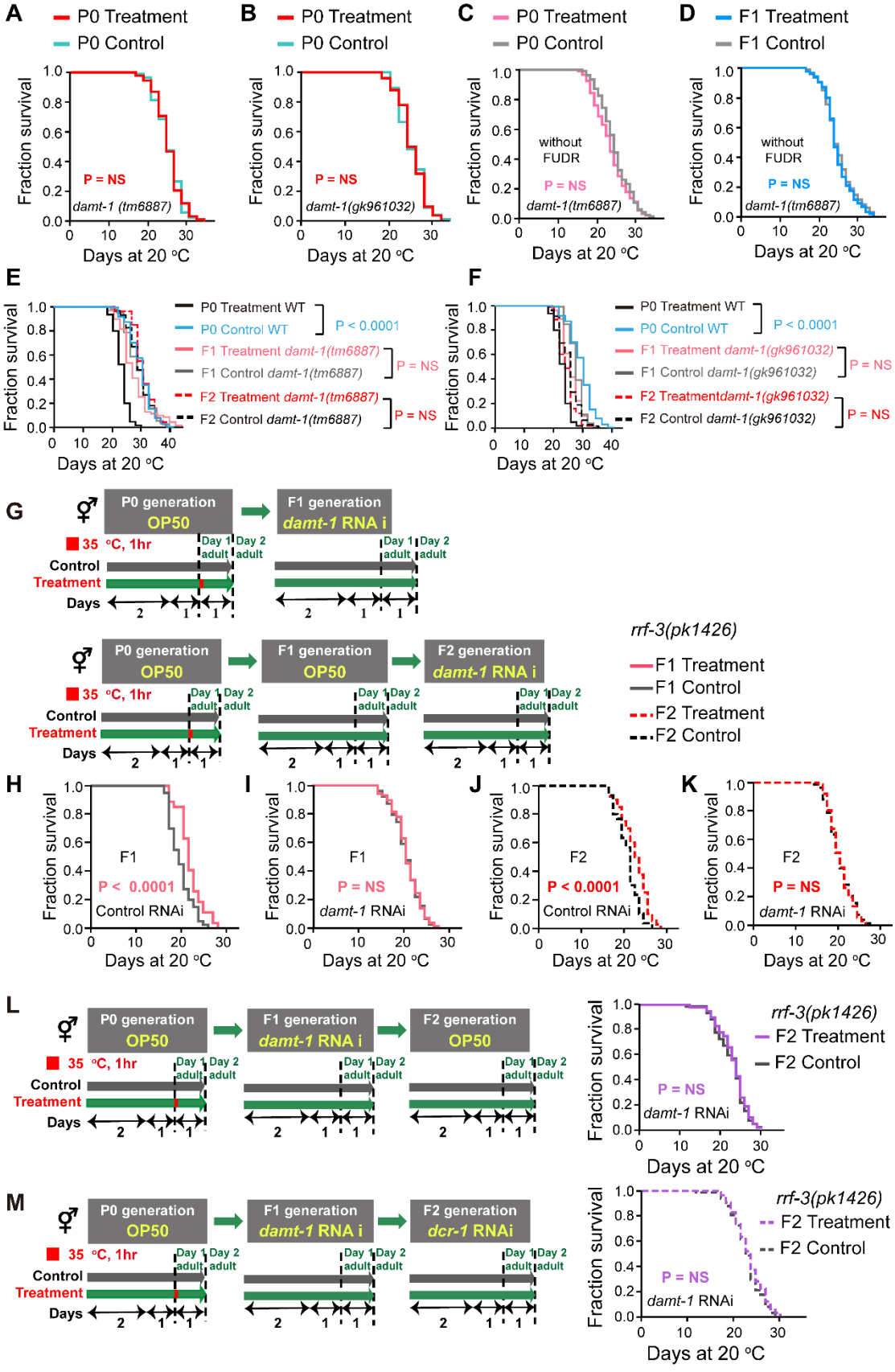


Fig. S7 DAMT-1 is required for TEI induced by heat stress. Lifespan analyses of

heat shocked P0 **(A)** *damt-1(tm6887)* mutants and **(B)** *damt-1(gk961032)* mutants. **(C, D)** Lifespan analyses of **(C)** *damt-1 (tm6887)* mutants (heat shocked P0) and **(D)** *damt-1 (tm6887)* mutants (recovered F1) (all worms treated without FUDR). **(E, F)** Trans-generational inheritance tested in **(E)** the *damt-1 (tm6887)* mutant (recovered F1 and F2) and **(F)** the *damt-1 (gk961032)* mutant (recovered F1 and F2). **(G)** Experimental scheme. **(H, I)** Lifespan analyses recovered F1 progeny exposed to **(H)** control (HT115) and **(I)** *damt-1* RNAi. **(J, K)** Heat shocked P0 and their recovered F1 worms were raised at OP50, and then F2 progeny were exposed to **(J)** control (HT115) and **(K)** *damt-1* RNAi before lifespan analyses. **(L, M)** Heat shocked P0 animals were raised at OP50, F1 progeny were exposed to *damt-1* RNAi, F2 progeny were fed with **(L)** OP50 or **(M)** *dcr-1* RNAi *E. coli*, and then lifespan of F2 progeny were analyzed. Lifespan was analyzed using the Kaplan-Meier test, P values were calculated using the log-rank test, and the lifespan values of the replicated tests are listed in Supporting File Table 1.

Fig. S8

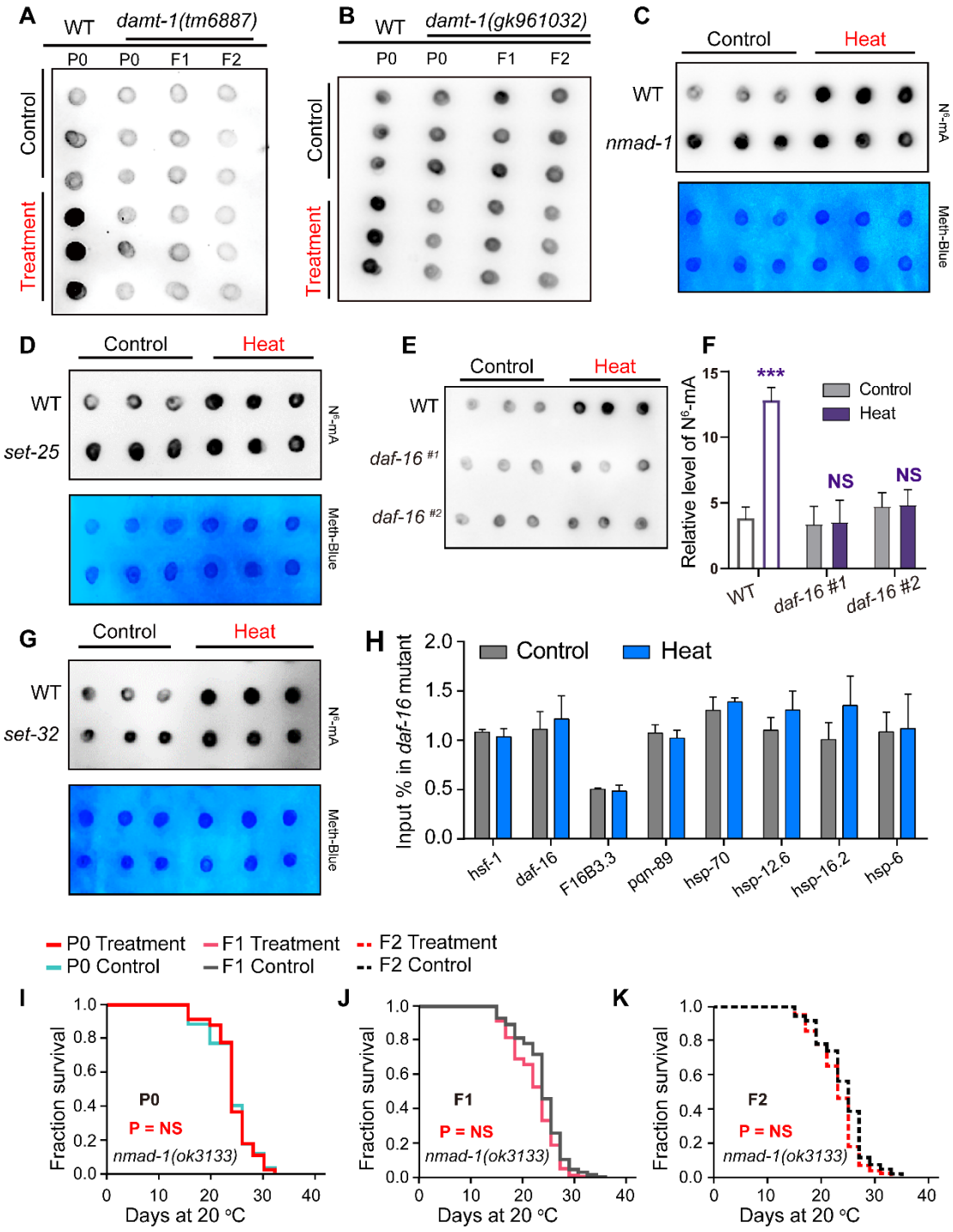


Fig. S8 DAMT-1 promotes global induction of N^6-mA upon P0 animal heat shock.

Dot blots of heat-shocked and untreated wild-type (WT) and those of the **(A)** *damt-1(tm6887)* mutants, **(B)** *damt-1(gk961032)* mutants, **(C)** *nmad-1(OK3133)* mutants, **(D)**

set-25(n5021) mutants, **(E, F)** *daf-16 (mu86)* mutants (#1 and #2 represent two independent repeated experiments) (means ± SD, two-tailed Student's t-test, *** P < 0.001, NS, not significant) and **(G)** *set-32(ok1457)* mutants. Methylene blue was used as a DNA loading control. **(H)** MeDIP-qPCR analyses of N^6-mA occupied on *daf-16* and *hsf-1*, and corresponding target genes of *daf-16*, *hsf-1* and *daf-12* in the *daf-16(mu86)* mutants (no statistical significance in all comparisons). **(I-K)** Lifespan analyses of *nmad-1 (ok3133)* in the P0 **(I)**, F1 **(J)** and F2 **(K)** generation from parents exposed or not exposed to high temperatures (P value by log-rank test), detailed lifespan values are listed in Supporting File Table 1. For P0 (left 2 panels in (A, B) and (I)), treatment meant day 1 adult worms exposed to 35 ℃ for 1 h; for F1 or F2 (right 2 panel in (A, B) and (J, K)), treatment meant they were recovered progeny of heat shocked P0.

Fig. S9

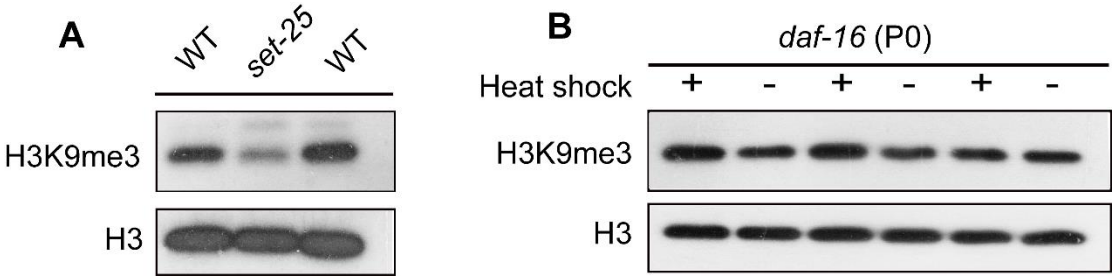
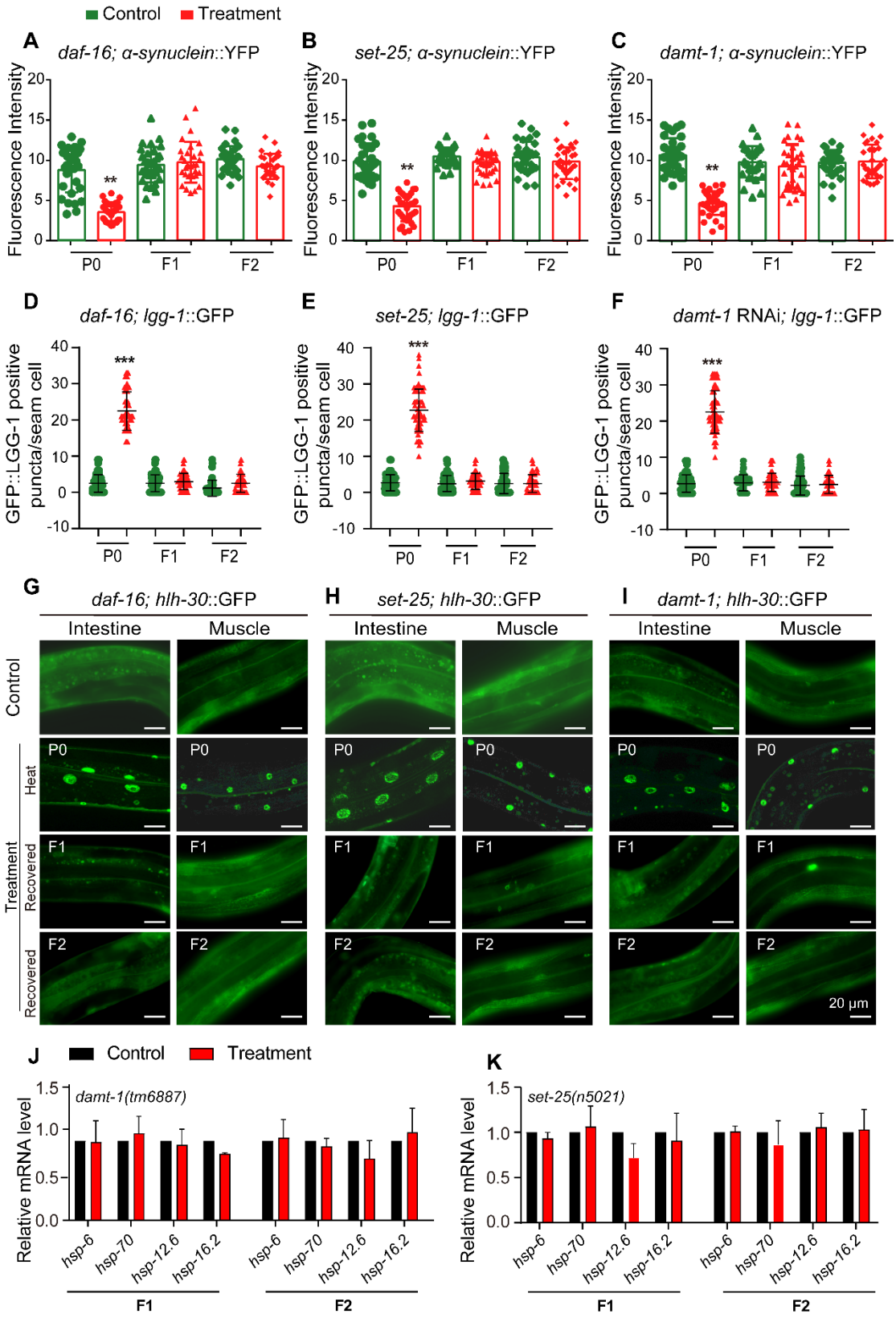


Fig. S9 No difference detected in *daf-16* worms' H3K9me3 modification between control and heat-shocked groups. (A) The level of histone H3K9me3 modification in *set-25* mutant and wild-type N2. **(B)** H3K9me3 modification level in *daf-16* mutant with or without heat shock.

Fig. S10


Control
Treatment
A
daf-16; α-synuclein::YFP
B
set-25; α-synuclein::YFP
C
damt-1; α-synuclein::YFP
Fluorescence Intensity
P0
F1
F2
D
daf-16; lgg-1::GFP
E
set-25; lgg-1::GFP
F
damt-1 RNAi; lgg-1::GFP
GFP::LGG-1 positive puncta/seam cell
G
daf-16; hlh-30::GFP
H
set-25; hlh-30::GFP
I
damt-1; hlh-30::GFP
Intestine
Muscle
Control
Treatment
Heat
Recovered
20 μm
J
damt-1(tm6887)
K
set-25(n5021)
Relative mRNA level
hsp-6
hsp-70
hsp-12.6
hsp-16.2

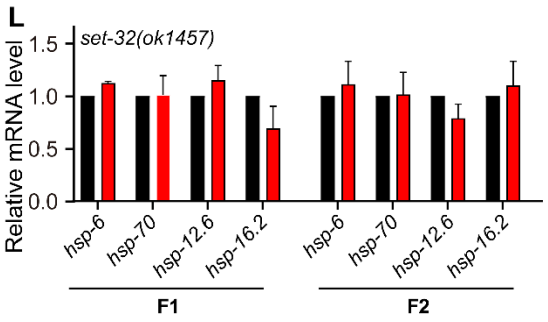


Fig. S10 Heritable fitness of *C. elegans* induced by heat shock require *daf-16, set-25* and *damt-1*. **(A-C)** Quantification of muscle α-synuclein aggregates of α-synuclein::YFP worms with or without treatment in **(A)** *daf-16(mu86)*; *α-synuclein::YFP*, **(B)** *set-25(n5021)*; *α-synuclein::YFP* and **(C)** *damt-1(tm6887)*; *α-synuclein::YFP* strains. **(D-F)** Quantification of GFP::LGG-1/Atg8 puncta in the hypodermal seam cells with or without treatment in **(D)** *daf-16 (mu86)*; *lgg-1*::GFP, **(E)** *set-25 (n5021)*; *lgg-1*::GFP and **(F)** *damt-1*(RNAi); *lgg-1*::GFP transgenic animals. The mean ± SD.; n ≥ 30 per condition; P value was calculated for Student's t tests; ** P < 0.01, *** P < 0.001. **(G-I)** Representative images of the nuclear localization of HLH-30::GFP in worms with or without treatment in **(G)** *daf-16 (mu86)*; *hlh-30*::GFP, **(H)** *set-25 (n5021)*; *hlh-30*::GFP and **(I)** *damt-1(tm6887)*; *hlh-30*::GFP. Each experiment was repeated at least three times. For P0 worms, treatment meant day 1 adult worms exposed to 35 ℃ for 1 h; for F1 or F2 worms, treatment meant they were recovered progeny of heat shocked P0. **(J-L)** Transcription level of heat shock protein genes (i.e., *hsp-6, hsp-70, hsp-12.6* and *hsp-16.2*) in **(J)** *damt-1* mutants, **(K)** *set-25* mutants and **(L)** *set-32* mutants. For F1 or F2 worms, treatment meant they were recovered progeny of heat shocked P0.