Supplemental materials

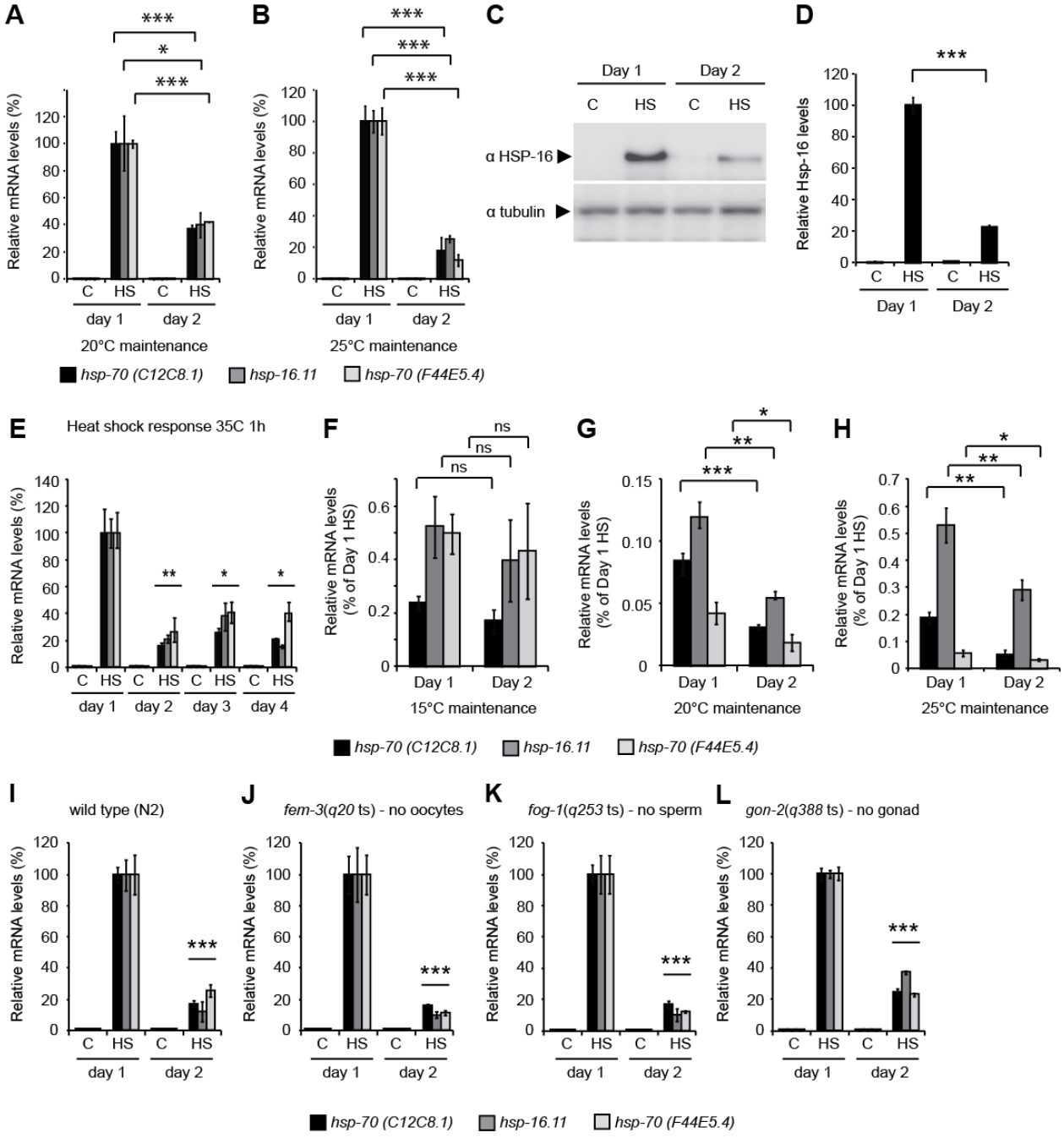

Figure S1, related to figure 1. Collapse of the HSR is independent of maintenance temperature and is not due to increased embryo mass.

C. elegans were maintained at (**A**) 20°C or (**B**) 25°C and exposed to control conditions (c) or heat shock (HS) at 33°C for 30 min at day 1 (4h post L4) or day 2 (28h post L4) of adulthood. Expression of *hsp-70 (C12C8.1* and *F44E5.4)* and *hsp-16.11* was calculated relative to *cdc-42* and *rpb-2*. (**C**) Representative western blot of HSP-16 and tubulin 24 hours after exposure to control or heat shock conditions at day 1 or day 2 of adulthood. (**D**) Quantification of HSP-16 protein levels relative to tubulin at day 1 or day 2 of adulthood (n=4 per group). (**E**) Heat shock response gene expression relative to *rpb-2* and *cdc-42* in adult animals subjected to control (c) conditions or heat shock (HS) at 35°C for 1 hour. (**F - H**) Basal expression of heat shock genes at day 1 or day 2 of adulthood after maintenance at (**F**) 15°C, (**G**) 20°C or (**H**) 25°C. Values are plotted relative to levels after heat shock (33°C, 30 min) on day 1 of adulthood (Figure 1A, S1A and B respectively for comparison). (**I – L**) Stress gene expression in (**I**) wild type (N2) or temperature sensitive mutants of (**J**) *fem-3* (*q20ts gf*), (**K**) *fog-1* (*q253ts*) or (**L**) *gon-2* (*q388ts*) maintained at the restrictive temperature (25°C) and subjected to control (c) conditions or heat shock (HS) at 33°C for 30 min at day 1 or day 2 of adulthood. Values plotted are the mean of 4 biological replicates and error bars represent SEM. Statistical significance was calculated using Students *t*-test except in panel C where one-way ANOVA with Tukey post analysis comparison was used. * $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$, not significant (ns) $p > 0.05$.

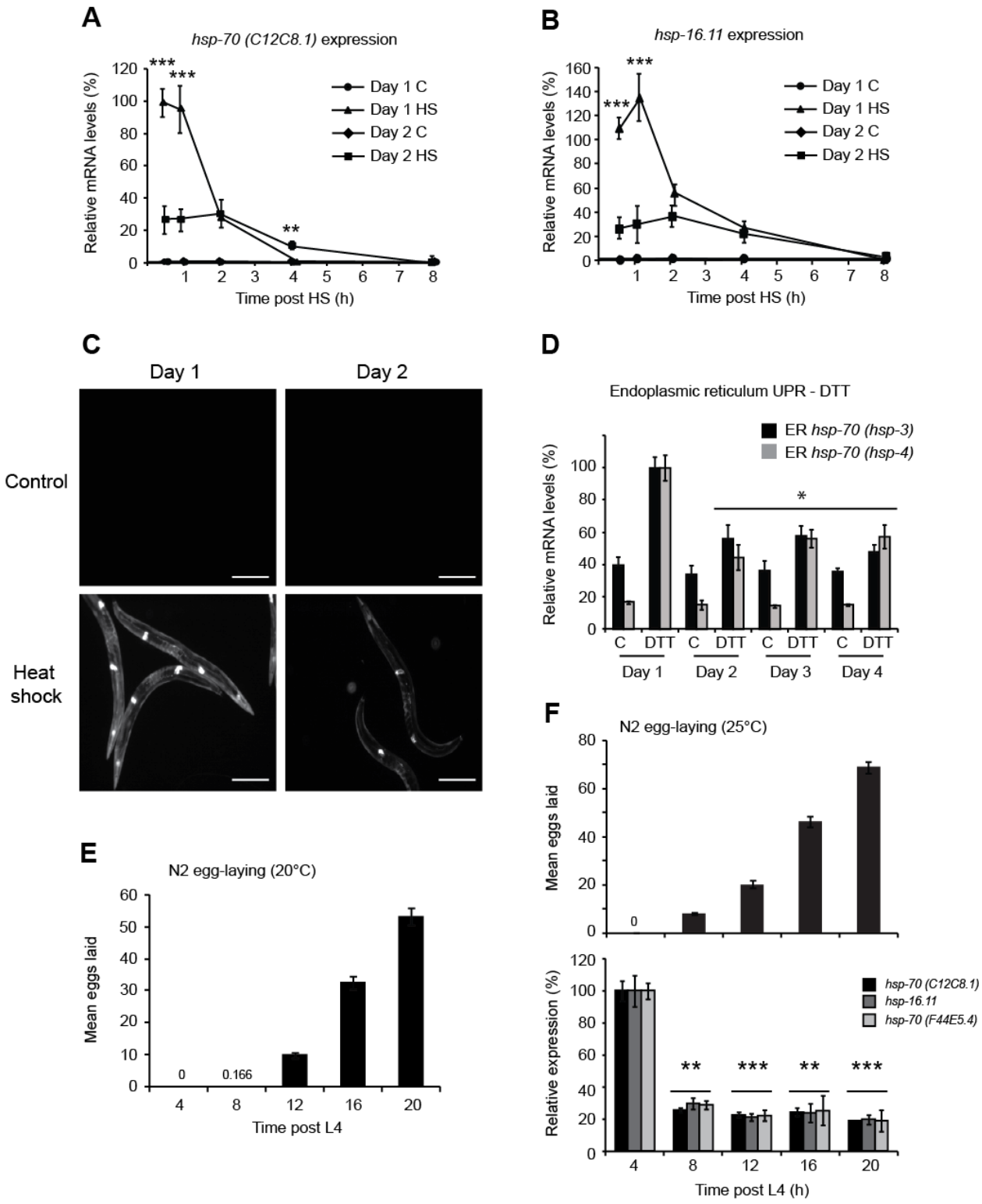
A
hsp-70 (C12C8.1) expression
Relative mRNA levels (%)
Time post HS (h)
Day 1 C
Day 1 HS
Day 2 C
Day 2 HS
B
hsp-16.11 expression
Relative mRNA levels (%)
Time post HS (h)
C
Day 1
Day 2
Control
Heat shock
D
Endoplasmic reticulum UPR - DTT
ER hsp-70 (hsp-3)
ER hsp-70 (hsp-4)
Relative mRNA levels (%)
C
DTT
Day 1
Day 2
Day 3
Day 4
E
N2 egg-laying (20°C)
Mean eggs laid
Time post L4
F
N2 egg-laying (25°C)
Mean eggs laid
Relative expression (%)
hsp-70 (C12C8.1)
hsp-16.11
hsp-70 (F44E5.4)
Time post L4 (h)

Figure S2, related to figure 2. Repression of heat shock gene induction is altered throughout the population and across the soma in early adulthood.

Expression of (**A**) *hsp-70 (C12C8.1)* and (**B**) *hsp-16.11* after 0.5, 1, 2, 4 or 8 hours recovery at 20°C following heat shock (HS) at 33°C for 30 min at day 1 (4h post L4) or day 2 (28h post L4) of adulthood. Expression at each time point was normalized to *rpb-2* and *cdc-42* and the values plotted are the mean of 4 biological replicates. (**C**) *C. elegans* expressing mCherry under the control of the *hsp-70* (*C12C8.1*) promoter were heat shocked at 33°C for 30 min and allowed to recover at 20°C for 8 hours. Animals were imaged using identical exposure times and alterations to brightness and contrast were applied linearly across all images. Images are representative of greater than 30 worms. Scale bar = 250 μM. (**D**) Expression of canonical UPR^{ER} genes relative to *rpb-2* and *cdc-42* following control (c) or DTT treatment (3 mM for 7 hours) at different days of adulthood. Time course of egg-laying in N2 worms at (**E**) 20°C and (**F**) 25°C (n=30). Eggs detectable 8 hours post L4 in (E) correspond to one worm within the population laying eggs early. All worms were laying eggs 12 and 8 hours post L4 when maintained at 20°C or 25°C respectively. (**F**) Expression of HSR genes in N2 adults following 30 min HS at 33C°. Times post L4 correspond with time points for egg-laying experiments. Values plotted are the mean of 4 biological replicates and error bars represent SEM. Statistical significance was calculated by two-way ANOVA with Bonferroni post analysis comparison (A and B) or one-way ANOVA with Tukey post analysis comparison (D and F) (*p*-value represents the largest value compared to day 1 DTT or HS treatment groups). Values are the mean of 4 biological replicates and error bars denote SEM * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

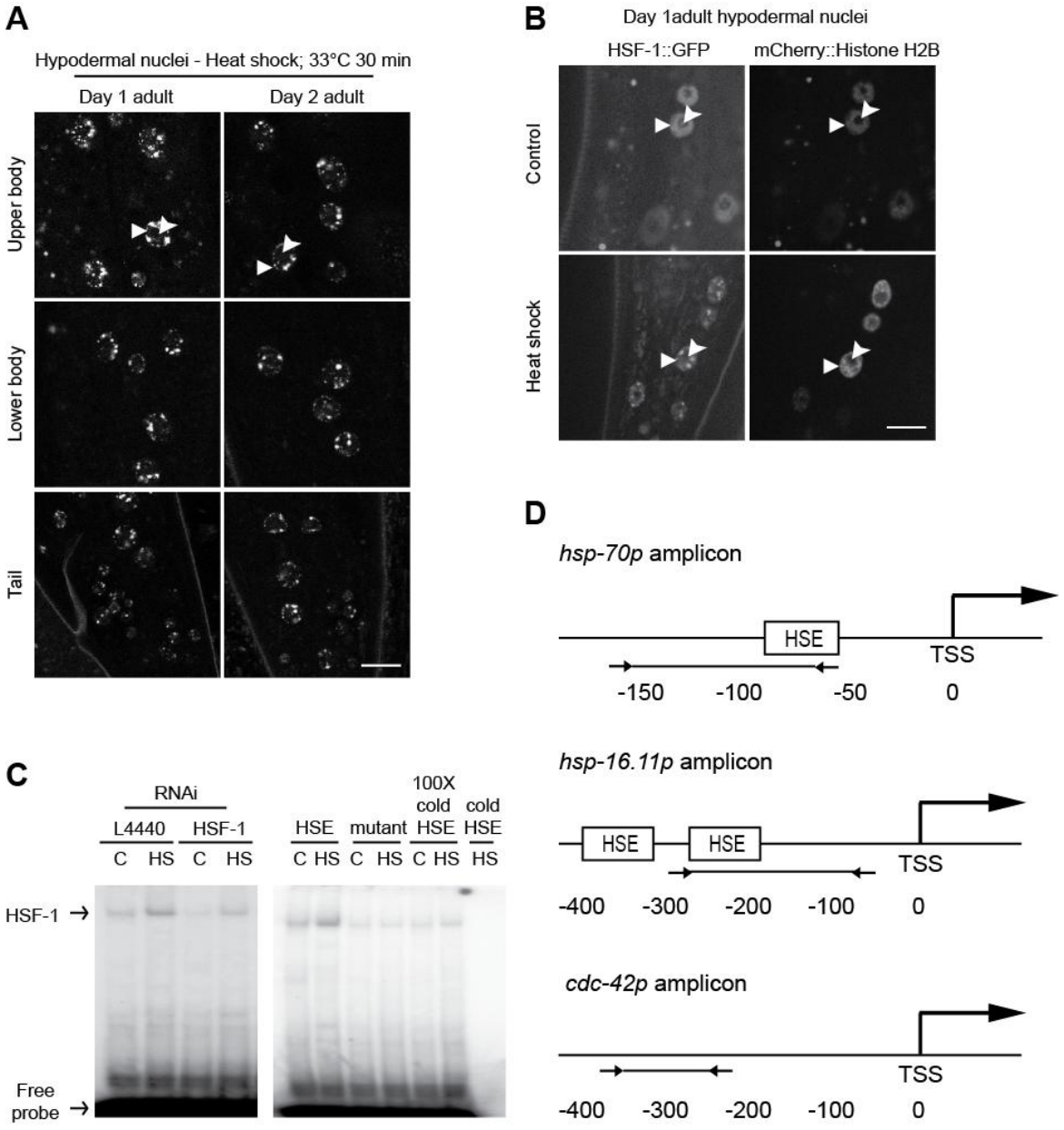
A
Hypodermal nuclei - Heat shock; 33°C 30 min
Day 1 adult
Day 2 adult
Upper body
Lower body
Tail
B
Day 1adult hypodermal nuclei
HSF-1::GFP
mCherry::Histone H2B
Control
Heat shock
C
RNAi
L4440
HSF-1
HSE
mutant
100X cold HSE
cold HSE
C HS C HS C HS C HS C HS
HSF-1
Free probe
D
hsp-70p amplicon
HSE
TSS
-150 -100 -50 0
hsp-16.11p amplicon
HSE HSE
TSS
-400 -300 -200 -100 0
cdc-42p amplicon
TSS
-400 -300 -200 -100 0
E
GFP ChIP: cdc-42p qPCR
% recovery of input
0.18
0.12
0.06
0
Control
Heat shock
Day 1
Day 2
Day 2
HSF1::GFP
N2
F
RNA Pol-II ChIP: cdc-42p
% recovery of input
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
ns
ns
Control
Heat shock
Day 1
Day 2
Day 2
α-RNA pol-2 (ama-1)
IgG

Figure S3, related to figure 3. HSF-1 forms nuclear foci following heat shock, complexes with a heat shock element containing DNA probe *ex vivo* and does not bind the *cdc-42* promoter.

(**A**) *C. elegans* expressing a single copy of HSF-1 C-terminally tagged with GFP (AM1060) were exposed to heat shock at 33°C for 30 min and then imaged within 20 min post heat shock at day 1 or day 2 of adulthood. (**B**) Confirmation of the nuclear localization of HSF-1::GFP in animals also expressing the nuclear marker mCherry::H2B. Triangles and arrow heads mark nuclei and nucleoli of select hypodermal cells. All images are representative of at least 4 animals. Scale bar = 10 µM. (**C**) Nuclear extracts from control (c) or heat shocked (HS) worms were incubated with radio-labelled intact heat shock element (HSE) or mutant HSE DNA probe or with an 100-fold excess of unlabelled HSE containing probe. Gel shifts were also performed using nuclear extracts from animals maintained on control (L4440) or HSF-1 RNAi. (**D**) Schematic of regions amplified in ChIP-qPCR assays relative to heat shock elements (HSE) and the transcription start site (TSS) as determined by modENCODE. ChIP using (**E**) anti-GFP or (**F**) anti-*ama-1* (Pol-II) antibody was performed and the levels of recovered *cdc-42* promoter were determined by quantitative PCR. Signal from wild type (N2) animals represents background for GFP pulldowns. IgG antibody was used to determine levels of non-specific signal in *ama-1* pulldowns. Values plotted are the mean from at least 3 biological replicates. Error bars represent SEM. Statistical significance was calculated by two-way ANOVA with Bonferroni post analysis comparison. Not significant (ns) $p > 0.05$.

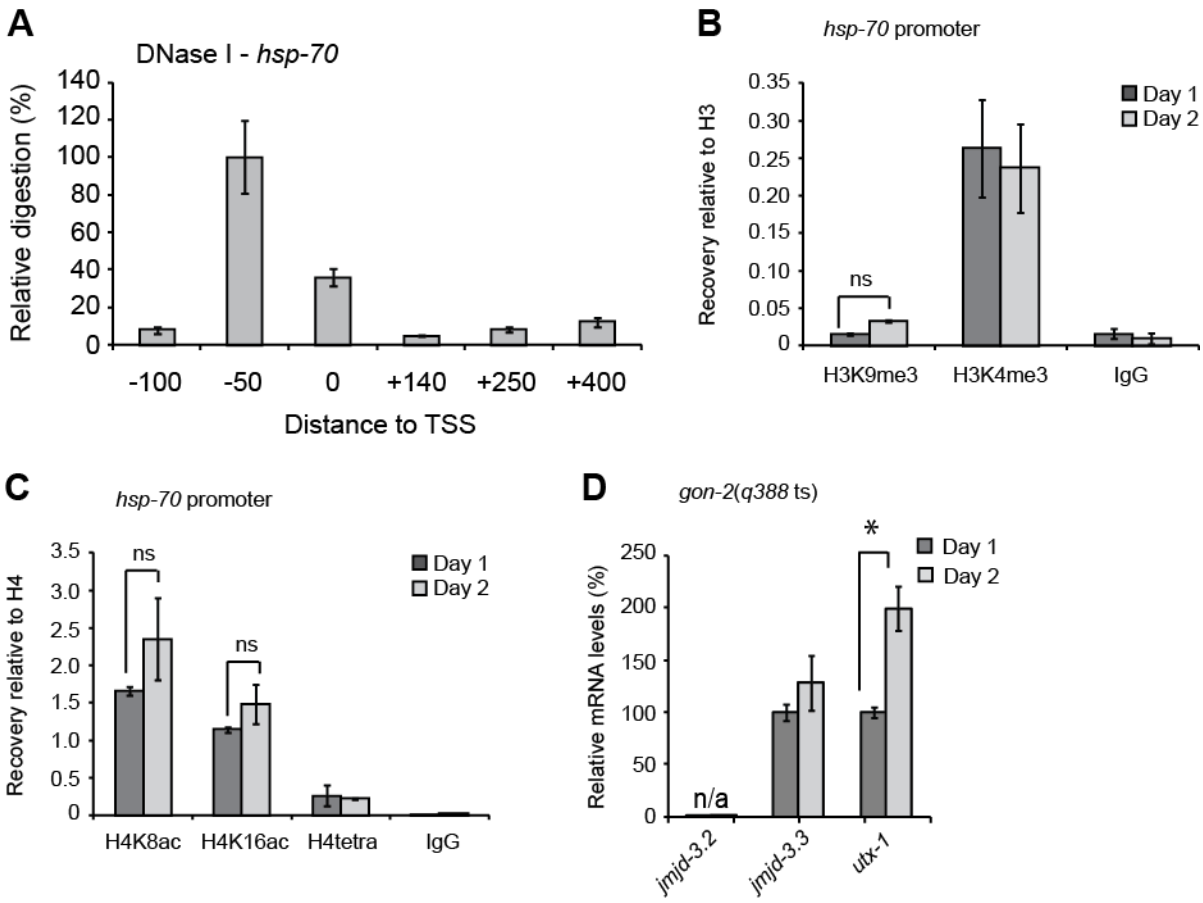
A
DNase I - hsp-70
Relative digestion (%)
140
120
100
80
60
40
20
0
-100
-50
0
+140
+250
+400
Distance to TSS
B
hsp-70 promoter
Recovery relative to H3
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
ns
Day 1
Day 2
H3K9me3
H3K4me3
IgG
C
hsp-70 promoter
Recovery relative to H4
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
ns
ns
Day 1
Day 2
H4K8ac
H4K16ac
H4tetra
IgG
D
gon-2(q388 ts)
Relative mRNA levels (%)
250
200
150
100
50
0
n/a
*
Day 1
Day 2
jmjd-3.2
jmjd-3.3
utx-1

Figure S4, related to figure 4. Optimization of DNase I treatment and chromatin immunoprecipitation of histone marks in gonad-less animals during early adulthood.

(**A**) Chromatin isolated from *gon-2* (*q388ts*) mutant animals was subjected to DNase I digestion or mock treatment and relative accessibility of genomic regions spanning the *hsp-70* promoter and gene body was determined by quantitative PCR. Levels of DNase I digestion are plotted relative to that observed for the promoter region containing the heat shock element (HSE) (TSS -50bp). Higher values indicate regions of greater accessibility. (**B** and **C**) ChIP with antibodies specific for (**B**) histone H3, H3K9me3, H3K4me3 or (**C**) histone H4, H4K8ac, H4K16ac, H4 tetra acetylation, and IgG was performed on chromatin extracted from *gon-2*(*q388ts*) mutants at day 1 or day 2 of adulthood. Levels of *hsp-70* promoter recovered with each antibody were plotted relative to (**B**) total H3 or (**C**) total H4. (**D**) Expression of H3K27me3 demethylases in *gon-2* worms at day 1 and day 2 of adulthood. Values plotted are the mean of 3 biological replicates except panel D which are the means of 4 replicates. Error bars represent SEM and statistical significance was calculated using Student's *t*-test. * $p < 0.05$; not significant (ns) $p > 0.05$. TSS denotes transcription start site. *hsp-70* refers to *C12C8.1*. n/a denotes no signal obtained.

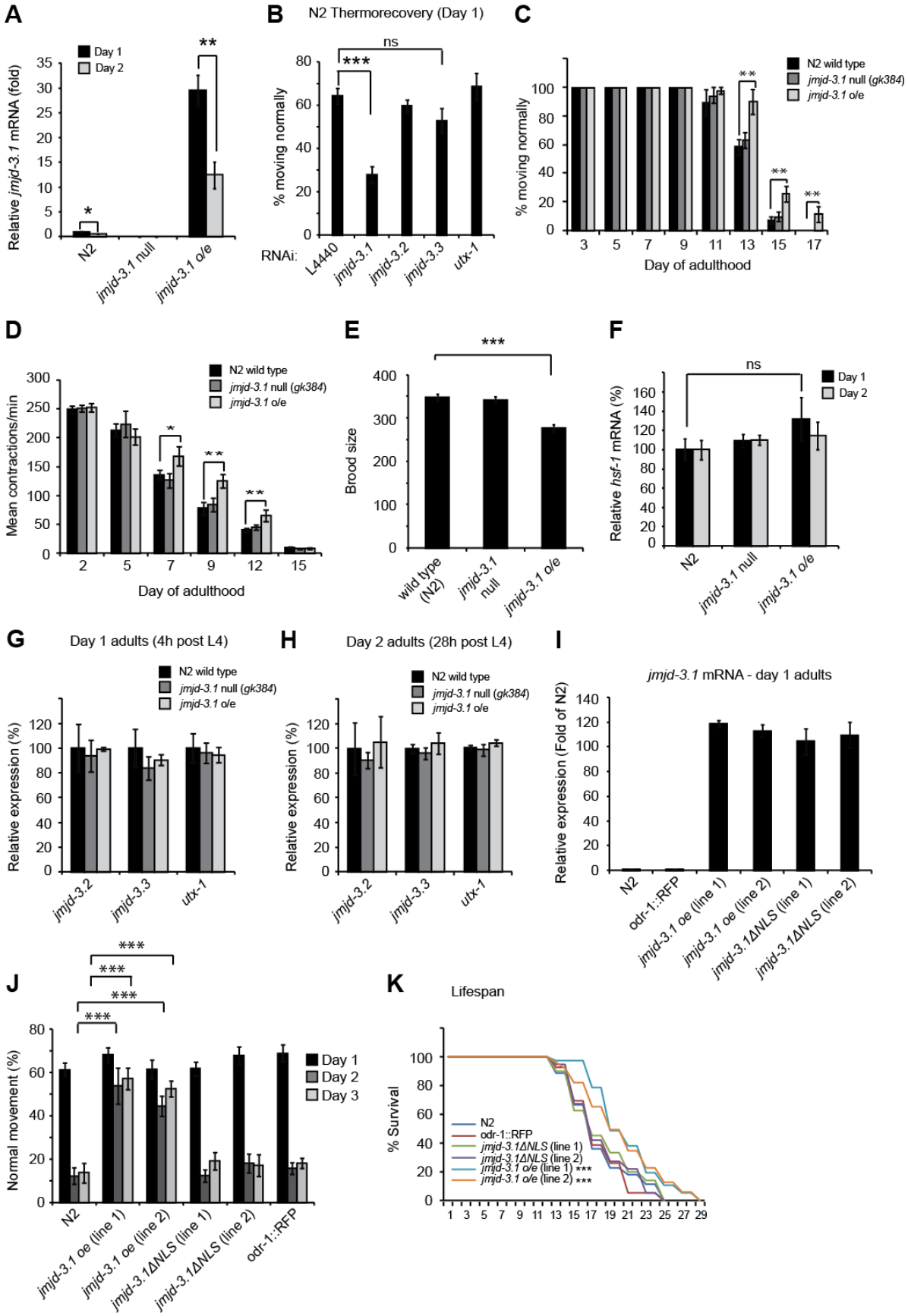
A
Relative jmjd-3.1 mRNA (fold)
Day 1
Day 2
N2
jmjd-3.1 null
jmjd-3.1 o/e
B
N2 Thermorecovery (Day 1)
% moving normally
ns
RNAi:
L4440
jmjd-3.1
jmjd-3.2
jmjd-3.3
utx-1
C
% moving normally
N2 wild type
jmjd-3.1 null (gk384)
jmjd-3.1 o/e
Day of adulthood
D
Mean contractions/min
N2 wild type
jmjd-3.1 null (gk384)
jmjd-3.1 o/e
Day of adulthood
E
Brood size
wild type (N2)
jmjd-3.1 null
jmjd-3.1 o/e
F
Relative hsf-1 mRNA (%)
Day 1
Day 2
G
Day 1 adults (4h post L4)
Relative expression (%)
N2 wild type
jmjd-3.1 null (gk384)
jmjd-3.1 o/e
H
Day 2 adults (28h post L4)
Relative expression (%)
I
jmjd-3.1 mRNA - day 1 adults
Relative expression (Fold of N2)
odr-1::RFP
jmjd-3.1 oe (line 1)
jmjd-3.1 oe (line 2)
jmjd-3.1ΔNLS (line 1)
jmjd-3.1ΔNLS (line 2)
J
Normal movement (%)
Day 1
Day 2
Day 3
K
Lifespan
% Survival
N2
odr-1::RFP
jmjd-3.1ΔNLS (line 1)
jmjd-3.1ΔNLS (line 2)
jmjd-3.1 o/e (line 1) ***
jmjd-3.1 o/e (line 2) ***

Figure S5, related to figure 5. *Jmjd-3.1* over expression suppresses age-related decline in pharangeal pumping and motility without effects on *hsf-1* or H3K27me3 demethylase expression. (**A**) Levels of *jmjd-3.1* in N2 wild type, *jmjd-3.1*(gk384) null or *jmjd-3.1* over-expressing (o/e) animals. (**B**) Thermorecovery of wild type (WT) N2 worms at day 1 (4 hours post L4) of adulthood following maintenance on control (L4440) or H3K27me3 specific RNAi. (**C**) Motility defects in worms during aging (n = 30 per group) (**D**) Pharangeal pumping rate. Mean number of contractions per minute was plotted (n = 10 per group). (**E**) Total brood size of N2 wild type, *jmjd-3.1* null or *jmjd-3.1* over-expressing worms. At least 20 worms were used per genotype. (**F)** *hsf*-1 expression in WT, *jmjd-3.1* null and *jmjd-3.1 o/e* worms at day 1 and day 2 of adulthood. (**G** and **H**) Expression of H3K27me3 demethylases in *jmjd-3.1* null or over-expressing animals at (**G)** day 1 or (**H**) day 2 (28 hours post L4) of adulthood. (**I**) Expression levels of *jmjd-3.1* in wild type (N2), *odr-1*::RFP (co-injection marker alone), two independent integrated *jmjd-3.1::*mCherry o/e lines (AM1110 and AM1111) and two independent integrated *jmjd-3.1delNLS*::mCherry o/e lines (AM1112 and AM1114) at day 1 of adulthood. (**J**) Thermorecovery at different days of adulthood of wild type worms and worms over-expressing *jmjd-3.1*::mCherry, *jmjd-3.1*delNLS::mCherry or co-injection marker alone. (**K**) Lifespan of worms over-expressing *jmjd-3.1*::mCherry, *jmjd-3.1*delNLS::mCherry or co-injection marker alone. Lifespan statistics from all trials (trials 1-5) can be found in Table S1. Statistical comparisons presented in (**K**) are relative to N2. Values plotted are the mean of at least 4 biological replicates (unless stated in the legend) and error bars represent SEM. Statistical significance was calculated by two-way ANOVA with Bonferroni post analysis comparison (C, D and J), one-way ANOVA with Tukey post analysis comparison (A, B, E and F), Student's *t*-test (G and H) or Log-rank test (K). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns $p > 0.05$.

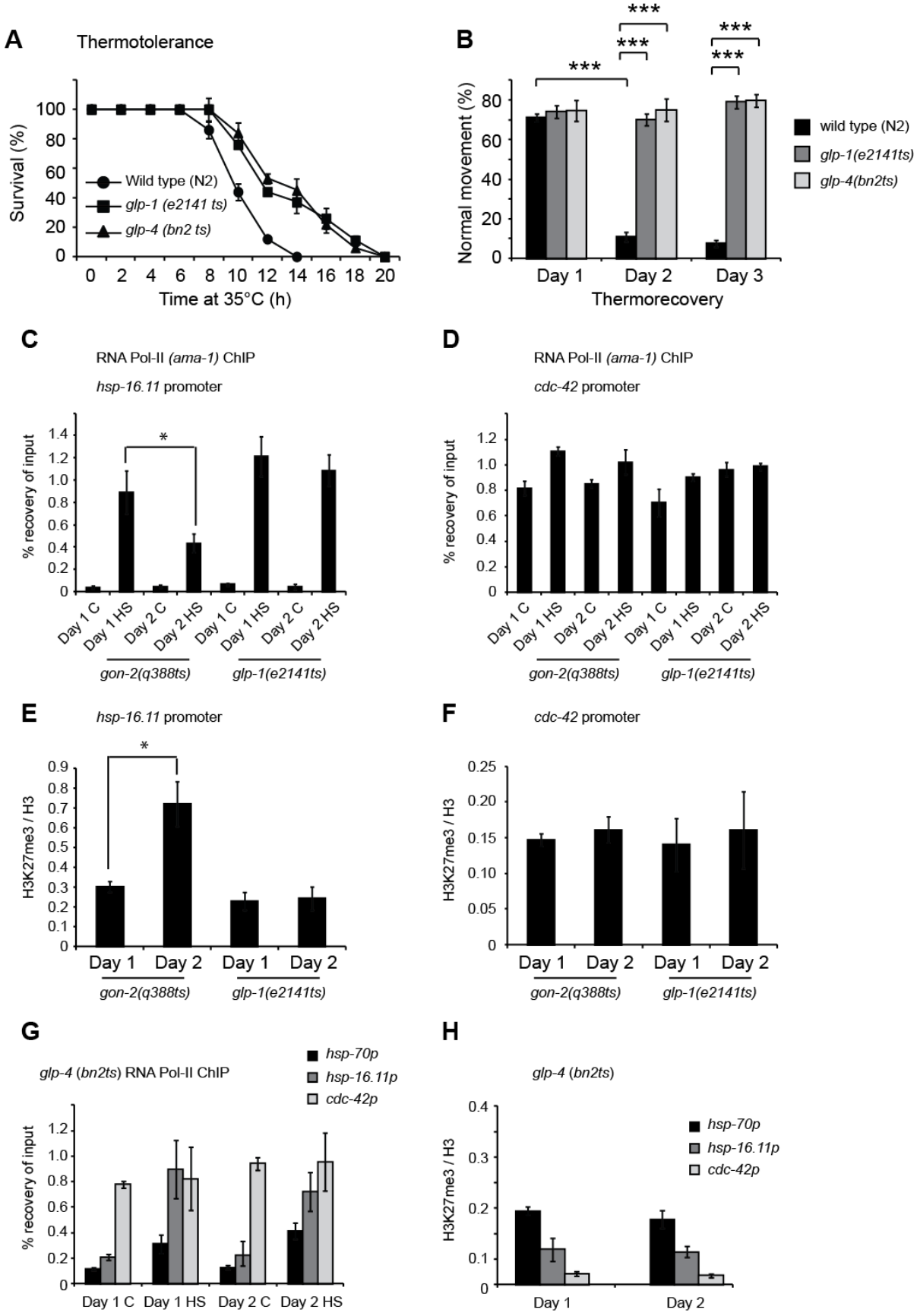
A
Thermotolerance
Survival (%)
Time at 35°C (h)
Wild type (N2)
glp-1 (e2141 ts)
glp-4 (bn2 ts)
B
Normal movement (%)
Thermorecovery
Day 1
Day 2
Day 3
wild type (N2)
glp-1(e2141ts)
glp-4(bn2ts)
C
RNA Pol-II (ama-1) ChIP
hsp-16.11 promoter
% recovery of input
Day 1 C
Day 1 HS
Day 2 C
Day 2 HS
gon-2(q388ts)
glp-1(e2141ts)
D
RNA Pol-II (ama-1) ChIP
cdc-42 promoter
E
hsp-16.11 promoter
H3K27me3 / H3
F
cdc-42 promoter
G
glp-4 (bn2ts) RNA Pol-II ChIP
hsp-70p
hsp-16.11p
cdc-42p
H
glp-4 (bn2ts)

Figure S6, related to figure 6. Removal of germ line stem cells suppresses H3K27me3 accumulation and restores RNA pol-II occupancy at heat shock promoters following stress.

(**A**) Survival of animals at 35°C. N2 wild type (WT) vs *glp-1*, $p < 0.001$; WT vs *glp-4*, $p < 0.001$. See Table S2 for complete statistical comparison of groups. (**B**) Thermorecovery of animals 48 hours post heat shock at 33°C for 6 hours. (**C - H**) ChIP was performed on chromatin from day 1 or day 2 adult *gon-2*(*q388ts*), *glp-1*(*e2141ts*) or *glp-4(bn2 ts)* worms using antibodies specific for (**C**, **D** and **G**) RNA pol-II (*ama-1*) or (**E**, **F** and **H**) histone H3 and H3K27me3. Levels of H3K27me3 were quantified relative to total histone H3 levels. Values plotted are the mean of at least 3 biological replicates and error bars represent SEM. Statistical significance was calculated using two-way ANOVA with Bonferroni post analysis comparison (A-G) or Student's t-test (H). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

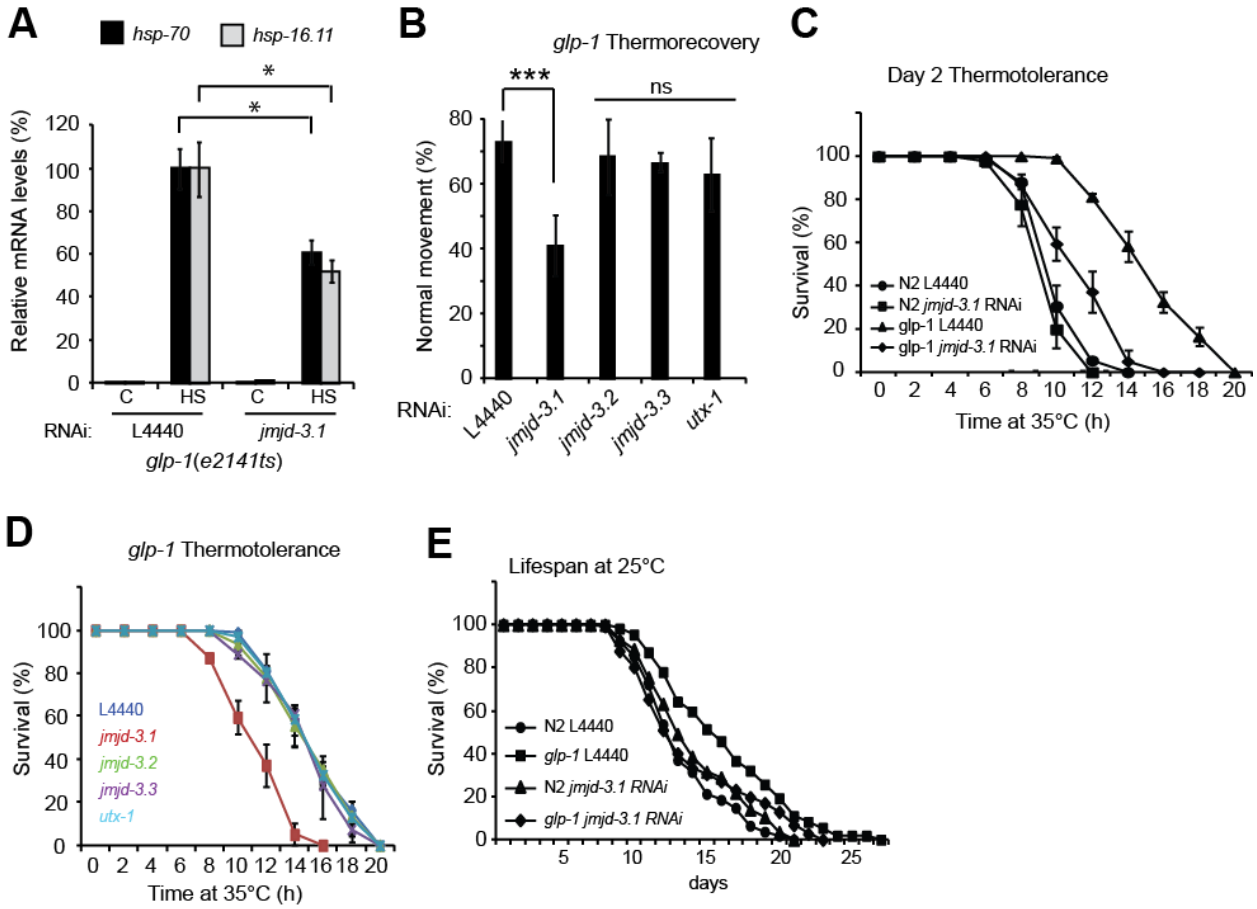
A
hsp-70
hsp-16.11
Relative mRNA levels (%)
C
HS
C
HS
RNAi:
L4440
jmjd-3.1
glp-1(e2141ts)
B
glp-1 Thermorecovery
Normal movement (%)
***
ns
RNAi:
L4440
jmjd-3.1
jmjd-3.2
jmjd-3.3
utx-1
C
Day 2 Thermotolerance
Survival (%)
N2 L4440
N2 jmjd-3.1 RNAi
glp-1 L4440
glp-1 jmjd-3.1 RNAi
Time at 35°C (h)
D
glp-1 Thermotolerance
Survival (%)
L4440
jmjd-3.1
jmjd-3.2
jmjd-3.3
utx-1
Time at 35°C (h)
E
Lifespan at 25°C
Survival (%)
N2 L4440
glp-1 L4440
N2 jmjd-3.1 RNAi
glp-1 jmjd-3.1 RNAi
days

Figure S7, related to figure 7. *Jmjd-3.1* is necessary for maintenance of the HSR, enhanced stress resistance and increased lifespan of germ line stem cell deficient animals. (**A**) Expression of HS genes in *glp-1* animals grown on control (L4440) or *jmjd-3.1* RNAi and exposed to control or heat shock at day 2 of adulthood. (**B**) Thermorecovery of *glp-1* worms at day 2 of adulthood after growth on control (L4440) or H3K27me3 specific RNAi. (**C** and **D**) Survival of day 2 adults maintained at 35°C. Data are from parallel experiments and for clarity are presented as (**C**) glp-1 vs N2, L4440 and jmjd-3.1 only, or (**D**) *glp-1* RNAi only. (**E**) Lifespan analysis of wild type (N2) and *glp-1* mutants grown on L4440 or *jmjd-3.1* RNAi at 25°C. Statistical significance was calculated by Student's *t*-test (A and B), two-way ANOVA (C and D) or Log-rank test (E). * $p < 0.05$, *** $p < 0.001$. For complete statistical comparison of data in panels C-E see Tables S1 and S2.

Supplemental tables

Table S1, related to figures 5 and 7. Statistics associated with lifespan assays.

Experiment 1			
Treatment group (n)	**Mean lifespan**	**Standard error**	**LOG-RANK *p*-value (to N2)**
N2 (77)	17.08	0.37	n/a
jmjd-3.1 gk384 (85)	17.11	0.32	0.78
jmjd-3.1 o/e (65)	19.36	0.50	0.0006
Experiment 2			
Treatment group (n)	**Mean lifespan**	**Standard error**	**LOG-RANK *p*-value (to N2)**
N2 (87)	15.94	0.41	n/a
jmjd-3.1 gk384 (93)	16.01	0.40	0.965
jmjd-3.1 o/e (90)	17.76	0.52	0.0045
Experiment 1			
Treatment group (n)	**Mean lifespan**	**Standard error**	**LOG-RANK *p*-value (to glp-1)**
N2 (62)	12.67	0.35	< 0.0001
glp-1 (70)	15.61	0.46	n/a
glp-1;jmjd-3.1 (69)	14.20	0.28	0.0001
Experiment 2			
Treatment group (n)	**Mean lifespan**	**Standard error**	**LOG-RANK *p*-value (to glp-1)**
N2 (80)	12.15	0.31	< 0.0001
glp-1 (65)	16.2	0.52	n/a
glp-1;jmjd-3.1 o/e (74)	14.20	0.48	0.0001
Experiment 1			
Treatment group (n)	**Mean lifespan**	**Standard error**	**LOG-RANK *p*-value (to N2 L4440)**
N2 L4440 (119)	12.97	0.27	n/a
N2 *jmjd-3.1* (101)	13.94	0.32	0.015
glp-1 L4440 (54)	17	0.57	> 0.0001
glp-1 *jmjd-3.1* (64)	13.92	0.48	0.007
Experiment 2			
Treatment group (n)	**Mean lifespan**	**Standard error**	**LOG-RANK *p*-value (to N2 L4440)**
N2 L4440 (127)	13.5	0.3	n/a
N2 *jmjd-3.1* (115)	14.05	0.29	0.568
glp-1 L4440 (54)	18.17	0.78	> 0.0001
glp-1 *jmjd-3.1* (66)	14.76	0.64	0.003
Experiment 3			
Treatment group (n)	**Mean lifespan**	**Standard error**	**LOG-RANK *p*-value (to N2 L4440)**
N2 L4440 (108)	13.38	0.29	n/a
N2 *jmjd-3.1* (114)	14.18	0.32	0.05
glp-1 L4440 (109)	16.24	0.4	> 0.0001

glp-1 *jmjd-3.1* (147)	13.97	0.34	0.05
Experiment 4			
Treatment group (n)	**Mean lifespan**	**Standard error**	**LOG-RANK *p*-value (to N2 L4440)**
N2 L4440 (88)	14.23	0.33	n/a
N2 *jmjd-3.1* (113)	14.65	0.33	0.17
glp-1 L4440 (119)	18.5	0.43	> 0.0001
glp-1 *jmjd-3.1* (124)	15.28	0.48	0.002
Experiment 1			
Treatment group (n)	**Mean lifespan**	**Standard error**	**LOG-RANK *p*-value (to N2)**
N2 (94)	16.8	0.46	n/a
*jmjd-3.1*o/e line 1(63)	20.7	0.68	< 0.0001
*jmjd-3.1*o/e line 2 (64)	20.5	0.89	<0.0001
odr-1::RFP (65)	16.8	0.55	0.8553
Experiment 2			
Treatment group (n)	**Mean lifespan**	**Standard error**	**LOG-RANK *p*-value (to N2)**
N2 (45)	17.8	0.53	n/a
*jmjd-3.1*o/e line 1(43)	20.6	0.66	0.0071
*jmjd-3.1*o/e line 2 (45)	20.6	0.63	0.0056
odr-1::RFP (49)	17.7	0.47	0.767
Experiment 3			
Treatment group (n)	**Mean lifespan**	**Standard error**	**LOG-RANK *p*-value (to N2)**
N2 (56)	18.2	0.59	n/a
*jmjd-3.1*ΔNLS line 1 (51)	18.5	0.55	0.676
*jmjd-3.1*ΔNLS line 2 (54)	18.3	0.45	0.964
odr-1::RFP (48)	17.7	0.55	0.658
Experiment 4			
Treatment group (n)	**Mean lifespan**	**Standard error**	**LOG-RANK *p*-value (to N2)**
N2 (57)	17.54	0.74	n/a
*jmjd-3.1*ΔNLS line 1 (56)	18.33	0.80	0.462
*jmjd-3.1*ΔNLS line 2 (56)	18.56	0.74	0.444
odr-1::RFP (60)	18.15	0.62	0.454
Experiment 5			
Treatment group (n)	**Mean lifespan**	**Standard error**	**LOG-RANK *p*-value (to N2)**
N2 (56)	17.4	0.69	n/a
*jmjd-3.1*ΔNLS line 1 (61)	17.6	0.56	0.89
*jmjd-3.1*ΔNLS line 2 (50)	17.6	0.67	0.73
*jmjd-3.1*o/e line 1(54)	19.6	0.79	0.012
*jmjd-3.1*o/e line 2 (60)	19.5	0.69	0.006

Table S2, related to figures 5 and 7. Additional statistical comparisons from thermotolerance assays. *P*-values were calculated by two-way ANOVA with Bonferroni post analysis comparison of groups.

Thermotolerance (Figure 5C)		
Genotype	***vs* genotype**	***p*-value**
N2 wild type, day 1	N2 wild type, day 2	0.01
N2 wild type, day 1	*jmjd-3.1(gk384)*, day 1	0.01
N2 wild type, day 1	*jmjd-3.1(gk384)*, day 2	< 0.001
N2 wild type, day 1	*jmjd-3.1* oe, day 1	> 0.05
N2 wild type, day 1	*jmjd-3.1* oe, day 2	> 0.05
N2 wild type, day 2	*jmjd-3.1(gk384)*, day 1	> 0.05
N2 wild type, day 2	*jmjd-3.1(gk384)*, day 2	> 0.05
N2 wild type, day 2	*jmjd-3.1oe*, day 1	0.009
N2 wild type, day 2	*jmjd-3.1oe*, day 2	0.002
jmjd-3.1(gk384), day 1	*jmjd-3.1(gk384)*, day 2	> 0.05
jmjd-3.1(gk384), day 1	*jmjd-3.1oe*, day 1	< 0.001
jmjd-3.1(gk384), day 1	*jmjd-3.1oe*, day 2	< 0.001
jmjd-3.1(gk384), day 2	*jmjd-3.1oe*, day 1	< 0.001
jmjd-3.1(gk384), day 2	*jmjd-3.1oe*, day 2	0.002
jmjd-3.1oe, day 1	*jmjd-3.1oe*, day 2	> 0.05
Thermotolerance (Figure 7D)		
Genotype	***vs* genotype**	***p*-value**
N2 wild type	*glp-1(e2141ts)*	< 0.0001
N2 wild type	*glp-1(e2141ts);jmjd-3.1(gk384)*	0.08
glp-1(e2141ts)	*glp-1(e2141ts);jmjd-3.1(gk384)*	0.001
Thermotolerance (Figure S6A)		
Genotype	***vs* genotype**	***p*-value**
N2 wild type	*glp-1(e2141ts)*	< 0.0001
N2 wild type	*glp-4(bn2ts)*	< 0.0001
glp-1(e2141ts)	*glp-4(bn2ts)*	> 0.05

Table S2. cont.

Thermotolerance (Figure S7C)		
genotype, RNAi	***vs* genotype, RNAi**	***p*-value**
N2 L4440	*glp-1* L4440	< 0.001
N2 L4440	N2 *jmjd-3.1*	> 0.05
N2 L4440	*glp-1 jmjd-3.1*	< 0.001
glp-1 L4440	N2 *jmjd-3.1*	< 0.001
glp-1 L4440	*glp-1 jmjd-3.1*	< 0.001
N2 *jmjd-3.1*	*glp-1 jmjd-3.1*	< 0.001
Thermotolerance (Figure S7D)		
***glp-1(e2141ts)*, RNAi**	***vs glp-1*, RNAi**	***p*-value**
L4440	*jmjd-3.1*	< 0.001
L4440	*jmjd-3.2*	> 0.05
L4440	*jmjd-3.3*	> 0.05
L4440	*utx-1*	> 0.05
jmjd-3.1	*jmjd-3.2*	< 0.001
jmjd-3.1	*jmjd-3.3*	< 0.001
jmjd-3.1	*utx-1*	< 0.001
jmjd-3.2	*jmjd-3.3*	> 0.05
jmjd-3.2	*utx-1*	> 0.05
jmjd-3.3	*utx-1*	> 0.05

Table S3, related to all main figures. Primers and probes used in this study

Target	Forward	Reverse
C12C8.1	CTACATGCAAAGCGATTGGA	GGCGTAGTCTTGTTCCCTTC
hsp-16.11	GGCTCAGATGGAACGTCAA	GCTTGAACTGCGAGACATTG
F44E5.4	TGATACCCATCTCGGAGGAG	GTGGATTGGGTGAAATGTCC
hsp-4	GGGGACAATCATTGGTATCG	ACGCAACGTATGATGGAGTG
hsp-3	GAACCATCGCTGGATTGAAC	CCTCCAAGATCGAAGACGAG
hsp-6	GTTATCGAGAACGCAGAAGGAG	CATCCTTAGTAGCTTGACGCTG
hsp-60	CATGCTCGTCGGAGTCAAC	TTTGTGATCTTTGGGCTTCC
gcs-1	GTGCAAGTGTCGACGATCGTAC	GCGAATATGTTTTGCCAGTGGCTC
gst-4	CCGTTTTCTATGGAAGTGACG	GCCCAAGTCAATGAGTCTCC
mtl-1	TGCAAGTGTGACTGCAAAAA	TTTTTCTCACTGGCCTCCTC
jmjd-3.1	ACGCCTGATGTTCTCATTCGCA	TATGCTGCACAGGCACAGGTTTC
jmjd-3.2	ATGCCTGGGCTGTCTTCGATTT	AATGCGGAAGGATTGCGTAGGA
jmjd-3.3	CGACCGAACGTATCCACTCACCTC	CGCCCGCTTCTTTGCCATTT
utx-1	TTCGGGTTAGGTCTTGTCTACTTG	TCATCATTATTCTCGTGGCTCAG
hsf-1	TGTACAAGGACGTCCCGAAT	TCCAAATTTTGTTGCGTCTG
cdc-42	GGTTGCTCCAGCTTCATTC	AACAAGAATGGGGTCTTTGA
rpb-2	AACTGGTATTGTGGATCAGGTG	TTTGACCGTGTCGAGATGC
HSE probe	TAAATTGTAGAAGGTTCTAGAAGATGCCAGA	TCTGGCATCTTCTAGAACCTTCTACAATTTA
Mutant probe	TAAATTGTAAAAGGAAATAAAAGATGCCAGA	TCTGGCATCTTTTATTTCCTTTTACAATTTA
hsp-70 TSS -50bp	AACTCAAATCTTATGCAGAAT	CGTAGTACCCAAGTCGATTCCA
hsp-70 TSS +0bp	ACGTACTCATGTGTCGGTAT	TCTTCTTCCAGTTTACATAATCCT
hsp-70 TSS+60bp	ACGTACTCATGTGTCGGTAT	TCTTCTTCCAGTTTACATAATCCT
hsp-70 TSS +120bp	TTCCGTTTTCAGGTTGAAATCCTC	TAAAAGCCACGTAGGAAGGCG
hsp-70 TSS +1Kb	GCCGGTTGAAAAGGCACTTC	TACGAGTTGATCCCCCAACC
hsp-16.11 promoter	CTGAATGTGAGTCGCCCTCC	GAGAGCCTCTGCAAACTGGA
cdc-42 promoter	ATGGTAAAGAAACGCTCGTG	TGAAAAATACGGATGAGTCACA
hsp-4 promoter	CGAAAAGTCTCGATTTCTCCA	ACACGTCGCAAAGAAGGAGT
gcs-1 promoter	GCCTTCAGGAGAGATGTAGAGAC	AATGCGAGCGTTTTTCCCAT
hsp-6 promoter	GGGCGTAACTGCAATGTGTG	TCACGGGCTTTTGCATCAAC
C12C8.1 promoter	ATAGCATAGGCGACCCACAG	ACGTTCCTCTGGCATCTTCT

Table S4, related to figures 3, 4, 6 and 7. Antibodies used in this study

Target	Supplier / Catalogue no.
Pan H3	Abcam ab1791
Pan H4	Abcam ab10158
H3K4me3	Millipore 07-473
H3K27me3	Millipore 07-449
H3K9me3	Millipore 07-442
H4K8ac	Abcam ab15823
H4K16ac	Millipore 07-329
H4 tetra acetyl	Millipore 06-866
Tubulin	Sigma T5168
Hsp-16	Lithgow lab

Supplemental experimental procedures

Worm maintenance

Worm strains used in this study were: CF1903 *glp-1* (*e2141ts*), CF2253 *gon-2* (*q388ts*), JK560 *fog-1*(*q253ts*), JK816 *fem-3* (*q20gf*), VC936 *jmjd-3.1* (*gk384*), AM726 *rmIs291*[*hsp-70p*::*mCherry*::p*myo-2::CFP*], OG497 *unc-119(ed3);drSi13*[*hsf-1p::hsf-1::GFP::unc-54utr;Cb-unc-119+*], AM1060 rmSi1[*hsf-1p*::*hsf-1::GFP::hsf-1utr*], AM1063 *unc-119(ed9)III, rmSi1 II; ltIs37 [pie-1*p*::mCherry::his-58 (pAA64) + unc-119(+)]* and AM1107 *Ex355*[*jmjd-3.1p*::*jmjd-3.1::mCherry ; odr-1::RFP]*, AM1110 rmIs370[jmjd-3.1p::jmjd-3.1::mCherry ; *odr-1*::dsRed], AM1111 rmIs371[jmjd-3.1p::jmjd-3.1::mCherry ; *odr-1*::dsRed], AM1112 rmIs372[jmjd-3.1p::jmjd-3.1delNLS::mCherry ; *myo-2*::mCherry)] and AM1114 rmIs374[jmjd-3.1p::jmjd-3.1delNLS::mCherry ; *myo-2*::mCherry)].

Chromatin immunoprecipitation

ChIP was performed as previously described (Mukhopadhyay et al., 2008) with slight modifications. Cross-linking was achieved by gently douncing 15 -20,000 worms (grown on solid NGM plates) for 30 minutes in 2% formaldehyde at room temperature. Worms were washed 3 times in 1 x PBS before being re-suspended in lysis buffer. Worms were dounced vigorously on ice and briefly sonicated on high power (30s “on”, 1 min “off”) using a Bioruptor sonicator until all carcasses were completely destroyed (as determined under a light microscope). Extracts were sonicated as above to yield 500bp – 1kb size DNA fragments (as determined by agarose gel electrophoresis). For pulldowns, chromatin (2 µg DNA) was incubated with 25 µl of protein g-dynabeads (pre-blocked with salmon sperm DNA and bovine serum albumin) and 2 µg of antibody at 4°C with rotation over night. After low to high salt washes, DNA was eluted from

beads by agitation at 65°C in 1% SDS and 0.1 M sodium bicarbonate for 1 hour. Cross linking was reversed by incubation at 65°C with 0.2 M NaCl overnight and DNA was recovered using Qiagen PCR purification kit as per manufacturer's instructions. Strain OG497 was used for HSF-1 ChIP to minimize signal from the germ line (Morton and Lamitina, 2013; Tatum et al., 2015). A complete list of primers and antibodies used in these experiments can be found in supplementary tables 1 and 2.

DNase I digestion assay

DNase I digestion assays were performed as previously described with some modifications (Dorschner et al., 2004). *C. elegans* were dounced vigorously on ice in Buffer A to release nuclei which were then washed and incubated with 200U of DNase I (Thermoscientific) for 10 minutes at 37°C in nuclease digestion buffer. Digested DNA was prepared for qPCR by standard phenol/chloroform ethanol precipitation. Real time quantitative PCR was used to quantify the digestion of genomic regions of interest in DNase treated versus untreated samples. The amount of DNA remaining following DNase digestion was compared to the starting amount (undigested) in order to calculate the relative amount of digestion that had taken place in each sample. The relative amount of digestion at genomic regions in chromatin from day 2 animals relative to day 1 adults was calculated and plotted in order to provide a less counter-intuitive view of the data.

Protein extraction and western blotting

Standard methods for western blotting were used for the detection of HSP-16 and tubulin. Briefly, 100 worms were collected in 20 µl of lysis buffer [50 mM Tris pH 7.4, 5 mM $MgCl_2$, 2% Triton X-100, 0.2 mM PMSF, 1 µg/µl Leupeptin, protease inhibitor tablet] and freeze thawed three times. Worms were then sonicated twice on high power for 15 minutes total (30s on, 1 min off) using a Bioruptor sonicater and cell debris was removed by centrifugation. 5 µg of protein

lysate in Laemmli loading buffer was separated by SDS-PAGE and transferred to nitrocellulose membranes. Blots were blocked for 1 hour with 5% milk in PBS and probed using anti HSP-16 (1:1000; overnight at 4°C) or anti tubulin primary antibodies (1:5000; 1 hour at room temperature). Promega anti mouse (W4021) and anti rabbit (W4011) HRP conjugated secondary antibodies were used at 1:5000 dilution for 1 hour at room temperature. Proteins were detected using ECL plus reagent (Perkin Elmer) and signal was captured using a Syngene PXi imager and Genesys software.