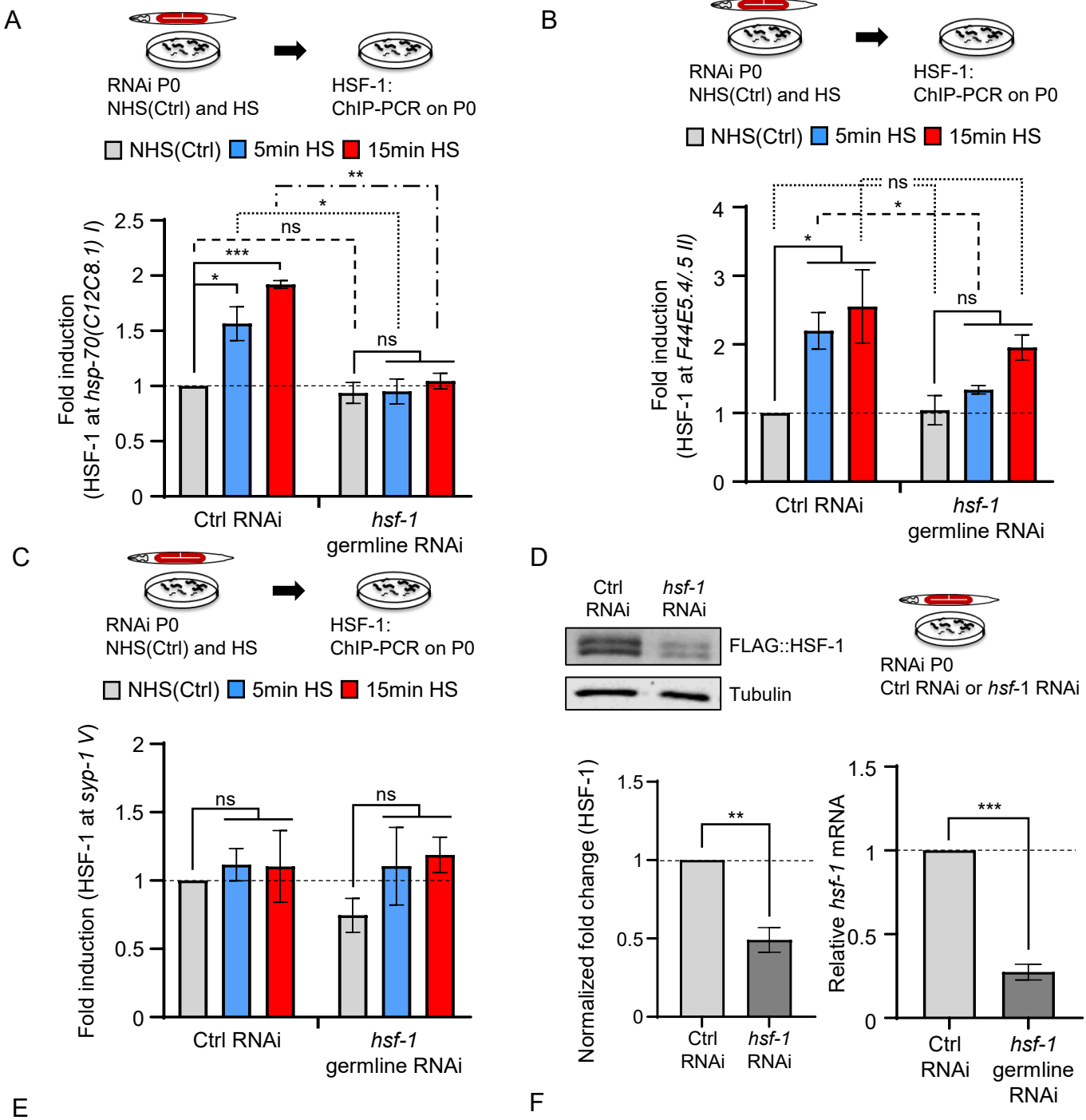
Figure S 1



CONDITION	Average number of eggs in uterus	Onset of laying of oocytes fertilized post-HS
NHS(Ctrl)	9.7 ± 0.5/animal	2 hours
5min HS	5.2 ± 0.8/animal	2 hours
30min HS	6.4 ± 0.6/animal	4 hours
60min HS	4.7 ± 0.5/animal	8 hours

Figure S1: Experimental protocol to activate HSF-1 in germ cells of *C. elegans* to examine the later-life phenotypes of offspring generated from these germ cells, Related to **Figures 1 and 2**.

(A) HSF-1 occupancy at the Promoter regions of *hsp-70(C12C8.1) I* under non-heat shock control conditions [NHS(Ctrl)] and upon a short (5 minute), and long (15 minute) heat-shock, determined in wild-type animals and animals where *hsf-1* was knocked down in the germline. (n=2-4 experiments). Data presented in **A** are normalized to non-heat shocked control [NHS(Ctrl)] control RNAi values. Mean induction when values are normalized to non-heat shocked control [NHS(Ctrl)] *hsf-1* RNAi values are 0.99 ± 0.07 and 1.20 ± 0.28 at 5 and 15 minute heat-shock respectively, and also not significantly different from NHS(Ctrl) *hsf-1* RNAi animals. For exact positions assayed in the ChIP-PCR experiments, see **STAR Methods**.

(B) HSF-1 occupancy at the Promoter region of *F44E5.4/.5 II* under non-heat shock control conditions [NHS(Ctrl)] and upon a short (5 minute), and long (15 minute) heat-shock, determined in wild-type animals and animals where *hsf-1* was knocked down in the germline. (n=2-4 experiments). Upon a long (15 minute) heat shock, HSF-1 binding at the *F44E5.4/.5* promoter is decreased, but is significantly ($p<0.001$) higher than non-heat shocked control [NHS(Ctrl)] *hsf-1* RNAi values, presumably because the HSF-1 promoter occupancy in somatic cells rapidly overrides contribution from the germline. Data presented in **B** are normalized to non-heat shocked control [NHS(Ctrl)] control RNAi values. Mean induction at 5 and 15 minutes when values are normalized to non-heat shocked control [NHS(Ctrl)] *hsf-1* RNAi values are 1.24 ± 0.16 and 2.35 ± 0.06 respectively. For exact positions assayed in the ChIP-PCR experiments, see **STAR Methods**.

(C) Specificity of HSF-1 binding in **A**, and **B**, verified by assessing HSF-1 occupancy at the *syp-1* promoter which does not possess a HSE, and is not known to bind HSF-1. For exact positions assayed in the ChIP-PCR experiments, see **STAR Methods**. (n=2-4 experiments).

(D) Left: Anti-FLAG::HSF-1 validated using RNAi knockdown of *hsf-1*. **Right:** *hsf-1* mRNA levels determined relative to *pmp*-3 and compared to that in animals subjected to control RNAi (L4440) treatment following germline RNAi to validate *hsf-1* RNAi knockdown.

(E) Y-axis: Total cumulative number of embryos laid by non-heat shocked control P0 mothers [NHS(Ctrl)] and P0 mothers subjected to 5 minute, 30 minute and 60 minute heat shock, at different times (X-axis) following heat shock. These numbers were calculated by counting the number of embryos laid every 2 hours for upto 12 hours.

(F) **Table Column 1**: P0 mothers' treatment conditions. **Table Column 2**: Average numbers of already fertilized eggs in the uterus of mothers under control conditions [NHS(Ctrl)], and immediately after heat shock exposure. The number of unfertilized oocytes in all animals (8.6 ± 0.5/animal). **Table Column 3**: The time post-heat shock at which oocytes resident in the maternal germline during heat shock will start being laid post-fertilization, as calculated from the information in **E**, and **Column 2**.

A-D: Data show Mean ± Standard Error of the Mean. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, ns: non-significant; Unpaired Student's t-test.

Figure S 2

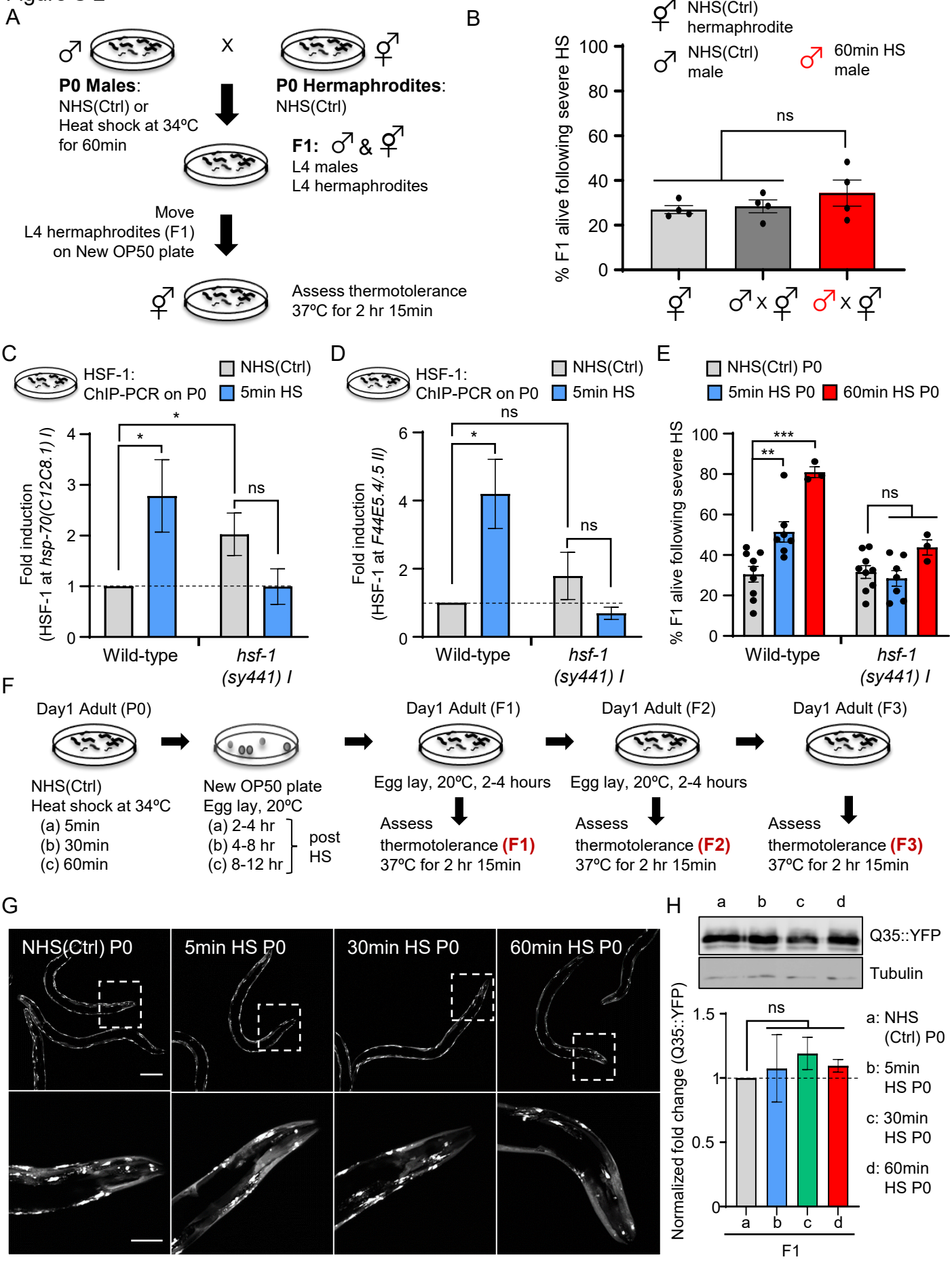

A
P0 Males:
NHS(Ctrl) or
Heat shock at 34ºC
for 60min
X
P0 Hermaphrodites:
NHS(Ctrl)
F1:
L4 males
L4 hermaphrodites
Move
L4 hermaphrodites (F1)
on New OP50 plate
Assess thermotolerance
37ºC for 2 hr 15min
B
NHS(Ctrl)
hermaphrodite
NHS(Ctrl)
male
60min HS
male
% F1 alive following severe HS
ns
C
HSF-1:
ChIP-PCR on P0
NHS(Ctrl)
5min HS
Fold induction
(HSF-1 at hsp-70(C12C8.1) I)
Wild-type
hsf-1
(sy441) I
D
HSF-1:
ChIP-PCR on P0
NHS(Ctrl)
5min HS
Fold induction
(HSF-1 at F44E5.4/.5 II)
Wild-type
hsf-1
(sy441) I
E
NHS(Ctrl) P0
5min HS P0
60min HS P0
% F1 alive following severe HS
Wild-type
hsf-1
(sy441) I
F
Day1 Adult (P0)
NHS(Ctrl)
Heat shock at 34ºC
(a) 5min
(b) 30min
(c) 60min
New OP50 plate
Egg lay, 20ºC
(a) 2-4 hr
(b) 4-8 hr
(c) 8-12 hr
post HS
Day1 Adult (F1)
Egg lay, 20ºC, 2-4 hours
Assess thermotolerance (F1)
37ºC for 2 hr 15min
Day1 Adult (F2)
Egg lay, 20ºC, 2-4 hours
Assess thermotolerance (F2)
37ºC for 2 hr 15min
Day1 Adult (F3)
Assess thermotolerance (F3)
37ºC for 2 hr 15min
G
NHS(Ctrl) P0
5min HS P0
30min HS P0
60min HS P0
H
a b c d
Q35::YFP
Tubulin
Normalized fold change (Q35::YFP)
a: NHS (Ctrl) P0
b: 5min HS P0
c: 30min HS P0
d: 60min HS P0
F1

Figure S2: Maternal HSF-1 protects progeny stress resilience and proteostasis, Related to **Figure 1**.

(A) Schematic of experimental procedure to test whether thermotolerance is transmitted through oocytes or sperm. Wild-type day one old males were heat shocked at 34°C for 60 minute, transferred onto plates containing non-heat shocked [NHS(Ctrl)] L4 wild-type hermaphrodites and allowed to mate. F1 hermaphrodite progeny were transferred onto a new plate and as day one old adult hermaphrodites, subjected to a severe heat shock of 37°C for 2 hours, 15 minutes.

(B) Stress resilience of F1 hermaphrodite cross-progeny generated through the mating of heat shocked males with non-heat shocked hermaphrodites, F1 hermaphrodite progeny from non-heat shocked males mated with non-heat shocked hermaphrodites, and self-progeny of non-heat shocked hermaphrodites. (n=4 experiments).

(C) HSF-1 occupancy at the Promoter regions of *hsp-70(C12C8.1) I* under non-heat shocked control conditions [NHS(Ctrl)] and upon a short (5 minute) heat shock in wild-type animals and *hsf-1(sy441) I* mutants. (n=3-5 experiments). Data are normalized to non-heat shocked control [NHS(Ctrl)] wild-type values. When normalized to *hsf-1(sy441) I* non-heat shocked control [NHS(Ctrl)] values, the mean occupancy of a 5 minute heat shock is 0.46 ± 0.12, and is significantly lower ($p<0.05$) than non-heat shocked [NHS(Ctrl)] *hsf-1(sy441) I*].

(D) HSF-1 occupancy at the Promoter regions of *F44E5.4/.5 II* under non-heat shocked control conditions [NHS(Ctrl)] and upon a short (5 minute) heat shock in wild-type animals and *hsf-1(sy441) I* mutants. (n=3-5 experiments). Data are normalized to non-heat shocked control [NHS(Ctrl)] wild-type values. When normalized to *hsf-1(sy441) I* non-heat shocked control [NHS(Ctrl)] values, the mean occupancy of a 5 minute heat shock is 0.57 ± 0.23, and is not significantly different from non-heat shocked [NHS(Ctrl)] *hsf-1(sy441) I*.

(E) Stress resilience of wild-type and *hsf-1(sy441) I* mutant F1 progeny of non-heat shocked control [NHS(Ctrl)] P0 mothers and P0 mothers subjected to a short (5 minute) or long (60 minute) heat shock. (n=3-9 experiments).

(F) Schematic of experimental procedure to test whether thermotolerance is inherited by the F2 and F3 generation of heat shocked P0 hermaphrodites.

(G) Representative micrographs showing projections of confocal z-sections through day four old adult F1 progeny of non-heat shocked control [NHS(Ctrl)] P0 mothers, and P0 mothers subjected to a short (5 minute) heat shock and long (30 minute or 60 minute) heat shock. Boxed regions in images on top are magnified below. The regions of high intensity represent aggregates as determined by previous publications in the field.

(H) Top: Representative Western blot showing polyglutamine protein levels (above) in day one adult F1 progeny of non-heat shocked control [NHS (Ctrl)] P0 mothers, and P0 mothers subjected to a short (5 minute) heat shock and long (30 minute or 60 minute) heat shock. Tubulin was the loading control (below). Day one adults were chosen as the majority of polyglutamine is still soluble at that age. **Bottom:** Quantitation of polyglutamine protein levels in day one adult F1 progeny. (n=3 experiments).

B-E, H: Data show Mean ± Standard Error of the Mean. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, ns: non-significant; Unpaired Student's t-test.

G: Scale bar: (top)150μm, (bottom)50μm.

Figure S 3

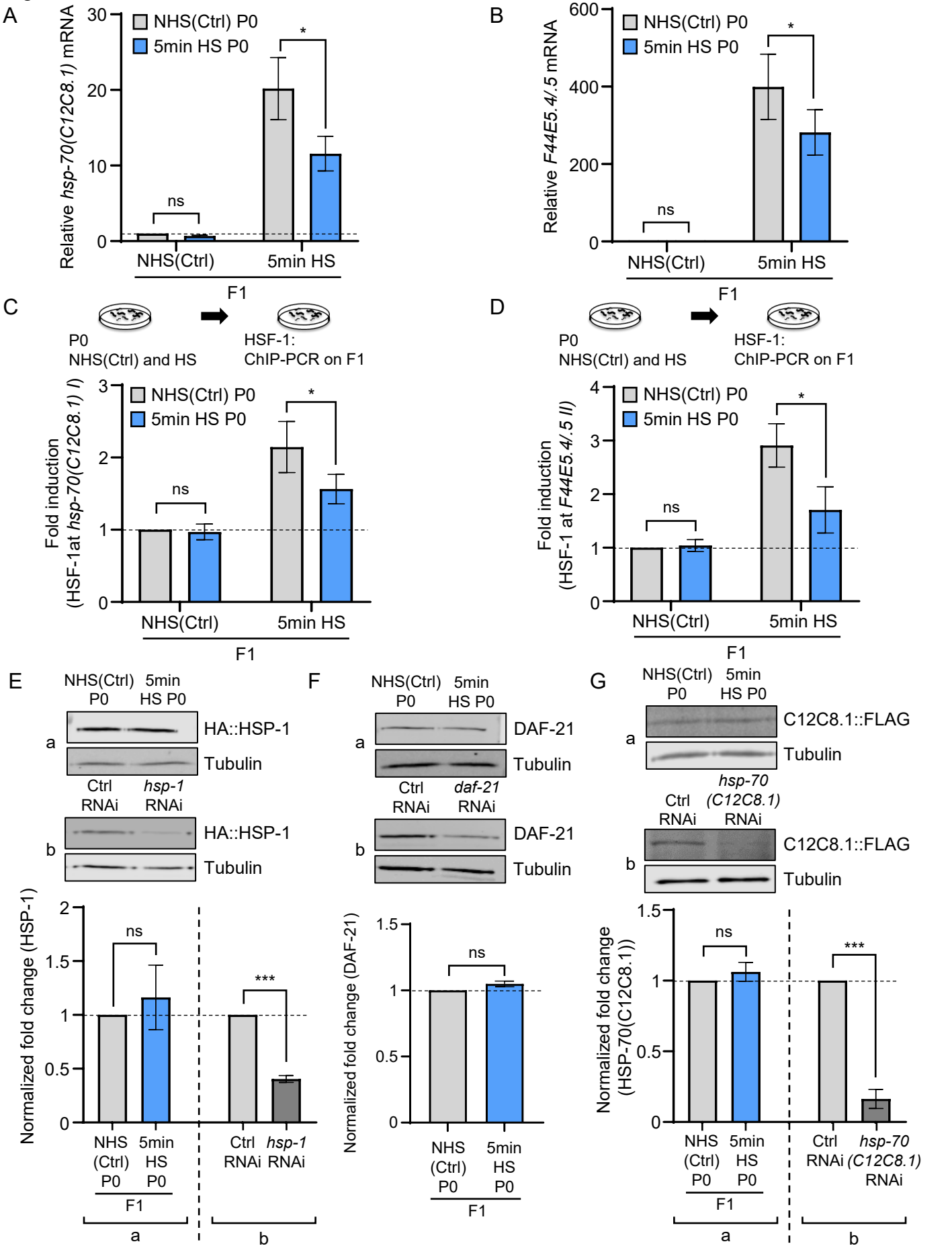

A
Relative hsp-70(C12C8.1) mRNA
NHS(Ctrl) P0
5min HS P0
ns
*
NHS(Ctrl)
5min HS
F1
B
Relative F44E5.4/.5 mRNA
C
P0
NHS(Ctrl) and HS
HSF-1:
ChIP-PCR on F1
Fold induction
(HSF-1at hsp-70(C12C8.1) I)
D
(HSF-1 at F44E5.4/.5 II)
E
HA::HSP-1
Tubulin
Ctrl RNAi
hsp-1 RNAi
Normalized fold change (HSP-1)
***
F
DAF-21
daf-21 RNAi
Normalized fold change (DAF-21)
G
C12C8.1::FLAG
hsp-70 (C12C8.1) RNAi
Normalized fold change (HSP-70(C12C8.1))

Figure S3: HSF-1 activity in the mother results in a decrease in the inducible heat-shock response in progeny, Related to **Figure 2**

(A) Average *hsp70*(*C12C8.1*) mRNA levels and **(B)** average *F44E5.4/.5* mRNA levels in F1 progeny of non-heat shocked control P0 mothers [NHS(Ctrl)], and F1 progeny of P0 mothers subjected to a 5 minute heat shock. mRNA levels in F1s were assessed under non-heat shocked control conditions [NHS(Ctrl)] and after they were also subjected to a 5 minute heat shock. mRNA levels were determined relative to *pmp-3* and normalized to that in non-heat shocked F1 progeny of non-heat shocked control P0 mothers [NHS(Ctrl)]. (n=4 experiments each).

(C) HSF-1 occupancy at the promoter region of *hsp-70(C12C8.1) I,* and **(D)** at the promoter region of *F44E5.4/.5 II* in F1 progeny of non-heat shocked control P0 mothers [NHS(Ctrl)], and F1 progeny of P0 mothers subjected to a short (5 minute) heat shock. HSF-1 occupancy in F1s was assessed under non-heat shock control conditions [NHS(Ctrl)] and after they were subjected to a 5 minute heat shock. HSF-1 occupancy was normalized to that in non-heat shocked F1 progeny of non-heat shocked control P0 mothers [NHS(Ctrl)]. (n=6 experiments each).

(E) Top: a, Representative Western blot showing HSP-1 protein levels (above) in day one adult F1 progeny of non-heat shocked control P0 mothers [NHS(Ctrl)], and P0 mothers subjected to a short (5 minute) heat shock. Tubulin was the loading control (below). **b,** Representative Western blot showing HSP-1 protein levels following *hsp-1* RNAi to validate the antibody. **Bottom: a,** Quantitation of HSP-1 protein levels in day one adult F1 progeny. (n=3 experiments). **b,** Quantitation of HSP-1 protein levels in day one adult F1 progeny following knockdown by *hsp-1* RNAi. Control RNAi was L4440. (n=3 experiments).

(F) Top: a, Representative Western blot showing DAF-21/HSP-90 protein levels (above) in day one adult F1 progeny of non-heat shocked control P0 mothers [NHS(Ctrl)], and P0 mothers

subjected to a short (5 minute) heat shock. Tubulin was used as the loading control (below). **b,** The antibody was validated using RNAi knockdown of *daf-21*. **Bottom: a,** Quantitation of DAF-21 protein levels in day one adult F1 progeny. (n=3 experiments).

(G) Top: a, Representative Western blot showing HSP-70(C12C8.1) protein levels (above) in day one adult F1 progeny of non-heat shocked control P0 mothers [NHS(Ctrl)], and P0 mothers subjected to a short (5 minute) heat shock. Tubulin was used as the loading control (below). **b,** The antibody was validated using RNAi knockdown of *hsp-70(C12C8.1)*. **Bottom: a,** Quantitation of HSP-70(C12C8.1) protein levels in day one adult F1 progeny. (n=2 experiments). **b,** Quantitation of HSP-70(C12C8.1) protein levels in day one adult F1 progeny following knockdown by *C12C8.1* RNAi. Control RNAi was L4440. (n=3 experiments).

Data show Mean ± Standard Error of the Mean. *: $p<0.05$, ***: $p<0.001$, ns: non-significant; **A-D** Paired Student's t-test. **E-G** Unpaired Student's t-test.

Figure S 4

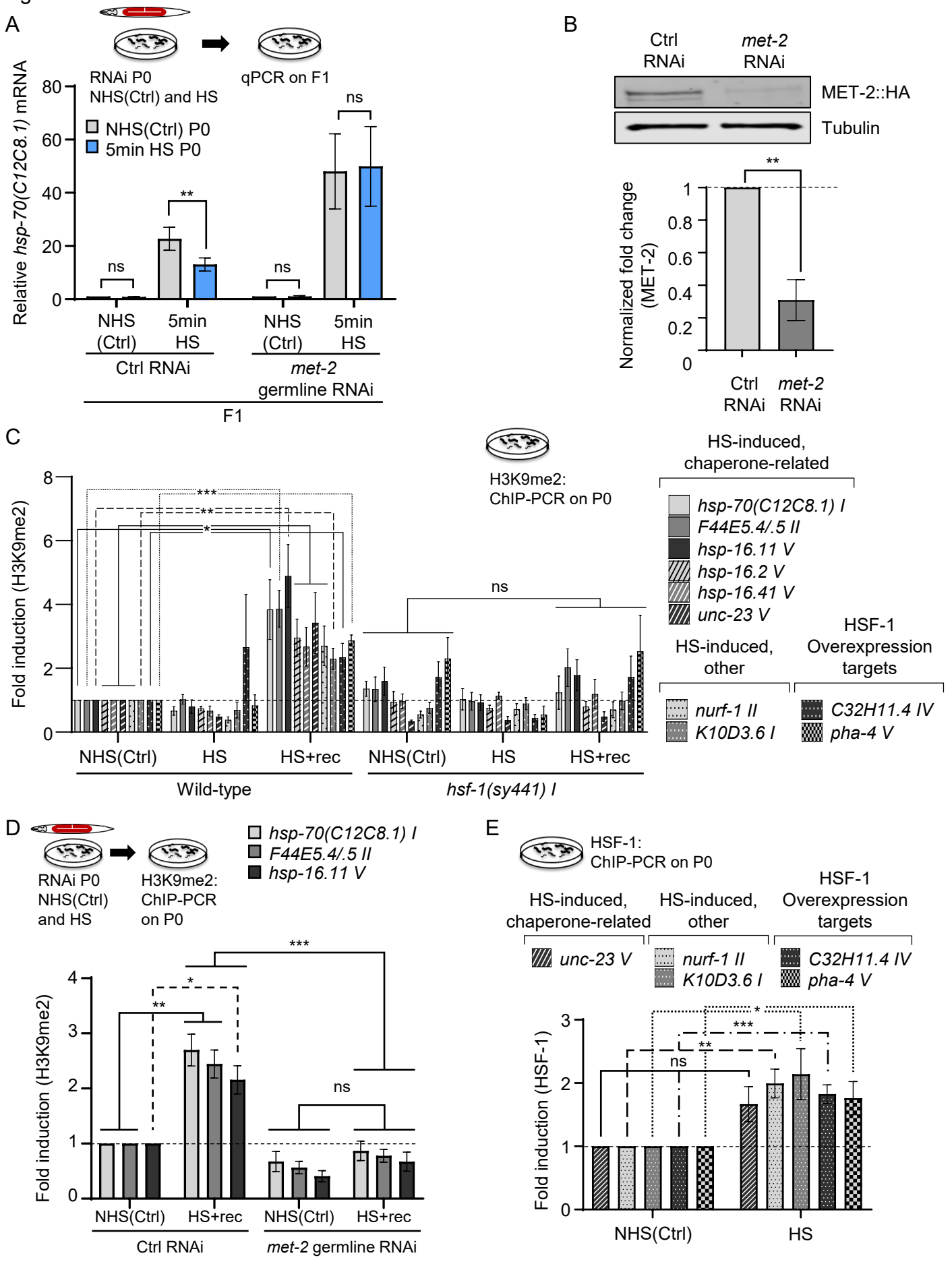

A
RNAi P0
NHS(Ctrl) and HS
qPCR on F1
NHS(Ctrl) P0
5min HS P0
Relative hsp-70(C12C8.1) mRNA
ns
**
NHS (Ctrl)
5min HS
Ctrl RNAi
met-2 germline RNAi
F1
B
Ctrl RNAi
met-2 RNAi
MET-2::HA
Tubulin
Normalized fold change (MET-2)
C
H3K9me2: ChIP-PCR on P0
HS-induced, chaperone-related
hsp-70(C12C8.1) I
F44E5.4/.5 II
hsp-16.11 V
hsp-16.2 V
hsp-16.41 V
unc-23 V
HS-induced, other
nurf-1 II
K10D3.6 I
HSF-1 Overexpression targets
C32H11.4 IV
pha-4 V
Fold induction (H3K9me2)
NHS(Ctrl)
HS
HS+rec
Wild-type
hsf-1(sy441) I
D
RNAi P0
NHS(Ctrl) and HS
H3K9me2: ChIP-PCR on P0
hsp-70(C12C8.1) I
F44E5.4/.5 II
hsp-16.11 V
Ctrl RNAi
met-2 germline RNAi
E
HSF-1: ChIP-PCR on P0
Fold induction (HSF-1)

Figure S4: Maternal germline *met-2* is reponsible for the increasing H3K9me2 levels at HSF-1 target genes in F1 progeny of heat shocked mothers, Related to **Figure 2**.

(A) Average *hsp70*(*C12C8.1*) mRNA levels in F1 progeny of non-heat shocked control P0 mothers [NHS(Ctrl)] and P0 mothers subjected to a 5 minute heat shock. P0 were subjected to germline RNAi using either control RNAi (L4440) or *met-2* RNAi. mRNA levels in F1s were assessed under non-heat shock conditions [NHS(Ctrl)] and after they were also subjected to a 5 minute heat shock. (n=3-7 experiments). mRNA levels were determined relative to *pmp-3*. Data are normalized to the respective values of non-heat shocked controls [NHS(Ctrl)] from each P0 RNAi condition.

(B) Validation of antibody and RNAi knockdown for *met-2*. **Top:** Representative Western blot showing MET-2 protein following RNAi knockdown of *met-2* (above). Tubulin was the loading control (below). **Bottom:** Quantitation of MET-2 protein levels in day one adults following knockdown by *met-2* RNAi. Control RNAi was L4440. (n=3 experiments).

(C) H3K9me2 occupancy [all data are normalized to non-heat shocked control [NHS(Ctrl)] wild-type samples; also see **Figure 2A**] at the promoter proximal 5'-UTR regions of *hsp-70(C12C8.1) I, F44E5.4/.5 II, hsp-16.11 V*, *hsp-16.2 V*, *hsp-16.41 V* , *unc-23 V* , *nurf-1 II, K10D3.6 I*, *C32H11.4 IV and pha-4 V* in P0 wild-type animals and *hsf-1 (sy441) I* animals under non-heat shocked control conditions [NHS(Ctrl)], immediately (HS), and 2 hours after (HS+rec) a 5 minute heat shock. (n=4-7 experiments). See **Methods** for details regarding the 5'-UTR regions assayed.

(D) H3K9me2 occupancy [all data are normalized to non-heat shocked control [NHS(Ctrl)] wild-type values; also see **Figure 2B**] at the promoter proximal 5'-UTR regions of *hsp-70(C12C8.1) I, F44E5.4/.5 II* and *hsp-16.11 V* in P0 animals subjected to RNAi induced knockdown of *met-2* only in the germline. L4440 empty vector was used as the control RNAi. H3K9me2 occupancy was

assessed in non-heat shocked animals [NHS (Ctrl)] and at 2 hours recovery post-5 minute heat shock (HS+rec), corresponding to the time point where H3K9me2 levels increase at these regions. (n=4-8 experiments).

(E) HSF-1 occupancy [normalized to non-heat shocked control samples [NHS(Ctrl)]] at the promoter proximal 5'-UTR regions of *unc-23 V*, *nurf-1 II, K10D3.6 I*, *C32H11.4 IV and pha-4 V* in P0 wild-type animals under non-heat shocked control conditions [NHS(Ctrl)] and upon a short (5 minute) heat shock. (n=5-11 experiments).

Data show Mean ± Standard Error of the Mean. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, ns: non-significant **A, B:** Paired Student's t-test. **C-E:** ANOVA with Tukey's correction.

Figure S 5

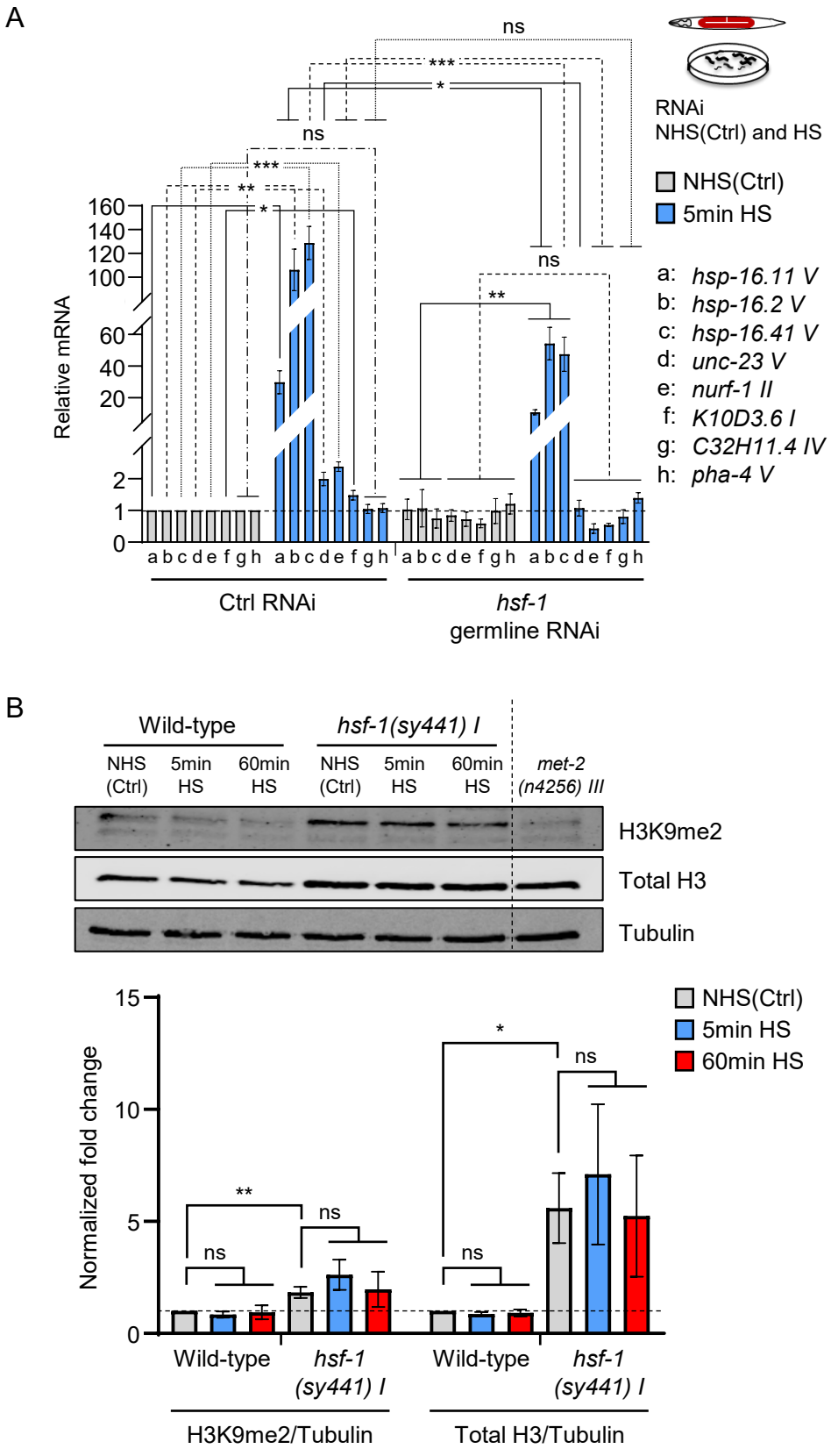

A
ns
***
*
ns
***
**
*
ns
**
160
140
120
100
60
40
20
2
1
0
Relative mRNA
a b c d e f g h a b c d e f g h a b c d e f g h a b c d e f g h
Ctrl RNAi
hsf-1
germline RNAi
RNAi
NHS(Ctrl) and HS
NHS(Ctrl)
5min HS
a: hsp-16.11 V
b: hsp-16.2 V
c: hsp-16.41 V
d: unc-23 V
e: nurf-1 II
f: K10D3.6 I
g: C32H11.4 IV
h: pha-4 V
B
Wild-type
hsf-1(sy441) I
NHS (Ctrl)
5min HS
60min HS
NHS (Ctrl)
5min HS
60min HS
met-2 (n4256) III
H3K9me2
Total H3
Tubulin
15
10
5
0
Normalized fold change
**
ns
ns
*
ns
ns
NHS(Ctrl)
5min HS
60min HS
Wild-type
hsf-1 (sy441) I
Wild-type
hsf-1 (sy441) I
H3K9me2/Tubulin
Total H3/Tubulin

Figure S5: Elevated H3K9me2 levels at HSF-1 germline target genes are not due to chromatin-wide alterations in the levels of H3K9me2 or histone H3 upon heat-shock, Related to **Figure 2**.

(A) Average *hsp-16.11*, *hsp-16.2*, *hsp-16.41*, *unc-23*, *nurf-1, K10D3.6*, *C32H11.4 and pha-4* mRNA levels in P0 wild-type animals subjected to control RNAi (L4440) or *hsf-1* germline RNAi under non-heat shocked control conditions [NHS(Ctrl)], and upon a 5 minute heat shock. (n=4-6 experiments). mRNA levels were measured by qPCR and determined relative to *pmp-3*. All data were normalized to non-heat shocked control wild-type samples [NHS(Ctrl)] subjected to control RNAi (L4440).. Significance: **a,b (***: $p<0.05$) **c,d,e,f,** (, ***: $p<0.001$), **g, h** (ns).

(B) Top: Representative Western blot using anti-H3K9me2 antibody (above), anti-Histone H3 (middle), and anti-tubulin (below) on wild-type and *hsf-1 (sy441) I* non-heat shocked P0 mothers [NHS(Ctrl)], and P0 mothers subjected to a short (5 minute) and long (60 minute) heat shock. P0 mothers were harvested for western blot analysis as day one adults. Note the lack of visible changes in H3K9me2, or total H3 levels in wild-type animals. This is confirmed by quantitation (**Bottom**). In contrast, *hsf-1(sy441) I* mutant animals have visibly elevated levels of H3K9me2, and total H3 both under non-heat shocked control conditions [NHS(Ctrl)] and upon heat-shock. This is confirmed by quantitation (**Bottom**). Equal numbers of animals were loaded per lane. *met-2(n4256)III* animals were used to show specificity of H3K9me2 antibody. (n=4-5 experiments).

Data show Mean ± Standard Error of the Mean. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, ns: non-significant; ANOVA with Tukey's correction, and Unpaired Student's t-test.

Figure S 6

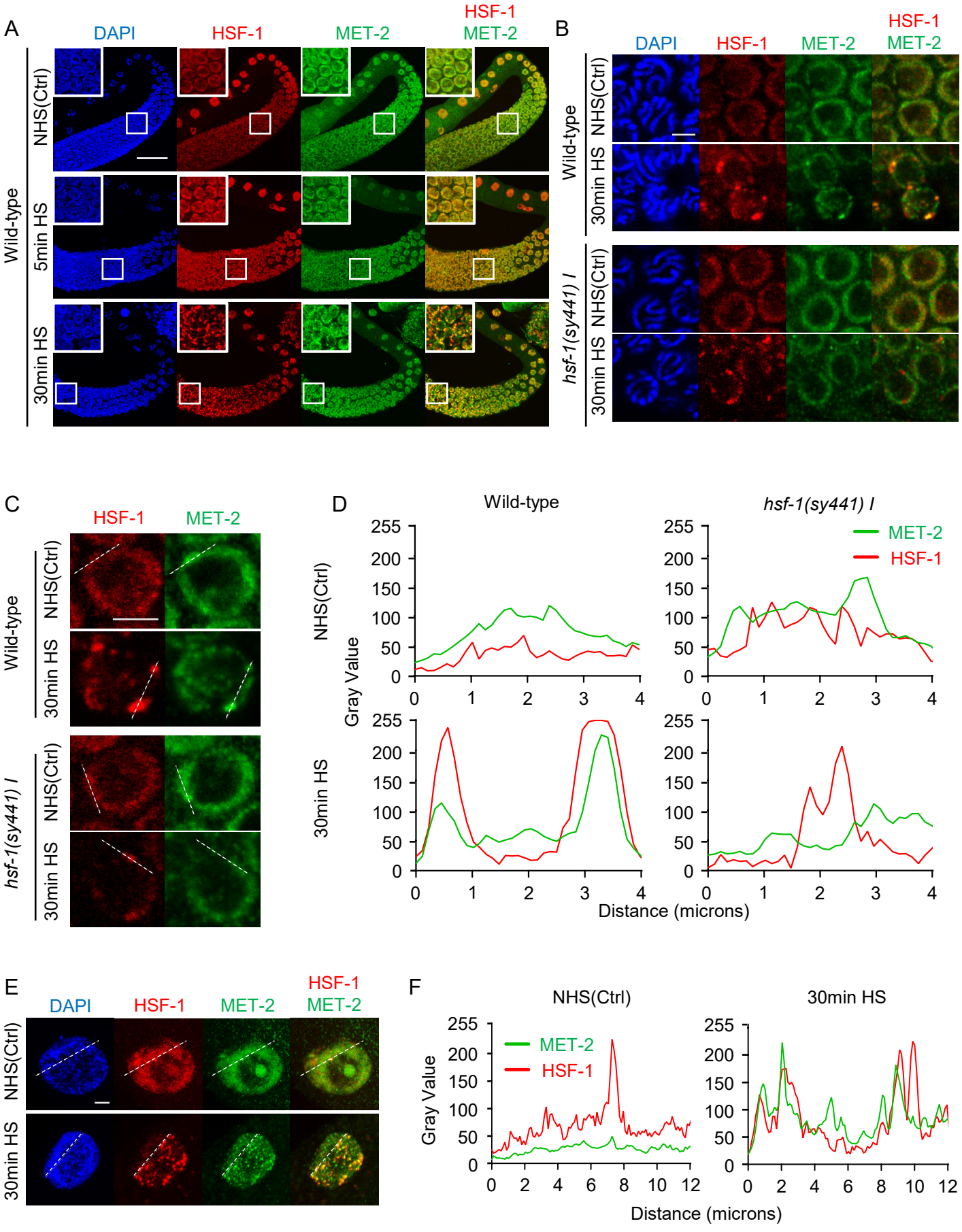

A
DAPI
HSF-1
MET-2
HSF-1
MET-2
Wild-type
NHS(Ctrl)
5min HS
30min HS
B
DAPI
HSF-1
MET-2
HSF-1
MET-2
Wild-type
NHS(Ctrl)
30min HS
hsf-1(sy441) I
NHS(Ctrl)
30min HS
C
HSF-1
MET-2
Wild-type
NHS(Ctrl)
30min HS
hsf-1(sy441) I
NHS(Ctrl)
30min HS
D
Wild-type
hsf-1(sy441) I
MET-2
HSF-1
NHS(Ctrl)
30min HS
Gray Value
Distance (microns)
E
DAPI
HSF-1
MET-2
HSF-1
MET-2
NHS(Ctrl)
30min HS
F
NHS(Ctrl)
30min HS
MET-2
HSF-1
Gray Value
Distance (microns)

Figure S6: Wild-type HSF-1, but not the truncated HSF-1 in *hsf-1 (sy441) I* mutant animals colocalizes with MET-2 in nuclear stress bodies (nSBs) upon heat shock, Related to **Figure 2**.

(A) Representative micrographs showing projections of confocal z-sections through dissected germlines of wild-type animals under non-heat shocked control conditions [NHS(Ctrl)] and upon short heat-shock (5 minute) and long heat shock (30 minute). Insets show maginified regions from pachytene.

(B) Representative micrographs of a confocal z-sections through pachytene germ cells in wild-type animals and *hsf-1(sy441) I*, under non-heat shocked control conditions [NHS(Ctrl)] and upon 30 minute heat-shock.

(C) Magnified image of one nucleus from **B**.

(D) Intensity profile graphs of HSF-1 colocalization with MET-2 in nuclei from **C**. Line scan graphs were generated by plotting the immunofluorescence intensity along the freely positioned line at the periphery of the nucleus in **C**.

(E) Representative micrographs showing projections of confocal z-sections through intestinal nuclei in wild-type animals under non-heat shocked control conditions [NHS(Ctrl)] and upon 30 minute heat-shock.

(F) Intensity profile graphs of HSF-1 colocalization with MET-2 (plotted as immunofluorescence intensity along the freely positioned line) of nuclei in **E**.

A, B, C, E: Red:HSF-1, Green:MET-2, DAPI:DNA.

A: Scale bar:30μm; **B, C, E:** Scale bar:3μm.

Figure S 7

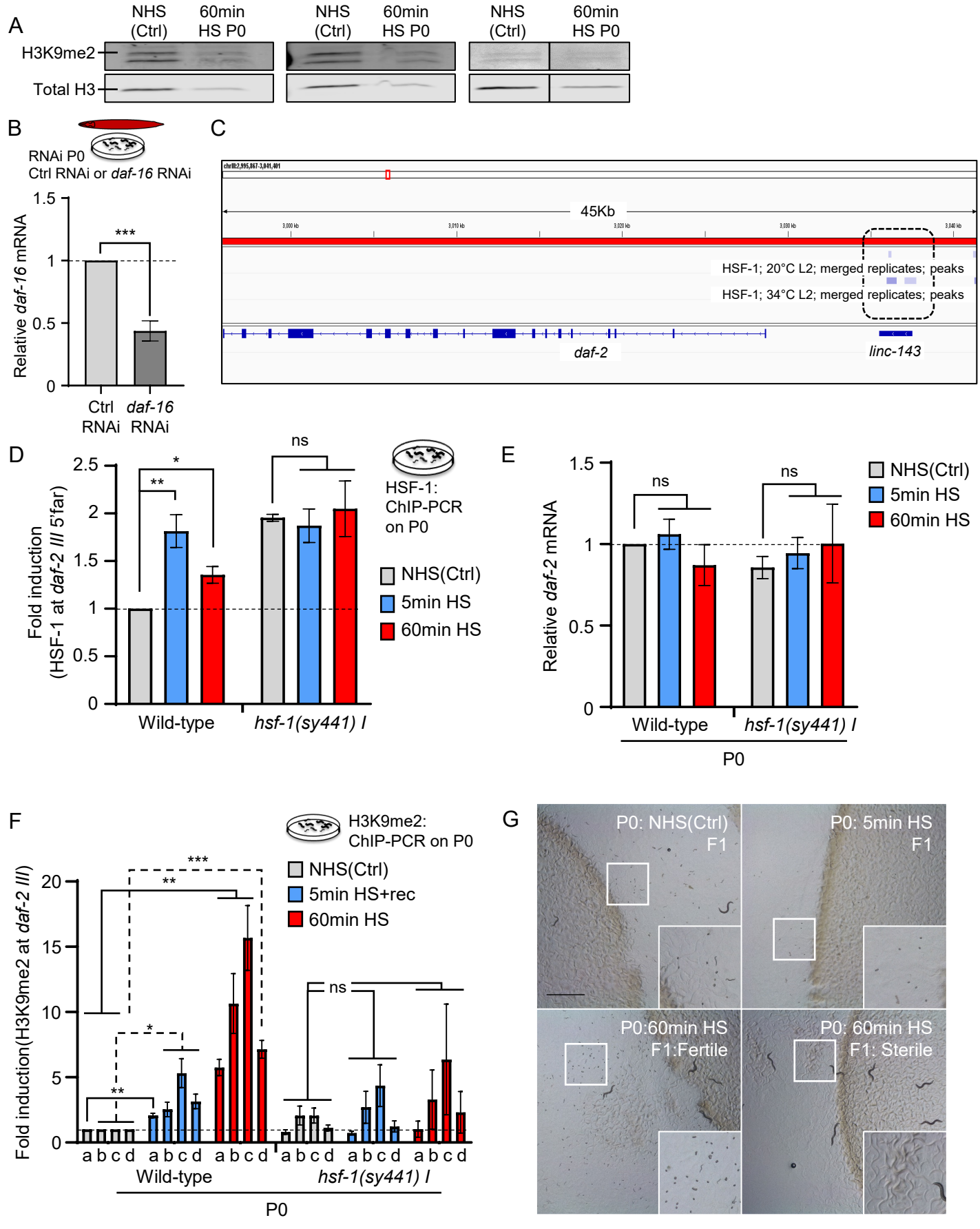

A
NHS (Ctrl)
60min HS P0
H3K9me2
Total H3
B
RNAi P0
Ctrl RNAi or daf-16 RNAi
Relative daf-16 mRNA
Ctrl RNAi
daf-16 RNAi
C
45Kb
HSF-1; 20°C L2; merged replicates; peaks
HSF-1; 34°C L2; merged replicates; peaks
daf-2
linc-143
D
Fold induction (HSF-1 at daf-2 III 5'far)
HSF-1: ChIP-PCR on P0
NHS(Ctrl)
5min HS
60min HS
Wild-type
hsf-1(sy441) I
E
Relative daf-2 mRNA
P0
F
H3K9me2: ChIP-PCR on P0
Fold induction(H3K9me2 at daf-2 III)
5min HS+rec
G
P0: NHS(Ctrl) F1
P0: 5min HS F1
P0:60min HS F1:Fertile
P0: 60min HS F1: Sterile

Figure S7: Negative bookmarking of *daf-2* upon maternal heat shock modulates the physiology of F1 progeny, Related to **Figures 3, 5, 6, and 7**.

(A) Representative Western blot using anti-H3K9me2 antibody (above) and anti-Histone H3 (below) on F1 progeny that develop from oocytes of P0 non-heat shocked mothers [NHS(Ctrl)], mothers subjected to a long (60 minute) heat shock. F1 progeny were harvested for western blot analysis after they became day one adults. Three separate biological repeats are shown. Note the visible decrease in total H3 levels compared to non-heat shocked control [NHS(Ctrl)] samples. Equal numbers of animals were loaded per lane. Also see **Figure 3D** for quantitation of H3K9me2 levels, and for representative western blots from non-heat shocked mothers [NHS(Ctrl)], and mothers subjected 5 minute and 30 minute heat shock where this decrease in H3 levels is not apparent.

(B) *daf-16* knockdown using RNAi was validated using qPCR to measure *daf-16* mRNA levels determined relative to *pmp*-3 and compared to that in animals subjected to control RNAi (L4440) treatment.

(C) IGV screen shot showing the 45-kb region on chromosome III (chr III: 2,995,867-3,041,401) containing *daf-2* and its 5'-UTR regions. HSF-1 ChIP-seq peaks in non heat-shocked [NHS(Ctrl)] and heat-shocked larval stage 2 animals identified from published ChIP seq data. Boxed region indicates region where HSF-1 occupancy was assayed in **Figure 5D**.

(D) HSF-1 occupancy [normalized to wild-type non-heat shocked control [NHS(Ctrl)] values] at the 5'-far region (-9071 to -8954) in wild-type animals and *hsf-1(sy441) I* mutants after a short (5 minutes), or long (60 minute) heat shock. (n=3 experiments).

(E) Average *daf-2* mRNA levels in wild-type and *hsf-1(sy441) I* P0 animals under non-heat shocked control conditions [NHS(Ctrl)] and upon a short (5 minute) and long (60 minute) heat-shock. mRNA levels were determined relative to *pmp*-3 and normalized to that in non-heat shocked control [NHS(Ctrl)] wild-type animals. (n=3 experiments).

(F) Alternative normalization to **Figure 5E** where H3K9me2 occupancy in wild-type P0 mothers and *hsf-1(sy441) I* P0 mothers under non-heat shocked control conditions [NHS(Ctrl)] and upon a short (5 minute) and long (60 minute) heat-shock is shown relative to non-heat shocked control samples [NHS(Ctrl)] from wild-type animals. (n=3-4 experiments).

(G) Represntative micrographs of agarose plates of F1 progeny from non-heat shocked control P0 mothers [NHS(Ctrl)], and mothers subjected to a short (5 minute) and long (60 minute) heat shock. Note the presence of eggs in F1 from NHS(Ctrl) and 5min HS mothers. Some F1 progeny from 60 minute heat-shocked mothers are fertile (bottom left), but 52.5±8.4% (n=3 experiments, 17-101 F1s scored/experiment) are sterile (bottom right).

B, D-F: Data show Mean ± Standard Error of the Mean. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, ns: non-significant; ANOVA with Tukey's correction, and Unpaired Student's t-test.
G: Scale bar:2mm

Figure S 8

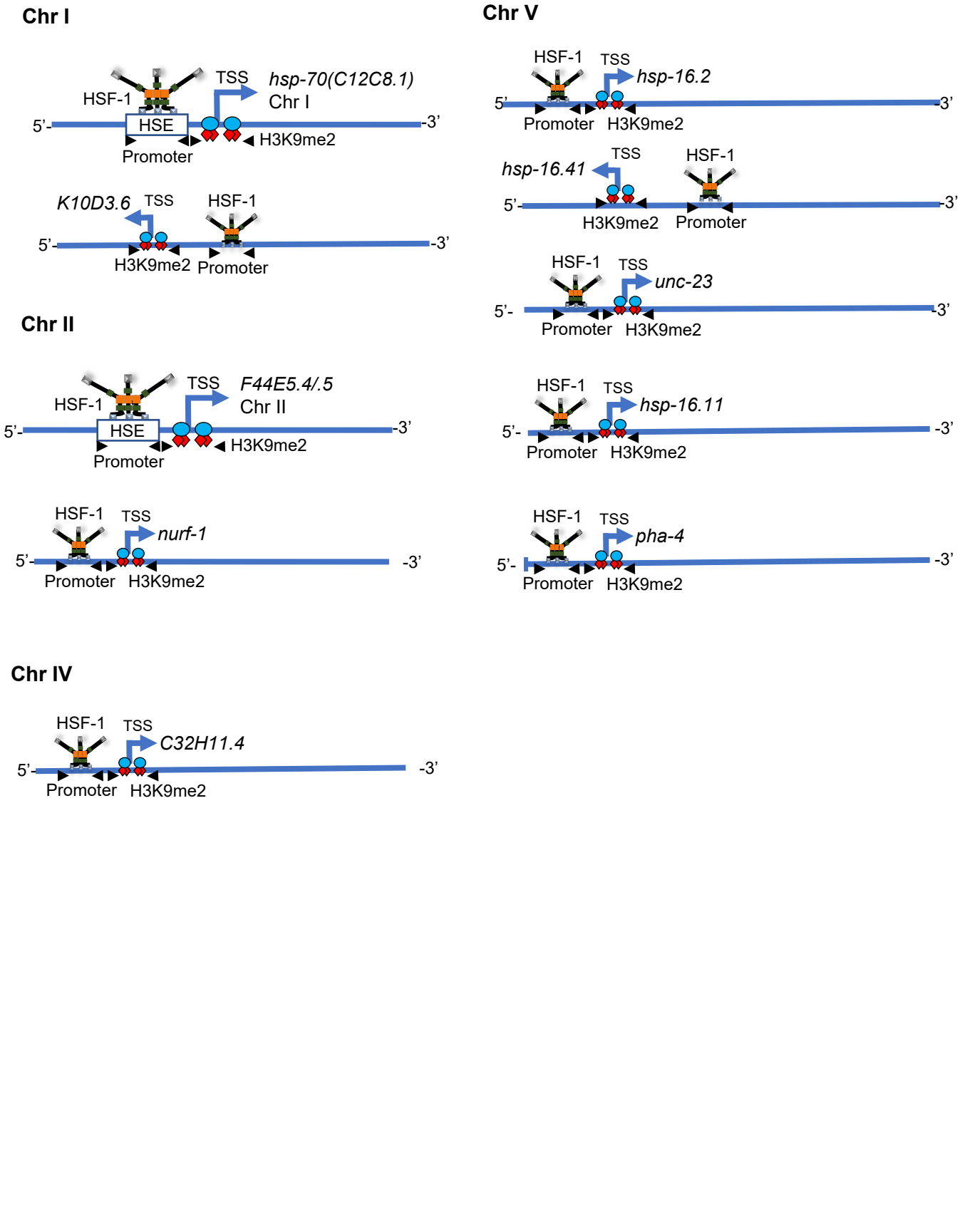

Chr I
HSF-1
TSS
hsp-70(C12C8.1)
Chr I
5'-
HSE
-3'
Promoter
H3K9me2
K10D3.6
TSS
HSF-1
5'-
H3K9me2
Promoter
-3'
Chr II
HSF-1
TSS
F44E5.4/.5
Chr II
5'-
HSE
-3'
Promoter
H3K9me2
HSF-1
TSS
nurf-1
5'-
Promoter
H3K9me2
-3'
Chr IV
HSF-1
TSS
C32H11.4
5'-
Promoter
H3K9me2
-3'
Chr V
HSF-1
TSS
hsp-16.2
5'
Promoter
H3K9me2
3'
hsp-16.41
TSS
HSF-1
5'-
H3K9me2
Promoter
-3'
HSF-1
TSS
unc-23
5'-
Promoter
H3K9me2
-3'
HSF-1
TSS
hsp-16.11
5'-
Promoter
H3K9me2
-3'
HSF-1
TSS
pha-4
5'-
Promoter
H3K9me2
-3'

Figure S8: The sequence and position of the primers used for ChIP experiments, and the expected amplicon sizes are listed in Table S3 and depicted in the cartoon below. See **STAR Methods.**