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Supplemental information

Insulin/IGF-1 signaling and heat stress differentially regulate HSF1 activities in germline development

Stacey L. Edwards, Purevsuren Erdenebat, Allison C. Morphis, Lalit Kumar, Lai Wang, Tomasz Chamera, Constantin Georgescu, Jonathan D. Wren, and Jian Li

Supplemental files_Cell Report

Insulin/IGF-1 signaling and heat stress differentially regulate HSF1 activities in germline development

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Figure S1

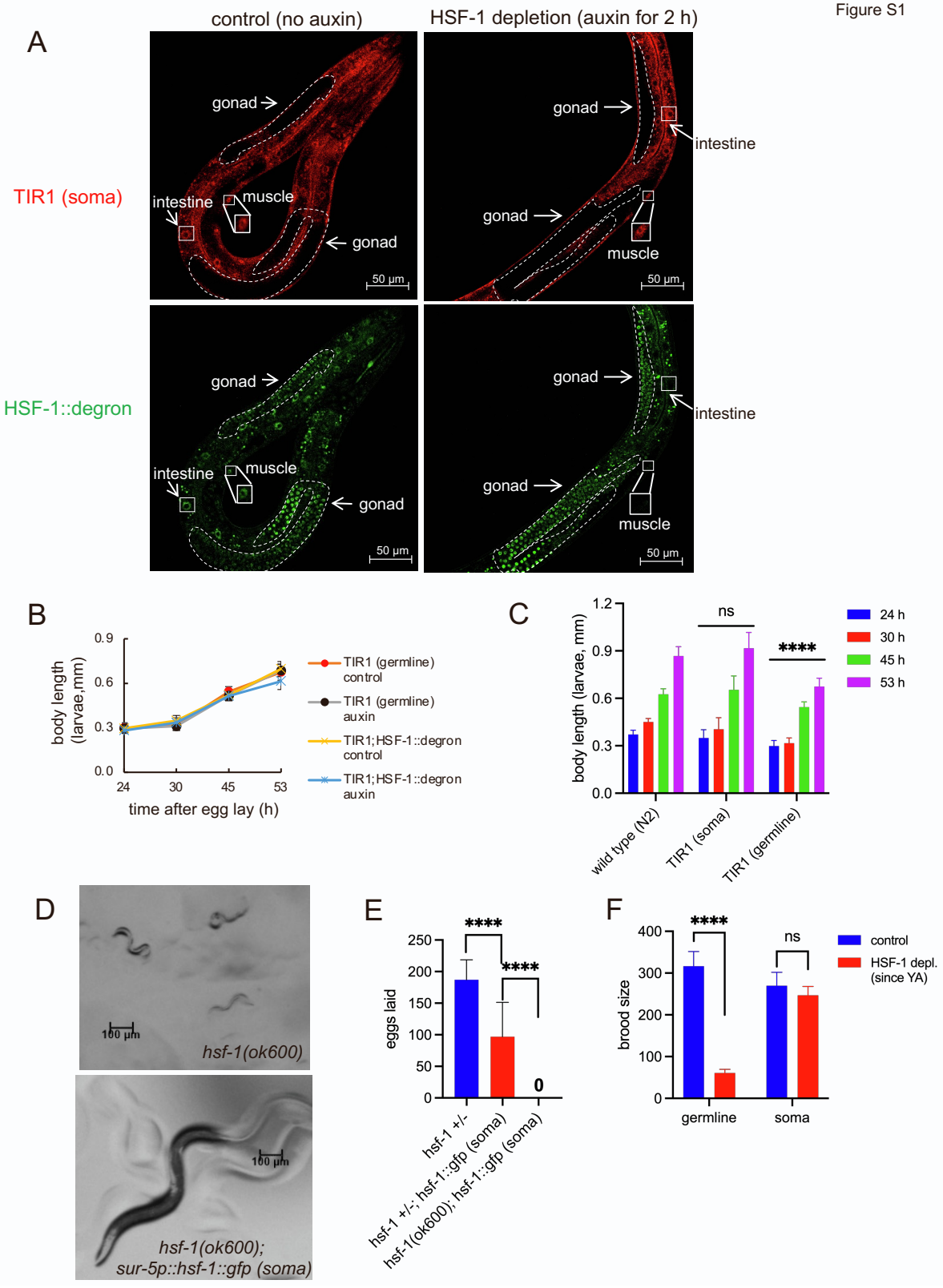
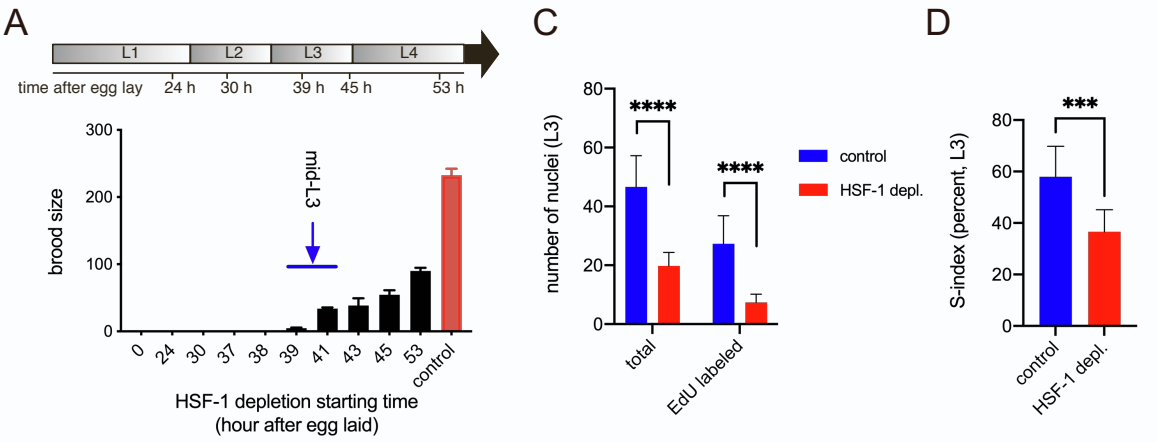
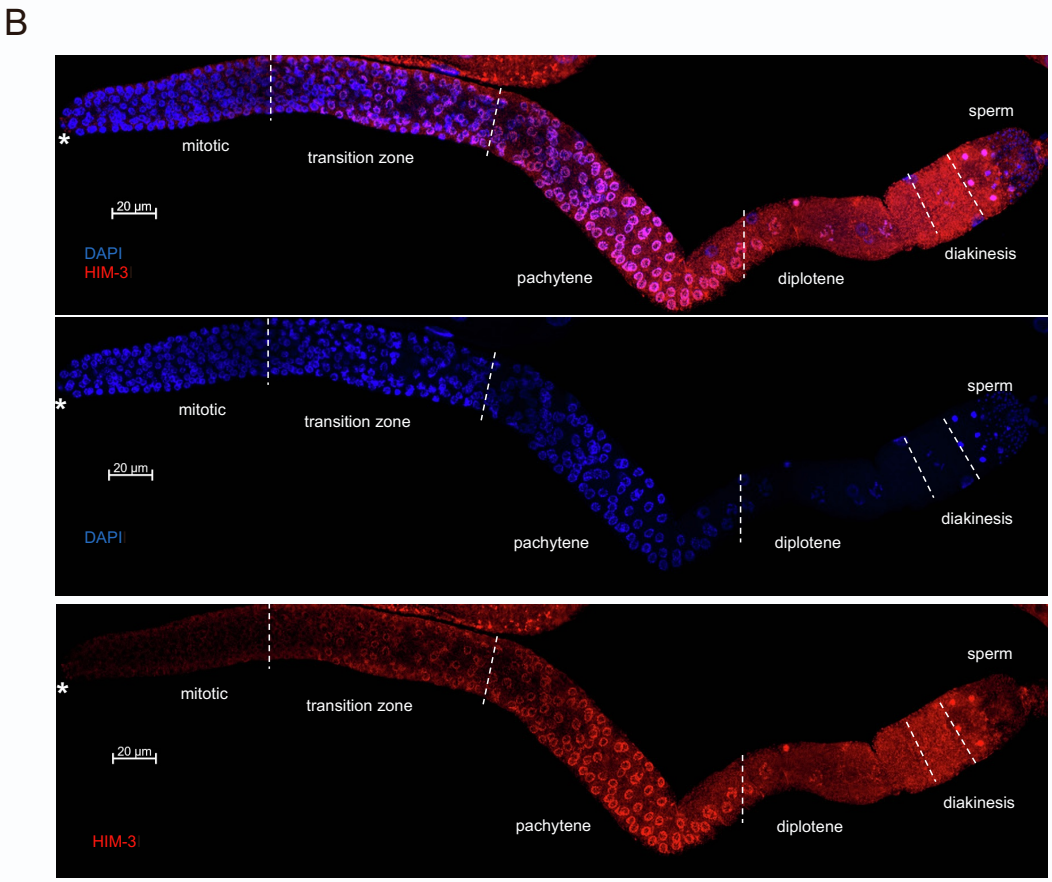

A
control (no auxin)
HSF-1 depletion (auxin for 2 h)
TIR1 (soma)
HSF-1::degron
gonad
intestine
muscle
50 µm
B
body length (larvae,mm)
time after egg lay (h)
TIR1 (germline) control
TIR1 (germline) auxin
TIR1;HSF-1::degron control
TIR1;HSF-1::degron auxin
C
body length (larvae, mm)
ns
****
24 h
30 h
45 h
53 h
wild type (N2)
TIR1 (soma)
TIR1 (germline)
D
100 µm
hsf-1(ok600)
hsf-1(ok600); sur-5p::hsf-1::gfp (soma)
E
eggs laid
hsf-1 +/-
hsf-1 +/-; hsf-1::gfp (soma)
hsf-1(ok600); hsf-1::gfp (soma)
F
brood size
control
HSF-1 depl. (since YA)
germline
soma

Figure S1, related to Figure 1. The auxin-inducible degron (AID) system reveals tissue-specific roles of HSF-1 in *C. elegans* larval development and reproduction

(A) Representative live animal images of young adults with soma-specific HSF-1 depletion by AID for 2 hours. The boxed images show HSF-1::degron::GFP (green) and TIR1::mRuby (red) from intestine and body-wall muscle cells with higher magnification. Dashed lines outline gonads. Scale bars, 50 μM.
(B) Size tracking of developing larvae with HSF-1 depletion in the germline initiated at egg lay. Data are represented as mean ± standard deviation (n>=15).
(C) Size tracking of developing larvae of wildtype (N2) animals, or transgenic animals expressing TIR1 in the soma (CA1200) or in the germline (CA1199). Data are represented as mean ± standard deviation (n>=15). While CA1200 developed at a similar pace as N2, CA1199 displayed delay of larval development. This developmental delay was not caused by depletion of germline HSF-1 (as shown in S1B) but expression of TIR1 in germ cells. Statistical significance was calculated by two-way ANOVA test (n>=17). ****p<0.0001, ns: not significant (p>=0.05).
(D) Representative brightfield images of the *hsf-1* null mutant <*hsf-1(ok600)*> and the *hsf-1* null animal that expresses the *hsf-1::gfp* transgene from the pan-soma *sur-5* promoter <*hsf-1 (ok600);sur-5p::hsf-1::gfp*>. Images were taken at 70 hours after egg lay when the *hsf-1 (ok600);sur-5p::hsf-1::gfp* animal developed into adult, but the *hsf-1(ok600)* null mutant was arrested at the L2 stage. Scale bars, 100 μM.
(E) Total number of eggs from animals with different tissue expression patterns of HSF-1. Data are represented as mean ± standard deviation. "*hsf-1 +/-":* heterozygous animals that carry both the *hsf-1 (wild type)* allele on a balancer and the *hsf-1(ok600)* allele (n=13); "*hsf-1 +/-; hsf-1::gfp (soma)*": heterozygous animals that also express the *sur-5p::hsf-1::gfp* transgene (n=21); "*hsf-1(ok600);hsf-1::gfp (soma)":* homozygous *hsf-1* (null) animals that express the *hsf-1::gfp* transgene from the pan-soma *sur-5* promoter (n=20). Homozygous expression of the balancer caused embryonic lethality so numbers of eggs instead of viable progenies were scored. Statistical significance was calculated by unpaired, two tailed t test. ****p<0.0001.
(F) Brood size of animals with HSF-1 depleted from either the soma or the germline since young adult stage. Data are represented as mean ± standard deviation (n>=12). Statistical significance was calculated by unpaired, two tailed Student's t test. ****p<0.0001.

Figure S2

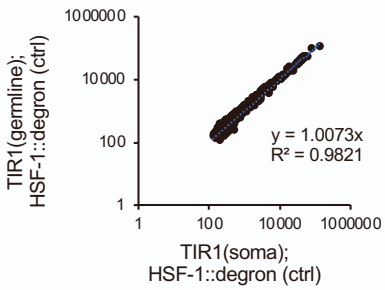




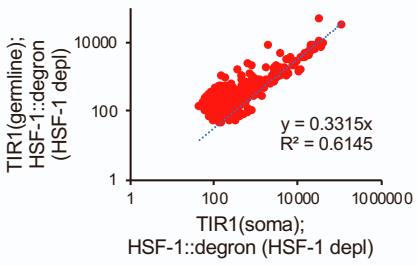
control (no HSF-1 depletion)

Figure S2, related to Figure 2. HSF-1 is required for mitotic proliferation of germline progenitor cells and early meiosis.
(A) Brood size of animals with depletion of HSF-1 from the germline by AID initiated at different time points after egg lay. Mean and SEM of three biological replicates are plotted (n>=10 in each replicate). Timeline represents larval stages (L1-L4) at given time points in the control animals JTL621 without HSF-1 depletion.
(B) Representative image showing immunofluorescence (IF) of the meiotic marker, HIM-3 in dissected gonads with DAPI staining of DNA as in Figure 2A. Young adult animals without auxin treatment (control) were used. Compared to Figure 2A, higher exposure was used to show HIM-3 in the transition zone. Scale bars, 20 μM.
(C) Quantification of total and EdU labeled nuclei (per gonad arm) at the L3 stage. EdU labeling was started at 38 hours after egg lay. Significant decreases were observed upon HSF-1 depletion from the germline initiated at egg lay in both total nuclei and EdU positive nuclei. Mean and standard deviation are plotted (n>=10). Statistical significance was calculated by unpaired, two-tailed Student's t test. ****p<0.0001.
(D) Histograms showing S-index calculated as the ratio of EdU positive nuclei to total nuclei based on the data of (C). HSF-1 depletion from the germline initiated at egg lay significantly decreased S-index. Mean and standard deviation are plotted. Statistical significance was calculated by unpaired, two-tailed Student's t test. ***p<0.001.

A



B

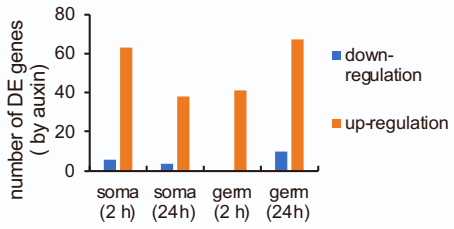


C

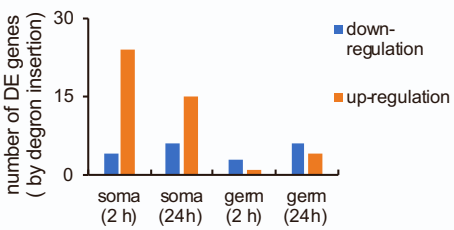
EtOH (ctrl)	Auxin
TIR1 (A)	TIR1 (C)
HSF-1::degron;TIR1 (B)	HSF-1::degron;TIR1 (D)

1. DE genes caused by auxin treatment: C vs. A
2. DE genes caused by degron insertion: B vs. A
3. DE genes caused by HSF-1 depletion: D vs. B or D vs. C
(with filtering of non-specific effects from 1. and 2. respectively)

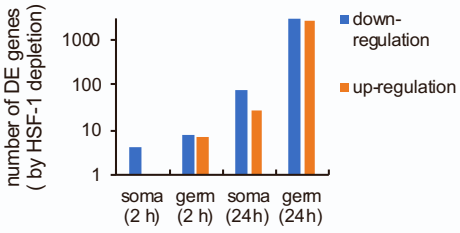
D



E



F



G

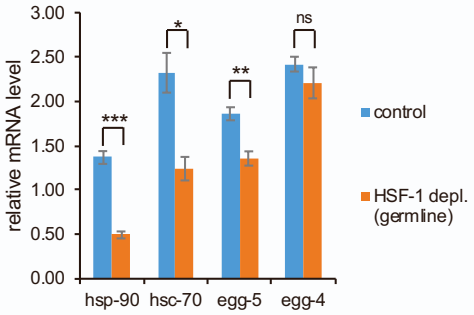


Figure S3, related to Figure 3. HSF-1 directs a compact transcriptional program important for homeostasis and development of the germline.
(A&B). Scatter plots of HSF-1 occupancy at ChIP-seq peaks (normalized HSF-1 reads mapped to ±500 bp from peak summits) from the two AID models without auxin treatment (ctrl) (A) or after auxin treatment for 2 hours (HSF-1 depl) (B). Worms from the two AID models were grown to young adults at 20°C (non-heat-shock condition). Linear regression was done, and the slopes and R^2 values are shown.
(C) Table of four experimental conditions used at each time point of RNA-seq analyses. The control group was treated with the vehicle, ethanol (0.25%).
(D&E). Histograms showing the number of differentially expressed (DE) genes (FDR: 0.05) caused by auxin treatment (D) and insertion of the *degron::gfp* sequence to the endogenous *hsf-1* locus (E). Since the control experiments for auxin (D) were done in the presence of TIR1, they also control for any effects caused by non-specific activity of TIR1 at proteins other than HSF-1.
(F) Histograms showing the number of DE genes (FDR: 0.05) caused by HSF-1 depletion for 2 and 24 hours. The DE genes were determined by comparing the ethanol treated to the auxin treated animals that express both TIR1 and HSF-1::degron, and filtered against the DE genes by auxin treatment in animals that only express TIR1.
(G) Relative expression of HSF-1 target genes by qRT-PCR in animals with depletion of HSF-1 from the germline for 16 hours since young adults (HSF-1 depl) or the control. Mean and SEM from biological triplicates are plotted. Statistical significance was calculated by unpaired, two-tailed Student's t test. *p<0.05; **p<0.01; ***p<0.001; ns: not significant (P>=0.05).

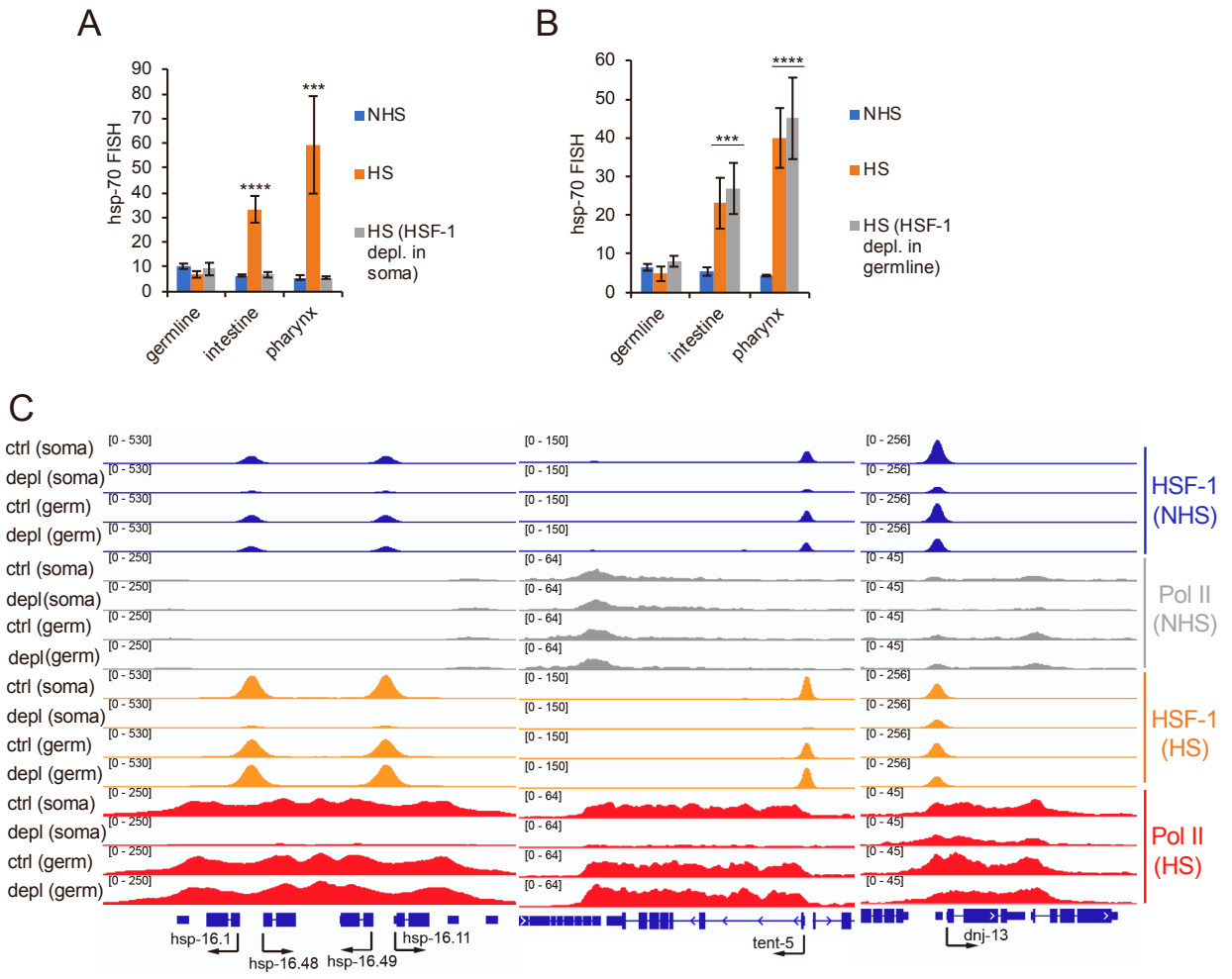
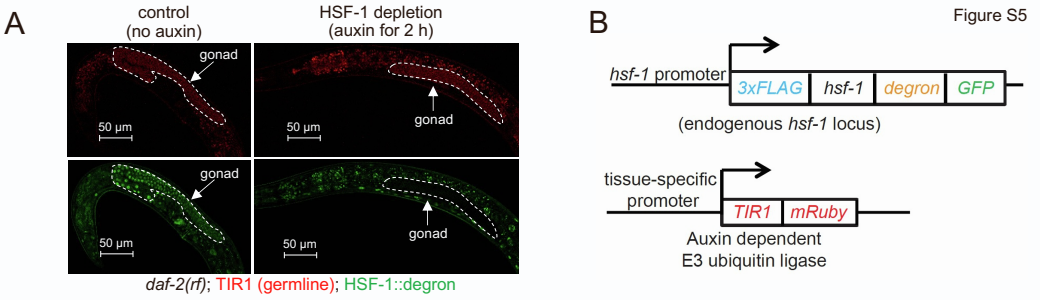
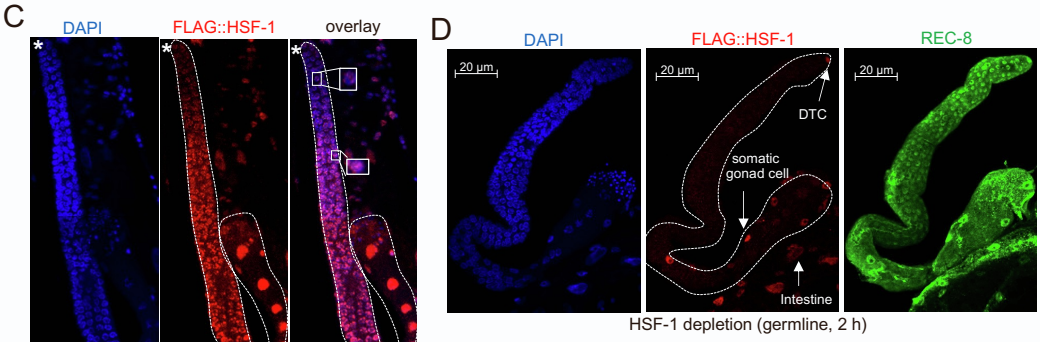
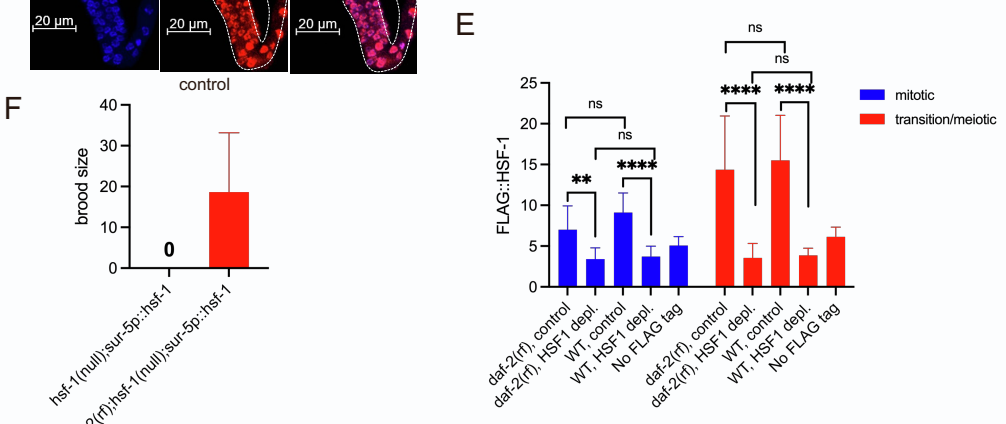


Figure S4, related to Figure 4. HSF-1 exhibits germline-specific response to heat stress.
(A&B). Quantification of fluorescence intensity of *hsp-70* (F44E5.4/.5) RNA-FISH in the germline, the intestine cells and the pharynx from young adults of AID animals. The two AID models were grown to young adults at 20°C without heat shock (NHS), or subjected to a heat shock (HS) at 34 °C for 15 min following 2 hours of mock treatment as the control (ctrl) or auxin treatment to deplete HSF-1 (depl) from the soma (A) or the germline (B). Mean and standard deviation are plotted (n=5). Statistical significance was calculated by unpaired, two-tailed Student's t test against the NHS controls. ***p<0.001; ****p<0.0001.
(C) Gbrowser views of HSF-1 and Pol II occupancy at the cluster of *hsp-16* genes, the *tent-5* gene and the *dnj-13* gene in NHS and HS conditions with the control (ctrl) and HSF-1 depletion (depl) from either the somatic (soma) or the germline (germ) cells.

Figure S5


A
control
(no auxin)
HSF-1 depletion
(auxin for 2 h)
gonad
50 µm
daf-2(rf); TIR1 (germline); HSF-1::degron
B
hsf-1 promoter
3xFLAG
hsf-1
degron
GFP
(endogenous hsf-1 locus)
tissue-specific
promoter
TIR1
mRuby
Auxin dependent
E3 ubiquitin ligase


C
DAPI
FLAG::HSF-1
overlay
20 µm
control
D
DAPI
FLAG::HSF-1
REC-8
DTC
somatic
gonad cell
Intestine
HSF-1 depletion (germline, 2 h)


E
FLAG::HSF-1
mitotic
transition/meiotic
ns
**
****
daf-2(rf), control
daf-2(rf), HSF1 depl.
WT, control
WT, HSF1 depl.
No FLAG tag
F
brood size
0
hsf-1(null);sur-5p::hsf-1
daf-2(rf);hsf-1(null);sur-5p::hsf-1

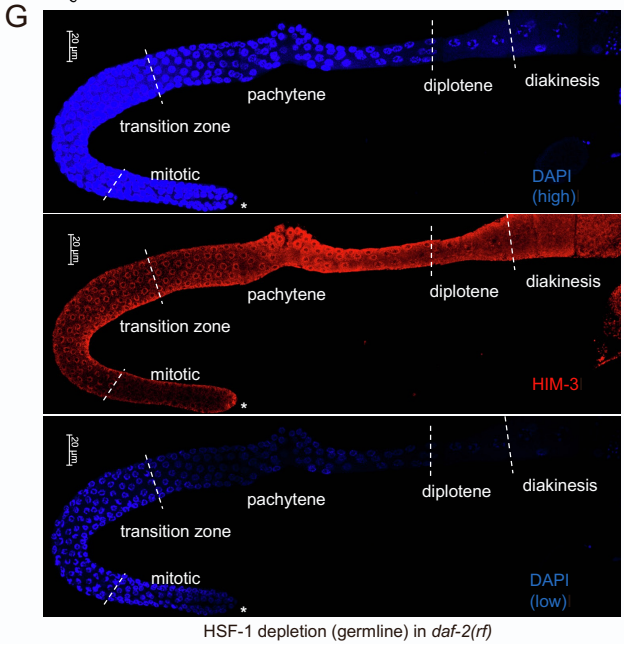

G
pachytene
diplotene
diakinesis
transition zone
mitotic
DAPI
(high)
HIM-3
DAPI
(low)
HSF-1 depletion (germline) in daf-2(rf)

Figure S5, related to Figure 5. HSF-1 is required for insulin/IGF-1 signaling (IIS)-promoted reproduction.
(A) Representative live animal images of the *daf-2 (rf)* mutant at young adult stage with depletion of HSF-1 from the germline by AID for 2 hours. Dash lines outline gonads. Scale bars, 50 μM.
(B) Schematic of the AID system with additional insertion of 3xFLAG tag to the N-terminus of endogenous HSF-1.
(C&D) Representative images of immunofluorescence (IF) of HSF-1 by the 3xFLAG tag (red) and DAPI staining of DNA (blue) in young adult *daf-2(rf)* animals that express 3XFLAG::HSF-1::degron. Animals were either mock treated (control, C) or treated with auxin for 2 hours to deplete HSF-1 from the germline (D). In panel C, the white asterisks (*) indicate the distal end of gonad, and the boxed images show the nuclear localization of HSF-1 in one nucleus in mitotic zone (distal side) and one in transition/meiotic zone. IF of REC-8, a cohesion protein was done in (D) to show the negative staining of HSF-1 in the gonad was not due to inaccessibility to antibody. White arrows indicate the distal tip cell (DTC), a somatic gonad cell and an intestinal cell respectively in (D). Scale bars, 20 μM.
(E) Quantification of HSF-1 IF by the 3xFLAG tag in the mitotic zone (relatively low signal) and the transition zone/meiotic cells (relatively high signal). Animals that express 3XFLAG::HSF-1::degron and TIR1 (germline) in the *daf-2(rf)* mutant (as in C&D) or in the genetic background of wild type *daf-2* gene (WT) were used. These animals were either mock treated (control) or treated with auxin for 2 hours to deplete HSF-1 from the germline (HSF-1 depl). We also included a negative control <*daf-2(rf)*; TIR1 (germline); HSF-1::degron> that does not have the 3XFLAG tag inserted to the N-terminus of HSF-1 (No FLAG tag) to show the background level of FLAG staining. Mean and standard deviation are plotted (n=10).
(F) Brood size of transgenic animals expressing HSF-1 only in the soma, and carrying either the wild type *daf-2* gene or the *daf-2(rf)* mutant allele. These animals are homozygous for the *hsf-1(ok600)* null allele and express the *hsf-1::gfp* transgene from the pan-soma *sur-5* promoter. Mean and standard deviation are plotted (n=20).
(G) Representative image showing immunofluorescence (IF) of the meiotic marker, HIM-3 in dissected gonad with DAPI staining of DNA. Young adult *daf-2(rf)* mutant was used, in which HSF-1 depletion from the germline was initiated at egg lay and continued through entire development. Different stages of germline development are labeled. The white asterisks (*) indicate the distal end of gonad. Two exposures of DAPI were used to show the nuclei either in diakinesis or in mitotic/transition zones. Scale bars, 20 μM.

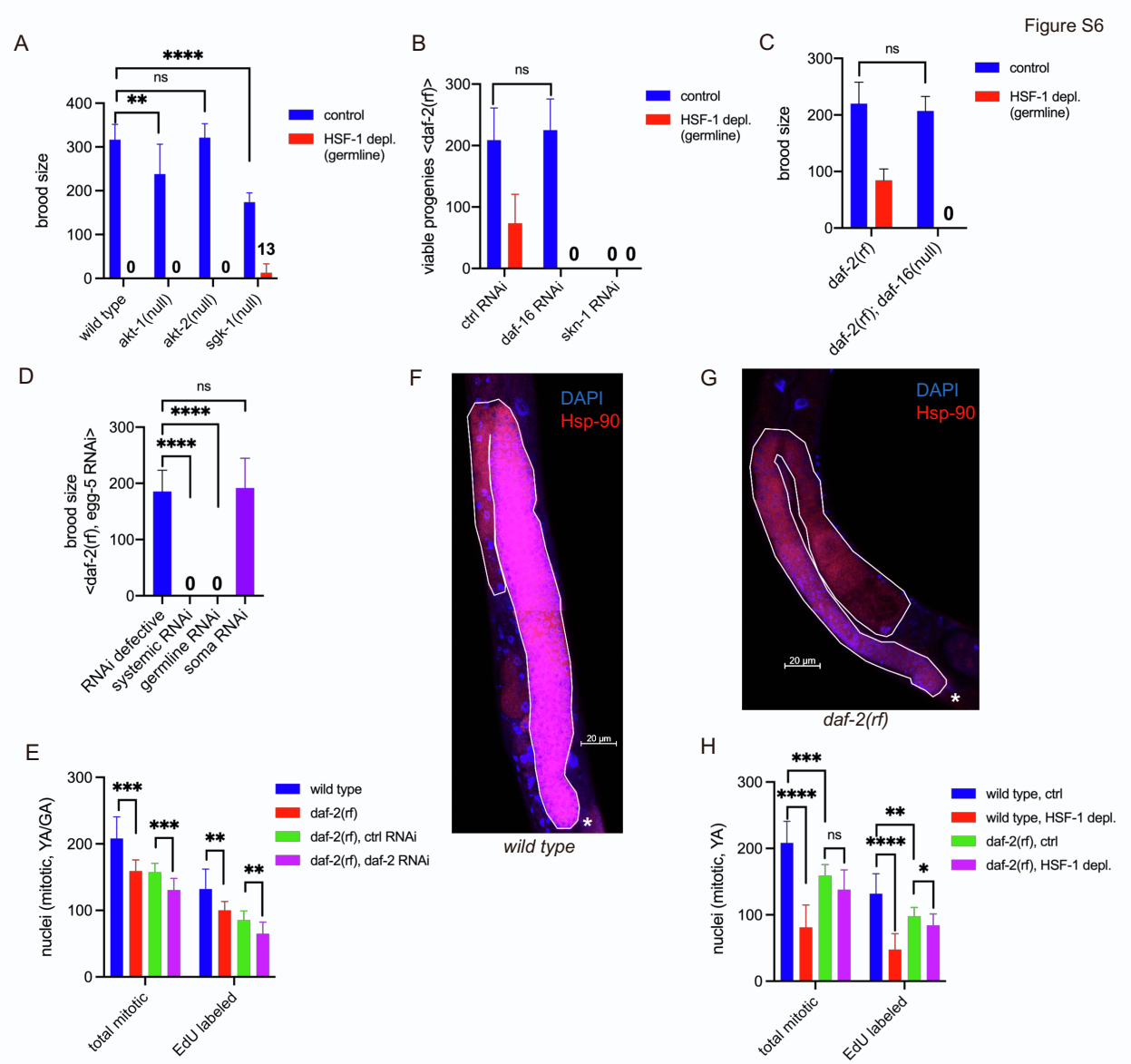


Figure S6, related to Figure 6. Insulin/IGF-1 signaling activates HSF-1 dependent expression of *hsp-90* and *hsc-70* in germline development.

(A) Histograms showing brood size of the wild type and null mutants of *akt-1*, *akt-2* or *sgk-1*. Depletion of HSF-1 from the germline started since egg lay. Data are represented as mean ± standard deviation (n>=12). Statistical significance was calculated by unpaired, two-tailed Student's t test. **p<0.01, ****p<0.0001, ns: not significant (p>=0.05).

(B) Histograms showing the number of viable progenies by the *daf-2(rf)* animals with systemic RNAi treatment against DAF-16 or SKN-1. The empty vector of L4440 was used as the 'control RNAi'. Depletion of HSF-1 from the germline and RNAi both started since egg lay. Data are represented as mean ± standard deviation (n>=15).

(C) Histograms showing brood size of the *daf-2(rf)* mutant and the *daf-2(rf)*; *daf-16(null)* double mutant animals. Depletion of HSF-1 from the germline started since egg lay. Data are represented as mean ± standard deviation (n>=15).

(D) Histograms showing brood size of the *daf-2(rf)* animals with RNAi treatment against EGG-5. RNAi was done in genetic models that are either RNAi defective (as a negative control) or enable RNAi to occur systemically, only in the germline or in the soma. RNAi started since egg lay. Data are represented as mean ± standard deviation (n>=15). Statistical significance was calculated by unpaired, two-tailed Student's t test. ****p<0.0001, ns: not significant (p>=0.05).

(E) Quantification of total mitotic nuclei and EdU labeled nuclei (per gonad arm) in age-synchronized Day 1 adult worms that were at the young adult (YA) and gravid adult (GA) transition (adult vulva and a few if any embryos in the uterus). The wild type and *daf-2(rf)* animals and *daf-2(rf)* animals treated with *daf-2* RNAi or the empty vector of L4440 as control (ctrl RNAi) were used. RNAi started since egg lay. Data are represented as mean ± standard deviation (n>=10). Statistical significance was calculated by unpaired, two-tailed Student's t test. **p<0.01, ***p<0.001, ns: not significant (p>=0.05).
(F&G) Representative images of RNA-FISH of *hsp-90* in the wild type animals (F) or the *daf-2(rf)* mutant (G) at the young adult stage. Scale bars, 20 μM.
(H) Quantification of total mitotic nuclei and EdU labeled nuclei (per gonad arm) in age-synchronized Day 1 adult worms that were at the young adult (YA) and gravid adult (GA) transition. The wild type and *daf-2(rf)* animals were used. Depletion of HSF-1 started since egg lay. Data are represented as mean ± standard deviation (n>=10). Statistical significance was calculated by unpaired, two-tailed Student's t test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns: not significant (p>=0.05).

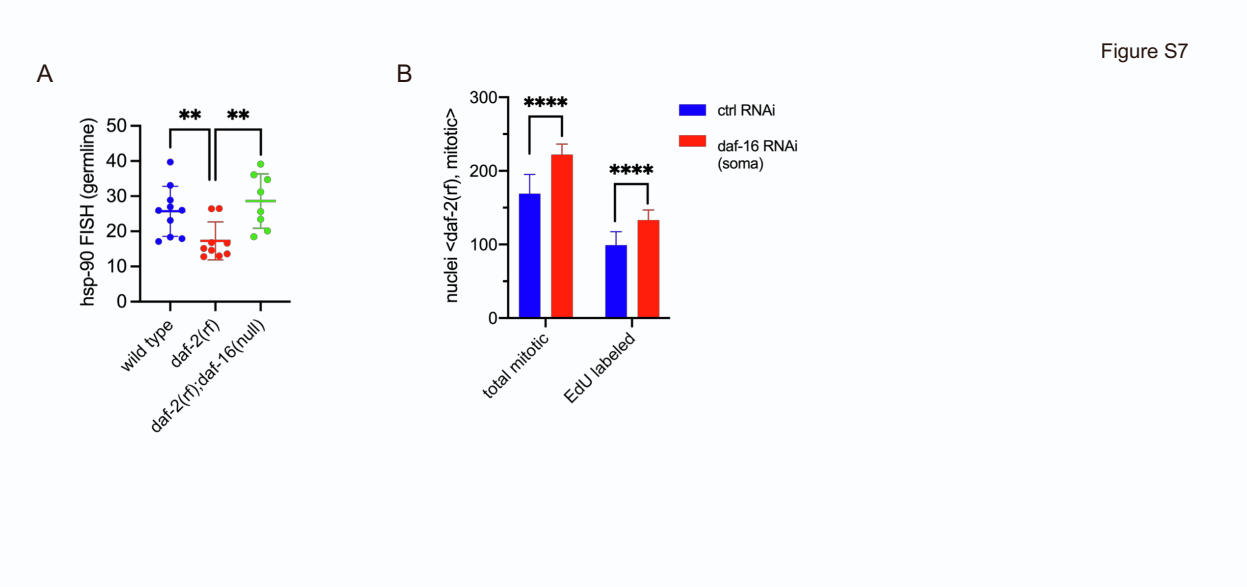


Figure S7, related to Figure 7. Insulin/IGF-1 signaling and FOXO/DAF-16 cell-non-autonomously regulate HSF-1 activities in the germline.

(A) Scatter dot plot showing the fluorescence intensity of *hsp-90* RNA-FISH in the germline of the wild type, the *daf-2(rf)* mutant and the *daf-2(rf)*; *daf-16(null)* double mutant animals. Mean and standard deviation are plotted. Statistical significance was calculated by unpaired, two-tailed Student's t test. **p<0.01.

(B) Quantification of total mitotic nuclei and EdU labeled nuclei (per gonad arm) in age-synchronized Day 1 adult worms that were at the young adult (YA) and gravid adult (GA) transition. The *daf-2(rf)* animals were treated with *daf-16* RNAi or the empty vector of L4440 as control (ctrl RNAi) only in the somatic tissues. RNAi started since egg lay. Data are represented as mean ± standard deviation (n>=10). Statistical significance was calculated by unpaired, two-tailed Student's t test. ****p<0.0001.