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Supplementary Materials for

Early-life stress triggers long-lasting organismal resilience and longevity via tetraspanin

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplemental Figures

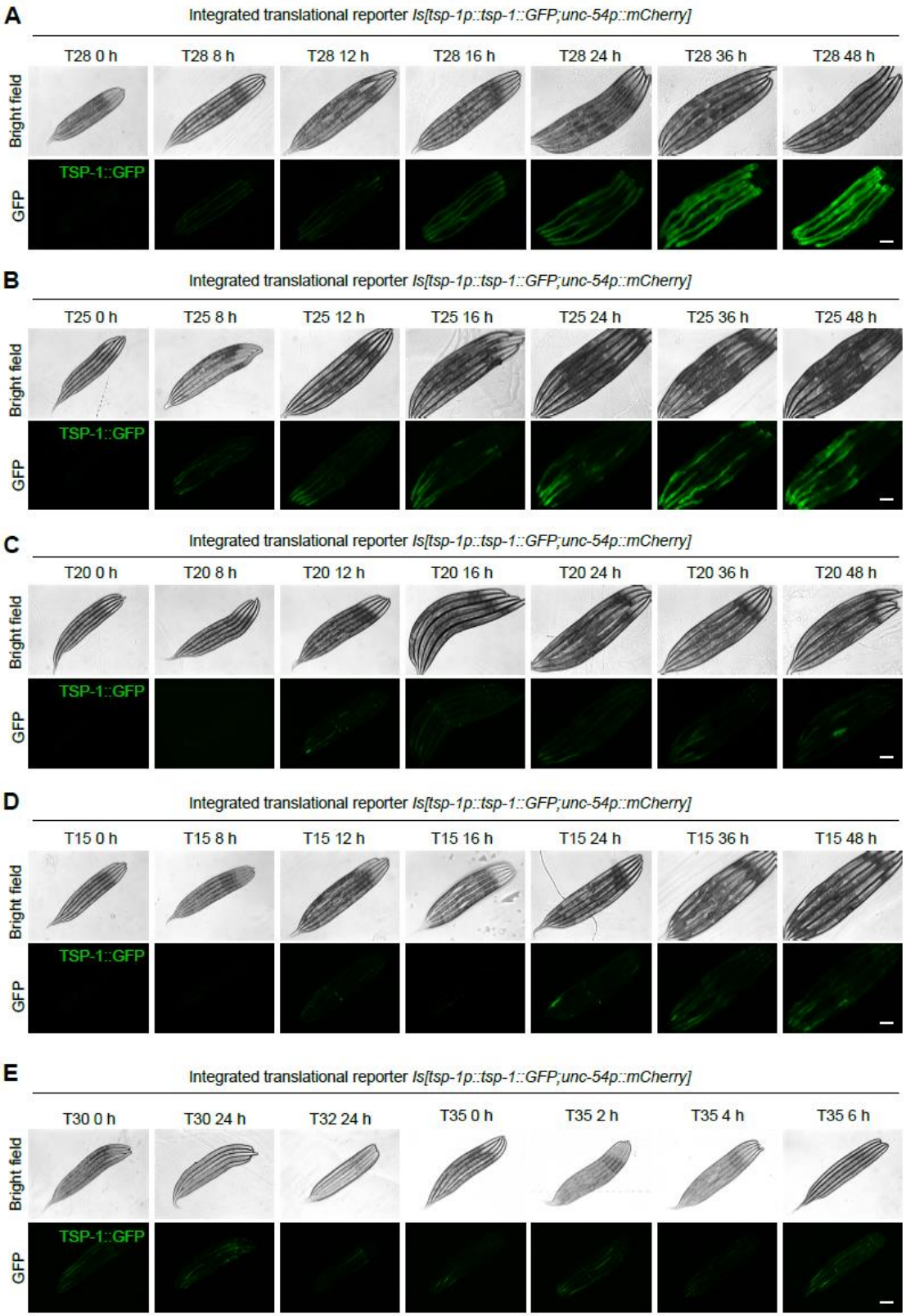

fig. S1. Duration and temperature-dependent induction of TSP-1::GFP. (A-E) Representative bright-field and epifluorescence images showing expression of *tsp-1p::tsp-1::GFP* under temperatures and durations indicated. Scale bars: 100 μm.

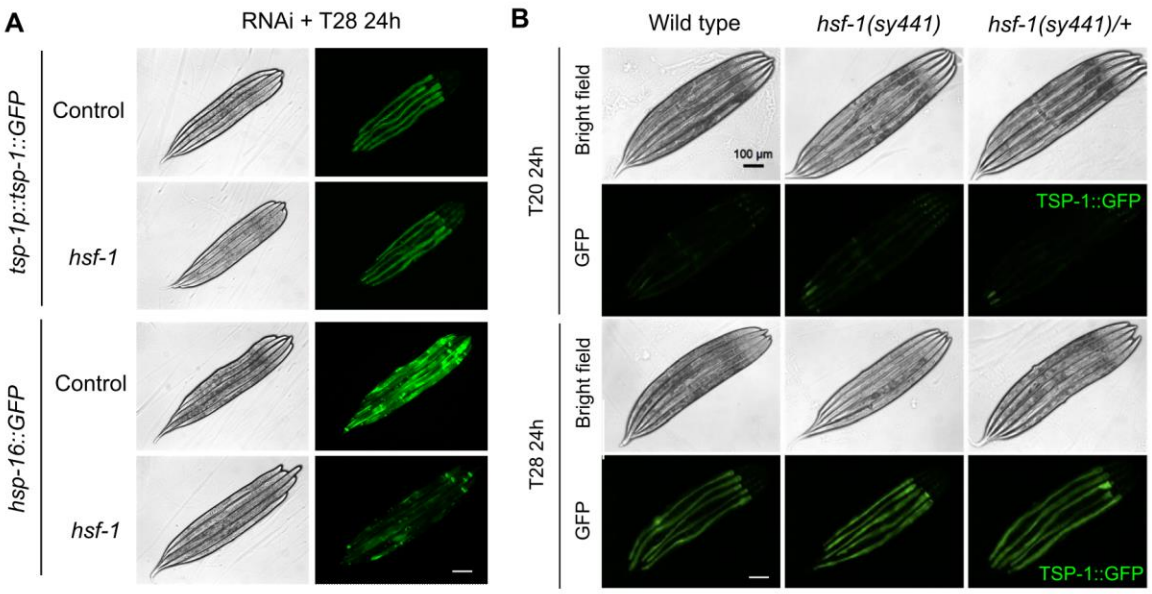

fig. S2. TSP-1 induction by T28 is independent of HSF-1. (A) Representative bright-field and epifluorescence images showing expression of *tsp-1p::tsp-1::GFP* or *hsp-16p::GFP* at 28 ºC for 24 hrs, with control and RNAi against *hsf-1*. (B) Representative bright-field and epifluorescence images showing expression of *tsp-1p::tsp-1::GFP* at 28 ºC for 24 hrs in wild type, *hsf-1(sy441)* reduction-of-function heterozygous or homozygous mutants. Scale bars: 100 µm.

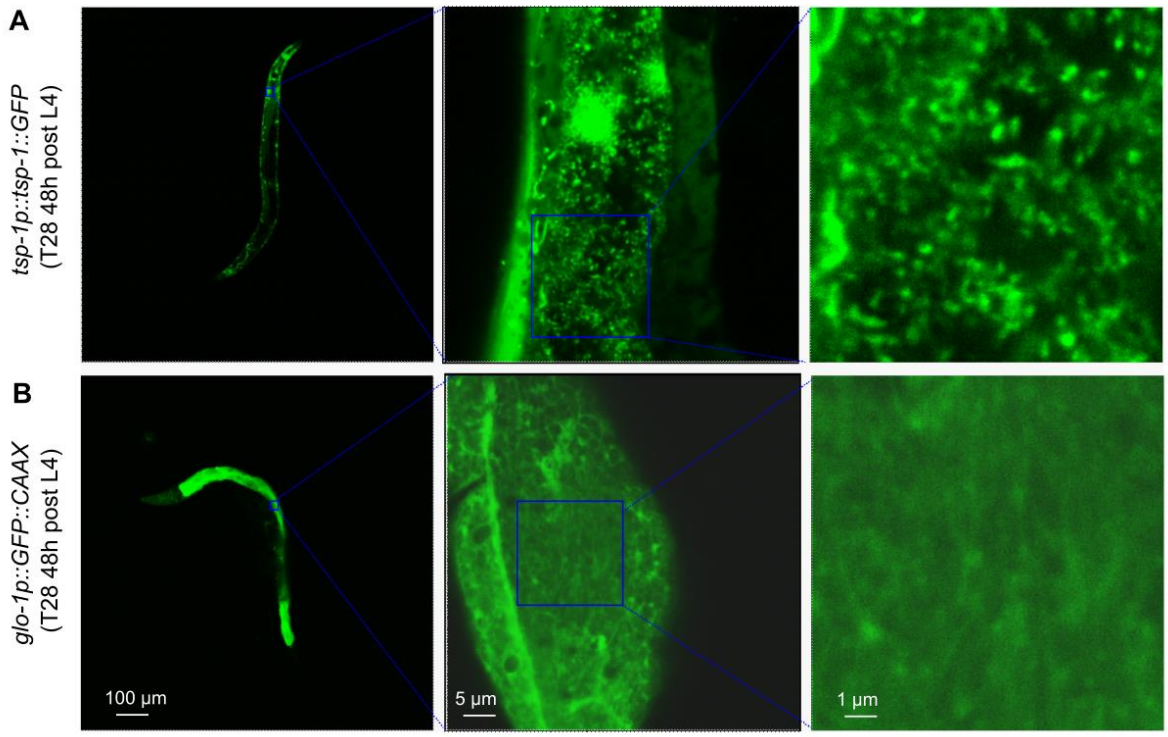

fig. S3. Tetraspanin web regulation by heat is specific for TSP-1. (A) Representative confocal fluorescence images showing T28-induced tetraspanin web structure formation by *tsp-1p::tsp-1::GFP* transgenes. (B), Representative confocal fluorescence images showing intestinal membrane GFP from *glo-1p::GFP::CAAX* under identical conditions (T28 48 hrs).

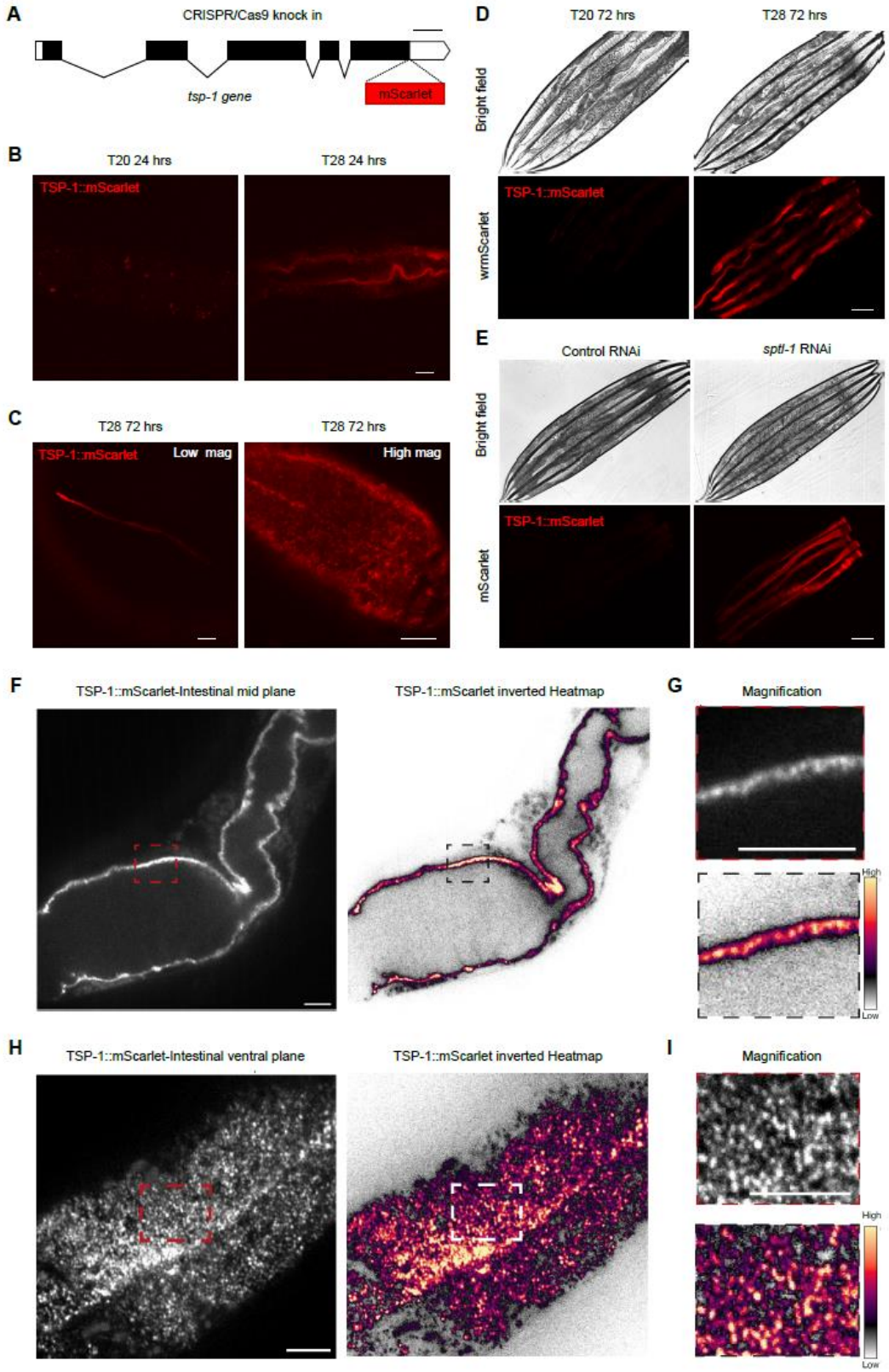
A
CRISPR/Cas9 knock in
tsp-1 gene
mScarlet
B
T20 24 hrs
T28 24 hrs
TSP-1::mScarlet
C
T28 72 hrs
T28 72 hrs
TSP-1::mScarlet
Low mag
High mag
D
T20 72 hrs
T28 72 hrs
Bright field
wrmScarlet
TSP-1::mScarlet
E
Control RNAi
sptl-1 RNAi
Bright field
mScarlet
TSP-1::mScarlet
F
TSP-1::mScarlet-Intestinal mid plane
TSP-1::mScarlet inverted Heatmap
G
Magnification
High
Low
H
TSP-1::mScarlet-Intestinal ventral plane
TSP-1::mScarlet inverted Heatmap
I
Magnification
High
Low

fig. S4. Heat-induced *tsp-1* endogenously tagged with wrmScarlet. (A) Schematic of tsp-1 gene structure showing CRISPR-mediated knock-in of wrmScarlet at the C-terminus of TSP-1. (B) Representative confocal fluorescence images showing up-regulation of endogenous TSP-1::wrmScarlet by 28 ^{o}C for 24 hrs. (C) Representative confocal fluorescence images showing low and high-mag views of endogenous TSP-1::wrmScarlet induced by 28 ^{o}C for 72 hrs. (D)Representative epifluorescence images showing up-regulation of endogenous TSP-1::wrmScarlet by 28 ^{o}C for 72 hrs. (E) Representative epifluorescence images showing up-regulation of endogenous TSP-1::wrmScarlet by RNAi against *sptl-1*, loss of which disrupts the biosynthesis of sphingolipids mimicking heat-induced membrane effects. (F-I) Representative spinning disc confocal images showing high-resolution views of tetraspanin web structures.

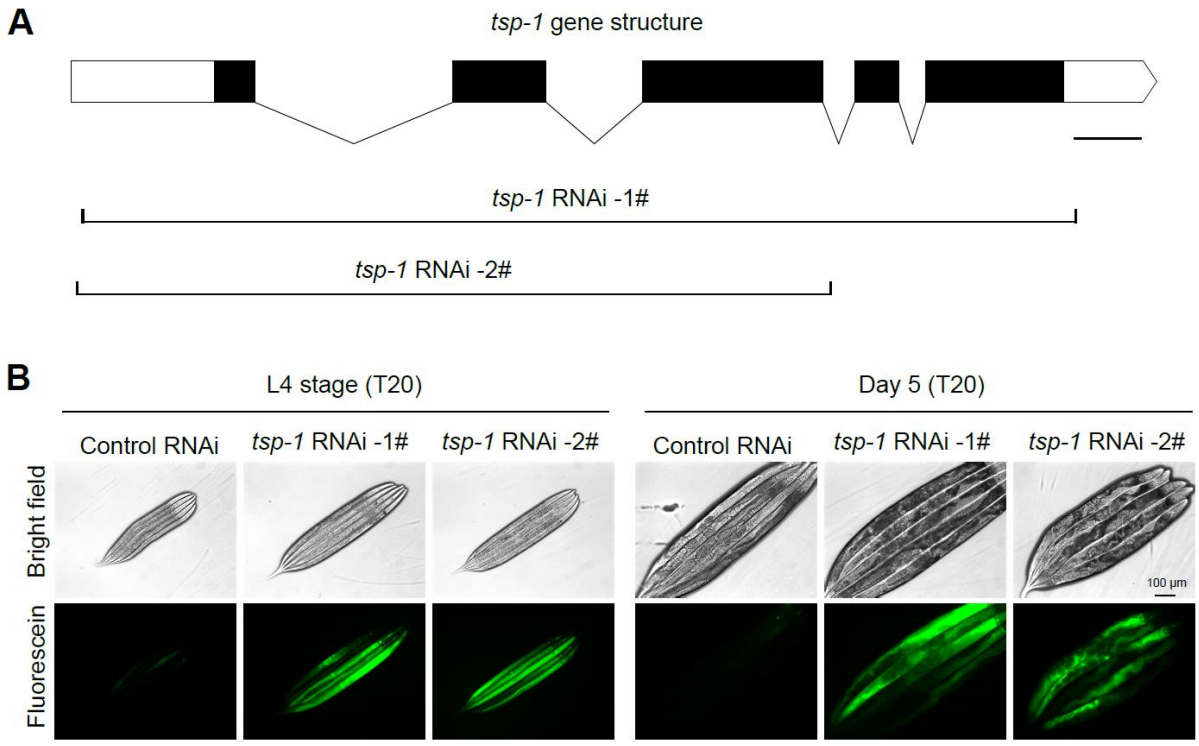

fig. S5. RNAi against *tsp-1* recapitulates mutant phenotype in membrane barrier functions. (A) Schematic of *tsp-1* gene structure showing two genomic regions used to construct RNAi for expression in *E. Coli* and feeding to *C. elegans*. (B) Representative epifluorescence images showing enhanced fluorescein uptake in *tsp-1* RNAi-treated animals, at both L4 stage and Day 5-old animals. Scale bars: 100 µm.

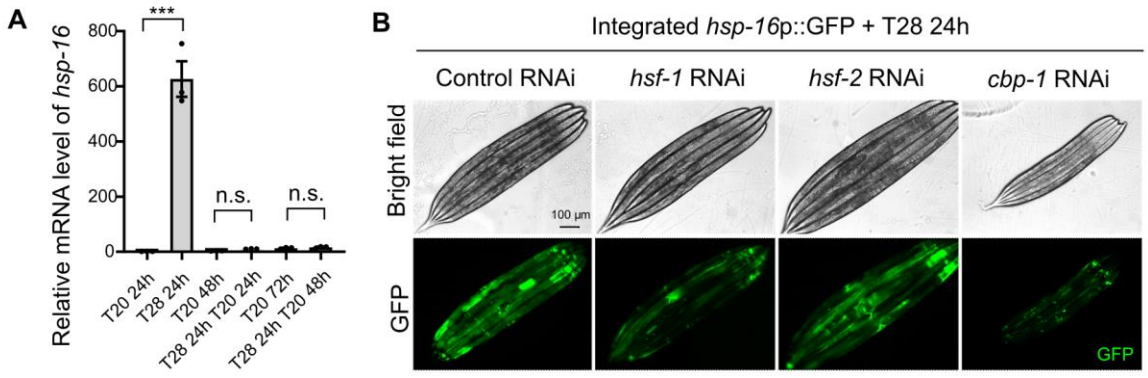

fig. S6. Heat shock protein induction by T28 requires both HSF-1 and CBP-1. (A) Quantitative RT-PCR measurements of *hsp-16* expression levels showing its transient induction by ELTS (T28 for 24 hrs at L4). *** indicates $P < 0.001$ (three independent biological replicates). (B) Representative epifluorescence images of animals with RNAi against *hsf-1* or *cbp-1* showing up-regulation of *hsp-16*p::GFP by ELTS that depends on both HSF-1 and CBP-1, but not HSF-2. Scale bars: 100 µm.

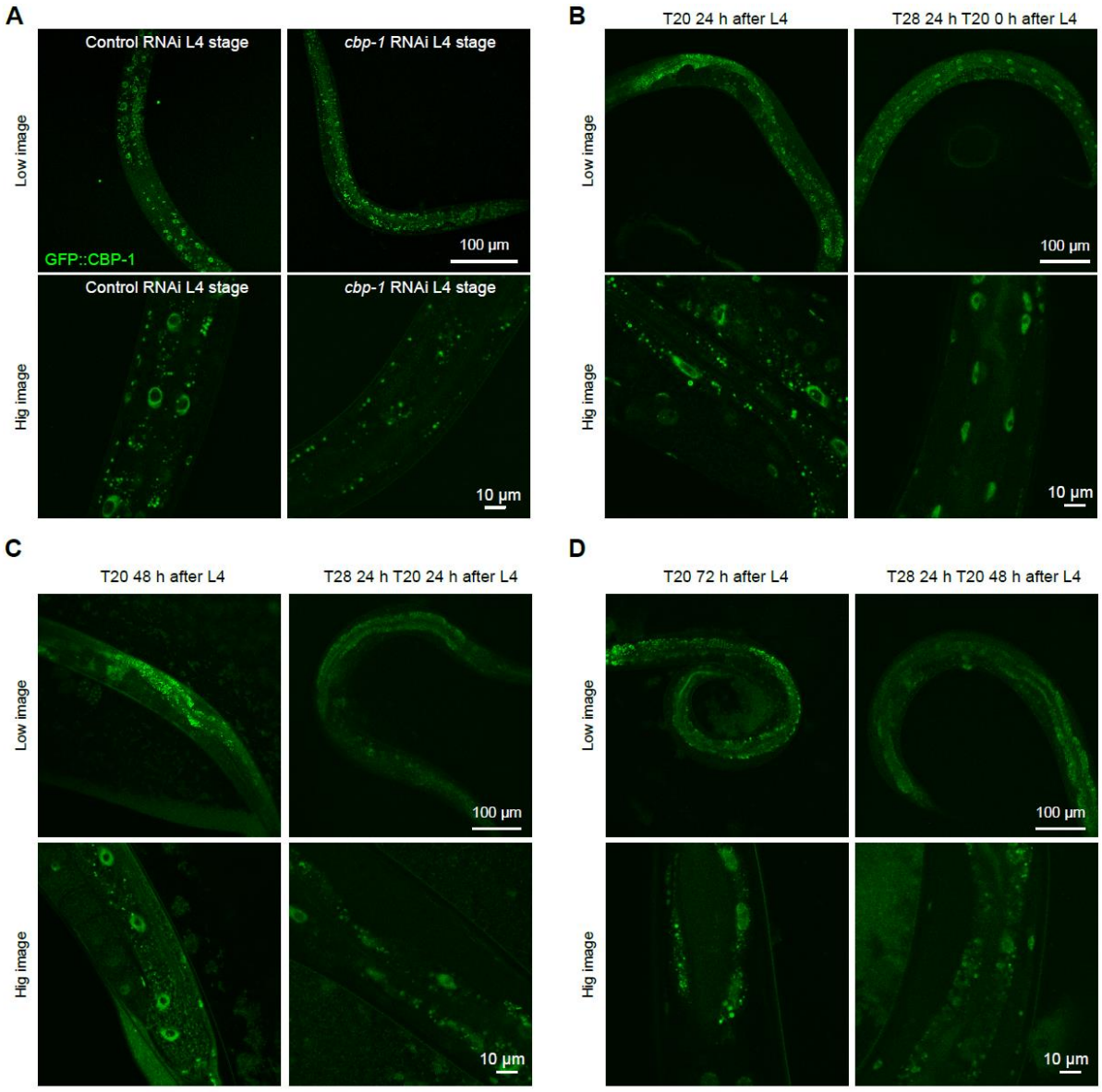

fig. S7. Heat-induced nuclear entry of CBP-1 endogenously tagged with GFP. (A) Representative confocal fluorescence images showing specific nuclear signals of GFP::CBP-1 (by CRISPR knock-in at the endogenous *cbp-1* locus) that were diminished by RNAi against *cbp-1*. (B) Representative confocal fluorescence images showing increased nuclear entry of endogenous GFP::CBP-1 by 28 ^{o}C for 24 hrs. (C) Representative confocal fluorescence images showing unaltered GFP::CBP-1 by 28 ^{o}C for 24 hrs and 20 ^{o}C for 24 hrs. (D) Representative

confocal fluorescence images showing unaltered GFP::CBP-1 by 28 ºC for 24 hrs and 20 ºC for 48 hrs. Shown are both high and low-magnification views. Scale bars are indicated.

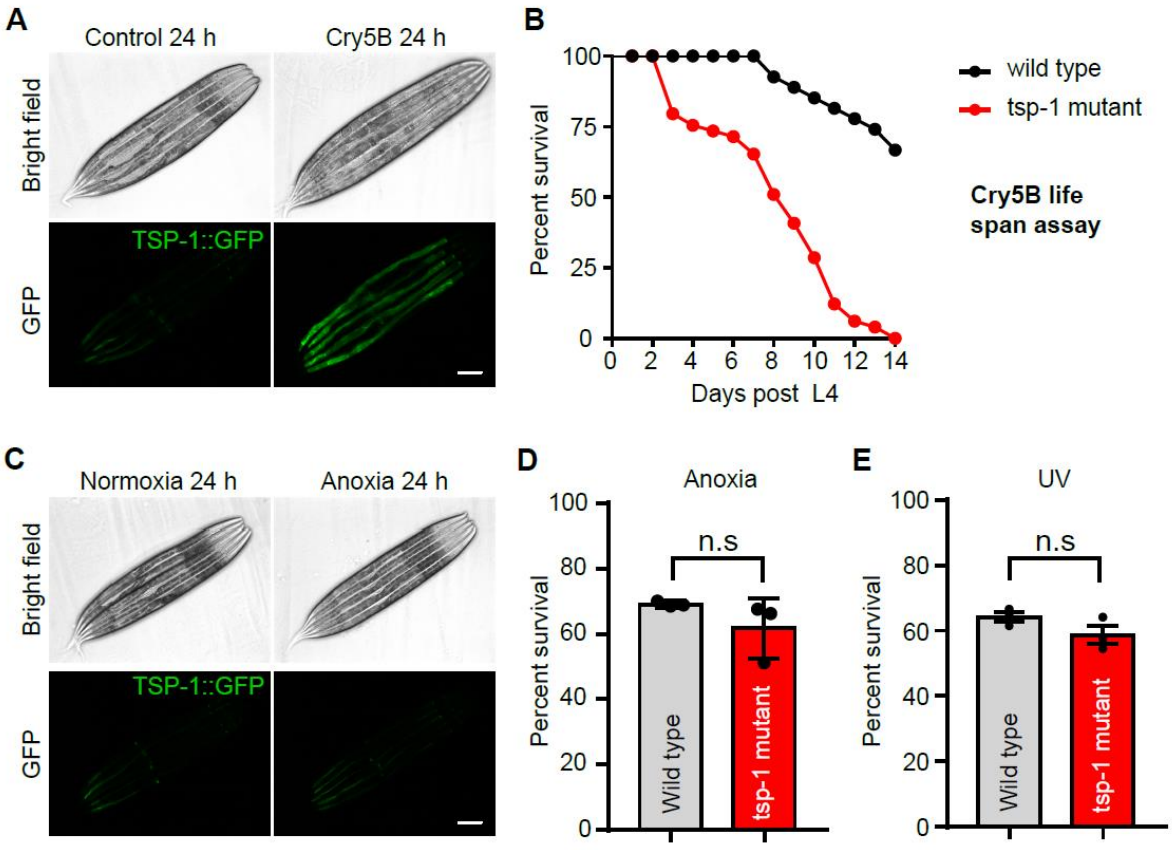

fig S8. Cry5B, UV, and anoxia effects on TSP-1 and organismal stress resilience. (A) Representative brightfield and epifluorescence images showing expression of an integrated transgene *tsp-1*p::*tsp-1*::GFP treated with cry5B expressing bacteria or control. Scale bars: 100 µm. (B) Lifespan curves of wild type and *tsp-1* deletion mutant allele *ok3594* with cry5B or control starting at L4. (C) Representative bright field and epifluorescence images showing expression of an integrated transgene *tsp-1p::tsp-1*::GFP under normoxia or anoxia for 24 h. Scale bars: 100 µm. (D) Percentage survival of wild type and *tsp-1* deletion mutants after exposure to anoxia at L4. (E) Percentage survival of wild type and *tsp-1* deletion mutants after exposure to UV at L4.

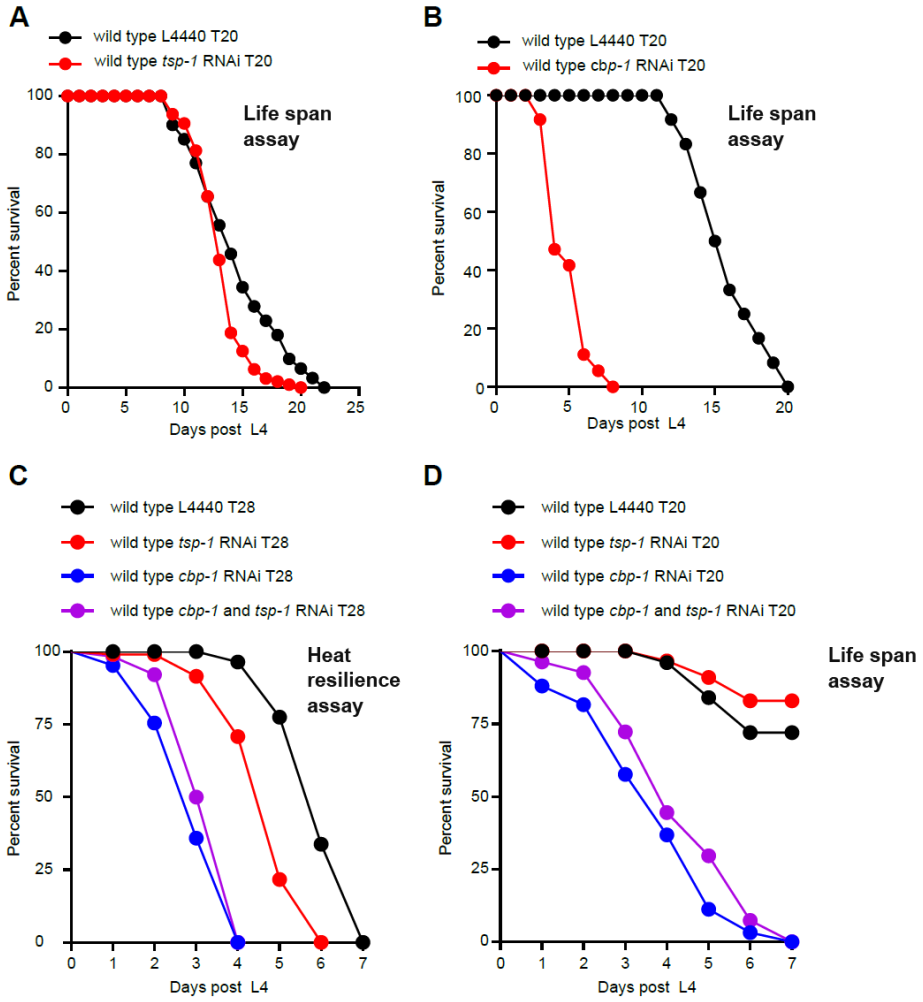

fig S9. Effects of co-application of *cbp-1* RNAi and *tsp-1* RNAi in *C. elegans*. (A) Lifespan curves of wild type with *tsp-1* RNAi or control starting at L4. (B) Lifespan curves of wild type with *cbp-1* RNAi or control at constant 20 ℃. (C) Lifespan curves of wild type with *tsp-1* RNAi, *cbp-1* RNAi or *tsp-1*+ *cbp-1* RNAi exposure to 28 ℃ starting at L4. (D) Lifespan curves of wild type with *tsp-1* RNAi, *cbp-1* RNAi or *tsp-1*+ *cbp-1* RNAi at constant 20 ℃ starting at L4.

Table S1.
Customized RNAi screen for candidate nuclear regulators of heat-induced TSP-1::GFP.

Table S2.
Quantitative summary of lifespan assay statistics and results.

Movie S1.
Tetraspanin webs exhibit stability from 120-min imaging of TSP-1::mScarlet. Scale bar: 10 μm.