Expanded View Figures

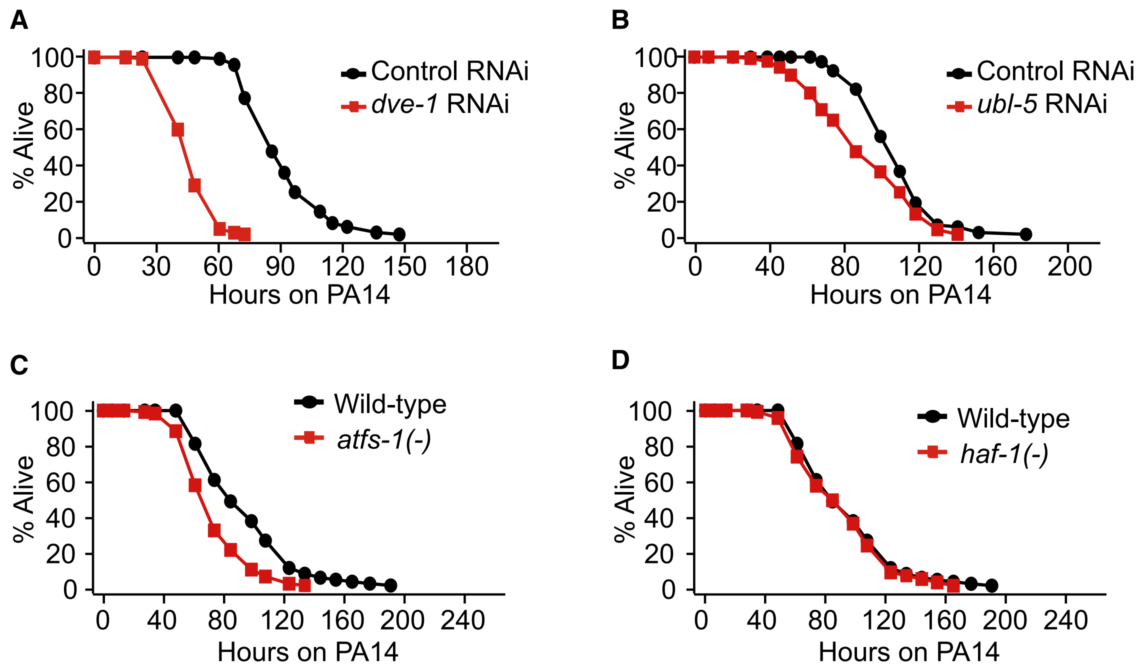

Figure EV1. The effects of genetic inhibition of various UPR^{MT} components on the survival of animals infected with PA14.

A, B *dve-1* RNAi (A)- or *ubl-5* RNAi (B)-treated animals displayed increased susceptibility to PA14 infection.
C, D *atfs-1(gk3094)* mutant animals (C) displayed increased susceptibility to PA14 infection, whereas *haf-1(ok705)* mutant animals (D) did not.

Data information: See Appendix Table S4 for additional repeats and statistical analysis for survival and lifespan data shown in this figure.

Figure EV2. The effects of genetic inhibition of various UPR^{MT} components on the expression of PMK-1 target genes.

A Upon PA14 infection, RNAi knockdown of *dve-1* or *ubl-5* decreased the expression of *T24B8.5p::GFP*, a downstream reporter of PMK-1.
B Quantification of data in panel (A) ($n \geq 23$ from three independent experiments). The data for control RNAi and *pmk-1* RNAi are the same as the ones shown in Fig 4B and shown here for comparison.
C *hsp-60* RNAi decreased the elevated level of *F35E12.5p::GFP*, a downstream reporter of PMK-1 upon PA14 infection.
D Quantification of data in panel (C) ($n \geq 20$ from three independent experiments).
E Mutations in *atfs-1* or *haf-1* did not affect the level of *T24B8.5p::GFP* on PA14, but *atfs-1* mutations induced *T24B8.5p::GFP* without PA14 infection.
F Quantification of data in panel (E) ($n \geq 25$ from three independent experiments).
G Quantification of Western blot data in Fig 4F ($n \geq 4$).

Data information: *pmk-1* RNAi (A–D) or mutation (E, F) was used as a positive control. Scale bars indicate 200 μm. Error bars represent SEM. Two-tailed Student's *t*-test was used for calculating *P*-values (**P* < 0.05, ***P* < 0.01, ****P* < 0.001).

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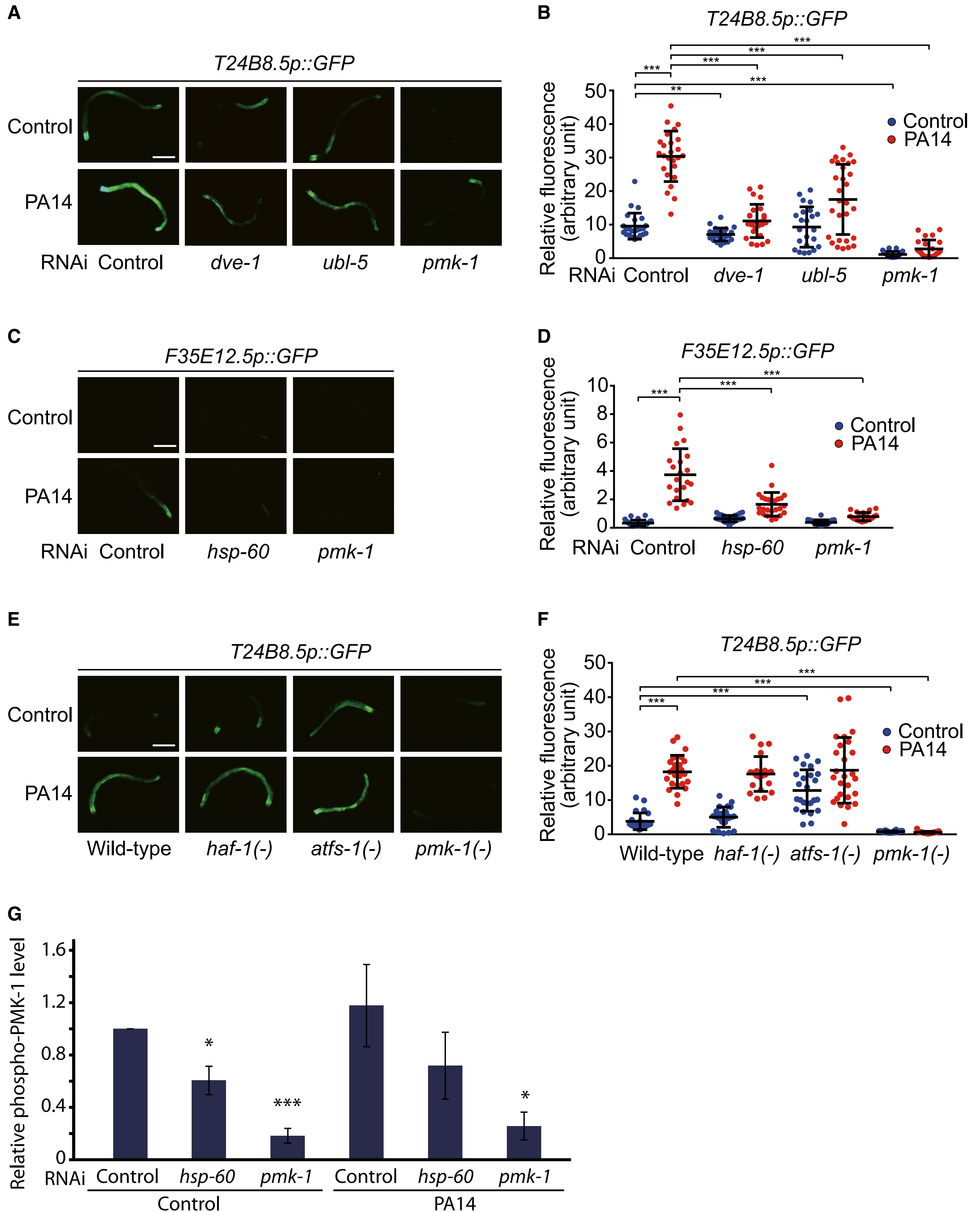

Figure EV2.

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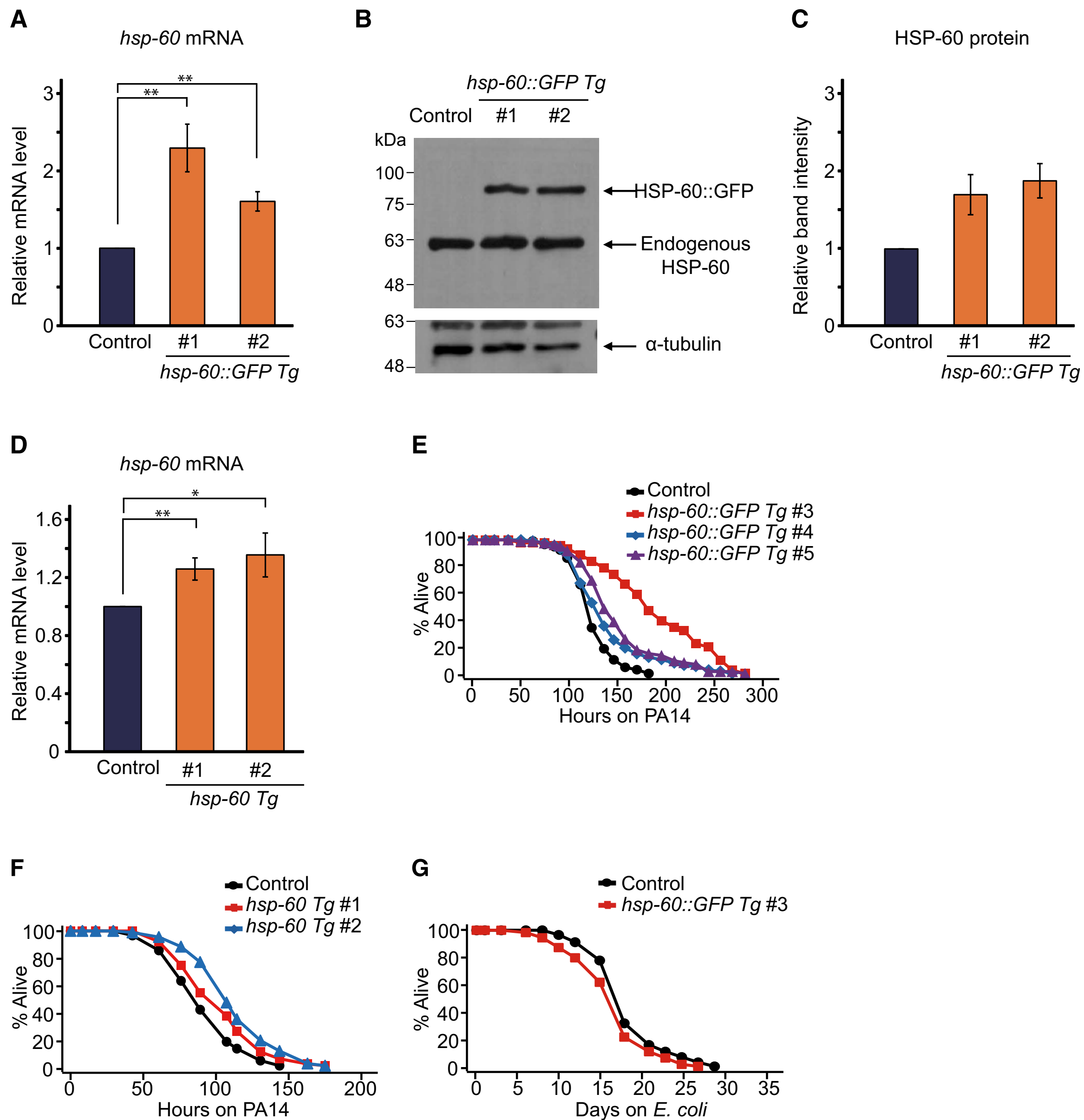

Figure EV3. ***hsp-60::GFP* expression increases the survival of animals infected with PA14, while not extending lifespan.**

A qRT–PCR results indicate that transgenic *hsp-60::GFP* expression (*hsp-60::GFP Tg*) increased the level of *hsp-60* mRNA in the two independent transgenic animals.

B, C Western blot data show the levels of both endogenous HSP-60 and GFP-fused HSP-60 in two independent transgenic lines (B). HSP-60 antibody was used for the detection of both endogenous and GFP-fused HSP-60. Total HSP-60 protein levels were quantified by adding band intensities of endogenous HSP-60 and GFP-fused HSP-60 ($n = 2$) (C).

D qRT–PCR data indicate that transgenic expression of *hsp-60* without a tag (*hsp-60 Tg*) slightly but significantly increased the level of *hsp-60* mRNA in two independent lines of transgenic animals (#1 and #2; see Materials and Methods).

E Three independent lines (#3, #4, and #5; see Materials and Methods) of transgenic animals that carried extrachromosomal arrays of *hsp-60::GFP* (*hsp-60::GFP Tg*) displayed significant increases in resistance to PA14 ($P < 0.001$). We noticed that the effects of *hsp-60::GFP Tg* #4 and #5 on the survival of worms on PA14 were smaller than those of *hsp-60::GFP Tg* #3. We currently do not know the basis of this variability among the transgenic lines.

F Two independent lines of transgenic animals with extrachromosomal arrays of *hsp-60* (*hsp-60 Tg* #1 and #2) showed significant increases in PA14 resistance ($P < 0.01$ and $P < 0.001$, respectively). The effects of *hsp-60 Tg* on PA14 resistance tend to be smaller than those of *hsp-60::GFP Tg* (Fig 6G). Note that the levels of *hsp-60* mRNA were significantly increased by 1.2- to 1.4-fold in *hsp-60 Tg* animals and by 2.3-fold in *hsp-60::GFP Tg* animals (A and B). Thus, it seems likely that the levels of *hsp-60* mRNA correlate with the survival time of animals on PA14.

G A line of transgenic animals with an extrachromosomal array of *hsp-60::GFP* (#3) lived slightly shorter than control animals on *Escherichia coli* (OP50).

Data information: Error bars represent SEM. *P*-values were calculated by using two-tailed Student's *t*-test (*$P < 0.05$, **$P < 0.01$) (A, B, and D), or by using log-rank test (E–G). See Appendix Tables S7 and S8 for additional repeats and statistical analysis for the survival and the lifespan data shown in this figure.

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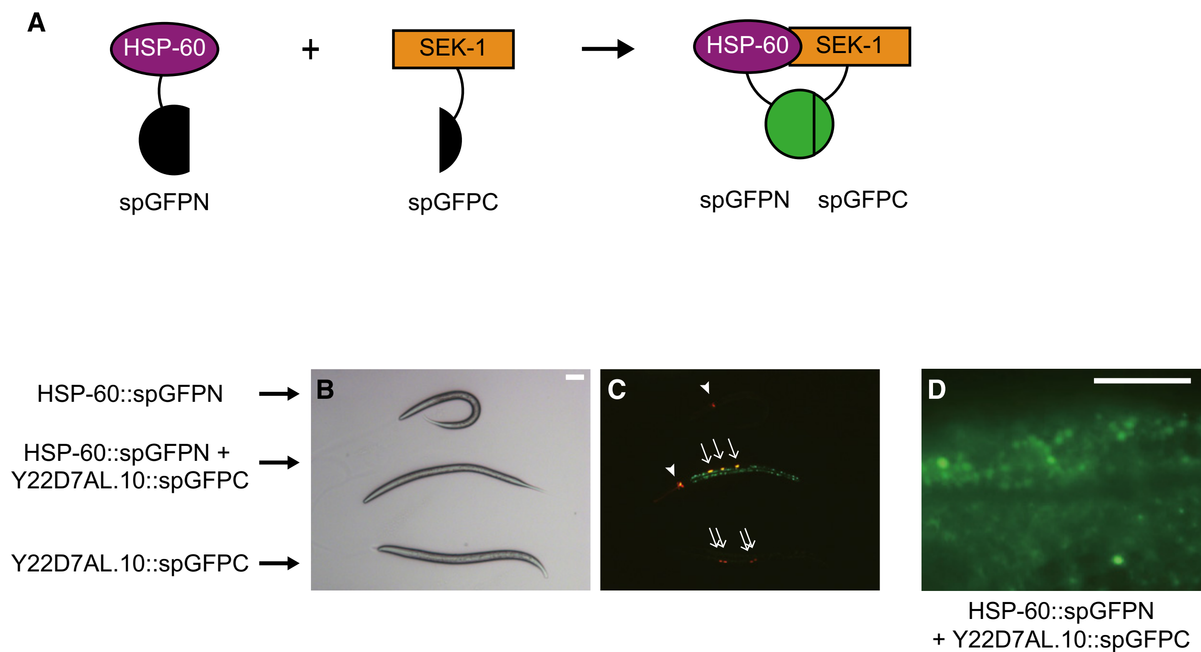

Figure EV4. HSP-60 and Y22D7AL.10/HSPE1 physically interact with each other.

A A schematic showing *in vivo* protein interaction using split GFP (spGFP) systems shown in Fig 9G–I. Physical interaction between HSP-60 and SEK-1 leads to the emission of green fluorescence.

B–D Bright field (B) and fluorescence (C) images of the animals that expressed an N-terminal GFP fragment fused with HSP-60 (HSP-60::spGFPN) and/or a C-terminal GFP fragment fused with Y22D7AL.10 (Y22D7AL.10::spGFPC) driven by an intestine-specific *vha-6* promoter. Among siblings from the same hermaphrodite, transgenic animals that expressed both HSP-60::spGFPN and Y22D7AL.10::spGFPC displayed GFP signals (C) (n = 5). The GFP signals were concentrated in punctae (D), which appear to be mitochondria. Arrowheads indicate *odr-1p::RFP*, a co-injection marker for HSP-60::spGFPN. Arrows indicate *coel::RFP*, a co-injection marker for Y22D7AL.10::spGFPC. Scale bars indicate 50 μm.

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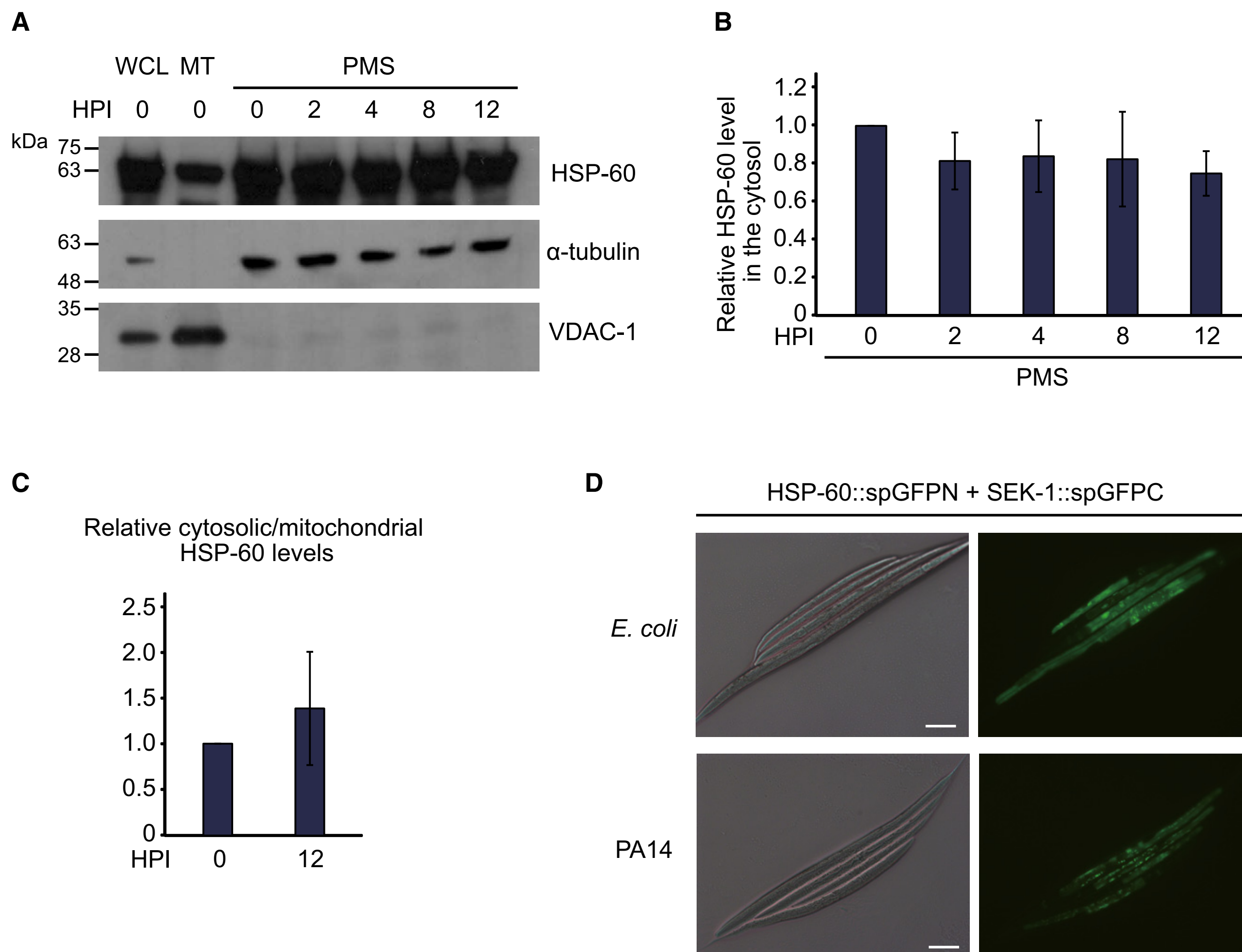

Figure EV5. PA14 infection does not alter the subcellular distribution of HSP-60.

A, B Time-course data of the PA14 infection followed by subcellular fractionation and Western blot analyses indicate that the level of HSP-60 in post-mitochondrial supernatant (PMS) was not significantly changed by PA14 infection (n = 4). The levels of HSP-60/α-tubulin in the PMS were quantified at different time points of PA14 infection as indicated. WCL and MT indicate whole-cell lysate and mitochondria, respectively. α-tubulin and VDAC-1 were used as markers for the PMS and mitochondria, respectively. HPI: hours postPA14 infection.

C Relative cytosolic/mitochondrial HSP-60 levels were quantified from the Western blot data in Fig 9A (n = 3). The ratio of cytosolic and mitochondrial HSP-60 was not significantly changed by PA14 infection (12 h).

D Bright field (left) and fluorescence (right) images of double transgenic animals that expressed both HSP-60::spGFPN and SEK-1::spGFPC in the intestine. PA14 infection for 12 h did not increase the fluorescent signals. Scale bars indicate 50 μm.

Data information: Error bars represent SEM. Two-tailed Student's t-test was used for calculating P-values.

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