Expanded View Figures

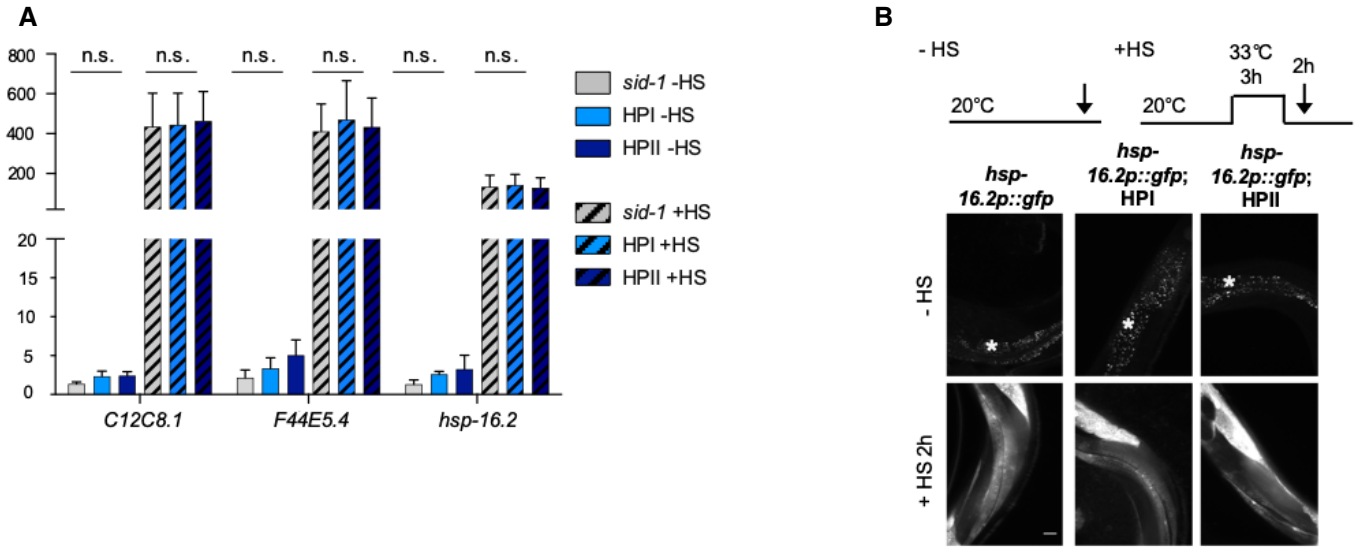

Figure EV1. KD of HSP-110 does not result in a compensatory activation of the heat shock response.

A Quantification of mRNA levels in 4-day-old animals expressing the indicated transgenes or harboring the indicated mutation relative to the −HS control of major HS-inducible genes (2 HSP70s, *C12C8.1* and *F44E5.4*, and the small HSP *hsp-16.2*) by real-time (RT)–PCR (mean ± SEM, $N = 4$). Statistical analysis was done using two-way ANOVA with Bonferroni's multiple comparison test. n.s. = not significant.

B Experimental set-up: Age-synchronized 4-day-old animals were subjected to heat stress for 3 h at 33°C (+HS) and then returned to 20°C or left at 20°C (−HS), respectively. Arrows indicate imaging time points. The −HS controls were imaged at the same time point as the +HS animals after 2 h at 20°C. Fluorescent microscopy images of animals expressing GFP under the HS-inducible *hsp-16.2* promoter in the control and HSP-110 KD background. Scale bars: 25 µm. The KD of HSP-110 did not evoke an heat shock response under ambient growth conditions (only autofluorescence of the gut granules (*) is visible in −HS strains), but animals were still able to mount an HSR under acute heat stress (+HS). All strains harbor the *sid-1(pk3321)* allele.

Figure EV2. WT and ATPase-deficient yeast SSE1 can compensate the loss of *Caenorhabditis elegans* HSP-110.

A Maximum intensity projections of confocal microscopy *z*-stacks of 5-day-old animals expressing the indicated transgenes. Scale bar: 10 μm. The expression of yeast WT and K69M mutant SSE1 but not FES1 reversed the reduction of α-Syn::YFP foci in the *hsp-110* hairpin expressing animals.

B Quantification of motility as a measure for transgene toxicity. Displayed is the mean ± SEM ($N = 3$) amount of body bends/30 s of animals expressing the indicated transgenes. Statistical analysis was done using two-way ANOVA with Bonferroni's multiple comparison test. n.s. = not significant, $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$. The presence of yeast WT SSE1 and mutant $SSE1^{K69M}$ but not FES1 suppressed the rescue of α-Syn::YFP toxicity in *hsp-110* hairpin expressing animals.

C Maximum intensity projections of fluorescent microscopy *z*-stacks of 5-day-old nematodes expressing the indicated transgenes. White dashed lines outline the borders of muscle cells. M: muscle, H: hypodermis. Scale bars: 10 μm. Signal outside of muscle cells reveals spreading of α-Syn.

D Quantification of animals showing α-Syn transmission into the hypodermis at indicated ages. Displayed is the mean ± SEM (in %) ($N = 3$). Statistical analysis was done using two-way ANOVA with Bonferroni's multiple comparison test. n.s. = not significant, $***P \leq 0.001$.

E Experimental set-up: Age-synchronized 4-day-old animals were subjected to heat stress for 3 h at 33°C (+HS) and then returned to 20°C or left at 20°C (−HS), respectively. Arrows indicate imaging time points. The −HS controls were imaged at the same time point as the +HS animals after 24 h at 20°C. Maximum intensity projections of fluorescent microscopy *z*-stacks of animals expressing the indicated transgenes. Scale bar: 20 μm. The impaired clearance of HS-induced FLUCSM::GFP foci in the *hsp-110* hairpin expressing cells is rescued by co-expression of WT SSE1 and $SSE1^{K69M}$.

F Quantification of FLUCSM::GFP foci disaggregation. % disaggregation is calculated as 100—ratio of FLUCSM::GFP foci area relative to total muscle area at the +HS 24 h time point to the +HS 2 h time point. Data are displayed as mean ± SEM (in %) ($N = 3$). Statistical analysis was done using one-way ANOVA with Dunnett's multiple comparison test. n.s. = not significant, $**P \leq 0.01$.

Data information: All strains harbor the *sid-1(pk3321)* allele.

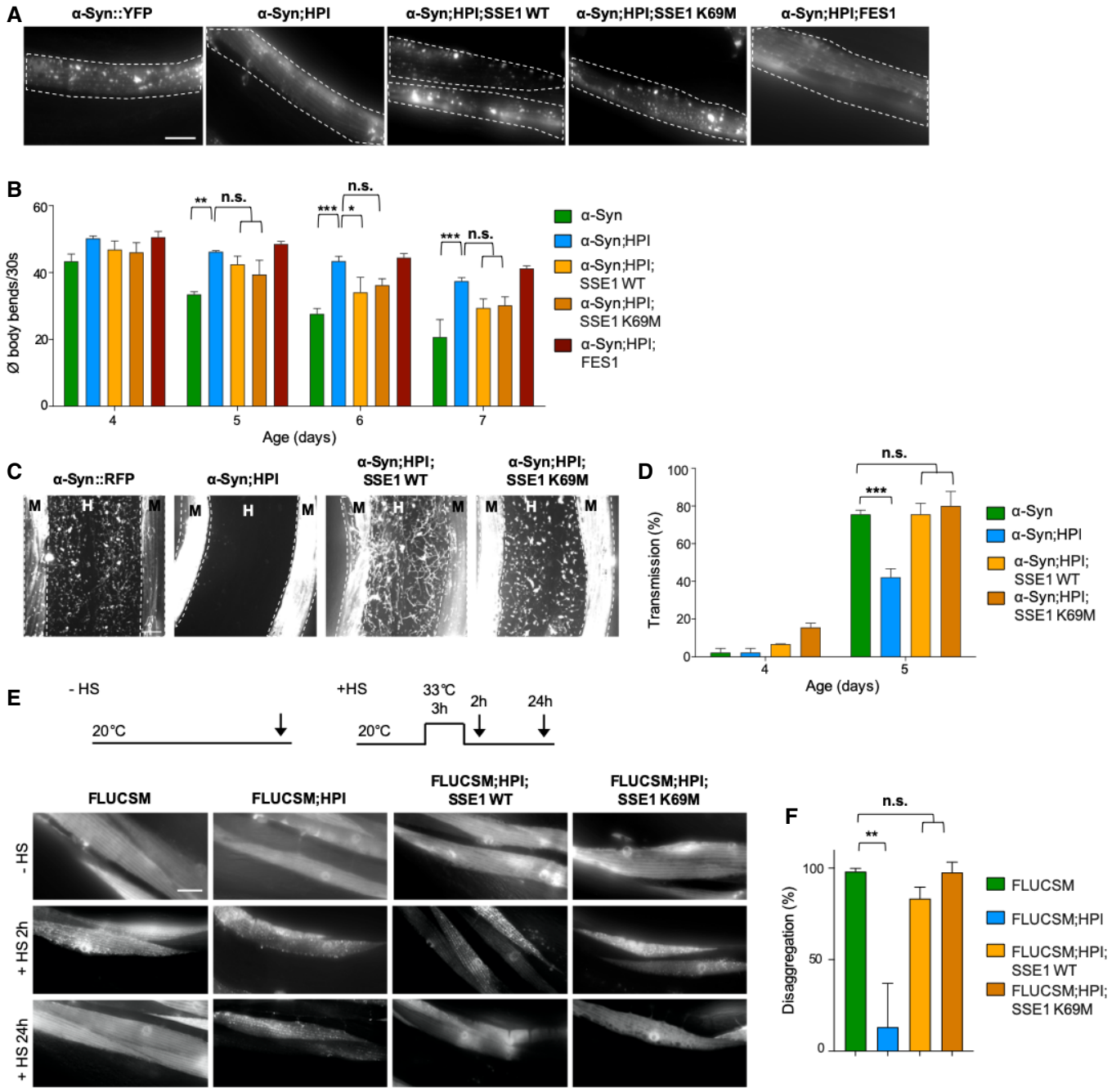
A
α-Syn::YFP
α-Syn;HPI
α-Syn;HPI;SSE1 WT
α-Syn;HPI;SSE1 K69M
α-Syn;HPI;FES1
B
Ø body bends/30s
Age (days)
n.s.
α-Syn
α-Syn;HPI
α-Syn;HPI; SSE1 WT
α-Syn;HPI; SSE1 K69M
α-Syn;HPI; FES1
C
α-Syn::RFP
α-Syn;HPI
α-Syn;HPI; SSE1 WT
α-Syn;HPI; SSE1 K69M
M
H
D
Transmission (%)
Age (days)
E
- HS
+HS
20°C
33°C
3h
2h
24h
FLUCSM
FLUCSM;HPI
FLUCSM;HPI; SSE1 WT
FLUCSM;HPI; SSE1 K69M
- HS
+ HS 2h
+ HS 24h
F
Disaggregation (%)

Figure EV2.

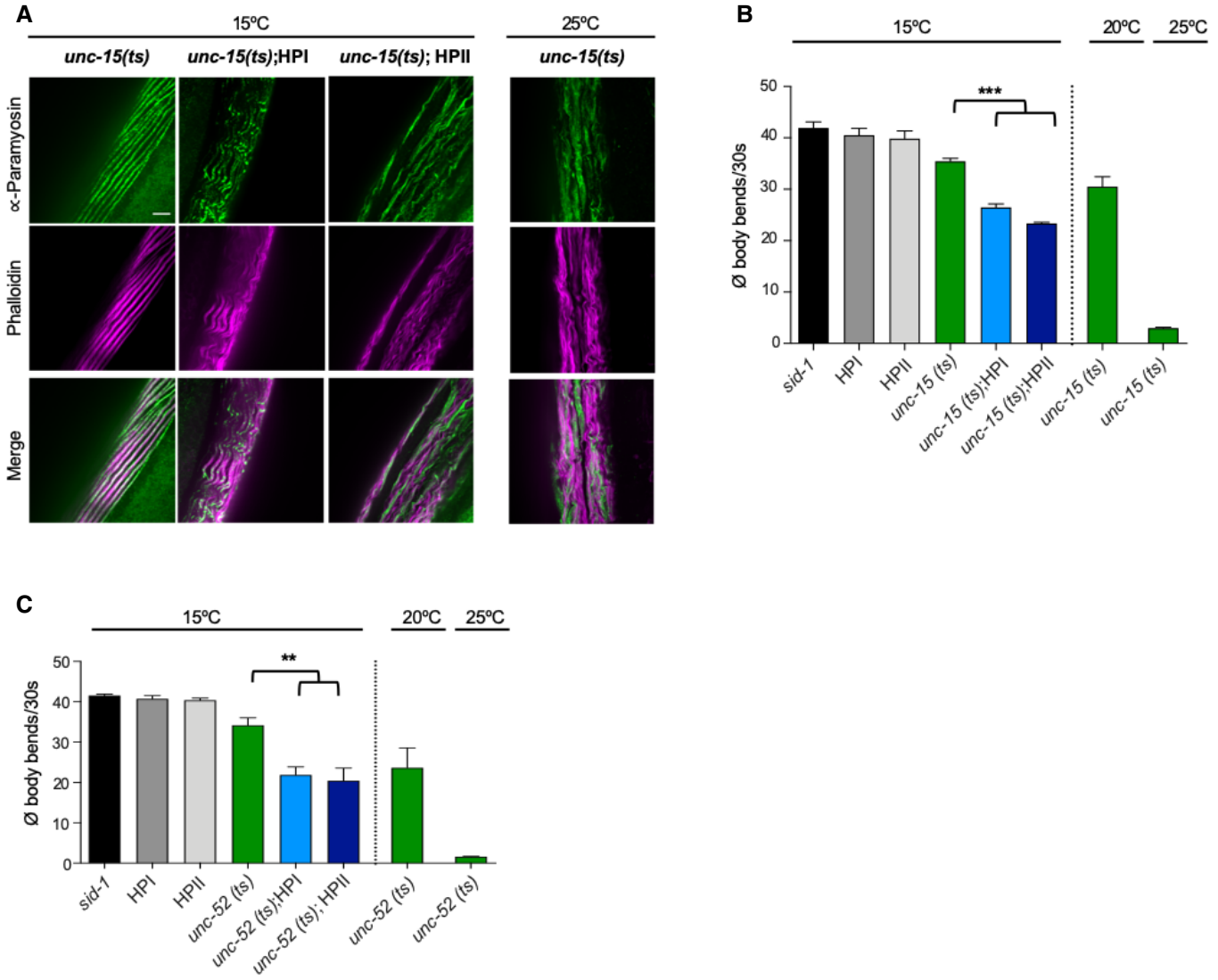

Figure EV3. HSP-110 HP expression impairs cellular protein folding homeostasis.

A Maximum intensity projections of confocal *z*-stacks of 4-day-old animals expressing the indicated transgenes or harboring the indicated temperature-sensitive (ts) mutations. Muscle cells were stained with anti-paramyosin (green) and Alexa Fluor 647-phalloidin (purple). Scale bar: 5 μm. Paramyosin misfolding revealed that co-expression of the *hsp-110* hairpins exposed the ts mutant phenotype of paramyosin(ts) [*unc-15*(*e1402*)] at the permissive temperate of 15°C.

B, C Quantification of motility as a measure for toxicity. Displayed is the mean ± SEM (N = 3) of body bends/30 s of animals expressing the indicated transgenes or harboring the indicated mutations. Statistical analysis was done using one-way ANOVA with Dunnett's multiple comparison test. $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. Co-expression of the *hsp-110* hairpins exposed the ts mutant phenotypes already at the permissive temperate of 15°C, resulting in a significant increase in movement defects. All strains harbor the *sid-1(pk3321)* allele.

Figure EV4. HSP-110 HP expression does not affect WT myosin or paramyosin folding.

A Quantification of motility as a measure for transgene toxicity. Displayed is the mean ± SEM (N = 3) of body bends/30 s of animals expressing the indicated transgenes or harboring the indicated mutations. Statistical analysis was done using one-way ANOVA with Dunnett's multiple comparison test. Animals that express the *hsp-110* hairpins show normal movement at 15 and 25°C.

B–E Maximum intensity projections of confocal *z*-stacks of 4-day-old animals expressing the indicated transgenes or harboring the indicated mutations. Muscle cells were stained with (B) and (C) anti-myosin (green) or (D) and (E) anti-paramyosin (green), and Alexa Fluor 647-phalloidin (purple). Scale bar: 5 μm. Animals expressing the *hsp-110* hairpins exhibited a normal myosin and paramyosin structure at 15 and 25°C. All strains harbor the *sid-1(pk3321)* allele.

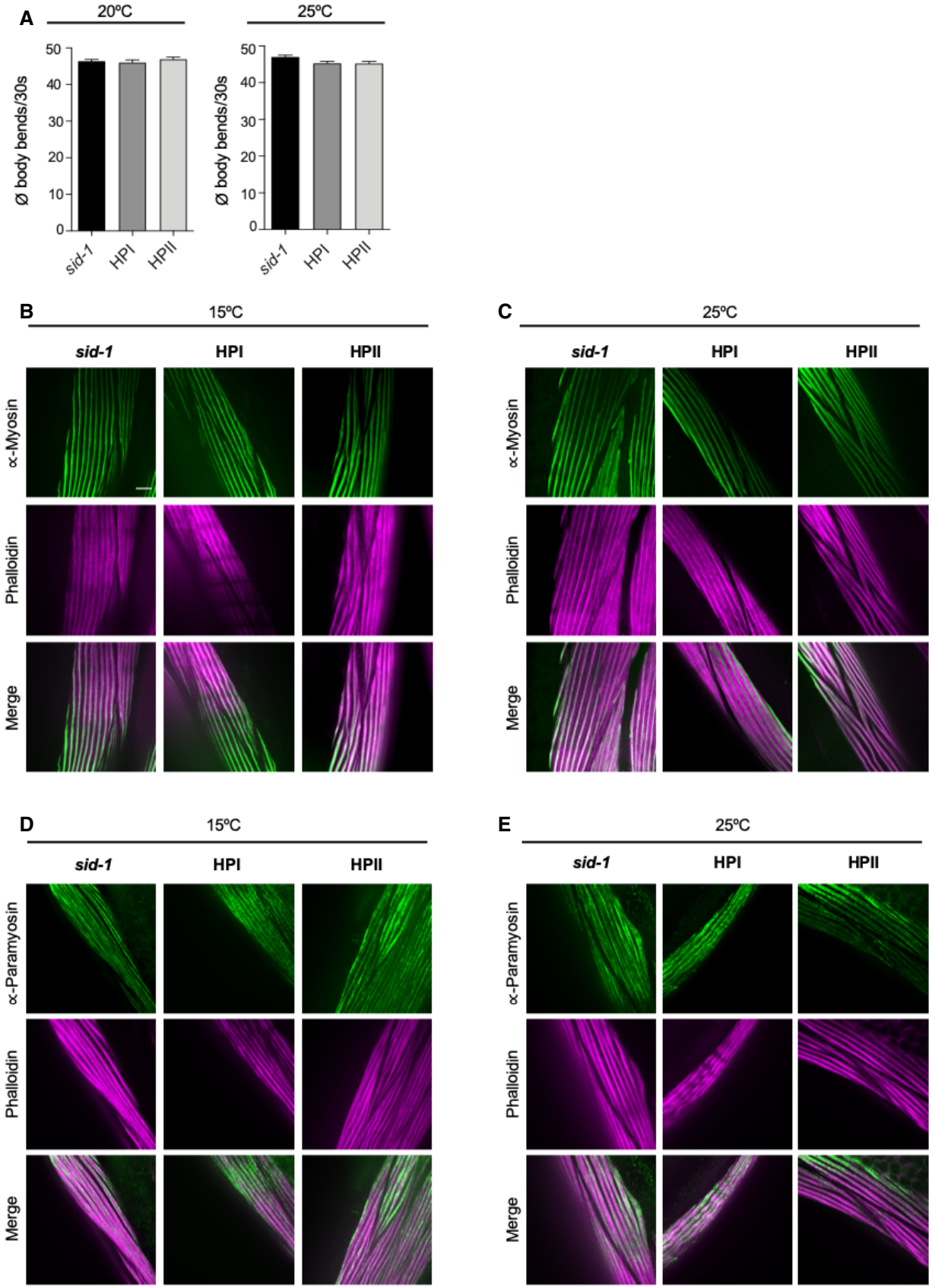

Figure EV4.

Figure EV5. HSP-110 HP expression impairs cellular protein folding homeostasis during aging.

A Quantification of motility as a measure for transgene toxicity. Displayed is the mean ± SEM (N = 3) amount of body bends/30 s of animals expressing the indicated transgenes or harboring the indicated mutations. Statistical analysis was done using two-way ANOVA with Tukey's multiple comparison test. *$P \leq 0.05$, **$P \leq 0.01$. Expression of the hsp-110 hairpins causes movement defects with increasing age at 25°C.

B Single plane confocal fluorescent microscopy images of 10-day-old animals harboring the indicated transgenes or endogenously tagged proteins. Muscle cells are outlined. Muscle cell-specific HSP-110::GFP depletion persisted during aging in HP animals. M: muscle, H: hypodermis. Scale bars: 10 μm.

C Maximum intensity projections of confocal z-stacks of 12-day-old animals expressing the indicated transgenes or mutations. White dashed lines outline the borders of muscle cells. Scale bar: 10 μm. α-Syn::YFP foci number remained low and did not increase in old HPI and HPII animals.

D Quantification of the ratio of α-Syn::YFP foci in 12-day-old animals to 5-day-old animals. The ratio of the product of mean foci fluorescence and foci area relative to muscle area from 12-day-old to 5-day-old animals is displayed (relFluoFoci). Data are shown as mean ± SEM (N = 3). All strains harbor the *sid-1(pk3321)* allele.

E Single plane fluorescent microscopy images of 12-day-old animals harboring the indicated transgenes.

F Quantification of Q35::YFP foci in WT and *hsp-110* hairpin background. Shown is the mean for each strain and age ± SEM (N = 3). Statistical analysis was done using two-way ANOVA with Dunnett's multiple comparison test. n.s. = not significant.

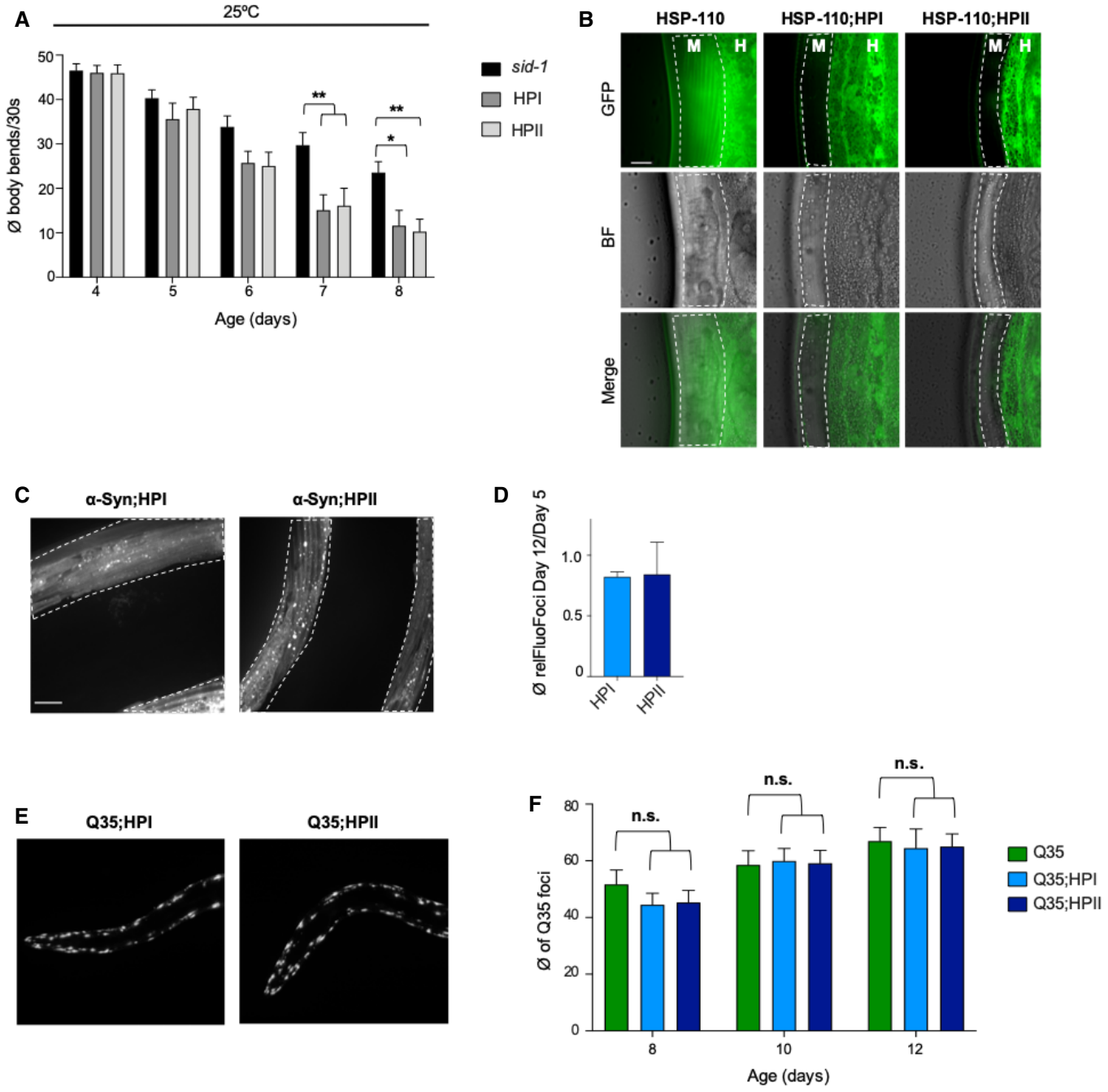

Figure EV5.