

## Supplementary Information

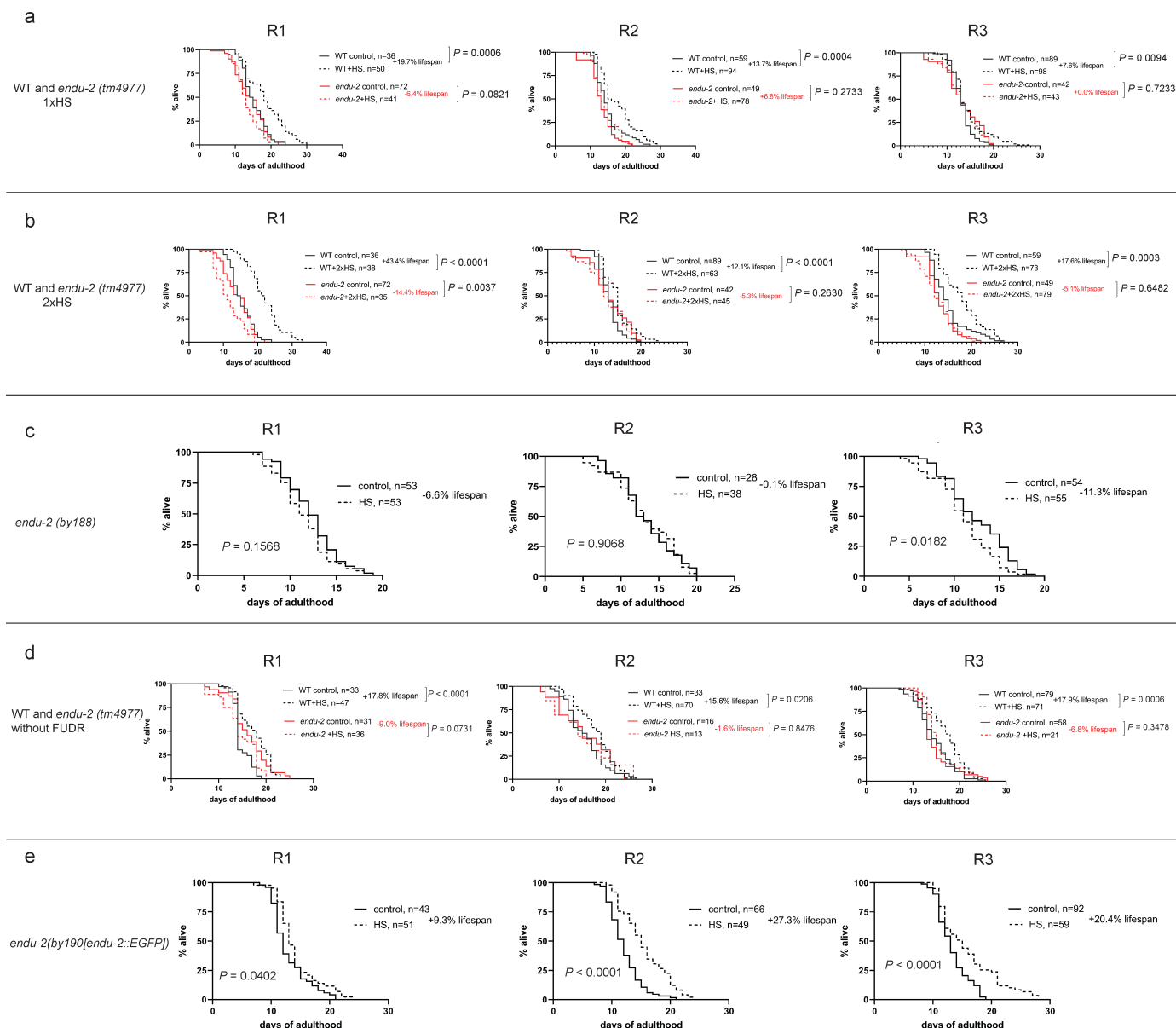
### **Reprogramming of the transcriptome after heat stress mediates heat hormesis in *Caenorhabditis elegans***

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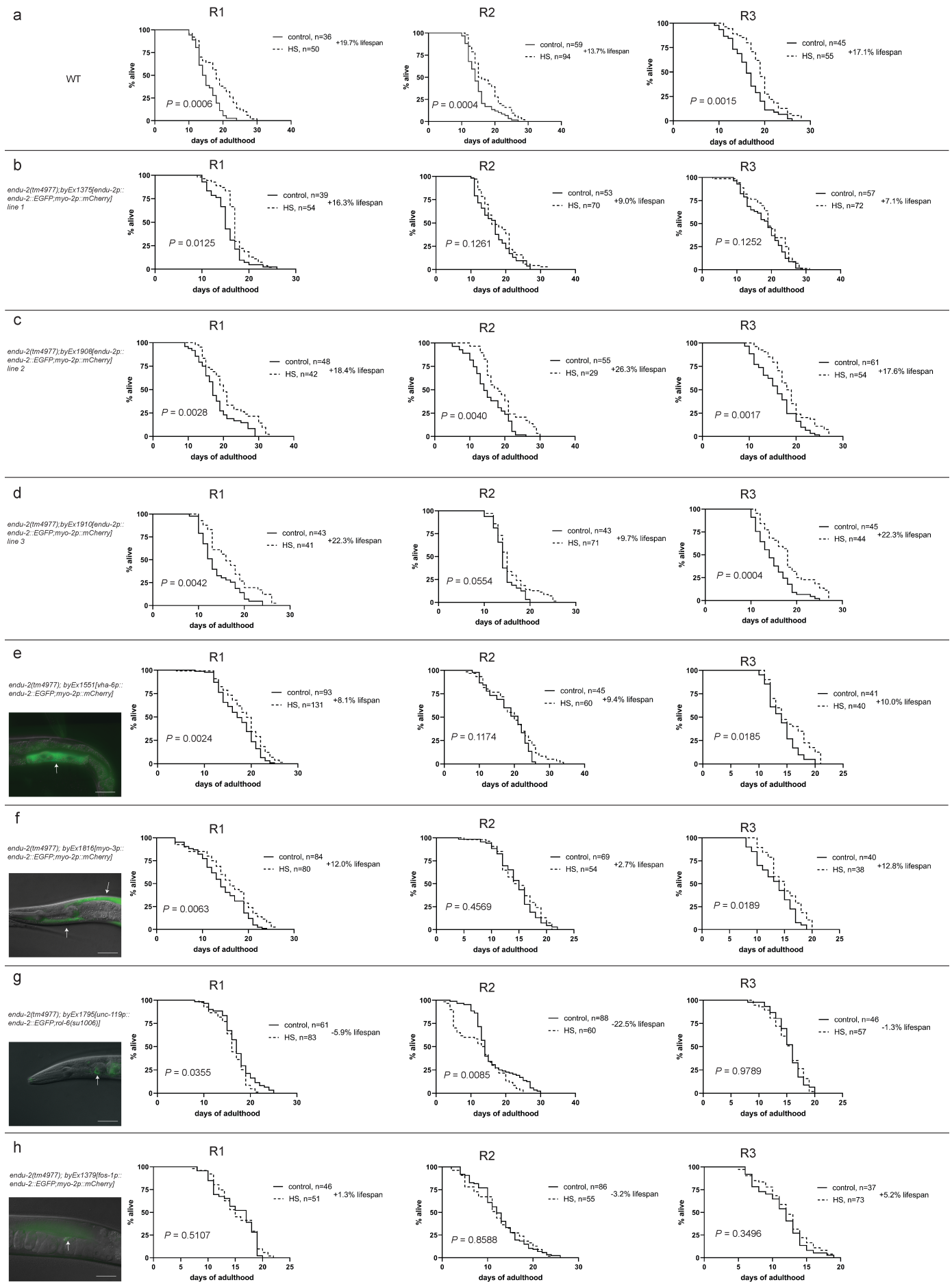
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**Supplementary Fig. 1 Summary of all biological replicates for the lifespan of WT and *endu-2* loss of function mutant animals**

**a**, Lifespan analysis of WT and *endu-2*(*tm4977*) animals upon one hormetic HS treatment (1 h at 35°C) on day 1 of adulthood. **b**, Lifespan analysis of WT and *endu-2*(*tm4977*) animals upon hormetic HS on day 1 and day 3 of adulthood. **c**, Lifespan analysis of *endu-2*(*by188*) loss of function animals upon hormetic HS on day 1 and day 3 of adulthood. **d**, Lifespan analysis of WT and *endu-2*(*tm4977*) animals upon a 45-min HS at 36°C on day 1 of adulthood. The experiments were performed without FUDR. **e**, Lifespan analysis of *endu-2*(*by190*)[*endu-2::EGFP*] CRISPR knock-in animals upon hormetic HS on day 1 and day 3 of adulthood.

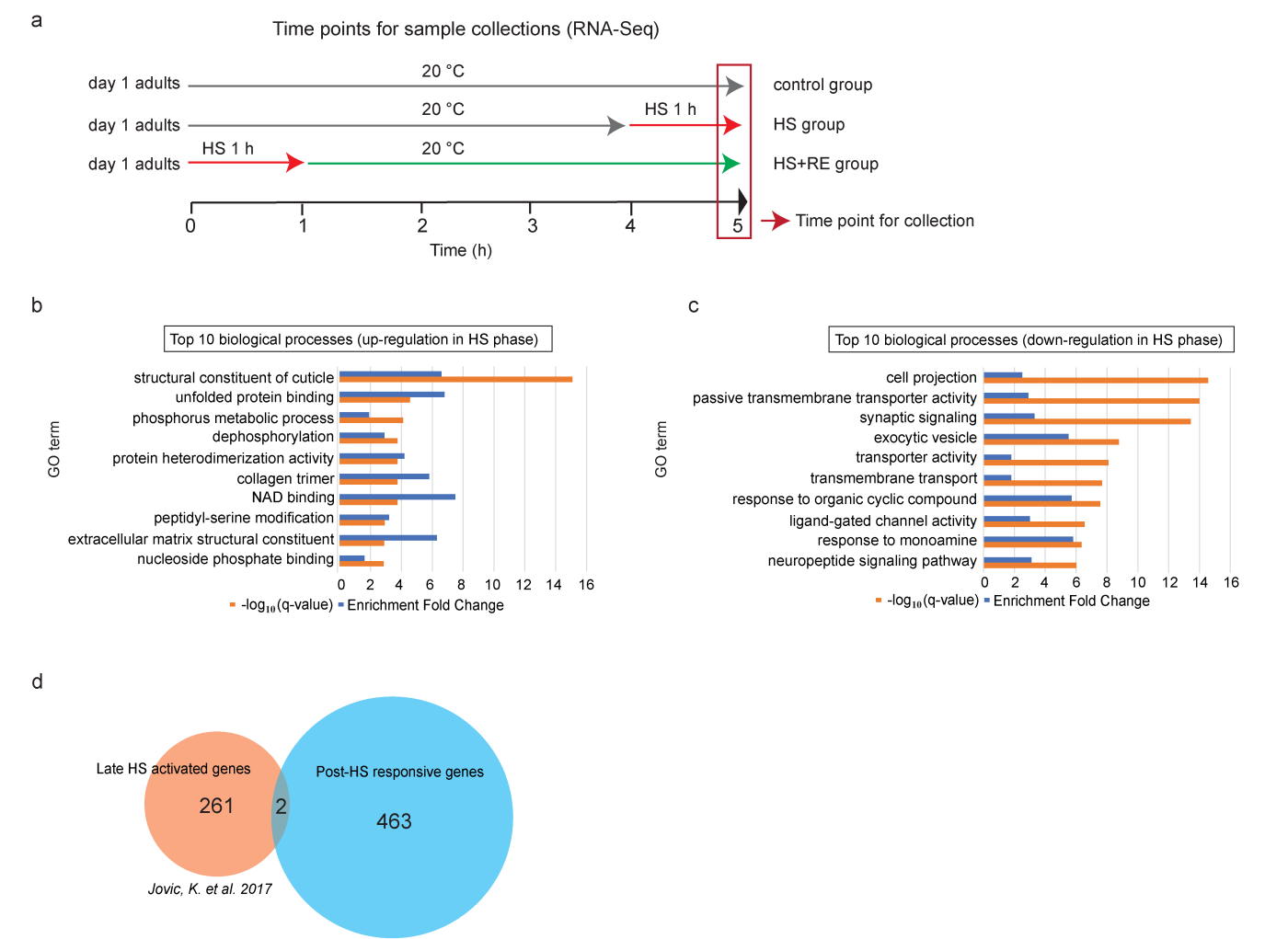
The  $P$  values were calculated using Log-rank (Mantel-Cox) test. This figure is related to **Fig. 1**.



**Supplementary Fig. 2 Summary of all biological replicates for Lifespan of WT and *endu-2::EGFP* rescued animals upon hormetic HS on day 1 and day 3 of adulthood.**

**a-h** Lifespan analysis of WT and different *endu-2::EGFP* rescue strains upon 2x hormetic HS. The ENDU-2::EGFP fluorescence in each tissue-specific rescue strain is indicated with the white arrows. Scale bar = 50  $\mu$ m. Shown are the three independent replicates. *P* values were calculated using Log-rank (Mantel-Cox) test.

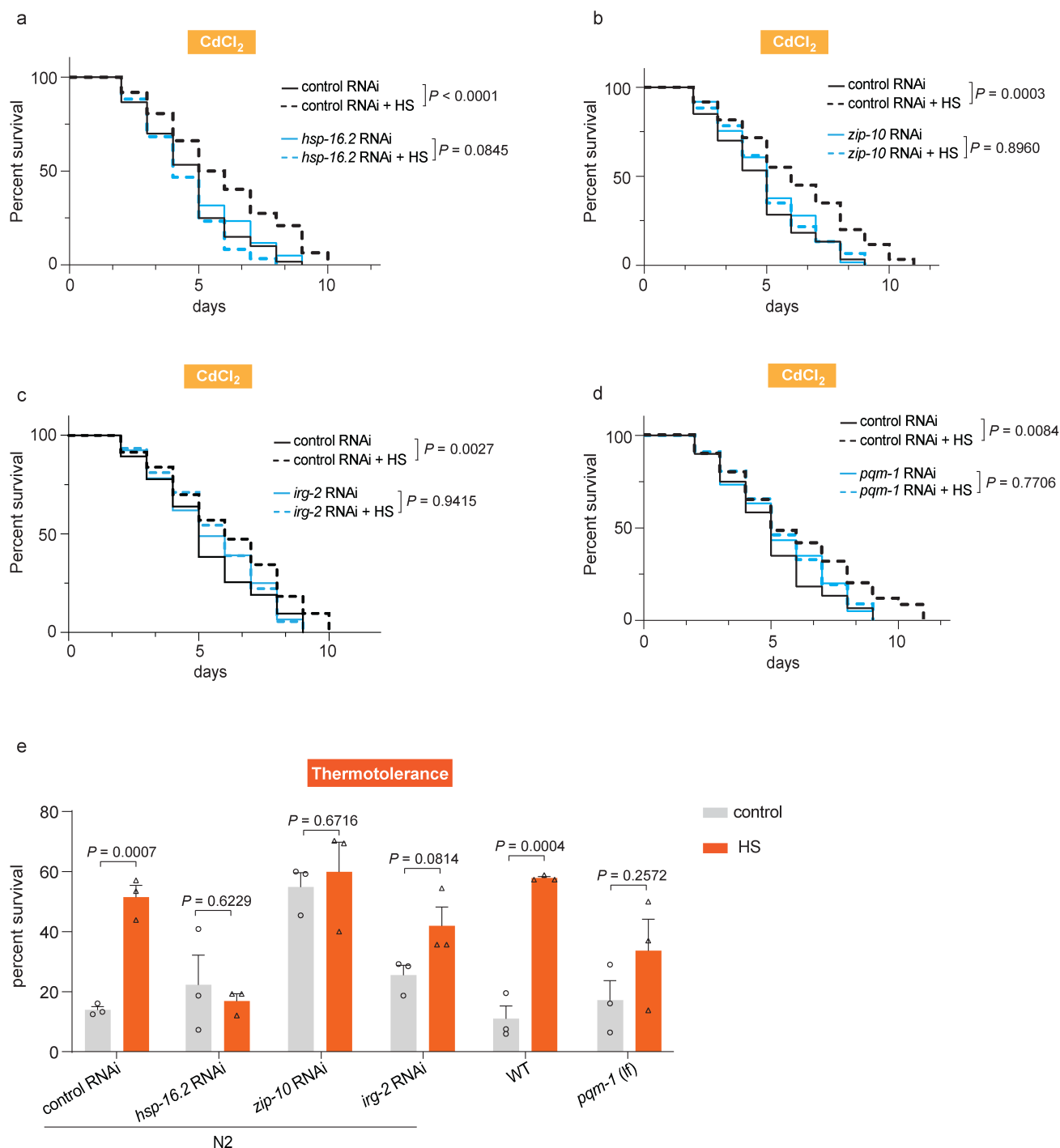
**a**, WT animals; **b**, *endu-2(tm4977);byEx1375[endu-2p::endu-2::EGFP]* (*endu-2::EGFP* rescue line 1) animals; **c**, *endu-2(tm4977);byEx1908[endu-2p::endu-2::EGFP]* (*endu-2::EGFP* rescue line 2) animals; **d**, *endu-2(tm4977);byEx1910[endu-2p::endu-2::EGFP]* (*endu-2::EGFP* rescue line 3) animals; **e**, *endu-2(tm4977);byEx1551[vha-6p::endu-2::EGFP::3xFlag]* (intestinal *endu-2* rescue); **f**, *endu-2(tm4977);byEx1816[myo-3p::endu-2::EGFP::3xFlag]* (muscular *endu-2* rescue); **g**, *endu-2(tm4977);byEx1795[unc-119p::endu-2::EGFP::3xFlag]* (neuronal *endu-2* rescue); **h**, *endu-2(tm4977);byEx1379[fos-1p::endu-2::EGFP::3xFlag]* (somatic gonadal *endu-2* rescue). This figure is related to **Fig. 1**.



**Supplementary Fig. 3 Gene Ontology (GO terms) enrichment analysis of DEGs upon HS.**

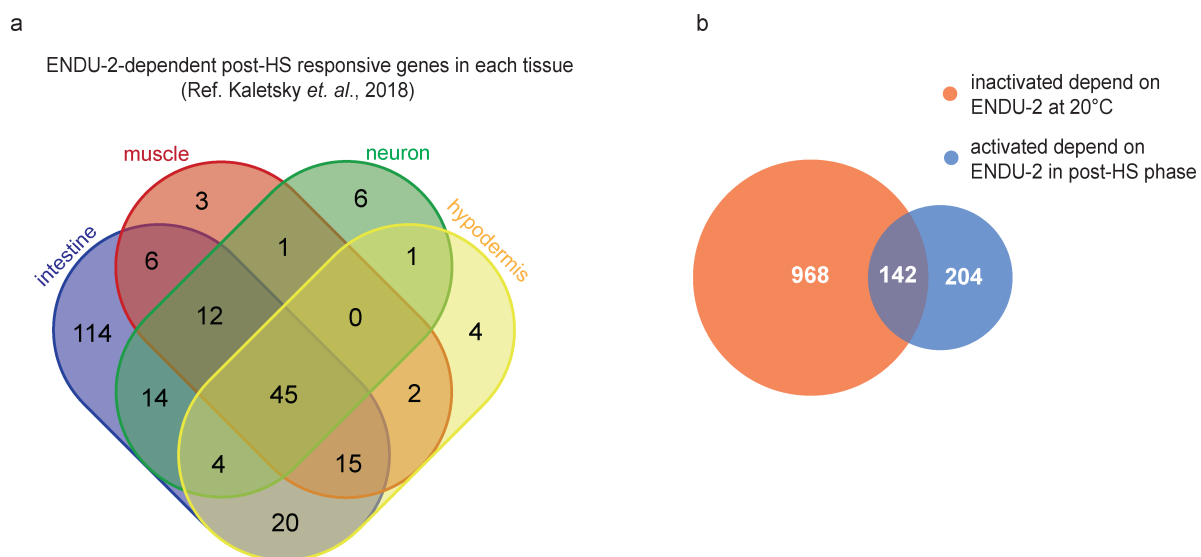


**a**, Graphic illustration of stage matched sample collection for RNA-Seq. **b**, Top 10 most enriched GO terms of the activated genes upon 1 h HS in WT animals. **c**, Top 10 most enriched GO terms of the inactivated genes upon 1 h HS in WT animals. **d**, The Venn diagram shows the overlap between post-HS responsive genes and the late-responsive genes upon continuous HS. This figure is related to **Fig. 2**.



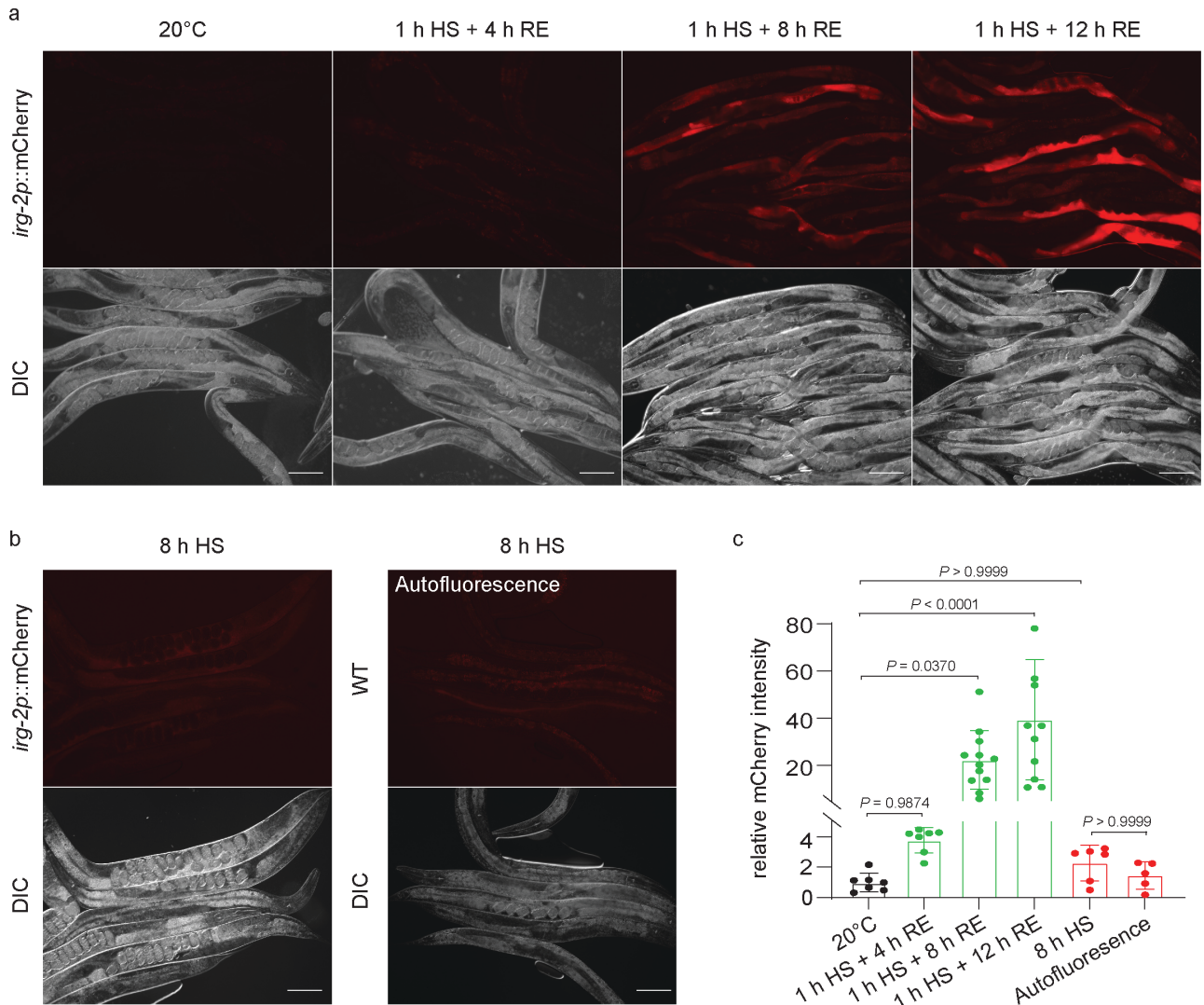
**Supplementary Fig. 4 Activation of the post-HS responsive genes contributes to the beneficial effect of hormetic heat stress.**

**a-d**, RNAi known-down of selected Class I (*hsp-16.2*), Class II (*zip-10*) and Class III (*irg-2* and *pqm-1*) post-HS responsive genes abolishes 1 h HS mediated beneficial effect against Cd<sup>2+</sup> toxicity. Shown are pooled data from *N* = 3 independent experiments for all experiments. *P* values were calculated using Log-rank (Mantel-Cox) test. **a**, Mean survival of RNAi control without HS: 4.6 days, *n* = 60. RNAi control with HS: 6.2 days, *n* = 60. *hsp-16.2* RNAi knockdown without HS: 4.8 days, *n* = 59. *hsp-16.2* RNAi knockdown with HS: 4.9 days, *n* = 61. **b**, Mean survival of RNAi control without HS: 4.9 days, *n* = 58. RNAi control with HS: 6.6 days, *n* = 60. *zip-10* RNAi knockdown without HS: 5.2 days, *n* = 29. *zip-10* RNAi knockdown with HS: 5.2 days, *n* = 60. **c**, Mean survival of RNAi control without HS: 5.1 days, *n* = 114. RNAi control with HS: 6.3 days, *n* = 113. *irg-2* RNAi knockdown without HS: 5.4 days, *n* = 112. *irg-2* RNAi knockdown with HS: 5.6 days, *n* = 120. **d**, Mean survival of RNAi control without HS: 5.2 days, *n* = 60. RNAi control with HS: 6.3 days, *n* = 58. *pqm-1* RNAi knockdown without HS: 5.1 days, *n* = 58. *pqm-1* RNAi knockdown with HS: 5.4 days, *n* = 62. **e**, Thermotolerance of animals upon RNAi known-down of selected Class I (*hsp-16.2*), Class II (*zip-10*) and Class III (*irg-2* and *pqm-1*) animals after hormetic HS. For thermotolerance after hormetic heat stress, day one adult animals were incubated at 35°C for 1 h, followed by a 12 h recovery at 20°C before exposure to 35°C for 8 h. *N* = 3 for all experiments. Data are the mean ± SEM, *P* values were calculated with two-tailed multiple unpaired t-test. This figure is related to **Fig. 2**.



**Supplementary Fig. 5 Intestine-only genes are dominant in the ENDU-2 dependent post-HS responsive genes**

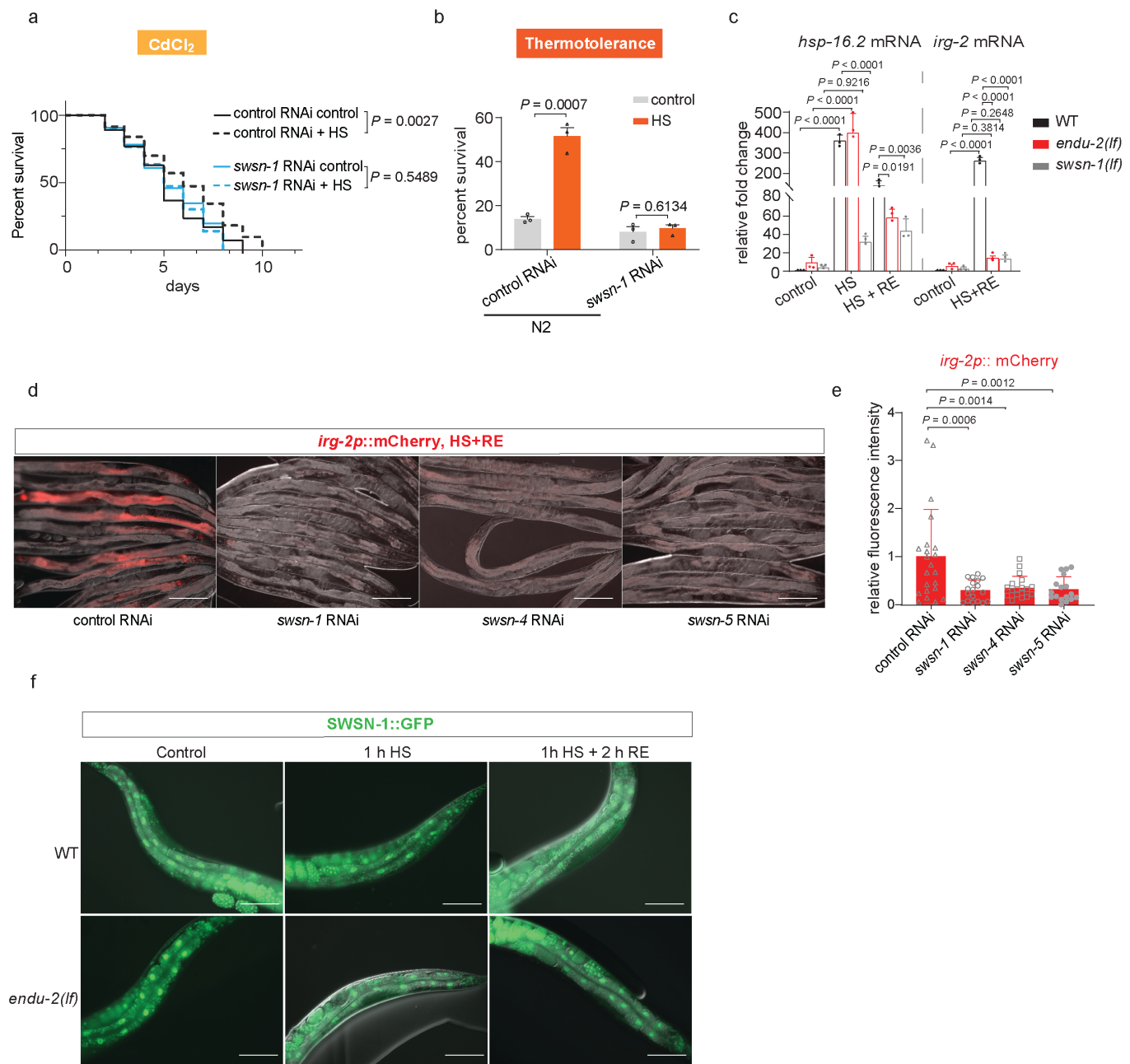
**a**, Venn diagram displaying the overlap between ENDU-2 dependent post-HS responsive genes and genes are expressed in each somatic tissue (intestine, muscle, neuron and hypodermis). **b**, Venn diagram displaying the overlap between genes inactivated by ENDU-2 at 20°C and genes activated by ENDU-2 in the post-HS phase. This figure is related to **Fig. 2**.



**Supplementary Fig. 6 Transcriptional activation of *irg-2* occurs specifically in the post-HS phase.**

**a**, Fluorescence micrographs of *byIs296[irg-2p::mCherry]* transgenic animals under indicated conditions. Scale bar = 100  $\mu$ m. **b**, Fluorescence micrographs of *byIs296[irg-2p::mCherry]* transgenic animals upon 8 h continuous HS. Fluorescence in WT N2 animals treated under the same condition serves as a negative control to exclude possible interference of gut autofluorescence in mCherry signal. Scale bar = 100  $\mu$ m. **c**, Quantification of the relative fluorescence intensity of mCherry in *byIs296[irg-2p::mCherry]* transgenic animals under the indicated conditions in the **(a)** and **(b)**. Transgenic animals at control (20°C):  $n = 7$ .

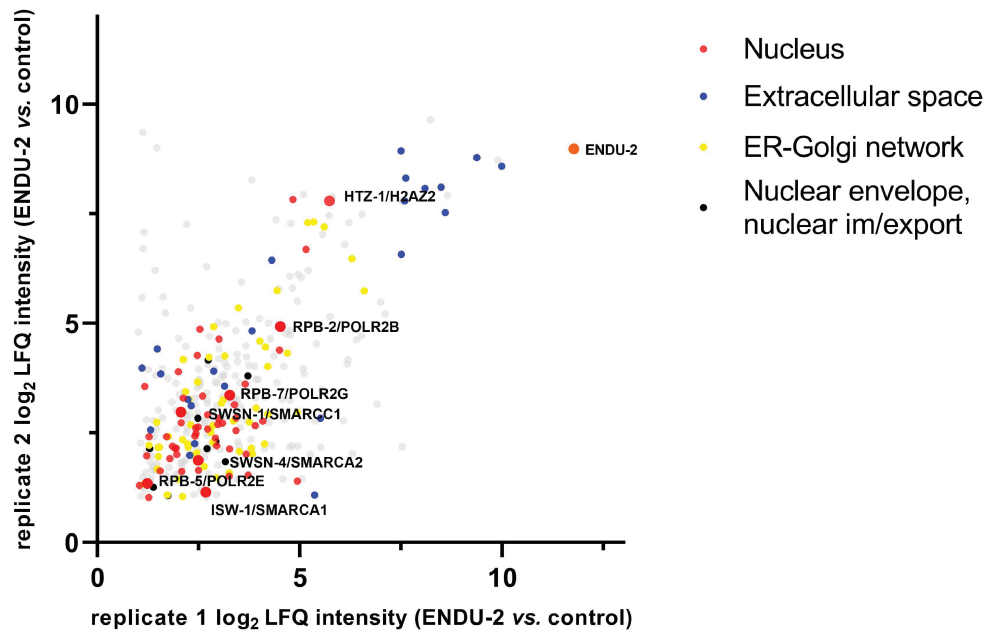
Transgenic animals with 1 h HS plus 4 h recovery:  $n = 7$ . Transgenic animals with 1 h HS plus 8 h recovery:  $n = 12$ . Transgenic animals with 1 h HS plus 12 h recovery:  $n = 10$ . Transgenic animals with 8 h HS without recovery:  $n = 6$ . WT animals with 8 h HS without recovery:  $n = 5$ . Data are the mean  $\pm$  SD,  $P$ -values were calculated using one-way ANOVA with Tukey's multiple comparisons test. This figure is related to **Fig. 3**.



**Supplementary Fig. 7 SWI/SNF nucleosome remodeling complex affects both HS and post-HS responses to mediate heat hormesis.**

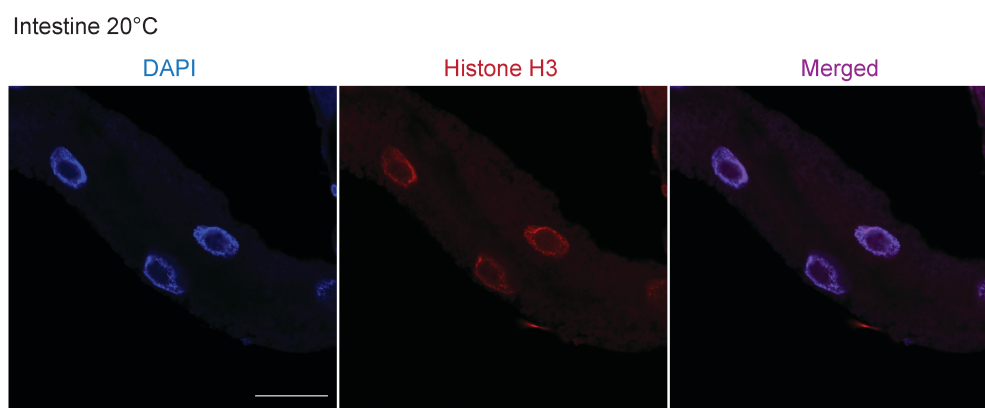
**a**, RNAi knock-down of *swn-1* abolishes hormetic HS mediated protective effect against Cd<sup>2+</sup> toxicity. Mean survival of RNAi control without HS: 5.2 days,  $n = 94$ . RNAi control with HS: 6.1 days,  $n = 93$ . *swn-1* RNAi

without HS: 5.3 days,  $n = 87$ . *swn-1* RNAi with HS: 5.2 days,  $n = 93$ . Shown are pooled data of  $N = 3$  independent experiments.  $P$  values were calculated using Log-rank (Mantel-Cox) test. **b**, RNAi knock-down of *swn-1* abolishes improved thermotolerance by hormetic HS in WT animals. For thermotolerance after hormetic heat stress, day one adult animals were incubated at 35°C for 1 h, followed by a 12 h recovery at 20°C before exposure to 35°C for 8 h. Survival rates for each group: WT with control RNAi without HS:  $(13.9 \pm 3.2) \%$ ,  $n = 92$ . WT with control RNAi with HS:  $(51.5 \pm 5.5) \%$ ,  $n = 97$ . WT with *swn-1* RNAi without HS:  $(8.1 \pm 4.2) \%$ ,  $n = 111$ . WT with *swn-1* RNAi with HS:  $(9.6 \pm 3.5) \%$ ,  $n = 107$ .  $N = 3$  for all groups. Data are the mean  $\pm$  SEM,  $P$  values were calculated with two-tailed multiple unpaired t-test. **c**, qRT-PCR quantification of relative *hsp-16.2* and *irg-2* mRNA levels of the representative post-HS responsive genes in WT, *endu-2(tm4977)* and *swn-1(os22)* animals in indicated conditions.  $N = 3$  independent experiments. Data are the mean  $\pm$  SD.  $P$ -values were calculated using two-way ANOVA with Tukey's multiple comparisons test. **d**, Fluorescence micrographs of *byIs296[irg-2p::mCherry]* transgenic animals under RNAi knock-down of *swn-1*, *swn-4* and *swn-5* in post-HS phase. Scale bar = 100  $\mu\text{m}$ . **e**, Quantification of the relative fluorescence intensity of mCherry in *byIs296[irg-2p::mCherry]* transgenic animals under the indicated condition in the **(d)**. RNAi control:  $n = 24$ . *swn-1* RNAi knock down:  $n = 18$ . *swn-4* RNAi knock down:  $n = 21$ . *swn-5* RNAi knock down:  $n = 17$ . Data are the mean  $\pm$  SD,  $P$ -values were calculated using one-way ANOVA with Tukey's multiple comparisons test. **f**, SWSN-1::GFP protein is localized in the nucleus independent of ENDU-2. Shown are fluorescence micrographs of *st12187[swn-1::TY1::EGFP::3xFLAG]* animals in WT or *endu-2(tm4977)* background. SWSN-1::GFP was detected in day 1 adult animals subjected to 1 h HS or 1 h HS followed by 2 h recovery at 20°C.  $N = 3$  independent experiments. Scale bar = 50  $\mu\text{m}$ . This figure is related to **Fig. 3**.



**Supplementary Fig. 8 Scatter Plot comparing two biological replicates of ENDU-2 MS interactor analysis**

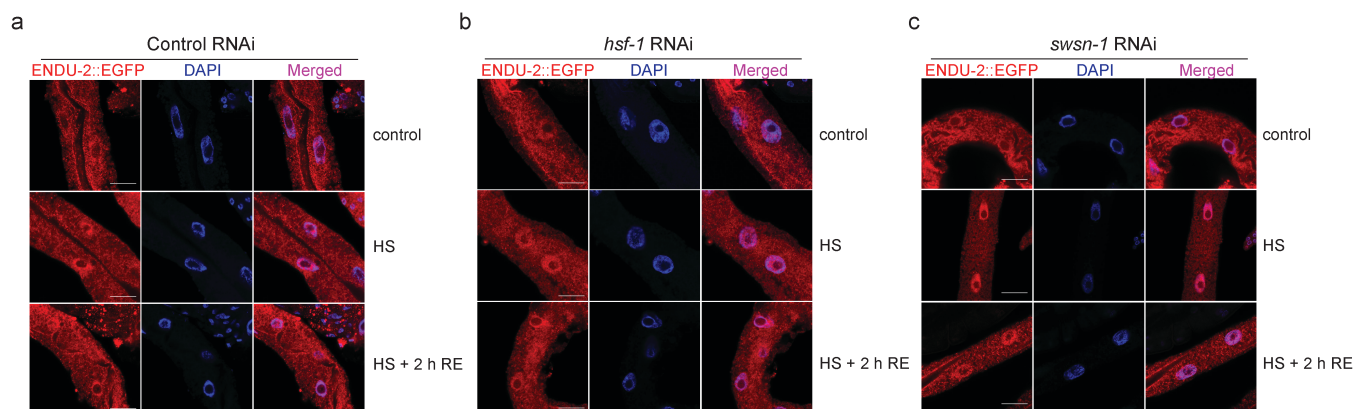
ENDU-2 (orange dot) associates with multiple proteins affecting in ER-Golgi transport (yellow dots), proteins in the extracellular matrix (blue dots), nuclear proteins (red dots) as well as factors affecting nuclear import/export (black dots) ( $\log_2\text{LFD intensity} > 1$ ). In the plot, histone protein HTZ-1, components of SWI/SNF complex: SWSN-1, SWSN-4 and ISW-1, and three subunits of RNA polymerase II (Pol II) (RPB-2, RPB-5 and RPB-7) are highlighted by larger red dots. This figure is related to **Fig. 4**.



**Supplementary Fig. 9 Histone H3 colocalizes with the DAPI-stained DNA**

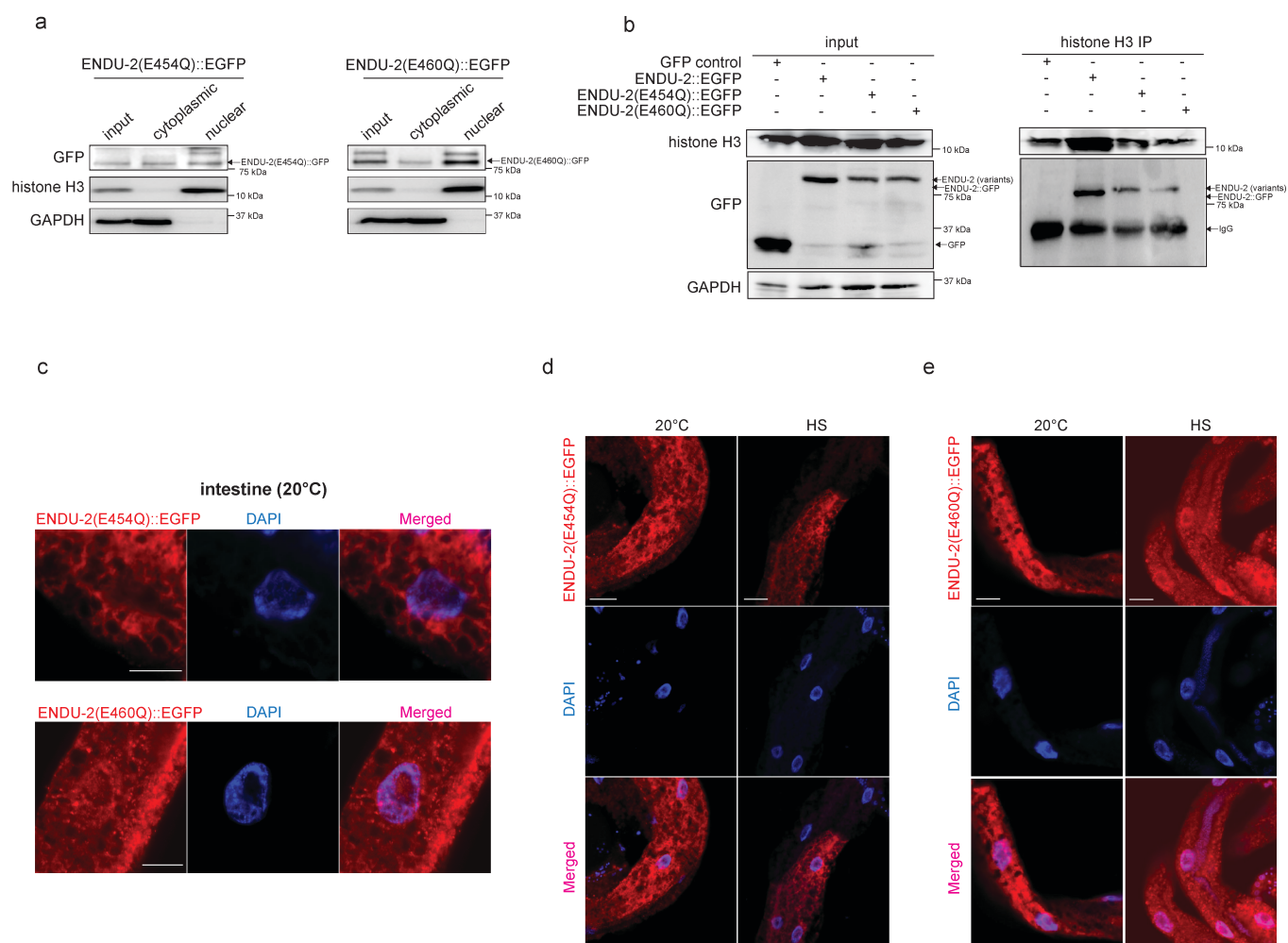
Immunofluorescence staining of histone H3 in *endu-2(by190[endu-2::EGFP])* CRISPR knock-in animals at 20°C. Scale bar = 20  $\mu\text{m}$ .  $N = 2$  independent experiments. This figure is related to **Fig. 4**.





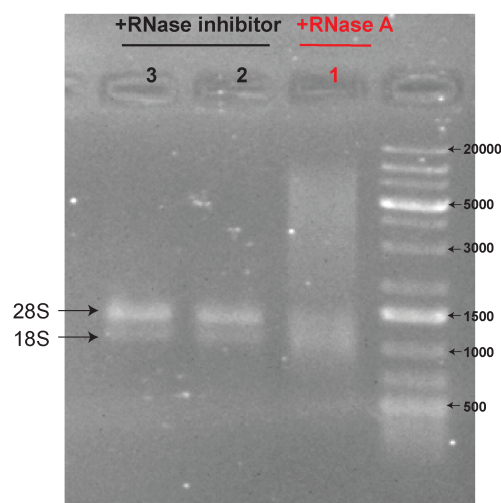
**Supplementary Fig. 10 RNAi knock-down of neither *hsf-1* nor *swsn-1* impairs chromatin localization of ENDU-2 during and after HS**

**a-c**, Immunofluorescence staining of ENDU-2::EGFP with GFP antibody in *endu-2(by190[endu-2::EGFP])* animals upon *hsf-1* or *swsn-1* RNAi and subjected to 1 h HS or 1 h HS followed by 2 h recovery. Scale bar = 20  $\mu$ m.  $n = 6$  for each condition. This figure is related to **Fig. 4**.



**Supplementary Fig. 11 ENDU-2 mutant variants show different subcellular localization**

**a**, Western Blot detection of ENDU-2(E454Q)::EGFP and ENDU-2(E460Q)::EGFP in different subcellular fractions. ENDU-2(E454Q)::EGFP and ENDU-2(E460Q)::EGFP are detected in whole cell lysis (input), cytoplasmic fraction and nuclear fraction. Histone H3 and GAPDH serve as controls for nuclear and cytoplasmic fractions, respectively.  $N = 3$  independent experiments. **b**, ENDU-2(E454Q)::EGFP and ENDU-2(E460Q)::EGFP is co-immunoprecipitated with histone H3, respectively. A transgenic *Is[sod-3p::gfp]* strain serves as a negative control to exclude the interaction between GFP and histone H3.  $N = 3$  independent experiments. **c**, Immunofluorescence staining of ENDU-2(E454Q)::EGFP and ENDU-2(E460Q)::EGFP with GFP antibody. The upper panel shows that ENDU-2(E454Q)::EGFP is diffusely distributed both in the cytoplasm and nucleus. The lower panel shows that ENDU-2 (E460Q)::EGFP is formed into puncta structures in addition to diffused distribution both in cytoplasm and nuclei. Scale bar = 10  $\mu\text{m}$ .  $n > 20$  for each group.  $N = 3$  independent experiments. **d**, HS does not significantly alter the subcellular localization of ENDU-2(E454Q)::EGFP. Shown are immunofluorescence of unstressed and 1 h HS stressed ENDU-2(E454Q)::EGFP day 1 adult animals stained with GFP antibody. Scale bar = 20  $\mu\text{m}$ .  $n = 5$  for unstressed condition,  $n = 17$  for HS. **e**, HS enhances chromatin localization of ENDU-2(E460Q)::EGFP. Shown are immunofluorescence of unstressed and 1 h heat stressed ENDU-2(E460Q)::EGFP day 1 adult animals stained with GFP antibody. Scale bar = 20  $\mu\text{m}$ .  $n = 13$  unstressed condition,  $n = 5$  for HS. This figure is related to **Fig. 5**.



**Supplementary Fig. 12 Analyzing RNA integrity after treatment with RNase A or RNase inhibitor**



1% TAE agarose gels containing 1% Sodium Hypochlorite (Carl Roth) was used for RNA electrophoresis. The absence of 28S, 18S ribosomal RNA (rRNA) bands indicates successful RNA decay with RNase A treatment (lane 1). Intact rRNA bands in the RNase inhibitors treated samples (lanes 2 and 3) indicate preservation of RNA integrity for ENDU-2/AMA-1 co-IP experiment.  $N = 3$  independent experiments. This figure is related to **Fig. 6**.