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Supplemental Information

Maintenance of Proteostasis by P Body-Mediated Regulation of eIF4E Availability during Aging in *Caenorhabditis elegans*

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SUPPLEMENTAL INFORMATION

Table S1. Lifespan Data, Related to Figures 2, 4 and 5 and to Figures S4 and S5.

Unless noted otherwise, all ageing experiments were performed on plates seeded with HT115(DE3) *E. coli* bacteria, carrying the indicated RNAi plasmid constructs (SEM: standard error of the mean; *P* values were calculated using the log-rank test, as described in Materials and Methods). Mean and max values refer to days after hatching.

Strain	Mean(±SEM) in days	Max*(±SEM) in days	Number of animals that died/total†	*P* value
Wild-type (N2)	20.23(±0.20)	29.69(±0.31)	1807/1956 (13)	
edc-3(RNAi)	22.11(±0.18)	32.67(±0.52)	1221/1332 (9)	<0.0001 #
ife-2(RNAi)	26.63(±0.26)	40.63(±0.57)	1017/1093 (8)	<0.0001 #
clk-1(e2519)	21.33(±0.88)	37.00(±1.16)	383/461 (3)	<0.0001 #
edc-3(RNAi);clk-1(e2519)	24.00(±1.00)	39.00(±1.16)	397/452 (3)	<0.0001 ∇
eat-2(ad465)	24.67(±1.20)	37.67(±1.33)	351/423 (3)	<0.0001 #
edc-3(RNAi);eat-2(ad465)	28.00(±1.00)	39.67(±1.45)	376/431 (3)	<0.0001 ∇
edc-3(RNAi);ife-2(RNAi)	22.50(±0.50)	34.50(±0.50)	282/302 (2)	<0.0001 #
edc-3(ok1427)	25.36(±0.36)	32.73(±0.54)	1507/1622 (11)	<0.0001 #
ife-2(ok306)	24.50(±0.5)	38.50(±0.50)	492/534 (4)	<0.0001 #
lsm-3(ok3635)	24.50(±0.5)	30.5(±0.5)	241/278 (2)	<0.0001
edc-3(ok1427);dcap-2(RNAi)	23.67(±0.33)	30.00(±0.00)	274/315 (3)	<0.0001 ∇
dcap-2(RNAi)	19.67(±0.33)	29.67(±0.33)	443/478 (3)	ns #
dcap-2(ok2023)	19.67(±0.33)	29.67(±0.33)	390/486 (3)	ns #
edc-3(RNAi);dcap-2(ok2023)	19.67(±0.33)	25.33(±0.33)	369/446 (3)	ns ∇
edc-3(ok1427);ife-2(ok306)	26.50(±0.50)	38.50(±0.50)	273/287 (2)	ns ∇
edc-3(RNAi);ife-2(ok306)	23.33(±0.33)	37.33(±0.67)	429/453 (3)	ns ∇

edc-3(ok1427);ife-2(RNAi)	33.86(±0.26)	47.43(±0.43)	893/967 (7)	<0.0001 ∇
N2;Ex[p*edc-3*EDC-3;pRF4]	18.00(±0.0)	26.50(±0.50)	275/302 (2)	<0.0004 #
N2;Ex[p*lsm-3*LSM-3::DsRed;pRF4]	18.50(±0.5)	25.00(±0.50)	231/293 (2)	<0.0004 #
N2;Ex[p*unc-119*EDC-3;pRF4]	18.67(±0.33)	26.67(±0.87)	425/476 (3)	<0.0004 #
N2;Ex[p*edc-3*EDC-3::DsRed;pRF4]	17.50(±0.50)	24.50(±0.5)	287/321 (2)	<0.0001 #
N2;Ex[p*edc-3*EDC-3;pRF4];*ife-2(RNAi)*	21.50(±0.50)	36.5(±0.50)	314/337(2)	<0.0001 ‡
N2;Ex[p*unc-119*EDC-3;pRF4];*ife-2(RNAi)*	26.67(±0.33)	41.00(±0.58)	426/463 (3)	ns ‡
***edc-3(ok1427);*Ex[p*edc-3*EDC-3;pRF4]**	21.67(±0.33)	29.00(±0.58)	399/443 (3)	<0.0001 ∇
***edc-3(ok1427);*Ex[p*unc-119*EDC-3;pRF4]**	21.50(±0.87)	30.00(±0.41)	534/587 (4)	<0.0001 ∇
edc-3(ok1427);*Ex[p*unc-119*EDC-3;pRF4];*ife-2(RNAi)	26.50(±0.50)	39.00(±1.00)	264/312 (2)	<0.0001 ‡
daf-16(RNAi)	19.00(±0.00)	24.33(±0.33)	392/471 (3)	<0.0001 #
edc-3(ok1427);daf-16(RNAi)	19.67(±0.33)	24.67(±0.33)	373/457 (3)	<0.0001 ∇
skn-1(RNAi)	17.33(±0.67)	21.67(±0.67)	372/412 (3)	<0.0001 #
edc-3(ok1427);skn-1(RNAi)	14.67(±0.33)	21.4(±0.38)	389/428 (3)	<0.0001 ∇ <0.0001 §
hsf-1(RNAi)	15.00(±0.00)	20.33(±0.68)	260/287 (2)	<0.0001 #
edc-3(ok1427);hsf-1(RNAi)	15.50(±0.50)	21.40(±0.38)	266/289 (2)	<0.0001 ∇ ns §
mev-1(kn1)	15.00(±0.58)	22.33(±1.20)	321/458 (3)	<0.0001 #
edc-3(RNAi);mev-1(kn1)	18.00(±0.58)	25.00(±0.58)	383/443 (3)	<0.0001 ∇
gas-1(fc21)	16.50(±0.50)	27.50(±0.50)	274/332 (2)	<0.0001 #
edc-3(RNAi);gas-1(fc21)	19.00(±0.00)	33.50(±0.50)	291/342 (2)	<0.0001 ∇
N2^{++}	20.00(±0.00)	27.67(±0.33)	412/441 (3)	ns ¶
***skn-1(RNAi)*$^{++}$**	16.33(±0.33)	23.67(±0.33)	369/432 (3)	ns ¶
***edc-3(RNAi)*$^{++}$**	20.67(±0.33)	27.67(±0.33)	403/439 (3)	<0.0004 ¶
***edc-3(ok1427)*$^{++}$**	21.33(±0.33)	28.67(±0.33)	412/435 (3)	<0.0001 ¶
***edc-3(ok1427);skn-1(RNAi)*$^{++}$**	18.50(±0.29)	25.67(±0.33)	383/429 (3)	<0.0001 ¶, ¥
N2**	15.67(±0.33)	22.33(±0.67)	358/397 (3)	

edc-3(RNAi)**	17.00(±0.58)	22.33(±0.67)	379/423 (3)	<0.0004 #
edc-3(ok1427)**	21.33(±0.33)	28.67(±0.33)	389/428 (3)	<0.0001 #

*Maximum lifespan shown is the median lifespan of the longest-lived 10% of the animals assayed.

†The total number of animals included in lifespan assays equals the number of animals that died plus the number of animals that were censored (see Materials and Methods). The number of independent lifespan assays for each strain is shown in parentheses. The least number of animals followed for any strain or condition tested was 140.

++Aging experiment performed on plates containing NAC.

#Compared to wild-type animals subjected to control RNAi, assayed at the same temperature.

▽Compared to the corresponding mutant subjected to control RNAi.

§Compared to N2 grown on the same RNAi.

‡Compared to the corresponding mutant subjected to *ife-2(RNAi)*.

¶Compared to animals of the same genetic background, but not treated with NAC.

¥Compared to *edc-3(ok1427)* grown under standard conditions.

**Aging experiment performed at 25°C.

ns: no significant difference compared to control (P>0.05).

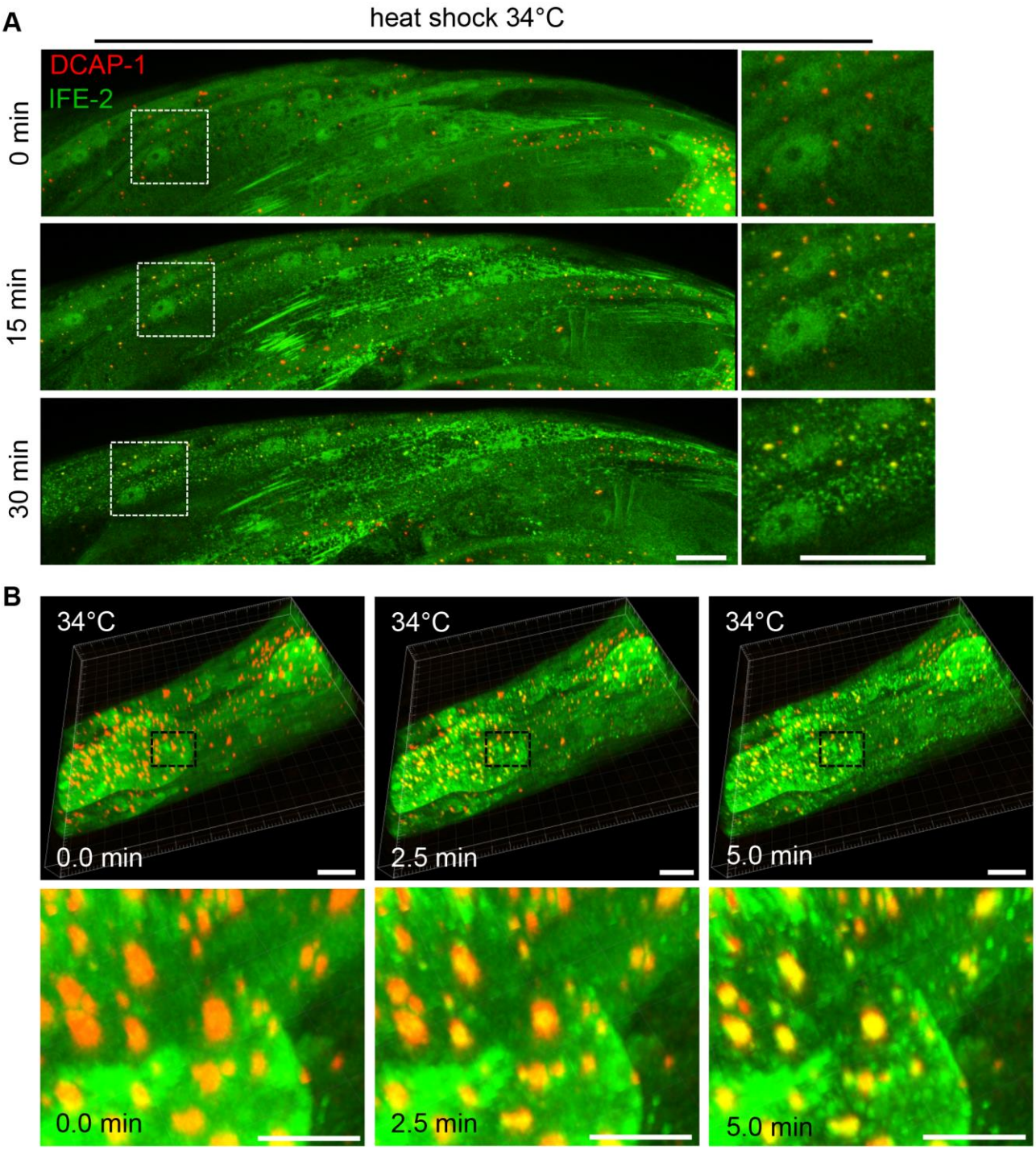


Figure S1. PBs rapidly colocalize with IFE-2 upon heat shock, Related to Figure 1

(**A**) Representative time-lapse images of animals co-expressing $p_{ife\text{-}2}$IFE-2::GFP and $p_{dcap\text{-}1}$DCAP-1::DsRed during heat shock at 34°C (see also Supplementary Movie 1). Scale bars, 20μm.

(**B**) Representative time-lapse images of the pharynx area of animals co-expressing $p_{ife\text{-}2}$IFE-2::GFP and $p_{dcap\text{-}1}$DCAP-1::DsRed during heat shock at 34°C (see also Supplementary Movie 2). Scale bars, 20μm.

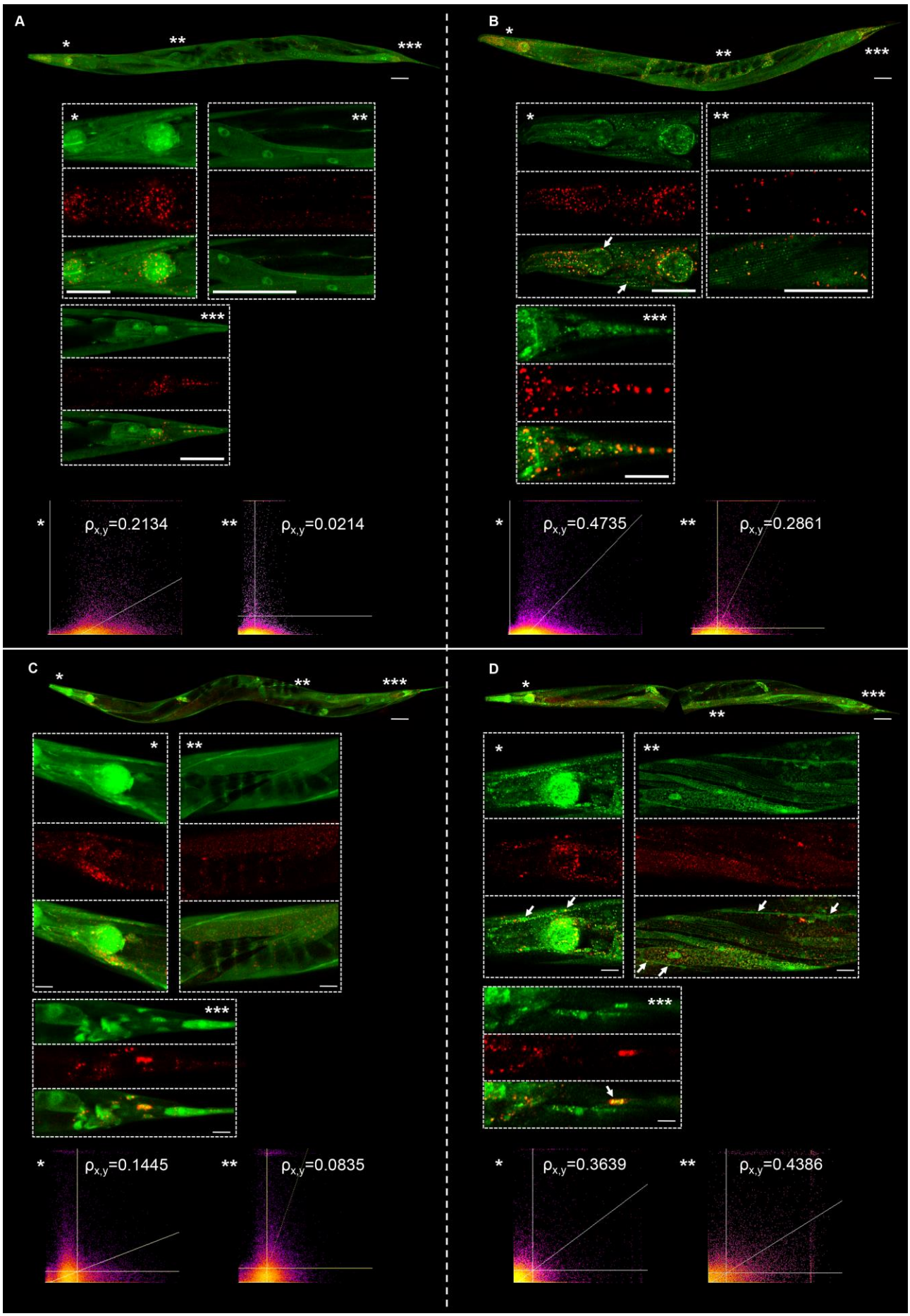

A
*
**
***
$\rho_{x,y}$=0.2134
$\rho_{x,y}$=0.0214
B
$\rho_{x,y}$=0.4735
$\rho_{x,y}$=0.2861
C
$\rho_{x,y}$=0.1445
$\rho_{x,y}$=0.0835
D
$\rho_{x,y}$=0.3639
$\rho_{x,y}$=0.4386

Figure S2. The PB-specific components DCAP-1 and EDC-3 colocalize with IFE-2 upon heat shock, Related to Figure 1

(**A**) and (**B**) Merged maximum intensity projections of confocal images of animals co-expressing p_{dcap-1}DCAP-1::DsRed and p_{ife-2}IFE-2::GFP at day 3 of adulthood. Representative images of (**A**) animals prior to heat shock and (**B**) after 1.5 hour of heat shock at 35^{o}C. The inlays show three confocal z stacks summarized in one image depicting IFE-2::GFP, DCAP-1::DsRed, and merged expression patterns. Higher magnification confocal view of the pharyngeal region (*), muscle and intestinal cells around the midsection of the animal (**) and the tail region (***). Scale bars, 50μm. White arrows indicate possible docking between PBs and SGs. Quantification of IFE-2 and DCAP-1 colocalization of ROIs within the head (*) and midsection (**) is summarized in 2D histograms and the Pearson's correlation coefficient $\rho_{x,y}$.

(**C**) and (**D**) Merged maximum intensity projections of confocal images of animals co-expressing the p_{edc-3}EDC-3::DsRed and p_{ife-2}IFE-2::GFP transgenes at day 3 of adulthood. Scale bar, 50μm. (**C**) A transgenic animal before heat shock. (**D**) An animal after 1.5 hour heat shock at 35^{o}C. Three z confocal stacks are summarized in images showing IFE-2::GFP or EDC-3::DsRed, and merged channels of selected regions, including the head region around the posterior bulb (*), muscle cells, the intestine and part of axon of the ventral nerve cord (VNC) around the midsection of the animal (**) and the tail region of the animal (***). Scale bars in the inlays, 20μm. White arrows indicate neuronal colocalization of PBs and SGs. Quantification of IFE-2 and EDC-3 colocalization of ROIs within the head (*) and midsection (**) is summarized in 2D histograms and the Pearson's correlation coefficient $\rho_{x,y}$.

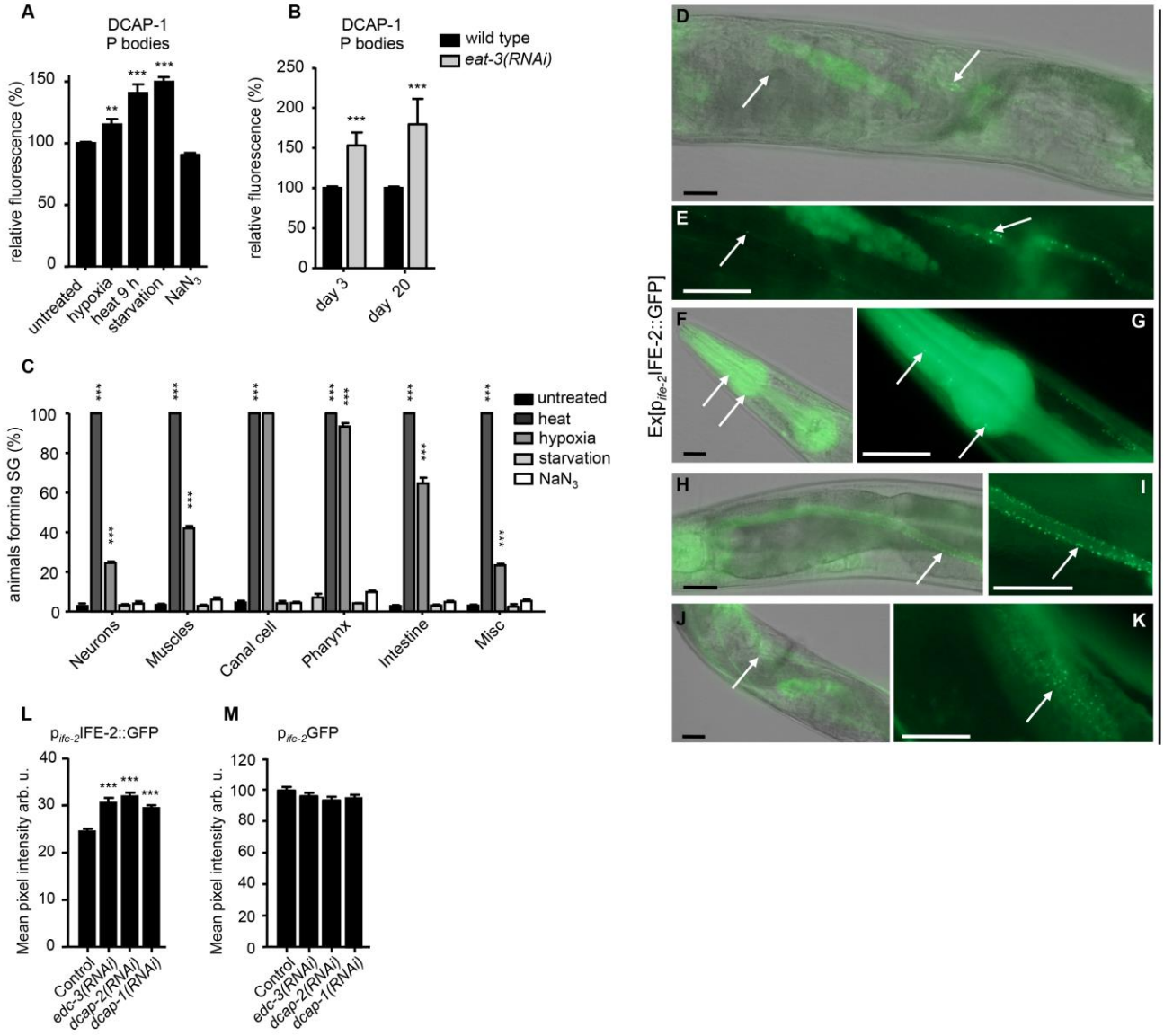
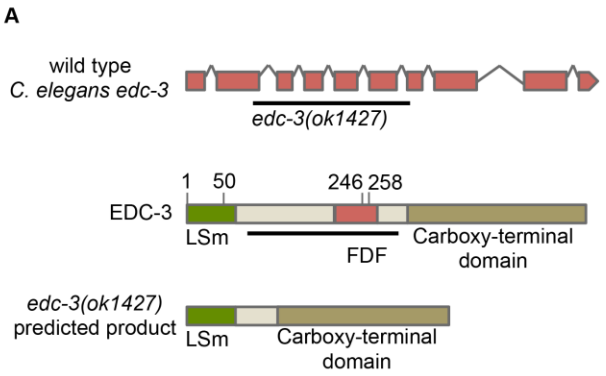
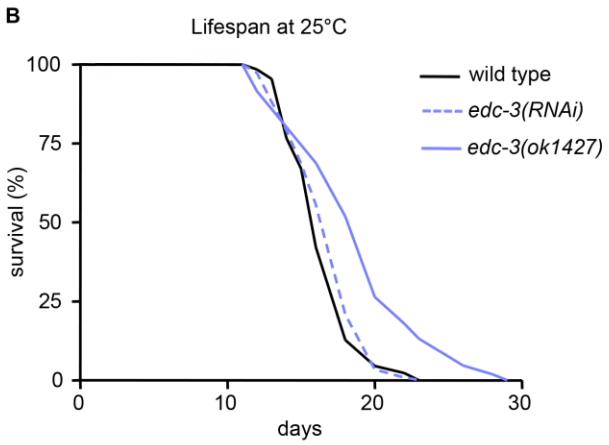


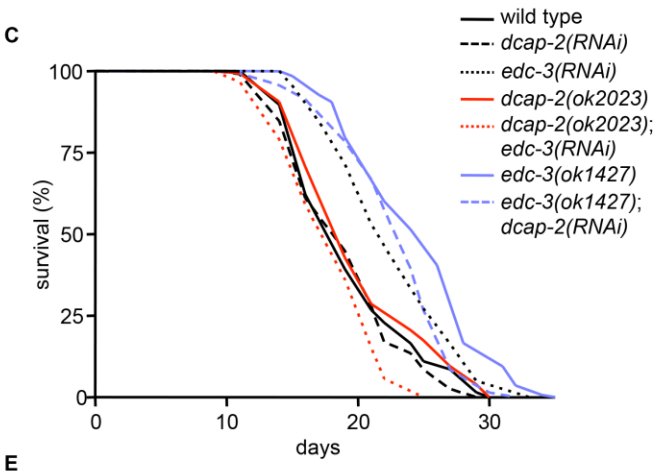
Figure S3. PBs and SGs accumulate upon stress and during ageing in *C. elegans,* Related to Figure 1

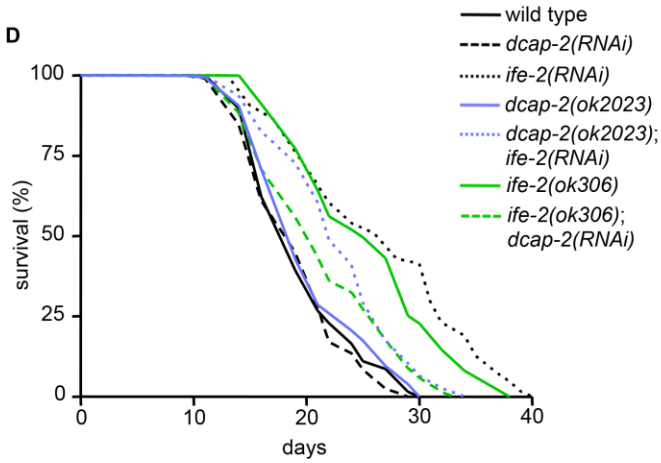
(**A**) Diverse stress insults influence PB abundance. DCAP-1::DsRed relative fluorescence levels in animals under normal conditions and after 6h exposure to low oxygen concentrations in a sealed hypoxia chamber, 9h exposure to 35°C, 24h deprivation of food and 30 min incubation with 20μM sodium azide (NaN_3). Fluorescence intensity is normalized to mean pixel intensity of p_{dcap-1}DCAP-1::DsRed at day 2 of adulthood. (Error bars denote SEM; n=40 animals per assay; **P<0.01, ***P<0.001, one-way ANOVA). (**B**) RNAi-mediated knockdown of *eat-3* markedly increases PB formation in 3-day- and 20-day-old adults compared to control animals. (Error bars represent SEM; n=40 animals per trial; ***p<0.0001, unpaired *t*-test, wild-type vs. *eat-3(RNAi)*). (**C**) Percentage of animals (day 1 of adulthood) forming stress granules under heat stress, hypoxia, starvation or treatment with sodium azide. Stress granules are quantified in neurons, muscles, the canal cell, the pharynx, the intestine and other tissues (Error bars denote SEM; n>50 animals per assay; *p<0.01, **p<0.001, ***p<0.0001, unpaired *t* -test to measure statistical significance between stress-induced

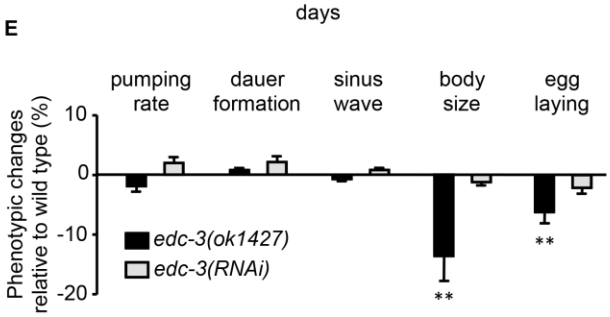
animals versus a non-treated control in the respective tissue). (**D**-**K**) SGs in animals expressing the p_{ife-2}IFE-2::GFP transgene at day 8 of adulthood, grown under physiological conditions. Images were acquired at an epifluorescence microscope using an x40 objective lens. Scale bars, 20μm. Representative images of animals displaying SGs (**D** and **E**) in neurons, such as the ventral nerve cord (VNC) or (**F** and **G**) in the pharynx, (**H** and **I**) in the canal cell and (**J** and **K**) in muscle tissue close to the mid-section of the animal. (**L**) Downregulation of the mRNA decapping components DCAP-1, DCAP-2 or EDC-3 increases IFE-2::GFP levels (Error bars show SEM; n=40 animals per experiment; ***p<0.0001, unpaired *t*-test, compared to control RNAi). (**M**) GFP expression of an *ife-2* transcriptional reporter is not affected by knockdown of *dcap-1*, *dcap-2* or *edc-3* (Error bars show SEM; n=40 animals per experiment; ^{ns}p>0.05, unpaired *t*-test, compared to control RNAi).

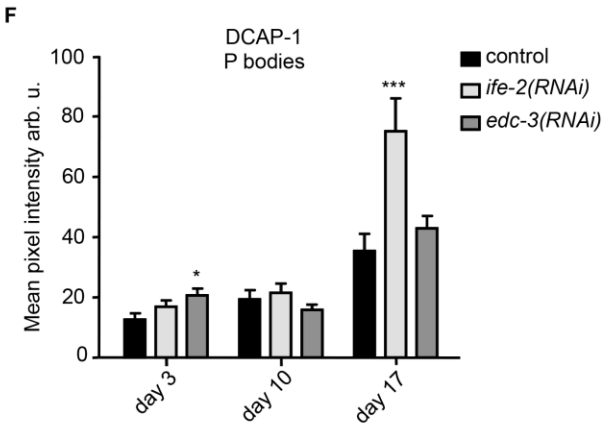

A
wild type
C. elegans edc-3
edc-3(ok1427)
1 50 246 258
EDC-3
LSm
FDF
Carboxy-terminal domain
edc-3(ok1427)
predicted product
LSm
Carboxy-terminal domain


B
Lifespan at 25°C
survival (%)
days
wild type
edc-3(RNAi)
edc-3(ok1427)


C
survival (%)
days
wild type
dcap-2(RNAi)
edc-3(RNAi)
dcap-2(ok2023)
dcap-2(ok2023); edc-3(RNAi)
edc-3(ok1427)
edc-3(ok1427); dcap-2(RNAi)


D
survival (%)
days
wild type
dcap-2(RNAi)
ife-2(RNAi)
dcap-2(ok2023)
dcap-2(ok2023); ife-2(RNAi)
ife-2(ok306)
ife-2(ok306); dcap-2(RNAi)


E
pumping rate
dauer formation
sinus wave
body size
egg laying
Phenotypic changes relative to wild type (%)
edc-3(ok1427)
edc-3(RNAi)
**
**


F
DCAP-1
P bodies
Mean pixel intensity arb. u.
control
ife-2(RNAi)
edc-3(RNAi)
*
***
day 3
day 10
day 17

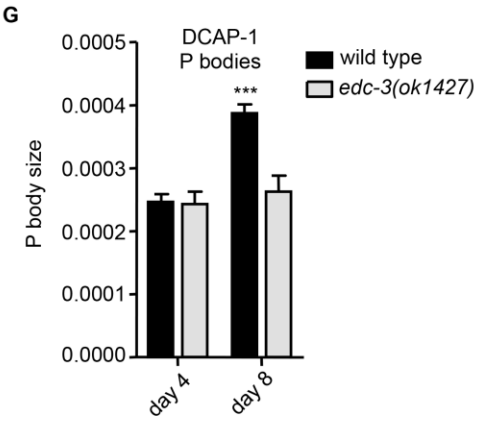

G
DCAP-1
P bodies
P body size
wild type
edc-3(ok1427)
***
day 4
day 8

Figure S4. Components of the mRNA decapping complex regulate longevity in *C. elegans,* Related to Figures 2 and 3

(**A**) Schematic representation of the *C. elegans edc-3* locus and EDC-3 protein. The extent of the gene and protein lesions in animals harboring the *edc-3*(*ok1427)* allele is indicated by a black bar. The structure of EDC-3 is inferred by sequence alignment with homologous proteins. EDC-3 contains an LSm (like-Sm) domain, an FDF (FDF motif, residues 247-249) domain and a carboxy-terminal domain. The predicted deletion product of the *ok1427* allele is also shown.

(**B**) Survival of *edc-3* deletion mutants is also increased at 25^{o}C. The percentage of animals remaining alive is plotted against age.

(**C**) DCAP-2 and EDC-3 genetically interact to modulate longevity. Downregulation of the *edc-3* gene slightly shortens the lifespan of animals carrying the *dcap-2(ok2023)* allele. Vice versa, *dcap-2* knockdown slightly but significantly shortens the lifespan of *edc-3(ok1427)* mutants. All lifespan assays were carried out at 20°C. The percentage of animals remaining alive is plotted against age.

(**D**) RNAi-mediated downregulation of *ife-2* extends *dcap-2(ok2023)* lifespan, while knocking down of *dcap-2* significantly decreases the lifespan of animals harboring the *ife-2(ok306)* allele.

(**E**-**G**) Phenotypic and PB-related changes upon loss of EDC-3.

(**E**) Behavioral or anatomical changes of animals subjected to *edc-3* RNAi or *edc-3* mutant worms in comparison with wild-type animals. Pharyngeal pumps per minute, percent dauer formation upon starvation, variance in locomotion of sinusoidal wave, body length of gravid adults, and egg laying rate within 4 hours, at day 1 of adulthood.

(**F**) PB-specific fluorescence signal robustly increases during ageing (day 17) upon *ife-2* knockdown. Downregulation of *edc-3* by RNAi results in a slight but significant increase of DCAP-1::DsRed intensity at day 3 of adulthood (Error bars denote SEM; n=10 animals per trial; *p<0.01, ***p<0.0001, unpaired *t*-test).

(**G**) The size of DCAP-1::DsRed-tagged PBs is decreased in *edc-3* mutants compared to wild-type animals at older age. (Error bars denote SEM; n=25 animals per trial; ***p<0.0001, unpaired *t*-test).

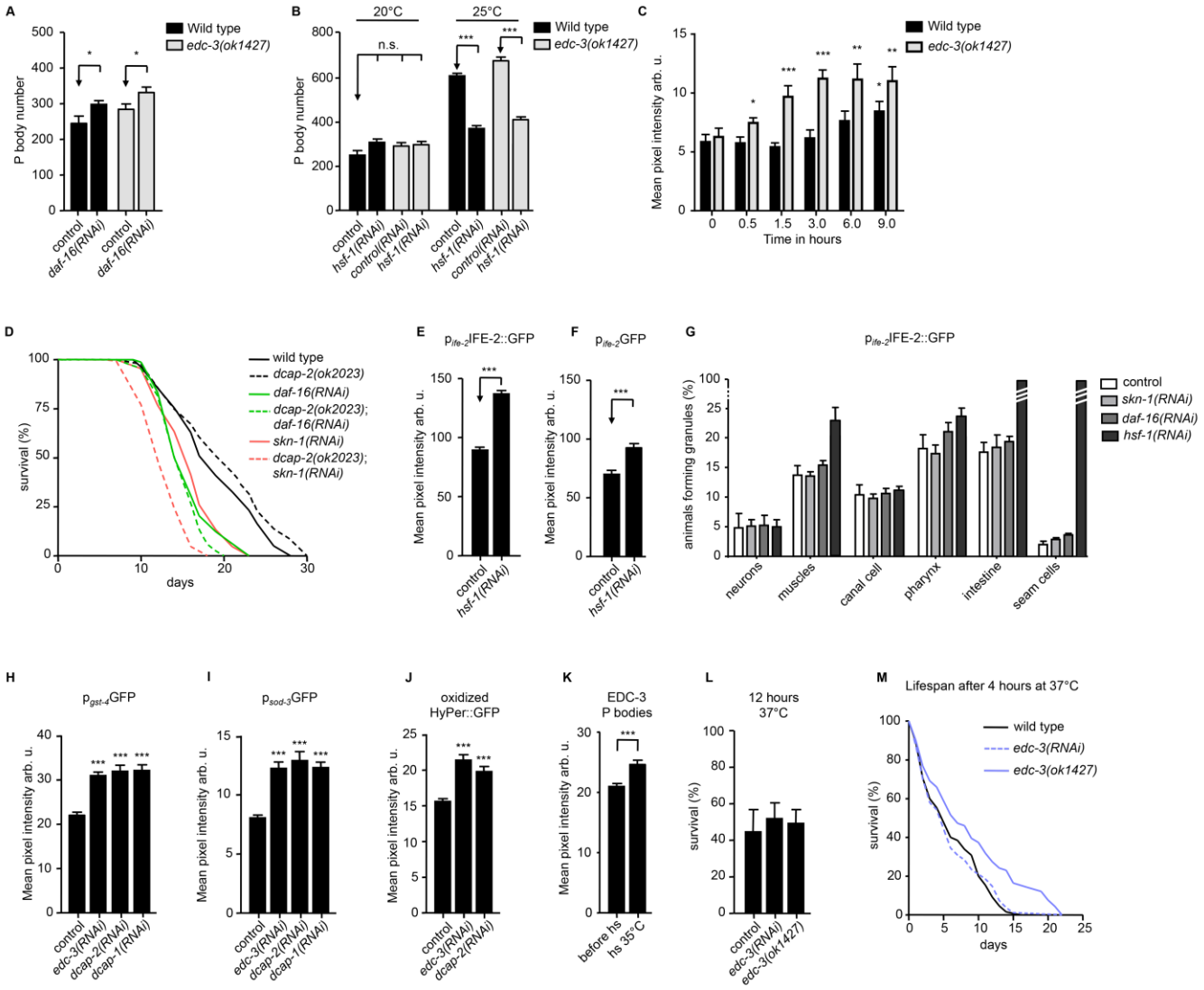


Figure S5. Key transcription regulators influence PB accumulation, Related to Figure 4

(**A**) PB quantification in whole animals expressing p_{dcap-1}DCAP-1::DsRed upon downregulation of *daf-16*.

(**B**) PB number in wild-type and *edc-3* mutant animals expressing the DCAP-1::DsRed fusion upon downregulation of *hsf-1* at 20°C or 25°C (Error bars represent SEM; n=25 per experiment; ^{ns}p>0.05, one way ANOVA).

(**C**) Time lapse study monitoring PB-intensities in wild-type and *edc-3* mutant strains expressing p_{dcap-1}DCAP-1::DsRed upon constant heat exposure of 1-day–old animals at 35°C (n=20 animals; *p<0.05, **p<0.005, ***p<0.0005, unpaired *t*- test in reference to the untreated control to evaluate significance within the wild-type, and the *edc-3* mutant compared to the time-matched control).

(**D**) Downregulation of *daf-16* shortens the lifespan of *dcap-2(ok2023)* mutants to the same extent as in wild-type animals, while *skn-1* knockdown shortens *dcap-2* mutant lifespan below control levels.

(**E**-**G**) HSF-1 transcription factor modulates PB accumulation (**E**) Knockdown of *hsf-1* increases the levels of IFE-2::GFP fusion protein at day 8 of adulthood. (Error bars denote SEM; n=40 animals per experiment;

***p<0.0001, unpaired *t*- test). (**F**) Knockdown of *hsf-1* increases the expression of p_{ife-2}GFP at day 8 of adulthood. (Error bars show SEM; n=40 animals per experiment; ***p<0.0001, unpaired *t*- test). (**G**) Knockdown of *hsf-1*, but not *daf-16* or *skn-1*, promotes SG formation at day 8 of adulthood. (**H**- **M**) Loss of PB components induces stress response genes and increases heat stress resistance (**H**) Knockdown of the PB-specific genes *dcap-1*, *dcap-2* and *edc-3* induces expression of the p_{gst-4}GFP reporter (Error bars show SEM; n=40 animals per experiment; ***p<0.0001, unpaired *t*- test). (**I**) Expression of the p_{sod-3}GFP reporter is significantly increased upon downregulation of *dcap-1*, *dcap-2* or *edc-3* (Error bars show SEM; n=40 animals per experiment; ***p<0.0001, unpaired t test). (**J**) Quantification of cellular H_2O_2 levels in animals harboring the HyPer::GFP reporter is shown upon downregulation of *edc-3* or *dcap-2* (Error bars denote SEM; n=40 animals per experiment; ***p<0.0001, unpaired *t*- test). (**K**) Abundance of EDC-3::DsRed fusion protein in whole animals increases after 1.5 hours of exposure to heat (35^{o}C) at day 1 of adulthood (Error bars represent SEM, n=40 animals per trial; ***p<0.0001, unpaired *t*- test). (**L**) Survival of animals after 12 hours of continuous exposure to 37°C (n=100 per trial, error bars denote SEM, unpaired *t*-test does not show significant differences). (**M**) Effect of *edc-3* downregulation or deletion on the survival of animals exposed to heat shock for 4 hours at 37°C, at day 2 of adulthood and then continuously maintained at 20^{o}C.

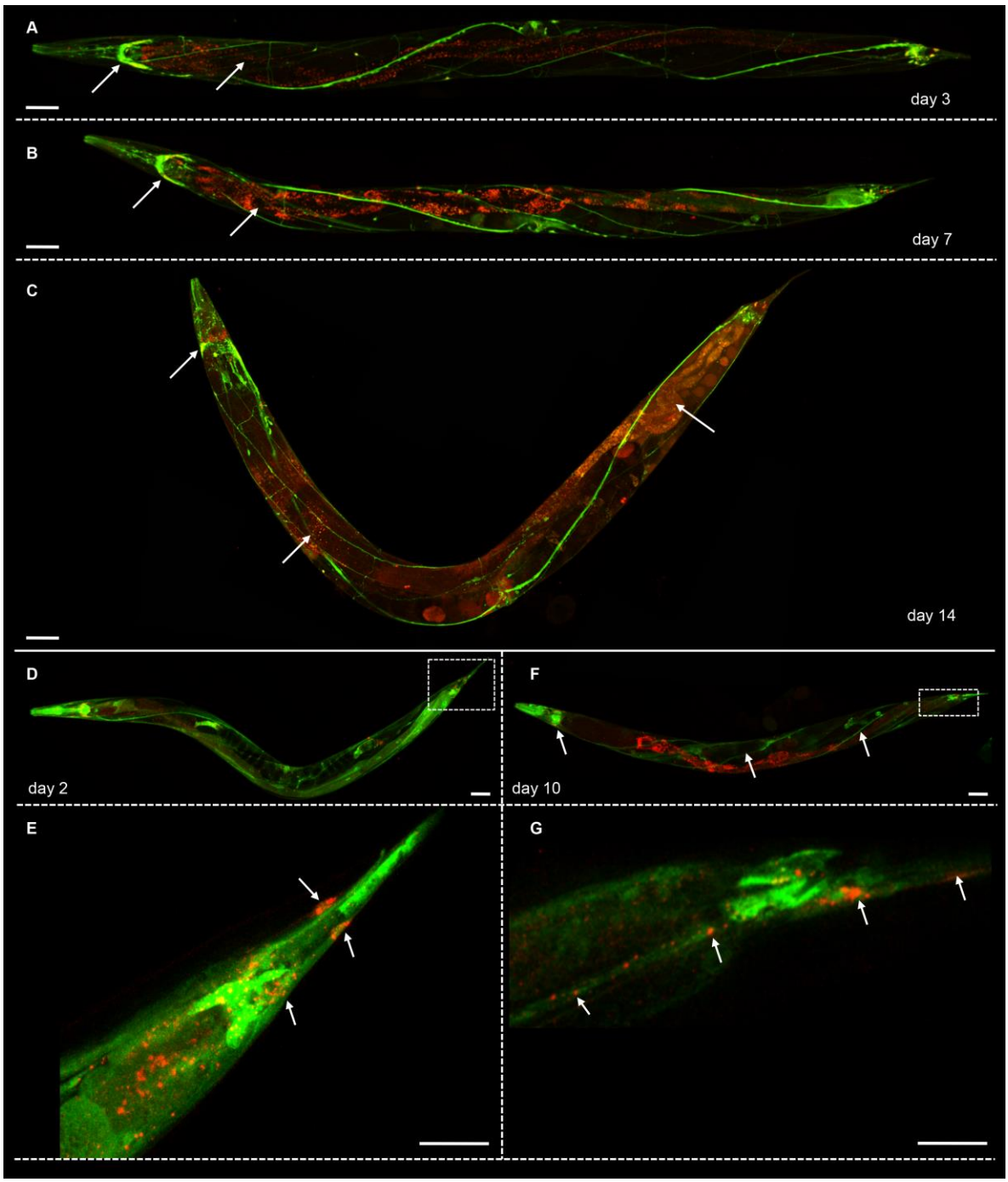


Figure S6. PBs accumulate and entrap IFE-2 in somatic tissues during ageing, Related to Figure 5.

(**A**-**C**) Merged maximum intensity projections of representative confocal images of animals co-expressing p_{edc-3}EDC-3::DsRed and $p_{unc-119}$GFP at (**A**) day 3, (**B**) day 7 and (**C**) day 14 of adulthood. PBs increasingly form in the intestine and the nervous system. Scale bars, 50μm. (**D**-**G**) IFE-2::GFP localizes to PBs during ageing. Merged maximum intensity projections of representative confocal images of animals co-expressing p_{edc-3}EDC-3::DsRed and p_{ife-2}IFE-2::GFP at (**D**) day 2 and (**F**) day 10 of adulthood. (**E**) and (**G**) Single-plane confocal images from the region of the inlays in D and F, respectively, presenting merged expression patterns of the tail area. Colocalizations along axons and in neuronal cell bodies are depicted with white arrows. Scale bars, 50μm**.**