

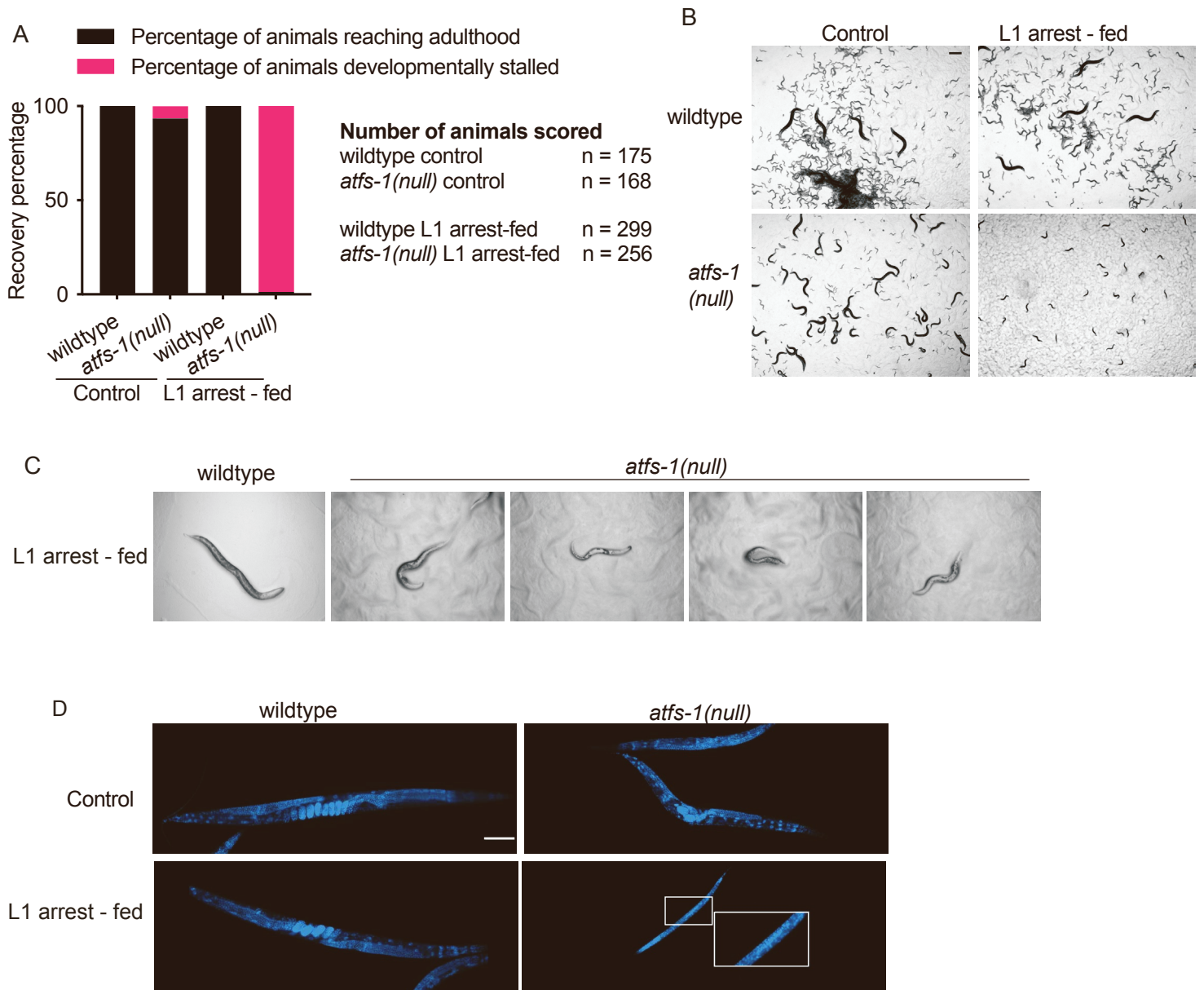
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**Supplemental information**

**Mitochondrial genome recovery by ATFS-1  
is essential for development after starvation**

**Nandhitha Uma Naresh, Sookyung Kim, Tomer Shpilka, Qiyuan Yang, Yunguang Du, and Cole M. Haynes**

**Figure S1**



**Figure S1: Upon feeding following prolonged L1 arrest, *atfs-1(null)* worms develop slower than wildtype worms and are infertile, Related to Figure 1.**

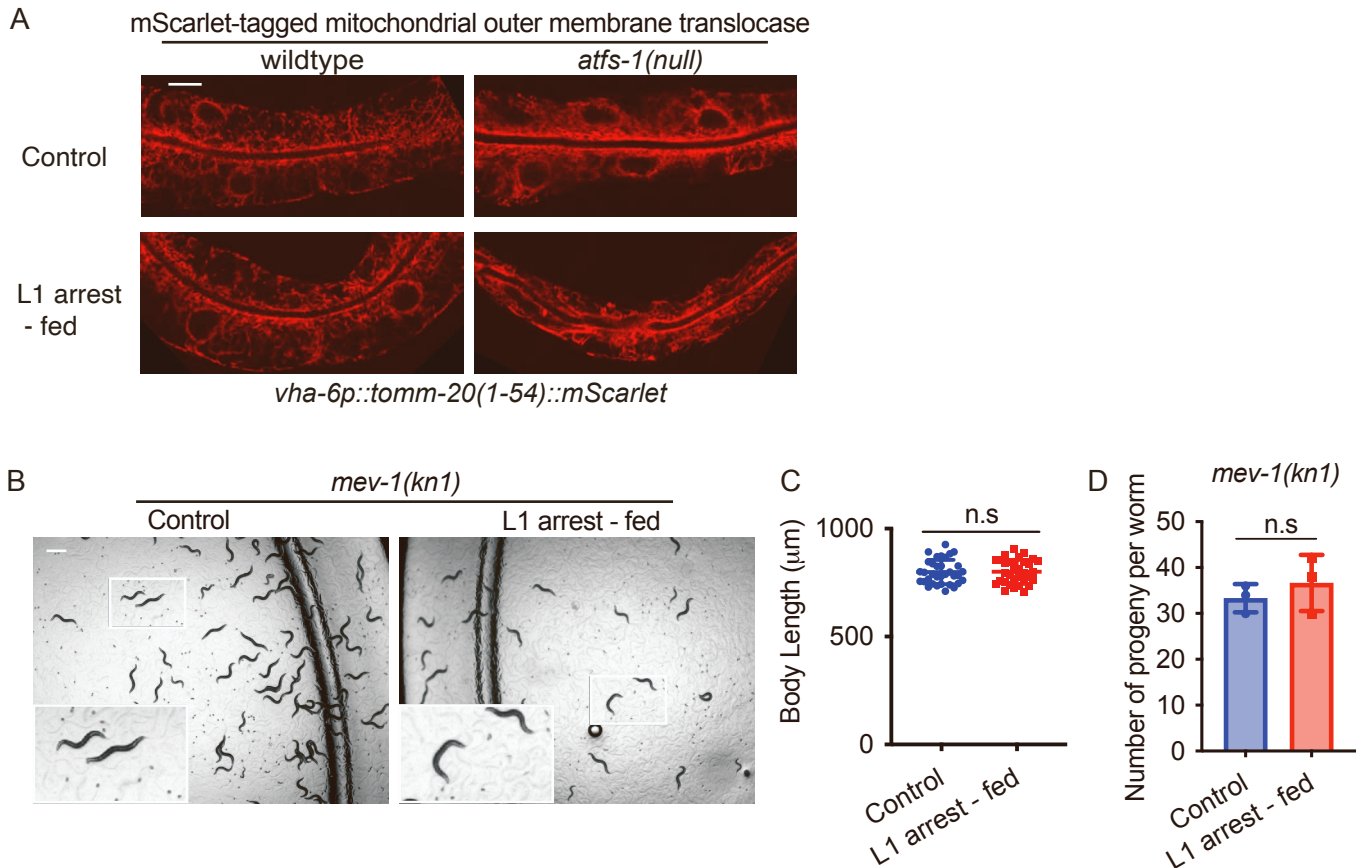
(A) Percentage of wildtype and *atfs-1(null)* mutant worms that develop into egg-laying adults following prolonged L1 arrest.

(B) Images of wildtype and *atfs-1(null)* worms in control and L1 arrest-fed conditions obtained 4 days after food was introduced (Scale bar, 0.5 mm).

(C) Images of wildtype and *atfs-1(null)* worms in L1 arrest-fed conditions obtained at the time wildtype worms reached L4 stage.

(D) Whole-worm DAPI staining images obtained from wildtype and *atfs-1(null)* in control and L1 arrest-fed conditions (Scale bar, 0.1 mm)

**Figure S2**



**Figure S2: ATFS-1 activity is required to maintain or establish mitochondrial morphology following L1 arrest, Related to Figure 2.**

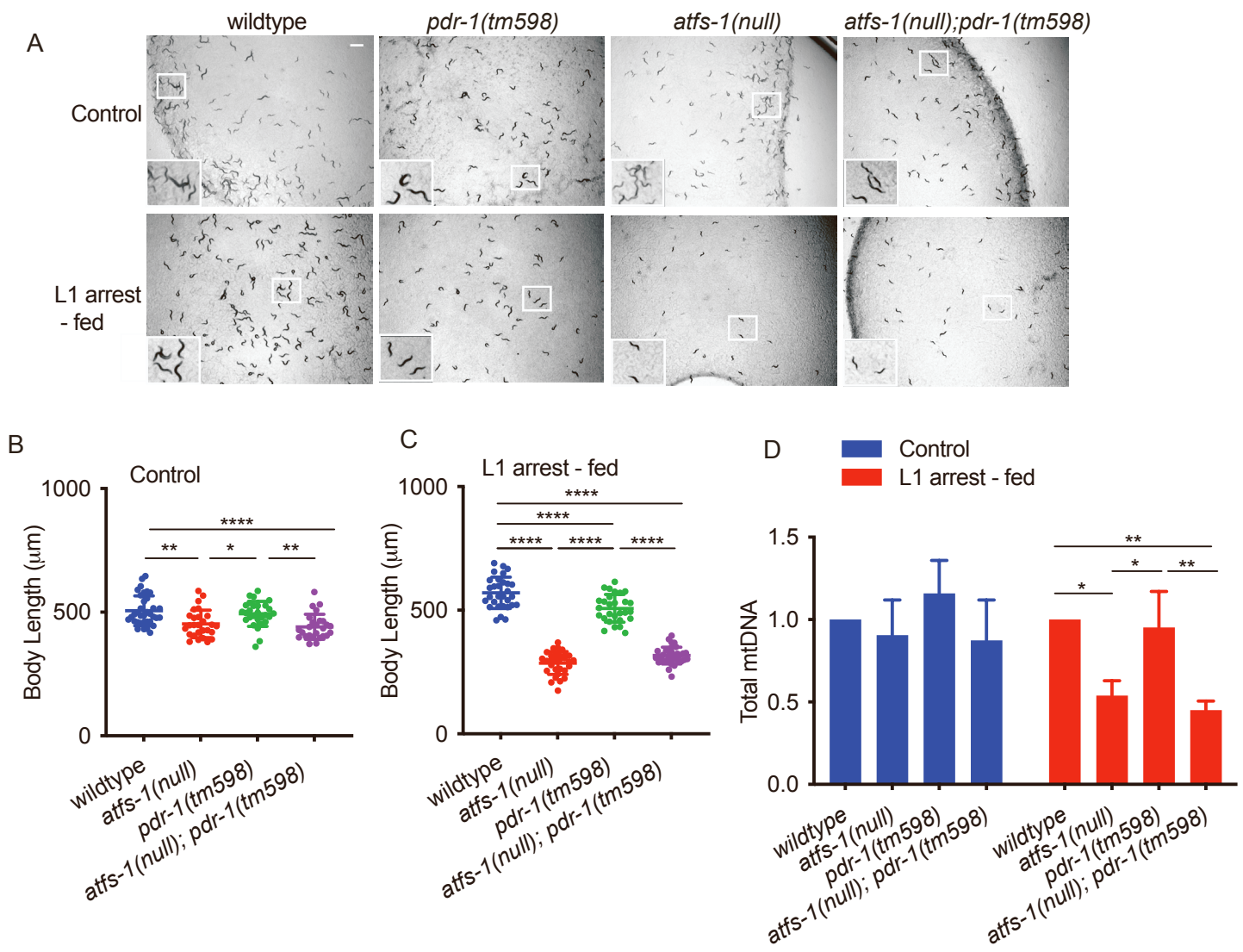
(A) Images of wildtype and *atfs-1(null)* worms carrying *vha-6p::tomm-20(1-54)::mScarlet* transgene in control and L1 arrest-fed conditions taken when control worms reached the L4 stage (Scale, 0.01 mm).

(B) Images comparing development of *mev-1(kn1)* worms in control and L1 arrest - fed conditions on day 1 of adulthood (Scale bar, 0.5 mm).

(C) Quantification of body lengths of *mev-1(kn1)* worms in control and L1 arrest - fed conditions measured on day 1 of adulthood ( $n = 3 \pm \text{SD}$ , Student's t-test).

(D) Brood size quantification of *mev-1(kn1)* worms in control and L1 arrest - fed conditions ( $n = 3 \pm \text{SD}$ , Student's t-test).

**Figure S3**



**Figure S3: Mitophagy does not impair mtDNA accumulation in *atfs-1(null)* worms following L1 arrest.**

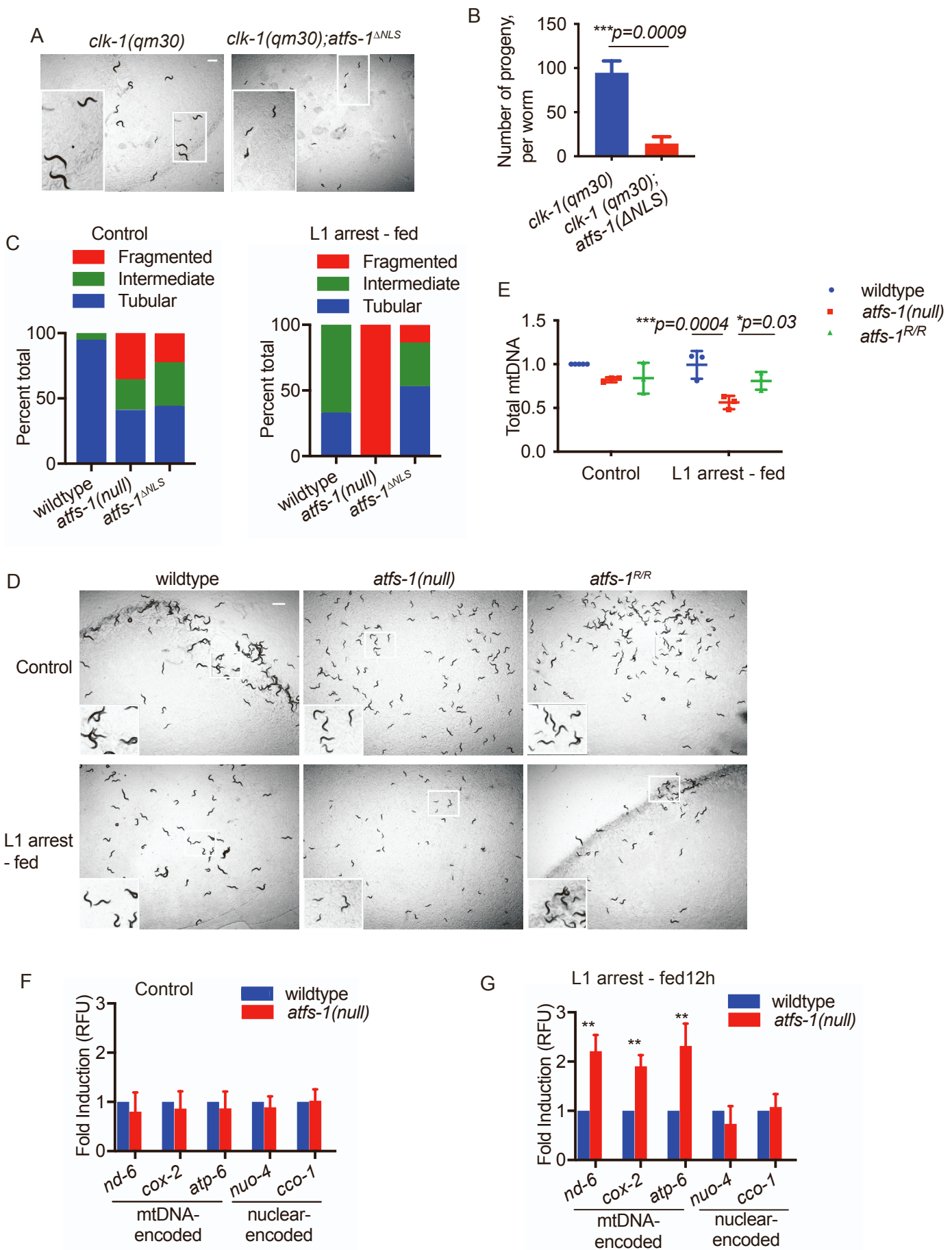
(A) Images of wildtype, *atfs-1(null)*, *pdr-1(tm598)* and *atfs-1(null);pdr-1(tm598)* worms in control and L1 arrest - fed conditions obtained when control worms reached the L4 stage (Scale bar, 0.5 mm).

(B) Quantification of body length of wildtype, *atfs-1(null)*, *pdr-1(tm598)* and *atfs-1(null);pdr-1(tm598)* in control condition when the worms L4 stage ( $n = 3 \pm \text{SD}$ , \*\*\*\* $p \leq 0.0001$ , one-way ANOVA with post-hoc Tukey's multiple comparison test).

(C) Quantification of body length of L1 arrest - fed wildtype, *atfs-1(null)*, *pdr-1(tm598)* and *atfs-1(null);pdr-1(tm598)* when the control condition worms of each strain reached the L4 stage ( $n = 3 \pm \text{SD}$ , \*\*\*\* $p \leq 0.0001$ , one-way ANOVA with post-hoc Tukey's multiple comparison test).

(D) mtDNA quantification of control and L1 arrest-fed wildtype, *atfs-1(null)*, *pdr-1(tm598)* and *atfs-1(null);pdr-1(tm598)* worms when the control condition worms of each strain reached the L4 stage ( $n = 5 \pm \text{SD}$ , two-way ANOVA with post-hoc Sidak's multiple comparison test).

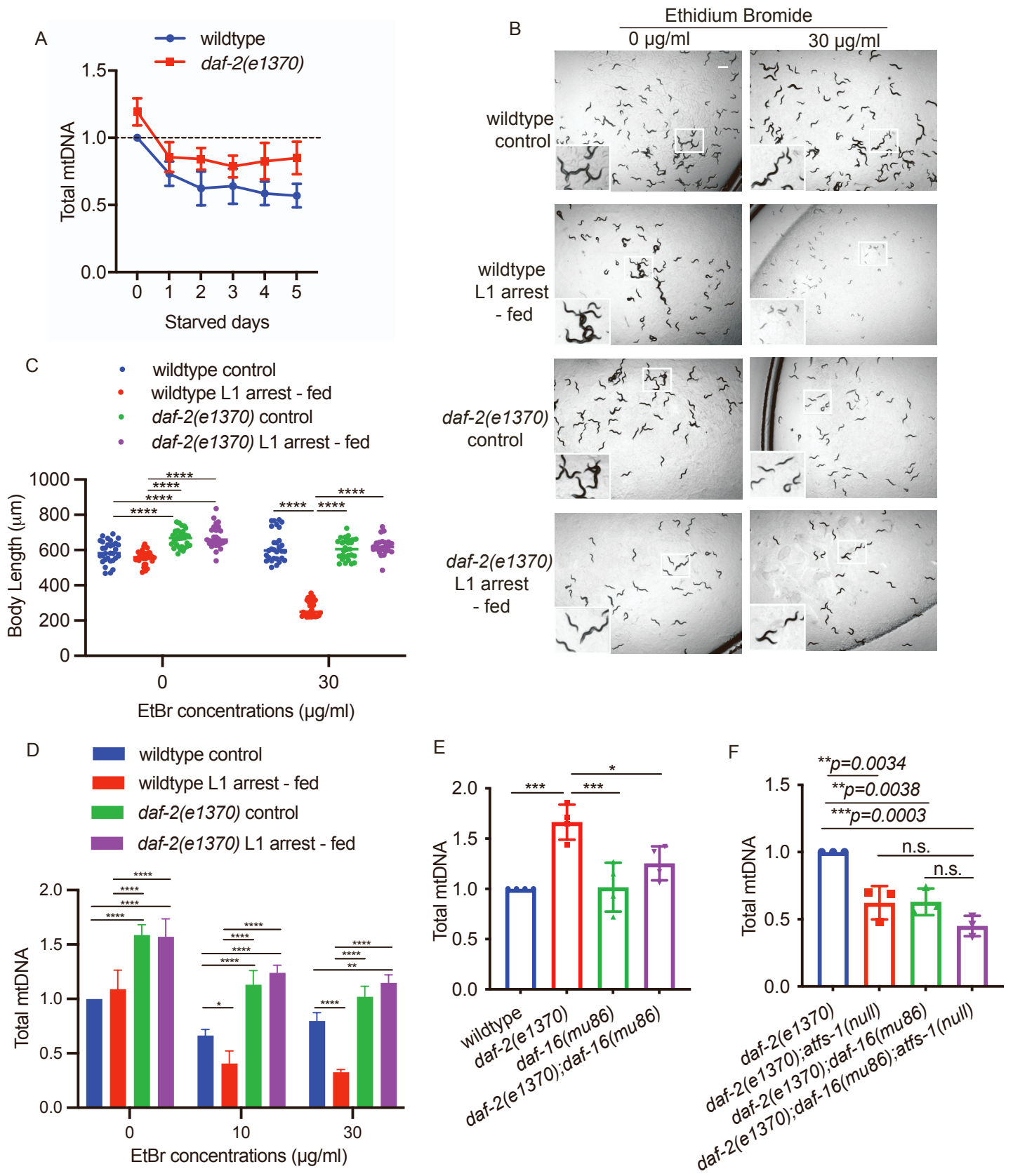
**Figure S4**



**Figure S4. Mitochondrial-localized ATFS-1 mediates growth, mitochondrial network recovery and repression of mtDNA gene transcription following prolonged L1 arrest, Related to Figure 3.**

- (A) Images comparing the size of *clk-1(qm30)* and *clk-1(qm30);atfs-1 $\Delta$ NLS* worms hatched on food obtained when the *clk-1(qm30)* reached day 1 of adulthood (Scale bar, 0.5 mm).
- (B) Brood size comparison of *clk-1* or *clk-1(qm30);atfs-1 $\Delta$ NLS* worms hatched onto food ( $n = 3 \pm \text{SD}$ ,  $***p = 0.0009$ , Student's t-test).
- (C) Quantification of TMRE staining morphology of wildtype, *atfs-1(null)* and *atfs-1 $\Delta$ NLS* worms at the L4 stage in control or L1 arrest - fed conditions ( $n = 25$  to  $30$  worms).
- (D) Images of wildtype, *atfs-1(null)* and *atfs-1 $^{RR}$*  worms in control and L1 arrest - fed conditions obtained when control worms reached L4 stage (Scale bar, 0.5 mm).
- (E) mtDNA quantification of control and L1 arrest - fed wildtype, *atfs-1(null)* and *atfs-1 $^{RR}$*  worms when the control condition worms reached the L4 stage ( $n = 3 \pm \text{SD}$ ,  $*p = 0.03$  comparing *atfs-1(null)* and *atfs-1 $^{RR}$*  worms,  $***p = 0.0004$  comparing wildtype and *atfs-1(null)* worms, two-way ANOVA with post-hoc Sidak's multiple comparison test).
- (F) Transcript levels of mtDNA- and nuclear DNA-encoded OXPHOS genes in wildtype and *atfs-1(null)* control worms 24 hours after hatching on food ( $n = 3 \pm \text{SD}$ , Student's t-test).
- (G) Transcript levels of mtDNA and nuclear DNA-encoded genes in wildtype and *atfs-1(null)* L1 arrest-fed worms at 12 hours following the introduction of food ( $n = 3 \pm \text{SD}$ ,  $**p \leq 0.01$ , Student's t-test).

**Figure S5**



**Figure S5: DAF-2 inhibition promotes mtDNA accumulation and recovery from prolonged L1 arrest, Related to Figure 4.**

- (A) mtDNA quantification of wildtype and *daf-2(e1370)* starved L1 worms over a period of 5 days ( $n = 3 \pm \text{SD}$ ,  $*p = 0.03$  on day 5 comparing wildtype and *daf-2(e1370)*, Student's t-test).
- (B) Images of wildtype and *daf-2(1370)* worms in control and L1 arrest - fed conditions exposed to 0 or 30  $\mu\text{g/ml}$  of ethidium bromide obtained when the control condition worms reached L4 stage (Scale bar, 0.5 mm).
- (C) Quantification of body lengths of wildtype and *daf-2(e1370)* worms in control and L1 arrest - fed condition exposed to 0 or 30  $\mu\text{g/ml}$  of EtBr obtained when the control condition worms reached L4 stage ( $n = 3 \pm \text{SD}$ ,  $****p \leq 0.0001$ , two-way ANOVA with post-hoc Tukey's multiple comparison test).
- (D) mtDNA quantification of wildtype, *daf-2(e1370)* in control and L1 arrest - fed condition exposed to 0, 10 or 30  $\mu\text{g/ml}$  of EtBr obtained when the control condition worms reached L4 stage ( $n = 3 \pm \text{SD}$ ,  $****p \leq 0.0001$ , two-way ANOVA with post-hoc Tukey's multiple comparison test).
- (E) mtDNA quantification of wildtype, *daf-2(e1370)*, *daf-16(mu86)*, *daf-2(e1370);daf-16(mu86)* worms at the L4 stage raised in control conditions ( $n = 4 \pm \text{SD}$ ,  $***p = 0.0004$ , one-way ANOVA with post-hoc Tukey's multiple comparison test).
- (F) mtDNA quantification of wildtype, *daf-2(e1370)*, *daf-2(e1370);daf-16(mu86)*, *daf-2(e1370);atfs-1(null)* and *daf-2(e1370);daf-16(mu86);atfs-1(null)* worms at the L4 stage raised in control conditions ( $n = 3 \pm \text{SD}$ ,  $***p \leq 0.001$ ,  $**p \leq 0.01$ , one-way ANOVA with post-hoc Tukey's multiple comparison test).



**Table S1. Data from survival assay of L1s arrested in liquid. Related to Figure 1.**

The following table shows the results obtained for survival assays performed with L1 larvae maintained in solution. The figures the data refer to are indicated in the table.  $n > 100$  worms were used at every time point in every batch.

Strain	Experiment batch no.	Showed in Figure	Temperature (°C)	Mean survival rate <sup>#</sup>	Area under the curve - Alive <sup>##</sup>
wildtype N2	1	1C	20	$35.26 \pm 0.09$	3094
wildtype N2	2	-	20	$23.82 \pm 0.14$	2116
wildtype N2	3	-	20	$33.02 \pm 0.17$	2887
<i>atfs-1(null)</i>	1	1C	20	$34.56 \pm 0.09$	2990
<i>atfs-1(null)</i>	2	-	20	$23.52 \pm 0.18$	2051
<i>atfs-1(null)</i>	3	-	20	$31.42 \pm 0.21$	2724

<sup>#</sup> Mean survival rate for each replicate was determined using OASIS2 platform (See also Methods).

<sup>##</sup> Area under the curve was calculated with GraphPad Prism.

Strain	Average mean survival rate of all replicates	<i>p</i> -value (Student's t-test between the average mean survival rates)
wildtype N2	$30.7 \pm 3.5$	NA
<i>atfs-1(null)</i>	$29.83 \pm 3.284$	0.9364 (n.s)

**Table S3. Statistics for lifespan. Related to Figure 1.**

The following table shows the results obtained for adult lifespan assay performed on plates. The figures the data refer to are indicated in the table.  $n = \text{total } 100 \text{ to } 120$  worms for each biological replicate.

Strain	Experiment batch #	Showed in Figure	Temperature (°C)	Number of worms <sup>#</sup>	Log-rank test $p$ -value
wildtype N2 vs. <i>atfs-1</i> (null)	1	1B	20	wildtype: 96/100 <i>atfs-1</i> (null): 79/100	**** $p < 0.0001$
wildtype N2 vs. <i>atfs-1</i> (null)	2	-	20	wildtype: 113/120 <i>atfs-1</i> (null): 101/120	**** $p < 0.0001$
wildtype N2 vs. <i>atfs-1</i> (null)	3	-	20	wildtype: 105/110 <i>atfs-1</i> (null): 89/110	**** $p < 0.0001$
wildtype N2 vs. <i>atfs-1</i> <sup>ΔNLS</sup>	1	1B	20	wildtype: 96/100 <i>atfs-1</i> <sup>ΔNLS</sup> :97/100	n.s
wildtype N2 vs. <i>atfs-1</i> <sup>ΔNLS</sup>	2	-	20	wildtype: 113/120 <i>atfs-1</i> <sup>ΔNLS</sup> :73/100	n.s
wildtype N2 vs. <i>atfs-1</i> <sup>ΔNLS</sup>	3	-	20	wildtype: 105/110 <i>atfs-1</i> <sup>ΔNLS</sup> :96/110	n.s

<sup>#</sup>Number of worms represents the number of dead worms scored relative to total number of worms initially started with. The difference in number is indicative of censored worms