1 **FSHR-1/GPCR activates the mitochondrial unfolded protein**

2 **response in *Caenorhabditis elegans***

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13   **Abstract**

14   The mitochondrial unfolded protein response (UPR^{mt}) is an evolutionarily conserved

15   adaptive response that functions to maintain mitochondrial homeostasis following

16   mitochondrial damage. In *C. elegans*, the nervous system plays a central role in

17   responding to mitochondrial stress by releasing endocrine signals that act upon

18   distal tissues to activate the UPR^{mt}. The mechanisms by which mitochondrial stress

19   is sensed by neurons and transmitted to distal tissues is not fully understood. Here,

20   we identify a role for the conserved follicle-stimulating hormone G protein coupled

21   receptor (GPCR), FSHR-1, in promoting UPR^{mt} activation. Genetic deficiency of *fshr-*

22   *1* severely attenuates UPR^{mt} activation and organism-wide survival in response to

23   mitochondrial stress. FSHR-1 functions in a common genetic pathway with SPHK-

24   1/sphingosine kinase to promote UPR^{mt} activation, and FSHR-1 regulates the

25   mitochondrial association of SPHK-1 in the intestine. Through tissue-specific rescue

26   assays, we show that FSHR-1 functions in neurons to activate the UPR^{mt}, to promote

27   mitochondrial association of SPHK-1 in the intestine, and to promote organism-wide

1 survival in response to mitochondrial stress. We propose that FSHR-1 functions cell

2 non-autonomously in neurons to activate UPR[mt] upstream of SPHK-1 signaling in the

3 intestine.

4

Introduction

6   The mitochondrial unfolded protein response (UPR[mt]) functions to maintain

7 mitochondrial protein homeostasis in response to mitochondrial dysfunction caused

8 by mitochondrial DNA damage, incorrect mitochondrial protein folding, or impaired

9 oxidative phosphorylation. Failure to appropriately control and maintain protein

10 homeostasis in the mitochondria is associated with the development of numerous

11 diseases, neurodegeneration, and ageing (DURIEUX *et al.* 2011; LIU *et al.* 2014;

12 PELLEGRINO *et al.* 2014; NARGUND *et al.* 2015; FIORESE *et al.* 2016; MARTINEZ *et al.*

13 2017). The UPR[mt] is initiated when mitochondrial proteostasis is disrupted, detection

14 of which by mitochondrial and cytosolic factors leads to epigenetic modifications and

15 transcriptional responses in the nucleus that restore mitochondrial function. A critical

16 sensor and activator of the UPR[mt] in *C. elegans* and in mammals is the leucine

17 zipper transcription factor ATSF-1/ATF5 (FIORESE *et al.* 2016). ATFS-1 is normally

18 targeted to mitochondria where it is degraded, but upon mitochondrial stress,

19 mitochondrial import is disrupted and ATFS-1 is targeted instead to the nucleus

20 where it regulates the expression of a cascade of genes including the conserved

21 HSP70-like chaperone, *hsp-6* which is targeted to the mitochondria to restore protein

22 folding (NARGUND *et al.* 2012). The intestinal UPR[mt] can be activated by

23 mitochondrial stress originating either cell autonomously in the intestine or cell non-

1   autonomously in the nervous system. Mitochondrial stress in neurons activates the

2   UPR^{mt} in intestine through the release of neuropeptides, serotonin and/or Wnt

3   ligands (BERENDZEN *et al.* 2016; SHAO *et al.* 2016; ZHANG *et al.* 2018).

4   Genetic screens for additional factors that activate the UPR^{mt} have revealed

5   important roles for mitochondrial ceramide produced by SPTL-1/serine

6   palmitoyltransferase and sphingolsine-1-phosphate (S1P) produced by SPHK-

7   1/sphingosine kinase in the activation of the UPR^{mt} (LIU *et al.* 2014; KIM and

8   SIEBURTH 2018a). SPHK-1 recruitment to mitochondria from cytoplasmic pools may

9   serve as an early signal to activate UPR^{mt} (KIM and SIEBURTH 2018a). SPHK-1

10   mitochondrial recruitment is positively regulated by mitochondrial stress originating

11   either from the intestine or from the nervous system. Neuronal mitochondrial stress

12   activates intestinal SPHK-1 by a mechanism that involves neuropeptide but not

13   serotonin signaling (KIM and SIEBURTH 2018a). Neuropeptides exert their biological

14   functions primarily through activating G protein coupled receptors (GPCRs) on target

15   cells to trigger downstream signaling events (FROONINCKX *et al.* 2012). However, the

16   specific neuropeptides and the GPCRs functioning in SPHK-1-mediated UPR^{mt}

17   activation have not been identified.

18   FSHR-1 is a GPCR containing extracellular Leucine Rich Repeats (LRRs)

19   homologous to the follicle-stimulating hormone receptor (POWELL *et al.* 2009). FSHR-

20   1 plays a critical role in activating innate immunity in response to infection by

21   pathogenic bacteria, and functions in the intestine to promote protection to

22   pathogenic infection and to regulate anti-microbial gene expression. (CHO *et al.* 2007;

23   POWELL *et al.* 2009; MILLER *et al.* 2015). Interestingly, infection by pathogenic

24   bacteria leads to multiple cellular responses in the intestine, including the activation

4

1   of the UPR[mt] (LIU *et al.* 2014; PELLEGRINO *et al.* 2014).

2   In this study, through the analysis of *fshr-1* null mutants, we show that FSHR-

3   1 positively regulates UPR[mt] activation in intestine and promotes the mitochondrial

4   association of SPHK-1. Through tissue-specific rescue experiments, we find that

5   FSHR-1 functions primarily in the nervous system, and also in the intestine, to exert

6   its function in UPR[mt] activation. We propose that FSHR-1 is part of a neuroendocrine

7   signaling network that functions to activate the UPR[mt] through inter-tissue signaling.

8

9   **Materials and Methods**

10

11 ***C. elegans* strains**

12 Strains used in this study were maintained at 22°C following standard methods.

13 Young adult hermaphrodites derived from the wild type reference strain N2 Bristol

14 were used for all experiments. The following mutant strains were used. SJ4100:

15 *zcIs13[Phsp-6::GFP],* OJ4113*: vjIs138[Pges-1::sphk-1::gfp],* OJ4143: *vjIs148[Pges-*

16 *1::tomm-20::mCherry],* OJ2329*: vjIs208[Pgst-4::gfp],* OJ997 *sphk-1(ok1097),*

17 KP3397: *fshr-1(ok778).* The *sphk-1(ok1097)* and *fshr-1(ok778)* strains were

18 outcrossed at least 6 times with wild type animals prior to analysis.

19 **Molecular biology**

20 *fshr-1* cDNA was cloned from *C. elegans* wild type cDNA and then inserted into the

21 pPD49.26 expression vector using standard molecular biology techniques. The

22 following plasmids were generated *pSK55[Pges-1::fshr-1] , pSK56[Prab-3::fshr-1].*

1   Sequence files of plasmids are available upon request.

2   **Transgenic lines**

3   Transgenic strains were generated by injecting expression constructs (10–25 ng/ul)

4   and the co-injection marker; *pJQ70 [Pofm-1::mCherry, 40 ng/ul]*, *KP#708[Pttx-3::rfp,*

5   *40 ng/ul] or KP#1106 [Pmyo-2::gfp, 10 ng/ul]* into N2 or indicated mutants using

6   standard techniques (MELLO *et al.* 1991). At least three lines for each transgene were

7   tested and a representative transgene was used for further experiments. The

8   following transgenic lines were made: *vjEx1448[Prab-3::fshr-1], vjEx1449[Pges-*

9   *1::fshr-1]*

10   **Microscopy and analysis**

11   Fluorescence microscopy experiments were performed following previous methods

12   (KIM and SIEBURTH 2018a). Briefly, L4 stage or young adult worms were immobilized

13   by using 2,3-butanedione monoxime (BDM, 30 mg/mL; Sigma) in M9 buffer then

14   mounted on 2% agarose pads for imaging. To quantify the fluorescence intensity of

15   P*hsp-6::GFP* or P*gst-4::GFP*, *Z* stacks of the intestine posterior to the vulva were

16   selected as a representative region because of low basal expression in the absence

17   of stress. Images were captured with the Nikon eclipse 90i microscope equipped

18   with a Nikon PlanApo 40 x or 60x or 100x objective (NA = 1.4) and a

19   PhotometricsCoolsnap ES2 or a Hamamatsu Orca Flash LT+ CMOS camera.

20   Metamorph 7.0 software (Universal Imaging/Molecular Devices) was used to capture

21   serial image stacks, and the maximum intensity was measured (KIM and SIEBURTH

22   2018b). Intensity quantification analysis was performed on the same day to equalize

23   the absolute fluorescence levels between samples within same experimental set.

6

RNA Interference

Feeding RNAi knockdown assay was performed following established protocols (KAMATH and AHRINGER 2003). Briefly, gravid adult animals were placed on RNAi plates seeded with HT115(DE3) bacteria transformed with L4440 vector containing a genomic fragment of the gene to be knocked down (or empty L4440 vector as a control), to collect eggs then removed after 4 hours to obtain an age-matched synchronized worm population. Young adult animals were used for subsequent assays.

Stress induction assays

For drug-induced stress, transgenic L4 animals were transferred to fresh NGM plates seeded with HB101 bacteria, then 80 ul of stock solutions dissolved in M9 buffer of paraquat were added to plates for a final concentration of 0.4 mM paraquat. 24 hours later, adults were selected for fluorescence microscopy analysis. For imaging of animals that had been subjected to RNAi-induced knockdown, L4 animals grown on RNAi plates were transferred to new RNAi plates to obtain synchronized animals and imaged 24 hours later. For P*gst-4::GFP* imaging, young adult animals are incubated with 5mM arsenite or 50mM paraquat in liquid solution (in M9 buffer) for 1 hour then drug was washed out and worms were transferred onto new NGM plates for 4 hours before imaging. M9 buffer was used as a control for paraquat and arsenite treatment.

For paraquat survival assays, young adult animals were placed onto NGM plates containing 10mM paraquat for 17 hours. After 17 hours, the percentage of surviving animals was counted every 3 hours over the course of 15 hours. Survival assay were done in experimental duplicate for each biological duplicate.

1   **Statistical Analysis**

2   Student's t test (two-tailed) was used to determine the statistical significance. P

3   values less than 0.01 or 0.001 are indicated with asterisks **(p< 0.01), ***(p<0.001),

4   respectively. Error bars in the figures indicate the standard error of the mean

5   (±S.E.M). The exact numbers of sample size (n) are indicated in each figure.

6   **Data Availability**

7   Strains and plasmids used in this study are available upon request. The authors

8   confirm that all data necessary for confirming the conclusions of the findings are

9   present within the article, figures.

10

11   **Results**

12   **FSHR-1/GPCR signaling regulates the UPR^{mt}**

13   Prior studies found that *fshr-1* mutants have normal lifespans (POWELL *et al.*

14   2009), but exhibit enhanced sensitivity to lethality caused by the mitochondrial toxin

15   and UPR^{mt} activator, paraquat (MILLER *et al.* 2015). Because impairing UPR^{mt}

16   activation causes sensitivity to paraquat-induced lethality (NARGUND *et al.* 2012;

17   GATSI *et al.* 2014; LIU *et al.* 2014; KIM and SIEBURTH 2018a), we speculated that

18   FSHR-1 may positively regulate the UPR^{mt}. To monitor UPR^{mt} activation, we

19   quantified intestinal fluorescence of the UPR^{mt} transcriptional reporter, *zcIs13*, in

20   which GFP is expressed under control of the *hsp-6* promoter (P*hsp-6::GFP*).

21   Paraquat is an oxidant that interferes with electron transport at the inner

22   mitochondrial membrane, and has been used widely to acutely activate the UPR^{mt} in

8

1   both *C. elegans* and in mammalian cells (NARGUND *et al.* 2012; RUNKEL *et al.* 2013;

2   FIORESE *et al.* 2016; KIM and SIEBURTH 2018a). Wild type animals treated with

3   paraquat for 24 hours exhibited a greater than ten-fold increase in P*hsp-6::GFP*

4   expression in the intestine compared to non-treated controls. In contrast, *fshr-1*

5   mutants showed either no significant change or a small increase in P*hsp-6::GFP*

6   expression following paraquat treatment (Figure 1A and 2A). *cco-1* (aka *cox-5B*),

7   encodes the cytochrome *c* oxidase subunit in complex IV, which is the terminal

8   electron acceptor of the electron transport chain, and RNA interference (RNAi)-

9   mediated *cco-1* knockdown is a potent activator of the UPR^{mt} (NARGUND *et al.* 2012;

10   BERENDZEN *et al.* 2016; MERKWIRTH *et al.* 2016; TIAN *et al.* 2016; KIM and SIEBURTH

11   2018a). *cco-1* RNAi by feeding significantly increased P*hsp-6::GFP* expression in

12   wild type animals but failed to increase P*hsp-6::GFP* expression in *fshr-1* mutants

13   (Figure 1C). *fshr-1* mutants exhibited normal induction of the antioxidant reporter

14   P*gst-4::GFP* (INOUE *et al.* 2005; CHOE *et al.* 2009; RUIZ-RAMOS *et al.* 2009; PRAKASH

15   *et al.* 2015; WU *et al.* 2016) in response to treatment with either the mitochondrial

16   ROS generator arsenite, or with paraquat (Figure 1D). Thus, FSHR-1 positively

17   regulates UPR^{mt} activation in response to mitochondrial stress.

18

19   **FSHR-1/GPCR functions cell non-autonomously in neurons to regulate UPR^{mt}**

20   FSHR-1 is expressed primarily in the intestine and in a subset of neurons

21   (SIEBURTH *et al.* 2005; CHO *et al.* 2007). To determine in which tissue FSHR-1

22   functions to regulate the UPR^{mt}, we examined P*hsp-6::GFP* expression in transgenic

23   *fshr-1* mutants expressing *fshr-1* cDNA in either the nervous system (using the *rab-3*

9

1   promoter) or the intestine (using the *ges-1* promoter). Pan-neuronal *fshr-1* nearly

2   fully rescued the paraquat-induced *hsp-6* expression defects of *fshr-1* mutants. In

3   contrast, intestinal *fshr-1* expression only partially rescued the paraquat-induced

4   *hsp-6* expression defects of *fshr-1* mutants (Figure 1A). P*hsp-6* expression was

5   increased 11 fold by pan-neuronal *fshr-1* compared to just 3.3 fold by intestinal *fshr-1*

6   expression (Figure 1A). Taken together, these results suggest that FSHR-1 primarily

7   functions cell non-autonomously in the nervous system to positively regulate UPR^{mt}

8   activation, and intestinal *fshr-1* has a minor role in contributing to UPR^{mt} activation.

9

10   **Neuronal FSHR-1 regulates baseline *hsp-6* expression.**

11   Under non-stressed conditions, *fshr-1* mutants exhibited a small but

12   significant increase in P*hsp-6::GFP* expression in the intestine compared to wild type

13   controls (Figure 1A and 1C). The increase in P*hsp-6::GFP* expression was blocked

14   by RNAi-mediated knockdown of *atfs-1* (Figure 1B), suggesting that FSHR-1 keeps

15   UPR^{mt} activation low under non-stressed conditions. The increase in baseline P*hsp-*

16   *6::GFP* expression of *fshr-1* mutants was restored to wild type levels by pan-

17   neuronal, but not by intestinal *fshr-1* cDNA expression (Figure 1A). Thus, neuronal

18   FSHR-1 functions cell non-autonomously to keep UPR^{mt} activity low in the absence

19   of stress.

20

21   **FSHR-1 and SPHK-1 function in a common pathway to activate the UPR^{mt}**

22   SPHK-1 functions in the intestine to activate the UPR^{mt} in response to a

23   variety of mitochondrial stressors, including paraquat (KIM and SIEBURTH 2018a). To

10

1   test whether FSHR-1 functions in a common pathway with SPHK-1 to activate the

2   UPR^mt, we examined genetic interactions between *sphk-1* and *fshr-1* mutants. *sphk-*

3   *1* mutants exhibit a significant reduction in paraquat-induced expression of P*hsp-*

4   *6::GFP,* (89% reduction) that is similar to that exhibited by *fshr-1* mutants (92%

5   reduction (Figure 2A and (KIM and SIEBURTH 2018a)). Double mutants lacking both

6   *sphk-1* and *fshr-1* displayed defects in paraquat-induced *hsp-6* induction that were

7   no more severe than those seen in single mutants (93% reduction, Figure 2A).

8   *sphk-1* mutants exhibit reduced survival rates when exposed to toxic levels of

9   paraquat (KIM and SIEBURTH 2018a), that are similar to those of *fshr-1* mutants

10   (Figure 2B). The survival rates of double mutants lacking both *sphk-1* and *fshr-1*

11   were no more severe than those of either single mutant (Figure 2B). Expression of

12   *sphk-1* cDNA in the intestine fully rescued the increased paraquat-induced lethality

13   of *sphk-1* mutants (KIM and SIEBURTH 2018a). In contrast, expression of *fshr-1* cDNA

14   in the nervous system fully rescued the increased paraquat-induced lethality of *fshr-1*

15   mutants. In addition, expression of *fshr-1* cDNA in the intestine restored near-normal

16   paraquat sensitivity to *fshr-1* mutants (Figure 2B and (MILLER *et al.* 2015)). Taken

17   together, FSHR-1 and SPHK-1 function in a common pathway to activate the UPR^mt

18   and to promote organism-wide protection from mitochondrial stress-induced lethality.

19   Moreover, FSHR-1 functions in both the nervous system and the intestine to activate

20   the UPR^mt and promote survival, whereas SPHK-1 functions exclusively in the

21   intestine.

22

23   **FSHR-1/GPCR regulates mitochondrial association of SPHK-1 in the intestine**

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1   SPHK-1 rapidly associates with intestinal mitochondria following mitochondrial

2   stress (KIM and SIEBURTH 2018a). To determine whether FSHR-1 regulates stress-

3   induced SPHK-1 mitochondrial association, we examined the mitochondrial

4   abundance of functional SPHK-1::GFP fusion proteins before and after paraquat

5   exposure. Wild type animals treated with paraquat exhibit a 1.8 fold increase in

6   SPHK-1::GFP mitochondrial fluorescence intensity (Figure 3C and (KIM and

7   SIEBURTH 2018a)). *fshr-1* mutants exhibited three defects in SPHK-1::GFP

8   mitochondrial association. First, *fshr-1* mutants exhibited a slightly smaller 1.5 fold

9   increase in paraquat-induced mitochondrial SPHK-1::GFP fluorescence compared to

10   wild type controls (Figure 3C). Second, *fshr-1* mutants exhibited a nearly two-fold

11   reduction in SPHK-1::GFP mitochondrial fluorescence compared to wild type

12   controls in the absence of paraquat (Figure 3A). Third, *fshr-1* mutants exhibited a

13   nearly two-fold reduction in SPHK-1::GFP mitochondrial fluorescence compared to

14   wild type controls following paraquat treatment (Figure 3C). The reduction in SPHK-

15   1::GFP mitochondrial fluorescence is likely not due to decreased expression of the

16   *sphk-1::gfp* transgene, or decreased mitochondrial mass since the fluorescence

17   intensity of the mitochondrial marker TOMM-20::mCherry expressed under the same

18   promoter (*ges-1*) was not reduced in *fshr-1* mutants either in the absence or

19   presence of paraquat (Figure 3A and (KIM and SIEBURTH 2018a)). Thus, *fshr-1*

20   mutations impair SPHK-1 association with mitochondria under both stressed and

21   non-stressed conditions.

22   Interestingly, we observed an increase in mitochondrial mass in *fshr-1*

23   mutants, as revealed by an increase in TOMM-20::mCherry mitochondrial

24   fluorescence compared to wild type controls (Figure 3A). Factoring in the increased

1   mitochondrial mass in *fshr-1* mutants, we found an approximately 5.9 fold decrease

2   in mitochondrial-associated SPHK-1 at baseline (Figure 3B). Thus, FSHR-1

3   negatively regulates mitochondrial mass in the intestine and positively regulates

4   SPHK-1 mitochondrial association, under normal conditions and following stress.

5

6   **Neuronal *fshr-1* cell non-autonomously regulates mitochondrial association of**

7   **SPHK-1 in intestine.**

8   To determine in which tissue FSHR-1 functions to regulate the mitochondrial

9   association of SPHK-1, we quantified SPHK-1::GFP mitochondrial fluorescence in

10 *fshr-1* mutants expressing *fshr-1* cDNA in either the nervous system or in the

11 intestine. We found that pan-neuronal expression of *fshr-1* cDNA in *fshr-1* mutants

12 restored mitochondrial SPHK-1::GFP fluorescence intensity to wild type levels, both

13 in the absence of paraquat and following paraquat treatment. In contrast, intestinal

14 *fshr-1* cDNA expression failed to rescue the SPHK-1::GFP fluorescence defects of

15 *fshr-1* mutants in the absence of paraquat and partially restored paraquat-induced

16 SPHK-1 mitochondrial association (Figure 3C). These results suggest that FSHR-1

17 functions exclusively in the nervous system to regulate SPHK-1 abundance in the

18 intestine in the absence of stress and that FSHR-1 function in both in the nervous

19 system and in the intestine is likely to promote paraquat-induced SPHK-1

20 mitochondrial association.

21 **Discussion**

22 The nervous system coordinates various stress responses by releasing diffusible

23 factors that act upon distal tissues to activate cellular defense programs (BERENDZEN

1   *et al.* 2016; SHAO *et al.* 2016). Here we show that the conserved GPCR FSHR-1 is

2   part of an integrated organism-wide response to mitochondrial stress that functions

3   to activate the UPR^{mt} in the intestine. FSHR-1 promotes UPR^{mt} activation in

4   response to either acute exposure to mitochondrial toxins or to chronic mitochondrial

5   dysfunction. FSHR-1 functions in a common pathway with SPHK-1 to promote

6   survival in response to toxic mitochondrial stress, and to promote UPR^{mt} activation.

7   FSHR-1 positively regulates the mitochondrial association of SPHK-1 in the intestine

8   in the absence of stress, as well as stress-induced SPHK-1 mitochondrial

9   recruitment. FSHR-1 functions in the nervous system and in the intestine to promote

10   UPR^{mt} activation, stress-induced SPHK-1 mitochondrial recruitment and survival in

11   the presence of mitochondrial stress. We propose a model whereby FSHR-1

12   activates the UPR^{mt} by two mechanisms: 1) neuronal FSHR-1 promotes cell non-

13   autonomous activation of the UPR^{mt} by regulating intestinal SPHK-1 activity, and 2)

14   intestinal FSHR-1 activates the UPR^{mt} by a cell-autonomous mechanism that does

15   not involve SPHK-1 (Figure 4). FSHR-1 also regulates mitochondrial homeostasis in

16   the absence of stress by regulating mitochondrial mass, baseline UPR^{mt} activity, and

17   SPHK-1 mitochondrial association.

18   Previous studies have established an important role for FSHR-1 in activating

19   the innate immune response following infection with pathogenic bacteria. FSHR-1

20   promotes both survival following infection, and the behavioral avoidance response to

21   pathogenic bacteria (POWELL *et al.* 2009; MILLER *et al.* 2015), and FSHR-1 activates

22   a number of antimicrobial and antioxidant genes in response to pathogens (POWELL

23   *et al.* 2009; MILLER *et al.* 2015). FSHR-1 is proposed to not participate in detection of

24   the pathogenic bacteria themselves, but instead to participate in the detection of

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1   cellular damage (e.g. ROS) resulting from bacterial infection (MILLER *et al.* 2015).

2   Pathogenic bacterial infection is also a potent activator of the UPR^{mt}, and UPR^{mt}

3   activation by pathogens promotes survival by inducing the expression of innate

4   immune genes (PELLEGRINO *et al.* 2014). Thus, FSHR-1 may activate the innate

5   immune response by contributing to activation of the UPR^{mt} following infection.

6   Interestingly, prior studies have found that expression of FSHR-1 in the nervous

7   system or the intestine can rescue survival defects of *fshr-1* mutants (POWELL *et al.*

8   2009), consistent with our studies showing a function for FSHR-1 in UPR^{mt} activation

9   in either tissue. We speculate that FSHR-1 may be a central component in the

10   cellular response to mitochondrial dysfunction caused by a broad array of insults (e.g.

11   pathogenic infection, ROS, and UPR^{mt} dysfunction).

12   FSHR-1 also regulates intestinal mitochondrial homeostasis in the absence of

13   stress. *fshr-1* mutants exhibit a nearly three-fold increase in mitochondrial mass in

14   the intestine, and a corresponding reduction in mitochondrial-associated SPHK-1.

15   FSHR signaling negatively regulates mitochondrial biogenesis in mammals (LIU *et al.*

16   2017). Enhanced mitochondria biogenesis is correlated with UPR^{mt} activation.

17   Inducers of mitochondria biogenesis, such as nicotinamide riboside (NR) and

18   poly(ADP-ribose) polymerase inhibitors (PARPi) disrupt the mito-nuclear protein

19   homeostasis resulting in UPR^{mt} activation via the sirtuin 1 (SIRT1) pathway in

20   mammals and *C. elegans*. (MOUCHIROUD *et al.* 2013). In addition, rapamycin and

21   resveratrol, which increase mitochondrial content, also induce UPR^{mt} activation in *C.*

22   *elegans* (UNGVARI *et al.* 2011; HOUTKOOPER *et al.* 2013; LERNER *et al.* 2013).

23   Consistent with this, *fshr-1* mutants exhibit increased baseline *hsp-6* expression,

24   which is dependent upon *atfs-1*, suggesting a potential function for FSHR-1 in

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1   suppressing UPR^{mt} activation by reducing mitochondrial mass and/or biogenesis. We

2   speculate that the defects in paraquat-induced UPR^{mt} activation in *fshr-1* mutants

3   may be due to an underlying defect in the mitochondrial association of SPHK-1

4   under normal conditions.

5   Since FSHR-1 functions to protect animals from diverse stressors, the ligand

6   for FSHR-1 is likely to originate from the host rather than from an exogenous source

7   (POWELL *et al.* 2009). FSHR-1 shares homology with three human GPCRs: FSHR,

8   TSHR and LHCGR by virtue of nine LRRs found in its extracellular domain (DOLAN *et*

9   *al.* 2007). These GPCRs are activated by the heterodimeric glycopeptide hormones

10   FSHα/β, TSHα/β, and LHα/β, respectively. In humans, FSH induces the generation

11   of S1P by stimulating SphK1 activity for granulosa cell proliferation (HERNANDEZ-

12   CORONADO *et al.* 2016), suggesting a potential functional conservation of ligand-

13   activated FSHR-1 signaling. However, the worm genome does not encode obvious

14   orthologues of any of these ligands (POWELL *et al.* 2009; MILLER *et al.* 2015),

15   suggesting divergence of an ancestral FSHR-1 ligand. A number of GPCR ligands

16   have been identified that function non-autonomously to either activate the UPR^{mt} or

17   the innate immunity response (SHAO *et al.* 2016; KIM and EWBANK 2018). Among

18   these are a set of neuropeptide-like proteins that function in a subset of

19   sensory/interneurons to activate the UPR^{mt}. These peptides are proposed to function

20   in a neuronal circuit that transduces mitochondrial stress signals originating in the

21   nervous system to the intestine to activate the UPR^{mt} (SHAO *et al.* 2016). Because of

22   the requirement for FSHR-1 in the nervous system in activating the UPR^{mt}, we

23   propose that FSHR-1 functions as part of this neuroendocrine signaling network,

24   possibly as a receptor for one or more of these peptides. Consistent with a role for

16

1 FSHR-1 in regulating neuron function, FSHR-1 positively regulates synaptic

2 transmission at motor synapses (SIEBURTH *et al.* 2005). Identifying the ligand(s) for

3 FSHR-1 will help to clarify both the cell autonomous and cell non-autonomous

4 mechanisms by which FSHR-1 activates the UPR^{mt} pathway.

5

Acknowledgements

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18   Response Is Mediated Cell-Non-autonomously by Retromer-Dependent Wnt
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20

Figure legends

Figure 1 FSHR-1 functions in the nervous system to activate the UPR^{mt}. (A) *Left*,

Representative images of posterior intestines of young adult animals or *fshr-1* mutants

expressing the *zcIs13* [P*hsp-6::GFP*] transgene (*GFP* driven by the *hsp-6* promoter)

following treatment with M9 (control) or paraquat (PQ) for 24 hours. "*fshr-1* neuronal rescue"

denotes *fshr-1* mutants expressing *fshr-1* cDNA under the neuron-specific promoter, *rab-3*.

"*fshr-1* intestine rescue" denotes *fshr-1* mutants expressing *fshr-1* cDNA under the intestine-

specific promoter, *ges-1*. *Middle*, Average GFP fluorescence intensity is quantified. *Right*,

Fold changes of P*hsp-6::GFP* expression of the indicated strains by paraquat treatment are

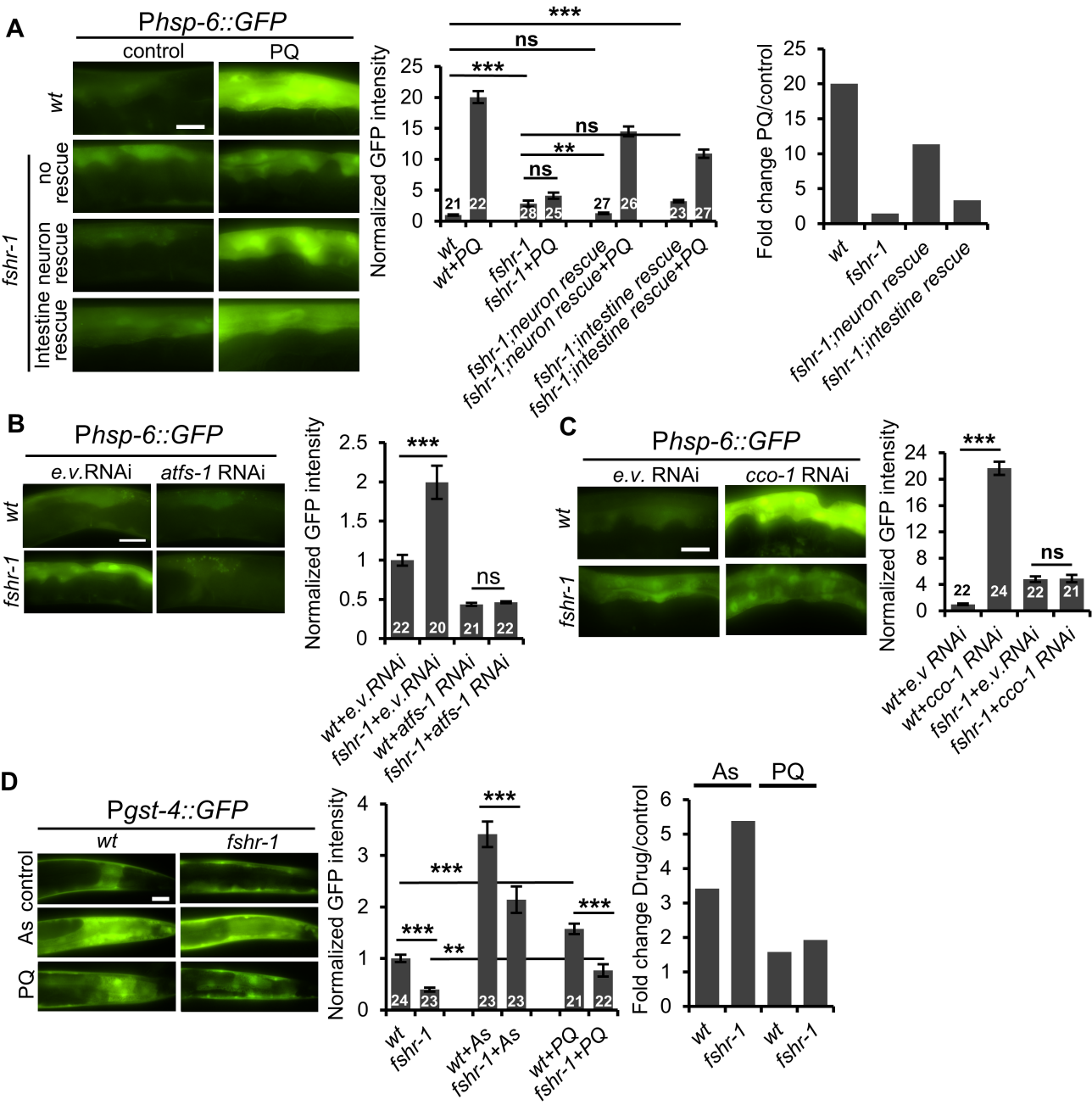measured. (B) *Left*, Representative images of intestines of wild type or *fshr-1* mutants

1   expressing P*hsp-6::GFP* following empty vector (e.v.) control or *atfs-1* RNAi treatment. *Right*,

2   Average GFP fluorescence intensity is quantified. (C) *Left*, Representative images of P*hsp-*

3   *6::GFP* fluorescence in intestines of wild type or *fshr-1* mutants following empty vector (e.v.)

4   control or *cco-1/cox-5B* RNAi treatment. *Right*, Average GFP fluorescence intensity is

5   quantified. (D) *Left*, Representative images of posterior intestines from animals expressing

6   the antioxidant reporter transgene [P*gst-4::GFP*] in wild type or *fshr-1* mutants in the

7   absence or presence of arsenite (As), or paraquat (PQ). *Middle*, Average GFP fluorescence

8   intensity is quantified. *Right*, Fold changes of *gst-4* expression of indicated strains by

9   arsenite or paraquat treatment are measured. Scale bars represent 10 μm. Error bars

10   indicate ±S.E.M. The number of animals tested are indicated. Student's t-test **$p<0.01$,

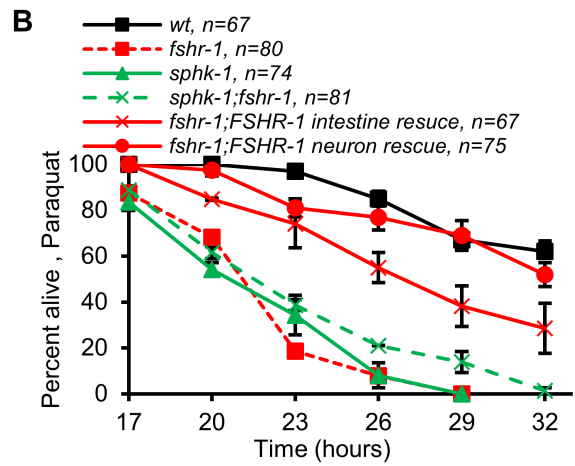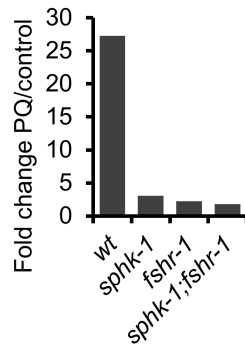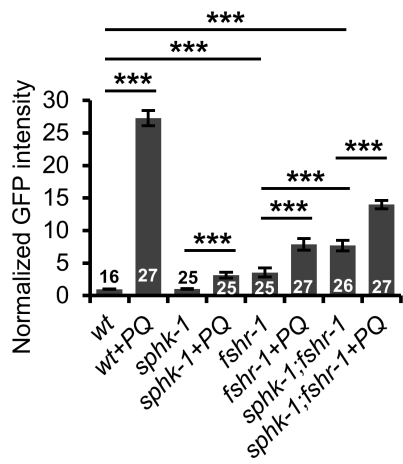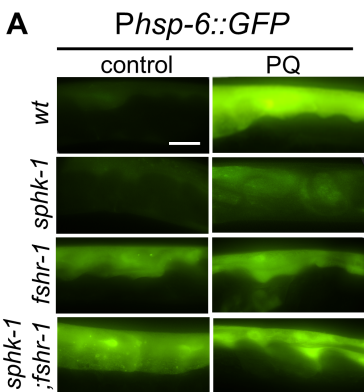11   ***$p<0.001$. "ns" denotes "not statistically significant"

12

13   **Figure 2** FSHR-1 functions in a common pathway with SPHK-1 to activate the UPR^{mt}. (A)

14   *Left*, Representative images of P*hsp-6::GFP* expression in the intestines of wild type, *sphk-1*

15   mutants, *fshr-1* mutants, and *sphk-1;fshr-1* double mutants in the absence or the presence

16   of paraquat (PQ) for 24 hours. *Middle*, Average GFP fluorescence intensity is quantified.

17   *Right*, Fold change denotes the ratio of average fluorescence intensity of animals treated

18   with paraquat (PQ) and control animals treated with buffer (M9). (B) Survival rate curves of

19   the indicated strains on plates containing 10mM paraquat. The number of animals tested are

20   indicated. Scale bar represents 10 μm. Student's t-test ***$p<0.001$.

21

22   **Figure 3** FSHR-1 regulates mitochondrial association of SPHK-1. (A) *Left*, Representative

23   posterior intestine images of wild type or *fshr-1* mutants expressing SPHK-1::GFP or the

24   mito-marker TOMM-20::mCherry under the intestinal-specific promoter, *ges-1*. *Right*,
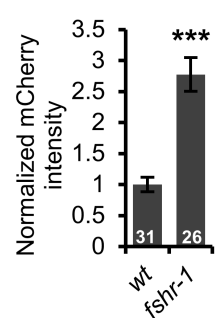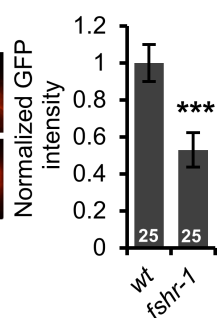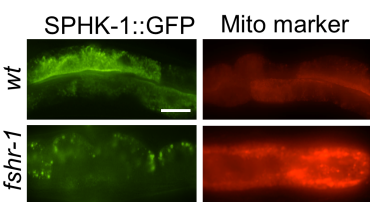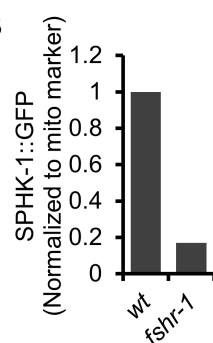
21

1   Average GFP and mCherry fluorescence intensities are quantified. (B) Ratio of SPHK-

2   1::GFP expression in wild type or *fshr-1* mutants (normalized to the intensity of each of the

3   mitochondria markers). (C) *Left*, Representative images of the indicated mutants expressing

4   intestinal SPHK-1::GFP in the absence or presence of paraquat for 24 hours. *fshr-1* neuron,

5   intestine rescue denotes transgenic *fshr-1* mutants expressing *fshr-1* cDNA under the pan-

6   neural promoter, *rab-3,* or the intestinal promoter, *ges-1,* respectively. *Middle*, Average

7   fluorescence intensities in the indicated strains expressing SPHK-1::GFP are quantified.

8   *Right,* Fold changes of SPHK-1::GFP expression of the indicated strains by paraquat

9   treatment are measured. Scale bar represents 10 µm. The number of animals tested are

10   indicated. Student's t-test ***p<0.001. "ns" denotes "not statistically significant"

11

12   **Figure 4** Working model for UPR^{mt} activation by FSHR-1 and SPHK-1. Upon mitochondrial

13   stress, neuroendocrine signaling involving FSHR-1 signaling from the nervous system

14   activates the UPR^{mt} by regulating intestinal SPHK-1 abundance on mitochondria, where

15   SPHK-1 catalyzes the production of S1P from SPH. S1P then activates the UPR^{mt}

16   transcriptional response. FSHR-1 also functions cell autonomously in the intestine to

17   promote activation of the UPR^{mt} by a mechanism that may be independent of SPHK-1.

18

22

A

P*hsp-6::GFP*

control | PQ

wt

fshr-1
no rescue

Intestine neuron rescue

Intestine rescue

Normalized GFP intensity

***
ns
***
**
ns
ns

25
20
15
10
5

21 22 28 25 27 26 23 27

wt
wt+PQ
fshr-1
fshr-1+PQ
fshr-1;neuron rescue
fshr-1;neuron rescue+PQ
fshr-1;intestine rescue
fshr-1;intestine rescue+PQ

Fold change PQ/control

25
20
15
10
5

wt
fshr-1
fshr-1;neuron rescue
fshr-1;intestine rescue

B

P*hsp-6::GFP*

*e.v.*RNAi | *atfs-1* RNAi

wt

fshr-1

Normalized GFP intensity

***
ns

2.5
2.0
1.5
1.0
0.5

22 20 21 22

wt+e.v.RNAi
fshr-1+e.v.RNAi
wt+atfs-1 RNAi
fshr-1+atfs-1 RNAi

C

P*hsp-6::GFP*

e.v. RNAi | *cco-1* RNAi

wt

fshr-1

Normalized GFP intensity

***
ns

24
20
16
12
8
4

22 24 22 21

wt+e.v RNAi
wt+cco-1 RNAi
fshr-1+e.v.RNAi
fshr-1+cco-1 RNAi

D

P*gst-4::GFP*

wt | *fshr-1*

As control

As

PQ

Normalized GFP intensity

***
***
***
***
**
***

4
3
2
1

24 23 23 23 21 22

wt
fshr-1
wt+As
fshr-1+As
wt+PQ
fshr-1+PQ

Fold change Drug/control

As | PQ

6
5
4
3
2
1

wt
fshr-1
wt
fshr-1

A

P*hsp-6::GFP*

control   PQ

wt

sphk-1

fshr-1

*sphk-1
;fshr-1*

B

- *wt, n=67*
- *fshr-1, n=80*
- *sphk-1, n=74*
- *sphk-1;fshr-1, n=81*
- *fshr-1;FSHR-1 intestine resuce, n=67*
- *fshr-1;FSHR-1 neuron rescue, n=75*

A

B

C

SPHK-1::GFP