Mitochondrial UPR negatively regulates wounding-induced innate immune response in *C. elegans* epidermis

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**Highlights:**
— ATFS-1 inhibits the wounding-induced innate immune response in *C. elegans*
— mitoUPR inhibits wounding-induced innate immune response
— Epidermal wounding induces downregulation of ATFS-1 in the epidermis
— mitoUPR acts upstream of p38MAPK and TGF-β pathways to regulate innate immunity

**Keywords:**
Innate immune response, Epidermal wounding, Mitochondrial UPR, ATFS-1, AMPs p38MAPK, TGF-β, NLPs, Caenacins

**Abstract**

Tissue damage elicits a rapid innate immune response that is essential for efficient wound healing and survival of metazoans. It is well known that p38 MAPK kinase, TGF-β, and hemidesmosome signaling pathways have been involved in wounding-induced innate immunity in *C. elegans*. Here, we find that loss of function of ATFS-1 increased innate immune response while an elevated level of
mitochondrial unfolded protein response (mitoUPR) inhibits the innate immune response upon epidermal wounding. Epidermal wounding triggers the nucleus export of ATFS-1 and inhibits themitoUPR in *C. elegans* epidermis. Moreover, genetic analysis suggests that ATFS-1 functions upstream of the p38 MAP kinase, TGF-β, and DAF-16 signaling pathways in regulating AMPs induction. Thus, our results suggest that the mitoUPR function as an intracellular signal required to fine-tune innate immune response after tissue damage.

**INTRODUCTION**

Wounding-induced immunity is a key feature for wound healing in all eukaryotic organisms. It is known that perturbations to protein synthesis, proteolysis, and mitochondrial activity are sufficient to activate innate immune responses after pathogen infection (Dunbar et al., 2012; Melo and Ruvkun, 2012; Richardson et al., 2010). *Caenorhabditis elegans* epidermis constantly encounters myriad environmental insults, including skin-puncturing pathogens and mechanical damage, which can break the epidermal barrier and activate self-defense responses by elevating the production of antimicrobial peptides (AMPs) (Pujol et al., 2008; Xu and Chisholm, 2011). The damage to the skin triggers a rapid innate immune response by inducing multiple AMPs such as neuropeptide-like proteins (NLPs) and caenacins (CNCs) to defend against sterile infection (Pujol et al., 2008; Zugasti and Ewbank, 2009), and this process is regulated by p38 MAP kinase, TGF-β, and hemidesmosome signaling pathways (Kim et al., 2002; Pujol et al., 2008; Zhang et al., 2015; Zugasti and Ewbank, 2009).

Cells respond to mitochondrial dysfunction by activating the mitochondrial unfolded protein response (mitoUPR) to detect pathogenic or toxic bacteria by monitoring mitochondrial function and initiating an innate immune response accordingly (Liu et al., 2014; Nargund et al., 2015; Nargund et al., 2012; Pellegrino et al., 2014). ATFS-1 is a key transcription factor required for the activation of mitoUPR. In healthy animals, ATFS-1 is efficiently imported into mitochondria and degraded. Under mitochondrial stress, mitochondrial import efficiency is reduced, allowing ATFS-1 to accumulate in the nucleus, where it can activate a protective
transcriptional response (Haynes and Ron, 2010; Lin and Haynes, 2016; Nargund et al., 2012). A recent study reported that defective ATFS-1 makes C. elegans susceptible to opportunistic P. aeruginosa, while constitutive activation of ATFS-1 improves the scavenging capacity of this pathogen in the intestine (Pellegrino et al., 2014). However, it is still unclear whether mitoUPR involves in the tissue damage-induced innate immune response.

RESULTS

ATFS-1 inhibits wounding-induced innate immune response

We investigated the wounding-induced innate immunity by examining the expression of the AMPs using a transcriptional reporter strain Pnlp-29::GFP, which can be strongly induced upon wounding (Pujol et al., 2008). We observed that an obvious upregulation of Pnlp-29-GFP expression in wild-type (WT) animals 4 and 7 hours post-wounding (h.p.w.) (Figure 1A). We then examined the expression of Pnlp-29-GFP in atfs-1(tm4525) mutant, in which a larger fragment of genomic DNA was deleted and reduced the mitoUPR (Nargund et al., 2012; Pellegrino et al., 2014). The expression of Pnlp-29-GFP in atfs-1(tm4525) without wounding is similar to WT but significantly enhanced after wounding (Figure 1A), suggesting the possibility that ATFS-1 functions in the wounding-induced innate immune response.

To confirm this observation, we used quantitative PCR (qPCR) to measure the relative expression of other AMPs, including nlp-29, nlp-30 (Pujol et al., 2008) and cnc-1, cnc-11 (Zugasti and Ewbank, 2009). Upon wounding, these two types of AMPs were expressed at significantly higher levels in both atfs-1(tm4525) mutants compared to WT (Figure S1A). Knockdown of atfs-1 by RNAi also enhanced the AMPs expression after wounding compared to L4440 (empty vector) control (Figure S1B). As we compared the expression change of AMPs before and after wounding, we thus quantified the AMPs expression by measuring the fold induction (Zugasti and Ewbank, 2009). Consistently, both NLPs and CNCs were significantly upregulated in atfs-1(tm4525) and atfs-1(tm4919) two loss-of-function mutants as well as RNAi knockdown animals after wounding (Figure 1B-E, Figure S1C-F). In addition, the
expression levels of fipr-22 and ilys-3, which are known to function in defense against fungal pathogen infections in the epidermis (Gravato-Nobre et al., 2016; Kim and Ewbank, 2018), were also significantly increased in atfs-1 mutants and RNAi knockdown animals after wounding (Figure 1F-1G, Figure S1G-J). Collectively, these results suggest that ATFS-1 is required for negative regulation of innate immune response upon skin damage.

We then examined if ATFS-1 is sufficient to inhibit the wounding-induced innate immune responses. Gain-of-function mutation of atfs-1(et18gf), a point mutation by which mitoUPR is constitutively activated (Rauthan et al., 2013), displayed a reduction in the extent of induction of Pnlp-29::GFP reporter (Figure 1A). Quantitative analysis nlp-29, nlp-30, cnc-11, CNCs, ilys-3, and fipr-22 showed a lower level of induction after wounding (Figure S2A-E). To further confirm this observation, we expressed ATFS-1 specifically in the epidermis under the control of the col-19 promoter. Overexpression of ATFS-1 in the epidermis significantly activated the mitoUPR (Figure S2F), and inhibited the induction of NLPS, CNCs, ilys-3, and fipr-22 innate immune response genes after wounding (Figure 1H-M, Figure S2G). Together, these observations support the notion that over-expression of ATFS-1 inhibits the wounding-induced innate immune responses.

**mitoUPR inhibits wounding induced innate immunity**

ATFS-1 is a key transcriptional factor required for mitoUPR (Nargund et al., 2012), we thus determine whether mitoUPR inhibits the wounding-induced innate immune responses. To this end, we knocked down spg-7, which encodes a mitochondrial protease required to inhibit mitoUPR (Haynes and Ron, 2010) (Figure S3A). We observed a significant inhibition of Pnlp-29::GFP induction 4 and 7 h.p.w. (Figure 2A). Moreover, qPCR analysis showed that knockdown of spg-7 also inhibited the induction of nlp-29, nlp-30, cnc-1, cnc-11, fipr-22, and fipr-23 after wounding (Figure 2B-I). In contrast, knockdown of ubl-5, which is required for mitoUPR activation (Benedetti et al., 2006; Haynes et al., 2007a; Haynes and Ron, 2010) (Figure S3A), significantly increased the induction of AMPs by wounding (Figure 2A-I). Furthermore, knockdown of clpp-1(RNAi), which encodes an ATP-
dependent mitochondrial protease required for the induction of mitochondrial chaperone genes in response stress (Haynes et al., 2007a), increased the induction of AMPs (Figure S3B-E). Together, these results indicate that mitoUPR signal negatively regulates innate immune response after epidermal wounding.

**Epidermal wounding inhibits mitochondrial unfolded protein responses**

We then determine how epidermal wounding affects the activity of mitoUPR. To this end, we examined the expression of a Phsp-6::GFP, an indicator of mitoUPR activation in *C. elegans* (Haynes et al., 2007b). However, we did not observe an apparent increase or reduction of Phsp-6::GFP expression after wounding (Figure 3A, Figure S4A). As needle puncture damaged the small region of the epidermis, the live imaging system may not be able to detect the local response of mitoUPR at the protein level. To further test this, we measured the transcriptional expression of hsp-6 and hsp-60, mitochondrial chaperone protein-coding genes that is activated by mitoUPR (Yoneda et al., 2004). Surprisingly, we observed a significant reduction in either hsp-6 or hsp-60 as early as 15 minutes to 3 hours after wounding (Figure 3B and Figure S4B), suggesting that the epidermal wounding may inhibit the mitoUPR. To further test this, we examined the expression of Phsp-6::GFP in animals with an elevated level of mitoUPR by ATFS-1 epidermal overexpression (Figure 3A). We observed an apparent reduction of Phsp-6::GFP fluorescence signal after wounding (Figure 3A, Figure S4A). Together, these results suggest that epidermal wounding inhibits mitoUPR response.

To further determine how wounding triggers the reduction of mitoUPR, we examined the localization of ATFS-1::GFP before and after wounding. Single-copy insertion of ATFS-1::GFP was localized in both nuclei and mitochondria in the epidermis before wounding (Figure 3C, Figure S4C). Interestingly, the ATFS-1::GFP signal in the nucleus was significantly reduced, starting from 15 minutes after wounding (Figure 3C, D, Figure S4C, D). The ATFS-1::GFP signal almost diminished from the nucleus, but not from mitochondria, 3 hours after wounding (Figure 3C, D, Figure S4C, D). To confirm this observation, we performed western blotting analysis to examine the ATFS-1::GFP protein level and detected a reduction
of overall ATFS-1::GFP protein after wounding (Figure 3E). In contrast, we did not observe a significant change of DVE-1::GFP, a transcription factor that acts during stress to induce mitoUPR after wounding (Figure S4E, F). Collectively, these results suggest that epidermal wounding downregulated the nuclear localization of ATFS-1 to reduce the level of mitoUPR.

**mitoUPR plays upstream of p38 MAPK and TGF-β pathways to regulate innate immune response**

To determine how mitoUPR regulates wounding-induced innate immune response, we performed a genetic analysis of *atfs-1* with *nsy-1* and *sek-1*, which encode key components of the p38 MAP kinase pathway. Loss-of-function mutants *nsy-1(ok593)* and *sek-1(km4)* abolished the p38 MAP kinase cascade (Kim et al., 2002; Pujol et al., 2008), exhibited significant reductions in the extent of *nlp-29* and *nlp-30* induction after wounding (Figure 4A-4D, Figure S5A). Loss-of-function of *atfs-1(tm4525)* enhanced the expression of NLPs in WT but not in *nsy-1(ok593)* or *sek-1(km4)* mutants. In addition, *atfs-1(tm4525)* did not suppress the expression of *tir-1*, *nsy-1*, *pmk-1*, *sek-1* (Figure S5B-S5E). Collectively, these results suggest that mitoUPR signaling plays upstream of the p38 MAP Kinase pathway in regulating NLPs expression.

Epidermal wounding-induced expression of CNCs is known to be dependent on DBL-1 TGF-β signal pathways (Zugasti and Ewbank, 2009). Similar to the epistatic experiment with the p38 MAP kinase pathway, we observed that *dbl-1(ok3749)* mutation suppressed *cnc-1* and *cnc-11* induction both in WT and *atfs-1(tm4525)* mutant after wounding (Figure 4E-4F, Figure S5A). This result indicates that mitoUPR signaling functions upstream of the TGF-β pathway to regulate the wounding-induced CNCs expression.

Previous work has demonstrated that the p38 MAP kinase cascade activates the STAT-like transcription factor STA-2 to induce an innate immune response (Dierking et al., 2011) and showed that abrogation of STA-2 blocked the defense response to infection and epidermal damage (Dierking et al., 2011; Zhang et al., 2015). To determine how ATFS-1 affects p38 MAP kinase cascade, we examined whether
ATFS-1 changes the nuclear localization of the transcription factor STA-2. STA-2::GFP was localized to both cytosolic and nucleus in the unwounded epidermis. However, strikingly, the STA-2::GFP signal intensity was significantly increased in the nucleus as early as 15 minutes after wounding (Figure 4G, H), suggesting that epidermal wounding triggers rapid STA-2 translocation. The loss-of-function of atfs-1(tm4525) increased the STA-2::GFP signal in the nucleus within 30 minutes after wounding (Figure 4G, H), indicating that ATFS-1 may inhibit STA-2 translocation in the nucleus and negatively regulates the induction of AMPs after epidermal damage.

Discussion

In conclusion, our results suggest wounding induces downregulation of mitoUPR to regulate the innate immune response in *C. elegans* epidermis. ATFS-1 and mitoUPR play upstream of well-known p38 MAP kinase and TGF-β pathways to regulate the expression of AMPs (Figure 4I). Mitochondria are protective organelles that have evolved basic physiological processes to maintain homeostasis and defend against environmental insults (Banoth and Cassel, 2018). Our results indicate a previously unknown injury response process in the epidermis that reduces the mitoUPR level, which in turn supports the fine-tuning of the regulation of multiple antimicrobial genes; this process acts upstream of known innate immune pathways upon epidermal wounding.

Stress and infection usually cause increased mitoUPR, a process in which key chaperone proteins clear misfolded and unfolded proteins (Banoth and Cassel, 2018; Lin and Haynes, 2016; Taffoni and Pujol, 2015). One possible explanation for these apparently contradictory conclusions from infection and wounding is that there are no dedicated immune cells in *C. elegans* (Kim and Ewbank, 2018), so specific forms of tissue damage may engage innate immune responses via distinct strategies. Or there could be separate signaling cascades for controlling gene expression after tissue damage vs. pathogen infection by secreting similar antimicrobial peptide genes (Pellegrino et al., 2014; Pujol et al., 2008).

Although the mitoUPR is known to regulate innate immune responses in both the intestine and the epidermis, the signal for induction of mitoUPR might be
identified by different receptors and transduced by specific pathways (Nargund et al., 2015; Pellegrino et al., 2014). Furthermore, whether the intestinal endothelial tissue can exhibit a similar immune phenotype as the epidermis upon wounding remains to be determined. Likewise, it is conceivable that the skin has evolved a unique host defense mechanism since it also acts as a first barrier to the surrounding environment in metazoans.

**Materials and Methods**

**Strains and transgenic animals**

Strains of *C. elegans* were cultured on the nematode growth medium (NGM) plates seeded with *E. coli* OP50 following standard protocols at 20°C (Brenner, 1974). The N2 Bristol strain was used as the wild type (WT) strain. Standard microinjection methods were used to generate transgenic animals carrying extrachromosomal arrays (zjuEx). Single-copy insertion of arrays (zjuSi) was accomplished by CRISPR-Cas9 mediated gene editing. Strains that were used in this work are summarized in the supplementary Table 1.

**Needle wounding**

NGM plates were cooled down on the ice for 20 minutes and transferred 30-40 young adult animals onto each plate. Needle wounding was then performed by microinjection needle on the hyp7 as described previously (Xu and Chisholm, 2011).

**RNAi**

The dsRNA bacteria were recovered from -80°C and incubated on the Ampicillin-resistant L.B. plate at 37°C overnight. The next day, the single clone was picked into 1ml Ampicillin-resistant L.B. liquid and incubated for about 6 hours. When the OD reached 0.4-0.6, we sed the bacteria solution on prepared RNAi plates and kept them in the dark. 72 hours later, we prepared a plate of adult animals and collected eggs by bleaching. We then put fresh eggs on the RNAi plate with specific dsRNA and waited for them to grow until L4 at 20°C. We then transferred about 200 synchronized late-L4 animals to a new RNAi plate and collected 30 D1 animals for each RNA sample. The *atfs-1* CDS dsRNA clone was made by PCR from the cDNA.
RNA isolation and real-time RT–PCR

Worms were plated onto NGM plates and incubated at 20°C. Synchronized late-L4 animals were then transferred to a new plate. Next day, 30 young adult animals for each RNA sample were collected and total RNA was obtained in 500μl Trizol reagent (Invitrogen, Carlsbad, CA, USA). cDNA was then synthesized via the cDNA Synthesis Kit (TOYOBO TRT-101). Using act-1 as the internal control to normalize expression and determine differences, qRT–PCR was performed according to the SYBR green kit instructions (Vazyme, China). Primer sequences used for qRT–PCR were listed.

Western blot

For Pcol-19-ATFS-1::GFP single-copy insertion transgenic animals, we collected 300 synchronized D1 animals for each protein sample and got the total cell lysate for detection. Before detection, the protein samples were normalized to the same concentration. Tublin was used as the internal control. Western blot was performed according to the Bio-Rad system.

Imaging analysis

All the images were analyzed using ImageJ software (https://imagej.nih.gov/ij/).

Statistical analysis

All statistical analyses used GraphPad Prism (La Jolla, CA). Two-way comparisons used Student’s t-test and multiple comparisons used one-way ANOVA Bartlett’s test.

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Author contributions: S.X. and J.Z. conceived the study and designed experiments. J.Z. designed and performed the experiments. H.Z. made constructs, performed wounding assay, X.R. performed Western Blot assay. S.X. and J.Z. wrote the
manuscript.

**Competing interests:** The authors declare no competing financial interests.
Figures and Figure legends:

Figure 1. ATFS-1 inhibits epidermal wounding-induced innate immune responses

(A) The green fluorescence in transgenic worms carrying a Pnlp-29::GFP reporter was observed post wounding in worms generated by crossing with WT, atfs-1 loss-of-function mutant atfs-1(tm4525), atfs-1(et18gf) or epidermis specific overexpression mutant atfs-1(juSi234). Scale bars, 500 μm.

(B-G) nlp-29, nlp-30, cnc-1, cnc-4, ilys-3, and fipr-22 expression levels as determined by qPCR in WT and atfs-1(tm4525) animals. N = 3 replicates. Bars indicate the mean ± SEM. *, P < 0.05, **, P < 0.01, ***, P < 0.001 (Student’s t-test).

(H-M) nlp-29, nlp-30, cnc-1, cnc-11, ilys-3, and fipr-22 expression levels as determined by qPCR in WT and atfs-1(juSi234) animals. N = 3 replicates. Bars indicate the mean ± SEM. *, P < 0.05, **, P < 0.01, *** P < 0.001 (Student’s t-test).
Figure 2. mitoUPR inhibits the induced expression innate immune responses genes upon epidermal wounding

(A) The green fluorescence in transgenic worms carrying a Pnlp-29::GFP reporter was observed in the wounded animals fed with an L4440 empty vector, atfs-1, spg-7, or ubl-5 RNAi. Scale bars, 500 μm.

(B-I) nlp-29, nlp-30, cnc-1, cnc-11, ilys-3, lys-3, fipr-22, and fipr-23 expression levels as determined by qPCR in WT animals fed with an L4440 empty vector, spg-7, or ubl-5 dsRNA. N = 3 replicates. Bars indicate the mean ± SEM. ns, P > 0.05, *, P < 0.05, **, P < 0.01 (Student's t-test).
Figure 3. Wounding reduces mitoUPR by inhibition of nuclear localization of ATFS-1

(A) Representative microscopy images of the HSP-6::GFP signal before and at early time points after needle wounding in both WT and Pcol-19-ATFS-1::GFP(juSi234) transgenic animals. Scale bars, 200 μm.

(B) hsp-60 expression levels as determined by qPCR in WT animals. 6-8 stabs throughout the whole hypodermis were performed on each animal and 30 D1 animals were collected for each RNA sample. N = 3 replicates. Bars indicate the mean ± SEM. ***, P < 0.001 (Student's t-test).

(C) Representative confocal microscopy images of ATFS-1::GFP before and at different time points after needle wounding. Pcol-19-ATFS-1::GFP(juSi234) single-copy insertion transgenic animals were used for wounding and imaging. Animals were wounded with one anterior stab and one posterior stab. Scale bars, 10 μm.

(D) Quantification of ATFS-1::GFP fluorescence in the nucleus post wounding. N ≥ 20 animals. Bars indicate the mean ± SEM. ***, P < 0.001, One-way ANOVA and Bartlett’s test.

(E) Western blot analysis of ATFS-1::GFP protein levels before and 3 hours after needle wounding. Protein samples were prepared from the total lysate and detected using an anti-GFP antibody.
Figure 4. mitoUPR plays upstream of p38MAPK and TGF-β pathways in regulating innate immune response upon wounding

(A-D) *nlp-29* and *nlp-30* expression levels as determined by qPCR in WT, *atfs-1*, *nsy-1*, *sek-1*, and double mutants *atfs-1; nsy-1* and *atfs-1; sek-1*. N = 3 replicates. Bars indicate the mean ± SEM. *, P < 0.05, **, P < 0.01, *** P < 0.001 (Student's t-test).

(E-F) *cnc-1* and *cnc-11* expression levels as determined by qPCR in WT or *dbl-1* on control versus *atfs-1*(RNAi). N = 3 replicates. Bars indicate the mean ± SEM. *, P < 0.05, **, P < 0.01 (Student's t-test).

(G) Representative confocal microscopy images of STA-2::GFP fluorescence before and after needle wounding. *Psta-2-STA-2::GFP* transgenic animals were used for wounding and imaging. Scale bars, 10 μm.

(H) Quantification of STA-2::GFP fluorescence intensity in the nucleus before and post wounding after feeding with an L4440 empty vector or *atfs-1* dsRNA. N ≥ 20 animals. Bars indicate the mean ± SEM. ns, P > 0.05, **, P < 0.01, One-way ANOVA and Bartlett’s test.

(I) A proposed model for mitoUPR senses the epidermal damage and regulates the wounding-induced innate immune response by inhibiting known innate immune response pathway upon wounding.
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Mitochondrial UPR negatively regulates wounding-induced innate immune response in C. elegans epidermis

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Supplemental Information
Including 5 Supplementary Figures and Figure Legends
Figure 1. Loss-of-function of ATFS-1 enhances the epidermal wounding-induced innate immune response in C. elegans epidermis

(A) Relative expression of nlp-29, nlp-30, cnc-1, cnc-4, ilys-3 and fipr-22 as determined by qRT–PCR in the wounded animals in both wild type (WT) and atfs-1(tm4525) mutant. N = 3. Bars indicate mean ± SEM.

(B) Relative expression of nlp-29, nlp-30, cnc-1, cnc-4, ilys-3 and fipr-22 as determined by qRT–PCR in the wounded animals in both atfs-1 RNAi and control L4440 (empty vector) RNAi animals. N = 3. Bars indicate mean ± SEM.

(C-J) Fold induction analysis of nlp-30, cnc-11, ilys-3 and fipr-22 transcripts as determined by qRT–PCR in WT and atfs-1(4919) or atfs-1 RNAi animals. N = 3. Bars indicate mean ± SEM. *, P < 0.05, **, P < 0.01, ***, P < 0.001 (student’s t-test).
Figure 2. Elevated mitoUPR inhibits the induction of wounding-induced innate immune response genes.

(A) Relative expression of nlp-29, nlp-30, cnc-1, cnc-4, ilys-3 and fipr-22 as determined by qRT–PCR in the wounded animals in both WT and atfs-1(et18gf) mutant. N = 3. Bars indicate mean ± SEM.

(B-E) nlp-30, cnc-11, ilys-3 and fipr-22 transcripts as determined by qRT–PCR in both WT and atfs-1(et18gf) mutants. N = 3. Bars indicate mean ± SEM. *, P < 0.05, **, P < 0.01 (student's t-test).

(F) The fluorescence signal of Phsp-6::GFP in both WT and Pcol-19-ATFS-1::GFP(juSi234) overexpressed animals. Scale bars, 200 μm.

(G) Relative expression of nlp-29, nlp-30, cnc-1, cnc-4, ilys-3 and fipr-22 as determined by qRT–PCR in the wounded animals in both WT and atfs-1(juSi234) overexpressed animals. N = 3. Bars indicate mean ± SEM.
Figure S3

Figure 3. Low level of mitoUPR enhance the induction of AMPs after wounding.

(A) Representative microscopy images of the HSP-6::GFP signal in the unwounded animals fed with an L4440 empty vector, *atfs-1*, *spg-7*, or *ubl-5* dsRNA. Scale bars, 500 μm.

(B) *nlp-29*, *nlp-30*, *cnc-1* and *cnc-11* transcripts as determined by qRT–PCR in the wounded animals fed by L4440 empty vector or *clpP-1* dsRNA. N = 3. Bars indicate mean ± SEM. *, P < 0.05, **, P < 0.01 (student’s t-test).
Figure 2. Epidermal wounding inhibits ATFS-1-dependent UPR\textsuperscript{mt}

(A) Representative microscopy images of HSP-6::GFP signal before and at later times after needle wounding in both wild-type and \textit{Pcol-19-ATFS-1::GFP(juSi234)} transgenic animals. HSP-6::GFP signal became weaker following wounding. Scale bars, 200 μm.

(B) \textit{hsp-6} expression levels as determined by qPCR in WT animals. 6-8 stabs throughout the whole hypodermis were performed on each animal and 30 young adult animals were collected for each RNA sample. N = 3 replicates. Bars indicate the mean ± SEM. ***, P < 0.001 (Student's t-test).

(C) Representative confocal microscopy images of ATFS-1::GFP and mito::mKate2 before and 3 hours post wounding. \textit{Pcol-19-ATFS-1::GFP(juSi234)}; \textit{Pcol-19-mito::mKate2(zjuSi47)} transgenic animals were used for wounding and imaging. Scale bars, 10μm.
(D) Statistic quantification of ATFS-1::GFP fluorescence intensity in mitochondria 3 hours post wounding. *Pcol-19-ATFS-1::GFP(juSi234);Pcol-19-mito::mKate2(zjuSi47) transgenic animals were used for wounding and statistics. The result was the ratio of green fluorescence to red fluorescence. N ≥ 20. Bars indicate mean ± SEM. *, P < 0.05 (Student's t-test).

(E) Representative confocal microscopy images of DVE-1::GFP in nucleus before and 3 hours post wounding. *Pdve-1-DVE-1::GFP(zcIs39) transgenic animals were used for wounding and imaging. Scale bars, 10μm.

(F) Statistic quantification of DVE-1::GFP fluorescence intensity in nucleus. N ≥ 20. Bars indicate mean ± SEM. ns, P > 0.05 (student's t-test).
Figure 5. ATFS-1 acts upstream of p38MAP kinase and TGF-β pathway

(A) The green fluorescence in transgenic worms carrying a Pnlp-29::GFP reporter was observed post wounding in WT, atfs-1, nsy-1, sek-1 and double mutants atfs-1; nsy-1 and atfs-1; sek-1. Scale bars, 500 mm.

(B-E) tir-1, nsy-1, sek-1 and pmk-1 transcripts as determined by qRT–PCR in WT and atfs-1(tm4525). N = 3. Bars indicate mean ± SEM. ns, P > 0.05 (student's t-test).