The Evolutionarily Conserved ESRE Stress Response Network is Activated by ROS and Mitochondrial Damage

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This PDF includes:
Figures S1-S14
Figure S2

A) Bar graph showing normalized GFP levels for different reporters. The x-axis represents the reporters: 3XESRE::GFP, Phsp-6::GFP, Ptbb-6::GFP, Phsp-16.1::GFP. The y-axis represents normalized GFP values. Different conditions are shown with 'EV(RNAi)' and 'spg-7(RNAi)' conditions. Significance levels are indicated by *** (p < 0.001) and NS (not significant).

B) Images of Phsp-16.1::GFP (ESRE) under different conditions: DMSO, Rotenone, CCCP, Na$_2$SeO$_3$, Phenantholine.
Figure S3
Figure S4
Figure S5

A

Phsp-6::GFP (UPR<sup>mt</sup>)

| Treatment       | DMSO | + Asc | Rotenone | + Asc |
|-----------------|------|-------|----------|-------|
| + NAC           |      |       |          |       |
| + NAC/Asc       |      |       |          |       |

B

Ptbb-6::GFP (MAPK<sup>mt</sup>)

| Treatment       | DMSO | + Asc | Rotenone | + Asc |
|-----------------|------|-------|----------|-------|
| + NAC           |      |       |          |       |
| + NAC/Asc       |      |       |          |       |

C

UPR<sup>mt</sup>

| Treatment       | DMSO | Rotenone |
|-----------------|------|----------|
| None            |      |          |
| NAC             |      |          |
| Asc             |      |          |
| NAC/Asc         |      |          |

D

MAPK<sup>mt</sup>

| Treatment       | DMSO | Rotenone |
|-----------------|------|----------|
| None            |      |          |
| NAC             |      |          |
| Asc             |      |          |
| NAC/Asc         |      |          |
Figure S6
Figure S7
Figure S8
Figure S9
Figure S10

**A** 3XESRE::GFP  |  Phsp-6::GFP  |  Ptbb-6::GFP

- **DMSO**
- **H₂O₂**

**B**

|          | DHE Level |
|----------|-----------|
| Control  | 0         |
| H₂O₂     | 24        |

**C**

|          | Relative H₂O₂ Level |
|----------|---------------------|
| Control  | 0                   |
| H₂O₂     | 24                  |

**D**

| Treatment     | Relative H₂O₂ Level |
|---------------|---------------------|
| DMSO          | NS                  |
| Rotenone      | NS                  |
| TTFA          | NS                  |
| Antimycin A   | NS                  |
| Sodium azide  | NS                  |
| CCCP          | NS                  |

**E**

- **Reporters**
  - ESRE
  - MAPK<sup>mt</sup>
  - UPR<sup>mt</sup>

- **Regression lines**
  - ESRE vs. UPR<sup>mt</sup>: $R = 0.23$, $p = 0.38$
  - ESRE vs. MAPK<sup>mt</sup>: $R = 0.31$, $p = 0.25$
  - UPR<sup>mt</sup> vs. MAPK<sup>mt</sup>: $R = 0.006$, $p = 0.98$

- **p-values**
  - ESRE – UPR<sup>mt</sup>, $p = 0.24$
  - ESRE – MAPK<sup>mt</sup>, $p = 0.36$
  - UPR<sup>mt</sup> – MAPK<sup>mt</sup>, $p = 0.66$
Figure S11
Figure S12

A

RNAi

DMSO

Rotenone

Normalized GFP

EV

atfs-1

pmk-3

pmk-3;atfs-1

***

NS

***

***

***

NS

***

***

***

***

B

3XESRE::GFP

Phsp-16.1::GFP

EV(RNAi)

atfs-1(RNAi)

EV(RNAi)

atfs-1(RNAi)

spg-7(RNAi)

spg-7; atfs-1(RNAi)

spg-7(RNAi)

spg-7; atfs-1(RNAi)
Figure S13
Figure S14

(A) Graph showing normalized fold change for UPR<sup>mt</sup> genes. hsp-6, cyp-14A1, pgp-9, ugt-2, cdr-4, and clec-17 are displayed with NS (not significant) and * (significant) markers.

(B) Graph showing normalized fold change for MAPK<sup>mt</sup> genes. C08E.4, F45E4.1, tbb-6, Y34F4.4, and comt-3 are displayed with * (significant) and ** (very significant) markers.