Acute-stress impairs cytoprotective mechanisms through neural inhibition of the insulin pathway

Maria José De Rosa[1 *], Tania Veuthey[1 *], Jeremy Florman[2], Jeff Grant[2], Gabriela Blanco[1], Natalia Andersen[1], Jamie Donnelly[2], Diego Rayes[1,2 #] and Mark J. Alkema[2 #]

1. Instituto de Investigaciones Bioquímicas de Bahía Blanca (CONICET), Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur,- Bahía Blanca, Argentina.

2. Department of Neurobiology, University of Massachusetts Medical School, Worcester, MA, USA

*Equal contribution, #Corresponding author

SUMMARY

Animals face both acute and long-term stressors that require behavioral and cellular defense strategies. How different stress responses are coordinated remains unclear. We show that the repeated induction of the *C. elegans* flight response reduces its ability to cope with environmental stressors. This acute-stress response triggers the activation of a single pair of neurons that release tyramine, the invertebrate analog of adrenaline/noradrenaline. Tyramine stimulates the DAF-2/Insulin/IGF-1 pathway through the activation of the adrenergic-like receptor TYRA-3 in the intestine. In contrast, exposure to long-term stressors reduces tyramine and DAF-2/IIS signaling, allowing the DAF-16/FOXO transcription factor to translocate to the nucleus and induce the transcription of cytoprotective genes. Tyramine signaling provides a state-dependent neural signal that controls the switch between acute and long-term stress responses to regulate the trade-off between behavioral and cellular defense mechanisms. These findings provide mechanistic insights how acute stress can impair the response to other environmental challenges.

Keywords: Stress, Hormesis, “Fight-or-Flight”, *C. elegans*, Neuromodulation, Tyramine, Insulin Signaling, G-coupled Receptors

INTRODUCTION

Stress consists of the elicited biological response when an animal perceives a threat to its homeostasis (Cannon, 1929; Selye, 1974). The animal's stress response requires different adaptive strategies depending on the nature and duration of the stressor. For instance, when confronted with a potentially harmful stressor the first and most efficient response is a behavioral one. An animal can avoid the negative impacts by removing itself from the threat; *e.g.* by seeking the shade if body temperatures become elevated or by fleeing to avoid predators. An attack, or even the perception of a predator elicits a rapid "fight-or-flight" response to enhance the animal's chance of survival. In mammals, the acute fight-or-flight response leads to the activation of the sympathetic nervous system and the release of "stress hormones", such as adrenaline and noradrenaline (Everly, 2013). These neurohormones trigger an increase in heart rate, blood flow, respiration and release of glucose from energy stores, which prepare the animal for vigorous muscle activity and physical exertion. The high energy-demand of a flight response is short-lived and has no significant impact on the animal's welfare once the threat disappears. However, a behavioral response may be insufficient in some cases. Stressful situations such as temperature challenges, food shortage, hypoxia or oxidative stress generally occur on longer time scales and need more gradual and persistent strategies for adaptation (Harada and Goto, 2017; Morimoto, 2011). The response to long-term environmental stressors triggers the activation of highly conserved cytoprotective processes. Stress-induced activation of transcription factors such as the Forkhead box O (FOXO), Heat Shock Factors (HSFs) and Hypoxia-Inducible Factors (HIFs), drives the expression of antioxidant enzymes and protein chaperones, to cope with protein misfolding and aggregation (Essers et al., 2004; Prahlad et al., 2008). Perhaps counterintuitively, exposure to a low dose of a harmful stressor can make an organism more resistant to higher and otherwise detrimental doses of the same stressor (Wiegant et al., 2012). The induction of improved stress tolerance, called hormesis, has been observed in response to oxidative stress, heat and radiation, in organisms ranging from microbes to plants to humans (Calabrese, 2008; Calabrese and Blain, 2009). This

adaptive response to cellular stress, can even lead to lifespan extension and cross adaptation to other stress factors (Cypser et al., 2006). Improved stress tolerance is thought to depend on protective activation of cytoprotective mechanisms that permit a more effective response to stronger environmental stressors (Wiegant et al., 2012). However, the concept of hormesis, and the nature of its underlying molecular mechanisms, remains contentious (Axelrod et al., 2004; Kaiser, 2003).

While moderate amounts of stress may improve health and increase lifespan (Kouda and Iki, 2010; Shore and Ruvkun, 2013), repeated activation of the acute stress response can have negative impacts. For instance repeated exposure to predators increases oxidative stress, reduces disease resistance and shortens lifespan in insects (McCauley and Dang, 2014). In mammals, the chronic activation of the fight-or-flight response results in immunosuppression (Glaser and Kiecolt-Glaser, 2005). When the acute stress response is perpetuated, as is the case of patients with post-traumatic stress disorder (PTSD), it can lead to accelerated aging and increased susceptibility to metabolic, cardiovascular and infectious diseases (Ahmadi et al., 2011; Wolf et al., 2016). The acute stress response can be triggered by an actual or perceived threat, suggesting a neuronal, non-cell autonomous regulation of cellular defense mechanisms. This is supported by studies in the nematode *Caenorhabditis elegans* in which neuronal perception of harmful situations is needed for the coordination of the systemic stress response (Kang and Avery, 2009; Prahlad et al., 2008). However, how the nervous system affects long-term stress response in animals remains largely obscure.

Like other animals, *C. elegans* faces challenges in its environment that occur either abruptly (*e.g.* predation) or more progressively (food shortage, osmotic stress, oxidation, high or low environmental temperatures). Much like in mammals, the nematode stress response to *e.g.* heat, starvation and oxidative stress features a central role for the DAF-2/ Insulin/IGF-1 signaling (IIS) pathway and the activation of DAF-16/FOXO, SKN-1/NRF and HSF-1 transcription factors (reviewed in Murphy and Hu, 2013). In response to acute challenges *C. elegans* can engage in an escape-response where it rapidly moves away from

a harmful stimulus. The escape response triggers the release of tyramine (Alkema et al., 2005; Pirri et al., 2009), which is structurally and functionally related to adrenaline/noradrenaline (Roeder et al., 2003). Tyramine plays a crucial role in the coordination of independent motor programs, which increases the animal's chances to escape predation (Alkema et al., 2005; Donnelly et al., 2013; Maguire et al., 2011; Pirri et al., 2009).

In this study we examine how the nervous system controls and coordinates behavioral and cellular defense mechanisms. We find that sub-lethal exposure to long-term stressors inhibits tyramine signaling and protects the animal from subsequent stronger stressors. In contrast, acute-stressors trigger tyramine signaling, making animals more sensitive to long–term stressors. Tyramine activation of an adrenergic-like receptor, TYRA-3, in the intestine stimulates the DAF-2/IIS pathway and impairs the cellular cytoprotective mechanisms.

RESULTS

Exposure to mild environmental stressors increases resistance to subsequent stronger challenges

In their natural habitats, animals have to cope with different types of life-threatening challenges (Fig. 1A). Prolonged exposure to environmental stressors can lead to death. For example, *C. elegans* dies within hours when exposed to strong oxidative stress or high temperature (Lithgow et al., 1994; Scott et al., 2002) (Fig. 1B-C, black line). To study the interaction between different stress responses we analyzed whether a mild stress can affect *C. elegans* resistance to subsequent stronger stressors. We induced mild oxidative stress in *C. elegans* by exposing animals (L4 stage) to low non-lethal Fe^{2+} concentrations (1 h at 1mM). Fe^{2+} is a classic inductor of reactive oxygen species (ROS) in multiple biological systems, including *C. elegans* (Almeida Fagundez et al., 2015). After one hour of recovery, animals were subsequently exposed to a stronger oxidative stress (3 mM Fe^{2+}). To quantify possible hormetic effects we calculated a survival index (SI = fraction of surviving pre-treated

animals - fraction of surviving naïve animals). This value is positive when pre-exposure increases stress resistance compared to naïve animals. Animals pre-exposed to mild oxidative stress were more resistant to subsequent strong oxidative stress than naive animals (SI = 0.24 ± 0.09, Fig. 1B). Next, we analyzed if a short non-lethal exposure to high temperature (20 min at 35°C) enhanced the resistance to a subsequent prolonged exposure to the same high temperature. Similar to our observations with oxidative stress, short pre-exposure to heat increased animal survival at high temperatures (SI = 0.19 ± 0.04, Fig. 1C). Our results indicate that pre-exposure of *C. elegans* to a mild stressor enhances the animal's resistance to subsequent exposure to the same stressor, which confirms the existence of hormesis in *C. elegans* (Cypser et al., 2006).

To determine whether pre-exposure with one stressor can induce cross-resistance to other types of stressors, animals were briefly exposed to high temperature (20 min at 35°C) and analyzed for subsequent resistance to strong oxidative stress (Fig. 1B). Strikingly, pre-exposure to heat resulted in an increased resistance to strong oxidative stress (Fig. 1B, SI = 0.20 ± 0.06). This enhanced survival to oxidative stress is similar to that achieved after iron pre-exposure. In the reciprocal experiment animals were pre-exposed to low oxidative stress, followed by exposure to high temperature. Pre-exposure to low oxidative stress also increased thermotolerance to a similar extent to that of mild-heat pretreatment (Fig. 1C, SI = 0.18 ± 0.03). This indicates that mild heat and oxidation provide cross-resistance to subsequent stronger forms of these stressors.

Does this hormetic cross-resistance extend to other forms of stress? Another major stressor limiting animal survival, growth, and reproduction is food deprivation. To analyze whether nutritional stress can affect tolerance to either oxidative or thermal stress, animals were food-deprived for eight hours and, after recovery, exposed to either oxidative or thermal stress. Food-deprived animals were more resistant to oxidative (SI = 0.37 ± 0.05) and thermal stress (SI = 0.20 ± 0.06) compared to untreated worms (Fig. 1B and C). Therefore, our results indicate that mild chemical-, physical- and nutritional-stressors can

confer cross-protection to subsequent challenges. This suggests that hormesis relies on common protective mechanisms that allow animals to better resist subsequent challenges independently of the nature of the environmental stressor.

The *C. elegans* escape response impairs the resistance to subsequent stressors

In addition to long-term stressors, *C. elegans* also encounters acute stressors in its environment (Fig. 1A). For instance, *C. elegans* engages in a flight response to escape from predacious fungi that ensnare nematodes with hyphal nooses (Maguire et al., 2011). This escape response, in which the animals reverse and turn away from the stimulus, is prompted by the activation of mechanosensory neurons (Chalfie and Sulston, 1981; Chalfie et al., 1985). To analyze if the escape response affects tolerance to subsequent environmental stressors, we repetitively triggered the escape response by applying a vibrational stimulus to the side of the plate ("tap") every 5 minutes for 2.5 hours. This spaced protocol elicited an escape response after every mechanical tap (Rankin, 1991; Wicks and Rankin, 1995), with no obvious adverse consequences on behavior or survival (see also Fig. 3B). After a 1 hour recovery period, animals were subjected to either oxidative or thermal stress (Fig. 1B and 1C). In sharp contrast to the protective effects observed with mild environmental stressors, the repetitive induction of the escape response markedly reduced resistance to subsequent oxidative (Fig. 1B, SI = -0.19 ± 0.03) or thermal stress (Fig. 1C, SI = -0.12 ± 0.01). These results suggest that the escape response impairs resistance to subsequent long-term environmental stressors.

Tyramine deficient animals are resistant to environmental stress

Tyramine plays a crucial role in the coordination of independent motor programs of the *C. elegans* escape response (Donnelly et al., 2013; Maguire et al., 2011). Since the escape response impaired resistance to subsequent environmental stressors we analyzed the role of tyramine in the animal's environmental stress response. We found that tyramine-deficient *tdc-1* mutants are more resistant to both Fe^{2+} and H_2O_2-induced oxidative stress

than wild-type animals (Fig. 2A and Fig. S1B-C). Furthermore, *tdc-1* mutants have increased thermotolerance compared to wild-type animals (Fig. 2A and Fig. S1D). *tdc-1* mutants have no obvious defects in pharyngeal pumping (Fig. S1E), indicating that dietary restriction is not the cause of the enhanced stress resistance. Tyramine is also a precursor for octopamine biosynthesis (Alkema et al., 2005) (Fig. S1A). *tbh-1* mutants, which still can produce tyramine, but fail to synthesize octopamine, displayed normal sensitivity to oxidative stressors (Fig. S1B, C). Octopamine-deficient mutants were slightly more heat resistant than wild-type animals, albeit not at the level of tyramine/octopamine deficient *tdc-1* mutants (Fig. S1D). Moreover, rescue of *tdc-1* expression in only the octopaminergic neurons *(tdc-1; Ptbh-1::*TDC -1*)* failed to reduce thermoresistance of *tdc-1* mutants (Fig. S1D). These results indicate that the lack of tyramine underlies the oxidative and thermal resistant phenotype of *tdc-1* mutants.

Tyramine inhibits hormesis

Since the escape response impairs resistance to subsequent long-term environmental stressors and because tyramine-deficient animals are more resistant to stressors, we asked whether the release of tyramine during the escape response underlies the impaired resistance to subsequent stressors. To address this question, we analyzed if repeated induction of the escape response affected the subsequent oxidative and thermal stress resistance of *tdc-1* mutants. In contrast to wild-type, pre-tapped *tdc-1* animals, did not exhibit impairments in their resistance to either strong oxidative (SI = -0.03 ± 0.02) or thermal stress (SI = -0.03 ± 0.01) (Fig. 2A and B). This suggests that, tyramine release during the escape response impairs the animal's capacity to respond to subsequent environmental stressors.

To further test our hypothesis that tyramine inhibits hormesis, we repeated the hormesis experiments in the presence of exogenous tyramine during exposure to the first minor stressor (Fig. 2C). Exogenous tyramine reversed, or strongly reduced the hormetic

effects of animals exposed to mild oxidative, thermal, or nutritional pre-stressors (Fig. 2C). Pre-incubation with exogenous tyramine in the absence of environmental pre-stressors did not produce significant differences in subsequent thermal and oxidative stress resistance (Fig. S2). While tyramine signaling increases the animal's chances to survive acute predatory challenges (Maguire et al., 2011), our experiments indicate that tyramine inhibits hormesis.

Activation of tyraminergic neurons impairs resistance to environmental stress

Acute and long-term stressors may pose conflicting challenges for the organism. Acute stressors, such as predation, trigger a rapid, energy demanding response, while prolonged environmental stressors, like starvation, require a more sustained strategy that conserves energy. What are the consequences of simultaneous activation of these opposing demands? To answer this question, we analyzed whether an animal's response to environmental stress is affected while subjected to an acute stressor. Repeated activation of the escape response by mechanical tap, during exposure to oxidative or heat stress reduced survival of wild-type animals compared to animals that were subjected to oxidative or heat stress alone (Fig. 3, Fig. S3A). While the repeated mechanical tap by itself has no obvious consequences on viability (Fig. 3B), we could not exclude that this vibrational stimulus caused physical damage that impairs the animal's defense mechanisms. To avoid mechanical stress on the animal, we triggered the escape response by optogenetic activation of mechanosensory neurons in transgenic animals expressing the red-shifted channelrhodopsin, ChRimson, under the P*mec-4* promoter. Transgenic P*mec-4*::ChRimson animals that were grown on plates containing the ChRimson co-factor all-*trans* retinal (ATR) showed robust behavioral responses to red light, indistinguishable from the escape response triggered by mechanical plate tap (Fig. 3D). Optogenetic activation of the escape response in P*mec-4*::ChRimson animals showed reduced survival to oxidative stress and heat compared to animals raised in the absence of ATR (Fig. 3D; Fig. S3B). The mere addition of

ATR, without optogenetic activation, caused no effect on the survival of animals exposed to either oxidative or thermal stress (Fig. S3C). This indicates that the activation of the escape response itself, instead of tap-induced mechanical impact, impairs resistance to environmental stressors.

Previous studies proposed that stimulation of the ALM/AVM touch receptor neurons (TRN) initiates the escape response through activation of the AVD/AVA backward pre-motor interneurons (Chalfie and Sulston, 1981; Chalfie et al., 1985) (Fig. 3A). The AVA neurons in turn activate the single pair of tyraminergic RIM neurons (Pirri et al., 2009; Pirri et al., 2015). Tyramine signaling coordinates head movements, reversal length and ventral bending during the escape response. Is activation of the tyraminergic RIM neurons sufficient to impair stress resistance? Optogenetic activation of RIM, in transgenic animals that express channelrhodopsin (ChR2) in the tyraminergic neurons (P*cex-1*::ChR2), was not sufficient to trigger an escape response (Fig. 3E). However, RIM activation did reduce resistance to oxidative stress in animals raised on ATR compared to those lacking ATR (Fig. 3E). Furthermore, exposure to simultaneous tapping and oxidative or heat stress did not affect survival of tyramine-deficient *tdc-1* mutants (Fig. 3C, Fig. S3A). Finally, we analyzed the stress resistance of transgenic animals in which tyramine release is inhibited through the expression of a *Drosophila* histamine-gated chloride channel in the RIM neurons (*Pcex-1*::HisCl1) (Pokala et al., 2014; Jin et al., 2016). Similar to tyramine-deficient mutants, silencing of RIM neurons by exogenous histamine increased resistance to oxidative and heat stress compared to control animals (Fig. 3F-G). These results indicate that tyramine release from the RIM neurons impairs resistance to long-term stressors.

Dynamic changes in tyraminergic signaling underlie the response to acute *vs* long-term stressors

While tyramine signaling is beneficial during the escape response (Maguire et al., 2011), tyramine signaling negatively impacts the response to environmental stress. This suggests that the up- or down-regulation from basal levels of tyramine signaling may provide

a neural signal to switch between acute and the long-term stress responses. To test this possibility, we monitored calcium levels in the tyraminergic RIM neurons as a proxy for neural activity. Calcium transients were analyzed by expressing GCaMP6 in the RIM neurons of freely-moving animals that were subjected to either acute or long-term stress. In response to mechanical plate tap, there was an immediate rise in RIM calcium levels (Fig. 4A). This increase in RIM activity upon an acute stress stimulus is consistent with neuronal models of the escape response (Alkema et al., 2005; Pirri et al., 2009; Pirri et al., 2015). To examine the effect of environmental stress on RIM neurons, animals were loaded into a microfluidic device and exposed to either oxidative stress or food deprivation after an initial 20-40 min acclimation period. Upon exposure to 6 mM Fe^{2+}, there was a gradual but sustained reduction of calcium levels in RIM neurons, reaching maximum reduction after roughly 20 minutes of oxidative stress (Fig. 4B). Similarly, removal of food also resulted in a gradual decrease in calcium levels in RIM neurons (Fig. 4C). Conversely, when animals that were food-deprived overnight were re-exposed to food, there was a sustained increase in RIM calcium levels. This increase was initiated within 10 minutes after food addition indicating that RIM activity quickly recovers and is likely not due to GCaMP expression changes (Fig. S4). Furthermore, fluorescence levels of a RIM-expressed fluorescence marker that is insensitive to calcium (mCherry) were not decreased upon oxidation or starvation (Fig. 4B and C). This indicates that exposure to environmental stress results in the active down regulation of RIM activity and tyramine signaling.

Activation of adrenergic-like TYRA-3 receptor inhibits the long-term stress response

What are the targets of tyramine? *C. elegans* has four different receptors that are activated by tyramine: three adrenergic-like GPCRs (SER-2, TYRA-2 and TYRA-3) (Bendesky et al., 2011; Komuniecki et al., 2004; Tsalik et al., 2003) and a tyramine-gated chloride channel (LGC-55) (Pirri et al., 2009; Ringstad et al., 2009). We analyzed mutants for each of these receptors to determine which tyramine receptor(s) underlies the effect of tyramine on stress resistance. Like tyramine-deficient animals, *tyra-3* mutants were resistant

to oxidative stress, heat and starvation (Fig. 5A; Fig. S6). Other receptor mutants did not display obvious increases in resistance. Moreover, exogenous tyramine, which impairs the stress resistance of wild-type and *tdc-1* animals (Fig. 5B), did not reduce the stress resistance of *tyra-3* mutants (Fig. 5B). This shows that tyraminergic activation of TYRA-3 is required to modulation the stress response.

Where does TYRA-3 act? A *Ptyra-3*::GFP reporter, driven by a 3.7 kb promoter is expressed in a subset of neurons in the nervous system and the intestine (Fig. 6A). We identified a 1.8 kb *tyra-3* promoter fragment, which drives reporter expression in the neuronal subset, but not the intestine (*Ptyra-3*$_{short}$*::mCherry;* Fig. S5). Expression of *tyra-3* in neurons (*Ptyra-3*$_{short}$*::*TYRA-3) failed to rescue the stress resistant phenotype in *tyra-3* mutants. However, expression of *tyra-3* in the intestine (*Pelt-2::*TYRA-3) was sufficient to restore the stress sensitivity of *tyra-3* mutants to wild-type levels (Fig. 6B-D, Fig. S6). Intestinal expression of *tyra-3* also restored the negative impact of exogenous TA on heat, oxidative stress and starvation survival assays (Fig. S6). Furthermore, like tyramine deficient mutants, repeated induction of the escape response in *tyra-3* mutants did not increase sensitivity to subsequent heat or oxidative stress (Fig. 6D). The tap-induced increase in sensitivity to environmental stressors was restored when *tyra-3* is expressed in the intestine (Fig. 6D). Since the RIM neuron has no direct synaptic outputs onto the intestine, these experiments show that tyramine acts as a neurohormone to inhibit the long-term stress response through the activation of TYRA-3 in the intestine.

Tyraminergic inhibition of systemic stress response depends on the DAF-2/IIS pathway

The insulin/IGF-1 signaling (IIS) pathway regulates growth, reproduction, metabolic homeostasis, lifespan and stress resistance from nematodes to humans (reviewed in Fontana et al., 2010; Shore et al., 2012)). In *C. elegans*, loss-of-function mutations in the insulin/IGF-1 receptor ortholog, DAF-2, increase stress resistance and longevity (Kenyon et al., 1993; Arantes-Oliveira et al., 2003). To test whether tyramine acts through the DAF-2/IIS

pathway, we analyzed the effect of tyramine signaling on the stress resistance of *daf-2* mutants. Stress resistance of *tdc-1; daf-2* and *tyra-3; daf-2* double mutants was similar to that of *daf-2* single mutants (Fig. 7A). Furthermore, exogenous tyramine did not impair the stress resistance of *daf-2* mutants (Fig. S7). This suggests that tyramine inhibition of the stress response is dependent on DAF-2/IIS pathway.

The transcription factor DAF-16/FOXO mediates a large portion of the physiological processes that are controlled by the DAF-2/IIS pathway. Environmental stressors reduce the activity of the DAF-2/IGFR, leading to the dephosphorylation and activation of DAF-16/FOXO. Upon activation, DAF-16/FOXO translocates to the nucleus where it induces the expression of stress response genes (Hsu et al., 2003; Yanase et al., 2002). In *C. elegans,* the inhibition of DAF-2/IIS signaling and nuclear translocation of DAF-16/FOXO has been described for a variety of environmental stressors (Essers et al., 2004; McElwee et al., 2003). The striking cross-resistance between heat, oxidative stress and starvation we observed in hormesis experiments (Fig. 1) could be explained by the protective activation of a common defense mechanism. Indeed, DAF-16/FOXO localized to the nucleus in response to a short exposure to heat, oxidative or nutritional stress (Fig. 7B). However, DAF-16/FOXO largely localized to the cytoplasm in response to repeated tap stimulus. To determine how the induction of the escape response and tyramine release affect the DAF-2/IIS pathway, we compared the localization of the translational reporter DAF-16::GFP in wild-type, *tdc-1* and *tyra-3* mutant animals. Under normal growth conditions DAF-16 is mostly cytoplasmic and no differences in its subcellular localization were detected (Fig. S7B). However, after a short heat exposure (35˚C, 10 min), *tdc-1* and *tyra-3* mutants exhibited significantly higher levels of DAF-16/FOXO nuclear accumulation when compared to wild-type background (Fig. 7C). Upon extended heat exposure, DAF-16/FOXO (35˚C, 35 min) is predominantly localized to the nucleus in both the wild-type and tyramine signaling mutants. Nevertheless, exogenous tyramine inhibits DAF-16/FOXO nuclear localization in wild-type and *tdc-1* animals, but not in *tyra-3* mutant animals (Fig. 7D). Intestinal expression of *tyra-3* rescued the DAF-16/FOXO localization phenotype of *tyra-3* mutants (Fig. 7D).

These observations are consistent with the notion that tyramine signaling inhibits DAF-16/FOXO phosphorylation, and the activation of stress response genes.

DISCUSSION

In this study we show that the acute stress response reduces worm ability to cope with environmental challenges. Acute stressors trigger the release of tyramine, which stimulates the DAF-2/IIS pathway through the activation of the adrenergic-like receptor TYRA-3 in the intestine. Exposure to long-term stressors reduces tyramine signaling, allowing DAF-16/FOXO translocation to the nucleus and induction of cytoprotective mechanisms. Tyramine signaling provides a state dependent neural signal that regulates the switch between the acute and long-term stress responses.

Hormesis

Adaptive responses to low levels of environmental challenges have been shown to increase the resistance to stronger stressors from bacteria to plants to animals (Calabrese et al., 2007). If the first signs of stress are indications of worse to come, the protective activation of cellular defense mechanisms may improve stress tolerance under ensuing adversity. This phenomenon, called hormesis, has also been reported for *C. elegans* (Cypser et al., 2006; Przybysz et al., 2009). In accordance, we show that low-dose stressors confer protection to stronger versions of the same stressor. Moreover, we find that a low-dose of one stressor also confers cross-protection to other types of environmental stressors. The occurrence of cross-resistance indicates that cytoprotective and restorative intracellular processes are shared among different environmental stressors (Le Bourg and Rattan, 2008; Rattan, 2004). Either brief food-deprivation, low-dose oxidative or heat stress, lead to the inactivation of DAF-2/IIS, and the translocation of DAF-16/FOXO to the nucleus. DAF-16 nuclear translocation activate stress response genes required for tolerance to heat, radiation, oxidative damage, anoxia as well as pathogen infection (Henderson and Johnson,

2001; Kawli and Tan, 2008; Mendenhall et al., 2006). The shared involvement of the DAF-2/IIS pathway in response to a wide variety of harmful challenges explains, at least in part, the cross-hormetic effect of environmental stressors. Cross-resistance may be a byproduct of the overlapping effects of different stressors. For example, osmotic, ionizing stress and heat can produce oxidative damage (Azzam et al., 2012; Davidson and Schiestl, 2001; Pastor et al., 2009). Alternatively, cross-resistance may have evolved to prepare organisms for impending stress if successive stressful environments are frequently encountered in nature such as heat and UV radiation and desiccation.

Neural modulation of the acute and the long-term stress response

The protective effect of the activation of systemic defense mechanisms is long lasting and can even extend the lifespan of the organism (Fontana et al., 2010; Shore et al., 2012). In contrast, acute stressors require actions on a much shorter time scale. Fight-or-flight responses against a predatory attack or aggression must occur within seconds to avoid imminent harm or even death. In *C. elegans* touch can elicit a flight- or escape-response where the animal reverses and quickly turns away from the mechanical stimulus. In sharp contrast to the hormetic cross-resistance of environmental stressors, the repetitive induction of the escape response impairs animal survival to subsequent stressors. Tyramine, which is structurally and functionally related to vertebrate adrenaline, plays a crucial role in the escape response (Maguire et al., 2011; Pirri et al., 2009). While the activity of the tyraminergic RIM neuron rapidly increases during the escape response, RIM activity decreases upon exposure to environmental stressors such as oxidative stress or food deprivation. These findings support computational models that predict inhibition of RIM activity upon food deprivation (Ghosh et al., 2016) and are consistent with decreased RIM activity in animals exposed to pathogens (Jin et al., 2016). Furthermore, optogenetic activation of the RIM reduces, whereas RIM silencing increases resistance to environmental stressors. This indicates that while tyramine release is needed for the escape response to avoid predation (Maguire et al., 2011), the down-regulation of tyraminergic signaling is

required to deal with long-term stressors. Tyramine may thus provide a neural switch for the animals' response to acute *vs* long-term stressors. Although it is currently unclear how sensory perception of environmental stressors leads to the inhibition of the RIM neuron, it is worth noting that RIM receives synaptic inputs from AIB and AIY neurons, key players in the *C. elegans* starvation- and thermal-response (Kang and Avery, 2009; Mori and Ohshima, 1995; Ohnishi et al., 2011).

Tyramine stimulates the DAF-2 insulin pathway

We show that tyramine acts as a neurohormone that inhibits the systemic stress response through the activation of the adrenergic-like GPCR, TYRA-3, in the intestine. During the acute stress response TYRA-3 stimulates the DAF-2/IIs pathway and inhibits DAF-16/FOXO translocation to the nucleus (Fig. 8). Stimulation of DAF-2/IIs pathway is linked to increased metabolic rate (Braeckman et al., 2002; Van Voorhies and Ward, 1999;,reviewed in Ashrafi, 2007). The metabolic rate also rises during the flight response in many animals (Hawlena and Schmitz, 2010; Lagos and Herberstein, 2017). Therefore, the extra-synaptic activation of TYRA-3 may induce a metabolic shift to provide the fuel needed for the high-energy demand of the *C. elegans* escape response. TYRA-3, a predicted Gq-coupled GPCR (Wragg et al., 2007), may promote phospholipase C (PLC) activity and stimulate PKC and inositol 1,4,5-trisphosphate (IP3)-mediated intracellular calcium release (Berridge, 1993). Gq-coupled adrenergic receptors have been shown to stimulate the PKC-PI3K-AKT signaling pathways (Guo et al., 2006; Strittmatter et al., 2012). Further studies are needed to elucidate if TYRA-3 promotes DAF-16 phosphorylation in the intestine by direct stimulation of AKT. It is noteworthy that TYRA-3 activation inhibits DAF-16 translocation, not only in the intestine, but also in other tissues. This suggests that tyramine-dependent Gq activation may also stimulate the release of Insulin-Like Peptides (ILPs) from the intestine to further inhibit cytoprotective pathways in non-intestinal cells. The fact that activation of Gq-receptors is critical for insulin secretion in mammals supports this idea (Latour et al., 2007; Sassmann et al., 2010).

Acute stress and cytoprotection

While isolated cells have the intrinsic capacity to respond to stress (e.g. HSPs, SOD and/or autophagy induction), the coordination of these cytoprotective mechanisms in multi-cellular organisms depends on the neural perception of the stressors (McEwen, 2007). In *C. elegans* for instance, the systemic heat shock response is regulated by the AFD thermosensory neuron, which stimulates the systemic activation of HSF1 in remote tissues (Prahlad et al., 2008; Tatum et al., 2015). Although the activation of cytoprotective pathways safeguard against the potentially greater costs of repair, replacement, and death, it drains resources from animal development and reproduction. For instance, *C. elegans* stress resistant mutants display slow developmental rates and reduced brood sizes (Crawford et al., 2007; Swanson and Riddle, 1981). An animal benefits most from limiting the activation of cytoprotective pathways when they are not necessary, while retaining inducible capacity for emergency defense. Tyramine thus provides a neural signal to inhibit the activation of cell-intrinsic defenses. While tyramine release facilitates and prepares the animal's escape from a threatening stimulus, the down regulation of tyramine signaling is crucial to deal with environmental stressors in which a behavioral response fails to alleviate the exposure to the stressor.

In vertebrates, acute stress stimulates the release of adrenaline and noradrenalin: key inducers of the animal's fight-or-flight response (Cannon, 1929; Selye, 1974). Frequent exposure to acute stressors, such as predators or even predator odors leads to the chronic release of these stress hormones (Boonstra, 2013; Sabban and Kvetnansky, 2001). Chronic activation of the fight-or-flight response weakens the animals defense systems to other stressors (Odio et al., 1987; Travers et al., 2010). For instance, salmon previously exposed to predators had significantly higher mortality when exposed to an osmotic stressor (Jarvi, 1990) and sparrows subject to frequent nest predation exhibit lower antioxidant capacity (Travers et al., 2010). In humans, persistent experience of either real or perceived danger

has been associated with adverse health, and premature death (Aamodt et al., 1991; Dhabhar, 2014; Glaser and Kiecolt-Glaser, 2005). For example, patients that suffer from post-traumatic stress disorder (PTSD) exhibit high risks of neurodegenerative, cardiovascular, respiratory and gastrointestinal diseases, immune dysfunction and premature aging (Groer et al., 2015; Kempuraj et al., 2017; Miller and Sadeh, 2014; Porhomayon et al., 2014). Severity of PTSD symptoms correlates with high levels of noradrenaline, suggesting that increased sympathetic arousal may be closely linked to the dysregulation of body fitness (Pietrzak et al., 2013; Strawn and Geracioti, 2008; Yehuda et al., 1992). How increased levels of stress neurohormones impair general health and defense mechanisms in humans is currently unclear. Given the striking conservation in neural control of stress responses, it will be interesting to determine whether adrenaline and noradrenaline negatively impact health and aging through the inhibition of insulin-dependent cytoprotective pathways.

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AUTHOR CONTRIBUTIONS

MJDR, TV, JF, JG, GB, NA, JD and DR performed the experiments and analyzed the data. MJDR, TV, JF, JG, DR and MJA designed the experiments. DR and MJA conceived the study, and wrote the paper.

EXPERIMENTAL PROCEDURES

Standard *C. elegans* culture and molecular biology methods were used. A strain list and detailed information are provided in Supplemental Experimental Procedures

Stress resistance assays

Heat Stress- The thermotolerance assays were performed as described (Prahlad et al., 2008) with a few modifications. For each strain, four 35 mm NGM agar plates containing 20 animals (with or without 10 mM exogenous tyramine) were incubated at 35ºC for 4 h. To ensure proper heat transfer, 6 mm thick NGM agar plates were used. Animals were synchronized as L4s and used 14 h later. Plates were sealed with Parafilm in zip-lock bags, and immersed in a water bath equilibrated to the appropriated temperature. After heat

treatment, surviving animals were counted after 20 h at 20ºC. Animals were scored as dead if they failed to respond to prodding with a platinum-wire pick to the nose. For longitudinal thermotolerance assays, we used a room at 35ºC and survival was quantified every 20 min on NGM plates containing 30-40 young adults.

Oxidative stress- Iron sulfate ($FeSO_4$) was used as an oxidative stressor. For Fe^{2+} treatment, 20 L4 worms were transferred to 35 mm agar plates containing $FeSO_4$ at the indicated concentration and time (for example 1 mM, 1 h for mild stress and 15 mM, 1 h for strong stress). Animals were scored as dead if they failed to respond to gentle prodding with a platinum-wire pick in the nose. For longitudinal assays we scored the survival every 20 minutes in agar plates containing 3 mM Fe^{2+}.

Food deprivation- L4 animals were washed off with liquid M9 buffer and then were seeded in a 96 multiwell plate (one worm per well) containing M9 with or without tyramine (10 mM). Survival was monitored every day until no living animals were observed. Animals that did not move in response to touch and displayed no pharyngeal pumping were considered as dead. Animals with larvae in their uterus ("bag of worms") were only occasionally observed, and those animals were discarded from the assay.

Hormesis assays

Young adult worms were exposed to mild oxidation (1 mM Fe^{2+} for 1 h), fasting (8 h without food), heating (35ºC for 20 min) or chronic acute stress (by tapping plates every 5 min during 2.5 h). All the pretreatments were performed both in the presence or absence of exogenous tyramine (10 mM). After a recovery time (1 h) the animals were exposed either to high Fe^{+} concentration (3 mM) or heat (35ºC). Animal survival was evaluated every 20 min.

Results are expressed as the survival index (SI) calculated as follow:

SI = (number of surviving pretreated worms/ total number of pretreated animals) - (number of non-pretreated surviving animals/total number of non-pretreated animals). To calculate the SI, the number of survivors was scored after 2 h of exposition to 3 mM Fe^{2+} or 4 h of exposition to 35ºC.

Simultaneous acute and oxidative stress assays

L4 worms were placed on 3 mM Fe^{2+} plates, seeded with a thin lawn of 3 mM Fe^{2+} OP50, and subjected to either a plate tap or a 5 sec light pulse every 5 min for 2.5 h. tap stimuli and LED were delivered using custom Matlab script to an Arduino Uno microcontroller driving a Mightex compact universal LED controller (Mightex SLC-MA02-U). Tap stimuli were delivered using a linear push solenoid (Saia-Burgess195205-127 / STA / 75L STA 0º PUSH). Light pulses for optogenetic experiments (617 nm for ChRimson activation and 470 nm for ChannelRhodopsin) were delivered using Mightex High-Power LED Collimator Sources (Mightex, LCS-0617-03-11, LCS-0470-03-11). Animals were tracked using Multi-Worm Tracker software (Swierczek et al., 2011) and single frames were captured every minute for use in scoring survival. Survival curves were generated by examining the captured frames and marking worm death events in a custom MATLAB script (Mathworks). Worms were considered dead when they were completely motionless for extended period. Velocity was analyzed using Choreography (Swierczek et al., 2011; Yemini et al., 2011) and custom MATLAB scripts.

RIM activity measurements

Microfluidic devices used for imaging Ca^{2+} dynamics in freely moving animals were prepared using soft lithography courtesy of D. Albrecht (Worcester Polytechnic Institute) (Larsch et al., 2013). Staged L4 animals that expressed GCaMP in the RIM (*lin-15(n765ts);zfEx758[pcex-1::NLSwCherry::SL2::GCaMP6]* were picked and allowed to grow overnight to young adults. Animals were then placed in S Basal buffer, aspirated into a 1 mL glass syringe, and loaded into the microfluidic chamber. Imaging was performed at 2.5 x magnification using an AxioObserver A1 inverted microscope (Zeiss) connected to a Sola SE Light Engine (Lumencor) and an ORCA-Flash 4.0 digital CMOS camera (Hamamatsu). Micromanager Software (Edelstein et al., 2014) was used to control fluorescence timing and image capture. For chronic stress experiments, fluorescent images were captured at a rate of 5 sec/capture,

while for acute mechanical stress at 500 ms/capture. RIM GCaMP 6.0 fluorescence values were analyzed using ImageJ software. A 4 x 4 pixel region of interest (ROI) was drawn around the RIM neuron in each animal, as well as for a background region. The integrated density was then calculated for the background area and subtracted from the image, followed by calculation of integrated density for each ROI. These values were extracted for 6 consecutive image captures starting at the indicated time points. The ROI values for individual animals at a given capture frame were pooled and averaged. The averaged integrated density values for each capture were normalized to the average of the 6 captures of the first time point.

Subcellular DAF-16 localization

DAF-16 translocation was analyzed using the TJ356 strain containing the translational *Pdaf-16*::DAF-16a/b::GFP reporter in a wild-type or tyramine signaling mutant background (*Pdaf-16*::DAF-16a/b::GFP; *tdc-1*; *Pdaf-16*:: DAF-16a/b::GFP; *tyra-3, Pdaf-16*:: DAF-16a/b::GFP; *tyra-3*; *Pelt-2::*TYRA-3). Young adult animals in basal conditions or exposed to either mild (35ºC, 10 min) or strong stress (35ºC, 30 min), with or without exogenous tyramine, were mounted to analyze DAF-16 distribution under a fluorescence microscope. The number of GFP labeled nuclei per animal was quantified by using Image J FIJI software.

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FIGURE LEGENDS

Figure 1. The escape response impairs the resistance to subsequent environmental stressors.

(A) Different types of stress induce behavioral and cellular responses. **(B)** Scheme for the sequential stress experiments. Pre-stressors: 1 h 1 mM Fe^{2+} (Oxidation), 0.5 h 35ºC (Heat), 8 h food deprivation (Fasting), or vibrational stimulus every 5 min for 2.5 h (Tap). After 1 hour of recovery, survival to strong oxidative stress was evaluated. Representative Kaplan-Meier survival curves of pre-stressed nematodes exposed to subsequent strong oxidative stress (3 mM Fe^{2+}). Curves are representative of three independent replicates. Right: Survival index at 2 h of exposure to strong oxidative stress. Survival index= (number of pre-stressed worms that survived/total number of pre-stressed worms tested) – (number of non-pre-stressed worms that survived/total number of non-pre-stressed worms tested). Positive values mean protection while negative values arise for impairment of oxidative stress resistance compared to non-treated animals. Each bar represents the mean ± standard error of the mean (SEM) from three independent experiments (n = 35-50 worms per condition per experiment). . *p<0.05, ***p<0.001, One-way ANOVA followed by Holm-Sidak's test for multiple comparisons against pre-oxidation **(C)** Pre-stressors are identical to B. Second stressor is heat (35ºC). Left: Representative Kaplan-Meier survival curves of pre-stressed nematodes exposed to subsequent thermal stress. The curves are representative of three independent replicates. Right: Survival index of worms exposed to heat (4 h at 35ºC) after different pre-treatments. Each bar represents the mean ± SEM from four independent experiments (n=35-50 worms per condition per experiment).). . *p<0.05, ***p<0.001, One-way ANOVA followed by Holm-Sidak's test for multiple comparisons against pre-heat. Summary of the lifespan experiments is presented in Table S1.

Figure 2. The negative effect of the escape response on stress resistance 9s medited by tyramine.

(A) Representative Kaplan-Meier survival curves of naïve and pre-stressed (mechanical tap) animals exposed to either oxidation (3 mM Fe^{2+}, left) or heat (35ºC, right). The curves are representative of three independent replicates. Summary of the lifespan experiments are presented in Table S1. Tyramine-deficient mutants, *tdc-1*, are more resistant to environmental stressors and exhibit no impairment of their survival capacity after tapping. **(B)** Survival index of naïve and pre-stressed, wild-type and *tdc-1* mutant for oxidation and heat. Each bar represents the mean ± SEM from three independent experiments (n=30-45 worms per condition per experiment). Statistical significance from wild-type in each type of stress: * $p< 0.05$, ***$p<0.001$, Student's t-test. **(C)** Exogenous Tyramine inhibits the hormetic effects of weak environmental stressors. Pre-stressors are as described in Fig. 1 with the addition of exogenous tyramine at 10 mM during exposure to the mild stressor. Survival index after pre-exposure to mild stressor followed by exposure to strong oxidation (2 h, 3 mM Fe^{2+}, left) or thermal stress (4 h, 35ºC, right). Bars represent SI in the absence (shaded) and presence of tyramine (dark) during the pre-stress treatment, respectively. Error bars represent the SEM from 4-5 independent experiments (n=40-80 worms per experiment per condition). Statistical significance to control (same pre-stressor without tyramine): * $p< 0.05$, **$p< 0.01$, ***$p<0.001$, Student's t-test

Figure 3. Optogenetic activation of RIM neuron is sufficient for impairing the response to environmental stressors.

(A) Neural circuit for *C. elegans* escape response triggered by mechanical stimulation. **(B-C)** Velocity traces and survival curves during strong oxidative stress (3 mM Fe^{2+}) for wild-type (B), or *tdc-1* mutants (C), in the absence or presence of a mechanical stimulus (tap). Velocity remains constant over the 2.5 h duration of recording in the absence of a stimulus (top), but increases rapidly in response to a mechanical plate tap (middle). When animals are placed on oxidative stress plates, tap delivery reduces stress resistance in the wild-type but not in *tdc-1* mutants (bottom). Black squares indicate timing of tap delivery. Red line with tap, grey line without tap. **(D)** Optogenetic activation of the mechanosensory neurons results

in velocity increases when animals are exposed to a 5 seconds light stimulus (top) and a reduction of oxidative stress resistance (bottom). Blue line represents survival curve of animals that were raised in the presence of all-trans retinal (ATR); grey line, animals raised without ATR. Blue squares indicate timing of light delivery. The strain used was: QW1649 *zfIs144[Pmec-4::Chrimson::wCherry, pL15EK].* **(E)** Optogenetic activation of the RIM neuron does not result in velocity changes (top), but is sufficient to reduce oxidative stress resistance in animals raised in the presence of all-trans retinal (bottom). Blue squares indicate timing of light delivery. The strain used was: QW1602 *lin-15(n765ts); zfEx758*[P*cex-1*::NLSwCherry::SL2::GCaMP6]. **(F-G)** Stress survival of animals expressing the histamine-gated chloride channel HisCl in the RIM neuron (RIM::HisCl). Animals were exposed to 10 mM histamine (Hist) prior and during oxidation (1 h, 15 mM Fe^{2+}, F) or heat (4 h at 35°C, G). Specific silencing of the RIM neuron leads to increased animal resistance to environmental stress. Error bars represent the SEM from 5 independent experiments (n=80-100 worms per experiment per condition). Statistical difference from experiments without exogenous histamine: ***$p \leq 0.001$, Student's t-test.

Figure 4. The tyraminergic RIM neuron exhibits inversed activation patterns on response to acute and gradual stressors.

(A) (Top) Acute mechanical stress induces a rapid increase in calcium levels in RIM neurons. The traces on the top represent the average RIM GCaMP6 Ca^{2+} activity (ΔF/F) 5 seconds before and 20 seconds after an mechanical stimulus to the plate in freely-moving animals (Red trace, ~1 sec tap stimulus at black arrow, n=13 animals) or in control animals with no stressor (Grey trace, n=14 animals). Shaded areas around the traces represent SEM for the averaged traces. Bottom: Each row from the heat maps represents the GCaMP6 fluorescence from individual animals that make up the average traces. Traces were sorted by showing the highest change to the lowest change in calcium levels. **(B)** Oxidative stress (6 mM Fe^{2+}) reduces calcium levels in RIM neurons. Overall RIM calcium levels (ΔF/F) are significantly decreased after exposure to oxidation (red trace, n=36 for each data point, 6

independent experiments). Oxidation does not affect mCherry fluorescence intensity in RIM neurons (grey trace, n=15 for each data point, 5 independent experiments). $p<0.0001$, one-way ANOVA, *$p<0.05$, ***$p<0.001$ compared to initial time point, Dunnett's multiple comparison. **(C)** Food-deprivation reduces Ca^{2+} levels in RIM neurons. Overall RIM Ca^{2+} levels (ΔF/F) are significantly decreased after food deprivation (red trace, n=30 for each data point, 5 independent experiments). Food-deprivation does not affect mCherry fluorescence intensity in RIM neurons (grey trace, n=6 for each data point, two independent experiments). $p<0.0001$, two-way ANOVA, *** $p < 0.001$, compared to initial time point, Dunnett's multiple comparison.

Figure 5. Tyraminergic coordination of the stress response is performed through the activation of the GPCR TYRA-3.

(A) Resistance of wild-type and tyramine receptor null-mutants exposed to (Left) oxidation (1 h, 15 mM Fe^{2+}) or (Middle) heat (4 h at 35 ºC). Only *tyra-3* mutant animals are as resistant as *tdc-1* mutant animals to oxidative and heat stress. Bars represent the mean ± SEM from 3-6 independent experiments (n= 80-100 animals per experiment, per condition). Statistical significance from wild-type, *$p<0.05$, ***$p<0.001$, One-way ANOVA followed by Holm-Sidak's test for multiple comparisons. (Right) Representative Kaplan-Meier survival curves of wild-type, *tdc-1* and *tyra-3* mutants exposed to starvation. Animals were food deprived as L4s. The curves are representative of three independent replicates. Summary of the lifespan experiments are presented in Table S1. **(B)** Resistance of wild-type, *tdc-1* and *tyra-3* mutant animals exposed to oxidation het or starvation in the absence and presence of exogenous tyramine (10 mM). Detrimental effects of exogenous tyramine on stress resistance are abolished in *tyra-3* mutant animals. Error bars mean SEM from 3-4 independent experiments (n=60-80 worms per experiment per condition). Statistical differences were evaluated by using Two-way ANOVA, followed by Holm-Sidak's test for multiple comparisons. From wild-type: † $p<0.05$, †† $p<0.01$, ††† $p<0.001$ and from the absence of tyramine within each strain **$p<0.01$ and ***$p<0.001$.

Figure 6. TYRA-3 intestinal expression is essential for the tyraminergic modulation of the stress response.

(A) Representative confocal fluorescence image of transgenic animals expressing GFP driven by a 3.6 kb promoter of *tyra-3a* (*Ptyra-3long*::GFP). GFP expression is observed in both nervous system and intestine. Scale bar: 150 µm. **(B-C)** Survival percentages of *tyra-3* null mutant animals expressing either the neuronal (*Ptyra-3s::*TYRA-3) or the intestinal (*Pelt-2::* TYRA-3) rescue constructs exposed to oxidative stress (1 h, 15 mM Fe^{2+}) (B) or heat (4 h at 35ºC) (C) . Bars represent the mean ± SEM from 3-6 independent experiments (n = 80-100 animals per experiment, per condition). Statistical significance from wild-type, ***p<0.001, One-way ANOVA followed by Holm-Sidak's test for multiple comparisons. **(D)** Left: Representative Kaplan-Meier survival curves of naïve (solid line) or pre-exposed to tapping (dashed line) exposed to strong oxidative stress (3 mM Fe^{2+}). The curves are representative of three independent replicates. Summary of the lifespan experiments are presented in Table S1.

Right: Survival Index to heat exposure (4 h, 35ºC) of animals that have been pre-exposed to tapping. Intestinal expression of *tyra-3* restores the detrimental effect of tapping on the stress response. Bars represent the mean ± SEM from 3 independent experiments (n = 30-40 animals per experiment per condition). Statistical significance, **p<0.01, *p<0.0.5, One-way ANOVA followed by Holm-Sidak's test for multiple comparisons.

Figure 7. Tyramine signaling inhibits stress-dependent nuclear translocation of DAF-16.

(A) Survival percentage of animals exposed to oxidative stress (3 h, 15 mM Fe^{2+}, top) or heat (7 h, 35ºC, bottom). *tdc-1; daf-2* and *tyra-3; daf-2* double mutants are as resistant as *daf-2* single mutant worms. Bars represent the mean ± SEM from 3-4 independent experiments (n~80 animals per experiment per condition). Statistical significance from *daf-2*, ***p<0.001, One-way ANOVA followed by Holm-Sidak's test for multiple comparisons. **(B)**

Top: Representative fluorescence images of young adults expressing the translational reporter *Pdaf-16*::*DAF-16a/b*::GFP after exposure to the mild stressors as tested in the hormesis experiment in Fig. 1 (1h, 1 mM Fe^{2+} (Oxidation), 0.5 h at 35ºC (Heat), 8 h food deprivation (Fasting) and vibrational stimulus every 5 min for 2.5 h (Tap)). Scale bar: 150 µm. Bottom: Scatter dot plot (line at the median) with the number of nuclear DAF-16 per worm (normalized to untreated or naïve animals). Nuclear localization of DAF-16 is significantly higher in animals exposed to long-term stressors compared to animals exposed to an acute stressor (tap). n~ 10-15 worms per condition. Statistical significance from naïve, ***p<0.001, One-way ANOVA followed by Holm-Sidak's test for multiple comparisons. **(C)** Top: Representative fluorescence images of wild-type animals expressing the translational reporter *Pdaf-16::DAF-16a/b::GFP* after heat exposure (10 min at 35ºC). Scale bar: 150 µm. Bottom: Scatter dot plot (line at the median) with the number of nuclear DAF-16 per worm (normalized to wild-type animals). Nuclear localization of DAF-16 is significantly higher in *tdc-1* and *tyra-3* mutants compared to both wild-type animals and animals in which *tyra-3* expression is rescues in the intestine. n~30-60 animals per condition. Statistical significance from wild-type, ***p<0.001, One-way ANOVA followed by Holm-Sidak's test for multiple comparisons. **(D)** Left: Representative fluorescence images (20x magnification) of animals expressing the translational reporter *Pdaf-16*::DAF-16a/b::GFP after heat exposure (30 min at 35ºC) in the absence or presence of exogenous tyramine (10 mM). Animals are shown with the anterior region to the left. Scale bar: 150 µm. Right: Scatter dot plot (line at the median) with the corresponding quantification of the number of DAF-16 positive nuclei per animal (normalized to untreated animals). Exogenous tyramine decreases DAF-16 nuclear localization in the wild-type, *tdc-1* mutants and animals expressing *tyra-3* solely in the intestine. Statistical significance from untreated worms, ***p<0.001, One-way ANOVA followed by Holm-Sidak's test for multiple comparisons.

Figure 8. Model: Tyraminergic modulation of the DAF-2/IIs pathway

Left: Exposure to acute stressors triggers the escape response and the release of tyramine from the RIM neuron. Tyramine activates the adrenergic-like Gq-coupled receptor TYRA-3 in the intestine. Gq-protein coupled receptor signaling activates the IIS pathway, possibly through AKT1. As a result, DAF-16 remains phosphorylated and its translocation to the nucleus is prevented. In parallel, TYRA-3 activation may also stimulate the release of ILPs that can activate the DAF-2 receptor in the intestine and other tissues. Right: Upon exposure to long-term stressors tyramine release from the RIM is inhibited. TYRA-3 becomes inactive leading thus no longer stimulating the DAF-2/IIS pathway. This allows for nuclear translocation of DAF-16/FOXO and, probably, the induction of other DAF-2/IIS dependent factors (HSF-1, SKN-1) resulting in the transcription of cellular stress response genes.

Figure 1

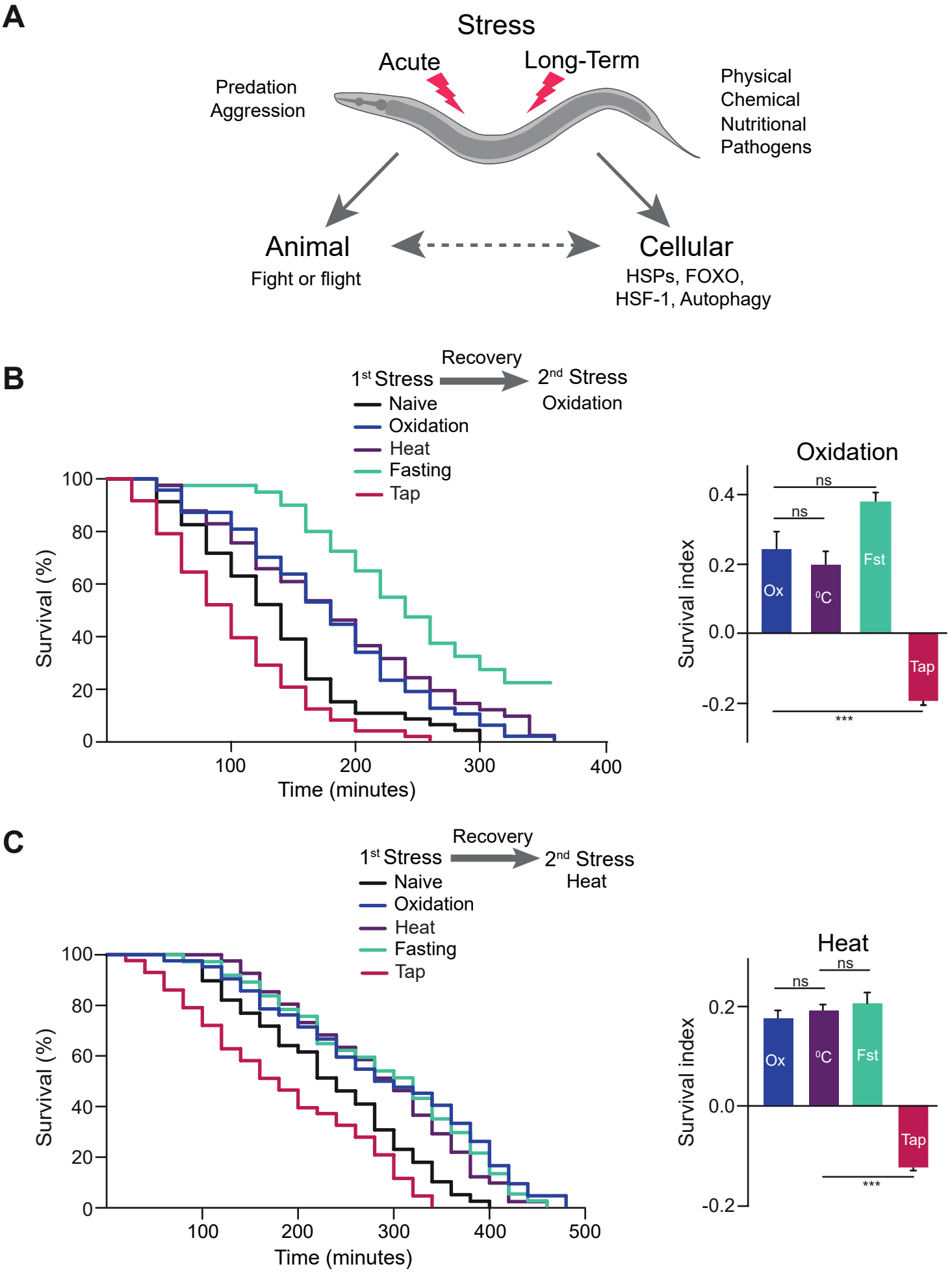

A
Stress
Acute
Long-Term
Predation
Aggression
Physical
Chemical
Nutritional
Pathogens
Animal
Fight or flight
Cellular
HSPs, FOXO,
HSF-1, Autophagy
B
Recovery
1st Stress
2nd Stress
Oxidation
Naive
Oxidation
Heat
Fasting
Tap
Survival (%)
Time (minutes)
Oxidation
Survival index
ns
ns
***
Ox
0C
Fst
Tap
C
Recovery
1st Stress
2nd Stress
Heat
Naive
Oxidation
Heat
Fasting
Tap
Survival (%)
Time (minutes)
Heat
Survival index
ns
ns
***
Ox
0C
Fst
Tap

Figure 2

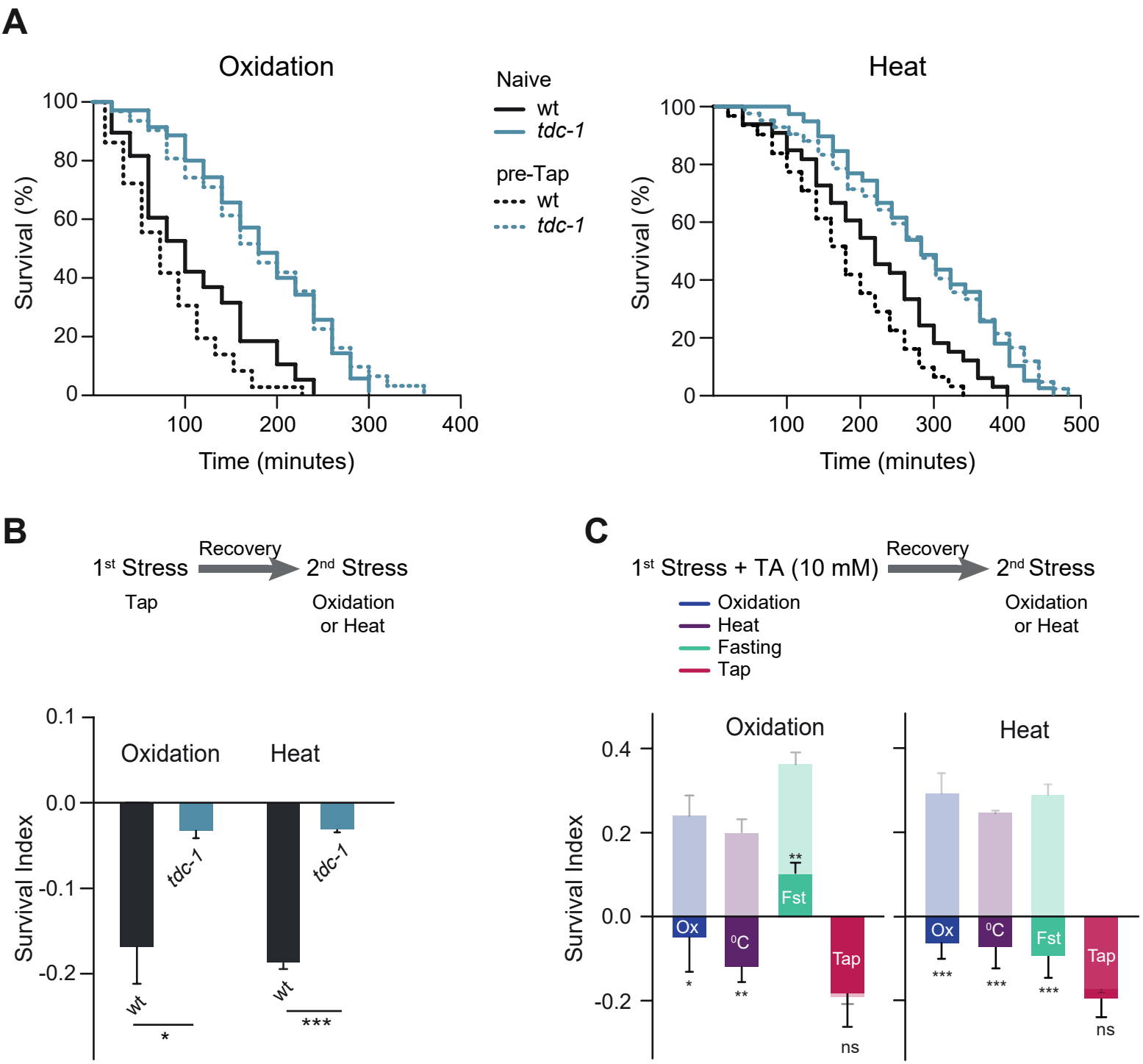
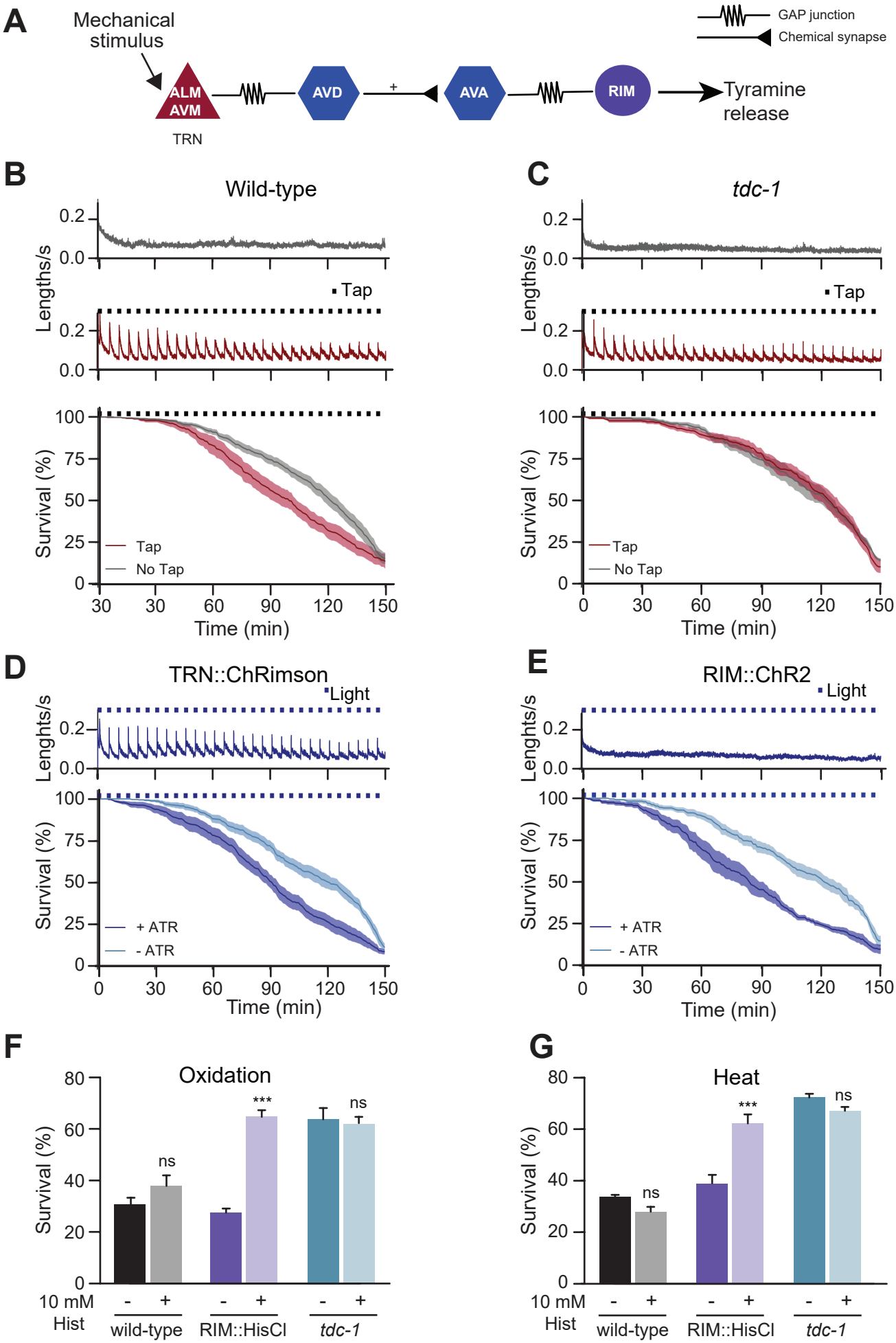

A
Oxidation
Heat
Naive
wt
tdc-1
pre-Tap
wt
tdc-1
Survival (%)
Time (minutes)
B
1st Stress
Recovery
2nd Stress
Tap
Oxidation
or Heat
Survival Index
Oxidation
Heat
tdc-1
wt
*
***
C
1st Stress + TA (10 mM)
Recovery
2nd Stress
Oxidation
or Heat
Oxidation
Heat
Fasting
Tap
Survival Index
Ox
0C
Fst
Tap
ns

Figure 3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Mechanical stimulus
ALM
AVM
TRN
AVD
AVA
RIM
Tyramine release
GAP junction
Chemical synapse
B
Wild-type
Lengths/s
Tap
Survival (%)
Tap
No Tap
Time (min)
C
tdc-1
Lengths/s
Tap
Survival (%)
Tap
No Tap
Time (min)
D
TRN::ChRimson
Lenghts/s
Light
Survival (%)
+ ATR
- ATR
Time (min)
E
RIM::ChR2
Lenghts/s
Light
Survival (%)
+ ATR
- ATR
Time (min)
F
Oxidation
Survival (%)
ns
***
ns
10 mM Hist
wild-type
RIM::HisCl
tdc-1
G
Heat
Survival (%)
ns
***
ns
10 mM Hist
wild-type
RIM::HisCl
tdc-1

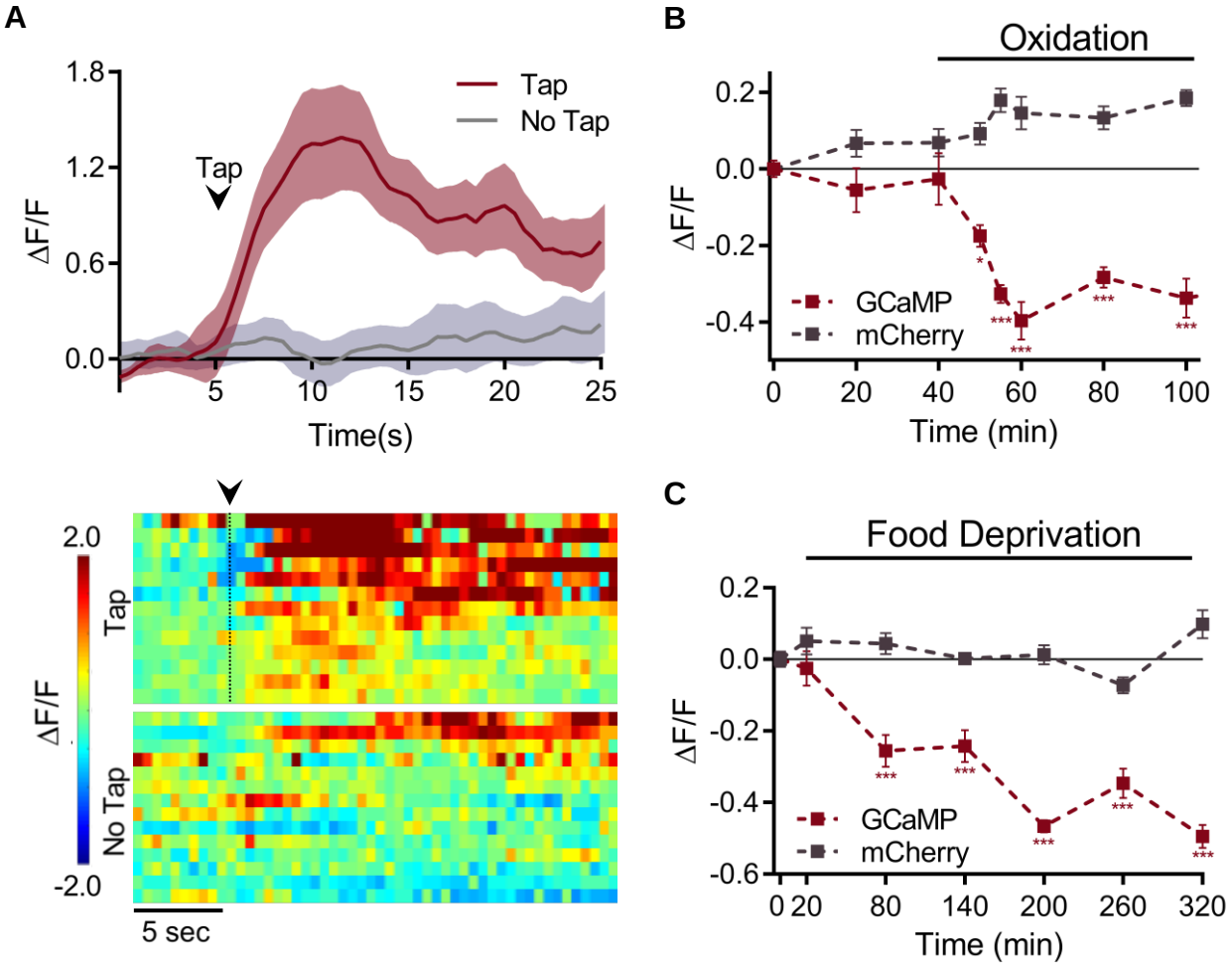

A
1.8
1.2
0.6
0.0
ΔF/F
Tap
Tap
No Tap
5
10
15
20
25
Time(s)
2.0
-2.0
ΔF/F
Tap
No Tap
5 sec
B
Oxidation
0.2
0.0
-0.2
-0.4
ΔF/F
GCaMP
mCherry
0
20
40
60
80
100
Time (min)
C
Food Deprivation
0.2
0.0
-0.2
-0.4
-0.6
ΔF/F
GCaMP
mCherry
0 20
80
140
200
260
320
Time (min)

Figure 5

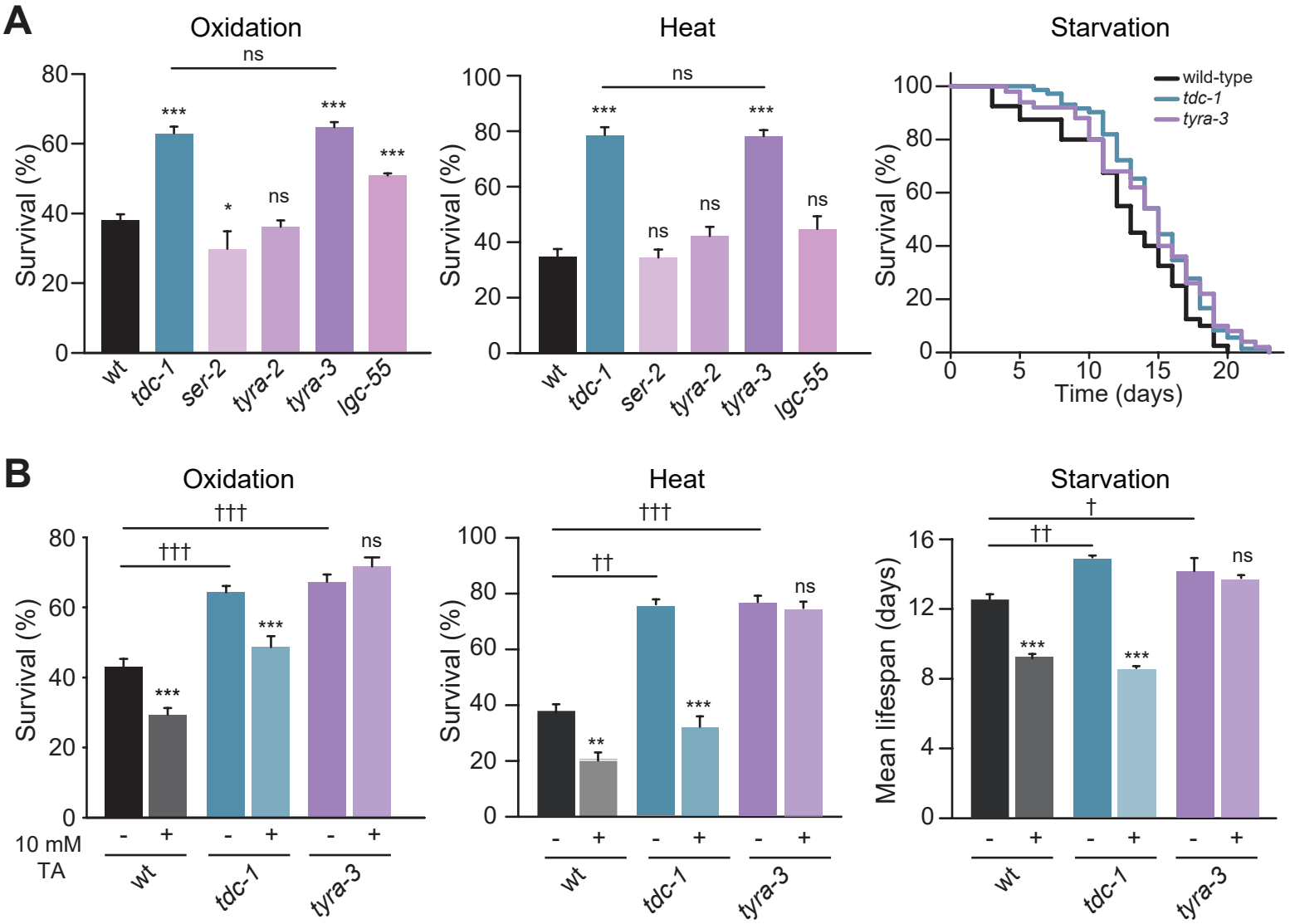

A
Oxidation
Heat
Starvation
Survival (%)
wt
tdc-1
ser-2
tyra-2
tyra-3
lgc-55
ns
***
*
wild-type
tdc-1
tyra-3
Time (days)
B
Oxidation
Heat
Starvation
Survival (%)
Mean lifespan (days)
10 mM TA
†††
††
†
**

Figure 6

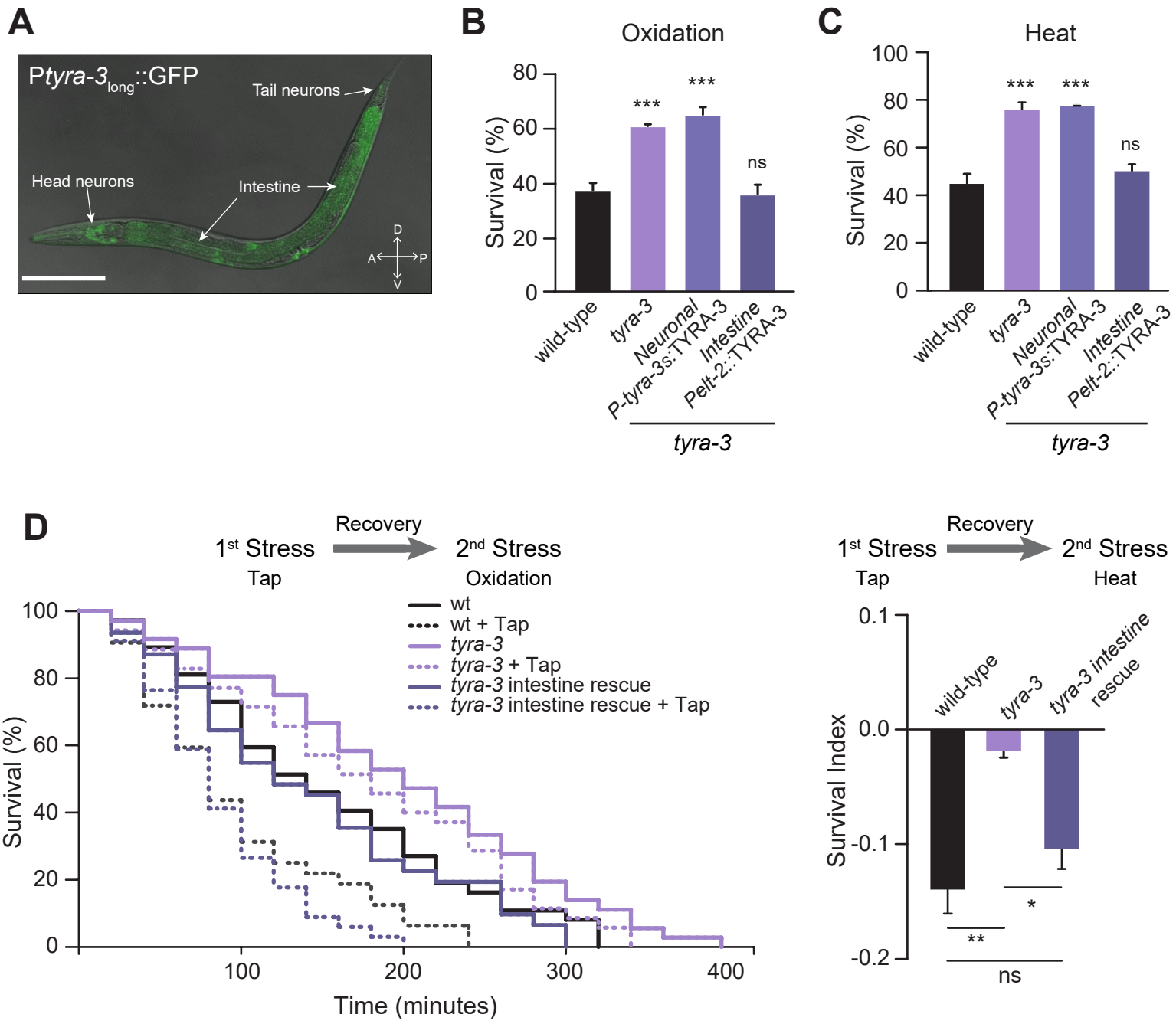
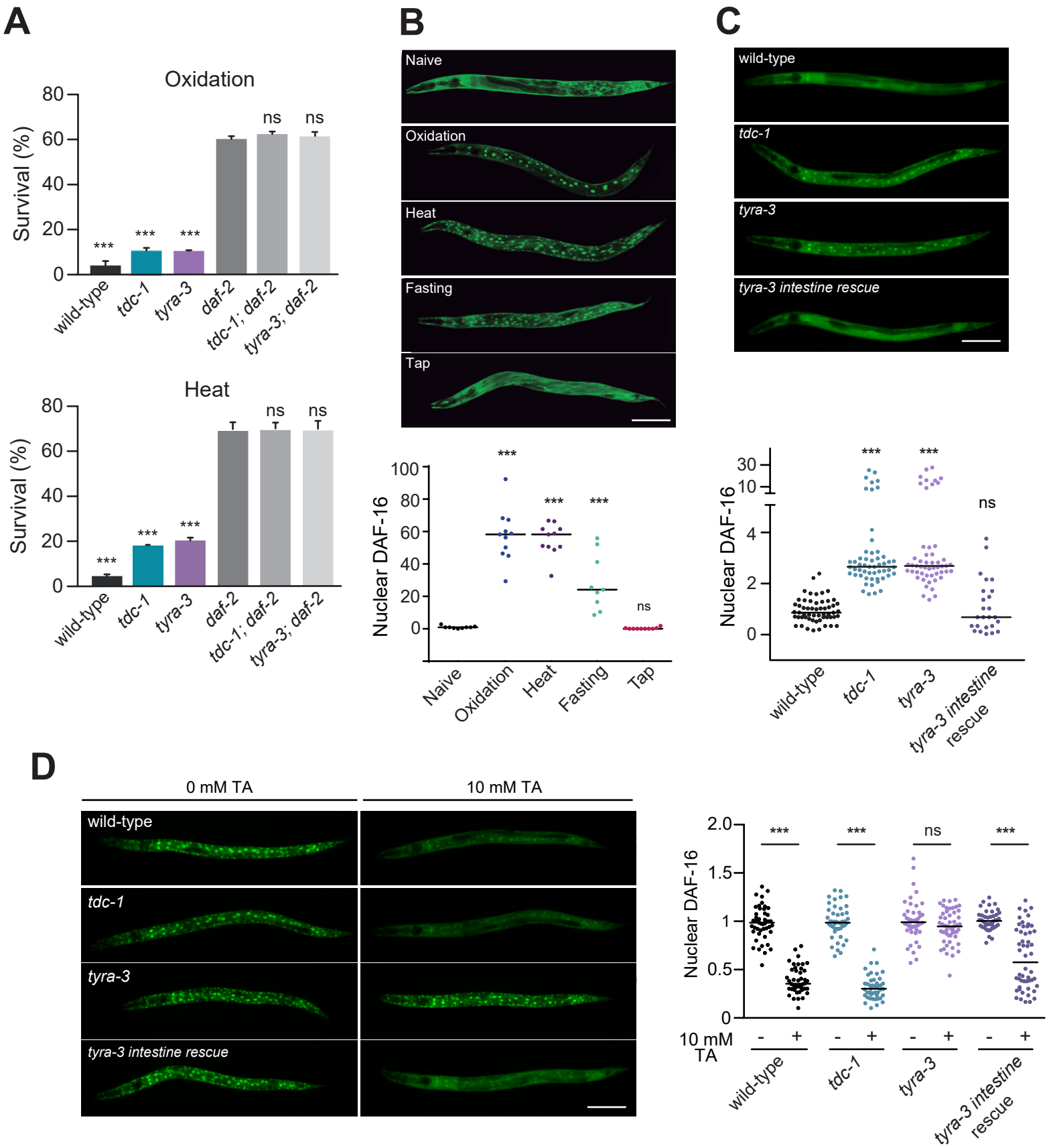
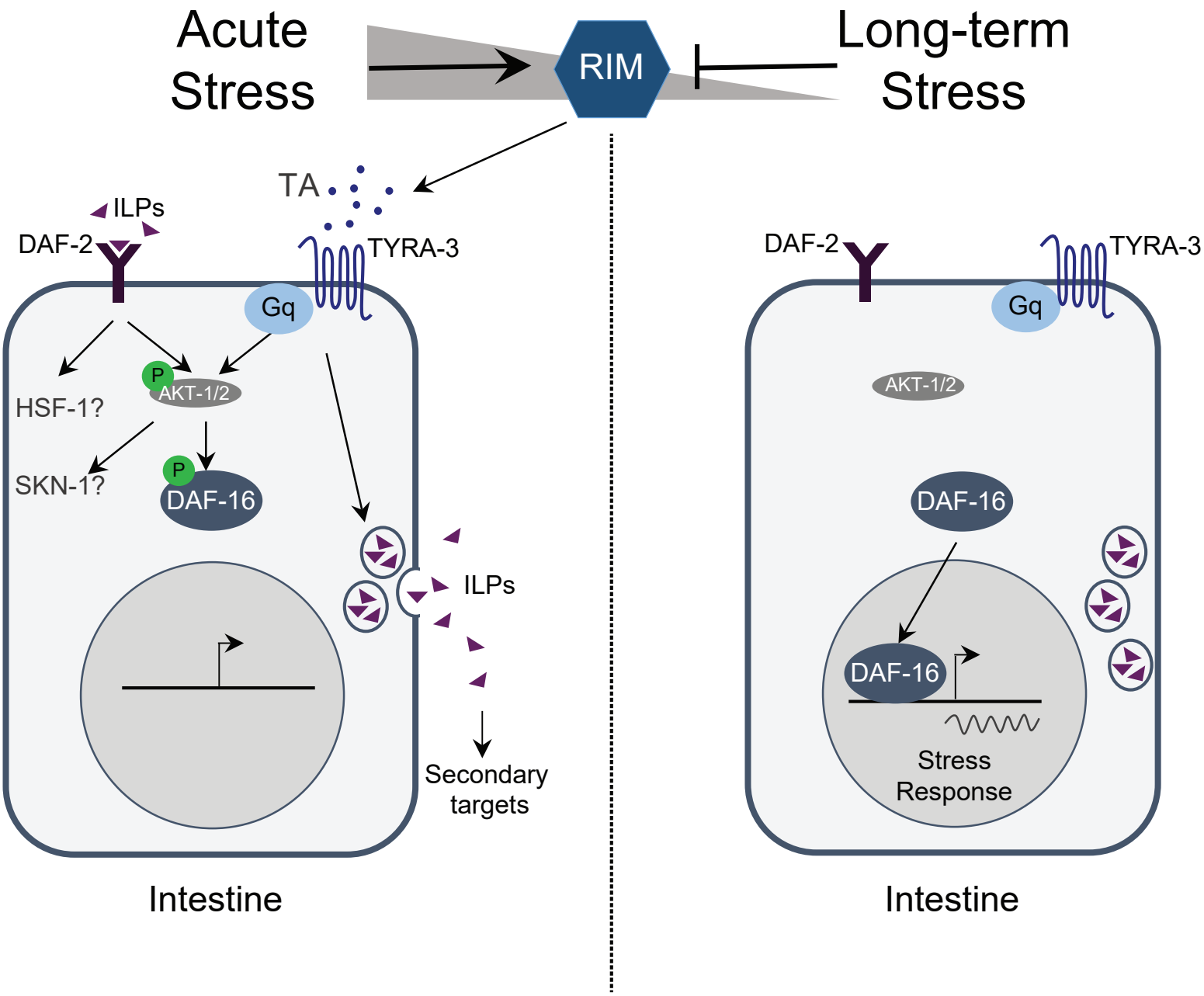

A
Ptyra-3long::GFP
Tail neurons
Head neurons
Intestine
D
A
P
V
B
Oxidation
Survival (%)
80
60
40
20
0
***
***
ns
wild-type
tyra-3
Neuronal P-tyra-3S::TYRA-3
Intestine Pelt-2::TYRA-3
tyra-3
C
Heat
Survival (%)
100
80
60
40
20
0
***
***
ns
wild-type
tyra-3
Neuronal P-tyra-3S::TYRA-3
Intestine Pelt-2::TYRA-3
tyra-3
D
1st Stress
Tap
Recovery
2nd Stress
Oxidation
wt
wt + Tap
tyra-3
tyra-3 + Tap
tyra-3 intestine rescue
tyra-3 intestine rescue + Tap
Survival (%)
100
80
60
40
20
0
100
200
300
400
Time (minutes)
1st Stress
Tap
Recovery
2nd Stress
Heat
Survival Index
0.1
0.0
-0.1
-0.2
wild-type
tyra-3
tyra-3 intestine rescue
**
*
ns

Figure 7


A
Oxidation
Survival (%)
80
60
40
20
0
***
***
***
ns
ns
wild-type
tdc-1
tyra-3
daf-2
tdc-1; daf-2
tyra-3; daf-2
Heat
Survival (%)
80
60
40
20
0
***
***
***
ns
ns
wild-type
tdc-1
tyra-3
daf-2
tdc-1; daf-2
tyra-3; daf-2
B
Naive
Oxidation
Heat
Fasting
Tap
Nuclear DAF-16
100
80
60
40
20
0
***
***
***
ns
Naive
Oxidation
Heat
Fasting
Tap
C
wild-type
tdc-1
tyra-3
tyra-3 intestine rescue
Nuclear DAF-16
30
10
4
2
0
***
***
ns
wild-type
tdc-1
tyra-3
tyra-3 intestine rescue
D
0 mM TA
10 mM TA
wild-type
tdc-1
tyra-3
tyra-3 intestine rescue
Nuclear DAF-16
2.0
1.5
1
0.5
0
***
***
ns
***
10 mM TA
-
+
-
+
-
+
-
+
wild-type
tdc-1
tyra-3
tyra-3 intestine rescue


Acute Stress
RIM
Long-term Stress
TA
ILPs
DAF-2
TYRA-3
Gq
P
AKT-1/2
HSF-1?
SKN-1?
P
DAF-16
ILPs
Secondary targets
Intestine
DAF-2
TYRA-3
Gq
AKT-1/2
DAF-16
DAF-16
Stress Response
Intestine