A Conserved GEF for Rho-Family GTPases Acts in an EGF Signaling Pathway to Promote Sleep-like Quiescence in *Caenorhabditis elegans*

Amanda L. Fry,* Jocelyn T. Laboy,* Huiyan Huang,† Anne C. Hart,† and Kenneth R. Norman*,1
*Center for Cell Biology and Cancer Research, Albany Medical College, New York 12208, and †Department of Neuroscience, Brown University, Providence, Rhode Island 02912

ABSTRACT Sleep is evolutionarily conserved and required for organism homeostasis and survival. Despite this importance, the molecular and cellular mechanisms underlying sleep are not well understood. *Caenorhabditis elegans* exhibits sleep-like behavioral quiescence and thus provides a valuable, simple model system for the study of cellular and molecular regulators of this process. In *C. elegans*, epidermal growth factor receptor (EGFR) signaling is required in the neurosecretory neuron ALA to promote sleep-like behavioral quiescence after cellular stress. We describe a novel role for VAV-1, a conserved guanine nucleotide exchange factor (GEF) for Rho-family GTPases, in regulation of sleep-like behavioral quiescence. VAV-1, in a GEF-dependent manner, acts in ALA to suppress locomotion and feeding during sleep-like behavioral quiescence in response to cellular stress. Additionally, VAV-1 activity is required for EGF-induced sleep-like quiescence and normal levels of EGFR and secretory dense core vesicles in ALA. Importantly, the role of VAV-1 in promoting cellular stress–induced behavioral quiescence is vital for organism health because VAV-1 is required for normal survival after cellular stress.

KEYWORDS behavioral quiescence; *Caenorhabditis elegans*; sleep; Vav

DESPITE being a subject of formal study for over 150 years, sleep is not clearly understood. Moreover, various explanations for the functional role of sleep have been proposed, such as allowing "recharging" of cells following high metabolic activity (Benington and Heller 1995; Tu and McKnight 2006; Scharf *et al.* 2008) and remodeling of synapses built during wakefulness (Tononi and Cirelli 2006). Yet sleep problems have a significant impact on human health and are a leading reason for seeking medical attention (Mahowald and Schenck 2005). Therefore, there is a great need for understanding the cellular and molecular mechanisms that regulate sleep–wake cycles.

Simple model organisms such as *Caenorhabditis elegans* have the potential to provide valuable information regarding sleep regulation. *C. elegans* is known for its easily manipulated genetics, small nervous system with mapped neuronal connectivity, stereotypical behaviors, and the ability to be studied efficiently in large numbers. Numerous studies have shown that *C. elegans* lethargus, a restful period that occurs before each molt of the cuticle during larval development, is neuronally regulated and likely orthologous to sleep in mammals (Van Buskirk and Sternberg 2007; Raizen *et al.* 2008; Van Buskirk and Sternberg 2010; Choi *et al.* 2013; Iwanir *et al.* 2013; Nelson *et al.* 2013; Turek *et al.* 2013; Cho and Sternberg 2014; Singh *et al.* 2014). Lethargus quiescence in *C. elegans* shares several characteristics with mammalian sleep: inactivity (decreased locomotion and cessation of pharyngeal pumping, or feeding), a specific posture, reduced response to aversive stimuli, and rapid reversibility (Cassada and Russell 1975; Raizen *et al.* 2008; Schwarz *et al.* 2012; Iwanir *et al.* 2013; Cho and Sternberg 2014). In addition, lethargus quiescence is under homeostatic regulation (*e.g.*, depriving animals in lethargus or rest by stimulation increases sleep drive), and prolonged deprivation of rest is lethal (Raizen *et al.* 2008; Driver *et al.* 2013), as it is in rats (Rechtschaffen and Bergmann 2002). Recently, *C. elegans* has further been shown to display sleep-like quiescence following

doi: 10.1534/genetics.115.183038
Manuscript received September 23, 2015; accepted for publication January 18, 2016; published Early Online January 21, 2016.
Supporting information is available online at www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.183038/-/DC1.
[1]Corresponding author: Center for Cell Biology and Cancer Research, Albany Medical College, 47 New Scotland Ave., Albany, NY 12208. E-mail: normank@mail.amc.edu

exposure to noxious stimuli that result in cellular stress (Hill *et al.* 2014; Nelson *et al.* 2014). Fascinatingly, molecules that regulate behavioral quiescence in *C. elegans* and *Drosophila* are conserved and influence sleep in mammals, indicating evolutionarily conserved origins of sleep and the potential utility of studying sleep in such simple animals (Zimmerman *et al.* 2008; Sehgal and Mignot 2011; Nelson and Raizen 2013). These molecules include the cAMP protein kinase A and cAMP response element-binding protein (CREB) signaling axis (Graves *et al.* 2003; Nelson *et al.* 2013), cGMP-dependent protein kinase (Raizen *et al.* 2008; Langmesser *et al.* 2009), neuropeptides (van den Pol 2012; Choi *et al.* 2013; Nelson *et al.* 2013, 2014), dopamine (Singh *et al.* 2014), transcription factor AP2 (Mani *et al.* 2005; Turek *et al.* 2013), and epidermal growth factor receptor (EGFR) (Snodgrass-Belt *et al.* 2005; Van Buskirk and Sternberg 2007). Strikingly, EGFR has been shown to be required in only one neuron of *C. elegans* (the ALA interneuron) for proper induction of sleep-like quiescence behavior during lethargus and cellular stress–induced sleep-like quiescence (Van Buskirk and Sternberg 2007; Hill *et al.* 2014).

Previously, we demonstrated that VAV-1, an evolutionarily conserved guanine nucleotide exchange factor (GEF) for Rho-family GTPases, is an important factor in a neural circuit that regulates *C. elegans* locomotion (Fry *et al.* 2014). We found that VAV-1 is required in the ALA interneuron to reduce locomotory speed in active adult animals. Here we show that VAV-1 in the ALA interneuron also serves a critical function to suppress locomotion and feeding behavior during sleep-like quiescence. VAV-1 is required for LIN-3/EGF-induced quiescence and cellular stress–induced quiescence in adulthood. We also show that VAV-1 regulates levels of LET-23/EGFR and IDA-1, which is a conserved dense core vesicle (DCV) transmembrane protein implicated in peptide release (Cai *et al.* 2004, 2009) in the ALA interneuron. Importantly, the role of VAV-1 in quiescence induction after cellular stress is biologically significant because loss of *vav-1* results in animals with impaired quiescence that correlates with impaired survival after stress. We also find that overexpression of constitutively active VAV-1 is sufficient to induce behavioral quiescence in adult animals. Together our results suggest that VAV-1 is a component of ALA neuronal signaling that mediates behavioral quiescence, which is critical for survival of the animal after cellular stress.

Material and Methods

Strains and maintenance

C. elegans strains were maintained and handled according to standard procedures (Stiernagle 2006). Animals were grown on nematode growth medium (NGM) plates seeded with OP50 *Escherichia coli* at 20°, and experiments were performed at ~24° unless otherwise noted. Day 1 adult hermaphrodites were selected for analysis in all experiments except for lethargus assays, for which mid-L4 larval-stage animals were selected. N2 (Bristol) was the wild-type strain. *vav-1* mutants are *vav-1(ak41)* homozygotes with selective expression of wild type *vav-1* in the pharynx, which restores normal pharyngeal activity, unless otherwise noted (Norman *et al.* 2005). The following mutant and transgenic strains were used in this study: N2: Bristol wild-type strain; AML10: *otIs355* (*Prab-3*::*NLS*::*mCherry*); *otIs45* (*Punc-119*::*GFP*); BL5752: *inIs181*; *inIs182* (*Pida-1*::*GFP*); CG21: *egl-30(tg26)*; *him-5(e1490)*; CL2070: *dvIs70* (*Phsp-16*::*GFP*); DA521: *egl-4(ad450sd)*; KM246: *Pida-1*::*ida-1*::*GFP*; KRN118: *vav-1(ak41) takIs5* (*Ppha-4*::*vav-1*::*GFP*); KRN149: *takEx6* (*Pvav-1*::*2xNLS*::*mCherry*); KRN314: *takEx81* [*Pvav-1*::*vav-1(GEF Dead)*::*GFP*]; KRN320: *takEx84* [*Pvav-1(minimal promoter)*::*vav-1ΔSH3B*::*GFP*]; KRN350: *vav-1(ak41)*; *takEx84* [*Pvav-1(minimal promoter)*::*vav-1ΔSH3B*::*GFP*]; KRN396: *vav-1(ak41)*; *takEx101* [*Pvav-1(minimal promoter)*::*vav-1ΔSH3A*::*GFP*]; KRN407: *vav-1(ak41)*; *takEx106* [*Pvav-1(minimal promoter)*::*vav-1*::*GFP*]; KRN423: *vav-1(ak41) takIs5*; *takEx81* [*Pvav-1*::*vav-1 (GEF dead)*::*GFP*]; KRN459: *vav-1(ak41) takIs5*; *takEx107* [*Pvav-1(minimal promoter)*::*vav-1ΔSH2*::*GFP*]; KRN595: *vav-1(ak41) takIs5*; *takEx67* (*Pver-3*::*vav-1*::*GFP*); KRN663: *rgEx235* (*Pplc-3*::*YFP*); KRN738: *vav-1(ak41)*; *takEx215* (*Pvav-1*::*vav-1*); KRN875: *takEx295* [*Phsp-16*::*vav-1(gf)*::*GFP*]; NY2066: *ynIs66* (*Pflp-7*::*GFP*); *him-5(e1490)*; OH10690: *otIs356* (*Prab-3*::*NLS*::*mCherry*); PS4886: *plc-3(tm1340)/mIn1*; PS5970: *syIs197(hs::lin-3C) him-5(e1490)*; TJ375: *gpIs1(hsp-16*::*GFP)*; and VM4240: *vav-1(ak41)*; *akEx87* (*Pvav-1*::*vav-1*::*GFP*).

Molecular biology and transgenesis

To generate SH-domain deletion constructs of VAV-1, we first generated a *vav-1* construct that consisted of 3 kb of the 5′ *cis*-regulatory region of *vav-1* gene that drives a *vav-1* complementary DNA (cDNA) containing a C-terminal GFP tag [the *vav-1* cDNA and GFP tag were isolated from the *Ppha-4*::*vav-1*::*GFP* construct (pRF84) previously described (Norman *et al.* 2005)]. Next, we used site-directed mutagenesis using the QuikChange II XL Site-Directed Mutagenesis Kit (Agilent) to generate SH-domain deletion constructs. Primers used for deletion generation are listed in Supporting Information, Table S1. Plasmids were sequenced to rule out PCR-induced errors. Correct expression of these constructs was confirmed by GFP expression in the pharynx, ALA neuron, and somatic gonad.

To generate an *hsp-16.41*::*vav-1(gf)* construct, we swapped the *myo*-3 5′ *cis*-regulatory region out of pKN19 [*Pmyo-3*::*vav-1(gf)*::*GFP*] (Norman *et al.* 2005; Spooner *et al.* 2012) for the *hsp-16.41* found in pPD49.83 (kind gift from A. Fire) using *Sph*I and *Nhe*I sites.

Transgenic strains were generated by microinjection using standard protocols (Jin 1999). The host strain was wild type (N2, Bristol).

Fluorescence microscopy

Animals were immobilized using 300 μM sodium azide on 2% agarose pads. Fluorescence was determined and differential

interference contrast (DIC) imaging was conducted with an inverted Zeiss AxioObserver microscope equipped with an Andor Clara charged-couple device (CCD) camera and a 40× or 60× objective lens (NA1.4). Images of the ALA neuron cell body were taken specifically in animals with ALA oriented toward the objective (cell body between the objective and midwidth of the animal but not deeper) for fluorescence of the highest intensity. Images of the ALA axon were taken in the posterior region of the animal (between the vulva and the bend of the gonad), and 20 μm was selected in which the *ida-1*::*ida-1*::*GFP* puncta were most in focus (because the strain carries a dominant roller mutation that complicates selection of regions of interest). In each imaging session, mutant strains were analyzed along with wild-type animals. For analysis of ALA translational markers (GFP fusion proteins), in comparisons between wild-type, *vav-1*, and *vav-1*; *vav-1 rescue* strains (Figure 2), images were analyzed using ImageJ (Schneider *et al.* 2012) and a Wacom Bamboo tablet and stylus. Freehand regions of interest (ROIs) were drawn around ALA neuron cell bodies or axon puncta, and the mean fluorescence (for axons, area of puncta) was measured. For each image, mean background fluorescence of a nearby ROI was subtracted from the ALA cell body or punctal mean fluorescence. Mutant data were normalized to wild type to correct for differences in light-source strength because imaging was conducted over months. Between 21 and 49 animals were analyzed per genotype (displayed in figure legends).

Quiescence assays

Lethargus quiescence assays were performed essentially as described previously with microfluidic chambers (Singh *et al.* 2011). Briefly, overnight OP50 *E. coli* culture was concentrated (sevenfold), treated with kanamycin, and resuspended in liquid NGM. This OP50 medium was applied to the microfluidic chambers (6- or 10-well format), and mid-L4 stage animals were loaded into chambers with a worm pick (only one animal per chamber and at least one blank chamber). Animals were staged based on L4 vulval morphology, active locomotion, and active feeding (pharyngeal pumping). A coverslip was placed over the chambers and sealed with molten 2% agarose. Time-lapse video recording (1 frame/10 sec) of all chambers was conducted using an Olympus dissecting microscope, Sony CCD camera, and Wormlab software. Quiescence data were generated using a Matlab image-analysis program that uses pixel differences to denote movement between frames (value of 0) or no movement (value of 1) and plots a rolling average (over 60 frames) called *fractional quiescence* (Singh *et al.* 2011). Recordings were 6- to 12-hr long, and for each animal, 6 hr of data was centered on clear peaks of fractional quiescence surrounded on either side by low background fractional quiescence values (below 0.2). Total quiescence was the total time an animal was quiescent during lethargus (each frame with a quiescence value of 1 represents 10 sec), while the duration of quiescence was the number of minutes within each 6-hr plot with a fractional quiescence value of 0.2 or more (in a consolidated bout of quiescence).

Heat shock–induced overexpression of LIN-3/EGF was carried out essentially as described previously (Van Buskirk and Sternberg 2007). Briefly, parafilmed agar plates bearing test animals were fully submerged in a 34.5° water bath for 30 min (topped with weights). Young-adult animals were then immediately transferred to fresh NGM plates seeded with OP50 to recover at 20° for 2 hr, and all quiescence measurements were conducted within the following 3 hr. For locomotory quiescence assays, each plate of heat-shocked and recovered animals was placed on an Olympus SZ61 stereomicroscope equipped with a Sony XCD-V60 CCD camera, and animals were allowed to equilibrate for 2 min. A 1-min movie was taken (at 7.5 frames/sec) of each plate. An animal was considered quiescent if its behavior fulfilled two criteria: the animal did not move three body bends or more within 1 min (both forward and backward movements were considered), and the animal spent at least one period of 10 sec or more absolutely still (not crawling and not foraging, *i.e.*, moving just the head). For Figure 3, 50–120 total animals were analyzed per genotype in at least three independent experiments (except for non-heat-shocked hs:LIN-3 and hs:LIN-3; *vav-1* controls, of which 10–20 animals were analyzed in one or two independent experiments). For Figure S2, 50–60 animals were analyzed per genotype.

Feeding quiescence was determined by cessation of pharyngeal pumping, observed by eye using a dissecting microscope (Van Buskirk and Sternberg 2007). Each animal was observed for 5 sec, and if any full contraction of the pharyngeal terminal bulb occurred, the animal was scored as "pumping." In total, 120–150 animals per strain were scored for pharyngeal pumping in four to six independent experiments. For Figure S1 and Figure S2, 50–60 animals were analyzed per genotype.

Feeding quiescence following heat shock–induced overexpression of constitutively active VAV-1 [*hsp-16.41*::*vav-1(gf)*] was performed as described earlier (Van Buskirk and Sternberg 2007). Young-adult animals were exposed to 33° for 30 min and allowed to recover for 2 hr before scoring for feeding quiescence. Between 40 and 60 animals were analyzed per genotype.

Heat stress–induced quiescence was induced essentially as described previously (Hill *et al.* 2014). Parafilm-sealed plates bearing test animals were fully submerged in a 35.5° water bath for 30 min. Heat shock of each strain in an assay was staggered by 2.5–3 min to allow precise 30-min exposure and assessment at recovery time points. After heat exposure, animals were quickly removed from plates to fresh ∼24° plates seeded with OP50 *E. coli* and assessed for feeding quiescence (lack of pharyngeal pumping observed for ∼5 sec per animal) every 10 min (at earlier time points), 30 min, or 60 min (at later time points) during recovery. Between 80 and 210 animals per strain were tested in total in four to seven independent experiments.

Heat stress survival

Parafilmed plates bearing test animals were fully submerged in a 40° water bath for 20 min (Hill *et al.* 2014). Survival of each strain was assessed daily after heat exposure. Animals were tested in three independent experiments. Thirty animals for each genotype were assayed per experiment.

Acute heat response

Unseeded NGM agar plates were warmed to 37°. Each test animal was transferred to the warmed plate, and body bends were immediately counted for 1 min. Control animals were transferred to room temperature (~24°) unseeded NGM plates, and body bends were counted for 1 min. Ten animals were analyzed for each group (genotype + temperature).

Heat shock reporter analysis was carried out by placing animals containing *hsp-16.2*::*GFP* reporter constructs in a 33° water bath for 30 min and allowing them to recover for 2 hr. Subsequently, animals were mounted on agar pads, and whole-body fluorescence intensity was examined on a Zeiss AxioObserver microscope equipped with an Andor Clara CCD camera using a 10× objective lens.

Statistics and sharing

Statistical comparisons between two groups were conducted by unpaired two-tailed *t*-tests. Comparisons of three groups or more were done by one-way ANOVA followed by Tukey posttests. However, Figure 2, B, D, and F, and Figure 4, D–G display data analyzed by Kruskal-Wallis one-way ANOVA followed by Dunn's multiple comparisons because population variances differed significantly (in Figure 2, B, D, and F, due to normalization to wild type). *X-Y* curves of quiescence or survival (Figure 5 and Figure 6) were analyzed by repeated-measures two-way ANOVA and Tukey posttests.

Relevant statistical comparisons showing significant differences and *P*-values are listed in File S1. Asterisks indicating statistical differences are not displayed in Figure 5 and Figure 6 for simplicity of the graphs.

Data availability

All strains and computer resources are available on request.

Results

VAV-1 is critical for normal accumulation of LET-23/EGFR and DCVs in the ALA interneuron

The *C. elegans* genome encodes a single Vav family member, VAV-1. VAV-1 is essential for pharyngeal function, and complete loss of *vav-1* results in lethality in the first larval stage (L1). Pharynx-specific expression of *vav-1* rescues L1 lethality but reveals defects in other tissues (Norman *et al.* 2005). For example, VAV-1 was recently shown to regulate the rate of locomotion by functioning in the ALA interneuron to reduce the speed of animals crawling on an agar surface (Fry *et al.* 2014). Here, using several independent *vav-1* fluorescent reporter transgenic lines, we confirm the expression of *vav-1* in the ALA interneuron, which is readily identifiable by its shape and position in the dorsal ganglion and its colocalization with a transcriptional reporter of a known ALA signaling molecule, PLC-3/PLCγ (Figure 1).

ALA is a single interneuron that extends a small process into the dorsal nerve cord and sends two lateral processes down the entire length (of the animal) (White *et al.* 1986). Previously, ALA cell position and axon processes were shown to develop and differentiate normally in *vav-1* mutants (Fry *et al.* 2014). To investigate ALA differentiation in greater detail, we examined several transcriptional reporters in *vav-1* mutants. Unless otherwise stated, from this point onward, *vav-1* mutants described in this paper are animals that are homozygous for a *vav-1* deletion and carry an integrated transgene that expresses the *vav-1* cDNA in the pharynx [*vav-1(ak41) takIs5* (*Ppha-4*::*vav-1*::*GFP*)], which rescues L1 larval lethality (Norman *et al.* 2005; Fry *et al.* 2014). First, to test whether loss of *vav-1* affects neuronal differentiation of ALA in *vav-1* mutants, we examined the expression of two pan-neuronal markers, *rab-3* (Nonet *et al.* 1997) and *unc-119* (Maduro and Pilgrim 1995), in the ALA neuron of *vav-1* mutants. In agreement with VAV-1 not having a role in ALA neuronal development, we observed consistent ALA expression of mCherry driven by the 5′ *cis*-regulatory element of the *rab-3* gene and GFP driven by the 5′ *cis*-regulatory element of the *unc-119* gene in *vav-1* animals (Table 1). Next, using their 5′ *cis*-regulatory elements, we examined the expression of several ALA-expressed genes, *flp-7*, *ida-1*, and *plc-3*, that have more restricted neuronal expression patterns (Zahn *et al.* 2001; Cai *et al.* 2004; Kim and Li 2004; Van Buskirk and Sternberg 2007). Examination of *Pflp-7:GFP*, *Pida-1:GFP*, and *Pplc-3*:YFP in *vav-1* mutants revealed consistent ALA expression (Table 1), suggesting that VAV-1 does not have a role in ALA development or differentiation. To further explore the role of VAV-1 in ALA, we examined the cellular distribution of two proteins shown to function in ALA, LET-23 and IDA-1 (Van Buskirk and Sternberg 2010). *let-23* encodes an EGFR and has been implicated in the function of ALA in promoting behavioral quiescence during lethargus and on cellular stress (Van Buskirk and Sternberg 2007; Hill *et al.* 2014). *ida-1* encodes a phogrin/islet antigen-2b, which is a transmembrane DCV protein (Zahn *et al.* 2001; Cai *et al.* 2004) that is implicated DCV release (Zhou *et al.* 2007) and may have a role in EGF-induced quiescence (Van Buskirk and Sternberg 2010). To investigate the role VAV-1 may have in EGFR activity in ALA, we used a LET-23::GFP translational reporter driven by the 5′ *cis*-regulatory element of the *let-23* gene (Chang and Sternberg 1999; Van Buskirk and Sternberg 2010). From our examination of ALA, we found that while LET-23::GFP was expressed in all *vav-1* mutants analyzed, its fluorescence intensity was significantly reduced (Figure 2A). Importantly, this reduced fluorescence could be rescued by expression of wild-type *vav-1* by the 5′ *cis*-regulatory element of the *vav-1* gene (Figure 2, A and B). These data suggest that VAV-1 regulates the accumulation of LET-23/EGFR in ALA. Next, because the ALA neuron has

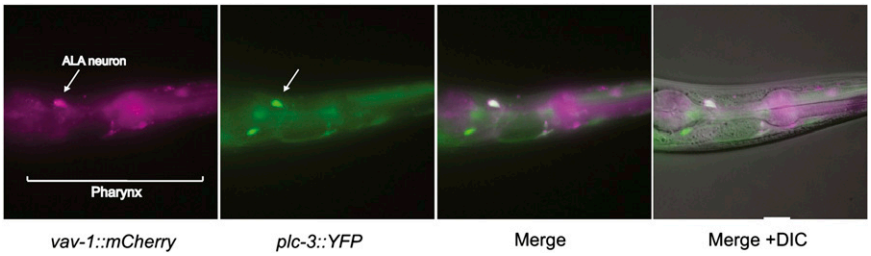

Figure 1 VAV-1 is expressed in the ALA interneuron. Fluorescence and differential interference contrast (DIC) images of an adult animal expressing a *vav-1* reporter (*Pvav-1::2xNLS-mCherry*) and a *plc-3* reporter (*Pplc-3::YFP*), which is known to be expressed in the ALA interneuron (Van Buskirk and Sternberg 2010). Bracket marks the pharynx, and arrow points to the ALA neuron. Anterior is to the right. Scale bar, 10 μm.

been shown to be peptidergic (Nelson *et al.* 2014) and IDA-1 has been shown to function in ALA, we used an IDA-1::GFP translational fusion protein expressed by the 5′ *cis*-regulatory element of the *ida-1* gene, which has been used to examine DCV function in ALA (Zhou *et al.* 2007), to investigate the localization and expression of DCVs in *vav-1* mutants. In addition to examining the localization and fluorescence intensity in the ALA cell body, we investigated IDA-1::GFP punctate distribution and fluorescence intensity in the ALA neuron lateral processes, which could uncover defects in DCV trafficking (Zahn *et al.* 2004). We found that IDA-1::GFP fluorescence in the cell body of ALA is increased in *vav-1* mutants and can be rescued by expression of wild-type *vav-1* by the 5′ *cis*-regulatory element of the *vav-1* gene (Figure 2, C and D). We hypothesized, therefore, that IDA-1::GFP might accumulate in the ALA cell body as a result of a perturbation in IDA-1::GFP trafficking to or within the axons. Surprisingly, we found that the fluorescence intensity of IDA-1::GFP axonal puncta is modestly increased in *vav-1* mutants, while the number of puncta (punctal density) and the size of the puncta along the ALA axon are not significantly different between wild-type and *vav-1* mutant animals (Figure 2, E–H). Unlike IDA-1::GFP fluorescence in the cell body of ALA (Figure 2, C and D), the change in fluorescence intensity of IDA-1::GFP axonal puncta is not rescued by wild-type *vav-1* expression (Figure 2F). Additionally, expression of the wild-type *vav-1* rescue construct leads to slight changes in punctal density and area (Figure 2, G and H). These data suggest that the changes in ALA axon puncta may not be related to the function of VAV-1 and that the changes in ALA cell body LET-23 and IDA-1 (which are more striking and rescued by wild-type *vav-1* expression) are more likely to be biologically relevant. Nevertheless, together our data indicate that VAV-1 regulates molecules within the ALA interneuron cell body that are critical for signal transduction within ALA and DCV signaling from ALA to other tissues (*i.e.*, released peptides).

VAV-1 is required for LIN-3/EGF-induced behavioral quiescence

The ALA interneuron and LET-23/EGFR were previously found to be required for lethargus and cellular stress– and EGF-induced sleep-like quiescence (Van Buskirk and Sternberg 2007; Hill *et al.* 2014). Because we have found consistent expression of *vav-1* in ALA (Figure 1) and altered accumulation of LET-23/EGFR in ALA of *vav-1* mutants (Figure 2, A and B), we hypothesized that VAV-1 is involved in behavioral sleep-like quiescence. We investigated this possibility by conditional expression of the EGF-like ligand LIN-3 using heat shock induction of LIN-3/EGF expression (Van Buskirk and Sternberg 2010). This method has been shown to induce sleep-like quiescence in adult animals. As expected, induced expression of LIN-3/EGF in young-adult animals resulted in both locomotory (Figure 3A) and feeding quiescence (Figure 3B), as measured by the proportions of animals exhibiting cessation of movement and lack of pharyngeal pumping behavior, respectively. Strikingly, the behavioral quiescence induced by LIN-3/EGF expression is strongly suppressed in *vav-1* mutants. On induction of LIN-3/EGF expression, *vav-1* mutants show significant locomotory activity and pharyngeal pumping (Figure 3, A and B). To confirm that the loss of quiescence behaviors is due to a loss of VAV-1 in ALA, we expressed VAV-1 specifically in the ALA neuron (and two muscle cells, the saucer cell of the pharynx and the anal sphincter, using the *ver-3* 5′ *cis*-regulatory element) of *vav-1* mutants. We found that restoring VAV-1 expression in ALA rescues sensitivity to LIN-3/EGF-induced quiescence because these animals exhibit similar quiescence to wild-type animals (Figure 3, A and B). These data indicate that VAV-1 acts in the ALA interneuron to mediate LIN-3/EGF-induced quiescence in adults.

Because ALA requires the secretion of FLP-13 peptides to mediate LIN-3/EGF-induced quiescence (Nelson *et al.* 2014) and we have observed abnormal accumulation of IDA-1::GFP, a marker that labels DCVs, in the ALA neuron of *vav-1* mutants (Figure 2, C and D), we investigated whether overexpression of IDA-1 can rescue the failure of *vav-1* mutants to respond to LIN-3/EGF-induced quiescence. Thus, we induced LIN-3/EGF in *vav-1* mutants overexpressing IDA-1 and examined quiescence. In these experiments, we did not observe a suppression of the quiescence behavior defect in *vav-1* mutants (Figure S1). These data indicate that simple overexpression of IDA-1 in *vav-1* mutants is not sufficient to rescue the *vav-1* defect in LIN-3/EGF-induced quiescence.

VAV-1 requires GEF activity and the C-terminal SH3 domain to mediate LIN-3/EGF-induced behavioral quiescence

VAV-1 is a multidomain signaling molecule that contains an evolutionarily conserved array of protein-protein interaction domains in addition to the classical Dbl homology–Pleckstrin (DH-PH) homology domains found in GEFs (Norman *et al.* 2005). In the C-terminal region of VAV-1, an SH2 domain is

Table 1 Percentage of animals showing reporter expression in ALA

ALA reporter	Wild type	*vav-1* mutants
unc-119::*GFP*	100% (20/20)	100% (40/40)
rab-3::*mCherry*	100% (40/40)	100% (80/80)
ida-1::*GFP*	100% (20/20)	100% (30/30)
flp-7::*GFP*	100% (25/25)	100% (45/45)
plc-3::*YFP*	96% (25/26)	97% (32/33)

flanked by two SH3 domains (SH3A and SH3B, respectively) (Figure 3C). Previously, GEF activity of VAV-1 was shown to be required for L1 larval survival (Norman *et al.* 2005) and in ALA for regulating locomotory behavior in active animals (Fry *et al.* 2014). Using this *vav-1* GEF-dead construct (Fry *et al.* 2014), we explored whether GEF activity was required for LIN-3/EGF-induced quiescence. We introduced the VAV-1 GEF-dead construct into the *vav-1* mutant background and analyzed LIN-3/EGF-induced quiescence. As shown in Figure 3 (D and E), although the wild-type *vav-1* rescuing construct restores locomotory and feeding quiescence to *vav-1* mutants expressing induced LIN-3/EGF, the GEF-dead *vav-1* construct does not. These data indicate that GEF activity of VAV-1 is required for mediating behavioral quiescence.

Next, we generated different *vav-1* domain deletion constructs to determine which, if any, protein-protein interaction domains are required for quiescence induction by LIN-3/EGF expression. First, we found that *vav-1* constructs with deletion of SH3A or SH3B were able to rescue the *vav-1* mutant L1 lethality, unlike the SH2 deletion construct. These data suggest that the SH2 domain is critical for the function of VAV-1 in the pharynx. While the SH2 deletion construct could not rescue L1 lethality, we found that it completely rescued the LIN-3/EGF-induced quiescence in *vav-1* mutants (Figure 3, D and E). Because the SH3A and SH3B deletion constructs rescued the *vav-1* mutant L1 lethality, analysis of LIN-3/EGF-induced quiescence was conducted in the *vav-1(ak41)* homozygous background lacking the *takIs5* (*Ppha-4*::*vav-1*::*GFP*) integrated transgene. From these analyses, we found that while the SH3A deletion could fully rescue the LIN-3/EGF-induced quiescence in *vav-1* mutants, the SH3B deletion could not (Figure 3, D and E). These data indicate that although the SH2 domain is important for VAV-1 pharyngeal activity, it is dispensable for LIN-3/EGF-induced quiescence. Conversely, the SH3B domain is required for LIN-3/EGF-induced quiescence but is dispensable for VAV-1 pharyngeal function.

GEF-dead and SH3B deletion *vav-1* constructs did not rescue LIN-3/EGF-induced quiescence in *vav-1* mutants, suggesting that these domains are required for VAV-1 function. However, it is possible that these constructs may function in a dominant-negative manner in ALA and disrupt normal quiescence induction by LIN-3/EGF. To investigate this possibility, we tested whether animals expressing either *vav-1* mutant construct in an otherwise wild-type background could respond to LIN-3/EGF-induced quiescence. Wild-type animals expressing the *vav-1* (GEF-dead) or the *vav-1* SH3B deletion construct responded normally to LIN-3/EGF-induced quiescence (Figure S2). These data indicate that these constructs likely do not have a dominant-negative impact on LIN-3/EGF-induced quiescence.

VAV-1 function is dispensable for sleep-like behavior during lethargus

The ALA interneuron has been shown to influence *C. elegans* behavioral quiescence in several different scenarios. For instance, ablation of ALA disrupts quiescence during lethargus (Van Buskirk and Sternberg 2007), the larval period characterized by cessation of movement and feeding (Raizen *et al.* 2008). Additionally, LET-23/EGFR function in ALA is required for normal quiescence during lethargus (Van Buskirk and Sternberg 2007). Therefore, because VAV-1 acts in ALA to regulate locomotion in adult animals (Fry *et al.* 2014), regulates the accumulation of LET-23/EGFR (Figure 2), and is critical for LIN-3/EGF-induced quiescence (Figure 3), we hypothesized that VAV-1 acts in ALA to suppress locomotion during lethargus. To analyze animals in lethargus, we selected animals in the middle of the L4 larval stage that were actively moving and feeding (pharyngeal pumping was observed). We then measured the locomotory activity of these animals across L4/adult lethargus, the transition period between the L4 larval and adult stages. We used specialized microfluidic chambers designed to keep animals confined to an area within a camera field of view for recording the entirety of the lethargus period while still allowing animals to move freely within the chamber (Singh *et al.* 2011). Surprisingly, when we observed locomotory quiescence during lethargus of wild-type animals (Figure 4A) and *vav-1* mutants (Figure 4B), we found no difference in the total quiescence (Figure 4D) or the duration of quiescence (Figure 4E), unlike lethargus quiescence defective *egl-30* mutants (Figure 4, C–E) (Schwarz and Bringmann 2013; Nagy *et al.* 2014). Because ALA has been reported to play a minor role in lethargus quiescence (Van Buskirk and Sternberg 2007; Trojanowski *et al.* 2015), we tested a mutant, *plc-3*, that has been shown previously to have a role in ALA to regulate quiescence during lethargus to determine whether our assay is sensitive enough to detect a minor lethargus quiescence defect. Indeed, *plc-3* mutants show a significant reduction in quiescence behavior during L4 to adult lethargus (Figure 4, F and G). Interestingly, when we examined *vav-1* mutants overexpressing wild-type *vav-1*, we found an extended lethargus quiescence period (Figure 4, F and G), indicating that excessive VAV-1 signaling can increase the lethargus quiescence period.

VAV-1 is required for sleep-like quiescence and survival after cellular stress

Recent publications have revealed an additional type of sleep-like quiescence behavior mediated by ALA: on exposure to cellular stress (*e.g.*, noxious heat, 35–40°), *C. elegans* displays sleep-like quiescence (Hill *et al.* 2014; Nelson *et al.*

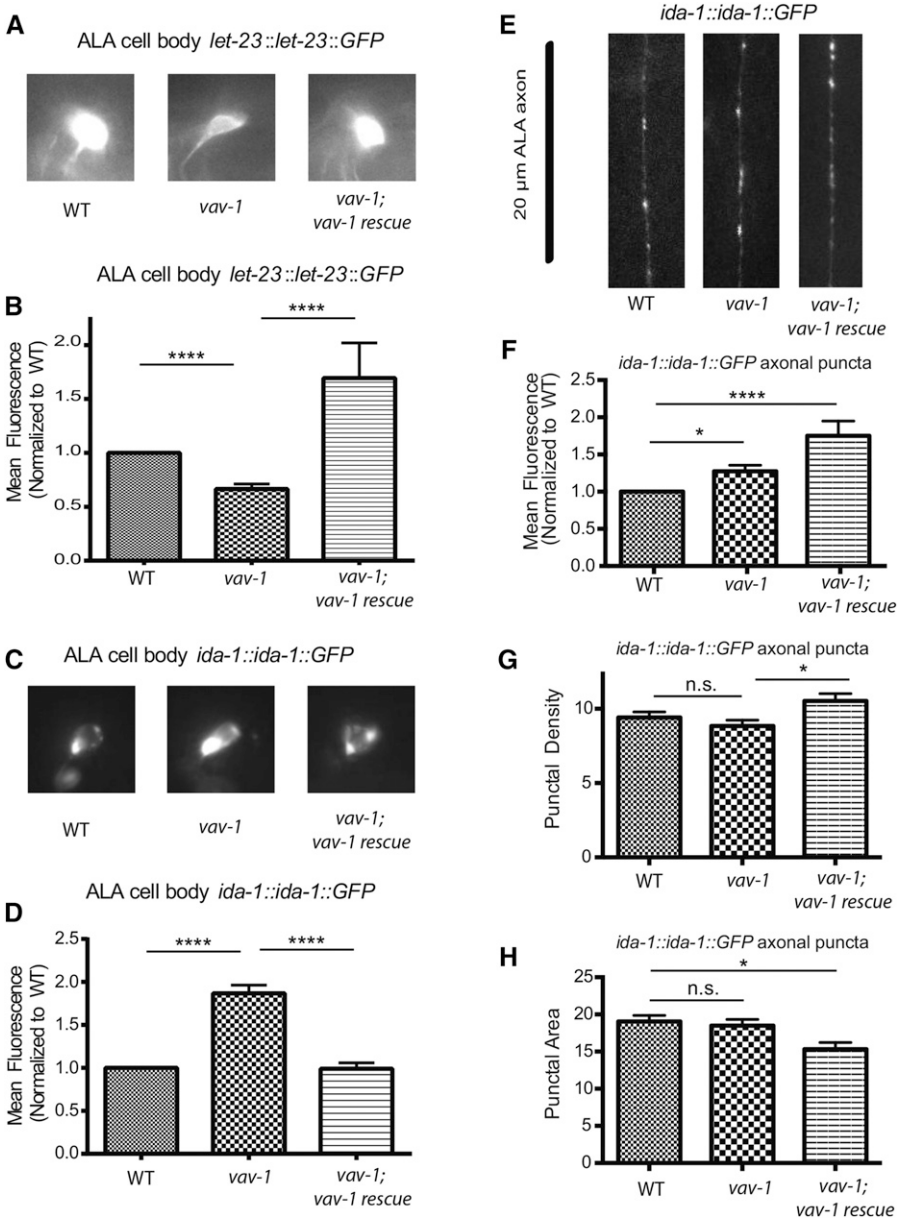

Figure 2 VAV-1 regulates LET-23/EGFR and IDA-1/phogrin in the cell body of ALA. (A) Representative images of LET-23::GFP in the ALA neuron cell body. (B) Quantification of the mean fluorescence of LET-23::GFP in the ALA cell body of wild-type animals, *vav-1* mutants, and *vav-1* mutants expressing a rescuing *vav-1* construct (*vav-1*; *vav-1 rescue* = *vav-1*; *takEx215*). *n* = 43 (wild type), 49 (*vav-1*), and 26 (*vav-1*; *vav-1 rescue*). (C) Representative images of IDA-1::GFP in the cell body of ALA. (D) Quantification of the mean fluorescence of IDA-1::GFP in the ALA cell body of wild-type animals, *vav-1* mutants, and *vav-1* mutants expressing a rescuing *vav-1* construct (*vav-1*; *vav-1 rescue* = *vav-1*; *takEx215*). *n* = 25 (wild type), 27 (*vav-1*), and 18 (*vav-1*; *vav-1 rescue*). (E) Representative images of IDA-1::GFP along 20 µm of one ALA axon in the posterior of the animal. (F–H) Quantification of the mean fluorescence, density, and area of ALA axon puncta in wild-type animals, *vav-1* mutants, and *vav-1*; *vav-1 (takEx215)* rescue animals. *n* = 44 (wild type), 37(*vav-1*), and 21 (*vav-1*; *vav-1* rescue). Data are represented as mean ± SEM. Data in B, D, and F were normalized to wild type and analyzed by Kruskal-Wallis one-way ANOVA followed by Dunn's multiple comparisons (****$P < 0.0001$). Data in G and H were analyzed by one-way ANOVA followed by Tukey's multiple comparisons (*$P < 0.05$; ****$P < 0.0001$; n.s., not significant).

2014). Indeed, when wild-type animals are exposed to noxious heat, sleep-like behavioral quiescence is readily observed, and recovery occurs over 60–80 min after heat shock (Figure 5, A and B). Importantly, a second, longer bout of ~2–5 hr (depending on heat stress temperature) of sleep-like behavioral quiescence occurs and has been proposed to be required for maintaining cellular proteostasis and survival after cellular stress (Figure 5, A and B) (Hill *et al.* 2014). Like lethargus quiescence and LIN-3/EGF-induced quiescence, proteins expressed in ALA are required to mediate this type of quiescence, including CEH-17, a transcription factor needed for differentiation of ALA, as well as LET-23/EGFR and PLC-3/PLCγ (Hill *et al.* 2014). Therefore, we investigated whether VAV-1 is required for this type of cellular stress–induced quiescence. We found that *vav-1* mutants are indeed impaired in both the early and later, long phase of quiescence following noxious heat stress (Figure 5, A and B). The longer phase of impaired quiescence observed in *vav-1* mutants can be rescued by wild-type *vav-1* gene expression but not the GEF-dead *vav-1* gene under the control of its 5′ *cis*-regulatory element (Figure 5A). Additionally, the second, longer phase defect observed in *vav-1* mutants also can be rescued by driving expression of VAV-1 in the ALA neuron using the *ver-3* 5′ *cis*-regulatory element (Figure 5B), indicating a cell-autonomous role of VAV-1 in ALA in regulating cellular stress–induced sleep-like behavior. The failure of rescue in the initial phase may be due to transgene expression efficiency. While *vav-1* mutants show defective quiescence induced by noxious heat, they sense noxious heat and respond normally by increasing the rate of locomotion on transfer to prewarmed 37° plates (Figure S3A). Furthermore, *vav-1* mutants undergo a normal heat shock response, as

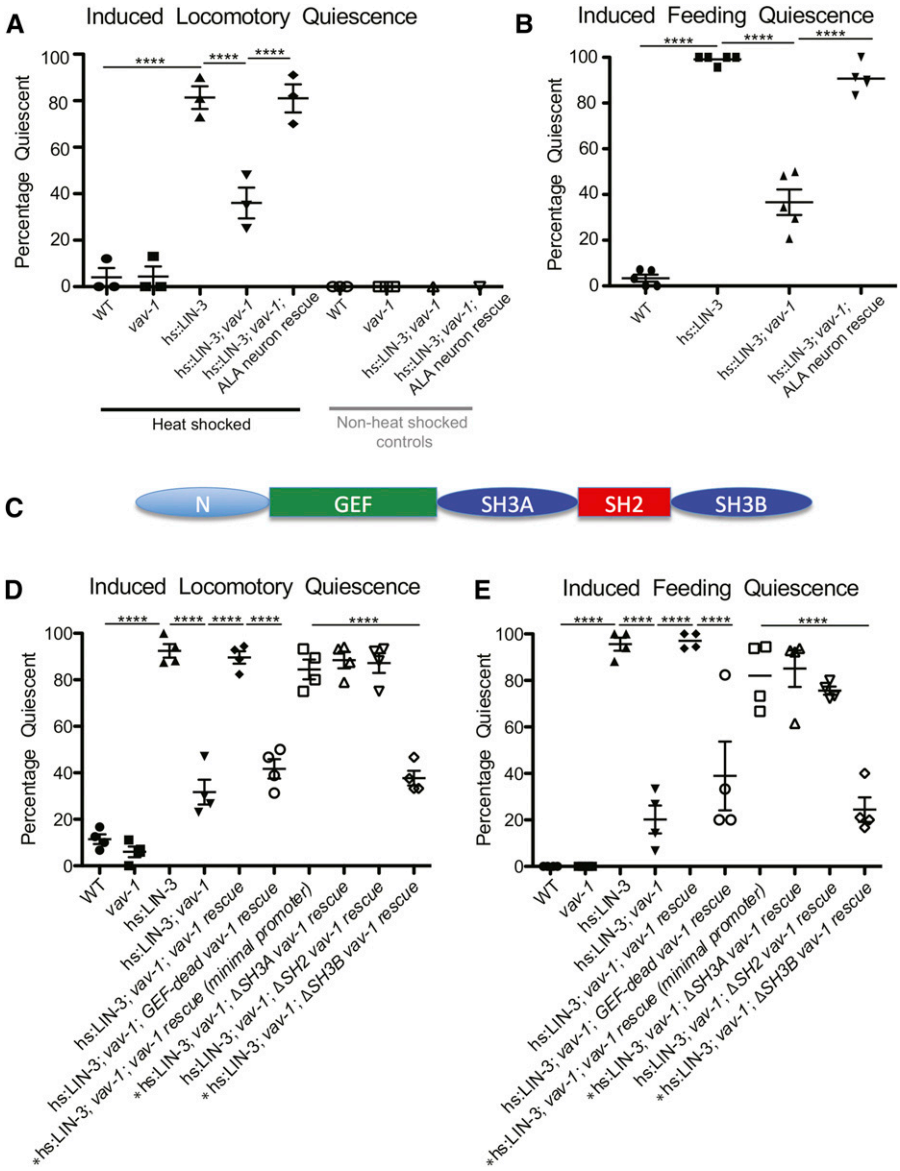

Figure 3 VAV-1 is required in the ALA interneuron for EGF-induced sleep-like quiescence. LIN-3/EGF was conditionally expressed by a heat shock–induced construct, and behavioral quiescence (locomotory and feeding quiescence) was measured. (A) Percentage of animals exhibiting locomotory quiescence and (B) percentage of animals displaying feeding quiescence (lack of pharyngeal pumping) are drastically increased on expression of LIN-3/EGF. The LIN-3/EGF-induced quiescence is disrupted by *vav-1* mutation and restored in *vav-1* mutants by VAV-1 expression in the ALA neuron (driven by the *ver-3* 5′ *cis*-regulatory element). Non-heat-shocked animals do not display sleep-like quiescence. (C) The multidomain structure of the VAV-1 protein is shown. (D) Percentages of animals exhibiting locomotory quiescence and (E) feeding quiescence show that the defective EGF-induced quiescence of *vav-1* mutants can be restored on expression of *vav-1* by the full ∼8-kb *vav-1* 5′ *cis*-regulatory element (*vav-1* rescue) or a minimal ∼3kb *vav-1* 5′ *cis*-regulatory element [*vav-1* rescue (minimal promoter)]. Rescue of quiescence behavior in *vav-1* mutants requires GEF activity and the SH3B domain of VAV-1. An asterisk (*) before the genotype label indicates *vav-1* mutants that do not contain *takIs5* (*Ppha-4*::*vav-1*::*GFP*). Three to four separate trials of 20–30 animals per genotype were analyzed for locomotory quiescence (except for non-heat-shocked hs:LIN-3 and hs:LIN-3; *vav-1* controls, for which one to three separate trials with 10–20 animals were analyzed), and four to five separate trials of > 30 animals were analyzed per genotype for feeding quiescence. Data are represented as mean ± SEM and were analyzed by one-way ANOVA with Tukey posttests (****$P < 0.0001$).

visualized by GFP, under the control of the heat shock response element (5′ *cis*-regulatory element of the *hsp-16.2* gene) (Figure S3, B–D).

Interestingly, it was shown previously that impairment in the longer phase of quiescence is correlated with decreased organism survival after cellular stress from noxious heat (Hill *et al.* 2014). Thus, we tested the survival of *vav-1* mutants after noxious heat exposure. We found that *vav-1* mutants have significantly impaired survival after heat stress (like positive-control *plc-3/PLC*γ mutants) (Hill *et al.* 2014), which we rescued by expression of wild-type *vav-1* (Figure 6A). None of these strains had decreased survival without heat stress (Figure 6B). Together our results suggest that VAV-1 is an important factor mediating quiescence from the ALA neuron and that the inability of *vav-1* mutants to rest after cellular stress, as observed in wild-type animals, is detrimental to their long-term survival. In agreement with this conclusion, we found that introducing a mutation that increases the frequency of sleep-like behavior can increase the survival of *vav-1* mutants after noxious heat stress. This mutation, a gain-of-function allele of the cyclic GMP–dependent protein kinase, *egl-4*, causes spontaneous bouts of sleep-like quiescence (Raizen *et al.* 2008). Moreover, *egl-4* is proposed to act downstream of ALA to promote LIN-3/EGF- and cellular stress–induced quiescence (Van Buskirk and Sternberg 2007; Hill *et al.* 2014). Analysis of *egl-4(gf)*; *vav-1* double mutants showed a significant increased survival over *vav-1* mutants after exposure to noxious heat (Figure 6C).

Induction of activated VAV-1 is sufficient to induce quiescence behavior

Our data thus far indicate that VAV-1 is required to promote ALA-mediated quiescence. However, it could be that VAV-1 has a more general role in ALA function and is not directly involved in quiescence signaling. To test the hypothesis that activated VAV-1 has a direct role in inducing sleep-like

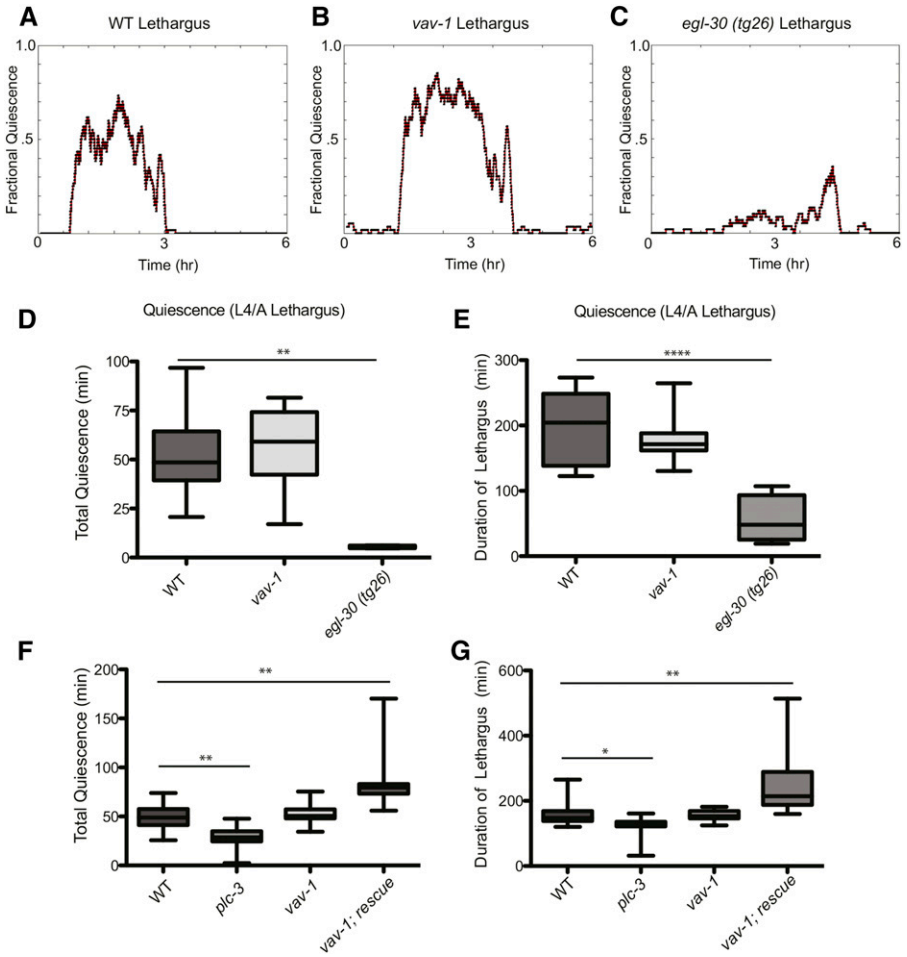

Figure 4 VAV-1 is dispensable for normal lethargus quiescence. (A–C) Representative plots of fractional quiescence during L4/adult lethargus of wild-type animals, *vav-1* mutants, and *egl-30 (tg26, gf)* animals in microfluidic chambers. Six hours of movement data are shown, centered on a consolidated bout of locomotory quiescence before transition to active adulthood. (D) Quantification of the total lethargus quiescence over 6 hr, and (E) quantification of the duration of lethargus quiescence of wild-type animals, *vav-1* mutants, and *egl-30(tg26)* animals show that *vav-1* mutants do not have impaired locomotory quiescence during lethargus, unlike lethargus quiescence defective *egl-30* mutants. n = 12 (wild type), 10 (*vav-1*), and 5 (*egl-30*). (F) Quantification of the total lethargus quiescence and (G) duration of lethargus in wild-type animals and *plc-3*, *vav-1*, and *vav-1*; *rescue* (*vav-1*; *akEx87–vav-1* overexpression) mutants. n = 22 (wild type), 15 (*plc-3*), 14 (*vav-1*), and 11 (*vav-1*; *rescue*). Data are represented as mean ± SEM and were analyzed by Kruskal-Wallis one-way ANOVA followed by Dunn's multiple comparisons (*P < 0.05; **P < 0.01; ****P < 0.0001).

quiescence behavior, we generated an inducible transgene using the *hsp-16.41* 5′ *cis* control element (Jones *et al.* 1989) to induce the expression of a constitutively active VAV-1 mutant. The constitutively active VAV-1 protein contains three tyrosine-to-phenylalanine mutations in the regulatory region, which have a conserved role in regulating GEF activity in Vav family members (Aghazadeh *et al.* 2000; Norman *et al.* 2005). Heat shock activation of this construct in otherwise wild-type young-adult active animals caused a significant increase in quiescence behavior, whereas non-heat-shocked animals showed normal active behavior (Figure 7). Moreover, 10 hr after heat shock induction, the heat-shocked animals resumed normal active behavior (Figure 7). These results indicate that VAV-1 has a direct role in promoting sleep-like quiescence.

Discussion

VAV-1 was shown previously to have a crucial role in modulation of rhythmic behaviors in *C. elegans* (*e.g.*, the feeding behavior of pharyngeal pumping, ovulation, and the defecation cycle) (Norman *et al.* 2005). Recently, VAV-1 also was shown to regulate the rhythmic behavior of locomotion, providing an inhibitory input from the ALA interneuron to the neural circuit regulating locomotion and thus reducing locomotory speed in wakeful, active adults (Fry *et al.* 2014). We show here that VAV-1, in a GEF-dependent manner, is necessary for sleep-like quiescence behaviors; VAV-1 is needed for suppression of rhythmic locomotion and feeding during EGF-induced and cellular stress–induced behavioral quiescence. Further, we demonstrate that activation of VAV-1 is sufficient to induce quiescence behavior in active adult animals. Moreover, we discovered that VAV-1 requires GEF activity and acts in the neurosecretory ALA interneuron, a neuron previously shown to be critical for sleep-like behavior, to mediate behavioral quiescence (Van Buskirk and Sternberg 2007; Hill *et al.* 2014). We also found that VAV-1 regulates the protein levels of two critical ALA signaling molecules, LET-23/EGFR and IDA-1/phogrin, required for ALA function (Van Buskirk and Sternberg 2007; Zhou *et al.* 2007; Van Buskirk and Sternberg 2010). Significantly, we demonstrate that loss of VAV-1–dependent behavioral quiescence results in reduced organism survival after cellular stress.

Previous work has shown that ablation of ALA results in a reduction of behavioral quiescence during lethargus (Van Buskirk and Sternberg 2007). However, because removal of ALA produces only a minor defect in lethargus quiescence, this suggests that ALA is not the only cell required for inducing

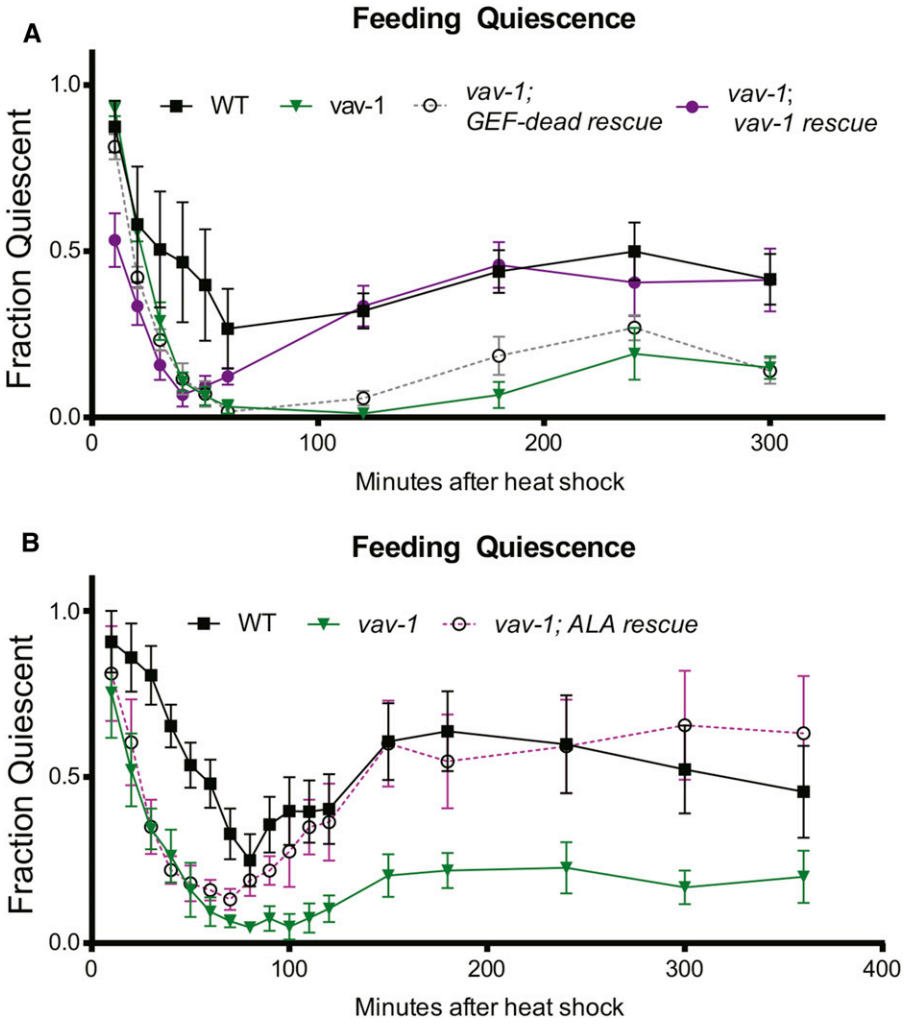

Figure 5 VAV-1 is required in the ALA interneuron for heat stress–induced quiescence. Feeding quiescence after a 30-min exposure to 35.5° heat stress is shown; the curves display behavioral quiescence immediately on transfer to room temperature and slow recovery (10 min to ~80 min), followed by a second, longer bout of behavioral quiescence (~120–300 min). (A) *vav-1* mutants are defective in behavioral quiescence after heat stress. The extended second bout of behavioral quiescence can be rescued by expression of wild-type *vav-1*, but this rescue requires VAV-1 GEF activity. (B) Similarly, expression of *vav-1* in the ALA interneuron rescues the *vav-1* mutant defect in the extended second bout of behavioral quiescence. Data are represented as mean ± SEM, and statistical analyses were conducted using repeated-measures two-way ANOVA followed by Tukey posttests. *P*-values are detailed in File S1.

behavioral quiescence associated with lethargus (Van Buskirk and Sternberg 2007). Indeed, RIA and RIS neurons, for instance, are also involved in lethargus quiescence (Nelson *et al.* 2013; Turek *et al.* 2013). Moreover, subsequent publications have shed light on perhaps a more vital role of ALA: induction of cellular stress–induced quiescence that is correlated with survival rates after cellular stress (*e.g.*, noxious heat) (Hill *et al.* 2014; Nelson *et al.* 2014). Mutation of *ceh-17*, a transcription factor required for LET-23/EGFR and PLC-3/PLCγ expression in ALA, and mutation of *flp-13*, a gene encoding FMRFamide-like neuropeptides expressed in ALA, both disrupt EGF- and cellular stress–induced quiescence. Interestingly, FLP-13, like VAV-1, is not required for endogenous lethargus quiescence. These observations suggest that the signaling mechanisms within ALA mediating sleep-like quiescence behaviors diverge at some point, with requirements of separate signaling molecules for the regulation of lethargus sleep-like quiescence and cellular stress–induced sleep-like quiescence and that FLP-13 and VAV-1 are crucial in the cellular stress–induced quiescence pathway. Interestingly, we found that overexpression of *vav-1* results in an extended quiescent bout in animals undergoing lethargus, suggesting that VAV-1, if overly abundant, can affect endogenous lethargus quiescence and prolong this quiescent state. Furthermore, our data demonstrate that IDA-1::GFP levels in the ALA cell body are regulated by VAV-1, suggesting that VAV-1 may be involved in DCV function in this neuron. Perhaps VAV-1 influences localization or release of FLP-13 (and/or other neuropeptides); further studies are needed to investigate these possibilities.

Cellular stress induced by noxious heat causes perturbation of cellular homeostasis, which drives sleep-like behavioral quiescence (Hill *et al.* 2014; Nelson *et al.* 2014). The sleep-like behavioral quiescence induced by cellular stress results in an initial short-lived quiescent state (~1 hr) followed by brief recovery and a second, prolonged quiescent state (several hours) (Hill *et al.* 2014). Hill *et al.* (2014) proposed that the prolonged bout of quiescence is critical for regulating cellular homeostasis. From our analyses, we have found that while *vav-1* mutants are defective in both cellular stress–induced sleep-like behavioral quiescence periods, we observed complete rescue of the second bout only on wild-type *vav-1* expression from either its 5′ *cis*-regulatory control element or the ALA-specific 5′ *cis*-regulatory element. It is unclear why

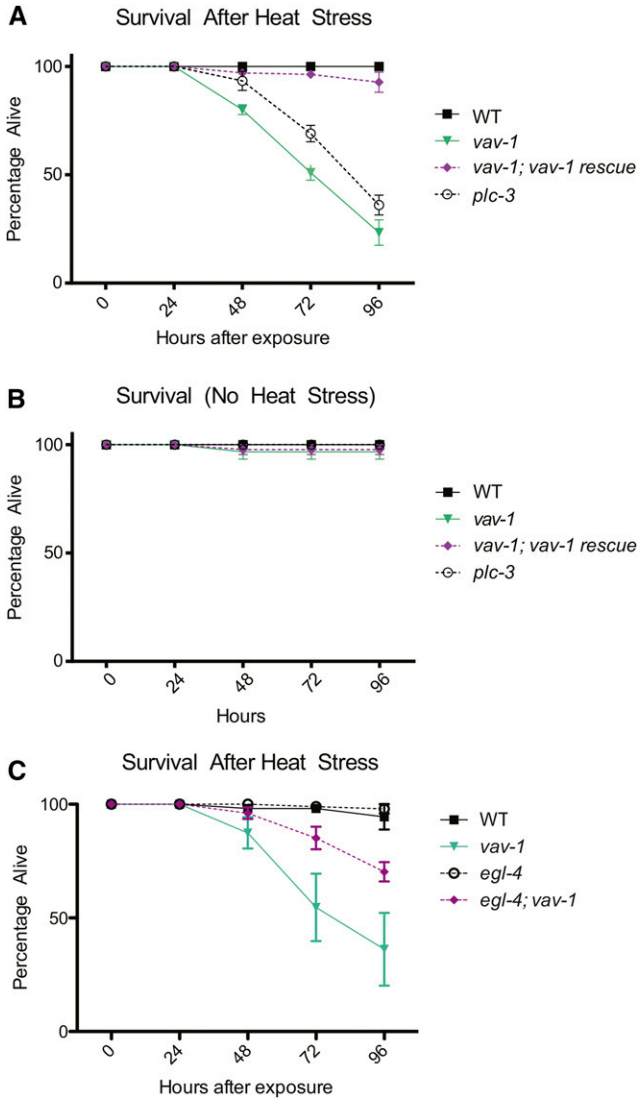

Figure 6 VAV-1 is required for survival after noxious heat stress. (A) Animals were exposed to 40° heat stress for 20 min, and survival was assessed over the following 4 days. Like *plc-3(tm1340)* mutants, which are defective for heat stress–induced quiescence (Hill *et al.* 2014), *vav-1* mutants show a loss of survival after exposure to noxious heat. This survival defect is rescued by expression of wild-type *vav-1*. (B) None of the tested strains showed differences in survival without heat stress exposure. (C) Survival of wild-type animals and *vav-1*, *egl-4(ad450sd)*, and *egl-4(ad450sd); vav-1* animals after 20 min of exposure to 40° heat stress. *egl-4(ad450sd)* significantly suppresses the survival defect observed in *vav-1* mutants. Data are represented as mean ± SEM, and statistical analyses were conducted using repeated-measures two-way ANOVA followed by Tukey posttests. *P*-values are detailed in File S1.

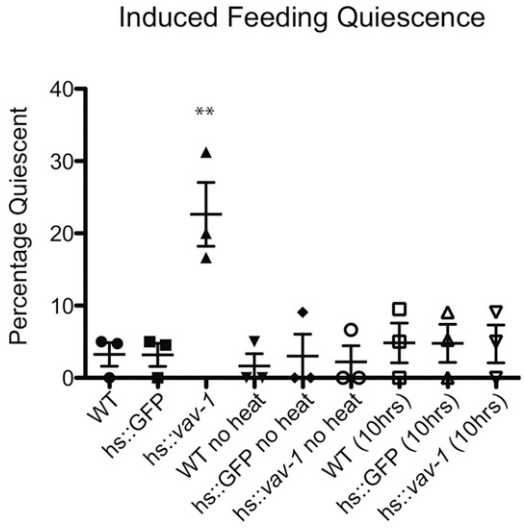

Figure 7 Induction of constitutively active VAV-1 is sufficient to induce behavioral quiescence. A constitutively active VAV-1 mutant was placed under the control of the 5′ *cis*-regulatory element of the inducible heat shock promoter gene *hsp-16.41*. Young-adult animals were exposed to 33° for 30 min and allowed to recover for 2 hr. While wild-type and hs::GFP animals displayed normal active behavior, induction of constitutively active *vav-1* (hs::*vav-1*) produces a significant increase in the percentage of animals displaying quiescence behavior. At 10 hr after heat shock, hs::*vav-1* animals showed a complete recovery. Non-heat-shocked animals (no heat) do not display quiescence behavior. Three separate trials with 20–30 animals for each genotype were analyzed. Data are presented as mean ± SEM and were analyzed by one-way ANOVA with Tukey posttest analysis (**$P < 0.01$).

we did not observe rescue in the initial phase of quiescence. This could be due to transgene expression levels and/or genetic background differences that lead to defects in the initial period of quiescence. Nevertheless, our studies confirm the notion that the second, prolonged bout of sleep-like behavioral quiescence is critical for cellular homeostasis because the rescued animals, unlike *vav-1* mutants, showed wild-type survival after exposure to noxious heat stress.

Of the three mammalian *Vav* genes, *Vav2* and *Vav3* are expressed in the nervous system (Cowan *et al.* 2005; Sauzeau *et al.* 2010a). Although the functions of Vav proteins in the nervous system are far from clear, several studies have found critical roles for Vav family members in nervous system function. For example, Vav proteins have been shown to be important for axonal development (Cowan *et al.* 2005; Malartre *et al.* 2010; Sauzeau *et al.* 2010a; Fernandez-Espartero *et al.* 2013), synaptic plasticity (Hale *et al.* 2011), and negative regulation of sympathetic nervous system activity (Sauzeau *et al.* 2006, 2007, 2010b, 2011; Zhu *et al.* 2015). Moreover, *Vav3* has been identified as a candidate schizophrenia gene (Ikeda *et al.* 2011; Aleksic *et al.* 2013). Here we have found that VAV-1 is required in the ALA interneuron for promoting both EGF- and cellular stress–induced sleep-like behavioral quiescence. We provide evidence implicating VAV-1 in neuronal EGF signaling: (1) *vav-1* mutants fail to respond to an EGF quiescence signal and (2) VAV-1 regulates levels of the EGFR/LET-23 in the ALA neuron. Previous studies have demonstrated that EGFR/LET-23 and its downstream effector phospholipase C gamma, PLC-3/PLCγ, act in the ALA neuron to promote both EGF- and stress-induced sleep-like behavioral quiescence (Van Buskirk and Sternberg 2007; Hill *et al.* 2014). Our data are consistent with VAV-1 acting in this LET-23–PLC-3 signaling pathway. Notably, Vav proteins have been identified as positive effectors of EGFR signaling

pathways in other tissues of *C. elegans*, as well as in *Drosophila* and mammalian cells (Margolis *et al.* 1992; Dekel *et al.* 2000; Pandey *et al.* 2000; Hornstein *et al.* 2003; Norman *et al.* 2005). However, our data are the first to suggest Vav interaction with EGFR or PLCγ in neurons. While our analyses demonstrate that *vav-1* mutants are defective in responding to EGF-induced quiescence and show reduced EGFR levels in the ALA cell body, the role of VAV-1 in mediating EGFR signaling is unclear. Notably, Vav proteins have been implicated in promoting EGFR and other receptor tyrosine kinase (RTK) signaling by regulating the endocytosis of the RTKs (Cowan *et al.* 2005; Thalappilly *et al.* 2010). Indeed, *Vav2*, in a GEF-dependent manner, has been shown to have a critical role in EGFR signaling by delaying EGFR receptor internalization and degradation via an interaction with endosomal-associated proteins (Thalappilly *et al.* 2010). Whether VAV-1 has a role in mediating endocytosis and degradation of the LET-23/EGFR in the ALA interneuron will require further studies.

Although this is the first study to uncover a role for a Vav protein in regulation of a sleep-like or quiescence behavior, a Rho-family GTPase guanine nucleotide–activating protein encoded by the *crossveinless-c* gene was recently implicated in sleep homeostasis in *Drosophila* (Donlea *et al.* 2014), suggesting a conserved role for Rho-family GTPases in sleep behavior. Furthermore, *Vav2* and *Vav3* transcripts are expressed in regions of the human and mouse brain associated with sleep (Lein *et al.* 2007; Hawrylycz *et al.* 2012), such as the thalamus (Fuentealba and Steriade 2005). Because EGF has a conserved role in sleep (Kushikata *et al.* 1998; Snodgrass-Belt *et al.* 2005; Foltenyi *et al.* 2007; Van Buskirk and Sternberg 2007) and we demonstrate that VAV-1 acts in an EGFR signaling pathway and has a role in sleep-like behaviors in *C. elegans*, it would be interesting to explore whether *Vav2* and/or *Vav3* has an evolutionarily conserved role in human sleep.

Acknowledgments

We thank the people in the Norman laboratory for help with cloning and transgenesis and valuable discussions. Many strains were provided by the Caenorhabditis Genetics Center, which is funded by the National Institutes of Health (NIH) Office of Research Infrastructure Programs (P40-OD010440). This work was supported by NIH grants GM-088213 (K.N.) and NS-055813 (A.H.), as well as a Brown Institute for Brain Science and the Norman Prince Neurosciences Institute Postdoctoral Fellowship in Translational Neuroscience (H.H.).

Literature Cited

Aghazadeh, B., W. E. Lowry, X. Y. Huang, and M. K. Rosen, 2000 Structural basis for relief of autoinhibition of the Dbl homology domain of proto-oncogene Vav by tyrosine phosphorylation. Cell 102: 625–633.

Aleksic, B., I. Kushima, R. Hashimoto, K. Ohi, M. Ikeda *et al.*, 2013 Analysis of the VAV3 as candidate gene for schizophrenia: evidences from voxel-based morphometry and mutation screening. Schizophr. Bull. 39: 720–728.

Benington, J. H., and H. C. Heller, 1995 Restoration of brain energy metabolism as the function of sleep. Prog. Neurobiol. 45: 347–360.

Cai, T., T. Fukushige, A. L. Notkins, and M. Krause, 2004 Insulinoma-associated protein IA-2, a vesicle transmembrane protein, genetically interacts with UNC-31/CAPS and affects neurosecretion in Caenorhabditis elegans. J. Neurosci. 24: 3115–3124.

Cai, T., H. Hirai, T. Fukushige, P. Yu, G. Zhang *et al.*, 2009 Loss of the transcriptional repressor PAG-3/Gfi-1 results in enhanced neurosecretion that is dependent on the dense-core vesicle membrane protein IDA-1/IA-2. PLoS Genet. 5: e1000447.

Cassada, R. C., and R. L. Russell, 1975 The dauerlarva, a post-embryonic developmental variant of the nematode Caenorhabditis elegans. Dev. Biol. 46: 326–342.

Chang, C., and P. W. Sternberg, 1999 *C. elegans* vulval development as a model system to study the cancer biology of EGFR signaling. Cancer Metastasis Rev. 18: 203–213.

Cho, J. Y., and P. W. Sternberg, 2014 Multilevel modulation of a sensory motor circuit during C. elegans sleep and arousal. Cell 156: 249–260.

Choi, S., M. Chatzigeorgiou, K. P. Taylor, W. R. Schafer, and J. M. Kaplan, 2013 Analysis of NPR-1 reveals a circuit mechanism for behavioral quiescence in *C. elegans*. Neuron 78: 869–880.

Cowan, C. W., Y. R. Shao, M. Sahin, S. M. Shamah, M. Z. Lin *et al.*, 2005 Vav family GEFs link activated Ephs to endocytosis and axon guidance. Neuron 46: 205–217.

Dekel, I., N. Russek, T. Jones, M. A. Mortin, and S. Katzav, 2000 Identification of the Drosophila melanogaster homologue of the mammalian signal transducer protein, Vav. FEBS Lett. 472: 99–104.

Donlea, J. M., D. Pimentel, and G. Miesenbock, 2014 Neuronal machinery of sleep homeostasis in Drosophila. Neuron 81: 860–872.

Driver, R. J., A. L. Lamb, A. J. Wyner, and D. M. Raizen, 2013 DAF-16/FOXO regulates homeostasis of essential sleep-like behavior during larval transitions in *C. elegans*. Curr. Biol. 23: 501–506.

Fernandez-Espartero, C. H., D. Ramel, M. Farago, M. Malartre, C. M. Luque *et al.*, 2013 GTP exchange factor Vav regulates guided cell migration by coupling guidance receptor signalling to local Rac activation. J. Cell Sci. 126: 2285–2293.

Foltenyi, K., R. J. Greenspan, and J. W. Newport, 2007 Activation of EGFR and ERK by rhomboid signaling regulates the consolidation and maintenance of sleep in Drosophila. Nat. Neurosci. 10: 1160–1167.

Fry, A. L., J. T. Laboy, and K. R. Norman, 2014 VAV-1 acts in a single interneuron to inhibit motor circuit activity in *Caenorhabditis elegans*. Nat. Commun. 5: 5579.

Fuentealba, P., and M. Steriade, 2005 The reticular nucleus revisited: intrinsic and network properties of a thalamic pacemaker. Prog. Neurobiol. 75: 125–141.

Graves, L. A., K. Hellman, S. Veasey, J. A. Blendy, A. I. Pack *et al.*, 2003 Genetic evidence for a role of CREB in sustained cortical arousal. J. Neurophysiol. 90: 1152–1159.

Hale, C. F., K. C. Dietz, J. A. Varela, C. B. Wood, B. C. Zirlin *et al.*, 2011 Essential role for vav Guanine nucleotide exchange factors in brain-derived neurotrophic factor-induced dendritic spine growth and synapse plasticity. J. Neurosci. 31: 12426–12436.

Hawrylycz, M. J., E. S. Lein, A. L. Guillozet-Bongaarts, E. H. Shen, L. Ng *et al.*, 2012 An anatomically comprehensive atlas of the adult human brain transcriptome. Nature 489: 391–399. Available at: Allen Institute for Brain Science. Allen Human Brain Atlas, 2015, http://human.brain-map.org.

Hill, A. J., R. Mansfield, J. M. Lopez, D. M. Raizen, and C. Van Buskirk, 2014 Cellular stress induces a protective sleep-like state in *C. elegans*. Curr. Biol. 24: 2399–2405.

Hornstein, I., M. A. Mortin, and S. Katzav, 2003 DroVav, the Drosophila melanogaster homologue of the mammalian Vav proteins, serves as a signal transducer protein in the Rac and DER pathways. Oncogene 22: 6774–6784.

Ikeda, M., B. Aleksic, Y. Kinoshita, T. Okochi, K. Kawashima *et al.*, 2011 Genome-wide association study of schizophrenia in a Japanese population. Biol. Psychiatry 69: 472–478.

Iwanir, S., N. Tramm, S. Nagy, C. Wright, D. Ish *et al.*, 2013 The microarchitecture of *C. elegans* behavior during lethargus: homeostatic bout dynamics, a typical body posture, and regulation by a central neuron. Sleep 36: 385–395.

Jin, Y., 1999 Transformation, pp. 66–96 in *Caenorhabditis elegans: A Practical Approach*, edited by I. A. Hope. Oxford University Press, New York.

Jones, D., D. K. Dixon, R. W. Graham, and E. P. Candido, 1989 Differential regulation of closely related members of the hsp16 gene family in Caenorhabditis elegans. DNA 8: 481–490.

Kim, K., and C. Li, 2004 Expression and regulation of an FMRFamide-related neuropeptide gene family in Caenorhabditis elegans. J. Comp. Neurol. 475: 540–550.

Kushikata, T., J. Fang, Z. Chen, Y. Wang, and J. M. Krueger, 1998 Epidermal growth factor enhances spontaneous sleep in rabbits. Am. J. Physiol. 275: R509–R514.

Langmesser, S., P. Franken, S. Feil, Y. Emmenegger, U. Albrecht *et al.*, 2009 cGMP-dependent protein kinase type I is implicated in the regulation of the timing and quality of sleep and wakefulness. PLoS One 4: e4238.

Lein, E. S., M. J. Hawrylycz, N. Ao, M. Ayres, A. Bensinger *et al.*, 2007 Genome-wide atlas of gene expression in the adult mouse brain. Nature 445: 168–176. Available at: Allen Institute for Brain Science. Allen Mouse Brain Atlas, 2015, http://mouse.brain-map.org.

Maduro, M., and D. Pilgrim, 1995 Identification and cloning of unc-119, a gene expressed in the Caenorhabditis elegans nervous system. Genetics 141: 977–988.

Mahowald, M. W., and C. H. Schenck, 2005 Insights from studying human sleep disorders. Nature 437: 1279–1285.

Malartre, M., D. Ayaz, F. F. Amador, and M. D. Martin-Bermudo, 2010 The guanine exchange factor vav controls axon growth and guidance during Drosophila development. J. Neurosci. 30: 2257–2267.

Mani, A., J. Radhakrishnan, A. Farhi, K. S. Carew, C. A. Warnes *et al.*, 2005 Syndromic patent ductus arteriosus: evidence for haploinsufficient TFAP2B mutations and identification of a linked sleep disorder. Proc. Natl. Acad. Sci. USA 102: 2975–2979.

Margolis, B., P. Hu, S. Katzav, W. Li, J. M. Oliver *et al.*, 1992 Tyrosine phosphorylation of vav proto-oncogene product containing SH2 domain and transcription factor motifs. Nature 356: 71–74.

Nagy, S., D. M. Raizen, and D. Biron, 2014 Measurements of behavioral quiescence in *Caenorhabditis elegans*. Methods 68: 500–507.

Nelson, M. D., and D. M. Raizen, 2013 A sleep state during *C. elegans* development. Curr. Opin. Neurobiol. 23: 824–830.

Nelson, M. D., N. F. Trojanowski, J. B. George-Raizen, C. J. Smith, C. C. Yu *et al.*, 2013 The neuropeptide NLP-22 regulates a sleep-like state in *Caenorhabditis elegans*. Nat. Commun. 4: 2846.

Nelson, M. D., K. H. Lee, M. A. Churgin, A. J. Hill, C. Van Buskirk *et al.*, 2014 FMRFamide-like FLP-13 neuropeptides promote quiescence following heat stress in Caenorhabditis elegans. Curr. Biol. 24: 2406–2410.

Nonet, M. L., J. E. Staunton, M. P. Kilgard, T. Fergestad, E. Hartwieg *et al.*, 1997 *Caenorhabditis elegans rab-3* mutant synapses exhibit impaired function and are partially depleted of vesicles. J. Neurosci. 17: 8061–8073.

Norman, K. R., R. T. Fazzio, J. E. Mellem, M. V. Espelt, K. Strange *et al.*, 2005 The Rho/Rac-family guanine nucleotide exchange factor VAV-1 regulates rhythmic behaviors in *C. elegans*. Cell 123: 119–132.

Pandey, A., A. V. Podtelejnikov, B. Blagoev, X. R. Bustelo, M. Mann *et al.*, 2000 Analysis of receptor signaling pathways by mass spectrometry: identification of vav-2 as a substrate of the epidermal and platelet-derived growth factor receptors. Proc. Natl. Acad. Sci. USA 97: 179–184.

Raizen, D. M., J. E. Zimmerman, M. H. Maycock, U. D. Ta, Y. J. You *et al.*, 2008 Lethargus is a *Caenorhabditis elegans* sleep-like state. Nature 451: 569–572.

Rechtschaffen, A., and B. M. Bergmann, 2002 Sleep deprivation in the rat: an update of the 1989 paper. Sleep 25: 18–24.

Sauzeau, V., M. A. Sevilla, J. V. Rivas-Elena, E. de Alava, M. J. Montero *et al.*, 2006 Vav3 proto-oncogene deficiency leads to sympathetic hyperactivity and cardiovascular dysfunction. Nat. Med. 12: 841–845.

Sauzeau, V., M. Jerkic, J. M. Lopez-Novoa, and X. R. Bustelo, 2007 Loss of Vav2 proto-oncogene causes tachycardia and cardiovascular disease in mice. Mol. Biol. Cell 18: 943–952.

Sauzeau, V., J. A. Horta-Junior, A. S. Riolobos, G. Fernandez, M. A. Sevilla *et al.*, 2010a Vav3 is involved in GABAergic axon guidance events important for the proper function of brainstem neurons controlling cardiovascular, respiratory, and renal parameters. Mol. Biol. Cell 21: 4251–4263.

Sauzeau, V., M. A. Sevilla, M. J. Montero, and X. R. Bustelo, 2010b The Rho/Rac exchange factor Vav2 controls nitric oxide-dependent responses in mouse vascular smooth muscle cells. J. Clin. Invest. 120: 315–330.

Sauzeau, V., J. M. Carvajal-Gonzalez, A. S. Riolobos, M. A. Sevilla, M. Menacho-Marquez *et al.*, 2011 Transcriptional factor aryl hydrocarbon receptor (Ahr) controls cardiovascular and respiratory functions by regulating the expression of the Vav3 proto-oncogene. J. Biol. Chem. 286: 2896–2909.

Scharf, M. T., N. Naidoo, J. E. Zimmerman, and A. I. Pack, 2008 The energy hypothesis of sleep revisited. Prog. Neurobiol. 86: 264–280.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri, 2012 NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9: 671–675.

Schwarz, J., and H. Bringmann, 2013 Reduced sleep-like quiescence in both hyperactive and hypoactive mutants of the Galphaq Gene *egl-30* during lethargus in *Caenorhabditis elegans*. PLoS One 8: e75853.

Schwarz, J., J. P. Spies, and H. Bringmann, 2012 Reduced muscle contraction and a relaxed posture during sleep-like lethargus. Worm 1: 12–14.

Sehgal, A., and E. Mignot, 2011 Genetics of sleep and sleep disorders. Cell 146: 194–207.

Singh, K., M. Y. Chao, G. A. Somers, H. Komatsu, M. E. Corkins *et al.*, 2011 *C. elegans* Notch signaling regulates adult chemosensory response and larval molting quiescence. Curr. Biol. 21: 825–834.

Singh, K., J. Y. Ju, M. B. Walsh, M. A. DiIorio, and A. C. Hart, 2014 Deep conservation of genes required for both *Drosophila melanogaster* and *Caenorhabditis elegans* sleep includes a role for dopaminergic signaling. Sleep 37: 1439–1451.

Snodgrass-Belt, P., J. L. Gilbert, and F. C. Davis, 2005 Central administration of transforming growth factor-alpha and neuregulin-1 suppress active behaviors and cause weight loss in hamsters. Brain Res. 1038: 171–182.

Spooner, P. M., J. Bonner, A. V. Maricq, G. M. Benian, and K. R. Norman, 2012 Large isoforms of UNC-89 (obscurin) are required for muscle cell architecture and optimal calcium release in Caenorhabditis elegans. PLoS One 7: e40182.

Stiernagle, T., 2006 Maintenance of *C. elegans* (February 11, 2006), pp. 1–11 in *WormBook*, ed. The *C. elegans* Research Community, doi/10.1895/wormbook.1.7.1, http://www.wormbook.org.

Thalappilly, S., P. Soubeyran, J. L. Iovanna, and N. J. Dusetti, 2010 VAV2 regulates epidermal growth factor receptor endocytosis and degradation. Oncogene 29: 2528–2539.

Tononi, G., and C. Cirelli, 2006 Sleep function and synaptic homeostasis. Sleep Med. Rev. 10: 49–62.

Trojanowski, N. F., M. D. Nelson, S. W. Flavell, C. Fang-Yen, and D. M. Raizen, 2015 Distinct mechanisms underlie quiescence during two *Caenorhabditis elegans* sleep-like states. J. Neurosci. 35: 14571–14584.

Tu, B. P., and S. L. McKnight, 2006 Metabolic cycles as an underlying basis of biological oscillations. Nat. Rev. Mol. Cell Biol. 7: 696–701.

Turek, M., I. Lewandrowski, and H. Bringmann, 2013 An AP2 transcription factor is required for a sleep-active neuron to induce sleep-like quiescence in *C. elegans*. Curr. Biol. 23: 2215–2223.

Van Buskirk, C., and P. W. Sternberg, 2007 Epidermal growth factor signaling induces behavioral quiescence in Caenorhabditis elegans. Nat. Neurosci. 10: 1300–1307.

Van Buskirk, C., and P. W. Sternberg, 2010 Paired and LIM class homeodomain proteins coordinate differentiation of the *C. elegans* ALA neuron. Development 137: 2065–2074.

van den Pol, A. N., 2012 Neuropeptide transmission in brain circuits. Neuron 76: 98–115.

White, J. G., E. Southgate, J. N. Thomson, and S. Brenner, 1986 The structure of the nervous system of the nematode *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. B Biol. Sci. 314: 1–340.

Zahn, T. R., M. A. Macmorris, W. Dong, R. Day, and J. C. Hutton, 2001 IDA-1, a *Caenorhabditis elegans* homolog of the diabetic autoantigens IA-2 and phogrin, is expressed in peptidergic neurons in the worm. J. Comp. Neurol. 429: 127–143.

Zahn, T. R., J. K. Angleson, M. A. MacMorris, E. Domke, J. F. Hutton *et al.*, 2004 Dense core vesicle dynamics in Caenorhabditis elegans neurons and the role of kinesin UNC-104. Traffic 5: 544–559.

Zhou, K. M., Y. M. Dong, Q. Ge, D. Zhu, W. Zhou *et al.*, 2007 PKA activation bypasses the requirement for UNC-31 in the docking of dense core vesicles from *C. elegans* neurons. Neuron 56: 657–669.

Zhu, S., C. Zhao, Y. Wu, Q. Yang, A. Shao *et al.*, 2015 Identification of a Vav2-dependent mechanism for GDNF/Ret control of mesolimbic DAT trafficking. Nat. Neurosci. 18: 1084–1093.

Zimmerman, J. E., N. Naidoo, D. M. Raizen, and A. I. Pack, 2008 Conservation of sleep: insights from non-mammalian model systems. Trends Neurosci. 31: 371–376.

Communicating editor: P. Sengupta

GENETICS

Supporting Information
www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.183038/-/DC1

A Conserved GEF for Rho-Family GTPases Acts in an EGF Signaling Pathway to Promote Sleep-like Quiescence in *Caenorhabditis elegans*

Amanda L. Fry, Jocelyn T. Laboy, Huiyan Huang, Anne C. Hart, and Kenneth R. Norman

DOI: 10.1534/genetics.115.183038

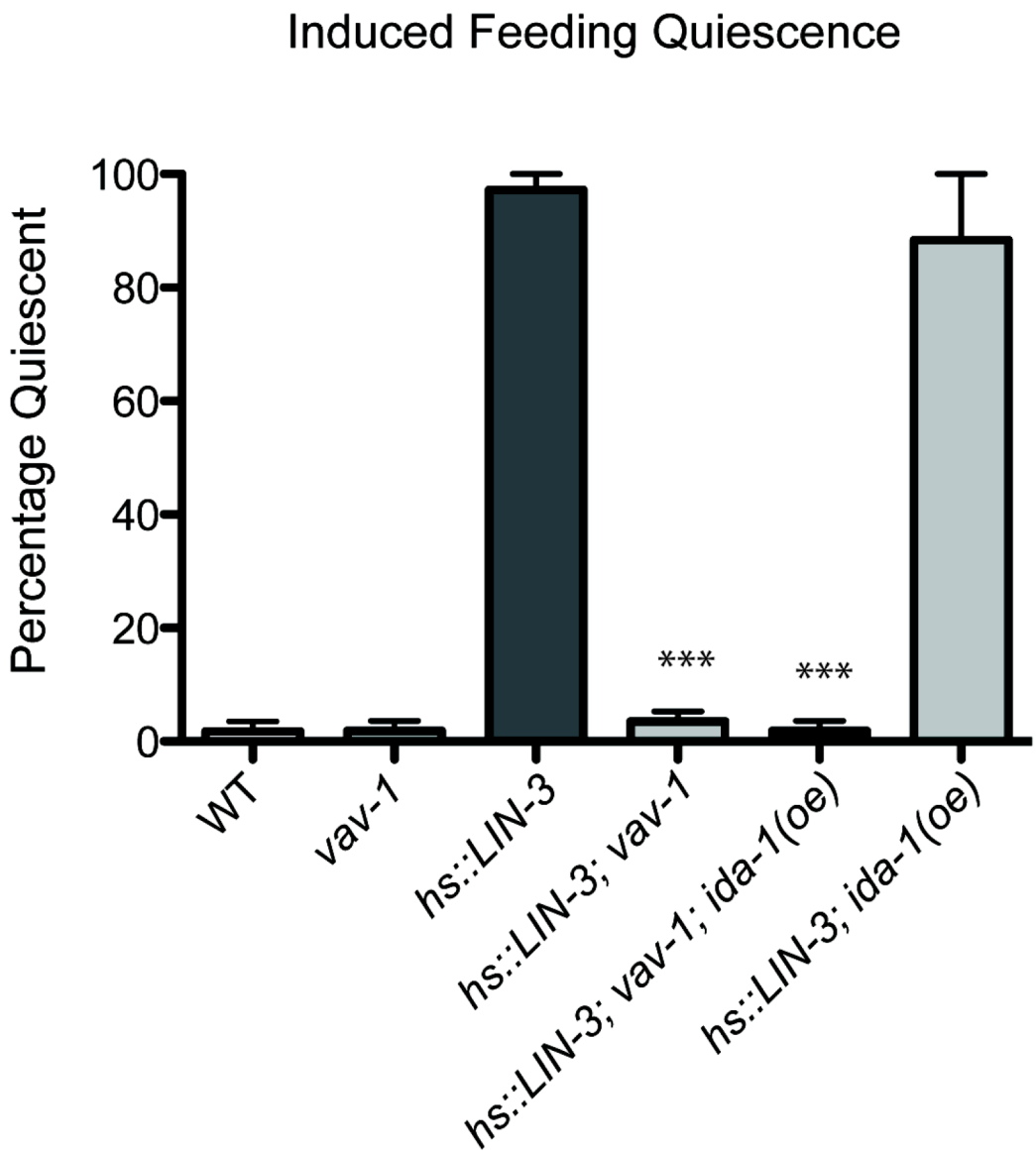

Figure S1 Overexpression of IDA-1 does not rescue the defective LIN-3/EGF induced quiescence phenotype of *vav-1* mutants. Percentage of animals showing LIN-3/EGF induced feeding quiescence. 50-60 animals were analyzed for each genotype. Data are presented as mean +/- SEM and were analyzed by one-way ANOVA with Tukey post-test analysis (*** $p<0.001$).

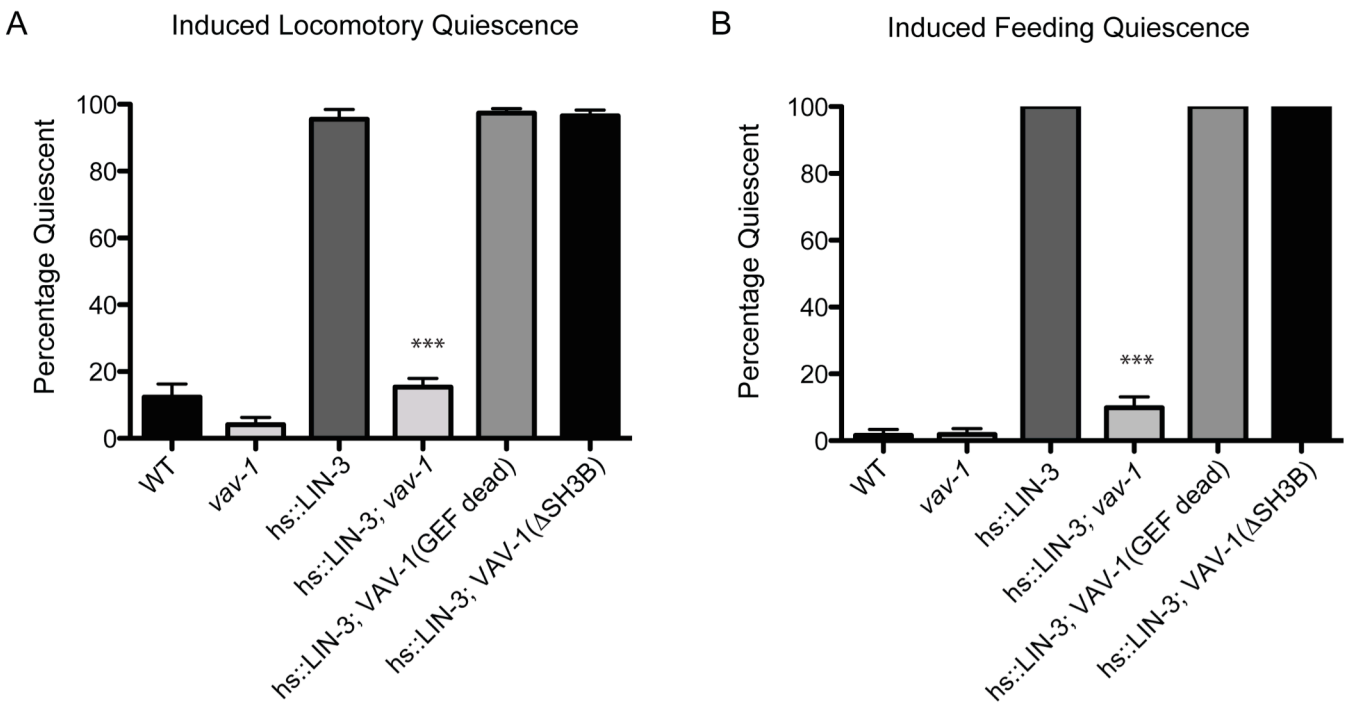

Figure S2 Expression of GEF dead or ΔSH3B does not cause a dominant negative effect on LIN-3/EGF induced quiescence. (A) Percentage of animals showing LIN-3/EGF induced locomotory quiescence. (B) Percentage of animals showing LIN-3/EGF induced feeding quiescence. While *vav-1* mutants fail to undergo LIN-3/EGF induced quiescence, both the GEF dead and the ΔSH3B construct display normal quiescent behavior upon LIN-3/EGF expression. 50-60 animals were analyzed for each genotype. Data are presented as mean +/- SEM and were analyzed by one-way ANOVA with Tukey post-test analysis (*** $p<0.001$).

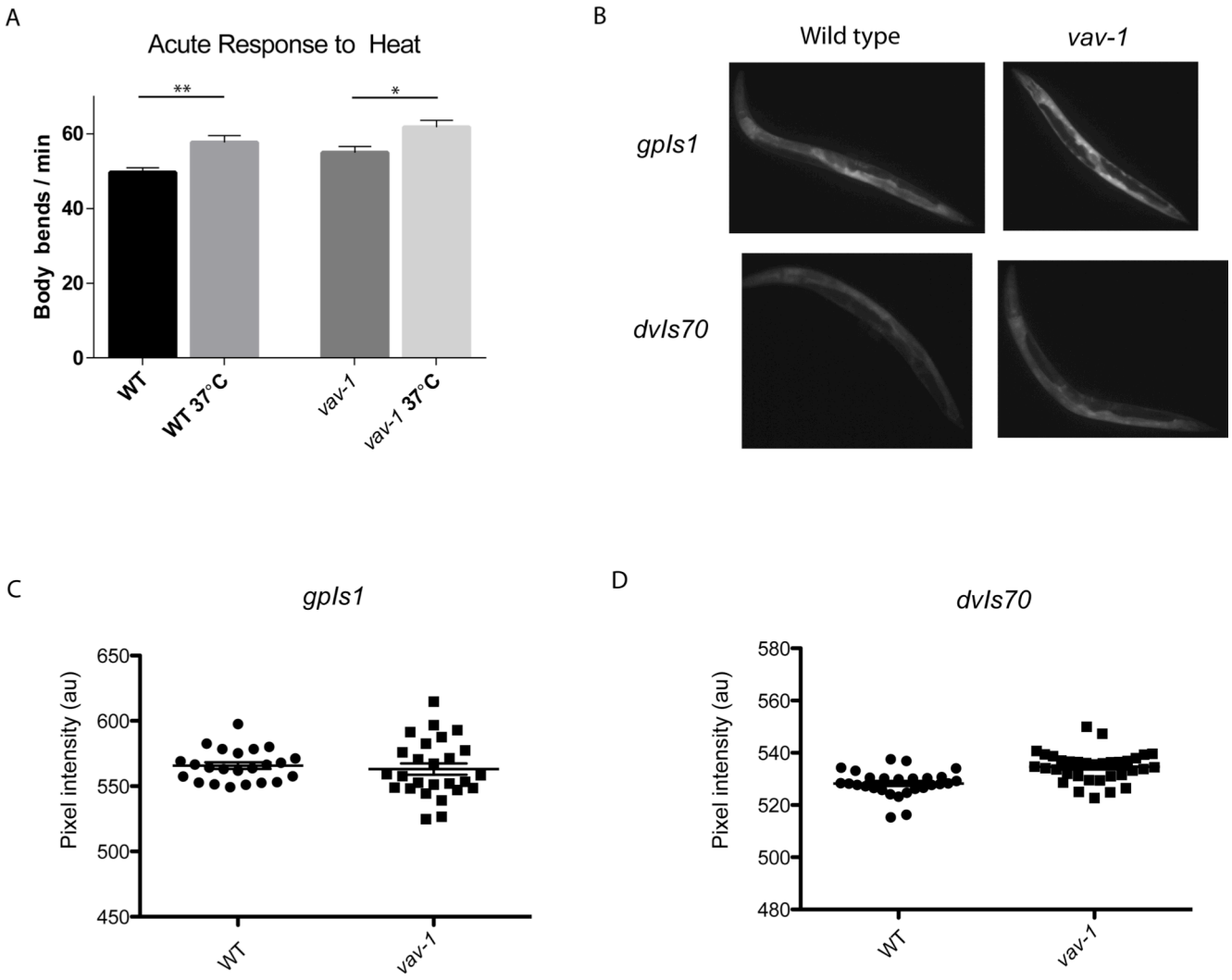

Figure S3 *vav-1* mutants respond normally to acute heat exposure. (A) Animals were individually transferred to fresh room temperature agar plates, or pre-warmed 37°C agar plates, and body bends were counted for one minute following transfer. Both WT and *vav-1* mutants move faster in response to heat. Data are represented as mean +/- SEM and were analyzed by unpaired two-tailed T-test (* $p<0.02$, ** $p<0.003$). (B-D) *vav-1* mutants heat stressed at 33°C for 30 minutes show robust heat shock reporter activity. (B) Representative images of wild type and *vav-1* mutant animals 2 hours after heat stress. *gpIs1* and *dvIs70* are two independently hsp-16.2::GFP chromosomally integrated transgenes. (C) Quantification of *gpIs1* heat shock reporter expression. n = 23 (WT) and 26 (*vav-1*). (D) Quantification of *dvIs70* heat shock reporter expression. n = 28 (WT) and 34 (*vav-1*). Data presented as mean +/- SEM.

Table S1. Primer list

Oligo Name	Sequence	Description
P247	5' CTTTAGTTCACGAAGGGGATAGAGTAAGCATGTTTTGTAGTTTAAAGG 3'	VAV-1 SH3A deletion Forward
P248	5' CCTTTAAACTACAAAACATGCTTACTCTATCCCCTTCGTGAACTAAAG 3'	VAV-1 SH3A deletion Reverse
P249	5' GGAAATATCGGAATTCCTATGGCGCAGTAACAATTTGATTGAAATTTTTGC 3'	VAV-1 SH2 deletion Forward
P250	5' GCAAAAATTTCAATCAAATTGTTACTGCGCCATAGGAATTCCGATATTTCC 3'	VAV-1 SH2 deletion Reverse
P251	5' CCTCAAAAATCCATATTCCCAATGCGATCCGGCCACAGAAGGGAGC 3'	VAV-1 SH3B deletion Forward
P252	5' GCTCCCTTCTGTGGCCGGATCGCATTGGGAATATGGATTTTTGAGG 3'	VAV-1 SH3B deletion Reverse
P349	5' TGCCTCTCACTTCATACTCTTCG 3'	3kb 5' *cis* regulatory element of *vav-1* gene Forward
P350	5' GCACCTGAAAATGCATTAAAAAATATTGG 3'	3kb 5' *cis* regulatory element of *vav-1* gene Reverse

File S1. Supplemental – Statistics

Figure 2 – ALA marker imaging

For Fig. 2B, 2D, and 2F, Kruskal-Wallis one-way ANOVA (since data is normalized to WT, standard deviations differ significantly) followed by Dunn's multiple comparisons.

For Fig. 2G and 2H, one-way ANOVA followed by Tukey's multiple comparisons.

	Strain Comparison	**P value**
Fig. 2B	WT v. *vav-1*	****p< 0.0001
	vav-1 v. *vav-1; vav-1 rescue*	****p< 0.0001
Fig. 2D	WT v. *vav-1*	****p< 0.0001
	vav-1 v. *vav-1; vav-1 rescue*	****p< 0.0001
Fig. 2F	WT v. *vav-1*	*p<0.05
	WT v. *vav-1*; *vav-1 rescue*	****p< 0.0001
Fig. 2G	*vav-1* v. *vav-1; vav-1 rescue*	*p<0.05
Fig. 2H	WT v. *vav-1*; *vav-1 rescue*	*p<0.05

Figure 3 – hs:LIN-3 Induced Quiescence

Fig. 3A, 3B, 3D, 3E One-way ANOVA with Tukey's multiple comparison post-tests

	Strain Comparison	**P value**
Fig. 3A	WT v. hs:LIN-3	****p< 0.0001
	hs:LIN-3 v. hs:LIN-3; *vav-1*	****p< 0.0001
	hs:LIN-3; *vav-1* v. hs:LIN-3; *vav-1*; *ALA neuron rescue*	****p< 0.0001
Fig. 3B	WT v. hs:LIN-3	****p< 0.0001
	hs:LIN-3 v. hs:LIN-3; *vav-1*	****p< 0.0001
	hs:LIN-3; *vav-1* v. hs:LIN-3; *vav-1*; *ALA neuron rescue*	****p< 0.0001
Fig. 3D	WT v. hs:LIN-3	****p< 0.0001
	hs:LIN-3 v. hs:LIN-3; *vav-1*	****p< 0.0001
	hs:LIN-3; *vav-1* v. *hs:LIN-3; *vav-1*; *vav-1 rescue*	****p< 0.0001
	*hs:LIN-3; *vav-1*; *vav-1 rescue* v. hs:LIN-3; *vav-1*; *GEF-dead vav-1 rescue*	****p< 0.0001
	*hs:LIN-3; *vav-1*; *vav-1 rescue (minimal promoter)* v. hs:LIN-3; *vav-1*; ΔSH3B *vav-1 rescue*	****p< 0.0001
Fig. 3E	WT v. hs:LIN-3	****p< 0.0001
	hs:LIN-3 v. hs:LIN-3; *vav-1*	****p< 0.0001
	hs:LIN-3; *vav-1* v. *hs:LIN-3; *vav-1*; *vav-1 rescue*	****p< 0.0001

	*hs:LIN-3; *vav-1*; *vav-1 rescue* v. hs:LIN-3; *vav-1*; *GEF-dead vav-1 rescue*	****p< 0.0001
	*hs:LIN-3; *vav-1*; *vav-1 rescue (minimal promoter)* v. hs:LIN-3; *vav-1*; ΔSH3B *vav-1 rescue*	****p< 0.0001

Figure 4 – Lethargus Quiescence

Fig. 4D, 4E, 4F, and 4G, Kruskal-Wallis one-way ANOVA (since standard deviations of populations differ significantly) followed by Dunn's multiple comparisons

	Strain Comparison	**P value**
Fig. 4D	WT v. *egl-30 (tg26)*	**p<0.01
	vav-1 v. egl-30 (tg26)	**p<0.01
Fig. 4E	WT v. *egl-30 (tg26)*	****p<0.0001
	vav-1 v. egl-30 (tg26)	****p<0.0001
Fig. 4F	WT v. *plc-3*	**p<0.01
	WT v. *vav-1; rescue*	**p<0.01
Fig. 4G	WT v. *plc-3*	*p<0.05
	WT v. *vav-1; rescue*	**p<0.01

Figure 5 – Heat Shock Recovery Quiescence

Fig. 5A and 5B, Two-way RM ANOVA, Tukey post-tests

	Strain Comparison	**Time Point (min)**	**p value**
Fig 5A	WT v. *vav-1*	20	* p<0.05
		30	** p<0.01
		40	* p<0.05
		50	* p<0.05
		60	* p<0.05
		100	* p<0.05
		150	** p<0.01
		180	** p<0.01
		240	* p<0.05
		300	* p<0.05
	WT v. *vav-1; ALA rescue*	30	**p<0.01
		40	** p<0.01
		50	* p<0.05
	vav-1 v. *vav-1; ALA rescue*	100	* p<0.05
		240	* p<0.05

		300	** p<0.01
		360	* p<0.05
Fig 5B	WT v. *vav-1*	40	** p<0.01
		50	* p<0.05
		120	* p<0.05
		180	** p<0.01
		240	* p<0.05
	WT v. *vav-1; GEF-dead rescue*	30	* p<0.05
		40	** p<0.01
		50	** p<0.01
		120	* p<0.05
		300	* p<0.05
	WT v. *vav-1*; *vav-1 rescue*	10	**p<0.01
		30	** p<0.01
		40	*** p<0.001
		50	* p<0.05
	vav-1 v. *vav-1*; *vav-1 rescue*	10	*** p<0.001
		120	* p<0.05
		180	** p<0.01
	vav-1; GEF-dead rescue v. *vav-1*; *vav-1 rescue*	10	* p<0.05
		120	* p<0.05
		180	* p<0.05
		300	* p<0.05

Figure 6 - Stress Survival

Fig. 6A, 6B, 6C, Two-way RM ANOVA, Tukey post-tests

	Strain Comparison	**Time Point (hours)**	**p value**
Fig. 6A	WT v. *vav-1*	48	****p< 0.0001
		72	****p< 0.0001
		96	****p< 0.0001
	WT v. *plc-3*	72	****p< 0.0001
		96	****p< 0.0001
	vav-1 v. *vav-1; vav-1 rescue*	48	***p<0.001
		72	****p<0.0001
		96	****p<0.0001
	vav-1 v. *plc-3*	48	**p<0.01
		72	****p<0.0001
		96	**p<0.01

Fig. 6C	WT v. *vav-1*	72	****p<0.0001
		96	****p<0.0001
	vav-1 v. *egl-4; vav-1*	72	**p<0.01
		96	**p<0.01

Figure 7 – Heat shock-induced VAV-1

One-way ANOVA with Tukey's multiple comparison post-tests

Strain Comparison	**P value**
WT v. hs::*vav-1*	**p<0.01
hs::GFP v. hs::*vav-1*	**p<0.01
hs::*vav-1* v. hs::*vav-1* no heat	***p<0.001
hs::*vav-1* v. hs::*vav-1* (10 hrs)	**p<0.01

Supplemental Figures

Fig. S1 and S2, one-way ANOVA with Tukey's multiple comparison post-tests

Fig. S3 Unpaired two-tailed T Tests

	Strain Comparison	**P value**
Fig. S1	hs::LIN-3 v. hs::LIN-3*; vav-1*	***p<0.001
	hs::LIN-3 v hs::LIN-3*; vav-1; ida-1 (oe)*	***p<0.001
Fig. S2A	hs::LIN-3 v. hs::LIN-3*; vav-1*	***p<0.001
Fig. S2B	hs::LIN-3 v. hs::LIN-3*; vav-1*	***p<0.001
Fig. S3	WT v. WT 37°C	**p<0.003
	vav-1 v. *vav-1* 37°C	*p<0.02