
Mechanical Stretch Inhibition Sensitizes Proprioceptors to Compressive Stresses

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Abstract

A repetitive gait cycle is an archetypical component within the behavioural repertoire of many if not all animals including humans. It originates from mechanical feedback within proprioceptors to adjust the motorprogram during locomotion and thus leads to a periodic orbit in a low dimensional space. Here, we investigate the mechanics, molecules and neurons responsible for proprioception in *Caenorhabditis (C.) elegans* to gain insight into how mechanosensation shapes the orbital trajectory to a well-defined limit cycle. We used genome editing, force spectroscopy and multiscale modeling and found that alternating tension and compression with the spectrin network of a single proprioceptor encodes body posture and informs TRP-4/NOMPC and TWK-16/TREK2 homologs of mechanosensitive ion channels during locomotion. In contrast to a widely accepted model of proprioceptive ‘stretch’ reception, we found that proprioceptors activated under compressive stresses *in vivo* and *in vitro*, and speculate that this property is conserved across function and species.

1 Introduction

2 Many, if not all, motile animals generate forward thrust that is powered by collective cell shape
3 changes due to antagonizing muscle activity. In *C. elegans*, the associated contraction/relaxation
4 cycles are performed with sub-maximal capacity and are driven by four classes of coupled excitatory
5 and inhibitory motor neurons [1], giving rise to the tailward propagating wave that bends the body with
6 a given curvature [2]. The amplitude of these optimal driving patterns is remarkably robust against
7 external and internal perturbations, due in part to mechanosensitive feedback from specialized sensory
8 cells and neurons that signal the mechanical deformation to the central nervous system [3]. Several
9 proprioceptor neurons have been identified in *C. elegans* that become activated upon spontaneous or
10 imposed body bending, such as DVA, PVD, SMD and the motor circuit itself [4–8], all of which express
11 mechano-electrical transduction (MeT) channels that are likely candidates to read out the mechanical
12 strains and stresses that arise during locomotion [9, 10]. However, we still have little knowledge
13 about the physiologically relevant mechanical stresses and deformations that lead to the activation of
14 mechanosensitive neurons during proprioception or visceral mechanosensation [11]. This is due in
15 part to the complexity of animal anatomy confounded by the coexistence of multiple MeT channels,
16 their differential sensitivity to specific stress tensors [12, 13], and the associated difficulty to record
17 from the mechanosensor in moving specimens. Contrary to intuition, structure-guided modeling
18 revealed that one of the best characterized MeT channels of the TRP family, NOMPC [14, 15], is
19 gated under compressive stresses [16]. In contrast, members of the ubiquitously expressed two-pore
20 potassium (K2P) channels, activate under mechanical tension [17]. Because TRP and K2P channels
21 have opposing roles on neuronal (de)polarization [15], strain selectivity of these ion channels could
22 fine tune neuronal responses in a dynamic environment [18].

23 Here, we identify that a single neuron, DVA in *C. elegans*, activates upon compression of the
24 spectrin cytoskeleton in a NOMPC/TRP-4 dependent manner, while mechanical tension attenuates
25 neuronal activity and prevents firing through the K2P homolog TWK-16. We speculate that this
26 mechanical interplay is particularly important during body movement to confine neuronal activity

27 within controlled regions when positive and negative stresses coincide in long sensory neurites.

28 **Results**

29 **β -spectrin curbs body bends** We previously identified UNC-70 β -spectrin, as a key cytoskeletal
30 component that is under mechanical tension in neurons in *C. elegans* [19]. We noticed that the same
31 mutations that lead to a failure to detect forces during gentle body touch, also increase body curvature
32 during locomotion. Thus, we conjectured that UNC-70 might have roles in proprioception during
33 locomotion. We first recorded short videos of freely moving wildtype (wt) and *unc-70* mutant animals,
34 and applied dimensionality reduction techniques (see Methods and Table S1 for details) to represent
35 the emergent locomotion pattern as a periodic orbit in a low dimensional phase space (Fig. 1A,B and
36 Ref. [20]). The first two modes describe the forward locomotion and form planar, phase locked orbit,
37 with a limit corresponding to the amplitude of the body bends. The third mode has been attributed to
38 turning behavior and deep body bends [20,21]. These modes, previously termed eigenworms, span
39 a parametric space that effectively describes the stability and dynamics of postural changes within a
40 simple 3D Cartesian coordinate system.

41 Except for omega-turns, wt animals show little deviations into the third dimension during forward
42 locomotion (Fig. 1A), leading to a planar, toroidal manifold. In contrast, *unc-70(e524)* animals
43 display a much larger orbit in the two forward modes, and significant excursions into the turning
44 mode (Fig. 1B), indicative for severely exaggerated body bends (Fig. 1C,D). With this analysis
45 it becomes apparent that UNC-70 is required to reach and adjust the curvature amplitude during
46 forward locomotion that is optimized for animal locomotion.

47 **The spectrin network has cell-specific roles during locomotion** Because β -spectrin is ex-
48 pressed in many if not all neurons and weakly in body wall muscles [19,22,23], a higher bending
49 amplitude could in principle reflect defects in muscle contraction or a loss of neuronal control. We
50 therefore sought to test tissue-specific roles of β -spectrin independently and generated a 'floxed'

Figure 1

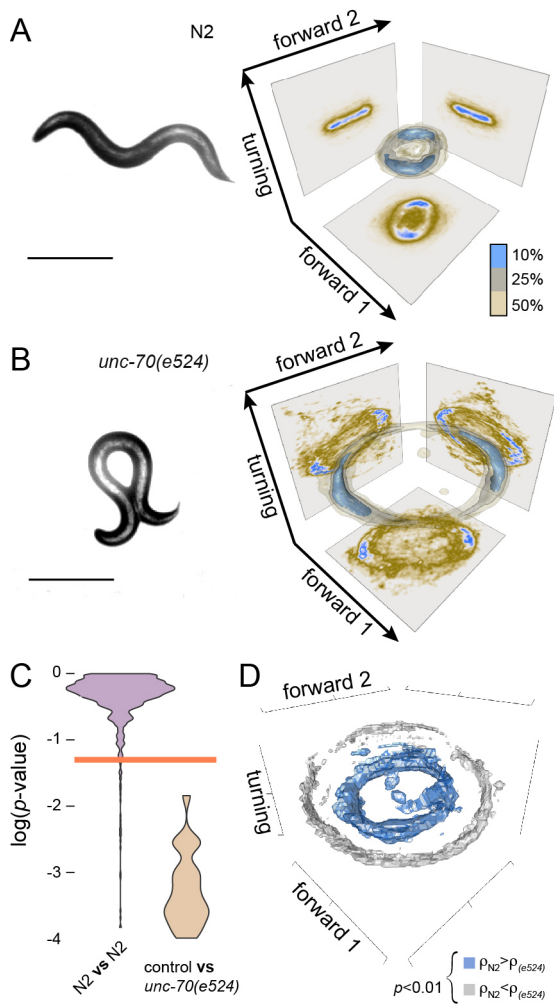


Figure 1. β -spectrin constrains the limit cycles during forward locomotion

A,B Representative still image of (A) wildtype animals and (B) *unc-70(e524)* animals and the 3D density estimate for the joint probability distribution (equivalent to a discrete 3D histogram) of the two forward and turning modes in the eigenworm space. Color scale = (brown, low density; blue, high density) **C** Violin plots of the p -value distributions for 1000 independent tests of a bootstrapped population estimate of wt 3D probability density function (ctrl) tested against itself or a bootstrapped population estimate of *unc-70(e524)*. Orange line indicates $\alpha=0.05$ level of significance for the hypothesis H_0 that bootstrapped density functions derived from a and b are equal (see Methods). **D** Color-coded, statistically significant differences in the local probability density functions shown in (A) and (B).

51 *unc-70* allele (Fig. 2A; see Methods for details) that allows for conditional excision upon cell-specific
 52 expression of the CRE recombinase in muscles or neurons and confirmed CRE activity using a
 53 fluorescent recombination reporter (Ref. [24] and Fig. S1, Table S2). Importantly, neither the CRE
 54 lines nor the floxed *unc-70* (Fig. 2B,F, Fig. S2A-C,J) allele had a phenotype on its own when tested
 55 separately. Having successfully confirmed CRE activity, we pan-neurally expressed CRE in the
 56 floxed *unc-70* background (Fig. 2C, Video S2) and rarely observed a regular orbit in the eigenworm
 57 space, indicative for severely uncoordinated locomotion behavior. When we expressed CRE under
 58 muscle-specific promoter [25], we did not observe any defect (Fig. 2D) suggesting that β -spectrin is

59 functionally restricted to neurons during crawling behavior in adults.

60 We then expressed CRE in either A, B or D-type motor neurons or previously proposed propriocep-
61 tors SMD, PVD, Touch Receptor Neurons (TRNs) or DVA and confirmed successful recombination
62 (Fig. S1, Table S2). Even though we neither detected a significant locomotion phenotype in A, B
63 and D-type motor neurons (Fig. S2D-F and Video S2) nor in TRNs (Fig. S2G) or PVD (Fig. S2H),
64 *unc-70* deletion in SMD caused subtle defects (Fig. S2I,K) and only the knockout of *unc-70* in DVA
65 (Fig. 2E,F) leads to an abnormally exaggerated body posture (Video 2). This observation motivated
66 us to ask if this DVA-dependent role of UNC-70 in movement was a general property of spectrin
67 fibers or specific to β -spectrin. Thus we knocked out SPC-1/ α -spectrin, using the well established
68 auxin-induced protein degradation (AID) system [26]. We used a previously published SPC-1::AID
69 animal [27] and first verified that the spatially restricted expression of TIR ligase in TRNs leads to
70 a reduction in protein levels upon addition of auxin (Fig. S3A-D). Then, we targeted TIR to DVA
71 and found that neither expression of the TIR ligase (Fig. S3E-H) nor the SPC-1::AID alone (Fig.
72 S3I-L), but only the co-expression of both transgenes in DVA lead to exaggerated body angles, visible
73 as a larger manifold in the eigenworm space (Fig. S3M-P), similar to the CRE-dependent *unc-70*
74 recombination observed before (Fig. 2E). Taken together, our conditional gene ablation strategy
75 unveiled an unexpected cell-specific role, of the otherwise widely expressed spectrin network, in
76 regulating the extent of body bending during locomotion.

77 **DVA activity correlated with compressive stresses *in vivo*** We next investigated whether changes
78 in body postures influenced neuronal activity in DVA in an *unc-70* dependent manner. We first gener-
79 ated a Ca^{2+} activity reporter using DVA specific expression of Gal4 driving a GCaMP6s effector [28].
80 Then, we performed live imaging and video tracking to correlate Ca^{2+} activity and body curvature in
81 proximity to the cell body (square in Fig. 2G), and normalized the Ca^{2+} sensitive by a Ca^{2+} insensitive
82 fluorophor (Fig. 2H,I). Even though the low Ca^{2+} signal exhibited occasional spontaneous activity
83 bursts and low-amplitude spontaneous signals in completely restrained animals (Fig. S4A), we
84 observed striking activity changes between the ventral and the dorsal side in animals undergoing full

Figure 2

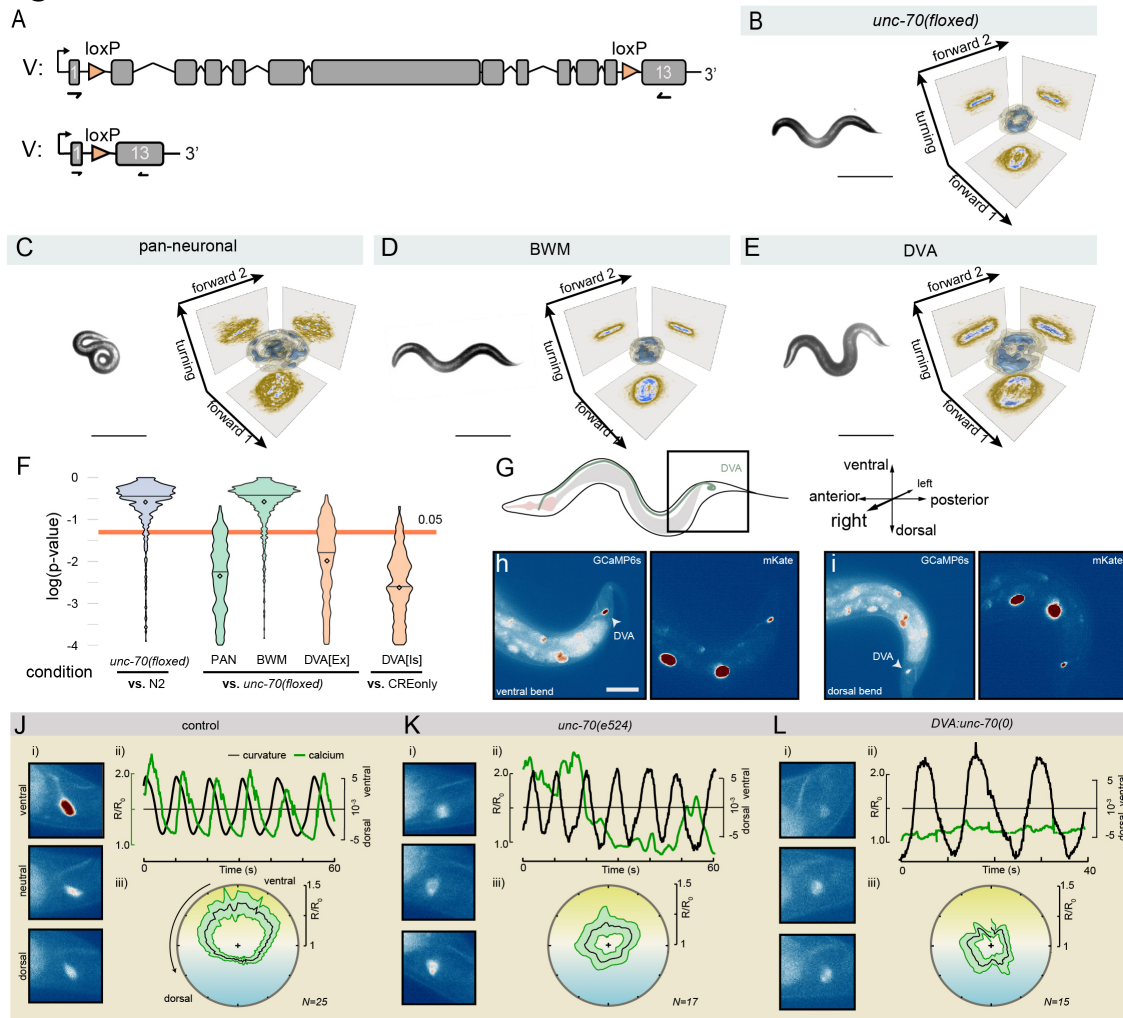


Figure 2. Fig. 2. β -spectrin controls body posture specifically in DVA

A Genetic strategy for cell-specific deletion of *unc-70* in individual cells and neurons by flanking the *unc-70* genomic fragment with two loxP sites and the resulting genomic scar after recombination. **B-E** Representative still image and the corresponding manifold in the three dimensional eigenworm space for **(B)** *unc-70(loxP)* control animals without CRE expression, **(C)** PAN-neuronal *unc-70* deletion, and *unc-70(loxP)* animals with CRE-expression in **(D)** body wall muscles and **(E)** DVA sensory interneurons. Scale bar = 300 μ m. **F** *p*-value distribution for 1000 independent tests for the hypothesis H_0 that the density function for indicated combinations are equal (see Methods). Orange line indicated $\alpha=0.05$ level of significance. **G** Schematic of an animal with the location of DVA cell body and its axon and the coordinate system of our analysis. For clarity and convenience, we display ventral up to emphasize positive calcium correlation with ventral postures. **H,I** Representative calcium-sensitive (GCaMP6s) and insensitive (mKate) images for **(H)** ventral and **(I)** dorsal body bends. Scale bar=50 μ m. **J-L** Representative calcium imaging data from moving **(J)** control, **(K)** *unc-70(e524)*, **(L)** DVA::*unc-70(0)* mutant animals expressing GCaMP6s calcium reporter in DVA subjected to ventral and dorsal bends. (i) Video stills of ventral, neutral and dorsal bends. (ii) Time series of the normalized GCaMP/mKate ratio (left axis) and body curvature (right axis). (iii) Average GCaMP/mKate ratio plotted against the phase angle of body curvature. Yellow shading indicates ventral bends (top), blue corresponds to dorsal body posture (bottom).

85 body swings (Video S3, Fig. 2J). To our very surprise, activation did not correlate with dorsal, but
86 strongly with ventral postures, under which the DVA neurites shorten dramatically (Fig. S5A,B).

87 To understand the role of UNC-70 in this process, we repeated these experiments in the *unc-*
88 *70(e524)* mutation (Fig. 2K) and in the DVA-specific *unc-70* knockout (Fig. 2L). In both conditions, we
89 did not observe curvature-correlated Ca^{2+} activity, suggesting that β -spectrin is needed for neuronal
90 activation as a response to body posture changes and that it acts cell-autonomously within DVA.
91 Rarely, however, signals also appeared during dorsal bends, indicating that UNC-70 directs the
92 preference of neuronal activation during ventral postures.

93 **β -spectrin is under compression during ventral body bends** The finding that DVA activates
94 during ventral bends primed us to investigate how mechanical stresses affect axon shape in moving
95 animals and how the spectrin network contributes to proprioceptive mechanosensitivity. We thus
96 recorded short videos and quantified the local length changes of DVA in each frame as a function of
97 body posture in flexing animals (Fig. S5). Similar to ventral touch receptor neurons (e.g. AVM [19,29]),
98 DVA locally shortens and elongates up to 40% (Fig. S5A,D) during dorsoventral swings in wildtype
99 animals. In *unc-70(e524)* and DVA-specific β -spectrin mutants, however, DVA extended under dorsal
100 body postures but failed to shorten during ventral bends (Fig. S5B-F). Instead, the axon showed a
101 shape change characteristic of a mechanical failure due to compressive stresses, known as buckling
102 instability.

103 Physical intuition teaches us that an elastic body subjected to bending experiences compression on
104 the convex and extensions on the concave side [30,31], with a stress σ that increases with the distance
105 to the central axis (d), elasticity of the worm's body (E) and the curvature assumed (c , Fig. 3A). To
106 directly visualize the stresses originating in the body of *C. elegans* in postures that are typical during
107 locomotion, we resorted to a previously characterized FRET tension sensor [19,32], with predominantly
108 neuronal expression (Fig. SA,B). The inherent mobility of *C. elegans* precluded unrestrained imaging,
109 thus we redesigned a microfluidic device [33] with channels of varying curvatures (Fig. 3B,C) that
110 could trap animals in different postures for 3D imaging (Fig. 3D). In completely straight positions,

Figure 3

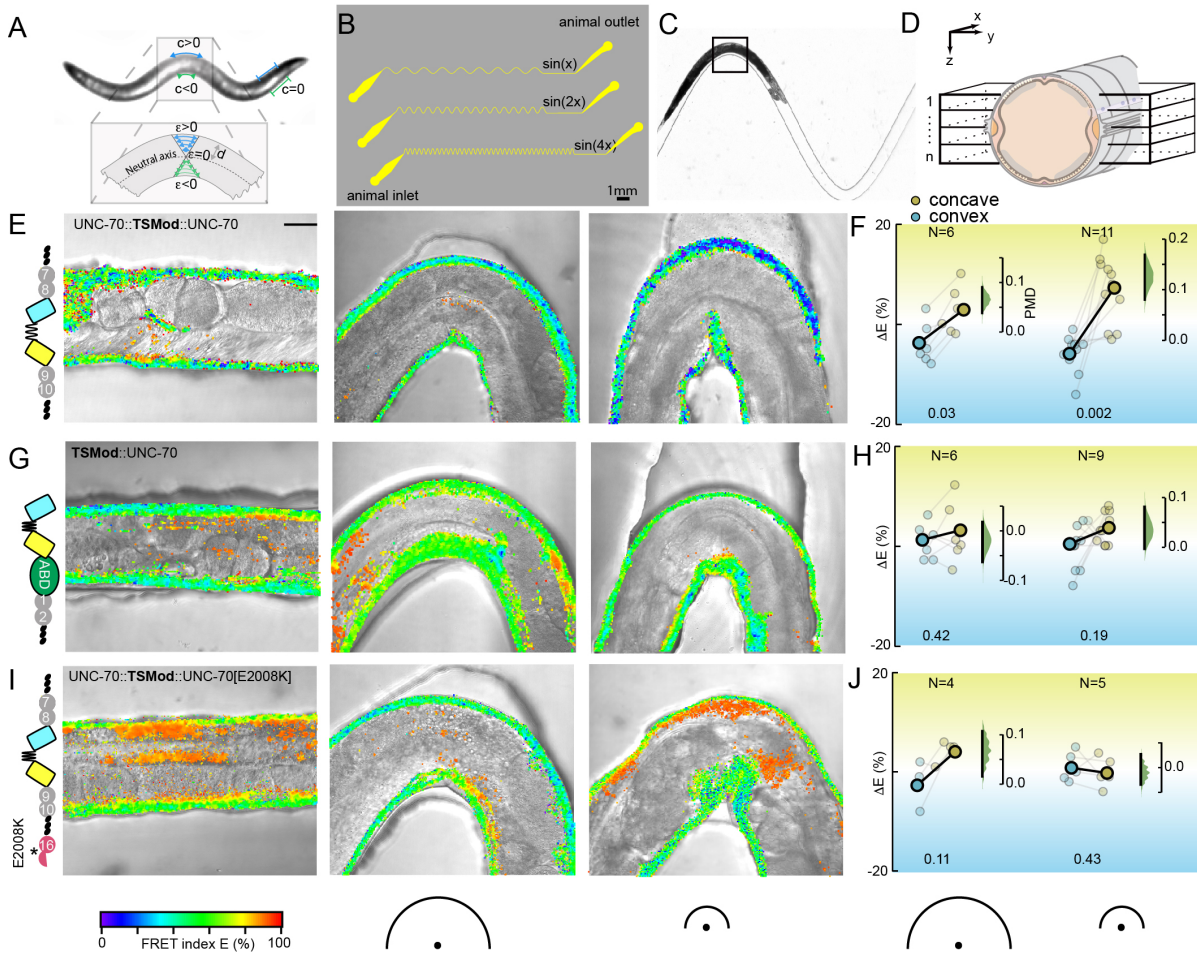


Figure 3. Changes in body mechanics encodes body posture curvature

A Schematic of a crawling animal with varying posture along its body and the associated Euler strains. c =curvature; ϵ =strain; d =thickness **B** Sketch of the mask layout with varying channel curvatures and periodicities. **C** Brightfield image of an animal inside the $\sin(2x)$ channel. **D** 3D representation of the image acquisition procedure. A volume consisting of 10-15 frames encompassing the ventral and dorsal nerve chord was acquired, omitting the lateral nerves. **E, G, I** Schematic and representative and FRET images of collapsed z-stacks for straight and increasingly curved channel of the WFS chip with the FRET index map overlaid on top of a brightfield image for **(E)** UNC-70::TSMoD force sensor with full-length UNC-70 β -spectrin bearing TSMoD embedded between spectrin repeat 8 and 9, **(G)** force-insensitive FRET control with the TSMoD fused to the N-terminus of full length β -spectrin and **(I)** UNC-70::TSMoD(E2008K) mutant force sensor. Orange pixels indicate autofluorescence from the gut. Colorscale indicating FRET indices; half circles indicating curvatures of the trapping channel, larger circle=smaller curvature. **F, H, J** Quantification of the FRET index difference between the convex and concave side of the body and an uncurved portion of the same animal. $\Delta E > 0$ indicates compression, $\Delta E < 0$ indicates tension. Each connected pair is derived from one image; bold connected dots indicate mean of the sample. The floating right axis shows the paired mean difference (PMD) as a bootstrap sampling distribution (green) and the 95% confidence interval as a vertical black line. Numbers below the graph indicate the p -values for the likelihoods of observing the effect size, if the null hypothesis of zero difference is true.

111 FRET values were evenly distributed on the ventral and dorsal side of the animals (Fig. 3E). When
112 the same animal was bent inside the channel, the concave side had a higher FRET efficiency than the
113 convex side in a curvature-dependent manner (Fig. 3E,F), indicative for a differential compression
114 and extension of the animal's body. However, these differences in FRET between the convex and
115 concave side were not observed in a stretch-insensitive sensor that was fused to the N-terminus of
116 the protein, such that it could not be pulled apart and report tension (Fig. 3G,H), or in a constitutive
117 high-FRET and low-FRET construct in which the elastic force sensor domain was replaced with a stiff
118 5 or 200 aminoacid linker domain (Fig. S6E-H). Likewise, after performing FRET imaging in E2008K
119 spectrin mutant animals (Fig. S6C,D), we failed to detect differences in FRET efficiencies between
120 the compressed (concave) and stretched (convex) side at high curvatures (Fig. 3I,J), indicative for a
121 failure of the mutant β -spectrin to sustain compressive and tensile mechanical stresses. Importantly,
122 we conclude that the E2008K mutation does not interfere with formation of the ubiquitous α/β -spectrin
123 network (see Fig. S6E,F and Ref. [29]), as judged by the α -spectrin periodicity in the β -spectrin *unc-*
124 *70(e524)* point mutation. Together, this shows that the spectrin cytoskeleton sustains compressive
125 AND tensile stresses during body bending. We further conclude that changes in body curvature are
126 encoded in the mechanical state of the spectrin network, which conveys mechanical stresses within
127 DVA.

128 **UNC-70 genetically interacts with TRP-4 mechanosensitive ion channel** Our notion that DVA-
129 specific mutations of *unc-70* increase body curvature (Fig. 2E) is shared by the function of the
130 mechanosensitive NOMPC homolog TRP-4 in DVA [4], suggesting a functional relation between
131 UNC-70 and TRP-4. Indeed, animals bearing the *trp-4(sy695)*, and occasionally in *trp-4(ok1605)*
132 single mutation (not shown), have significantly different locomotion phenotypes as compared to wt
133 animals (Fig. 4A,D). In order to determine whether or not UNC-70 and TRP-4 function together in
134 determining the body posture during locomotion, we generated animals carrying *trp-4(sy695)* and
135 *unc-70(e524)* double mutations, and *trp-4(sy695)* and the conditional allele with a DVA-specific defect
136 of *unc-70*. In both conditions, the double allele generally did not show a more exaggerated body

137 posture than either single mutation alone (Fig. 4D). Importantly, the distribution and trafficking of
138 endogenously tagged TRP-4 into the distal part of the sensory endings is unaffected by the E2008K
139 spectrin mutation. Expression levels of TRP-4 in DVA remained below background autofluorescence
140 (Fig. 4E) and we restricted our analysis to the cilia of CEP or sensilla of ADE, which locally enrich the
141 protein in sensory endings. Taken together, our data suggests that *unc-70* acts epistatic with *trp-4* to
142 limit body bending amplitude.

143 **TRP-4 is not essential to elicit curvature-dependent calcium activity** We next analyzed whether
144 compression induced Ca^{2+} signals depend on the TRP-4/NOMPC ion channel. Indeed, spontaneous
145 activity in immobile *trp-4* mutant animals was strongly reduced, with occasional decreases in Ca^{2+}
146 signals (Fig. S4B). However, when we performed Ca^{2+} imaging in flexing animals, we still observed
147 DVA Ca^{2+} activity during ventral body bends in *trp-4(sy695)* mutant animals, even though, these DVA
148 signals were less modulated by swings in body curvature (Fig. 4F). This analysis shows that TRP-4
149 expression is dispensable for compression induced Ca^{2+} signal during body bending. We were thus
150 wondering if TRP-4 is sufficient to sensitize otherwise motion-insensitive neurons lacking endogenous
151 TRP-4 expression [34], to activate during dorso-ventral body bends. We hence expressed full-length
152 *trp-4* cDNA and an N-terminally truncated construct in TRNs, the gentle body touch mechanoreceptors.
153 In wildtype animals and transgenics expressing the truncated isoform, basal Ca^{2+} levels measured in
154 PLM rarely changed and are seemingly uncorrelated with body curvature in slowly moving animals
155 (Fig. S4C,G). However, upon expression of full-length TRP-4 in TRNs, we observed a strong periodic
156 signal in PLM during body bending that correlated well with body bends (Fig. 4H). Interestingly, we
157 also frequently observed increases in PLM activity when the animal bent towards both, the dorsal
158 and ventral sides (Fig. 4H). Taken together, TRP-4 can, in principle, endow curvature sensitivity in
159 heterologous neurons, but is dispensable for curvature induced DVA activity.

160 **DVA responds to tension and relaxation gradients *in vitro*** We then established a primary
161 culture of *C. elegans* neurons [19, 35] on compliant PDMS surfaces and verified that DVA expressing

Figure 4

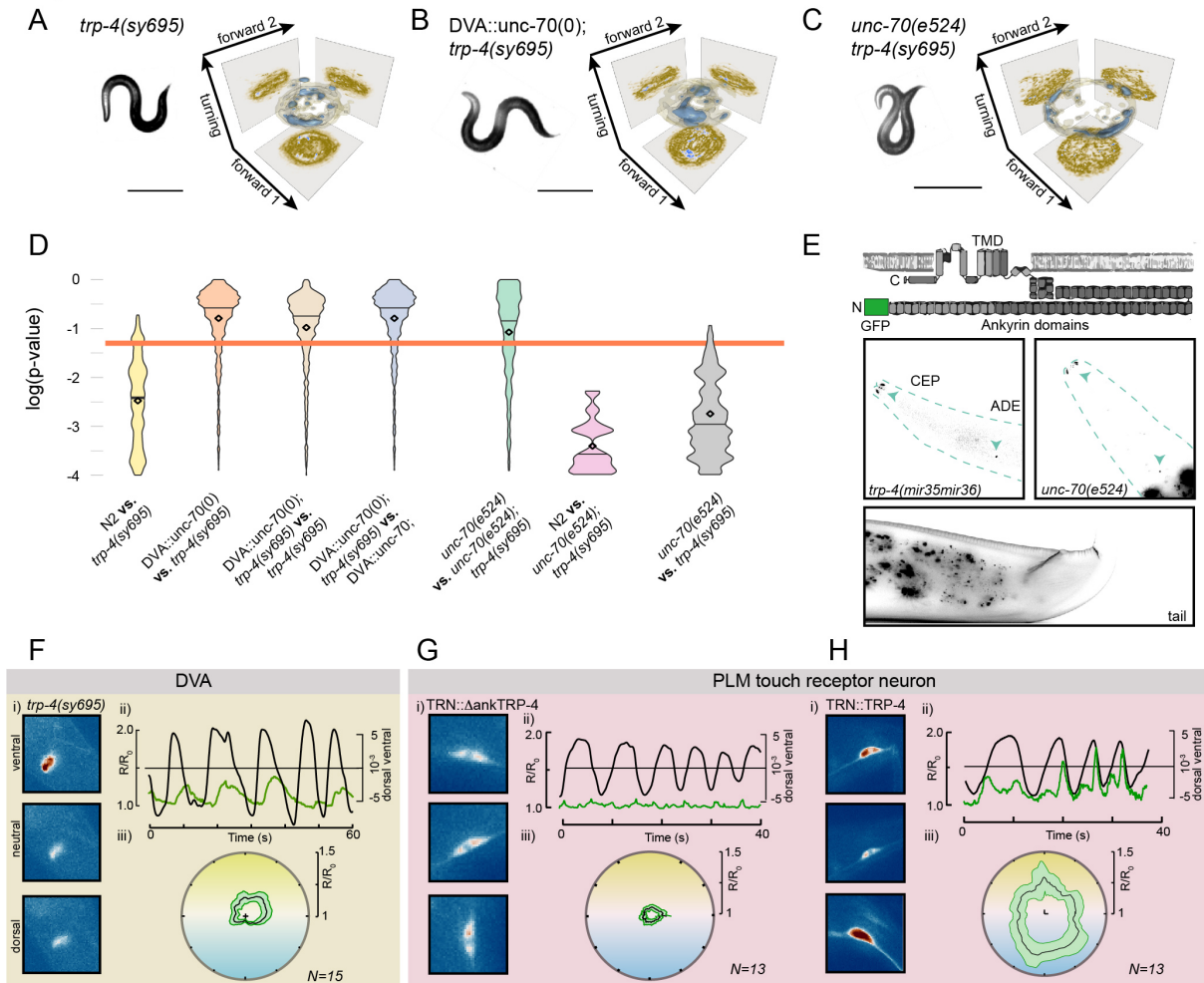


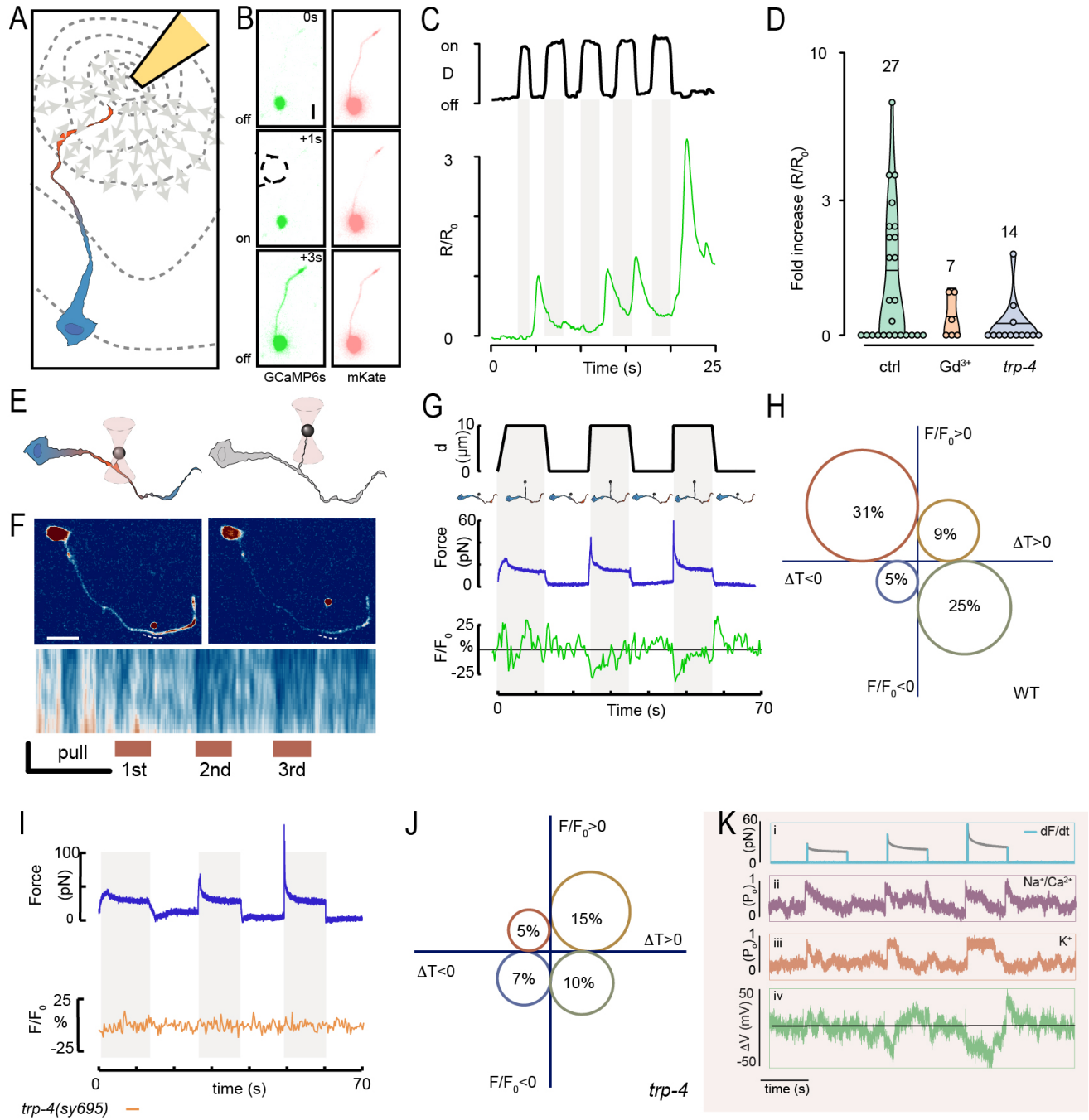
Figure 4. TRP-4 calcium activity peaks under compressive stresses

A-C Representative still image and the corresponding manifold in the three dimensional eigenworm analysis for **(A)** *trp-4(sy695)* mutant animals, **(B)** *trp-4(sy695);DVA::unc-70* double mutants **(C)** *trp-4(sy695);unc-70(e524)* double mutation. **D** *p*-value distribution for 1000 independent tests for the hypothesis H_0 that the density function for indicated combinations are equal (see Methods). Orange line indicated $\alpha=0.05$ level of significance. **E** Schematics of the TRP-4::GFP CRISPR knock-in and representative images of the head and the tail in wildtype and *unc-70(e524)* background. **F-H** Calcium imaging data from moving **(F)** DVA neuron in *trp-4(sy695)* mutant, **(G)** PLM in an animal expressing N-terminally truncated TRP-4, **(H)** PLM in animals expressing fulllength TRP-4. (i) Video stills of ventral, neutral and dorsal bends. (ii) Time series of the normalized GCaMP/mKate ratio (left axis) and body curvature (right axis). (iii) Average GCaMP/mKate ratio \pm SE plotted against the phase angle of body curvature.

162 GCaMP6s robustly activated in response to substrate deformation (Fig. 5A). Clearly, cultured DVA
163 neurons repetitively elicited high Ca^{2+} transients (Fig. 5B,C Video S4), that depended on functional
164 TRP-4 expression and could be partially blocked by non-specific cation channel inhibitor GdCl_3 (Fig.
165 5D). Most notable, we frequently found that DVA neuron became active during the force offset (Fig.
166 5B,C) and buckled during indentation, suggesting that they sensed negative tension gradients or
167 compression. Because PDMS substrate deformation induces compressive and tensile stresses along
168 the axon, we specifically applied positive and negative tension gradients to isolated axons (Fig. 5E,F)
169 and visualized their resultant Ca^{2+} transients using confocal microscopy (Fig. S7A). To do so, we
170 used optically trapped microspheres and extruded single membrane tethers [36] from DVA axons
171 (Fig. 5E) with varying velocity. The resulting tether force increased with the pulling velocity but quickly
172 relaxed to a static value (Fig. 5G) when the movement ceased. Using this approach, we measured
173 local gradients that were 100x higher than the resting membrane tension (2mN/m, Fig. S6B), resulting
174 in a rich behavior in the Ca^{2+} dynamics. Strikingly, Ca^{2+} signals increased preferentially during the
175 relaxation phase of the force-distance cycle directly at the tether neck (Video S5 and Fig. 5F-H),
176 indicating that negative tension gradients, similar to axon compression *in vivo* (Fig. S5A), can induce
177 neuronal activity (Fig. 2J). In *trp-4* mutants, however, we did not observe an increase in Ca^{2+} activity
178 during tension relaxation (Fig. 5I,J). In addition to the Ca^{2+} increases during tension relaxation in
179 wildtype neurons, we also frequently observed a transient reduction in Ca^{2+} signals during tether
180 extrusion especially at higher velocities (Fig. 5F-H), suggesting that the concomitant increase in
181 membrane tension (Fig. 5E-G) suppresses neuronal activity.

182 How does an increase in membrane tension lead to decrease in calcium signal? This observation,
183 in principle, can be explained in part due to the existence of a stretch activated potassium channel
184 (SAPC) that deactivates the neuron under tension. To understand the observed Ca^{2+} dynamics,
185 we set up a computational model [37], in which positive and negative forces selectively activate
186 hyperpolarizing and depolarizing ion channels, respectively. We illustrate this scenario with two
187 hypothetical, mechanosensitive ion channels acting as primarily sodium/calcium conductive (depo-

Figure 5



188 larizing) and a potassium conductive (hyperpolarizing) and model channel gating under force as a
189 thermally driven escape over a potential barrier [38] (Fig. S7E, for details see Methods). Consistent
190 with previous results derived from electrophysiological recording of TRP-4 expressing CEP [39], we
191 modeled sodium activity at the force onset and offset by sensitizing it to the loading rate (Fig. 5Kii)
192 while K^+ channel displayed activity only during force onset (Fig. 5Kiii). With realistic ion channel
193 parameters taken from the literature (Methods) and assuming a constant input resistance, the open
194 probability of the K^+ channel was able to completely suppress the Ca^{2+} channel induced neuron
195 activity at the force onset but not when the force was released. Even though our calculation does
196 not include the complex regulation and spontaneous Ca^{2+} dynamics that likely take place inside a
197 cell, our reductionist model is in good match to the experimentally determined DVA activity (Fig. 5G),
198 such as a transient reduction during extension and transient rise due to relaxation. We next sought
199 to test this prediction.

Figure 5 (preceding page). Opposing membrane tension gradients modulate DVA activity *in vitro* through TRP-4 and TWK-16

A Schematic of the PDMS stretching experiments. **B** Three successive images of the Ca^{2+} -sensitive (left) and Ca^{2+} -insensitive dye (right) of an isolated DVA subjected to substrate deformations. **C** PDMS substrate deformation D (black line) and GCaMP/mKate ratio (R/R_0 , green line) plotted against time. **D** Violin plot of the Ca^{2+} activity derived from wt, wt treated with Gd^{3+} and *trp-4(sy695)* mutant DVA neurons. **E** Schematics of the dynamic tether extrusion experiments. **F** Representative still images of the GCaMP signal from an isolated DVA neuron before and during tether extrusion. Dotted line indicates the location of the kymograph displayed below showing the temporal evolution of the Ca^{2+} signal at the tether neck. **G** Representative displacement (d), force and normalized Ca^{2+} signal extracted from the experiment in (F). **H** Bubble plot of the probability showing that a tether extrusion or relaxation leads to an increase or decrease in GCaMP activity in wt DVA neurons. ΔT indicates increases or decreases in measured tension gradient. N=48 cells. **I** Representative force trace and normalized Ca^{2+} transients for *trp-4* (orange) mutant cells. **J** Bubble plot of the probability showing that a tether extrusion or relaxation leads to an increase or decrease in GCaMP activity in DVA neurons mutant for *trp-4*, N=38, mutant cells. **K** *In silico* study of the optical trapping experiment. i) Simulated force (black trace) and temporal tension gradients (light blue). ii,iii) Open probability of the hypothetical Na^+ and K^+ channel. iv) Sum of a current through a linear combination of its average open probability x single channel conductance. Note the double peak in the purple trace becomes suppressed due to simultaneous activation of the inhibitory signal, giving rise to the experimentally observed behavior.

200 **The K2P homolog TWK-16 suppresses Ca²⁺ activity during dorsal posture** The results of our
201 simple kinetic model predicts that the Ca²⁺ signals at the force onset becomes unmasked in absence
202 of inhibitory, potassium activity. DVA is one of the few neurons that expresses the mechanically
203 regulated leak potassium channel TWK-16 [40,41], a TREK2 homolog of mechanosensitive, two-pore
204 K⁺ channels [42] in *C. elegans*. We thus repeated the dynamic tether extrusion experiment in a *twk-16*
205 null mutant background (Fig. 6A,B) and measured the concomitant change in GCaMP intensity.
206 Consistent with our hypothesis, we did not observe a Ca²⁺ decrease during tether pull-out, indicating
207 that mechanical de-activation at high membrane tension gradients is *twk-16* dependent. Instead, we
208 frequently observed a Ca²⁺ increase at the onset of the tether extrusion (Fig. 6A,B), as if TWK-16
209 functions in suppressing tension-induced activity normally observed in TRP-4 expressing neurons [39].
210 The notion of stretch-induced suppression of Ca²⁺ activity through TWK-16 in isolated DVA neurons,
211 raises the question of whether or not TWK-16 is a functional component during proprioception. To
212 answer this, we first repeated the Ca²⁺ imaging of DVA in moving *twk-16* mutant animals. Compared
213 to wt animals, we found that the deletion of *twk-16* caused a subtle increase in Ca²⁺ activity in
214 DVA during spontaneous body bending (Fig. 6C) with an overall unchanged ventral preference.
215 Nevertheless, we frequently observed GCaMP intensity changes during dorsal posture and even
216 during both, dorsal and ventral posture (Fig. 6C), a signature we never observed in DVA of wt animals
217 but occasionally in *unc-70* mutants (Fig. 2L) and ectopic expression of TRP-4 in TRNs (Fig. 4H).
218 Together, this suggests that TWK-16 is able to suppress Ca²⁺ transient at dorsal posture, when DVA
219 experiences mechanical tension, similar to how tension suppresses calcium activity in our *in-vitro*
220 experiment (Fig. 6A).

221 We then asked if TWK-16 expression in DVA is required for animal locomotion. In contrast to *trp-4*
222 animals, *twk-16* null mutants, we observed a subtle, but significant difference between the wt and
223 mutant in 3D densities in the eigenworm space, as an indicator for a smaller body posture (Fig. 6D,
224 S8A-C). TWK-16 is also expressed in other neurons, including AVK that counteracts DVA activity [21].
225 First, we inserted a AID:wScarlet tag [26] at the TWK-16 C-terminus, verify expression in DVA axon

Figure 6

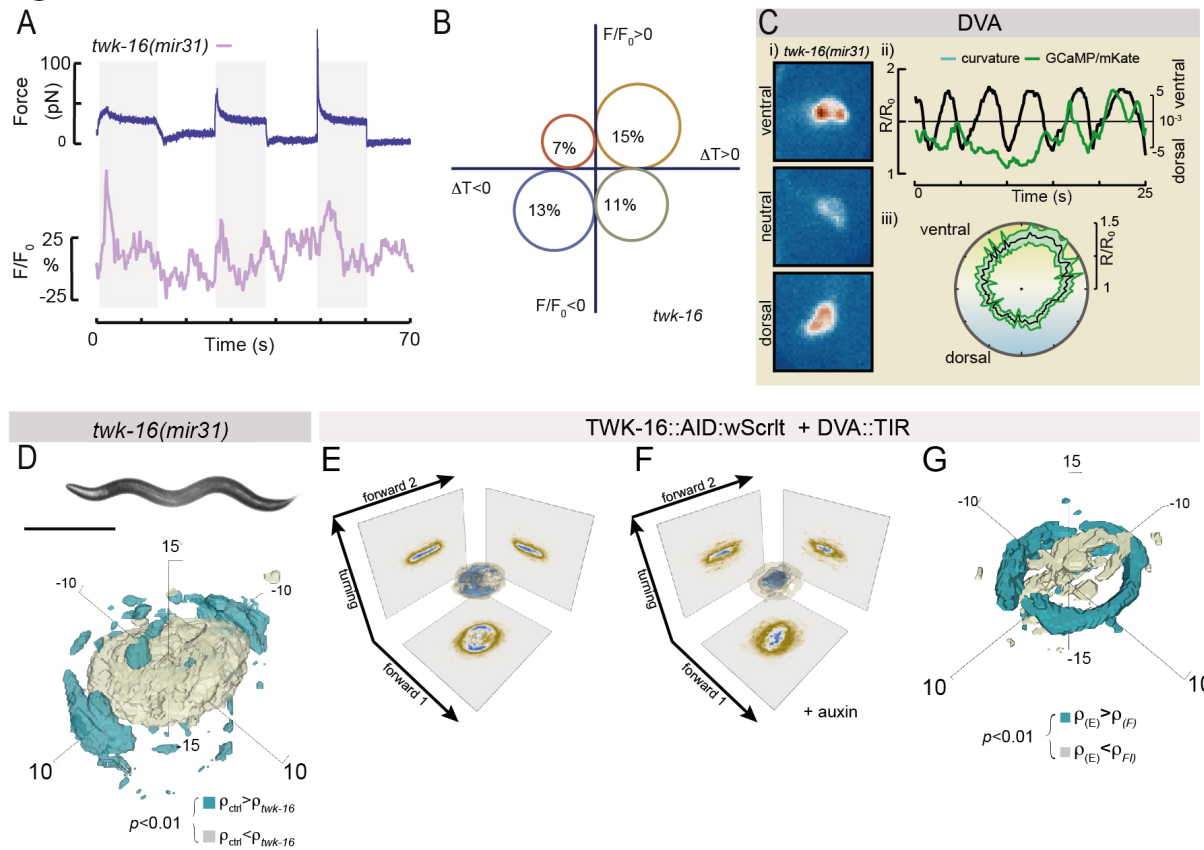


Figure 6. TWK-16 suppresses bending excursions through modulation of DVA activity

A Representative force trace and normalized Ca^{2+} transients for *twk-16* (purple) mutant cells. **B** Bubble plot of the probability showing that a tether extrusion or relaxation leads to an increase or decrease in GCaMP activity in DVA neurons mutant for *twk-16*, N=31, mutant cells. **C** DVA Ca^{2+} data of *twk-16(mir31)* animal lacking functional TWK-16. (i) Video stills of DVA cell body during ventral, neutral and dorsal bends. (ii) Time series of the normalized GCaMP/mKate ratio (left axis, green) and body curvature (right axis, black). (iii) Average GCaMP/mKate ratio \pm SE plotted against the phase angle of body curvature. **D** Representative image of a *twk-16(mir31)* animal and the color-coded, statistically significant differences in the local probability density functions between N2 and *twk-16*. Scale bar = $300\mu m$. **E, F** 3D kernel density function (histogram) in the three dimensional eigenworm space for TWK-16::AID::wScarlet animals expressing TIR in DVA in (E) absence and (F) presence of auxin. **G** Color-coded, statistically significant differences in the local probability density functions between (E) and (F). Blue voxels indicate higher local density for untreated, beige voxels indicate higher density for auxin-treated animals on the $\alpha=0.01$ level.

Figure 7

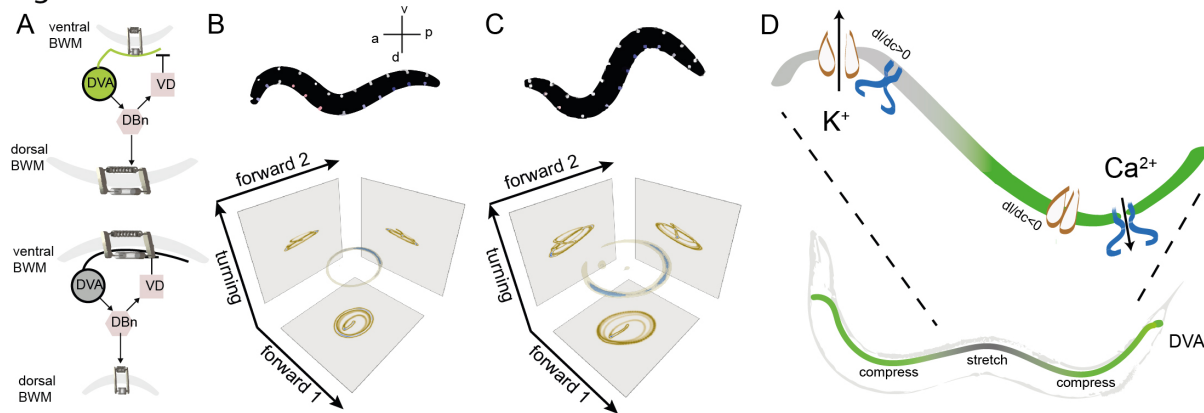


Figure 7. Compression in DVA provides proprioceptive feedback to the motorcircuit

A Schematic of the model formulation. A passive mechanical body is modeled as a lightly damped elastic beam (passive Kelvin-Voigt material) [44] in parallel with the muscles, as an ‘active’ Kelvin-Voigt material with stiffness governed by the state variable of the motorcircuit (through neuronal input). DVA senses compression during ventral bends and informs the DB neurons, which in turn activate dorsal muscle contraction and at the same time connect to inhibitory VD motor neurons, resulting in the relaxation of the ventral muscles. Under dorsal bends, DVA activity is suppressed (lower panel). **B,C** A representative snapshot from the simulation of **(B)** ‘wildtype’ and **(C)** ‘mutant’ with reduced sensitivity to compressive stresses and the corresponding manifold in the eigenworm state space. Dorsoventral circles depict motorneurons and their stretch receptor input. Blue=off, red=on. Ventral motorneurons are white, as they do not receive stretch receptor input. **D** Illustration of the mechanical scenario under which neuronal tension hyperpolarizes (activates TWK-16/TREK potassium channels, orange) and compression depolarizes (TRP-4/NOMPC, blue) neuronal segments. Hyperpolarized domains thus limit the extend and spread of the signal along the length thus leading to effective compartmentalization.

226 and cell body (Fig. S8D), and confirmed that the fusion protein did not cause any locomotion defects
 227 in absence and presence of auxin (Fig. S8E-G). Then, we expressed the previously characterized
 228 TIR ligase (Fig. S3I-L) together with the TWK-16::AID and repeated the behavior experiment. Even
 229 though we already noticed an auxin-independent effect [43] on body bending amplitude (Fig 6E) the
 230 addition of 1mM auxin caused a markable reduction of body bending amplitude compared to wt and
 231 a clearly distinguishable eigenworm representation (Fig. 6D,E; S8H-J; Video S6). Together, TWK-16
 232 inactivates DVA under tension and counteracts TRP-4 depolarization with DVA-specific function in
 233 locomotion.

234 **Compression-induced activity adjusts motor output in a neuronal network model** The known
235 wiring diagram of *C. elegans* offers the opportunity to ask how the observed neuronal activity changes
236 are processed on the nervous systems level. To interrogate how the compressive proprioception
237 of DVA adjusts the motorcircuit (Fig. 7A) and affects *C. elegans* locomotion, we adapted a well-
238 established neuromechanical model [44] that was previously deployed to simulate the effect of
239 stretch-sensitive feedback embedded directly into the motoneurons. In our modification, we consider
240 a single compression sensitive current during ventral bends, matching our notion that DVA is excited
241 during ventral curvatures. In agreement with the connectivity of the *C. elegans* network, DVA
242 directly informs the motor neurons on the dorsal side. Indeed, in this scenario, the simulation of
243 compressive-sensitivity during ventral bends, but not stretch-sensitivity during dorsal bends produced
244 a crawling pattern seemingly similar to experimental results (Fig. 7B). With the aim to understand
245 how the compression-current sensitivity influences crawling pattern, we reduced the responsiveness
246 to compression, compatible to the *unc-70* and *trp-4* mutations in the experiments (Fig. 4). Strikingly,
247 the simulation recapitulated the crawling behavior observed in the mutant conditions, visible as an
248 expansion of the manifold in the eigenworm state space (Fig. 7C, Video S7).

249 Discussion

250 Proprioceptive and visceral mechanosensation are vitally important processes ensuring body home-
251 ostasis and organ function. Here, we genetically, mechanically and computationally deciphered
252 the molecular underpinnings that determine the shape of the limit cycle attractor during *C. elegans*
253 locomotion. We used a well characterized mechanical stress sensor [19, 45] in combination with neu-
254 ronal activity indicators and revealed that DVA activates under compressive stresses in an UNC-70
255 β -spectrin dependent manner. This apparent discrepancy in light of the classical view of mechanical
256 stretch reception is not a sole feature of DVA but has previously been observed in other *C. elegans*
257 proprioceptors such as SMD [6], *Drosophila melanogaster* multidendritic class III proprioceptors [46]

258 which preferentially activate in a direction-selective manner when the contracting segment gets com-
259 pressed [46], and mechanosensory neurons regulating thirst in the mammalian brain [47]. Latter is
260 directly sensed by volume changes through a specific TRPV1-microtubule interaction when neurons
261 lose water the membrane-bound ion channels push against the highly interweaved microtubule
262 cytoskeleton, thus activating the neurons [47]. It seems plausible that this ‘push-activation’ model
263 also accounts for the activation of the pentascolopedial chordotonal organs in the fruitfly, which have
264 been shown to activate under compressive stresses during locomotion [48] in a NOMPC dependent
265 manner. Intriguingly, the TRP family member NOMPC also interacts with a specialized microtubule
266 cytoskeleton [49,50] which conveys mechanical stress to the gating pore through a conserved ankyrin
267 spring. Whether or not tension [51] or compression [16,52] of the ankyrin repeats leads to mechanical
268 gating of the pore, remains to be determined, nonetheless, the application of membrane tension
269 was not sufficient to activate mammalian TRP channel homologs [53]. Likewise, our results from the
270 truncated TRP-4 lacking ankyrin domain (Fig. 4G), suggests a membrane-independent activation
271 mechanisms. TRP and TMC channels are not the only ones implicated in compression sensing.
272 Recent work demonstrated that a normal force of 50pN is sufficient to cause conformational change in
273 PIEZO1 [54], providing a plausible mechanism for compressive mechanosensitivity [55,56]. Our work
274 showing compressive mechanosensitivity *in-vitro* and *in-vivo* thus motivates us to rethink the operation
275 of ‘stretch’ sensitive mechanoreceptors. Future work needs to directly address if TRP-4/NOMPC ion
276 channels are gated by compression of their ankyrin spring.

277 What interferes with DVA mechanosensing in the spectrin mutation? Our genetic analysis suggests
278 that the spectrin network and TRP-4 act in the same process, e.g. during force transmission. We
279 performed a co-immunoprecipitation of TRP-4 and UNC-70 as well as SPC-1 from CHO cells, but
280 could not detect a direct biochemical interaction in this assay (not shown). Hence, we propose that
281 indirect force transmission takes place involving stress propagation through local and global axon
282 mechanics. We showed that DVA axons in *unc-70* mutations undergo buckling instabilities (Fig.
283 S5), which could, similar to events in TRNs, cause a dramatic reduction in the number and length of

284 microtubules [29], consistent with curvature-induced fracture during buckling [57]. This disintegration
285 and disassembly of the load bearing microtubule filaments during cyclic strains conforms to fluidization
286 of the cytoskeleton [58], which constitutes a plausible mechanism of how a single point mutation in
287 spectrin could interfere with efficient force transmission to the ion channel: the loss of tension sets
288 the origin of the fluidization with vanishing stiffness [59] and thus a failure to built-up and convert
289 compressive stresses from the cytoskeleton into ion channel opening.

290 If a single mechanosensory neurite integrates information from mechanical compression and
291 extension, that also need to be processed locally, mechanisms need to be in place to restrict
292 spread of depolarization. DVA has a single neurite that spans the entire length of the animal's
293 body, such that tensile and compressive stresses coexist during dorso-ventral body bends. Under
294 the notion of a high input resistance inherent to *C. elegans* neurons [60], a small input current
295 during compression could in principle delocalize and depolarize the entire axon, hence disrupting the
296 proprioceptive coordination along the length of the animal. We propose that stretch activates TREK-
297 2/TWK-16 and hence suppresses or at least dampen the NOMPC/TRP-4-related transduction current
298 to achieve compartmentalized alternating 'active zones' that correspond to the compressed state
299 of the neurite (Fig. 7D). Such a mechanism would not only facilitate gain control during mechanical
300 signaling subjected to stochastic ion channel noise but also allows to sample the full dynamic range
301 of proprioceptor activity.

302 At least two alternative processes could plausibly explain the observed Calcium increase during
303 ventral bending: First, TRP-4 and TWK-16 could both be activated under mechanical tension during
304 neurite extension on dorsal bends, but close with different kinetics. Under the assumption that TRP-4
305 closing kinetics are slower than TWK-16, they would remain open for a longer time and an apparent
306 ventral activity is obtained. Our in vitro data showing that Ca^{2+} activity increased during tension
307 relaxation can be interpreted as an argument against this scenario. Second, a single synapse from
308 the ventral motor neurons to DVA has been described [61]. This notion allows us to hypothesize
309 that the ventral Ca^{2+} increases in DVA could be due to corollary discharge from VB motor neurons

310 that provide a copy of the motor state to the central nervous system [62]. Our data showing that
311 DVA-specific mutations in *unc-70* lead to decorrelated Ca^{2+} dynamics contradicts this interpretation.
312 However, whole animal Ca^{2+} imaging experiments are needed to further investigate and completely
313 disprove this scenario.

314 In summary, our data revealed that compressive and tensile stresses in the spectrin network
315 modulate two opposing, excitatory and inhibitory ion channels of DVA proprioceptors, TRP-4 and
316 TWK-16 respectively, that are critical to confine the full, deep modulation of Ca^{2+} activity in moving
317 animals. We suggest that this may be a general mechanism by which long mechanosensory dendrites
318 achieve local computation through mechanical compartmentalization. Future experiments will need
319 to directly address how this mechanism transcends the animals kingdom and human gait adaptation.

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335 and locomotion, analysis and writing. LL: building animal behavior tracker, writing software for Calcium
336 and locomotion analysis, first draft. FCC: microscopy, optical trapping, tissue culture, analysis, code
337 writing, first draft. NM: animal husbandry, molecular biology, behavior assay and calcium imaging,
338 first draft. NS: animal husbandry, molecular biology. MPR: animal husbandry, molecular biology. AP:
339 Neuromechanical modeling. MK: Concept and acquisition of funding, analysis, programming and
340 writing.

341 **Competing Interests** The authors declare that they have no competing financial interests.

342 **Code and Material availability** All reagents produced are freely available upon reasonable request
343 to the corresponding author. Some strains will be deposited to the CGC. Scripts developed supporting
344 the analysis can be accessed under Gitlab::NMSB and all relevant behavioral recordings will be
345 deposited to Zenodo upon publication.

References

- 347 1. J. Gjorgjieva, D. Biron, G. Haspel, *BioScience* **64**, 476 (2014).
- 348 2. N. A. Croll, *Advances in parasitology* **13**, 71 (1975).
- 349 3. U. Proske, S. C. Gandevia, *Physiological Reviews* **92**, 1651 (2012).
- 350 4. W. Li, Z. Feng, P. W. Sternberg, *Nature* **440**, 684 (2006).
- 351 5. L. Tao, *et al.*, *Developmental cell* pp. 1–15 (2019).
- 352 6. J. Yeon, *et al.*, *PLOS Biology* **16**, e2004929 (2018).
- 353 7. Q. Wen, *et al.*, *Neuron* **76**, 750 (2012).
- 354 8. P. Liu, B. Chen, Z.-w. Wang, *Nature Communications* **11**, 5076 (2020).
- 355 9. W. R. Schafer, *Pflugers Archiv European Journal of Physiology* **467**, 39 (2014).
- 356 10. R. Das, S. Wieser, M. Krieg, *Experimental Cell Research* **378**, 104 (2019).
- 357 11. B. D. Umans, S. D. Liberles, *Trends in Neurosciences* **xx**, 1 (2018).
- 358 12. S. H. Woo, *et al.*, *Nature Neuroscience* **18**, 1756 (2015).
- 359 13. S. H. Lin, *et al.*, *Nature Communications* **7**, 1 (2016).
- 360 14. P. Jin, *et al.*, *Nature* **547**, 118 (2017).
- 361 15. P. Jin, L. Y. Jan, Y.-n. Jan, *Annual Review of Neuroscience* **43**, 207 (2020).
- 362 16. D. Argudo, S. Capponi, N. P. Bethel, M. Grabe, *Journal of General Physiology* **151**, 316 (2019).
- 363 17. S. G. Brohawn, *Annals of the New York Academy of Sciences* **1352**, 20 (2015).
- 364 18. J. M. Kefauver, A. B. Ward, A. Patapoutian, *Nature* **587**, 567 (2020).
- 365 19. M. Krieg, A. R. Dunn, M. B. Goodman, *Nature Cell Biology* **16**, 224 (2014).
- 366 20. G. J. Stephens, B. Johnson-Kerner, W. Bialek, W. S. Ryu, *PLoS Computational Biology* **4**
367 (2008).
- 368 21. I. Hums, *et al.*, *eLife* **5**, 1 (2016).
- 369 22. S. Moorthy, L. Chen, V. Bennett, *The Journal of Cell Biology* **149**, 915 (2000).
- 370 23. M. Hammarlund, W. S. Davis, E. M. Jorgensen, *The Journal of Cell Biology* **149**, 931 (2000).
- 371 24. S. Ruijtenberg, S. Van Den Heuvel, *Cell* **162**, 300 (2015).
- 372 25. E. Kage-Nakadai, *et al.*, *PloS one* **9**, e114680 (2014).
- 373 26. L. Zhang, J. D. Ward, Z. Cheng, A. F. Dernburg, *Development (Cambridge)* **142**, 4374 (2015).
- 374 27. A. C. E. Wirshing, E. J. Cram, *Molecular Biology of the Cell* **29**, 2433 (2018).

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- 375 28. C. M. Chai, *et al.*, *Nature Methods* **14**, 145 (2016).
- 376 29. M. Krieg, *et al.*, *eLife* **6**, e20172 (2017).
- 377 30. M. Krieg, A. R. Dunn, M. B. Goodman, *BioEssays* **37**, 335 (2015).
- 378 31. O. Bauchau, J. Craig, *Structural Analysis* pp. 173–221 (2009).
- 379 32. C. Grashoff, *et al.*, *Nature* **466**, 263 (2010).
- 380 33. S. R. Lockery, *et al.*, *Journal of Neurophysiology* **99**, 3136 (2008).
- 381 34. D. Lockhead, *et al.*, *Molecular biology of the cell* **27**, 3717 (2016).
- 382 35. K. Strange, M. Christensen, R. Morrison, *Nature Protocols* **2**, 1003 (2007).
- 383 36. F. M. Hochmuth, J. Y. Shao, J. Dai, M. P. Sheetz, *Biophys J* **70**, 358 (1996).
- 384 37. A. Nekouzadeh, Y. Rudy, *Mathematical biosciences* **210**, 291 (2007).
- 385 38. G. I. Bell, *Science* **200**, 618 (1978).
- 386 39. L. Kang, J. Gao, W. R. Schafer, Z. Xie, X. Z. S. Xu, *Neuron* **67**, 381 (2010).
- 387 40. L. Salkoff, *et al.*, *Neuroscience* **103**, 853 (2001).
- 388 41. C. Puckett Robinson, E. M. Schwarz, P. W. Sternberg, *PLoS ONE* **8**, 1 (2013).
- 389 42. S. G. Brohawn, Z. Su, R. MacKinnon, *Proceedings of the National Academy of Sciences* **111**,
390 3614 (2014).
- 391 43. E. Schiksnis, *et al.*, *microPublication biology* **2020**, 3 (2020).
- 392 44. J. H. Boyle, S. Berri, N. Cohen, *Frontiers in Computational Neuroscience* **6**, 1 (2012).
- 393 45. M. Kelley, *et al.*, *eLife* **2015**, 1 (2015).
- 394 46. L. He, *et al.*, *Current Biology* pp. 1–12 (2019).
- 395 47. M. Prager-Khoutorsky, A. Khoutorsky, C. Bourque, *Neuron* (2014).
- 396 48. A. Hassan, *et al.*, *Cell Reports* **27**, 2272 (2019).
- 397 49. X. Liang, *et al.*, *Current Biology* **23**, 755 (2013).
- 398 50. W. Zhang, *et al.*, *Cell* **162**, 1391 (2015).
- 399 51. J. Howard, S. Bechstedt, *Current Biology* **14**, 224 (2004).
- 400 52. Y. Wang, *et al.*, *bioRxiv* pp. 1–15 (2019).
- 401 53. Y. A. Nikolaev, *et al.*, *Journal of Cell Science* **132** (2019).
- 402 54. Y.-C. Lin, *et al.*, *Nature* (2019).
- 403 55. J. M. Romac, R. A. Shahid, S. M. Swain, S. R. Vigna, R. A. Liddle, *Nature Communications* **9**,
404 1 (2018).

-
- 405 56. N. Srivastava, D. Traynor, M. Piel, A. J. Kabla, R. R. Kay, *Proceedings of the National Academy*
406 *of Sciences of the United States of America* **117**, 2506 (2020).
- 407 57. C. M. Waterman-Storer, E. D. Salmon, *Journal of Cell Biology* **139**, 417 (1997).
- 408 58. T. Wu, J. J. Feng, *Biophysical Journal* **108**, 43 (2015).
- 409 59. M. Schenk, S. D. Guest, *Proceedings of the Institution of Mechanical Engineers, Part C: Journal*
410 *of Mechanical Engineering Science* **228**, 1701 (2014).
- 411 60. M. B. Goodman, D. H. Hall, L. Avery, S. R. Lockery, *Neuron* **20**, 763 (1998).
- 412 61. J. G. White, E. Southgate, J. N. Thomson, S. Brenner, *Philosophical transactions of the Royal*
413 *Society of London. Series B, Biological sciences* **314**, 1 (1986).
- 414 62. T. B. Crapse, M. A. Sommer, *Nature Reviews Neuroscience* **9**, 587 (2008).

415 **Supplementary Material: Mechanical Stretch Inhibition**
416 **Sensitizes Proprioceptors to Compressive Stresses**

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1 Materials & methods

1.1 Soft lithography and PDMS replica molding

SU-8 soft lithography and PDMS replica molding have been done as described before [63]. In short, the fabrication of the molds was undertaken in-house as a single layer process using standard SU-8 photolithography techniques. We first applied a 5 μm thick adhesion layer to reduce lift-off of the patterned structure during device fabrication. Piranha cleaned 4 inch wafers were used to create an adhesion layer using SU-8 50 before photo patterning in SU-8 2000. After fabrication, molds were vapor-phase silanized in chlorotrimethylsilane to prevent adhesion of the PDMS to the substrate. A 10:1 mixture of Sylgard 184 prepolymer/curing agent was degassed ($\approx 30\text{min}$ in vacuum desiccator) and poured onto the silanized molds. After settling, the PDMS/wafer were baked at 80°C for two hours. Devices were then cut using a scalpel, lifted off and punched with a biopsy punch (1mm or 0.75mm). The procedure of animal insertion into the trapping channel has been described in detail elsewhere [63]. In brief, to load individual in the chip, one young adult worm transgenic for TSMOD in UNC-70 and its derivatives (GN517, GN519, GN600, MSB233; Ref. [19]) were picked from an NGM plate containing OP50 bacteria and transferred to a 5 μl droplet of 30% Optiprep (to reduce scattering from PDMS due to refractive index mismatches between the animal and the surrounding [65, 66]) placed onto a hydrophobic substrate (25cm² Parafilm) to swim for 30 sec and rid themselves from bacteria. Then, using a stereo dissecting scope at 60x total magnification (Leica S80), the animals were aspirated into a 23 gauge metal tube (Phymep) connected to a 5 ml syringe (VWR) with a PE tube (Phymep, BTPE-50, 0.58x0.97mm) pre-filled with 30% Optiprep buffer. The loading tube was inserted in the inlet port of the device, while a gentle pressure onto the plunger of the syringe released the animals into waveform sampler. Channel with a thickness of 60 μm were used to firmly hold animals immobile during the 3-channel FRET imaging procedure without stretching or confining them visibly. After each image, the animals were pushed for a distance of π into the device to probe the opposite curvature at the same body coordinate.

1.2 Animal maintenance

Animals were maintained using standard protocols [67, 68] and grown at 20°C, unless indicated otherwise. For Gal4 expressing transgenes, animals were maintained and raised at 25°C to ensure consistent expression [28].

1.3 FRET imaging

Three channel FRET imaging of animals transgenic for *unc-70*(TSMOD), *unc-70E2008K*(TSMOD), no-force and high-FRET controls was carried out as described previously [19]. Importantly, the 2000 basepair *unc-70* promoter fragment drives our *unc-70* cDNA predominantly in neurons, with little expression in muscles and no expression in the hypodermis. In contrast, visualizing *unc-70* expression from the endogenous locus with a c-terminal slowtimer fluorescent protein [70] showed

471 significantly expression in the hypodermis and muscles (not shown). In short, animals were either
 472 immobilized on agar pads [71] or in microfluidic chips with varying curvature as described above.
 473 Imaging was carried out on a Leica DMI6000 SP5 confocal microscope through a 63x/1.4 NA oil
 474 immersion lens. Three images were acquired, the direct donor (mTFP2) excitation and emission,
 475 donor excitation and acceptor emission and the direct acceptor (mVenus) excitation and acceptor
 476 emission. mTFP2 was excited using the 458nm, while mVenus was excited with the 514nm line of an
 477 Argon ion laser at 80% and 11% transmission respectively (with 25% of its full available power). The
 478 fluorescent light was collected using two hybrid GaAs avalanche photodiodes with 100% gain through
 479 an acusto-optical beam splitter with the donor emission window set between 465-500nm and the
 480 acceptor emission window set between 520-570nm. Linearity of the detector and the ratio $\frac{\psi_D}{\psi_A}$ (ratio
 481 of collection efficiency of the two APD) was determined experimentally by imaging a homogenous
 482 fluorescein solution with increasing gain at constant laser power. Each image was acquired as an
 483 average of 4 frames acquired at a 400Hz line-rate with 512x512 pixels at a digital zoom 3 (for Figure
 484 S6) or zoom 1-1.5 (for Figure 3) in the microfluidic chip. For each imaging session, the donor->FRET
 485 bleedthrough and acceptor cross-excitation by the donor laser was determined using a sample
 486 with mTFP (ARM101 or MSB60; [72]) or mVenus (GN498) expression only, respectively, [19]. The
 487 bleedthrough and crosstalk factor were calculated as described and assumed to be constant across
 488 all intensities and images (for a given set of parameters). The raw FRET intensity was then corrected
 489 according to

$$cF(i, j) = I_F(i, j) - \alpha \cdot I_A(i, j) - \delta \cdot I_D(i, j)$$

490 and the FRET efficiency calculated using

$$E = 100 \cdot \frac{\left(cF \cdot Q_D \cdot \frac{\psi_D}{\psi_A} \right)}{qD + \left(cF \cdot Q_D \cdot \frac{\psi_D}{\psi_A} \right)}$$

491 .

492 For whole animal images in the WFS, collapsed maximum intensity projections from 15 images of a
 493 3D stack undersampled with $\approx 2 \mu\text{m}$ interplane distance were background subtracted with a constant
 494 value determined in an ROI that did not contain information from the animal. Before FRET calculation,
 495 the individual images were binned to 256x256 pixels to increase signal/noise ratio. For final display,
 496 the FRET map was overlaid on top of the corresponding brightfield image and displayed in Figure 3.
 497 In contrast, individual neurons were traced using a Gaussian fit to the intensity profile and processed
 498 as described [19].

499 1.4 Neuron morphology imaging and analysis

500 Animals expressing mKate in DVA were embedded in the low percentage agar (1-2%), which allowed
 501 animals to undergo dorso-ventral body bends without moving out of field of view. Imaging was
 502 performed on a Leica DMI8 through a 40x/1.1 water immersion lens using the 575 nm line of a
 503 Lumencor SpectraX LED lightsource. Fluorescence was collected through a 641/75nm emission
 504 bandpass filter (Semrock, FF02-641/75-25) and recorded using a Hamamatsu Orca Flash 4 V3 for

505 1-2 minutes at 10Hz with an exposure time of 50ms. Out-of-focus frames were discarded and the
506 resulting videos were post-processed as previously described [19].

507 Locomotion behavior analysis

508 **Animal tracking platform and data acquisition:** Animals were synchronized using standard
509 alkaline hypochlorite (bleaching solution) treatment method. Arrested L1 were seeded onto OP50
510 NGM plates and incubated at 20°C. Without a lack of generality and to facilitate automated tracking
511 and post-processing, we recorded videos of the locomoting animals without food (OP50). To ensure
512 that the absence of food did not alter the outcome of the experiment, we sampled the mutant genotypes
513 and wt on food ($p_{off}^{N2-vs-CB524} = 3.4e - 5$; $p_{on}^{N2-vs-CB524} = 5.6e - 4$). Each video containing a single
514 animal was 1-2 minutes in length and was taken at 25 frames per second (fps). In total, ≈ 1 million
515 frames were collected and processed (Table S1). Videos of the young-adult animals were taken
516 within 5 min after picking using a home-built animal behavior tracking platform. The custom build
517 microscope is composed of an sCMOS camera (IDS UI-3080 CP Rev. 2) coupled to a Navitar
518 6.5x zoom lens at 2x zoom. A closed loop stage tracking algorithm, implemented in C#, kept the
519 animal in the field of view for the duration of the recording, or was manually followed with a motorized
520 xz-scanning stage (102x102mm travel, Standa), trans-illuminated with a diffused white light LED
521 array.

522 **Auxin experiment and quantification of the knockdown:** Auxin plates were prepared by adding
523 100 mM stock of 1-Naphthaleneacetic acid (Auxin, Sigma Aldrich 317918) dissolved in 100% ethanol
524 to cooled NGM right before pouring the plates at a final concentration of 1mM auxin. Once solidified the
525 auxin containing plates or only ethanol (1%) containing control plates were seeded with concentrated
526 10X OP50. Young-adult animals containing TIR plasmid injected at 10 ng/ μ l were transferred to
527 the auxin or control plates and incubated at RT for 2 hours prior to the video acquisition of their
528 locomotion behavior.

529 **Data analysis using eigenworms:** Imaging processing was performed using custom MATLAB
530 scripts based on Ref. [73] using the following methods:

- 531 1. All video frames were converted to grayscale images showing only the worm itself with a white
532 background, which was achieved by background subtraction and two thresholding procedures.
 - 533 (a) Background subtraction
534 Each video was divided into several equal blocks. Then a background image was created
535 by taking the brightest pixel from the images, which was used to remove the background
536 from the frames from that block.
 - 537 (b) First thresholding
538 After the background subtraction, the first threshold value was applied to the images that
539 resulted in removal of most of the dark non-worm pixels.

- 540 (c) Second thresholding
541 Subsequently, an adaptive thresholding, also known as Bradley method [74], was applied
542 to remove the remaining small clutters, specially close to the worms.
- 543 2. Fast tracking pipeline, adapted from Ref. [73], was applied. In brief, all frames were passed
544 through the fast tracking pipeline, however only simple (non-self-overlapping posture) frames
545 were successfully processed, but concurrently labeling the unprocessed frames as 'crossed'
546 (self-overlapping posture) frames which required further processing. The results from the
547 tracking pipeline for the simple frames include the head/tail position, backbone, thickness and
548 modes (a_1, a_2, a_3).
- 549 3. Crossed frames tracking
- 550 (a) Manual tracing
551 First, 'crossed' frames were divided into a number of blocks where each block consists of
552 continuous 'crossed' frames. Then, each 'crossed' block was down sampled at 5 Hz to
553 perform manual annotation of the backbone.
- 554 (b) Comparative reconstruction
555 For comparative reconstruction, we used either the first manually traced or the last image
556 of the simple block (if any) to align with the first image of the crossed block using cross
557 correlation, and to calculate the difference between the two images. Then, we applied
558 the image thinning process to find the backbone of this difference. This backbone was
559 then connected to the rest of the worm's backbone for that corresponding image. The
560 comparative reconstruction step was repeated four times until the next manually traced
561 frame. Now, the new manually traced frame served as the reference for next four frames
562 and the process continued until the end of the crossed block. After the comparative
563 reconstruction we only got backbone of the worms, thus we used the backbone to calculate
564 the required modes (a_1, a_2, a_3).

565 Calcium imaging and analysis

566 **Imaging:** Young adults animals were mounted onto 1-1.5% of agar pad with 3-5 μ L of latex beads
567 (Polybeads, 0.2 μ m, PolySciences) to facilitate body movement and stabilize the position of field
568 of view [71]. Calcium imaging was performed using a Leica DMI8 microscope equipped with a
569 25x/0.95 water immersion lens, Lumencor SpectraX LED lightsource, and a Hamamatsu Orca Flash
570 4 V3 sCMOS camera. The GCaMP6s calcium sensor was excited with 30% of the cyan LED of the
571 SpectraX with a 488 nm excitation filter (\approx 12mW) and the calcium insensitive signal was excited
572 with the 50% of the green/yellow LED through a 575/25 nm excitation filter (\approx 33mW) using a triple
573 bandpass dichroic mirror in the filter turret (FF409/493/596-Di02-25x36, Semrock). The incident
574 power of the excitation light was measured with a Thorlabs microscope slide power meter head
575 (S170C) attached to PM101A power meter console. Emission was split with a Hamamatsu Gemini
576 W-View with a 538 nm edge dichroic (Semrock, FF528-FDi1-25-36) and collected through two single
577 band emission filters, 512/25 nm for GCaMP (Semrock, FF01-512/25-25) and 670/30 nm for mKate
578 (Semrock, FF01-670/30-25), respectively. Both emission signals were split onto top/bottom of the
579 image sensor, enabling differential exposure times optimized for imaging. Individual frames were
580 acquired at 10Hz for 40-60 seconds (depending on worm movement) with an 88 ms and 50 ms
581 exposure time, using the master pulse from the camera to trigger the light source through Hamamatsu

582 HImage software. Because it was impossible to resolve Ca^{2+} signals throughout the long DVA axon
583 in moving animals, we restricted our curvature analysis to the posterior region close to the cell body.

584 **Image analysis** Images were processed using custom built MATLAB routines to extract the mean
585 intensity of the cell body as a function of body centerline curvature near the tail. Due to the omnipresent
586 autofluorescent signal in the GFP channel, adaptive thresholding was able to separate the animal
587 backbone from the background signal. First, the raw images from the GCaMP channel were binarized
588 and eroded to find the skeleton of the worm in each frame. An iterative approach was chosen to prune
589 the branches to get the longest path describing the centerline of the worm [73]. Next, a segment of
590 the centerline enough to capture one bend of the worm in the tail region was chosen, which was
591 further divided into two equal segments using three points. These three points were used to construct
592 a triangle, and subsequently a circumcircle. Finally, the curvature is calculated at the middle point of
593 the three points using the radius of the circumcircle by the formula $\kappa = 1/R$, where R is the radius of
594 the circumcircle and κ is the curvature, and directionality of the curvature was determined by the sign
595 of the tangential angle at the point where the curvature was calculated.

596 The neuron was labelled manually for the first frame in the mKate, calcium insensitive channel,
597 and automatically tracked in subsequent frames, based on a local search in the vicinity of the location
598 in the previous frames. The area, position and intensity was collected in the mKate channel and its
599 position was mapped onto the GCaMP channel to extract the intensity in each frame. Finally, the
600 calcium sensitive signal was divided by the insensitive signal after background correction (for Fig.
601 S4) to obtain the ratio R which was normalized to the baseline ratio R_0 . The trough of the periodic
602 signals was taken as R_0 . The background subtracted calcium traces were divided by the background
603 subtracted mKate signal to yield the ratiometric intensity signal:

$$R = \frac{I^{\text{Ca}^{2+}}(t) - I_{bg}^{\text{Ca}^{2+}}(t)}{I^{\text{mKate}}(t) - I_{bg}^{\text{mKate}}(t)}$$

604 The fact that not all animals could be recorded with the same curvature change velocity and not
605 all worms bent their bodies to the same extent, precluded the calculation of an average calcium
606 signal *vs.* time. We thus transformed the periodic curvature c into a phase angle coordinate θ of 360
607 degrees [46] along the ventral-dorsal-ventral bending trajectory according to

$$\theta = \begin{cases} (c_{\max} - c_t) \cdot \left(\frac{\pi}{c_{\max} - c_{\min}} \right) & \text{for } c' = \frac{\partial c}{\partial t} < 0 \\ 2\pi - (c_{\max} - c_t) \cdot \left(\frac{\pi}{c_{\max} - c_{\min}} \right) & \text{for } c' = \frac{\partial c}{\partial t} > 0 \end{cases}$$

608 in which $c(t)$ is the curvature at a given point in time, c_{\max} and c_{\min} is the maximum curvature and
609 minimum curvature respectively, while c' is the first derivative of c with respect to time t . We arbitrarily
610 assigned ventral to 0 and 360 degrees (start and end of the cycle) while dorsal corresponded to 180
611 degrees. The average calcium intensity ratio R/R_0 was calculated and plotted against the phase
612 angle.

613 Molecular biology and transgenesis

614 **Construction of CRE driver lines:** To drive CRE expression in target neurons, we constructed
615 plasmids carrying CRE recombinase (synthesized from TWIST BioScience) and *F49H12.4p* for
616 PVD [76], *nlp-12p* for DVA [21], *acr-5p* for B-type motorneurons [7] and *flp-22p* for SMD neurons [6].
617 All promoters were amplified using primers as listed below from genomic DNA isolated from N2 wt lab
618 strains and cloned into a vector carrying codon-optimized CRE (TWIST BioScience) and synthetic
619 introns designed according to [80] terminated with a *tbb-2* 3' UTR using Gibson assembly. All clones
620 were verified by Sanger sequencing.

621 **Generation of *unc-70*(flox) using CRISPR:** CRISPR/Cas9 genome editing was performed using
622 previously described method with some modifications [81]. In brief, Cas9-crRNA-tracrRNA RNP
623 complex together with repair templates (HDR) was assembled in IDT Nuclease-Free Duplex Buffer
624 (30 mM HEPES, pH 7.5; 100 mM potassium acetate) and Mili-Q water. As in the co-CRISPR method
625 we co-injected Cas9 complexes and repair templates targeting the marker gene *dpy-10* to incorporate
626 the semi-dominant *cn64* allele. 20-30 young adult hermaphrodites were injected with the injection mix
627 containing Cas9-crRNA-tracrRNA-HDR, and recovered onto individual plates. In some cases more
628 than one crRNA were used. After 3 days post-injection, successful edits were identified based on
629 the dominant *dpy-10*(*cn64*) roller phenotype and picked to individual plates to produce self-progeny.
630 Mothers were then lysed and screened by PCR for the corresponding edits using following primers
631 as indicated in the following table. To generate the final genotypes, the CRE driver strains were bred
632 with EG7944 [82] to mark the position of *unc-70* on chromosome 5 before crossing into *unc-70* floxed
633 strain to establish the cell-specific CRE in the *unc-70*(flox) background. Successful recombination
634 of the *unc-70* locus was confirmed by PCR.

635 Generation of mutant TWK-16 allele

636 **Conditional allele:** We tagged *twk-16* locus directly after the first ATG with worm optimized
637 mScarlet, wScarlet, and a degron tag borrowed from the AID system, using Sunybiotech's CRISPR
638 services. We could identify wScarlet::*TWK-16* expression in a single neuron in the tail (DVA) and
639 two neurons in the head (probably AVK and ADE). Importantly, the fusion of *TWK-16* with the
640 AID::wScarlet tag did not cause any locomotion defects as compared to wt worms (Fig. S8E-G),
641 indicating full functionality of the transgene.

642 **Constitutive mutation:** We removed 1672-bp from the genomic locus of *twk-16*, the region
643 spanning the first two exons and the intron in between them. Two crRNAs were used to cut right
644 before the start codon and in the intron region after the second exon, with a donor consisting of an
645 ssODN containing the two 35-bp homology arms flanking the PAM sequence of the two crRNAs.

646 ***mec-4p*::TRP-4:** The *mec-4* promoter was amplified from pMH686 (gift of M. Harterink [83]) and
647 cloned together using Gibson assembly with synthetic cDNA fragments produced by TWIST Bio-
648 science, containing full-length TRP-4 (residues 1-1924) or truncated TRP-4 lacking the N-terminal

649 domain with the ankyrin repeats (residues 1433-1924) to yield pNM2. The resulting plasmids were
650 injected at 20 ng/ μ l into GN692 [84] to yield MSB382 and MSB279 respectively.

651 **DVA-Gal4 driver:** The DVA-Gal4 driver was constructed using Gibson assembly of the backbone
652 amplified from pHW393 (*rab-3p::GAL4-SK(DBD)::VP64::let-858* 3'UTR, addgene, plasmid # 85583)
653 [28] and insert fragment containing *nlp-12p* amplified from pRD1 (*nlp-12p::CRE::tbb-2* 3'UTR) plasmid
654 to yield pRD10. The resulting plasmid was injected at 5 ng/ μ l together with *unc-122p::mCherry* as
655 coinjection marker and integrated by UV irradiation using standard procedures.

656 **DVA and TRN-TIR driver:** The TRN-TIR driver was constructed using Gibson assembly of the
657 backbone amplified from pUN1020 (*fln-1p::TIR::F2A::mCherry::H2B*, a gift from the Cram lab [27])
658 and insert fragment containing *mec-4p* amplified from pNM2 (*mec-4p::TRP-4::tbb-2* 3'UTR) plasmid
659 to yield pNS30. The resulting plasmid was injected at 30 ng/ μ l together with *unc-122p::mCherry* as
660 coinjection marker. The DVA-TIR driver was constructed using Gibson assembly of the backbone
661 amplified from pNS30 and insert fragment containing *nlp-12p* amplified from pRD1 plasmid to yield
662 pNS43. The resulting plasmid was injected at 10 ng/ μ l together with *unc-122p::mCherry* as coinjection
663 marker.

664 **Generation of the E2008K allele in UNC-70(TSMod):** pRD7 was constructed using Gibson as-
665 sembly of the backbone amplified from pMK35(*pExprunc-70R8TSmodR9::unc-54* 3'UTR) and insert
666 synthetic fragment (TWIST bioscience) containing E2008K mutation. The resulting plasmid was
667 injected at 20 ng/ μ l.

668 1.5 Extrachromosomal array and integration

669 Extrachromosomal arrays were generated by injecting the aforementioned amount of DNA into the
670 appropriate strain and selecting for the F1 progenies with the co-injection marker. Three independent
671 lines were generated whenever possible. The integration of the extrachromosomal array was per-
672 formed using UV/TMP method. In brief, late L4-young adult animals carrying the array were picked
673 onto a NGM plate without OP50. These animals were fed TMP at a final concentration of 50 μ g/ml
674 for 20 minutes. Then, they were UV irradiated for 30 seconds at 450 $mJ \cdot cm^{-2}$ and expanded for 3-4
675 weeks before selection. Three independent integrated lines were recovered whenever possible.

676 1.6 Primers and gRNA sequences

No	Gene	Sequence	Comment
1	unc-70	gRNA-1(GCAACGGCGCGAAACGTCGT) gRNA-2(GCGAAACGTCGTCGGCAATA) gRNA-3(CGTCGTCGGCAATATGGCTA)	5' edit
2	unc-70	gRNA(GCTACCAGGTAACGATTA)	3' edit
3	twk-16	gRNA-1(TTGCAGAATAAACATCATTG) gRNA-2(TTATATGTAGCACACTTTTG)	deletion exon 1+2
4	trp-4	gRNA (ACGTGGCGAATCCATAACCG)	GFP tag
677 5	nlp-12p	FWD:CTGACCTtaaattcaggtgtgtgatcgagaacgccgagcagttgaagctcgtg REV:TTTTGATGAACAGTGAGAAGATTTGACATtttgcggaggcaattgaaataagtttcgc	minimal promoter in DVA
6	F49H12.4p	FWD: caggtgtgtttgaaaaatgattacgataaacctga REV:CAGTGAGAAGATTTGACATcatgtctattttcttgaggatgaagt	PVD driver
7	acr-5p	FWD:CTGACCTtaaattcaggtgtgtggcaatggaattggcaattgt REV: GAACAGTGAGAAGATTTGACATATgctgaaaatt	B-type MN driver
8	flp-22Δ4p	FWD: CCTtaaattcaggtgtgccccaaaaattttaac REV:CAGTGAGAAGATTTGACATtgcaagcttagagta	SMD driver

678 CRISPR reagents and primers for isolation of promoter sequences from genomic DNA

679 Primary culture and mechanical stimulation

680 **DVA primary cell culture:** Embryonic cell isolation was performed using a previously described
681 method with some modifications [35]. Briefly, synchronized worms seeded onto peptone enriched
682 plates and incubated at RT until the plates were populated with eggs. Then, the plates were washed
683 off and the eggs were collected using milli-Q H₂O. The worm and egg pellets were resuspended in
684 the freshly prepared bleaching solution and rocked gently for 4-7 minutes, once 70-80% of the worms
685 were lysed the reaction was stopped using egg buffer (118 mM NaCl, 48 mM KCl, 2 mM CaCl₂, 2 mM
686 MgCl₂, 25 mM HEPES, pH 7.3 and an osmolarity of 340 mOsm.). The collected eggs were washed 3
687 times with fresh egg buffer. Then the eggs were separated using 30% final concentration of sucrose
688 by centrifuging at 1200 rpm for 20 minutes. The separated top layers of eggs were collected in a new
689 tube and washed 2-3 times with egg buffer. Then, the eggs were treated with chitinase (0.5 U/ml) for
690 40 minutes to dissociate embryonic cells. The chitinase reaction was stopped using L15 medium.
691 After chitinase treatment the embryos with the digested egg cells were passed through 25 G needle
692 10-15 times for dissociating into single cells. The dissociated cell suspension was filtered through 5
693 μm Durapore filter (Millipore). Then the single cell solution was centrifuged for 3 minutes at 3200 rpm
694 and the pellet was resuspended in L15 medium.

695 Optical trapping chambers were based on two parallel glass surfaces spaced with a 50-μm-thick
696 polydimethylsiloxane (PDMS) layer (1:10 curing agent, Sylgard). A glass bottom Petri dish (BD,
697 Wilco Glass, #1.5) was spin-coated for 1 min at 750 rpm [87] and cured for 1h at 65°C. A 1x1 cm
698 square cavity was gently peeled off with a scalpel and the bottom dishes were then sterilized by
699 UV irradiation for 1h. The cavity was then incubated with a 100 μL drop of peanut lectin (Medicago,

700 Sweden) diluted 1:10 into phosphate-buffered saline (PBS) for 20 min. After that, the plate was rinsed
701 with L15 medium and a 150- μ L drop containing the cells was seeded. Cell density was $1.5 \cdot 10^5 \text{L}^{-1}$ to
702 ensure enough number of DVA neurons per plate, while keeping an appropriate space for the tether
703 extrusion experiments. After 60 min, 0.5 mL of L15 medium was added and the cells were cultured
704 at 25°C overnight. Fresh medium was changed (0.5 – 1 mL) every 24h. All experiments were carried
705 out between one and four days post isolation.

706 **Stimulation of DVA neurons cultured on elastic substrates:** For the axonal deformation ex-
707 periments, the neurons were directly seeded on the PDMS surface. The latter was obtained by
708 spin-coating a bottom glass (Wilco Glass, #1.5) for 1 min at 750 rpm. The coated dish was then
709 plasma-treated for 5 min (Plasma Surface Technology, Diener Electronic), UV-irradiated for 1h and
710 incubated with 500 μ L of peanut lectin (1:10 in PBS, Sigma Aldrich) for 24h. Axonal deformation
711 was undertaken by indentation of the substrate with a 1-mm-thick microinjection needle held with a
712 4D-control micromanipulator (uMP-4, Sensapex). The needle tip was brought \approx 5 mm away from
713 the axonal tip and pushed vertically to induce substrate indentation. Substrate deformation was
714 determined from the average perpendicular displacement at the middle of the axon. GCaMP activity
715 was measured from the cell body and was normalized to the mKate, Ca^{2+} -independent fluorescence
716 signal (see below). A total of 25 cells were tested, 18 of which showed reproducible responses to
717 substrate indentation.

718 **Optical measurement of membrane mechanics and calcium activity:** The optical tweezer (OT)
719 platform (SensoCell, Impetux Optics, Spain) consists of a continuous wave laser ($\lambda=1064$ nm, 5
720 W nominal output power, Azur Light) steered with a pair of acousto-optic deflectors (AOD 1 and 2)
721 and a force detection module that captures the forward-scattered light from the optical traps (Fig.
722 S7A). This is mounted around an inverted research microscope (Nikon Eclipse Ti2) equipped with a
723 spinning disk confocal microscope (Andor DragonFly 502, Oxford Instruments) on top of an active
724 isolation table (Newport). The laser is directed onto a microscope objective (MO, 60x/NA=1.2, water
725 immersion, Nikon) after being expanded with a telescope (lenses L1 and L2) to fill the MO entrance
726 pupil, through the epifluorescence port. A short-pass dichroic mirror (D1) reflects the IR trapping
727 beam and transmits both the excitation and emission light for fluorescence microscopy, as well
728 as bright-field illumination (BF). To prevent from IR light leaking towards the detector, a neutral,
729 shortpass filter (IR-F) was placed at the imaging optical path. The optical traps were positioned using
730 LightAce software (Impetux Optics, Spain), in synchronization with a CMOS camera (BlackFly S,
731 FLIR Systems) recording bright-field images of the sample. The force detection module of our optical
732 tweezers platform operates by detecting light-momentum changes, after capturing the scattered
733 trapping beam through an NA = 1.4, oil immersion collecting lens (CL), with a position-sensitive
734 detector (PSD) placed at plane optically equivalent to the back focal plane (BFP) [88]. This allowed
735 us to measure forces with no previous trap calibration and beyond the linear trapping regime, thus
736 covering the full spectrum until the escape force of \approx 200 pN, hence allowing working with lower laser
737 power (250 mW), as compared to standard back focal plane interferometry [89]. The module enables
738 the BF illumination (BF) to pass through, which is properly filtered to avoid leakage into the PSD.

739 **Membrane tether extrusion:** Prior to force measurements, red-fluorescent, 1- μm polystyrene
740 (PS) microspheres (FluoSpheres F8816, Thermo Fischer) were washed with cell culture medium by
741 centrifuging at 10^4 rpm for 3 min and added to the sample chambers at a concentration of $2 \cdot 10^5 \mu\text{L}^{-1}$
742 and was then covered with a 1x1 inch coverglass (CG, #1.5, Ted Pella). The microspheres were
743 captured in a 250-mW optical trap (laser power at the sample plane) and brought close to a DVA
744 neuronal axon for 1 second before retraction to produce a stable membrane tether. The tethering
745 force, F_{tether} , was balanced with the trapping force, F_{OT} , resulting in a change in the trapping beam
746 momentum detected with the force detection module. Pulling routines were pre-defined with LightAce
747 software (Impetux Optics) as follows (Fig. 5G): first, bead was contacted the axon and a pulled a
748 distance of 10 μm , reaching a peak in the tether tension value; second, the bead stopped for 10 s,
749 letting the tether tension to relax down to a static value arising from the stored stress. Third, the bead
750 was brought back to the initial position, releasing F_{tether} to almost zero; finally, the bead stopped
751 again for 10 s, letting the tether tension load up to a similar value. This routine was repeated three
752 times for every DVA neuron tested at increasing velocities, 5, 20, 80 μms^{-1} . Finally, every force
753 measurement was compensated for initial momentum variation by subtracting a baseline trajectory
754 taken on the same positions without a bead. The appearance of the Ca^{2+} transients is likely caused
755 by the mechanical perturbation as opposed to local heating, as tether-free trials were unable to elicit
756 reproducible changes (Fig. S6C,D). During each tether extrusion sequence, GCaMP and mKate
757 fluorophores were simultaneously excited using the 488 nm and 561 nm laser lines of an Andor
758 DragonFly Spinning Disk Confocal microscope, respectively. The excitation beam is corrected for
759 non-uniformity and throughput using a Borealis Illuminator (BI, Andor, Oxford Instruments), before it
760 passes through a quadband dichroic mirror (D2, 405-488-561-637 nm, Andor). After illuminating the
761 sample plane, fluorescence emission is reflected at D2 and split with a 565-nm longpass dichroic
762 mirror (D3) to image GCaMP and mKate emissions using F1: $\lambda = 521$ nm, F2: $\lambda = 647$ nm at two
763 identical, back-illuminated scientific CMOS cameras (sCMOS 1&2, Andor, Oxford Instruments).

764 **Data analysis of tether pulling:** Peak (F_{peak}) and storing (F_{base}) force values were obtained from
765 the trapping force signals during the tether extrusion experiments (Fig. S7B). Tension was calculated
766 from the force according to Ref. [36]. GCaMP emission was measured both from the cell body (CB)
767 and tether neck (TN) by setting a region of interest (ROI) with Fiji [91]. The background contribution
768 was measured far from the neuron and subtracted from the intensity profile. The photobleaching
769 trend was corrected by normalization over an exponential fit, providing the background subtracted
770 and baseline normalized GCaMP signal (F/F_0). Tension modulated calcium activity was observed in
771 $\approx 30\%$ of all extrusion events and is thus within the single molecule approximation [92]. To determine
772 if the changes in neuronal membrane tension induced a significant increment in the GCaMP intensity,
773 this was measured for a 3-second timeframe before and after the tether was extruded. Because
774 the two, synchronized cameras recorded videos with a 10-frame rate, GCaMP intensity values were
775 averaged over $N=30$ data points. The two intensity values (before and after pulling) were t-tested
776 and thresholded within a p -value of <0.01 . When significant, the pulling events were classified
777 into GCaMP-increasing ($\Delta I > 5\%$) and GCaMP-decreasing ($\Delta I < 5\%$). To rule out that the Ca^{2+}
778 transient were caused by heating of the trapping laser, we carried out a series of measurements on
779 wt DVA neurons in the absence of a membrane nanotube tethering the trapped microbead and the

780 neuronal axon (without prior contact of the bead).

781 1.7 Monte Carlo simulation of force-gated ion channel ensembles

782 To capture the dynamics and the statistical behavior resulting from the stochastic activation of an
783 ensemble of mechanosensitive ion channels, subjected to a mechanical force, we set up a continuous
784 time Markov chain Monte Carlo simulation [93]. We choose to model a pair of mechanosensitive
785 ion channels, which we conceptualize as an excitator, sodium or calcium conductive in channel
786 and an inhibitory, potassium or chloride conductive ion channel. Our model is agnostic of the
787 force transmission pathway and does not differentiate between membrane and cytoskeletal force
788 delivery. To simulate the behavior in absence of external noise, we assumed that each channel
789 acts independent, activities are uncoupled, and each channel is characterized by an open and a
790 closed state that is separated by a potential barrier with height E (Fig. S7). The lifetime of each
791 state depends on the height of the energy barrier separating the closed from the open states and
792 the loading conditions. Opening is driven by thermal fluctuations, and, as a result, is a stochastic
793 process. Application of force to the channel tilts the energetic landscape, thus reducing the energy
794 barrier that separates the closed from the open state by an amount $F \cdot \gamma$, in which γ is the distance to
795 the transition state [38]. If a load is applied to the channel for durations that are much shorter than
796 the intrinsic lifetime of the closed state, the channel resists opening. Importantly, channels do not
797 confer resistance to force on timescales that are larger than the intrinsic lifetime of the particular
798 closed state [95, 96]. In agreement with previous data on whole cell recordings from TRP-4 [39],
799 we assumed that the excitatory channel activates at the onset and the offset of the force. Such
800 behavior is consistent with a strain-rate sensitivity [98], thus, we model the channel sensitive to the
801 first derivative of the force, $\frac{\partial F}{\partial t}$. The forward transition rate was model using the modified Evans-Bell
802 model of time-dependent bond-strength, as determined by the force-rate or loading rate r_f [96].
803 Loading rate was calculated from the stiffness of the ankyrin domain [99] multiplied by the pulling
804 velocity in the experiment. We start the simulation with all states closed, and are interested in the
805 evolution of the ensemble to the open state.



806 The lifetime of the closed state is governed by the spontaneous opening constant k_o^0 according to

$$p(t) = \exp(-k_o^0 \cdot t) \quad (2)$$

807 For an open channel, the probability of finding the channel open after time t decays exponentially and
808 will spontaneously revert back to the closed state stochastically if the random sampling parameter
809 ($r \in \mathbb{R}_{>0}^{\leq 1}$) is smaller than $p(t)$. Thus, if a channel is in the open state at time t , the probability of finding
810 it in the open state $t + 1$ decreases e -fold:

$$p(t) = \exp(-k_C^0 \cdot t) \quad (3)$$

811 Force sensitivity is achieved by applying Bell's model to the forward rate constant. We likewise
 812 assume that the channel cannot sustain the open state as long as force is acting. This assumption has
 813 the physical manifestation in a force-transmission pathway through a weak protein-ligand interaction
 814 (slip bond). After time t , we apply a force to the channels. Thus, the probability of a closed channel
 815 responding to the external forces changes to

$$p(t, F) = p_0 \cdot \exp(-k_o(\mathbf{F}) \cdot t) \quad (4)$$

816 in which

$$k_o(\mathbf{F}) = k_o^0 \cdot \exp\left(-\frac{F\gamma}{k_B T}\right) \quad (5)$$

817 Evan's modification for a finite loading rates was implemented to capture the strain-rate dependence
 818 of the Calcium channel (TRP-4), known to respond to the change in force.

$$k_o(\mathbf{F}) = \frac{r_f}{\exp\left(\frac{F}{f_\beta}\right) \cdot f_\beta} \quad (6)$$

819 with $\frac{\gamma}{k_B T}$ as the force scale.

820 We implemented the simulation in R, with a timestep of 1e-5 s and the following kinetic constant.

821 Inhibitory Channel: $\gamma=2.93$, $k_o^0=120$, $k_C^0=600$, $k_C^F=700$

822 Excitatory channel: $\gamma=2.10$, $k_o^0=100$, $k_C^0=300$, $k_C^F=100$

823 The physical representation of the values k_o^0 , k_C^0 correspond to the spontaneous opening constants.
 824 For $k_o^0 > k_C^0$, ion channel remain statistically open, otherwise they spent more time in the closed state
 825 on average. Without a lack of generality, the concept can be applied for lipid bilayer tension-gated
 826 ion channels in which the free energy profile of the energy landscape is altered by a external tension
 827 σ according to $\Delta\Delta G = -\Delta G - \sigma \cdot \Delta A$, in which ΔA equals to the increase in cross sectional area of
 828 the gated ion channel, e.g. $\Delta A=4.7\text{nm}^2$ for TREK2 [17]. Thus, the tension dependent k_o conforms to
 829 $k_o(\sigma) = k_o^0 \cdot \exp\left(-\frac{\sigma \cdot A}{k_B T}\right)$. It can be readily seen that without an increase in cross-sectional area, the
 830 open state is not preferred. Finally, the average current was calculated by $I = cNP_o$, where c is the
 831 single-channel current taken from the literature (TRP-4, 18pA; 140pS [101]; K2P, 13pA; 90pS [102]),
 832 N the number of channels, and P_o the average probability of finding the channel open derived from
 833 the simulations. Under assumption of a high input resistance typical for *C. elegans* neurons [60], the
 834 K signal was then subtracted from the Ca signal to yield a macroscopic 'observable', distantly related
 835 to a Calcium signal. The picture that is emerging from this simulation is that ventral DVA activity
 836 emerges in part from TRP-4 activation under compression and the suppression of 'stretch' currents
 837 under dorsal side. Whereas this describes a plausible explanation for our findings, two other possible
 838 scenarios could give rise to the observed ventral activity *in vivo*: 1) TWK-16 and TRP-4 both activate
 839 under tension, and close with different rates such that a remaining Ca^{2+} activity is visible during
 840 ventral bouts (Fig. S7F); and 2) TRP-4 is constitutively active and only modulated by TWK-16 leading
 841 to Ca^{2+} suppression during tension (Fig. S6G). The combined results from our *in-vitro* (Fig. 5), *in vivo*
 842 (Fig. 6) and *in-silico* (Fig. S6e-g) experiments favor a scenario in which mechanosensitive TWK-16
 843 activity suppresses stretch-induced depolarization. Because TRP-4 is a pore-forming sub-unit of a

844 mechanosensitive ion channel that activates at the force onset and offset [39] we should expect DVA
845 activity during dorsal AND ventral bends, but we exclusively recorded Ca^{2+} increases during force
846 relaxation/offset *in vitro* and compressed axons during ventral bends *in vivo*. In absence of TWK-16,
847 however, the biphasic TRP-4 activity is unveiled.

848 1.8 Neuromechanical model

849 Without exception, all parameters and assumptions have been reproduced as outlined in Ref. [44].
850 In short, the framework consists of a 2D structural skeleton composed of 46 segments and 98
851 discrete joints distributed on the ventral and dorsal sides. These joints are vertically connected
852 by incompressible rods and lateral connections embody passive forces modeled as a Kelvin-Voigt
853 material with a spring and a dashpot in parallel owing a constant material property. Diagonal elements,
854 connecting neighboring joints on opposing sides, represent the effect of pressure and ensure volume
855 conservation. In parallel to the passive force, ‘muscle’ forces are computed as an active Kelvin-Voigt
856 material with varying spring constants and viscosities, whose parameters are slaved to neuronal
857 state variable of the motor circuit (active, inactive). For details see ref [44].

858 The minimal motorcircuit was embedded into that active/passive framework, consisting of a pair
859 of excitatory (DB,VB) and inhibitory motoneurons (DD,VD). The current within the motor ventral
860 motorcircuit has contribution from stretch receptors, in addition to the input current from AVB and motor
861 current driving muscle actuators. The current in dorsal motor neuron does not contain adjustments
862 from the stretch receptor. The modified the model according to our experimental data: We eliminated
863 dorsal stretch receptor currents $\frac{\partial I}{\partial \epsilon} = 0$ (with $\epsilon = dc$ as the strain) and inverted the relationship of
864 the stretch receptor current on the ventral side and deliver it to B-class motor neurons on the dorsal
865 side. No other stretch receptors were considered on the ventral side. In this model a compression on
866 the ventral side generates a polarising response, which results in polarising current flowing into the
867 B-class motor neurons on the dorsal side, and *vice versa*, the stretch on the ventral side generates
868 a hyperpolarizing response. The DVA neuron is implemented indirectly, as a compression/stretch
869 sensor, sending either positive or negative current to DB neurons. (A similar effect can be achieved
870 by modifying the firing rate of DVA, which is non-zero at resting length). The factor modifying the
871 effective stretch receptor activation function ($-\mathbf{S}$) was implemented in the original [44, Eq. 11]:

$$h_m^k = -\mathbf{S}\lambda_m\gamma_m^k \frac{L_{L,m}^k - L_{0L,m}}{L_{0L,m}} \quad (7)$$

872 where \mathbf{S} scales the effective stretch receptor activation function. This may be understood as decreas-
873 ing the number of effective stretch/compression sensitive ion channels, or a failure to recruit active
874 ion channels (equivalent to a failure in force transmission).

875 1.9 Statistics

876 Statistical analyses were performed in R and Igor. To construct and visualize the 3D distributions of
877 the modes (a_1, a_2, a_3) , the 3D kernel density estimate of the first three modes was calculated in R
878 using the `ks` package [105] with an unconstrained plug-in selector bandwidth. We choose to indicate
879 the 10, 25 and 50% contours of the highest density regions in the manifold and the 2D projections
880 of the floating data cloud along the corresponding planes. To compare two different data sets and
881 test for the null hypothesis that the two kernel density functions are similar, we resampled the highly
882 oversampled population by bootstrapping to avoid spurious significance due to long tailed outliers.
883 The resampling and testing was performed 1000 times to yield a distribution of p -values which is
884 displayed as a violin plot summarizing each figure of locomotion data. Importantly, the resampling
885 does not lead to significant discrimination of the downsampled and original dataset within the same
886 population. Alternatively, the local density between two distributions was tested using a binned kernel
887 density estimator. For this, we used the `kde.local.test` function from the `ks` package in **R** [105]
888 to a) converted all n data points of each distribution to counts on a $100 \times 100 \times 100$ binning grid and
889 embedded into a matrix C , b) evaluate the kernel function at these grid points to embed them into a
890 matrix K and c) then obtain the binned density estimator f from a sequence of discrete convolutions
891 of C and K . The exact formulation of that procedure and a detailed presentation of the algorithm can
892 be found in ref. [106]. For all grid points in which $p > 0.01$, we accept the null hypothesis that the two
893 densities at this grid point for the two distributions is the same. For all other grid points, a polarity is
894 assigned and plotted as two different colors in a 3D voxelgram (e.g. Fig. 1D), indicating that $x_1 > x_2$
895 or $x_1 < x_2$. Swarm plots and estimation statistics have been calculated using the methods described
896 in ref. [107].

897 **Supplementary References**

- 898 63. H. Fehlaue, *et al.*, *Journal of Visualized Experiments* **2018**, 1 (2018).
- 899 64. M. Krieg, A. R. Dunn, M. B. Goodman, *Nature Cell Biology* **16**, 224 (2014).
- 900 65. T. Boothe, *et al.*, *eLife* **6**, 1 (2017).
- 901 66. A. L. Nekimken, B. L. Pruitt, M. B. Goodman, *Molecular Biology of the Cell* **31**, 1735 (2020).
- 902 67. M. Porta-de-la Riva, L. Fontrodona, A. Villanueva, J. Cerón, *Journal of Visualized Experiments*
903 p. e4019 (2012).
- 904 68. T. Stiernagle, *WormBook : the online review of C. elegans biology* pp. 1–11 (2006).
- 905 69. C. M. Chai, *et al.*, *Nature Methods* **14**, 145 (2016).
- 906 70. F. V. Subach, *et al.*, *Nature Chemical Biology* **5**, 118 (2009).
- 907 71. E. Kim, L. Sun, C. V. Gabel, C. Fang-Yen, *PLoS ONE* **8**, e53419 (2013).
- 908 72. B. Sands, *et al.*, *Translational Medicine of Aging* **2**, 1 (2018).
- 909 73. O. D. Broekmans, J. B. Rodgers, W. S. Ryu, G. J. Stephens, *eLife* **5**, 1 (2016).
- 910 74. D. Bradley, G. Roth, *Journal of Graphics Tools* **12**, 13 (2007).
- 911 75. L. He, *et al.*, *Current Biology* pp. 1–12 (2019).
- 912 76. A. Albeg, *et al.*, *Molecular and cellular neurosciences* **46**, 308 (2011).
- 913 77. I. Hums, *et al.*, *eLife* **5**, 1 (2016).
- 914 78. Q. Wen, *et al.*, *Neuron* **76**, 750 (2012).
- 915 79. J. Yeon, *et al.*, *PLOS Biology* **16**, e2004929 (2018).
- 916 80. S. Redemann, *et al.*, *Nature Methods* **8**, 250 (2011).
- 917 81. A. Paix, A. Folkmann, G. Seydoux, *Methods* **121-122**, 86 (2017).
- 918 82. C. Frøkjær-Jensen, *et al.*, *Nature methods* **11**, 529 (2014).
- 919 83. M. Harterink, *et al.*, *Current Biology* **26**, R153 (2016).
- 920 84. A. L. Nekimken, *et al.*, *Lab Chip* **17**, 1116 (2017).
- 921 85. A. C. E. Wirshing, E. J. Cram, *Molecular Biology of the Cell* **29**, 2433 (2018).
- 922 86. K. Strange, M. Christensen, R. Morrison, *Nature Protocols* **2**, 1003 (2007).

-
- 923 87. J. H. Koschwanetz, R. H. Carlson, D. R. Meldrum, *PLoS ONE* **4**, 2 (2009).
- 924 88. A. Farré, M. Montes-Usategui, *Optics Express* **18**, 11955 (2010).
- 925 89. A. Farre, F. Marsa, M. Montes-Usategui, *Methods in enzymology* **1486**, 41 (2017).
- 926 90. F. M. Hochmuth, J. Y. Shao, J. Dai, M. P. Sheetz, *Biophys J* **70**, 358 (1996).
- 927 91. J. Schindelin, *et al.*, *Nature methods* **9**, 676 (2012).
- 928 92. K. C. Johnson, W. E. Thomas, *Biophysical Journal* **114**, 2032 (2018).
- 929 93. A. Nekouzadeh, Y. Rudy, *Mathematical biosciences* **210**, 315 (2007).
- 930 94. G. I. Bell, *Science* **200**, 618 (1978).
- 931 95. E. Evans, *Annu Rev Biophys Biomol Struct* **30**, 105 (2001).
- 932 96. E. A. Evans, D. A. Calderwood, *Science* **316**, 1148 (2007).
- 933 97. L. Kang, J. Gao, W. R. Schafer, Z. Xie, X. Z. S. Xu, *Neuron* **67**, 381 (2010).
- 934 98. A. L. Eastwood, *et al.*, *Proceedings of the National Academy of Sciences* **113**, E2471 (2015).
- 935 99. S. Bechstedt, J. Howard, *Current Topics in Membranes* (Elsevier, 2007), vol. 59, pp. 399–424.
- 936 100. S. G. Brohawn, *Annals of the New York Academy of Sciences* **1352**, 20 (2015).
- 937 101. Z. Yan, *et al.*, *Nature* **493**, 221 (2013).
- 938 102. J. T. Brennecke, B. L. de Groot, *Biophysical Journal* **114**, 1336 (2018).
- 939 103. M. B. Goodman, D. H. Hall, L. Avery, S. R. Lockery, *Neuron* **20**, 763 (1998).
- 940 104. J. H. Boyle, S. Berri, N. Cohen, *Frontiers in Computational Neuroscience* **6**, 1 (2012).
- 941 105. T. Duong, *Journal of Statistical Software* **21**, 1 (2007).
- 942 106. J. Chacon, T. Duong, *Monographs on Statistics and Applied probability 160: Multivariate*
943 *Kernel Smoothing and its application* (Taylor & Francis, 2018).
- 944 107. J. Ho, T. Tumkaya, S. Aryal, H. Choi, A. Claridge-Chang, *Nature Methods* **16**, 565 (2019).
- 945 108. E. Kage-Nakadai, *et al.*, *PloS one* **9**, e114680 (2014).
- 946 109. M. Harterink, *et al.*, *Journal of Cell Science* **131** (2018).
- 947 110. S. Ruijtenberg, S. Van Den Heuvel, *Cell* **162**, 300 (2015).
- 948 111. W. Li, Z. Feng, P. W. Sternberg, *Nature* **440**, 684 (2006).
- 949 112. J. Kubanek, P. Shukla, A. Das, S. A. Baccus, M. B. Goodman, *The Journal of Neuroscience*
950 **38**, 3081 (2018).
- 951 113. M. Kelley, *et al.*, *eLife* **2015**, 1 (2015).

952 2 Supplementary Videos

953 **Video S1: Locomotion behavior in wt and *unc-70* mutants** Representative video of wildtype
954 (N2) and *unc-70(e524)* (CB524) mutant animal. Acquired at 25Hz

955 **Video S2: Crawling behavior of conditional *unc-70* alleles.** Representative video of conditional
956 CRE/loxP mutant strains in the order as they appear in Figure 3. Scale bar = 300 μ m, acquired at 25Hz.
957 *unc-70*(floxed) is the control animal without CRE expression denoting the background for all other
958 genotypes. Pan-neuronal, *rgef-4p::CRE*; BWM, body wall muscle restricted *myo-3p::CRE*; D-type
959 MN, GABAergic motoneuron directed *unc-25p::CRE*; B-type MN, cholinergic forward motoneurons
960 directed *acr-5p::CRE*; A-type MN, cholinergic backwards motoneurons directed *unc-4p::CRE*; TRN,
961 touch receptor neuron specific *mec-17p::CRE*; SMD, SMD-directing *flp-22 Δ 4p::CRE*; PVD, PVD-
962 directing *F49H12.4p::CRE*; DVA, DVA-specific *nlp-12p::CRE* in the *unc-70*(floxed) background.

963 **Video S3: DVA Calcium activity in *unc-70* mutants** Representative video of DVA calcium activity
964 in wildtype (left), *unc-70(e524)* (middle) and conditional DVA CRE/loxP mutant strains (right). Upper
965 panel shows the calcium sensitive GCaMP6s, lower panel a calcium-insensitive mKate as a movement
966 and defocussing control. Playback speed, 30 frames/s.

967 **Video S4: DVA responds to substrate deformation** False color labeling of a GCaMP6s express-
968 ing DVA neuron cultured on PDMS, subjected to a mechanical deformation. Yellow shows the calcium
969 sensitive GCaMP6s, magenta a calcium-insensitive mKate as a movement and defocussing control.
970 Scale bar = 5 μ m. Acquired at 10Hz.

971 **Video S5: Calcium activity in DVA during dynamic membrane tether extrusion** Representa-
972 tive video of DVA neuron in the dynamic optical trapping assay. Scale bar = 5 μ m. Acquired at
973 10Hz.

974 **Video S6: Locomotion behavior of conditional *twk-16* mutant animals** Representative video
975 of a TWK-16::AID animal in presence of 1mM auxin; left animal without (MSB555) and right with
976 (MSB526) DVA::TIR expression. Scale bar = 300 μ m. Acquired at 25Hz.

977 **Video S7: Compression induced proprioceptor current coordinates locomotion behavior** Left:
978 Animation derived from the results of the neuromechanical model for input parameters giving rise

979 to wildtype-like animal locomotion pattern implementing DVA as a compression sensitive proprio-
980 ceptor. Right: Same model with lower sensitivity to curvature induced compression current in DVA,
981 representing *trp-4* and *unc-70* mutations.

982 3 Supplementary Figures

983 **Supplementary Fig. S1. Reporting CRE recombination efficiency**

984 **A** Strategy of the CRE recombination reporter. A floxed tagBFP with a nuclear localization signal
985 (NLS) under the control of the ubiquitous *rps-18* promoter is visible before recombination. After
986 CRE expression in specific cells and tissues, the tagBFP gets excised and brings an NLS::mCherry
987 construct under the control of the *rps-18p*, enabling the identification of targeted cells. For details and
988 number of animals investigated see Table S2. **B** Schematic of the predicted pattern and representative
989 picture of a reporter animal without CRE expression showing only BFP expressing cells. **C** Schematic
990 of the predicted pattern and representative picture of a panneuronal CRE activity under *rgef-1p*. **D**
991 Schematic of the predicted pattern and representative picture of a CRE activity in body wall muscles
992 under *myo-3p*. **E** Expected pattern for motoneurons. **F** Representative picture of a CRE activity
993 in A-type motoneurons under *unc-4p*. The red dot in the tail is due to *lin-44::DsRed* coinjection
994 marker. **G** Representative picture of a CRE activity in B-type motoneurons under *acr-5p*. The
995 pharyngeal signal is due to *myo-2p::Cherry* coinjection reporter. **H** Representative picture of a CRE
996 activity in D-type motoneurons under *unc-25p*. The pharyngeal signal is due to *myo-2p::Cherry*
997 coinjection reporter. **I** Schematic of the predicted pattern and representative picture of a CRE activity
998 in DVA neuron under *nlp-12p*. The pharyngeal signal is due to *myo-2p::Cherry* coinjection reporter.
999 **J** Schematic of the predicted pattern and representative picture of a CRE activity in SMD under
1000 *flp-22Δp*. The six red spots belong to *unc-122p::RFP* coinjection reporter. **K** Expected recombination
1001 pattern for touch receptor neurons (TRNs). Representative picture of a CRE activity in TRNs visible
1002 in four (on the left side) out of the six neurons. The right side is not imaged. **L** Schematic of the
1003 predicted pattern and representative picture of a CRE activity in PVD under the control of the *des-2p*
1004 and *F48H12.2p*.

1005 **Supplementary Fig. S2. DVA-specific mutation of the spectrin network causes aberrant** 1006 **body postures**

1007 **A-C** Still image and the corresponding 3D eigenworm orbit for **(A)** pan-neuronal CRE, **(B)** *nlp-*
1008 *12p::CRE* and **(C)** *flp-22Δ4p::CRE* expressing animals. Only CRE drivers are shown that showed a
1009 phenotype in combination with the *unc-70(flox)* allele. **D** Distribution of *p*-values for the hypothesis
1010 test H0 that the two data sets indicated in the graph are sampled from the same population. Orange
1011 line indicates $\alpha=0.05$ level of significance, black diamond represents the mean and horizontal line the
1012 median *p*-value of the distribution. **F-K** Representative still image and the corresponding manifold
1013 in the three dimensional eigenworm space for **(F)** D-type, **(G)** B-type, **(H)** A-type motoneurons, **(I)**
1014 in TRNs, **(J)** SMD, and **(K)** PVD. **L,M** Distribution of *p*-values for the combinations indicated in the
1015 figure. Orange line indicates $\alpha=0.05$ level of significance.

1016 **Supplementary Fig. S3. SPC-1 shares function during locomotion in DVA with UNC-70**

1017 **A-C** Representative images of an **(A)** UN1823 (*SPC-1::AID::mKate*) expressing control animal
1018 (without TIR ligase) and **(B)** MSB453 (*mec-4p::TIR->SPC-1::AID::mKate*) with nuclear mCherry
1019 localization indicating TIR expression in TRNs in absence and **(C)** presence of 1mM auxin. Due to the
1020 overlap of DVA axons with other neurites in the ventral nerve chord, we choose to estimate the effect
1021 in TRNs. **D** Quantification of the neurite intensity of TRNs without TIR (ctrl, N=17 animals) and with

1022 TIR ligase in absence (N=19) and presence of auxin (N=20) in the SPC-1::AID::mKate background,
1023 normalized by the intensity of motorneuron commissure (that do not express the TIR ubiquitin
1024 ligase). **E-G** Representative snapshot of a (**E**) SPC-1::AID::mKate animal without TIR ligase and the
1025 corresponding quantification of its behavior in (**F**) absence and (**G**) presence of auxin. **H** Distribution
1026 of *p*-values as described above for the combinations indicated in the figure. **I-K** Representative
1027 snapshot of a (**I**) MSB503 animal expressing TIR exclusively in DVA (*nlp-12p::TIR::F2A::H2BmKate*)
1028 and the corresponding quantification of its behavior in (**J**) absence and (**K**) presence of auxin. **L**
1029 Distribution of *p*-values for the combinations indicated in the figure **M-O** Representative snapshot
1030 of a (**M**) MSB464 animal expressing TIR exclusively in DVA together with the SPC-1::AID::mKate
1031 degron and the corresponding quantification of its behavior in (**N**) absence and (**O**) presence of
1032 auxin. **P** Distribution of *p*-values for the combinations indicated in the figure. Note, due to the auxin-
1033 independent TIR activity, the addition of 1 mM auxin does not further increase the auxin-independent
1034 loss of coordination.

1035 **Supplementary Fig. S4. Cell autonomous calcium activity in immobilized animals and TRNs**

1036 **A,B** Single still images of a tail from (**A**) control and (**B**) *trp-4* mutant animals and the quantification
1037 of curvature and spontaneous calcium activity displayed as normalized GCaMP6s/mKate ratio on
1038 the left. Scale bar = 50 μ m. Images and traces representative for 12 and 11 animals, respectively.
1039 **C** Calcium activity in control PLM touch receptor neuron (without ectopic TRP-4 expression). i)
1040 Representative images of the calcium-sensitive GCaMP6s expressing in PLM cell body under ventral,
1041 neutral and dorsal body bends. False colored Vik palette. ii) Curvature and ratiometric calcium signal
1042 plotted against experimental time, showing little to no modulation of calcium transients under modest
1043 curvatures. iii) Quantification of the average GCaMP6s/tagRFpT ratio as a function of the phase angle
1044 of the dorso-ventral body curvature.

1045 **Supplementary Fig. S5. DVA is under compression during ventral bends**

1046 **A-C** Normalized length change in DVA vs body curvature in (**A**) wildtype, (**B**) *unc-70(e524)* and (**C**)
1047 DVA::unc-70(0) animals. Black line indicates the running average of the individual datapoints shown
1048 in colored circles with the slope corresponding to the compliance of the neuron. Representative
1049 morphologies corresponding to DVA under compressive and tensile body curvatures are depicted in
1050 the inset epifluorescence micrograph of a DVA::mKate expressing animal.

1051 **Supplementary Fig. S6. β -spectrin organization and mechanics**

1052 **A** Maximum intensity projection of high resolution confocal images of N-terminal β -spectrin fusion
1053 under the control of the endogenous 2kB *unc-70* promoter used for TSMoD expression (for details
1054 about construction, see Ref. [19]), showing predominant expression in neurons and faint expression
1055 in muscles. Scalebar = 20 μ m. **B** Posterior image of the same animals as in (**A**). **C** Representative
1056 ROIs of different neurons (of untracked identity) expressing the tension sensor module embedded into
1057 wildtype and E2008K mutant β -spectrin compared to the N-terminal no force control. **D** Swarm plot
1058 of the average FRET efficiency per neuronal ROI analysed for the three transgenes. The Cummings
1059 plot on the right indicates the bootstrapped distribution of the Cohen's *d* as calculated from the mean
1060 difference taken from 5000 trials divided by the combined standard deviation comparing control
1061 vs E2008K and control vs N-term. The vertical black bar indicates the 95% confidence interval.
1062 **E,F** FRET measurement in a transgenic line expressing a constitutive high FRET construct (mTFP-

1063 5aa-mVenus) embedded between repeats 8 and 9. **G,H** FRET measurement in a transgenic line
1064 expressing a constitutive low FRET construct (mTFP-TRAF-mVenus) embedded between repeats 8
1065 and 9. **I,J** Representative STED images and autocorrelation of **(I)** SPC-1::GFP expressing neurons
1066 and *unc-70(e524)* mutant animals expressing SPC-1::GFP [29]. Scalebar=1 μ m

1067 **Supplementary Fig. S7 Dynamic tether force spectroscopy of isolated proprioceptor neurons**

1068 **A** Schematic of the set-up combining spinning-disk confocal microscopy and optical trapping.
1069 (ILE, integrated laser engine; BI, Borealis Illuminator; D, dichroic mirror; F, filter; IR-F, IR filter; CL,
1070 optical tweezers collecting lens; PSD, position-sensing detector; TL, transmitted light source; L, lens;
1071 AOD, acousto-optic deflector; LS, trapping laser source; AUX, eyepiece camera). **B** Membrane
1072 tension ($\Delta T = T_{\text{Peak}} - T_{\text{base}}$) gradient measured for each extrusion event as a function of velocity.
1073 Tension was derived from the difference between the peak force and the plateau force of the tether
1074 extrusion experiments according to $\frac{F_{\text{Peak}}^2 - F_{\text{base}}^2}{8\pi l^2 \kappa}$ with $\kappa = 2.7e - 19\text{Nm}$ as the bending rigidity of the
1075 axonal membrane [36]. **C** Representative displacement, force and bleach-corrected calcium trace
1076 for the tether-free no-force control. **D** GCaMP variation versus tension gradient bubble plot for
1077 the tether-free negative control. N=108 events on n=36 cells. **E** Schematic of how force tilts the
1078 hypothetical 1-D energy landscape, with the location of the transition state γ separating the closed
1079 and open conformation of the mechanosensitive ion channel. **F** Simulation of a cation (purple) and
1080 K⁺ selective (orange), mechanosensitive ion channel that solely respond to the force onset and
1081 close with different kinetics ($k_{\text{close}}^K > k_{\text{close}}^{Ca}$). The green trace resembles the Calcium dynamics under
1082 the assumption of an unchanged input resistance and single channel conductance. **G** Simulation of
1083 a constitutively active cation selective ion channel (purple) and a mechanosensitive K⁺ ion channel
1084 (orange). The forced activity of the K⁺ channel modulates the observable (combined open probability,
1085 green trace).

1086 **Supplementary Fig. S8. Suppression of DVA activity through TWK-16 modulated locomotion**
1087 **behavior**

1088 **A-C** 3D density estimate for joint probability distribution of the two forward and turning modes in
1089 the eigenworm space for **(A)** control and **(B)** *twk-16(mir31)* mutant animals and **(C)** the statistically
1090 significant differences in the local density functions ρ_{ctrl} and $\rho_{\text{twk-16}}$. Blue voxels indicate higher local
1091 density for ctrl, beige voxels indicate higher density for *twk-16* on the $\alpha=0.01$ level. **D** Micrograph of a
1092 DVA neuron expressing TWK-16::AID::wScarlet, representative for >20 animals. **E-G** Corresponding
1093 3D density functions for TWK-16::AID control animals in **(E)** absence and **(F)** presence of auxin, and
1094 **(G)** the statistically significant differences in the local density functions on the $\alpha=0.01$ level. Note,
1095 the discontinuity in the distribution of the p-values, indicates high similarity of the 3D probability
1096 functions. **H,I** 3D density functions for TWK-16::AID::wScarlet animals **(H)** WITHOUT TIR expression
1097 and **(I)** WITH DVA restricted TIR expression. Both distribution were recorded in presence of auxin. **J**
1098 Statistically significant differences between the local density functions ρ_H and ρ_I displayed in panel
1099 **(H)** and **(I)**.

Strain	genotype	condition	Fig.	Neuron	worms	frames
N2			1		41	75985
CB524	unc-70(e524)		1		20	20000
MSB187	unc-70 (mir6mir16) V; tmls1070	CRElox	S2	a-type MN	21	35933
MSB535	mirls37; unc-70 (mir6mir16) V;	CRElox	S2	b-type MN	20	44077
MSB239	tmls1087; unc-70 (mir6mir16) V	CRElox	2	BWM	19	38600
MSB186	unc-70 (mir6mir16) V;tmls1072	CRElox	S2	D-type MN	19	25702
MSB160	tmls777; unc-70 (mir6mir16) V	CRElox	2	PAN	20	20078
MSB536	mirls42; unc-70 (mir6mir16)	CRElox	S2	PVD	20	42268
MSB450	heSi208; hrtSi27 V;ll; unc-70(mir6mir16)V	CRElox	S2	PVD	11	19941
MSB295	mirEx98; unc-70 (mir6mir16) V	CRElox	S2	SMD	19	31331
MSB424	heSi317; hrtSi99; unc-70(mir6mir16)	CRElox	S2	TRN	10	21125
MSB188	mirEx13; unc-70(mir6mir16)	CRElox	2	DVA	38	62307
MSB539	mirls43;unc-70(mir6mir16)	CRElox	2	DVA	17	34406
MSB225	trp-4(sy695);unc-70(e524)		4		20	6694
MSB250	trp-4(sy695);unc-70(DVA)		4		6	11049
TQ296	trp-4(sy695)		4		23	28577
MSB115	unc-70(mir6mir16)	no CRE	2		10	35646
GN717	trp-4(ok1605)		ns		21	39086
MSB516	mirls43; he317	no floxP	ns	DVA	17	37668
MSB66	mirEx13	no floxP	S2	DVA	5	11147
FX14125	tmls777	no floxP	S2	PAN	12	25008
MSB340	mirEx98	no floxP	S2	SMD	17	23700
MSB464	mirEx194; spc-1::degron::mKate2	TIR+AID+au	S3		15	17840
MSB464	mirEx194; spc-1::degron::mKate2	ctrl	S3		15	16872
UN1823	spc-1::degron::mKate2	AID + auxin	S3		15	20574
UN1823	spc-1::degron::mKate2	AID ctrl	S3		15	20666
MSB503	mirEx197	TIR ctls	S3		15	22151
MSB503	mirEx197	TIR + auxin	S3		15	21781
MSB555	twk-16(syb2541)	AID ctrl	S8		10	21323
MSB555	twk-16(syb2541)	AID + auxin	S8		16	34971
MSB521	twk-16(mir31); mirls19; syls423		6, S8		20	40856
MSB526	mirEx197; syb2541	ctrl	6,S8		10	20154
MSB526	mirEx197; syb2541	auxin	6, S8		19	36135

Table S1. Locomotion data
Strains used for the data acquisition of animal locomotion behavior

Strain	Promotor	Ref.	Neuron	# Animal	# cells/ Animal	# expected cells	% Animal	comments
MSB205	<i>rgef-1p</i>	[25]	PanNeuro	20	many	300	100	individual cells cannot be counted reliably
MSB211	<i>unc-4p</i>	[25]	A-type	10	10-25	12+9	100	visible in the ventral nerve chord
MSB495	<i>acr-5p</i>	this study	B-type	24	18-22	11+7	100	visible in the ventral nerve chord, recombination also visible in a few cells in the tail.
MSB213	<i>unc-25p</i>	[25]	D-type	10	16-19	13+6	100	visible in the ventral nerve chord
MSB214	<i>myo-3p</i>	[25]	BWM	20	all?	95	100	individual cells cannot be counted reliably
MSB282	<i>flp-22Δ4p</i>	this study	SMD	20	4-8	2+2	100	In the majority of the animals the recombination is happening in the head neurons, SMD with a few other false positive in the other head neurons and a few animals with a false positive with 1-2 tail neurons.
STR335	<i>des-2p</i>	[109]	PVD	10	6	2+2	100	additional recombination observed in FLP; m4; tail neurons
STR655	<i>mec-17p</i>	[109]	TRN	10	6-8	6	100	false positive presumably due to transient expression of CRE in PVD in the <i>mec-17</i> promotor
MSB210	<i>nlp-12p</i>	this study	DVA	10	1-2	1	100	
MSB500	<i>F49H12.4p</i>	this study	PVD	28	3	4	100	possible recombination in AQR, but cannot be seen in these animals because of the <i>myo-2::mCherry</i> markers

Table S2. CRE activity reporter

Strains and their properties used as recombination reporter to study the efficiency and specificity of the cell-type specific CRE recombination.

Table S3. Strains used in this study

Strains	Genotype	Source	Used in Fig.
N2 Bristol	N2	CGC (*)	Fig1
CB524	unc-70[e524] V	CGC (*)	Fig1
MSB115	unc-70 [mir6(loxP)mir16(loxP)]V	This study	Fig2
FX14215	tmls777[rgef-1p::CRE; unc-119::VENUS] (?)	Mitani lab, [25]	Fig. S2
FX16634	tmls1087 [myo-3p::CRE; Pgcy-10::DsRed] (?)	Mitani lab, [25]	Marker
FX16658	tmls1072[unc-25p::CRE; myo-2::dsRED] (?)	Mitani lab, [25]	Marker
MSB510	mirIs37[acr-5p::CRE; myo2p:mCherry] (?)	This study	Marker
FX16655	tmls1068[unc-4p::CRE; lin-44:dsRED] (?)	Mitani lab, [25]	Marker
MSB340	mirEx96 [flp-22p::CRE; unc-122::mCherry]	This study	Fig.S2
MSB513	mirIs42[F49H12.4p::CRE; myo2p:mCherry] (?)	This study	Marker
MSB66	mirEx13[nlp-12p:CRE; myo-2p:mCherry]	This study	Fig.S2
MSB160	tmls777[rgef-1p::CRE; unc-119::VENUS]; unc-70 [mir6(loxP)mir16(loxP)]V	This study	Fig 2
MSB239	tmls1087 [myo-3p::CRE; Pgcy-10::DsRed]; unc-70 [mir6(loxP)mir16(loxP)]V	This Study	Fig 2
MSB186	tmls1072[unc-25p::CRE; myo-2::dsRED]; unc-70 [mir6(loxP)mir16(loxP)]V	This study	Fig S2
MSB535	mirIs37[acr-5p::CRE; myo-2p:mCherry]; unc-70 [mir6(loxP)mir16(loxP)]V	This study	Fig. S2
MSB187	tmls1070[unc-4p::CRE; lin-44::dsRED]; unc-70 [mir6(loxP)mir16(loxP)]V	This study	Fig S2
MSB424	hrtSi99[mec-17p::CRE]; heSi317[[Peft-3p::Lox2272-BFP-Lox2272::mCherry]; unc-70 [mir6(loxP)mir16(loxP)]V	This study	Fig S2
MSB295	mirEx96[flp-22p::CRE; unc-122::mCherry]; unc-70 [mir6(loxP)mir16(loxP)]V	This study	Fig S2
STR335	heSi208[Peft-3::LoxP::egl-13NLS::tagBFP2::tbb-2UTR::LoxP::egl-13NLS::mCherry::tbb-2UTR LGV]; hrtSi27[Pdes-2::CRE LGII]	M. Harterink [109]	Fig S2
MSB336	mirIs42[F49H12.4p::CRE; myo-2p:mCherry]; unc-70 [mir6(loxP)mir16(loxP)]V	This study	Fig 23
MSB188	mirEx13[nlp-12p:CRE; myo-2p:mCherry]; unc-70 [mir6(loxP)mir16(loxP)]V	This study	Fig. 2
EG7944	oxTi553 [eft-3p::tdTomato::H2B::unc-54 3'UTR + Cbr-unc-119(+)]	[82]	Marker
SV2049	heSi317[eft-3p::Lox2272-BFP-Lox2272::mCherry]	vdHeuvel lab, [24]	Fig S21
MSB205	tmls777[rgef-1p::CRE; unc-119::VENUS] ; heSi317[eft-3p::Lox2272-BFP-Lox2272::mCherry]	This study	FigS1
MSB214	tmls1087 [myo-3p::CRE; gcy-10p::DsRed]; heSi317[eft-3p::Lox2272-BFP-Lox2272::mCherry]	This study	FigS1
MSB213	tmls1072[unc-25p::CRE; myo-2p::dsRED]; heSi317[eft-3p::Lox2272-BFP-Lox2272::mCherry]	This study	FigS1
MSB495	mirIs37[acr-5p::CRE; myo2p:mCherry]; heSi317[eft-3::Lox2272-BFP-Lox2272::mCherry]	This study	FigS1
MSB211	tmls1068[unc-4p::CRE; lin-44p:dsRED] ; heSi317[eft-3p::Lox2272-BFP-Lox2272::mCherry]	This study	FigS1
STR655	hrtSi99[mec-17p::Cre]; heSi317[[Peft-3::Lox2272-BFP-Lox2272::mCherry]	M Harterink, [109]	FigS1
MSB282	mirEx91[flp-22p::CRE; unc-122::mCherry]; heSi317[eft-3::Lox2272-BFP-Lox2272::mCherry]	This study	FigS1

Table S3. Strains used in this study

Strains	Genotype	Source	Used in Fig.
MSB500	mirIs42[F49H12.4p::CRE; myo2p:mCherry]; heSi317[eff-3::Lox2272-BFP-Lox2272::mCherry]	This study	FigS1
MSB210	mirEx72[nlp-12p::CRE; myo-2p:mCherry] ; heSi317[eff-3p::Lox2272-BFP-Lox2272::mCherry]	This study	FigS1
TQ296	trp-4[sy695] I	CGC(*) [4]	Fig. 4
MSB225	trp-4[sy695] I; unc-70[e524] V	This study	Fig 4
MSB250	trp-4[sy695] I; unc-70 (mir6mir16) V; mirEx13	This study	Fig 4
MSB273	mirIs19[nlp-12p::GAL-4; unc-122p::mCherry]; syls423 [15xUAS::Δpes-10::GCaMP6s::SL2::mKate2::let-858 3'UTR] ; [myo-2p::NLS::mCherry + 1kb DNA ladder(NEB)].	This study	Fig2,5,S4
MSB306	mirIs19[nlp-12p::GAL-4; unc-122p::mCherry]; syls423 [15xUAS::Δpes-10::GCaMP6s::SL2::mKate2::let-858 3'UTR] ; myo-2p::NLS::mCherry + 1kb DNA ladder(NEB); unc-70[e524] V	This study	Fig2
MSB328	mirEx13[nlp-12p:CRE; myo-2p:mCherry] + mirIs19[nlp-12p::GAL-4; unc-122p::mCherry]; syls423 [15xUAS::Δpes-10::GCaMP6s::SL2::mKate2::let-858 3'UTR] ; [myo-2p::NLS::mCherry + 1kb DNA ladder(NEB)]; unc-70 [mir6(loxP)mir16(loxP)]V	This study	Fig2
MSB387	mirIs19[nlp-12p::GAL-4; unc-122p::mCherry]; syls423 [15xUAS::Δpes-10::GCaMP6s::SL2::mKate2::let-858 3'UTR] ; [myo-2p::NLS::mCherry + 1kb DNA ladder(NEB)] ; trp-4[sy695] I	This study	Fig4,5,S4
GN692	ljSi123[mec-7p:GCaMP6s::SL2::tagRFP];lite-1(ce314)	Goodman lab, [84]	Fig.S4
MSB382	ljSi123[mec-7p:GCaMP6s::SL2::tagRFP];lite-1[ce314] + mirEx144[mec-4p::TRP-4(full length)]; [myo-2p::mCherry]	This study	Fig 4
MSB379	ljSi123[mec-7p:GCaMP6s::SL2::tagRFP];lite-1[ce314] + mirEX141[mec-4p::Δank:TRP-4]; ; [myo-2p::mCherry]	This study	Fig. 4
GN716	trp-4(ok1605)	Goodman lab, [112]	
GN517	pgEx116 [unc-70p::UNC-70(1-1166)::TsMod::UNC-70(1167-2267); Pmyo-3::mCherry]	Goodman lab, [19]	Fig3
GN519	pgEx131 [UNC-70(1-1166)::mTFP-5aa-Venus::UNC-70(1167-2267) unc-122p::RFP]	Goodman lab, [19]	Fig. S6
GN600	pgIs22; oxIs95[pdi-2::unc-70(fl), myo-2::GFP, lin-15] IV	Goodman lab, [45]	Fig 3
MSB233	mirEx77 [unc-70p::UNC-70(1-1166)::TsMod::UNC-70(1167-2267)_E2008K; myo-2p:mCherry]	This study	Fig S6
MSB366			
MSB339	mirIs23 [unc-70p::UNC-70(1-1166)::mTFP-TRAF-Venus::UNC-70(1167-2267); unc-70(s1502); oxIs95 pdi-2::unc-70(fl), myo-2::GFP, lin-15(+)]	This study	Fig 3
PHX2541	syb2541[wrmScarlet::DEGRON::twk-16]	Sunny biotech	
MSB555	syb2541[wrmScarlet::DEGRON::twk-16], outcrossed 2x	This study	Fig S8
MSB526	mirEx197[nlp-12p::TIR,unc-122p::GFP]; syb2541[wrm-Scarlet::DEGRON::twk-16]	This study	Fig 6,S8
UN1823	spc-1::degron::mKate2	Cram lab, [27]	FigS3
MSB453	spc-1::degron::mKate2;mirIs34(mec4p::at-TIR1::F2A::mCherry::H2B + Punc -122::GFP)	This study	FigS3
MSB464	mirEx194[nlp-12p::TIR::P2A::mCherry, unc-122p::GFP]; spc-1::degron::mKate2	This study	FigS3

Table S3. Strains used in this study

Strains	Genotype	Source	Used in Fig.
MSB503	mirEx194[nlp-12p::TIR::P2A::mCherry, unc-122p::GFP]	This study	FigS3
MSB521	twk-16(mir31); mirIs19[nlp-12p::GAL-4; unc-122p::mCherry]; sYIs423 [15xUAS:: Δ pes-10::GCaMP6s::SL2::mKate2::let-858 3'UTR + myo-2p::NLS::mCherry + 1kb DNA ladder(NEB)]	This study	Fig. 6,S8
MSB591	trp-4 (mir35mir36[GFP::TRP-4]) I	This study	Fig.4
MSB539	mirIs43[nlp-12p:CRE; unc-122p:GFP] ; unc-70 (mir6mir16) V	This study	Fig. 2
MSB516	mirIs43[nlp-12p:CRE; unc-122p:GFP] ; he317[eff-3::Lox2272-BFP-Lox2272::mCherry]	This study	
MSB601	trp-4(mir35mir36) I; unc-70(e524) V;	This study	Fig. 3