Supplementary Materials for

An asymmetric mechanical code ciphers curvature-dependent proprioceptor activity

Ravi Das, Li-Chun Lin, Frederic Català-Castro, Nawaphat Malaiwong Neus Sanfeliu-Cerdán, Montserrat Porta-de-la-Riva, Aleksandra Pidde, Michael Krieg*

*Corresponding author. Email: michael.krieg@icfo.eu

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Videos S1 to S10
1 Supplementary Methods

1.1 Monte Carlo simulation of force-gated ion channel ensembles and assumptions

To capture the dynamics and the statistical behavior resulting from the stochastic activation of an ensemble of mechanosensitive ion channels, subjected to a mechanical force, we set up a continuous time Markov chain Monte Carlo simulation [88]. We choose to model a pair of mechanosensitive ion channels, which we conceptualize as an excitatory, sodium or calcium conductive in channel and an inhibitory, potassium or chloride conductive ion channel. Our model is agnostic of the force transmission pathway and does not differentiate between membrane and cytoskeletal force delivery. To simulate the behavior in absence of external noise, we assumed that each channel acts independent, activities are uncoupled, and each channel is characterized by an open and a closed state that is separated by a potential barrier with height $\Delta G$ (Fig. S7E). The lifetime of each state dependents on the height of the energy barrier separating the closed from the open states and the loading conditions. Opening is driven by thermal fluctuations, and, as a result, is a stochastic process. Application of force to the channel tilts the energy landscape, thus reducing the energy barrier that separates the closed from the open state by an amount $F \cdot \gamma$, in which $\gamma$ is the distance to the transition state [42]. If a load is applied to the channel for durations that are much shorter than the intrinsic lifetime of the closed state, the channel resists opening. Importantly, channels do not confer resistance to force on timescales that are larger than the intrinsic lifetime of the particular closed state [89]. In agreement with previous data on whole cell recordings from TRP-4 [43], we assumed that the excitatory channel activates at the onset and the offset of the force. Such behavior is consistent with a strain-rate sensitivity of common mechanoreceptors [10], thus, we model the channel sensitive to the first derivative of the force, $\frac{\partial F}{\partial t}$. The forward transition rate was model using the modified Evans-Bell model of time-dependent bond-strength, as determined by the force-rate or loading rate $r_f$ [89]. Loading rate was calculated from the stiffness of the ankyrin domain [90] multiplied by the pulling velocity in the experiment. We start the simulation with all states closed, and are interested in the evolution of the ensemble to the open state.

\[
[C] \xrightarrow{k_o} [O]
\]

The lifetime of the closed state is governed by the spontaneous opening constant $k^0_o$ according to

\[
p(t) = \exp \left( -k^0_o \cdot t \right)
\]  

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

\[
p(t) = \exp \left( -k^0_C \cdot t \right)
\]
Force sensitivity is achieved by applying Bell’s model to the forward rate constant. We likewise assume that the channel cannot sustain the open state as long as force is acting. This assumption has the physical manifestation in a force-transmission pathway through a weak protein-ligand interaction (slip bond). After time $t$, we apply a force to the channels. Thus, the probability of a closed channel responding to the external forces changes to

$$p(t, F) = p_0 \cdot \exp \left( -k_o(F) \cdot t \right)$$

in which

$$k_o(F) = k^0_o \cdot \exp \left( -\frac{F\gamma}{k_BT} \right)$$

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

$$k_o(F) = \frac{r_f}{\exp \left( \frac{F}{f_0} \right) \cdot f_\beta}$$

with $\frac{\gamma}{k_BT}$ as the force scale.

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

Inhibitory Channel: $\gamma=2.93$, $k^0_o=120$, $k^0_C=600$, $k^F_C=700$

Excitatory channel: $\gamma=2.10$, $k^0_o=100$, $k^0_C=300$, $k^F_C=100$

The physical representation of the values $k^0_o$, $k^0_C$ correspond to the spontaneous opening constants. For $k^0_o > k^0_C$, ion channel remain statistically open, otherwise they spent more time in the closed state on average. Without a lack of generality, the concept can be applied for lipid bilayer tension-gated ion channels in which the free energy profile of the energy landscape is altered by an external tension $\sigma$ according to $\Delta \Delta G = -\Delta G - \sigma \cdot \Delta A$, in which $\Delta A$ equals to the increase in cross sectional area of the gated ion channel, e.g. $\Delta A=4.7nm^2$ for TREK2 [18]. Thus, the tension dependent $k_o$ conforms to

$$k_o(\sigma) = k^0_o \cdot \exp \left( -\frac{\sigma \cdot A}{k_BT} \right)$$

It can be readily seen that without an increase in cross-sectional area, the open state is not preferred. Finally, the average current was calculated by $I = cNP_o$, where $c$ is the single-channel current taken from the literature (TRP-4, 18pA; 140pS [91]; K2P, 13pA; 90pS [92]), $N$ the number of channels, and $P_o$ the average probability of finding the channel open derived from the simulations. The K current was then subtracted from the Ca signal. Under assumption of a high input resistance typical for C. elegans neurons [66], the simulated current is representative for a macroscopic ‘observable’, related to a Calcium signal. The picture that is emerging from this simulation is that ventral DVA activity emerges in part from TRP-4 activation under compression and the suppression of ‘stretch’ currents under dorsal side. Whereas this describes a plausible explanation for our findings, two other possible scenarios could give rise to the observed ventral activity in vivo: 1) TWK-16 and TRP-4 both activate under tension, and close with different rates such that a remaining Ca$^{2+}$ activity is visible during ventral bouts (Fig. S7F); and 2) TRP-4 is constitutively active and only modulated by TWK-16 leading to Ca$^{2+}$ suppression during tension (Fig. S7G). The combined results from our in-vitro (Fig. 6), in vivo (Fig. 7) experiments on twk-16 mutations and the in-silico (Fig. S7E-G) experiments favor a scenario in which mechanosensitive TWK-16 activity suppresses stretch-induced depolarization. Because TRP-4 is a pore-forming sub-unit of a mechanosensitive ion channel that activates at the force onset and offset [43] we should expect DVA activity during dorsal...
AND ventral bends, but we exclusively recorded Ca$^{2+}$ increases during force relaxation/offset in vitro and compressed axons during ventral bends in vivo. In absence of TWK-16, however, the biphasic TRP-4 activity is unveiled.

## 2 Supplementary Videos

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

**Video S2: Crawling behavior of conditional unc-70 alleles.** Representative video of conditional CRE/loxP mutant strains shown in Fig. 2 and S2. Scale bar = 300µm, acquired at 25Hz. unc-70(floxed) is the control animal without CRE expression denoting the background for all other genotypes. Pan-neuronal, rgef-4p::CRE; BWM, body wall muscle restricted myo-3p::CRE; D-type MN, GABAergic motorneuron directed unc-25p::CRE; B-type MN, cholinergic forward motorneurons directed acr-5p::CRE; A-type MN, cholinergic backwards motorneurons directed unc-4p::CRE; TRN, touch receptor neuron specific mec-17p::CRE; SMD, SMD-directing flp-22Δ4p::CRE; PVD, PVD-directing F49H12.4p::CRE; DVA, DVA-specific nlp-12p::CRE in the unc-70(floxed) background. DVA::UNC-70 is DVA specific rescue with unc-70 (cDNA).

**Video S3A-C: DVA Calcium activity depends on UNC-70** Representative video of DVA calcium activity in (A) wildtype, (B) DVA-specific CRE/loxP mutant strains and (C) DVA-specific CRE/loxP mutant expressing an nlp-12::unc-70 rescue construct in DVA. Upper panel shows the calcium sensitive GCaMP6s, lower panel a calcium-insensitive mKate. Playback speed, 23 frames/s.

**Video S4: Calcium imaging of DVA under imposed bending in a microfluidic device** Calcium imaging sequence of an animal being pushed tail first through a channel, creating a single ventral (upper panel) and dorsal (lower panel) curvature close to the tail.

**Video S5: UNC-70 stabilizes DVA axons against mechanical compression** Live imaging of DVA axons in semi-restraint animals in 1.5% agar pads undergoing dorso-ventral body swings. Wildtype left, unc-70(e524) middle and DVA-specific CRE/loxP mutant (right). Scale bar = 200µm.

**Video S6: Locomotion behavior of trp-4(sy695) mutant** Representative video of a phenotypic trp-4(sy695) mutant animal (TQ296, left) and trp-4(sy695) animals expressing a TRP-4 rescue construct in DVA (MSB757, right).
**Video S7: DVA responds to substrate deformation**  False color labeling of a GCaMP6s expressing DVA neuron cultured on PDMS, subjected to a mechanical deformation. Yellow shows the calcium sensitive GCaMP6s, magenta a calcium-insensitive mKate as a movement and defocussing control. Scale bar = 5µm. Acquired at 10Hz.

**Video S8: Calcium activity in DVA during dynamic membrane tether extrusion**  Representative video of DVA neuron in the dynamic optical trapping assay. Scale bar = 5µm. Acquired at 10Hz.

**Video S9: Locomotion behavior of conditional twk-16 mutant animals**  Representative video of a TWK-16::AID animal in presence of 1mM auxin; left animal without (MSB555) and right with (MSB526) DVA::TIR expression. Scale bar = 300µm. Acquired at 25Hz.

**Video S10: Compression induced proprioceptor current coordinates locomotion behavior**  Left: Animation derived from the results of the neuromechanical model for input parameters giving rise to wildtype-like animal locomotion pattern implementing DVA as a compression sensitive proprioceptor. Right: Same model with lower sensitivity to curvature induced compression current in DVA, representing *trp-4* and *unc-70* mutations.
Figure S1

A

loxP
mCherry
rps-18p
3'

B

CRE
Promoter

I

DVA

J

rgef
p

myo-3p

Des-2p

F49H12.4p

K

TRNs

L

PVD (FLP)

unc-4p
acr-5p
unc-25p
A,B,D-type

unc-4p
acr-5p
unc-25p

DVA

rgef
p

myo-3p

Des-2p

F49H12.4p

PVD (FLP)
3 Supplementary Figures

Supplementary Fig. S1. Reporting CRE recombination efficiency

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

A  unc-70(loxP)

B  rgef-1p:CRE  forward 1

C  nip-12p:CRE  forward 1

D  flp-22Δ4p:CRE  forward 1

E  B-type

F  A-type

G  D-type

H  TRN  forward 1

I  PVD  forward 1

J  SMD  forward 1

K  CRE only

L  CRE+unc-70(loxP)

| Condition | unip-12p | rgef-1p | flp-22Δ4p | nip-12p:CRE | rgef-1p:CRE | flp-22Δ4p:CRE |
|-----------|----------|---------|------------|-------------|-------------|--------------|
| vs N2     |          |         |            |             |             |              |

| Condition | D-MN | A-MN | B-MN | TRN | SMD | PVD | PVD |
|-----------|------|------|------|-----|-----|-----|-----|
| vs unc-70(loxP) |      |      |      |     |     |     |     |
Supplementary Fig. S2. DVA-specific mutation of the spectrin network causes aberrant body postures

A-D Still image and the corresponding 3D eigenworm orbit for (A) unc-70(loxP), (B) pan-neuronal CRE, (C) nlp-12p::CRE and (D) flp-22Δ4p::CRE expressing animals. Only CRE drivers are shown that showed a phenotype in combination with the unc-70(loxP) allele. E-J Representative still image and the corresponding 3D eigenworm orbit for conditional knockdown of unc-70 after CRE expression with specific promotors for (E) B-type, (F) A-type, (G) D-type motorneurons, (H) TRNs, (I) SMD, and (J) PVD using the promoters indicated in Table S2 and panel (L). K,L Distribution of $p$-values after 1000 independent tests between bootstrapped distribution for the combinations of genotypes indicated in the figure. Orange line indicates $\alpha=0.05$ level of significance, black diamond represents the mean and horizontal line the median $p$-value of the distribution.
Figure S3

**SPC-1::AID::mKate**

- **A**: SPC-1::AID::mKate
- **B**: mec-4p::TIR; SPC-1::AID
- **C**: mec-4p::TIR; SPC-1::AID + 1mM auxin

**nlp-12p::TIR**

- **I**: nlp-12p::TIR
- **J**: nlp-12p::TIR
- **K**: nlp-12p::TIR + SPC-1::AID::mKate

**Data Analysis**

- **E**: SPC-1::AID::mKate
- **F**: SPC-1::AID::mKate
- **G**: SPC-1::AID::mKate
- **H**: SPC-1::AID::mKate

- **I**: nlp-12p::TIR
- **J**: nlp-12p::TIR
- **K**: nlp-12p::TIR + SPC-1::AID::mKate
- **L**: nlp-12p::TIR + SPC-1::AID::mKate

**Log(p-value)**

- **H**: Log(p-value) for SPC-1::AID::mKate
- **L**: Log(p-value) for nlp-12p::TIR + SPC-1::AID::mKate

**Scale Bars**

- **E**: Scale bar for auxin
- **I**: Scale bar for auxin
- **M**: Scale bar for auxin
Supplementary Fig. S3. SPC-1 shares function during locomotion in DVA with UNC-70

A-C Representative images of an (A) UN1823 (SPC-1::AID::mKate) expressing control animal (without TIR ligase) and (B) MSB453 (mec-4p::TIR->SPC-1::AID::mKate) with nuclear mCherry localization indicating TIR expression in TRNs in absence and (C) presence of 1mM auxin. Due to the overlap of DVA axons with other neurites in the ventral nerve chord, we choose to estimate the effect in TRNs. D Quantification of the neurite intensity of TRNs without TIR (ctrl, N=17 animals) and with TIR ligase in absence (N=19) and presence of auxin (N=20) in the SPC-1::AID::mKate background, normalized by the intensity of motorneuron commissure (that do not express the TIR ligase). E-G Representative snapshot of a (E) SPC-1::AID::mKate animal without TIR ligase and the corresponding quantification of its behavior in (F) absence and (G) presence of auxin. H Distribution of p-values as described above for the combinations indicated in the figure. I-K Representative snapshot of a (I) MSB503 animal expressing TIR exclusively in DVA (nlp-12p::TIR::F2A::H2B-mKate) and the corresponding quantification of its behavior in (J) absence and (K) presence of auxin. L Distribution of p-values for the combinations indicated in the figure M-O Representative snapshot of a (M) MSB464 animal expressing TIR exclusively in DVA together with the SPC-1::AID::mKate degron and the corresponding quantification of its behavior in (N) absence and (O) presence of auxin. P Distribution of p-values for the combinations indicated in the figure. Note, due to the auxin-independent TIR activity, the addition of 1 mM auxin does not further increase the auxin-independent loss of coordination.
Figure S4

A

DVA

control

Curvature
Calcium

R/R

Time (s)

B

unc-70(e524)

i)

ii)

iii)

Time (s)

C

trp-4(sy695)

N=17

D

TRN

i)

control

ii)

Time (s)

unc-70(e524)

N=12

trp-4(sy695)

N=17
Supplementary Fig. S4. Cell autonomous calcium activity in immobilized animals and TRNs

A Single still images of a tail from control animal and the quantification of curvature and spontaneous calcium activity displayed as normalized GCaMP6s/mKate ratio on the left. Scale bar = 50 \mu m. Images and traces representative for 12 animals, respectively. Green=Ca\textsuperscript{2+} activity; black=curvature  

B Calcium activity in DVA of unc-70(e524) mutant animal. i) Representative images of the calcium-sensitive GCaMP6s expressing in DVA cell body under ventral, neutral and dorsal body bends. False colored Vik palette. ii) Curvature and ratiometric calcium signal plotted against experimental time. Green=Ca\textsuperscript{2+} activity; black=curvature. iii) Quantification of the average GCaMP6s/mKate ratio as a function of the phase angle of the dorso-ventral body curvature.  

C Single still images of a tail from trp-4 mutant animal and the quantification of curvature and spontaneous calcium activity displayed as normalized GCaMP6s/mKate ratio on the left. Scale bar = 50 \mu m. Images and traces representative for 11 animals, respectively.  

D Calcium activity in control PLM touch receptor neuron (without ectopic TRP-4 expression). i) Representative images of the calcium-sensitive GCaMP6s expressing in PLM cell body under ventral, neutral and dorsal body bends. False colored Vik palette. ii) Curvature and ratiometric calcium signal plotted against experimental time, showing little to no modulation of calcium transients under modest curvatures. Green=Ca activity; black=curvature. iii) Quantification of the average GCaMP6s/tagRFPt ratio as a function of the phase angle of the dorso-ventral body curvature.
Figure S5
Supplementary Fig. S5. DVA is under compression during ventral bends

A-C Normalized length change in DVA vs body curvature in (A) wildtype, (B) unc-70(e524) and (C) DVA::unc-70(0) animals. Black line indicates the running average of the individual datapoints shown in colored circles with the slope corresponding to the compliance of the neuron. Representative morphologies corresponding to DVA under compressive and tensile body curvatures are depicted in the inset epifluorescence micrograph of a DVA::mKate expressing animal.
Supplementary Fig. S6. β-spectrin organization and mechanics

A Maximum intensity projection of high resolution confocal images of N-terminal β-spectrin fusion under the control of the endogenous 2kB unc-70 promoter used for TSMod expression (for details about construction, see Ref. [20]), showing predominant expression in neurons and faint expression in muscles. Scalebar = 20 µm. B Posterior image of the same animals as in (A). C Representative ROIs of different neurons (of untracked identity) expressing the tension sensor module embedded into wildtype and E2008K mutant β-spectrin compared to the N-terminal no force control. D Swarm plot of the average FRET efficiency per neuronal ROI analyzed for the three transgenes. The Cummings plot on the right indicates the bootstrapped distribution of the Cohen’s d as calculated from the mean difference taken from 5000 trials divided by the combined standard deviation comparing control vs E2008K and control vs N-term. The vertical black bar indicates the 95% confidence interval. E,F FRET measurement in a transgenic line expressing a constitutive high FRET construct (mTFP-5aa-mVenus) embedded between repeats 8 and 9. G,H FRET measurement in a transgenic line expressing a constitutive low FRET construct (mTFP-TRAF-mVenus) embedded between repeats 8 and 9. I,J Representative STED images and autocorrelation of (I) SPC-1::GFP expressing neurons and unc-70(e524) mutant animals expressing SPC-1::GFP [34]. Scalebar=1 µm
Supplementary Fig. S7. Dynamic tether force spectroscopy of isolated proprioceptor neurons

**A** Schematic of the set-up combining spinning-disk confocal microscopy and optical trapping. (ILE, integrated laser engine; BI, Borealis Illuminator; D, dichroic mirror; F, filter; IR-F, IR filter; CL, optical tweezers collecting lens; PSD, position-sensing detector; TL, transmitted light source; L, lens; AOD, acousto-optic deflector; LS, trapping laser source; AUX, eyepiece camera). **B** Membrane tension ($\Delta T = T_{\text{peak}} - T_{\text{base}}$) gradient measured for each extrusion event as a function of velocity. Tension was derived from the different between the peak force and the plateau force of the tether extrusion experiments according to $\frac{F_{\text{peak}}^2 - F_{\text{base}}^2}{8\pi\kappa}$ with $\kappa = 2.7\,\text{e}^{-19}\text{Nm}$ as the bending rigidity of the axonal membrane [40]. **C** Representative displacement, force and bleach-corrected calcium trace for the tether-free no-force control. **D** GCaMP variation versus tension gradient bubble plot for the tether-free negative control. N=108 events on n=36 cells. **E** Schematic of how force tilts the hypothetical 1-D energy landscape, with the location of the transition state $\gamma$ separating the closed and open conformation of the mechanosensitive ion channel. **F** Simulation of a cation (purple) and K+ selective (orange), mechananosensitive ion channel that solely respond to the force onset and close with different kinetics ($k_{K\text{close}} > k_{Ca\text{close}}$). The green trace resembles the Calcium dynamics under the assumption of an unchanged input resistance and single channel conductance. **G** Simulation of a constitutively active cation selective ion channel (purple) and a mechanosensitive K+ ion channel (orange). The forced activity of the K+ channel modulates the observable (combined open probability, green trace).
**Figure S8**

**twk-16(mir31)**

- **A** forward 1
- **B** forward 1
- **C** forward 1
- **D** forward 1

**TwK-16::AID:wScrlt ONLY**

- **E** forward 1
- **F** forward 1
- **G** forward 1

**TwK-16::AID:wScrlt + auxin**

- **H** forward 1
- **I** forward 1
- **J** forward 1
Supplementary Fig. S8. Suppression of DVA activity through TWK-16 modulated locomotion behavior

A-C 3D density estimate for joint probability distribution of the two forward and turning modes in the eigenworm space for (A) control and (B) twk-16(mir31) mutant animals and (C) the statistically significant differences in the local density functions $\rho_{\text{ctrl}}$ and $\rho_{\text{twk-16}}$. Blue voxels indicate higher local density for ctrl, beige voxels indicate higher density for twk-16 on the $\alpha=0.01$ level. D Micrograph of a DVA neuron expressing TWK-16::AID::wScarlet, representative for >20 animals. E-G Corresponding 3D density functions for TWK-16::AID control animals in (E) absence and (F) presence of auxin, and (G) the statistically significant differences in the local density functions on the $\alpha=0.01$ level. Note, the discontinuity in the distribution of the $p$-values, indicates high similarity of the 3D probability functions. H,I 3D density functions for TWK-16::AID::wScarlet animals (H) WITHOUT TIR expression and (I) WITH DVA restricted TIR expression. Both distribution were recorded in presence of auxin. J Statistically significant differences between the local density functions $\rho_H$ and $\rho_I$ displayed in panel (H) and (I).
A  Ctrl vs trp-4(sy695)  
B  trp-4(sy695) + DVA:TRP-4 vs trp-4(sy695)  
C  Ctrl vs trp-4(sy695) + DVA:TRP-4  
D  Ctrl vs DVA:unc-70(0)  
E  DVA:unc-70(0) + UNC-70 vs DVA:unc-70(0)  
F  Ctrl vs DVA:unc-70(0) + UNC-70  
G  TRN::AnkTRP-4 vs TRN:TRP-4  
H  Ctrl vs twk-16(mir31)
Supplementary Fig. S9. Statistical summary of the normalized calcium intensity changes during body bending Polar graphs showing the $p$-value as a function of the phase angle of the body bending cycle. The $\alpha=0.05$ level of significance is indicated as an orange dotted line. $p$-values lower than this value indicate that the Ca intensity of the test group is different to the control group. Test combinations are indicated inside the graph.
4 Supplementary Tables
| Strain   | genotype                        | condition         | Fig. | Neuron     | worms | frames  |
|---------|---------------------------------|-------------------|------|------------|-------|---------|
| N2      | unc-70(e524)                    | 1                 | 41   | 1          | 75985 |
| CB524   | unc-70(mir6mir16) V; tmls1070   | CRElox S2         | a-type MN | 21   | 35933 |
| MSB187  | mirls37; unc-70(mir6mir16) V;   | CRElox S2         | b-type MN | 20   | 44077 |
| MSB239  | tmls1087; unc-70(mir6mir16) V   | CRElox S2         | BWM   | 19   | 38600 |
| MSB186  | unc-70(mir6mir16) V; tmls1072   | CRElox S2         | D-type MN | 19   | 25702 |
| MSB160  | tmls777; unc-70(mir6mir16) V    | CRElox S2         | PAN   | 20   | 20078 |
| MSB536  | mirls42; unc-70(mi6mir16)       | CRElox S2         | PVD   | 20   | 42268 |
| MSB450  | heSi208; hrtSi27 V; unc-70(mi6mir16)V | CRElox S2        | PVD   | 11   | 19941 |
| MSB295  | mirEx98; unc-70(mi6mir16) V     | CRElox S2         | SMD   | 19   | 31331 |
| MSB424  | heSi317; hrtSi99; unc-70(mi6mir16) V | CRElox S2       | TRN   | 10   | 21125 |
| MSB188  | mirEx13; unc-70(mi6mir16)       | CRElox ns         | DVA   | 38   | 62307 |
| MSB765  | mirls71; unc-70(mi6mir16)       | CRElox 2          | DVA   | 15   | 17602 |
| 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(mi6mir16)                | no CRE S2         |      | 10   | 35646 |
| GN716   | trp-4(ok1605)                    | ns                |      | 21   | 39086 |
| MSB778  | mirls71                         | CRE only S2       |      | 20   | 32597 |
| MSB66   | mirEx13                         | no loxP ns        |      | 5    | 11447 |
| FX14125 | tmls777                         | no loxP S2        |      | 12   | 25008 |
| MSB340  | mirEx98                         | no loxP S2        |      | 17   | 23700 |
| MSB464  | mirEx194; spc-1::degron::mKate2 | TIR+AID+auxin     | S3   |      | 15    |
| MSB464  | mirEx194; spc-1::degron::mKate2 | ctrl              | S3   |      | 15    |
| UN1823  | spc-1::degron::mKate2           | AID + auxin       | S3   |      | 15    |
| UN1823  | spc-1::degron::mKate2           | AID ctrl          | S3   |      | 15    |
| MSB503  | mirEx197                        | TIR ctrl          | S3   |      | 15    |
| MSB503  | mirEx197                        | TIR + auxin       | S3   |      | 15    |
| MSB555  | twk-16(syb2541)                 | AID ctrl          | S8   |      | 10    |
| MSB555  | twk-16(syb2541)                 | AID + auxin       | S8   |      | 16    |
| MSB526  | twk-16(mir31); mirls19; syls423 | 6, S8             |      | 20   | 40856 |
| MSB526  | mirEx197; syb2541               | ctrl S8           |      | 10   | 20154 |
| MSB526  | mirEx197; syb2541               | auxin S8          |      | 19   | 36135 |
| MSB757  | mirls69; trp-4(sy695)           | TRP-4 rescue      | 4    |      | 16    |
| MSB750  | mirls71; unc-70(mi6mir16) V;    | UNC-70 rescue     | 2    |      | 17    |

**Table S1.** Locomotion data
Strains used for the data acquisition of animal locomotion behavior
| Strain     | Promoter | Ref. | Neuron | # cells/Animal | # expected cells | % Animal | comments |
|------------|----------|------|--------|----------------|------------------|----------|----------|
| MSB205     | rgef-1p  | [27] | PanNeuro | 20 | many | 300 | 100 | individual cells cannot be counted reliably |
| MSB211     | unc-4p   | [27] | A-type | 10 | 10-25 | 12+9 | 100 | visible in the ventral nerve chord |
| MSB495     | acr-5p   | 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     | unc-25p  | [27] | D-type | 10 | 16-19 | 13+6 | 100 | visible in the ventral nerve chord |
| MSB214     | myo-3p   | [27] | BWM    | 20 | all? | 95 | 100 | individual cells cannot be counted reliably |
| MSB282     | flp-22Δ4p | 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     | des-2p   | [93] | PVD    | 10 | 6 | 2+2 | 100 | additional recombination observed in FLP; m4; tail neurons |
| STR655     | mec-17p  | [93] | TRN    | 10 | 6-8 | 6 | 100 | false positive presumably due to transient expression of CRE in PVD in the mec-17 promoter |
| MSB215     | nlp-12p  | this study | DVA    | 10 | 1-2 | 1 | 100 | possible recombination in AQR, but cannot be seen in these animals because of the myo-2::mCherry markers |
| MSB500     | F49H12.4p | this study | PVD    | 28 | 3 | 4 | 100 | |

**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.
| Strains          | Genotype                                                                 | Source                  | Used in Fig. |
|------------------|---------------------------------------------------------------------------|-------------------------|--------------|
| N2 Bristol       | N2                                                                        | CGC (*)                 | Fig 1        |
| CB524            | unc-70[HSLP]mir[6[loxP]]mir[16[loxP]]V                                    | CGC (*)                 | Fig 1        |
| MSB115           | unc-70 [mir[6[loxP]]mir[16[loxP]]V                                       | This study              | Fig 2        |
| FX14215          | tmls777[rgef-1p::CRE; unc-119::VENUS] (? )                                 | Mitani lab, [27]        | Fig. 2       |
| FX16634          | tmls1087 [myo-3p::CRE; Pgcy-10::DsRed] (? )                                | Mitani lab, [27]        | Marker       |
| FX16658          | tmls1072[unc-25p::CRE; myo-2::dsRED] (? )                                  | Mitani lab, [27]        | Marker       |
| MSB510           | mirs37[acr-5p::CRE; myo2p::mCherry] (? )                                  | This study              | Fig S2       |
| FX16655          | tmls1068[unc-4p::CRE; lin-44::dsRED] (? )                                  | Mitani lab, [27]        | Marker       |
| MSB340           | mirEx96[flip-22p::CRE; unc-122::mCherry]                                  | This study              | Fig S2       |
| MSB513           | mirs42[F49H12.4p::CRE; myo2p::mCherry] (? )                               | This study              | Marker       |
| MSB66            | mirEx13[nlp-12p::CRE; myo-2p::mCherry]                                    | This study              | Methods, not shown |
| MSB160           | tmls777[rgef-1p::CRE; [mir[6[loxP]]mir[16[loxP]]V; unc-70] V              | This study              | Fig 2        |
| MSB239           | tmls1087 [myo-3p::CRE; [mir[6[loxP]]mir[16[loxP]]V; Pgcy-10::DsRed] V     | This Study              | Fig 2        |
| MSB186           | tmls1072[unc-25p::CRE; [mir[6[loxP]]mir[16[loxP]]V; myo-2::dsRED] V       | This study              | Fig S2       |
| MSB535           | mirs37[acr-5p::CRE; [mir[6[loxP]]mir[16[loxP]]V; myo-2p::mCherry] V       | This study              | Fig. S2      |
| MSB187           | tmls1070[unc-4p::CRE; [mir[6[loxP]]mir[16[loxP]]V; lin-44::dsRED] V       | This study              | Fig S2       |
| MSB424           | hrtSi99[mec-17p::CRE]; heSi317[Peft-3p::Lox2272-BFP-Lox2272::mCherry; unc-70 [mir[6[loxP]]mir[16[loxP]]V V | This study              | Fig S2       |
| MSB295           | mirEx96[flip-22p::CRE; unc-122::mCherry]; [mir[6[loxP]]mir[16[loxP]]V 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 [93]       | Fig S2       |
| MSB336           | mirs42[F49H12.4p::CRE; myo-2p::mCherry]; [mir[6[loxP]]mir[16[loxP]]V V | This study              | Fig S2       |
| MSB188           | mirEx13[nlp-12p::CRE; myo-2p::mCherry]; [mir[6[loxP]]mir[16[loxP]]V V | This study              | not shown    |
| EG7944           | oxTi553[eff-3p::tdTomato::H2B::unc-54 3’UTR + Cbr-unc-119(+) ]           | vdHeuvel lab, [26]      | Fig S1       |
| SV2049           | heSi317[Peft-3p::Lox2272-BFP-Lox2272::mCherry]                            | vdHeuvel lab, [26]      | Fig S1       |
| MSB205           | tmls777[rgef-1p::CRE; unc-119::VENUS]; heSi317[eff-3p::Lox2272-BFP-Lox2272::mCherry] | This study              | Fig S1       |
| MSB214           | tmls1087 [myo-3p::CRE; gcy-10p::DsRed]; heSi317[eff-3p::Lox2272-BFP-Lox2272::mCherry] | This study              | Fig S1       |
| MSB213           | tmls1072[unc-25p::CRE; myo-2::dsRED]; heSi317[eff-3p::Lox2272-BFP-Lox2272::mCherry] | This study              | Fig S1       |
| MSB495           | mirs37[acr-5p::CRE; myo2p::mCherry]; heSi317[eff-3p::Lox2272-BFP-Lox2272::mCherry] | This study              | Fig S1       |
| MSB211           | tmls1068[unc-4p::CRE; lin-44p::dsRED]; heSi317[eff-3p::Lox2272-BFP-Lox2272::mCherry] | This study              | Fig S1       |
| STR655           | hrtSi99[mec-17p::Cre]; heSi317[Peft-3::Lox2272-BFP-Lox2272::mCherry]      | M Harterink, [93]       | Fig S1       |
| Strains  | Genotype                                                                 | Source         | Used in Fig. |
|----------|---------------------------------------------------------------------------|----------------|--------------|
| MSB282   | mirEx91[fp-22p::CRE; unc-122p::mCherry]; heSi317[eft-3::Lox2272-BFP-Lox2272::mCherry] | This study     | Fig S1       |
| MSB500   | mirls42[F49H12.4p::CRE; myo2p::mCherry]; heSi317[eft-3::Lox2272-BFP-Lox2272::mCherry] | This study     | Fig S1       |
| MSB210   | mirEx72[nlp-12p::CRE; myo-2p::mCherry]; heSi317[eft-3::Lox2272-BFP-Lox2272::mCherry] | This study     | Fig S1       |
| TQ296    | trp-4[sy695] I                                                           | CGC(*) [4]     | Fig 5        |
| MSB225   | trp-4[sy695] I; unc-70[e524] V; mirEx13                                   | This study     | not shown    |
| MSB273   | mirls19[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     | Fig 2, 6, S4A |
| MSB306   | mirls19[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     | Fig S4B      |
| MSB328   | mirEx13[nlp-12p::CRE; myo-2p::mCherry] + mirls19[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     | Fig 2        |
| MSB387   | mirls19[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     | Fig 5, 6, S4 |
| GN692    | ls[123[mec-7p:GCaMP6s::SL2::tagRFP];lite-1(ce314)                           | Goodman lab.   | Fig. S4D     |
| MSB382   | ls[123[mec-7p:GCaMP6s::SL2::tagRFP];lite-1[ce314] + mirEx144[4ec-4p::TRP-4(full-length) + [myo-2p::mCherry] | This study     | Fig 4        |
| MSB379   | ls[123[mec-7p:GCaMP6s::SL2::tagRFP];lite-1[ce314] + mirEx141[4ec-4p::Δank:TRP-4]; [myo-2p::mCherry] | This study     | Fig 5        |
| GN716    | trp-4(ok1605)                                                             | gift from Miriam Goodman lab | not shown    |
| GN517    | pgEx116 [unc-70p::UNC-70(1-1166)::TsMod::UNC-70(1167-2267); Pmyo-3::mCherry] | Goodman lab.  | Fig. 4, S6   |
| GN519    | pgEx131 [UNC-70(1-1166)::mTFP-5aa-Venus::UNC-70(1167-2267) unc-122p::RFP]  | Goodman lab.  | Fig. 6       |
| GN600    | pgls22; oxls95[pdi-2::unc-70(fly), myo-2::GFP, lin-15] IV                  | Goodman lab.  | Fig. 4, S6   |
| MSB233   | mirEx77 [unc-70p::UNC-70(1-1166)::TsMod::UNC-70(1167-2267) E2008K, myo-2p::mCherry] | This study     | Fig 4, S6    |
| MSB339   | mirls23 [unc-70p::UNC-70(1-1166)::mTFP-TRAF-Venus::UNC-70(1167-2267); unc-70(s1502); oxls95 pdi-2::unc-70(fly), myo-2::GFP, lin-15(+) | This study     | Fig 4        |
| 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[wrmScarlet::DEGRON::twk-16] | This study     | Fig 6, S8    |
| UN1823   | spc-1::degron::mKate2                                                     | Cram lab. [31] | Fig. S3      |
| MSB453   | spc-1::degron::mKate2; mirls34[ mec4p::at-TIR1::F2A::mCherry::H2B + Punc -122::GFP] | This study     | Fig S3       |
### Table S3. Strains used in this study

| Strains   | Genotype                                                                                                                                                                                                 | Source          | Used in Fig. |
|-----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|--------------|
| MSB464    | mirEx194[nlp-12p::TIR::P2A::mCherry, unc-122p::GFP] ; spc-1::degron::mKate2                                                                                                                                 | This study      | Fig. S3      |
| MSB503    | mirEx194[nlp-12p::TIR::P2A::mCherry, unc-122p::GFP]                                                                                                                                                     | This study      | Fig S3       |
| MSB521    | twk-16(mir31); mirls19[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      | Fig 7,S8     |
| MSB591    | trp-4 (mir53mir36[GFP::TRP-4]) I                                                                                                                                                                        | This study      | Fig 5        |
| MSB539    | mirls43[nlp-12p::CRE; unc-122p::GFP] ; unc-70 (mir6mir16) V                                                                                                                                              | This study      | Fig 2        |
| MSB516    | mirls43[nlp-12p::CRE; unc-122p::GFP] ; he317[eft-3::Lox2272-BFP-Lox2272::mCherry]                                                                                                                                 | This study      |               |
| MSB601    | trp-4(mir35mir36) I; unc-70(e524) V;                                                                                                                                                                     | This study      | Fig 5        |
| MSB706    | mirEx280[nlp-12p::TRP-4cDNA; myo-2p::mCherry]; mirls19[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)] | This study      | Methods, not shown |
| MSB750    | mirls71[nlp-12p::CRE; myo-2p::mCherry] I (?) ; mirEx283[nlp-12p::unc-70cDNA; unc-122p::GFP] ; unc-70(mir6mir16) V                                                                                                                                              | This study      | Fig 2        |
| MSB765    | mirls71[nlp-12p::CRE; myo-2p::mCherry] I (?) ; unc-70(mir6mir16) V outcrossed 1x                                                                                                                                                                           | This study      | Fig 2        |
| MSB728    | mirls69[nlp-12p::TRP-4cDNA; myo-2p::mCherry]; mirls19[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)] | This study      | Fig 5, Fig 6 |
| MSB756    | mirEx283[nlp-12p::unc-70cDNA; unc-122p::GFP ]; unc-70(mir6mir16) V; mirEx13[nlp-12::CRE; myo-2p::mCherry]; mirls19[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      | Fig 2        |
| MSB757    | mirls69[nlp-12p::TRP-4cDNA; myo-2p::mCherry]; trp-4(sy695): outcrossed 2x                                                                                                                                 | This study      | Fig 5        |
| MSB778    | mirls71[nlp-12p::CRE; myo-2::mCherry] I (?) ; outcrossed 2x                                                                                                                                              | This study      | Fig S2       |
| MSB450    | heSi208[eft-3p::LoxP::egl-13NLS::tagBFP2::tbb-2UTR::LoxP::egl-13NLS::mCherry::tbb-2UTR::LGV]; hrtSi27[jdes-2p::CRE LGII] V;II; unc-70(mir6mir16)V                                                                                                                                 | This study      | Fig S2       |
| No | Gene | Sequence | Comment |
|----|------|----------|---------|
| 1  | unc-70 | gRNA-1(GCAACGGCGGAAACGTCGT) gRNA-2(GCGAAACGTCGTCGGCAATA) gRNA-3(CGTCGTCGGCAATATGGCTA) | 5’ edit |
| 2  | unc-70 | gRNA(GCTACCAGGTAACTGATTAA) | 3’ edit |
| 3  | twk-16 | gRNA-1(TTGCAGAATAAAACATCATTG) gRNA-2(TTATATGTAGCACACTTTTG) | deletion exon 1+2 |
| 4  | trp-4 | gRNA (ACGTGGCGAATCCCAAACCG) | GFP tag |
| 5  | GFP universal | gRNA: CGTCGAGCTCGACGGAGTCA | GFP KI |
| 6  | nlp-12p | FWD:CTGACCTtaaatcaggtttgatcgcagaagccggaggtttgtaagctcgtg REV:TTTTGATGAACAGTGAACGGAGATTTTACATtttctccgagctgcaatttgc | minimal promoter in DVA |
| 7  | F49H12.4 | FWD: caggtttttgaaaaatattgagcttacatcagaataaatcagtgcctagacttgtaaatt REV:CAGTGAGAAGATTTGACATctagctatatgtctattttctttttgaggtagtgaatgaagta | PVD driver |
| 8  | acr-5p | FWD:CTGACCTtaaatcaggtgttggccaatggaattggcaattgt REV:GAACAGTGAAGATTTTACATtgcaagcttagatgta | B-type MN driver |
| 9  | flp-22Δ4p | FWD: CCTtaaatcaggtgtgccccaaaaatatttaac REV:CAGTGAGAAGATTTTACATtgcaagcttagata | SMD driver |

Table S4: CRISPR reagents and primers for isolation of promoter sequences from genomic DNA
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