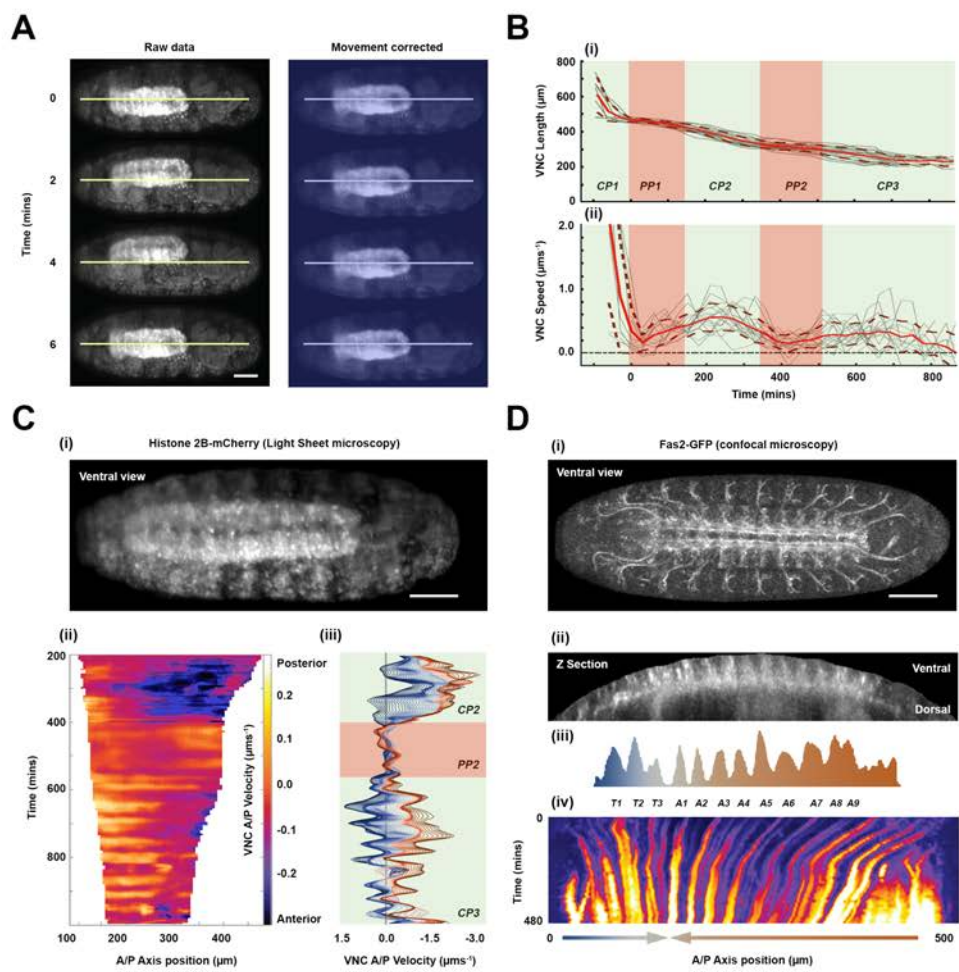


1 FIGURES AND LEGENDS

2

Figure 1



3

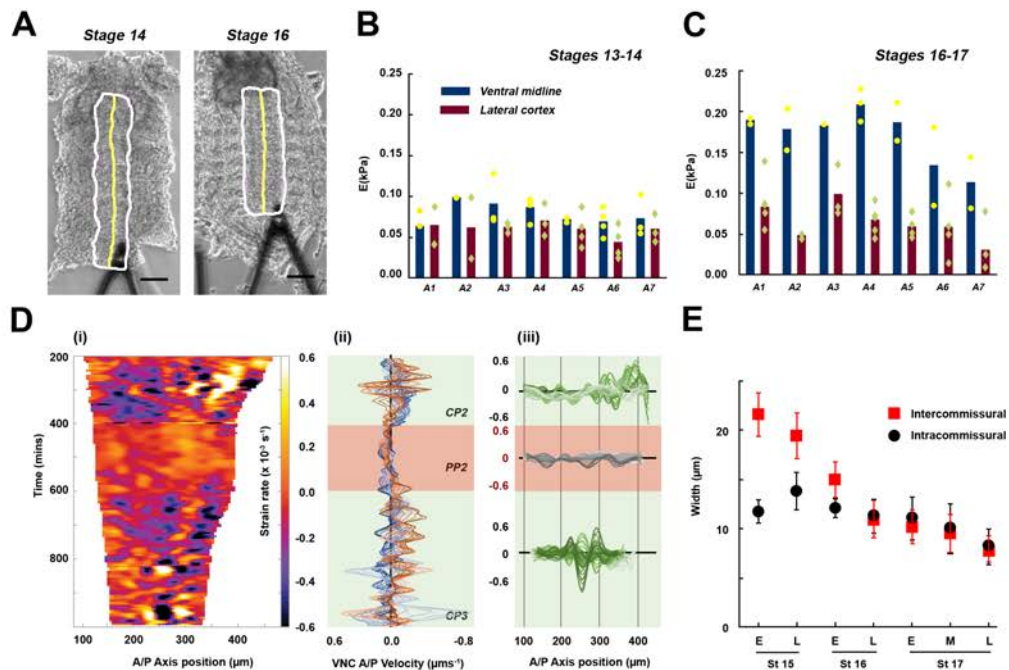
4

5 **Figure 1: Dynamics of VNC condensation**

6 **A)** Snapshots at 2-minute intervals from a time lapse (**Movie S3**) recorded by multi-view
7 light-sheet imaging of a live Histone 2Av-mCherry embryo (ventral view – late stage 17).
8 mCherry labeling marks all nuclei and was used to correct the embryo twitching (see
9 Experimental Procedures); raw data is shown on the left and “detwitched” images (blue
10 masked) on the right. In all images, anterior is to the left and posterior to the right. Lines
11 indicate the ventral midline. Scale bar 50 μm . **B)** Quantification of VNC length (**i**) and
12 condensation speed (**ii**) as function of time. Condensation (CP1, CP2 and CP3) and pause
13 (PP1 and PP2) phases are masked in pale green and red respectively. As a convention for
14 this and all subsequent figures (unless stated otherwise), $t=0$ corresponds to the onset of
15 the VNC pause phase (PP1), at the end of germ band retraction. Means (solid) and SD
16 (dashed) are represented by red lines. Gray lines represent individual embryos ($n=11$
17 embryos). **C)** Condensation velocity spatiotemporal dynamics. **(i)** Snapshot of a live
18 Histone 2Av-mCherry embryo monitored by light-sheet imaging at stage 16. Scale bar
19 50 μm . **(ii)** Velocity kymograph derived from PIV analysis (Experimental Procedures)
20 along the VNC. For this and all subsequent figures, position=0 along the AP axis
21 corresponds to the hinge between the brain lobes and the VNC proper. Time axis (top to
22 bottom) was defined as in **(B)**. Color-coded positive (posterior-ward - white/yellow) and
23 negative (anterior-ward - black/blue) values of velocity (neutral - red). **(iii)**
24 Representation of velocity profiles along the whole condensation process (CP2, PP2 and
25 CP3), with 5-minute resolution, for all points along the AP axis from the most anterior
26 (darkest blue) to the most posterior (darkest red lines) VNC positions. **D)** Kymograph
27 along the VNC length from a live embryo expressing Fas2-GFP. **(i)** Ventral view from a
28 confocal microscopy acquisition (**Movie S4**), at stage 16. Scale bar 50 μm . **(ii)** Stage 16
29 embryonic VNC, re-sliced over the Z-axis. **(iii)** Fluorescence intensity peaks mark
30 individual segments landmarks (color coded as in **(C)**). Time and AP axis positions are
31 as in **(B)** and **(C)**. **(iv)** Kymograph of condensation, with arrows denoting condensation
32 direction.

33

Figure 2



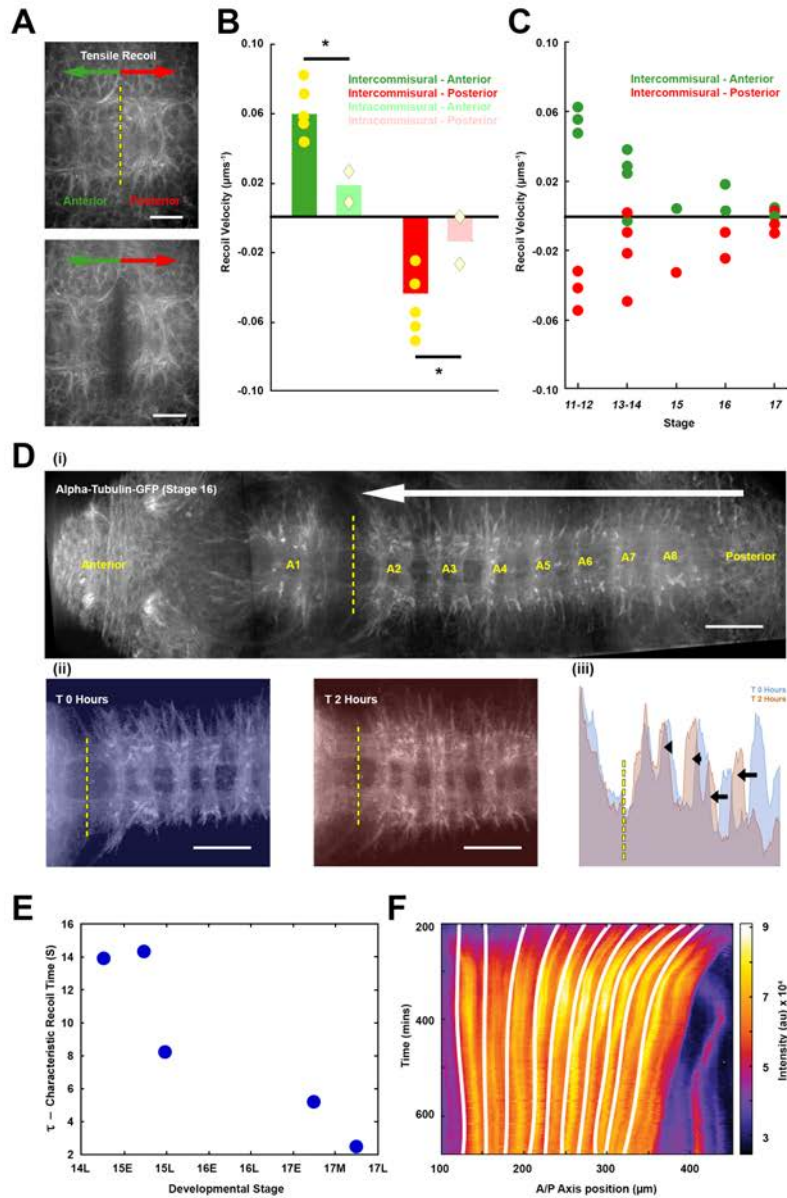
34

35 **Figure 2: Characterization of the VNC material properties and local dynamics along**
 36 **its condensation**

37 **A)** Representative images of flat dissected embryos in stages 14 and stages 16,
 38 respectively. VNC perimeter (white line) and midline (yellow line) are highlighted, with
 39 the AFM cantilever head shown. Anterior is to the top. Scale bar 50 μm . **B)** Measured
 40 tissue stiffness (E) for dissected VNC at early stages (13-14). Bars denote mean values at
 41 each abdominal segment A1 to A7. Mean tissue stiffness was measured at the midline
 42 (blue) and at lateral positions of the cortex (red). Dots and diamonds correspond to
 43 individual measurements. **C)** as **(B)** but for later, stage 16-17, samples. **D) (i)** Kymograph

44 of VNC strain rates, from data in **Figure 1C** (see Experimental Procedures). **(ii)**
45 Representation of strain rates profiles during condensation (CP2, PP2 and CP3), with 5-
46 minute resolution, from the most anterior (darkest blue line) to the most posterior (darkest
47 red line) VNC positions. **(iii)** Distribution of strains in VNC along the AP- axis for all
48 time points (earliest light to latest dark lines) during the phases CP2 (green), PP2 (gray)
49 and CP3 (green). **E**) Average size (and SD) of intra- and inter-commissural domains from
50 early (E) and late (L) stage 15, early (E) and late (L) stage 16 and early (E), middle (M)
51 and late (L) stage 17 embryos as the VNC condenses. Data was collected from 7-10
52 measurements per time point from two embryos.

Figure 3



53

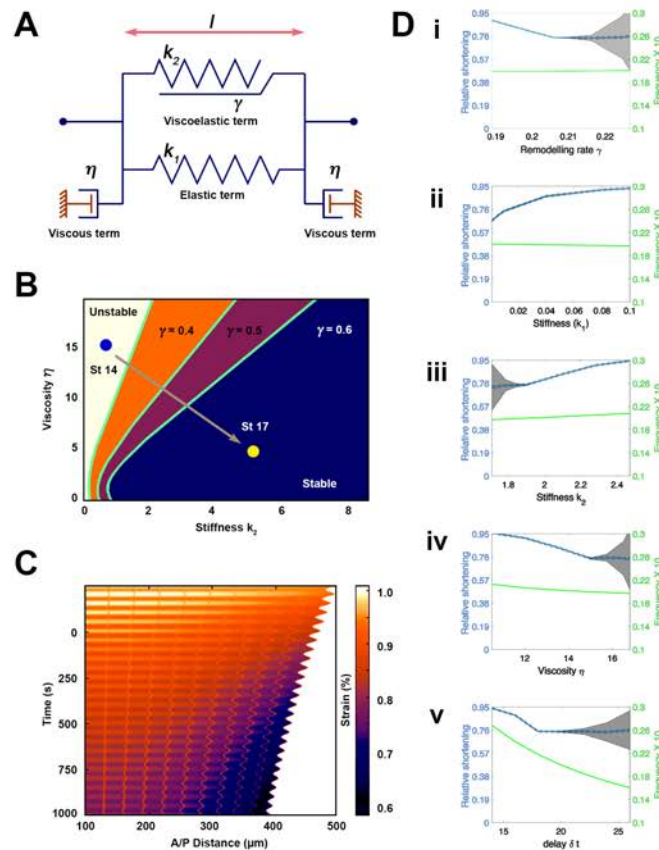
54 **Figure 3: VNC response to laser microsurgery during condensation and tissue**
 55 **tension**

56 **A)** Representative images of stage 14 embryos, expressing alpha Tubulin-GFP, before
 57 (top) and after (bottom) laser ablation. The yellow dashed line highlights the position of
 58 the laser cut (intercommissural), while green (anterior) and red (posterior) arrows indicate

59 tensile recoil directionality (**Movie S5**). Scale bar 10 μm . **B**) Tensile recoil velocity after
60 ablation at intercommissural (dark) and intracommissural (pale) domains, on stage 14
61 embryos. Bars represent mean recoil velocity of anteriorly (green) and posteriorly (red)
62 retracting tissue. Individual measurements are denoted by yellow dots (intercommissural)
63 and diamonds (intracommissural). * $p < 0.05$. **C**) Recoil velocity of anteriorly (green) and
64 posteriorly (red) retracting domains after VNC ablation at different stages of embryonic
65 development (n=12 embryos). **D**) **(i)** Tiled image of a stage 16 embryo expressing alpha
66 Tubulin-GFP after laser cutting the intercommissural domain between the abdominal
67 segments A1 and A2. The white arrow marks the direction of tissue condensation. The
68 anterior and posterior limits of the VNC and the different abdominal segments (A1 to A8)
69 are indicated (yellow). **(ii)** Snapshots, immediately post-ablation (masked blue), and 2
70 hours later (masked red), from **Movie S6**. Scale bar 20 μm . **(iii)** Superimposed
71 fluorescence intensity profiles of both time points. Black arrows indicate the magnitude
72 of the anterior-ward displacement of individual segmental landmarks over the analyzed
73 period. **E**) Characteristic recoil time τ at different embryonic stages computed from the
74 rate of recoil after laser ablation at the intercommissural domain. **F**) Kymograph of the
75 VNC during condensation (Fas2-GFP expressing embryo). White curves correspond to
76 fourth order polynomial fitting of the points of maximum compression as deduced from
77 the viscoelastic FE model of the VNC (Experimental Procedures). (See also **Figure S4D**
78 and **Movie S7**).

79

Figure 4



80

81 **Figure 4: Rheological model of VNC condensation**

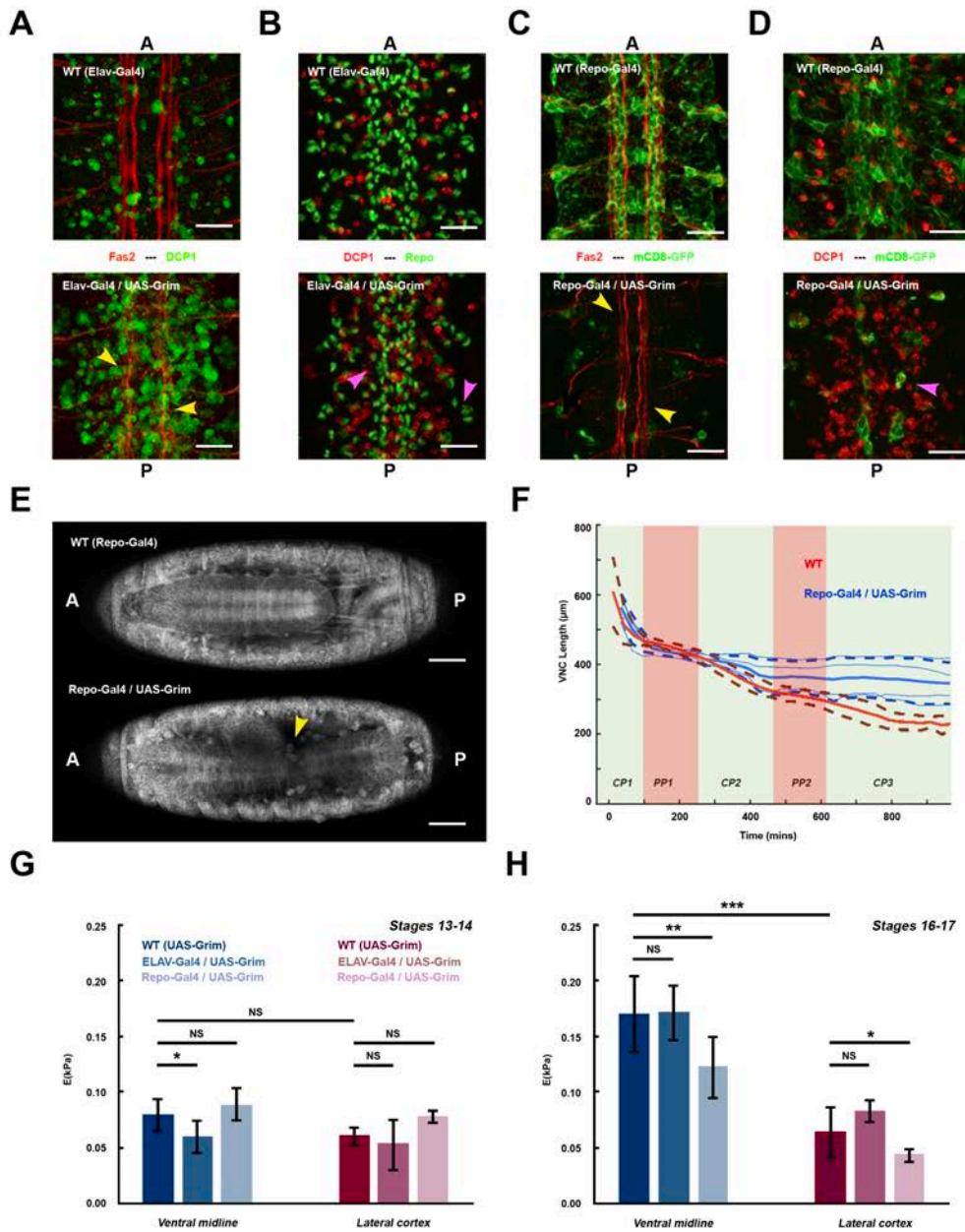
82 A) Scheme of one-dimensional rheological model. A viscoelastic term with variable rest-
 83 length l has stiffness k_2 and remodeling rate γ (see Eq. (1) in Results). The VNC is taken
 84 to have an elastic component in parallel, with stiffness k_1 . The model also includes viscous
 85 contact to the external environment, denoted by η . B) Phase diagram in the parameter
 86 space $k_2 - \eta$, showing that reduction of η and increase of k_2 stabilizes the oscillatory
 87 behavior. Points St 14 and St 17 represent material values and transition from early to
 88 later stages of VNC development, with a stabilizing effect. C) Kymograph of numerical
 89 simulation showing the oscillatory behavior of strains as a function of time. Simulations
 90 with other parameter values showing unstable responses are shown in **Figure S5. D)**

91 Sensitivity of VNC shortening and oscillatory frequencies to main model parameters on.
92 (i) Remodelling rate, γ . (ii) Stiffness, k_1 . (iii) Stiffness, k_2 . (iv) Viscosity, η . (v) Time
93 delay, δt . Shortening is measured as the relative final length, l_{final} / l_0 . The dotted blue line
94 indicates the initial amplitude of the oscillations for the model reference parameters (γ ,
95 k_1 , k_2 , η , δt) = (0.2, 0.01, 1.9, 15, 20), while the gray area represents the final amplitude
96 for the analyzed value of the parameter indicated on the horizontal axis. The green line
97 indicates the oscillations frequency as a function of the parameter values. Frequency is
98 measured in $\text{min}^{-1} * 10$.

99

100

Figure 5



101

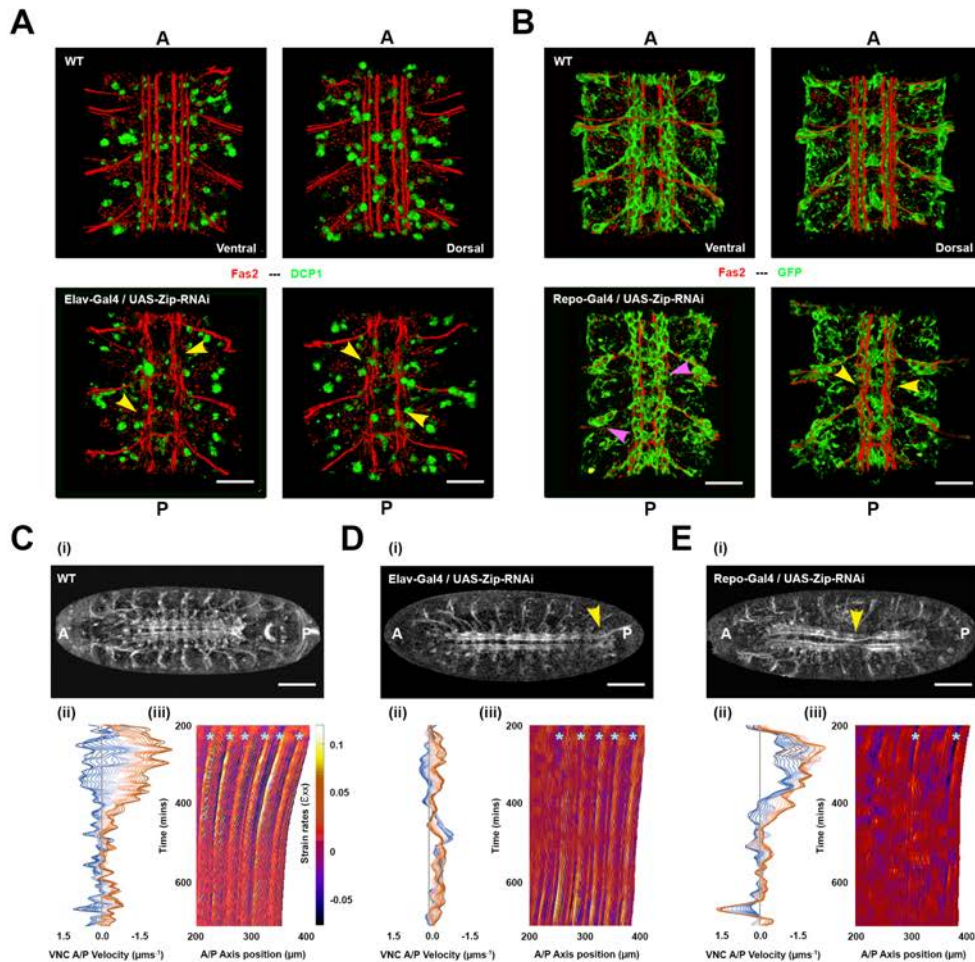
102 **Figure 5: Both neurons and glia participate in the architectural organization of the**
 103 **VNC and its condensation**

104 **A)** CNS Flat-preps of WT (top) and Elav-Gal4>UAS-Grim (bottom) embryos, at stage
 105 16, immunostained for Fas2 (red) and Dcp1 (green). Yellow arrowheads point to the
 106 disrupted axonal network. **B)** Embryos of the same genotype as in (A), immunostained
 107 for Dcp1 (red) and Repo (green). Pink arrowheads point to misplaced glia. **C)** CNS Flat-

108 preps of WT (top) and Repo-Gal4:UAS-mCD8-GFP>UAS-Grim (bottom) embryos, at
109 stage 16, immunostained for Fas2 (red) and GFP (green). Yellow arrowheads point to the
110 disrupted axonal network. **D**) Embryos of the same genotype as in **(C)**, immunostained
111 for Dcp1 (red) and GFP (green). Pink arrowhead points to surviving glia. Scale bar 10
112 μm in **A-D**. **E**) Snapshots from time lapse recordings of WT (Top) and Repo-Gal4>UAS-
113 Grim (bottom) embryos, in an alpha Tubulin-GFP background (ventral view –stage 17)
114 **(Movie S8)**. Yellow arrowhead points to the VNC misshaped buckling. Scale bar 50 μm .
115 AP axis orientation is indicated. **F**) Quantification of VNC length as a function of
116 developmental time in WT (red, n=11) and Repo-Gal4>UAS-Grim (blue, n=4) embryos
117 marked with *elav*:mCD8-GFP by confocal imaging. Solid and dashed lines show mean
118 and SD values respectively. **G**) Tissue stiffness (E) measured by AFM for dissected
119 VNCs at early stages (13-14), from WT, Elav-Gal4>UAS-Grim and Repo-Gal4>UAS-
120 Grim embryos. Bars denote mean values at the ventral midline (blue) and at lateral cortex
121 areas (red). * $p < 0.05$, ** $p < 10^{-2}$ and *** $p < 10^{-3}$. **H**) As **(G)** but for later stage 16-17
122 embryos.

123

Figure 6



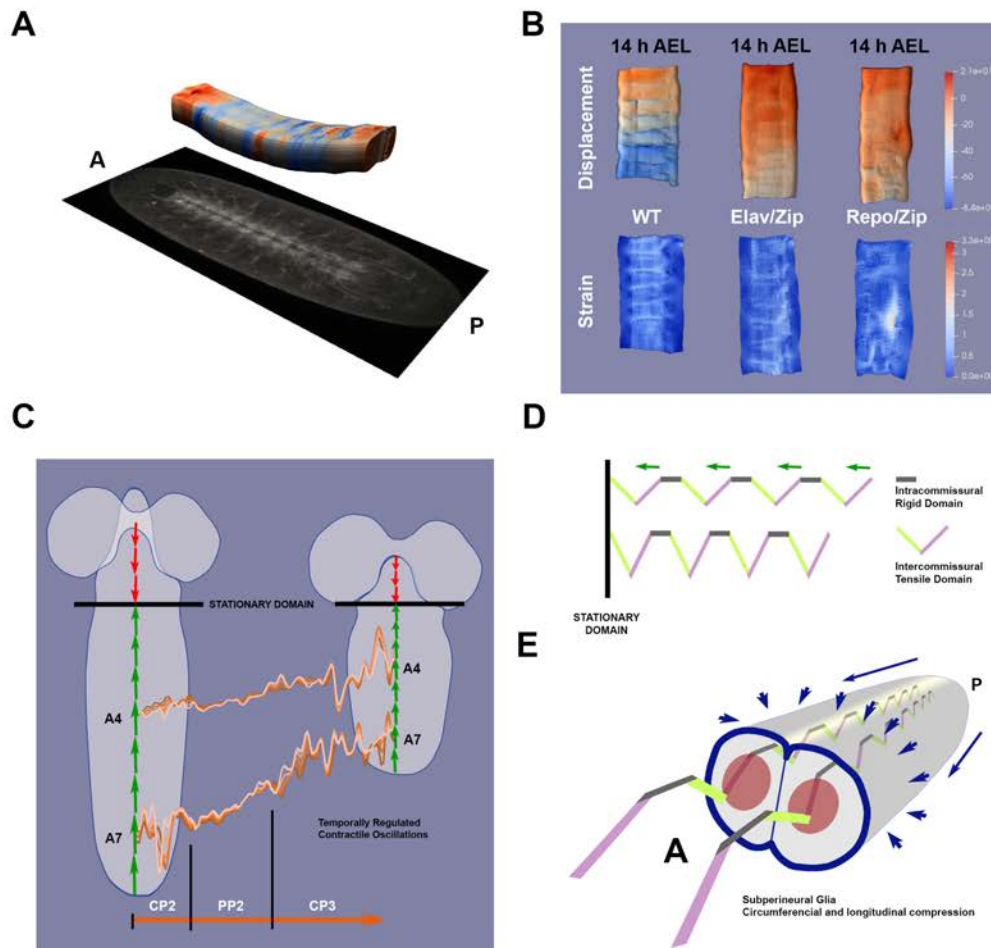
124

125 **Figure 6: Active contractility in neurons and glia have distinct roles for VNC**
 126 **architecture and condensation.**

127 **A)** Ventral and Dorsal 3D views of dissected, stage 16, WT (top) and Elav-Gal4>UAS-
 128 Zip-RNAi (bottom) embryos, immunostained for Fas2 (red) and Dcp1 (green). Yellow
 129 arrowheads point to the disrupted axonal network. **B)** Ventral and Dorsal 3D views,
 130 equivalent to (A), of stage 16, WT (top) and Repo-Gal4:UAS-mCD8-GFP>UAS-Zip-
 131 RNAi (bottom) embryos, immunostained for Fas2 (red) and GFP (green). Pink
 132 arrowheads point to misplaced glia. Yellow arrowheads point to the disrupted axonal

133 network. **A-B** Scale bar 10 μm . **C-E**) Condensation dynamics in control (**C**), Elav-
134 Gal4>UAS-Zip-RNAi (**D**) and Repo-Gal4>UAS-Zip-RNAi (**E**) embryos (**Movie S10**).
135 **(i)** Snapshots of live embryos, expressing Fas2-GFP, monitored by confocal imaging, at
136 stage 17. Yellow arrowheads point to the posterior tip of the uncondensed VNC (**D**) and
137 to the VNC misshaped buckling (**E**). Anterior is to the left. Scale bar 50 μm . **(ii)**
138 Representation of velocity profiles during condensation along the AP axis, from the most
139 anterior (darkest blue) to the most posterior (darkest red line) VNC positions (as in **Figure**
140 **1C**). **(iii)** Kymograph of strain rates along the VNC (as **Figure 2D**). Cyan marks point to
141 strain oscillations, which are strongly diminished upon reduction of glia contractility.

Figure 7



142

143 **Figure 7: Neurons and Glia cooperate to lead the oscillatory character of VNC**
 144 **condensation**

145 **A)** Snapshot from the 3D representation (**Movie S11**) of the segmental 2D strain pattern
 146 of the VNC during condensation, in WT animals. The actual 3D meshwork (top) is
 147 aligned to the corresponding raw image (bottom). AP axis orientation is indicated. **B)**
 148 Displacements and strains along the VNC, in WT embryos and in embryos with pan-
 149 neural (Elav-Gal4>UAS-Zip-RNAi) or pan-gial (Repo-Gal4>UAS-Zip-RNAi) non

150 muscle Myosin II knockdown, at equivalent developmental times [14 hours after egg
151 laying (AEL) at 29°C]. (Snapshots from **Movie S12**). **C**) Cartoon summarizing the VNC
152 condensation oscillatory regime during the CP2 and CP3 stages (examples at the level of
153 the abdominal segments A4 and A7, data from **Movie S3** – see **Figure 1C**), as well as
154 pointing to the opposing displacements of the thoracic (red) and abdominal (green)
155 segments towards the central stationary domain. **D**) Cartoon presenting the segmentally
156 iterated intercommissural and intracommissural domains of the axonal network before
157 (top) and after (bottom) condensation. Their mechanical properties (rigid or tensile) are
158 shown. This representation depicts the first three abdominal segments actively
159 contracting (green arrows) towards the thorax/abdomen stationary domain. **E**) Cartoon
160 presenting in 3D the VNC internal segmentally iterated axonal network (described in **D**)
161 surrounded by the glial shell (Subperineural Glia) with centripetal and longitudinal
162 contractile capability (blue arrows). AP axis orientation is indicated.

163

164 **REFERENCES**

165

166 Amourda, C., and Saunders, T.E. (2017). Gene expression boundary scaling and organ
167 size regulation in the *Drosophila* embryo. *Dev Growth Differ* 59, 21-32.

168 Anava, S., Greenbaum, A., Ben Jacob, E., Hanein, Y., and Ayali, A. (2009). The
169 regulative role of neurite mechanical tension in network development. *Biophys J* 96,
170 1661-1670.

171 Baines, R.A., and Bate, M. (1998). Electrophysiological development of central neurons
172 in the *Drosophila* embryo. *J Neurosci* 18, 4673-4683.

173 Barriga, E.H., Franze, K., Charras, G., and Mayor, R. (2018). Tissue stiffening
174 coordinates morphogenesis by triggering collective cell migration in vivo. *Nature* 554,
175 523-527.

176 Beckervordersandforth, R.M., Rickert, C., Altenhein, B., and Technau, G.M. (2008).
177 Subtypes of glial cells in the *Drosophila* embryonic ventral nerve cord as related to
178 lineage and gene expression. *Mech Dev* 125, 542-557.

179 Bertet, C., Sulak, L., and Lecuit, T. (2004). Myosin-dependent junction remodelling
180 controls planar cell intercalation and axis elongation. *Nature* 429, 667-671.

181 Boix-Fabres, J., Karkali, K., Martin-Blanco, E., and Rebollo, E. (2019). Automated
182 Macro Approach to Remove Vitelline Membrane Autofluorescence in *Drosophila*
183 Embryo 4D Movies. *Methods Mol Biol* 2040, 155-175.

184 Bullmore, E., and Sporns, O. (2012). The economy of brain network organization. *Nature*
185 reviews Neuroscience 13, 336-349.

186 Bullock, T.H., and Horridge, G.A. (1965). Structure and function in the nervous systems
187 of invertebrates (San Francisco: W. H. Freeman).

188 Buszczak, M., Paterno, S., Lighthouse, D., Bachman, J., Planck, J., Owen, S., Skora,
189 A.D., Nystul, T.G., Ohlstein, B., Allen, A., *et al.* (2007). The carnegie protein trap library:
190 a versatile tool for *Drosophila* developmental studies. *Genetics* 175, 1505-1531.

191 Cajal, S.R.y. (1899). *Textura del Sistema Nervioso del Hombre y de los Vertebrados*
192 (Madrid, Spain: Nicolas Moya).

193 Campos-Ortega, J.A., and Hartenstein, V. (1985). The embryonic development of
194 *Drosophila melanogaster* (Berlin ; New York: Springer-Verlag).

195 Cavanaugh, K.E., Staddon, M.F., Munro, E., Banerjee, S., and Gardel, M.L. (2020).
196 RhoA Mediates Epithelial Cell Shape Changes via Mechanosensitive Endocytosis. *Dev*
197 *Cell* 52, 152-166 e155.

198 Chen, P., Nordstrom, W., Gish, B., and Abrams, J.M. (1996). grim, a novel cell death
199 gene in *Drosophila*. *Genes & development* 10, 1773-1782.

200 Christley, S., Alber, M.S., and Newman, S.A. (2007). Patterns of mesenchymal
201 condensation in a multiscale, discrete stochastic model. *PLoS Comput Biol* 3, e76.

202 Clement, R., Dehapiot, B., Collinet, C., Lecuit, T., and Lenne, P.F. (2017). Viscoelastic
203 Dissipation Stabilizes Cell Shape Changes during Tissue Morphogenesis. *Curr Biol* 27,
204 3132-3142 e3134.

205 Dawi, M.A., and Munoz, J.J. (2021). Stability bounds of a delay visco-elastic rheological
206 model with substrate friction. *J Math Biol* 83, 71.

207 DeLise, A.M., Fischer, L., and Tuan, R.S. (2000). Cellular interactions and signaling in
208 cartilage development. *Osteoarthr Cartil* 8, 309-334.

209 Doubrovinski, K., Swan, M., Polyakov, O., and Wieschaus, E.F. (2017). Measurement of
210 cortical elasticity in *Drosophila melanogaster* embryos using ferrofluids. *Proc Natl Acad*
211 *Sci U S A* 114, 1051-1056.

212 Erneux, T. (2009). *Applied delay differential equations* (New York: Springer).

213 Evans, I.R., Hu, N., Skaer, H., and Wood, W. (2010). Interdependence of macrophage
214 migration and ventral nerve cord development in *Drosophila* embryos. *Development* 137,
215 1625-1633.

216 Franze, K. (2013). The mechanical control of nervous system development. *Development*
217 140, 3069-3077.

218 Franze, K., Janmey, P.A., and Guck, J. (2013). Mechanics in neuronal development and
219 repair. *Annu Rev Biomed Eng* 15, 227-251.

220 Frenz, D.A., Jaikaria, N.S., and Newman, S.A. (1989). The mechanism of precartilage
221 mesenchymal condensation: a major role for interaction of the cell surface with the
222 amino-terminal heparin-binding domain of fibronectin. *Developmental biology* 136, 97-
223 103.

224 Hall, B.K., and Miyake, T. (2000). All for one and one for all: condensations and the
225 initiation of skeletal development. *BioEssays* 22, 138-147.

226 Hartenstein, V., and Wodarz, A. (2013). Initial neurogenesis in *Drosophila*. Wiley
227 *Interdiscip Rev Dev Biol* 2, 701-721.

228 Heisenberg, C.-P., and Bellaïche, Y. (2013). Forces in Tissue Morphogenesis and
229 Patterning. *Cell* 153, 948-962.

230 Hogan, B.L. (1999). Morphogenesis. *Cell* 96, 225-233.

231 Ito, K., Urban, J., and Technau, G.M. (1995). Distribution, classification, and
232 development of *Drosophila* glial cells in the late embryonic and early larval ventral nerve
233 cord. *Roux Arch Dev Biol* 204, 284-307.

234 Jorba, I., Uriarte, J.J., Campillo, N., Farre, R., and Navajas, D. (2017). Probing
235 Micromechanical Properties of the Extracellular Matrix of Soft Tissues by Atomic Force
236 Microscopy. *J Cell Physiol* 232, 19-26.

237 Karkali, K., Saunders, T.E., Vernon, S.W., Baines, R.A., Panayotou, G., and Martín-
238 Blanco, E. (2020). JNK signaling in pioneer neurons directs the architectural organization
239 of the CNS and coordinates the motor activity of the *Drosophila* embryo. *bioRxiv*,
240 092486.

241 Khalilgharibi, N., Fouchard, J., Asadipour, N., Barrientos, R., Duda, M., Bonfanti, A.,
242 Yonis, A., Harris, A., Mosaffa, P., and Fujita, Y. (2019). Stress relaxation in epithelial
243 monolayers is controlled by the actomyosin cortex. *Nat Physics* 15, 839-847.

244 Kiehart, D.P., and Feghali, R. (1986). Cytoplasmic myosin from *Drosophila*
245 *melanogaster*. *J Cell Biol* 103, 1517-1525.

246 Kilinc, D. (2018). The Emerging Role of Mechanics in Synapse Formation and Plasticity.
247 *Front Cell Neurosci* 12, 483.

248 Krzic, U., Gunther, S., Saunders, T.E., Streichan, S.J., and Hufnagel, L. (2012).
249 Multiview light-sheet microscope for rapid in toto imaging. *Nat Methods* 9, 730-733.

250 Landgraf, M., Bossing, T., Technau, G.M., and Bate, M. (1997). The origin, location, and
251 projections of the embryonic abdominal motoneurons of *Drosophila*. *J Neurosci* 17,
252 9642-9655.

253 Landgraf, M., Sanchez-Soriano, N., Technau, G.M., Urban, J., and Prokop, A. (2003).
254 Charting the *Drosophila* neuropile: a strategy for the standardised characterisation of
255 genetically amenable neurites. *Dev Biol* 260, 207-225.

256 LeGoff, L., and Lecuit, T. (2015). Mechanical Forces and Growth in Animal Tissues.
257 *Cold Spring Harb Perspect Biol* 8, a019232.

258 Li, Y., Muffat, J., Omer, A., Bosch, I., Lancaster, M.A., Sur, M., Gehrke, L., Knoblich,
259 J.A., and Jaenisch, R. (2017). Induction of Expansion and Folding in Human Cerebral
260 Organoids. *Cell Stem Cell* 20, 385-396 e383.

261 Lin, D.M., Fetter, R.D., Kopczynski, C., Grenningloh, G., and Goodman, C.S. (1994).
262 Genetic analysis of Fasciclin II in *Drosophila*: defasciculation, refasciculation, and
263 altered fasciculation. *Neuron* 13, 1055-1069.

264 Lynch, H.E., Crews, S.M., Rosenthal, B., Kim, E., Gish, R., Echiverri, K., and Hutson,
265 M.S. (2013). Cellular mechanics of germ band retraction in *Drosophila*. *Developmental*
266 *biology* 384, 205-213.

267 Mammoto, T., and Ingber, D.E. (2010). Mechanical control of tissue and organ
268 development. *Development* 137, 1407-1420.

269 Martinek, N., Shahab, J., Saathoff, M., and Ringuette, M. (2008). Haemocyte-derived
270 SPARC is required for collagen-IV-dependent stability of basal laminae in *Drosophila*
271 embryos. *J Cell Sci* 121, 1671-1680.

272 Matsubayashi, Y., Sanchez-Sanchez, B.J., Marcotti, S., Serna-Morales, E., Dragu, A.,
273 Diaz-de-la-Loza, M.D., Vizcay-Barrena, G., Fleck, R.A., and Stramer, B.M. (2020).
274 Rapid Homeostatic Turnover of Embryonic ECM during Tissue Morphogenesis. *Dev*
275 *Cell* 54, 33-42 e39.

276 Mayer, M., Depken, M., Bois, J.S., Julicher, F., and Grill, S.W. (2010). Anisotropies in
277 cortical tension reveal the physical basis of polarizing cortical flows. *Nature* 467, 617-
278 621.

279 Meyer, S., Schmidt, I., and Klambt, C. (2014). Glia ECM interactions are required to
280 shape the *Drosophila* nervous system. *Mech Dev* 133, 105-116.

281 Miller, C.J., and Davidson, L.A. (2013). The interplay between cell signalling and
282 mechanics in developmental processes. *Nat Rev Genet* 14, 733-744.

283 Mongera, A., Rowghanian, P., Gustafson, H.J., Shelton, E., Kealhofer, D.A., Carn, E.K.,
284 Serwane, F., Lucio, A.A., Giammona, J., and Campas, O. (2018). A fluid-to-solid
285 jamming transition underlies vertebrate body axis elongation. *Nature* 561, 401-405.

286 Muñoz, J.J., Dingle, M., and Wenzel, M. (2018). Mechanical oscillations in biological
287 tissues as a result of delayed rest-length changes. *Phys Rev E* 98, 052409.

288 Notbohm, J., Banerjee, S., Utuje, K.J.C., Gweon, B., Jang, H., Park, Y., Shin, J., Butler,
289 J.P., Fredberg, J.J., and Marchetti, M.C. (2016). Cellular Contraction and Polarization
290 Drive Collective Cellular Motion. *Biophys J* *110*, 2729-2738.

291 Oliveira, M.M., Shingleton, A.W., and Mirth, C.K. (2014). Coordination of wing and
292 whole-body development at developmental milestones ensures robustness against
293 environmental and physiological perturbations. *PLoS Genet* *10*, e1004408.

294 Olofsson, B., and Page, D.T. (2005). Condensation of the central nervous system in
295 embryonic *Drosophila* is inhibited by blocking hemocyte migration or neural activity.
296 *Dev Biol* *279*, 233-243.

297 Page, D.T., and Olofsson, B. (2008). Multiple roles for apoptosis facilitating
298 condensation of the *Drosophila* ventral nerve cord. *Genesis* *46*, 61-68.

299 Pastor-Pareja, J.C., and Xu, T. (2011). Shaping cells and organs in *Drosophila* by
300 opposing roles of fat body-secreted Collagen IV and perlecan. *Dev Cell* *21*, 245-256.

301 Petridou, N.I., and Heisenberg, C.P. (2019). Tissue rheology in embryonic organization.
302 *EMBO J* *38*, e102497.

303 Petrolli, V., Le Goff, M., Tadrous, M., Martens, K., Allier, C., Mandula, O., Herve, L.,
304 Henkes, S., Sknepnek, R., Boudou, T., *et al.* (2019). Confinement-Induced Transition
305 between Wavelike Collective Cell Migration Modes. *Phys Rev Lett* *122*, 168101.

306 Peyret, G., Mueller, R., d'Alessandro, J., Begnaud, S., Marcq, P., Mege, R.M., Yeomans,
307 J.M., Doostmohammadi, A., and Ladoux, B. (2019). Sustained Oscillations of Epithelial
308 Cell Sheets. *Biophys J* *117*, 464-478.

309 Pipa, R.L. (1973). Proliferation, movement, and regression of neurons during the
310 postembryonic development of insects. In *Developmental Neurobiology of Arthropods*
311 (Cambridge: Cambridge University Press).

312 Redies, C., and Puelles, L. (2001). Modularity in vertebrate brain development and
313 evolution. *Bioessays* *23*, 1100-1111.

314 Roig-Puiggros, S., Vigouroux, R.J., Beckman, D., Bocai, N.I., Chiou, B., Davimes, J.,
315 Gomez, G., Grassi, S., Hoque, A., Karikari, T.K., *et al.* (2020). Construction and
316 reconstruction of brain circuits: normal and pathological axon guidance. *J Neurochem*
317 *153*, 10-32.

318 Sanchez-Soriano, N., Tear, G., Whittington, P., and Prokop, A. (2007). *Drosophila* as a
319 genetic and cellular model for studies on axonal growth. *Neural Dev* *2*, 9.

320 Saunders, T.E., and Ingham, P.W. (2019). Open questions: how to get developmental
321 biology into shape? *BMC Biol* *17*, 17.

322 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T.,
323 Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., *et al.* (2012). Fiji: an open-source
324 platform for biological-image analysis. *Nat Methods* *9*, 676-682.

325 Schulze, K.L., Broadie, K., Perin, M.S., and Bellen, H.J. (1995). Genetic and
326 electrophysiological studies of *Drosophila* syntaxin-1A demonstrate its role in
327 nonneuronal secretion and neurotransmission. *Cell* *80*, 311-320.

328 Schwabe, T., Li, X., and Gaul, U. (2017). Dynamic analysis of the mesenchymal-
329 epithelial transition of blood-brain barrier forming glia in *Drosophila*. *Biol Open* *6*, 232-
330 243.

331 Serwane, F., Mongera, A., Rowghanian, P., Kealhofer, D.A., Lucio, A.A., Hockenbery,
332 Z.M., and Campas, O. (2017). In vivo quantification of spatially varying mechanical
333 properties in developing tissues. *Nat Methods* *14*, 181-186.

334 Shellard, A., and Mayor, R. (2021). Collective durotaxis along a self-generated stiffness
335 gradient in vivo. *Nature* *600*, 690-694.

336 Shklyar, B., Sellman, Y., Shklover, J., Mishnaevski, K., Levy-Adam, F., and Kurant, E.
337 (2014). Developmental regulation of glial cell phagocytic function during *Drosophila*
338 embryogenesis. *Developmental biology* *393*, 255-269.

339 Shyer, A.E., Rodrigues, A.R., Schroeder, G.G., Kassianidou, E., Kumar, S., and Harland,
340 R.M. (2017). Emergent cellular self-organization and mechanosensation initiate follicle
341 pattern in the avian skin. *Science* *357*, 811-815.

342 Singh, P., and Schwarzbauer, J.E. (2012). Fibronectin and stem cell differentiation -
343 lessons from chondrogenesis. *J Cell Sci* *125*, 3703-3712.

344 Solon, J., Kaya-Copur, A., Colombelli, J., and Brunner, D. (2009). Pulsed forces timed
345 by a ratchet-like mechanism drive directed tissue movement during dorsal closure. *Cell*
346 *137*, 1331-1342.

347 Spedden, E., and Staii, C. (2013). Neuron biomechanics probed by atomic force
348 microscopy. *Int J Mol Sci* *14*, 16124-16140.

349 Staddon, M.F., Cavanaugh, K.E., Munro, E.M., Gardel, M.L., and Banerjee, S. (2019).
350 Mechanosensitive Junction Remodeling Promotes Robust Epithelial Morphogenesis.
351 *Biophys J* *117*, 1739-1750.

352 Stark, M.R., Sechrist, J., Bronner-Fraser, M., and Marcelle, C. (1997). Neural tube-
353 ectoderm interactions are required for trigeminal placode formation. *Development* *124*,
354 4287-4295.

355 Stépán, G. (1989). *Retarded dynamical systems : stability and characteristic functions*
356 (Harlow: Longman Scientific and Technical).

357 Sumi, A., Hayes, P., D'Angelo, A., Colombelli, J., Salbreux, G., Dierkes, K., and Solon,
358 J. (2018). Adherens Junction Length during Tissue Contraction Is Controlled by the
359 Mechanosensitive Activity of Actomyosin and Junctional Recycling. *Dev Cell* *47*, 453-
360 463 e453.

361 Swanson, L.W. (2007). Quest for the basic plan of nervous system circuitry. *Brain Res*
362 *Rev* *55*, 356-372.

363 Technau, G.M. (2008). *Brain development in Drosophila melanogaster*, Vol 628
364 (Springer Science+Business Media, LLC - Landes Bioscience).

365 Tessier-Lavigne, M., and Goodman, C.S. (1996). The molecular biology of axon
366 guidance. *Science* *274*, 1123-1133.

367 Thielicke, W., and Stamhuis, E.J. (2018). The effects of wing twist in slow-speed flapping
368 flight of birds: trading brute force against efficiency. *Bioinspir Biomim* *13*, 056015.

369 Tiwari, P., Rengarajan, H., and Saunders, T.E. (2021). Scaling of internal organs during
370 *Drosophila* embryonic development. *Biophys J* *120*, 4264-4276.

371 Vig, D.K., Hamby, A.E., and Wolgemuth, C.W. (2016). On the Quantification of Cellular
372 Velocity Fields. *Biophys J* *110*, 1469-1475.

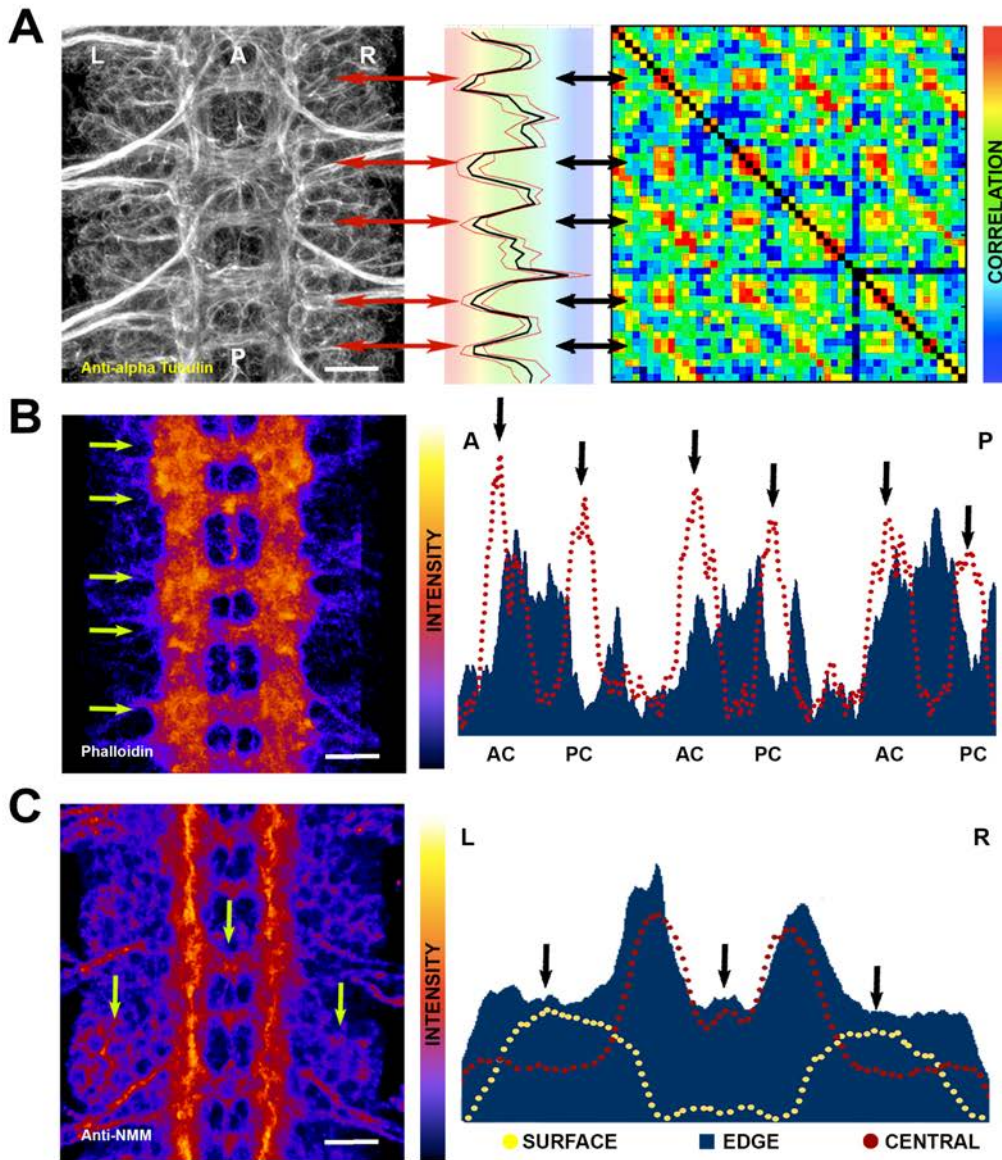
373 Weber, G.F., Bjerke, M.A., and DeSimone, D.W. (2011). Integrins and cadherins join
374 forces to form adhesive networks. *J Cell Sci* *124*, 1183-1193.

375 Zhang, H., and Labouesse, M. (2012). Signalling through mechanical inputs: a
376 coordinated process. *J Cell Sci* *125*, 3039-3049.

377 Zlatic, M., Li, F., Strigini, M., Grueber, W., and Bate, M. (2009). Positional cues in the
378 *Drosophila* nerve cord: semaphorins pattern the dorso-ventral axis. *PLoS Biol* *7*,
379 e1000135.

380

Figure S1



382

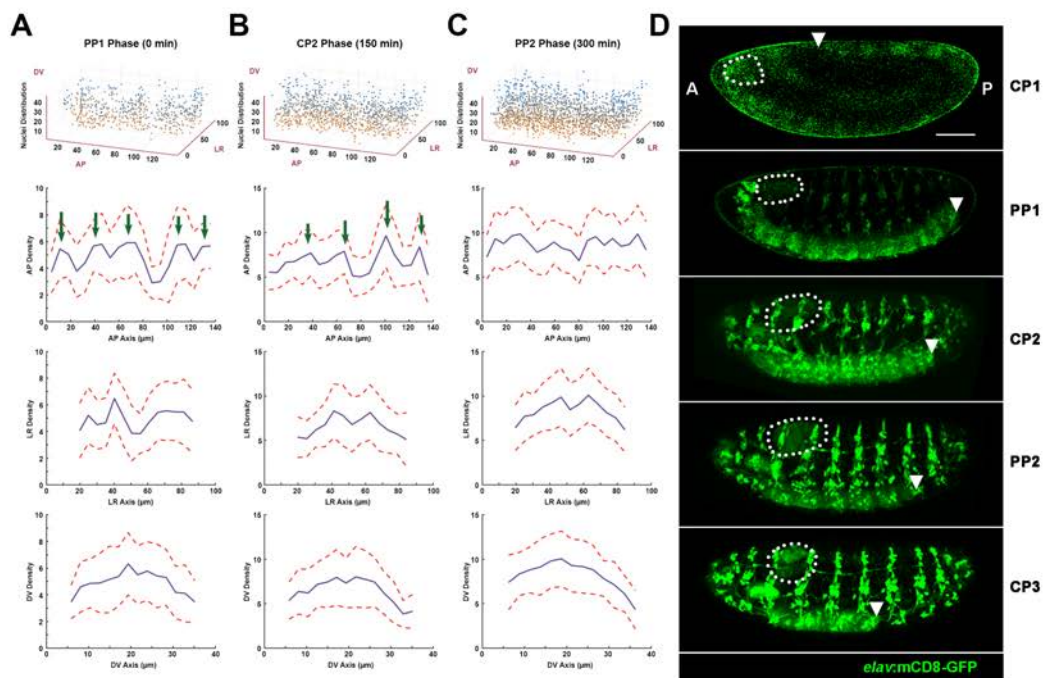
383 **Figure S1: VNC Cytoskeleton structural organization**

384 A) Acetylated α -Tubulin immunoreactivity. Left; maximum projection of a ventral view
 385 of three abdominal segments (A2 to A4) of the VNC of a 16-stage embryo. Left (L) and
 386 Right (R) and Anterior (A) and Posterior (P) orientation are labelled. Scale Bar is 10 μ m.
 387 Middle; image cross-correlation score [mean (black) and \pm standard deviation (red)]
 388 along the AP axis. Right; self cross-correlation matrix of the Z sections of the same image.

389 The color-coded representation shows the correlation level (red-maximum to blue-
390 minimum) for each possible cross comparison at each position of the image divided in 50
391 bins (see Experimental Procedures). Two axonal nodes with robust maximum correlation
392 are conserved from segment to segment. **B**) Phalloidin (Actin) distribution. Left;
393 maximum projection of a ventral view of three abdominal segments (A2 to A4) of the
394 VNC of a 16-stage embryo. Signal Intensity is color coded (Fire LUT). Arrows point to
395 the anterior and posterior commissures. Scale Bar is 10 μm . Right; Actin intensity profile
396 along the AP axis: Discontinued Red at the ventral midline highlighting the anterior (AC)
397 and posterior commissures (PC) (arrows). Solid Blue at the main contralateral trunks
398 uncovering a stereotyped segmentally iterated distribution. **C**) Non-Muscle Myosin
399 (NMM - Myosin II) immunoreactivity. Left; maximum projection of a ventral view of three
400 abdominal segments (A2 to A4) of the VNC of a 16-stage embryo. Signal Intensity is
401 color coded (Fire LUT). Arrows point to the lateral (left and right) and central neuropile
402 domains. Scale Bar is 10 μm . Right; NMM intensity profile transversal to the AP axis:
403 Discontinued Yellow at the ventral surface of the VNC highlighting the NMM
404 accumulation at the lateral domains around the neurons cell bodies; Solid Blue at the
405 medial edge of the longitudinal axonal trunks showing the preferential accumulation of
406 NMM at contralateral single-cell domains at the dorsomedial edge; and Discontinued Red
407 at the middle of the VNC trunk. See also **Movie S1**.

408

Figure S2



409

410 **Figure S2: VNC condensation temporal development and spatial distribution of cell**
 411 **density**

412 Density of neurons cell bodies along condensation progression: **A)** PP1 phase; **B)** CP2
 413 phase; **C)** PP2 phase. Top row: 3D representation of the spatial position of the neurons
 414 cell nucleus at the respective time points, with color coding representing height along the
 415 DV-axis. Second row: Average number of nuclei neighbouring each nucleus (AP density)
 416 along the AP axis. Third row: Average number of nuclei neighbouring each nucleus along
 417 the left-right (LR) axis. Fourth row: Average number of nuclei neighbouring each nucleus
 418 along the DV axis. In all panels the blue curve is mean and the dashed red line represent
 419 ± 1 s.d. Green arrows denote peaks in the density. **D)** Snapshots corresponding to the five
 420 phases of VNC condensation (CP1, PP1, CP2, PP2, CP3) from a time lapse (Movie S2)

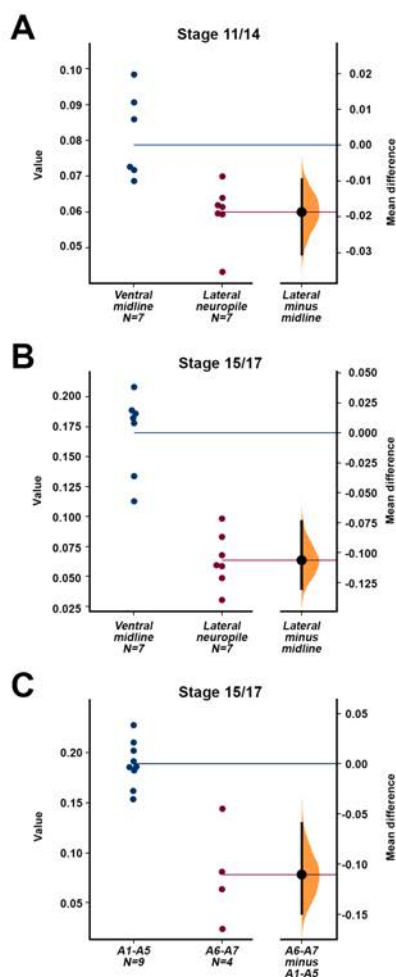
421 of an *Elav-Gal4>UAS-mCD8-GFP* embryo (lateral view) recorded by confocal
422 microscopy. mCD8-GFP labelling marks all neural derivatives. Dotted shapes indicate
423 the position of the brain lobes. Arrowheads denote the posterior tip of the VNC. AP axis
424 orientation is indicated. Scale bar 50 μ m. We calculated the nuclei density by quantifying
425 the number of nuclei that were within a sphere of radius 7.5 μ m from each nucleus. We
426 did not calculate the density for nuclei near the tissue edges to minimize boundary effects.

427

428

429

Figure S3



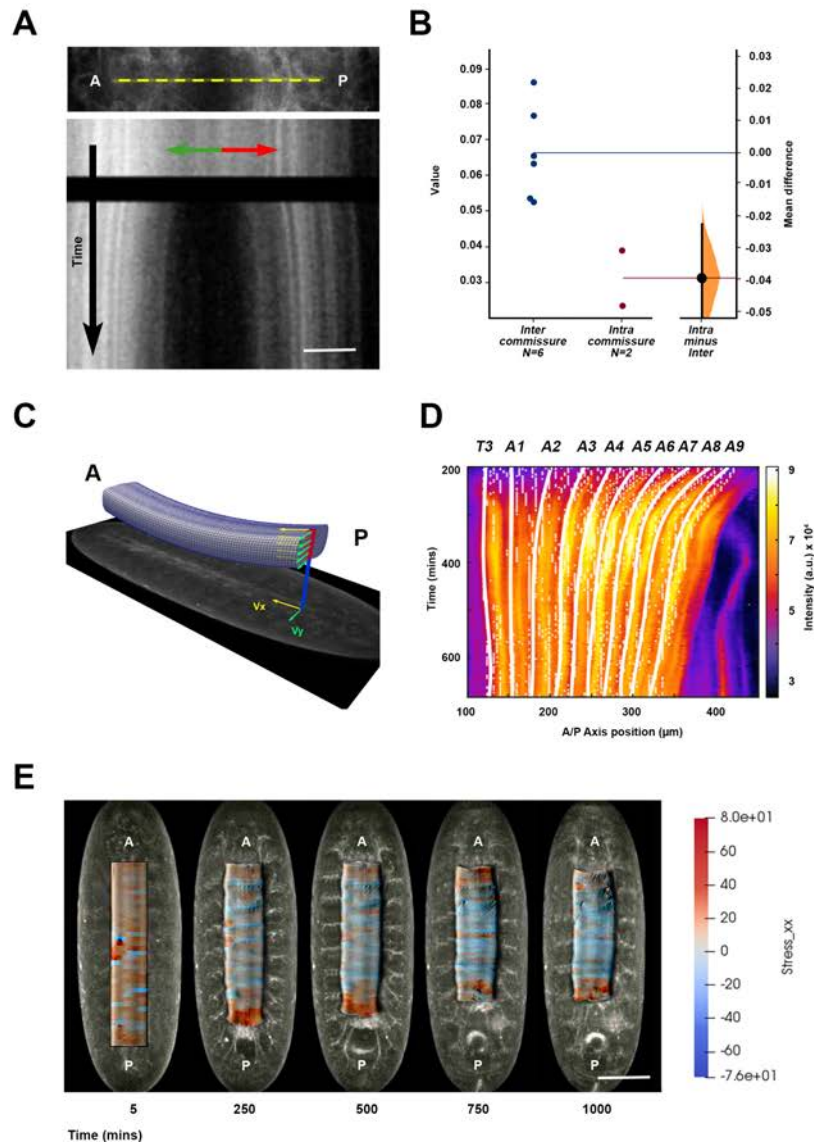
430

431 **Figure S3: Quantification of VNC material properties during condensation**

432 **A)** Statistical analysis of measured E (stiffness values) at different positions from stage
433 11-14 (estimationstats.com). Data points are shown on the left. The confidence interval
434 is shown on right. $p < 0.05$ from Mann-Witney test. **B)** As **(A)**, but for late stages 15-17.
435 $p < 10^{-2}$ from Mann-Witney test. **C)** As **(B)**, but comparing the E measured in anterior
436 domains (A1-A5) with those of posterior domains (A6-A7). $p < 10^{-2}$ from Mann-Witney
437 test.

438

Figure S4



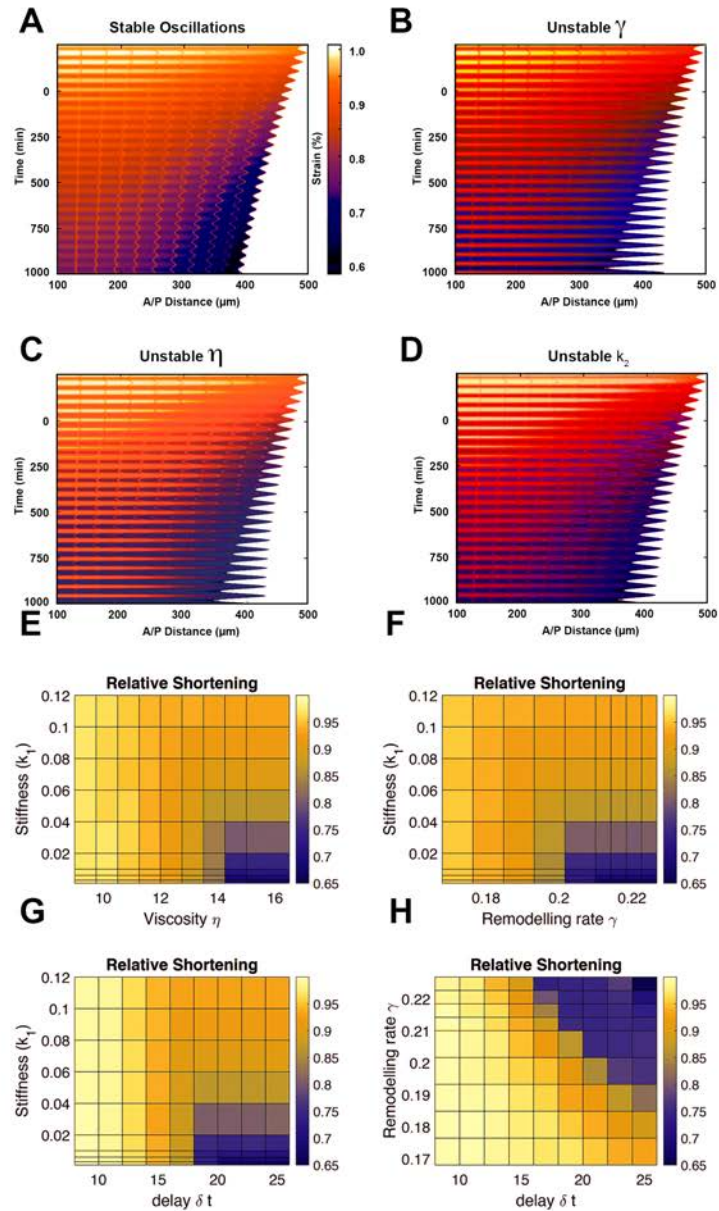
439

440 **Figure S4: Analyses of laser microsurgery of the VNC and details of the finite**
 441 **elements model**

442 **A)** (Top) VNC tissue recoil after generating a laser cut perpendicular to the AP axis of a
 443 stage 14 embryo expressing alpha Tubulin-GFP. Yellow dashed line indicates the region
 444 of analysis. (Bottom) Kymograph of VNC recoil after laser ablation. Green (anterior) and
 445 red (posterior) arrows indicate tissue recoil directionality. The black transversal domain
 446 spans the period of laser cutting. Scale bar 10 μm . **B)** Analysis of VNC recoil speed, at

447 inter- and intracommissural domains. Confidence interval on right. Generated using
448 estimationstats.com. **C)** Mapping of the measured velocities from PIV onto the FE model.
449 Each velocity on the (x, y) plane is mapped onto points of the deformed mesh with closer
450 (x, y) positions. Nodes with non-associated velocity were deformed according to
451 Cauchy's equilibrium equation for a viscoelastic material and discretized (see
452 Experimental Procedures). **D)** As **Figure 3F**, but showing the points of maximum
453 compression (minimum value of σ'''') before (white dots) and after smoothing (white
454 lines). **E)** Snapshots of deformed FE model showing contour plot of the AP normal stress
455 σ_{xx} superimposed over the corresponding images (ventral view) of Fas2-GFP embryos.
456 Scale bar 50 μm .

Figure S5



457

458 **Figure S5: Kymographs of rest-length and condensation diagrams on four planes of**
 459 **the parameter space**

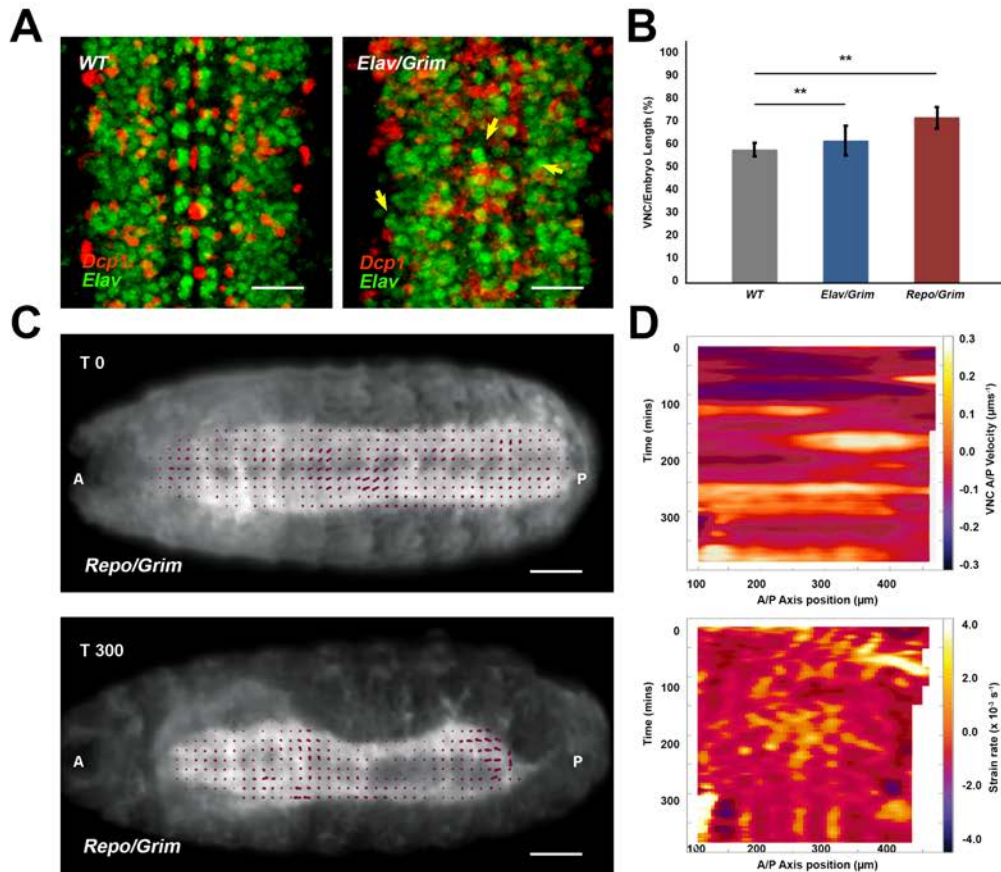
460 **A-D)** Rheological model with delay using different material parameters and numerical
 461 simulation **A)** Stable oscillation using reference values: remodeling rate $\gamma = 0.21 \text{ s}^{-1}$,
 462 viscous friction $\eta = 15 \text{ Pa}\cdot\text{s}$, delay $\Delta t = 20 \text{ s}$, and stiffnesses $k_1 = 0.01 \text{ Pa}$ and $k_2 = 1.9 \text{ Pa}$,
 463 respectively. Initial rest-length is $L_0 = 0.95l_0$, with l_0 being the initial apparent length. **B)**

464 Unstable oscillations due to increase of remodeling rate ($\gamma = 0.22 \text{ s}^{-1}$). **C)** Unstable
465 oscillations due to increase of viscosity $\eta=16 \text{ Pa.s}$. **D)** Unstable oscillations due to
466 decrease of stiffness $k_2=1.8 \text{ Pa}$. **E-H)** Relative shortening measured as the relative final
467 length, l_{final}/l_0 , for different combinations of perturbed values of model parameters: elastic
468 stiffness k_1 , viscosity η , delay δt , and remodeling rate γ . **A)** Stifness / Viscosity. **B)**
469 Stifness / Remodeling rate. **C)** Stifness / Delay time. **D)** Remodeling rate / Delay time.
470 Values have been chosen around the reference parameters $(k_1, \eta, \delta t, \gamma) = (0.01, 15, 20,$
471 $0.2)$. Effects of stiffness κ_2 of viscoelastic branch are similar to those of k_1 .

472

473

Figure S6



474

475 **Figure S6: Large-scale forces and local tissue dynamics are modulated by neurons**
476 **and glia**

477 **A)** CNS Flat-preps of WT (top) and Elav-Gal4>UAS-Grim (bottom) embryos, at stage
478 16, immunostained for Dcp1 (red) and Elav (green). Yellow arrowheads point to ELAV
479 positive, Dcp1 negative cells, which are neurons that have not engaged to apoptosis. Scale
480 bar 10 μm . **B)** Quantification of VNC length (VNC/Embryo Length %) of WT (gray),
481 Elav-Gal4>UAS-Grim (blue) and Repo-Gal4>UAS-Grim (red) embryos, at stage 16.
482 Bars represent mean values (n=6 embryos). ****** $p < 10^{-2}$. **C)** Snapshots from light-sheet

483 imaging recordings of a Repo-Gal4::UAS-mCD8-GFP::His2Av-mRFP>UAS-Grim
484 embryo (ventral view) at two different times of development (Stages 15-17) (**Movie S10**).
485 Magenta arrows denote local velocity trajectories from PIV analyses. AP axis orientation
486 is indicated. Scale bar 50 μm . **D**) Velocity (as in **Figure 1C**) and strain rate (as in **Figure**
487 **2D**) kymographs for a representative Repo-Gal4>UAS-Grim embryo. No periodic
488 oscillations were observed.

489

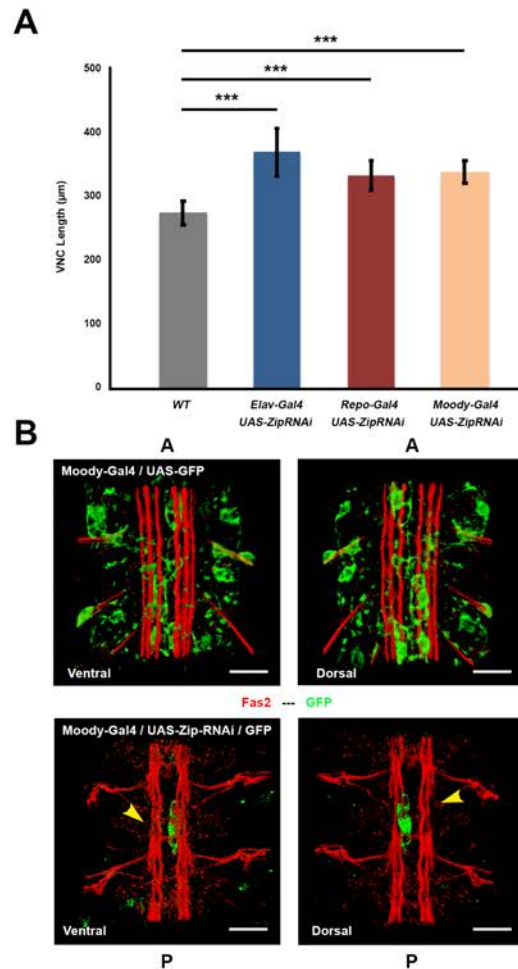
490

491

492

493

Figure S7



494

495 **Figure S7: Subperineural glia contractility is necessary for condensation and VNC**
 496 **organization.**

497 **A)** Quantification of VNC length of WT (gray), Elav-Gal4>UAS-Zip-RNAi (blue),
 498 Repo-Gal4>UAS-Zip-RNAi (red) and Moody-Gal4>UAS-Zip-RNAi embryos (orange),
 499 at stage 16. Bars represent mean values (n = 5 embryos). ***p < 10⁻³. **B)** Ventral and
 500 Dorsal 3D views of dissected, stage 16, control (top) and Moody-Gal4::UAS-mCD8-
 501 GFP>UAS-Zip-RNAi (bottom) embryos, immunostained for Fas2 (red) and GFP (green).
 502 Yellow arrowheads point to the disrupted axonal network. AP axis orientation is
 503 indicated. Scale bar 10 µm.

504

505 **SUPPLEMENTARY MOVIES LEGENDS**

506

507 **Movie S1. VNC Cytoskeleton structural organization**

508 Animated 3D reconstruction of a section of the VNC of a late *Drosophila* embryo (Stage
509 16) highlighting the levels of expression (Fire Lut) of different cytoskeletal components:
510 the axonal pattern stained with anti Acetylated α -Tubulin antibodies (left); the iterated
511 segmental distribution of actin (Phalloidin staining) along the AP axis (centre); and the
512 distribution of NMM accumulating at the longitudinal dorsomedial edges of the
513 neuropile. Scale bar 15 μ m.

514

515 **Movie S2. Dynamics of VNC condensation**

516 Time lapse of an *elav-Gal4>UASmCD8-GFP* embryo (lateral view) recorded by confocal
517 microscopy. mCD8-GFP labeling marks all neural derivatives. AP axis orientation is
518 indicated. Time in hours. Scale bar 50 μ m.

519

520 **Movie S3. Isotropic three-dimensional reconstructions of embryo images**

521 Time lapse recorded by multi-view light-sheet imaging of a live Histone2Av-mCherry
522 embryo (ventral view). mCherry labeling marks all nuclei and was used to correct the
523 embryo twitching. Raw data is shown on the top and “detwitched” images on the bottom.
524 AP axis orientation is indicated. Time in hours. Scale bar 50 μ m.

525

526 **Movie S4. Anterior and posterior contractile oscillations (stationary domain)**

527 Time Lapse recording of an embryo expressing Fas2-GFP (Top - ventral view; Bottom -
528 re-slice over the Z-axis) acquired by Confocal Microscopy. The double headed arrow
529 points to the stationary domain where converge anterior and posterior condensation. AP
530 axis orientation is indicated. Time in hours. Scale bar 50 μ m.

531

532 **Movie S5. VNC response to laser microsurgery during condensation**

533 Laser ablation of stage 14 embryos expressing alpha Tubulin-GFP. The recoil of
534 intercommissural (left) and intracommissural (right) cuts are compared. Yellow lines
535 highlight the position of the laser cuts. AP axis orientation is indicated. Time in seconds.
536 Scale bar 20 μ m.

537

538 **Movie S6. VNC condensation is segmentally autonomous**

539 Evolution over time of a laser cut at the intercommissural space between the abdominal
540 segments A1 and A2, of a stage 14 embryo, expressing alpha Tubulin-GFP. After
541 ablation, the individual neuromeres (color coded dots at the bottom mark the positions of
542 the anterior and posterior commissures of each neuromere at sequential times) continue
543 to condense autonomously. Yellow line highlights the position of the laser cut. AP axis
544 orientation is indicated. Time in hours. Scale bar 20 μ m.

545

546 **Movie S7. Three-dimensional Finite Element model (FE)**

547 The measured velocity field was mapped onto the FE model to reconstruct strain and
548 stress fields. Evolution through time of contour plots of AP stresses σ''' (FE model)
549 superimposed over experimental live images (ventral view) of an embryo expressing
550 Fas2-GFP. AP axis orientation is indicated. Time in hours. Scale bar 50 μ m.

551

552 **Movie S8. Glia participates in the architectural organization of the VNC and its**
553 **condensation**

554 Time lapse recordings of WT (Top) and Repo-Gal4>UAS-Grim (bottom) embryos in an
555 alpha Tubulin-GFP background (ventral view) acquired by Confocal Microscopy. AP
556 axis orientation is indicated. Time in hours. Scale bar 50 μ m.

557

558 **Movie S9. VNC condensation requires the mechanical contribution of glia**

559 Light-sheet imaging record of a Repo-Gal4::UAS-mCD8-GFP::His2Av-mRFP>UAS-
560 Grim embryo (ventral view) at different times of development (Stages 15-17). Magenta

561 arrows denote local velocity trajectories from PIV analyses. The VNC is significantly
562 elongated and misshaped. AP axis orientation is indicated. Time in hours. Scale bar 50
563 μm .

564

565 **Movie S10. Distinct roles for neurons and glia in VNC architecture and**
566 **condensation**

567 Time lapse recordings of embryos expressing Fas2-GFP, monitored by confocal imaging.
568 From top to bottom, condensation dynamics in control (WT); Elav-Gal4>UAS-Zip-RNAi
569 and Repo-Gal4>UAS-Zip-RNAi embryos. AP axis orientation is indicated. Time in
570 hours. Scale bar 50 μm .

571

572 **Movie S11. Finite Element model of VNC condensation**

573 Three-dimensional representation of VNC condensation. FE model showing the
574 evolution through time of contour plots of AP displacements (top) and experimental live
575 images (ventral view) of an embryo expressing Fas2-GFP (bottom). AP axis orientation
576 is indicated. Time in hours.

577

578 **Movie S12. Myosin-mediated contractility in neurons and glia is required for VNC**
579 **condensation**

580 Finite element simulations with mapped velocities of control (WT); Elav-Gal4>UAS-
581 Zip-RNAi and Repo-Gal4>UAS-Zip-RNAi embryos. Contour plots in the top row show
582 the AP displacement fields and in the bottom row the elastic strains ϵ_{xx} .

583

584