

1 Interkinetic nuclear movements promote apical expansion in pseudostratified
2 epithelia at the expense of apicobasal elongation

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15

16 Abstract

17 Pseudostratified epithelia (PSE) are a common type of columnar epithelia found in a wealth of
18 embryonic and adult tissues such as ectodermal placodes, the trachea, the ureter, the gut and
19 the neuroepithelium. PSE are characterized by the choreographed displacement of cells'
20 nuclei along the apicobasal axis according to phases of their cell cycle. Such movements,
21 called interkinetic movements (INM) have been proposed to influence tissue expansion and
22 shape and suggested as culprit in several congenital diseases such as CAKUT and esophageal
23 atresia. INM rely on cytoskeleton dynamics just as adhesion, contractility and mitosis do.
24 Therefore, longer term impairment of INM without affecting proliferation and adhesion is
25 currently technically unachievable. Here we bypassed this hurdle by generating a 2D agent-
26 based model of a proliferating PSE and compared its output to the growth of the chick
27 neuroepithelium to assess the interplay between INM and these other important cell
28 processes during growth of a PSE. We found that INM directly generates apical expansion and
29 apical nuclear crowding. In addition, our data strongly suggest that apicobasal elongation of

30 cells is not an emerging property of a proliferative PSE but rather requires a specific
31 elongation program. We then discuss how such program might functionally link INM, tissue
32 growth and differentiation.

33

34 Authors Summary

35 Pseudostratified epithelia (PSE) are a common type of epithelia characterized by the
36 choreographed displacement of cells' nuclei along the apicobasal axis during proliferation.
37 These so-called interkinetic movements (INM) were proposed to influence tissue expansion
38 and suggested as culprit in several congenital diseases. INM rely on cytoskeleton dynamics.
39 Therefore, longer term impairment of INM without affecting proliferation and adhesion is
40 currently technically unachievable. We bypassed this hurdle by generating a mathematical
41 model of PSE and compared it to the growth of an epithelium of reference. Our data show
42 that INM drive expansion of the apical domain of the epithelium and suggest that apicobasal
43 elongation of cells is not an emerging property of a proliferative PSE but might rather requires
44 a specific elongation program.

45

46 Introduction

47 Pseudostratified epithelia (PSE) are a special type of columnar epithelia in which cells
48 are thin and elongated. Nuclei packing is very high and forces cells to distribute their nuclei
49 along the apicobasal axis creating multiple layers of nuclei within a monolayer of cells, hence
50 the term pseudostratification. PSE are found across the animal kingdom from invertebrates to
51 vertebrates [1]. During development, several structures adopt a pseudostratified
52 configuration such as the placodes and the central nervous system in vertebrates or the
53 imaginal discs in Drosophila. In adults, PSE can be found along the respiratory, urinary and
54 digestive tracts (e.g trachea, ureter, midgut) [2, 3] and various organs such as the gonads (e.g.
55 epididymis) or the eye (lens, retina) [1]. One characteristic feature of PSE is the coordinated
56 movements of nuclei during the cell cycle called interkinetic nuclear movements (INM) [4].
57 INM are decomposed in several steps: an apical to basal movement occurring during the G1
58 and S phases of the cell cycle and a basal to apical nuclear movement occurring during the G2
59 and M phases. The apical-ward movement, sometimes referred to as PRAM (Pre-mitotic
60 Rapid Apical Movement), can be achieved via microtubules like in the chick neuroepithelium
61 [5] or in the brain of mouse and rat embryos [6, 7] as well as in the retina of post-natal mice
62 [8]. Such movements can also occur in an actomyosin-dependent manner as observed in the
63 retina of fish embryos [9]. The return of nuclei to basal positions after mitoses was initially
64 proposed to be passive and a direct consequence of nuclear crowding in the apical region of
65 PSE. However, there are numerous evidence indicating an active role of the cytoskeleton in
66 apical to basal nuclear displacement. For instance, Kif1A, an anterograde molecular motor of
67 microtubules, is required for the apical to basal movement of nuclei in rat brain [7]. In
68 addition, in mouse telencephalon, myosin II was shown to be essential for apical to basal
69 movement [10]. Further, in ferrets' brains apical to basal movements of nuclei are faster than

70 basal to apical movements suggesting that the nuclear movement towards basal regions of
71 the brain is active while the opposite is observed in mouse [11]. All these observations
72 indicate that INM is regulated by cytoskeleton-dependent mechanisms and that the actual
73 mechanism employed differs from species to species and organ to organ.

74 One consequence of the cytoskeleton-based regulation of INM is that it renders INM
75 difficult to study *in vivo* since it is far from the only cell process that relies on cytoskeleton
76 dynamics. Cell-cell, cell-matrix adhesions and cell contractility require normal microfilaments
77 and microtubules dynamics and mitosis relies on microtubules-driven separation of
78 chromosomes and actomyosin-dependent cytokinesis. Therefore, it is currently technically
79 impossible to study the specific roles that INM might have in PSE dynamics, growth and shape
80 over long periods of time (hours to days) without impairing adhesions, contractility or cell
81 division. This motivates the use of alternative approaches, such as computational modelling.

82 Many models of cell tissue mechanics can be found in the literature, ranging from
83 agent-based [12] to continuum models [13, 14]. Agent-based models describe the tissue at
84 the cell scale and have been used to study local phenomena, such as the influence of the
85 variation of spatial constraints in the cell cycle [15], how curvature of an epithelial sheet is
86 determined by mechanical tensions [16] or how contact inhibition of locomotion generates
87 forces in the tissue [17]. Continuum models instead describe the system at the tissue scale
88 (cell density) and study global properties, such as the tissue curvature, resistance to
89 deformation [18], contraction-elongation and tissue shear flow [13]. Despite being easier to
90 treat, both computationally and analytically, continuum models do not incorporate all
91 information about individual cell shape and position. An agent-based model instead is able to
92 provide detailed spatial information and, in particular, it can account for variability in cell
93 characteristics associated with the different stages of the cell cycle and variability in cell shape

94 associated with the dynamics of INM (see [19] for a comparison between the continuum and
95 agent-based frameworks). For these reasons, we opted for the agent-based approach. A large
96 number of agent-based models of cell tissues have been developed in the last decades. The
97 well-known Potts model [20] is a lattice-based model in which the cells may have complex
98 shapes with a desired resolution. However, it has been reported that grid artefacts occur in
99 cell movement and intercellular interactions [21] and they increase with particle density [22],
100 which makes this model unsuited to describe crowded systems. Off-lattice models include for
101 example the vertex model [15, 18, 23-25] and the Voronoi model [26, 27]. The tissue is
102 regarded as a partition of space where each part represents one cell that is contiguous to its
103 neighbours with no intercellular space between them. These models are able to describe
104 densely packed systems. However, congestion is encoded into the model. In a PSE, nuclear
105 crowding may not occur every time nor everywhere, so it should not be included in the model
106 but rather occur as an emergent phenomenon.

107 Therefore, we reasoned that an appropriate framework to model a PSE would be an
108 agent-based model where each cell moves in an off-lattice domain and interacts with its
109 neighbours. Using such model, and comparing it with the chick neuroepithelium as a
110 biological PSE of reference, we have explored the impact of INM, proliferation, adhesion and
111 contractility on tissue shape, position of mitoses, pseudostratification and growth. Our results
112 indicate that INM generates apical nuclear crowding, opposes apical shrinkage due to apical
113 contractility and directly favors tissue growth oriented perpendicularly to the apicobasal axis
114 (dorsoventrally, anteroposteriorly). Interestingly, all characteristics observed in the chick
115 neuroepithelium such as apical positioning of mitoses, apical straightness, apical nuclear
116 crowding and pseudostratification emerge from a combination of INM, proliferation, apical
117 contractility and cell adhesion. However, the sustained linear apicobasal growth observed

118 during development of the chick spinal cord cannot be reproduced with this combination of
119 parameters. We show that neuroepithelial cells undergo a dramatic change of shape
120 concomitantly to a reduction of cell volume while elongating along the apicobasal axis. This
121 change of cell shape exceeds what is needed to accommodate new nuclei added by
122 proliferation. Therefore, our simulations and in vivo observations strongly suggest that, while
123 INM contributes to the expansion of the apical domain, the observed in vivo apicobasal
124 elongation requires a specific elongation program. We then discuss whether such program,
125 together with INM promoting apical nuclear crowding, might be a way to coordinate tissue
126 growth and differentiation.

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130 Results

131 Evolution of the chick trunk neuroepithelium from two to four days of development

132 As a biological PSE of reference, we chose the trunk neuroepithelium of the chick
133 embryo. The neuroepithelium is a well-described PSE and has the advantage of being easily
134 accessible for observation and manipulation. We started by monitoring the evolution of the
135 neuroepithelium at the level of the prospective forelimb (somites 15-20) between two and
136 four days of development [28], corresponding to developmental stage HH13- (18 somites,
137 48h of incubation) to stage HH23 (96 hours of incubation) and performed transversal
138 cryosections (Fig 1A-B). Importantly, we focused on the intermediate region (half way
139 between the dorsal and ventral sides of the tissue) to avoid the effect of extensive neuronal
140 delamination and differentiation occurring in the ventral region of the tissue at these stages.
141 We then performed immunostaining for phospho-histone H3 to label cells in mitosis and

142 counterstained for actin and DNA (Fig 1C-D). We found that the dorsoventral size of the
143 neuroepithelium increases 2.45-fold over two days, going from an average of 133 μ m to
144 326 μ m (Fig 1E), while the apico-basal size of the tissue grows by 2.3-fold from 50 μ m to
145 115 μ m (Fig 1F). Over the same period, the antero-posterior distance from somite 15
146 caudalward increases 3.56-fold, going from 2.6 to 9.4mm (S1 Fig). This indicates that growth
147 of the posterior neuroepithelium between 2 and 4 days of development is biased toward
148 anteroposterior growth. The number of pseudolayers of nuclei from apical to basal increases
149 by 2.5-fold (Fig 1G). Interestingly, the distribution of nuclei changes from a homogenous
150 distribution along the apicobasal axis in 48h-old embryos (Fig 1H, light green curve) to an
151 accumulation of nuclei in the apical region in 72 and 96h-old embryos (Fig 1H, dark green
152 curves). Note that nuclei density is lower in the basal region of the epithelium (Fig 1D). A
153 change of average nuclear shape is also observed. The aspect ratio goes from 1.67 at 2 days
154 to 2.01 at 4 days (Fig 1I). Nuclei become more elongated along the apicobasal axis (Fig 1D).
155 This is due to a shortening of the nuclear length along the DV axis between 2 and 3 days of
156 development while the length along the AB axis remains constant. By contrast, other
157 parameters such as the straightness of the apical domain (Fig 1J), or the mean position of
158 mitoses along the apicobasal axis (Fig 1K-L) do not significantly change (fold change inferior to
159 1.1). From these observations, we next wondered whether the balance between cell
160 adhesion, proliferation and INM would be sufficient to drive the growth of the
161 neuroepithelium, the progressive apical accumulation of nuclei and the increased
162 pseudostratification, while apical positioning of mitoses and apical straightness remain
163 constant.
164

165 **Fig 1. Growth of caudal neuroepithelium of the chicken embryo from 2 to 4 days of**
166 **development.** A, Diagram of a chicken embryo at 48h of incubation (stage HH13-, 18 somites).
167 B, transversal cryosections at the level of the forelimb at 48h, 72 and 96h of incubation. C-D,
168 Confocal images of the intermediate region of the neuroepithelium at the level of the
169 forelimb at 48, 72 and 96h of incubation, nuclei are stained with DAPI (grey) and actin with
170 Phalloidin (green). Magenta line, apical domain; cyan line, basal domain. Note that the basal
171 region progressively becomes devoid of nuclei. E, net dorsoventral length of the neural tube.
172 F, net apicobasal length of the neural tube. G, number of pseudolayers of nuclei along the
173 apicobasal axis. H, distribution of DAPI intensity along the apicobasal axis, normalized to the
174 peak intensity in each dataset and to the apicobasal size so that the various stages can be
175 compared. I, ratio of apicobasal and dorsoventral length of nuclei. J, straightness of apical
176 domain (net distance between dorsal-most and ventral-most points of the apical surface
177 divided by the actual length of the apical surface between these two points). K, positions of
178 mitotic cells (either phospho-histone H3-positive cells or cells with condensed chromosomes
179 from DAPI staining), at scale with the actual apicobasal size of the neural tube. L, positions of
180 mitoses, raw data. Descriptive data collected from 20 embryos. Dots represent mean values.
181 Error bars show the standard deviation. Box and whiskers plot: the box extends from the 25th
182 to the 75th percentile; the whiskers show the extent of the whole dataset. The median is
183 plotted as a line inside the box. AB, apicobasal; DV, dorsoventral; HH, Hamburger-Hamilton
184 stages of chicken development; ss, somites.

185

186 **Agent-based model of PSE dynamics**

187 To be able to assess the impact of INM versus other cytoskeleton-dependent
188 processes (e.g. adhesion, mitosis), we built an agent-based model of the neuroepithelium.

189 The chick neuroepithelium is an elongated PSE meaning that cells are very thin tubes with a
190 large protruding nucleus giving them a watermelon-in-a-sock morphology (Fig 2A, S1 Movie).
191 Cells are polarized according to the apicobasal axis with most of the cell-cell junctions
192 localized apically and, conversely, the cell-matrix adhesions located basally [29]. In the model,
193 each cell is approximated to a nucleus, an apical point and a basal point. The two points are
194 attached to the nucleus via dynamic adjustable springs representing the viscoelastic
195 properties of the cytoplasm (Fig 2B). Cells are placed next to one another along a lateral axis
196 perpendicular to the apicobasal axis. Since the model is in 2D, this lateral axis can represent
197 either the dorsoventral axis or the anteroposterior axis. Importantly, cells cannot intercalate
198 nor swap positions. To model cell-cell and cell-matrix interactions we use simple mechanical
199 and behavioural rules. On the apical side, apical points are attached to each other by apical-
200 apical springs representing cell-cell adhesion. On the basal side, basal points are attached to a
201 fixed basal line representing the basal lamina of the epithelium. Basal points can only move
202 along the basal line and a maximum distance between adjacent basal points is implemented
203 to avoid uncontrolled flattening of the tissue along the basal line. As in the real epithelium,
204 apical points are only attached to their direct neighbors and thus can move within the 2D
205 domain. Neuroepithelial cells are known to keep a straight shape. Thus, an alignment
206 mechanism is set to prevent the apical point, the nucleus and the basal point of each cell from
207 deviating significantly from a straight line. Nuclei cannot overlap. In the literature, non-
208 overlapping constraints are approximated by a soft repulsion potential [30]. However, despite
209 being computationally less expensive, this approximation becomes less and less accurate as
210 the compression forces generated by congestion increase. Instead, we consider the nucleus
211 being formed by an inner sphere (the hard core) and an outer sphere (the soft core). This
212 representation allows soft cores to overlap with one another representing the deformation

213 that would occur when two nuclei are pressed against each other [31]. In the chick
214 neuroepithelium, nuclei are slightly compressed along the dorsoventral axis, giving them an
215 elongated form along the apicobasal axis (see Fig 1C-D). Overlap of soft cores leads to a
216 repulsive force which is controlled by the non-overlapping constraints imposed to the nucleus
217 inner core.

218

219 **Fig 2. Agent-based model of the pseudostratified epithelium.** A. 3D confocal image of a single
220 neuroepithelial cells expressing membrane-GFP, in a 2-day old chick neuroepithelium (see S1
221 Movie). B. Cells in the model are abstracted to a nucleus attached to a set of springs. C,
222 implementation of cell cycle and INM during the simulation. Cells in the model constantly
223 proliferate by going through a simplified cell cycle corresponding to three phases: a
224 G1/S/pассив G2 phase during which springs connected to the nucleus adjust to local
225 constraints, a PRAM/active G2 phase during which apical-nucleus springs shrink while
226 nucleus-basal springs elongate to recapitulate INM movements and the M phase during which
227 springs behave as in G2. In addition, the hard core of nuclei enlarges in M phase to account
228 for cell swelling and stiffening. Finally, at the end of the M phase each cell gives two daughter
229 cells. One is systematically kept within the 2D-plane, the other daughter cell can be excluded.
230 This parameter allows to control the rate of growth of the tissue independently of the pace of
231 the cell cycle. See Supplementary Information for a detailed description of the model.

232

233 A clock, representing a simplified cell cycle, rules the proliferation rate. This *in silico*
234 cycle has 3 phases. A first phase corresponding to G1, S and the part of G2 during which no
235 directed movements of nuclei take place (Fig 2C; G1/S/pассив G2). A second phase
236 accounting for the active nuclear movements occurring in G2 known as pre-mitotic rapid

237 apical movements (PRAM; Fig 2C, PRAM/active G2). A third phase representing Mitosis (Fig
238 2C; M). PRAM are implemented as follows: in cells entering active G2, the preferred rest
239 length of apical-nucleus springs is set to 0 and the preferred rest length of the nucleus-basal
240 spring is set to the current height of the cell. This drives an active apical-ward movement of
241 the nuclei. In addition, during mitosis, the hard core of the nucleus increases (Fig 2C). Given
242 that in our model cells have no cytoplasm, the increase of the hard core represents the
243 known swelling and stiffening of cells during mitoses [32, 33]. At the end of mitosis daughter
244 cells can be kept within the 2D-plane of the model or excluded. Thus, by systematically
245 excluding 50% of the daughter cells we can keep the size of the cell population constant. This
246 allows us to decouple the cell cycle and INM from actual proliferation (increase of cell number
247 over time). Importantly, given the current lack of consensus about systematic active basal-
248 ward movement of nuclei during G1 and S phases, we chose not to implement active
249 movements of nuclei toward the basal side of the tissue in our model.

250 Outside of the PRAM/active G2 and M phases, apical-nucleus and nucleus-basal
251 springs adjust their preferred rest length to their actual length, thus incorporating viscous
252 behavior into the cytoplasm dynamics. This allows cells to accommodate their nuclei all along
253 the apicobasal axis according to local constraints and forces (e.g., the position of the other
254 nuclei or the forces on the various springs). Furthermore, there is a noise factor that allows
255 nuclei to randomly move from their current location at each iteration of the simulation. In the
256 chick neuroepithelium, nuclei are not known to display large scale random movements
257 outside of PRAM. Thus, in our simulations, the noise is set very low compared to PRAM.

258 Each simulation is initialized with 30 cells. All cells have their apical point, nucleus and
259 basal point aligned. Apical points and basal points are evenly distributed. This can be seen in
260 the first frame of S2, S3 and S4 Movies. Then, at each time-iteration, the simulation runs as

261 follows: springs and nuclei are updated according to the position of each cell in the cell cycle,
262 cells that are in mitosis divide and noise is implemented. Mitosis and noise may lead to the
263 violation of the non-overlapping constraints on the nuclei hard cores. A minimization
264 algorithm developed in [34] is then used to obtain an admissible configuration. This
265 configuration corresponds to a local minimizer of the total mechanical energy in the system
266 associated to the springs, nuclei soft core and alignment forces. A complete description of the
267 mathematical model can be found in Supplementary Information and all parameters used for
268 the simulations presented in all figures hereafter are summarized in S1 Table.

269

270 **INM opposes apicobasal elongation, generates apical nuclear crowding and enlarges the**
271 **apical domain**

272 To start with, we checked the evolution of the tissue in absence of proliferation (no
273 INM, Fig 3) to assess the influence of cell-cell, cell-matrix adhesions and the non-overlapping
274 constraints between nuclei. To do so, we set the minimum duration of the cell cycle to 10000
275 hours making it unlikely that any cells would divide during the course of the 48-hour
276 simulation. The apical-apical springs were set to be passive, meaning that they do not adjust
277 their size in response to stretch or compression. In these conditions, there is no change in
278 apicobasal size of the tissue (Fig 3A, red curve, AB) with nuclei distributing homogenously
279 halfway along the apicobasal axis (Fig 3A, red curve, N). The number of pseudolayers remains
280 constant (Fig 3B, red curve) and the apical domain stays flat (Fig 3C, red curve). See S2 Movie.
281 In order to assess the effect of INM without adding more cells to the tissue, we set the
282 exclusion rate of daughter cells to 50%. This means that after mitosis only one of the
283 daughter cells was kept in the 2D-plane, keeping the total cell number constant. The total cell
284 cycle duration is set to a range of 10 to 21 hours corresponding to averages of the known

285 values for the duration of the cell cycle in the chick trunk neuroepithelium between 2 and 4
286 days of development [35]. G2 and M phases respectively last 90 and 30 minutes each,
287 however active nuclear movements corresponding to PRAM only occur in a fraction of the
288 total G2. Thus, in the model to generate normal INM conditions we set PRAM/active G2 and
289 M phases to 30 min each (normal INM, Fig 3A-C, black curves). To generate low INM
290 conditions where cells actively displace their nuclei for a shorter period, we set the duration
291 of PRAM/active G2 to 0 and M to 6 minutes (one iteration only) (low INM, Fig 3A-C, brown
292 curves). Introducing low or normal INM slightly reduces the apicobasal size of the tissue (Fig
293 3A, brown and black curves AB are below the corresponding red curve) and the average
294 position of nuclei shifted apically (Fig 3A, brown and black curves N are above the
295 corresponding red curve). Adding INM also leads to a slight decrease in terms of pseudolayers
296 (Fig 3B, black curve) and apical straightness (Fig 3C, black curve). Normal INM parameters
297 lead to apical mitosis whereas low INM lead to a widespread distribution of mitoses along the
298 apicobasal axis (Fig 3D). These data indicate that INM is sufficient to drive global nuclear
299 apical crowding (Fig 3A, black curve), to slightly destabilize apical straightness and of course
300 to control apical positioning of mitoses. In addition, we checked the lateral expansion of the
301 apical, nuclear and basal domains under each of these 3 conditions. Each domain is defined
302 by the length between the proximal and distal apical, nuclear and basal points along the
303 lateral axis, respectively (Fig 3E-G). These analyses reveal that INM promotes the expansion of
304 the apical domain, especially when all mitoses are apical (Fig 3E-G, magenta curves, arrow).
305 See S2 Movie.
306

307 **Fig 3. INM opposes apicobasal elongation, generates apical nuclear crowding and enlarges the**
308 **apical domain.** Simulations with passive apical-apical springs with INM (black and brown

309 curves) or without INM (red curves) with constant cell number (see S2 Movie). A, Apicobasal
310 length of the PSE (AB) and mean nuclear position along the AB axis, expressed in
311 micrometers. Note that INM reduces apicobasal length (black curve with open circles (INM) is
312 below the red curve (no INM)) and generates apical nuclear crowding (black curve with closed
313 circles (INM) is above the red curve (no INM)). B, Number of layers of nuclei along the AB axis.
314 C, straightness of apical domain (net distance between the first and last apical point divided
315 by the actual distance between these two points). D, Position of mitoses along AB axis, 1
316 being apical. E-F, mean width of apical (magenta), nuclear (black) and basal (cyan) domains of
317 the PSE over time. For each domain, the distance the between the first and last point along
318 the lateral axis is computed and its evolution plotted over time. Note that INM promotes
319 enlargement of the apical domain (panel 3G, arrow). Each simulation was performed over 480
320 iterations (48h of biological time) for 10 repetitions. Each curve represents the mean value of
321 each dataset for the parameter plotted. Box and whiskers plot: the box extends from the 25th
322 to the 75th percentile; the whiskers show the extent of the whole dataset. The median is
323 plotted as a line inside the box.

324

325 Apical domains of epithelia are known to be dynamic and to display actomyosin
326 contractility [36]. In addition, epithelial cells are known to resolve local imbalances in tension,
327 compression and shear by aligning their cytoskeleton [37]. Further, apical constriction is
328 known to be important for epithelial cell shape changes from squamous to columnar which
329 corresponds to an apicobasal elongation [16, 36]. Thus apical constriction may be an
330 important driving force for apicobasal elongation in PSE. To explore this hypothesis, we first
331 check that actomyosin contractility was important in the chick neuroepithelium by treating
332 samples with the ROCK inhibitor, a compound specifically blocking Rho-dependent myosin

333 contractility (S2 Fig). Indeed, a brief 2-hour treatment with the ROCK inhibitor leads to a
334 decrease of the apicobasal length, a reduction in pseudostratification, a rounding of nuclei
335 and a decrease of the apical straightness. In addition, mitoses are not systematically apical (S2
336 Fig). These data indicate that the chick neuroepithelium has a short-term reliance on
337 actomyosin contractility to maintain its shape and mitoses positions. Therefore, we ran the
338 same simulations as above but setting the preferred rest length of apical springs to 0 in order
339 to model overall apical contractility. Therefore, apical springs dynamically adjust their sizes to
340 reach this rest length (Fig 4). We found that introducing contractile apical springs leads to a
341 slight increase of apicobasal elongation (Fig 4A, AB curves) but overall nuclear positioning (Fig
342 4A, N curves), pseudostratification (Fig 4B) and tissue shape (Fig 4C) are not dramatically
343 affected by having a contractile apical domain. In the absence of INM, contractile apical
344 springs lead to a rapid shrinkage of the apical domain (Fig 4E, arrowhead). This effect is
345 prevented by adding INM (Fig 4G, arrow) indicating that apical mitoses can oppose apical
346 constriction (S2 Movie).

347

348 **Fig 4. INM opposes apical constriction.** Same simulations as in Fig 3 but with contractile
349 apical-apical springs with INM (black and brown curves) or without INM (red curve), with
350 constant cell number (see S2 Movie). A, Apicobasal length of the PSE (AB) and mean nuclear
351 position along the AB axis, expressed in micrometers. B, Number of layers of nuclei along the
352 AB axis. C, straightness of apical domain (net distance between the first and last apical point
353 divided by the actual distance between these two points). D, Position of mitoses along AB
354 axis, 1 being apical. E-G, mean width of apical (magenta), nuclear (black) and basal (cyan)
355 domains of the PSE over time. For each domain, the distance the between the first and last
356 point along the lateral axis is computed and its evolution plotted over time. Note that apical

357 contractility reduces the width of the apical domain (panel 4E, arrow), whereas introducing
358 INM opposes apical constriction (panel 4G, arrow; red curve in G is higher than in E). Each
359 simulation was performed over 480 iterations (48h of biological time) for 10 repetitions. Each
360 curve represents the mean value of each dataset for the parameter plotted. Box and whiskers
361 plot: the box extends from the 25th to the 75th percentile; the whiskers show the extent of
362 the whole dataset. The median is plotted as a line inside the box.

363

364 **Increase in cell number strongly increases pseudostratification but has a weak effect on**
365 **apicobasal elongation**

366 Since neither INM nor apical constriction are sufficient to drive extensive apicobasal
367 elongation, we next wanted to compare the impact of having passive or contractile apical-
368 apical springs in the context of increasing cell number by proliferation (allowing more than
369 one daughter cell to remain in the 2D plane after mitosis). For that, we ran two sets of
370 simulations with passive or contractile apical-apical springs and with different rates of
371 exclusion of daughter cells ranging from 0% (all cells generated by mitosis are added to the
372 2D plane) to 50% (one daughter cell is systematically excluded). INM is set to normal with
373 PRAM/active G2 and M phases lasting 30 min each in all conditions. All outputs of simulations
374 with passive apical-apical springs are plotted in shades of grey to black whereas outputs for
375 simulations with contractile apical-apical springs are plotted in shades of hot colors from
376 yellow to dark red. Contractile apical-apical springs have a slight positive impact on tissue
377 apicobasal elongation (Fig 5A, AB curves; all colored curves are above their corresponding
378 grey-to-black curves). This is accompanied by a slight apical shift of nuclei (Fig 5A, N curves).
379 Pseudostratification correlates directly with the number of cells retained in the 2D plane (Fig
380 5B). At maximal (50%) or intermediate (40%, 30%) exclusion rates of daughter cells,

381 contractile apical-apical springs further increase pseudostratification (Fig 5B, orange and red
382 curves are above their cognate grey-to-black curves; S3 Movie). This effect is lost when all
383 cells are retained (Fig 5B, 0% exclusion, yellow and grey curves overlap). With passive apical-
384 apical springs the PSE shape becomes very sensitive to an increase of cell number. The more
385 cells are kept within the 2D plane of the epithelium the faster apical straightness decreases
386 (Fig 5C). Introducing contractile apical-apical springs mitigates the effect of hyper-
387 proliferation (Fig 5C, orange and red curves stay close to straightness of 0.9). We then
388 monitored the lateral expansion of apical, nuclear and basal domains over time for all
389 conditions (Fig 5D-I). Increase in cell number induces a rapid expansion of the apical domain
390 (Fig 5D-F). Interestingly, the apical shrinkage induced by contractile apical springs (Fig 5G, red
391 curve) can be opposed by increasing the number of cells (Fig 5H-I, magenta curves). In
392 addition, apical contractility positively feeds back into basal expansion. Note that the cyan
393 curves in panels 5H and 5I increase faster than in panels 5E and 5F. These data indicate that
394 an increase in total cell number drives a slight increase in apicobasal length, strongly drives
395 pseudostratification and, in addition to INM, leads to an expansion of the apical domain.
396 Interestingly, in the context of increasing cell number, apical contractility promotes basal
397 rearrangements, an effect not seen with constant cell numbers (compare the cyan curves in
398 panels 3E-G and 4E-G, with cyan curves in panel 5D, 5G). Further, apicobasal constriction
399 slightly contributes to pseudostratification and helps maintain tissue shape during tissue
400 growth.

401
402 **Fig 5. Increase in cell number drives pseudostratification and apical contractility feeds back**
403 **into basal rearrangements.** Simulations with passive or contractile apical springs, normal INM
404 and various rates of exclusion of daughter cells (see S3 Movie). A, apicobasal length of the PSE

405 (AB) and mean nuclear position along the AB axis (N) over time expressed in micrometers. B,
406 Number of pseudolayers of nuclei along the AB axis. C, straightness of apical domain (net
407 distance between the first and last apical point divided by the actual distance between these
408 two points). D-F, mean width of apical (magenta), nuclear (black) and basal (cyan) domains of
409 the PSE with passive apical-apical springs over time with 50 (D), 40 (E) and 30% (F) of
410 daughter cells being excluded from the 2D plane. G-I, mean width of apical (magenta),
411 nuclear (black) and basal (cyan) domains of the PSE with contractile apical-apical springs over
412 time with 50 (G), 40 (H) and 30% (I) of daughter cells being excluded from the 2D plane. For
413 each domain, the distance the between the first and last point along the lateral axis is
414 computed and its evolution plotted over time. Note that apical contractility leads to basal
415 rearrangements (compare cyan curves in H-I grow faster than in E-F). Each simulation was
416 performed over 480 iterations (48h of biological time) for 10 repetitions. Each curve
417 represents the mean value of each dataset for the parameter plotted.

418

419 **Neuroepithelial cells undergo a dramatic change in shape that exceeds what is needed to**
420 **accommodate nuclei along the apicobasal axis**

421 So far, our simulations reveal that a progressive increase of pseudostratification, apical
422 nuclear accumulation and apical mitoses can emerge from cell-cell/cell-matrix adhesion,
423 proliferation and INM. However, none of the conditions tested allows a rapid apicobasal
424 elongation of the tissue over 48h. This suggests that something is missing in our model. To
425 achieve fast apicobasal elongation, cells could either get bigger (increase of cell volume) or
426 elongate beyond what is necessary to accommodate the increase in number of nuclei due to
427 proliferation. Our descriptive *in vivo* data (Fig 1A-B) show that a region of low nuclei density is
428 formed in the basal domain of the chick neuroepithelium between 48h to 96 hours of

429 incubation. This is driven by INM in our model (Fig 3A). Such low basal density of nuclei has
430 been seen in other elongated PSE as well [29]. This observation suggests that apicobasal
431 elongation of cells may not be driven by the piling of nuclei along the apicobasal axis. Could
432 elongation be caused by a change of cell shape or are cells also changing in size? To answer
433 this question, we dissected explants of the neuroepithelium from the forelimb region (facing
434 somites 15 to 20) in embryos at 2, 3 and 4 days of development (Fig 6A). Neural tubes were
435 enzymatically isolated from surrounding tissues and dissociated to produce a suspension of
436 single neuroepithelial cells (see material and methods). Cells were automatically counted and
437 their diameters retrieved using a cell counter. From these measurements, volumes were
438 calculated. This analysis reveals that the mean volume of neuroepithelial cells decreases
439 between 2 and 3 days of development and remains stable from 3 to 4 days (Fig 6B-C). We
440 next checked the size of the apical domains by performing *en face* microscopy on
441 neuroepithelia from the same stages (Fig 6A, D-E). We found that the mean area occupied by
442 the cells' apical sides is also significantly getting smaller between 2 and 3 days of
443 development but does not significantly change from 3 to 4 days (Fig 6D-E). These data
444 indicate that neuroepithelial cells undergo a dramatic change of cell shape, together with a
445 reduction of cell volume, which appear to exceed what would be needed to accommodate
446 the increase of cell nuclei along the apicobasal axis. This strongly suggests that
447 undifferentiated PSE cells specifically elongate rather than simply adjust to local nuclear
448 crowding.

449

450 **Fig 6. Mean volume and mean apical surface of chick neuroepithelial cells decreases between**
451 **2 and 3 days of development.** A, Diagram depicting the regions used for the preparation of
452 cell suspensions and open book histology from chicken embryos at 2, 3 and 4 days of

453 development (see Methods for details of the experimental procedures). Region monitored
454 (from somite 15 to 20, forelimb region) is indicated by a dotted line. B, representative images
455 of neuroepithelial cells in suspension after neural tube dissection and enzymatic dissociation.
456 C, mean volume of neuroepithelial cells over time ($n_{48h}=82$; $n_{72h}=568$; $n_{96h}=1168$). Cells get
457 significantly smaller from 2 to 3 days of development and remain stable. Box and whiskers
458 plot: the box extends from the 25th to the 75th percentile; the whiskers show the extent of
459 the whole dataset. The median is plotted as a line inside the box. One-way ANOVA (Kruskal-
460 Wallis) followed by Dunn's multiple comparisons. ****, $p<0.0001$; ns, $p>0.9999$. D, *en face*
461 view of the apical domain of the intermediate region of the neuroepithelium (actin is stained
462 by Phalloidin). E, mean area of the individual apical surfaces over time ($n_{48h}=67$; $n_{72h}=66$;
463 $n_{96h}=81$). Apical surfaces shrink from 2 to 3 days of development and remain stable. Dots
464 represent mean of the dataset, error bars represent S.D. One-way ANOVA followed by
465 multiple comparisons. ****, $p<0.0001$; ns, $p=0.1586$.

466

467 Apicobasal elongation requires a specific elongation force

468 Therefore, we wondered whether adding a global non-oriented expansion force might
469 account for the apicobasal elongation observed *in vivo*. To do that, we increased the amount
470 of noise on nuclear position at each iteration. All nuclei are allowed to move in a random
471 direction at each iteration of the simulation. Since apical-nucleus and nucleus-basal springs
472 are able to update their rest length to adjust to their actual size, any increase in random
473 nuclear movements forces the apical-nucleus and nucleus-basal springs to stretch. Given that
474 cells are attached to the basal line, to each other and that cells are prevented from bending
475 due to an imposed alignment force, increasing random nuclear noise should generate a linear
476 apicobasal elongation force. We ran simulations with conditions similar to those presented in

477 Fig 4 but with a 25-fold increase of random nuclear movements (Fig 7). Under these
478 conditions, we observed a 2-fold increase in apicobasal length (Fig 7A, S4 Movie) whereas the
479 number of pseudolayers (Fig 7B) and apical straightness (Fig 7C) were similar to the values
480 obtained with low noise (Fig 5B-C). In addition, increasing noise does not affect the overall
481 dynamics of lateral expansion of the tissue (Fig 7D-F). At low percentages of exclusion of
482 daughter cells (30%) the lateral expansion of the apical domain is faster than that of the basal
483 domain (Fig 7F, compare magenta and cyan curves). Thus, we attempted to solve this issue by
484 allowing basal points to update their positions at a faster rate. This was sufficient to allow an
485 isotropic expansion of the tissue (Fig 7G, black, cyan and magenta curves grow at the same
486 pace; S4 Movie). Finally, our previous simulations (Figs 3 and 4) hinted that INM was capable
487 of opposing apicobasal elongation. We wanted to check if this was still true under the
488 extensive apicobasal growth generated by increased noise. We repeated the same
489 simulations as shown in Fig 7, excluding 50% of daughter cells to keep total cell number
490 constant, but setting low INM conditions with PRAM/active G2 set to 0 and M to 6min. (S3
491 Fig). Under low INM conditions, the PSE elongates along the apicobasal axis faster than with
492 normal INM and there is a shrinkage of the apical domain (S5 Movie) confirming our previous
493 observations.

494
495 **Fig 7. Apicobasal elongation requires a specific elongation force.** Simulations with contractile
496 apical springs, normal INM and high noise. A, apicobasal length of the PSE (AB) and mean
497 nuclear position along the AB axis (N) over time expressed in micrometers (see S4 Movie). B,
498 Number of pseudolayers of nuclei along the AB axis. C, straightness of apical domain (net
499 distance between the first and last apical point divided by the actual distance between these
500 two points). D-G, mean width of apical (magenta), nuclear (black) and basal (cyan) domains of

501 the PSE with dynamic apical-apical springs over time with 50 (D), 40 (E) and 30% (F-G) of
502 daughter cells being excluded from the 2D plane. For each domain, the distance between
503 the first and last point along the lateral axis is computed and its evolution plotted over time.
504 D-F, slow update of basal point positions (as in all previous Figs). G, fast update of basal point
505 positions (twice faster). Each simulation was performed over 480 iterations (48h of biological
506 time) for 10 repetitions. Each curve represents the mean value of each dataset for the
507 parameter plotted.

508

509 In conclusion, our data indicate that: i/ pseudostratification is mainly controlled by the
510 increase of cell number, ii/ apical contractility is essential to maintain tissue shape in the
511 context of a high proliferation rate, iii/ INM promotes the expansion of the apical domain, iv/
512 INM opposes pseudostratification, apical constriction and apicobasal elongation whereas it
513 controls apical positioning of mitosis and apical nuclear crowding, v/ apicobasal elongation of
514 cells is likely to be due to an active elongation program and not a mere consequence of
515 increased nuclear density (Fig 8).

516

517 **Fig 8. Interplay between INM, proliferation and cell adhesion in the context of a specific**
518 **apicobasal elongation program are needed to recapitulate normal PSE dynamics.** Green
519 arrows indicate positive action, red arrows indicate negative/inhibitory action. Dotted line
520 indicate weaker effect than plain lines. PRAM, pre-mitotic rapid apical migration; PRAR, post-
521 mitotic rapid apical removal; INM, interkinetic nuclear movement.

522

523

524

525 **Discussion**

526 Our simulations clearly link INM to the rapid emergence of nuclear crowding in the
527 apical domain and to the formation of a region of low nuclear density in the basal part of the
528 PSE. Yet, *in vivo*, the low nuclear density observed in the basal domain of the chick trunk
529 neuroepithelium (forelimb level) only emerges in 3-to-4-day old embryos. This could be due
530 to a lack of INM at early stages of neural plate/tube development. This is unlikely since apical
531 mitoses have been observed even at open neural plate stages in chick and mouse embryos
532 [38, 39]. Alternatively, at early stages, there could be a counterbalancing post-mitotic rapid
533 apical removal (PRAR, Fig 8) of nuclei as part of the INM, as suggested in rats and ferrets'
534 brains [7, 11]. Such active basal-ward movement would prevent the early formation of a
535 crowded apical domain and that of a relatively loose basal domain. Neuroepithelial cells
536 always detach from the apical surface upon differentiation into neurons but they can also be
537 induced to detach from the apical surface by a local increase of the apical density of nuclei
538 [40, 41]. Thus, it may be important to delay apical nuclear crowding to prevent early
539 delamination of undifferentiated neural progenitors from the apical domain. Therefore, one
540 could propose that regulation of the intensity of INM might control the onset of neuron
541 delamination in order to synchronize neuronal differentiation with the development of the
542 spinal cord itself. The loose basal region may also be needed to accommodate the cell body of
543 delaminating neurons which accumulate in the basal side of the epithelium. In addition, or
544 alternatively, INM might control the onset of neuron delamination in order to synchronize
545 neuronal differentiation with the development of adjacent structures awaiting innervation.
546 For instance, it would be interesting to see if somites (or the myotome), which produce
547 muscles to be innervated, interfere with the patterns of INM in the adjacent neural tube.

548 Apical constriction is known to be essential to promote the apicobasal elongation
549 driving the switch from squamous to cuboidal epithelial sheets [36]. Yet, in our simulation
550 apical constriction was a poor driver of apicobasal elongation. This is likely due to the fact that
551 in elongated PSE the apical surface is small compared to the size of the basolateral membrane
552 of cells. In the trunk of a 2-day old chicken embryo, the diameter of the apical surface is
553 around 5 microns whereas cells are already 50 microns tall. Thus, any change of the apical
554 size will only have a marginal effect on cell height. At early stages, during the folding of the
555 neural plate, actomyosin contractility is important and Rho and myosin are detected in the
556 apical region of most neuroepithelial cells [42]. It is proposed that apical constriction may
557 drive cell shape changes contributing to neural plate bending. However, the region that
558 undergoes the most dramatic change of shape, the medial hinge point located at the midline
559 above the notochord, has little and only transient accumulation of Rho. In addition, in mice
560 with targeted defects in cytoskeletal genes, neurulation of the caudal neural tube is rarely
561 affected [43]. Following observations in chick [44] and mouse [38, 45] embryos, it was
562 alternatively proposed, that the change in cell shape at the medial hinge point could be driven
563 by local differences in INM in the neural plate, with cells above the notochord having longer
564 S-phase. The nuclei would spend more time in the basal regions favoring an enlargement of
565 the basal domain. This would let cells adopt a pyramidal shape promoting tissue folding. As
566 for the effect of apical constriction, a longer S-phase is unlikely to generate any significant
567 change in cell shape in elongated PSE since the width of the nucleus only represent a small
568 fraction of the total cell height. It is interesting to note that while the intermediate region of
569 the neuroepithelium exhibits a fast linear increase in apicobasal size from 2 to 4 days of
570 development, the cells located in the floor plate of the neural tube do not change significantly
571 in height during this period. If indeed their pyramidal shape is linked to a specific cell cycle

572 with a long S-phase, this relationship can only be maintained if the cells do not elongate
573 beyond a size that would mitigate the effect of the position of the nucleus.

574 Histological analyses in mice with CAKUT (congenital anomalies of the kidney and
575 urinary tract) or EA/TEF (Esophageal atresia/tracheoesophageal fistula) [2, 3] show a
576 correlation between tissue expansion defects and local lack of INM. The local loss of INM
577 (basal mitoses) correlates with problems of convergence extension and defects in tissue
578 separation which were proposed to be due to a loss of apicobasal polarity in these
579 syndromes. Interestingly, INM is not a permanent feature of all PSE. In mouse, INM stops
580 around E13 in the esophagus meaning that from this stage basal mitoses naturally occur in
581 this tissue [2]. By contrast, by E14, INM and apical mitoses can still be observed in the brain,
582 the trachea, the ureter and the intestine. As with the correlation between lack of expansion
583 and lack of INM in the aforementioned syndromes, it was proposed that INM might be a
584 driving force of tissue expansion, since the organs in which it persists expand at a faster rate
585 than the overall growth of the embryo at these stages. Here we show that INM directly
586 contributes to expansion of the apical domain and thus promotes growth in axes
587 perpendicular to the apicobasal axis (DV, AP). In vivo, other mechanisms likely contribute to
588 tissue expansion along the dorsoventral or anteroposterior axis. One possibility is cell
589 intercalation. This has been observed during neurulation in mouse [38]. The dorsal regions of
590 the neural plate grow faster than the ventral regions. This is due in part to a faster cell cycle
591 but also to significant cell intercalation from ventral to dorsal.

592 Another mechanism that could contribute to tissue expansion in one direction is the
593 orientation of mitotic spindles. It was observed in the chick neural tube that, at early stages
594 (HH7 to HH12, corresponding to 24 to 44h of incubation), most mitoses (circa 55%) were
595 oriented along the anteroposterior axis [39]. Such percentages would correspond to an

596 exclusion rate of daughter cells of 27% in our simulation. Therefore, with such a biased
597 orientation, proliferation would favor anteroposterior elongation of the neuroepithelium
598 compared to expansion in apicobasal and dorsoventral orientations. In addition, in most PSE
599 studied, the mean cell cycle length tends to change over time. It can get longer in the caudal
600 neural tube [35] and in the brain [46] or shorter as in the ureteric epithelium [3] and the
601 midgut [47]. Changes in cell cycle length will obviously change the rate at which a tissue
602 increases in terms of cell number but will also affect the frequency of INM events. Given that
603 apical localization of mitoses drives expansion of the apical domain by opposing apical
604 constriction, such changes in cell cycle pace may also affect tissue shape. Indeed, in our
605 simulations, imbalances in proliferation and apical contractility were sufficient to promote
606 either bending or buckling of the apical surface (see S4 Movie). To maintain tissue shape and
607 straightness of the apical domain, the rapid expansion of the apical domain needs to be
608 compensated by an equivalent expansion if the basal domain or hindered by extensive apical
609 constriction.

610 Further, defects in microtubule dynamics can lead to lissencephaly and microcephaly
611 two common neurodevelopmental defects due to improper growth of the brain. This
612 prompted some to suggest that impaired INM, a microtubule-dependent phenomenon, might
613 contribute to these pathologies [48, 49]. However, all of these microtubule-related defects
614 have also problems in neuronal migration, mitotic spindle positioning and proliferation
615 making it difficult to identify the effects specifically due to a lack of INM.

616 Finally, our data indicate that apicobasal elongation is likely to require a specific cell
617 elongation program. In the model, we generated the elongation force using an artificially
618 increased nuclear noise. Given that, outside of PRAM, such large scale random nuclear
619 movements were never observed in the chick neuroepithelium, it is very unlikely that *in vivo*

620 apicobasal elongation comes from a progressive increase in random nuclear movements.
621 Most likely, it comes from an extensive reorganization of the cytoskeleton. Experimentally
622 disentangling the various putative cytoskeleton-related mechanisms involved in INM (PRAM
623 or PRAR) and cell elongation will required the generation of new tools for the fine-tuning of
624 actin/tubulin dynamics over long periods of time (hours to days) without affecting
625 proliferation.

626

627 Materials and Methods

628 Chicken eggs

629 Fertilized chicken eggs were obtained from S.C.A.L (Société Commerciale Avicole du
630 Languedoc) and incubated at 38°C until the desired stage [28].

631

632 Histology and staining of chicken embryo samples

633 Embryos were soaked in Phosphate Buffer 15% sucrose overnight at 4°C. Then, embryos were
634 transferred for 2 hours in gelatin 7.5% / sucrose 15%. Small weighing boats are used as molds.
635 A small layer of gelatin/sucrose is deposited at the bottom and left to set. Embryos are then
636 transferred on the gelatin layer using a 2.5mL plastic pipette. Each embryo is placed in a
637 single drop and left to set. Once all drops are set, an excess of gelatin/sucrose solution is
638 poured on to the weighing boat to fill it. Once again gelatin is left to set on the bench. After
639 setting, the dish is placed at 4°C for 1 hour to harden the gelatin. Once ready, the block of
640 gelatin containing the embryos is placed under a dissecting microscope and individual blocks
641 are carved to position the embryos in the desired orientation for sectioning. Cryosections
642 were performed using a Leica CM1950 cryostat. Sections were incubated in PBS1X at 42°C for
643 30 minutes to remove the gelatin, treated with PBS1X, 1% triton, 2% serum for

644 permeabilization and blocking. Primary antibodies were diluted in PBS1X 2% newborn calf
645 serum and applied overnight at 4°C under a coverslip. Secondary antibodies were diluted in
646 PBS1X and applied for 2 hours at room temperature. Washes were done in PBS1X. Antibodies
647 used: mouse anti-phospho-histone 3 (Cell Signaling, MA312B). Counterstaining for actin and
648 DNA was done with Phalloidin (1/1000) and DAPI (1/1000).

649

650 **Suspension of neuroepithelial cells**

651 Samples of the whole trunk between somite 15 and 20 were taken from embryos at 2, 3 and
652 4 days of development. The explants were incubated in Dispase II (Stem Cell Technologies;
653 #07923, at 1U/mL) at 37°C for 20 minutes to degrade collagens and fibronectin. Tissues were
654 then separated using mounted needles. Neural tube explants were then incubated in a
655 trypsin solution (ThermoFisher, 25300054
656 Concentration) to generate single cells. From neural tube explants from 3-day and 4-day old
657 embryos, numerous cells (most likely neurons) did not adopt a round morphology after
658 dissociation, instead they maintained an elongated fiber-like morphology and accumulated at
659 the bottom of the tubes. They were not included in the supernatant used for cell diameter
660 analysis.

661

662 **Open book observation**

663 Samples of the whole trunk between somite 15 and 20 were taken from embryos at 2, 3 and
664 4 days of development. The neural tube was open from its dorsal side using forceps. The tip
665 of one forceps is inserted in the neural tube lumen and moved along the anteroposterior axis
666 to open the whole explant. Explants are then squeezed in between two coverslips to maintain
667 them open. Apical side is positioned face down on an inverted microscope for observation.

668

669 **Imaging**

670 Confocal images were taken on a Zeiss 710 confocal microscope. Whole mount images were
671 acquired on a Leica MZ10F.

672

673 **Statistics**

674 Statistical analyses of in vivo data were performed with Prism 6 (GraphPad). Datasets were
675 tested for Gaussian distribution. Student t-tests or ANOVA followed by multiple comparisons
676 were used with the appropriate parameters depending on the Gaussian vs non-Gaussian
677 characteristics of the data distribution. Significance threshold was set at p<0.05.

678

679 **In silico simulations and associated plots**

680 The code was written in Fortran90 in sequential mode and the simulations were performed
681 on a DELL Precision T7810 with windows 8.1, 64 bits of RAM, with two CPU Intel Xeon E52637
682 3.8 GHZ processors. The computational time of each simulation of the tissue evolution for 48
683 hours (480 time iterations) ranges from 8 minutes with 30 cells and no proliferation to
684 approximately 40 minutes with proliferation and the exclusion rate of daughter cells set to
685 0%. For each set of in silico conditions at least 10 repetitions were performed. Data were
686 processed using MatLab R2017b. Plots: for simplicity, mean values of each parameter were
687 plotted at each time-iteration over 40 iterations (4hours) and error bars were not displayed.
688 An example of error bars representing standard deviation can be seen on S3 Fig. To help
689 visualizing differences between each in silico conditions, min and max values of equivalent
690 graphs across the various Figs were kept constant.

691

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695

696 **Authors' contributions**

697 ET and PD conceived the project. MF and PD made the computational model with inputs from
698 ET. ET, EDG and FD generated the biological data. ET designed and performed in silico
699 simulations. ET, MF and PD interpreted and organized the data. ET, MF and PD wrote the
700 manuscript. All authors commented on the manuscript.

701

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718

719 **Data Availability Statement**

720 All relevant data are within the manuscript and its Supporting Information files.

721

722 **Competing interests**

723 The authors have declared that no competing interests exist.

724

725 **Ethics statement**

726 This research only used chicken embryos at early stages of development (before the 6th day).

727 None of the procedures fall under legal requirements for animal use and can be performed by

728 anyone without animal licence.

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907 **Supporting Information captions**

908 **S1 Figure. Anteroposterior growth from the forelimb to the tail bud from 2 to 4 days of**
909 **development.** A. Representative images of chicken embryos at stage HH13-, 18 and 23
910 corresponding to 48, 72 and 96 hours of incubation. The cephalic region of the embryo at
911 stage HH18 removed prior to the picture. The green dotted line indicates the regions that was
912 measured. B, plot of the mean length of the portion indicated in green in panel A. Dots
913 represent the mean, error bars indicate standard deviation.

914

915 **S2 Figure. Maintenance of neuroepithelial architecture requires actomyosin contractility.** A,
916 Explants of the trunk are incubated in suspension with culture medium, culture medium with
917 DMSO or culture medium with ROCK inhibitor (Y27632, 400 μ M). B, Transversal sections with
918 nuclear (DAPI, grey) and actin staining (Phalloidin, green). C, apicobasal length. D, number of
919 pseudolayers of nuclei. E, straightness of the apical domain. F, shape of nuclei. G, position of
920 mitoses, at scale with the tissue. H, position of mitoses, raw data (n48h=42, n72h=66,
921 n96h=153; Kruskal-Wallis followed by multiple comparisons; ****, p<0.0001). Box and
922 whiskers plot: the box extends from the 25th to the 75th percentile; the whiskers show the
923 extent of the whole dataset. The median is plotted as a line inside the box.

924

925 **S3 Figure. INM opposes apicobasal elongation, even with high random nuclear noise.** A, Mean
926 apicobasal (AB, open circle) and nuclear-basal (N, closed circles) lengths with normal INM
927 (black) and low INM (red). Note that mean tissue heights goes from 75 μ m to 89 μ m in absence
928 of INM. In the meantime, mean nuclear heights goes from 51 μ m to 48 μ m representing a shift
929 from being located within the most apical top third of the tissue with normal INM (51 μ m
930 /75 μ m =68%) to being located midway between the apical and basal domains

931 (48 μ m/89 μ m=54%). B-C, Mean width of apical (magenta), nuclear (black) and basal domain
932 (cyan) domains with normal INM (B) or low INM conditions (C). Note that low INM conditions
933 lead to a shrinkage of the apical domain (arrow). Error bars represent standard deviation.

934

935 **S1 Table. List of parameters used in each simulations presented on Figs 3-5, 7 and S3.** Yellow,
936 passive apical-apical springs; Green, contractile apical-apical springs; blue frame, fast update
937 of basal points; magenta, high random nuclear movement (noise); light grey, no proliferation
938 and no INM; dark grey, low INM; bold text, normal INM conditions. The following parameters
939 were common to all simulations: radius of nucleus soft core ($R_s=5\mu$ m), radius of nucleus hard
940 core ($R_h_S=1.5\mu$ m), radius of nucleus hard core during mitosis ($R_h_M=3.5\mu$ m), maximum
941 distance between two consecutive apical points ($a_0=1/6*R_s$), maximum distance between
942 two consecutive basal points ($b_0=1/6*R_s$). The relative strengths of the various forces was set
943 as follows: stiffness of soft core of the nucleus ($\alpha_X=1$), stiffness of apical-nucleus spring
944 ($\alpha_aX=2$), stiffness of nucleus-basal spring ($\alpha_bX=2$), stiffness of apical-apical spring
945 ($\alpha_aS=5$), stiffness of apical-apical spring during G2 phase and mitosis ($\alpha_aM=10$),
946 magnitude of the apical-nucleus-basal alignment force ($\alpha_{ab}=15$).

947

948 **S1 Movie (related to Figure 2).** Mosaic expression of membrane-GFP (green) and membrane-
949 mCherry (red) into the chick neuroepithelium at stage HH14 at the level of the intermediate
950 neural tube. Nuclei are counterstained with DAPI (grey).

951

952 **S2 Movie (related to Figures 3 and 4).** All simulations start with 30 cells. Exclusion rate of
953 daughter cells is set to 50% keeping the total cell number constant. Top panels: simulations
954 with passive apical-apical springs without INM (left), with low INM (middle), with normal INM

955 (right). Bottom panels: simulations with contractile apical-apical springs without INM (left),
956 with low INM (middle), with normal INM (right). Note that in absence of INM there is a rapid
957 shrinkage of the apical domain giving the tissue a pyramidal shape. Also, INM leads the rapid
958 emergence of a low nuclear density region basally. Only hard cores of nuclei, apical points and
959 basal points are represented. Soft cores of nuclei and springs are not displayed. Red, cells in
960 mitosis; yellow cells in PRAM/active G2; black line, tracking of nuclei in PRAM/G2 and M. Each
961 frame corresponds to one iteration of the simulation (circa. 6 minutes of biological time).
962 Total duration 480 iterations (48h of biological time).

963

964 **S3 Movie (related to Figure 5).** All simulations start with 30 cells with normal INM. From left to
965 right, exclusion rate of daughter cells is set to 50% (constant cell number), 40%, 30%, 0% (all
966 daughter cells added to the 2D plane). Top panels: simulations with passive apical-apical
967 springs. Bottom panels: simulations with contractile apical-apical springs. Note that
968 contractile apical springs mitigates buckling of the apical domain and feeds back into basal
969 rearrangements. Red, cells in mitosis; yellow cells in PRAM/active G2; black line, tracking of
970 nuclei in PRAM/G2 and M. Each frame corresponds to one iteration of the simulation (circa. 6
971 minutes of biological time). Total duration 480 iterations (48h of biological time).

972

973 **S4 Movie (related to figure 7).** All simulations start with 30 cells with normal INM and a 25-
974 fold increase of random nuclear displacement at each iteration (noise) compared to previous
975 simulations. From left to right, exclusion rate of daughter cells is set to 50% (constant cell
976 number), 40% and 30%. The fourth condition at the far-right is with 30% of exclusion rate and
977 fast update of basal points. Note that the global non-oriented force generated by increased
978 noise is converted into apicobasal elongation. Also, allowing fast reorganization of the basal

979 points promotes isotropic expansion of the apical and basal domain. Red, cells in mitosis;
980 yellow, cells in PRAM/active G2; black line, tracking of nuclei in PRAM/G2 and M. Each frame
981 corresponds to one iteration of the simulation (circa. 6 minutes of biological time). Total
982 duration 480 iterations (48h of biological time).

983

984 **S5 Movie (related to Supplementary Figure 3).** Simulations start with 30 cells. Exclusion rate is
985 set to 50% (constant cell number). Left panel, normal INM. Right panel, low INM conditions.
986 Note that without normal INM the apicobasal expansion is faster and that there is a shrinkage
987 of the apical domain.

988

989 **Supplementary Information.** This file contains the details about the mathematical model.

990

passive apical springs

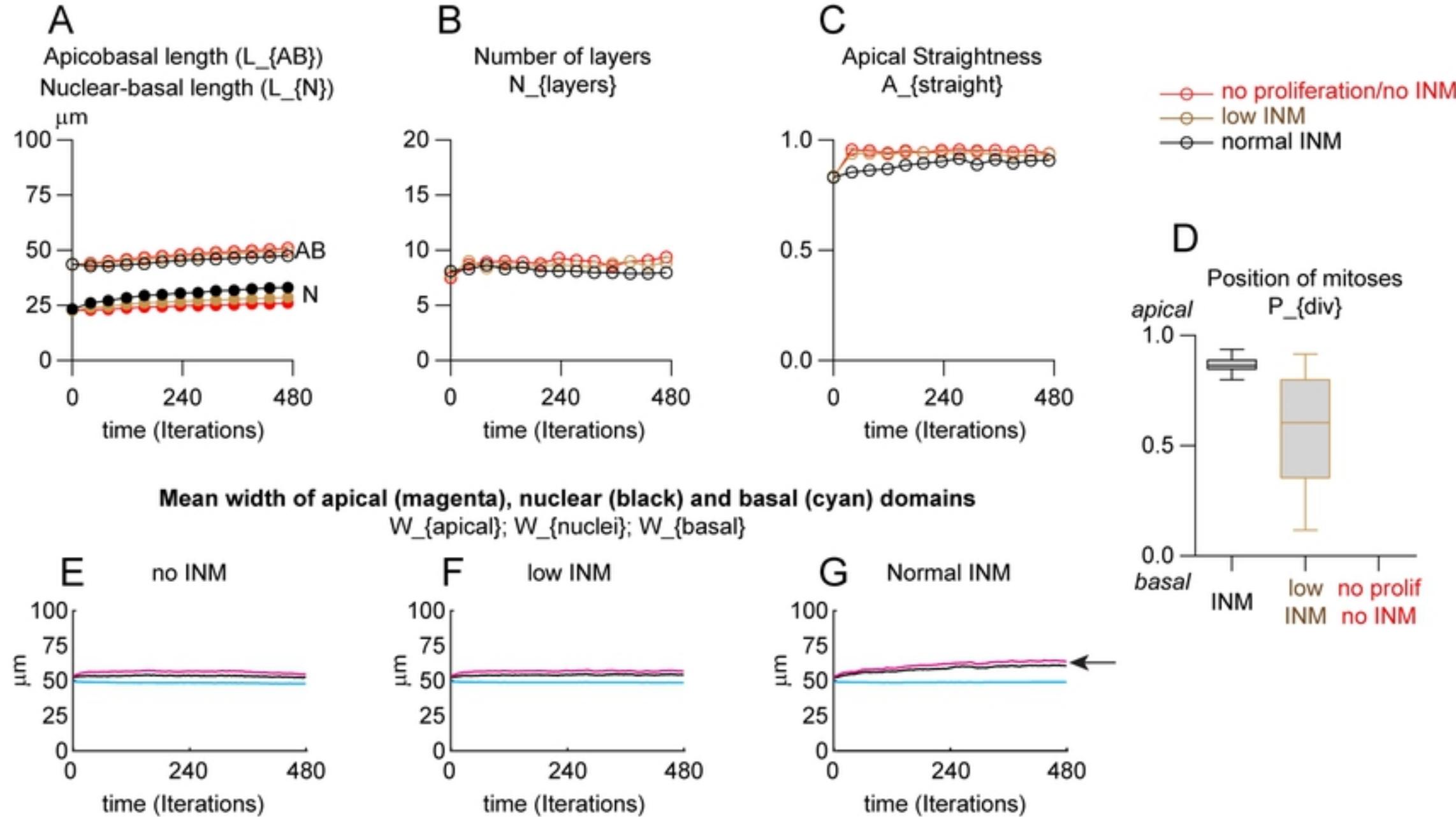


Figure 3

contractile apical springs

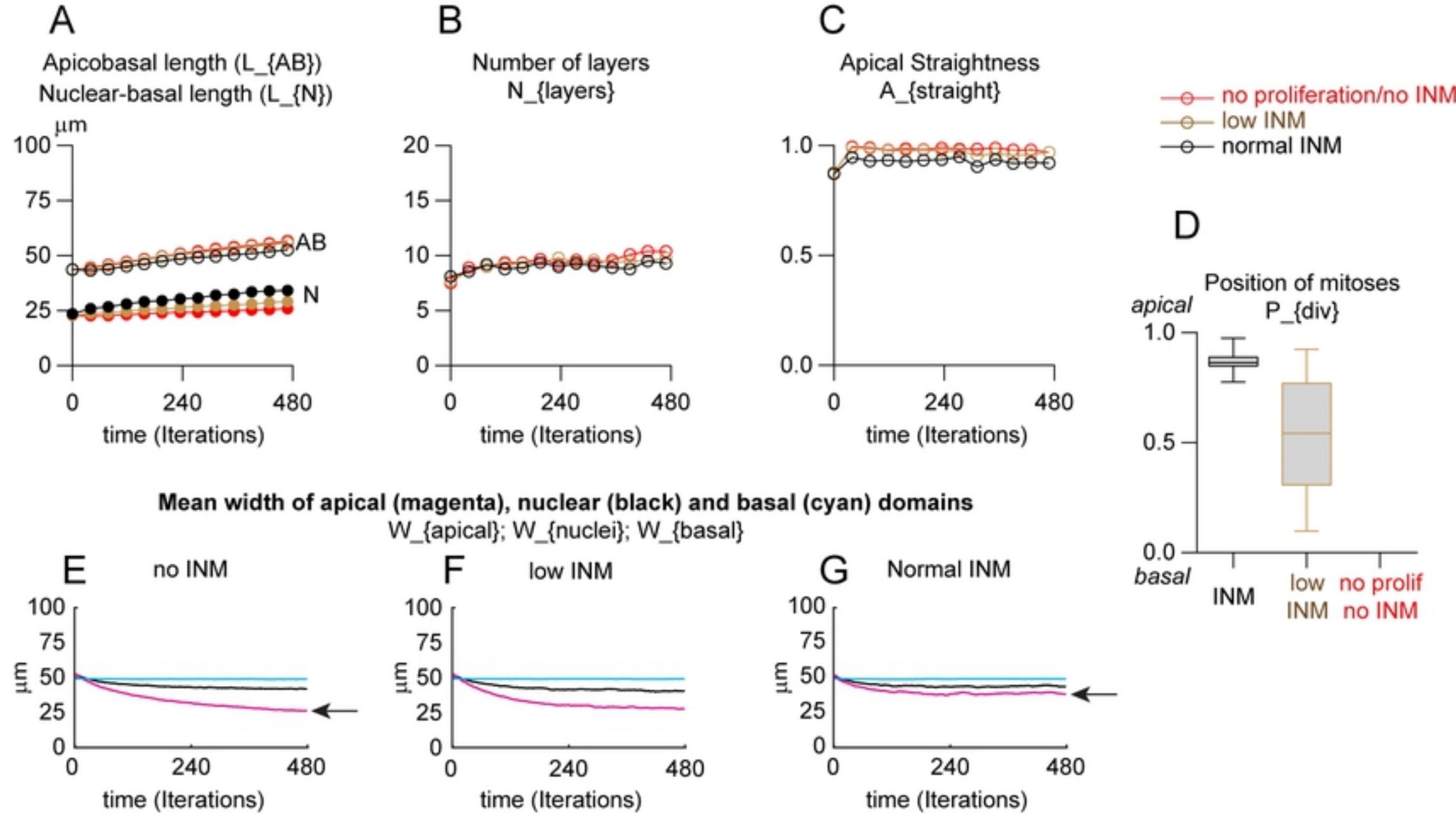


Figure 4

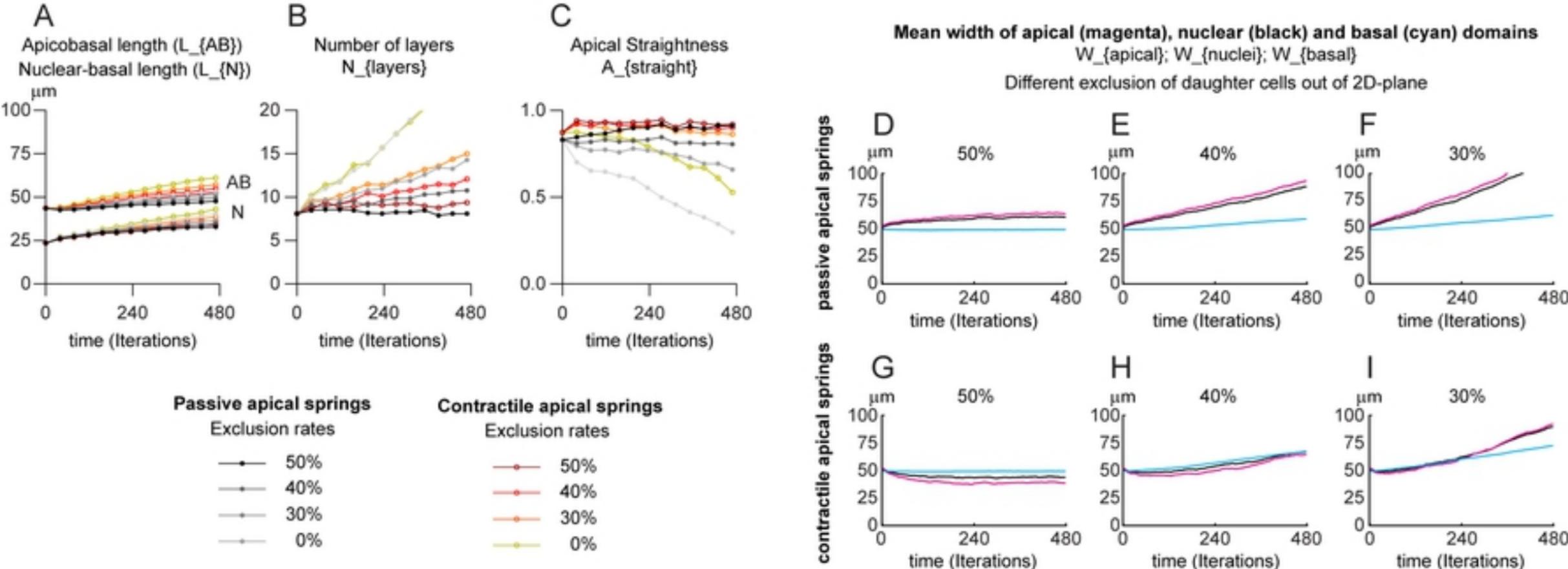


Figure 5

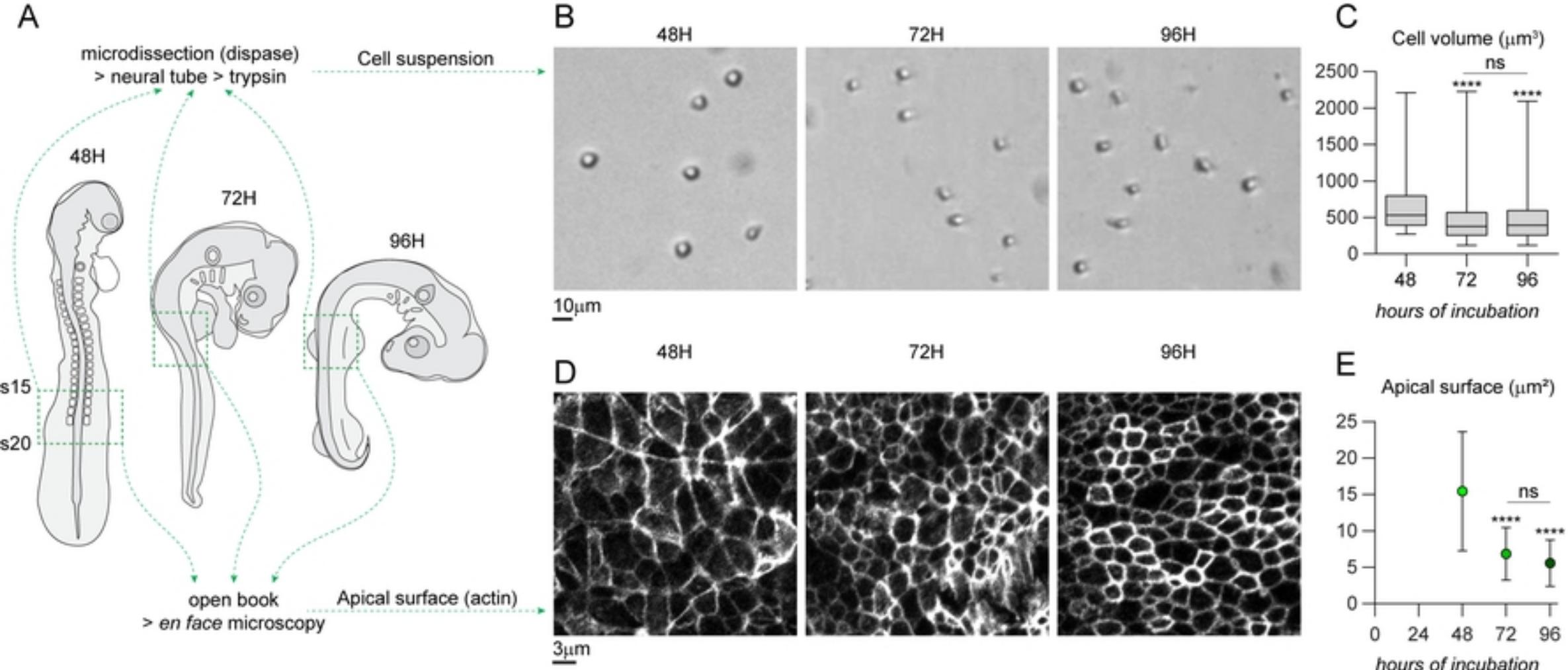


Figure 6

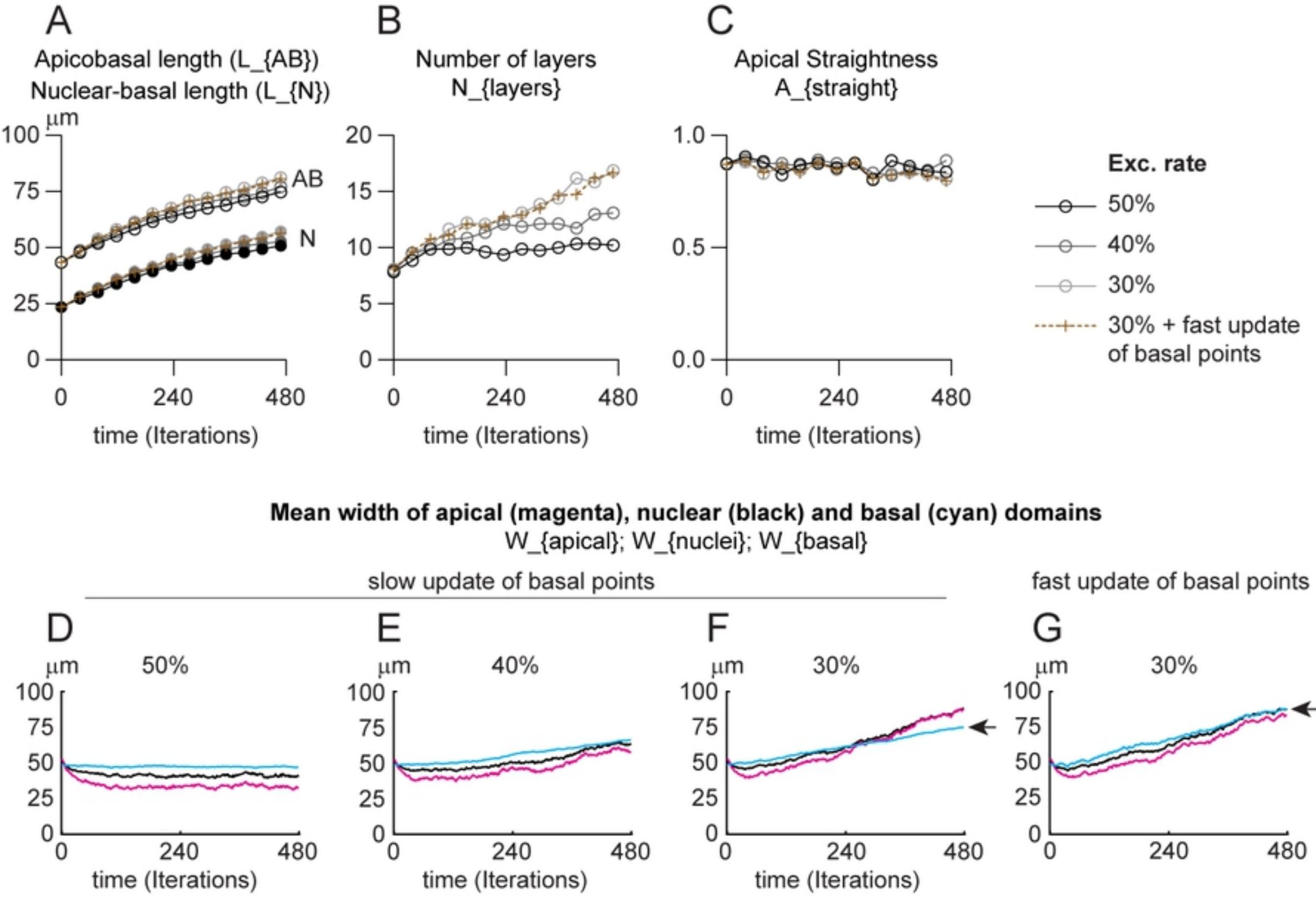


Figure 7

Proliferation

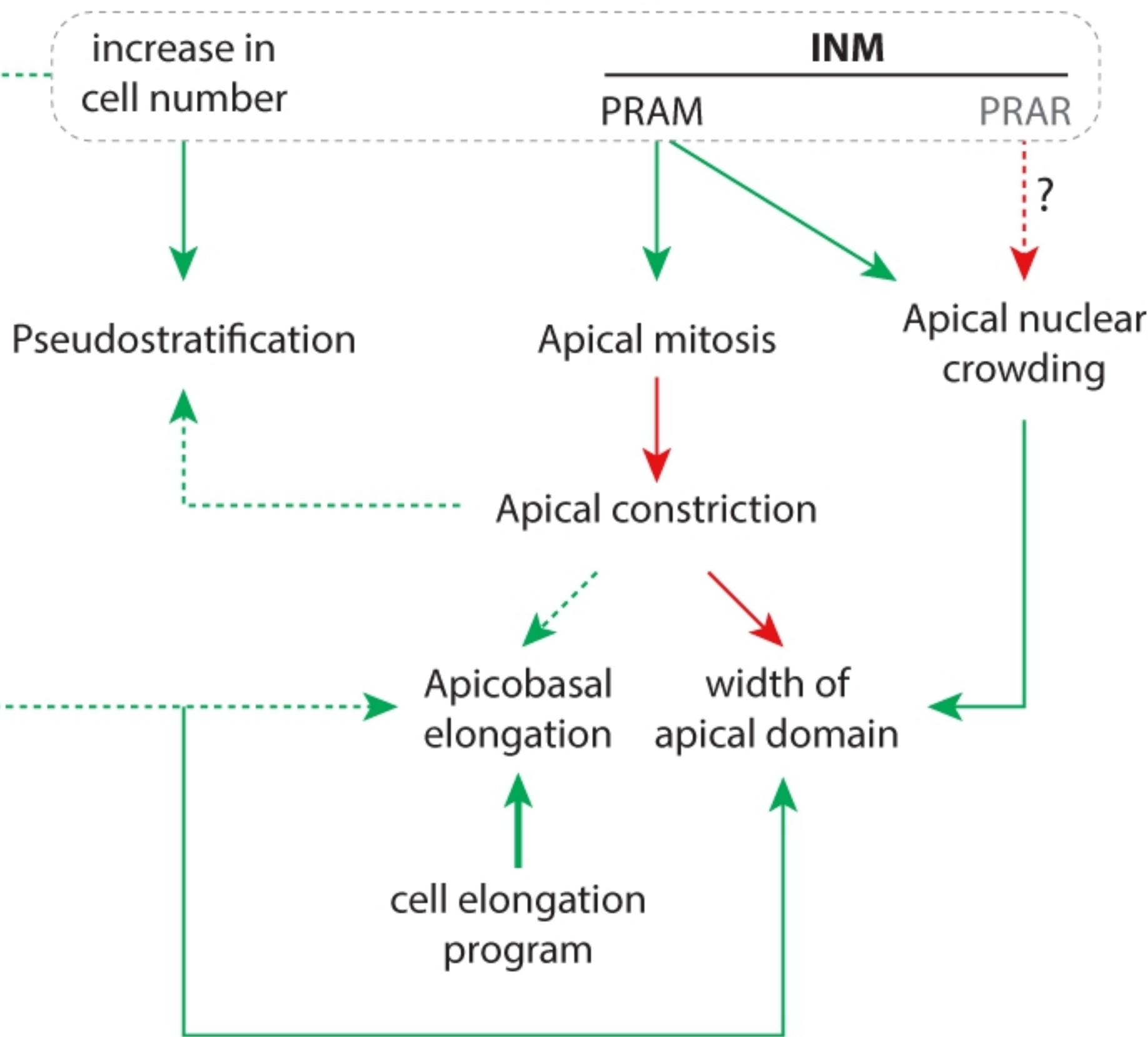


Figure 8

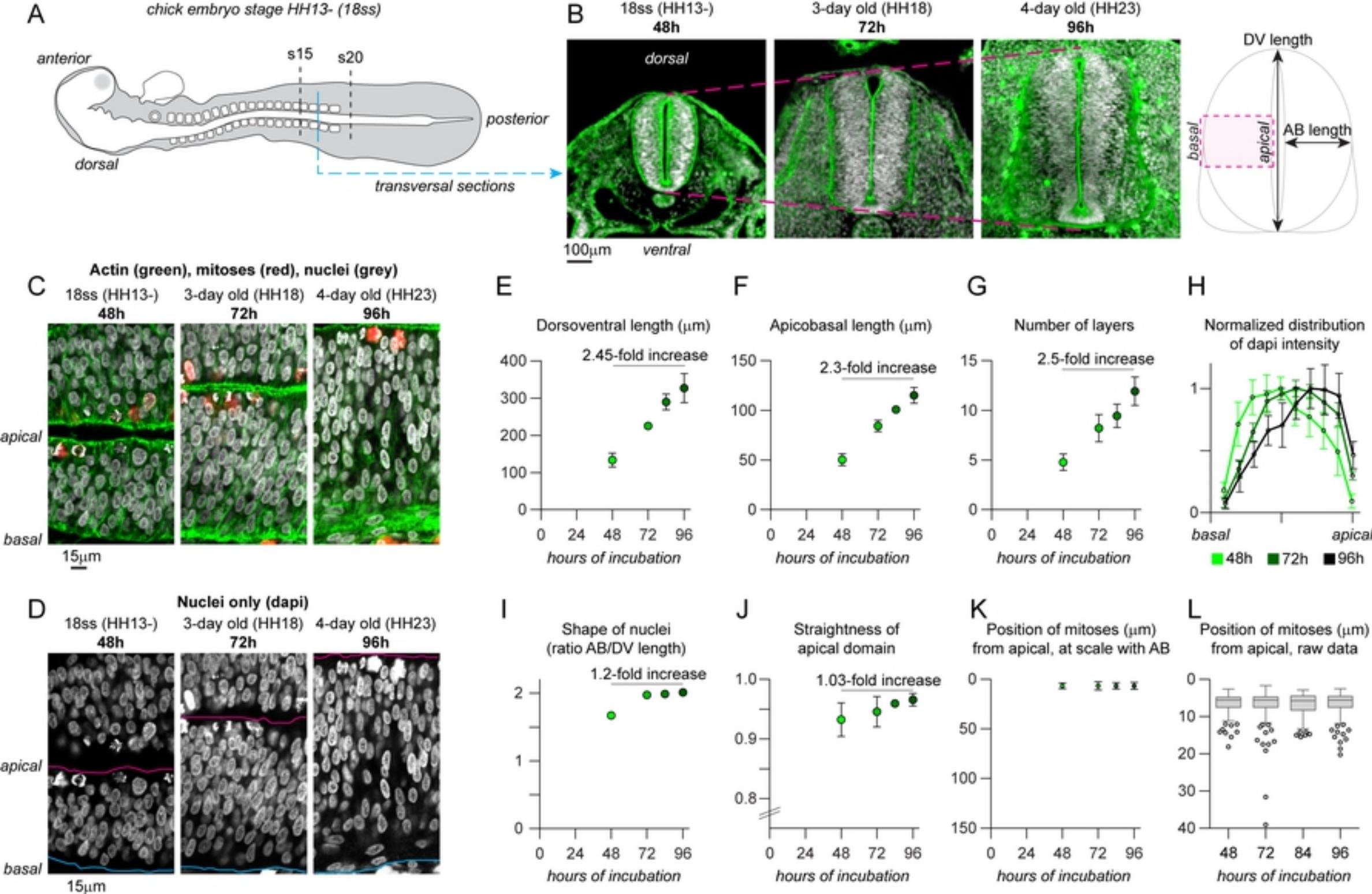


Figure 1

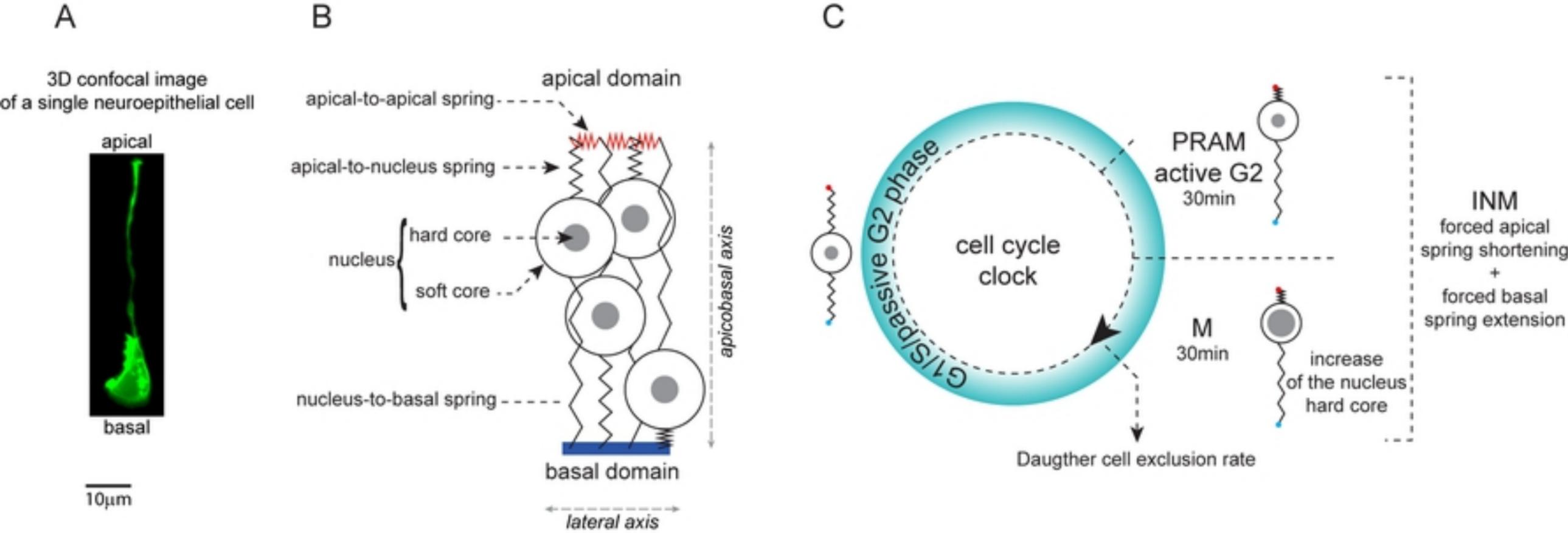


Figure 2

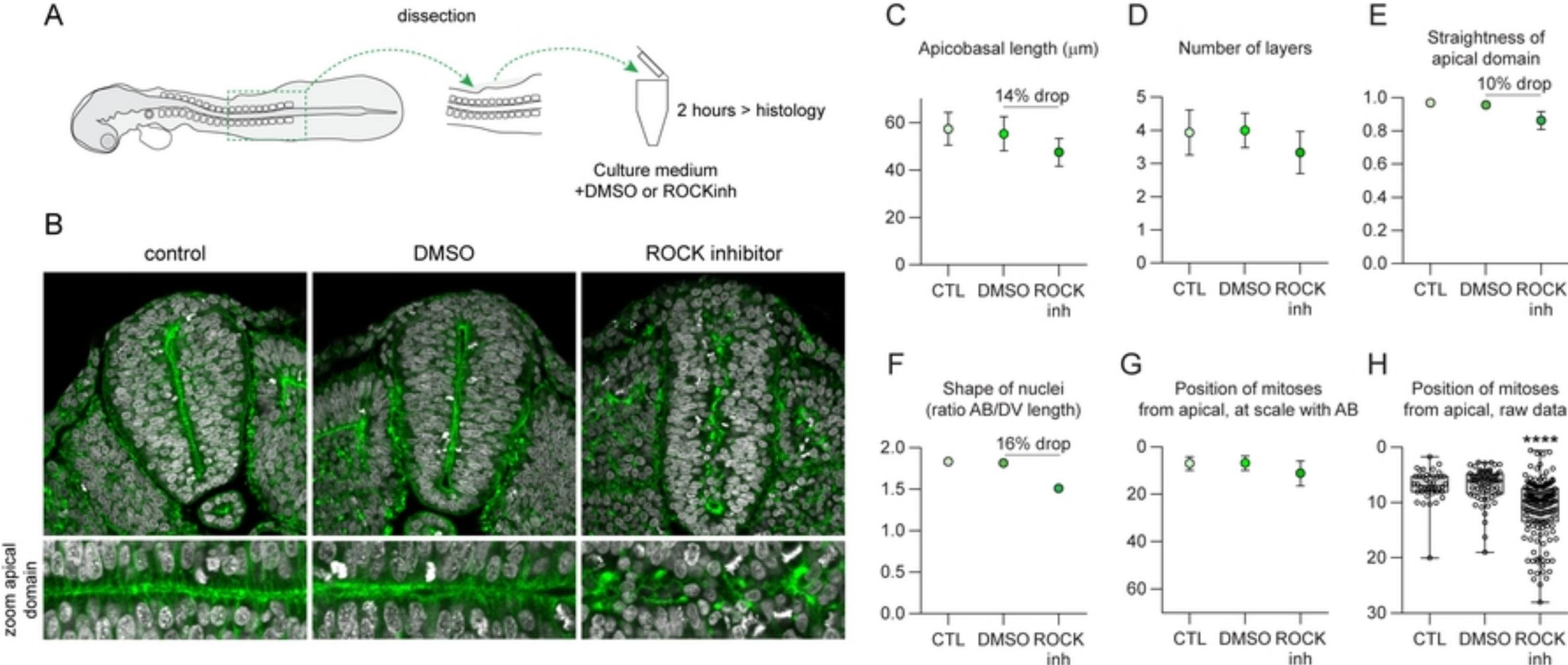
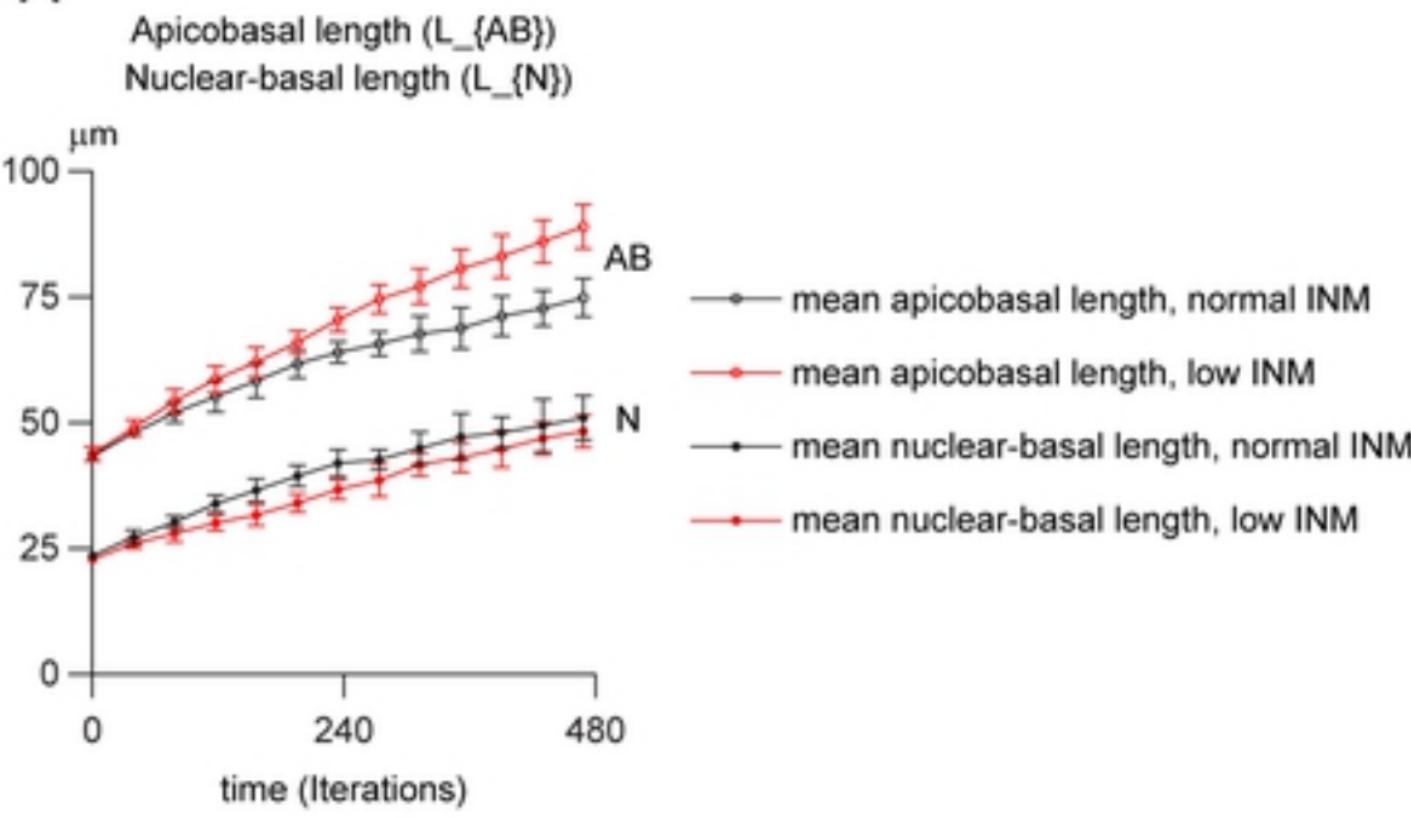


Figure S2

A

Mean width of apical (magenta), nuclear (black) and basal (cyan) domains
 W_{apical} ; W_{nuclei} ; W_{basal}

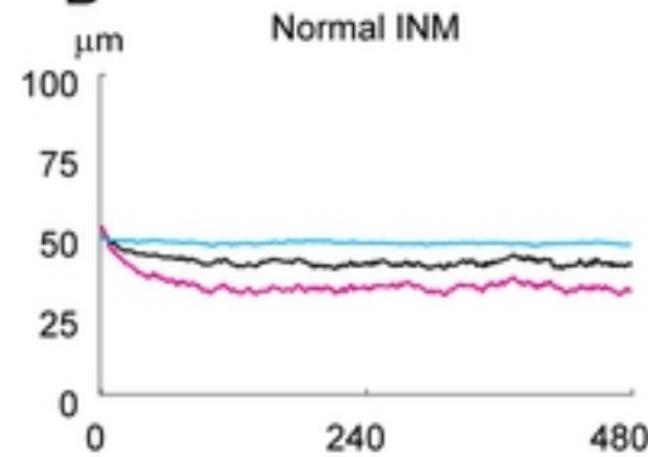
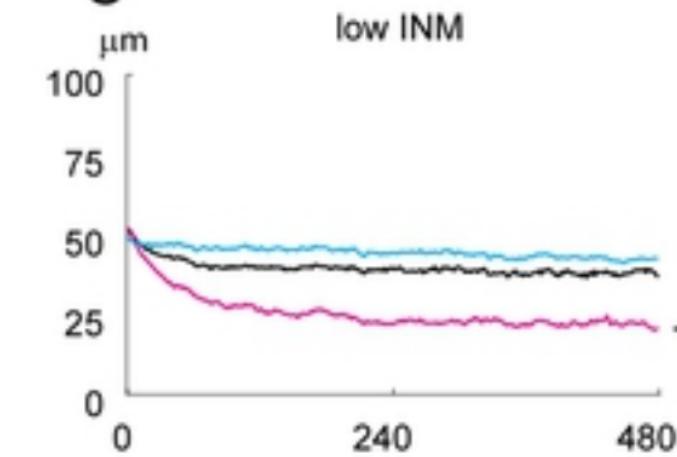
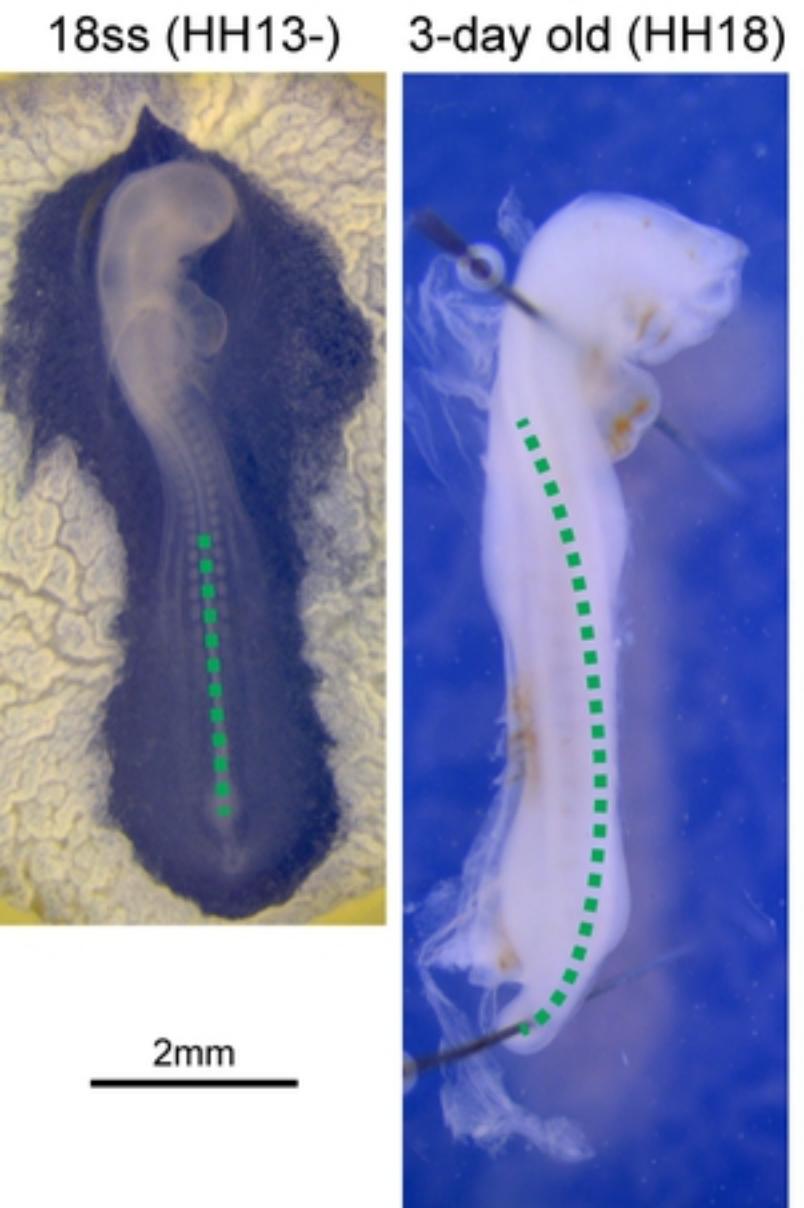
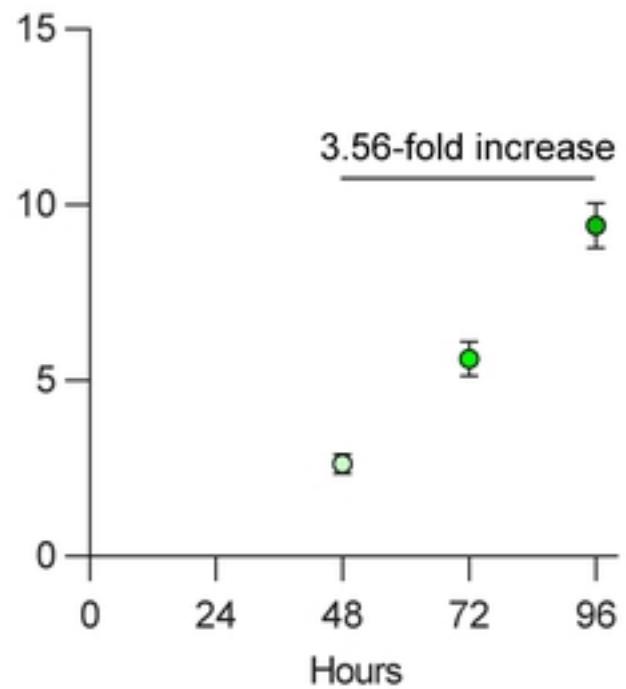
B**C**

Figure S3

A**4-day old (HH23)****B**

Trunk length (mm)
from somite 15 to posterior



- 18ss (HH13-)
- 3-day old (HH18)
- 4-day old (HH23)

Figure S1