Apical constriction is driven by a pulsatile apical myosin network in delaminating Drosophila neuroblasts

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Abstract

Cell delamination is a conserved morphogenetic process important for generation of cell diversity and maintenance of tissue homeostasis. Here we used Drosophila embryonic neuroblasts as a model to study the apical constriction process during cell delamination. We observe dynamic myosin signals both around the cell adherens junctions and underneath the cell apical surface in the neuroectoderm. On the cell apical cortex the non-junctional myosin forms flows and pulses, which are termed as medial myosin pulses. Quantitative differences in medial myosin pulse intensity and frequency are critical to distinguish delaminating neuroblasts from their neighbors. Inhibition of medial myosin pulses blocks delamination. The fate of neuroblasts is set apart from their neighbors by Notch signaling-mediated lateral inhibition. When we inhibit Notch signaling activity in the embryo, we observe that small clusters of cells undergo apical constriction and display an abnormal apical myosin pattern. Together, we demonstrate that a contractile actomyosin network across the apical cell surface is organized to drive apical constriction in delaminating neuroblasts.
Introduction

A single cell moving out of an epithelial layer is important for generation of diverse cell types during organogenesis. For example, single cells moving into the interior of the sea urchin blastula give rise to the primary mesenchymal cells; individual cells delaminating from the neural tube in vertebrates give rise to a variety of cell types such as peripheral neurons and glia. In adult epithelial tissues, single cell extrusions have been shown to counter balance growth (Eisenhoffer et al., 2012; Marinari et al., 2012). Single cells leaving the epithelia can have two different fates. The first type of cell fate is to differentiate and generate other cell types, such as the primary mesenchymal cells derived from the sea urchin blastula and the neural crest cells delaminated from the neural tube. The second type of cell fate is to undergo apoptosis or anoikis after leaving the epithelial layer; this is the default fate for apoptotic cells extruded from the epithelial layer (Rosenblatt et al., 2001; Toyama et al., 2008) or alive cells extruded due to tissue crowding (Eisenhoffer et al., 2012; Marinari et al., 2012). While the apoptotic single cell extrusion process has been studied in several contexts (Gu and Rosenblatt, 2012), the non-apoptotic single cell delamination process is less well characterized. It is unknown whether the molecular and cellular regulatory mechanisms for different types of single cell extrusions are conserved. Moreover, it is unclear how cells experience internal and external force, change shape, and move out of the epithelial layer without compromising the epithelial integrity.

The Drosophila embryo has been established as an excellent system to study epithelial morphogenesis, including invagination and intercalation, because of its optical and genetic accessibility (Lecuit et al., 2011). Here we developed Drosophila embryonic ventral nerve cord (VNC) neural stem cells (neuroblasts) as a model to study the mechanical processes of non-apoptotic single cell delamination. Several hundreds of VNC neuroblasts delaminate from the embryonic ventral neuroectoderm region within 4 hours after the onset of gastrulation (Campos-Ortega and Hartenstein, 1997). The highly predictable pattern therefore allows us to track delamination processes with high spatiotemporal resolution. Delaminating neuroblasts undergo apical constriction as a first step of leaving the epithelial layer. The apical constriction process is primarily driven by an apical myosin network (Martin and Goldstein, 2014). However, the organization and dynamics of the actin-myosin network varies in different contexts (Martin and Goldstein, 2014). The contractile actin-myosin machinery important for apical constriction can be put into two categories. First, the purse-string-like contraction of bundled actin-myosin fibers around the apical adherens junctions can generate force to drive apical constriction (Burgess, 1982). For example, when apoptotic cells are extruded from a MDCK cell monolayer, the actin-myosin ring is assembled in both the apoptotic cells and their neighbors during extrusion (Rosenblatt et al., 2001). It was later shown that the contraction of the actin-myosin ring assembled in the dying cells is important for the early stage of apical constriction in this system (Kuipers et al., 2014). Second, the actin-myosin meshwork underlying the apical plasma membrane has been shown to organize into cortical flows and pulses that drive apical constriction in several cell types. For example, in ventral furrow cells undergoing invagination as a group of cells in Drosophila embryos, the myosin foci and fibers arise across the apical surface, move to the center of the cells, form large spots with high intensity and disperse. This non-junctional myosin movement on the cell apical surface was defined as medial myosin pulses, and each pulse coincides with a rapid apical area constriction phase (Martin et al., 2009; Mason et al., 2013). The amnioserosa cells also assemble a dynamic medial actomyosin network that drives apical cell shape fluctuations during Drosophila dorsal closure process (Blanchard et al., 2010; David et al., 2010). Moreover, the salivary gland placode cells also undergo apical constriction driven
by a medial actomyosin network in *Drosophila* embryos (Booth et al., 2014; Roper, 2012). In all these contexts, the contraction of an actomyosin network across the epithelial apical surface is critical for the apical constriction process for a group of cells.

We tracked and analyzed the highly dynamic apical myosin network in delaminating neuroblasts. Combining quantitative analysis, drug inhibition and genetic experiments, we demonstrate that the apical constriction process in delaminating neuroblasts is dependent on a pulsatile actin-myosin meshwork underlying the apical plasma membrane, unlike the cases reported for single cells extruded from epithelia destined for apoptosis (Eisenhoffer et al., 2012; Hogan et al., 2009; Rosenblatt et al., 2001). Moreover, both experimental results and mathematical modeling suggest that quantitative differences in myosin pulse intensity and frequency are sufficient to generate different outcomes for delaminating neuroblasts and their neighbors. Finally, we demonstrate a role of the fate-determining Notch signaling activity in the apical constriction process in delaminating neuroblasts.

**Results**

*Drosophila* embryonic neuroblasts undergo apical constriction as single cells.

The Drosophila VNC neuroblasts are derived from the neurogenic region that bilaterally occupies the embryonic ventral-lateral surface (Campos-Ortega and Hartenstein, 1997). We used a membrane-mCherry transgenic line (Martin et al., 2010) to visualize the apical cell shape change in the neurogenic region. Before the ventral furrow closes, the epithelial cells are mostly hexagon-shaped and morphologically indistinguishable in the neurogenic region. As the ventral furrow closes and the germ band extension process starts, the cells in the neurogenic region start to exhibit morphological heterogeneity (Irvine and Wieschaus, 1994). Within 10 min after the ventral furrow closes, we started to observe clusters of cells adopting a characteristic “flower-like” structure, with one cell in the center decreasing its apical area while the apical area of neighboring cells undergoes fluctuation (Figure 1A-B, Supplemental Movie 1). The clusters of cells maintain the flower-like structure on the apical surface for another 15 to 30 minutes before the center cell drops out of the imaging plane. These center cells have been previously characterized as “bottle-like” cells expressing the neuroblast marker Insuteable (Figure 1C) and later become the first wave of neuroblasts detectable beneath the surface epithelium at embryonic stage 8 (Doe, 1992; Kraut and Campos-Ortega, 1996). Therefore, the reduction of apical area in neuroblasts is one of the earliest steps of delamination, which can be tracked and analyzed starting immediately after the ventral furrow closes (Figure 1A).

Using the ventral midline as the positional reference, we imaged 6-8 rows of cells from the ventral midline and quantitatively tracked the apical cell area change for these cells including delaminating neuroblasts which later become the first and second rows of neuroblasts from the ventral midline under the epithelial layer (Doe, 1992). A neuroblast is initially indistinguishable from its neighbors by their apical shape or area. As a neuroblast initiates the delamination program, we start to see that its apical area steadily decreases while the neighboring cells maintain their apical area with fluctuations (Figure 1D-E). The delaminating neuroblasts decrease their apical area at an average rate of \(5 \mu m^2/min\), a rate comparable with the apical area reduction rate of ventral furrow cells that undergo multicellular tissue invagination (Martin et al., 2009). Moreover, similar to ventral furrow cells, the delaminating neuroblasts also undergo pulses of rapid apical area constriction interrupted by phases of apical area stabilization (Figure 1F-G).
phase, the area constriction rate exceeds 10μm²min⁻¹. On average, a presumptive neuroblast undergoes about 4.6 pulses in 6 min and reduces its area to 50% of its original value.

Overall, the quantitative measurement shows that the apical constriction behavior of individual neuroblasts is similar to that of ventral furrow cells (Martin et al., 2009), in spite of the fact that delaminating neuroblasts undergo apical constriction as single cells and ventral furrow cells undergo apical constriction as a group of cells.

**A dynamic apical myosin network exists during the apical constriction process in delaminating neuroblasts.**

The similarity displayed in the apical constriction behavior of ventral furrow cells and delaminating neuroblasts prompted us to test whether a similar actin-myosin network exists to drive the apical constriction process in delaminating neuroblasts. Monitoring myosin dynamics in live embryos using a Myosin-GFP transgenic line (myosin regulatory light chain, Spaghetti squash, fused to GFP)(Martin et al., 2009; Royou et al., 2002), we observed that dynamic myosin foci and fibers are present on the apical cell cortex across the embryo surface. Importantly, the myosin foci and fibers display pulsatile coalescence movements in delaminating neuroblasts similar to those observed in ventral furrow cells, which were defined as medial myosin pulses (Figure 2A, 2B and Supplemental movie 2). It is noteworthy that a dynamic myosin signal is also present at adherens junctions in delaminating neuroblasts (Figure 2A, 2B and Supplemental movie 2), unlike the case in ventral furrow cells where myosin predominantly localizes to the apical cortex (Martin et al., 2009).

The role of medial myosin coalescence in driving apical constriction in ventral furrow cells was demonstrated through quantification of myosin intensity and correlation analysis of myosin intensity change rate with apical constriction rate (Martin et al., 2009). We applied this method to dissect the contribution of medial and junctional myosin to the apical constriction process in delaminating neuroblasts. The correlation analysis showed that the apical constriction rate is significantly correlated with the medial myosin intensity change rate rather than the junctional myosin intensity change rate (Figure 2C-D, Supplemental Figure 1). Notably, in both delaminating neuroblasts and ventral furrow cells the correlation analysis shows that the peak myosin intensity change rate and the peak apical constriction rate occur simultaneously (Supplemental Figure 2A and 2C), while the peak total myosin change rate precedes the peak apical constriction rate by 6s (Supplemental Figure 2B and 2D), indicating that the myosin-driven apical constriction behavior of delaminating neuroblasts and ventral furrow cells is similar.

The delaminating neuroblasts are surrounded by cells that do not undergo apical constriction and exhibit apical area fluctuation. In the non-delaminating neighboring cells, the medial and junctional myosin intensity also undergoes dynamic changes. Moreover, we also observed a correlation between the medial myosin intensity change rate and the cell area change rate in neighboring cells (Figure 2E-F, Supplemental Figure 1). Both the medial and junctional myosin intensity exhibits similar dynamic changes in amplitude and frequency for cells immediately next to a delaminating neuroblast (1st degree neighbors) and cells at least one cell distance away from a delaminating neuroblast (2nd degree neighbors) (Supplemental Figure 3).
To address how the dynamic apical myosin network is organized to generate different outcomes for delaminating neuroblasts and their neighboring cells, we compared the myosin intensity change in delaminating cells and neighboring cells. First, we observed that delaminating neuroblasts display higher medial myosin intensity in comparison with their neighboring cells (Figure 2G-I), while the junctional myosin intensity does not exhibit a significant difference between delaminating neuroblasts and their neighbors (Figure 2K-M). Second, the medial myosin pulses appear at a higher frequency in delaminating neuroblasts (0.8 pulse/min) compared to their neighboring cells (0.5 pulse/min) (Figure 2J), while the junctional myosin pulses appear at a similar frequency between delaminating neuroblasts and their neighbors (0.5 pulse/min) (Figure 2N). Together, these data raise the possibility that medial myosin pulses play an important role in driving apical constriction in delaminating neuroblasts.

**Medial myosin pulses are required for the apical constriction process in delaminating neuroblasts.**

The formation of medial myosin pulses is dependent on a radial-polarized actin network with the pointed ends of actin filaments enriched in the medioapical region of ventral furrow cells (Coravos and Martin, 2016; Mason et al., 2013; Vasquez et al., 2014). Low-dose of cytochalasin D (CytoD) treatment is a widely-used method to disrupt the radial polarized apical actin network and block medial myosin pulses in ventral furrow cells (Coravos and Martin, 2016; Mason et al., 2013) and in cells undergoing shape deformation during Drosophila germband extension (Munjal et al., 2015).

To test the role of medial myosin pulses in the apical constriction process in delaminating neuroblasts, we injected the embryo with a low-dose of cytochalasin D (CytoD). Using an Utrophin-GFP strain to visualize the F-actin network (Rauzi et al., 2010), we observed that the apical actin meshwork that also displays pulsatile coalescence movements is disrupted by the low-dose CytoD treatment while the junctional actin signal remains unaffected (Figure 3A, Supplemental movie 3 and 4). Consequently, the low-dose CytoD treatment disrupts the medial myosin pulses but does not appreciably lower the junctional myosin intensity (Figure 3B-C, Supplemental movie 5 and 6). Disruption of medial myosin pulses by a low dose of CytoD treatment lead to a global reduction of delaminating cells in the neuroectoderm region (Figure 3D). Moreover, while DMSO mock-injected embryos display orderly rows of delaminated neuroblasts marked by Achaete below the surface epithelial layer 90 min after the onset of gastrulation, the embryos injected by a low dose of CytoD show few delaminated neuroblasts at the same stage (Figure 3E).

RhoGEF2, a RhoA activator, is required to organize medial myosin pulses in ventral furrow cells (Mason et al., 2016) and cells in the ectoderm during Drosophila germband extension (Kerridge et al., 2016). In particular, it was reported that depletion of RhoGEF2 through RNAi lead to a specific reduction of medial myosin without affecting junctional myosin in epithelial cells located in the germband (Kerridge et al., 2016). The delaminating neuroblasts are located in the ectoderm undergoing germband extension. We therefore used *RhoGEF2 RNAi* to examine its effects in neuroblast delamination. In *RhoGEF2 RNAi* embryos, we observed a significant reduction of the medial myosin intensity but not the junctional myosin intensity, consistent with published results (Figure 3F-G, Supplemental Movie 7 and 8). Consequently, we observed a lower number of cells that undergo delamination in *RhoGEF2 RNAi* embryos (Figure 3H-I).
Taken together, these experiments suggest that medial myosin pulses are important for the apical constriction process in delaminating neuroblasts.

**Mathematical modeling reveals that myosin pulse intensity and frequency are key parameters for neuroblasts to overcome an energy barrier for delamination.**

The dynamic medial and junctional myosin flows and pulses are qualitatively similar in both delaminating neuroblasts and their neighbors. To address whether the quantitative differences in medial myosin pulses observed in our experiments are sufficient to generate different outcomes for delaminating cells and their neighbors, we propose a phase field model to simulate dynamics of the neuroblast delamination process. The phase field method has been successfully used in modeling in single cell events such as cell membrane bending and cell migration (Du et al., 2004; Shao et al., 2012; Wang et al., 2017). Here we extend this approach to study the morphodynamics of a multi-cellular system.

We chose six cells with the periodic boundary condition to model the delaminating neuroblast (the center cell in Figure 4) and its neighboring cells (Figure 4). We first computed the minimum energy path to quantify the process of neuroblast delamination (Figure 4A). Before the neuroblast delaminates, the initial state of six cells with an equal shape is found to be a steady metastable state. When the neuroblast starts to delaminate, the system needs to overcome an energy barrier by passing through a transition state, and as the apical area of the delaminating cell decreases to zero, the system reaches a final stable state with five cells. Such a path indicates that the neuroblast requires a driving force in order to delaminate. We use stochastic periodic signaling to simulate the myosin pulses observed in the experiments as the driving force. By using the statistical data of normalized medial myosin intensity and frequency in Figure 2H and 2I, our model is able to reproduce the dynamic change of apical area in delaminating cells (Supplemental Movie 9, Figure 4B and 4C). If we reduce the median myosin pulse frequency from 1pulse/min to 0.5pulse/min, the apical area fluctuates periodically (Figure 4D). Similarly, when the mean of the median myosin intensity is decreased by half, the apical area also shows oscillations, but the neuroblast cannot delaminate (Figure 4E).

The mathematical model suggests that the quantitative differences in medial myosin pulse intensity and frequency are indeed sufficient to generate different outcomes for delaminating cells and their neighbors. Only sufficient high frequency and large intensity of the median myosin pulse can drive the neuroblast to pass over the energy barrier in the minimum energy path and lead to delamination.

**Perturbation of cell fate-determining Notch signaling activity leads to an abnormal delamination pattern in the neuroectoderm.**

The Notch signaling activity is critical for inhibiting neuroblast fate in the neighboring cells surrounding a chosen neuroblast (Campos-Ortega and Knust, 1990; Jan and Jan, 1994). During *Drosophila* early embryogenesis, the action of axis-patterning genes turns on the expression of *proneural* genes in clusters of cells in the neuroectoderm. Every cell in a proneural cluster has the potential to become a neuroblast. Through a lateral inhibition process mediated by Notch-Delta signaling activity, one cell from a proneural cluster is specified as a neuroblast, while the rest of the cells in the cluster that have higher Notch signaling activity remain epithelial cells. During the formation of proneuromast rosette in
Zebrafish posterior lateral line primordium, the morphogenesis process of rosette formation is independent of cell fate-determining Notch-Delta signaling activity (Hava et al., 2009). In Drosophila embryos, it has been shown that overexpression of Notch intracellular domain (NICD) lead to premature cell division in the ventral neuroectoderm (Hartenstein et al., 1994). It remains unclear whether and how Notch signaling activity affects the cellular processes of neuroblast delamination including the apical constriction process.

To understand how differential Notch signaling activity in a proneural cluster affects the delamination process, in particular the apical constriction process, we lowered Notch signaling activity by injecting the embryos with Delta or Notch dsRNA (Supplemental Figure 4). We observed that clusters of cells, which all adopt neuroblast fate, undergo apical constriction (Supplemental Movie 10 and 11, Figure 5A and 5C). Individual cells from a delaminating cluster still undergo pulses of apical constriction correlated with the dynamic change of medial myosin intensity (Figure 5B, 5E and 5F, Supplemental Figure 5). In the Delta RNAi embryos, the delaminating neuroblasts exhibit higher medial myosin intensity than the non-delaminating cells, while the junctional myosin intensity in both delaminating cells and non-delaminating cells are similar (Figure 5G-H). However, on average, these cells in the Delta RNAi embryos reduce their apical area at a rate of 2.5 μm²min⁻¹, slower than the rate of delaminating neuroblasts in water-injected control embryos (Figure 5I). The delaminating cells in Delta RNAi embryos exhibit a reduction of medial myosin pulse frequency from 0.8 pulse/min to 0.5 pulse/min in comparison with control, while the non-delaminating cells also exhibit a reduction of medial myosin pulse frequency from 0.5 pulse/min to 0.2 pulse/min (Figure 5J). When the medial myosin pulse frequency is reduced in delaminating neuroblasts, we expect that three parameter changes can lead to effective apical constriction in Delta or Notch RNAi embryos: first, the overall energy barrier for neuroblast delamination is reduced; second, the medial myosin pulse intensity increases in delaminating neuroblasts; third, the apical constriction process can be driven by a compensatory mechanism such as an increase of junctional myosin contraction (Supplemental Figure 6). Quantitative analysis indicates that medial myosin intensity is higher in delaminating neuroblasts in Delta or Notch RNAi embryos and that junctional myosin intensity is significantly higher in delaminating neuroblasts in Delta or Notch RNAi embryos in comparison with water-injected control embryos (Figure 5K-L). On a close examination of the myosin network, we noticed a strong accumulation of myosin fibers and cables around the junctional region in the delaminating cells in the Delta or Notch RNAi embryos (Supplemental Movie 12, Figure 5M), supporting the possibility that an increase of junctional myosin activity compensates partially for the reduction of medial myosin pulse frequency in Delta or Notch RNAi embryos.

Discussion

The apical constriction process differs for delaminating cells destined for differentiation and extruding cells destined for apoptosis

Previous studies have shown that the formation of a junctional actin-myosin contractile ring is critical for single cells destined for apoptosis to be extruded from the epithelia (Eisenhoffer et al., 2012; Hogan et al., 2009; Rosenblatt et al., 2001). Here we demonstrated that a pulsatile medial myosin network is required for delaminating neuroblasts leaving the epithelia. It is possible that the type of myosin contractility (medial vs junctional) adapted to promote single cell leaving epithelia is cell type-specific. For cells leaving the epithelia for differentiation, our results support the conclusion that the apical constriction process is
primarily cell-autonomous and largely relies on the medial myosin network within the cell. For cells being extruded from the epithelia due to tissue crowding or apoptosis, the apical area reduction process is likely to be triggered by the neighbors and relies on the assembly of a junctional actin-myosin ring. It will be interesting to examine whether the two types of actin-myosin organization modes can be categorized into being cell-autonomous or being passively squeezed by the neighbors through studying more types of apical constriction processes during development.

The apical constriction process for delaminating neuroblasts and ventral furrow cells is largely similar with a few differences.

The apical constriction process in ventral furrow cells during invagination is well characterized (Martin and Goldstein, 2014). The delaminating neuroblasts display similarity with ventral furrow cells in apical constriction speed, duration, pulsatile behavior and the underlying actin-myosin network organizational mode. In both cell types, a pulsatile medial myosin network is critical for the apical constriction process. However, a few differences exist between the two cell types.

Intense and dynamic myosin signals can be detected in the junctional region of delaminating neuroblasts but not in the junctional region of ventral furrow cells. The delaminating neuroblasts reside in the elongating germband and the junctional myosin is undergoing dynamic rearrangements to drive cell-cell intercalation during germband extension (Bertet et al., 2004; Irvine and Wieschaus, 1994). While the correlation coefficient between junctional myosin change rate and apical constriction rate in delaminating neuroblasts is low, it does not exclude the possibility that the junctional myosin might play a role in apical constriction. Our data does not exclude a model that the feedback between medial and junctional myosin network exists to drive apical constriction process in delaminating neuroblasts.

Importantly, the upstream regulatory signal for ventral furrow cells and delaminating neuroblasts are different. Snail and Twist are two critical regulators for the apical constriction process in ventral furrow cells (Martin et al., 2009). In delaminating neuroblasts, snail and twist RNAi does not affect the apical constriction process (Supplemental Movie 13 and 14).

While a dynamic actomyosin network is present across all the cells in the neuroectoderm, the delaminating neuroblasts and their neighbors differ by the medial myosin pulse intensity and frequency. The neuroblast fate is set to be distinct from their neighbors by a lateral inhibition process mediated by the Notch-Delta signaling pathway. It is unclear how the fate-determining Notch signaling activity is translated into a patterned and dynamic myosin network in the neuroectoderm. The canonical Notch signaling pathway is dependent on enzymatic cleavage and release of Notch intracellular domain (NICD), which translocates into the nucleus and functions as a transcriptional regulator. The uncleaved Notch also has a NICD-independent noncanonical function of binding to beta-catenin and modulating beta-catenin level and activity (Andersen et al., 2012; Hayward et al., 2005). Since beta-catenin is an important cytoskeletal regulator, as a first step we examined whether the apical constriction process of delaminating neuroblasts is dependent on NICD-mediated transcription. We injected the embryos with a widely-used gamma-secretase inhibitor \( \text{N-\([3,5\text{-difluorophenacetyl-l-alanyl}\]-S-phenylglycine t-butyl ester (DAPT) to inhibit Notch cleavage and release of NICD (Geling et al., 2002; Vaccari et al., 2008). We observed that DAPT-injected embryos display phenotypes similar to those in Delta or Notch RNAi embryos (Supplemental Movie 11 and 15), indicating that the normal delamination pattern is}
dependent on NICD-release and the canonical Notch pathway. In the future it will be interesting to explore the molecular links and feedbacks between differential Notch signaling activity and a patterned myosin network existing in the neuroectoderm through experiments and modeling.

Methods

Fly stock

The fluorescent protein fusion stock sqh\textsuperscript{P}-Sqh::GFP; Gap43::mCherry (Martin et al., 2010) was obtained from Martin lab. Utrophin-GFP was obtained from Lecuit lab (Rauzi et al., 2010). The sqh\textsuperscript{P}-Sqh::mCherry mat67-Gal4; ECad-GFP mat15-Gal4 /TM3 line was obtained from Wieschaus lab. RhoGEF2 RNAi (Bloomington 34643) and TRiP background control line (Bloomington 36303) were obtained from Bloomington Drosophila Stock Center.

Immunofluorescence staining

Embryos are fixed and stained according to standard protocols (Sullivan et al., 2000). The primary antibodies used were rabbit anti-Insc (1:1000, gift from Doe lab) and mouse anti-Ac (1:20, Developmental Studies Hybridoma Bank). The secondary antibodies used were Alexa Fluor 405-conjugated goat anti-rabbit IgG and Alexa Fluor 405-conjugated goat anti-mouse IgG (Life Technology).

Fly embryo live imaging

Embryos of the correct stage were collected, dechorionated with bleach for 40s, washed with ddH\textsubscript{2}O and mounted with the ventrolateral side up onto a slide coated with embryo glue (30cm Scotch double-sided tape dissolved in 5ml heptane). No.1 coverslips were glued to the slide using double-sided tape as spacers. A No.0 coverslip was then added on top of the spacers to create a chamber so that embryos in the chamber are not compressed. The chamber was filled with Halocarbon 27 oil.

All images were acquired on a Leica TCS SP8 confocal microscope at room temperature with a 40×/1.1 NA water-immersion objective. The pinhole was set at 1.5 airy units. For two-color simultaneous excitation, a 488 nm OPAL laser was used to excite GFP and a 552 nm OPAL laser was used to excite mCherry. The band-pass filters for detection were set at 492-538 nm for GFP and 558-650 nm for mCherry. For wild type, water-injected and delta RNAi embryos, images were acquired at 6s interval for 30 min. For each time point, the images were taken as 4 z-slice stacks with a z-stack step size of 0.8\textmu m. For DMSO-injected, CytoD-injected, DAPT-injected, RhoGEF2 RNAi and TRiP line background control embryos, images were acquired at 10s interval for 50min as 7 z-slice stacks that are 0.8 \textmu m apart.

Double-stranded RNA synthesis

Primers for double-stranded RNA synthesis are listed in supplemental methods. Genomic DNA was extracted from sqh\textsuperscript{P}-Sqh::GFP; Gap43::mCherry flies. PCR products were amplified from the genomic DNA. The PCR products were purified using DNA Clean & Concentrator\textsuperscript{TM}-5 kit (ZYMO RESEARCH) and used as templates for in vitro transcription reaction using MEGAscript transcription kit (Ambion). The dsRNA was purified with RNA
Clean & Concentrator™-5 kit (ZYMO RESEARCH) and resuspended in DEPC treated water.

Embryo drug injection and dsRNA injection

To prepare embryos for drug injection or dsRNA injection, embryos of the appropriate stage were dechorionated in bleach, washed in water and mounted on a glass slide with the ventrolateral side up. The embryos were then desiccated for 9 min, and covered with halocarbon oil 700 for injection. The embryos were injected laterally at the posterior end. After injection, the halocarbon oil 700 was flushed away using the halocarbon oil 27 and the injected embryos are covered with a thin layer of halocarbon oil 27. CytoD (Enzo Life Sciences) was resuspended at 5 mg/ml, diluted in DMSO and injected at 0.25 mg/ml after ventral furrow closes immediately before imaging. DAPT (CAS208255-80-5, Santa Cruz) was kept as 30mM stock in DMSO and injected at 10 mM within 30min after egg laying. For dsRNA injection, dsRNA was injected at a concentration of 2-3mg/ml within 30min after egg-laying. The injected embryos were then put in a humid petri dish at 25 °C for about three hours prior to imaging.

Image processing and data analysis

The images were processed with Fiji and MATLAB. The cell membrane outline is extracted from the bottom slice of the GAP43-mCherry or E-Cadherin-GFP Z-stack. Myosin-GFP or Myosin-mCherry is the maximum Z project of all slices of the Myosin-GFP or Myosin-mCherry z-stack. Gaussian blur filter (radius=1 or 2 for myosin and membrane outline channel, respectively) was applied to reduce background noise. We used Tissue Cell Segment Movie macro from Fiji to detect cell boundary and set the junction width as 1 μm.

Embryo Development Geometry Explorer (EDGE) (Gelbart et al., 2012) was used to measure total/medial myosin (myosinT/M), total apical area (areaT) and cell perimeter (peri). Junctional myosin (myosinJ) was calculated by subtracting medial myosin (myosinM) from total myosin (myosinT). Medial (meanM) or junctional (meanJ) myosin intensity was calculated through dividing medial or junctional myosin by their respective areas. Junctional area (areaJ) equals the cell perimeter multiplied by 0.5μm; Medial area (areaM) equals total cell area (areaT) subtracted by junctional area (areaJ). Raw data at each time point were smoothed by averaging the values of three adjacent time points

\[
\text{areaJ} = \text{peri} \times 0.5;
\]
\[
\text{areaM} = \text{areaT} - \text{areaJ};
\]
\[
\text{myosinJ} = \text{myosinT} - \text{myosinM};
\]
\[
\text{meanM} = \frac{\text{myosinM}}{\text{areaM}};
\]
\[
\text{meanJ} = \frac{\text{myosinJ}}{\text{areaJ}}
\]

Apical constriction rate was calculated as the inverse value of the first derivative of apical area. Myosin intensity rate of change was the first derivative of myosin intensity. Pearson cross-correlation between apical constriction rate and myosin intensity change rate was calculated using MATLAB toolbox for different time offsets. Cell area was normalized by
setting the first frame value of each cell as 1, and the data of following frames were calculated proportionally (Fig. 1 and Fig. 5); The myosin intensity was normalized so that the maximum value of all cells in each cluster was 1 and the minimum value was 0, other frames were calculated proportionally (Fig. 2). Pulses are defined as smoothed data fragments above the threshold. For apical area constriction pulse, the threshold is 10 μm²min⁻¹. For medial and junctional myosin pulse, the threshold is the average myosin intensity of all cells in each cluster. For supplemental movies 2, 3, 4, 5, 7, 8, 11, 12, 13 and 14, clusters of cells move within the extending germband and tissue drift is corrected with StackReg Plugin in Image J for movie display. No tissue drift correction was performed for data quantification and analysis.

**Mathematical modeling**

We develop a phase field model to investigate the dynamics of delaminating neuroblasts. In the phase field model, a set of continuous phase field variables are used, i.e., \( \eta_i(x), \eta_2(x), ..., \eta_p(x), \) where \( x \) is the position and \( p \) represents the number of cells in space.

Then the shape of the \( i \)th cell is described by a narrow transition layer between the interior of cell (\( \eta_i(x) = 1 \)) and exterior of cell (\( \eta_i(x) = 0 \)).

The total free energy is

\[
E_{total} = \int \left[ \sum_{i=1}^{p} \frac{\kappa_i}{2} (\nabla \eta_i(x))^2 + f(\eta_1(x), \eta_2(x), ..., \eta_p(x)) \right] dx ,
\]

where the coefficient \( \kappa_i \) is the parameter reflecting the thickness of the cell membrane, and it can also be used to represent the interfacial anisotropy of different types of cells. To simulate the cell-cell interactions, we choose the following free energy density functional

\[
f(\eta_1(x), \eta_2(x), ..., \eta_p(x)) = \sum_{i=1}^{p} \alpha \eta_i^2 (\eta_i - 1)^2 + \beta \sum_{i=1}^{p} \sum_{j \neq i} \eta_i^2 \eta_j^2 ,
\]

where \( \alpha \) and \( \beta \) are two parameters. The general requirement for choosing parameters is that it has \( p \) degenerate minima with equal depth, \( f_{min} \), located at

\((\eta_1(x), \eta_2(x), ..., \eta_p(x)) = (1,0,...,0), (0,1,...,0),..., (0,0,...,1)\)

in \( p \) dimensional space. The last term in Eq. 2 represents the interactions of neighboring cells, such as cell-cell adhesion.

Disregarding cell growth and cell division, the cell area \( V = \int_\Omega \eta(x) dx \) is generally conserved during deformation and movement. So an apical area constraint term is introduced as follows:

\[
E_{area}(\eta_1, \eta_2, ..., \eta_p) = \sum_{i=1}^{p} M \left( \int \eta_i(x) dx - V_0 \right)^2 ,
\]

where \( V_0 \) is the average apical area of each cell. The penalty constant \( M \) is defined as \( M = M_0 \int \eta(x) dx \), so that it allows the cell to delaminate without changing the energy.

Next, we incorporate the myosin signals during the delamination process. Because the molecular origins of cortical forces and the detailed underlying mechanism are uncertain, we use a phenomenological and simple description of the contraction force caused by the myosin:
\[ F_\text{myosin} = -(\rho_M(t) + \lambda \rho_J(t))n, \]  
where \( n \) is the unit normal vector on the cell membrane. The total myosin is composed by the medial myosin \( \rho_M(t) \) and the junctional myosin \( \rho_J(t) \) with a scaling parameter \( \lambda \). In the simulations, we generate stochastic medial myosin pulse \( \rho_M(t) \) with square wave pulses at regular intervals with the mean media myosin intensity as the amplitude and 30 seconds as the width. For simplification, the junctional myosin \( \rho_J(t) \) is chosen to be a Gaussian random signal as the basal level of the total myosin intensity.

The evolution equations for phase field variables are then governed by

\[ \tau \frac{\partial \eta_i}{\partial t} = -\frac{\delta E_{\text{total}}}{\delta \eta_i} - \frac{\delta E_{\text{area}}}{\delta \eta_i} + F_{\text{myosin}}, \quad i = 1, ..., p. \]  

Here \( \tau \) is the relaxation time.

We estimate the approximation of the medial myosin pulse \( \rho_M(t) \) by fitting the curve of the distribution of medial myosin intensity for delaminating cells and none-delaminating cells respectively (Supplemental Figure 7). We obtain mean 0.5027 and standard deviation 0.2363 of medial myosin intensity for delaminating cells, and mean 0.222 and standard deviation 0.1712 for non-delaminating cells. The frequency of the medial myosin pulse is 0.8 pulse/min in the delaminating cells and 0.5 pulse/min in the non-delaminating cells (Figure 2J). The junctional myosin pulse has no essential difference between delaminating cells and non-delaminating cells (Figure 2N), so we set the junctional myosin \( \rho_J(t) \) as a constant for simplification.

In the simulations, we choose the number of cells to be six, i.e., \( p=6 \). The domain size is 15 \( \mu m \times 15 \mu m \). The period boundary condition is applied and the Fourier spectral method is implemented for space discretization. Some values of parameters are \( \kappa = 0.09, M_0 = 8 \times 10^{-4}, \lambda = 0.1, \alpha = 1 \) and \( \beta = 1 \).

We applied the constrained string method (Du and Zhang, 2009) to compute the minimum energy path in Figure 3A. 26 images are used to discrete the string in the simulation. The algorithm details of the constrained string method can be found in reference (Du and Zhang, 2009).

We applied the semi-implicit scheme for the time discretization to solve Eq. 4 to obtain the simulation in Figure 4B.
Author contributions:

Y.A. and Y.Y. designed the experiments. Y.A. performed all the experiments. Y.A., G.S.X. and I. T. analyzed the data and prepared all the Figures except Figure 4, Supplemental Figure 6 and Supplemental Movie 9. L.Z. performed the mathematical modeling and prepared Figure 4 and Supplemental Figure 6, and Supplemental Movie 9. X.W.Z., W.C.Y., Y.S., D.M. and I. T. provided data analysis tools. Y.Y., L.Z., Y.A. wrote the manuscript.

Acknowledgements

We thank Dr. Adam Martin, Dr. Eric Wieschaus, Dr. Chris Doe, Dr. Bing He, Dr. Thomas Lecuit, Bloomington Stock Center and Developmental Biology Hybridoma Bank for providing fly stocks and reagents. We thank Dr. Zilong Wen, Dr. Karl Herrup and Dr. Mingjie Zhang for sharing their confocal microscopes. We thank Dr. Trudi Schupbach for helpful comments on the manuscript. This work was supported by grants to Yan Yan from the Research Grants Council of the Hong Kong Special Administrative Region (grants GRF16103815, AoE/M-09/12) and to Zhang Lei from China NSFC (grants No. 11622102 and 91430217).
Reference:


**Figures**

(A) Illustration of the embryo developmental stage and orientation for live imaging. The embryo was mounted with the ventrolateral neurogenic region facing the objective. We started imaging as the ventral furrow closes.

(B) Time-lapse images of the apical area change of a neuroblast (marked by a magenta dot) and its surrounding cells (marked by blue dots) in a representative cluster of cells (from Supplemental Movie 2). The apical cell outline is visualized by a membrane-mCherry transgenic line. Scale bar: 5 μm.

(C) A cluster of cells with a delaminating neuroblast in the center stained for Insuteable (Insc, gray, n>50). The apical cell outline is visualized by a membrane-mCherry transgenic line (magenta). Scale bar: 5 μm.

(D-E) The apical area change (D-E) and normalized apical area change (D’-E’) of 25 neuroblasts (D, D’) and 67 neighboring cells (E, E’) during a 6-min delamination window from 12 embryos. In D’ and E’, at the imaging onset, the apical area of each cell is set to one. For the rest of time points, the apical area of each cell is shown as the its original value divided by its cell area at imaging onset, highlighting the relative cell area change over time for individual cells. Each row represents the normalized apical area for one cell over the 6-min time window. The normalized apical area at each time point for each individual cell is color-coded and the value varies between 0.2 and 1.8 (see color bars).

**Figure 1** The *Drosophila* ventral nerve cord (VNC) neuroblasts undergo pulsatile apical constriction.

(A) Illustration of the embryo developmental stage and orientation for live imaging. The embryo was mounted with the ventrolateral neurogenic region facing the objective. We started imaging as the ventral furrow closes.

(B) Time-lapse images of the apical area change of a neuroblast (marked by a magenta dot) and its surrounding cells (marked by blue dots) in a representative cluster of cells (from Supplemental Movie 2). The apical cell outline is visualized by a membrane-mCherry transgenic line. Scale bar: 5 μm.

(C) A cluster of cells with a delaminating neuroblast in the center stained for Insuteable (Insc, gray, n>50). The apical cell outline is visualized by a membrane-mCherry transgenic line (magenta). Scale bar: 5 μm.

(D-E) The apical area change (D-E) and normalized apical area change (D’-E’) of 25 neuroblasts (D, D’) and 67 neighboring cells (E, E’) during a 6-min delamination window from 12 embryos. In D’ and E’, at the imaging onset, the apical area of each cell is set to one. For the rest of time points, the apical area of each cell is shown as the its original value divided by its cell area at imaging onset, highlighting the relative cell area change over time for individual cells. Each row represents the normalized apical area for one cell over the 6-min time window. The normalized apical area at each time point for each individual cell is color-coded and the value varies between 0.2 and 1.8 (see color bars).
(F) The apical area constriction rate of the 25 neuroblasts tracked in (D). Each row represents the apical constriction rate for one cell over the 6-min time window. The apical constriction rate at each time point for each individual cell is color-coded and the value varies between -40 μm²/min (stretch) and 40 μm²/min (constriction) (see color bars).

(G) Plot of the apical area change (blue line) and the constriction rate (red line) of a representative delaminating neuroblast.
Figure 2 A dynamic apical myosin network composed of medial and junctional myosin is present in delaminating neuroblasts and their neighboring cells.

(A) Time-lapse images of a medial myosin pulse in one delaminating neuroblast from an embryo expressing Myosin-GFP (gray in upper panel, green in lower panel) and membrane-mCherry (magenta in lower panel). White arrowheads indicate that medial myosin foci coalescence and disassemble. Scale bar: 5 μm.

(B) Plot of the apical area (blue line), medial myosin intensity (red line) and junctional myosin intensity (dashed red line) as a function of time for one representative delaminating neuroblast.
(C-F) Plot of correlation between apical constriction rate and myosin intensity rate of change for individual delaminating neuroblasts (C-D, n=25, from 12 embryos) and their neighboring cells (E-F, n=67, from 12 embryos) against time offset for medial myosin (C and E) and junctional myosin (D and F), respectively. Mean cross-correlation against time offset for C-F is plotted in C’-F’.

(G) Plot of mean medial myosin intensity for delaminating neuroblasts (magenta line, n=25, from 12 embryos) and their neighboring cells (blue line, n=67, from 12 embryos) as a function of time. Error bars are standard deviation.

(H-I) Plot of normalized medial myosin intensity for individual delaminating neuroblasts (H, n=25, from 12 embryos) and their neighboring cells (I, n=67, from 12 embryos) as a function of time. To compare the myosin intensity for each delaminating neuroblast with its neighboring cells, the myosin intensity was normalized within each cluster of cells using the following method: the maximum myosin intensity value is set as 1; the minimum value is set as 0; the intensity values were calculated proportionally between 0 and 1. Each row represents the normalized medial myosin intensity (color-coded) for one cell over a 6-min time window.

(J) Quantification of medial myosin pulse frequency in delaminating neuroblasts (n=25, from 12 embryos) and their neighboring cells (n=67, from 12 embryos). Error bars are standard errors of the mean (s.e.m.). P value is calculated by one-way ANOVA.

(K) Plot of mean junctional myosin intensity for delaminating neuroblasts (magenta line, n=25, from 12 embryos) and their neighboring cells (blue line, n=67, from 12 embryos) as a function of time. Error bars are standard deviation.

(L-M) Plot of normalized junctional myosin intensity for individual delaminating neuroblasts (H, n=25, from 12 embryos) and their neighboring cells (I, n=67, from 12 embryos) as a function of time. The normalization method is described in (H-I). Each row represents the normalized junctional myosin intensity (color-coded) for one cell over the 6-min time window.

(N) Quantification of junctional myosin pulse frequency in delaminating neuroblasts (n=25, from 12 embryos) and their neighboring cells (n=67, from 12 embryos). Error bars are s.e.m. P value is calculated by one-way ANOVA.
Figure 3 Medial myosin pulses promote apical constriction in delaminating neuroblasts.

(A) Time-lapse images of the apical F-actin visualized by Utrophin-GFP in one presumptive neuroblast from a DMSO-injected embryo (upper panel) and an embryo injected with 0.25mg/ml CytoD (lower panel). Note that in embryos injected with a low dose of CytoD, the medial actin forms discrete foci and fails to form a meshwork that coalesces and disassembles. White arrowheads indicate that medial actin meshwork coalesces and disassembles. Scale bar: 5 μm.

(B) Time-lapse images of the apical myosin visualized by Myosin-GFP (green) in one presumptive neuroblast from a DMSO-injected embryo (upper panel) and an embryo injected with 0.25mg/ml CytoD (lower panel). The apical cell outline is visualized by membrane-mCherry (magenta). White arrowheads indicate that medial myosin foci coalesce and disassemble. Note that medial myosin pulses are depleted in embryos injected with a low dose of CytoD. Scale bar: 5 μm.

(C) Plot of mean medial myosin intensity (C) and mean junctional myosin intensity (C') as a function of time in delaminating neuroblasts (yellow line, n=15) from 5 DMSO-injected embryos and presumptive neuroblasts (green line, n=47) from 15 embryos injected with 0.25mg/ml CytoD. Error bars are standard deviation.

(D) Quantification of percentage of delaminating cells observed in DMSO-injected embryos (n=5) and embryos injected with 0.25mg/ml Cyto D (n=15) in a 10-min time window. Error bars are s.e.m. P value is calculated by one-way ANOVA.

(E) Embryos injected with DMSO and 0.25mg/ml CytoD were stained for Achaete (gray) 90min after the onset of gastrulation. The apical cell outline is visualized by a membrane-mCherry transgenic line (magenta). Scale bar: 20 μm.

(F) Time-lapse images of the apical myosin visualized by Myosin-mCherry (gray in upper panel and magenta in lower panel) in one neuroblast from a control embryo (F) and a RhoGEF2 RNAi embryo (F'). The apical cell outline is visualized by an Ecadherin-GFP line (green). White arrowheads indicate that medial myosin foci coalesce and disassemble. Embryo genotypes are described in the figure legends of Supplemental Movie 7 and 8. Scale bar: 5 μm.

(G) Plot of mean medial myosin intensity (G) and mean junctional myosin intensity (G') as a function of time in delaminating neuroblasts (yellow line, n=13) from 7 control embryos and presumptive neuroblasts (green line, n=21) from 5 RhoGEF2 RNAi embryos. Error bars are standard deviation.

(H) Quantification of percentage of delaminating cells observed in control embryos (n=8) and RhoGEF2 RNAi embryos (n=7) in a 10-min time window. Error bars are s.e.m. P value is calculated by one-way ANOVA.

(I) Control and RhoGEF2 RNAi embryos were stained for Achaete (gray) 90min after the onset of gastrulation. The apical cell outline is visualized by an Ecdaherin-GFP line (magenta). Scale bar: 20 μm.
Figure 4 Mathematical modeling of the neuroblast delamination process.

(A) Minimum energy path reveals that the delaminating cell needs to overcome an energy barrier by passing through a transition state.
(B) Time-lapse images of a cluster of cells with one cell in the center undergoing delamination in simulation.
(C) Plots of the apical area change as a function of time for delaminating cells from six simulations, and plots of the stochastic medial myosin intensity (blue dash curve) and the smooth medial myosin intensity (red solid curve) for delaminating cells (mean 0.5, frequency 1 pulse/min).
(D) Plots of the apical area change and the medial myosin intensity for non-delaminating cells with low-frequency myosin (mean 0.5, frequency 0.5 pulse/min).
(E) Plots of the apical area change and the medial myosin intensity for non-delaminating cells with low-intensity myosin (mean 0.25, frequency 1 pulse/min).
Figure 5 Small clusters of presumptive neuroblasts undergo apical constriction upon inhibition of Notch signaling activity.

(A) Time-lapse images of the apical area visualized by membrane-mCherry in clusters of neuroblasts (marked by magenta dots) and their surrounding cells (marked by blue dots) from a Delta RNAi embryo.
(B) Plot of the apical area (blue line), medial myosin intensity (red line) and junctional myosin intensity (dashed red line) as a function of time for one representative neuroblast from a Delta RNAi embryo. 

(C-D) The apical area change (C-D) and normalized apical area change (C’-D’) of 39 neuroblasts (C, C’) and 34 neighboring cells (D, D’) during a 12-min delamination window from 8 Delta RNAi embryos. In C’ and D’, at the imaging onset, the apical area of each cell is set to one. For the rest of time points, the apical area of each cell is shown as the its original value divided by its cell area at imaging onset, highlighting the relative cell area change over time for individual cell. Each row represents the normalized apical area for one cell over the 12 min time window. The normalized apical area at each time point for each individual cell is color-coded and the value varies between 0.1 and 2.5 (see color bars). 

(E-F) Plot of correlation between apical constriction rate and myosin intensity rate of change for individual delaminating neuroblasts (n=11, from 4 Delta RNAi embryos) against time offset for medial myosin (E) and junctional myosin (F), respectively. Mean cross-correlation against time offset for E-F is plotted in E’- F’. 

(G-H) Plot of mean medial (G) and junctional (H) myosin intensity for delaminating neuroblasts (magenta line, n=11, from 4 embryos) and their neighboring cells (blue line, n=10, from 4 embryos) in Delta RNAi embryos as a function of time. Error bars are standard deviation. 

(I) Quantification of apical area decrease rate for delaminating neuroblasts in water injected embryos (n=18, from 8 embryos) and Delta RNAi embryos (n=39, from 8 embryos). Error bars are s.e.m. P value is calculated by one-way ANOVA. 

(J) Quantification of medial myosin pulse frequency for delaminating neuroblasts (n=18, from 8 water-injected embryos, n=11, from 4 Delta RNAi embryos, light gray bar) and non-delaminating cells (n=52, from 8 water-injected embryos, n=10, from 4 Delta RNAi embryos, dark gray bar). Error bars are s.e.m. P value is calculated by one-way ANOVA. 

(K-L) Plot of mean medial myosin intensity (K) and mean junctional myosin intensity (L) as a function of time in delaminating neuroblasts from 8 water-injected embryos (yellow line, n=18) and 4 Delta RNAi embryos (green line, n=11). Error bars are standard deviation. 

(M) Time-lapse images of two delaminating neuroblasts from a Delta RNAi embryo expressing Myosin-GFP (gray in upper panel, green in lower panel) and membrane-mCherry (gray in middle panel, magenta in lower panel). Note that Myosin-GFP forms intense cables around the junctional region that fail to dissemble over time. Scale bar: 5 μm.
Supplemental Movies

**Supplemental Movie 1** Live imaging of an embryo expressing membrane-mCherry with three delaminating neuroblasts highlighted with magenta pseudo-color. Images are obtained at a time interval of 6s between frames. The video is displayed at 10 frames per second (fps). Scale bar: 10µm

**Supplemental Movie 2** Live imaging of a delaminating neuroblast (pseudocolored in magenta) and its neighboring cells (pseudocolored in blue) in an embryo expressing Myosin-GFP and membrane-mCherry. Images are obtained at a time interval of 6s between frames. The video is displayed at 10fps. Scale bar: 5µm
Supplemental Movie 3 Live imaging of a delaminating neuroblast (pseudocolored in magenta) and its neighboring cells in a DMSO-injected embryo expressing Utrophin-GFP. Images are obtained at a time interval of 10s between frames. The video is displayed at 10fps. Scale bar: 5µm
Supplemental Movie 4 Live imaging of cells in the neuroectoderm in an embryo injected with 0.25mg/ml CytoD expressing Utrophin-GFP. Images are obtained at a time interval of 10s between frames. The video is displayed at 10fps. Scale bar: 5µm
Supplemental Movie 5 Live imaging of a delaminating neuroblast (pseudocolored in magenta) and its neighboring cells in a DMSO-injected embryo expressing Myosin-GFP and membrane-mcherry. Images are obtained at a time interval of 10s between frames. The video is displayed at 10fps. Scale bar: 5µm

Supplemental Movie 6 Live imaging of cells in the neuroectoderm in an embryo injected with 0.25mg/ml CytoD expressing Myosin-GFP and membrane-mcherry. Images are obtained at a time interval of 10s between frames. The video is displayed at 10fps. Scale bar: 5µm
**Supplemental Movie 7** Live imaging of a delaminating neuroblast (pseudocolored in magenta) and its neighboring cells in a control embryo expressing Myosin-mCherry and E-cadherin-GFP. Embryo genotype: sqh<sup>P</sup>-Sqh::mCherry mat67-Gal4/+; ECad-GFP\mat15-Gal4 / P{y[+t7.7]=CaryP}attP2 (TRiP background line). Images are obtained at a time interval of 10s between frames. The video is displayed at 10fps. Scale bar: 5µm.

**Supplemental Movie 8** Live imaging of a presumptive neuroblast (pseudocolored in magenta) and its neighboring cells in an RhoGEF2 RNAi embryo expressing Myosin-mCherry and E-cadherin-GFP. Embryo genotype: sqh<sup>P</sup>-Sqh::mCherry mat67-Gal4/+; ECad-GFP mat15-Gal4/UAS-RhoGEF2RNAi. Images are obtained at a time interval of 10s between frames. The video is displayed at 10fps. Scale bar: 5µm.
**Supplemental Movie 9** Dynamics simulation of neuroblast delamination. Neuroblast is the center cell in the movie. The video is displayed at 5fps. The simulation box size: $15\mu m \times 15\mu m$.

**Supplemental Movie 10** Live imaging of a Delta RNAi embryo expressing membrane-mcherry with a cluster of delaminating neuroblasts highlighted with magenta pseudo-color. Images are obtained at a time interval of 6s between frames. The video is displayed at 10 frames per second (fps). Scale bar: 10µm
Supplemental Movie 11 Live imaging of a cluster of delaminating neuroblasts highlighted with magenta pseudo-color in a *Notch RNAi* embryo expressing membrane-mcherry and Myosin-GFP. Images are obtained at a time interval of 10s between frames. The video is displayed at 10 frames per second (fps). Scale bar: 5µm

Supplemental Movie 12 Live imaging of two delaminating neuroblasts (pseudocolored in magenta) in a *Delta RNAi* embryo expressing Myosin-GFP and membrane-mcherry. Images are obtained at a time interval of 6s between frames. The video is displayed at 10 frames per second (fps). Scale bar: 5µm
Supplemental Movie 13 Live imaging of a delaminating neuroblast highlighted with magenta pseudo-color in a snail RNAi embryo expressing membrane-mcherry and Myosin-GFP. Images are obtained at a time interval of 6s between frames. The video is displayed at 10 frames per second (fps). Scale bar: 5µm

Supplemental Movie 14 Live imaging of a delaminating neuroblast highlighted with magenta pseudo-color in a twist RNAi embryo expressing membrane-mcherry and Myosin-GFP. Images are obtained at a time interval of 10s between frames. The video is displayed at 10 frames per second (fps). Scale bar: 5µm
Supplemental Movie 15 Live imaging of a cluster of delaminating neuroblasts highlighted with magenta pseudo-color in an embryo injected with DAPT expressing membrane-mcherry and Myosin-GFP. Images are obtained at a time interval of 10s between frames. The video is displayed at 10 frames per second (fps). Scale bar: 5µm
Supplemental Figure 1 Quantification of apical constriction parameters in a representative delaminating neuroblast and its neighboring cells.

(A) Plot of the apical area of a delaminating neuroblast (magenta line) and its neighboring cells (blue lines) against time in a representative cluster shown in Supplemental Movie 2.

(B-C) Plot of the apical area constriction rate (blue line), medial myosin intensity change rate (red line, B) and junctional myosin intensity change rate (red line, C) for the delaminating neuroblast pseudocolored in magenta in Supplemental Movie 2 and shown in Figure 2B.

(D) Plot of the apical area (blue line), medial myosin intensity (red line) and junctional myosin intensity (dashed red line) as a function of time for one of the neighboring cells pseudocolored in blue in supplemental Movie 2.

(E-F) Plot of the apical area constriction rate (blue line), medial myosin intensity change rate (red line, E) and junctional myosin intensity change rate (red line, F) for the neighboring cell plotted in (D).
Supplemental Figure 2 Correlation analyses between apical constriction rate and myosin intensity and total myosin rate of change in ventral furrow cells and delaminating neuroblasts.

(A) Plot of mean cross-correlation between apical constriction rate and myosin intensity rate of change for individual ventral furrow cells against time offset (n=41, from 2 embryos).

(B) Plot of mean cross-correlation between apical constriction rate and total myosin rate of change for individual ventral furrow cells against time offset (n=41, from 2 embryos).

(C) Plot of mean cross-correlation between apical constriction rate and myosin intensity rate of change for individual delaminating neuroblasts against time offset (n=25, from 12 embryos, same as Figure 2C’).

(D) Plot of mean cross-correlation between apical constriction rate and total myosin rate of change for individual delaminating neuroblasts against time offset (n=25, from 12 embryos).
Supplemental Figure 3 Quantification of myosin intensity change in cells immediately next to a delaminating neuroblast (1st degree neighbors) and cells at least one cell distance away from a delaminating neuroblast (2nd degree neighbors).

(A-B) Plot of mean medial myosin intensity (A) and mean junctional myosin intensity (B) for 1st degree neighbors (light blue line, n=20, from 5 embryos) and 2nd degree neighbors (dark blue line, n=18, from 8 embryos) as a function of time. Error bars are standard deviation.

(C-F) Plot of medial myosin intensity (C-D) and junctional myosin intensity (E-F) for individual 1st degree neighbors (C, E) (n=20, from 5 embryos) and individual 2nd degree neighbors (D, F) (n=18, from 8 embryos) as a function of time.
Supplemental Figure 4 Western blotting analysis of knockdown efficiency in Notch RNAi (A) and Delta RNAi embryos (B).

(A) Lysates from 10 embryos injected with water and injected with Notch dsRNA are analyzed for Notch expression.
(B) Lysates from 10 embryos injected with water and injected with Delta dsRNA are analyzed for Delta expression.

Supplemental Figure 5 Quantification of apical constriction parameters in a representative delaminating neuroblast from a Delta RNAi embryo.

(A) Plot of the apical area of delaminating neuroblasts (magenta lines) and their neighboring cells (blue lines) against time in a representative cluster from a Delta RNAi embryo shown in Supplemental Movie 10.
(B-C) Plot of the apical area constriction rate (blue line), medial myosin intensity change rate (red line, B) and junctional myosin intensity change rate (red line, C) for a delaminating neuroblast pseudocolored in magenta from a Delta RNAi embryo shown in Supplemental Movie 10 and in Figure 5B.
Supplemental Figure 6 Mathematical modeling of the neuroblast delamination process in Delta RNAi embryos.

(A) Plots of the apical area change as a function of time for delaminating cells in three different cases. Case 1: Reduction of 2% energy barrier by decreasing area constraint constant (blue solid curve); Case 2: 20% increase of mean media myosin intensity (purple dash curve); Case 3: 25% increase of mean junctional myosin (red dot curve); (B-D) Plots of the stochastic myosin intensity for delaminating cells in Case 1: media myosin (mean 0.5, frequency 0.5 pulse/min), mean of junctional myosin 2 (B); Case 2: media myosin (mean 0.6, frequency 0.5 pulse/min), mean of junctional myosin 2 (C); and Case 3: media myosin (mean 0.5, frequency 0.5 pulse/min), mean of junctional myosin 2.5 (D). For (B-D), Neighboring cells: media myosin (mean 0.22, frequency 0.25 pulse/min), mean of junctional myosin 2.
Supplemental Figure 7 Distribution of normalized medial myosin intensity in delaminating neuroblasts and their neighbors.

(A) Plot of the distribution of normalized medial myosin intensity in delaminating neuroblasts (n=25 cells, 60 time points, from 12 embryos).
(B) Plot of the distribution of normalized medial myosin intensity in neighboring cells (n=67 cells, 60 time points, from 12 embryos).
Supplemental Method

Double stranded RNA synthesis primers

Primers for double-stranded RNA synthesis are:

\textit{Delta}-F, 5’- TAATACGACTCACTATAGGGGTGTGTGCCAATGGTTTCAG-3’;
\textit{Delta}-R: 5’- TAATACGACTCACTATAGGGCGACTTGTCCCAGGTGTTTT-3’;

\textit{Notch}-F, 5’- TAATACGACTCACTATAGGGCTACAAGGGCGTGGATTGTT-3’;
\textit{Notch}-R: 5’- TAATACGACTCACTATAGGGATATGTAGCCCGTGTAGCCG-3’;

\textit{Snail}-F, 5′-TAATACGACTCACTATAGGGCGGAACCGAAACGTGACTAT-3’;
\textit{Snail}-R, 5′-TAATACGACTCACTATAGGGCGGTAGTTTTTGGCATGAT-3’;

\textit{Twist}-F, 5′-TAATACGACTCACTATAGGGCCAAGCAAGATCACCAAAT-3’;
\textit{Twist}-R, 5′-TAATACGACTCACTATAGGGCACCTCGTTGCTGGGTATGT-3’;

Embryo western blotting

\textit{Drosophila} embryos are lysed and analysed by western blotting following standard protocol described in (Sullivan et al., 2000). The antibodies used are mouse anti-Notch (C17.9C6, DSHB), mouse anti-Delta (C594.9B, DSHB) and mouse anti-tubulin (12G10, DSHB).

Reference: