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\textsuperscript{P}-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\textsuperscript{P}-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: 15µm × 15µm.

Supplemental Movie 10  Live imaging of a Delta RNAi embryo expressing membrane-mcherry with a cluster of delaminating neuroblasts highlighted with magenta pseudocolor. 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:

**Delta-F**, 5′- TAATACGACTCACTATAGGGGTGTGTGCCAATGGTTTCAG-3’;
**Delta-R**: 5′- TAATACGACTCACTATAGGGCGACTTGTCCCAGGTGTTTT-3’;

**Notch-F**, 5′- TAATACGACTCACTATAGGGCTACAAGGGCGTGGATTGTT-3’;
**Notch-R**: 5′- TAATACGACTCACTATAGGGATATGTAGCCCGTGTAGCCG-3’;

**Snail-F**, 5′-TAATACGACTCACTATAGGGCGGAACCGAAACGTGACTAT-3’;
**Snail-R**, 5′-TAATACGACTCACTATAGGGCGGTAGTTTTTGGCATGAT-3’;

**Twist-F**, 5′-TAATACGACTCACTATAGGGGCCAAGCAAGATCACCAAAT-3’;
**Twist-R**, 5′-TAATACGACTCACTATAGGGGACCTCGTTGCTGGGTATGT-3’;

Embryo western blotting

*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 (1:1000, C17.9C6, DSHB), mouse anti-Delta (1:1000, C594.9B, DSHB) and mouse anti-tubulin (1:2000, 12G10, DHSB).

Reference: