

Figure S1. Basic anatomy and mechanics of germband extension. Cartoon depicting an embryo during early (left), mid (centre) or late (right) germband extension. The germband is coloured. Different colours indicate distinct cell populations: ventral (red), lateral (blue), and posterior (green). Anterior (A) left, posterior (P) right, dorsal (D) up, ventral (V) down. Black arrows indicate actomyosin-based contractile stresses generated in the germband. White arrows indicate tensile stresses caused by endoderm ingression in the posterior pole of the embryo.

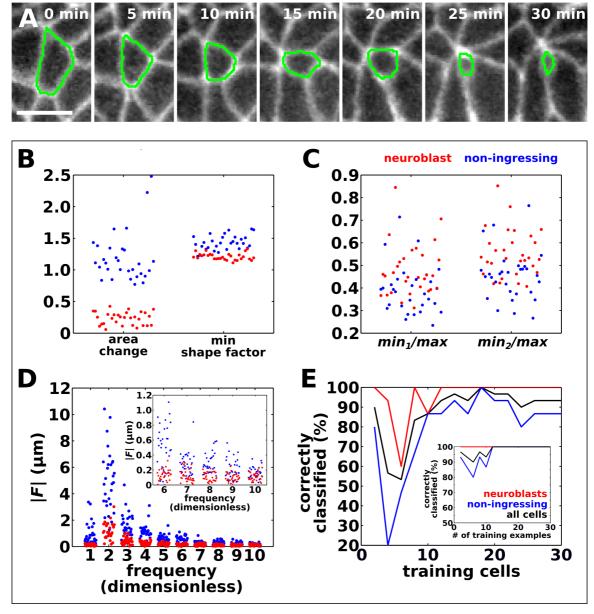
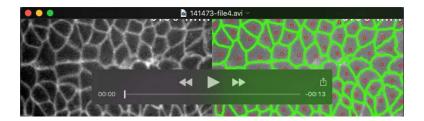
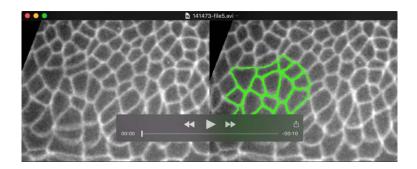


Figure S2. Ingressing neuroblasts display distinct morphological features. (A)

Neuroblast expressing Gap43:mCherry as it delaminates from the ectoderm. Green shows segmentation results. Bar, 20  $\mu$ m. Anterior left, ventral down. **(B-D)** Scatter plots of the area change ratio and minimum shape factor (B), minimum-to-maximum cell radii ratios (C), and 1-10 integer components of the Fourier transform of the centroid-polygon distance (D) both for ingressing neuroblasts (n = 30 in 10 embryos, red) and noningressing cells (n = 36 in 10 embryos, blue). **(D)** Inset shows the magnitude of frequency components 6-10 with a different Y-axis scale. **(E)** Percent correctly classified neuroblasts (red), non-ingressing (blue), and total (black) cells as a function of the number of cells in the training set (in a 1:1 ratio of neuroblasts to non-ingressing cells). Inset shows percent correctly classified cells using only 6 features (area change, minimum shape factor,  $min_1/max$ ,  $min_2/max$ , and frequency components 1 and 2).

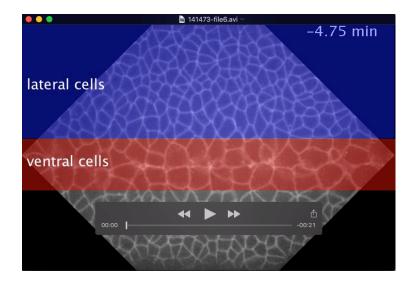


**Movie S1. Integrating image analysis and machine learning allows cell segmentation and tracking in developing embryos.** Germband cells expressing Gap43:mCherry at the final stages of axis elongation. Red circles represent watershed seeds. Green shows segmentation and tracking results. A stack was acquired every 15 s. Anterior left, ventral centre.

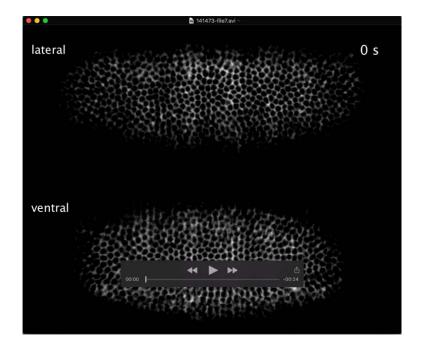


Movie S2. Image analysis and machine learning can detect ingressing neuroblasts.

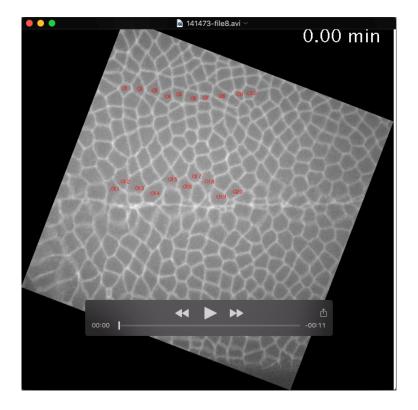
Cells in a stage 7 embryo expressing Gap43:mCherry. Green represents segmentation and tracking results. Red indicates detection of a neuroblast. A stack was acquired every 15 s. Anterior left, ventral down.



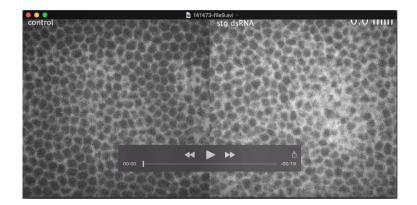
Movie S3. Cells on the anterior end of the germband divide during *Drosophila* axis elongation. Germband cells expressing Gap43:mCherry at the final stages of axis elongation. Red ventral, blue lateral. A stack was acquired every 15 s. Anterior left, ventral down.



**Movie S4. Ventral cells divide at the same time as lateral cells.** Germband cells expressing Gap43:mCherry during axis elongation acquired using dual-illumination, light sheet microscopy. Cyan arrow indicates first lateral division, red arrow shows first ventral division. A stack was acquired every 25 s. Anterior left, ventral down (top) or centre (bottom).



**Movie S5. Ventral cells do not intercalate during axis elongation.** Germband cells expressing Gap43:mCherry during axis elongation. Red circles track cells. Cells 1-10 are lateral cells, cells 11-20 are ventral cells. A stack was acquired every 15 s. Anterior left, ventral down.



**Movie S6.** *stg* dsRNA eliminates germband divisions. Germband cells expressing Gap43:mCherry during axis elongation in a water-injected control (left) or an embryo injected with dsRNA against *stg* (right). A stack was acquired every 30 s. Anterior left, ventral down.