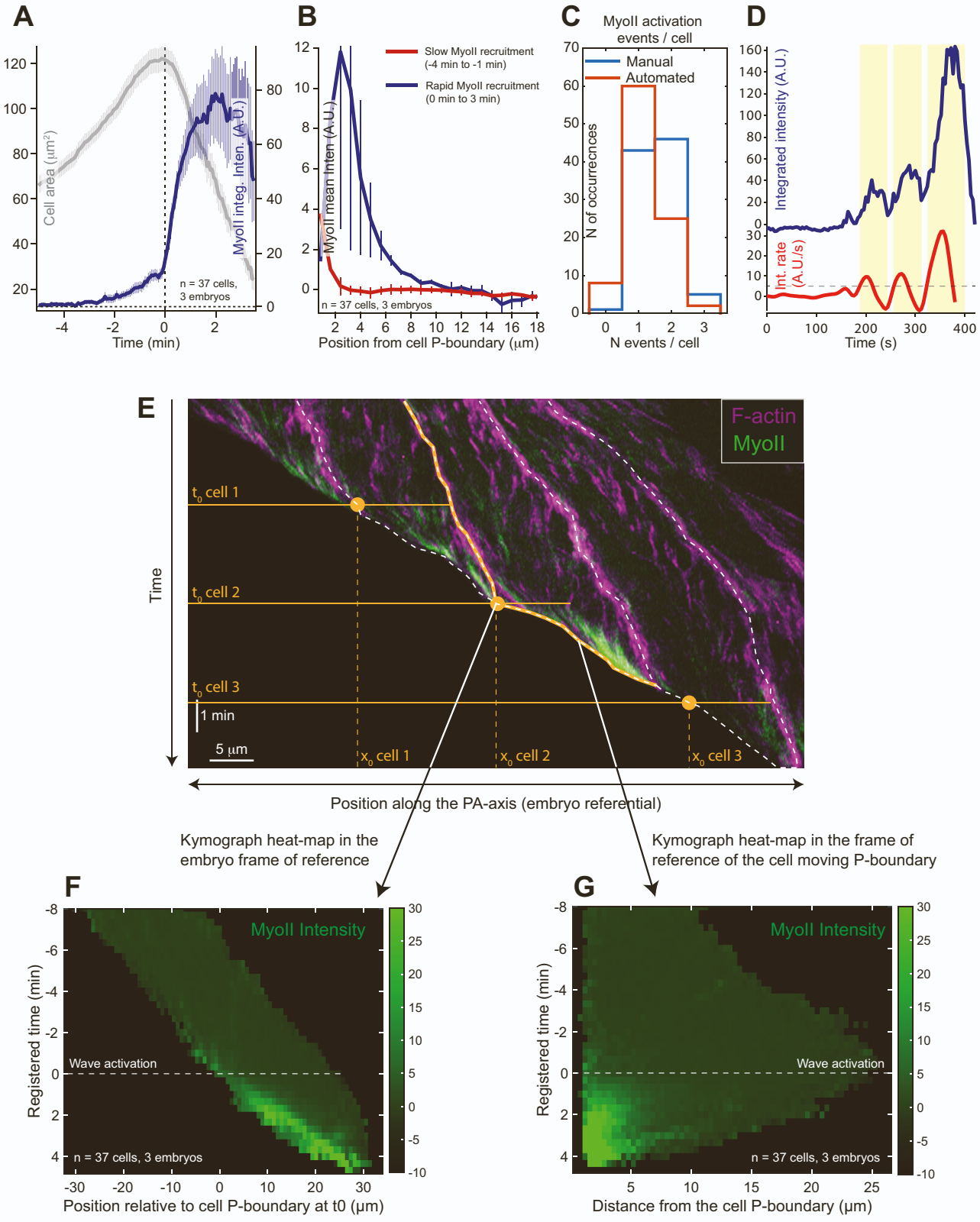
Developmental Cell, Volume *59*

Supplemental information

Mechanical regulation of substrate adhesion and de-adhesion drives a cell-contractile wave during *Drosophila* tissue morphogenesis

Claudio Collinet, Anaïs Bailles, Benoit Dehapiot, and Thomas Lecuit

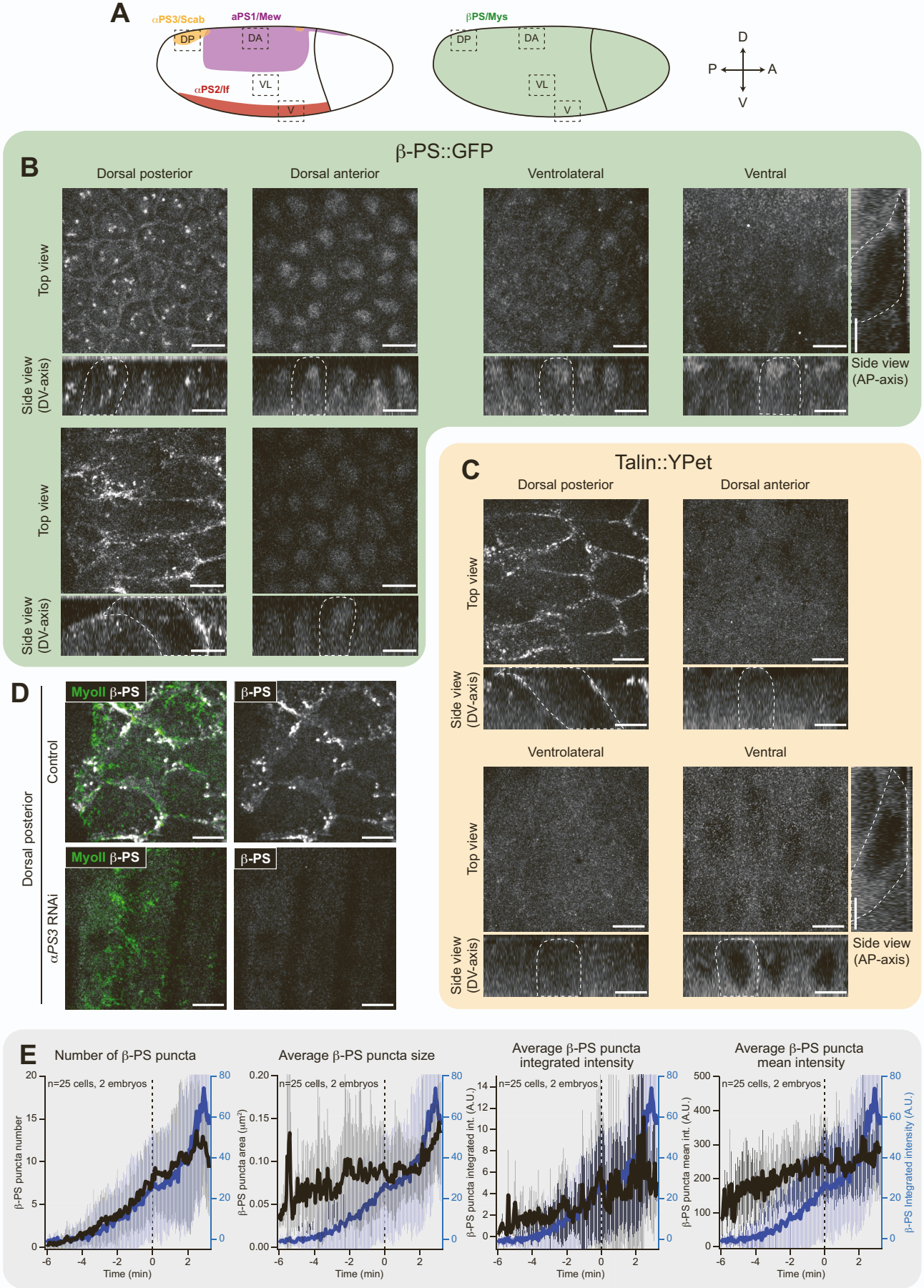
Supplementary Fig. S1



Supplementary Figure S1 (related to Fig.1): MyoII recruitment in cells and methods for cell registration. (**A**) Time trace of MyoII integrated intensity and cell area in cells of the propagation zone. Time 0 is the onset of rapid MyoII recruitment. Mean±s.e.m between cells, n=37 cells, 3

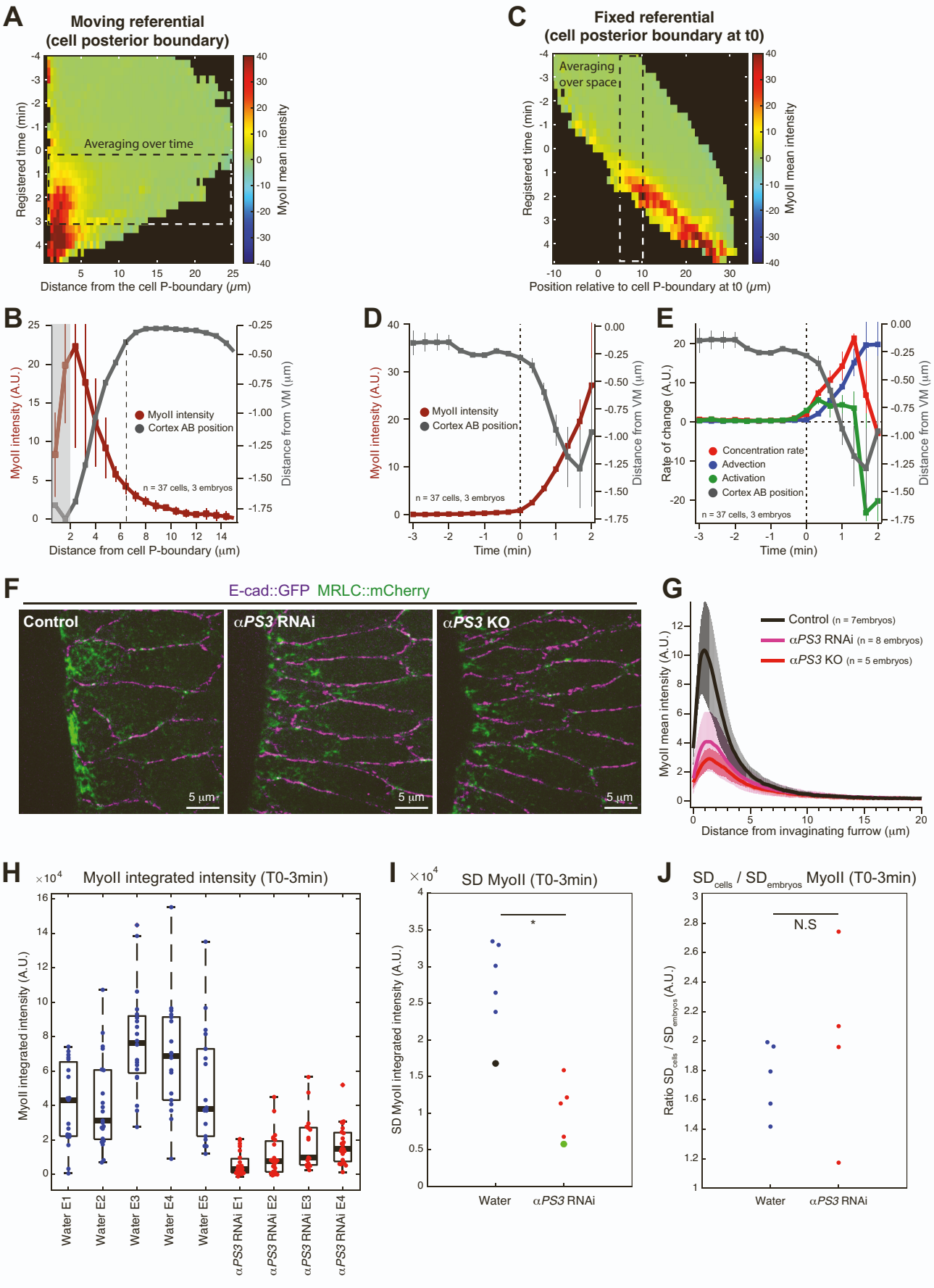
embryos. (**B**) Spatial distribution of MyoII mean intensity within the cell at the indicated times. Time 0 is defined as in **A**. Spatial cell registration is performed as illustrated in **G**. Mean±SDs. n=3 embryos (37 cells). (**C**) Histogram of the number of MyoII activation events in cells of the propagation zone with manual (blue) or automated (red) detection. n=95 cells, 5 embryos. (**D**) MyoII integrated intensity time trace (blue) along with its derivative (red) of the cell in Fig.1D. The detected MyoII recruitment events (manual method) are highlighted in yellow. (**E**) Kymograph along the posterior-anterior (PA) axis of a region of the dorsal epithelium during wave propagation. Illustrated are the 2 types of spatial cell registration used in this study. In all cases time 0 (t0) is defined as the onset of rapid MyoII recruitment. For quantifications in the fixed embryo referential (as in **F**) cells are registered spatially by defining x=0 the position of the cell posterior boundary (P-boundary) at time t=0. For quantifications in the referential of the moving P-boundary of the cell (as in **G**), x=0 is the position of the cell P-boundary at each time point t. The dashed white lines highlight the A- and P- boundaries of three consecutive cells along the AP-axis. The solid orange line highlights the moving P-boundary of the middle cell. Indicated are the time t0 of the three cells. The orange solid circles indicate the position of the P-boundary at t0 for the three cells. Scale bars, 5µm for space and 1 min for time. (**F-G**) Kymograph heatmap of MyoII mean intensity in the fixed referential of the embryo (**F**) and in the moving referential of the cell P-boundary (**G**). The same data are plotted in **F** and **G**, n=37 cells, 3 embryos.

Supplementary Fig. S2



Supplementary Figure S2 (related to Fig.2): Integrin localization in the embryo and time evolution of β-PS puncta during wave propagation. (**A**) Illustration of the expression patterns of the 5 *Drosophila* α-Integrins (left) and of β-PS (*mys*), the main β-Integrin, (right) in the embryo at gastrulation stage. The patterns were derived from Sawala et al.[52]. Dashed boxes: the imaged regions in **B** and **C**. DP is dorsal posterior, DA dorsal anterior, VL ventrolateral and V is ventral. (**B-C**) Representative top and side views of β-PS (**B**) and Talin (**C**) in the indicated regions. The top views are maximum projections covering a depth of 9 µm. Side views are slices along the DV-axis and for ventral regions also slices along the AP-axis are shown. In **B** for the DP and DA regions images at onset of gastrulation (top) and mid gastrulation during wave propagation (bottom) are shown. All other images are at mid gastrulation stage. (**D**) Representative stills of MyoII and β-PS in the DP region in a control (water injection) and *αPS3* RNAi injected embryo. In **B**, **C** and **D** the scale bars are 5µm. (**E**) Time traces of the average number of β-PS puncta per cell, their average size, their average integrated intensity and their average mean intensity (in black) together with the total β-PS integrated intensity per cell (in blue). Time 0 is the onset of rapid MyoII recruitment. Mean±SDs between cells. n=25 cells, 2 embryos.

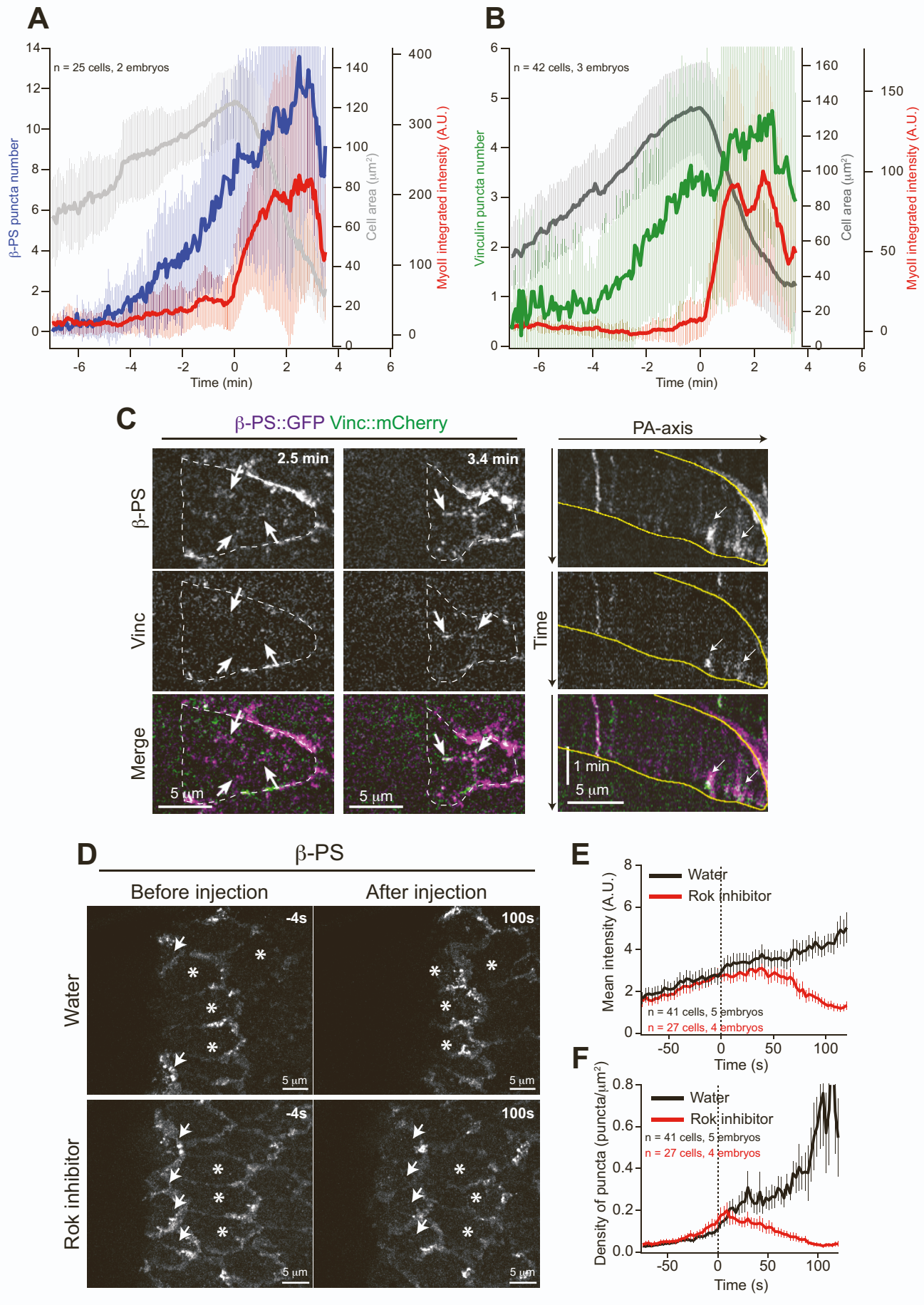
Supplementary Fig. S3



Supplementary Figure S3 (related to Fig.3): Generation of spatial and temporal profiles of MyoII intensity/MyoII rates and MyoII levels and cell/embryo variability in *αPS3* KD embryos. (**A** and **C**) Heatmap kymograph of MyoII intensity in WT embryos in the referential of the moving P-boundary of cells (**A)** or in the fixed referential of the embryo (**C**). Dashed boxes: region of the kymograph used to average along the time axis to obtain spatial profiles (as in **B**) or along the x-axis to obtain temporal profiles in a fixed region of the cortex (as in **D-E**). (**B**) Spatial profiles of MyoII mean intensity with the distance of the apical cortex from the vitelline membrane (cortex AB position) in WT cells. Averaging time window 0-3 min as illustrated in **A**. Dashed line: cortex detachment. Gray box: region of cortex disappearance from the field of view where measurements are less reliable. Mean±SDs. (**D-E**) Time evolution of MyoII mean intensity (**D**) and of the MyoII rates (**E**) with the distance from the vitelline membrane in a fixed region of the cortex (cortex AB position) in WT embryos. Mean±s.e.m. Averaging window: 5µm wide between position x=3µm and x=8µm, as illustrated in **C**. In **A-E** n=3 WT embryos (37 cells). (**F**) Stills of MyoII and E-cad during wave propagation in control (water injection in WT embryos), in *αPS3* RNAi (*αPS3* dsRNAs injected in WT embryos) and *αPS3* KO (water injection in *αPS3* null mutant embryos). Scale bars 5µm. (**G**) Spatial distribution of MyoII mean intensity within the tissue anterior to the invaginating furrow in the indicated conditions. n=7 embryos for control, 8 embryos for *αPS3* RNAi and 5 embryos for *αPS3* KO. Mean±SDs. (**H**) Individual cell measurements of the average MyoII integrated intensity during rapid MyoII recruitment in 5 control (water injected) and 4 *αPS3* RNAi injected embryos. Averaging time window: 0-3 min. Individual data points are superimposed to box plots. n = 19, 21, 20, 18, and 18 cells for the 5 water injected embryos and 23, 23, 17, 24 for the 4 αPS3 RNAi injected embryos. (**I**) Dot plot of the standard deviation (SD) between cells within individual embryos (small dots) and of the standard deviation between embryos (large dots) in the indicated conditions. The SD between cells within individual embryos were compared between water and *αPS3* RNAi injected embryos and the * indicates a $p<0.05$ at a Mann-Whitney test. (**J**) Dot plot of the ratio between the standard deviation between cells and that between embryos in the indicated conditions. Each dot represents an individual embryo. N.S. is a $p>0.05$ at a Mann-Whitney test.

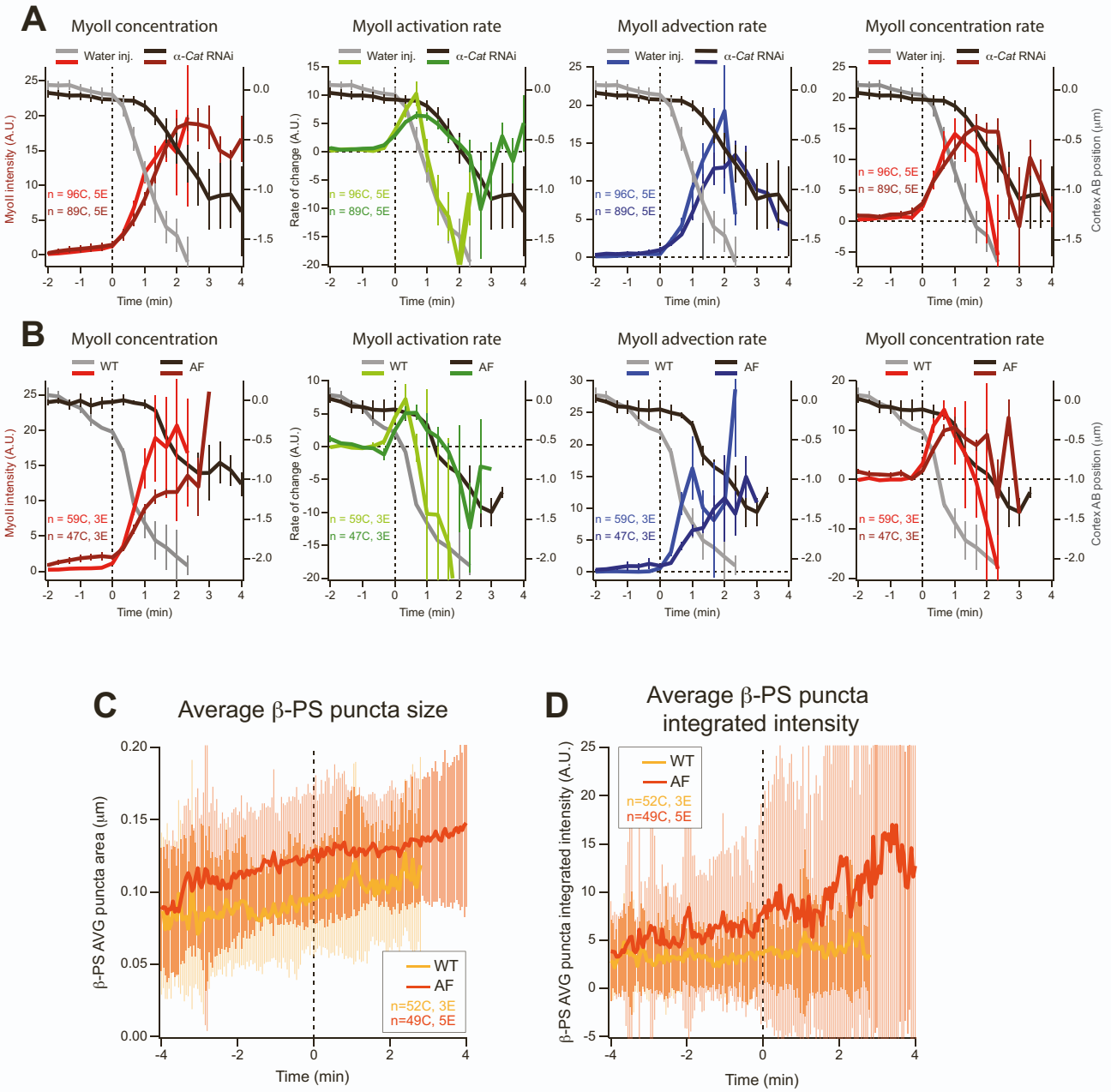
Time 0 is the onset of rapid MyoII recruitment.

Supplementary Fig. S4



Supplementary Figure S4 (related to Fig.4): Vinculin is recruited on β-PS puncta and MyoII sustains their assembly. (**A-B**) Time traces the number of β-PS (**A**) or Vinculin (**B**) puncta per cell (in blue β-PS and in green Vinculin) with the respective traces of the apical cell area (in gray) and of the MyoII integrated intensity (in red). Mean±SDs. In **A** n=25 cells (2 embryos) and in **B** n=42 cells (3 embryos). (**C**) Left: Stills of β-PS and Vinculin during wave propagation in a cell. Dashed white line: cell contour. Right: kymographs along the PA-axis of the cell on the left. Yellow: the A- and P-boundaries of the cell. White arrows: β-PS puncta recruiting Vinculin. Scale bars 5 μm. (**D**) Larger views of β-PS in the posterior endoderm before and after water or Rho kinase (Rok) inhibitor injection during wave propagation. White arrows: cells at the edge of the invaginating furrow where the intracellular wave of MyoII propagates (quantifications in Fig.4H-I), white asterisks: cells where MyoII increases slowly and the intracellular wave has not yet been activated at the time of injection. Scale bars 5 μm. (**E-F**) Time traces of β-PS mean intensity (**E**) and β-PS density of puncta (**F**) in cells where MyoII increases slowly (white asterisks in **D**). Mean±s.e.m. n=41 cells from 5 and 27 cells from 4 water and Rok inhibitor injected embryos, respectively. In **A** and **B** time 0 is the onset of rapid MyoII recruitment, in **E** and **F** time 0 is the time of injection.

Supplementary Fig. S5

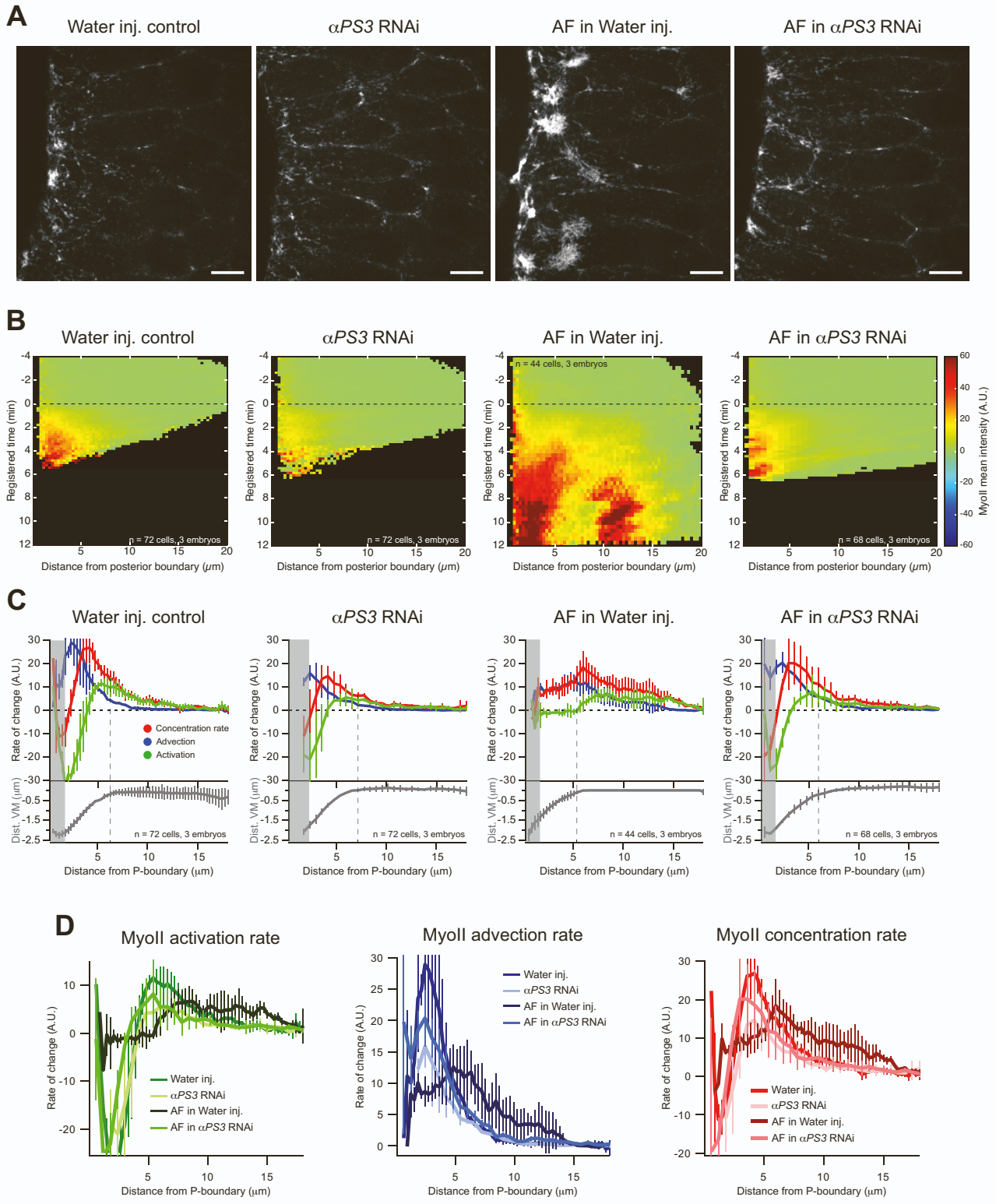


Supplementary Figure S5 (related to Fig.6): MyoII activation is prolonged and β-PS puncta grow larger when cortex detachment is impaired. (**A-B**) Time evolution of MyoII mean intensity and of the MyoII rates with the distance from the vitelline membrane in a fixed region of the cortex (cortex AB position, gray curves) in *α-Cat* RNAi (**A**) and embryos with anterior fences (AF in **B**) with their respective controls. n=5 embryos (96 cells) for water injection, 5 embryos (89 cells) for *α-Cat* RNAi, 3 embryos (59 cell) for WT control and 3 embryos (47 cells) for anterior fence. Mean±s.e.m. (**C-D**) Time traces of the average size (**C**) and average integrated intensity (**D**) of β-PS

puncta in cells in control (WT) and embryos with anterior fences (AF). n=52 cells from 3 WT and 49 cells from 5 embryos with anterior fences. Mean±SDs.

In all cases time 0 is the time of wave activation (rapid MyoII recruitment).

Supplementary Fig. S6

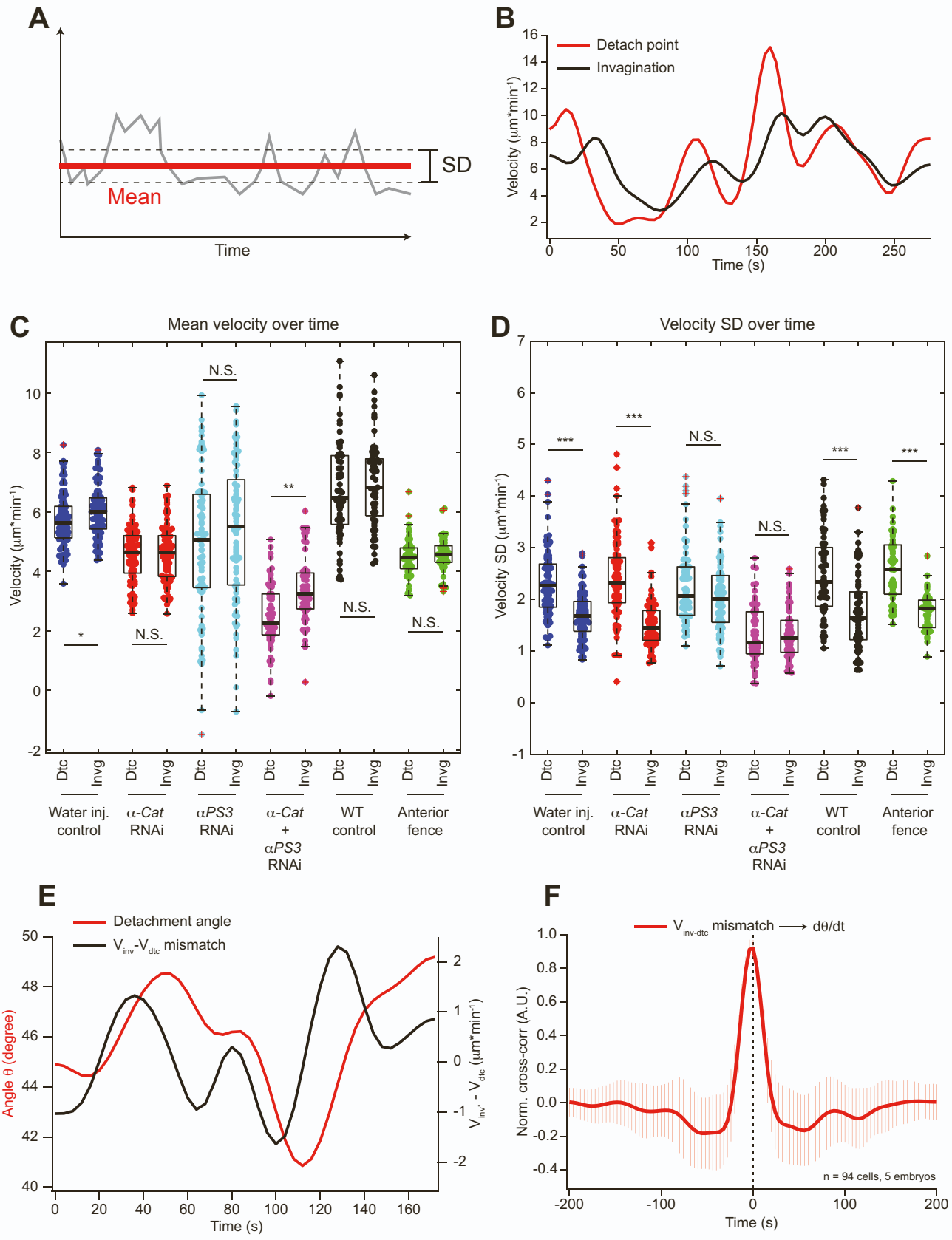


Supplementary Figure S6 (related to Fig.6): The elevated levels of MyoII in embryos with anterior fences depend on integrins. (**A**) Stills of MyoII during wave propagation in control (water injection), *αPS3* RNAi, control embryos with anterior fences (AF in water injection) and *αPS3* RNAi in embryos with anterior fences (AF in *αPS3* RNAi). Scale bars 5 μm. (**B**) Heatmap

kymographs of MyoII mean intensity in the indicated conditions. (**C**) Spatial profile of MyoII rates in the indicated conditions along with the distance of the apical cortex from the vitelline membrane, in gray (averaging time window 0-3 min for water injection controls and *αPS3* RNAi and 0-6 min for embryos with anterior fences). Dashed lines: cortex detachment. Gray boxes: region of cortex disappearance from the field of view where measurements are less reliable. (**D**) Separated plots of the activation rate (left), advection rate (middle) and concentration rate (right) of MyoII in the indicated conditions.

In **B, C** and **D** the data are plotted relative to the moving P-boundary of the cells and n=72 cells from 3 control (water injected in WT embryos), 72 cells from 3 *αPS3* RNAi in WT embryos, 44 cells from 3 water injected embryos with anterior fences and 68 cells from 3 *αPS3* RNAi injected embryos with anterior fences. In **C** and **D** mean±S.Ds. In all cases time 0 is the time of wave activation (rapid MyoII recruitment).

Supplementary Fig. S7



Supplementary Figure S7 (related to Fig.7): The detachment angle changes due to a mismatch between the velocity of the detachment point and the velocity of the basal furrow. (**A**) Illustration of a time trace (in gray) with its mean value (in red) and standard deviation (black dashed lines). The SD is used as an estimation of the variance along the time trace. (**B**) Representative smoothed time traces of the speed along the PA-axis of the detachment point (in red) and of the basal furrow (in black) estimated from side views as in Fig.7D. (**C-D**) Measurements of the mean velocity (**C**) and of the velocity standard deviation (**D**) from time traces of the speed of the detachment point (Dtc) and the basal furrow (Invg) along the PA-axis in the indicated conditions. Individual data points are superimposed to box plots. n=94 cells from 5 embryos for Water injection controls, 85 cells from 5 embryos for *α-Cat* RNAi, 76 cell from 4 embryos for *αPS3* RNAi, 59 cells from 5 embryos for *α-Cat*+ *αPS3* RNAi, 61 cells from 3 embryos for WT control and 47 cells from 3 embryos for Anterior fence. *** is a p-value <0.001, ** a p-value <0.01, * a p-value<0.05 and N.S. a p-value>0.05 at a Mann-Whitney test. (**E**) Representative smoothed time traces of the detachment angle θ (in red) and of the difference between the anterior velocity of the basal furrow and that of the apical detachment point (in black) in a WT embryo. (**F**) Average normalized cross-correlation between the velocity mismatch between the basal furrow and the apical detachment point, and the time derivative of the detachment angle θ in WT embryos. Mean±SDs between cells. n=94 cells from 5 WT embryos.