Simões et al., https://doi.org/10.1083/jcb.201608038

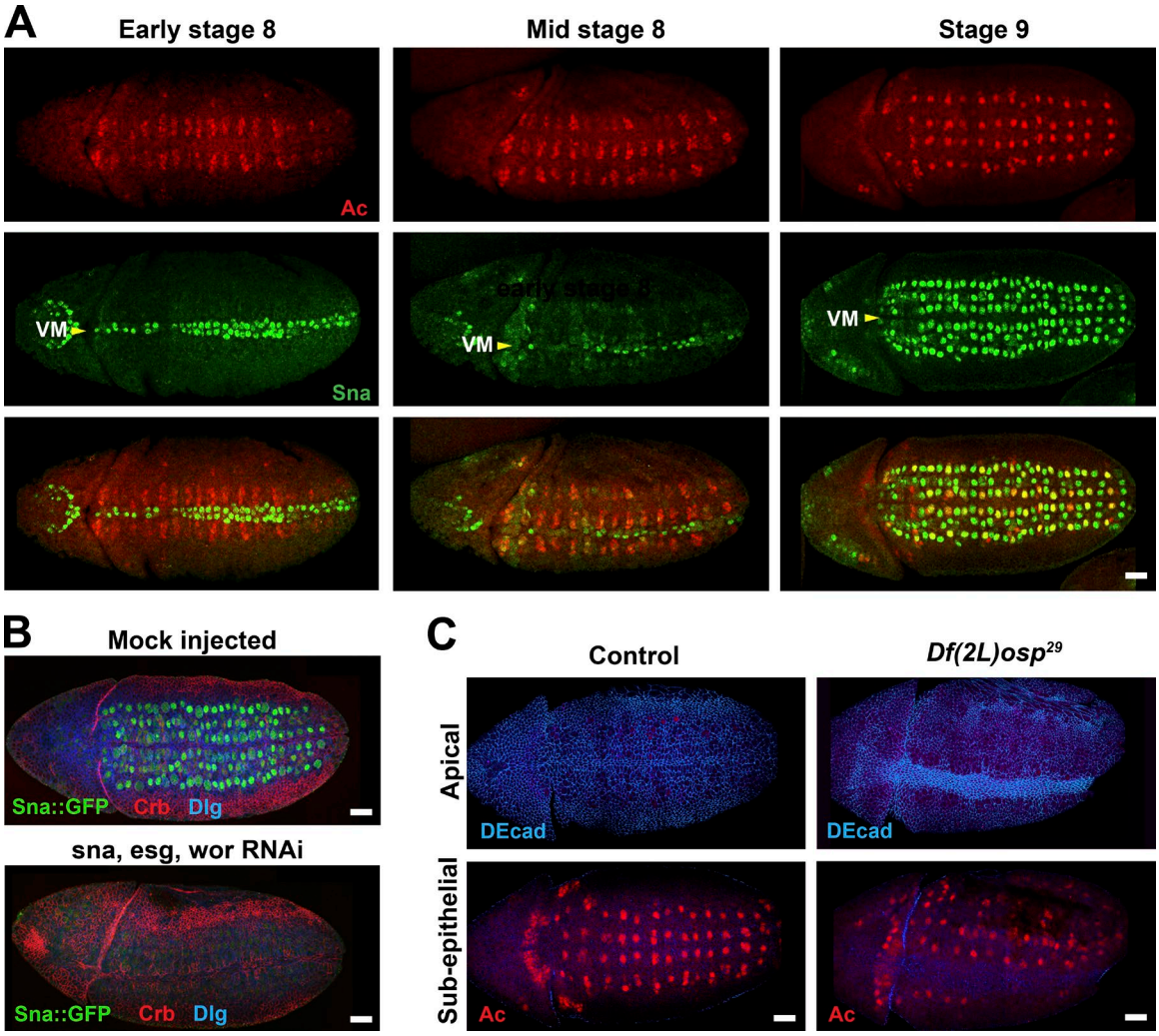


Figure S1. **NB ingression is independent of the Sna family of transcriptional repressors.** (A) Ventral views of early stage 8, mid–stage 8, and stage 9 embryos stained for endogenous Ac (red) and Sna (green). Sna is undetectable in Ac-positive proneural clusters during early stage 8 and is weakly detectable at mid–stage 8. Robust Sna expression is observed in fully ingressed NBs at stage 9. VM cells express Sna at stages 7–8. Anterior is to the left, dorsal is up. (B) Ventral views of stage 9 Sna::GFP-expressing embryos (green) injected with water (top) and *sna*, *wor*, and *esg* dsRNA (bottom). Crumbs (Crb, apical marker) is in red and Discs Large (Dlg, basolateral marker) is in blue. Notice the strong reduction in Sna::GFP levels in the *sna*, *esg*, *wor* triple RNAi embryos. (C) Ventral views of a stage 9 control embryo and an embryo homozygous for the deficiency *Df(2L)ops*^{29}, which uncovers *sna*, *esg*, and *wor* stained for DEcad (blue) and Ac (red). Apical and subepithelial panels are z projections of 0–1.7 µm and 4.8–8.5 µm, respectively, beneath the embryo surface. At stage 9, NBs occupy subepithelial positions in both genotypes. Bars, 50 µm.

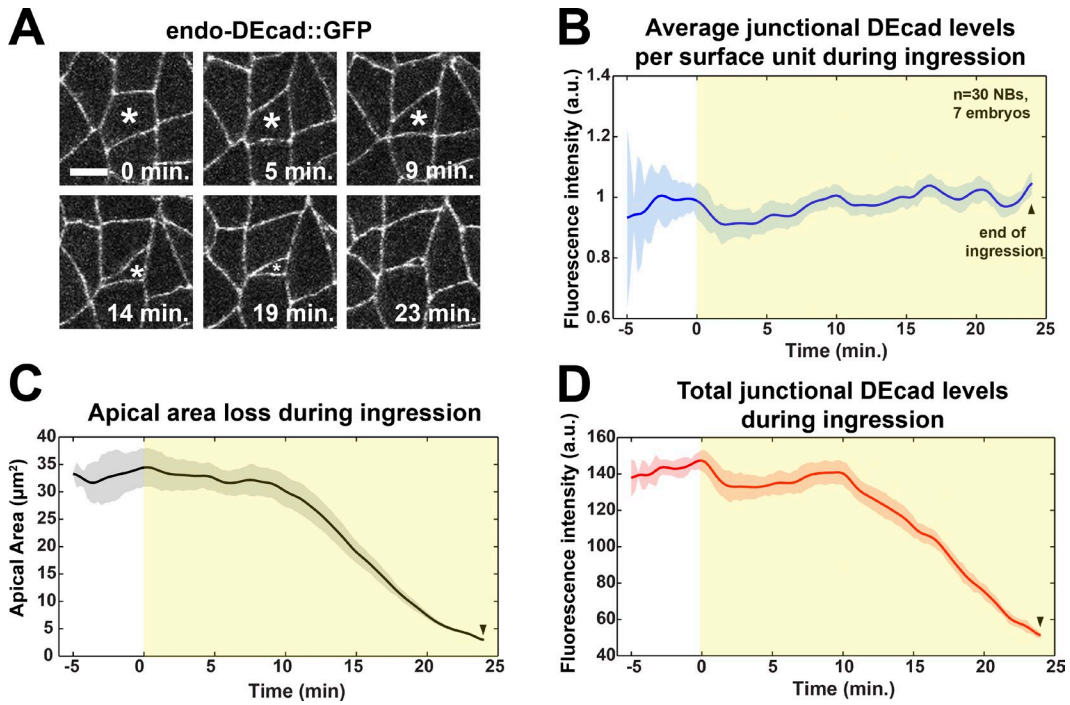


Figure S2. **DEcad concentration per surface unit is kept constant while its total levels decrease during ingression.** (A) Stills of a time-lapse video showing an ingressing NB (asterisks) labeled with endoDEcad::GFP. Bar, 5 µm. Time 0 depicts onset of ingression. (B) Normalized mean DEcad levels at the apical junctions per surface unit during ingression (yellow shade in B–D). (C) Mean apical area loss. (D) Total DEcad levels (mean level × junctional perimeter) during ingression of the same ingressing NBs as in B. Arrowheads in B–D indicate the end of ingression (time point at which apical area is 2.5 µm²). *n* = 30 NBs, seven embryos with endo-DEcad::GFP. Shaded error bars indicate SEM. a.u., arbitrary units.

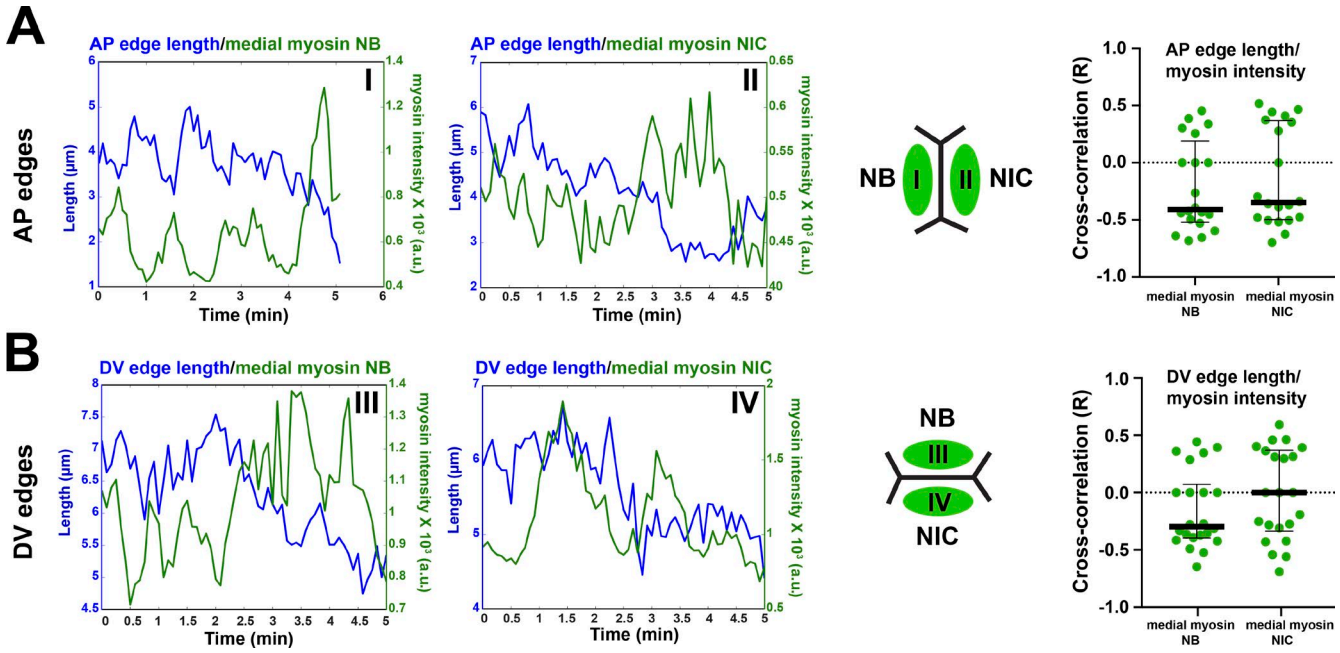


Figure S3. **AP edge disassembly correlates with medial myosin levels in NBs and neighboring NICs, whereas DV edge disassembly only correlates with medial myosin levels in NBs.** (A and B) Medial myosin levels anticorrelate with changes in AP edge length (A) and DV edge length (B) during ingression. Graphs representing cell edge length versus medial myosin levels in edge vicinity (<1.8 μm), either within the NB (plots I and III) or in the neighboring NIC (plots II and IV). Plots in A indicate disassembling AP edges; plots in B indicate disassembling DV edges. The scatter plots represent cross-correlation coefficients between cell edge length and medial myosin levels in their vicinity. Each dot is one cell edge, and bars are IQRs. AP edge length is significantly anticorrelated with medial myosin levels in NBs and in neighboring NICs (median cross-correlation coefficients: R = −0.41 [quartiles −0.52 and 0.19] and R = −0.34 [quartiles −0.49 and 0.37], respectively; *n* = 20 edges). In contrast, DV edge length is only anticorrelated significantly with medial myosin levels within NBs (R = −0.29 [quartiles −0.39 and 0.07]), but not with medial myosin in neighboring NICs (R = 0 [quartiles −0.33 and 0.37]; *n* = 22 edges). a.u., arbitrary units.

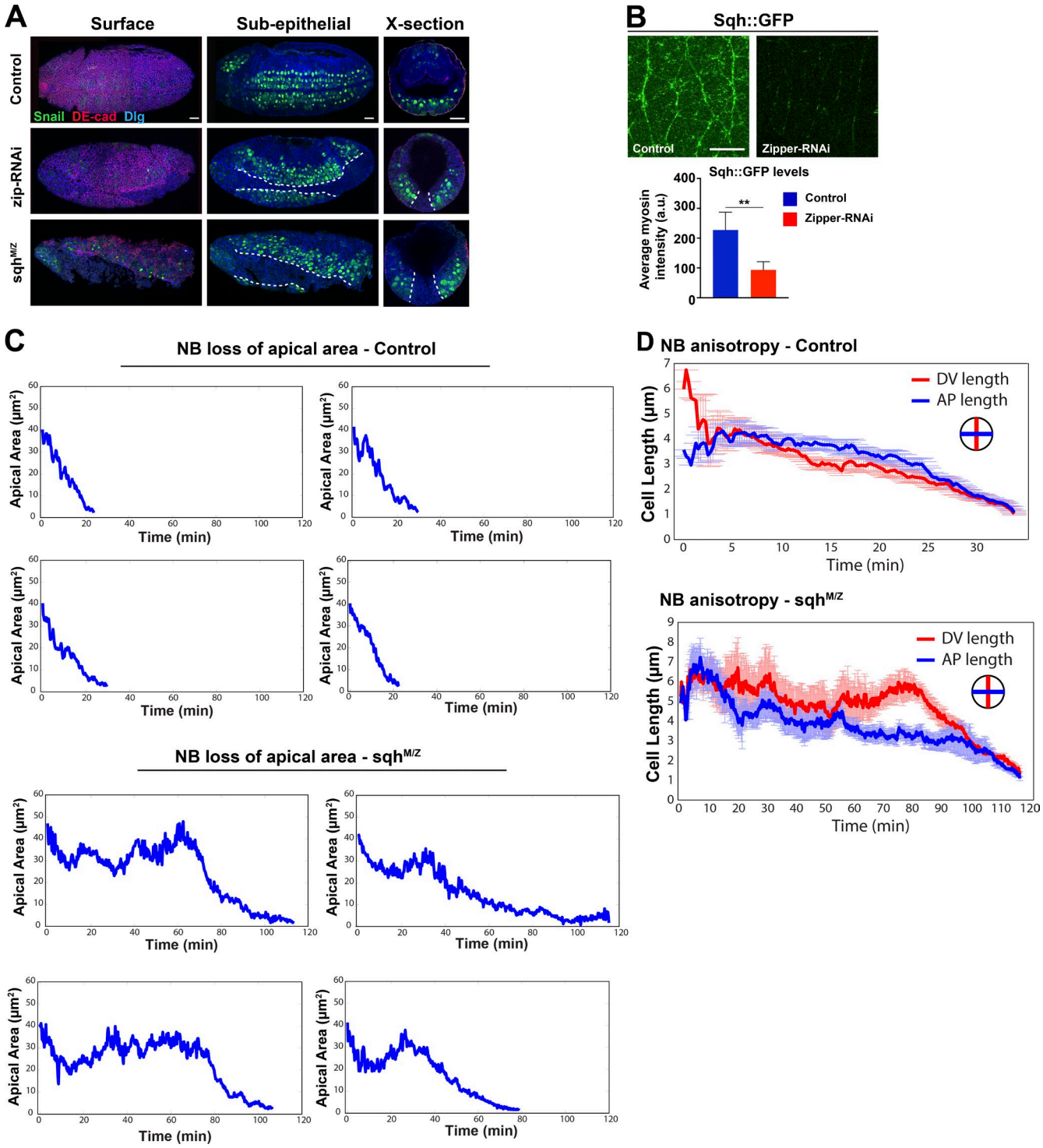


Figure S4. **Myosin controls the anisotropic disassembly of cell junctions and the ratchet mechanism of apical membrane loss during ingression.** (A) Projections of ventral surface (left, 0–1.75 µm beneath embryo surface), their corresponding subepithelial sections (middle, 7–12 µm beneath embryo surface), and cross sections (right) of stage 9 mCherry-RNAi control embryos, *zip-RNAi* embryos, and *sqh*$^{M/Z}$ mutants. Dashed lines delimit partially open ventral furrows. Embryos are labeled for Sna::GFP (green), DEcad (apical marker; red) and Discs Large (Dlg, lateral marker; blue). 97.7 ± 0.02%, 90.1 ± 0.09%, and 87.5 ± 0.08% of NBs are subepithelial in control, *zip-RNAi*, and *sqh*$^{M/Z}$ embryos, respectively (n = 102–242 NBs/embryo, six to eight embryos per genotype; $P < 0.05$, Mann–Whitney test). Bars, 25 µm. (B) Live myosin levels (Sqh::GFP) are 60% decreased in *zip-RNAi* embryos compared with control (*mCherry-RNAi*). Data presented are means ± SD. **, $P = 0.0022$ (KS test). Bar, 10 µm. n = six embryos/genotype. a.u., arbitrary units. (C) Representative plots of apical membrane loss of individual NBs during ingression in ubi-DEcad::GFP embryos (control) and *sqh*$^{M/Z}$ mutants expressing ubi-DEcad::GFP. Notice that loss of apical membrane in delayed *sqh*$^{M/Z}$ NBs is not monotonic and is frequently interrupted by variable apical expansions. (D) Maximum DV and AP apical cell length during ingression in control (n = 22 NBs, four embryos) and delayed *sqh*$^{M/Z}$ mutant NBs (n = 8, three embryos). *sqh*$^{M/Z}$ NBs shrink their apical surface along the DV axis 1.11× slower than along the AP axis. In contrast, controls shrink their apical surface 1.37× faster along the DV axis than along the AP axis. Time 0 indicates the onset of ingression. Data presented are means ± SEM.

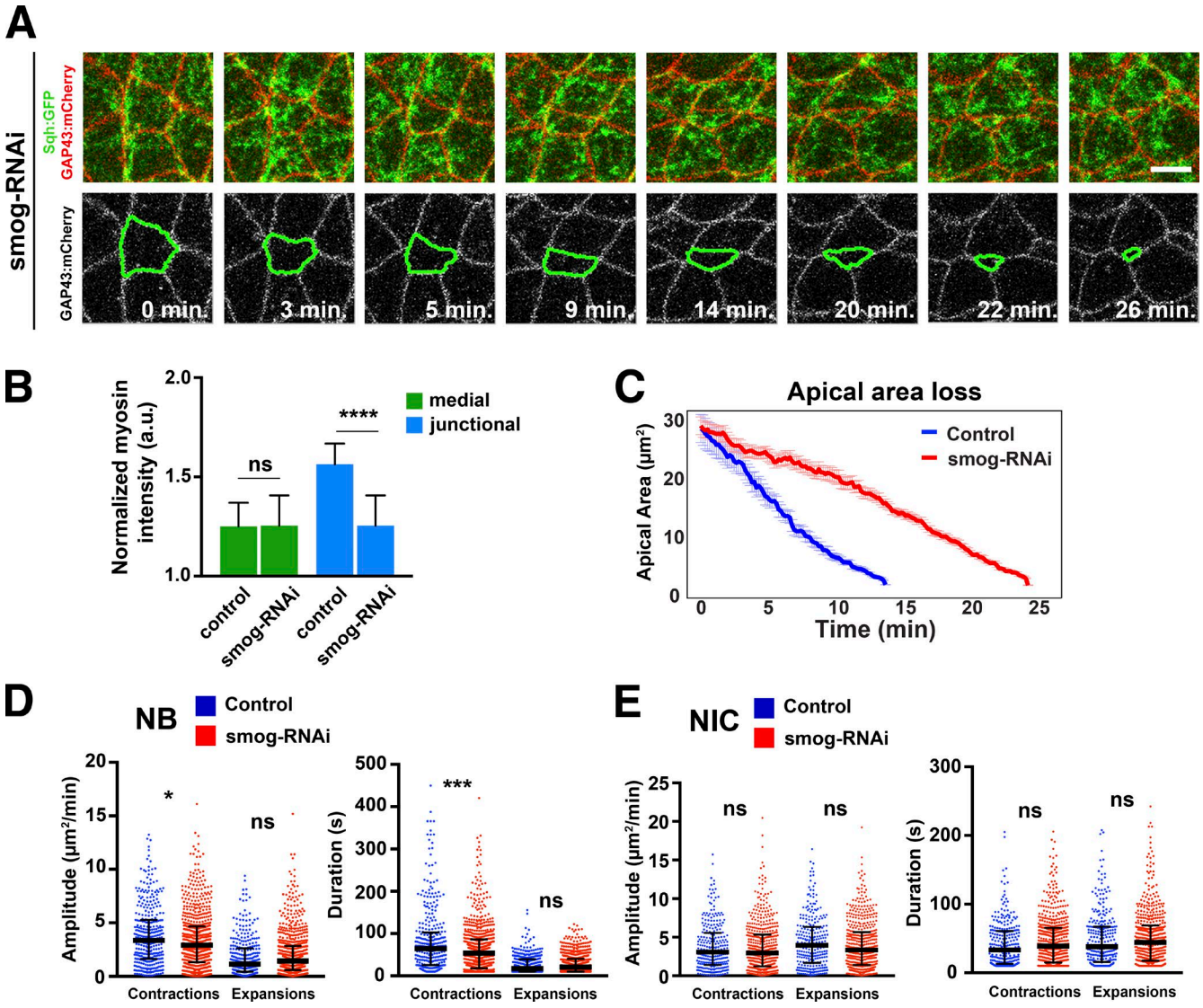
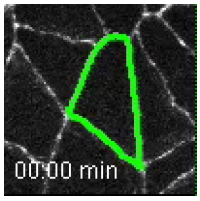
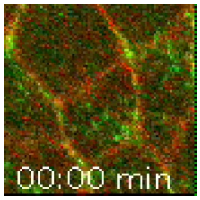


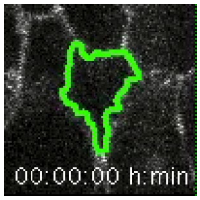
Figure S5. **A partial reduction in junctional myosin by Smog knockdown alters ratcheted contractions of ingressing NBs.** (A) Stills from time-lapse video of an ingressing NB in an Sqh::GFP GAP43::mCherry embryo injected with *smog* dsRNA. Notice the depletion in junctional myosin and reduction in ingression speed compared with controls (corresponding control shown in Fig. 8 A). The segmented NB is outlined in green in the bottom panels. Bar, 5 µm. (B) Medial and junctional mean myosin levels in NBs of control and *smog-RNAi* embryos. Smog depletion reduces junctional but not medial myosin in NBs and NICs. A mean intensity for medial and junctional myosin was calculated for each NB considering all time points during ingression. n = 18 NBs from two embryos in each condition. a.u., arbitrary units. ****, P = 3.5 × 10^{-8} (two-tailed t test). (C) Apical area loss during ingression in control (n = 40 NBs, six embryos) and *smog-RNAi* embryos (47 NBs, five embryos). Data presented are means ± SD (B) or SEM (C). (D) Depletion of Smog causes reduced amplitude and duration of contractions in NBs relative to controls. Maximum amplitude and duration of individual contractions/expansions in ingressing NBs of control and *smog-RNAi* embryos. Median amplitudes for control/mutant (squared micrometers/minute): contractions, 3.36/2.913; *, P = 0.05; n = 357/733; expansions, 1.14/1.44; ns (not significant), P = 0.17; n = 321/719. Median duration times for control/mutant (seconds): contractions, 64.3/53.6; ***, P = 8 × 10^{-4}; n = 353/759; expansions, 17.4/20.5; ns, P = 0.2; n = 347/729 (KS test). (E) Depletion of Smog does not affect pulsatile behaviors of NICs. Maximum amplitude and duration of individual contractions/expansions in NICs in control and *smog-RNAi* embryos. Median amplitudes for control/mutant (squared micrometers/minute): contractions, 3.09/2.95; ns, P = 0.73; n = 275/513; expansions, 3.94/3.33; ns, P = 0.13; n = 277/510. Median duration times for control/mutant (seconds): contractions, 33.3/38.8; ns, P = 0.32; n = 290/522; expansions, 38.1/44.3; ns, P = 0.46; n = 290/521 (KS test).



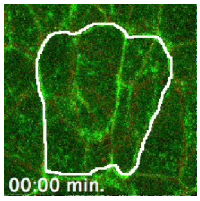
Video 1. **NB ingression.** Time lapse of a ubi-DEcad::GFP-expressing embryo tracking the apical surface of an ingressing NB during the first wave (S1) of early neurogenesis in the ventral ectoderm. The result of apical membrane segmentation is shown in green. Video is displayed at 20 frames per second.



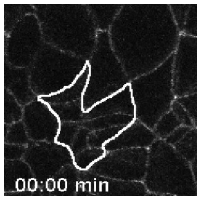
Video 2. **Myosin is planar polarized and pulsatile in ingressing NBs.** Time-lapse video showing an ingressing NB (cell in the center) expressing Sqh::GFP (myosin, green) and GAP43::mCherry (membrane outline, red). Notice the planar polarization of myosin at the cell junctions and periodic pulses of myosin assembly and disassembly coinciding with the ratcheted pattern of apical membrane loss. Video is displayed at 20 frames per second.



Video 3. **NB ingression in an *sqh*$^{M/Z}$ maternal mutant.** Time-lapse video of an NB showing delayed ingression in an *sqh*$^{M/Z}$ embryo. The green line represents the result of cell segmentation. DEcad (white) labels the cell junctions. Notice that the loss of apical surface follows a more variable contraction/expansion pattern compared with controls (Video 1). Video is displayed at 20 frames per second.



Video 4. **Myosin dynamics in NBs ingressing as clusters of cells in a *Delta-RNAi* embryo.** Time-lapse video of a *Delta-RNAi* embryo showing a cluster of ingressing NBs (encircled in white in the first time frame). Myosin is labeled in green (Sqh::GFP), and the cell membranes are labeled in red (GAP43::mCherry). Notice that myosin accumulates in persistent puncta as multiple cells attempt to ingress together. After a "tug of war" between cells, their apically constricted membranes snap back and disrupt collective ingression. Video is displayed at 20 frames per second.



Video 5. **DEcad dynamics in NBs ingressing as clusters of cells in a *Notch-RNAi* embryo.** Time-lapse video of a *Notch-RNAi* embryo showing a cluster of ingressing NBs (encircled in white in the first time frame). DEcad (white) labels AJs. Notice that DEcad is specifically down-regulated at junctions shared by ingressing cells. Video is displayed at 20 frames per second.