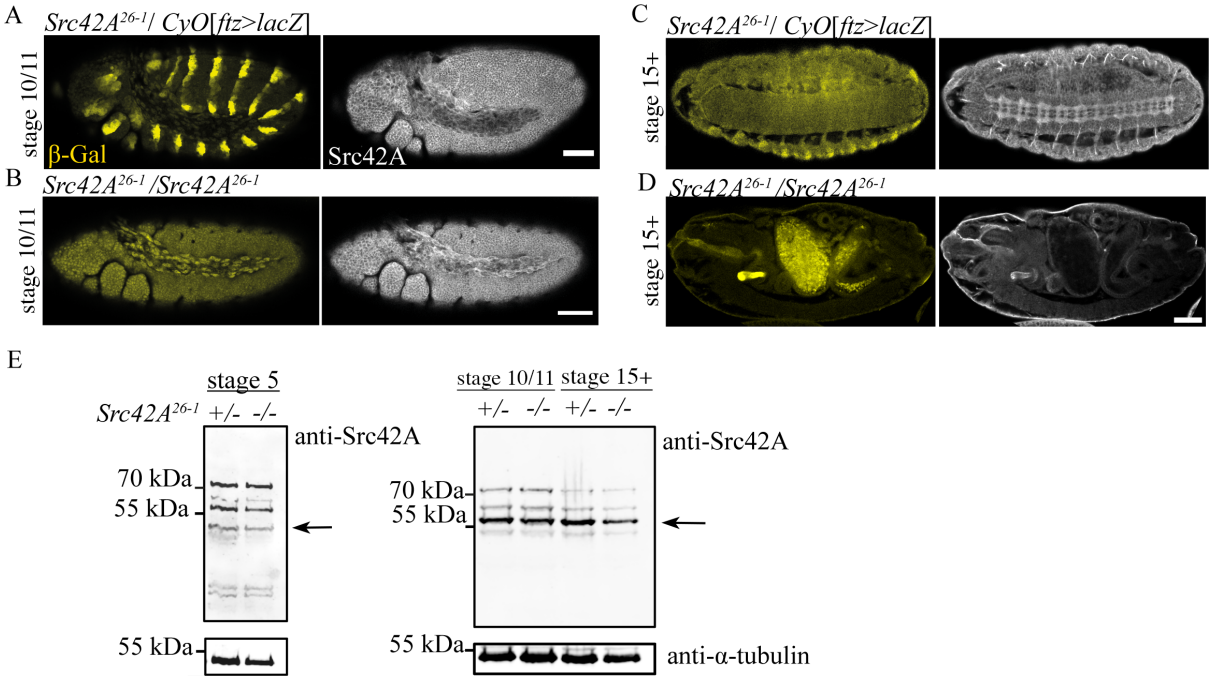


Fig. S1. Src42A antibody characterization in zygotic *Src42A$^{26-1}$* mutants. **(A-D)** *Src42A$^{26-1}$/ CyO*[*wg>lacZ*] is heterozygous viable mutant for *Src42A* used as a control for the *Src42A$^{26-1}$/ Src42A$^{26-1}$* homozygous zygotic mutant; the *wg>lacZ* balancer was used as a marker to discriminate the control and mutant embryos by anti-β-Gal staining (yellow). Scale bars represent 50 μm. **(E)** SDS-PAGE followed by immunoblotting show maternal Src42A protein in zygotic mutants in different stages as marked on top of the blot, for each lane lysate of 20 embryos were loaded. +/- and -/- denotes heterozygous and homozygous mutants, respectively. The predicted molecular weight for Src42A is 59kDa (marked with arrows). An additional band observed approximately at 70kDa. The identity of the 70kDa band is unclear but note that the intensity of this band is reduced in later stage embryos mutant for *Src42A*. Anti-α-tubulin antibody was used as a loading control.

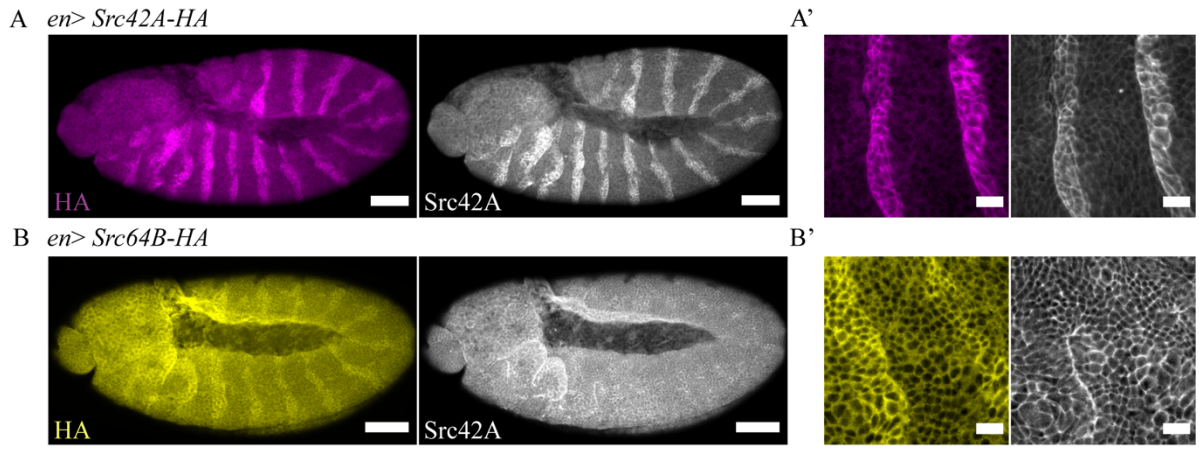


Fig. S2. Src42A antibody does not cross react with Src64B. (A) Stage 10 embryos expressing *UAS::Src42A-HA* transgene using *engrailed>Gal4* driver flies. **(B)** Stage 11 embryos expressing *UAS::Src64B-HA*. Scale bars shows 50 µm. Images were taken at 25x magnification. **A'** and **B'** show 63x magnification images of *Src42A-HA* and *Src64B-HA* expression respectively. HA antibody staining marked in magenta and yellow and Src42A staining marked in grey. Scale bars corresponds to 10 µm.

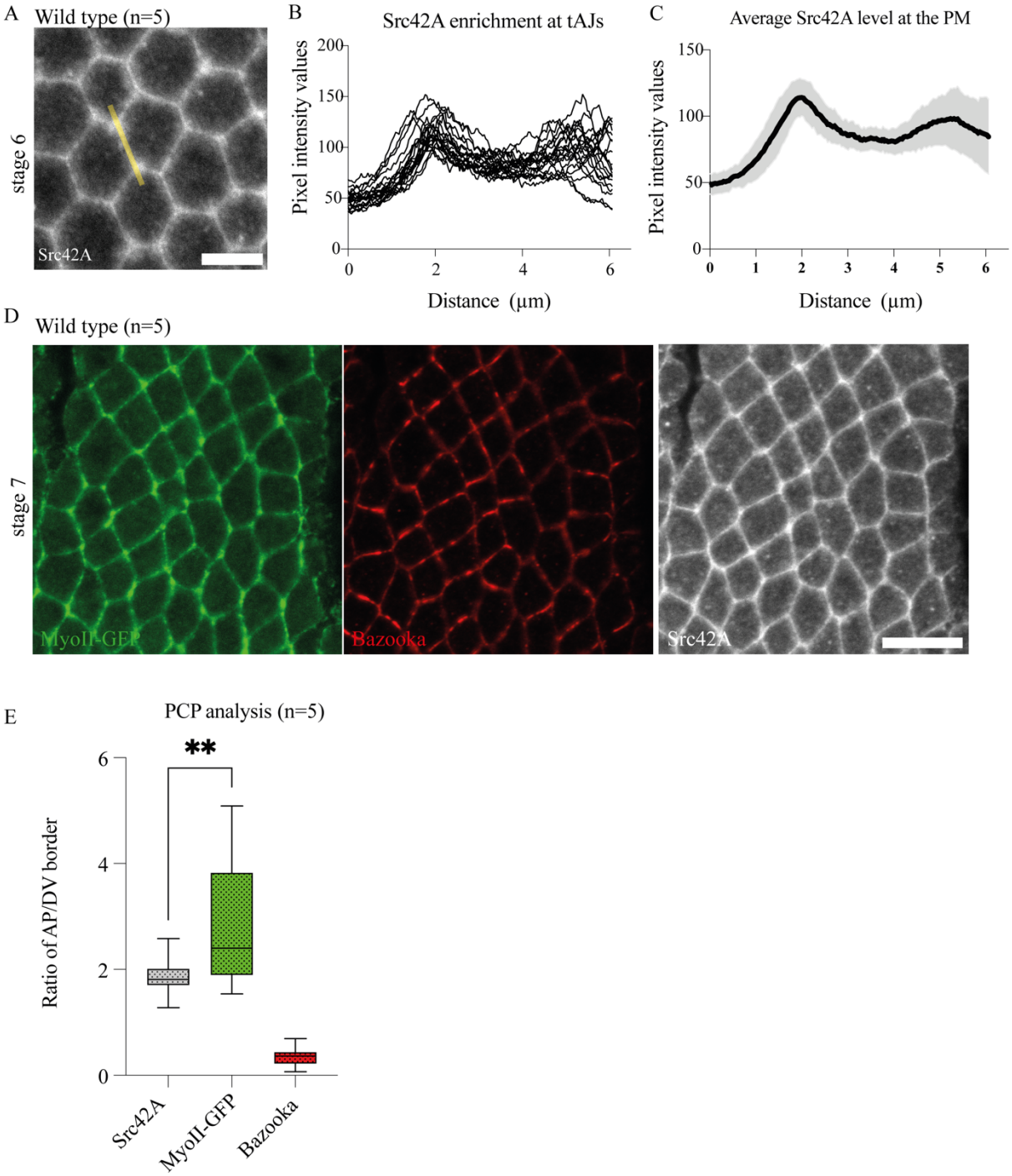


Fig. S3. Src42A enrichment at tAJs and AP cell border. (A) Confocal micrograph of stage 6 wildtype embryos stained for Src42A. Yellow line indicates the area used for measuring Src42A pixel intensity values. Scale bar denotes 5 µm. **(B)** Src42A enrichment at defined area plotted over distance. **(C)** Average level of Src42A at the plasma membrane was plotted over distance. Grey shade indicates standard deviation for each data point. **(D)** Confocal micrographs of stage 7 *MyoII::KI-GFP* embryos stained for Bazooka and Src42A. Scale bar indicates 10µm. **(E)** Planar polarity analysis of Src42A, MyosinII and Bazooka. Student t-test was performed between Src42A and MyoII-GFP showing a p value of 0.0017. 25 junctions were measured in 5 embryos; in each embryo 5 tAJs and 5 bAJs were measured). Error bars were drawn using range (min to max).

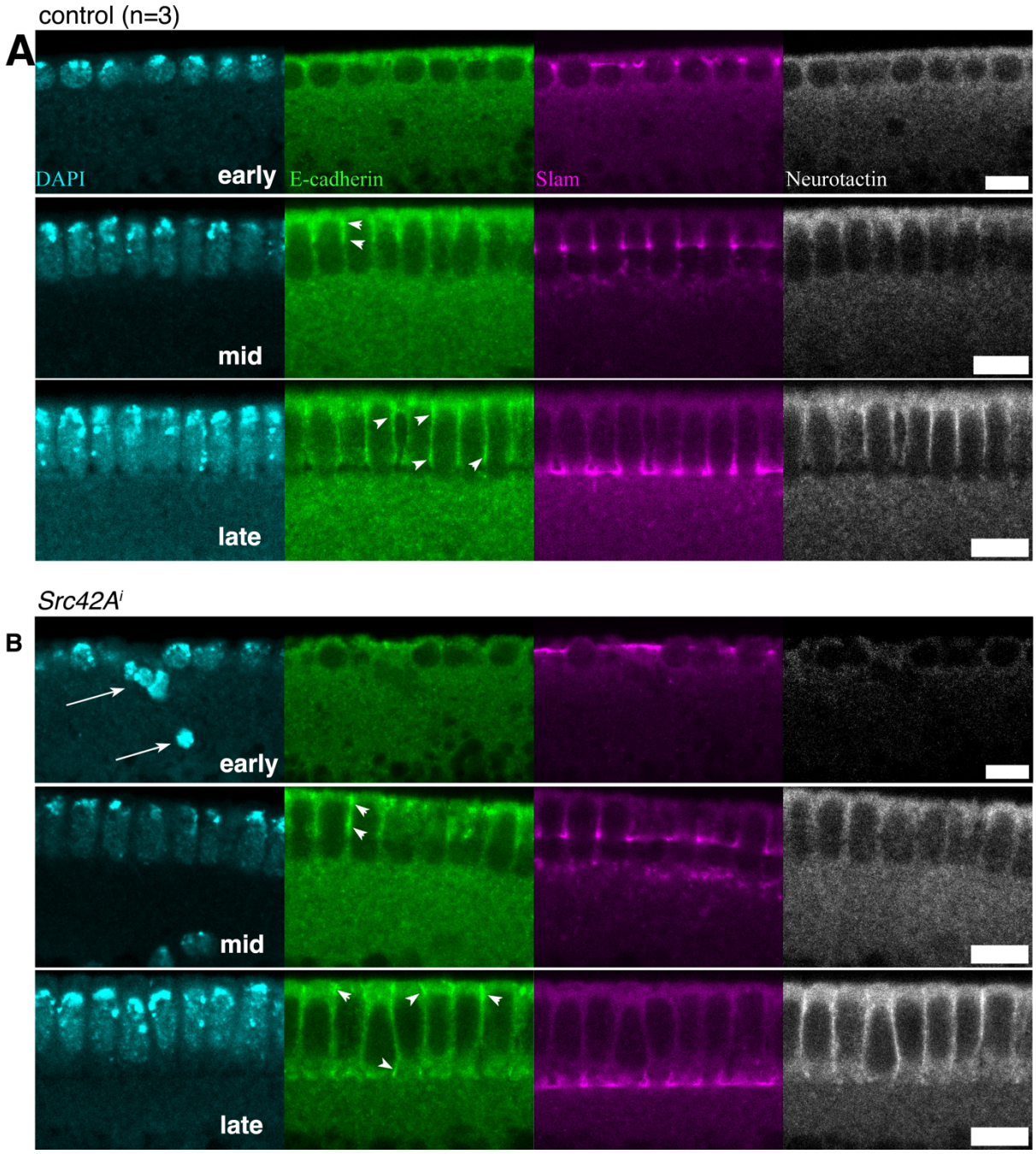


Fig. S4. Distribution of membrane domain markers in cellularization stage *Src42A*^{i} embryos compared to controls. (**A,B**) Confocal images of control and *Src42A*^{i} embryos in early, mid and late cellularization stages, fixed and stained for DAPI (cyan), E-cadherin (for adherens junctions in green), Slam (for furrow canals in magenta) and Neurotactin (for newly forming plasma membranes in grey). Optical sections were taken as transversal cross sections. Note a severe case of nuclear dropping from cell cortex in early cellularization stages in *Src42A*^{i} embryos (marked by arrows in B, first row). Note accumulation of E-cadherin at apical and basal junctions in mid and late cellularization stages in both control and *Src42A*^{i} embryos (arrowheads in A and B). Scale bar indicates 10 μm.

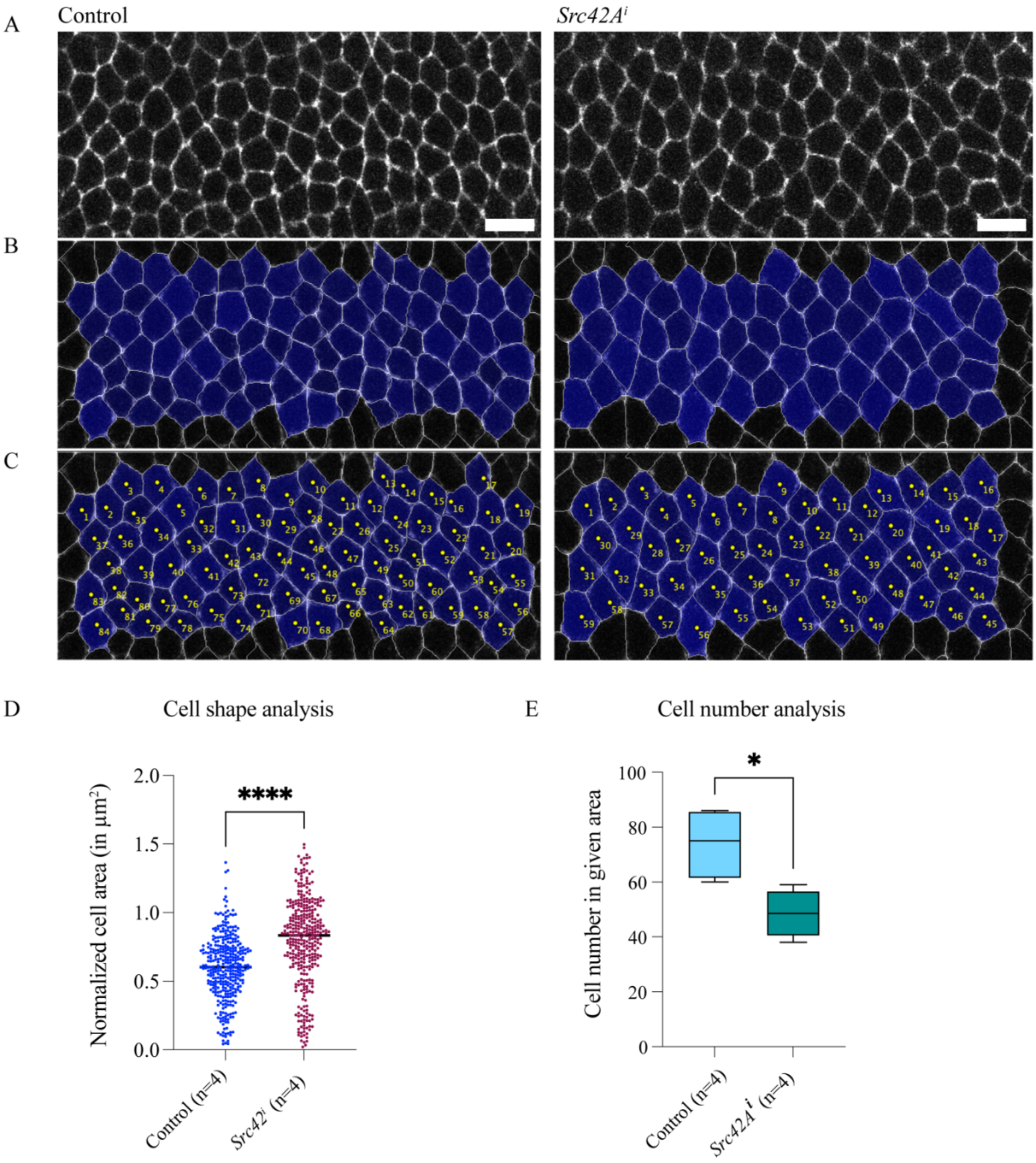


Fig. S5. Cell number and apical cell area analysis before germband extension. (A) Control and *Src42A*^{i} embryos expressing *Sqh::Utrophin-GFP* marking cell membrane. Scale bar denotes 10µm. **(B)** Segmented cells used to measure cell area. **(C)** Cell numbers were counted within 97.12x41.84 microns in control and *Src42A*^{i} embryos. **(D)** Cell area was measured before germ band extension in Control and *Src42A*^{i}. Student t-test was performed on normalized cell area showing a p value of <0.0001. **(E)** Cell numbers were counted in given area in Control and *Src42A*^{i} embryos. Student t-test show p value of 0.0207

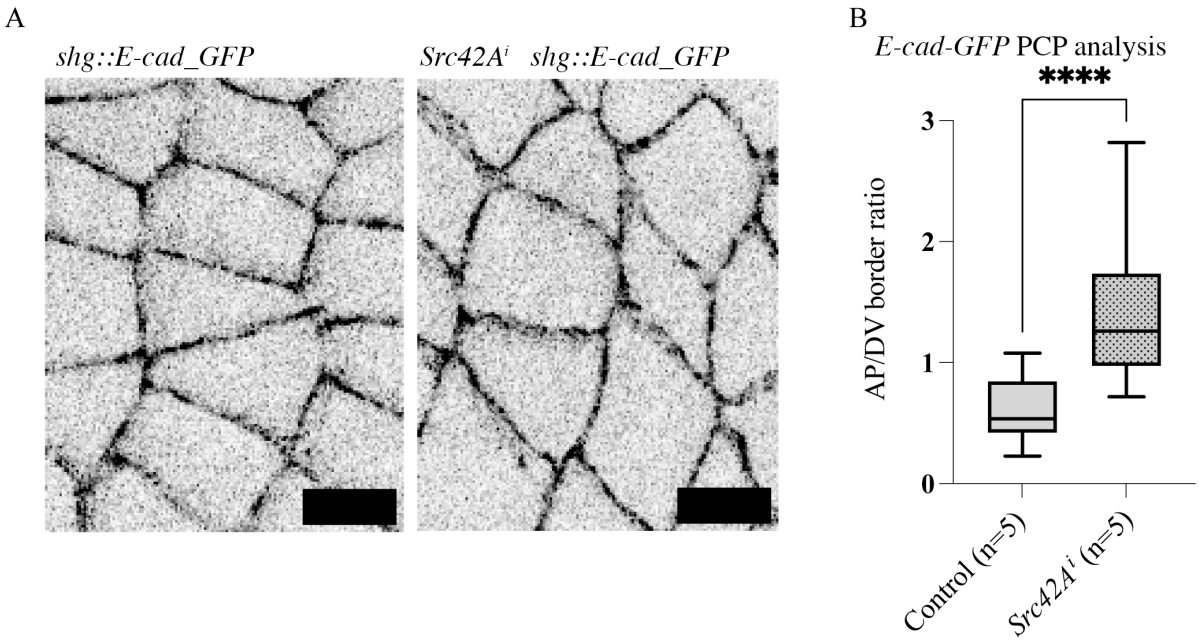


Fig. S6. E-cadherin-GFP planar cell polarity analysis. (A) Confocal micrographs of control and *Src42A*^{i} embryos showing planar cell polarity. Scale bar denotes 5µm. **(B)** Quantification showing planar cell polarity of E-cadherin-GFP in control and *Src42A*^{i}. Student t-test was performed to check the statistical significance showing p value of <0.0001.

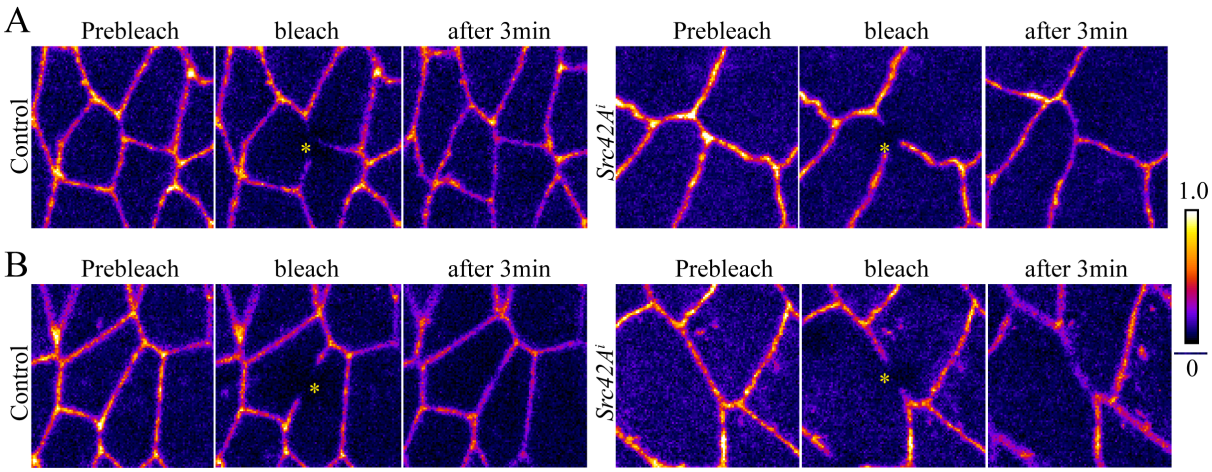


Fig. S7. FRAP experiments on E-cadherin-GFP in control and *Src42A*^{i} embryos. **(A,B)** Confocal images of germ band cells photobleached at tAJs (**A**) and bAJs. (**B**) from control and *Src42A*^{i} embryos expressing E-cadherin-GFP. Representative time points for pre-bleach, bleach and recovery after 3 minutes (min) are shown. Asterisks indicate the bleached region. Heat map represents a scale for fluorescence intensities.

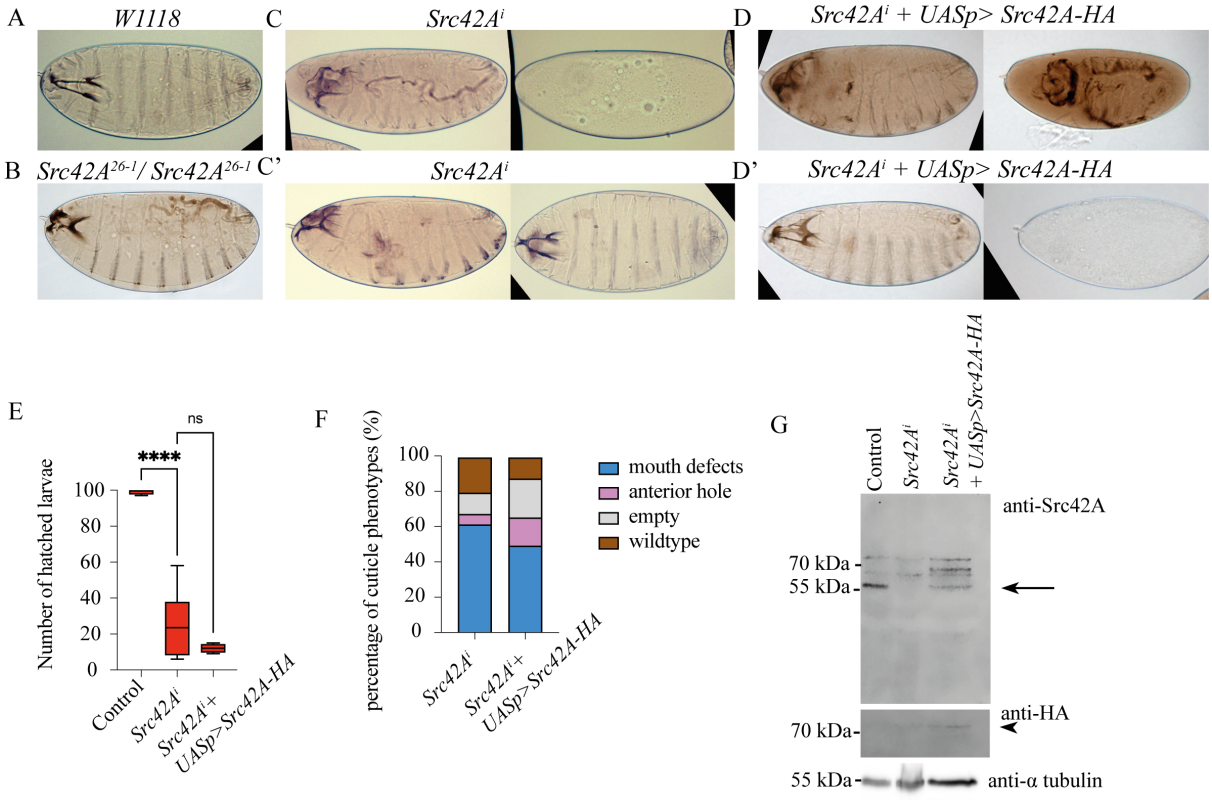


Fig. S8. *Src42A^{i}* rescue attempt with *UASp>Src42A-HA*. (A,B) Cuticle preparation from wild type (w^{1118}) and *Src42A* zygotic mutant ($Src42A^{26\text{-}1}/Src42A^{26\text{-}1}$), respectively. **(C,C')** *Src42A^{i}* cuticles showing variable phenotypes. (**D,D')** Various cuticle phenotypes of *Src42A^{i} +UASp>Src42A-HA* embryos display similar effects as *Src42A^{i}*. (**E)** Hatching rate analysis of *Src42Ai* and rescue. Statistics were done using Dunnett's multiple comparison test shows significant difference among the mean values. The P value (0.1114) show no significant difference between *Src42A^{i}* and *Src42A^{i}+UASp>Src42A-HA* rescue. (**F)** Cuticle classification from *Src42A^{i}* and rescue analysis. **(G)** Western blot analysis of *Src42A^{i}* rescue using *UASp>Src42A-HA*. The specific band for Src42A is marked by an arrow. Antibody against the Haemagglutinin (HA) epitope shows a faint expression at a higher molecular weight (~70kDa) range (marked by an arrowhead).

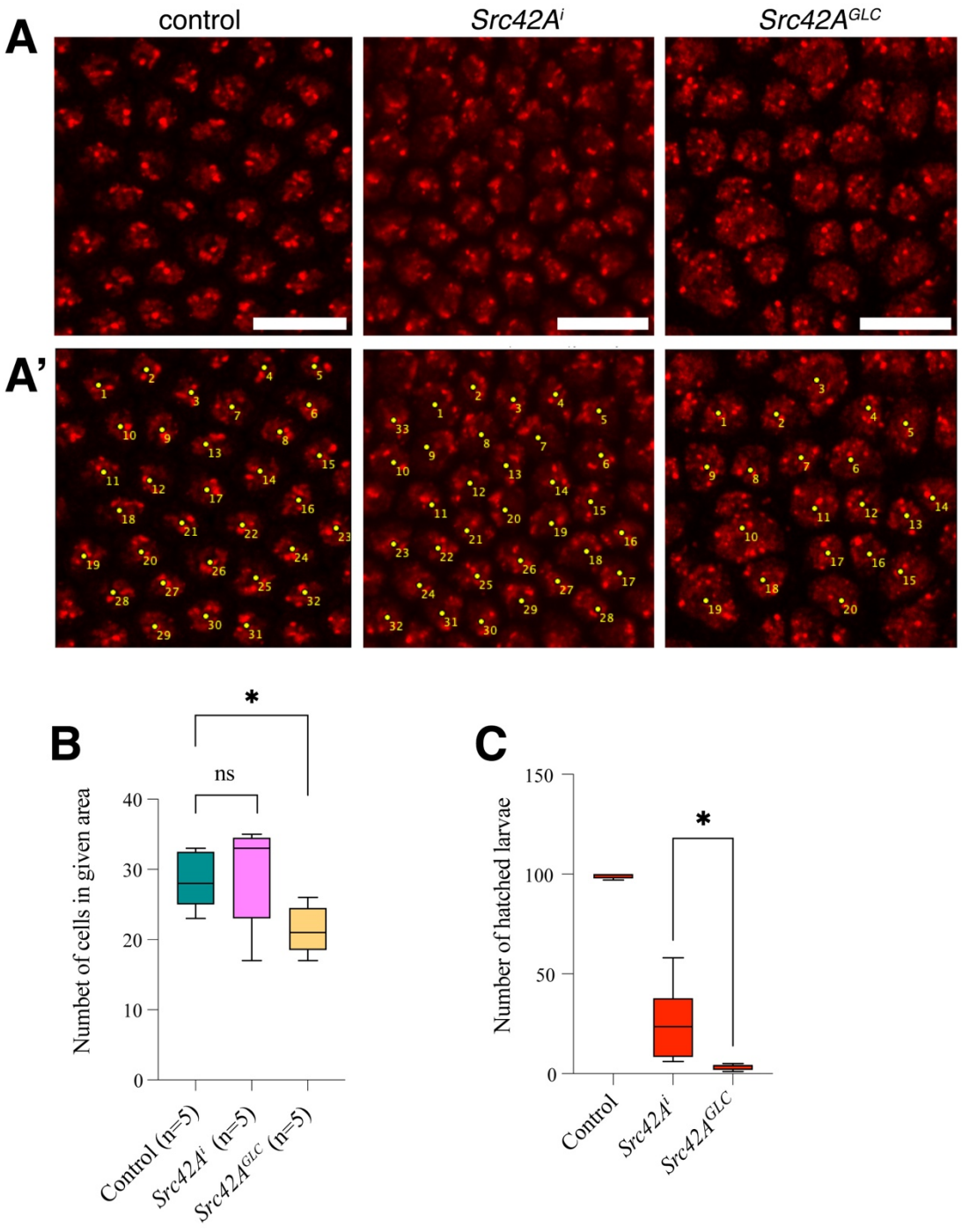
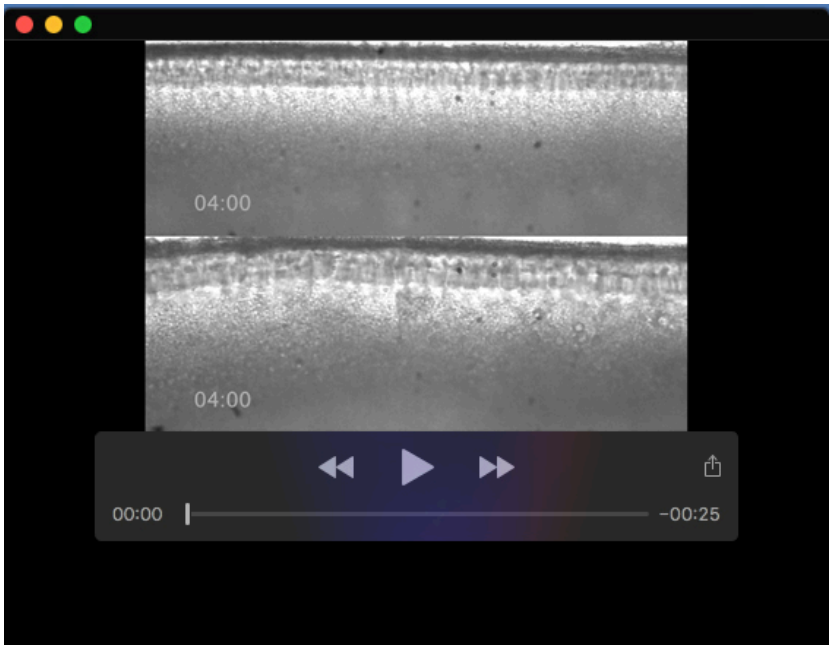
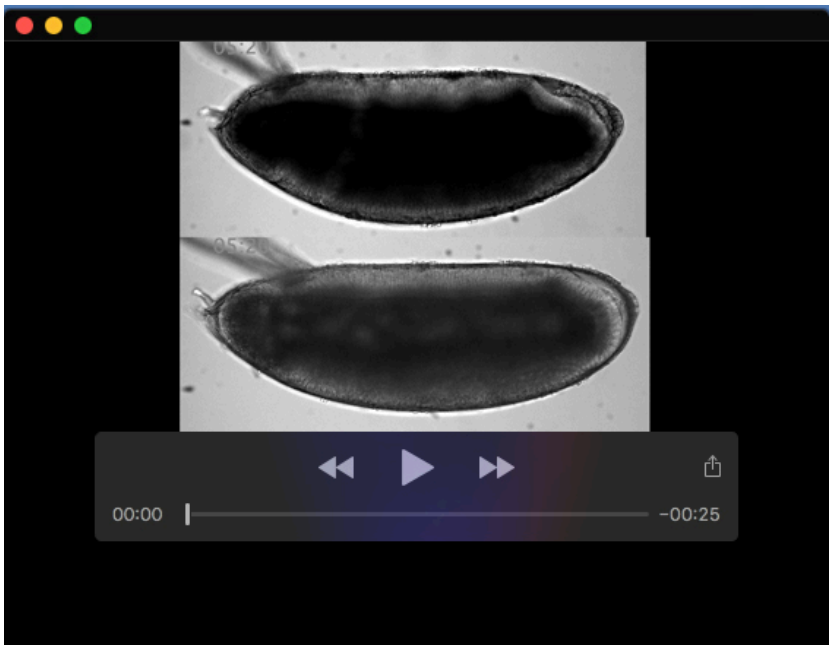


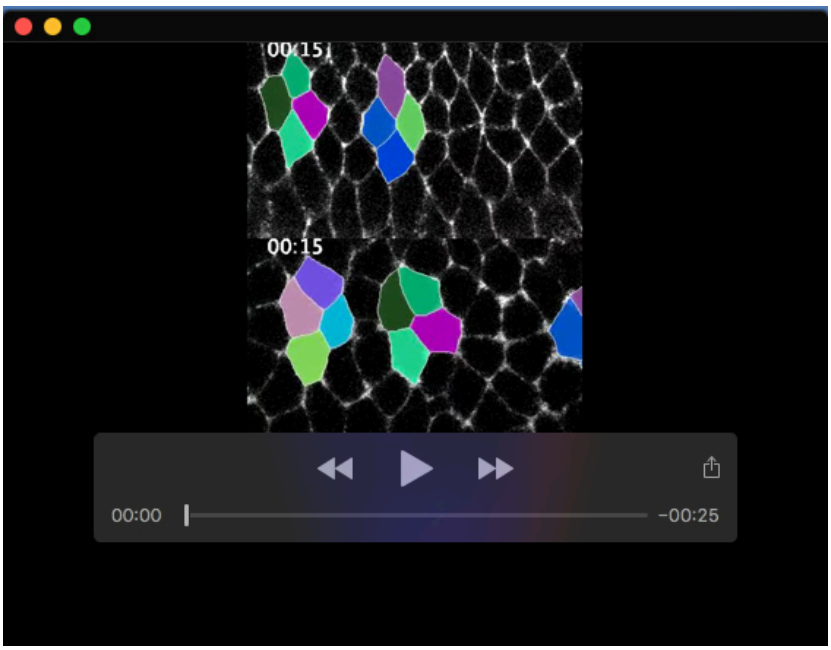
Fig. S9. Comparison of phenotypic expressivity between embryos derived from *Src42A^{i}* and *Src42A^{GLC}*. (A) Confocal images of representative areas (31.93 µm x 32.53 µm) of blastoderm embryos stained with DAPI to mark the nucleus in control, *Src42A^{i}* and *Src42A^{GLC}* embryos (scale bar is10µm). Maximum intensity projection images were generated using 6 Z-stack images and used for quantification (nuclei number indicated in (**A'**). **(B)** Number of cells in the given area were compared in control, *Src42^{i}* and *Src42A^{GLC}* embryos. Dunnett's multiple comparison test show no significant difference between Control and *Src42A^{i}*, in the case of *Src42A^{GLC}* the p value is 0.0288 (n = number of embryos analysed). **(D)** Hatching rate analysis comparing viability of embryos derived from *Src42Ai* and *Src42A^{GLC}*. Student's t-test show p value = 0.0373.



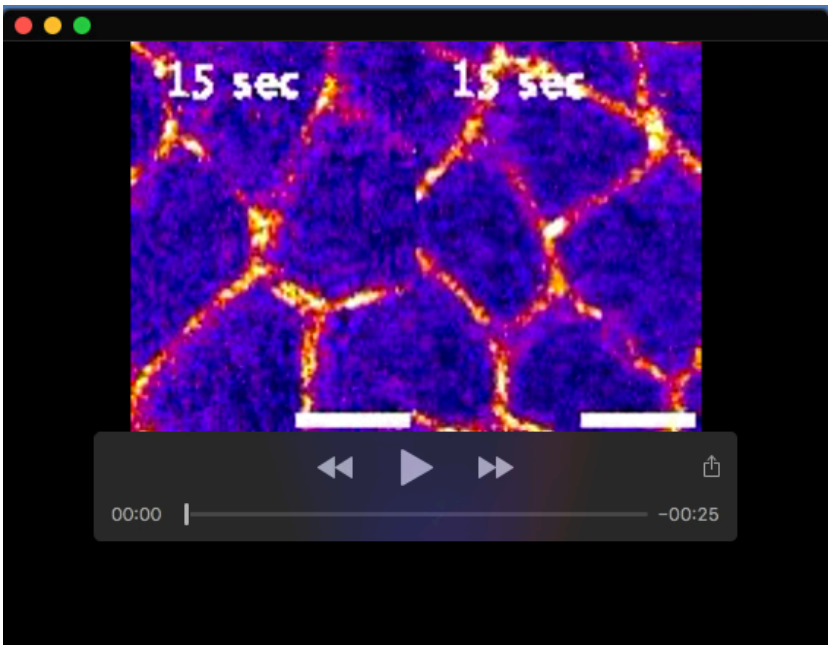
Movie 1. Bright field time-lapse movies of control (upper panel) and *Src42A*^{i} (lower panel) embryos in cellularization. Single frames were taken at 20 sec intervals. Time is indicated in mm:ss format.



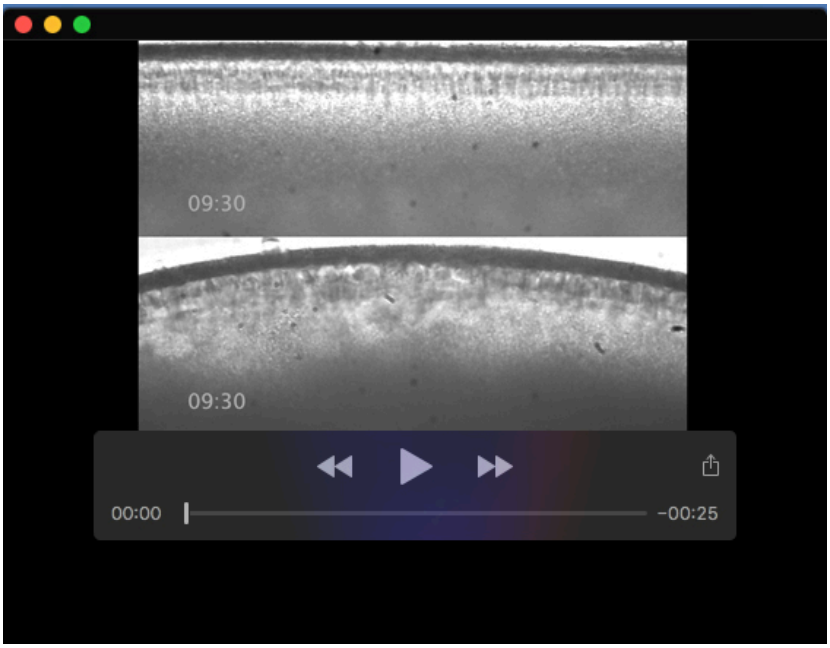
Movie 2. Bright field time lapse movies of control (upper panel) and *Src42A*^{i} (lower panel) embryos during germband extension. Time is indicated in mm:ss format.



Movie 3. Germband cells in control (upper panel) and *Src42A*^{i} (lower panel) embryos expressing Sqh::Utrophin-GFP. Germband cells that are undergoing T1-transitions are marked in colors. Time is in mm:ss format.



Movie 4. E-cadherin dynamics at tAJs. Time lapse sequence of representative vertex of *shg::E-Cad-GFP* (left panel) and *Src42A*^{i}*+shg::E-Cad-GFP* (right panel) embryos, respectively. Scale bar indicates 5 μm.



Movie 5. Bright field time-lapse movie of control (upper panel) and *Src42A*^{GLC} (lower panel) embryo in cellularization. Single frames were taken at 20 sec intervals. Time is indicated in mm:ss format.