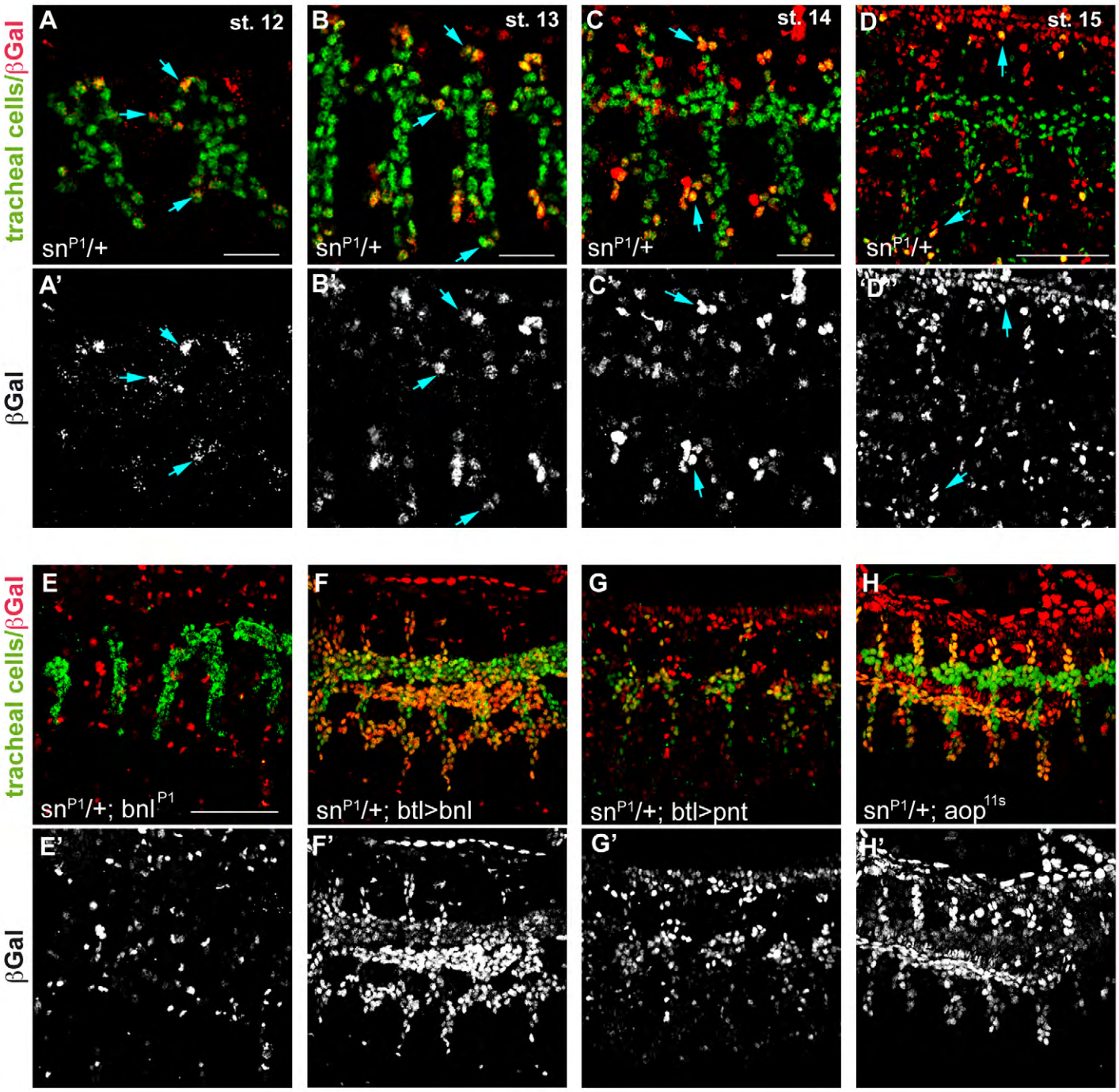


Figure S1: Expression of *sn* and its regulation during tracheal development

Embryos showing tracheal cells (green) and *sn* transcriptional pattern (red or white) visualised using a *sn-lacZ* line in heterozgous conditions (sn^{P1}). (A-D) Embryos at indicated stages showing *sn* expression. Note the expression at the tip of the branches (blue arrows). (E-H) Pattern of *sn* expression in embryos at stages 14-15 in the genetic backgrounds indicated. Note the absence of *sn* expression in *bnl* mutants and the generalised expression when *bnl* or *pnt* are overexpressed or in *aop* mutants.

Scale bar A,B,C 10 μm; D,E 25 μm.

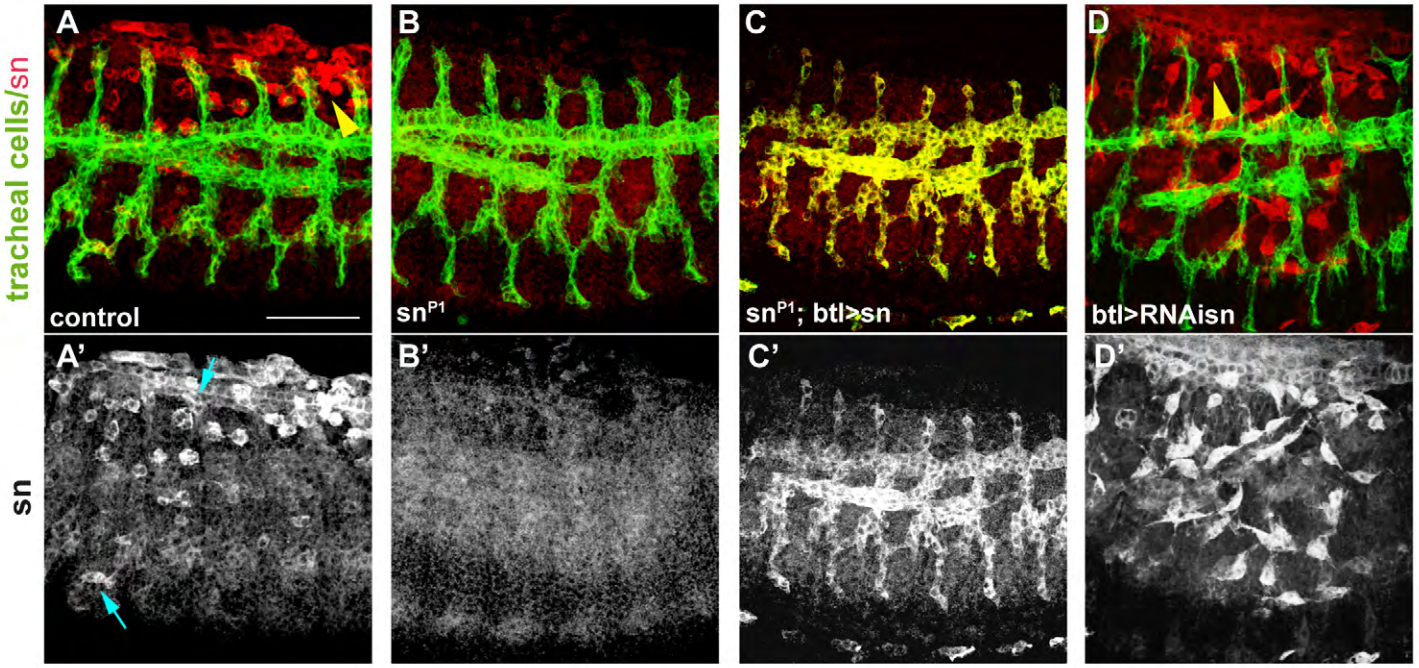


Figure S2: Pattern of Sn accumulation in *sn* mutant conditions

Stage 14-15 embryos carrying *btlGal4>UASsrcGFP* stained for tracheal cells (green) and Sn protein accumulation (red or white) in the genetic backgrounds indicated. Blue arrows point to tracheal tip cells and yellow arrowheads point to plasmatocytes.

Scale bar 25 μm.

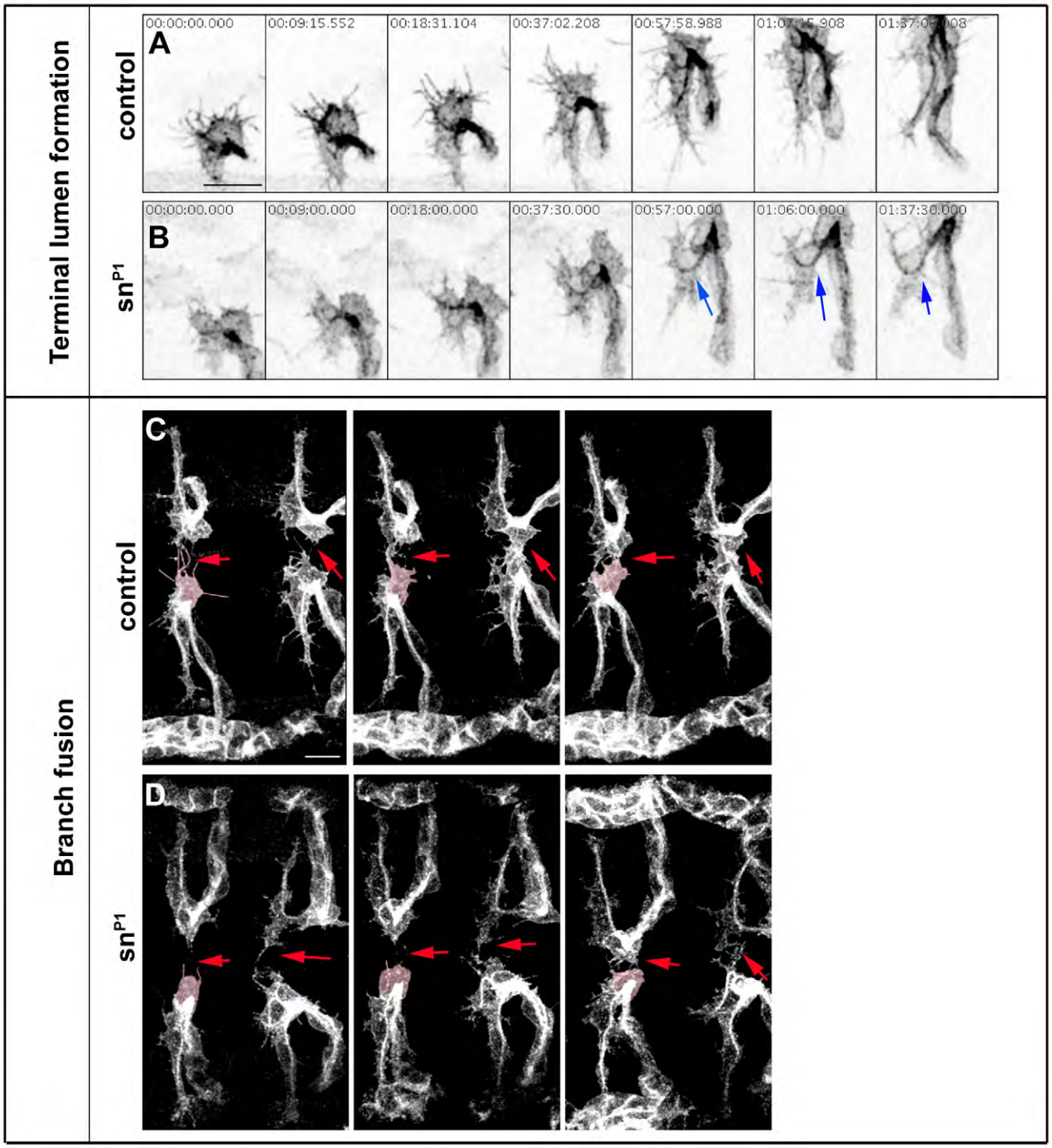


Figure S3: Fusion and terminal branching defects in *sn* mutants

(A,B) Images of time-lapse movies of control and *sn* mutants marked with *btl>srcGFP* showing a lumen missguidance in the mutant (blue arrows), which elongates dorsally instead of ventrally.

(C,D) Images of time-lapse movies of control and *sn* mutants marked with *btl>srcGFP* showing two pairs of contralateral DBs during branch fusion (red arrows). The shape of one of the fusion cells is highlighted. Note the abnormal filopodia contact and cell front advance in *sn* mutant fusion cells (D) as compared to wild type (C).

Scale bar 10 μm

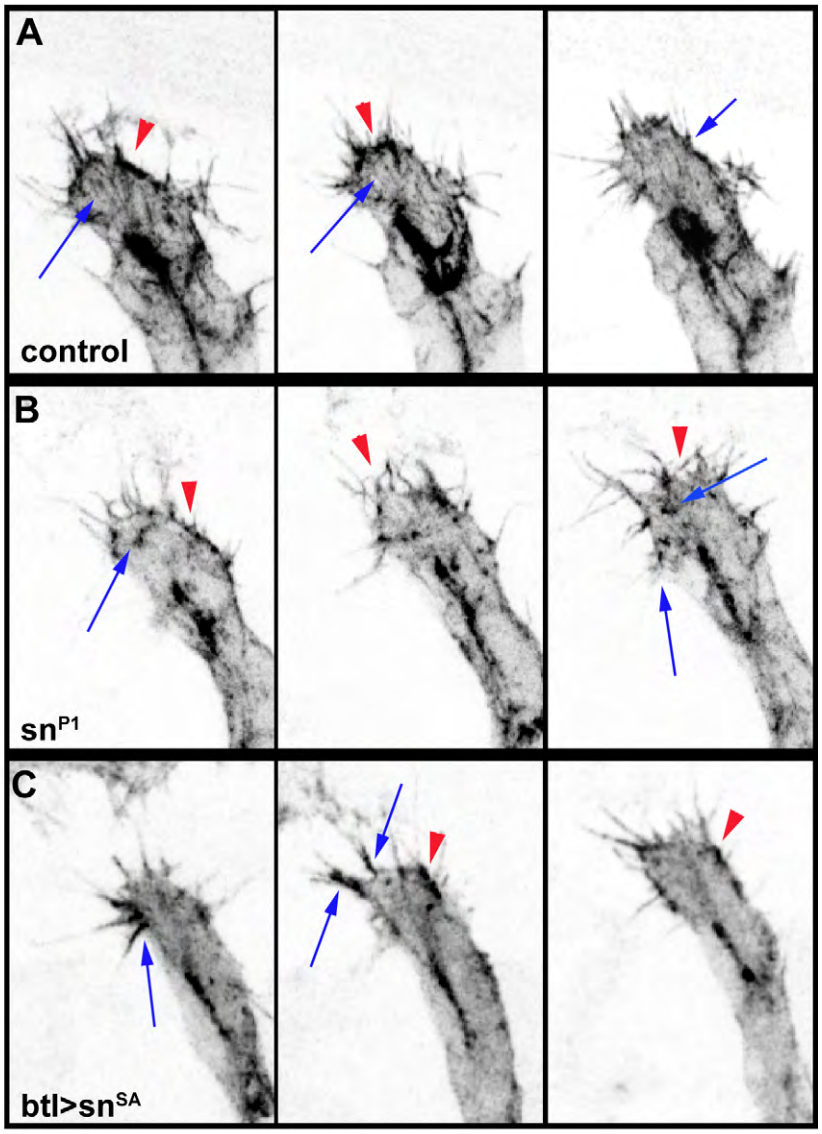


Figure S4: *sn* is required for proper actin organisation

Images from time-lapse movies of embryos with *btl>moeGFP* showing actin organisation. In *sn* mutants (B) the actin accumulation at the lamellipodia (red arrowhead) and filopodia (blue arrows) is less conspicuous as compared to the wild type (A). Note the abnormal accumulation of actin in punctae or short bundles in the mutants (blue arrows). In sn^{SA} conditions (C) high accumulation of actin can be detected along the filopodia, while in the wild-type filopodia actin accumulates at their base (A).

Scale bar 5 μm

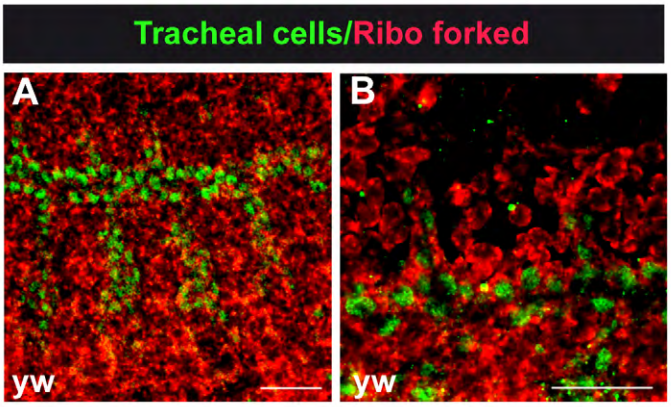
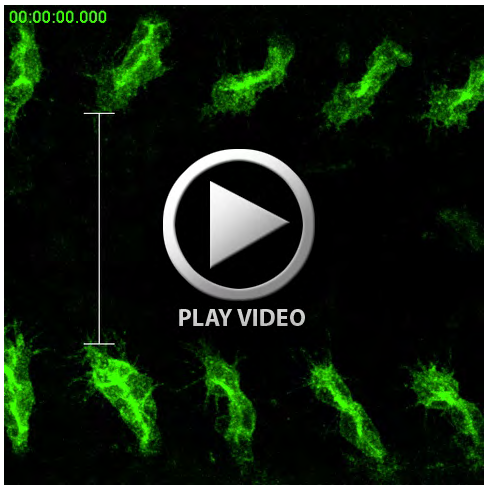
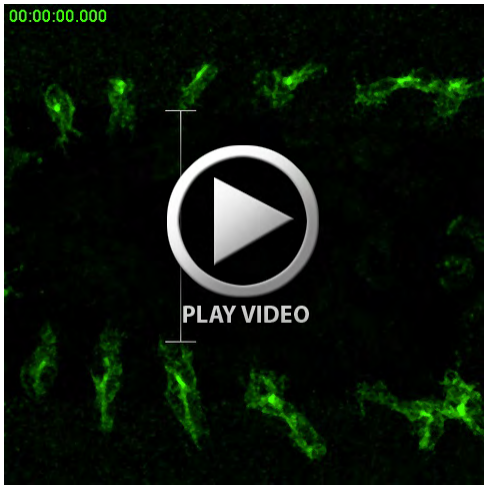


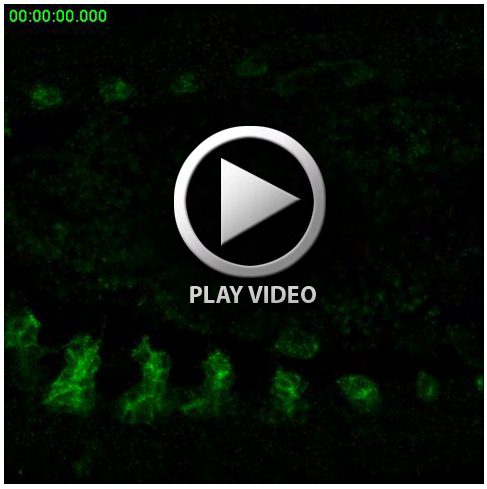
Figure S5: *f* expression
Whole mount in situ hybridisation showing the presence of *f* transcript in tracheal cells (green).
Scale bar A 25 µm; B 10 µm



Movie 1 (Figure 3D,D'): Approaching of dorsal branches during tracheal development
Embryo carrying *btlGal4>UASsrcGFP* visualised from a dorsal view using an inverted TCS-SP5 confocal microscope with a 63.0x1.40 Oil objective. Time-lapse images were taken every 1min30sec. Distance between contralateral DB4 is marked by a bar and the fusion point by an astersik at time point 0, 1 hour and 1 hour 25 min (indicated by an arrowhead).

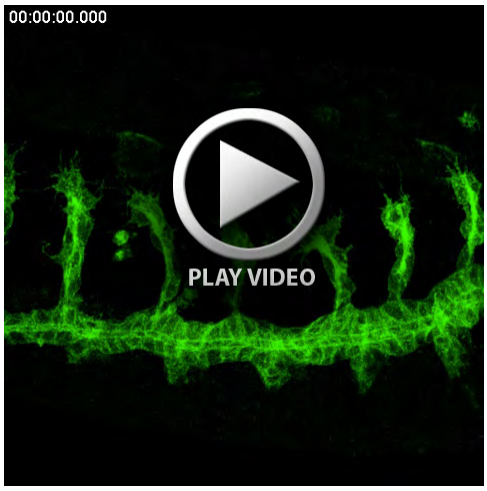


Movie 2 (Figure 3E,E'): Delayed approaching of dorsal branches in *sn* mutant
Embryo mutant for *sn* (sn^{P1}) and carrying *btlGal4>UASsrcGFP* visualised from a dorsal view using an inverted TCS-SP5 confocal microscope with a 63.0x1.40 Oil objective. Time-lapse images were taken every 1min30sec. Distance between contralateral DB4 is marked at time point 0, 1 hour and 1 hour 25 min (indicated by an arrowhead). Note that DB4 is several minutes delayed to cover the same distance (indicated by bars or asterisk) when compared to control embryos (Movie 1). Yellow arrow points to a DB missguidance.



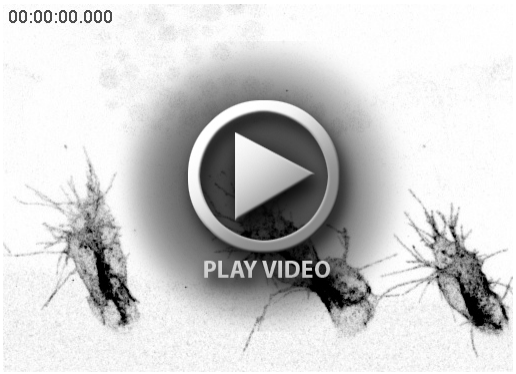
Movie 3: Branch outgrowth, branch fusion and terminal branching in control dorsal branches

Embryo carrying *btlGal4>UASsrcGFP* visualised from a lateral view using an inverted TCS-SP5 confocal microscope with a 63.0x1.40 Oil objective. Time-lapse images were taken every 1min14sec and 1min59sec and concatenated post-imaging. Note the formation of the cytoplasmic extension of terminal cells starting before dorsal branch fusion (white arrows). Branch fusion at the dorsal midline is marked by white asterisks.



Movie 4: Defective branch outgrowth, branch fusion and terminal branching in *sn* mutants

Embryo mutant for *sn* (sn^{P1}) and carrying *btlGal4>UASsrcGFP* visualised from a lateral view using an inverted TCS-SP5 confocal microscope with a 63.0x1.40 Oil objective. Time-lapse images were taken every 2min7sec. Note that cytoplasmic extensions are delayed (white arrows) when compared to the control embryos (Movie3). Several dorsal branches remain unfused at the end of embryogenesis (red asterisks). Note the formation of a misguided terminal lumen (yellow arrowhead) that turns dorsally.



Movie 5 (Figure 5A): Fast time-lapse imaging of filopodia and tip-cell shape in control dorsal branches

Live imaging of DB tip cells of control embryo (*btlGal4>UASsrcGFP*) was acquired using an inverted TCS-SP5 confocal microscope with a 63.0x1.40 Oil objective. Time-lapse images were taken every 10 sec.

Movie 6: Curved filopodia and irregular cell fronts in tip cells of *sn* mutant

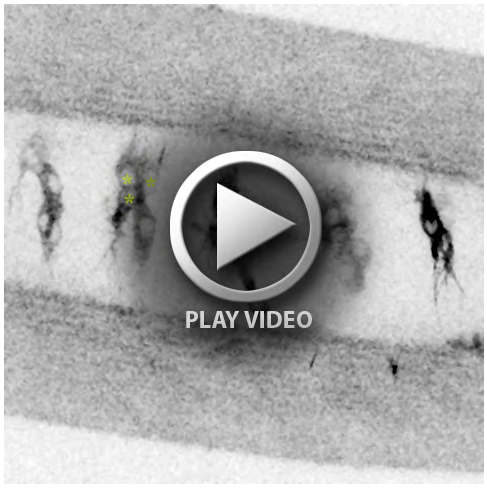
Live-imaging of DB tip cells of *sn* mutant (*sn^{P1};btlGal4>UASsrcGFP*) was acquired using an inverted TCS-SP5 confocal microscope with a 63.0x1.40 Oil objective. Time-lapse images were taken every 10 sec, revealing bent and flaccid filopodia, that often extend parallel to the irregular and disorganised cell front.

Movie 7: Organised actin dynamics in wild-type tip cells.

Utr-GFP in an otherwise wild-type background was visualised in an inverted Zeiss Lsm780 confocal with 63x Water objective and a 1,6 zoom. Images were taken every 13,56 and 14,04 seconds in 5-6µm Z-stack. Note the high and linear accumulation of actin that organises towards the protruding front of the leading cells (lamellipodium) and that reaches out at least until the base of the straight filopodia. Long bundles of actin extend from the cytoplasmic meshwork and reorganise to form the filopodia.

Movie 8: Disorganised actin cytoskeleton in *sn* mutant tip cells

Time-lapse imaging of Utr-GFP expressed in *sn* mutant cells, visualised in an inverted Zeiss Lsm780 confocal with 63x Water objective and a 1,8 zoom. Images were taken every 14,04 seconds in 5-6µm Z-stack. Actin accumulation appears in very short bundles or as punctae that moves apparently randomly trough the cytoplasm and that do not organise in a continuous and dense network at the lamellipodium. Note that filopodia form without detectable long and cytoplasmic-organised bundles of actin.



Movie 9: Live-imaging of dorsal branches overexpressing a non-phosphorytable form of Sn (Sn^{S52A})

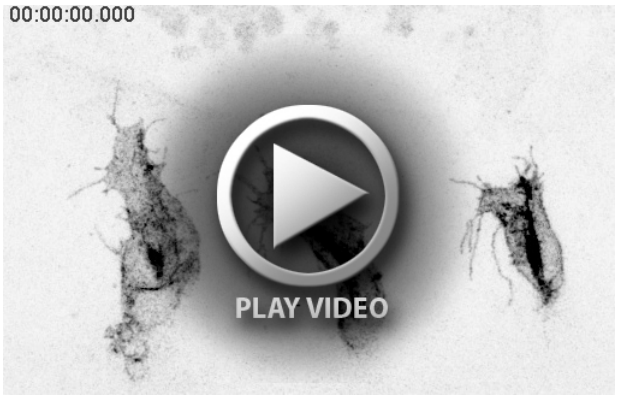
Embryo expressing *sn^{S52A}-GFP* under the *btlGal4* driver was visualized using an inverted TCS-SP5 confocal microscope with a 63.0x1.40 Oil objective. Time-lapse images were taken every 1min14sec. Note the presence of extra tip cells (yellow asterisks, three tip-cells instead of 2 in normal conditions.). Note the thick and robust cytoplasmic extensions of terminal cells (magenta arrowhead), which clearly display straight and thick filopodia.

Movie 10 (Figure 6D): Subcellular localisation of a non-phosphorytable form of Sn (Sn^{S52A}) in tip cells

Images of tracheal tip cells from a *sn* mutant embryo (*sn^{P1}*) carrying *btlGal4>UASSn^{S52A}-GFP* visualised using an inverted TCS-SP5 confocal microscope with a 63.0x1.40 Oil objective. Frames were taken every 10sec, revealing an enrichment of Sn^{S52A} in the filopodia, although it is also present in the cytoplasm.

Movie 11 (Figure 6E)*:* Subcellular localisation of a phosphomimetic form of Sn (Sn^{S52E}) in tip cells

Images of tracheal tip cells from a *sn* mutant embryo (*sn^{P1}*) carrying *btlGal4>UASSn^{S52E}-GFP* visualised using an inverted TCS-SP5 confocal microscope with a 63.0x1.40 Oil objective. Frames were taken every 10sec, showing a diffuse accumulation of Sn^{S52E} throughout the cytoplasm, although it also appears transiently in filopodia.



Movie 12 (Figure 7H): *sn* and *f* double mutant fast time-lapse imaging of filopodia and tip cell shape

Live-imaging of DB tip cells of $sn^{3}f^{36a}$ mutant ($sn^{3}f^{36a}$;*btlGal4>UASsrcGFP*) was acquired using an inverted TCS-SP5 confocal microscope with a 63.0x1.40 Oil objective. Time-lapse images were taken every 10sec, showing only few, short and sometimes bent filopodia, arising from very irregular and unproductive cell fronts.

Movie 13 (Figure 7I): Fast time-lapse imaging of filopodia and tip cell shape of a *sn* and *f* double mutant expressing wild-type Sn in the trachea

Live-imaging of DB tip cells of a $sn^{3}f^{36a}$ mutant carrying *btlGal4>UASsn-GFP* was acquired using an inverted TCS-SP5 confocal microscope with a 63.0x1.40 Oil objective. Time-lapse images were taken every 10sec, and show a clear rescue of the straightness of filopodia and of the irregular cell fronts of double mutants.