Supplementary Information

Supplementary Figures

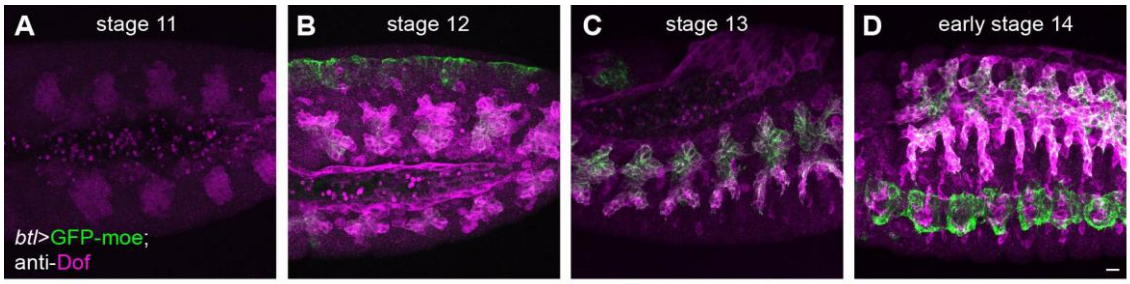


Fig. S1. Dof expression patterns: Anti-Dof staining of embryos carrying *btl>GFP-moe*. (A) At stage 11, Dof is expressed in all cells in the tracheal primordia that have completed invagination. (B) At stage 12, Dof is expressed in nearly all tracheal cells undergoing Branchless/FGF-induced primary branching. (C). At stage 13, Dof is downregulated in the middle of the trachea, which is populated by stalk cells of the dorsal trunk and dorsal branch (DB). (D). At stage 14, strong Dof expression is maintained in the DB tip and the ventral side (ganglionic branch and lateral trunk). Scale bar: 10 µm.

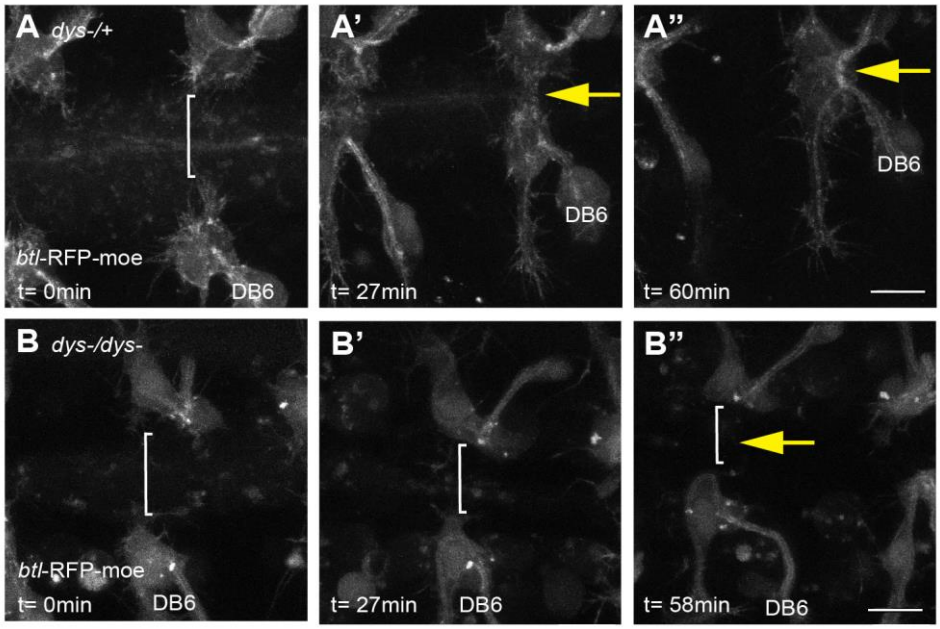


Fig. S2. *dys* mutant phenotype. DB movement was monitored by *btl-RFP-moe* marker. The number of filopodia in FC was reduded in *dys* mutant (B, identical to Fig. 2F, F', F''), compared with control trachea that showed abundant filopodia (A). This figure is presented to show control image of *btl-RFP-moe.* Scale bar: 10µm.

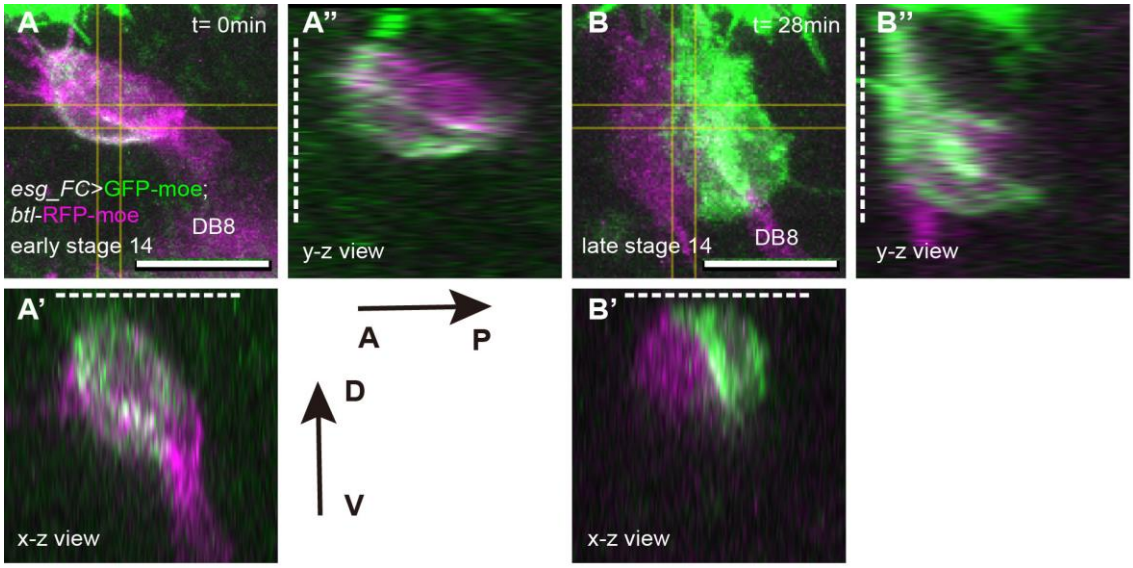


Fig. S3. Antero-posterior positional change of terminal cells at stage 14. Snapshots from live images of the DB8 tip in an embryo carrying the fusion-cell (FC) marker *esg_FC>GFP-moe* and the pan-tracheal marker *btl-RFP-moe*, which labels terminal cells (TC) and stalk cells (SCs). The x-z and y-z views show sections of the regions marked by yellow lines. (A) When GFP was first detected in the FC at early stage 14, the FC was closely associated with the epidermis (white dotted line) and the RFP single-positive TC was positioned below the FC. (B) Still at stage 14, 30 minutes later: the TC had completed anterior migration and the tip cells were positioned side-by-side next to the epidermis. Orientation of anterior-posterior and dorsal-ventral axis is indicated below A''. Scale bar: 10 µm.

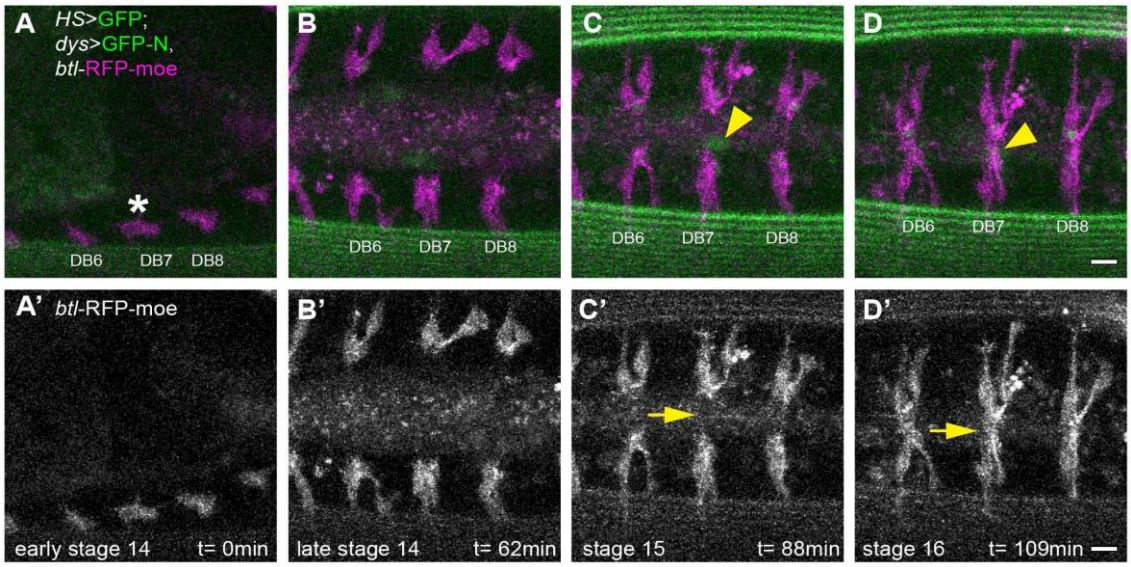


Fig. S4. HS-eGFP did not affect dorsal branch fusion. Snapshots from *HS-eGFP*, *dys>GFP-N*, *btl-RFP-moe* embryo show induction of eGFP did not inhibit the fusion process of dorsal branch. Asterisk: the heat-shock position. The strong green fluorescence is auto-fluorescence in yolk cells and vitelline membrane. Yellow arrowheads: HS-eGFP. Yellow arrows: prospective fusion points. Scale bar: 10μm.

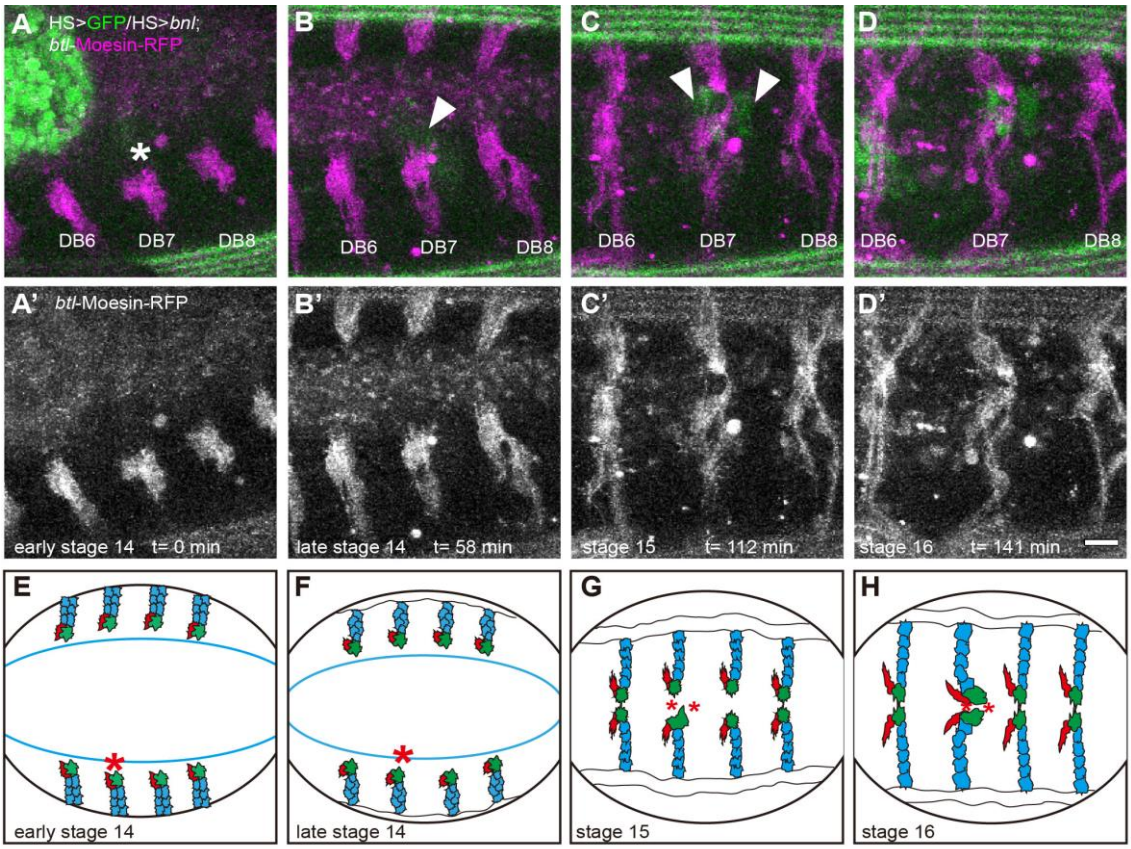
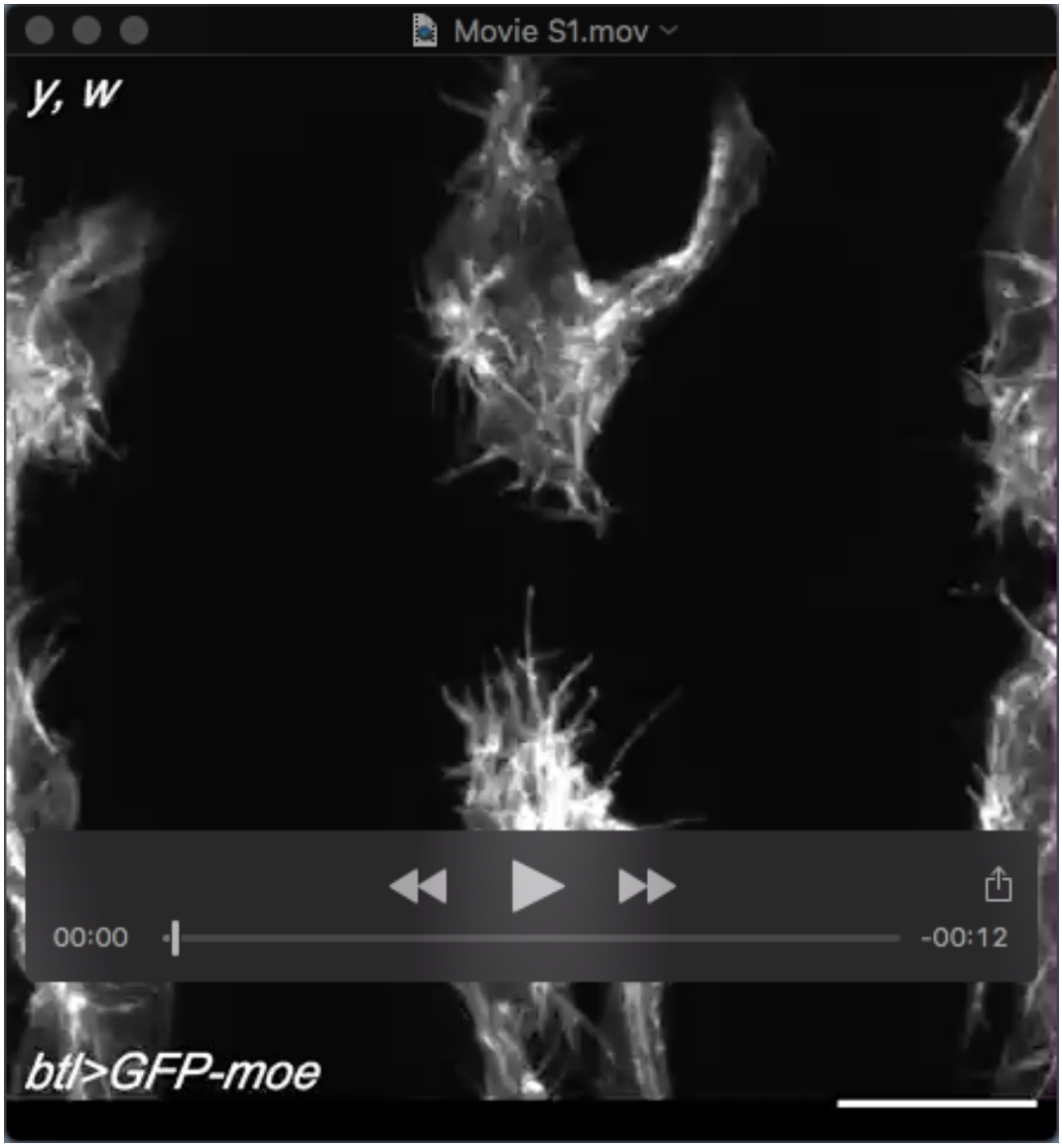


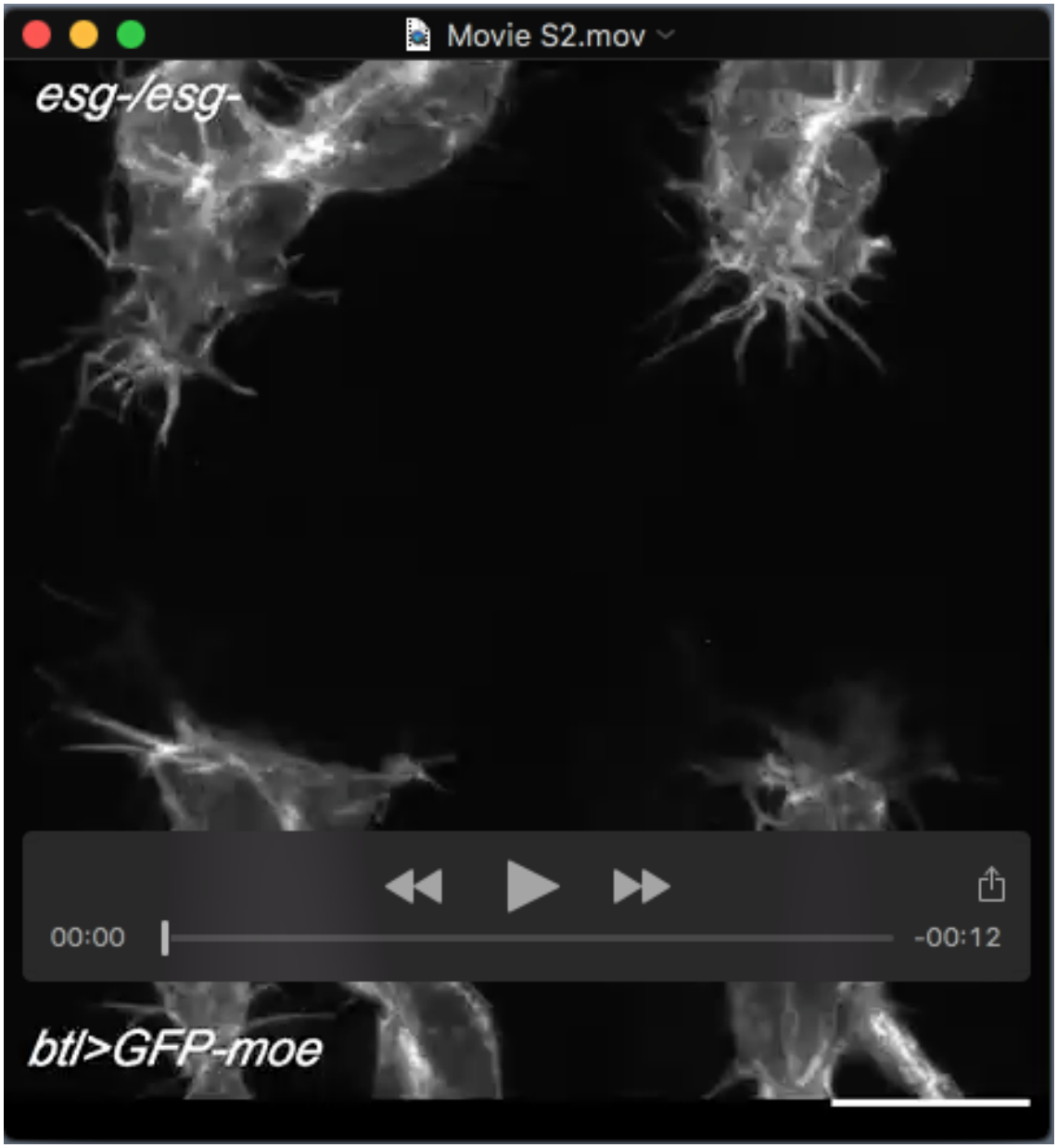
Fig. S5. Detour of FC around Bnl expressing cells. Snapshots from *HS-eGFP*, *HS-Bnl, btl-RFP-moe* embryo. Cells at the heat-shocked position (asterisk) were split into two clusters expressing HS-eGFP (arrowhead in C). Protrusions of the pair of FCs in DB7 navigated through the channel between the two GFP positive cells and contacted with each other. Arrowheads: HS-eGFP positive cells. Scale bar: 10µm.

Movies



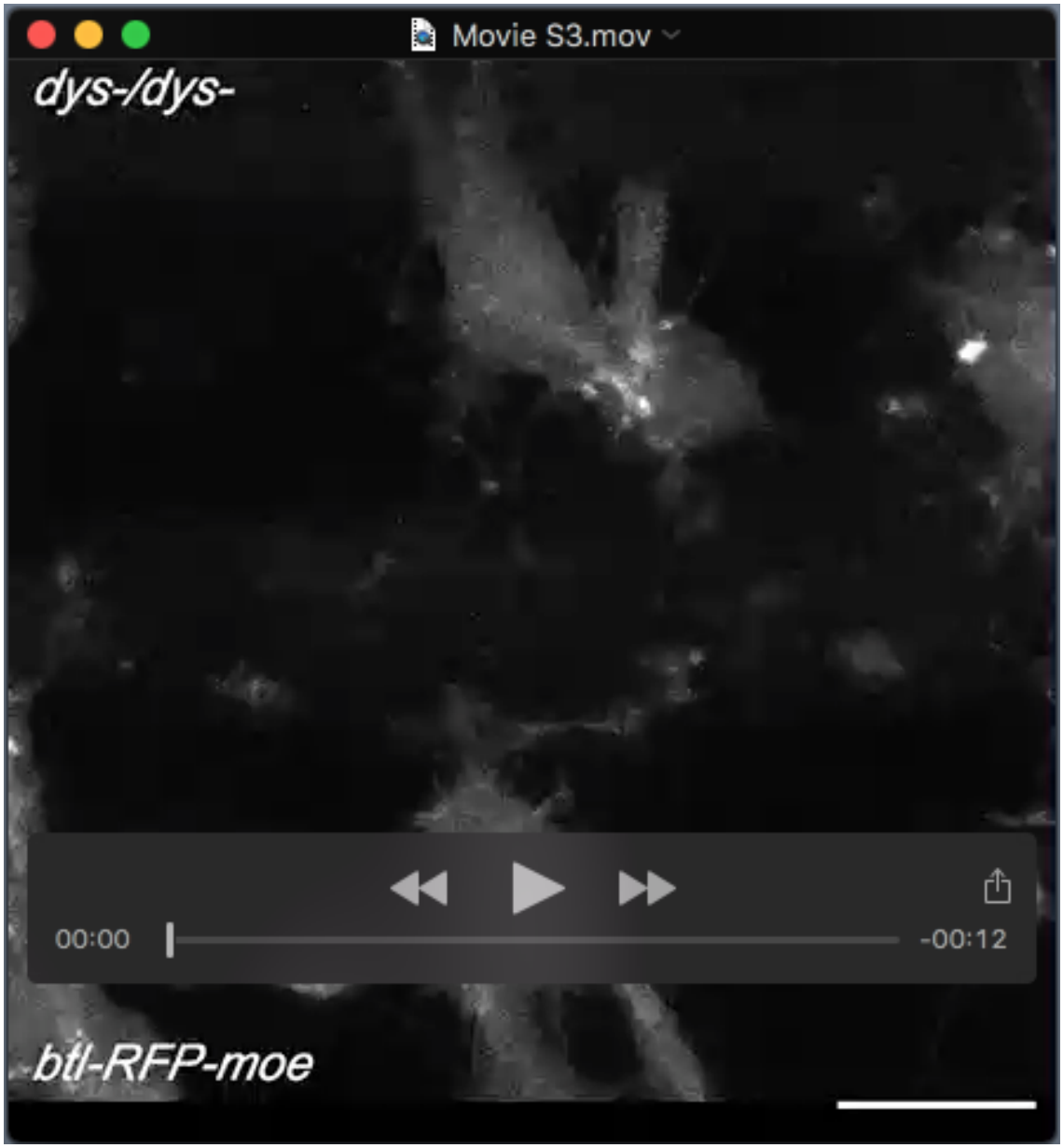
Movie S1.

Dorsal branch migration and fusion in a control *btl>GFP-moe* embryo; 10 confocal sections (1-µm intervals) were taken every 59 sec for 1 hour. Scale bar: 10 µm. Imaging conditions are the same for all movies unless otherwise stated.



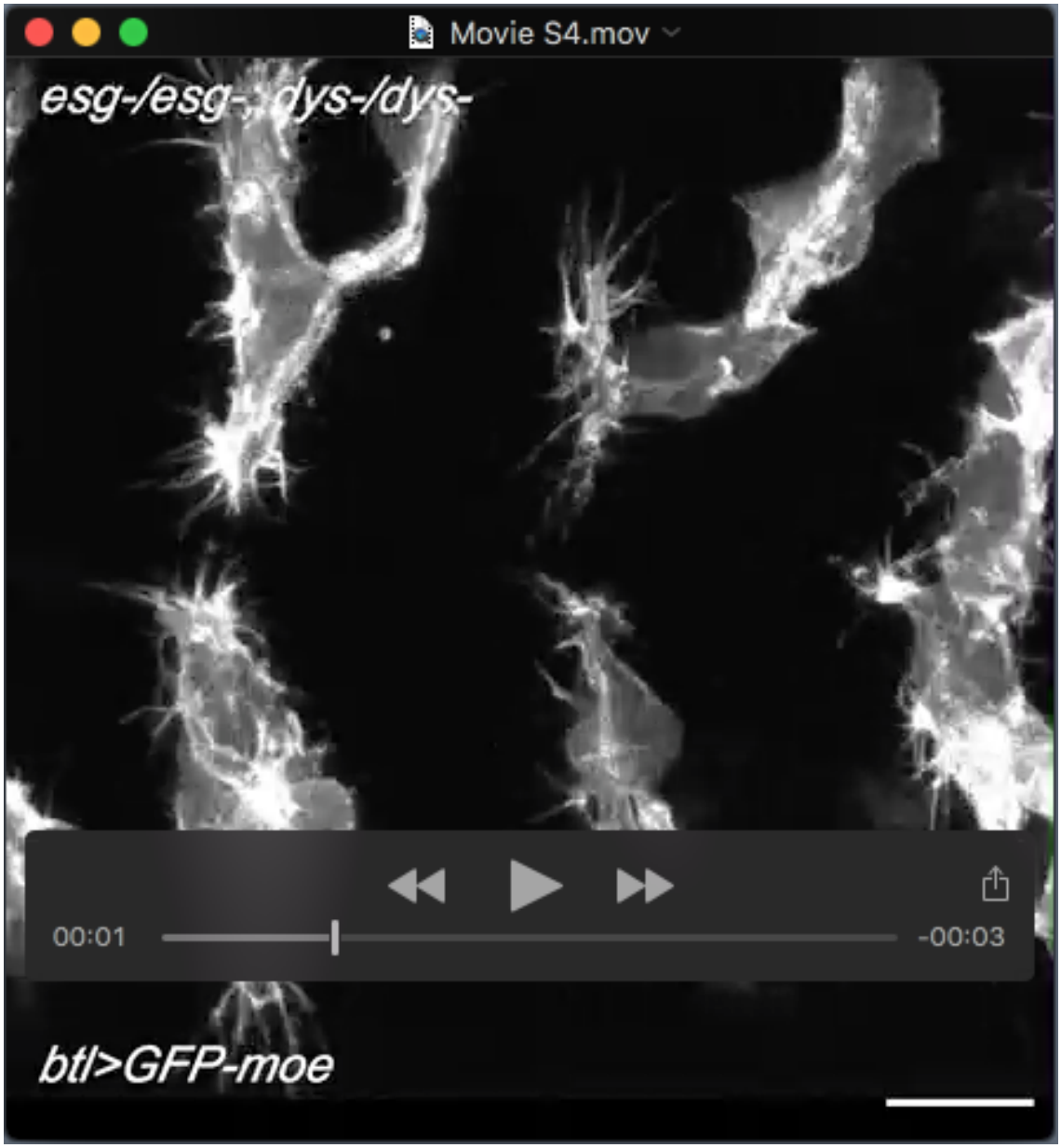
Movie S2.

An *esg*^{G66B} homozygote embryo.



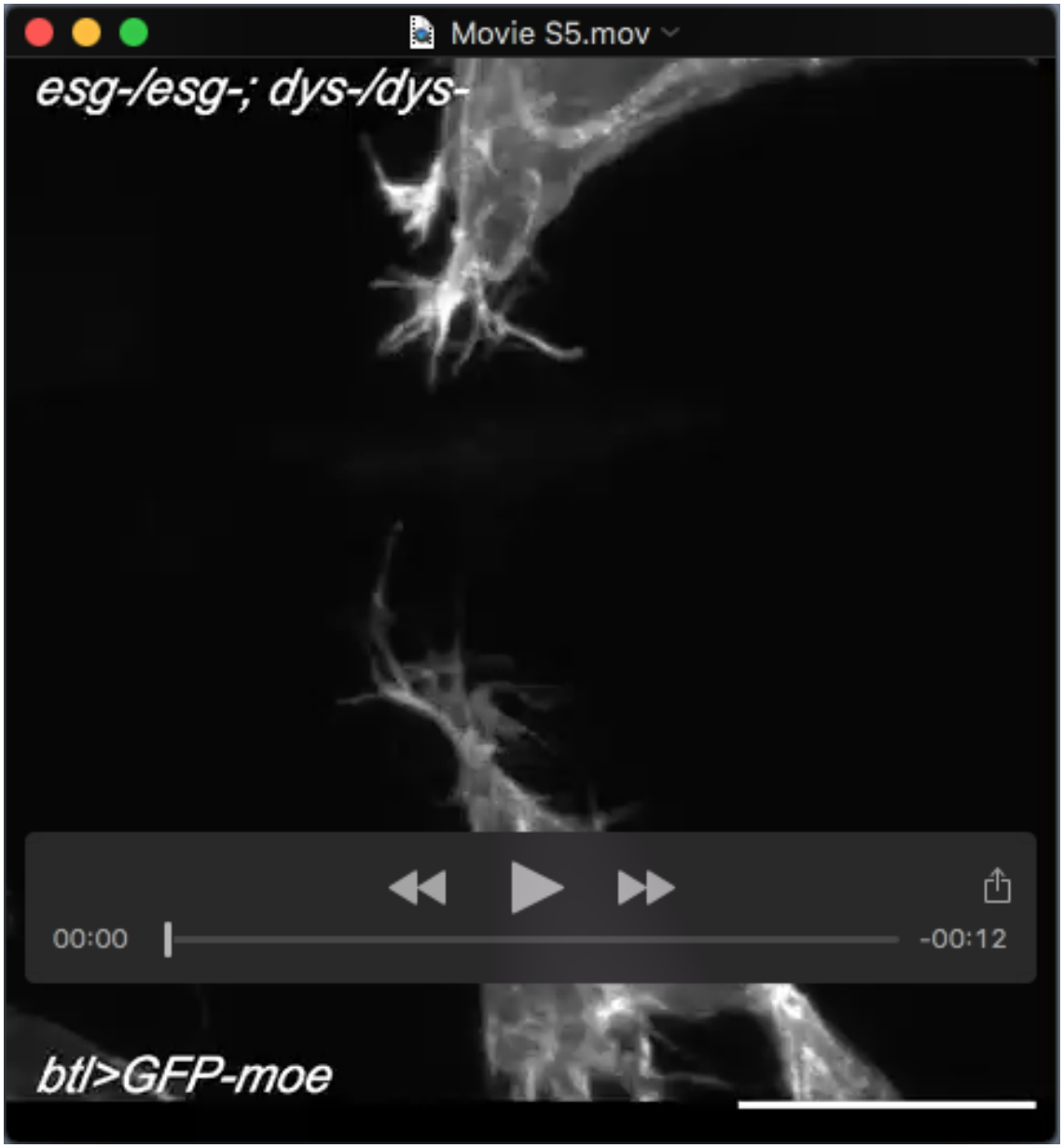
Movie S3.

A *dys*^{2} / *dys*^{3} embryo carrying *btl-RFP-moe.*



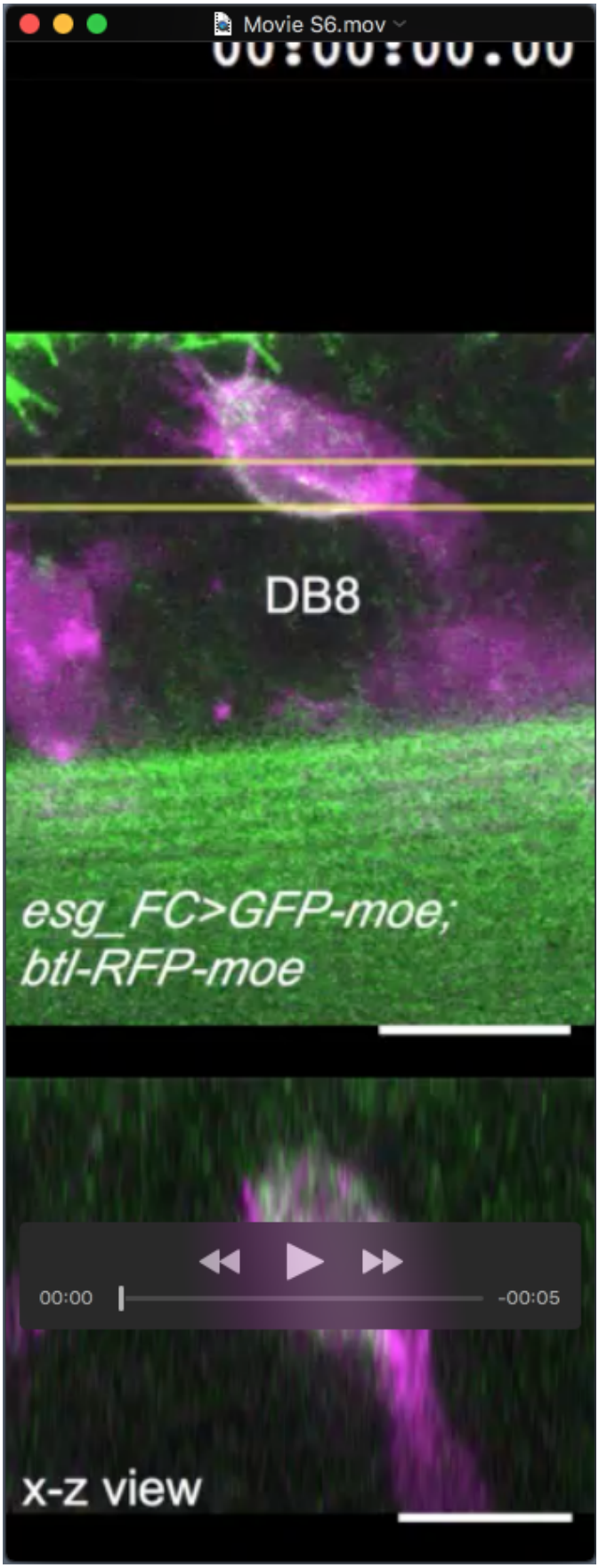
Movie S4.

An *esg*^{G66B} / *esg*^{G66B}*; dys*^{2} / *dys*^{3} embryo carrying *btl>GFP-moe*, showing the duplication of the terminal-cell (TC) phenotype (left-side pair, class 2) and single TC phenotype (central pair, class 3). Interval: 172 sec.



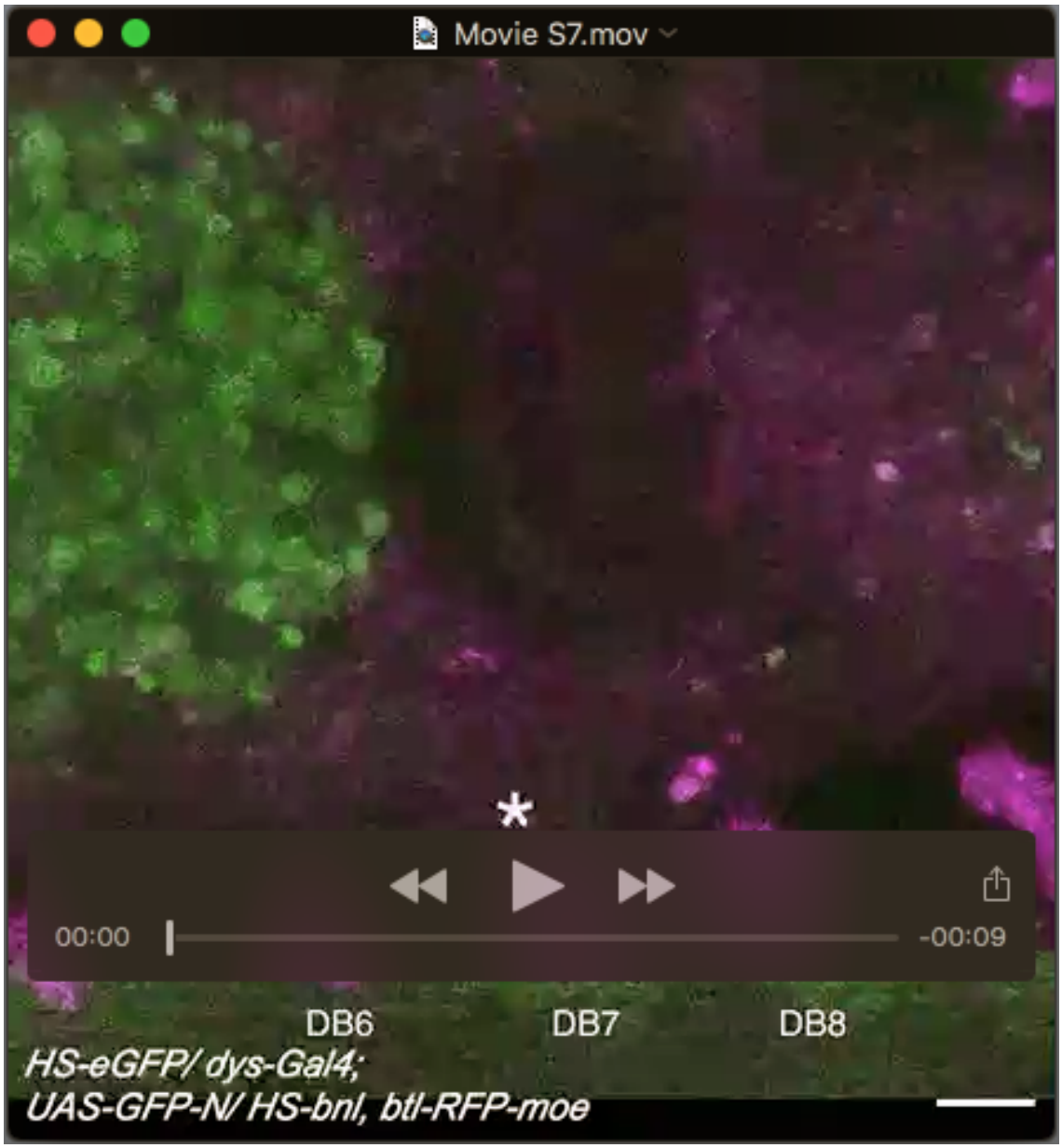
Movie S5.

A second example of an *esg*^{G66B} / *esg*^{G66B}; *dys*^{2} / *dys*^{3} embryo, showing the bifurcation of a single terminal cell.



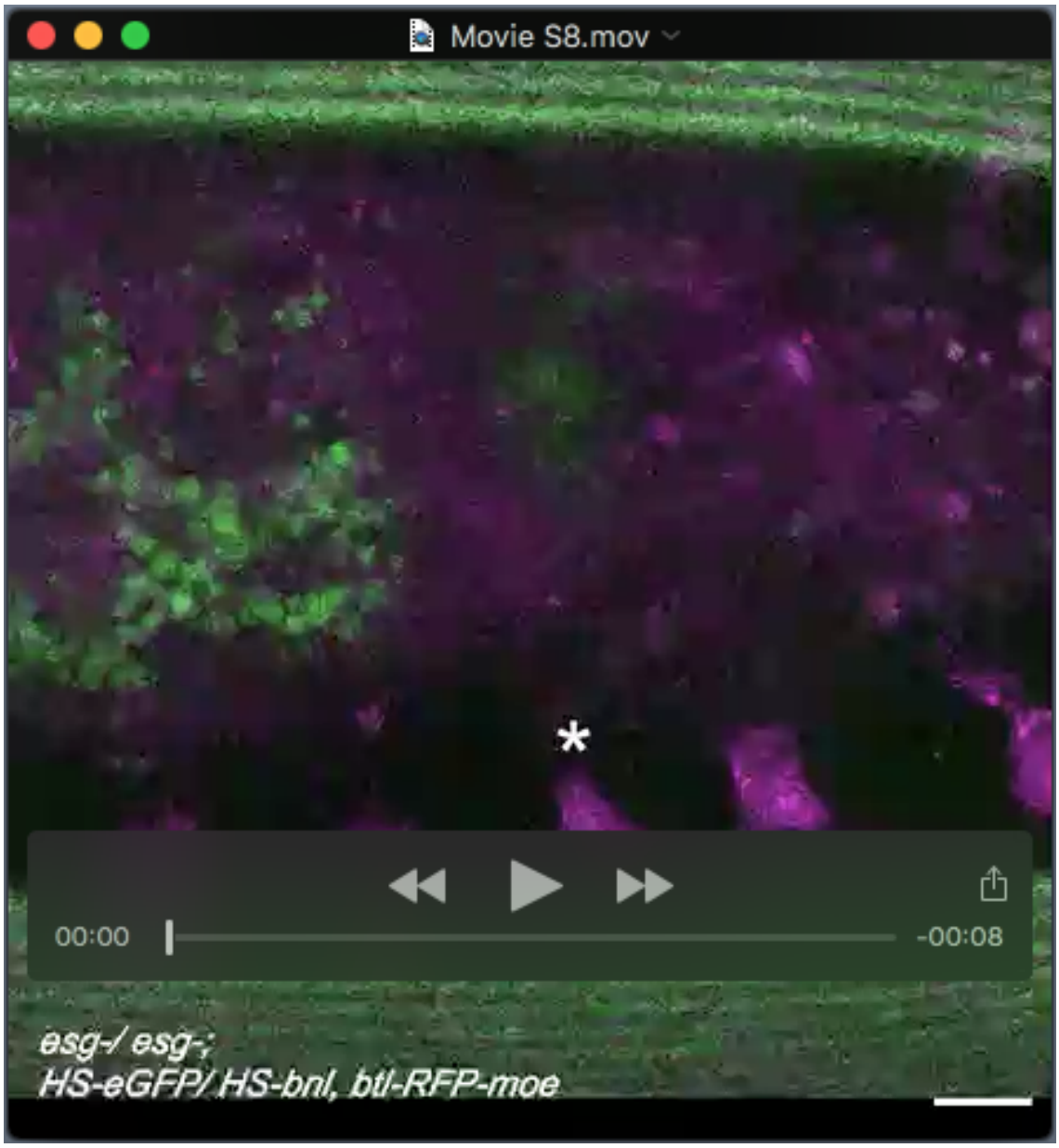
Movie S6.

The anterior migration of terminal cells (TCs) at stage 14. The TC (magenta) is labeled with *btl-RFP-moe*. Fusion cells were labeled with *esg_FC>GFP-moe*. Anterior, left; dorsal, up. Images were taken every 124 sec.



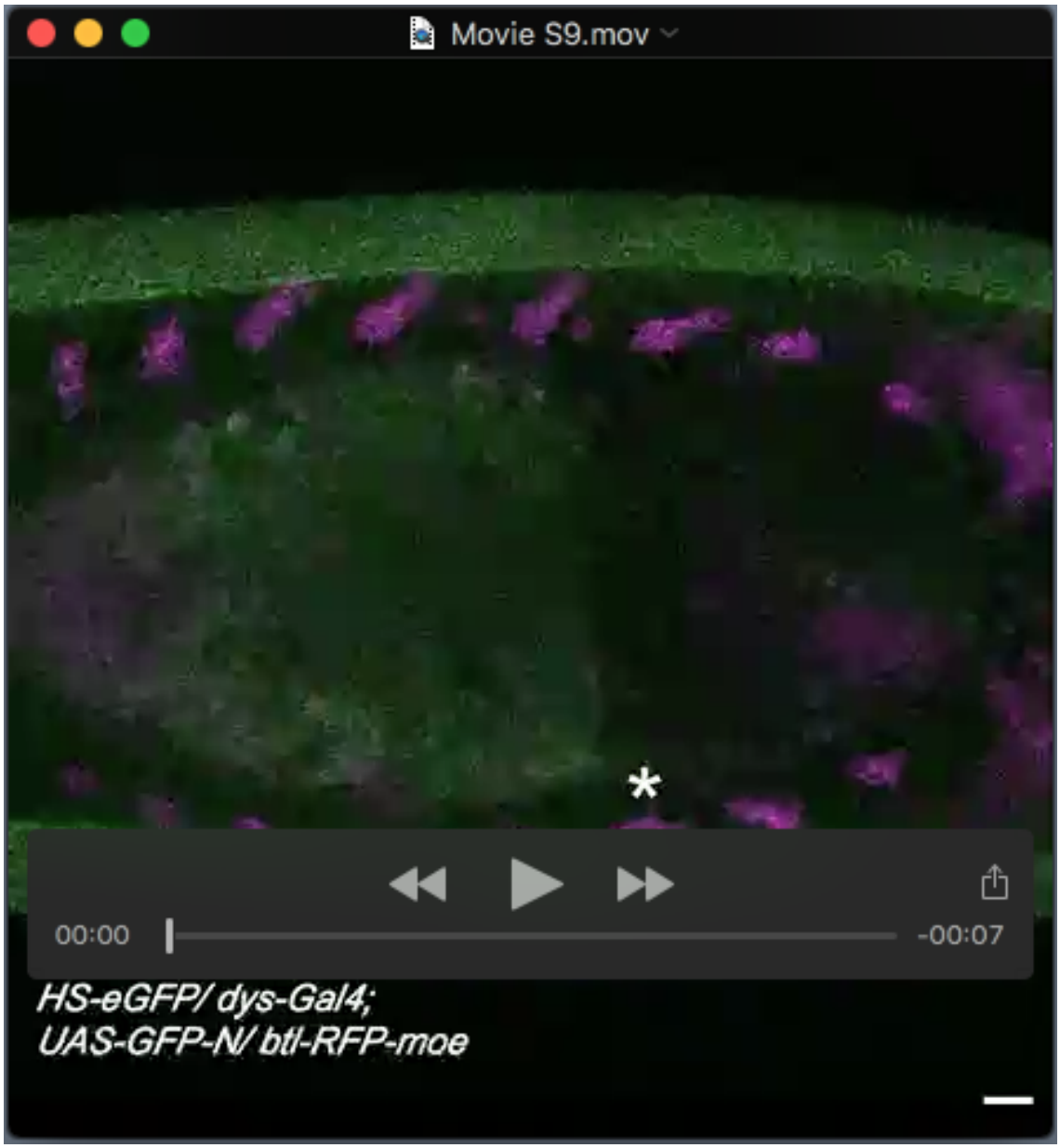
Movie S7.

Ectopic Branchless (Bnl)/FGF expression arrests fusion-cell (FC) migration. Local IR illumination was directed immediately dorsal to the dorsal-branch tip (asterisk at 0:00:00) in *HS-GFP/dys-Gal4; UAS-GFPN-lacZ / HS-Bnl, and btl-RFP-moe* embryos. Images were taken every 295 sec. Arrowhead: GFP (and Bnl/FGF). Arrow: the affected FC. Scale bar: 10 µm.



Movie S8.

Branchless (Bnl)/FGF's inhibition of fusion-cell (FC) migration requires *esg*. The laser heat-shock experiment was repeated on *esg*^{G66B} / *esg*^{G66B}; *HS-eGFP / HS-Bnl and btl-RFP-moe* embryos. FCs that had transformed into terminal cells were not arrested by ectopic Bnl/FGF. Interval: 176 sec.



Movie S9.

Control experiment without Ectopic Bnl. *HS-GFP/dys-Gal4; UAS-GFPN-lacZ / btl-RFP-moe* embryo received local IR illumination at the position immediately dorsal to the DB tip (asterisk in time 0:00:00) and time lapse images were taken every 313 sec. FC migration and fusion was not affected by HS-eGFP expression.