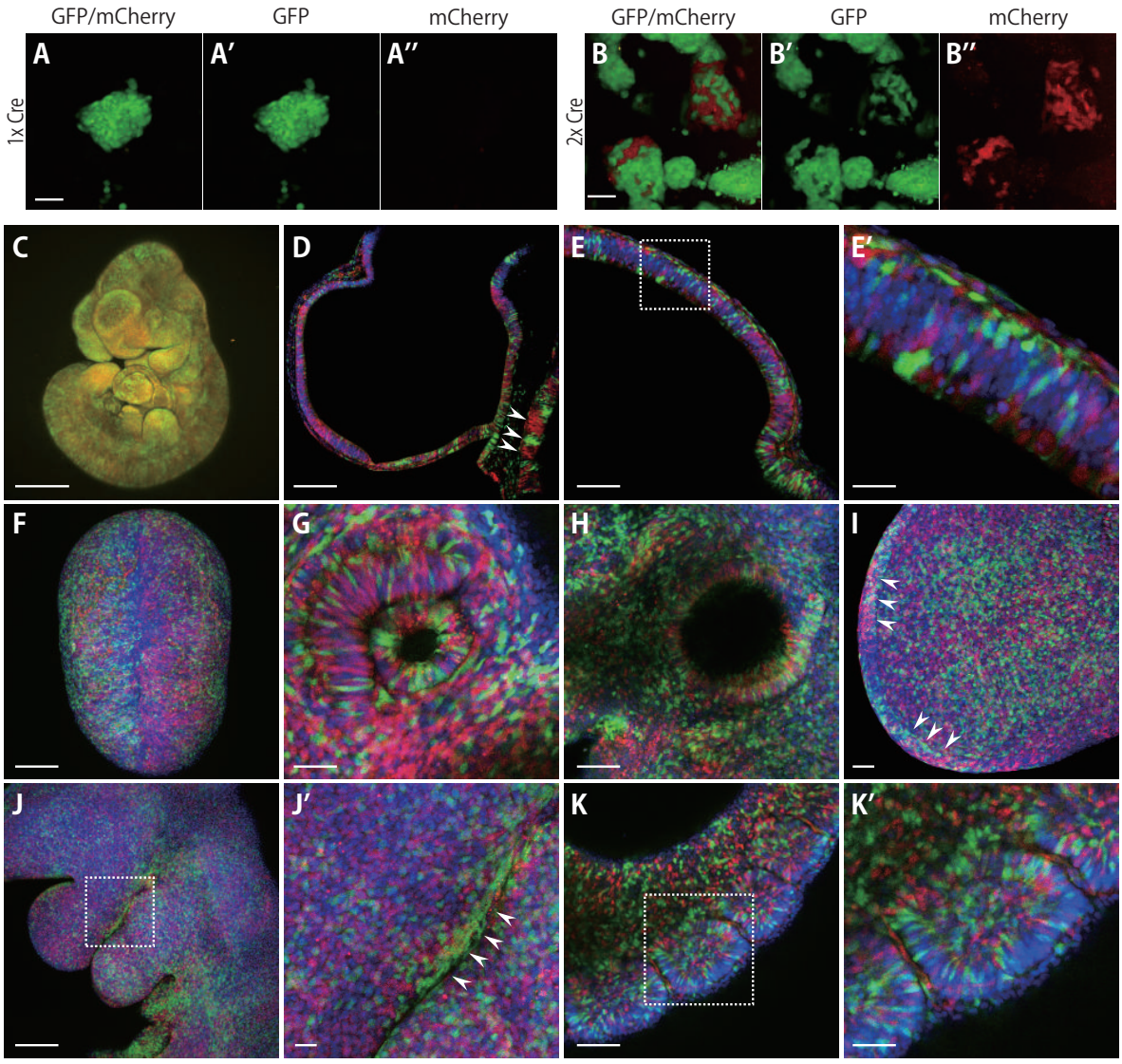


Figure S1. In vitro and in vivo Recombination in R26R-MGK allele (related to Figure 1A and B)(A–A") ES colony expressing EGFP exclusively due to excision of STOP and mKeimaRed modules. (B–B") R26R-MGK ES cells that originally expressed only EGFP were induced for secondary Cre recombination, which led to the expression of mCherry in the population, and cells showing stepwise expression of two fluorescent proteins. (C–K′) E10.5 R26R-MGK/R26CreER embryos expressing EGFP and mCherry treated with tamoxifen at E6.5. Shown are a whole embryo (C), sagittal views of the forebrain (D) and midbrain (E, E′), a top view of the (dorsal) midbrain (F), sagittal views of the optic (G) and otic (H) vesicles, the ventral forelimb (I), and sagittal views of branchial arches (J, J′) and somites (K, K′). (E'), (J'), and (K') are enlarged views of dotted boxes in E, J, and K, respectively. Arrowheads indicate labeled cells forming coherent clusters. Green, red, and blue indicate EGFP, mCherry, and DAPI fluorescence, respectively. Scale bar = 50 μm (B and C); 1 mm (C); 200 μm (D, F, J); 100 μm (E, K); 25 μm (E', G, H, I, J', K').

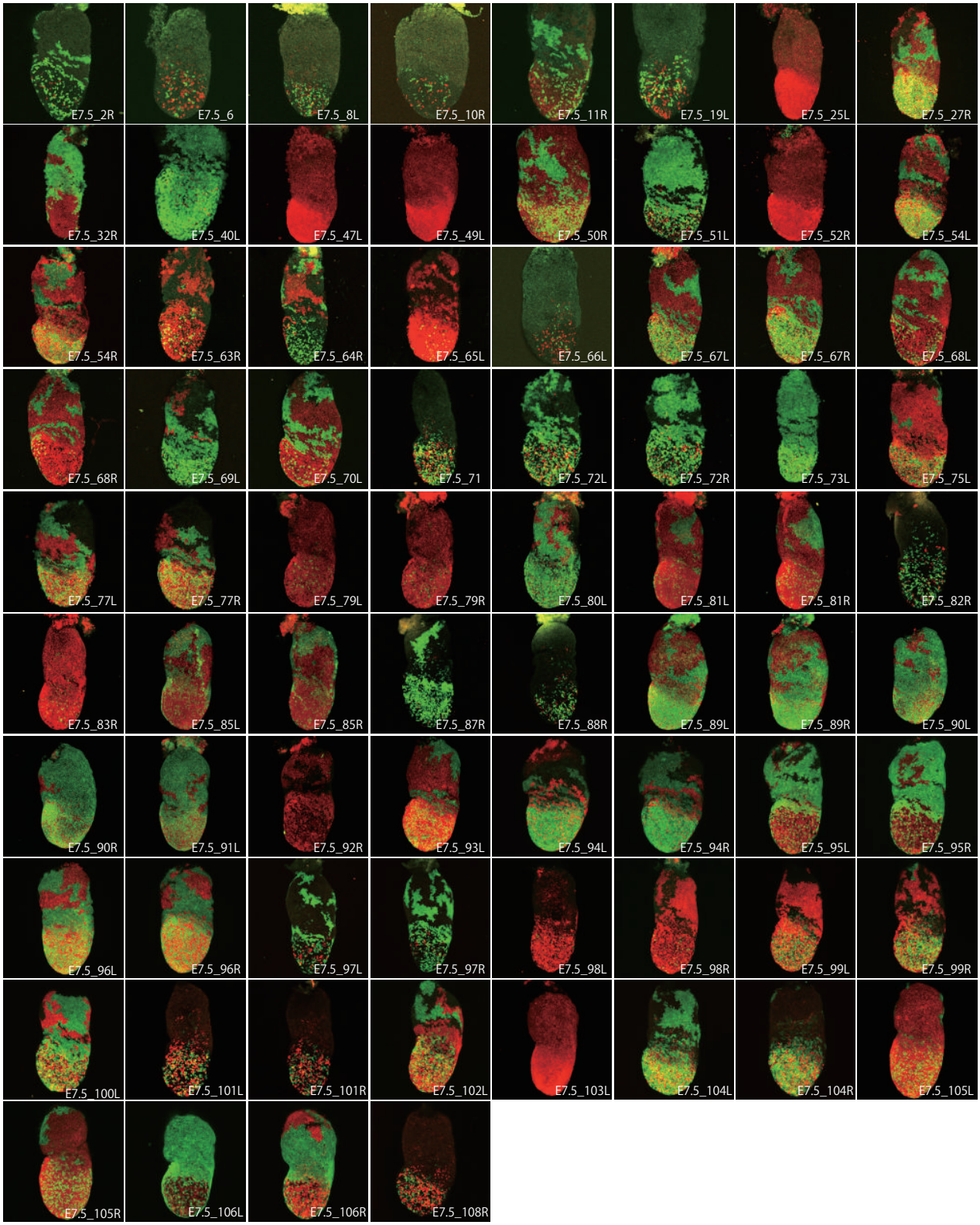


Figure S2. Lateral images of E7.5 EIIaCre/R26R-MGK embryos (related to Figure 1C–N)

Eighty-eight Z-stack images showing a lateral view of E7.5 embryos were collected. Seventy-six images are shown. The remaining 12 images are shown in Figure 1C–N. Anterior is towards the left. L and R indicate left and right side views. Green and red represent EGFP and mCherry signals, respectively.

Figure S3. Normalized binary images of E7.5 EIIaCre/R26R-MGK embryos (related to Figure 2)
Normalized binary images analyzed with LP-Clonal, an ImageJ plugin. Eighty-seven images from 32 embryos that have cell clusters in the ExEm region. Anterior is towards the left.

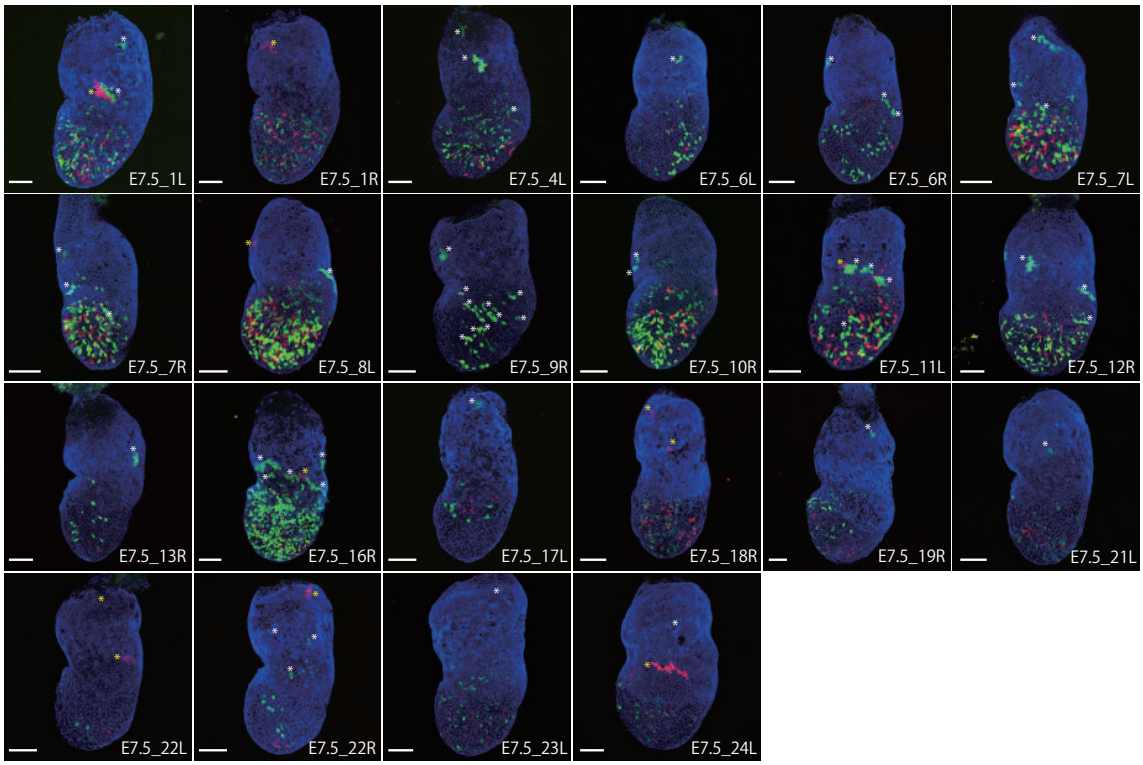


Figure S4. Lateral images of E7.5 R26CreER/R26R-MGK embryos (related to Figure 3)

Twenty-two Z-stack images of the lateral view of E7.5 R26CreER/R26R-MGK embryos treated with tamoxifen at E5.5 with merged GFP and mCherry channels. Asterisks indicate cell patches quantitatively analyzed. White and yellow asterisks are EGFP- and mCherry-expressing cells, respectively. Anterior is towards the left. L and R indicate left and right side views. Green, red, and blue represent EGFP, mCherry, and phalloidin signals, respectively. Scale bars = 100 μm.

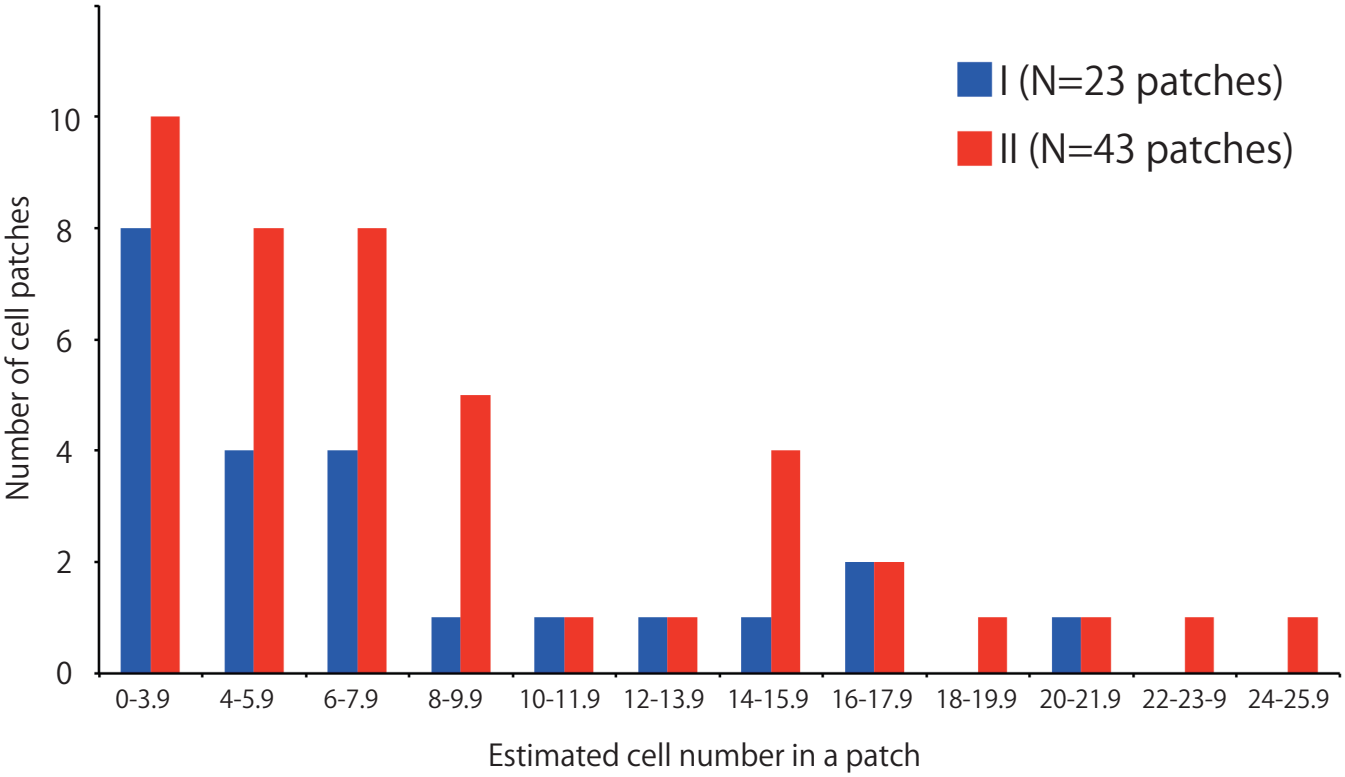


Figure S5. Estimated cell number in each cell patch (related to Figure 3)

A total of 23 and 43 patches were analyzed in regions I and II (shown in Fig. 3), respectively. X- and Y-axes indicate estimated cell number in each patch and number of cell patches counted, respectively.

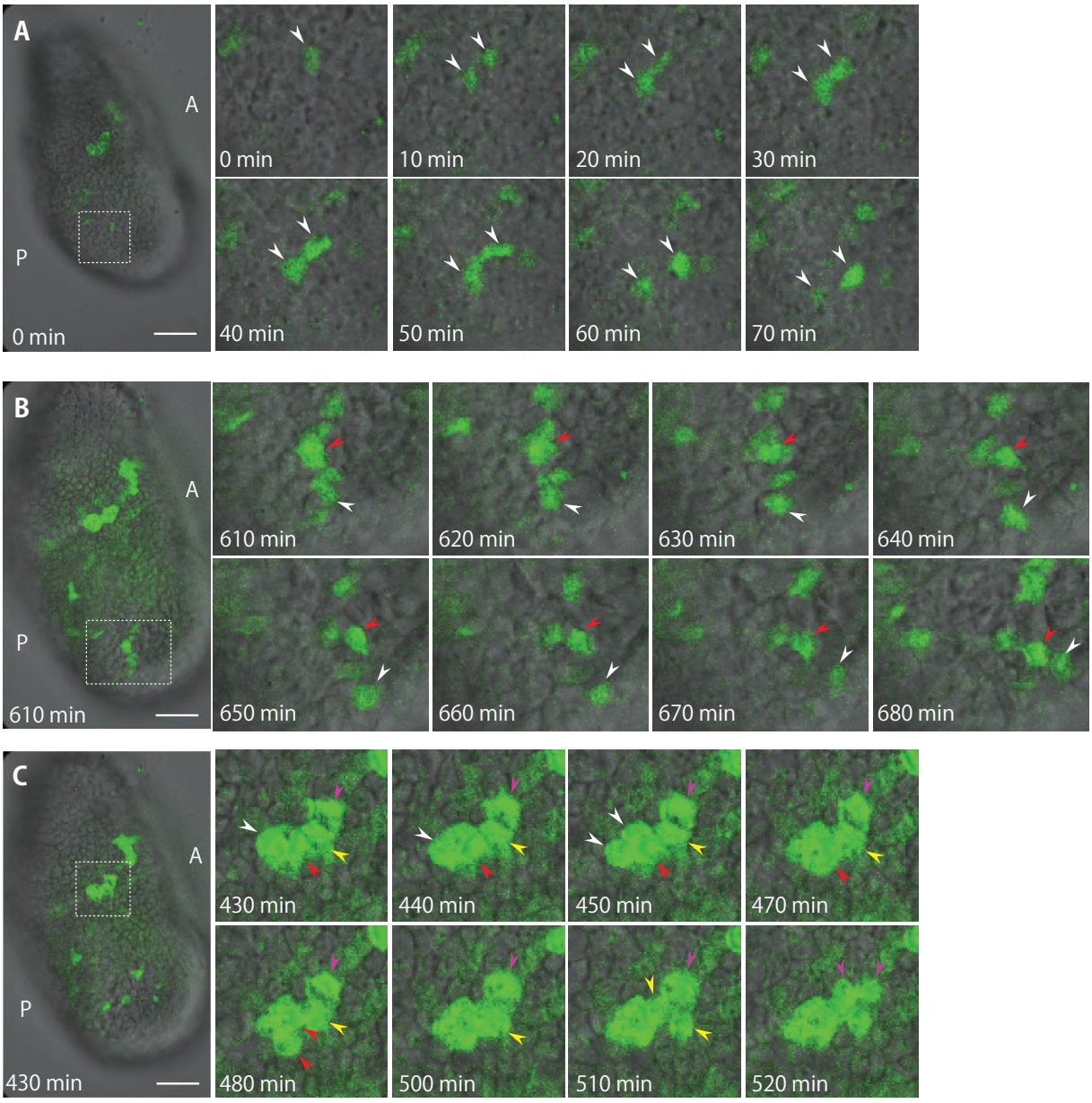


Figure S6. Time-lapse imaging of labeled cells from E6.5 (related to Movie 2,3)

(A–D) Lateral view snapshots of an E6.5 R26CreER/R26R-EGFP embryo that had been treated with tamoxifen at E5.5, carrying few EGFP-labeled cells. (A) Dividing cell in the EmVE layer. (B) Migrating cells in the EmVE layer. (C) Dividing cells in the ExEmVE. Arrowheads indicate dividing or migrating cells, with same colors for sister cells. Scale bar = 50 μm.

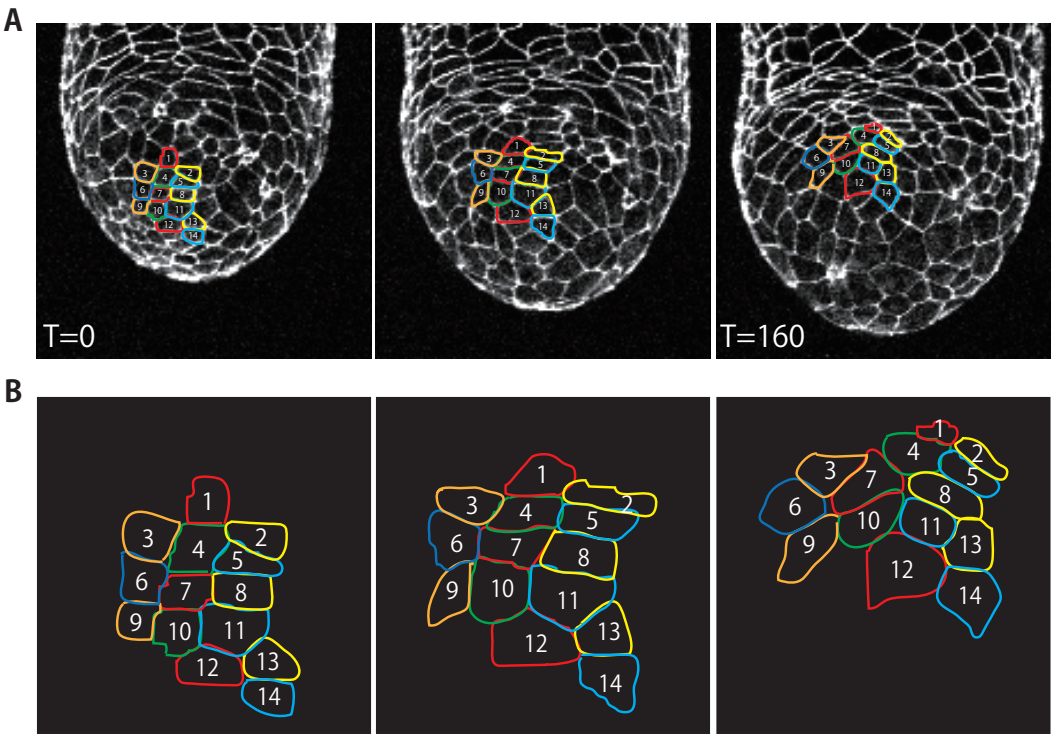
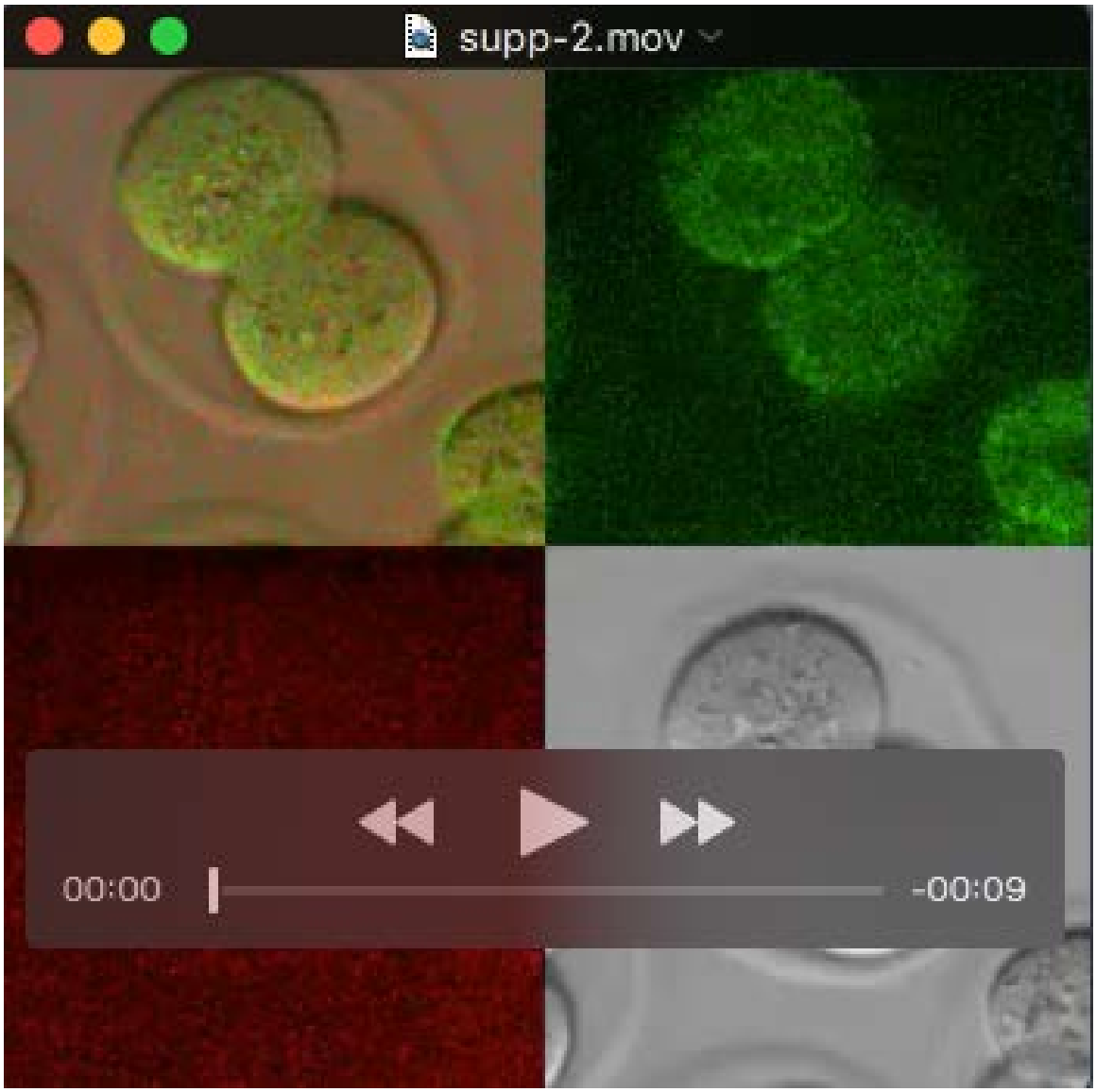


Figure S7. Reconstruction of contacts between adjacent cells (related to Figure 4G)

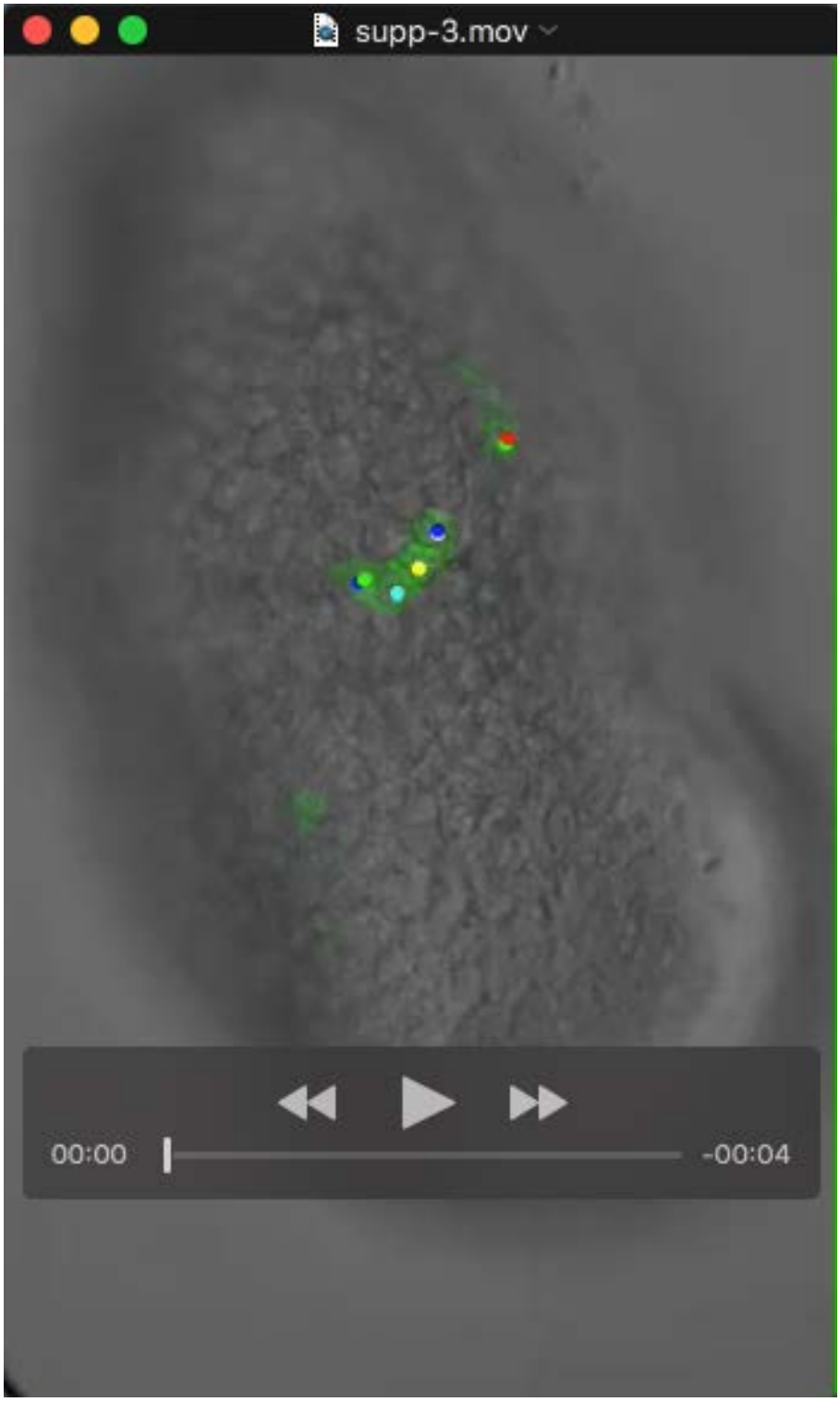
(A) Snapshot images of time-lapse recording of E6.25 R26-PHA7-EGFP embryos at 0, 60, and 160 min. Analyzed cells are encircled in different colors. (B) Enlarged view of (A), showing cell boundaries and ID# of cells.



Movie 1. Live imaging of *EIIaCre/R26R-MGK* embryo (related to Figure 1B)

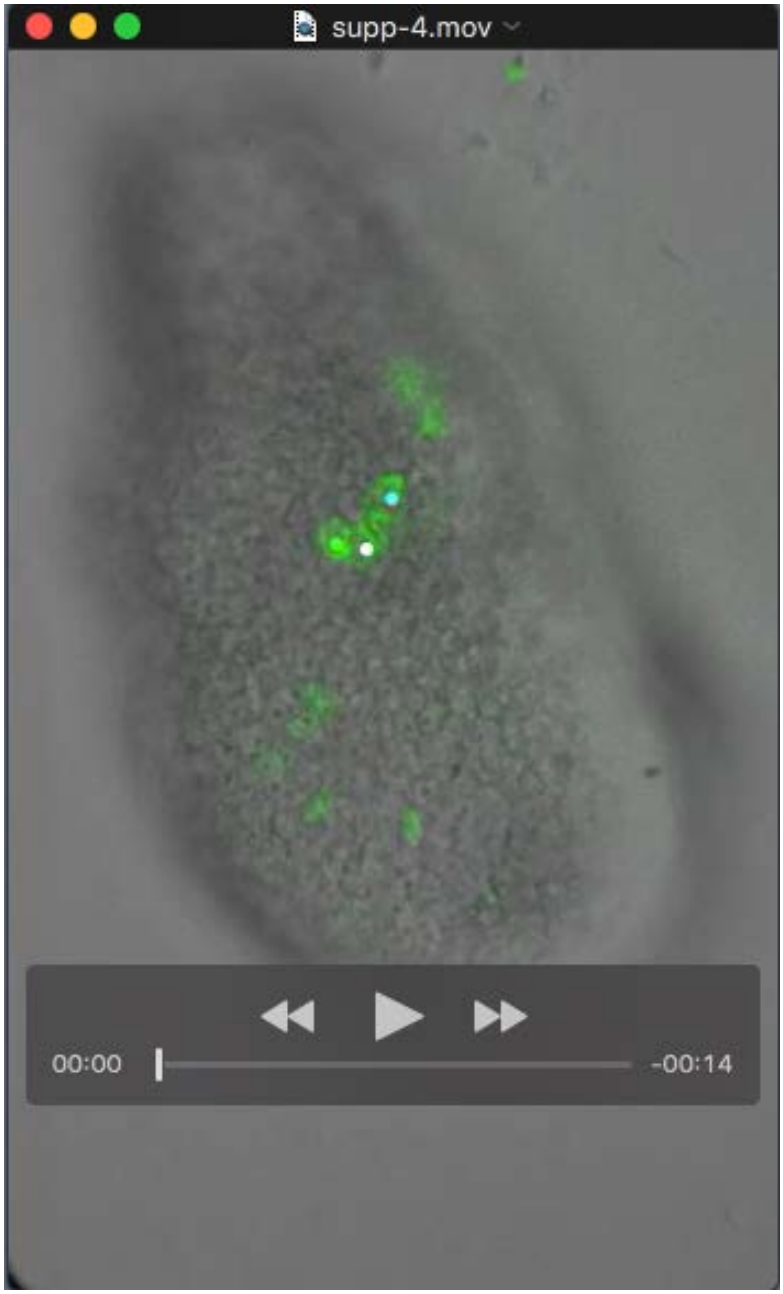
Time-lapse movie of the embryo shown in Fig. 1B from two-cell to blastocyst stage.

Green and red are EGFP and mCherry, respectively.



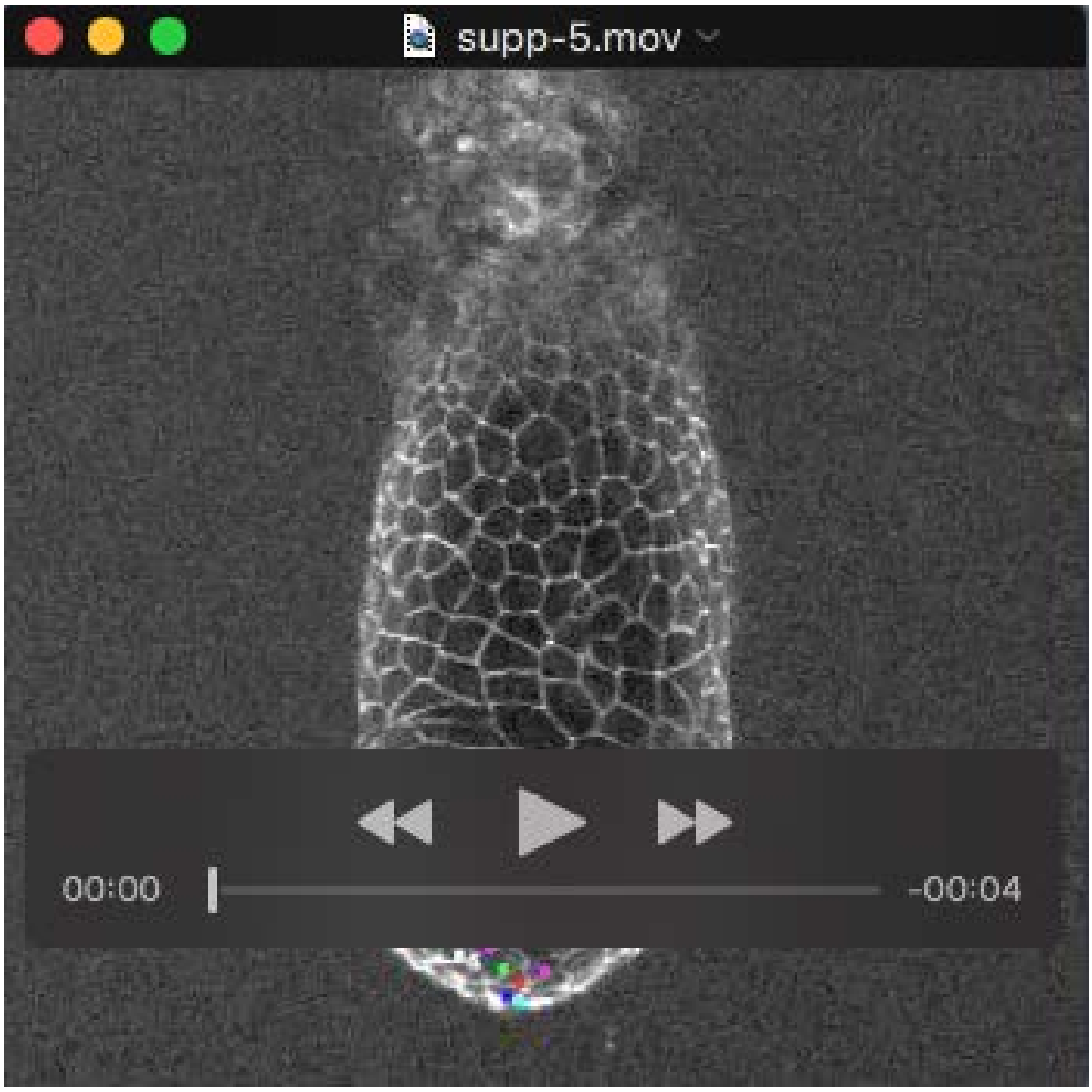
Movie 2. Time-lapse movie of *R26CreER/R26R-EGFP* embryo starting from E6.5: ExEm VE cells

Time-lapse movie of a Z-slice of *R26CreER/R26R-EGFP* embryo on E6.5 that was first treated with tamoxifen on E5.5, carrying a few EGFP-positive cells, showing coherent cell growth in the ExEm VE (colored dots). Anterior is towards the right.



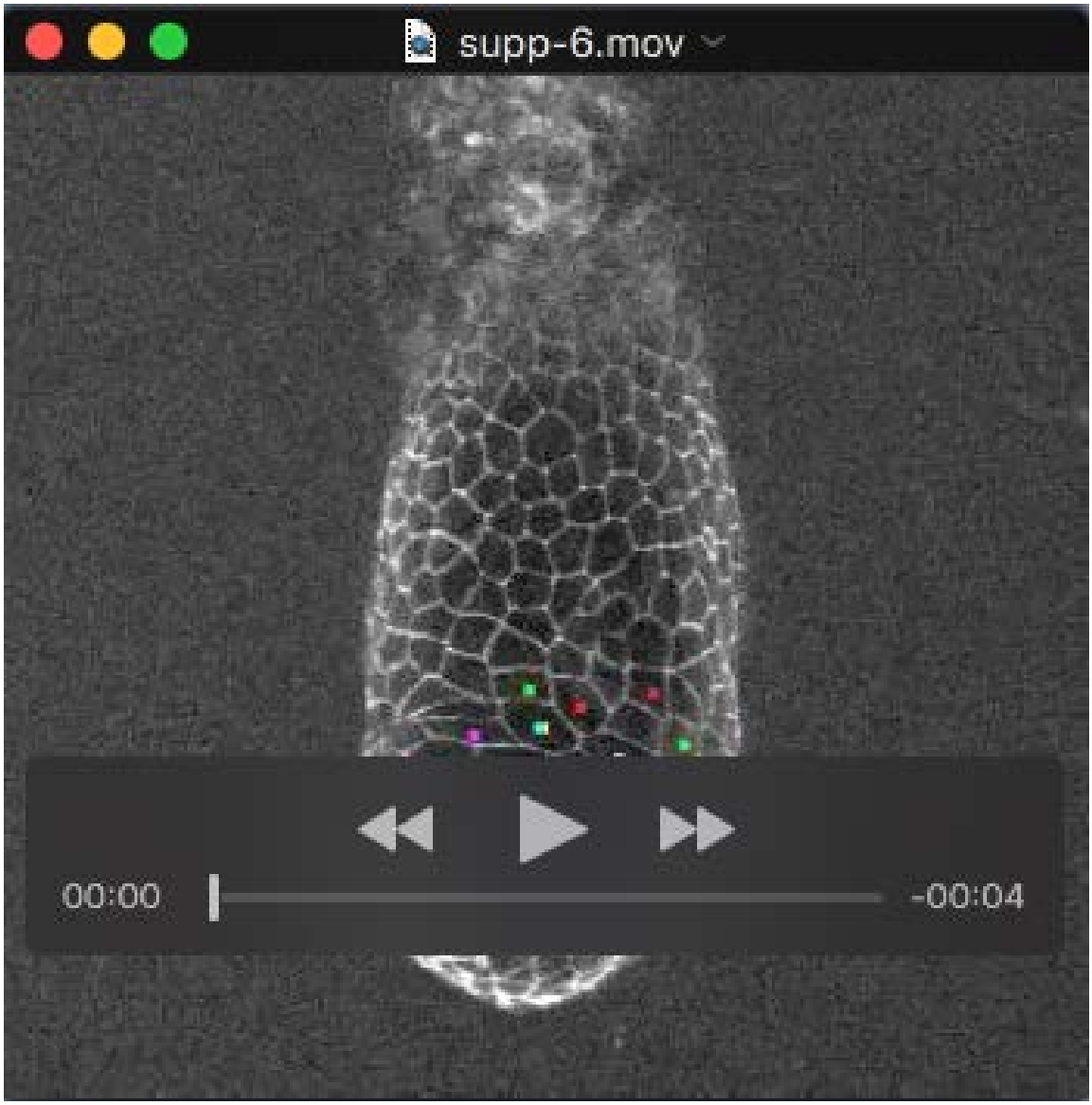
Movie 3. Time-lapse movie of *R26CreER/R26R-EGFP* embryo from E6.5: Em and ExEm VE cells

Time-lapse movie showing another Z-slice of *R26CreER/R26R-EGFP* embryo featured in Movie 3, which has a few EGFP-positive cells. The movie shows moving cells in the EmVE layer and coherent cell growth in the ExEm VE (colored dots). Anterior is towards the right.



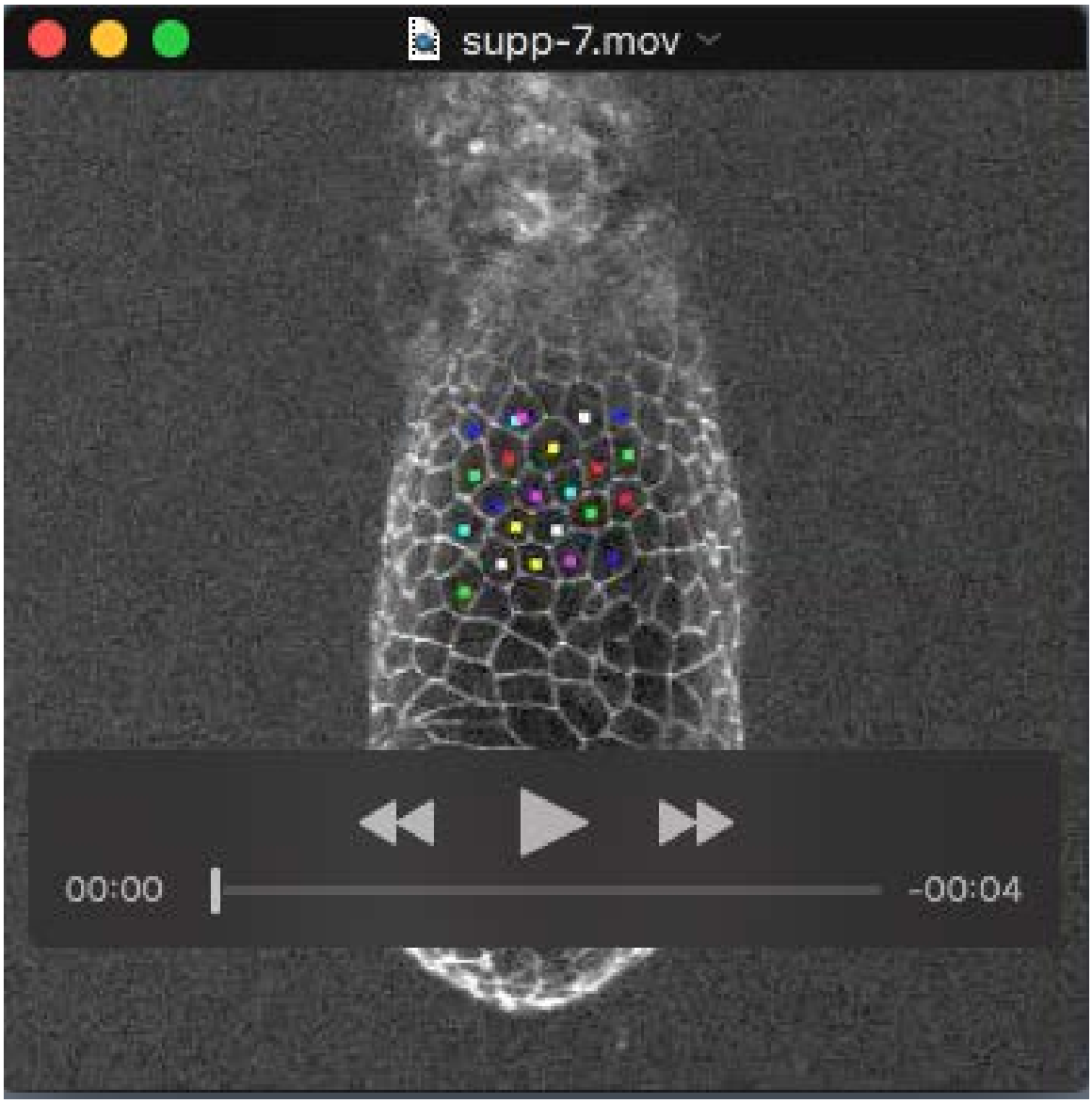
Movie 4. Time-lapse movie of *R26-PHA7-EGFP* embryo starting from E6.25: EmVE cells (related to Figure 4D)

Time-lapse movie of Z-stack images of *R26-PHA7-EGFP* embryo from E6.25. Tracked cells migrate from the distal tip to the anterior (colored dots). The trajectories are shown in Fig. 4D. Anterior is towards the left.



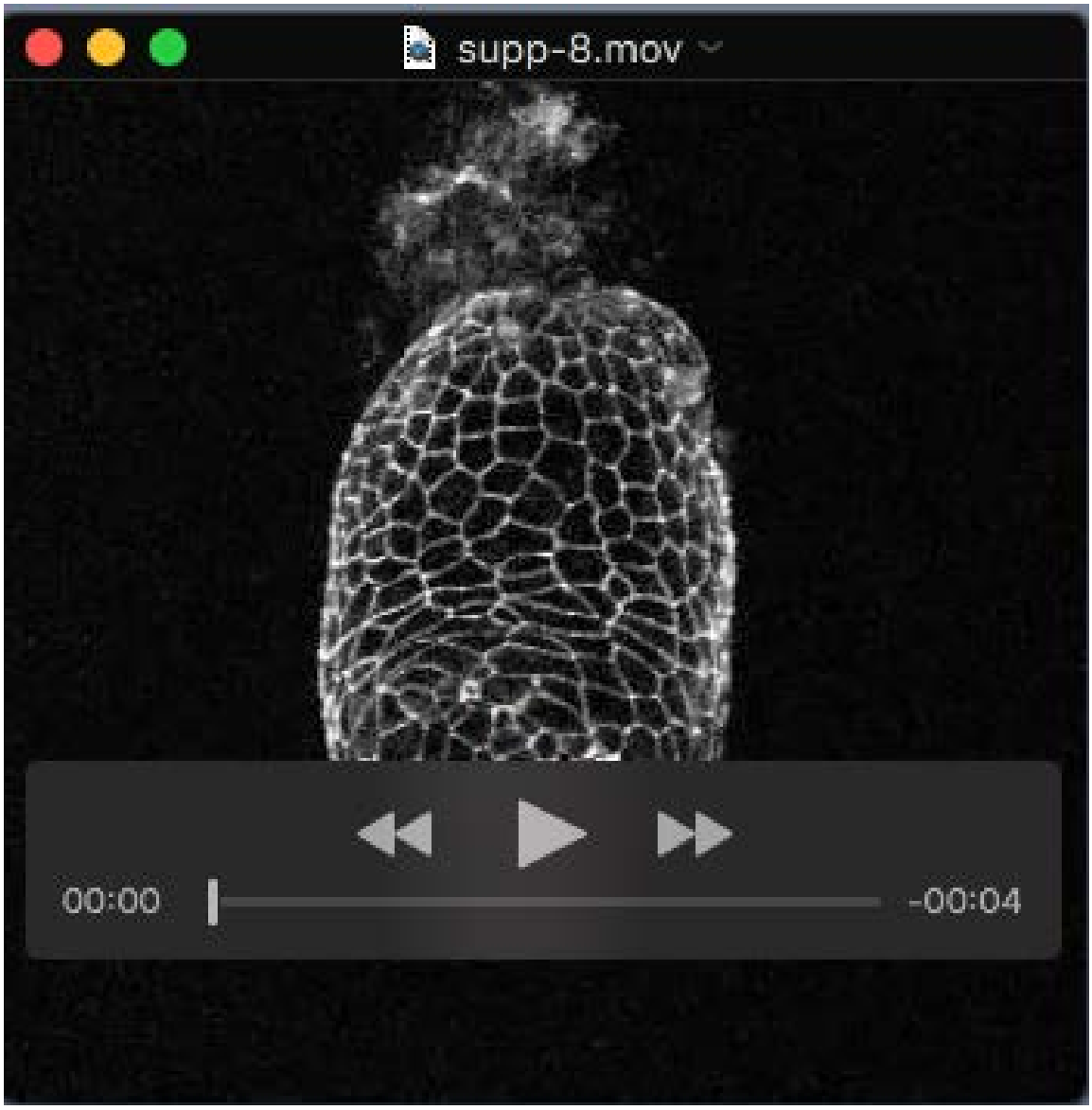
Movie 5. Time-lapse movie of an *R26-PHA7-EGFP* embryo starting from E6.25: cells near the border between Em and ExEm regions (Related to Figure 4E)

The same time-lapse movie shown as Movie 4 with added tracking of cells migrating from anterior to posterior (colored dots). The trajectories are shown in Fig. 4E.



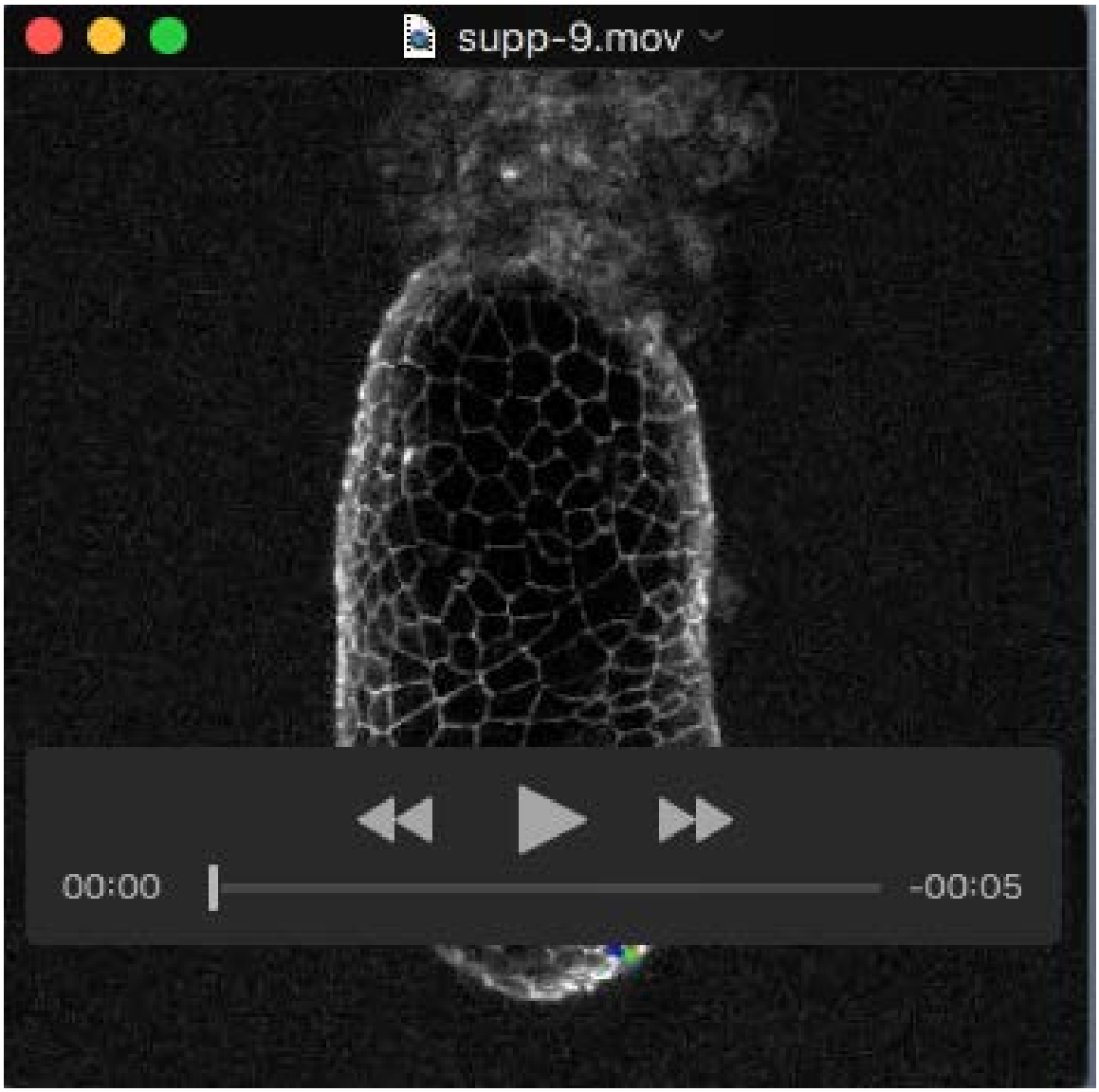
Movie 6. Time-lapse movie of *R26-PHA7-EGFP* embryo from E6.25: ExEm VE cells (related to Figure 4F)

The same time-lapse movie shown as Movies 4 and 5. Tracking (colored dots) show no apparent active migration within the ExEm VE. Trajectories are shown in Fig. 4F.



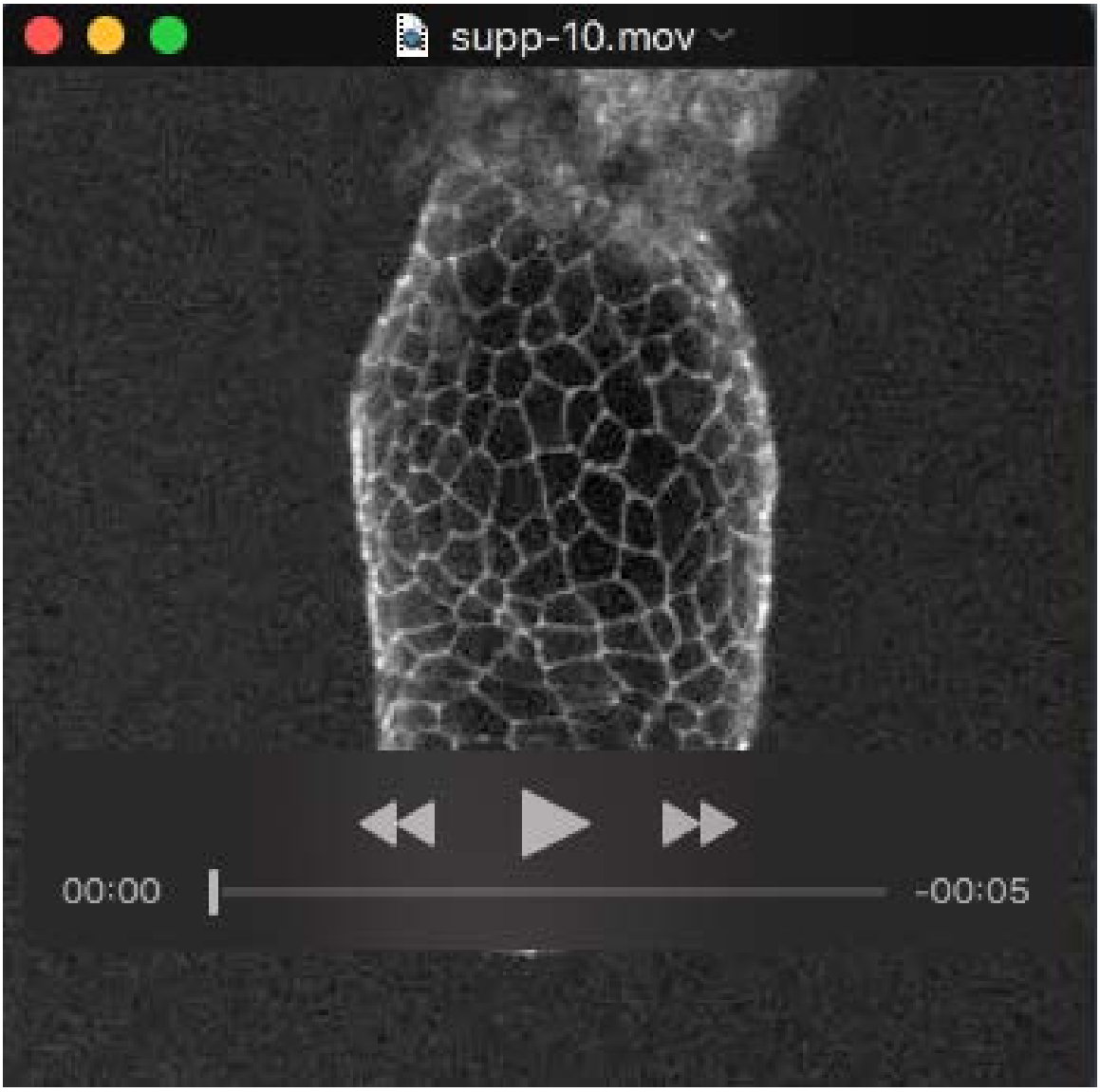
Movie 7. Time-lapse movie of another *R26-PHA7-EGFP* embryo starting from E6.25: Em VE cells (related to Figure 4G)

Time-lapse movie of Z-stack images of *R26-PHA7-EGFP* embryo viewed from its anterior starting from E6.25. Tracked cells (colored dots) migrate from the distal tip to the anterior. Trajectories are shown in Fig. 4G.



Movie 8. Time-lapse movie of *R26-PHA7-EGFP* embryo starting from E6.25

Time-lapse movie of Z-stack images of an *R26-PHA7-EGFP* embryo starting from E6.25. Tracked cells migrate from the distal tip to the anterior (colored dots). Anterior is towards the right.



Movie 9. Time-lapse movie of an *R26-PHA7-EGFP* embryo starting from E6.25

Time-lapse movie of Z-stack images of an *R26-PHA7-EGFP* embryo starting from E6.25. Tracked cells migrate from the distal tip to the anterior (colored dots). Anterior is towards the left.