

Fig. S1. Single channel images from Figure 1

(A-F''') Individual channels from panels I-J in Fig. 1 with EdU staining. Scale bars are identical as in Fig. 1.

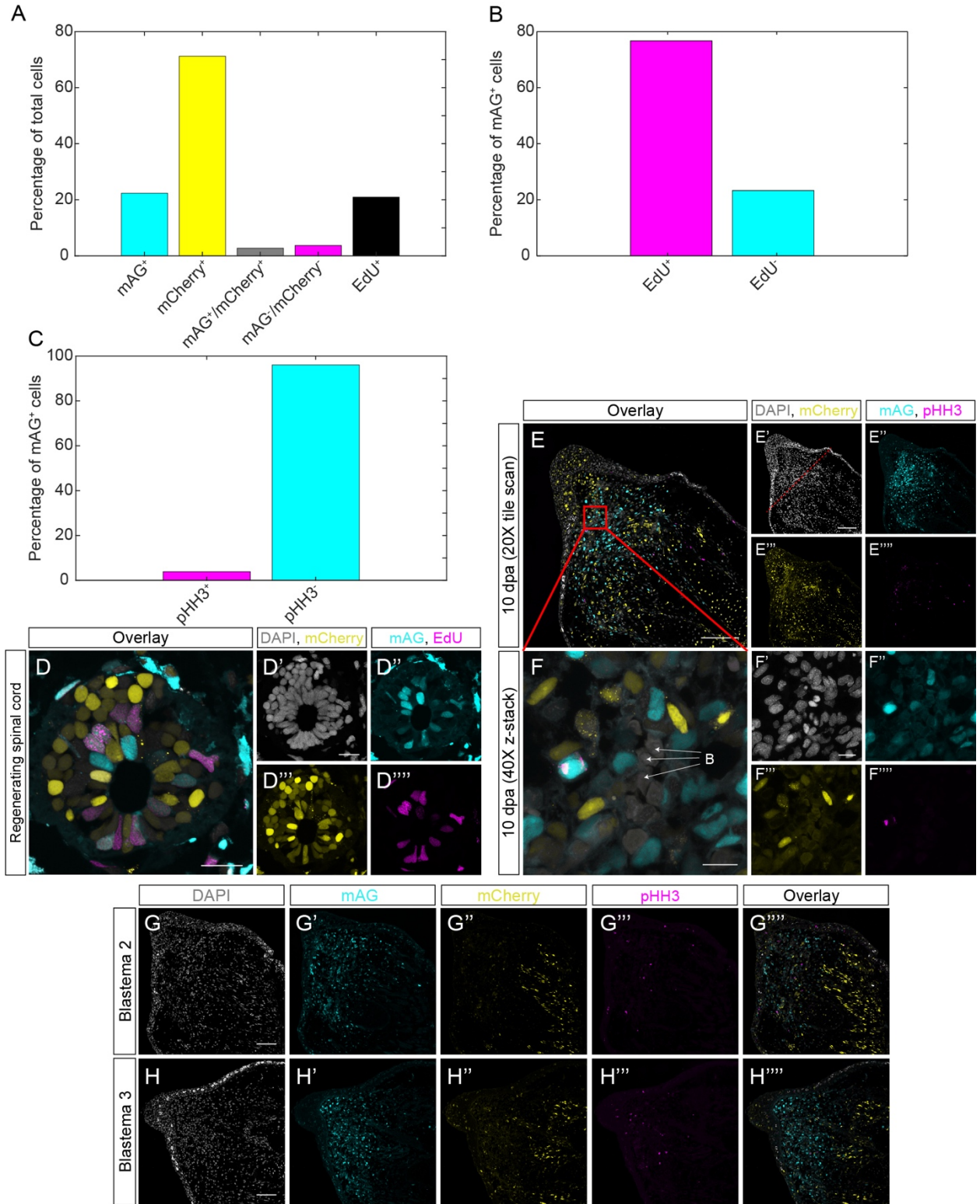


Fig. S2. Additional information from FUCCI validation

(A) Total cell characterization of the 2547 cells from the EdU pulsed 14 dpa regenerating spinal cords. (B) Quantification of the number of mAG⁺/EdU⁺ and mAG⁺/EdU⁻ cells from the EdU pulsed 14 dpa regenerating spinal cords. (C) Quantification of mAG⁺ cells that were pHH3⁺ or pHH3⁻ from 10 dpa limb blastemas. (D-D''') Representative image of an EdU pulsed spinal cord used for quantification in Fig. 2B and Fig. S2B. Scale bars= 25 μ m. (E-E''') 20X tile scan of a 10 dpa FUCCI blastema stained with pHH3. Scale bars= 150 μ m. (F-F''') 40X z-stack of pHH3 stained blastema mesenchyme. B= blood cells. Scale bars= 25 μ m. (G-H''') Two additional replicates of 10 dpa FUCCI limb blastemas stained for pHH3. Scale bars= 100 μ m.

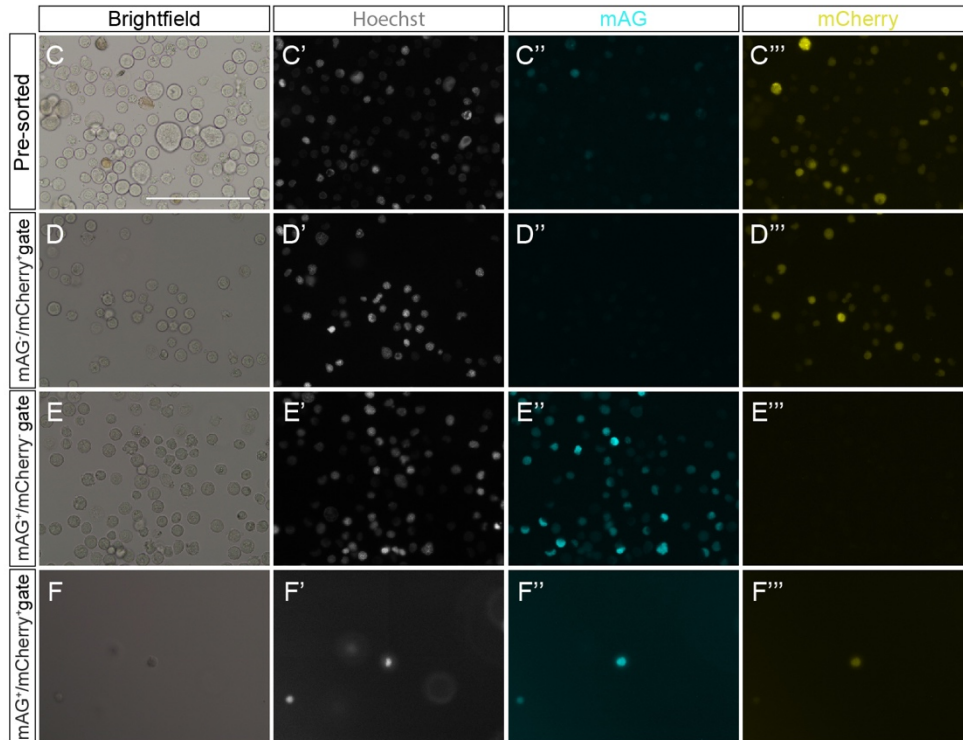
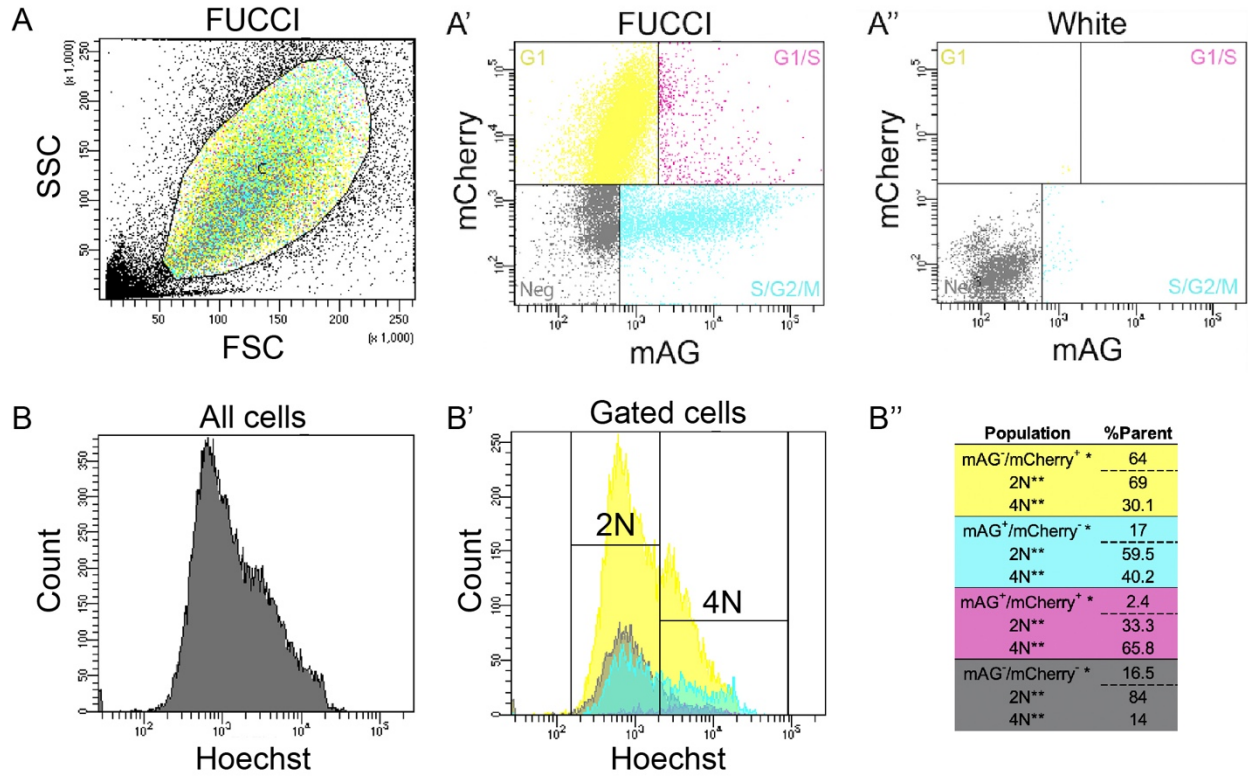


Fig. S3. DNA content correlates with FUCCI probe expression

(A) Scatter plot of FSC vs SSC on a blastema sample from FUCCI animals (n=11, 15-18 cm animals aged 1 year). Data from 10,000 events were collected. The parent gate is set around the blastema singlet population. (A', A'') Scatter plots of mAG versus mCherry fluorescence in blastema cells from FUCCI (A') and white strain (A'') animals. Quadrant gates delineating the G1 (mAG⁻/mCherry⁺, yellow), S/G2/M (mAG⁺/mCherry⁻, cyan), G1/S (mAG⁺/mCherry⁺, pink), and negative populations (mAG⁻/mCherry⁻, gray) were established as described in the materials and methods. (B-B'') Analysis of Hoechst staining on the FUCCI blastema cell population. (B) Histogram of Hoechst stain on all blastema cells. (B') Histogram of Hoechst stain in the G1 (mAG⁻/mCherry⁺), S/G2/M (mAG⁺/mCherry⁻), G1/S (mAG⁺/mCherry⁺), and negative (mAG⁻/mCherry⁻) blastema populations (colors as in A') with the 2N and 4N gates. Gating was performed as described in methods. (B'') Table of the percent of 2N and 4N cells from G1 (mAG⁻/mCherry⁺), S/G2/M (mAG⁺/mCherry⁻), G1/S (mAG⁺/mCherry⁺), and negative populations (mAG⁻/mCherry⁻). *= parent population is all cells. **=parent population is the gated population. (C-F''') Sorted cells using the gates established in A'. Scale bar= 200 μm.

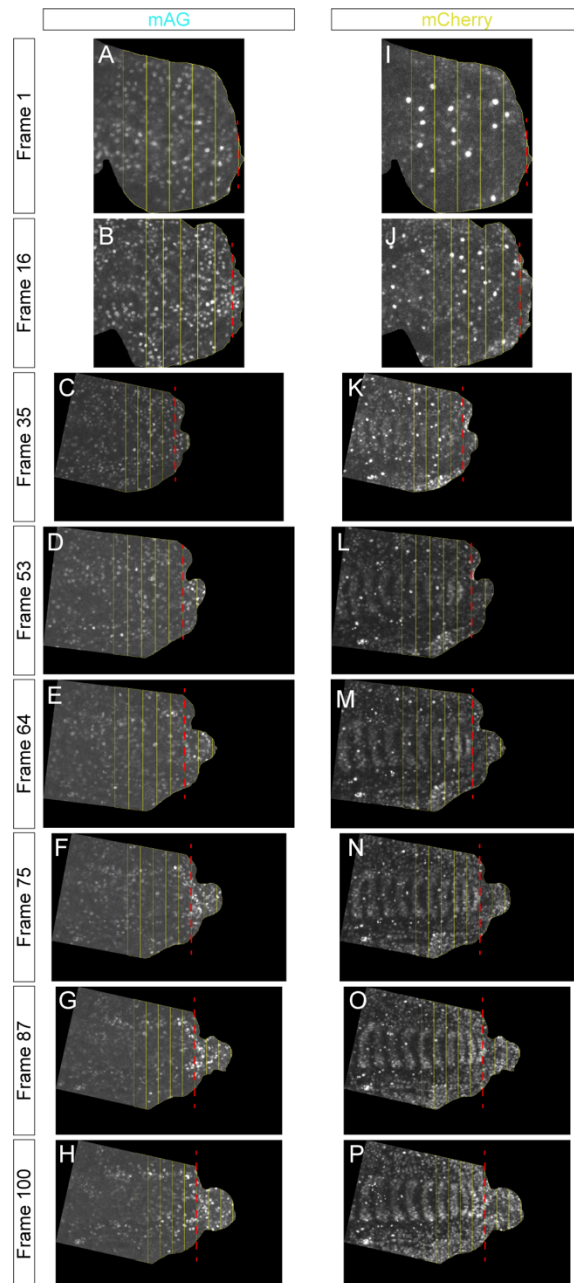


Fig. S4. Tail regeneration live image quantification boxes

(A-H) Frames with quantification boxes for mAG. (I-P) Frames with quantification boxes for mCherry. The red dashed line indicates the amputation plane on each frame. Raw integrated density was measured for each box and divided by the total box area.

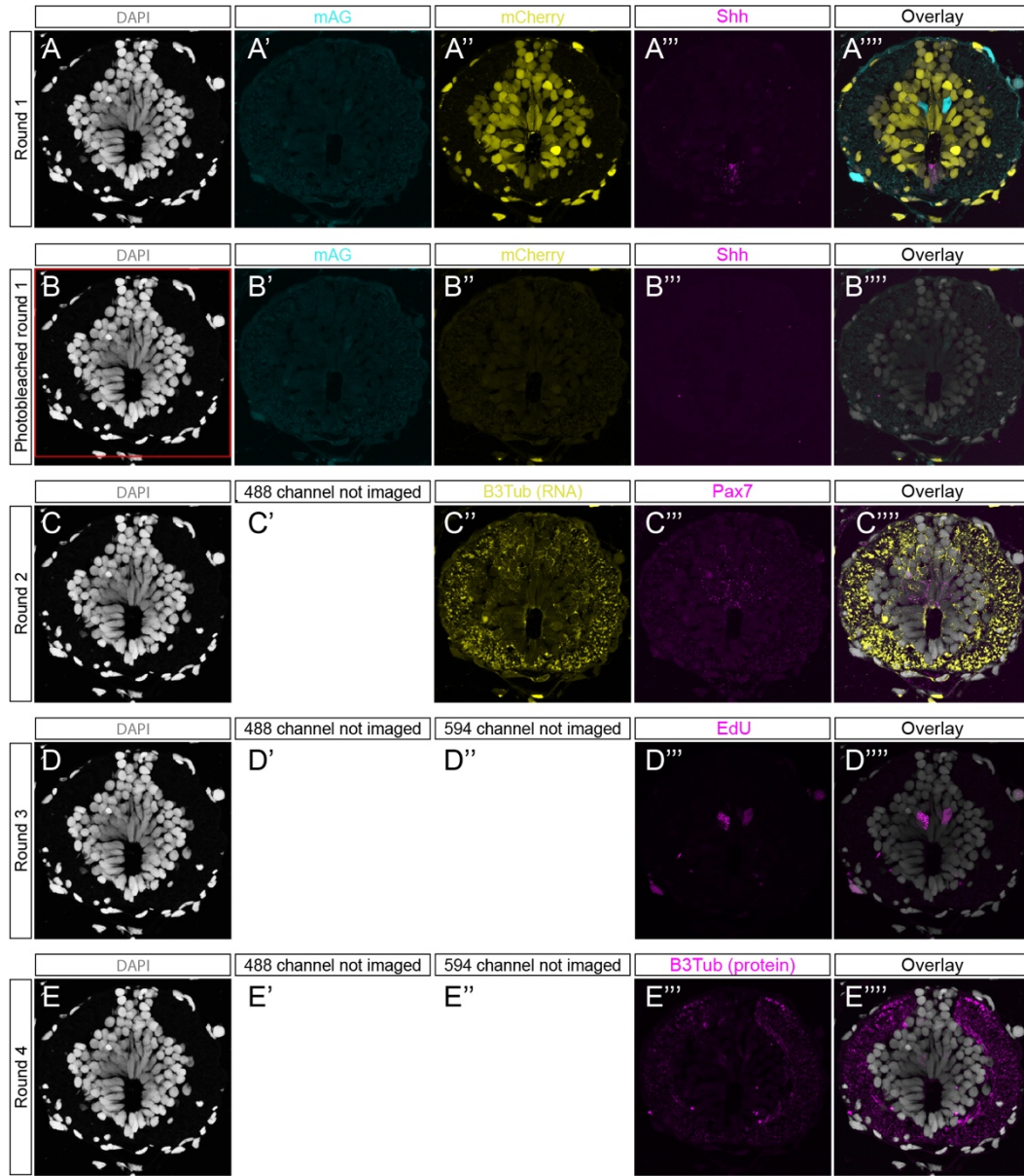


Fig. S5. Single channel images from Figure 4

(A-E'') Individual channels from each round of multimodal imaging. Scale bar= 50 μ m.

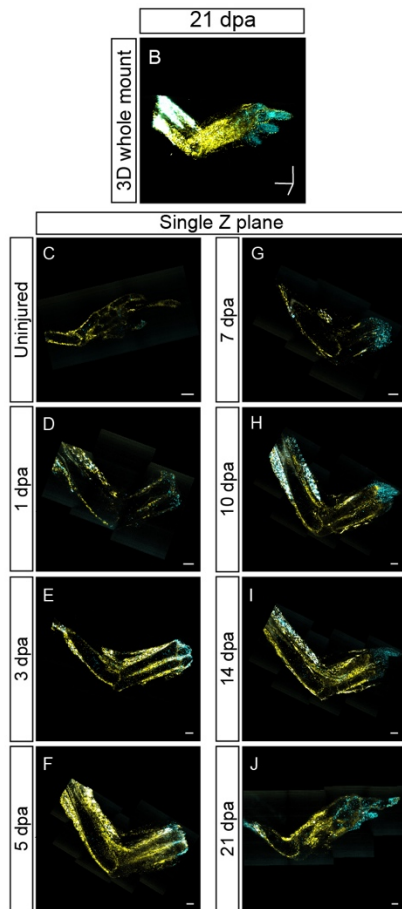
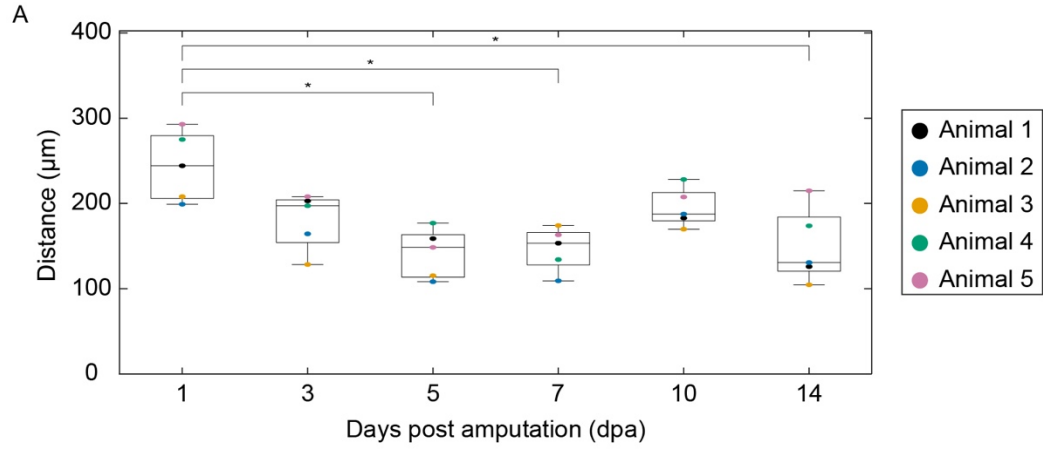


Fig. S6. Additional FUCCI limb regeneration information

(A) Quantification of the distance mAG fluorescence is observed from the amputation plane to the mCherry⁺ muscle line at each time point. Each dot color represents a replicate from a different animal. * = p-value < 0.05 (One-way ANOVA with a Tukey-Kramer multiple comparison test). (B) 3D, whole-mount image of 21 dpa FUCCI limb taken with light-sheet fluorescence microscopy. Scale bars = 600 μm in each axis. (C-J) 2D z-slices of whole-mount images from Fig. 5H-N and Fig. S6B. Scale bars = 200 μm.

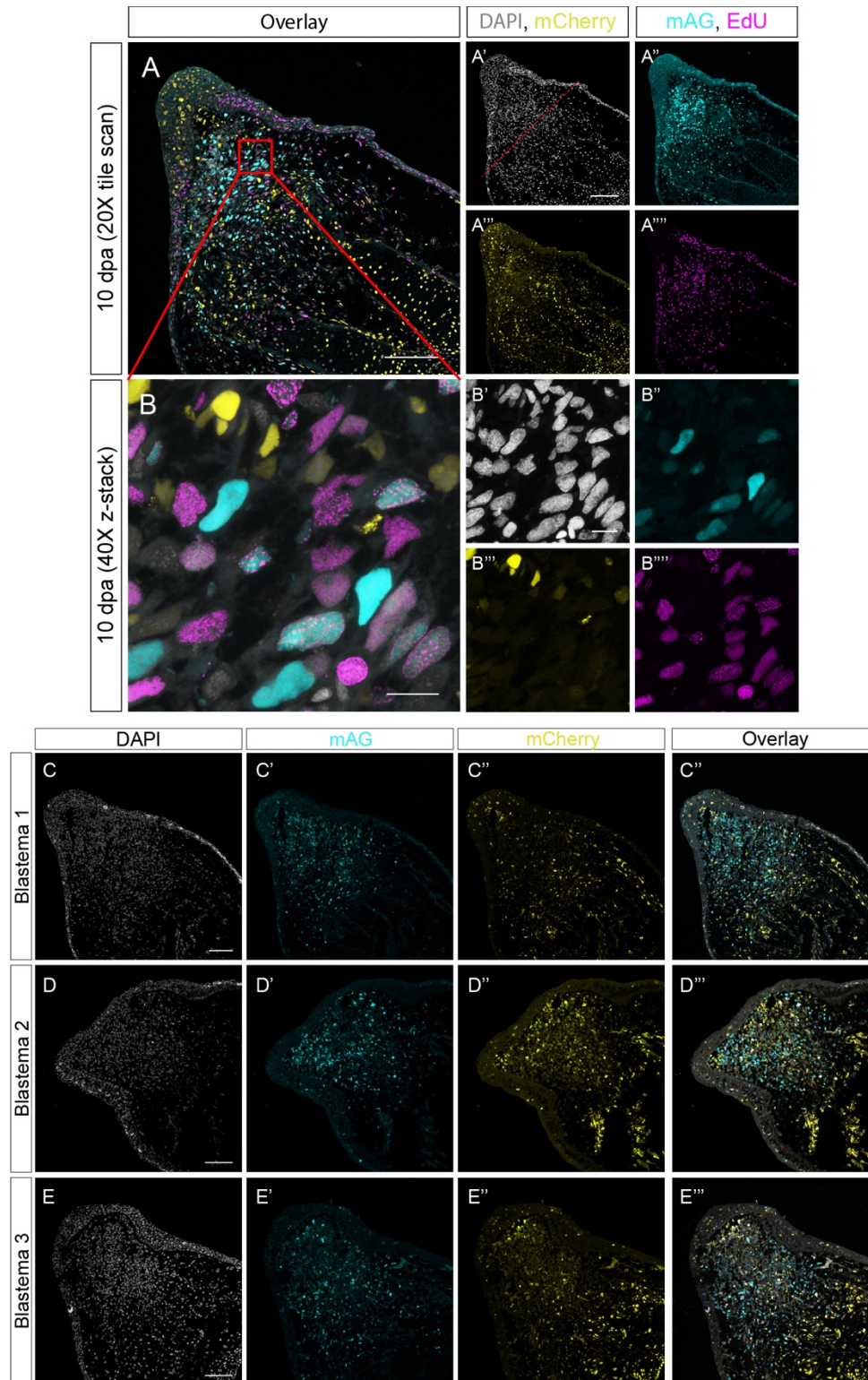


Fig. S7. Regenerating Fucci limbs tissue sections

(A-A'') 20X tile scan of a 10 dpa Fucci blastema pulsed with EdU for three hours.

Scale bars= 150 μm . (B-B'') 40X z-stack of the EdU pulsed blastema mesenchyme.

Scale bars= 25 μm . (C-E'') Single color channels for three replicates of 14 dpa Fucci limbs. Scale bars= 100 μm .

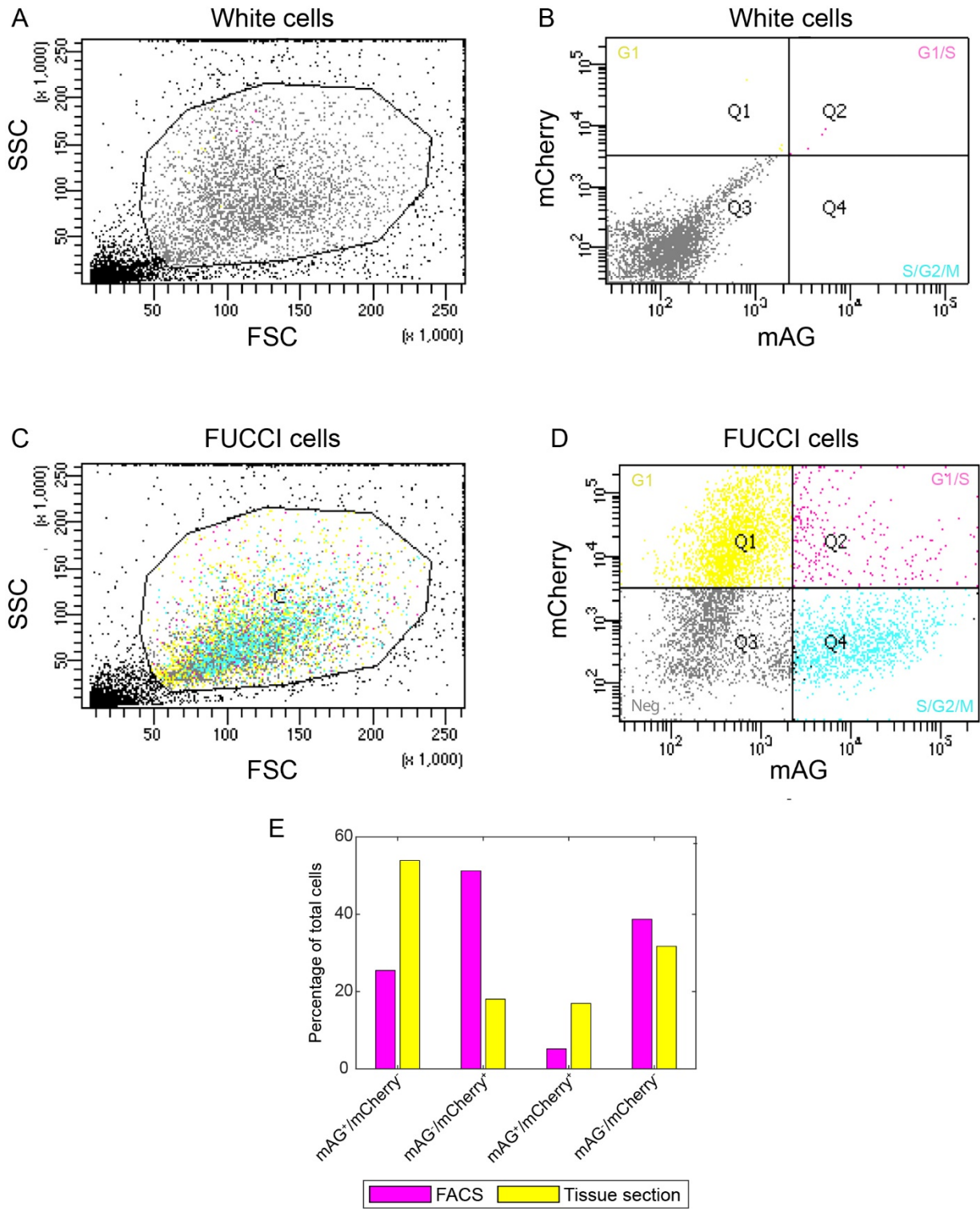


Fig. S8. Comparison between quantification in FACS and tissue sections

(A-D) Flow cytometry analysis of blastema cells from white strain (n=10, 4 cm animals aged 4 months) (A-B) and FUCCI (C-D) (n=10, 4 cm animals aged 4 months) axolotls. A forward scatter (FSC-A) and side scatter (SSC-A) plot was used to gate for the cell population (A, C). The stage of the cell cycle was determined using a FITC (mAG) versus PE-Texas Red (mCherry) scatter plot displaying only the gated cell population (B, D). Gates were established as described in methods. The cell population from the white blastema cells (A) represents 3,340 cells from a total of 10,000 events. Of these cells, 99.6% were included in the negative population and 0.2% fell in the red population (B). For the FUCCI blastemas, 5,682 cells were analyzed from the total 10,000 events (C). Of the cells, 32.1% were mAG⁻/mCherry⁻, 21.1% were mAG⁺/mCherry⁻, 42.5% were mAG⁻/mCherry⁺, and 4.3% were mAG⁺/mCherry⁺ (D). (E) Quantification of mAG⁺/mCherry⁻, mAG⁻/mCherry⁺, mAG⁺/mCherry⁺, and mAG⁻/mCherry⁻ cell populations in FACS sorted blastemas (n=10, 4 cm animals aged 4 months) and tissue sections (n=3, 7-8 cm animals aged 6 months).

Table S1. Crossing F1 FUCCI animals suggests the presence of multiple integrations

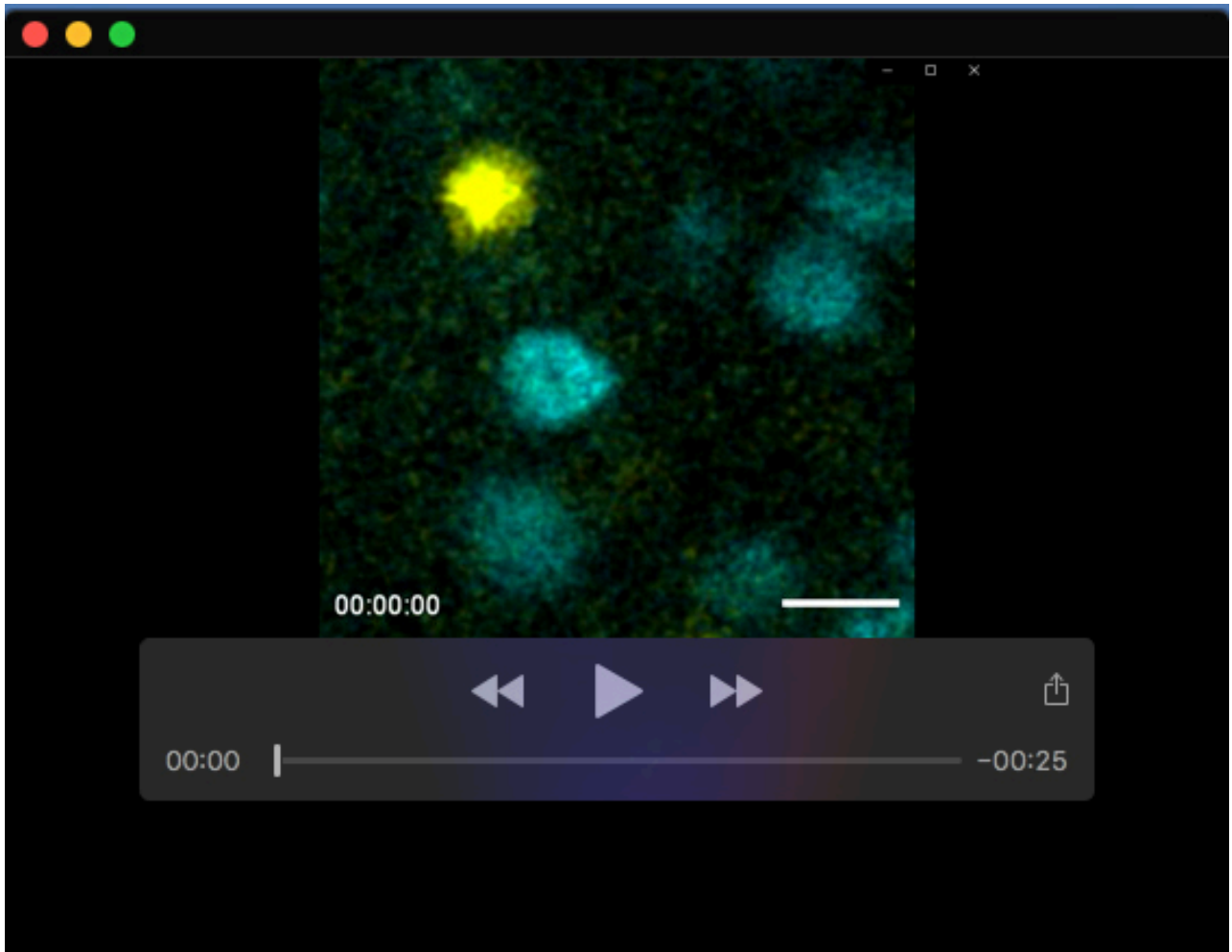
Cross	Number of offspring	FUCCI ⁺	FUCCI ⁻
F1 FUCCI male x d/d female	91	81 (89%)	10 (11%)
F1 FUCCI female x d/d male	26	22 (85%)	4 (15%)
F1 FUCCI x F1 FUCCI	263	251 (95%)	12 (5%)
F1 FUCCI x F1 FUCCI	132	132 (100%)	0 (0%)

Table S2. Number of animals and cells quantified at each anatomical position in Figure 6

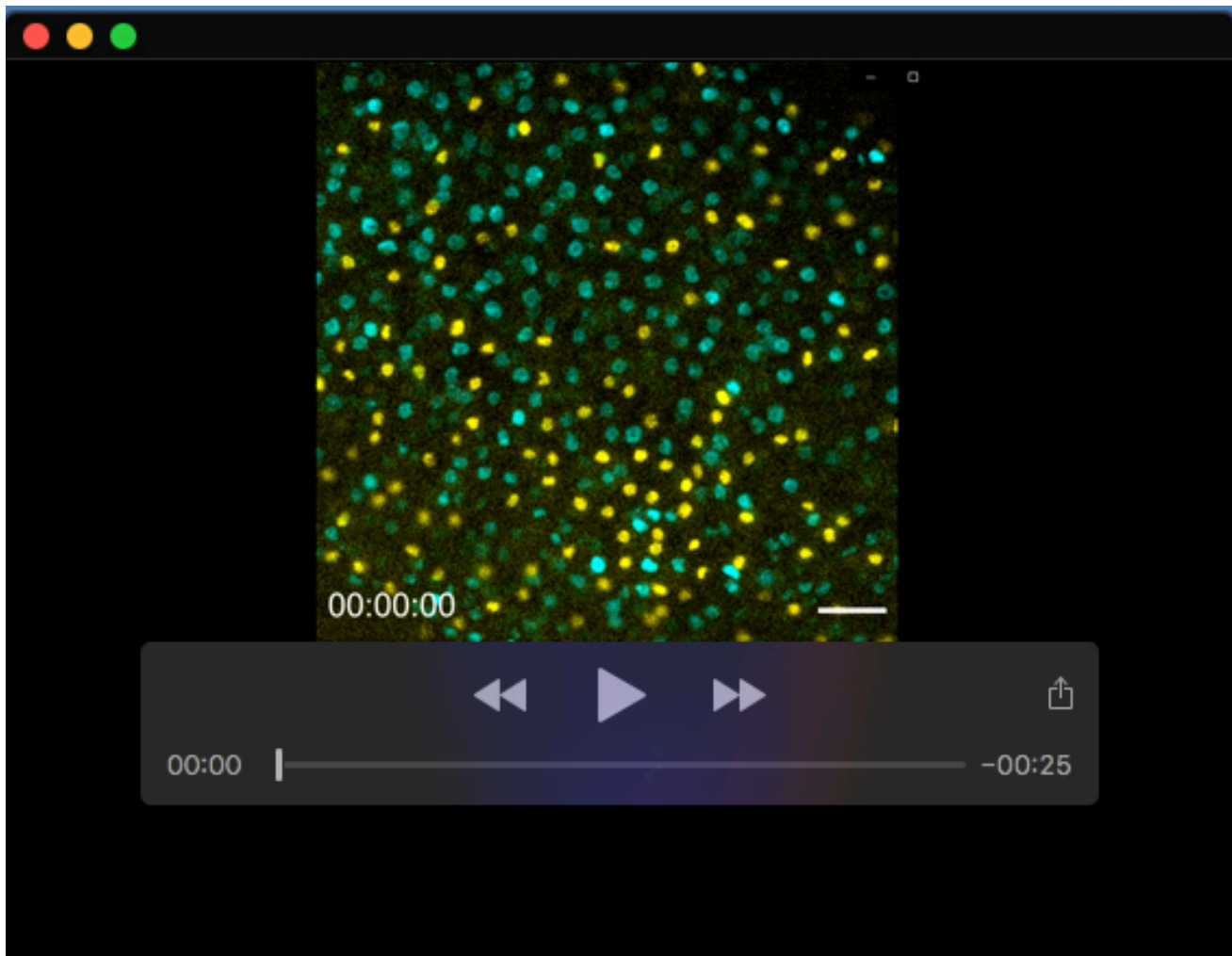
AP location	Number of animals	Number of cells
-250 μm	4	157
-150 μm	4	156
Rod end	4	167
250 μm	4	203
500 μm	4	251
750 μm	4	283
1000 μm	4	276
5000 μm	4	514
Uninjured	5	540

Table S3. V3.HCR FISH probe sequences

[Click here to download Table S3](#)



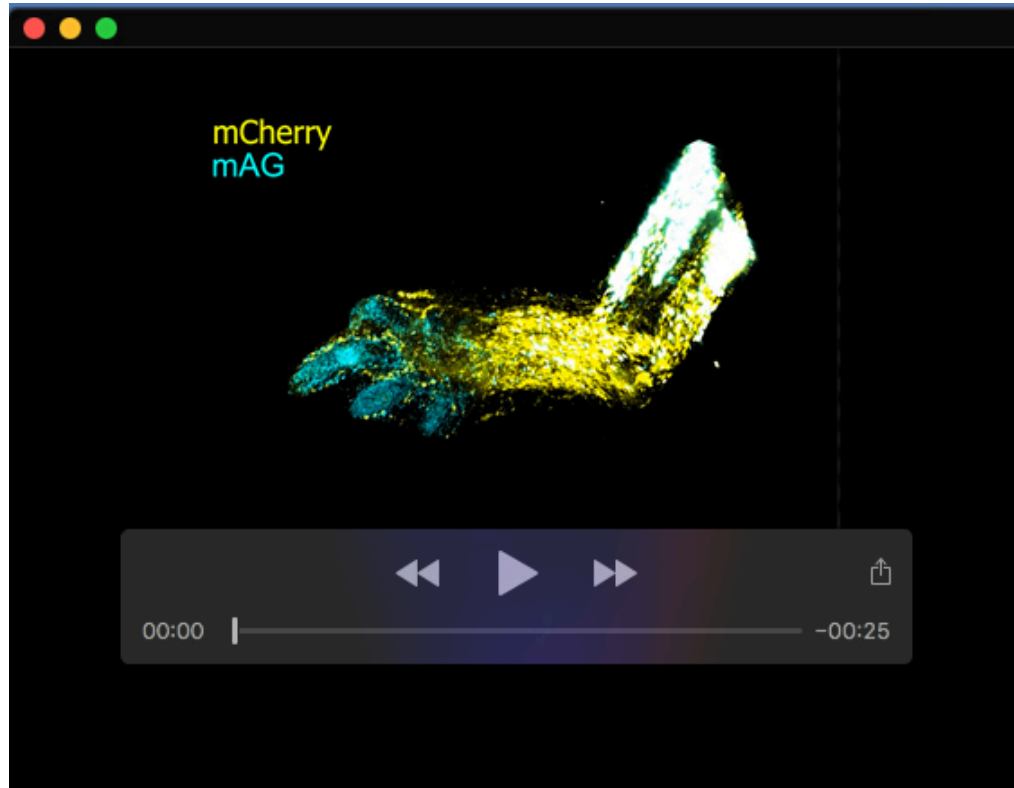
Movie 1. 16-hour live image of dividing epithelial cells in a stage 32 Fucci larva
Scale bar = 100 μ m.



Movie 2. Dividing mAG+ epithelial cell from a stage 32 larva Scale bar= 25 μ m.

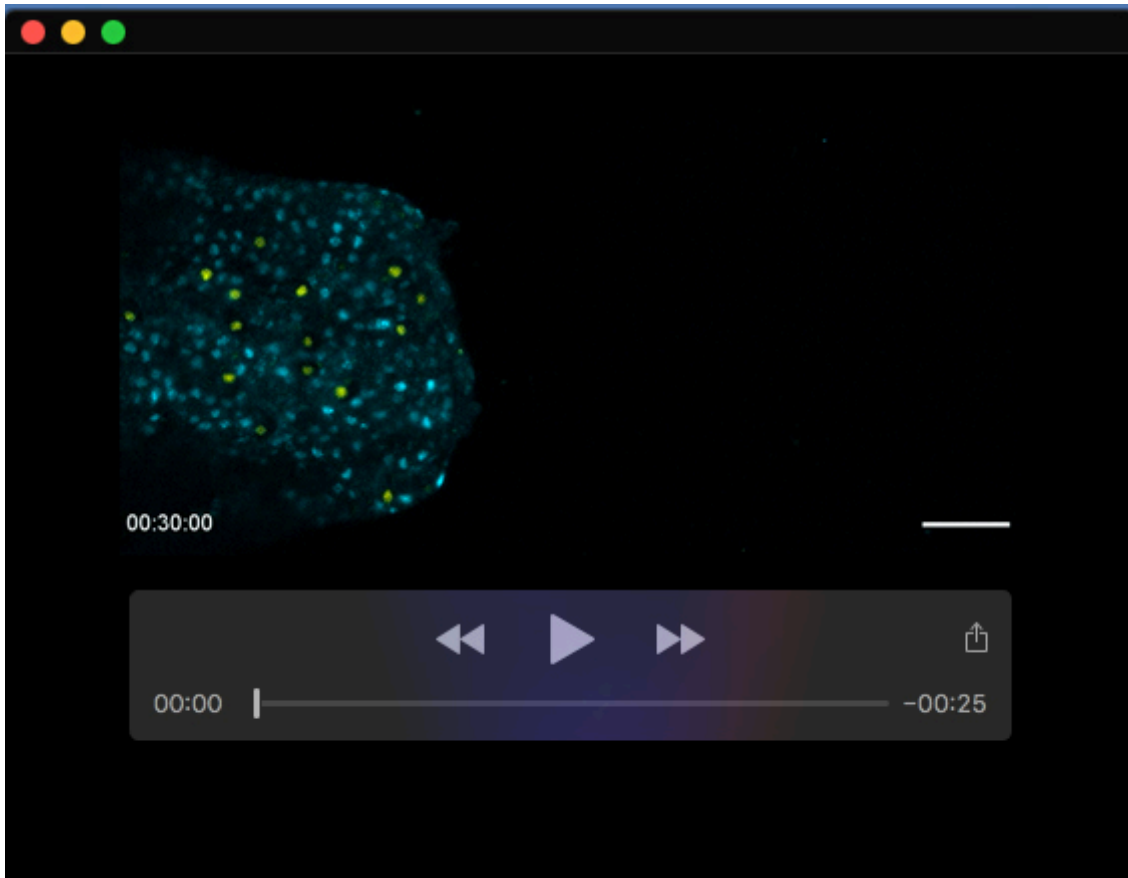


Movie 3. 60-hour live image of tail regeneration from a stage 36 Fucci larva
Animal mounted in 0.3% agarose immediately after amputation. Scale bar= 50 μ m.



Movie 4. mAG tracks for each frame from Movie 3

Animal mounted in 0.3% agarose immediately after amputation. Scale bar= 50 μ m.



Movie 5. Whole-mount uninjured Fucci limb.



Movie 6. Whole-mount 21 dpa regenerating Fucci limb.