Inventory of Supplemental Materials:

Movie Legends

Supplemental Figures

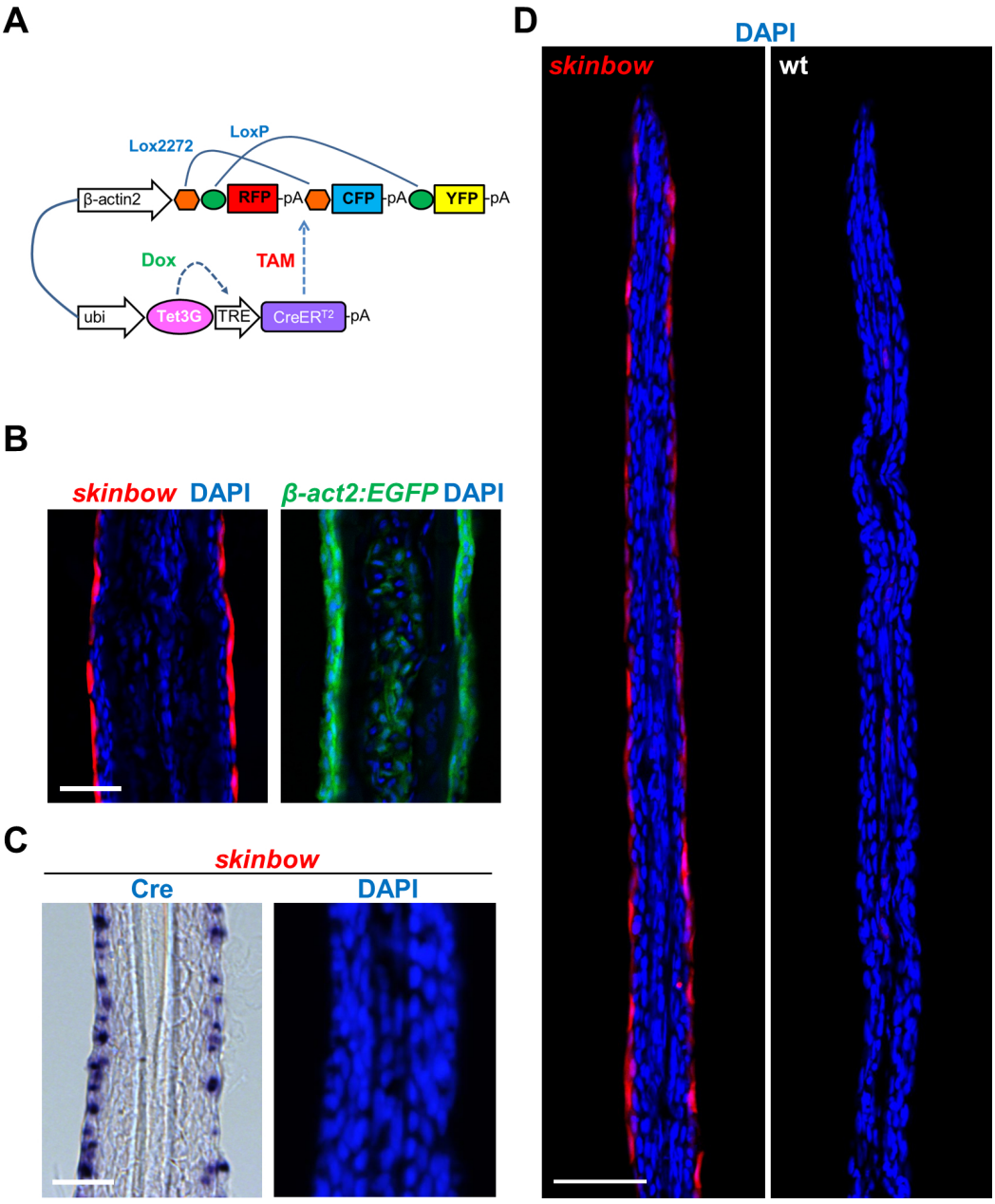

Figure S1. *skinbow* Labeling of the Outermost Layer of Adult Zebrafish Fin Epidermis, Related to Figure 1. (A) Single-transgene Brainbow-based construct. (B) Longitudinal sections of the caudal fin in *skinbow* and *β-act2:EGFP* lines. Although the Brainbow cassette is driven by the *β-actin2* promoter in the *skinbow* construct, the expression is restricted to the SEC layer, likely a consequence of the transgene integration site. By contrast, EGFP fluorescence in the *β-act2:EGFP* line is present in all fin epithelial cell layers. (C) Longitudinal sections of *skinbow* fins assessed by in situ hybridization, visualizing *CreER* RNA expression in the SEC layers. (D) Longitudinal sections of distal tips of *skinbow* and wild-type caudal fins. Only the red channel is excited in these images. Scale bars, 50 μm.

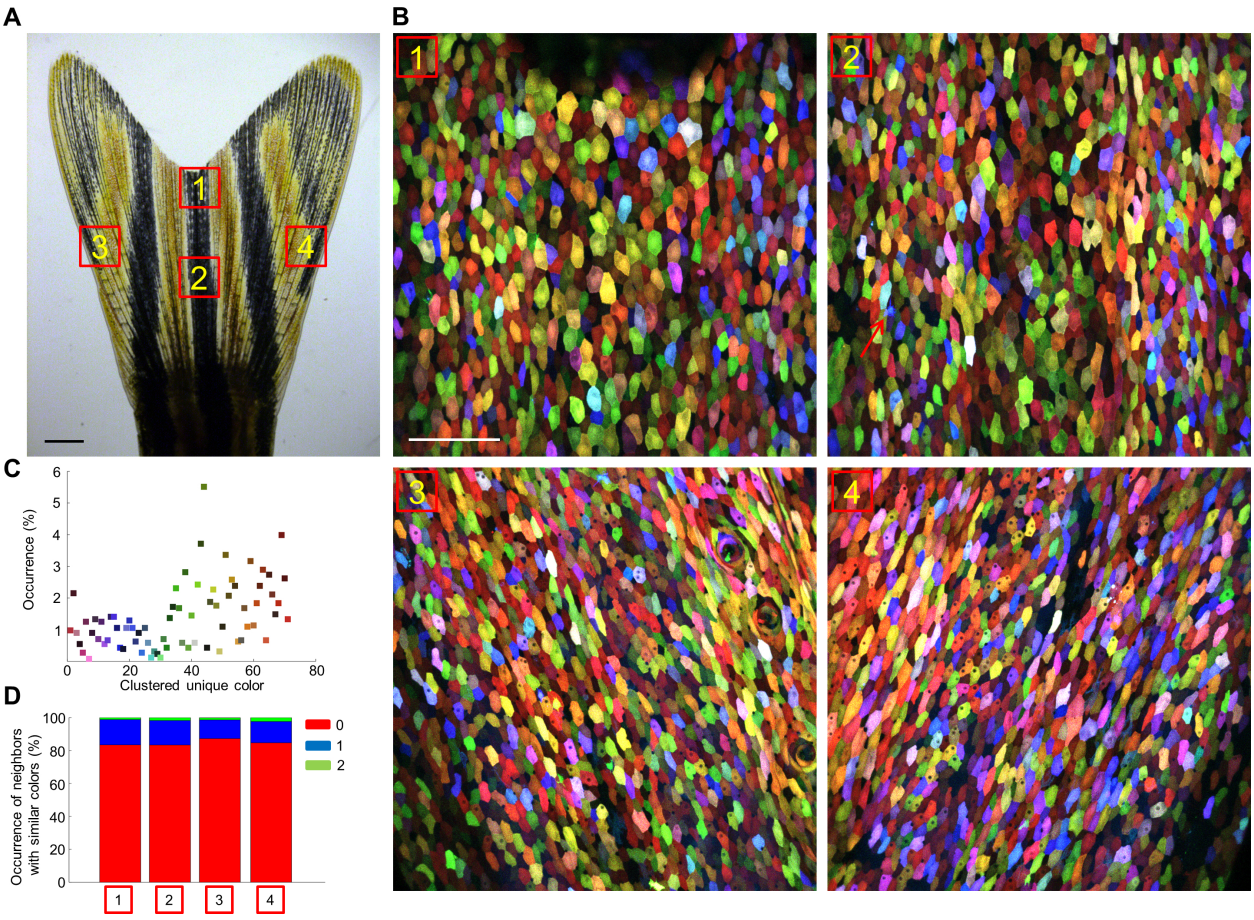

Figure S2. *skinbow* Labels the Entire Fin Surface with Diverse Colors, Related to Figure 2. (A, B) Brightfield view of adult zebrafish caudal fin. Scale bars, 1 mm. Red boxes in (A) indicate areas where z-stacked confocal images (B) were captured. Scale bars, 100 µm. Red arrows in Area-2 point to migratory immune cells, a minor labeled population. (C) Occurrence of each color cluster. Green, brown and red colors are slightly more frequent than blue and violet colors (see Experimental Procedures for details). Cells from all 4 areas shown in (B) were combined for analysis. (D) To evaluate the spatial randomness of the color, we quantified occurrence of cells from all 4 areas shown in (B) with nearest neighbors of the same color cluster. Red, blue, and green areas in the row stacked plot represent cases with 0, 1, or 2 same-color neighbors, respectively.

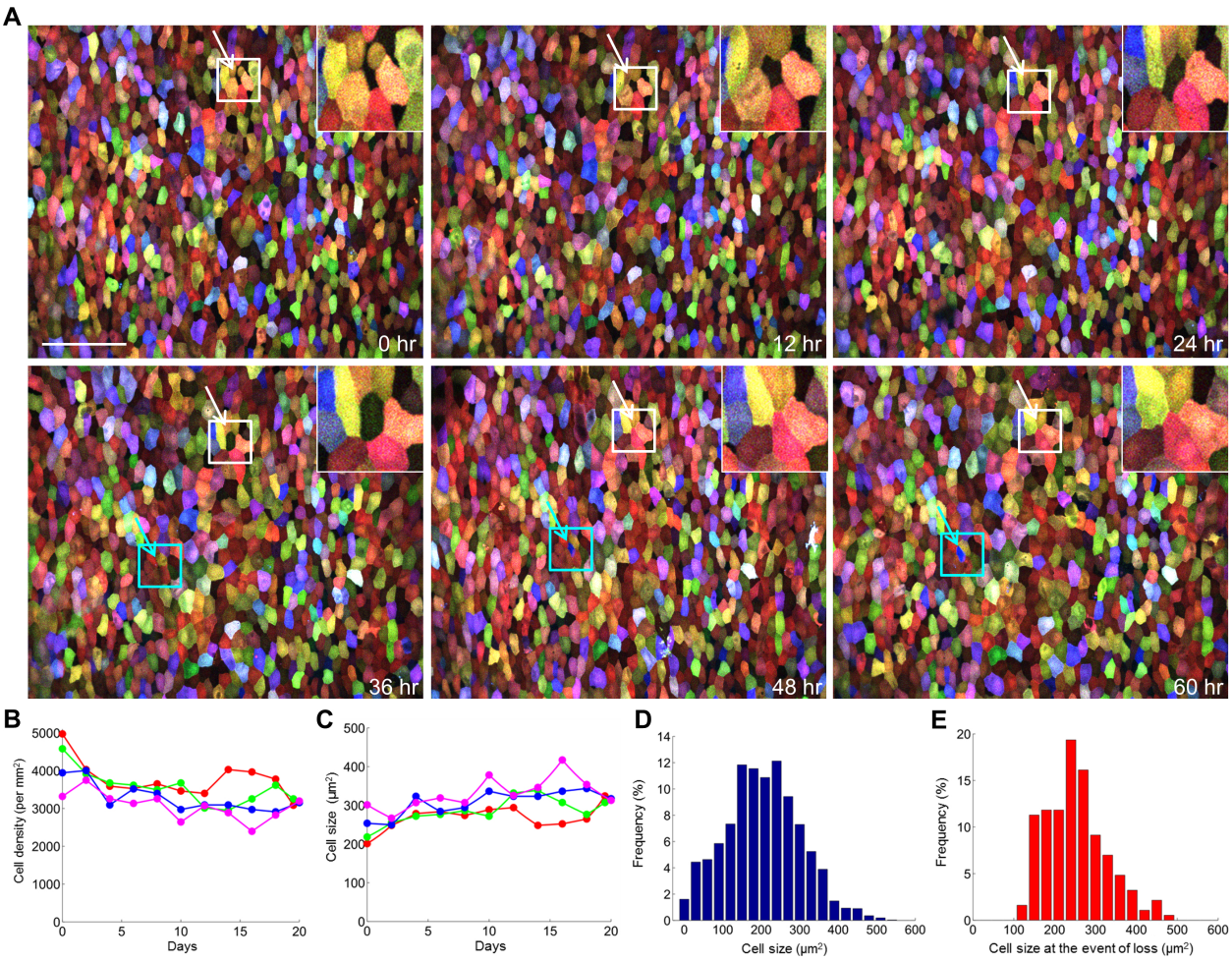

Figure S3. Precise Identification and Tracking of Individual SECs in a Large Field, Related to Figure 2. (A) Confocal images of the same fin area under homeostatic conditions, captured every 12 h over 20 consecutive days. Representative images are shown here. White and black arrows point to same cells in consecutive frames. White boxes indicate areas of enlarged views. Scale bar, 100 μm. (B, C) To evaluate cell density and number, we quantified the number of cells at different timepoints and calculated density as the number of cells divided by the labeled area in the images. Counting was performed manually on subimages (n = 4 over 41 time points). (D) Normalized histogram of cell size (3109 cells from 186 trajectories in 4 different animals.). (E) Normalized histogram of cell size at the frame prior to cell loss (186 cells from 4 animals).

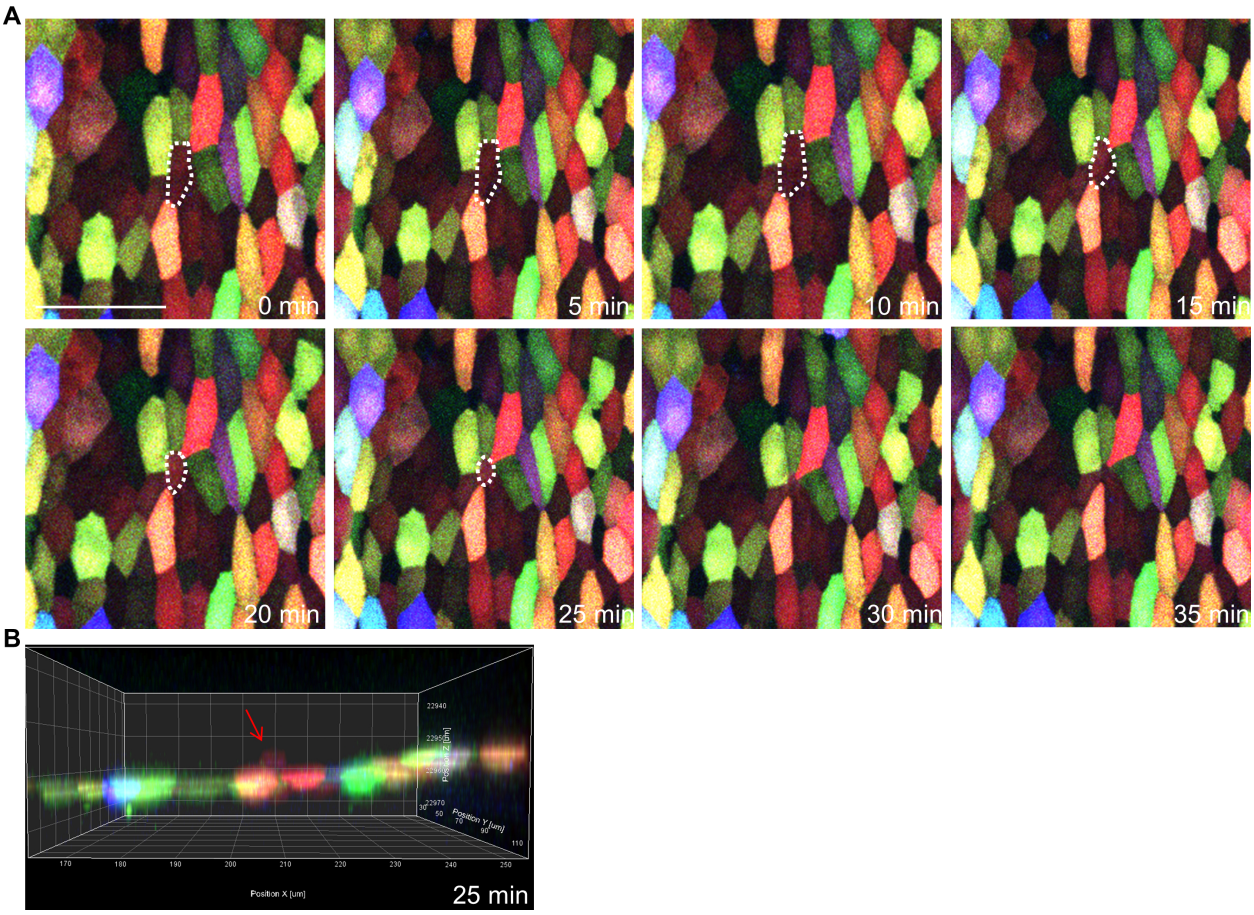

Figure S4. Cell Replacement by Reorganization of Neighboring Cells, a Process Taking Only Minutes, Related to Figure 3. (A) White dashed lines indicate a cell shed from the fin surface under homeostatic conditions. Images were captured every 5 minutes in uninjured fins. Scale bar, 50 μm. (B) Three-dimensional images were created using Imaris software to reveal surface release of the outlined cell in (A) (arrow).

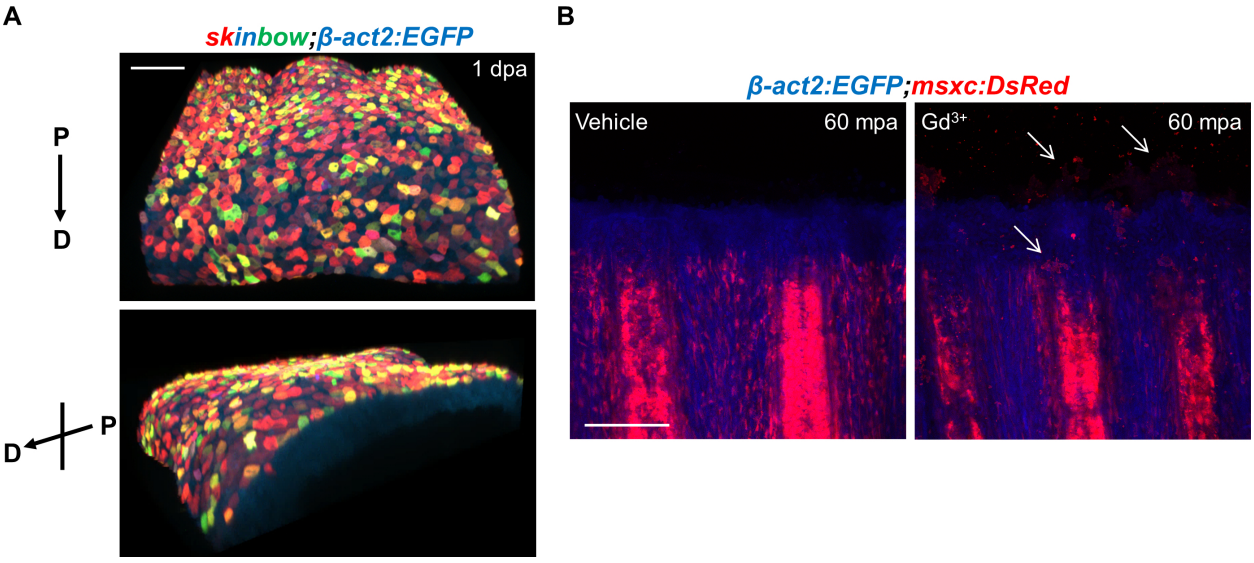

Figure S5. Transgenic Monitoring of Different Epithelial Layers and Mesenchymal Cells After Amputation Injury, Related to Figure 5. (A) Three-dimensional confocal images of the amputation plane in *skinbow; β-act2:EGFP* fins. Multicolor patches indicate fluorescent SEC labeling in the fin epithelium. *β-act2:EGFP* fluorescence labels all epithelial cells, and in these images is colored blue in all non-SEC epithelial layer. Images were acquired at 1 dpa. D: distal end. P: proximal end. Scale bar, 100 µm. (B) Maximum-projected images of the amputation plane in *β-act2:EGFP; msxc:DsRed* fins. *β-act2:EGFP* labels all epithelial cells, shown in blue. *msxc:DsRed* labels most mesenchymal cells, which are shown in red. Images were captured at 60 minutes post amputation. Animals were treated with either vehicle (water) or Gd^{3+} during imaging. White arrows point to leakage of mesenchymal tissue. Scale bars, 100 µm.

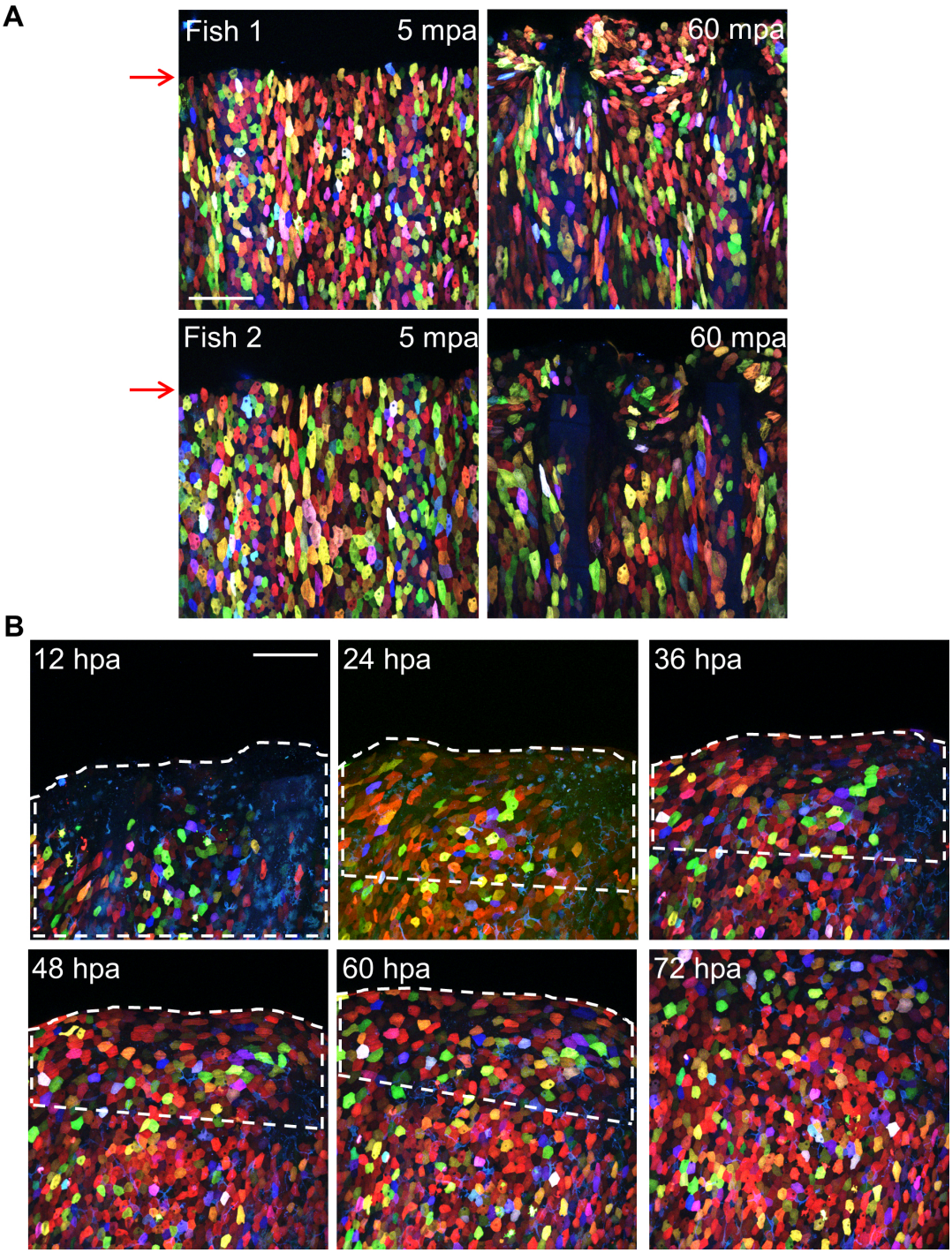

Figure S6. Rapid SEC Loss from Amputated Fins upon Return to Aquarium Water, Related to Figure 6. (A) Confocal images captured at 5 and 60 minutes post amputation (mpa). Animals were released back to the water between these two imaging time points, causing major SEC loss. Two animals were shown here. Red arrows indicate the amputation plane. Scale bars, 100 µm. (B) White-dashed lines enclose defined tissue areas that translocate and become deformed while pacing with the front of advancing tissue (as shown in Fig. 6B). By 72 hpa the defined area is out of the imaging range. Scale bars, 100 µm.

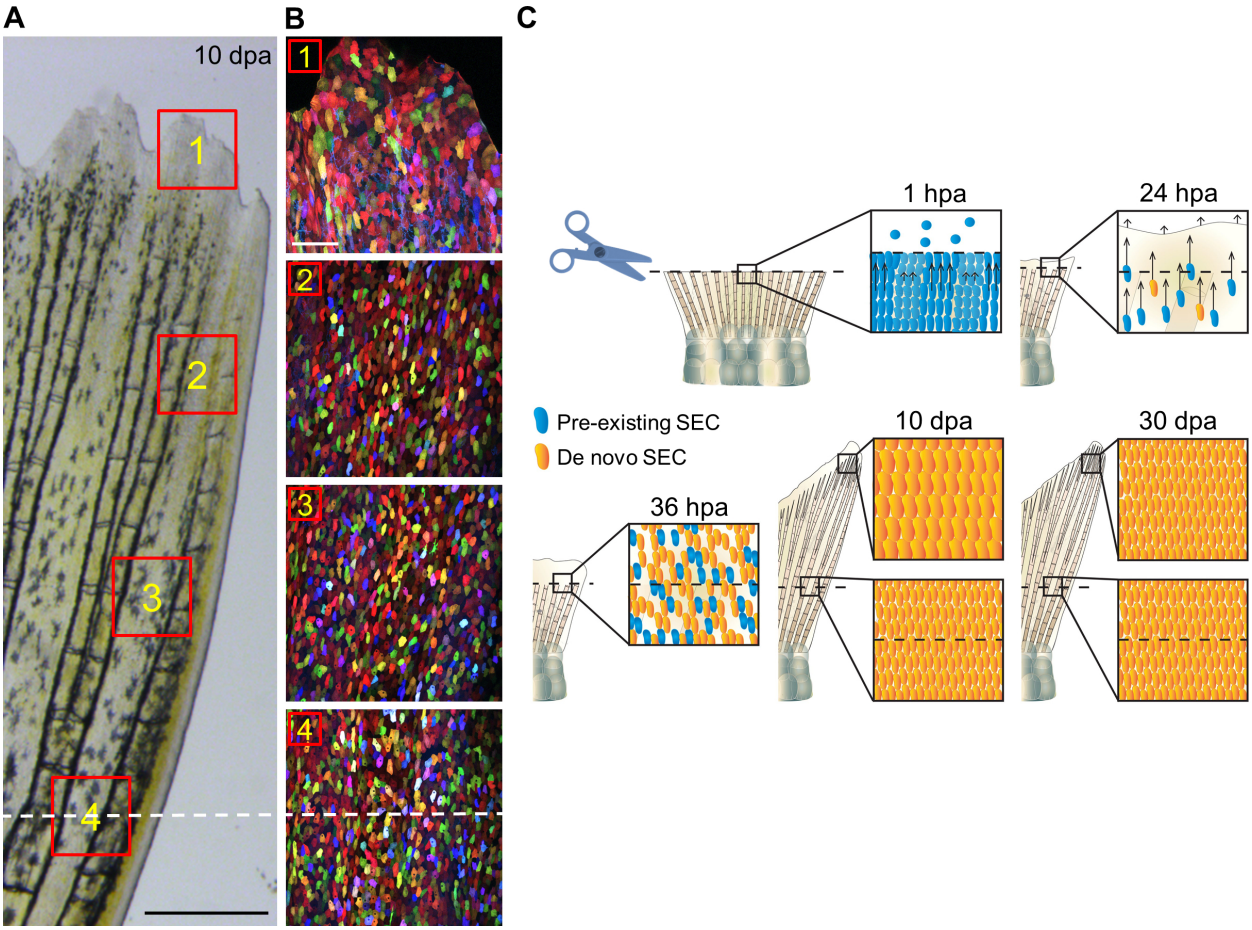

Figure S7. Sequence of Collective SEC Behaviors During Fin Regeneration, Related to Figure 6. (A, B) Brightfield view of adult zebrafish caudal fin at 10 dpa. Scale bars, 0.5 mm. Red boxes in (A) indicate areas where z-stacked confocal images (B) were captured. Scale bars, 100 µm. White-dashed lines indicate the amputation plane. Images in (A) and (B) were captured from the same animal. Scale bars, 100 µm. (C) Wounds seal in the first hour post amputation (hpa) by interactions between alternating SEC sheets with distinct cell displacement and shape responses. Many SECs are extruded at the amputation plane during this process. At 24 hpa, spared SECs are recruiting to the regenerating area over long distances. By 36 hpa, do novo SECs are well-integrated with pre-existing SECs, and new SEC creation becomes the predominant cellular supply of surface epithelium. At late stages in ongoing regeneration (10 dpa), fins retain many hypertrophic SECs at the distal regenerating tips. This phenotype becomes fully resolves after completion of appendage replacement (30 dpa), and SECs have a similar size distribution across the entire appendage. Pre-existing cells are labeled in blue; de novo SECs are labeled in yellow.

Movie Legends

Movie S1. Three-dimensional Image of *skinbow* Fin Epithelium, Related to Figure 1. Images and movies were created using Imaris software. *skinbow* cell labeling uniformly colors virtually the entire fin surface, but is not present in the underlying layers (i.e. suprabasal layer, basal layer, or fin mesenchyme). A few migratory immune cells are also labeled and detectable as a minor population in this line.

Movie S2. Time Lapse Images of *skinbow* Fin Epithelium Under Homeostatic Conditions, Related to Figure 2. The movie was created from series of confocal images captured every 12 h for 20 days from an uninjured animal (29 consecutive images were shown; 8 frames/second). New SECs acquired a stable color once on the fin surface. SEC division events were not observed in our imaging experiments, and cell migration within the organized epithelium, though detectable and quantifiable, was minimal.

Movie S3. Time Lapse Images of *skinbow* Fin Epithelium After Exfoliation Injury, Related to Figure 4. The movie was created from a series of confocal images captured at 0.2 hpa and every 12 h afterward to 96 hpa (10 consecutive images were shown; 4 frames/second). Injuries exposed large areas of non-fluorescent tissue that were quickly repopulated through a regenerative response.

Movie S4. Time Lapse Images of an Amputated *skinbow; β-act2:EGFP* Fin, Related to Figure 5. The *skinbow* line labels SECs, which are shown in multi-color. *β-act2:EGFP* labels all epithelial cells, which are shown in blue. Optical section images of the same z-position were captured every 2 minutes from 10 to 70 minutes post amputation (30 consecutive images were shown; 4 frames/second). The fin stump is rapidly covered first by basal and suprabasal cells, then closely trailed by SECs.

Movie S5. Time Lapse Images of an Amputated *skinbow* Fin after vehicle or Gd^{3+} Treatment, Related to Figure 5. Images were captured every 2 minutes from 10 to 40 minutes post amputation (15 consecutive images are included; 4 frames/second). After fin amputation, SECs mobilize in two distinct, alternating domains in the process of wound healing. In contrast, Gd^{3+} treatment polarizes the differences between movement velocities of interray and ray SEC sheets.

Movie S6. Time Lapse Images of an Amputated *β-act2:EGFP; msxc:DsRed* Fin After Vehicle or Gd^{3+} Treatment, Related to Figure 5. *β-act2:EGFP* labels all epithelial cells, shown in blue. *msxc:DsRed* labels most mesenchymal cells, which are shown in red. Images were captured every 2 minutes from 10 to 70 minutes post amputation (30 consecutive images are included; 4 frames/second). The fin heals and no significant mesenchymal leakage is observed. In contrast, Gd^{3+} treatment disrupts closure, and mesenchymal leakage occurs.

Movie S7. Three-dimensional Image of *skinbow; krtt1c19e:Venus-hGeminin* Fin Epithelium at 4 dpa, Related to Figure 7. Images and movies were created using Imaris software. The *skinbow* line labels SECs, which are shown in multi-color. *krtt1c19e:Venus-hGeminin* labels all dividing basal cells, which are shown in green. Many cycling basal cells were detected in vivo at 4 days after fin amputation.