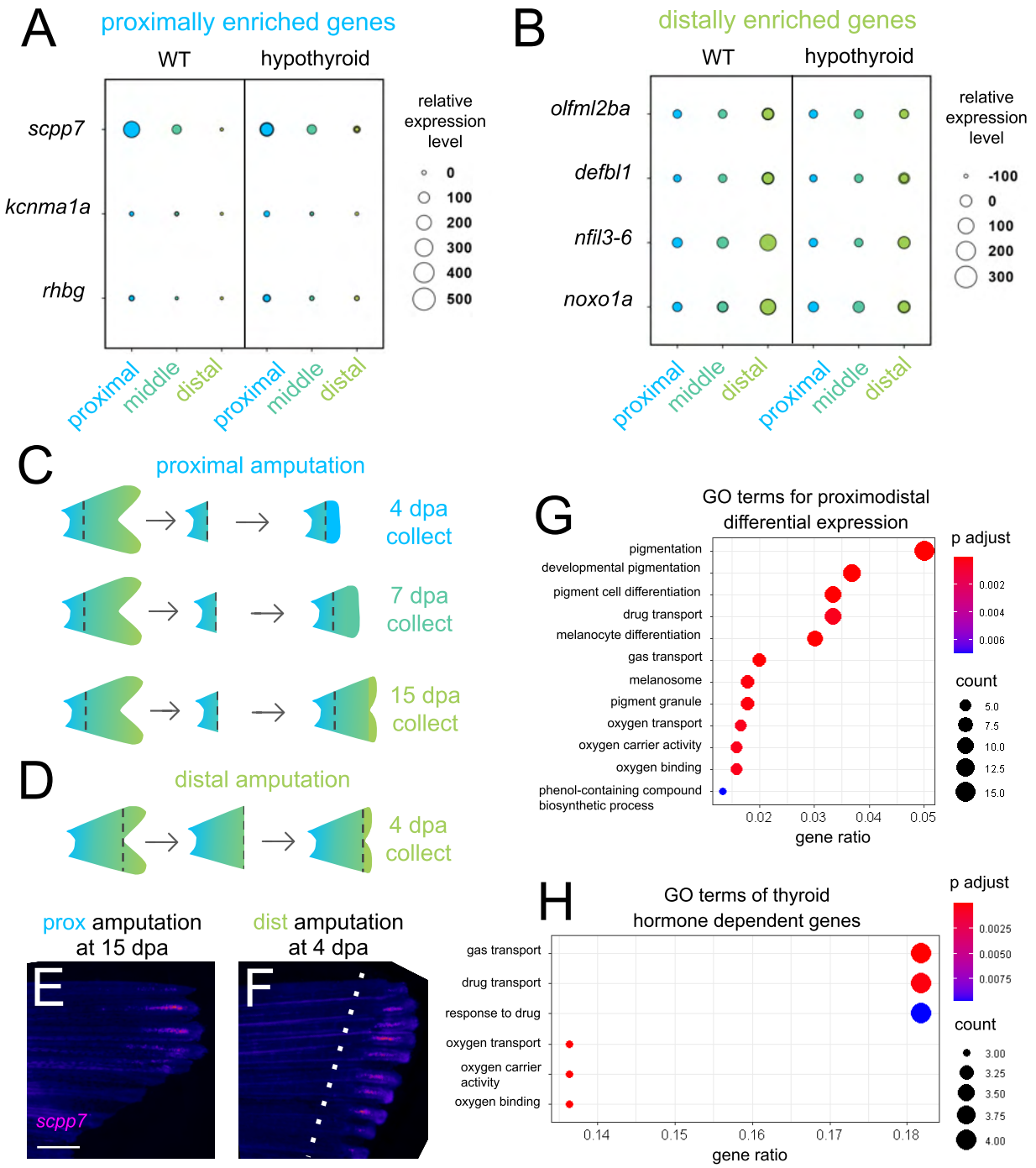
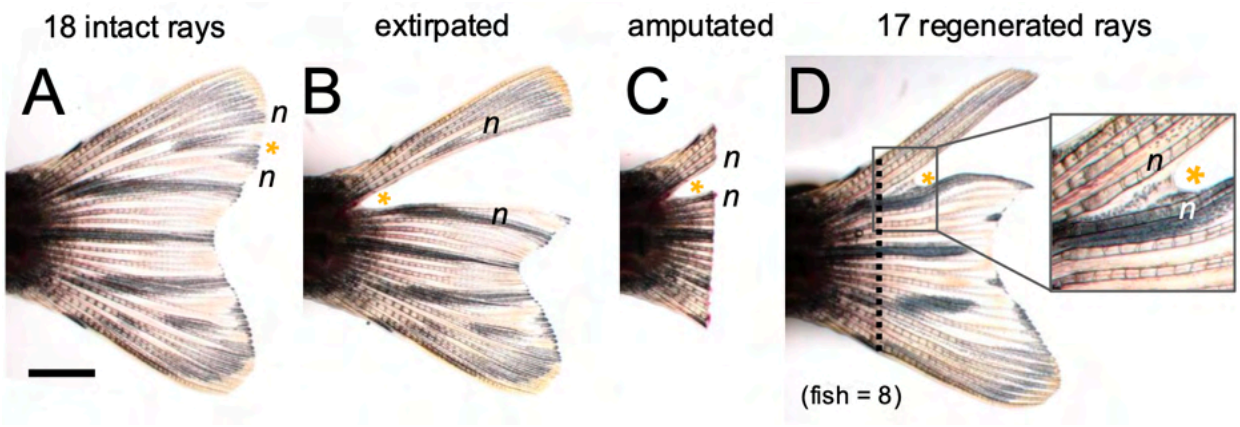
SUPPLEMENTARY FIGURES



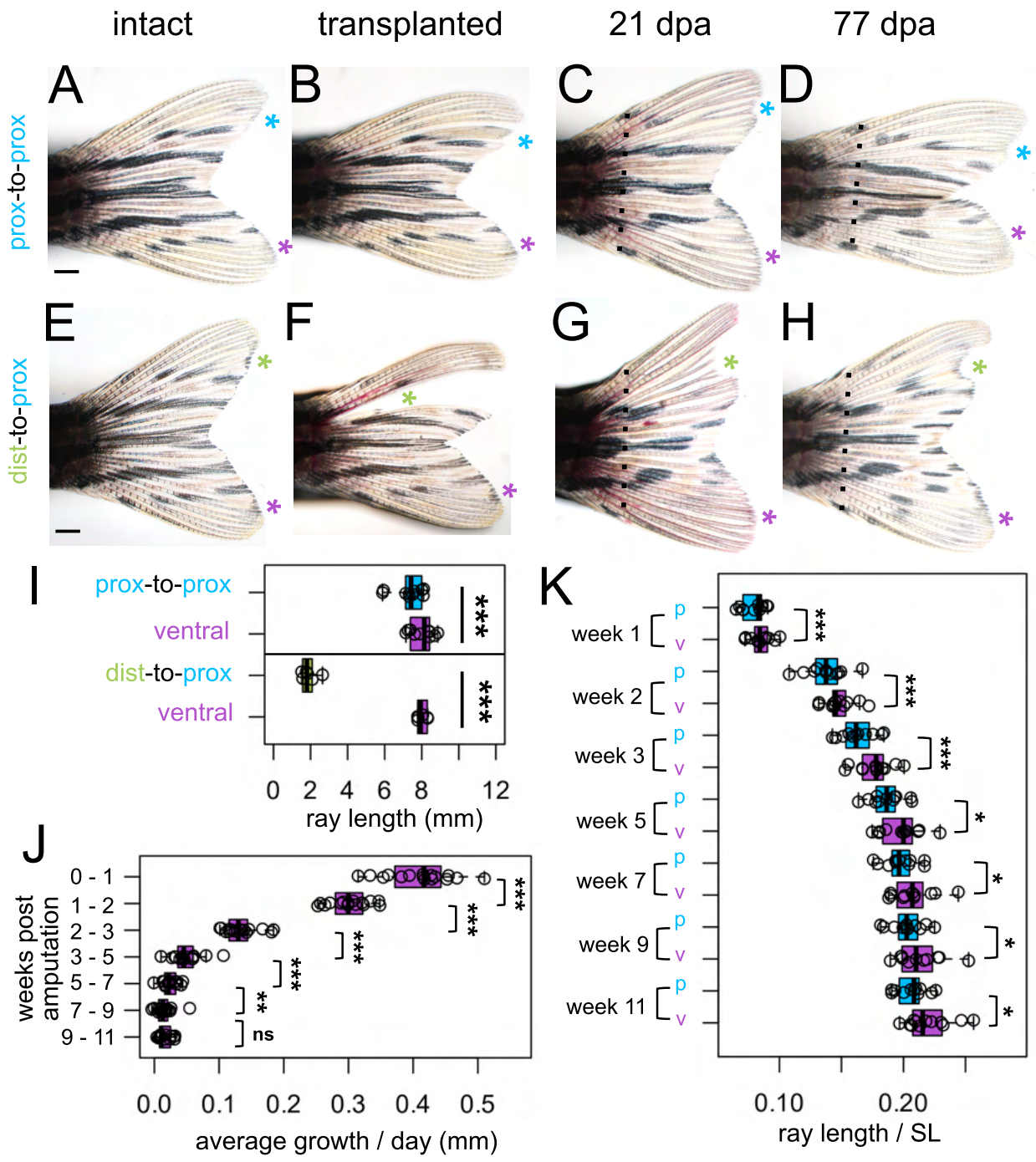
Supplementary Figure 1. Differentially expressed gene candidates for fluorescent *in situ* hybridization. Thyroid hormone-dependent gene candidates that are either (A) proximally enriched or (B) distally enriched in WT tissues. Custom RNAscope probes were made and tested for all genes, but only the *scpp7* probe showed specific staining. (C-D) Schematic

558 showing sample collection with (C) proximal or (D) distal amputation. (E) Proximally amputated
559 at 15dpa or (F) distally amputated 4dpa tissue stained for *scpp7*. Amputation plane, dashed line.
560 Warm colors indicate highest regions of expression. (G) GO enrichment of the 489 genes
561 proximodistal differentially expressed in WT. (H) GO enrichment of the 45 genes that were
562 thyroid hormone dependent and proximodistal differentially expressed in WT. Scale bar, 400 μm.

563



Supplementary Figure 2. Regeneration does not originate from an extirpated ray. (A) Intact fin with 18 rays, dorsal ray 4 (D4) marked with yellow asterisk. (B) Fin one day post D4 extirpation. (C) Freshly amputated fin, one day post D4 extirpation. (D) Fin regenerates with 17 rays (one-less ray than original, intact fin). n indicates neighboring dorsal rays 3 and 5. Amputation plane, dashed line. Scale bar, 2 mm.

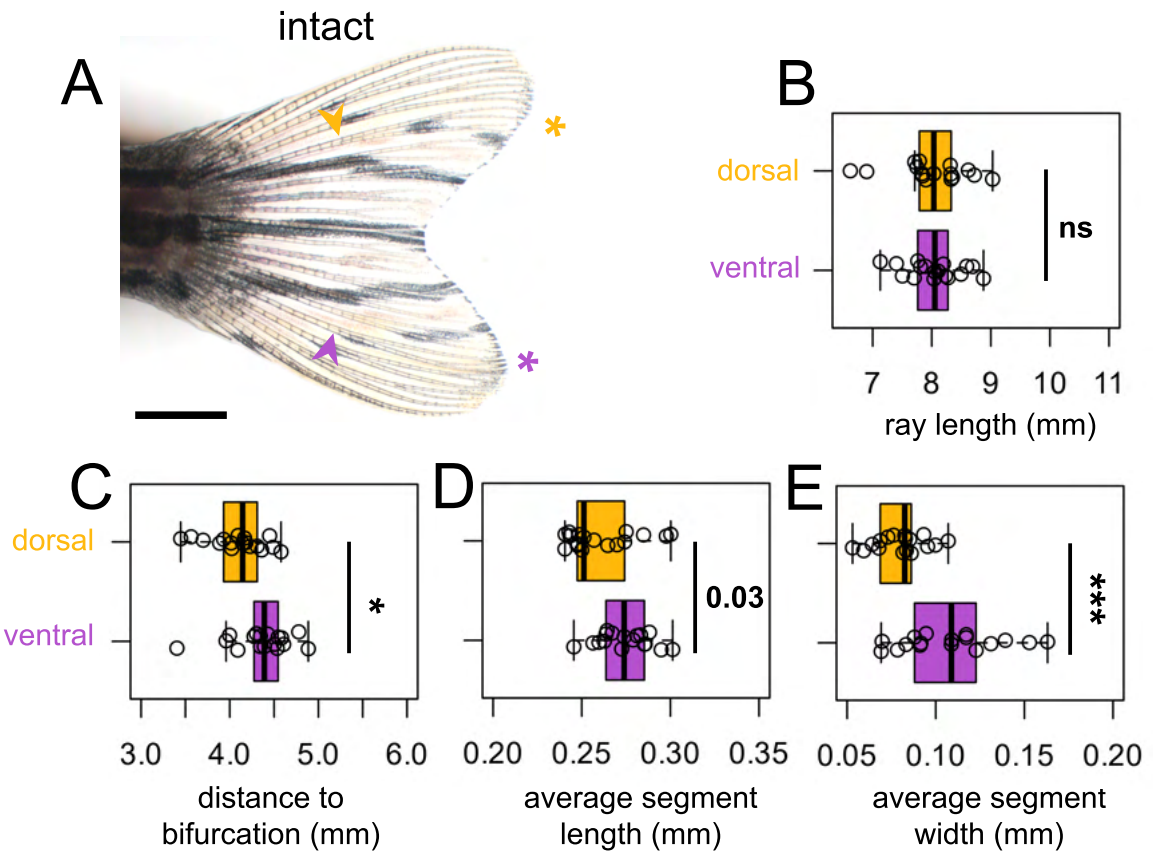


570

571 **Supplementary Figure 3. Non-transplanted rays regenerated faster than transplanted**
572 **rays.** Fins of (A-D) proximal-to-proximal (blue asterisk) or (E-H) distal-to-proximal (green
573 asterisk) transplantation: (A, E) intact pre-transplantation, (B-F) one day post-transplantation,
574 (C-G) regenerating at 21 dpa, (D, H) regenerating at 77 dpa. Ventral rays indicated with purple
575 asterisks. Amputation plane, dashed line. (I) Length of the rays after transplantation, as
576 measured from the peduncle. (J) Average amount of growth per day during a one/two week
577 periods for all the ventral ray comparisons. (K) Prox-to-prox rays versus ventral ray

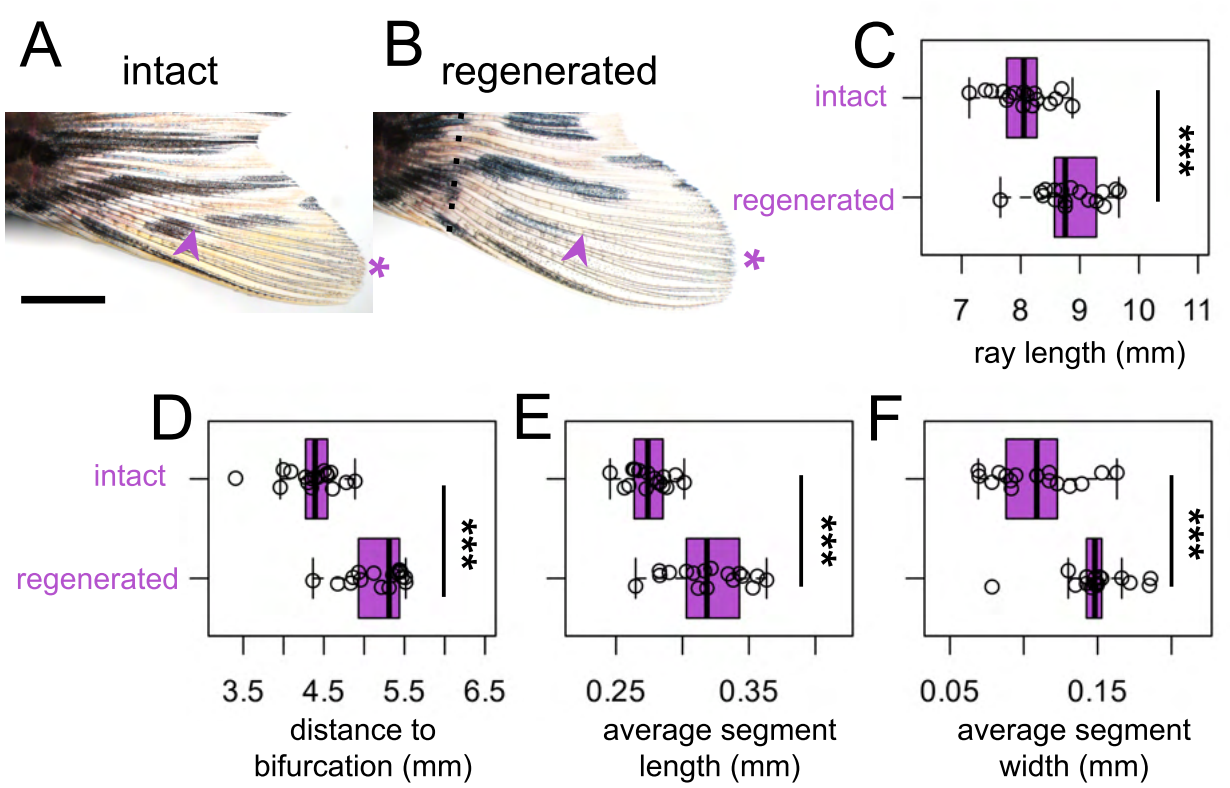
578 comparisons, ray length (measured from amputation plane) divided by SL at each week.
579 Significance determined by paired Welch two-sample t tests. Scale bar, 1 mm.

580



581

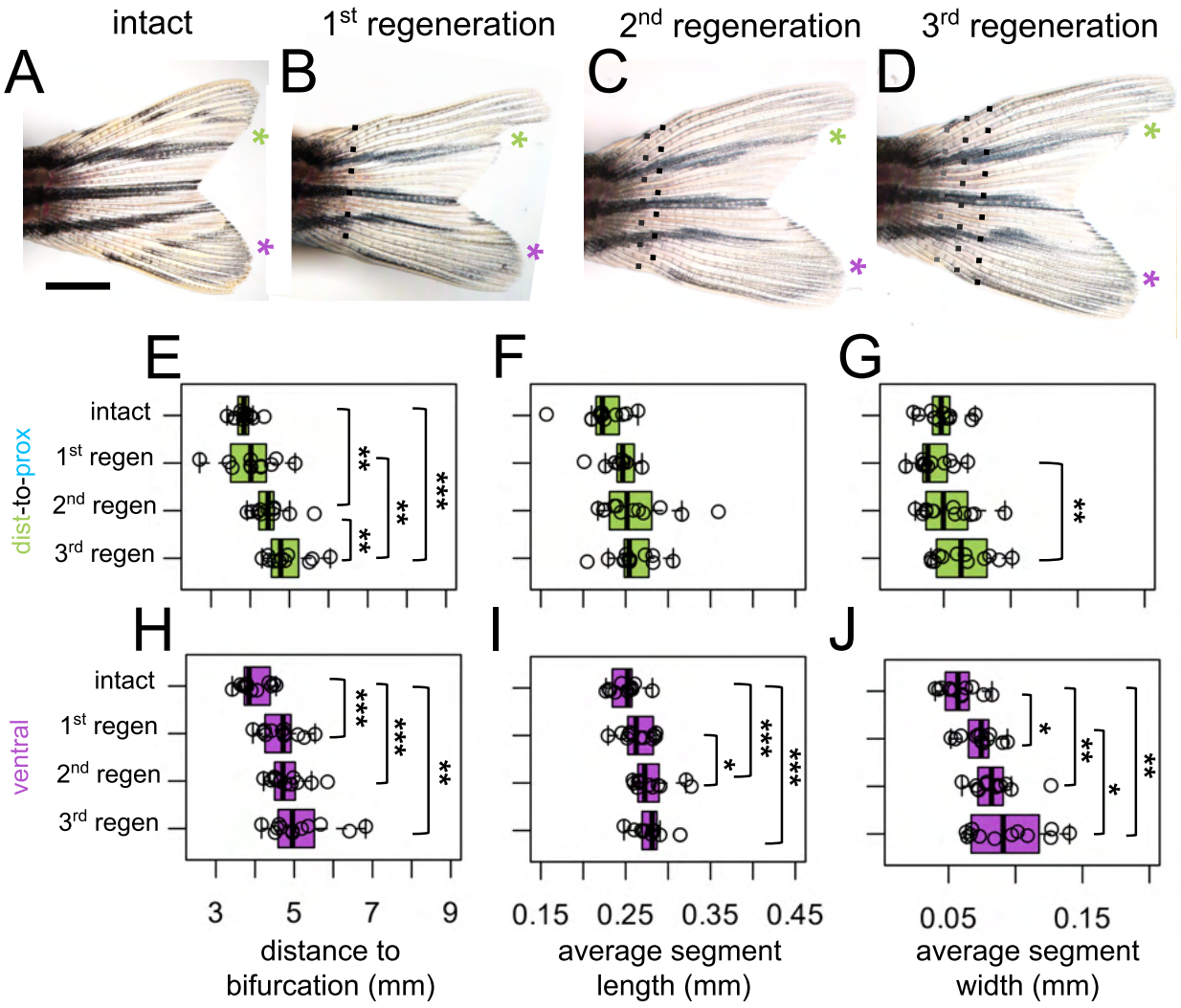
582 **Supplementary Figure 4. Dorsal ray patterning is unique from ventral ray patterning.** (A)
583 Intact fin. A yellow or purple asterisk indicates dorsal ray 4 or ventral ray 4, respectively.
584 Arrowheads, primary bifurcations. Boxplots showing the (B) total length of the ray, (C)
585 proximodistal position of the bifurcation, (D) average segment length, and (E) average segment
586 width measured from a set distance from the peduncle. Significance determined by a paired
587 Welch two-sample t test. Scale bar, 2 mm.



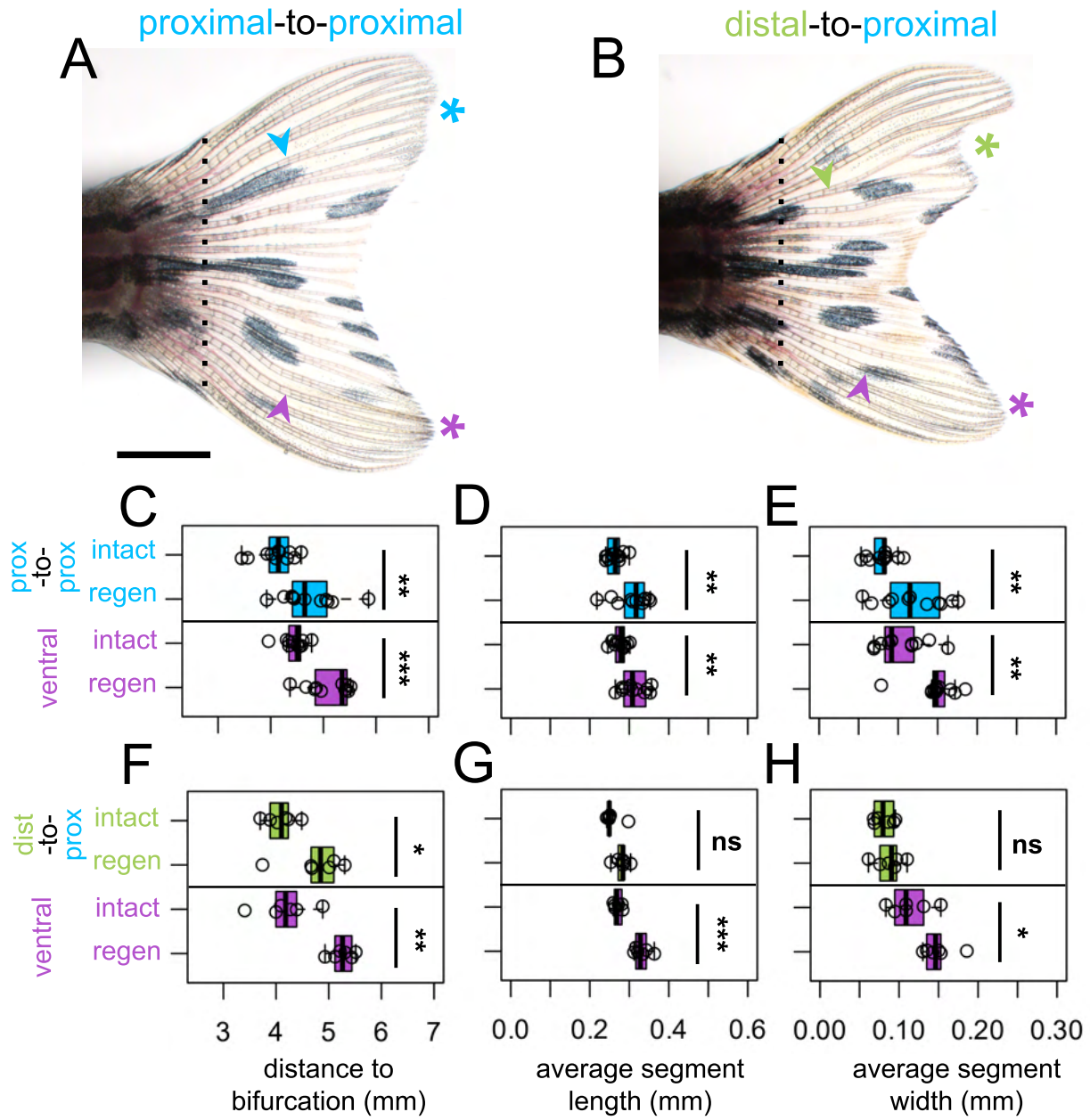
588

589 **Supplementary Figure 5. Intact and regenerated ray patterning are different.** (A-B) Ventral
590 lobe of (A) intact or (B) regenerating fin at 35dpa. Purple asterisks indicate ventral ray 4.
591 Arrowheads, primary bifurcations. Amputation plane, dashed line. Boxplots showing the (C) total
592 length of the ray, (D) proximodistal position of the bifurcation, (E) average segment length, and
593 (F) average segment width measured from a set distance from the peduncle. Significance
594 determined by a paired Welch two-sample t test. Scale bar, 2 mm.

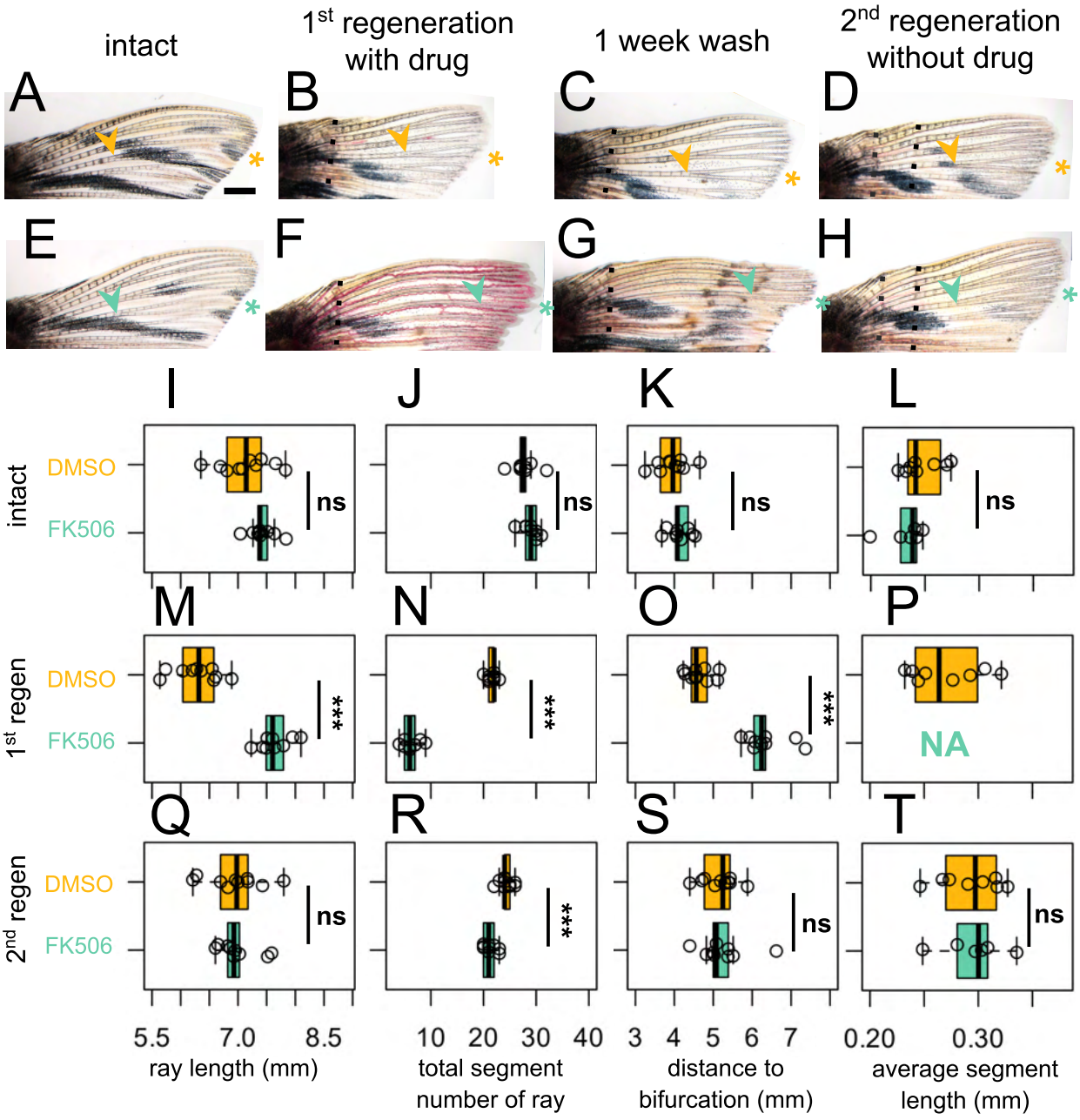
595



Supplementary Figure 6. Regenerative ray patterning differs from previous regenerated morphology. (A) Intact fin. (B-D) Regenerating fin after distal-to-proximal transplantation: (B) 28 days post first amputation, (C) 28 days post second amputation, (C) 28 days post third amputation. Green or purple asterisks indicate dist-to-prox or ventral ray, respectively. Black dashed line, most recent amputation. Grey dashed lines, previous amputation planes. (E, H) Boxplots showing the proximodistal position of the bifurcation. Note that bifurcations form at increasingly distal location after each amputation, as previously described . Boxplots showing (F, I) average segment length, and (G, J) average segment width. All measurements were taken from a set distance from the peduncle. Significance determined by paired repeated samples ANOVA followed by pairwise t tests. Scale bar, 2 mm.

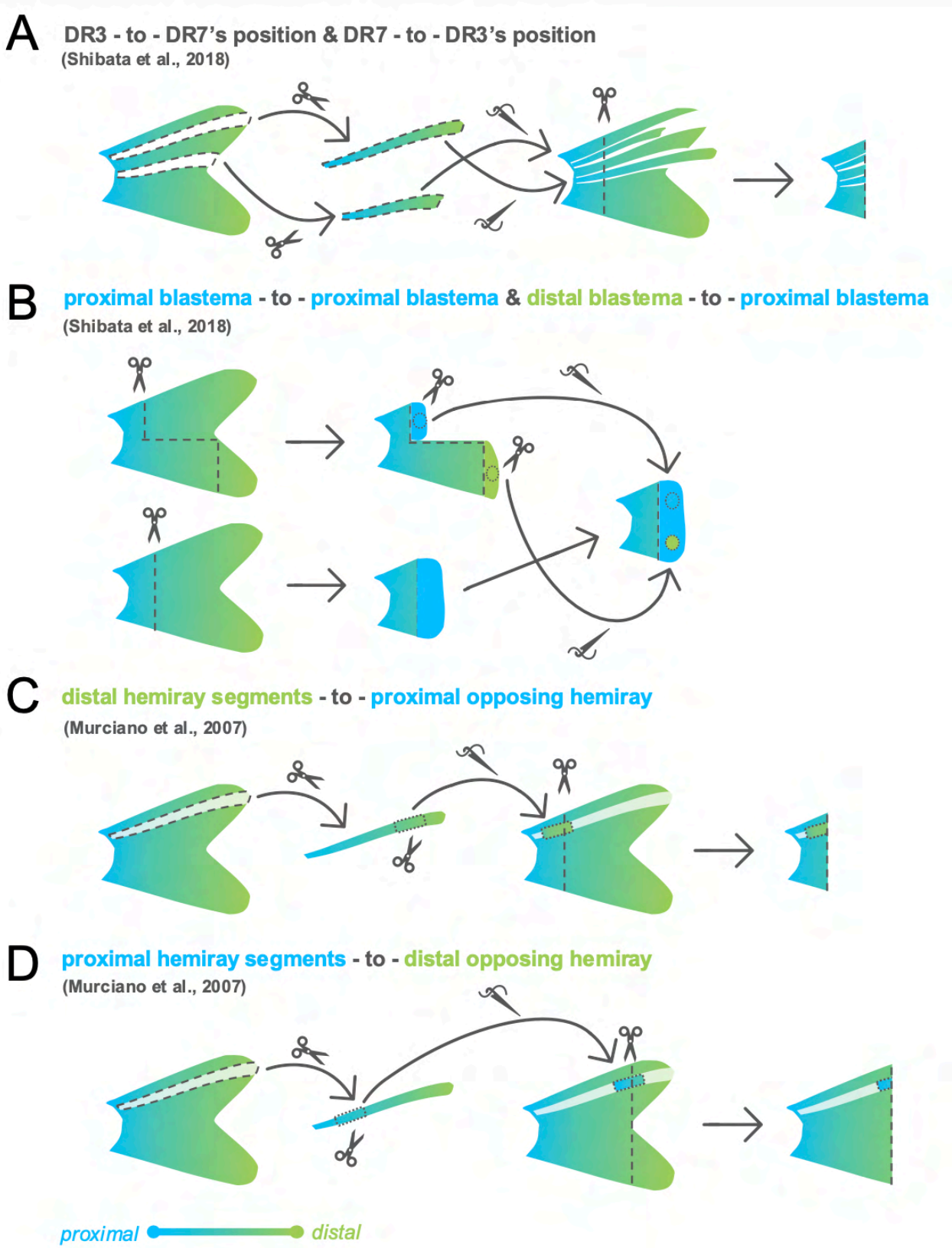


Supplementary Figure 7. Proximodistal patterning is dependent upon the current regenerative environment. Regenerating fins at 35dpa after either (A) proximal-to-proximal (blue asterisk) or (B) distal-to-proximal (green asterisk) transplantation. Purple asterisks indicate ventral ray comparison. Amputation plane shown with dashed line. Arrowheads indicate primary bifurcations. C-H) Boxplots showing the (C, F) proximodistal position of the bifurcation, (D, G) average segment length, and (E, H) average segment width of intact or regenerated rays, measured from a set distance from the peduncle. (C-E) Prox-to-prox or dist-to-prox ray measurements are shown alongside their ventral ray comparisons. Significance determined by a paired Welch two-sample t test. Scale bar, (A-B) 2 mm.

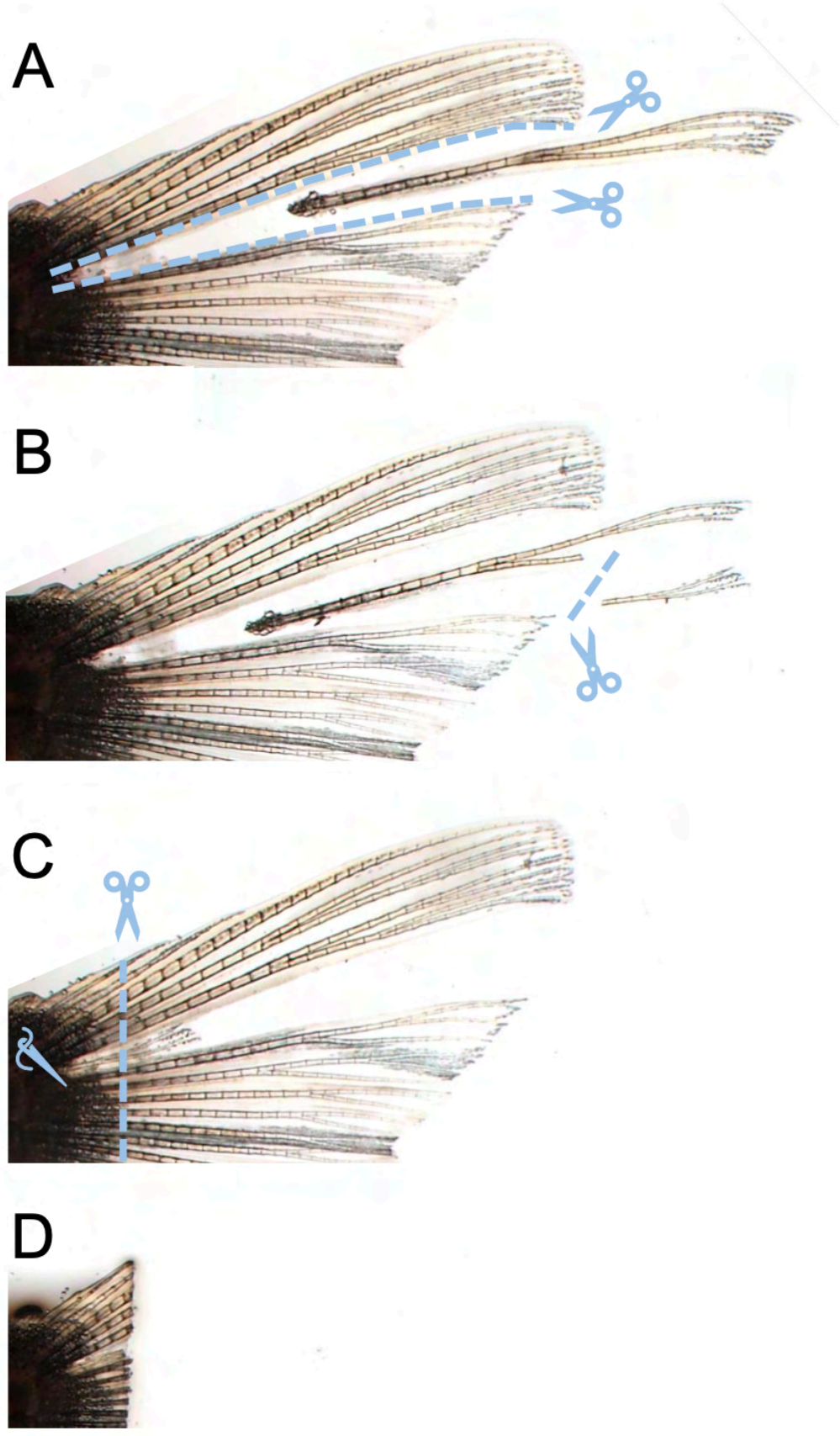


Supplementary Figure 8. Calcineurin inhibition-induced morphologies are not remembered in subsequent regeneration cycles. (A, E) Intact dorsal lobe before treatment. (B, F) Regenerated fin after (B) DMSO (yellow asterisk) or (F) 200 nM FK506 (turquoise asterisk) treatment, 21 days post amputation. (C, G) Fins after one week wash to clear remaining drug from water. (D, H) Regenerated fin 21 days post second amputation with no treatment. Black dashed line, most recent amputation. Grey dashed lines, previous amputation plane. Boxplots showing (I, M, Q) total ray length, (J, N, R) total number of segments of the ray, (K, O, S) bifurcation position, and (L, P, T) average segment length for (I-L) intact, (M-P) first regeneration with respective drug treatment, and (Q-T) second regeneration with no drug treatment. All measurements were taken from a set distance from the peduncle. Note in (P),

628 rays were built from only ~5 segments, making segments lengths so long that none were
629 contained by the standard region of interest measured. Significance determined by unpaired
630 Welch two-sample t test. Scale bar, 1 mm.



Supplementary Figure 9. Historical transplantation experiments. (A) Shibata et al., 2018 performed full ray transplantations, moving dorsal ray 3 into dorsal ray 7 position and vise versa. After successful grafting, they amputated the entire. (B) Shibata et al., 2018 also made a proximal and distal amputation in a fin, collected blastema tissue from each region, and then transplanted these tissues into a proximally regenerating fin. (C) Murciano et al., 2007 extirpated an entire distal hemiray from the fin. A distal hemiray segment was grafted onto a proximal region to appose a proximal hemiray segment, then the entire fin was amputated through the graft. (D) Murciano et al., 2007 further extirpated a single hemiray, then grafted a proximal hemiray segment onto a distal region to appose a distal hemiray, then the entire fin was amputated through the graft.



642

643 **Supplementary Figure 10. Distal-to-proximal transplantation.** (A) Interray tissue is cut
644 sliced on either side of dorsal ray 4, permitting the ray to be cleanly plucked out of the peduncle.
645 (B) Distal ray tissue is removed from the rest of the ray. (C-D) After allowing 24hrs for the
646 transplanted tissue to graft, the entire fin is amputated.