Changes in regeneration-responsive enhancers shape regenerative capacities in vertebrates

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Outline

- Background
- Results
  - Identification of regeneration-responsive enhancers (RREs)
  - Experimental validation of RREs
  - Identification of potential regulators
- Summary

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RESEARCH ARTICLE

Changes in regeneration-responsive enhancers shape regenerative capacities in vertebrates

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Wang et al., Science *369*, 1207 (2020) 4 September 2020

Corresponding author

- Scientific director of the Stowers Institute for Medical Research
- Investigator of the Howard Hughes Medical Institute
- Established a powerful new model system to study the molecular basis of regeneration, using the freshwater flatworm *Schmidtea mediterranea* (planaria)
- Current research: the molecular basis of regeneration

Stowers Institute for Medical Research

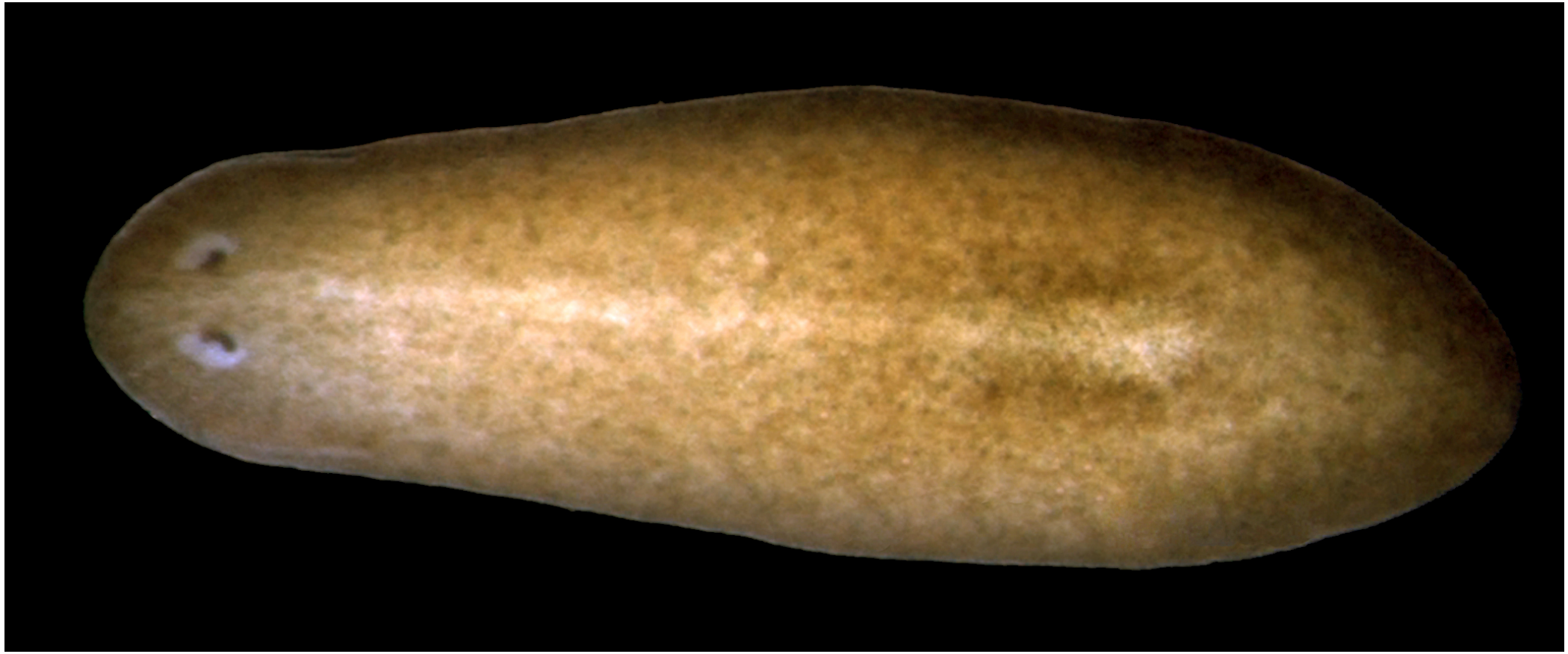
Howard Hughes Medical Institute

Prof. Alejandro Sánchez Alvarado

http://planaria.stowers.org/about/a-sanchez-alverado/

The freshwater flatworm *Schmidtea mediterranea* (planaria)

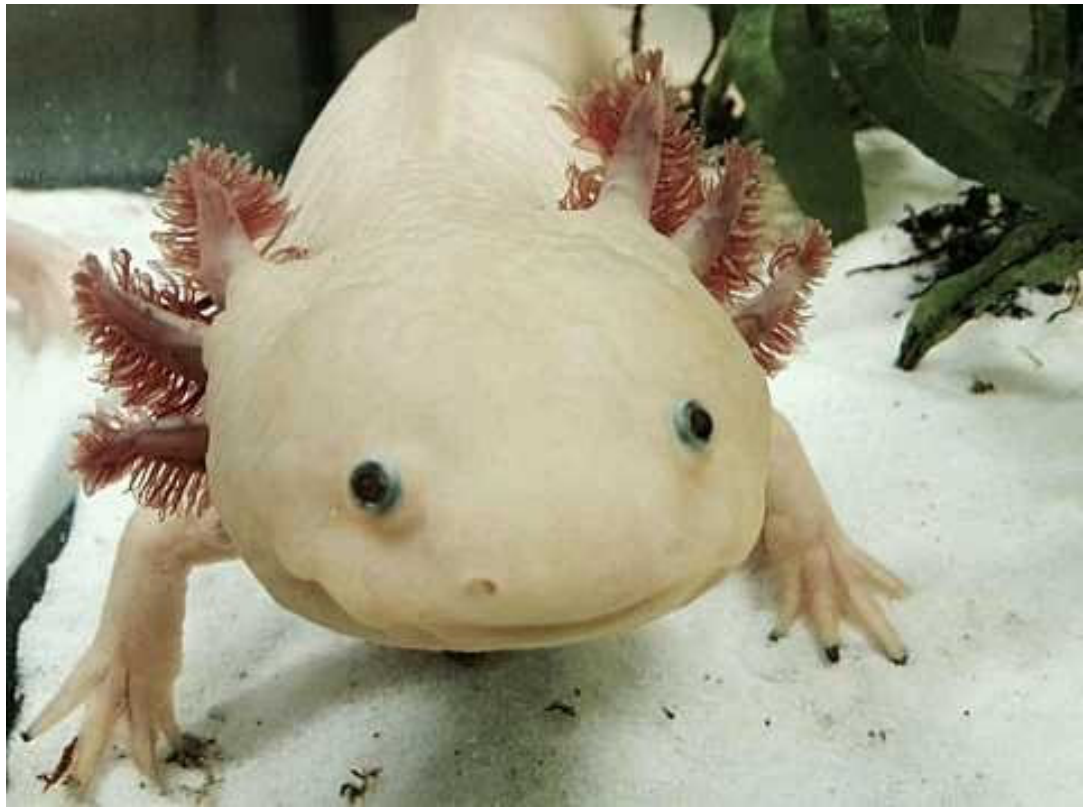
- Almost any piece from a planaria individual can regenerate an entire organism in a few days



https://en.wikipedia.org/wiki/Schmidtea_mediterranea

What is regeneration?

- ***Definition***: in biology, regeneration is the process of renewal, restoration, and tissue growth that makes genomes, cells, organisms, and ecosystems resilient to natural fluctuations or events that cause disturbance or damage (from Wikipedia)

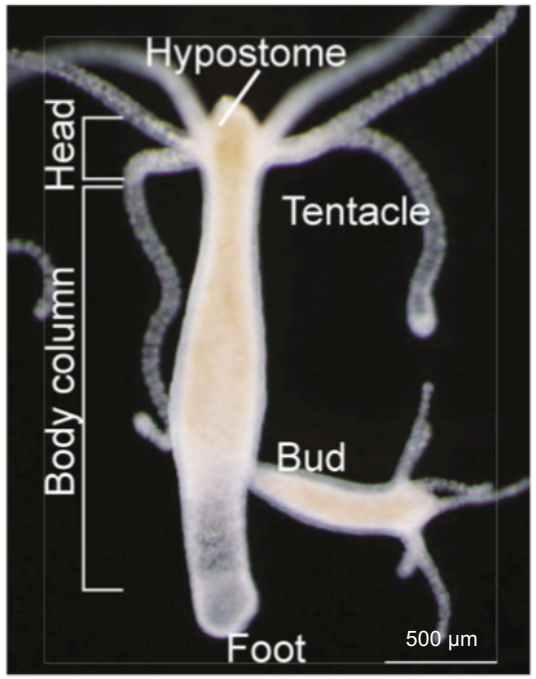


Axolotls can regenerate a variety of structures, including their limbs.

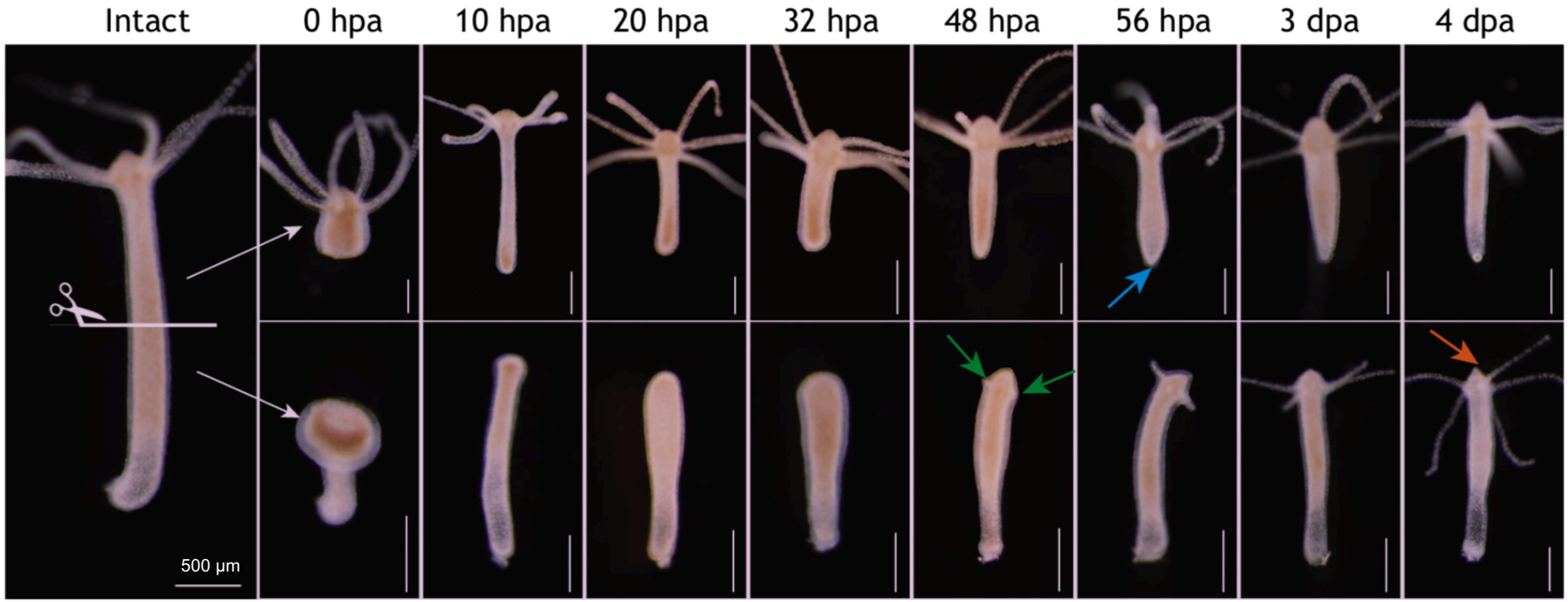
https://en.wikipedia.org/wiki/Regeneration_(biology)

Regenerative capabilities of *Hydra*

- *Hydra* is a freshwater polyp that exhibits remarkable regenerative capabilities
- When a *Hydra* polyp is bisected, the head and foot regenerate within a few days
- Blue arrow: the fully regenerated foot; green arrows: the emergence of tentacle rudiments; red arrow: a fully regenerated head



Hydra anatomy



Hydra head and foot regeneration

Vogg et al., *Development* (2019)

African killifish in this study

Features of African Killifish (*Nothobranchius furzeri*)

- Rapid sexual maturation (as short as 2 weeks)
- Diapause embryos
- Life span is extremely short (4 to 6 months)

Red morph

Yellow morph

Wang et al., *Science* (2020)

Cellerino et al., *Biol. Rev.* (2016)

Background

Known facts

- Changes in *cis*-regulatory elements have been shown to be a major source of morphological diversity (why they study enhancers)

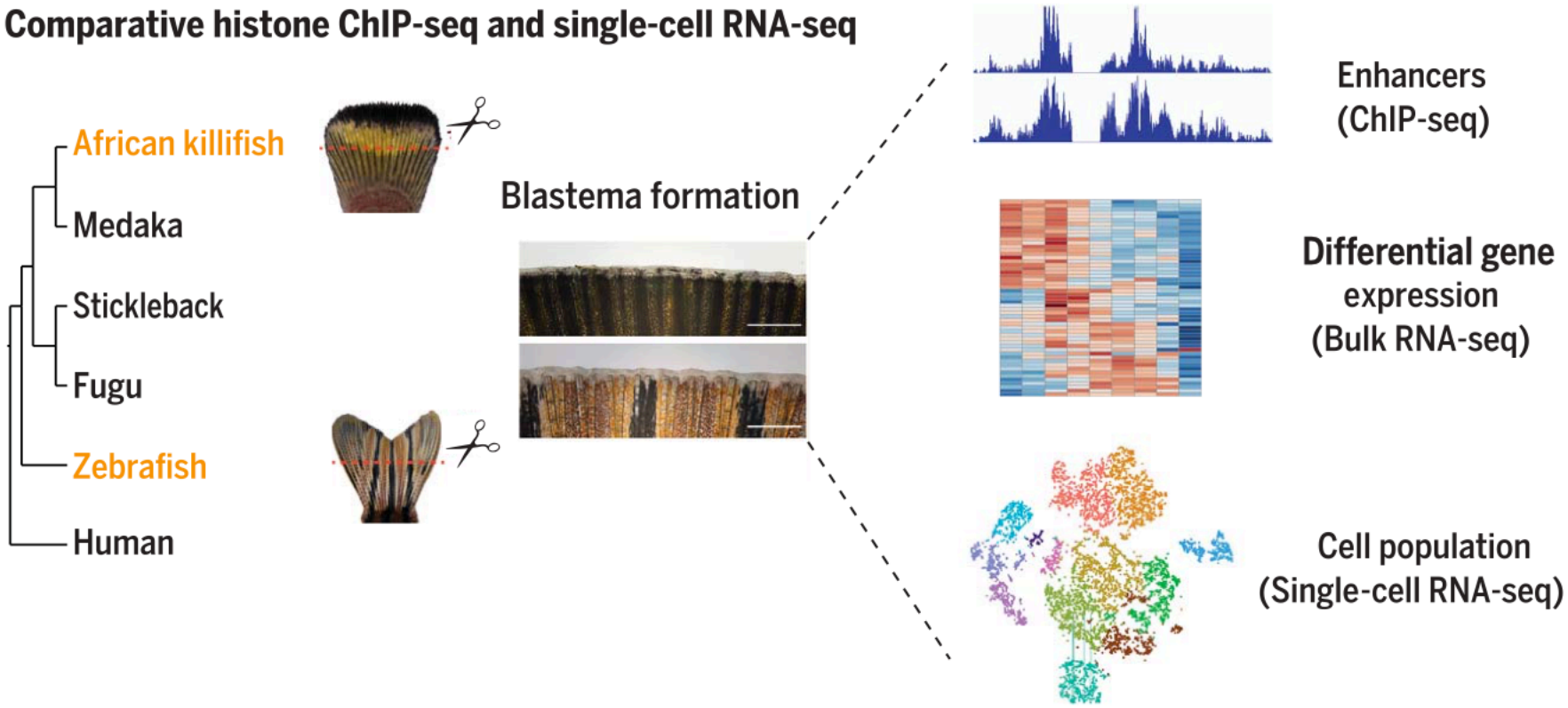
- Emerging evidence indicates that injury-dependent gene expression may be controlled by injury-responsive enhancer elements (why they study enhancers)

- African killifish and zebrafish are two related but evolutionarily distant species that are capable of regenerating their caudal fin (why they study African killifish)

- The gene *inhba* is required in both tail and heart regeneration in zebrafish and is differentially regulated between regenerating and non-regenerating tissue (why they study the enhancer regulating *inhba*)

Why authors selected the African killifish

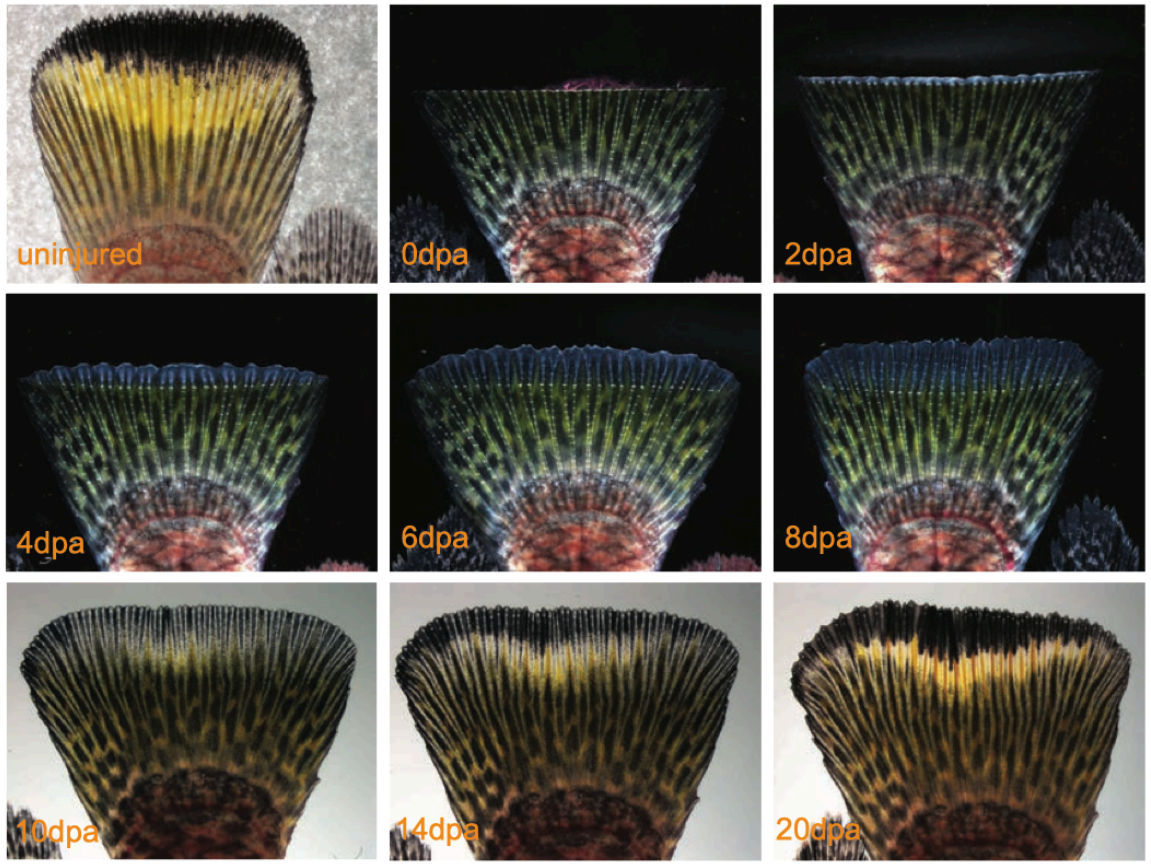
- Identification of conserved regeneration-responsive enhancers (RREs) requires two related but evolutionarily distant species that are capable of regeneration
- The authors took advantage of the fast sexual maturation of African killifish to rapidly generate transgenic reporter assays to validate predicted RREs and to facilitate their functional testing in adult regeneration



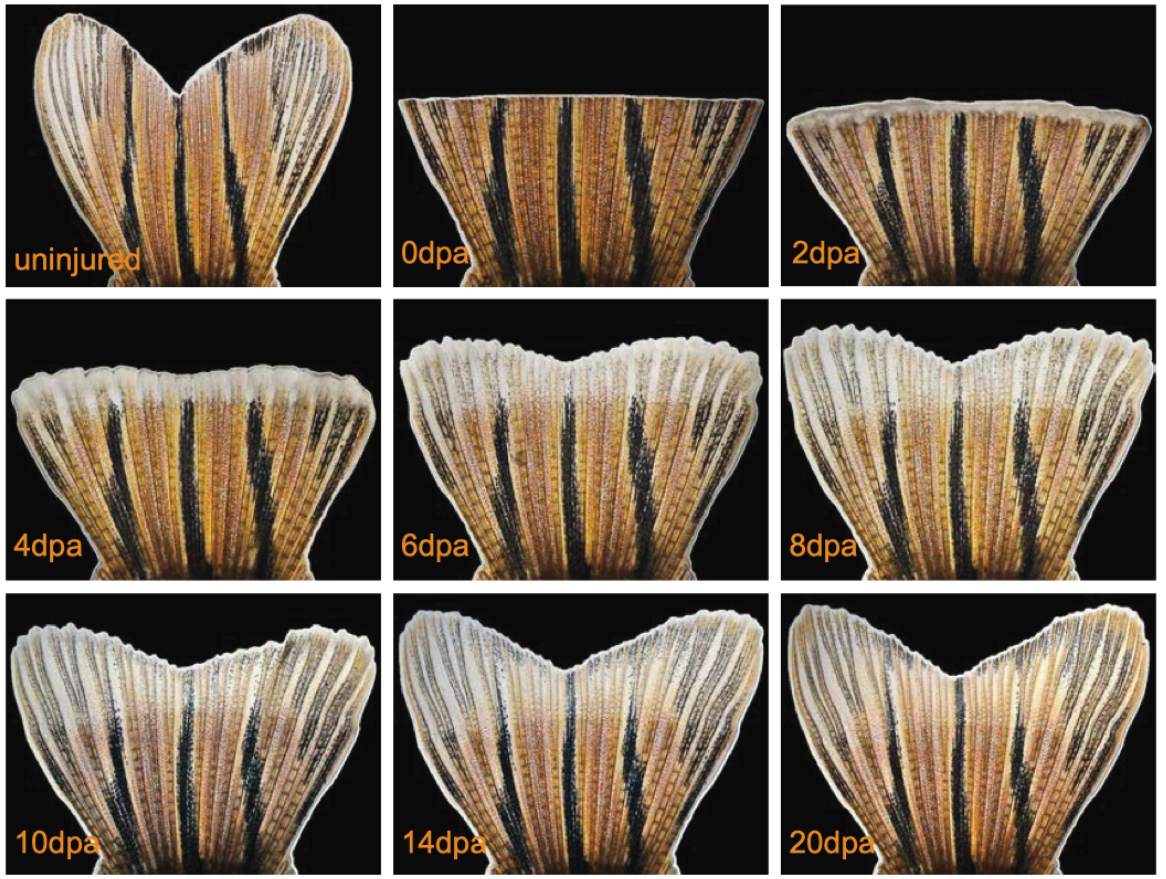
Wang et al., *Science* (2020)

Different stages of fin regeneration in killifish and zebrafish

- The morphology of the regenerated area becomes different at 3 dpa* in killifish and zebrafish
- Regeneration is completed at 20 dpa in both species



Killifish



Zebrafish

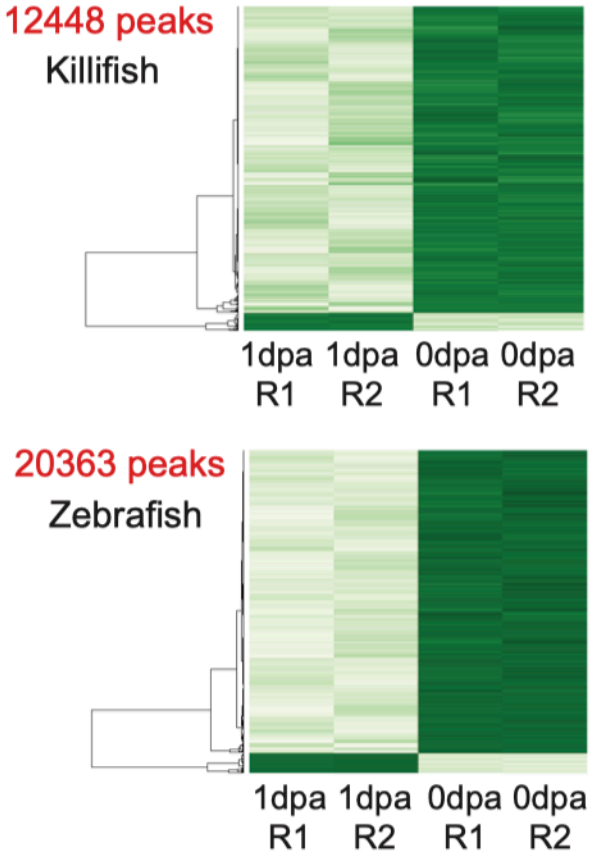
*dpa: day post amputation

Wang et al., *Science* (2020)

Dynamic change of H3K27ac peaks during fin regeneration in killifish and zebrafish

- Only a small portion of peaks are regeneration-activated
- More peaks were detected in the distal and intergenic regions after injury

A



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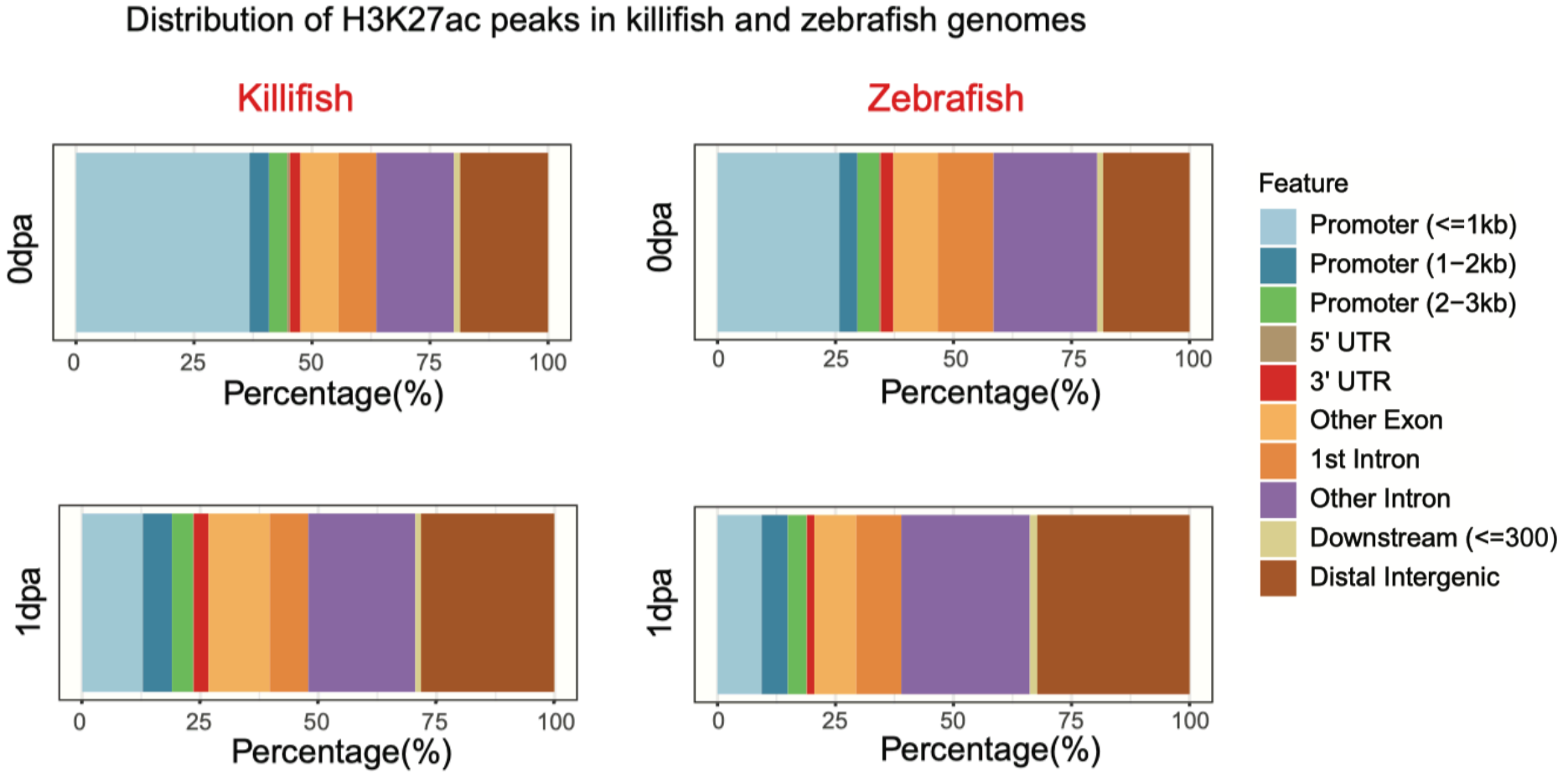
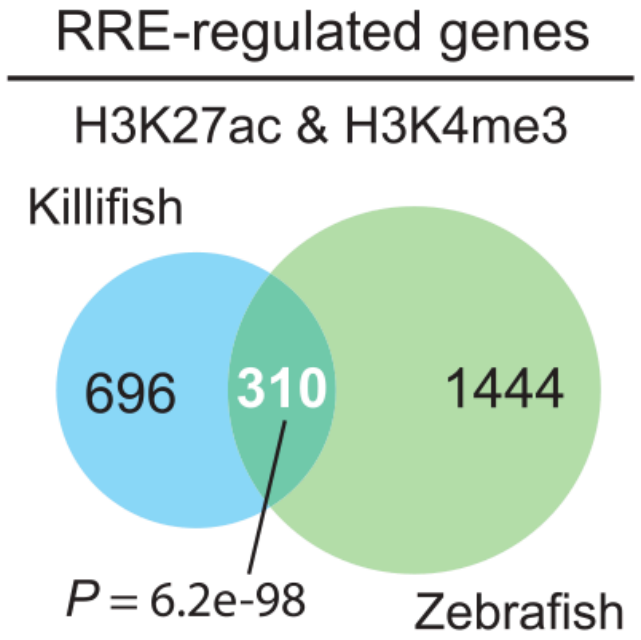
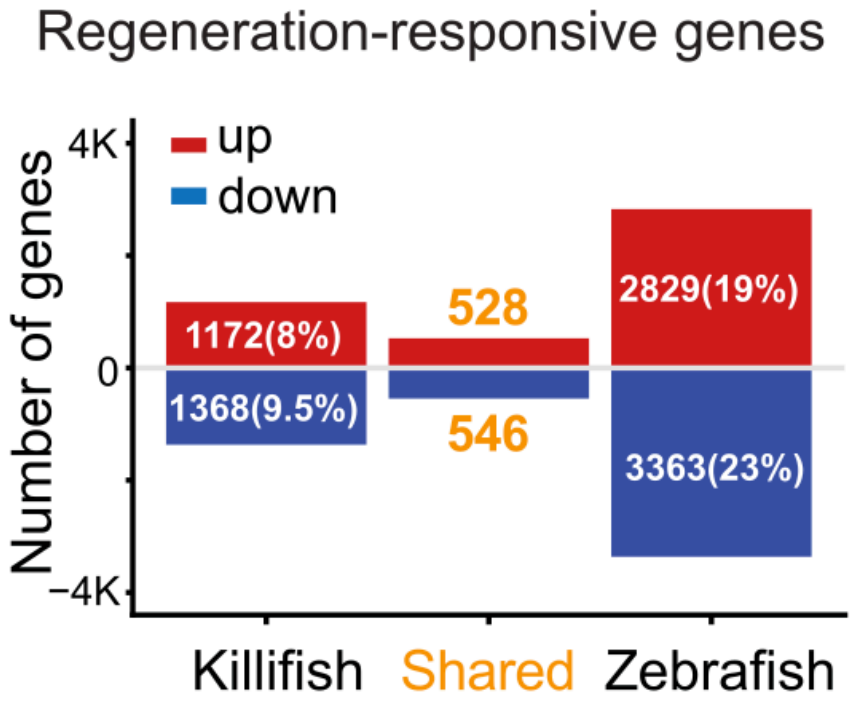
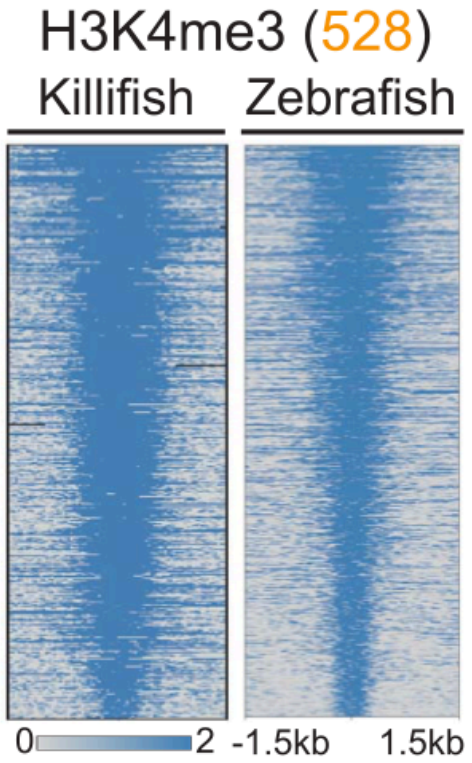


Identification of a conserved regeneration response program (49 genes)

- The authors identified shared 49 genes with H3K27ac-defined RREs, H3K4me3-marked active promoters, and elevated gene expression





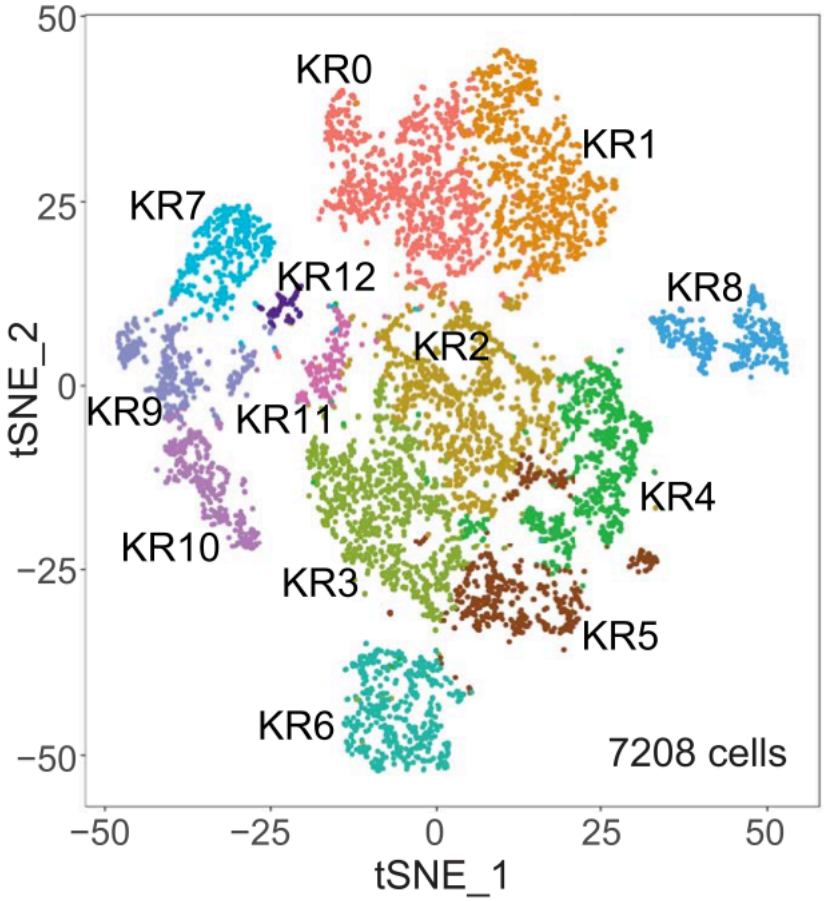


Where do these 49 genes express?

scRNA-seq identified shared cell types of early killifish and zebrafish regeneration

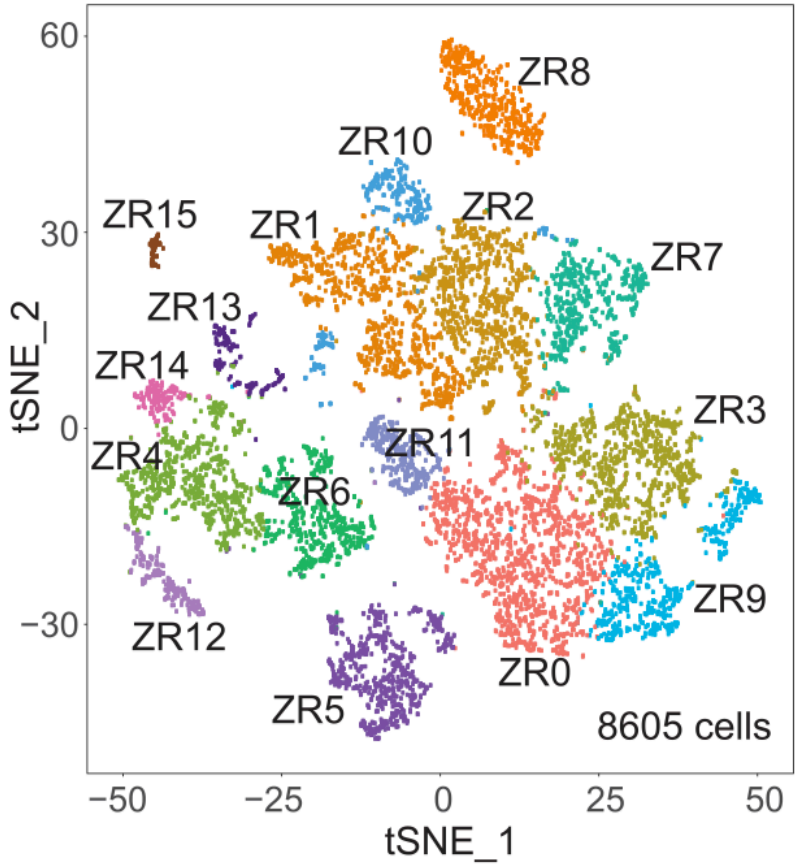
- A new early blastema marker, *fstl1*, was identified by scRNA-seq and confirmed by *in situ* hybridization

Killifish regeneration (KR)



13 clusters, 7,208 cells

Zebrafish regeneration (ZR)



16 clusters, 8,605 cells

Major cell types

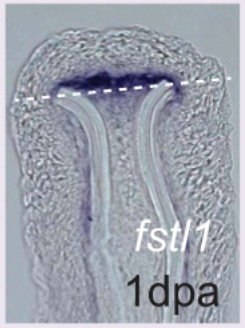
Non-blastema cells

	Killifish	Zebrafish
Macrophages	KR0, 1	ZR0, 3, 9, 11
Epidermal cells	KR7, 9	ZR4, 6, 12, 14
Neuronal cells	KR12	ZR13
Basal epidermal cells	KR10	ZR5

Blastema cells

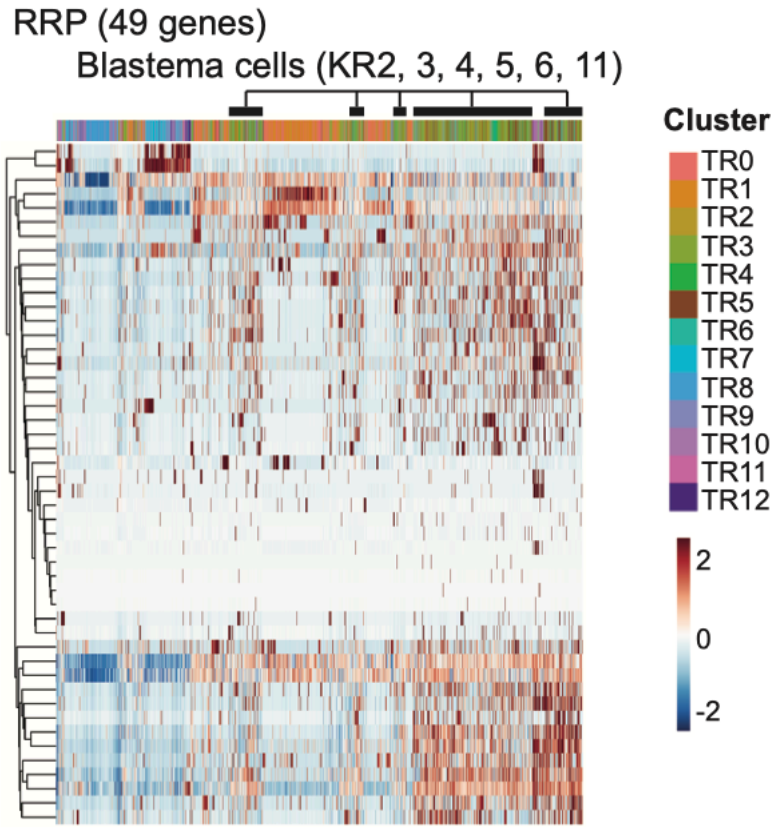
Killifish:
KR2, 3, 4, 5, 6, 11

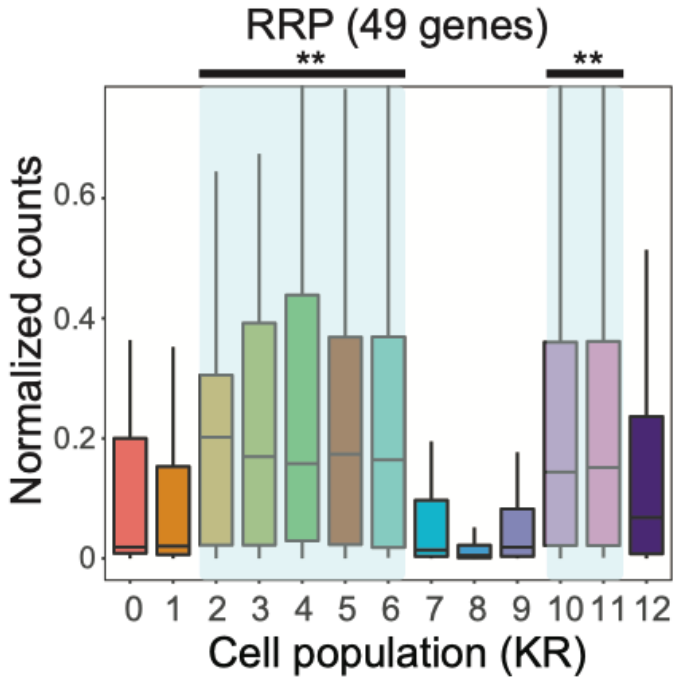
Zebrafish:
ZR1, 2, 7, 10



Blastema cells are the primary source of RRP gene expression

- The identified RRP genes were mainly expressed in regeneration-specific cells, i.e. blastema cells.

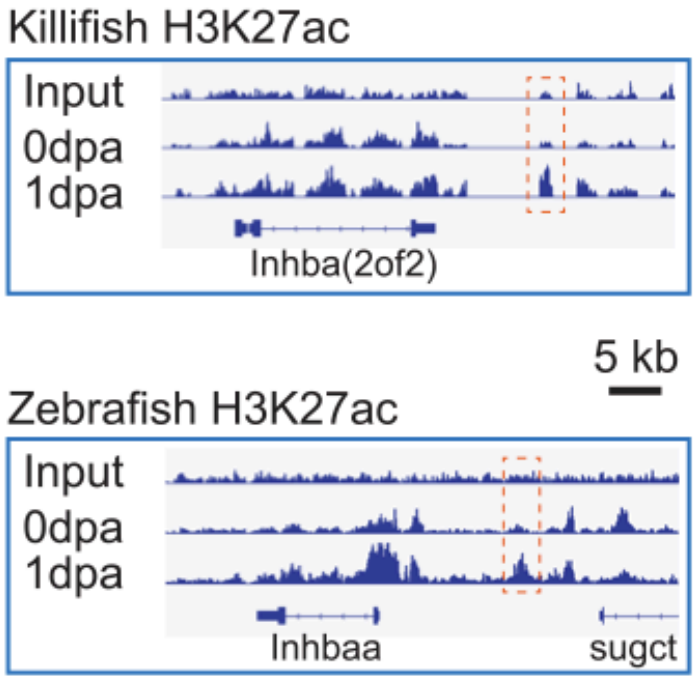


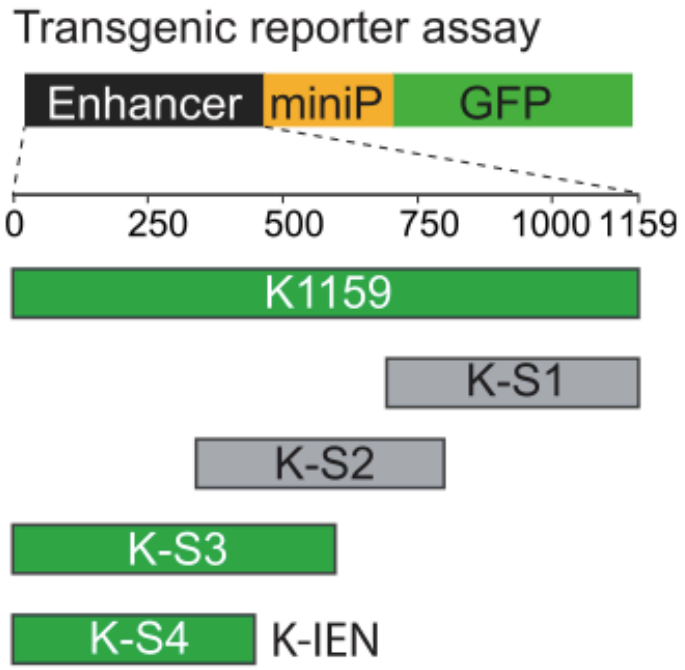


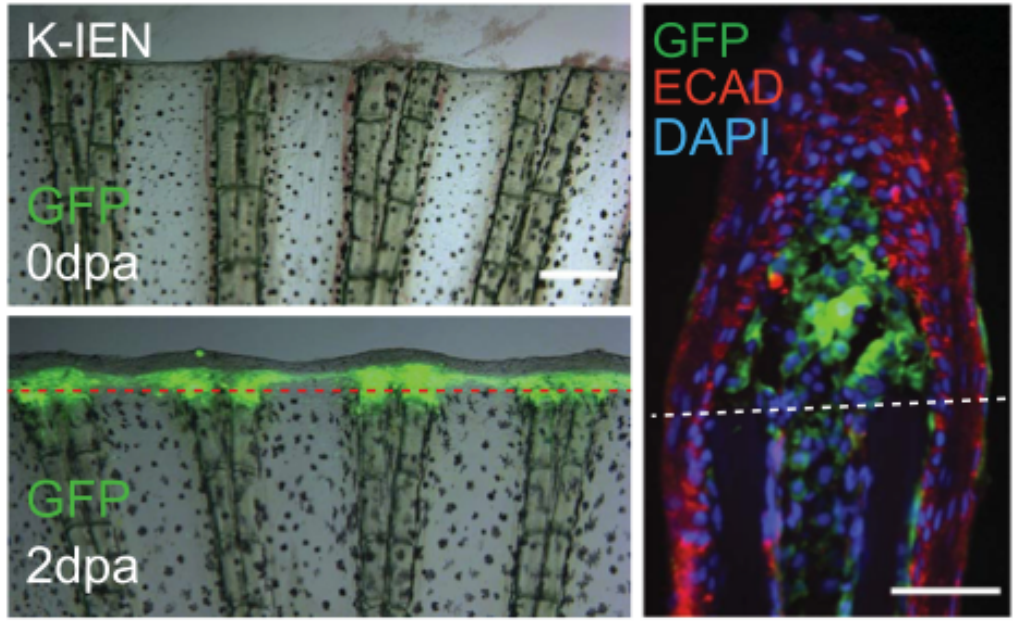
Experimental validation

The RRE *K-IEN* directs gene activation after amputation and is essential for regeneration

- *K-IEN*: killifish *inhba(2of2)* enhancer
- Robust reporter expression was detected in the blastema region after fin amputation in *K-IEN:GFP*-transgenic fish



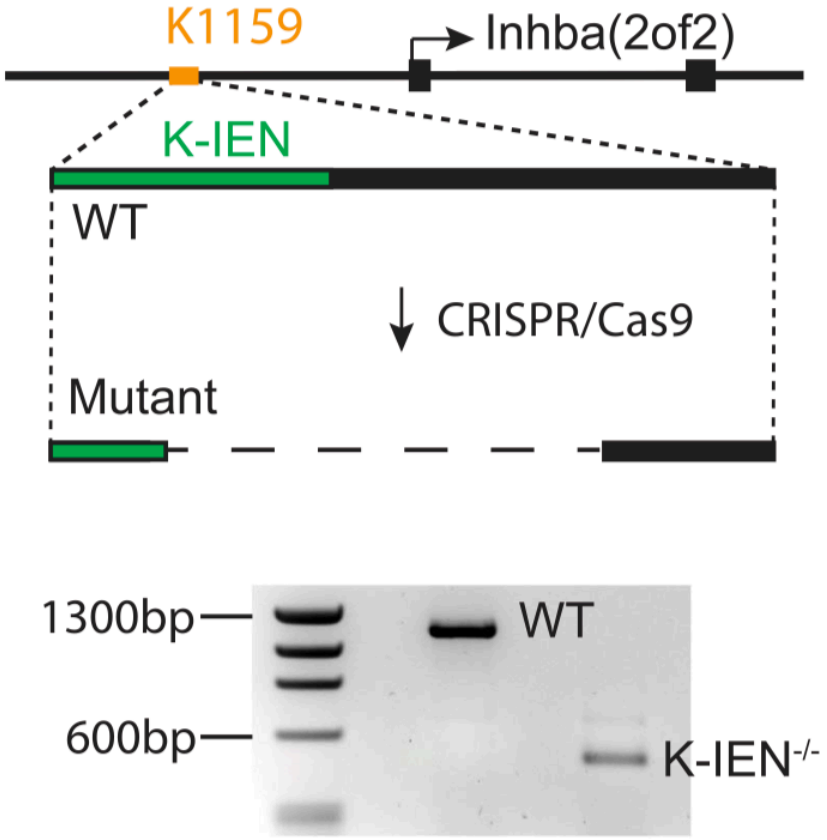




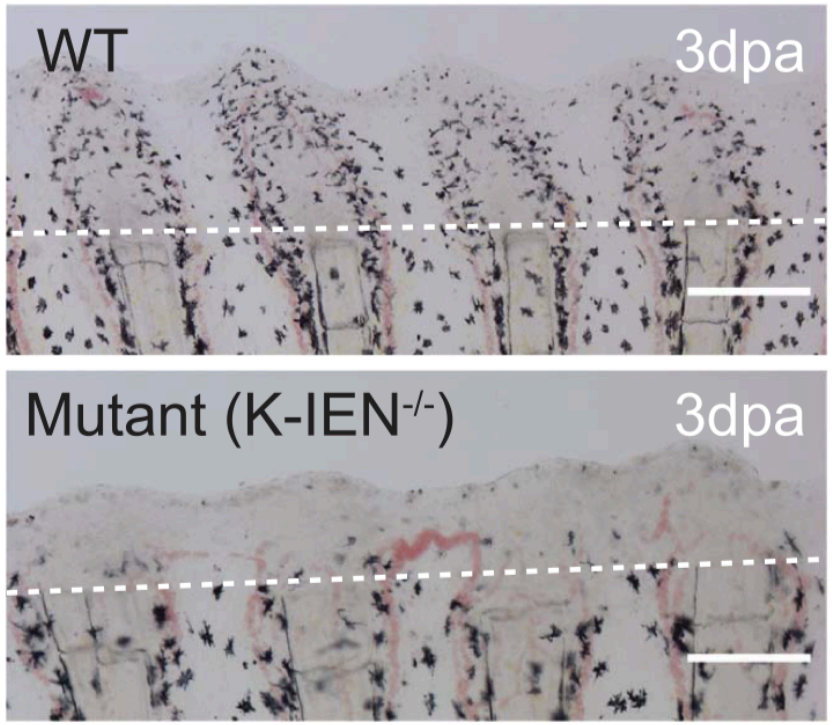
E-cadherin (ECAD) labels epithelial cells (red)

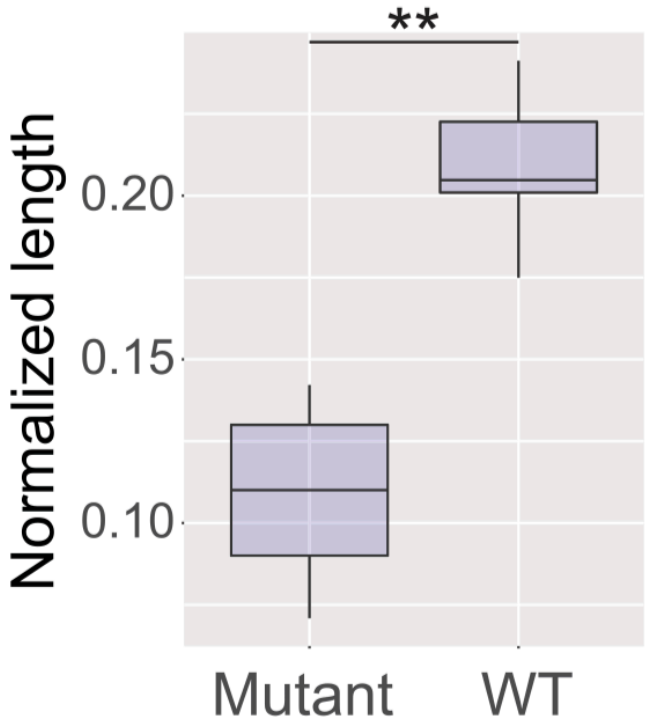
Fin regeneration is significantly delayed in *K-IEN* −/− mutants

- Disruption of *K-IEN* significantly delayed tail regeneration in homozygous mutants compared with wild-type animals



Disruption of *K-IEN*

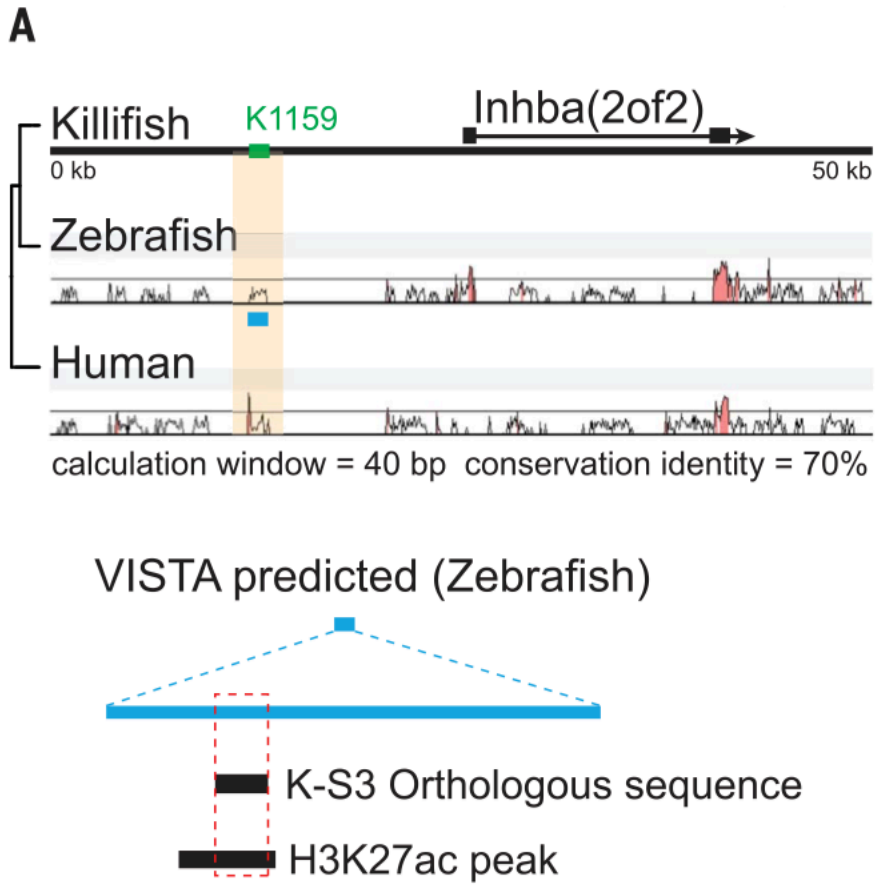


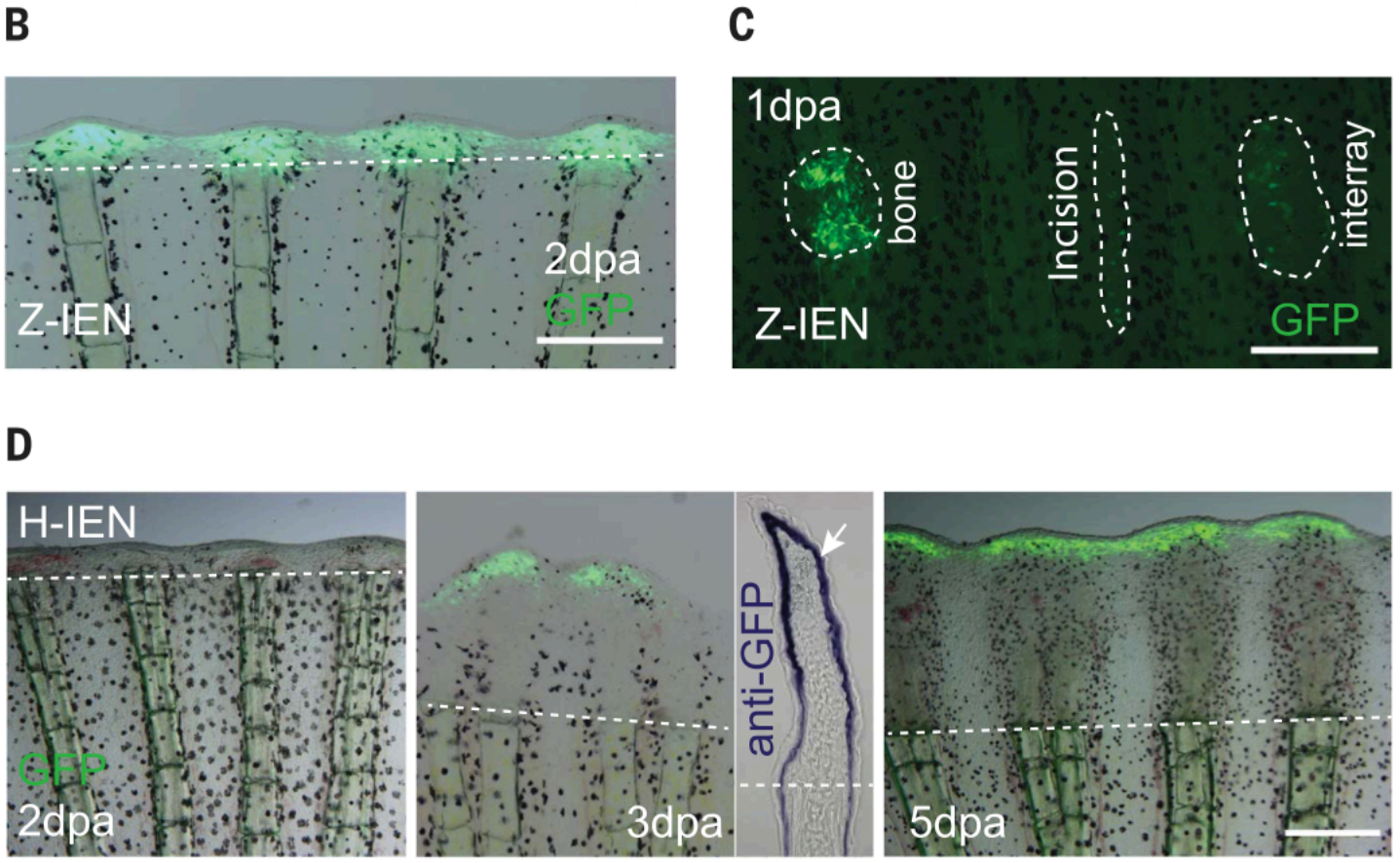


n = 10, ***P* < 0.01

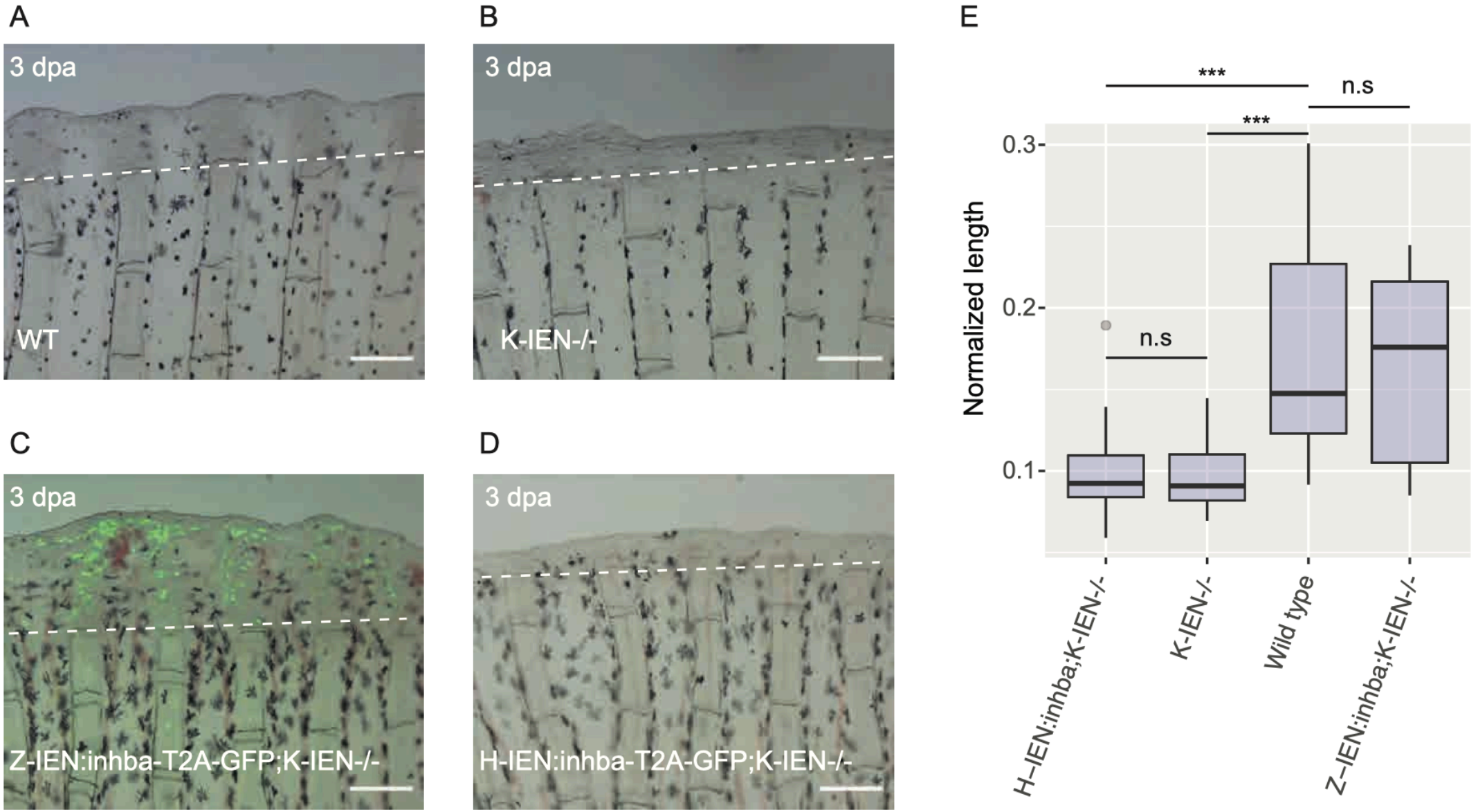
Evolutionary changes of *IEN* in vertebrates

- Multiple sequence alignments detected a relatively conserved noncoding block near the *inhba* loci in killifish, zebrafish, and human
- The expression of GFP directed by the predicted human *inhba* enhancer (*H-IEN*) was barely detectable before 2 dpa but was robustly observed by 3 dpa and persisted to 5 dpa (GFP was detected in the basal epidermal cells)



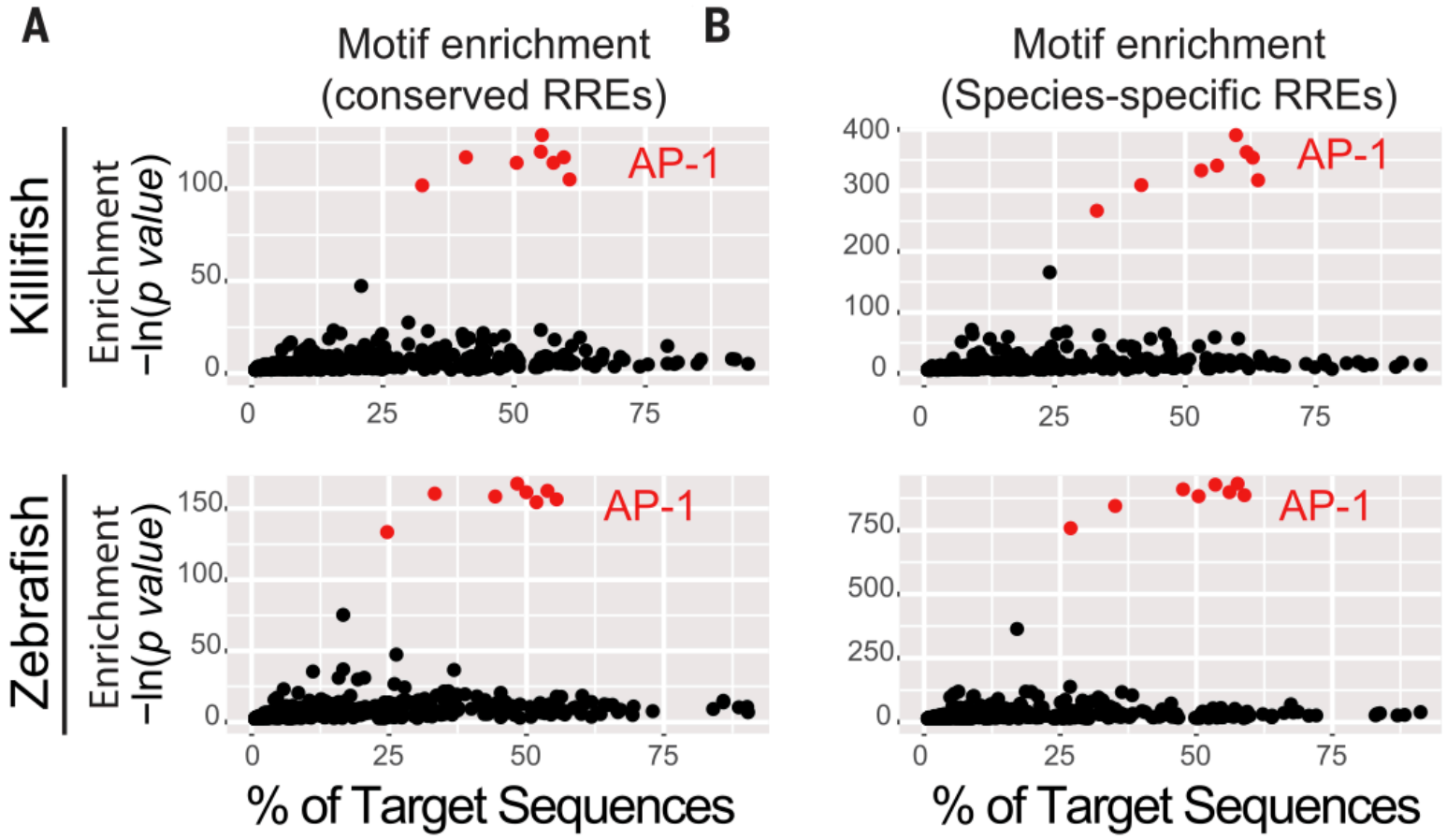


Re-expression of killifish *inhba* driven by *H-IEN cannot* rescue the fin regeneration phenotype in *K-IEN* −/− mutants



Identification of potential regulators

Regeneration-responsive enhancers (RREs) are enriched for AP-1 motifs



C

AP-1 motifs

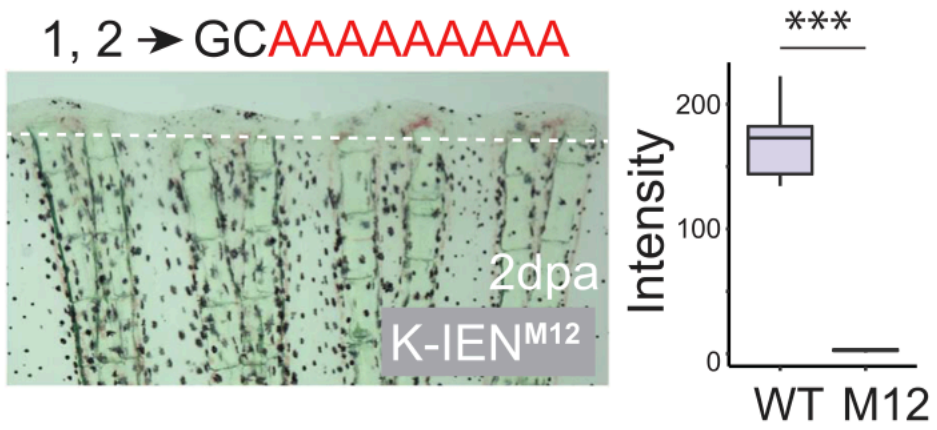
K-IEN
1, GCTGACTCAGA
2, GCTGACTCACT
448 bp

Z-IEN
1, GCTGACTCAT
2, GCTGACTCTA
594 bp

AP-1 motifs are required for the activation of RREs in response to amputation

- The expression of GFP driven by either the *K-IEN*^{M12} – mutated or *Z-IEN*^{M12} –mutated enhancers was completely abolished compared with the original enhancers

A



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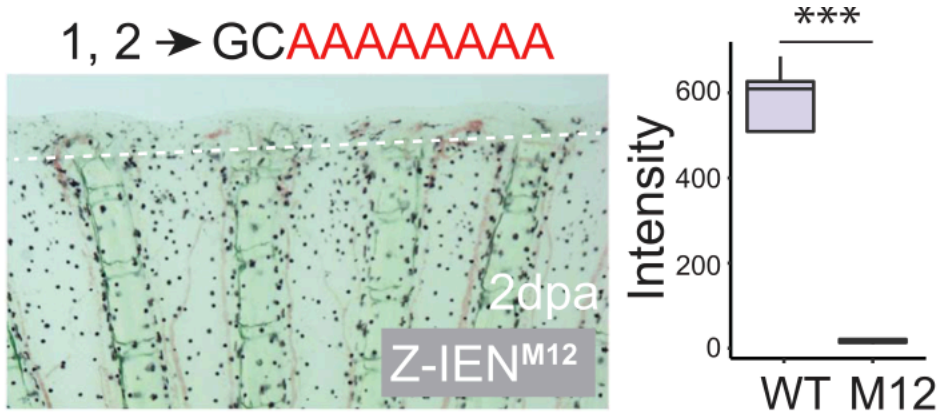
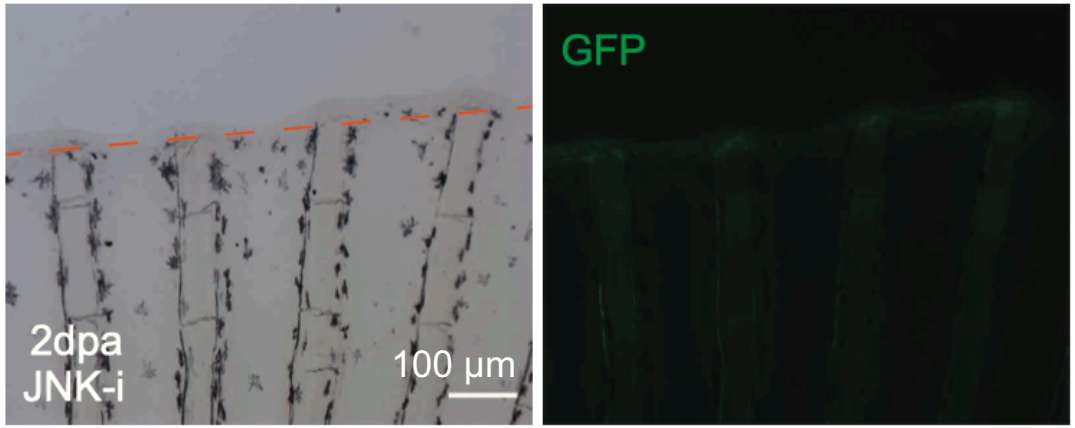


AP-1 motifs are required for the activation of RREs in response to amputation

- Fin regeneration and *K-IEN:GFP* were blocked by inhibiting the activation of AP-1 complex using JNK inhibitor SP600125 compared with the control

A



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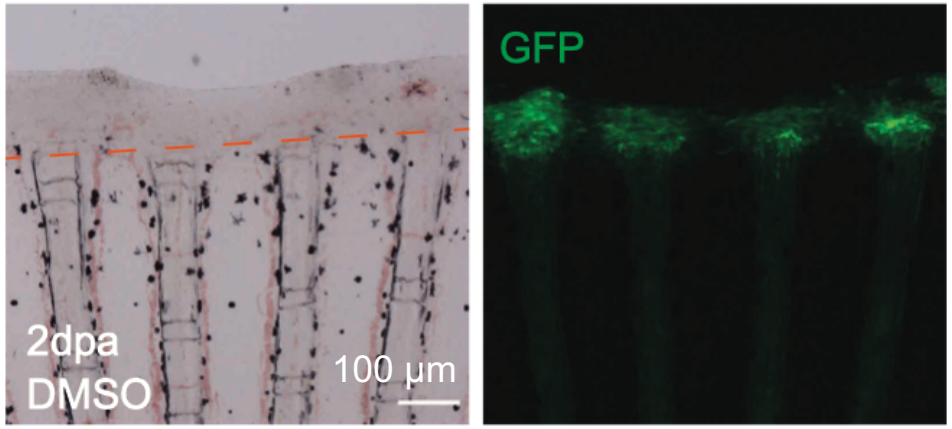
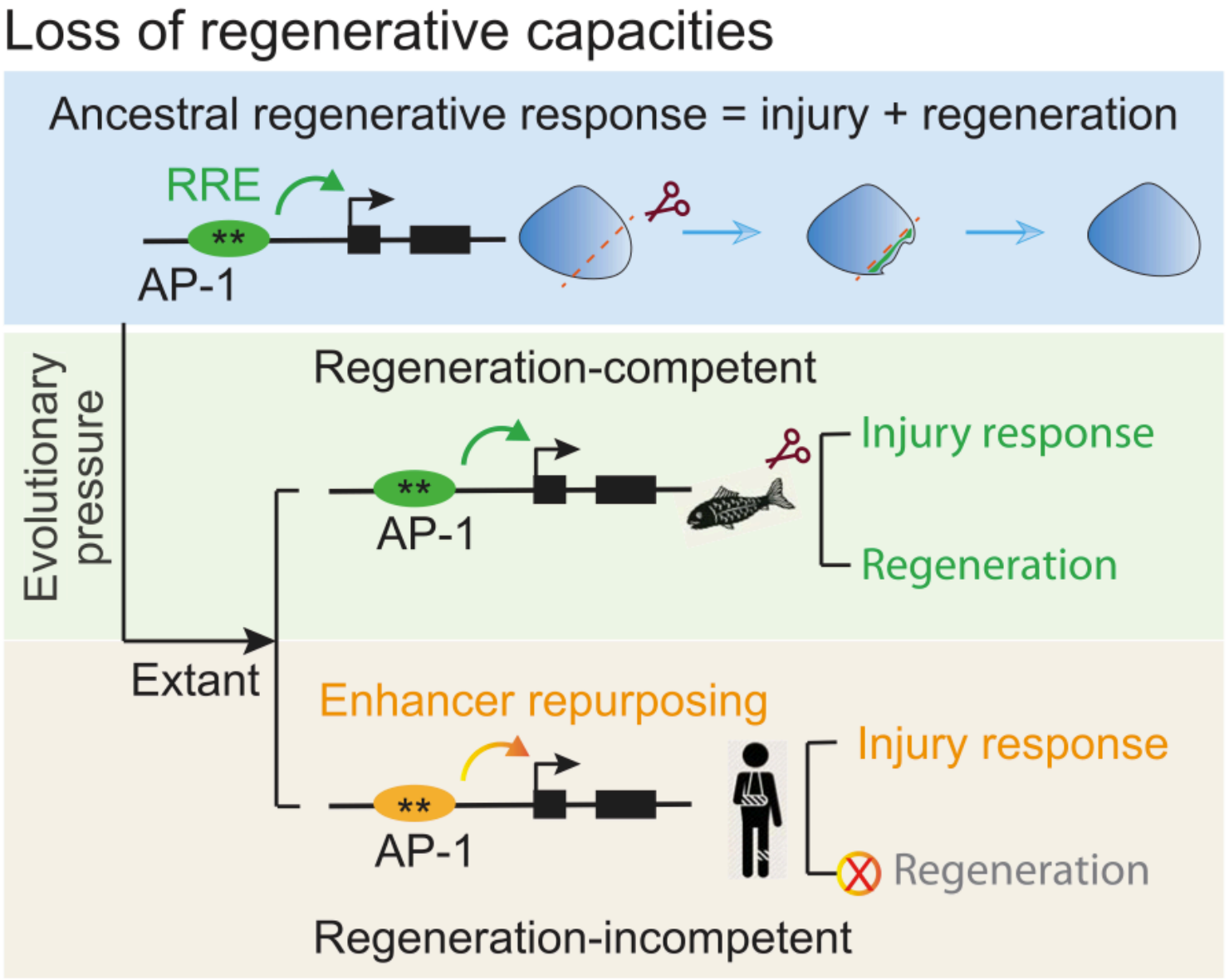


Summary

- The authors identified conserved regeneration-responsive enhancers (RREs), and validated their functions for regeneration
  - Deletion of the killifish *inhba* RRE significantly perturbed caudal fin regeneration
- AP-1 may be required for both injury and regeneration responses
- The function of those identified RREs has diversified in human during evolution

A comparative study of two fish species offers important clues about vertebrate regeneration



Thinking

Why were regeneration capacities lost during evolution?

Is there any potential side effect?

Thank you!

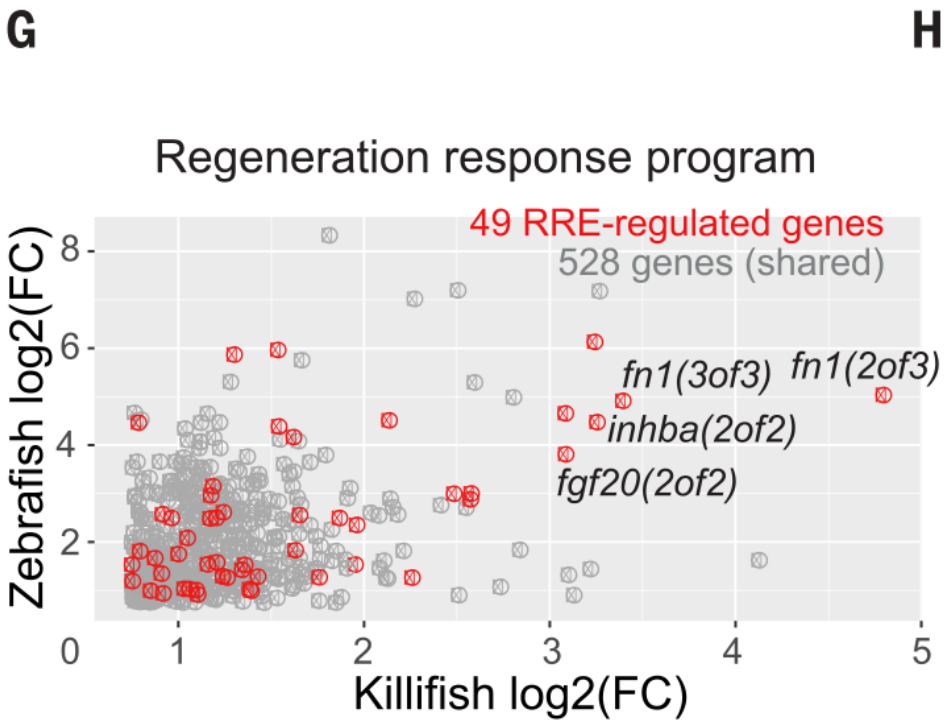
Discussion

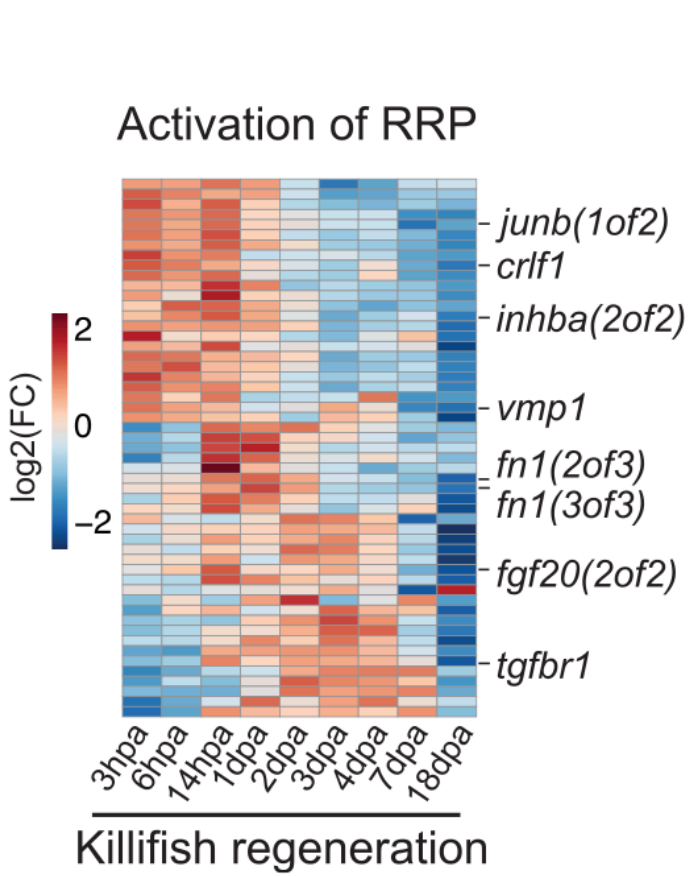
Thinking

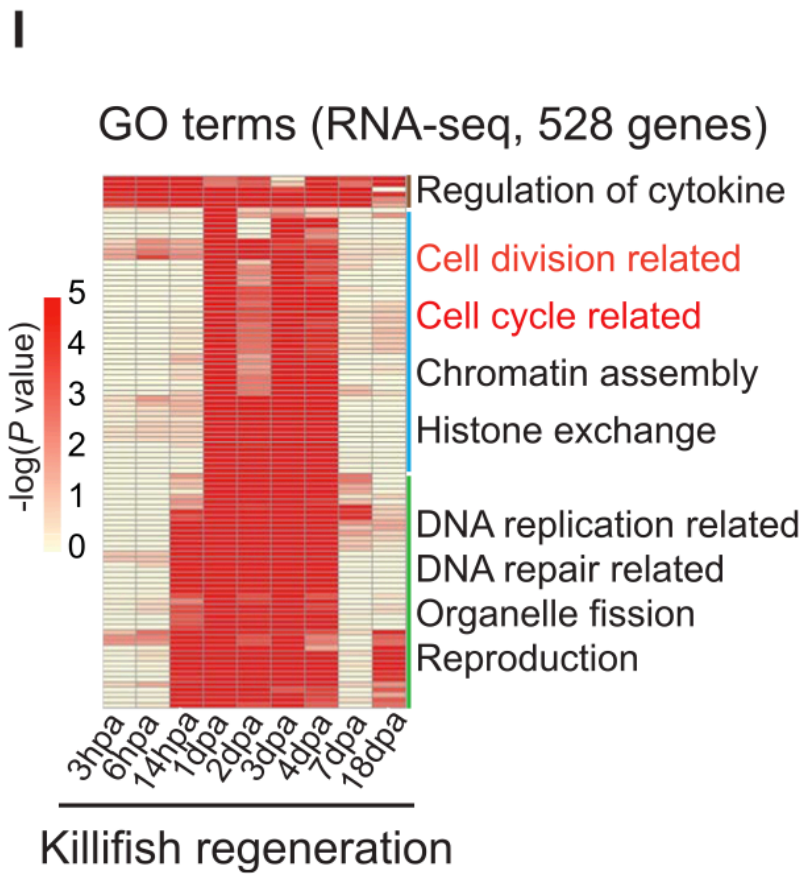
This study lacks direct evidence for the regulatory relationship between enhancer and gene.

Identification of a conserved regeneration response program (49 genes)

- Shared 49 genes: H3K27ac-defined RREs, H3K4me3-marked active promoters, and elevated gene expression
- Putative new regulators: e.g., *crlf1*, *vmp1*, and *tgfbr1*

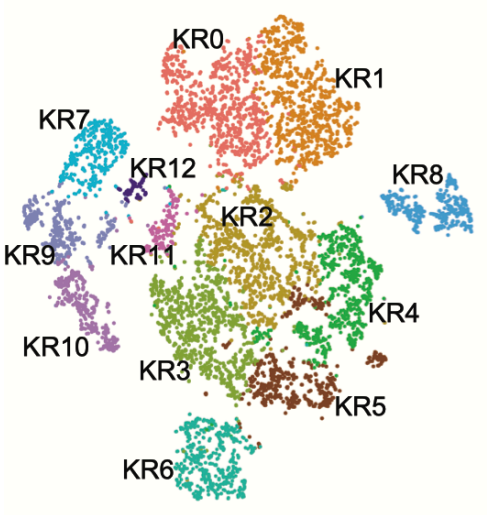




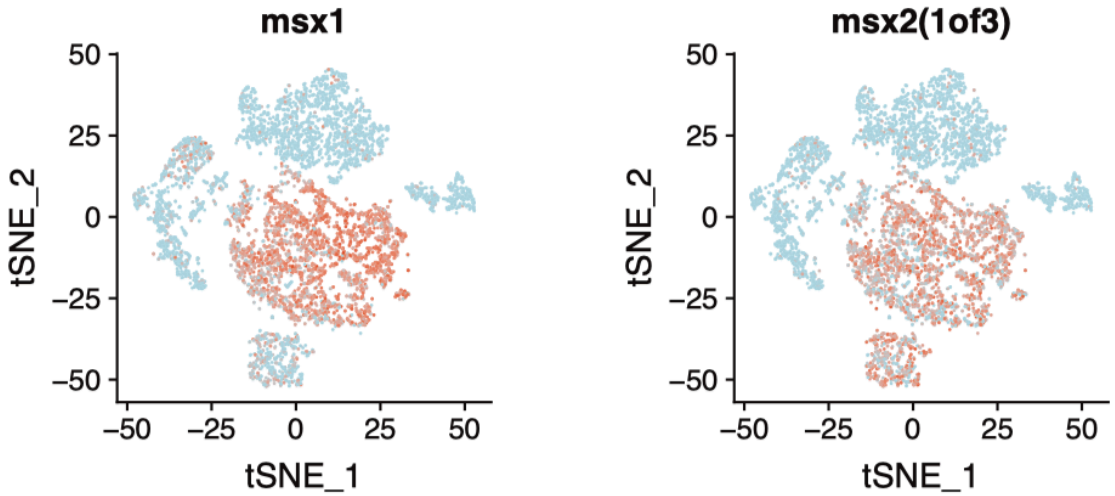


The blastema cell clusters were de-fined by the known blastema markers *msx* homeobox genes

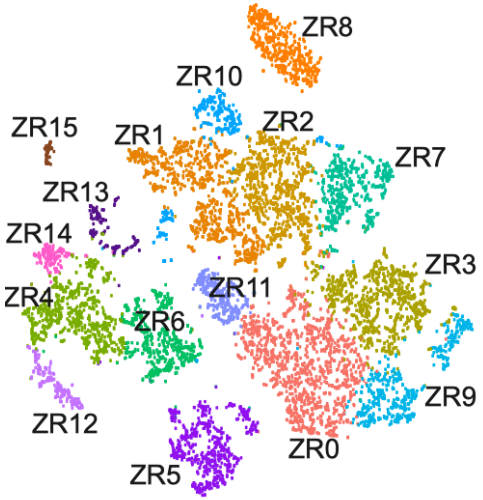
Killifish



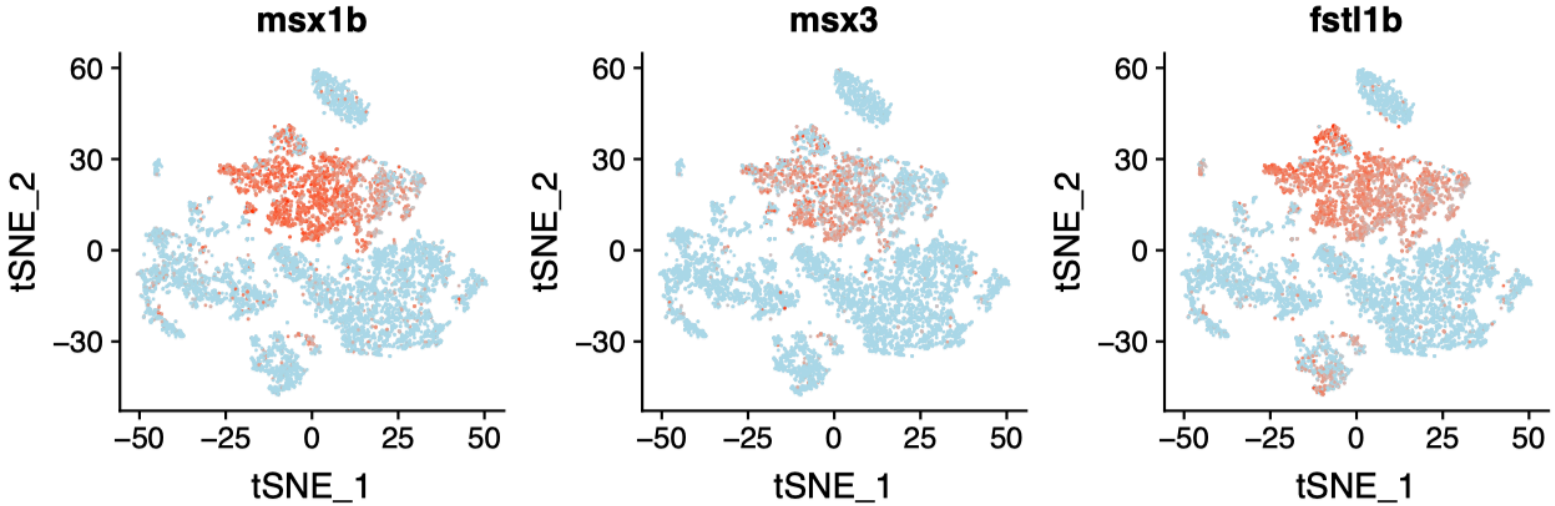
Blastema cells (KR2, 3, 4, 5, 6, 11)



Zebrafish

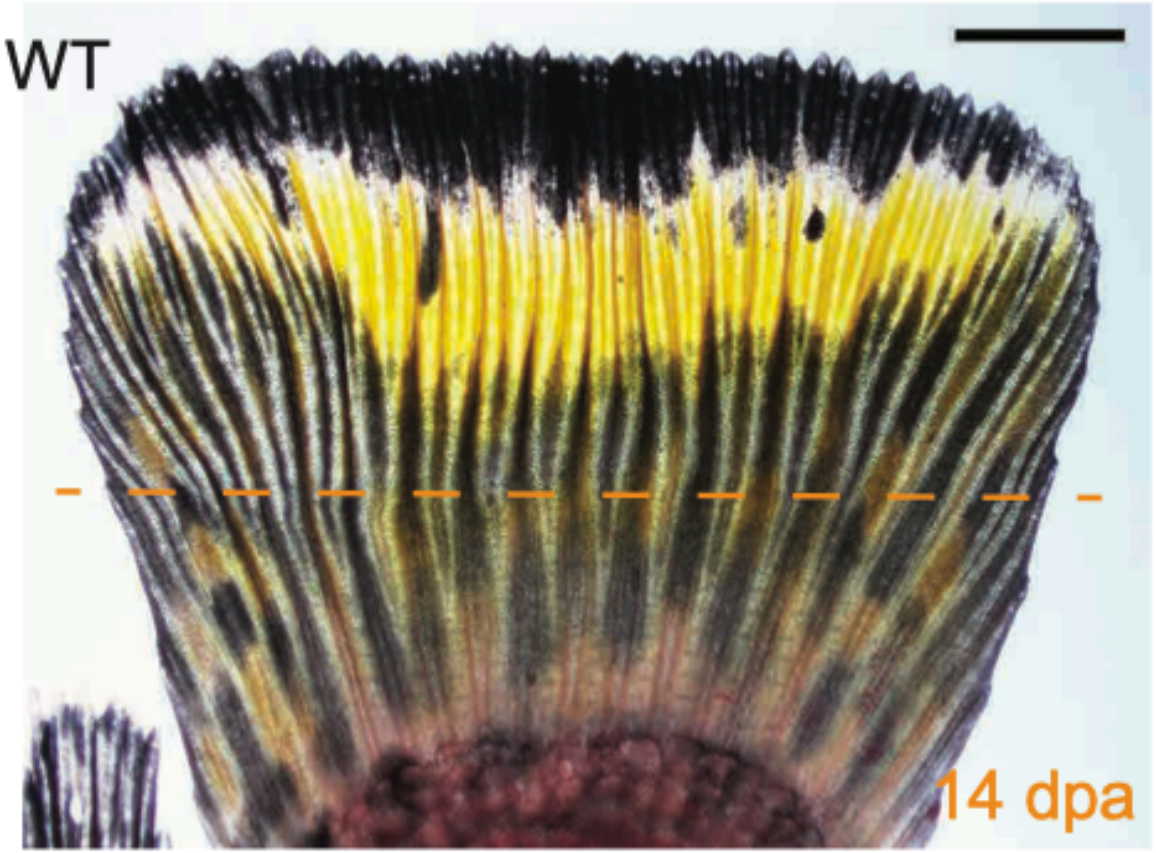


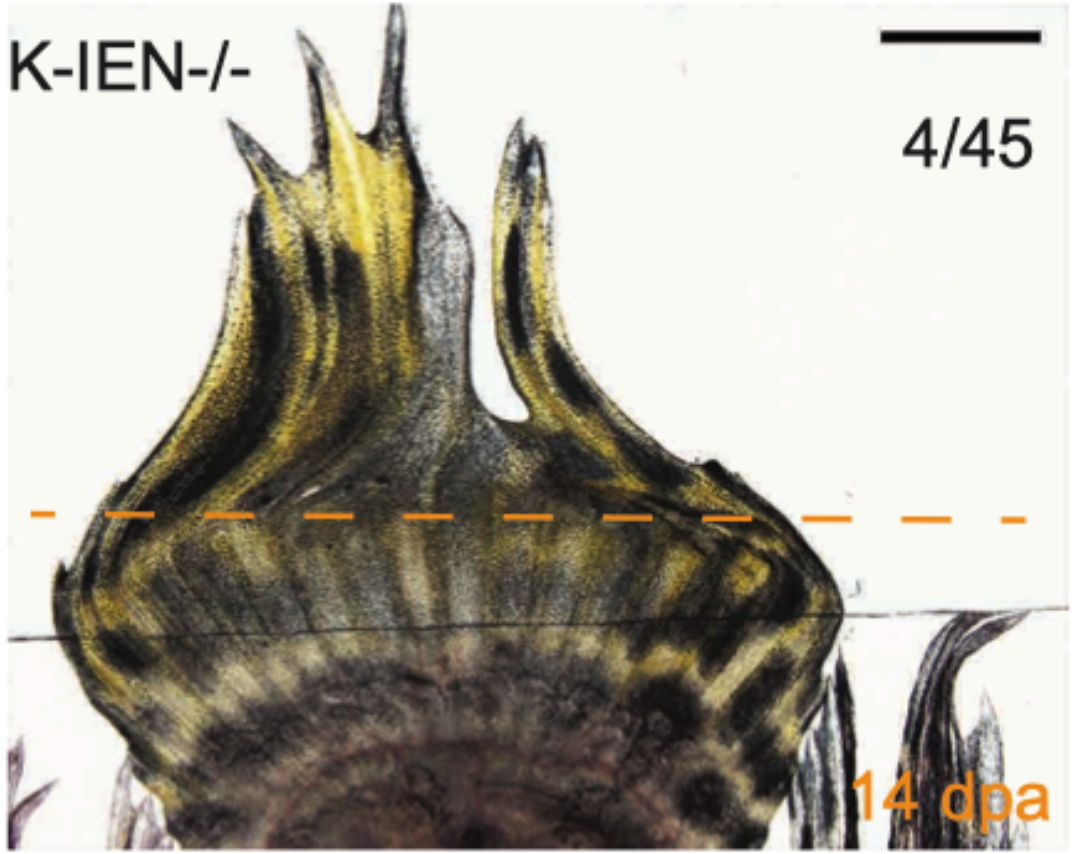
Blastema cells (ZR1, 2, 7, 10)



Fin regeneration is significantly delayed in *K-IEN −/− mutants*

- The regenerated caudal fin in a small percentage of *K-IEN -/-* mutants (4/45) is deformed compared with wild type animals





Regeneration-responsive enhancers (RREs) are enriched for AP-1 motifs

- The AP-1 complex is a heterodimer composed of members from different families of DNA-binding proteins, including the Jun, Fos, ATF, JDP, and Maf families

Enriched motifs in shared RREs

Killifish

Name	Motif	P-value	Presence
JunB		1e-55	54.61%
Fosl1		1e-51	54.39%
Fosl2		1e-50	40.13%
Atf3		1e-49	58.77%
BATF		1e-48	56.80%
AP-1		1e-44	59.87%

Zebrafish

Name	Motif	P-value	Presence
JunB		1e-71	47.67%
Atf3		1e-69	53.17%
Fosl1		1e-69	49.33%
Fosl2		1e-68	32.67%
AP-1		1e-66	54.83%
BATF		1e-65	51.17%

Enriched motifs in species-specific RREs

Killifish

Name	Motif	P-value	Presence
Fosl1		1e-167	59.02%
BATF		1e-155	61.05%
Atf3		1e-151	62.23%
JunB		1e-145	55.40%
Fosl2		1e-141	52.36%
AP-1		1e-135	63.24%

Zebrafish

Name	Motif	P-value	Presence
Atf3		1e-398	56.93%
Fosl1		1e-397	52.85%
Fosl2		1e-388	46.92%
BATF		1e-384	55.44%
AP-1		1e-378	58.19%
JunB		1e-377	49.77%