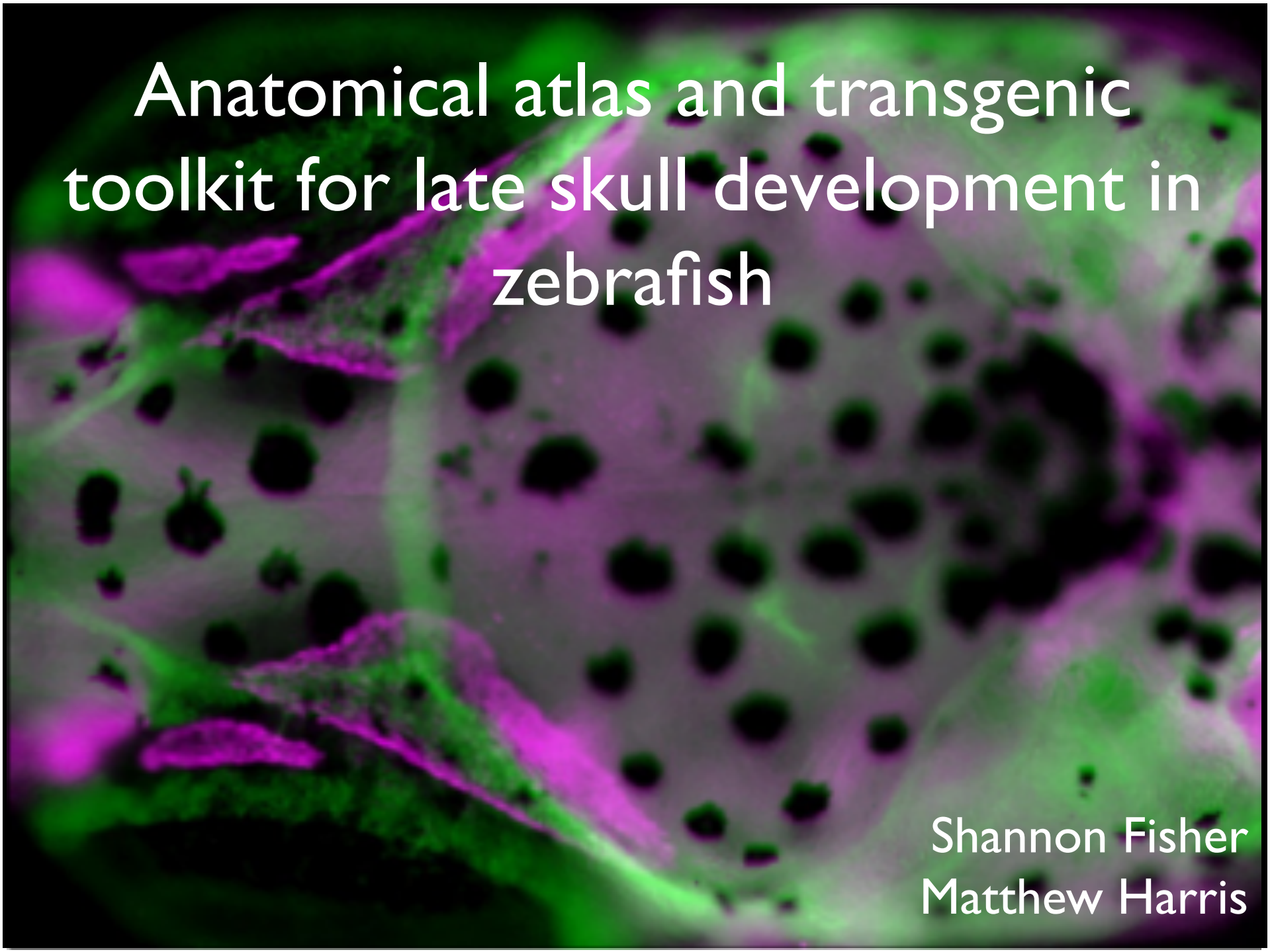
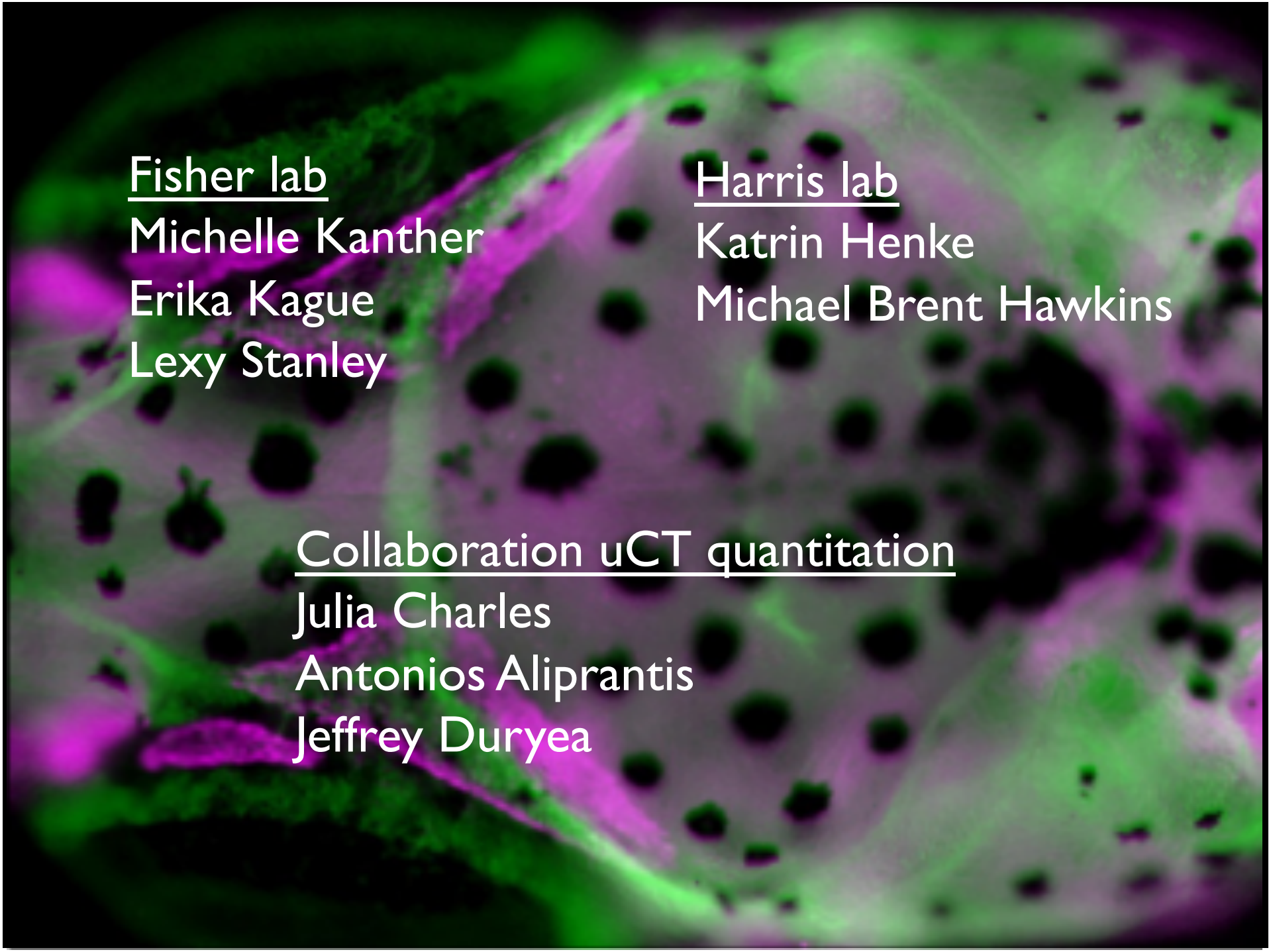

Anatomical atlas and transgenic toolkit for late skull development in zebrafish
Shannon Fisher
Matthew Harris

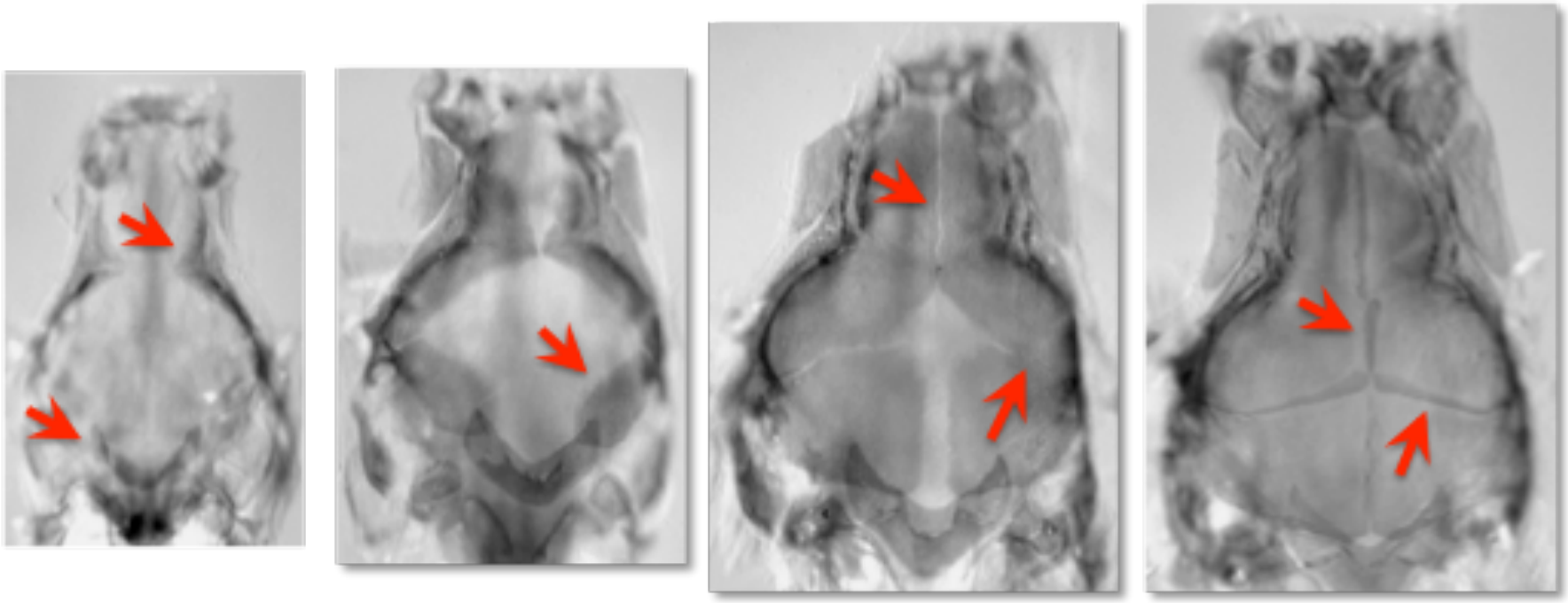

Fisher lab
Michelle Kanther
Erika Kague
Lexy Stanley
Harris lab
Katrin Henke
Michael Brent Hawkins
Collaboration uCT quantitation
Julia Charles
Antonios Aliprantis
Jeffrey Duryea

How do you build a fish skull?

Integrated approach to describe zebrafish skull formation

- Anatomic and molecular description of normal process
- Comparison to mutants
- Comparison to other species

Post-larval development of the cranial vault

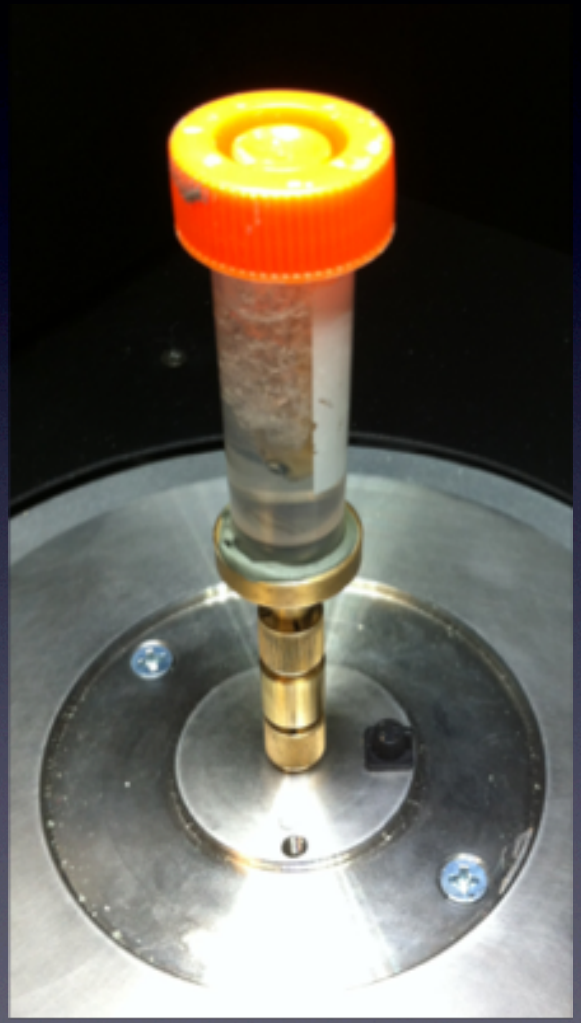


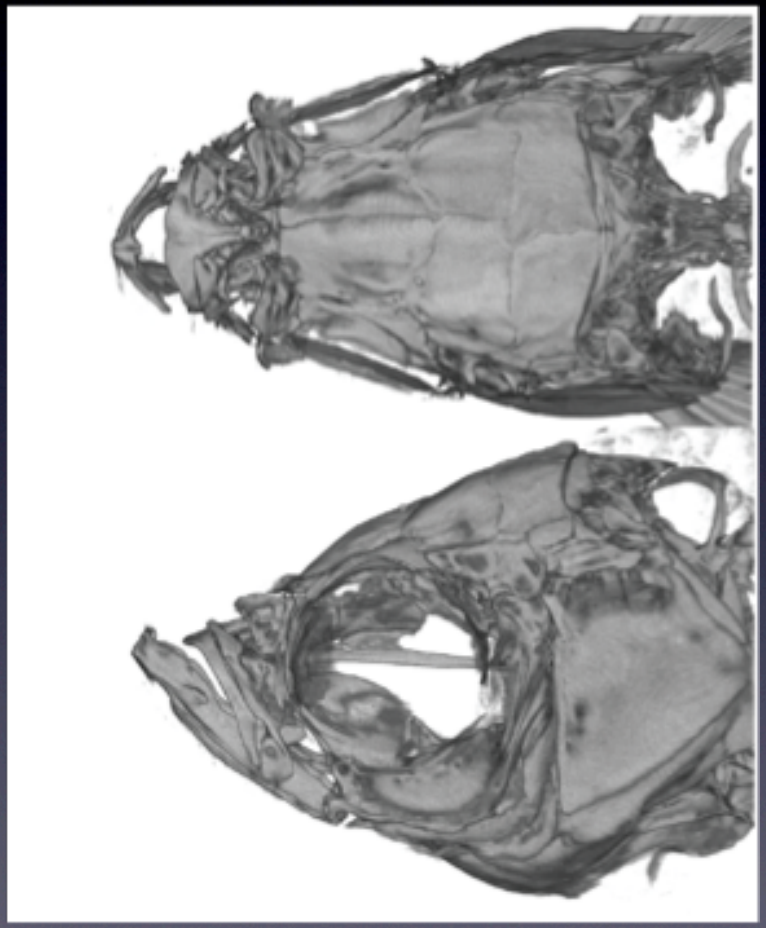
6.5 mm SL 9 mm SL 11 mm SL 13.5 mm SL

SL = standard length

6.5 mm ~ 2.5-3 weeks, 13.5 mm ~ 6 weeks

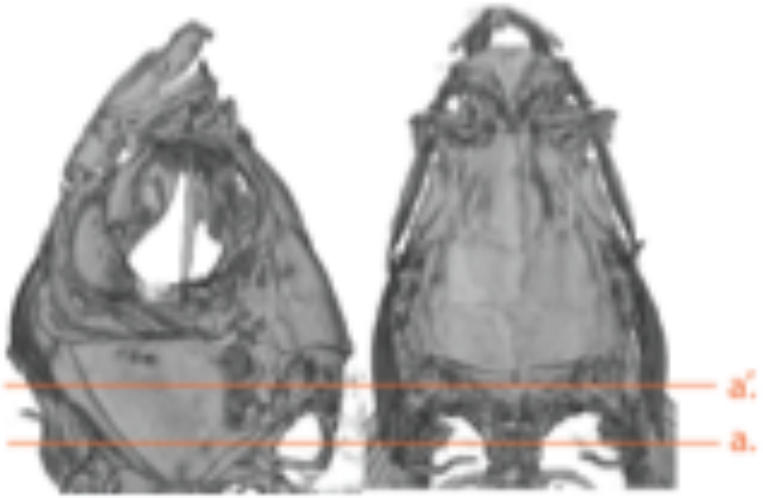
Detailed archived anatomy of the adult zebrafish skull: microcomputed tomography (μCT)

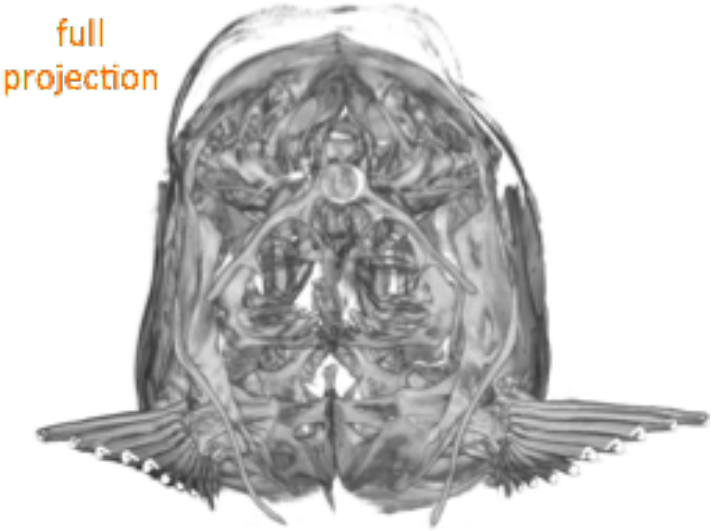


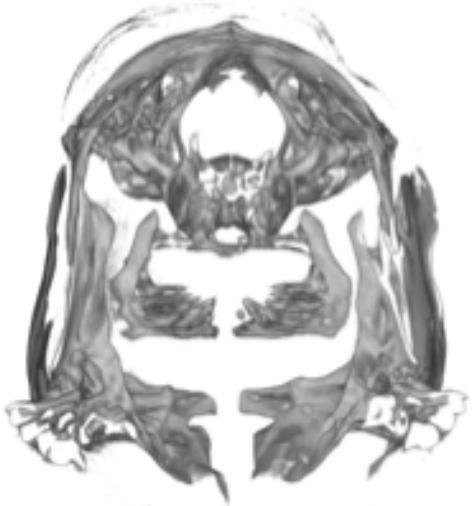


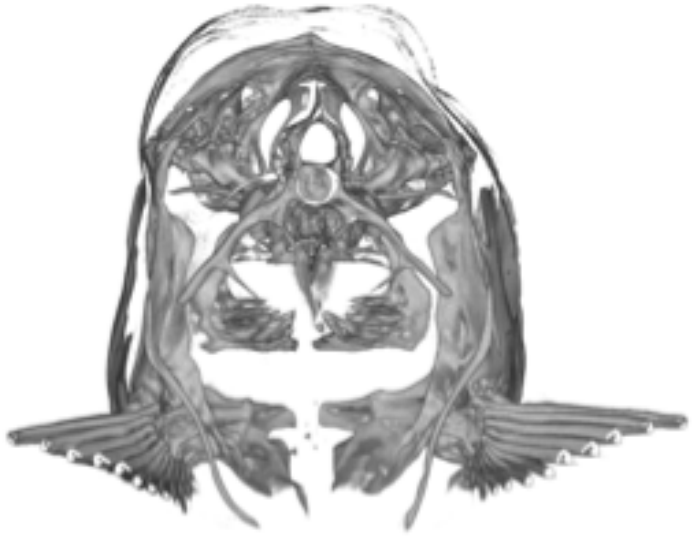
Visualization of complex form

Ability to dissect complex anatomy *in silico*



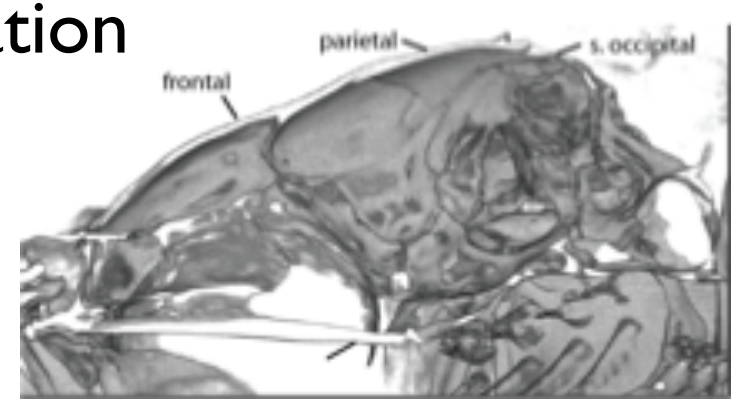


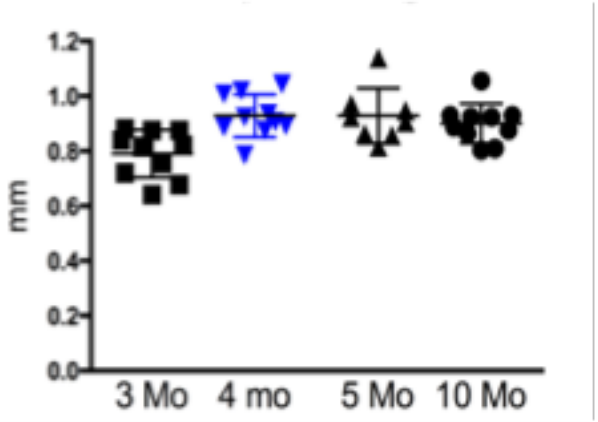


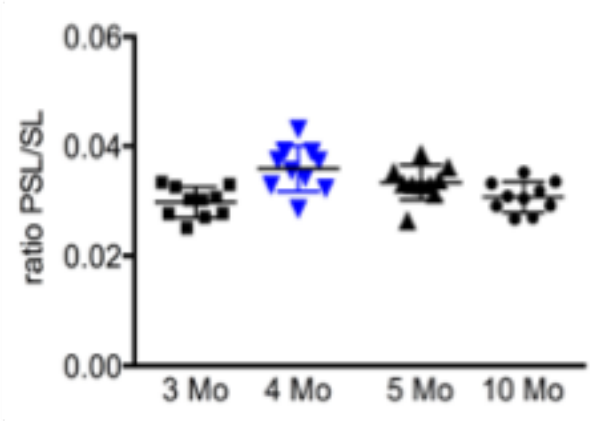


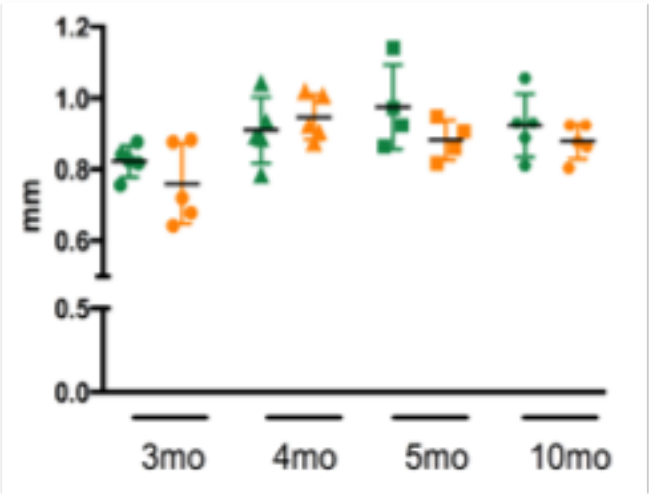
Standardization to minimize variation

Effects of rearing, age, and genetic variation



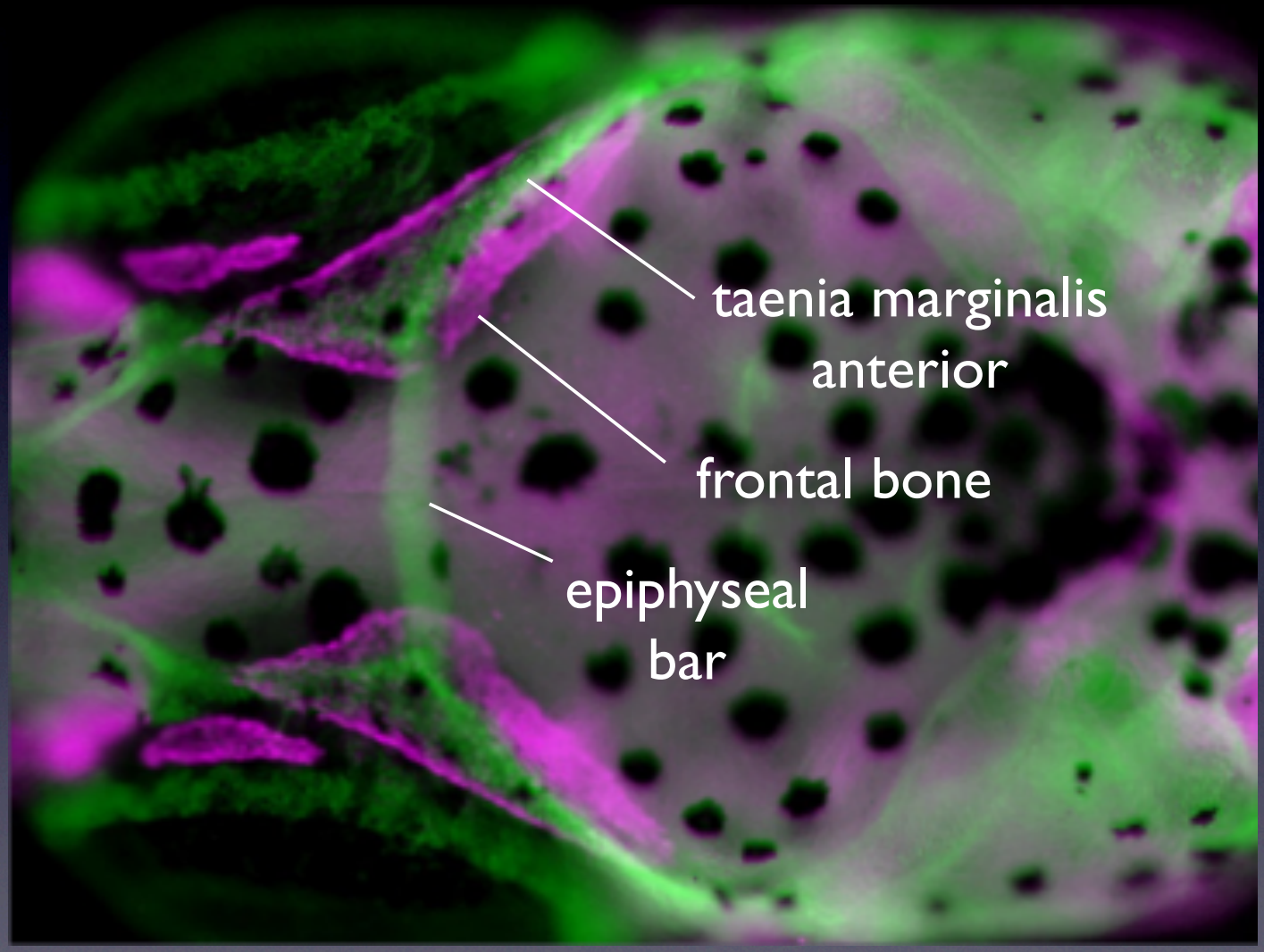






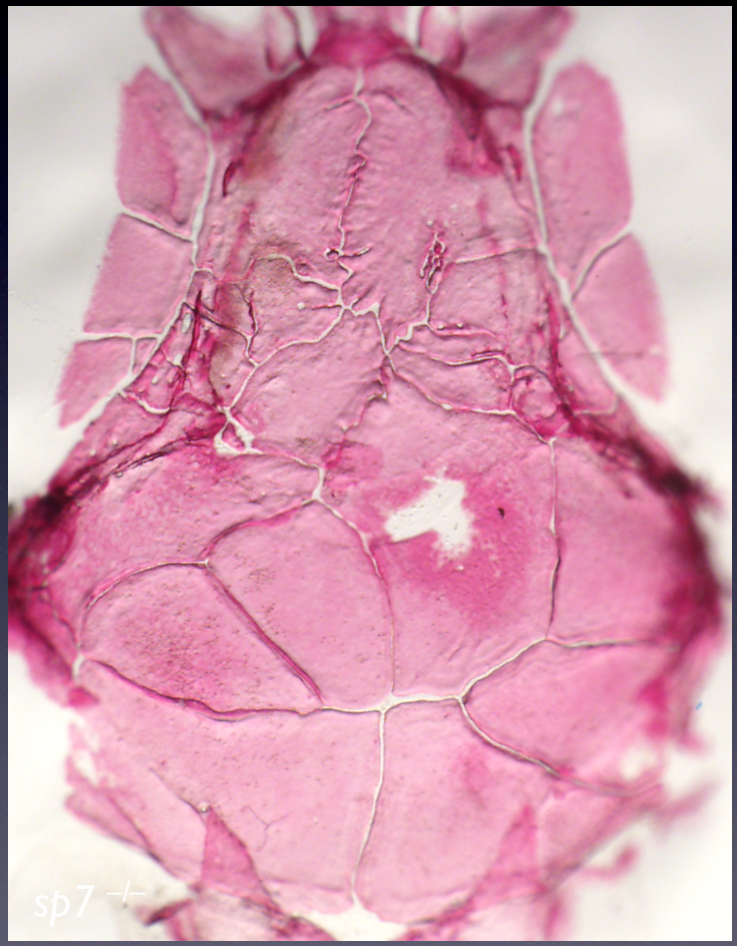
Julia Charles
Katrin Henke

Dynamic imaging of the post-larval skeleton

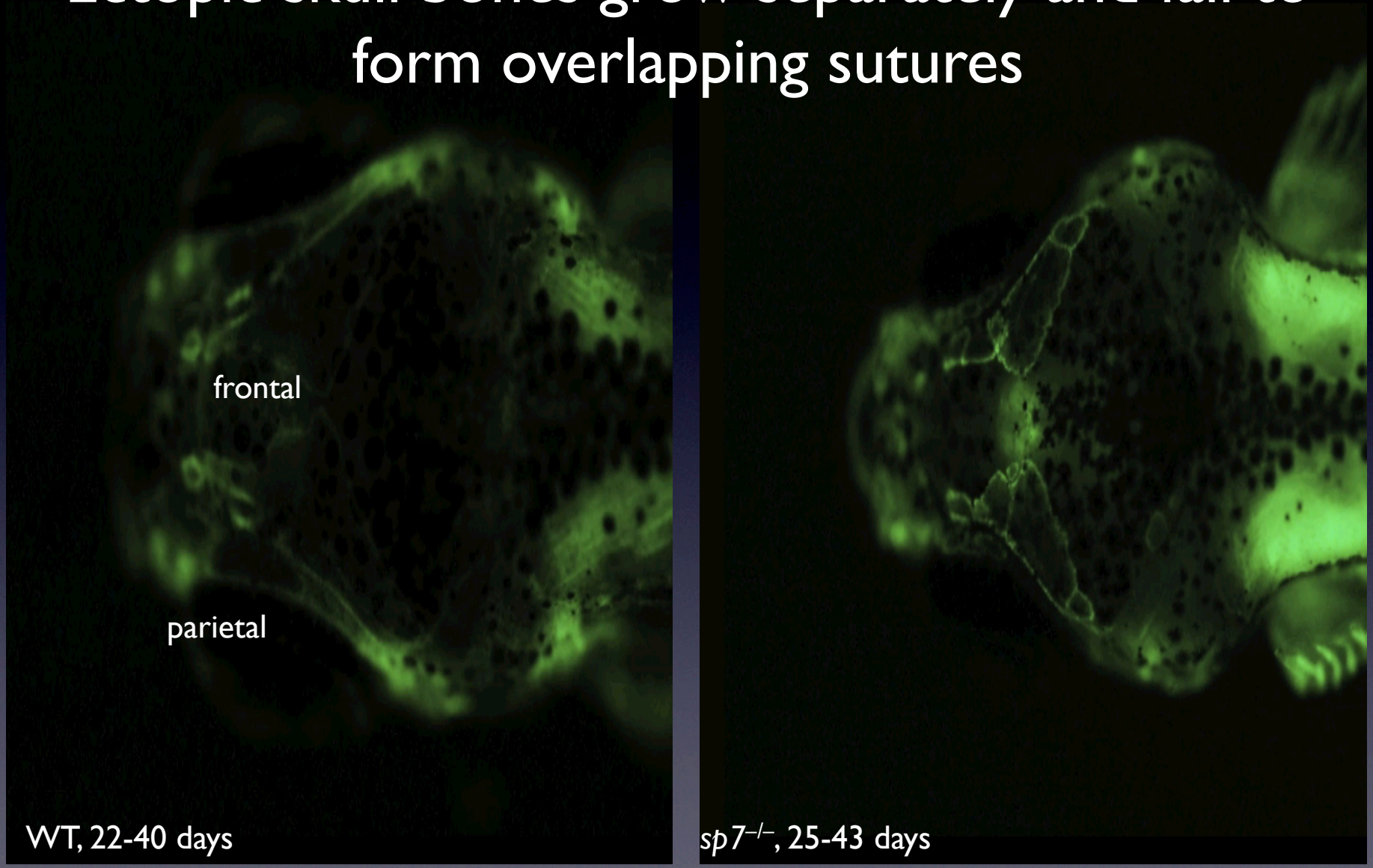


col1a1:egfp *sp7:mcherry*

Loss of general osteoblast gene *sp7* causes severe skull patterning defects



Ectopic skull bones grow separately and fail to form overlapping sutures



RUNX2:egfp (early osteoblasts)

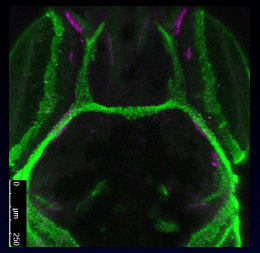
Erika Kague

Leica TSI LCS macro confocal

Sequential confocal imaging of skull formation

Michelle Kanther

col1a1:egfp *sp7:mcherry*



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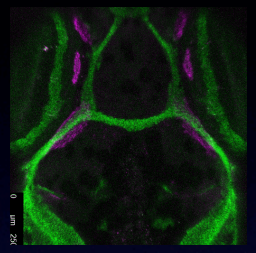

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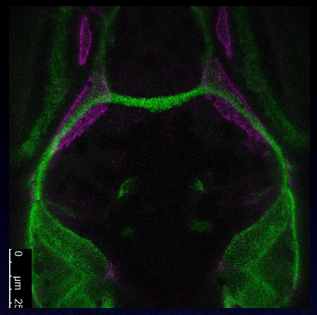

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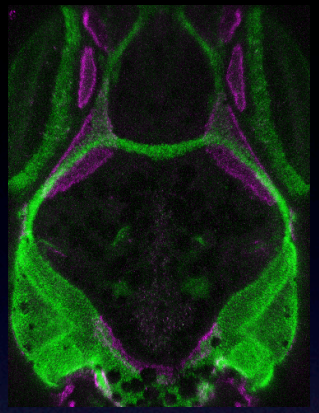

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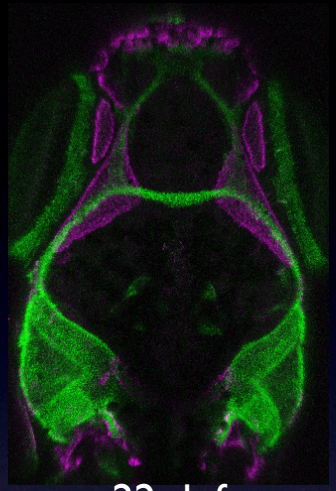

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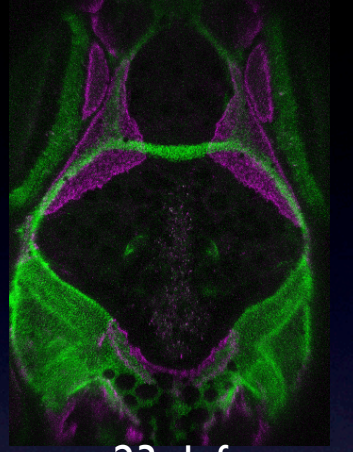

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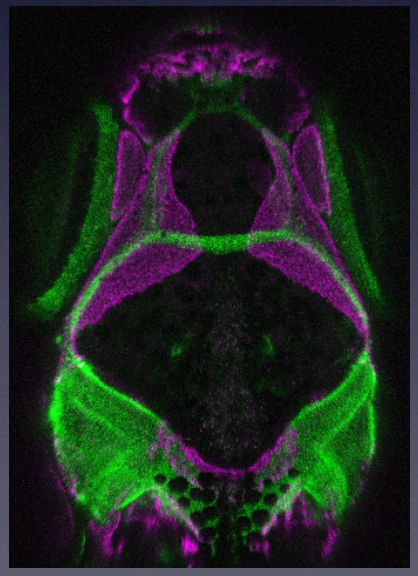

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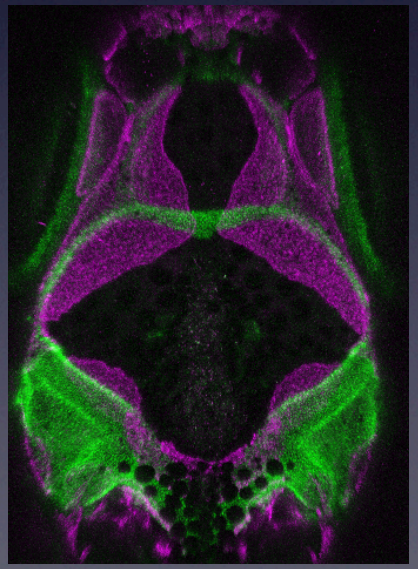

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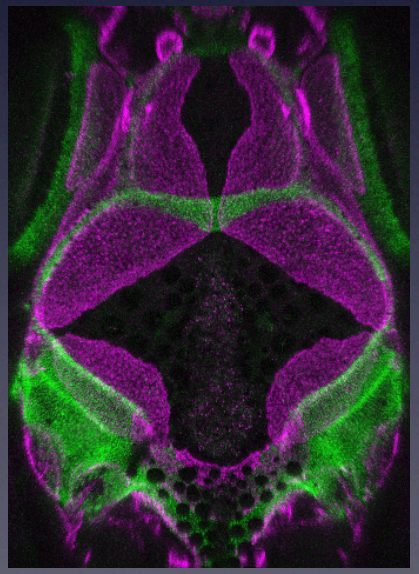

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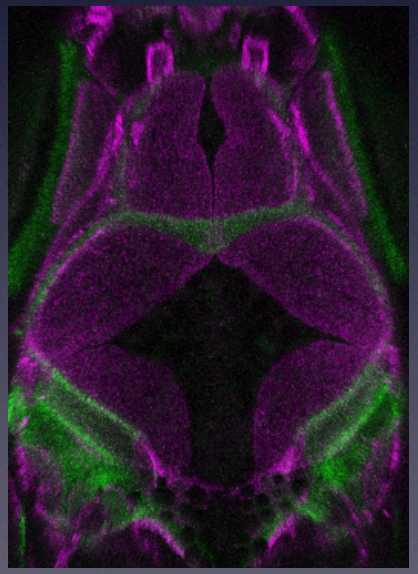

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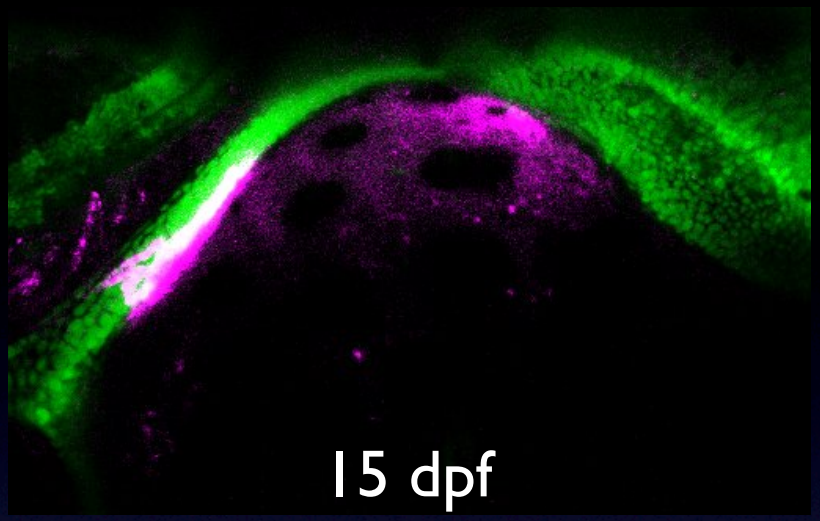
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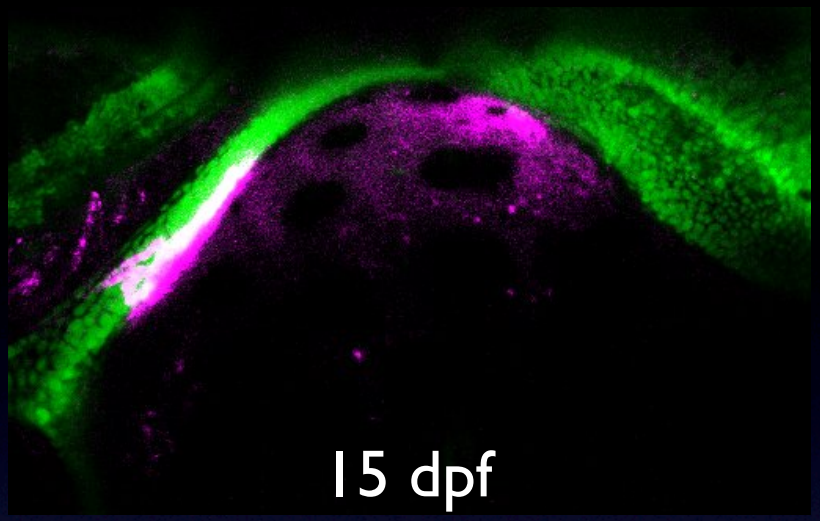
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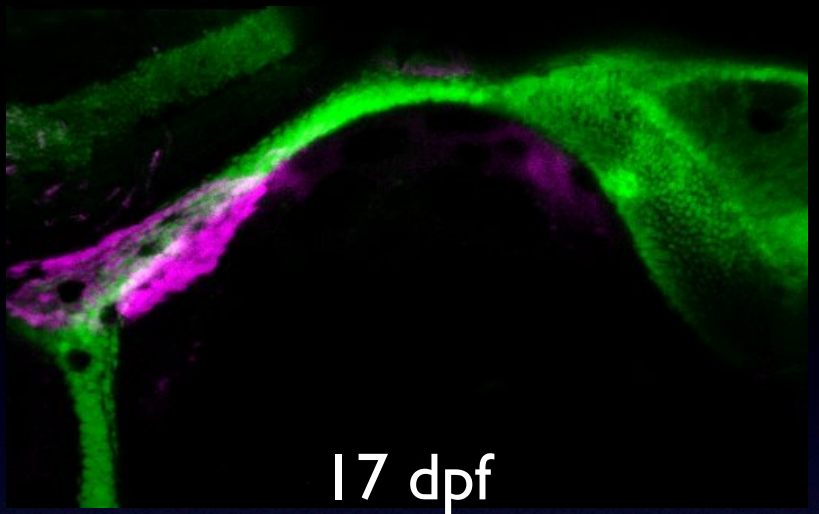
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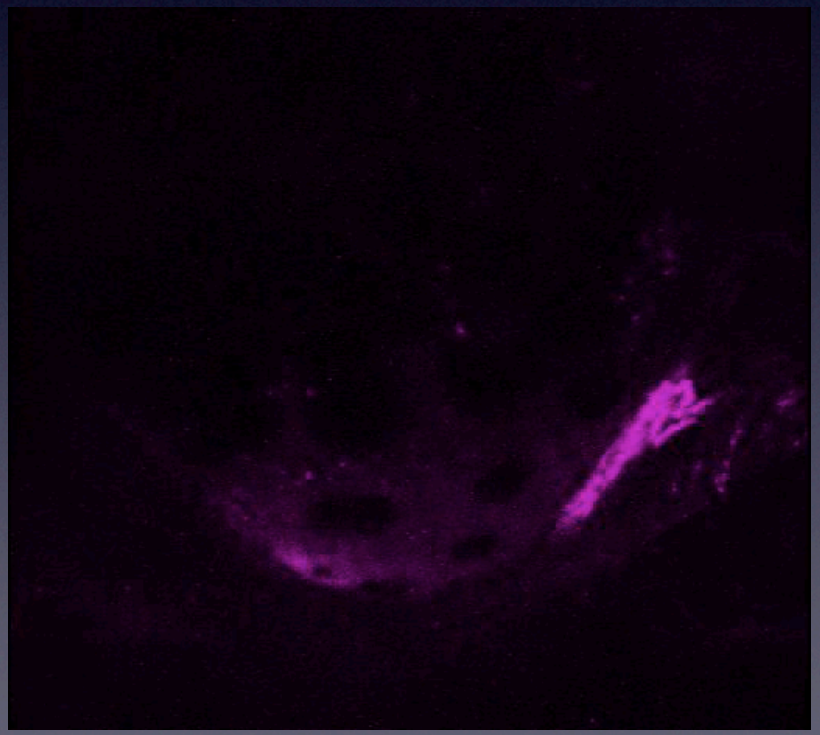

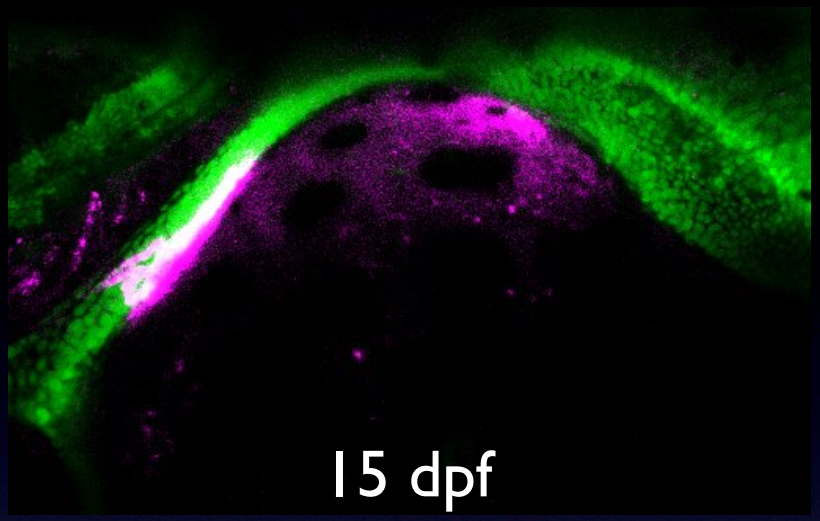
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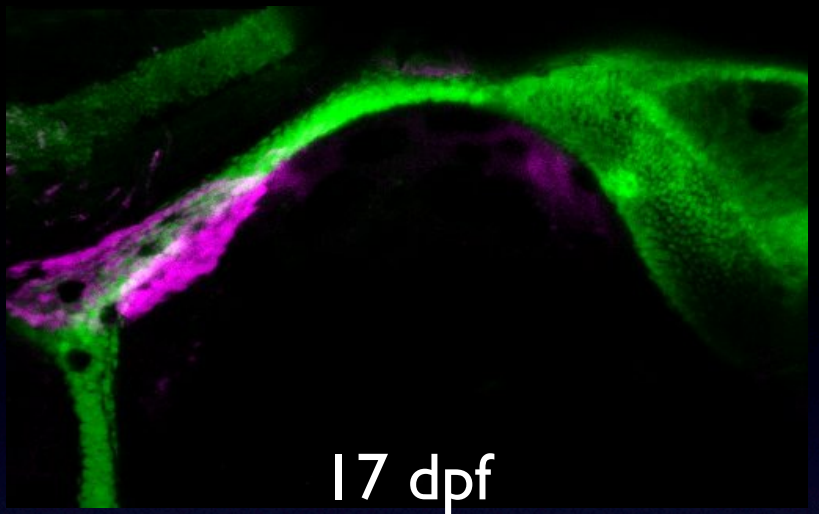
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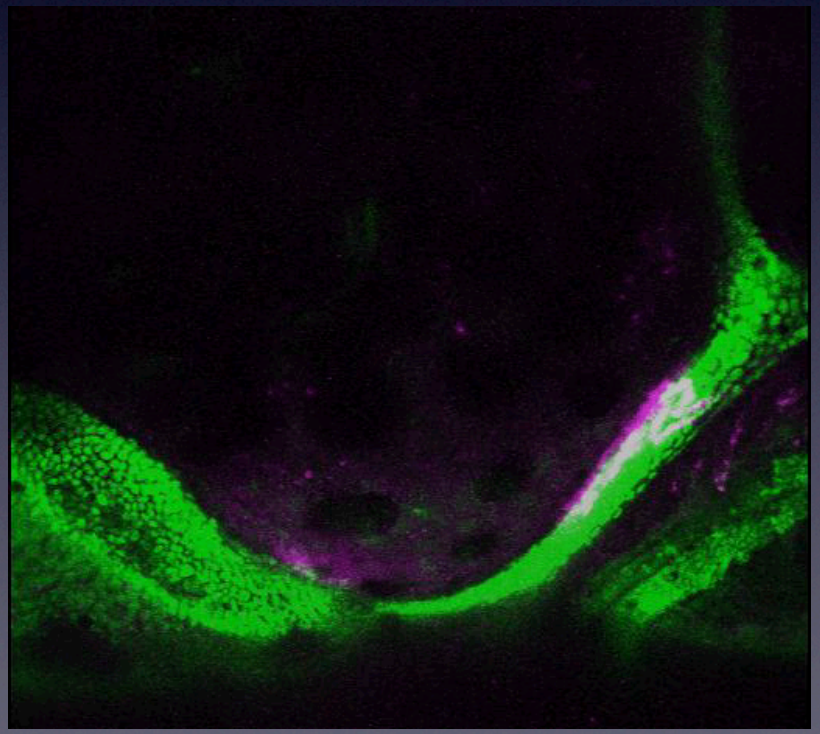
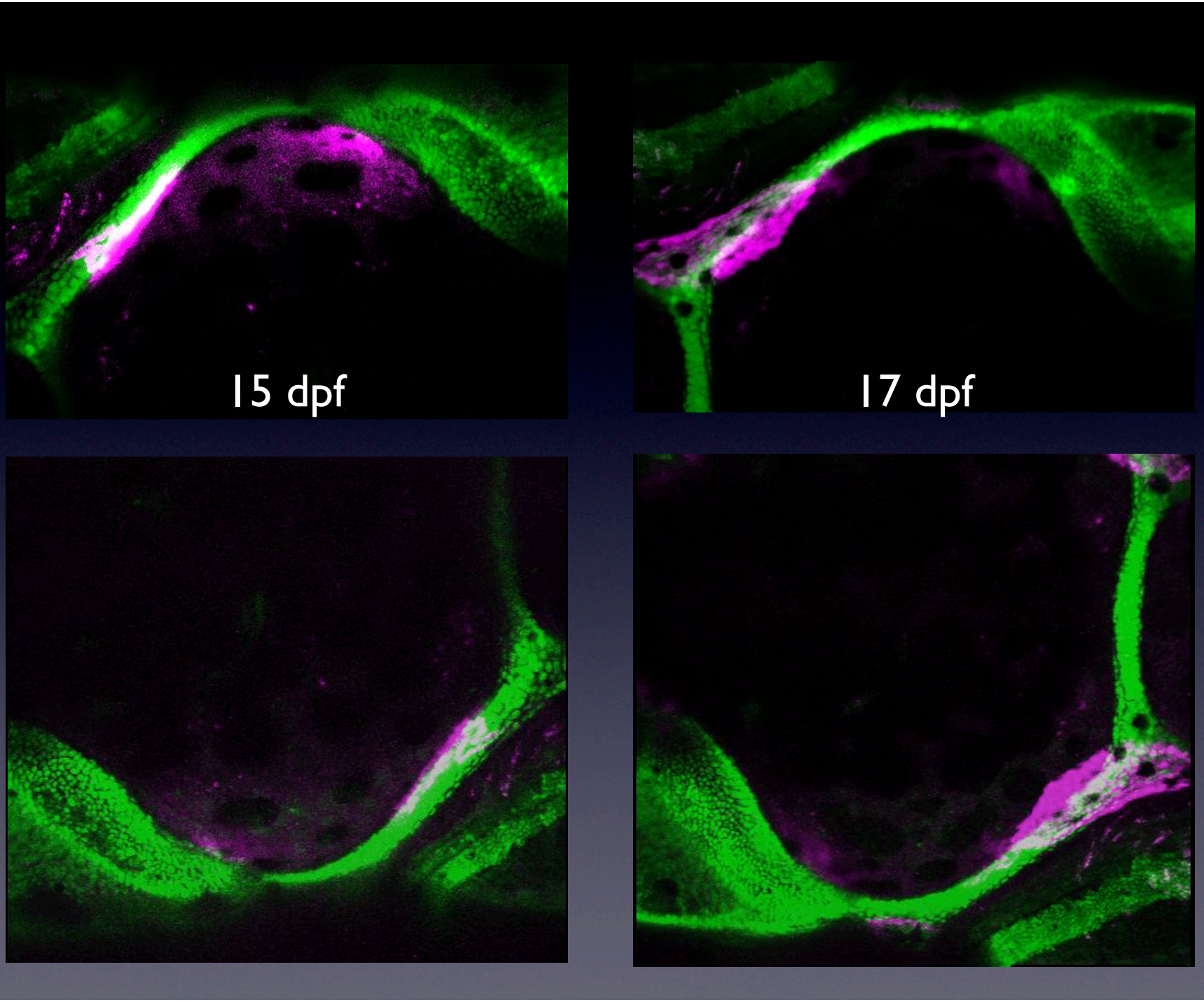
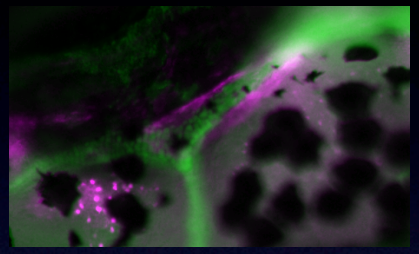
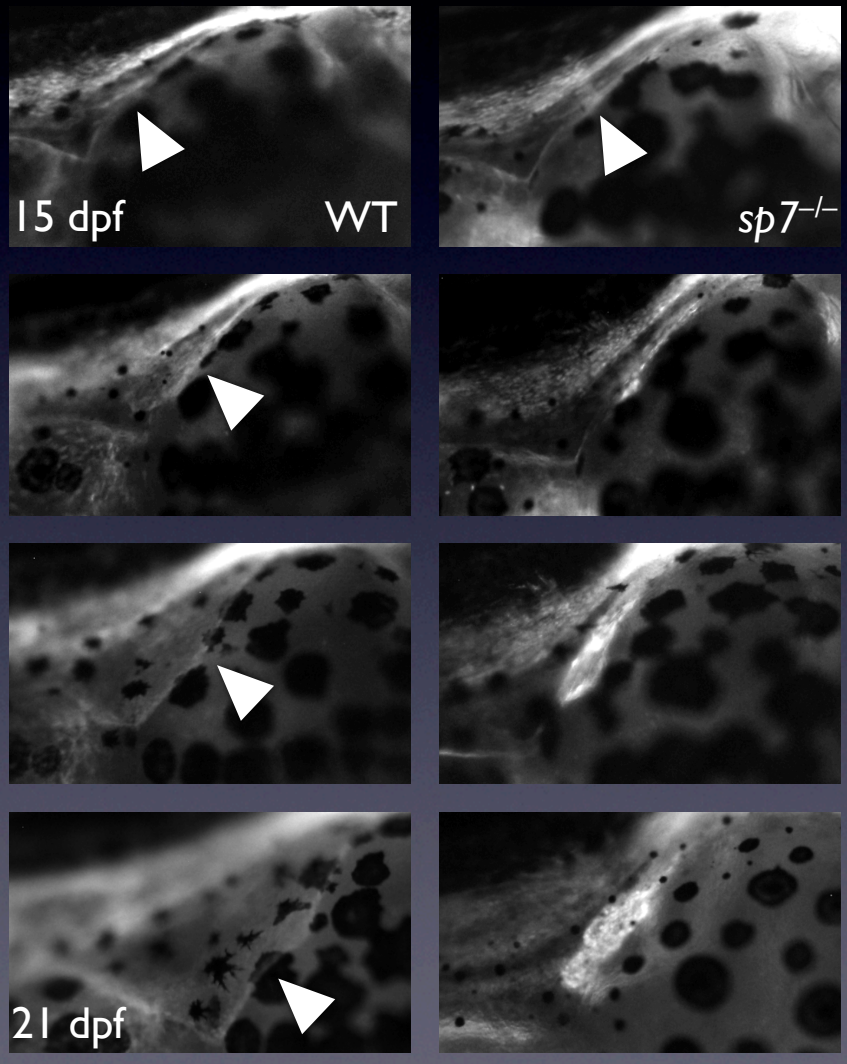



15 dpf
17 dpf

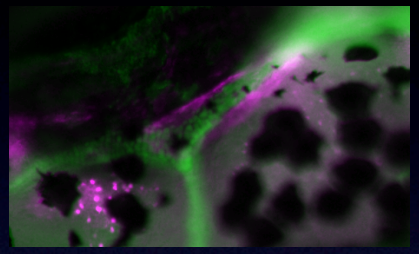
Ectopic bones form shortly after frontal bone initiation



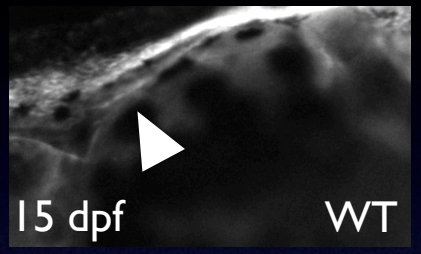
col1a1:egfp
RUNX2:mcherry

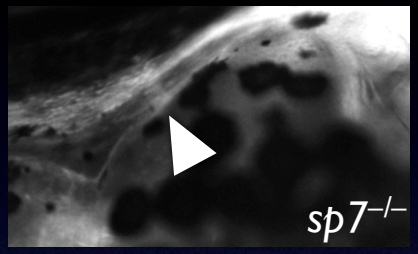


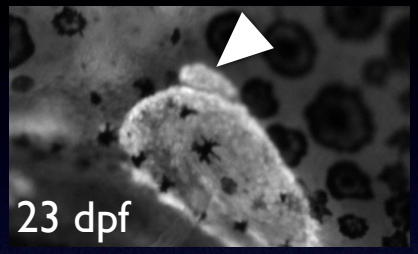
Ectopic bones form shortly after frontal bone initiation

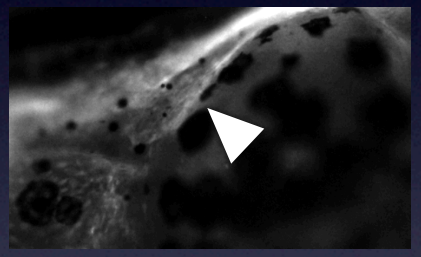


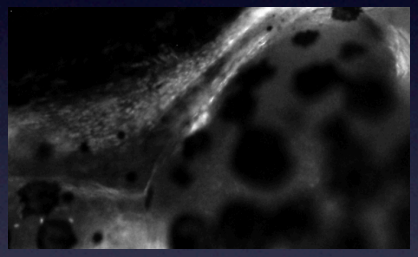
col1a1:egfp
RUNX2:mcherry

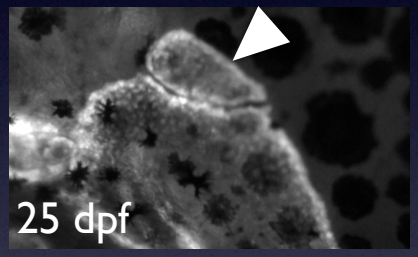


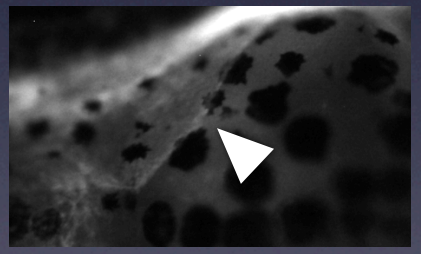


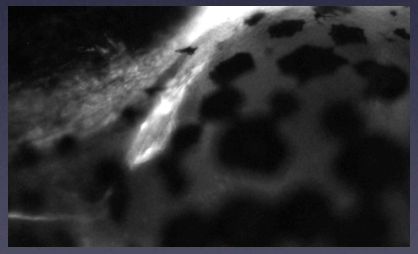


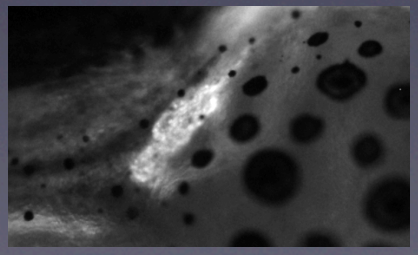




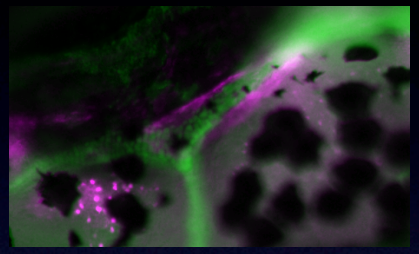




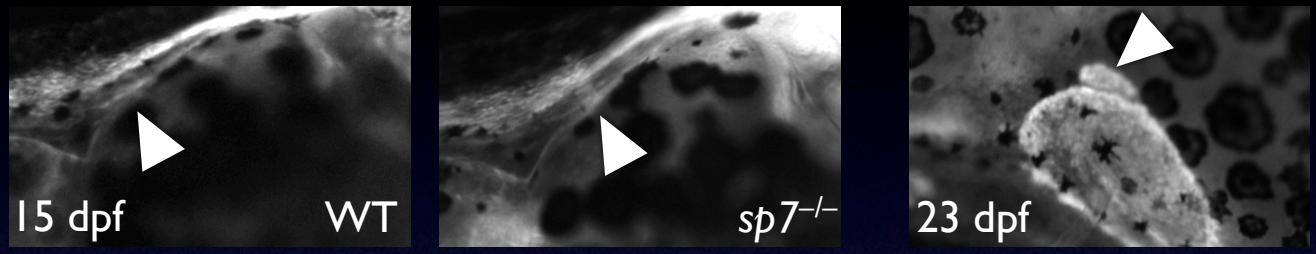


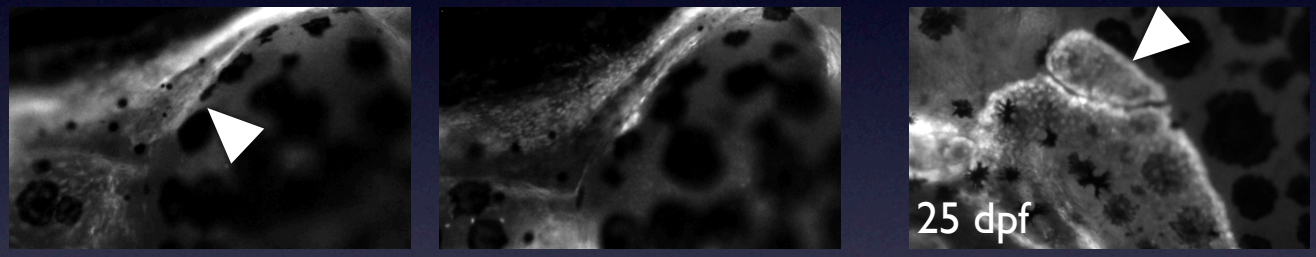


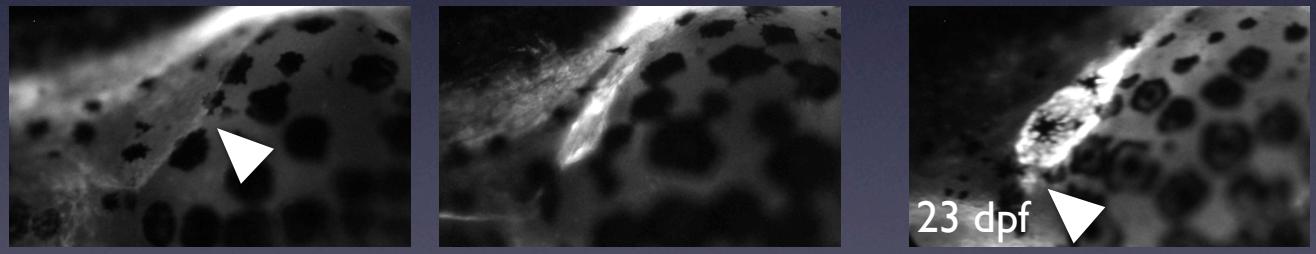
Ectopic bones form shortly after frontal bone initiation

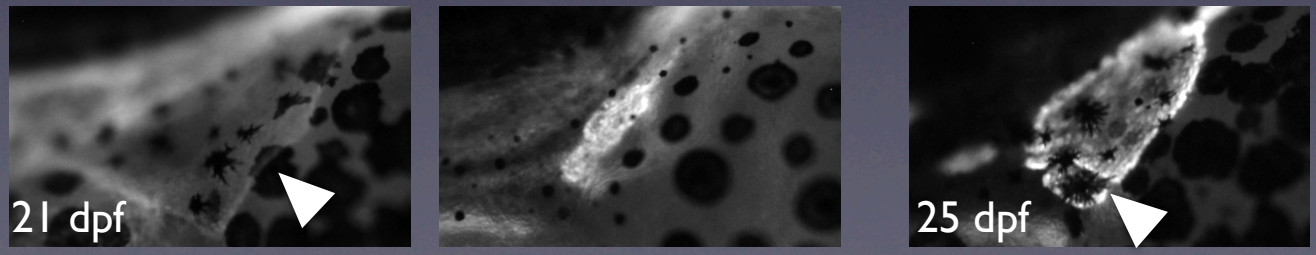


col1a1:egfp
RUNX2:mcherry





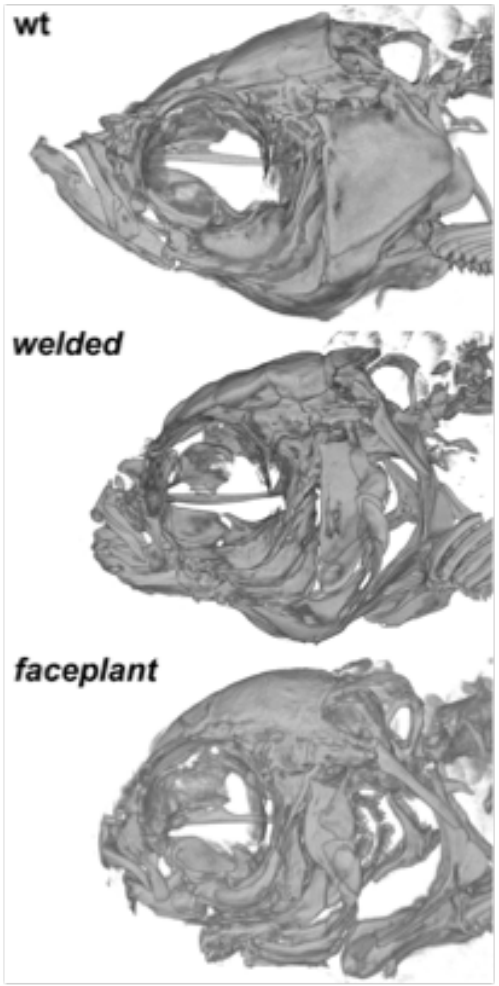


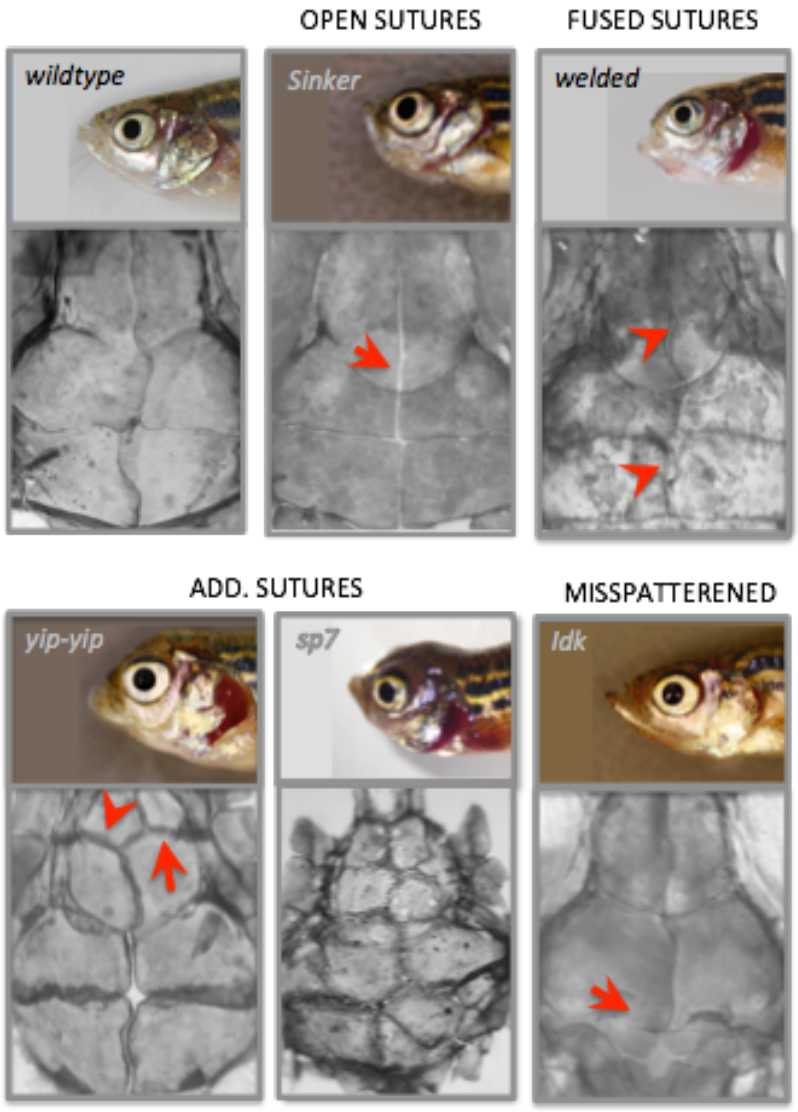


Dynamic imaging of skull development

- Frontal bone initiates in close contact with cartilage
- Initial lag followed by rapid planar growth
- Hypothesize three phases of frontal bone formation
  Initiation - similar to endochondral bone formation?
  Planar, directional growth (BMP signaling)
  Overlapping to form sutures - signals between bones?

Zebrafish mutants display abnormalities in multiple aspects of skull formation



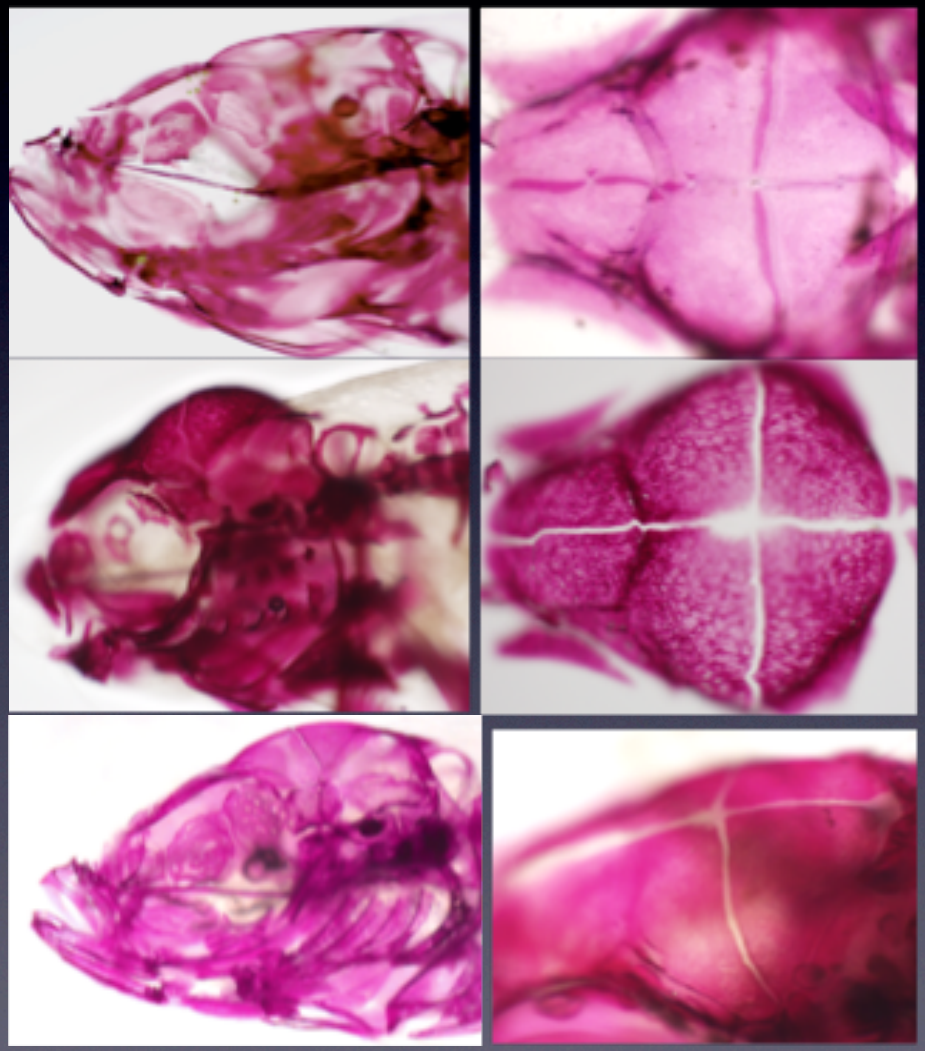


Mutations in *toth* and *lubber* lead to non-overlapping sutures

WT

toth $^{-/-}$

lubber $^{-/-}$



Short-term goals

- Integrate approaches
  - Expand period of time where both approaches work
    - Confocal on later stages, fixed samples
    - Sensitizing μCT to work on smaller fish
  - Fix transgenic fish for μCT
  - Image same mutants with both approaches
- Prepare WT data for upload
- Make first set of transgenic lines, plasmids available

Longer-term issues to resolve

- How to best analyze and present data
  - Confocal stacks in universal format
  - Same for μCT data?
  - Isosurface rendering for confocal data, with annotation
- Comparisons to other species
  - Ontology cannot be unified! (22 vs. 78 bones)
- Alternatives to strict anatomical ontology
  - Developmental homology
  - Functional equivalence