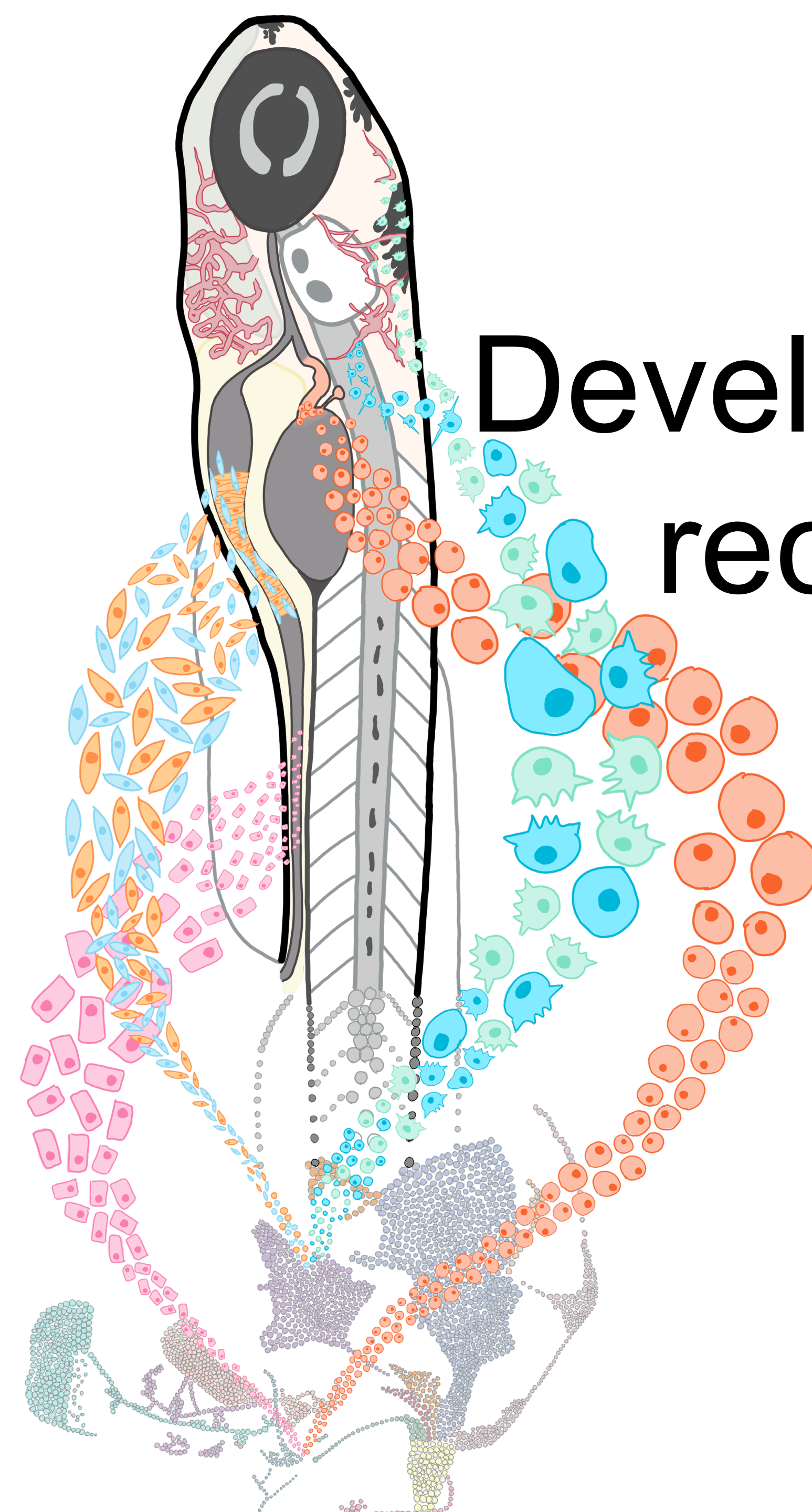




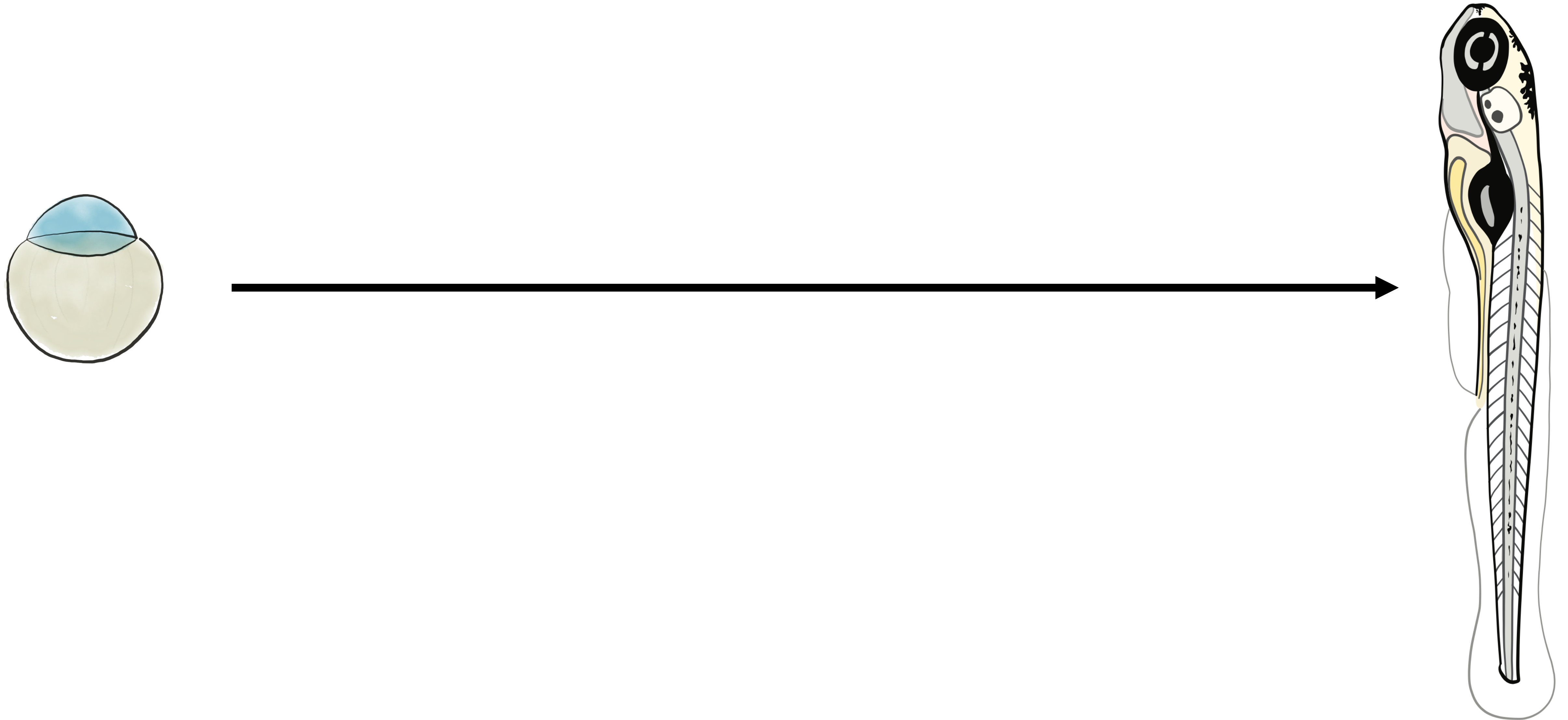
Developmental regulation of recently discovered *best4*+ intestinal epithelial cells

Jeffrey Farrell

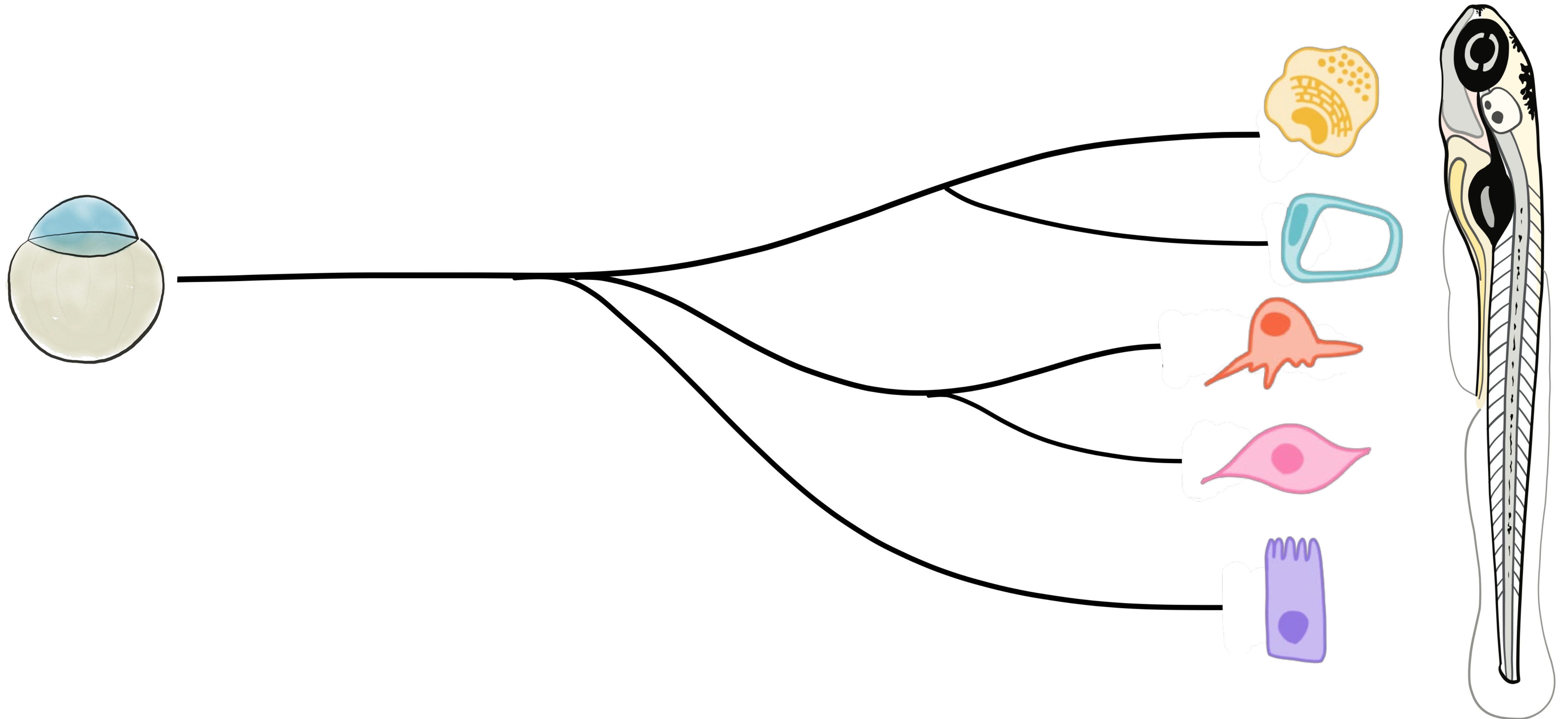
NICHD Division of Intramural Research

NICHD Advisory Council Meeting
September 2025

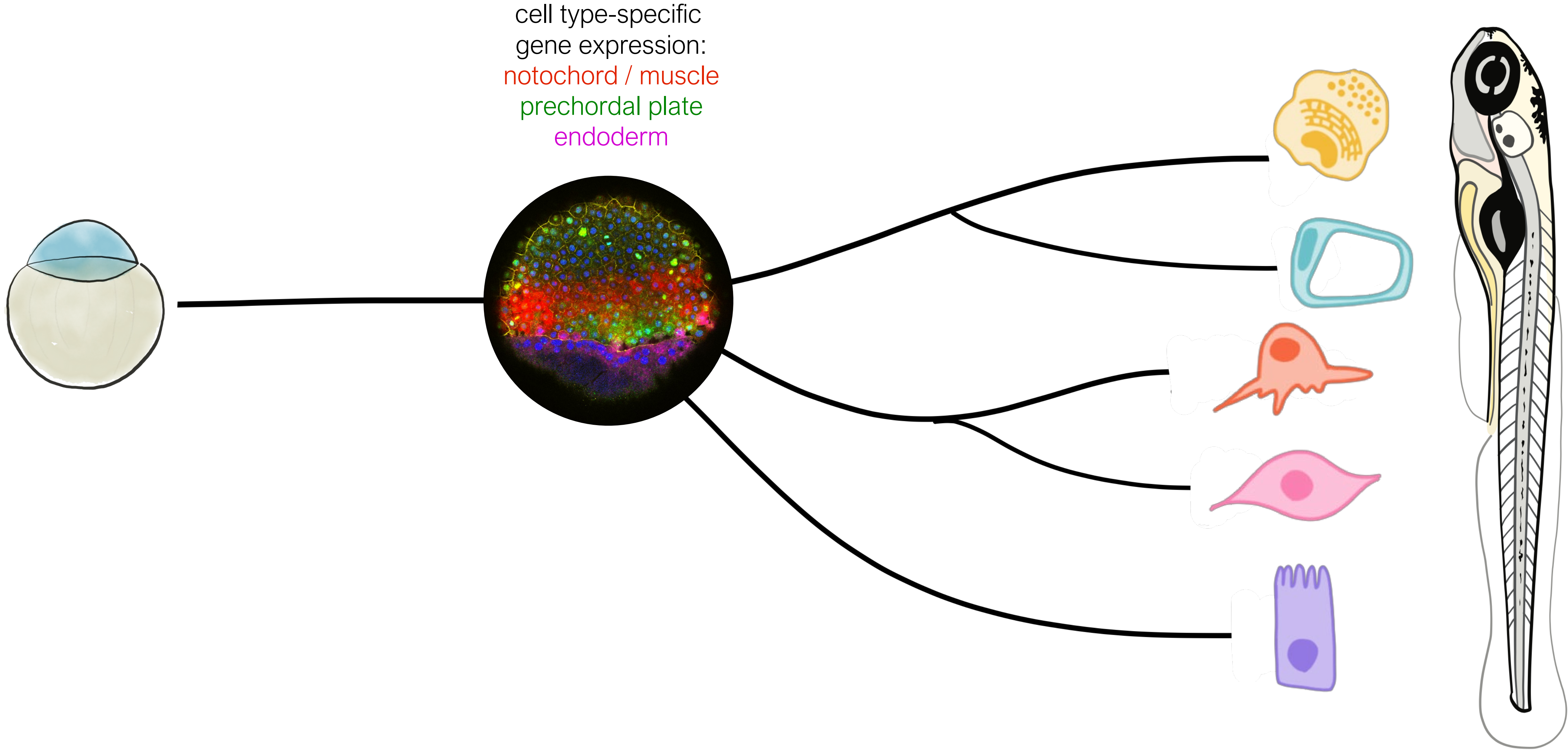
Every animal needs to assemble itself from scratch during development



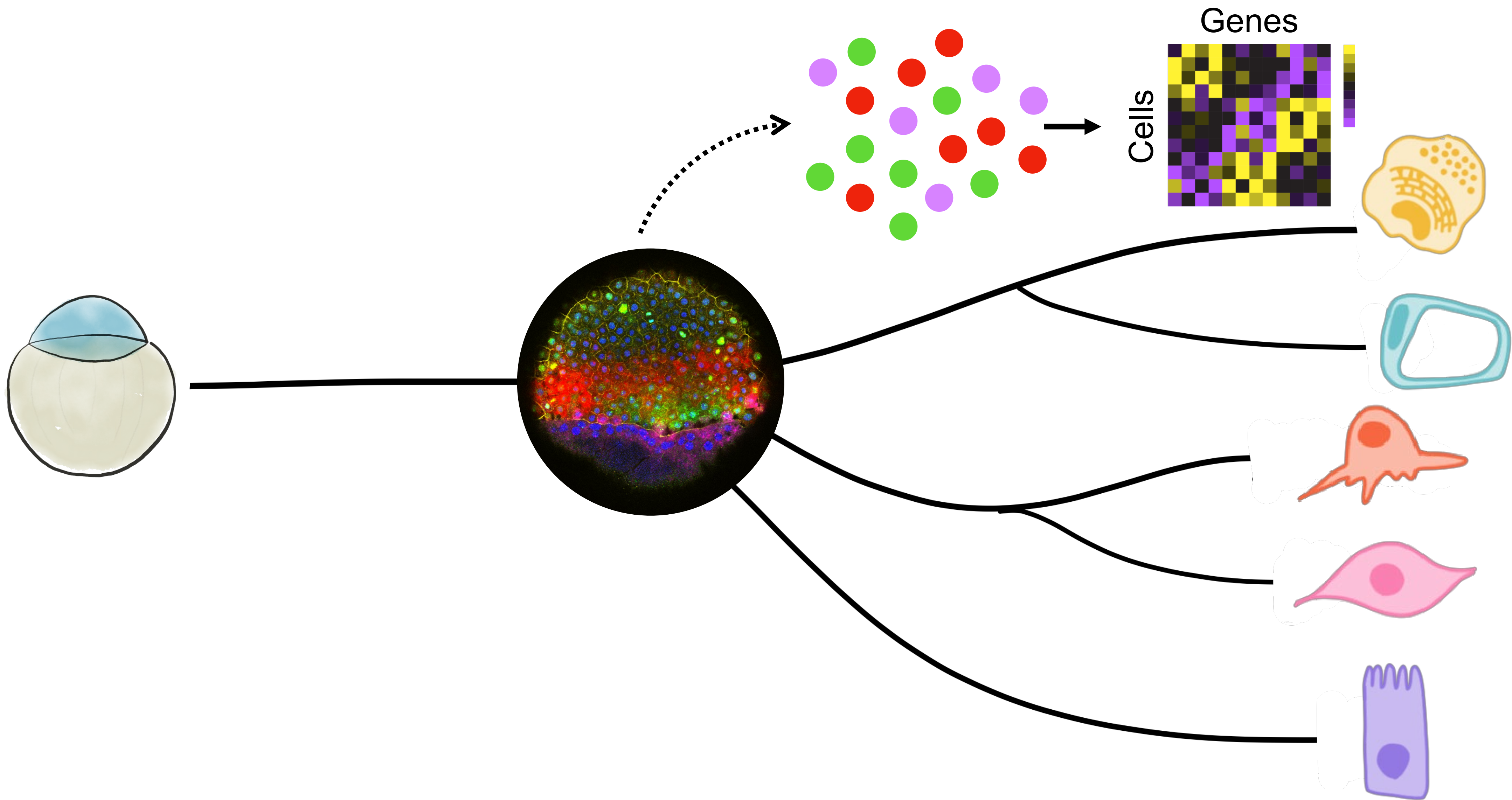
And that means re-creating an enormous diversity of cell types



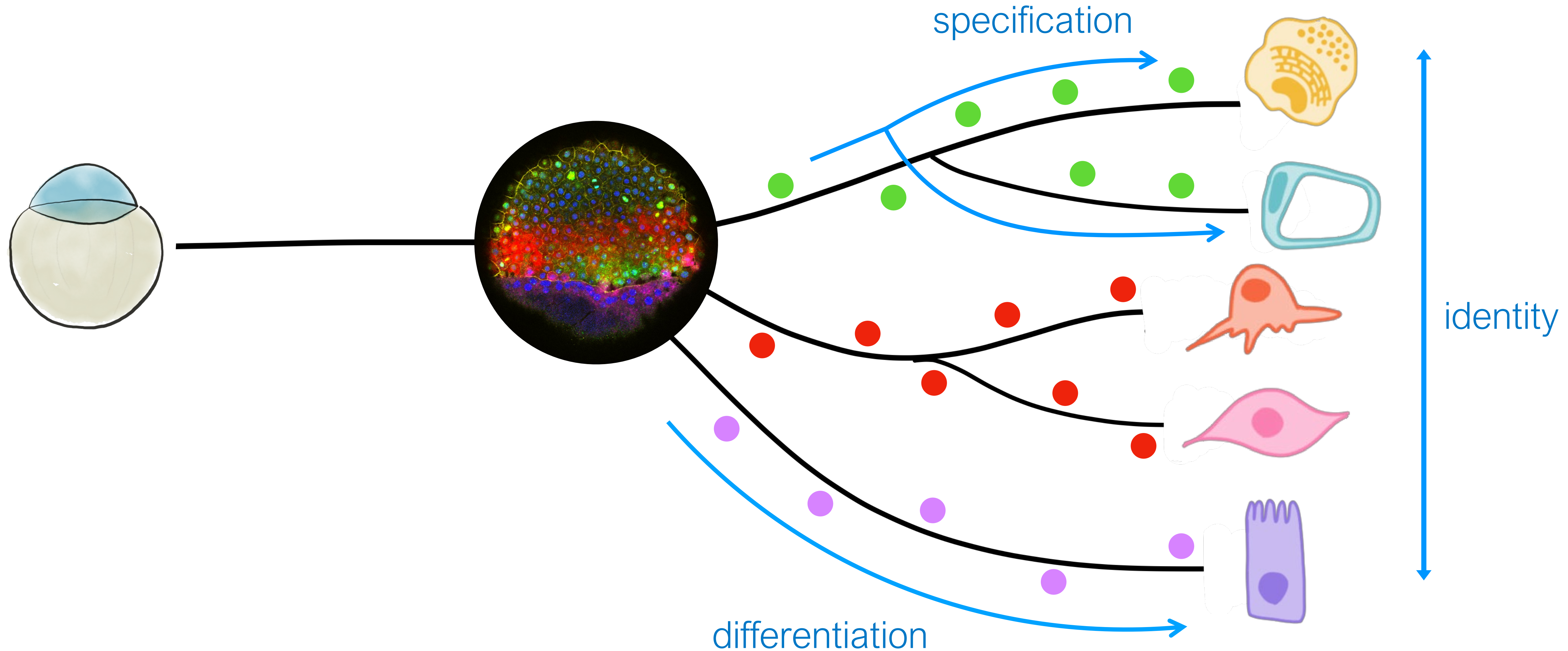
Our lab studies the genetic programs that regulate cell specification & differentiation



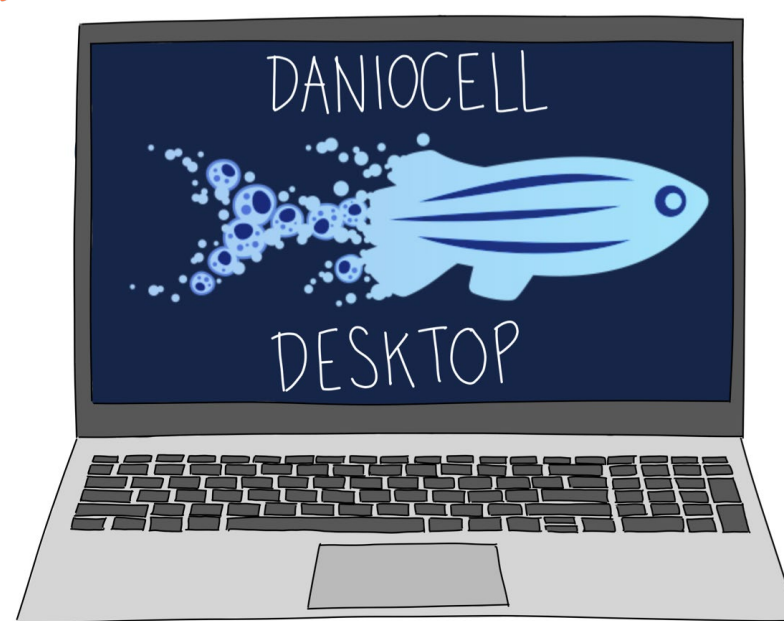
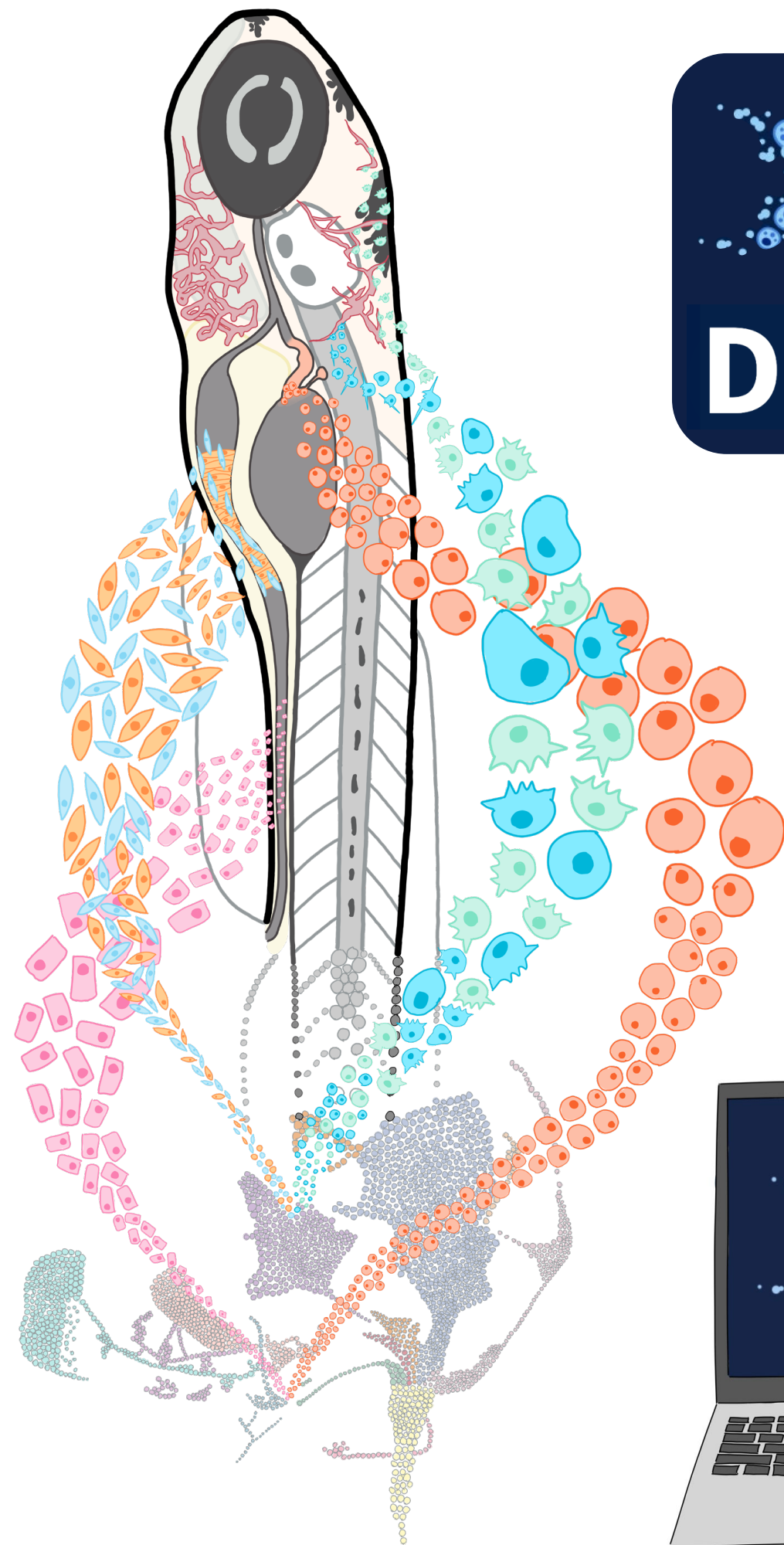
Our lab studies the genetic programs that regulate cell specification & differentiation



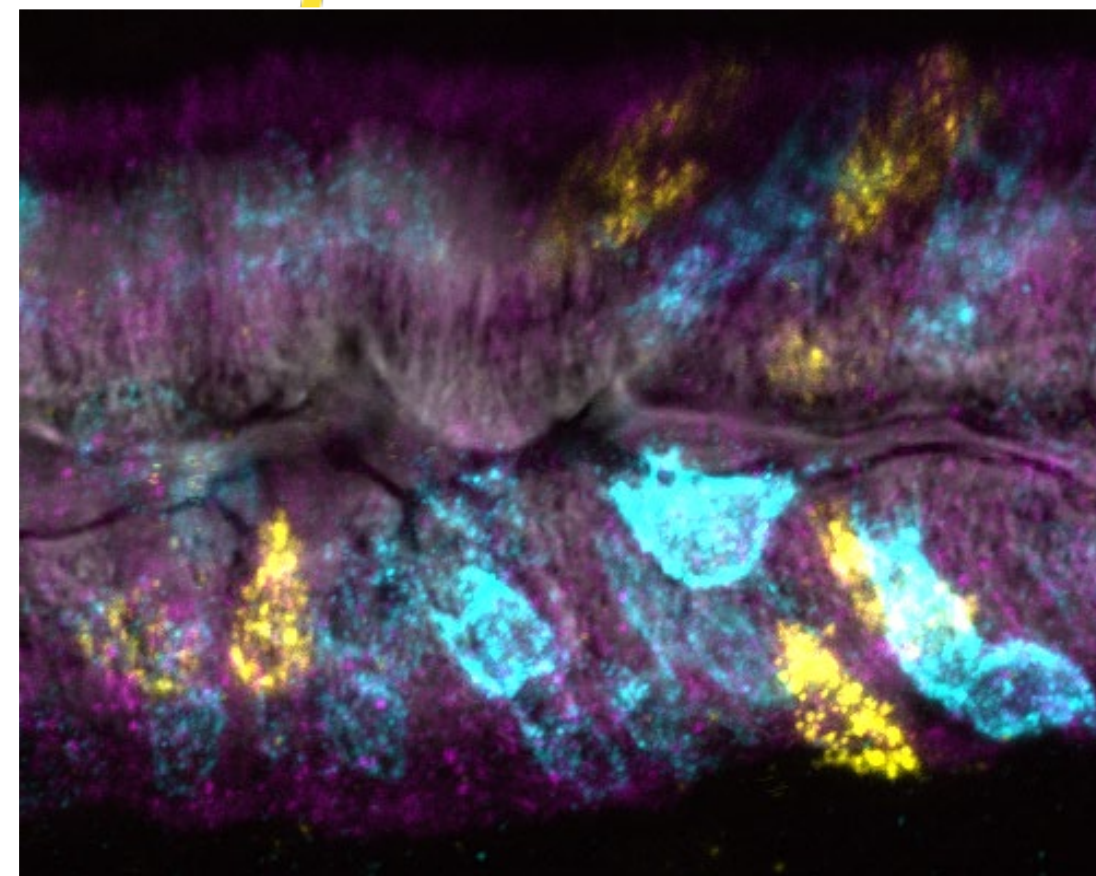
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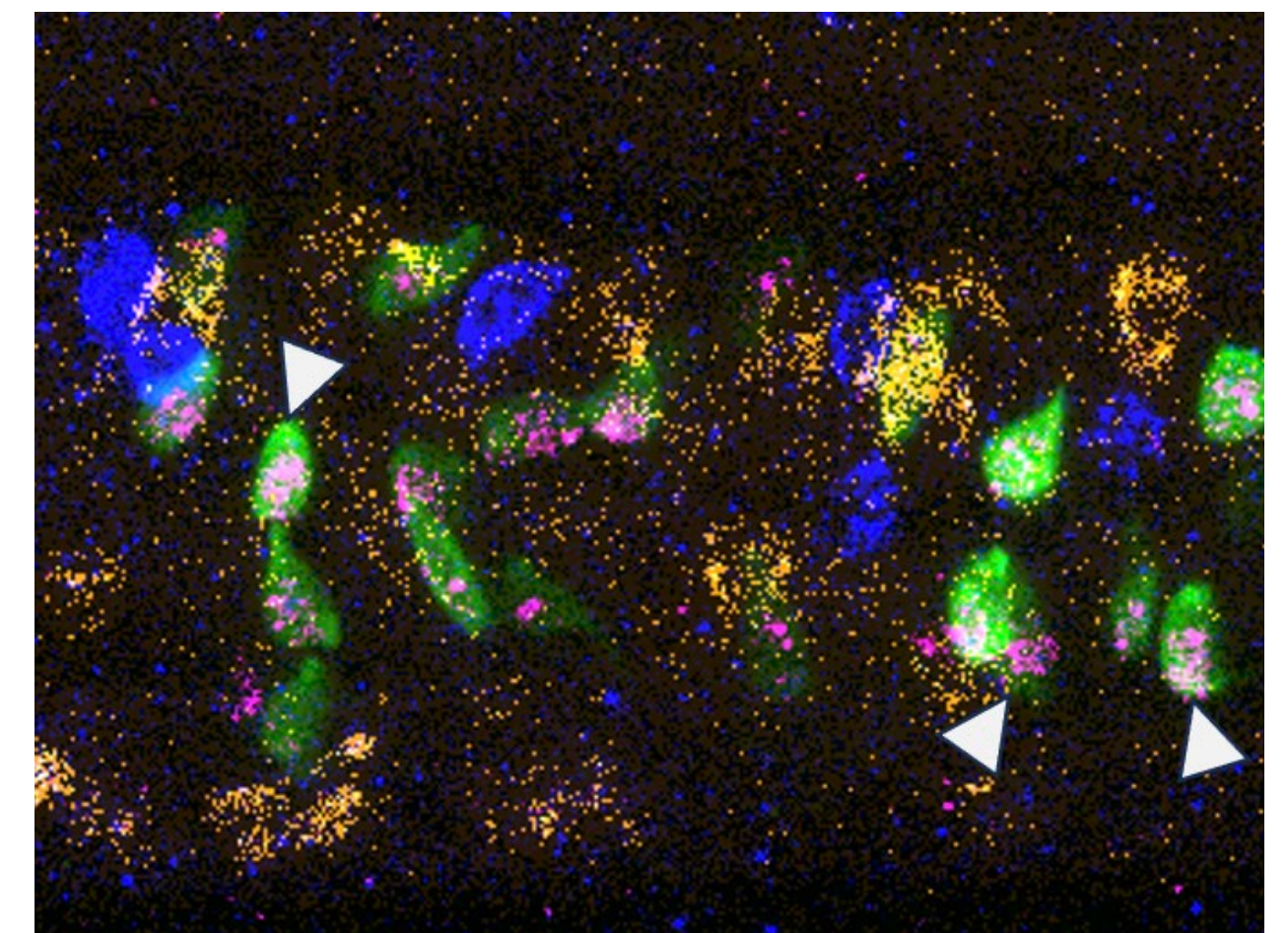
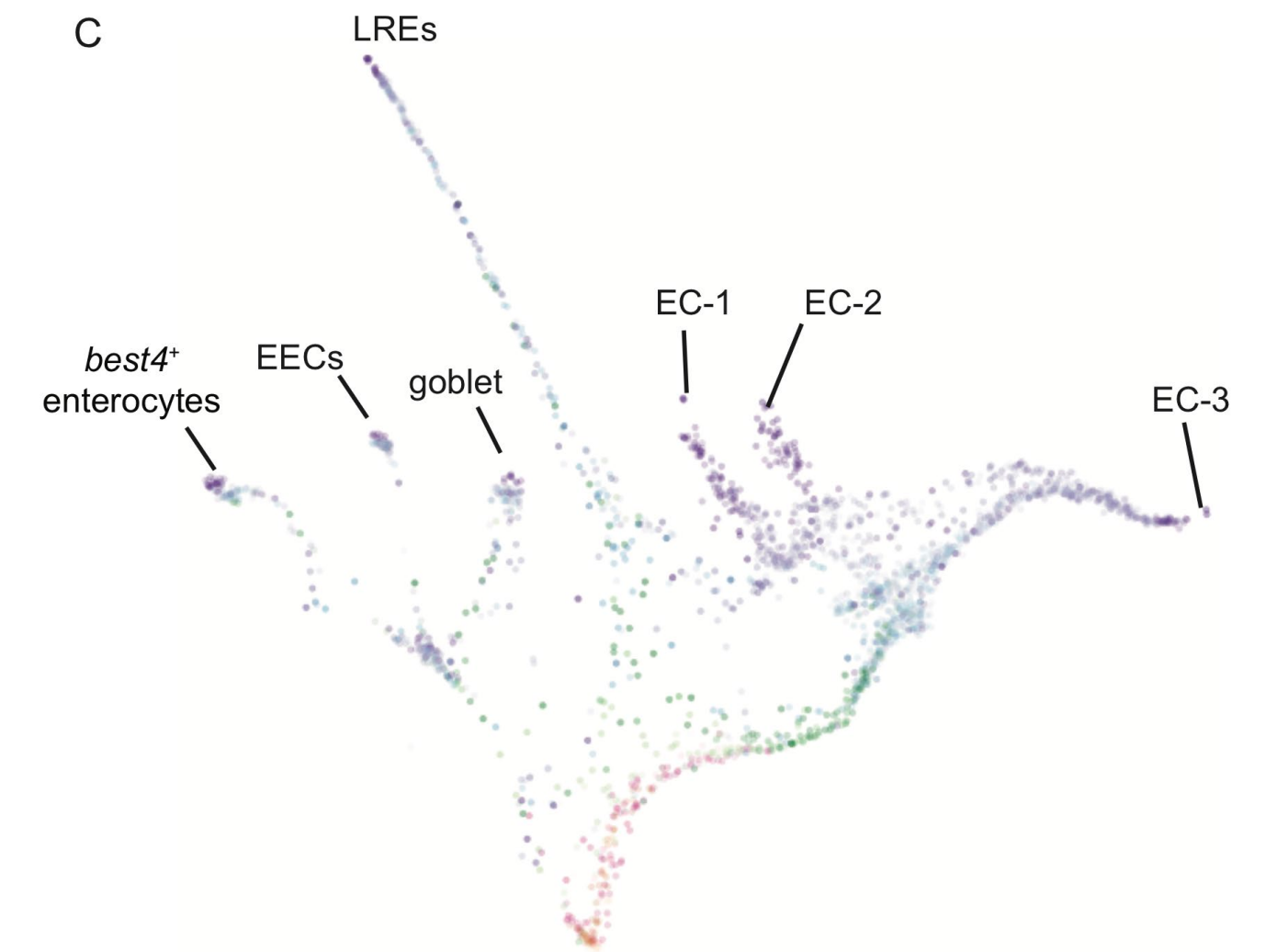
broad + genomic
profiling resources



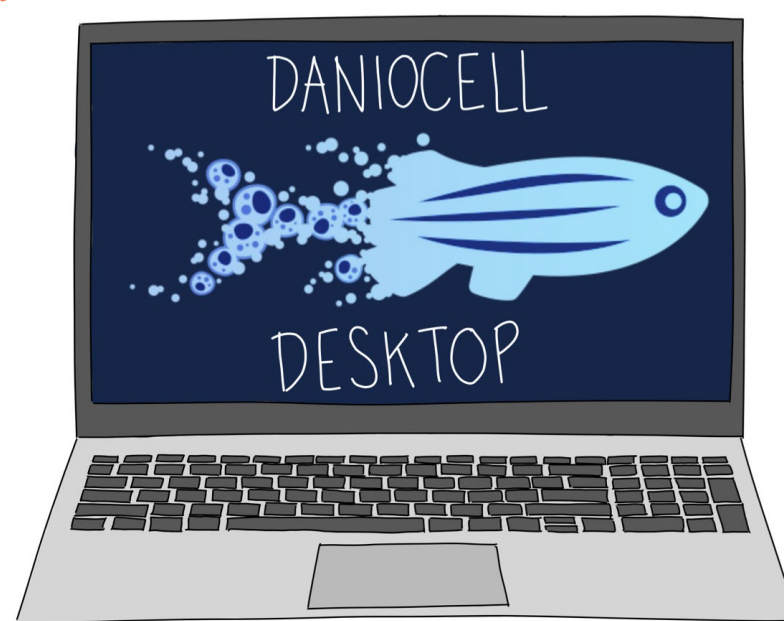
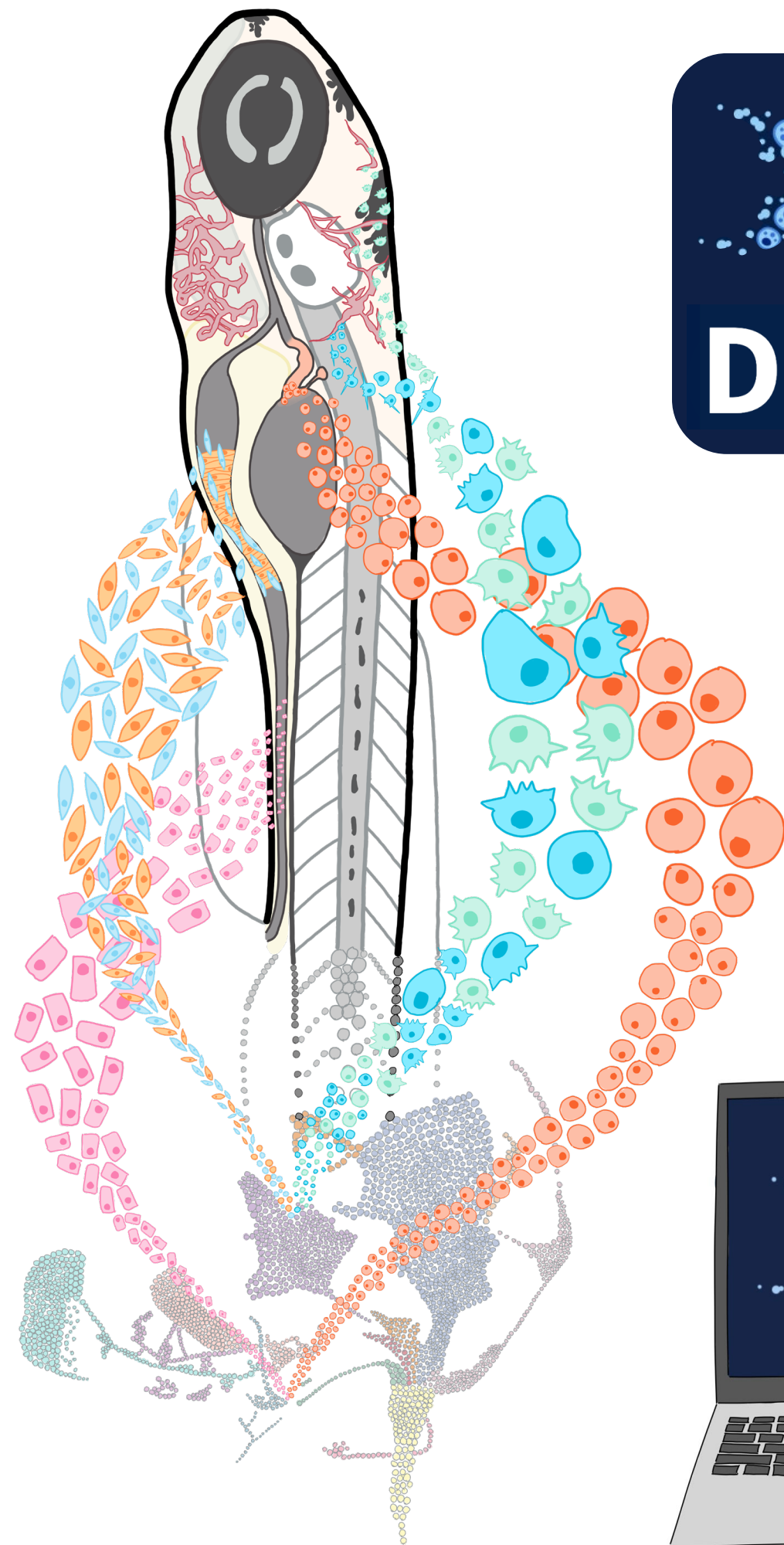
an unexpected
cell type



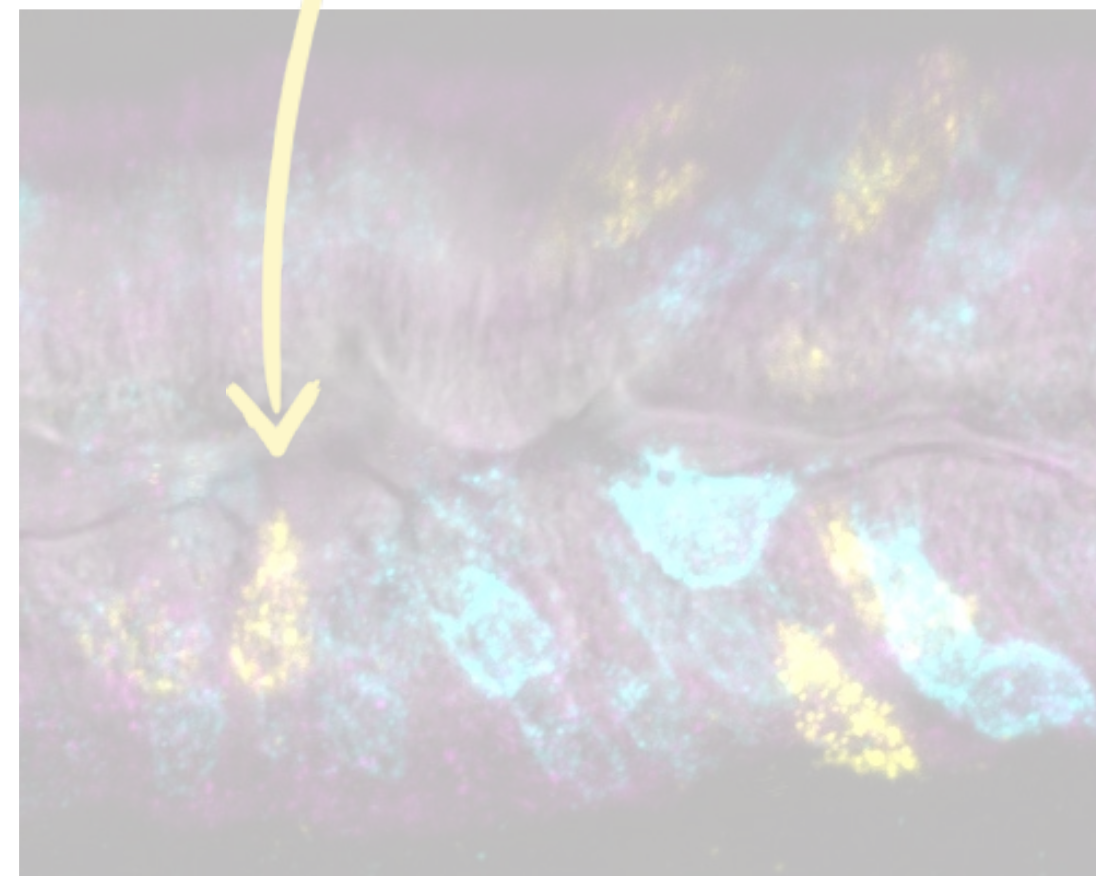
computationally +
experimentally determine
how that cell type
develops



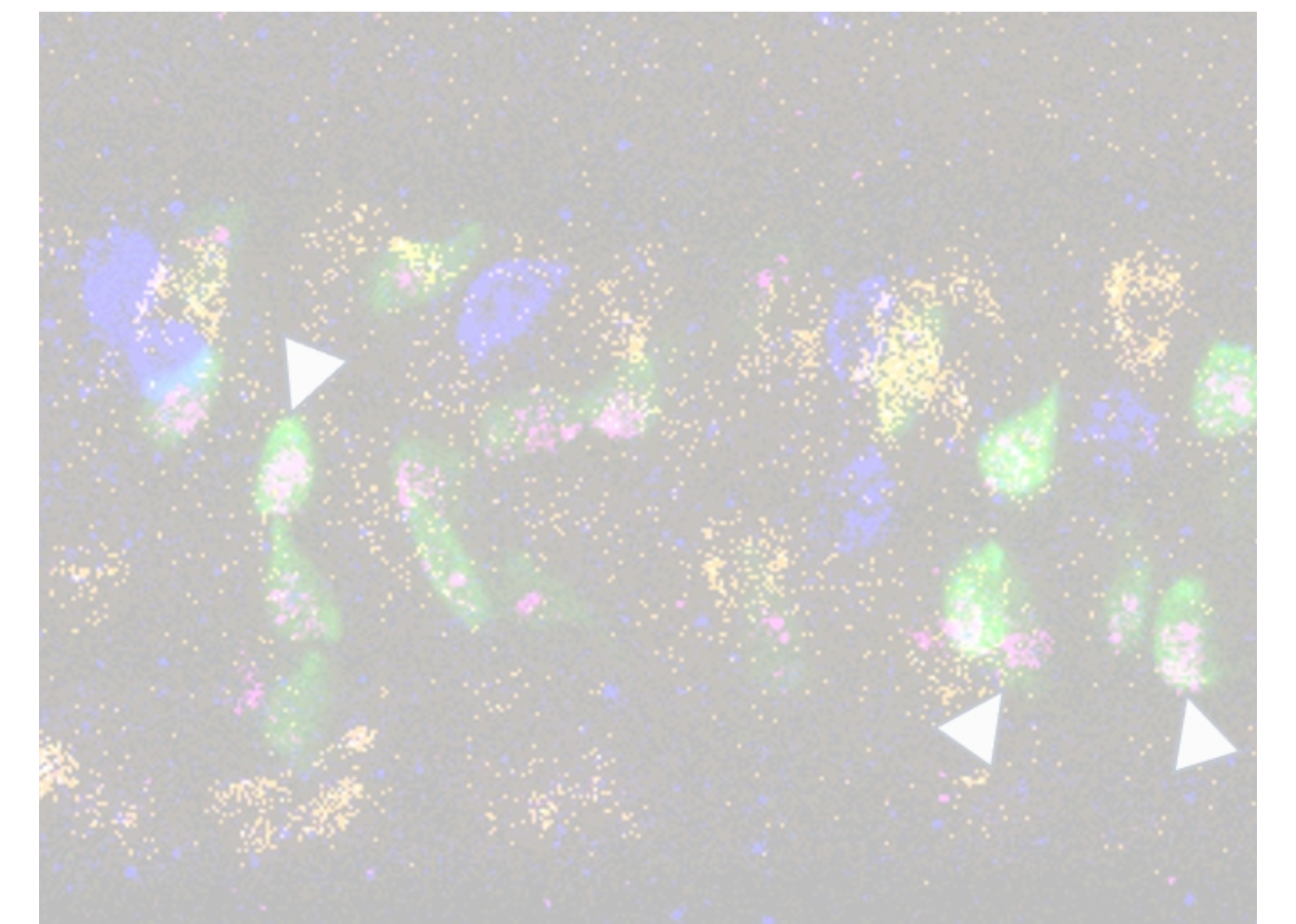
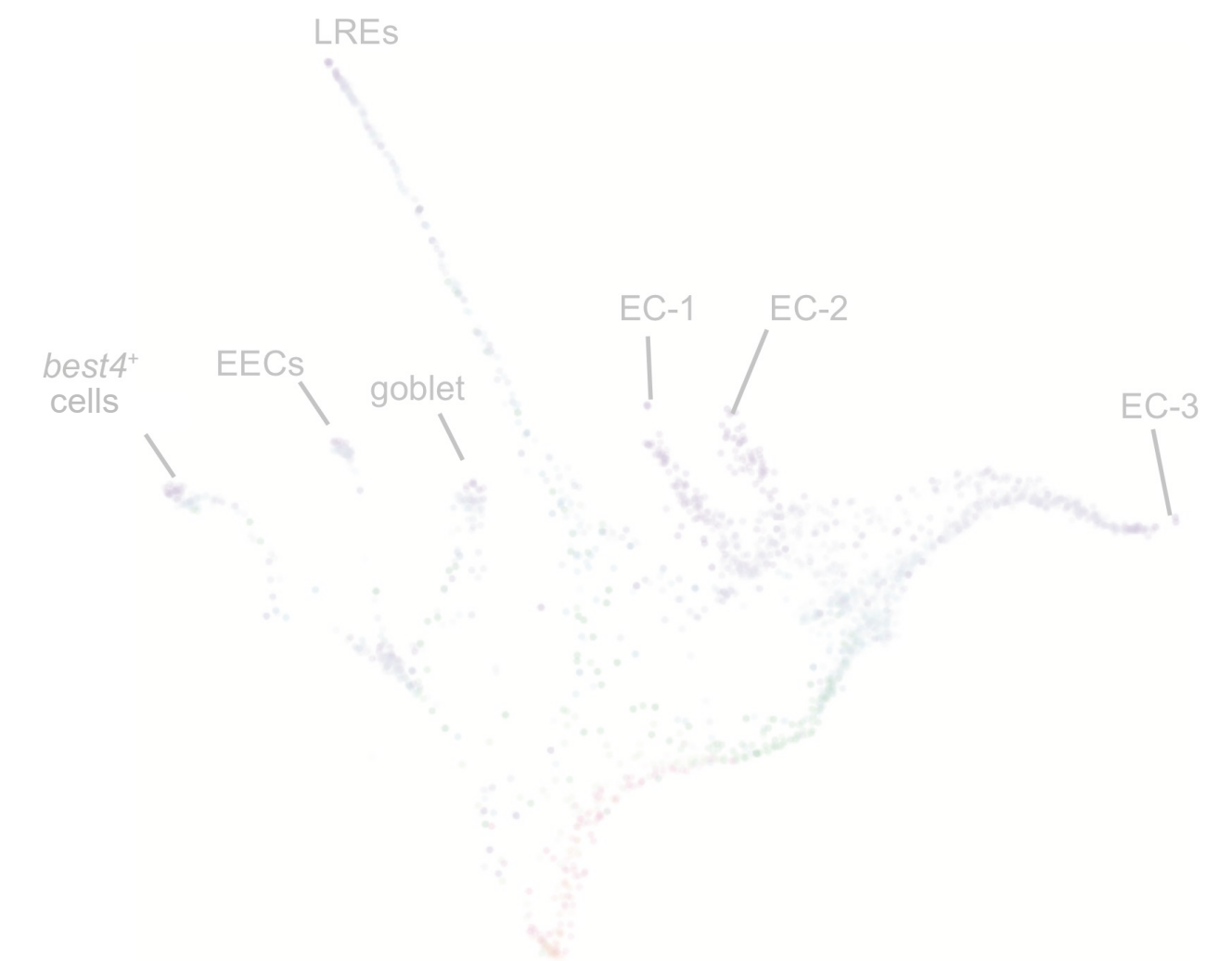
broad + genomic
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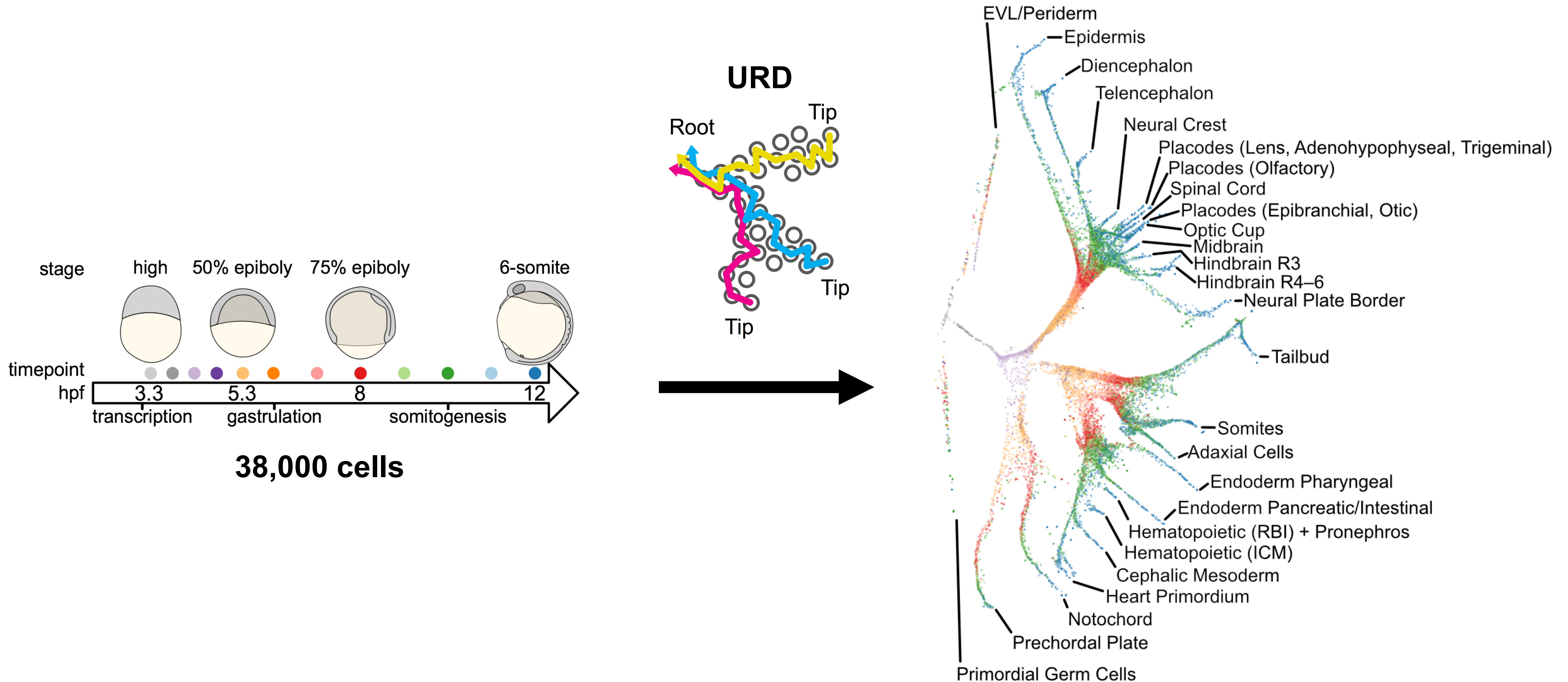
an unexpected
cell type



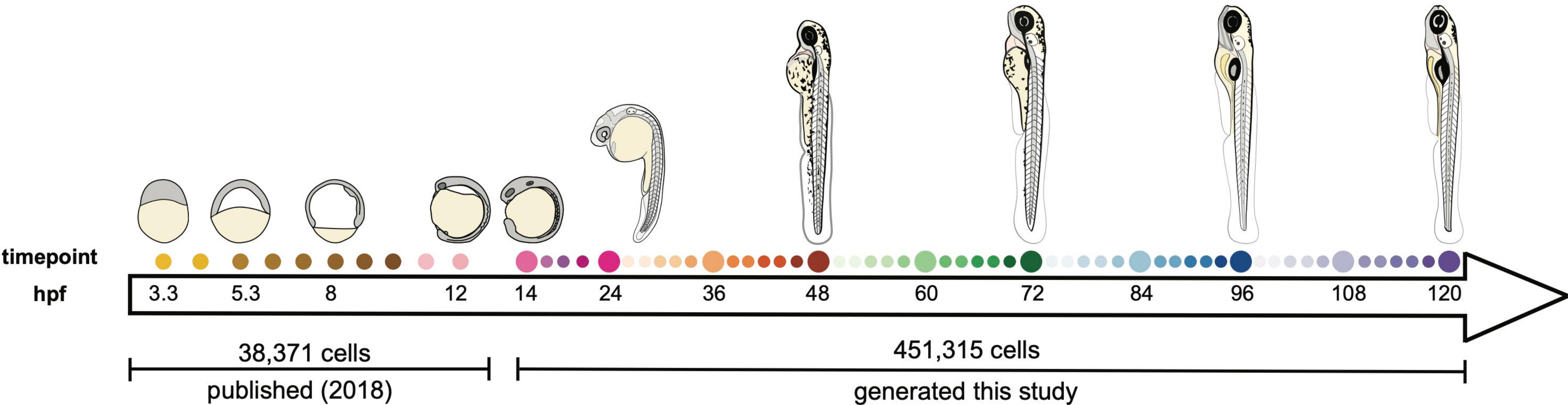
computationally +
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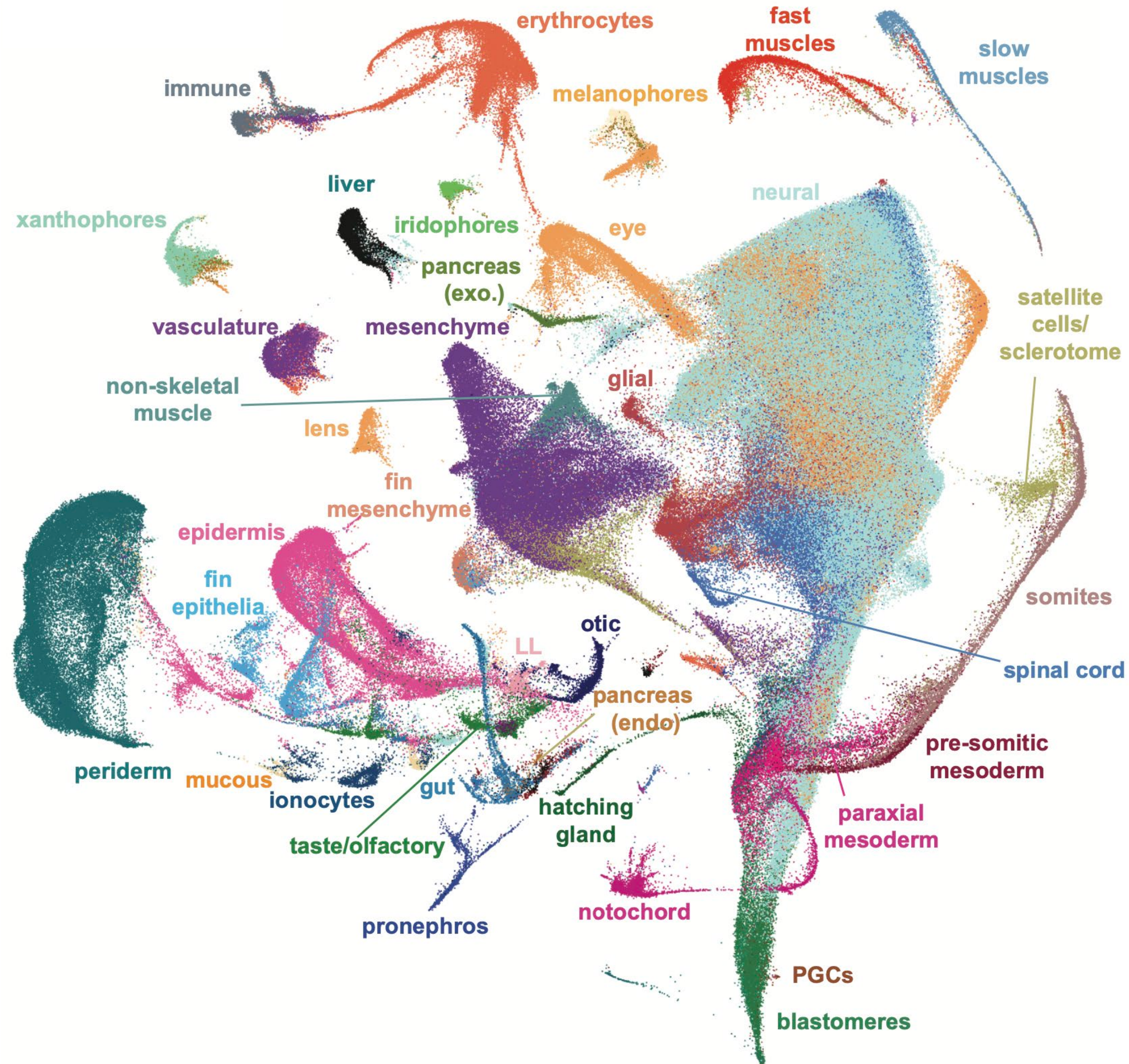
Specification tree of zebrafish embryogenesis



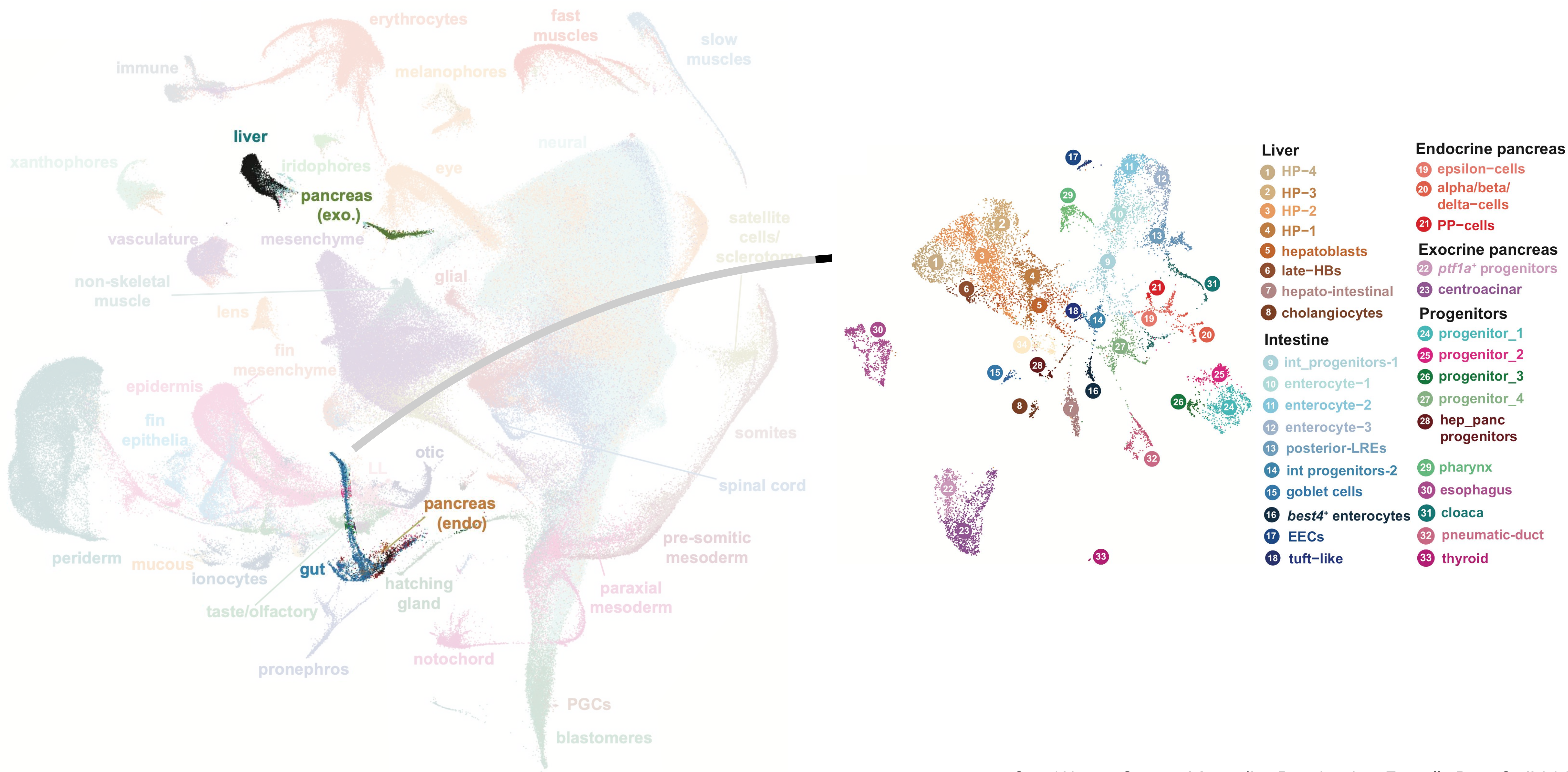
A molecular atlas of zebrafish late embryogenesis and early larval development



Cell identity annotation: 521 clusters, representing ~200 cell types and 43 tissues



Cell identity annotation: 521 clusters, representing ~200 cell types and 43 tissues



Daniocell

vil1 (all cells)



DaniocellDesktop: for further analysis of our data

Define your own cell populations

Add all cells from cluster(s):

Cluster(s)

(endo.16) intestine - best4+ cells

Limit to cells from stages:

Hours

376120

Limit to cells with gene expression:

Gene

otop2

<=

>=

Value

0.2

Perform custom differential expression

Choose Results to View

best4: otop2+ vs otop2-

Delete selected results

Filter Results

Filter Defaults:

Differentially ExpressedBest Markers

Set Your Own Filters:

Max. Wilcox p

-3

Min. log2FC

0.585

Mean.1 (Min)

0

Mean.2 (Min)

0

Min. auROC

0.3

Min. Diff. %

0

Mean.1 (Max)

50

Mean.2 (Max)

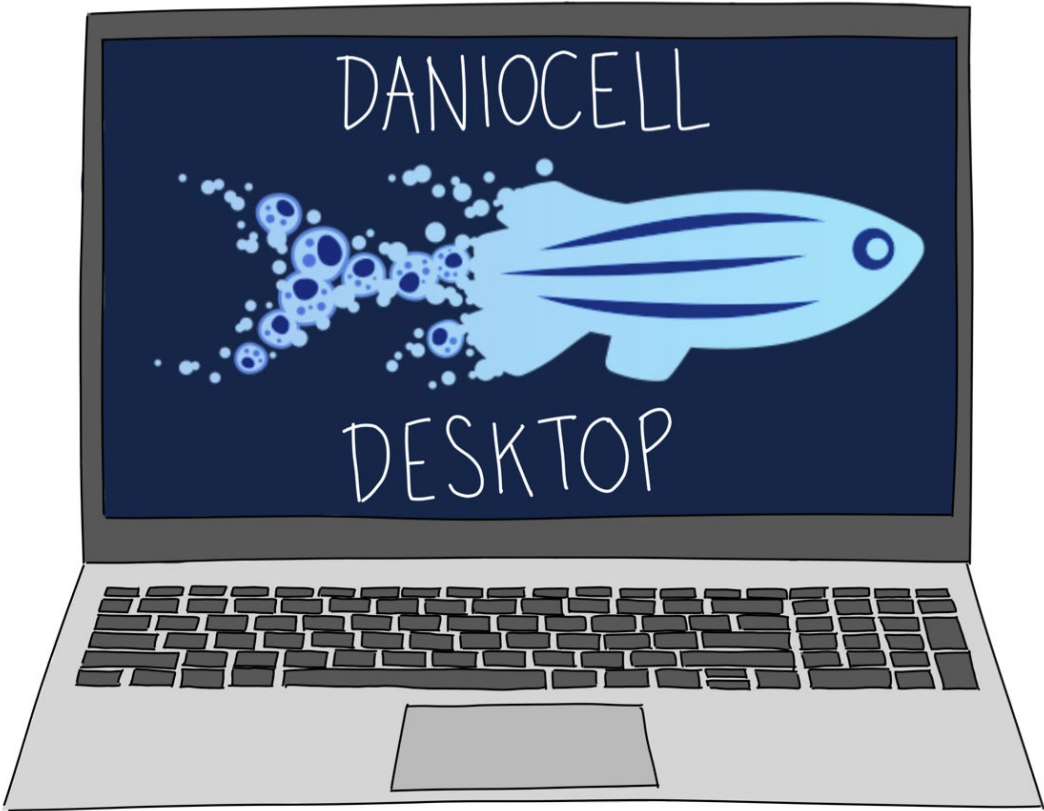
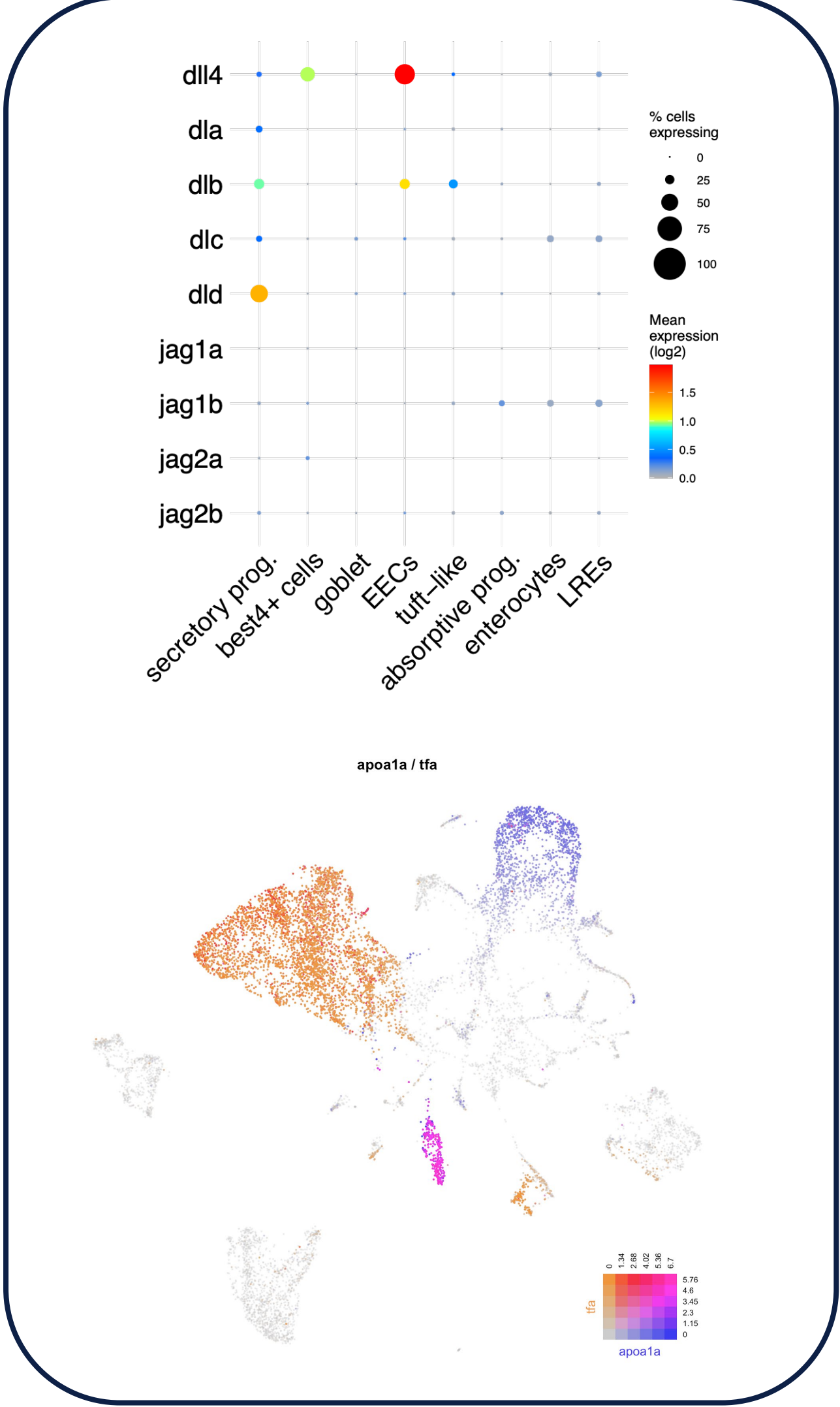
50

Display upregulated genes only

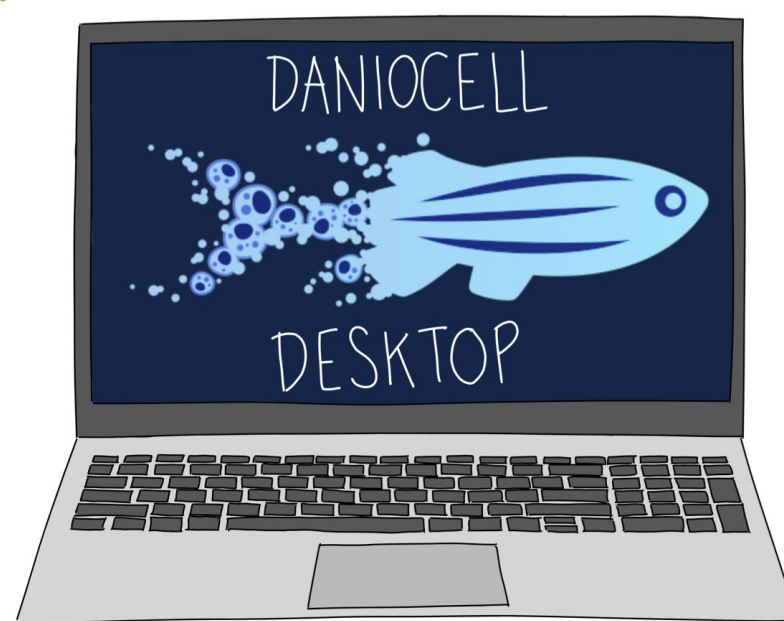
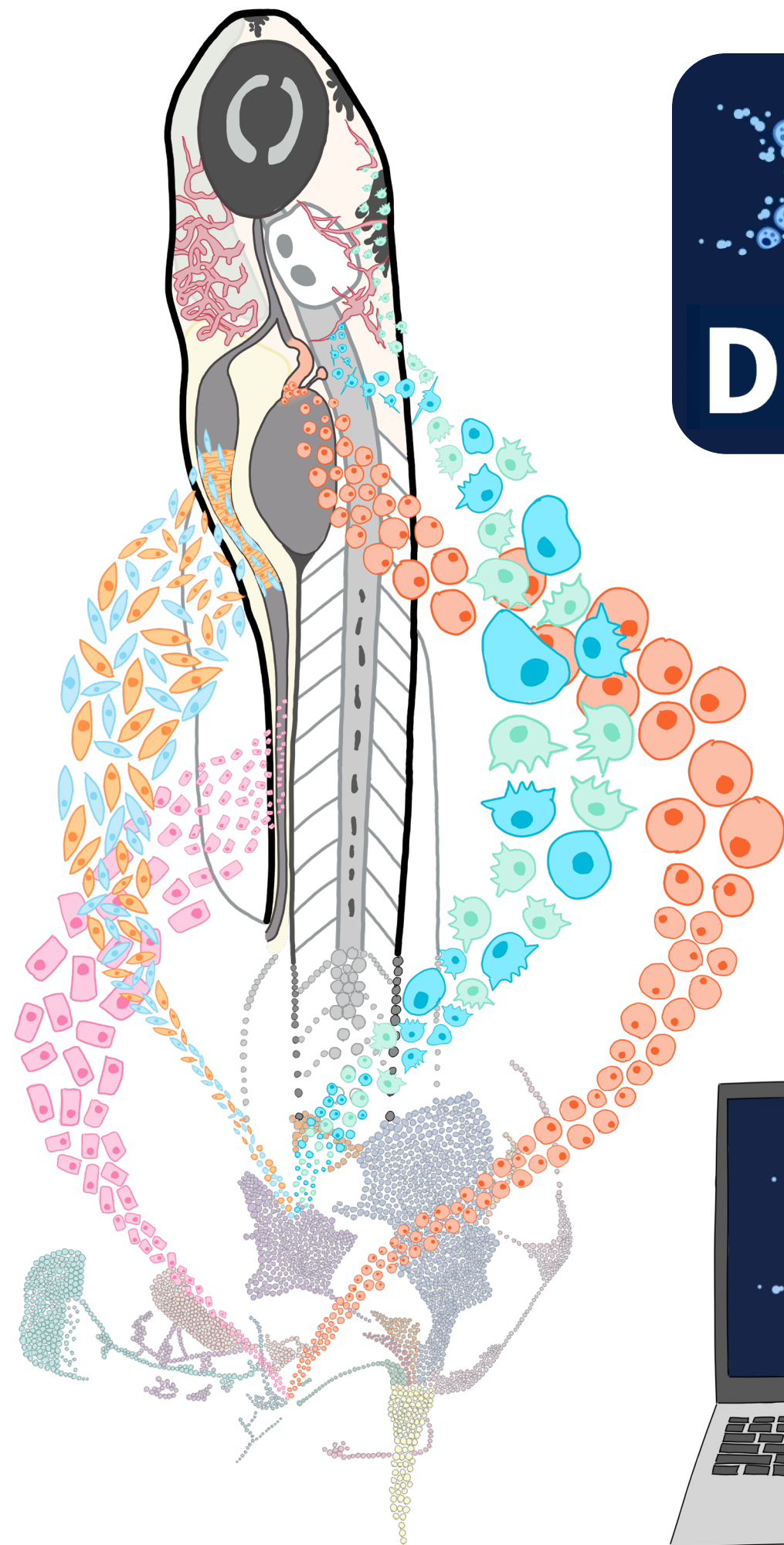
Search:

Gene	Pop. 1 Mean Expression (Log2)	Pop. 2 Mean Expression (Log2)	Pop. 1 % cells expressing	Pop. 2 % cells expressing	Log2 fold-change	auROC power	Wilcoxon adj. p-value
otop2	3.35	0	100	0	8.451	1	1.089e-13
bmp16	0.807	0.05	35.3	2.6	3.626	0.332	3.849e-1
slc43a2b	1.268	0.272	50	10.5	2.604	0.418	1.554e-1
ca4b	4.035	1.833	91.2	36.8	2.572	0.723	8.969e-6
zgc:77439	1.443	0.367	44.1	10.5	2.456	0.346	7.355e-1
dhhs9	1.464	0.446	54.4	15.8	2.19	0.4	3.601e-1
hsd11b1la	1.77	0.703	57.4	15.8	1.891	0.415	3.194e-1
LOC100536156	1.244	0.435	44.1	10.5	1.869	0.336	9.668e-1
zgc:77739	1.338	0.515	45.6	10.5	1.761	0.336	9.668e-1
eppk1	1.993	0.9	73.5	34.2	1.747	0.48	1.280e-1
tcnba	9.527	7.827	89.7	39.5	1.703	0.601	2.811e-3
cmah	1.77	0.816	58.8	26.3	1.623	0.371	9.668e-1
LOC100536213	1.808	0.857	63.2	26.3	1.588	0.391	7.355e-1
lmo7a	1.769	0.989	73.5	36.8	1.26	0.391	9.668e-1
zgc:92140	2.598	1.695	85.3	57.9	1.163	0.505	1.111e-1
adgrg4b	1.955	1.207	72.1	36.8	1.116	0.4	7.756e-1

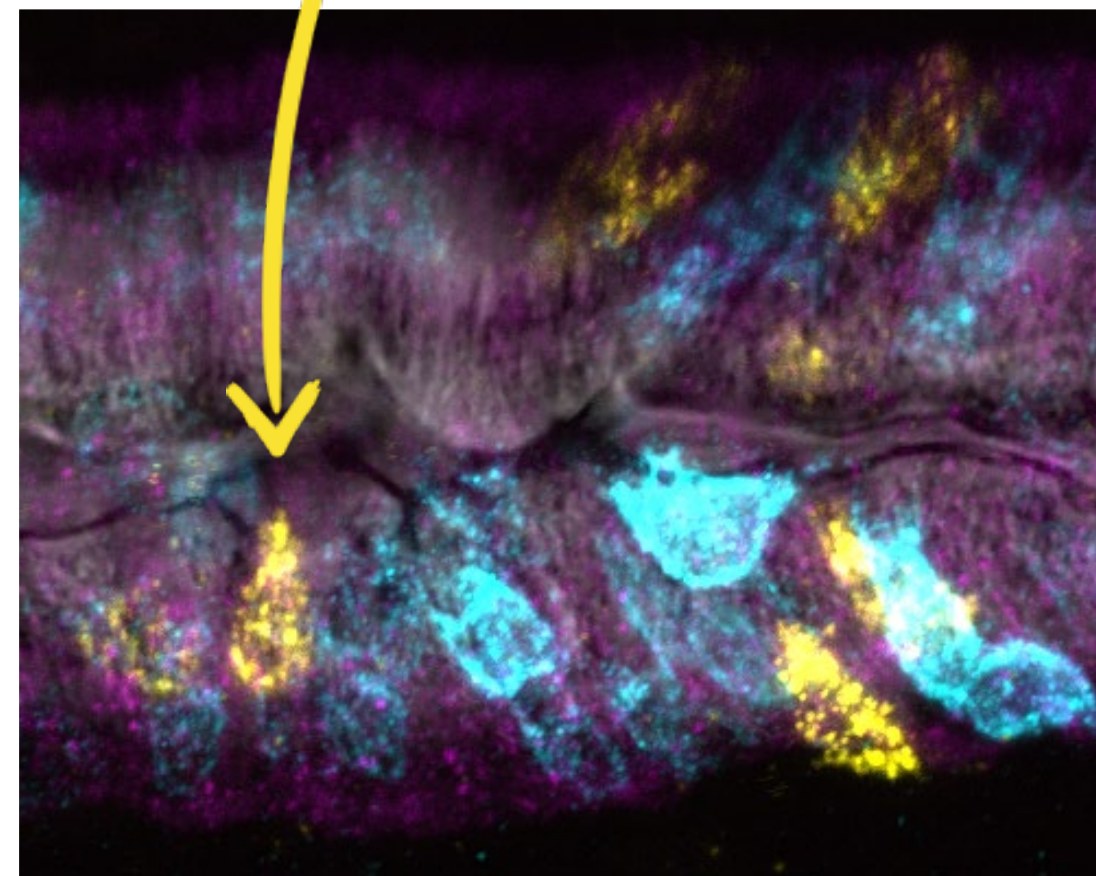
Customized, publishable plots



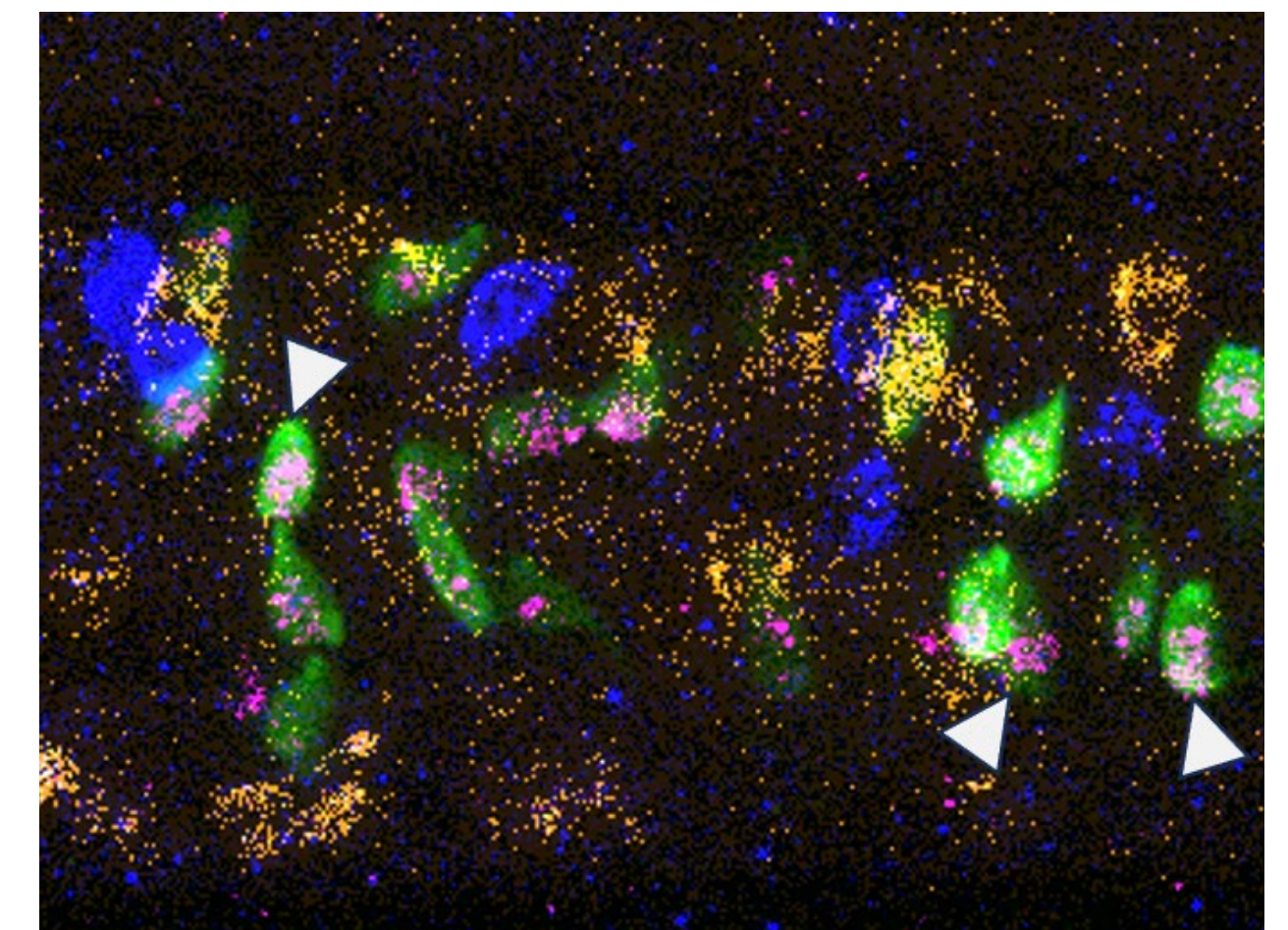
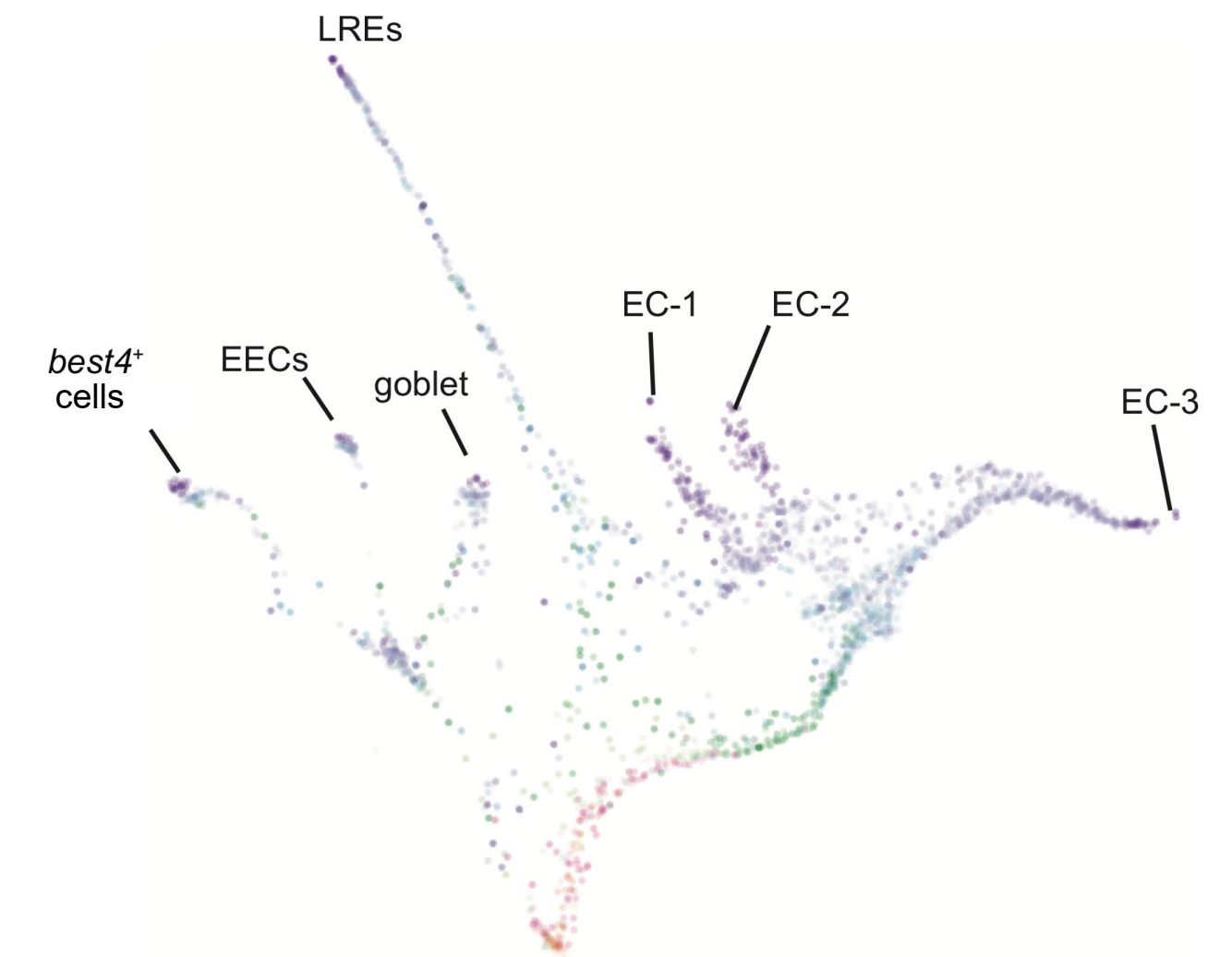
broad
profiling + genomic
resources



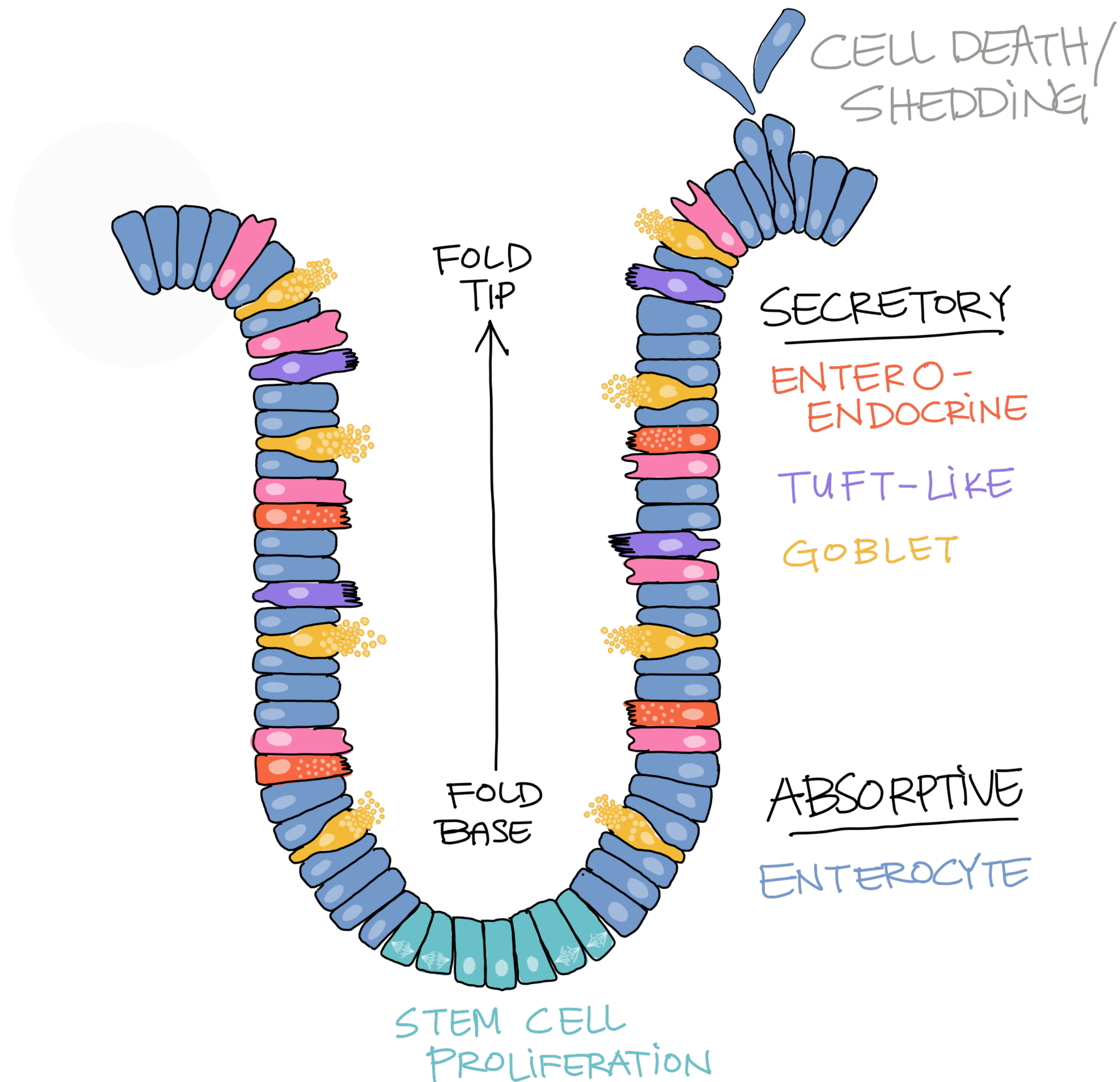
an unexpected
cell type



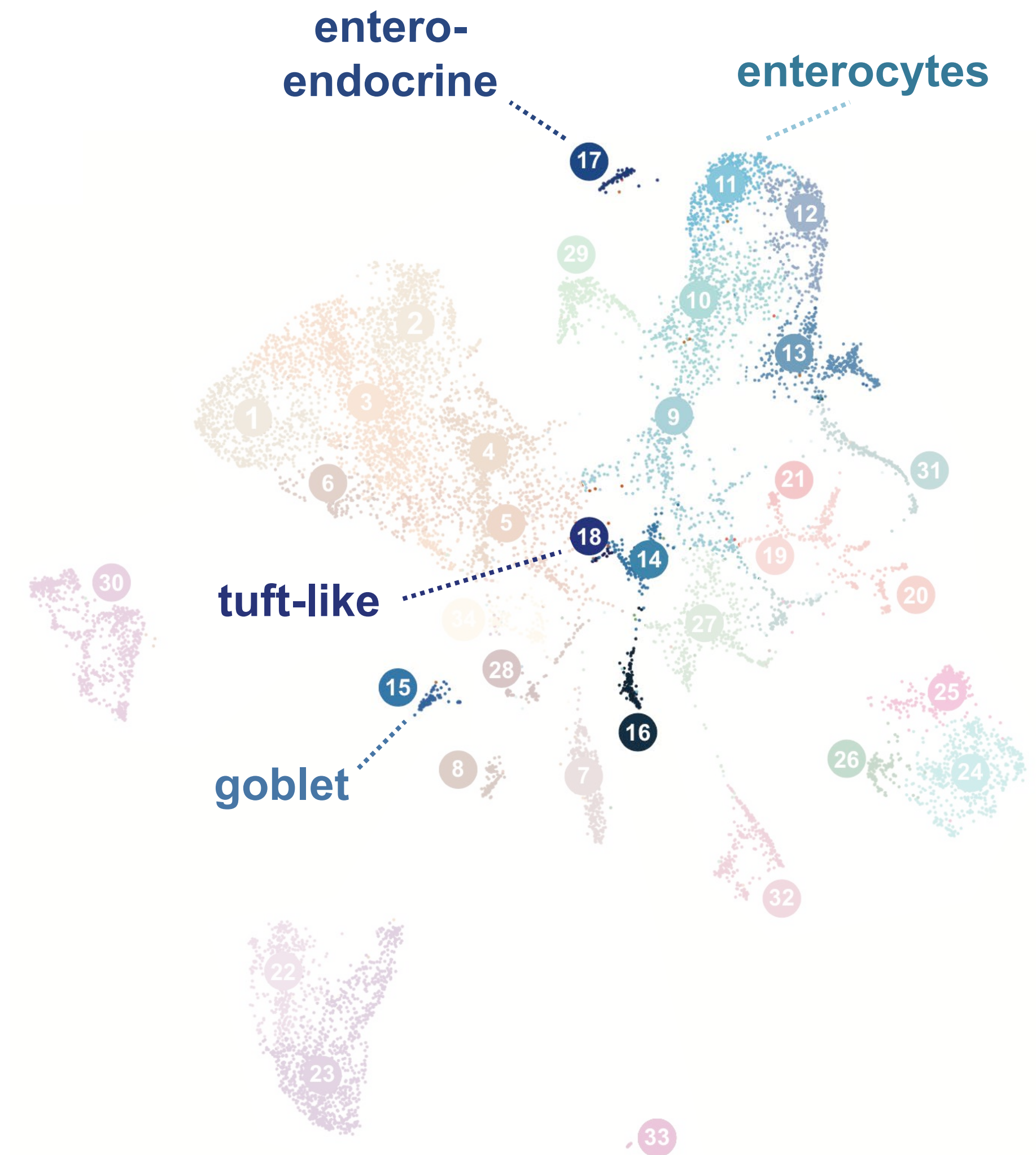
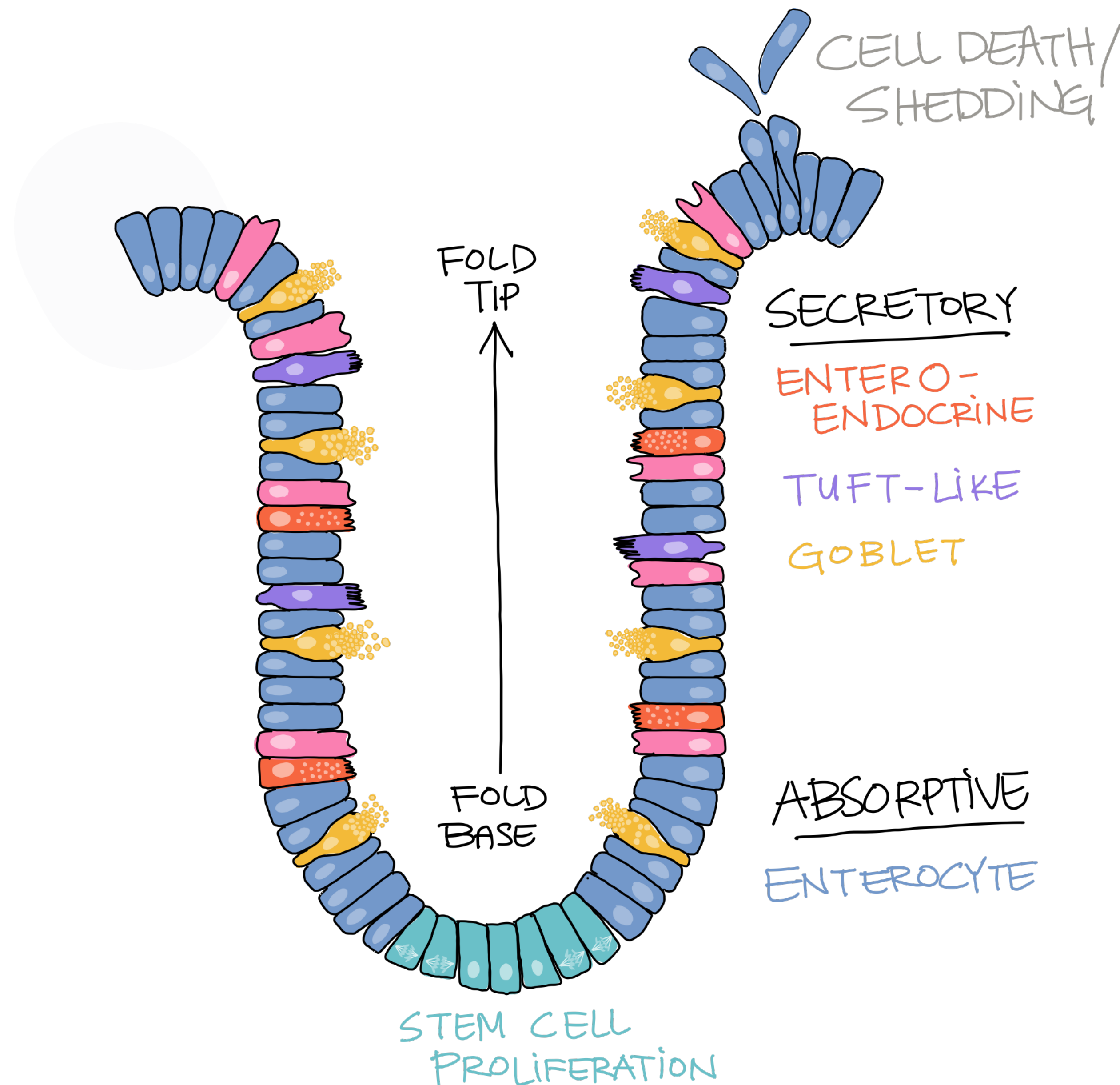
computationally +
experimentally determine
how that cell type
develops



Cell populations within the intestinal epithelium



Cell populations within the intestinal epithelium

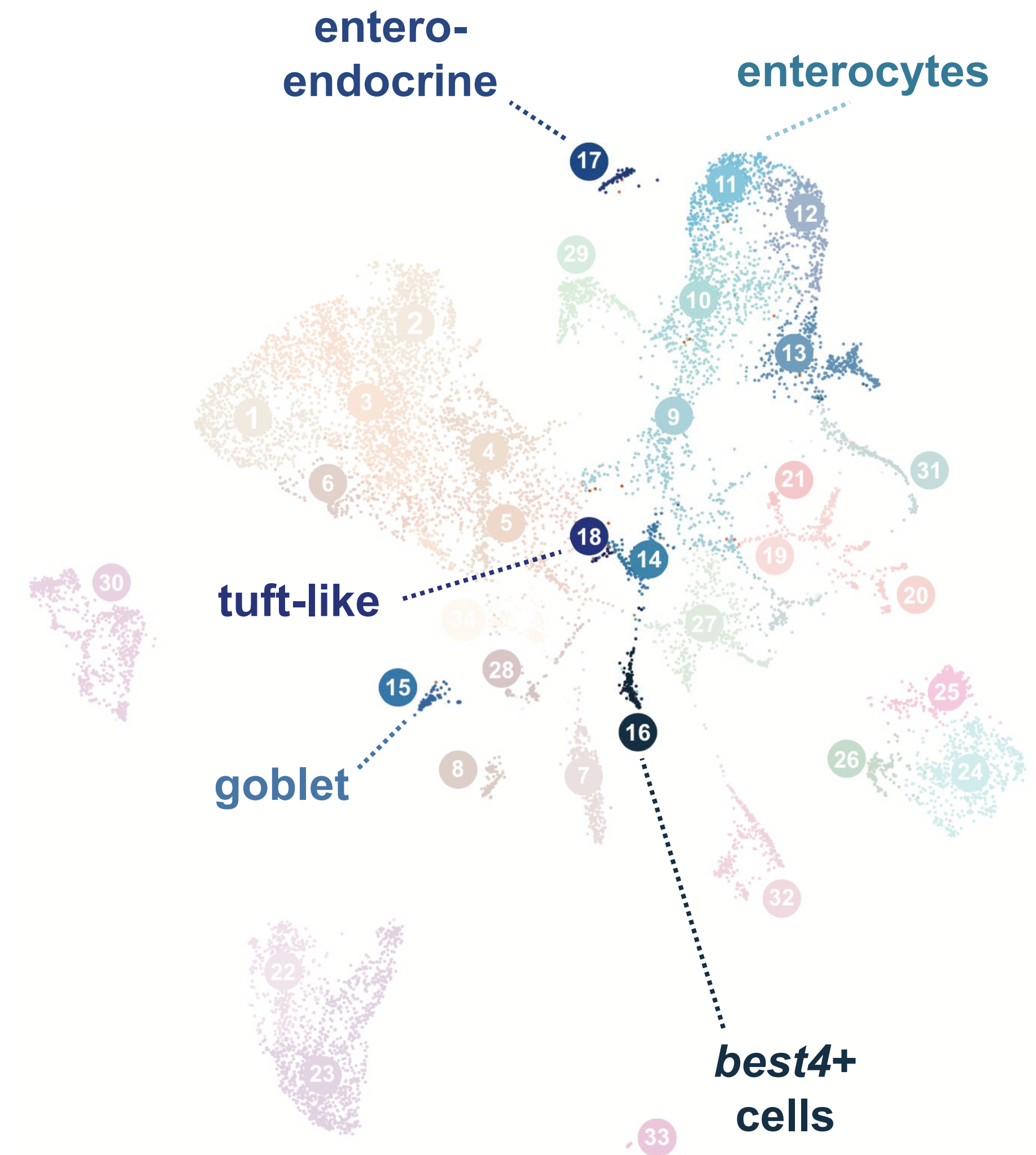
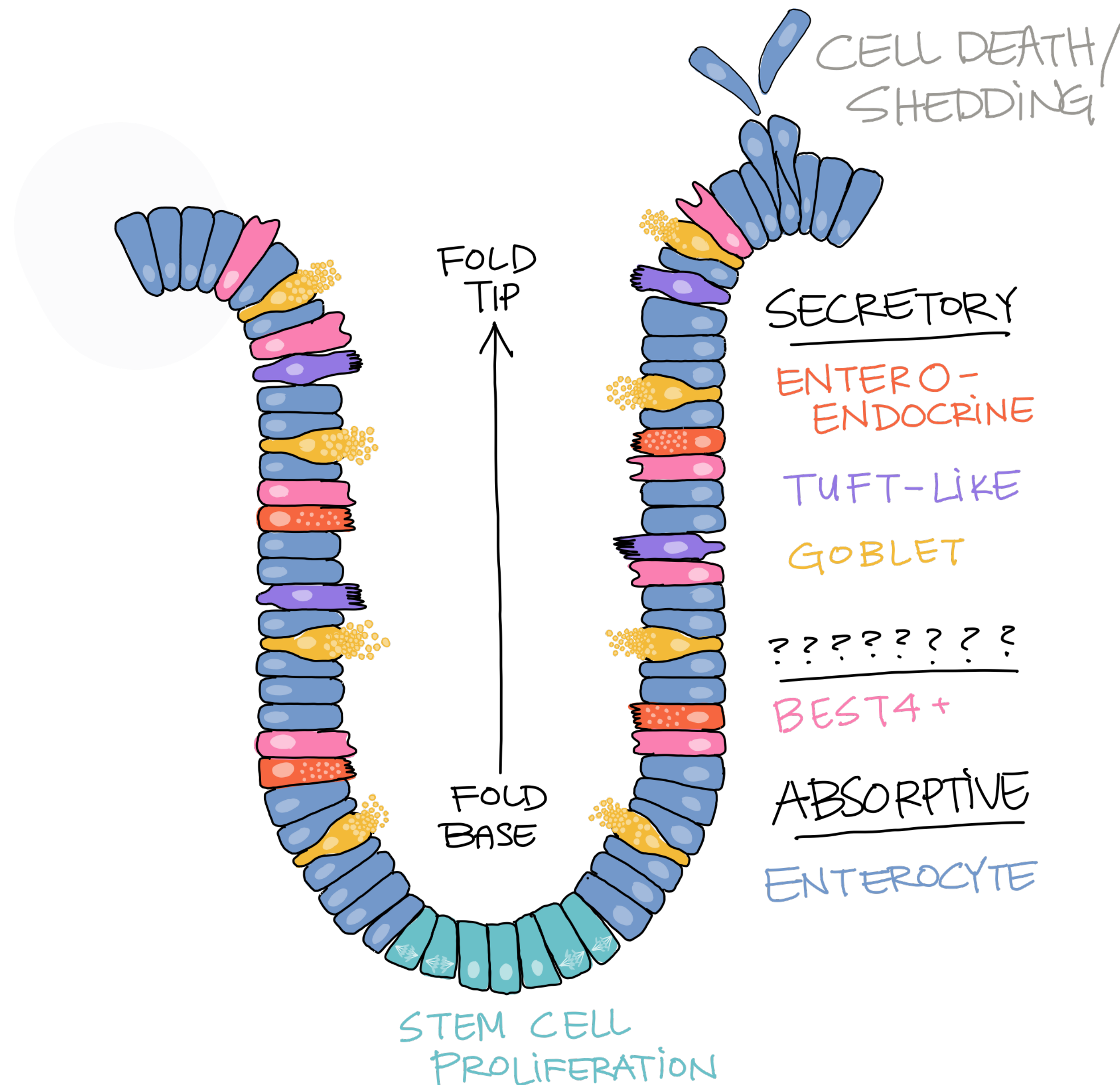


Wen ... Rawls: *Sci Adv* 2021

Willms, Jones, Hocking, Foley: *Cell Reports* 2022

Sur, Wang, Capar, Margolin, Prochaska, Farrell: *Dev Cell* 2023

Zebrafish homologs of *best4*+ intestinal cells



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Zebrafish homologs of *best4*+ intestinal cells

- ▶ Fewer *best4*+ cells in inflammatory bowel disease patients
- ▶ Increased expression of *best4*+ cell markers in some colorectal cancer patients
- ▶ Functionally uncharacterized
- ▶ Developmental program unknown



Parikh*, Antanaviciute*, Fawkner-Corbett* ... Simmons: *Nature* 2019

Smillie*, Biton*, Ordovas-Montanes* ... Regev: *Cell* 2019

Li *et al.*: *Cell Regen* 2022

Tang *et al.*: *BMC Biology* 2022

Malonga *et al.*: *AM J of Physiology* 2024

Westfall *et al.*: *PNAS* 2024

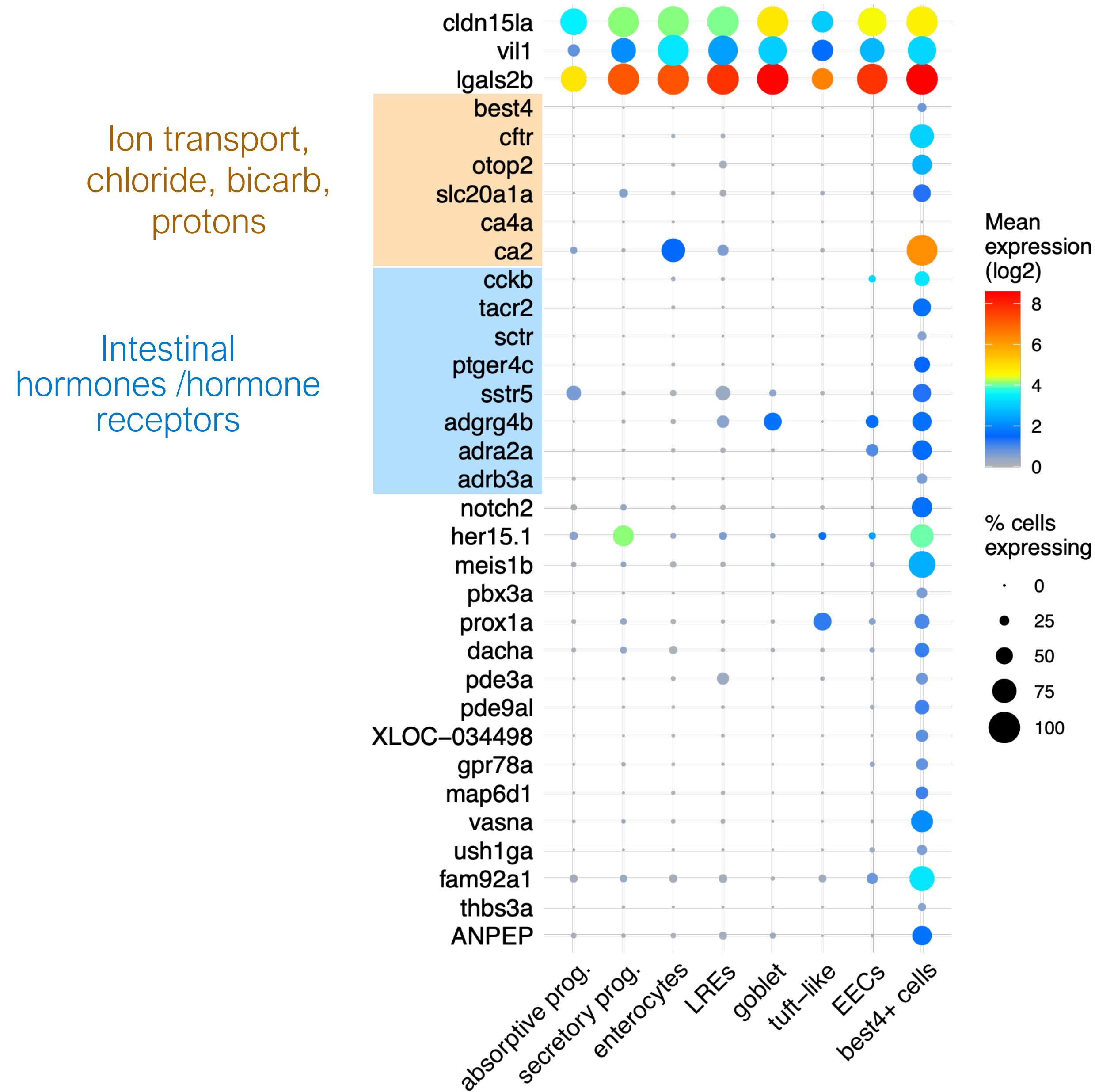
Trezíse & Buchwald: *Nature* 1991

Ameen *et al.*: *Gastroenterology* 1995

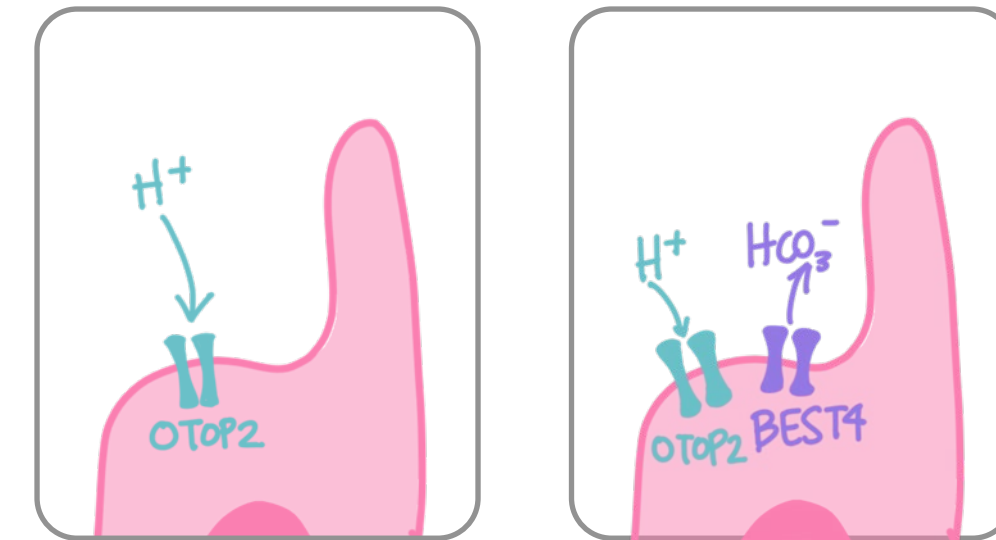
- ▶ Characterize *best4*⁺ cells
- ▶ Where do *best4*⁺ cells come from?
- ▶ What developmental program specifies *best4*⁺ cells?

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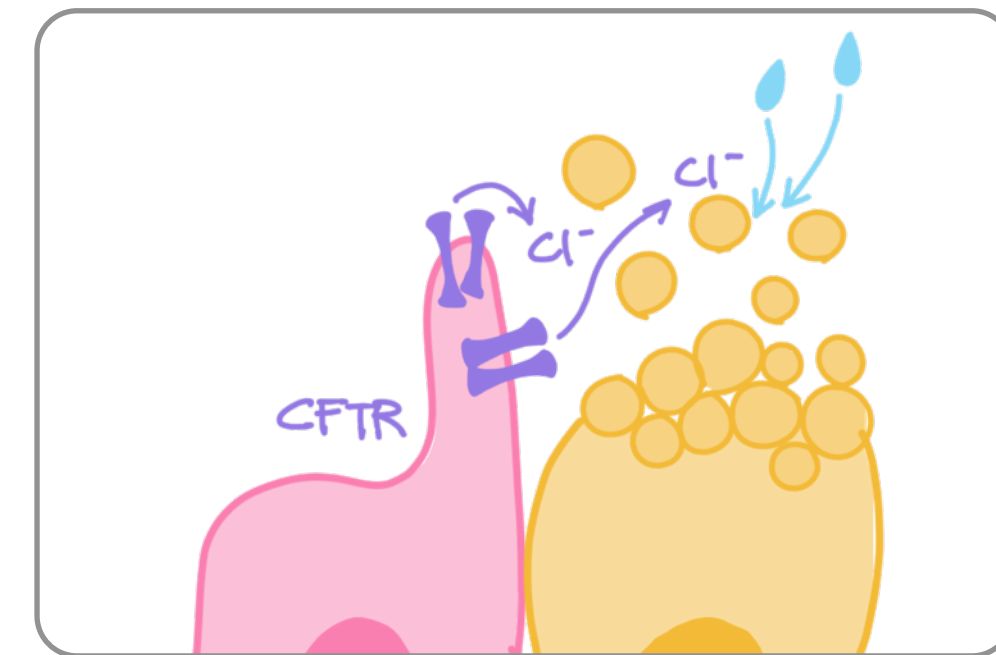
best4⁺ cells have a distinct gene expression program



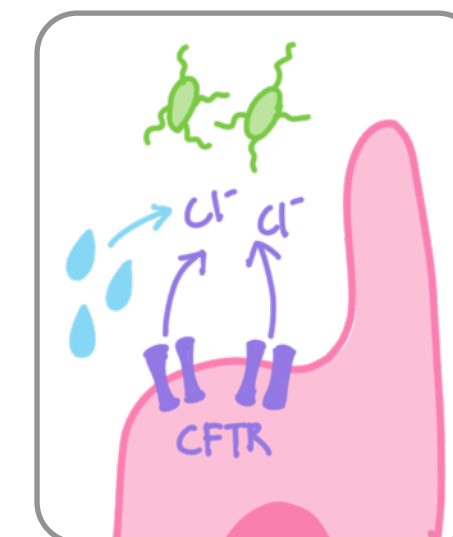
pH sensation pH regulation



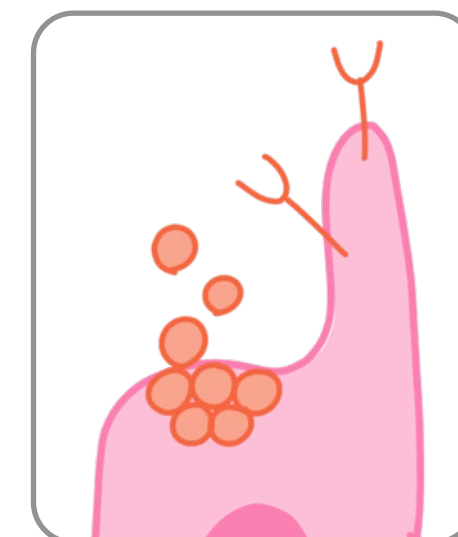
hydration of mucous layer



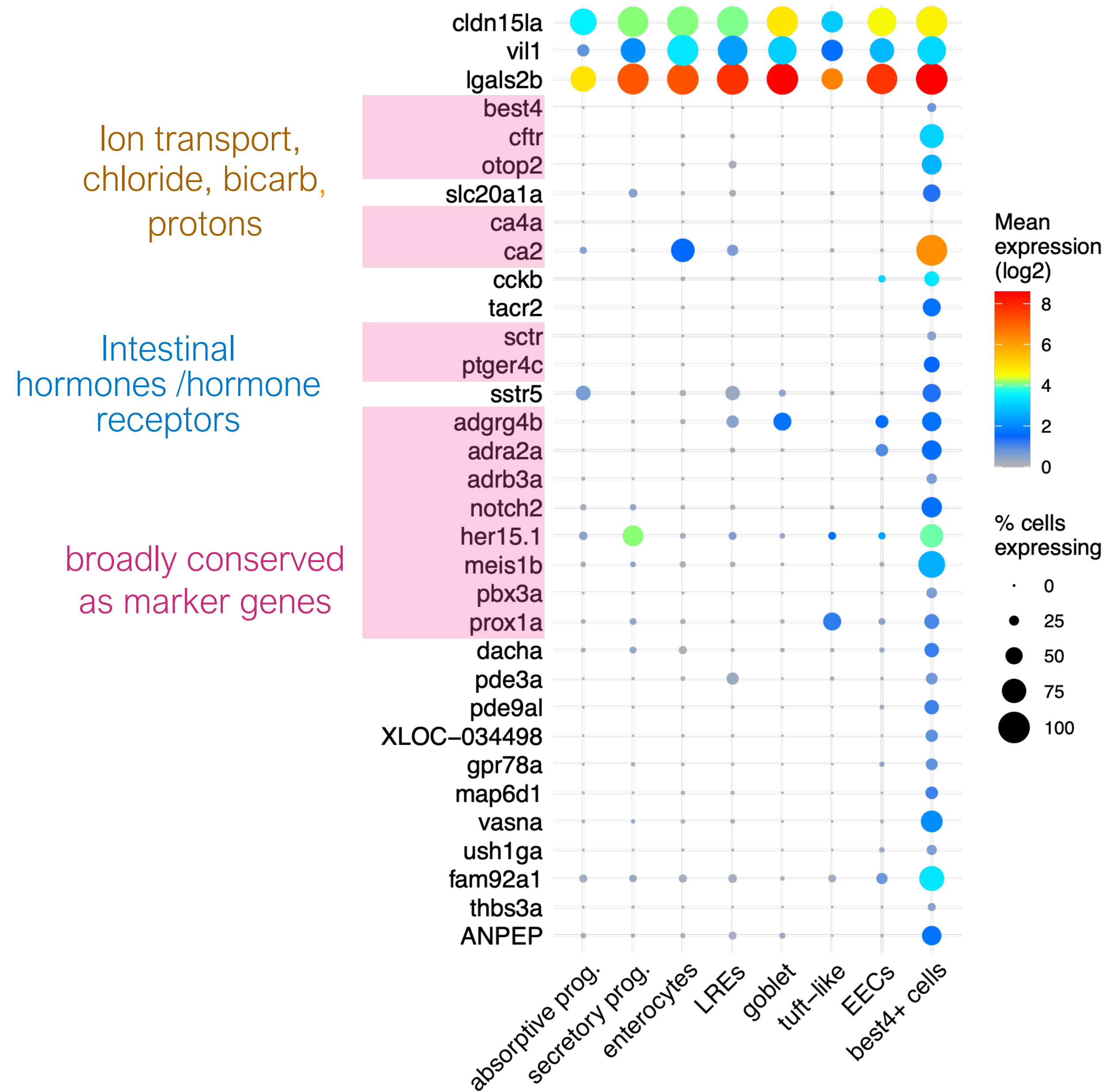
pathogen response



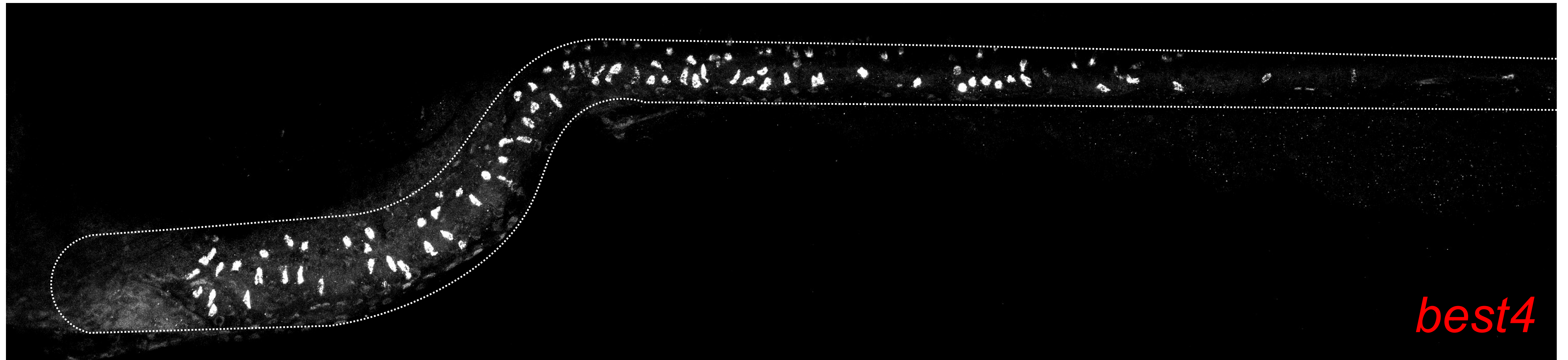
coordination of behavior



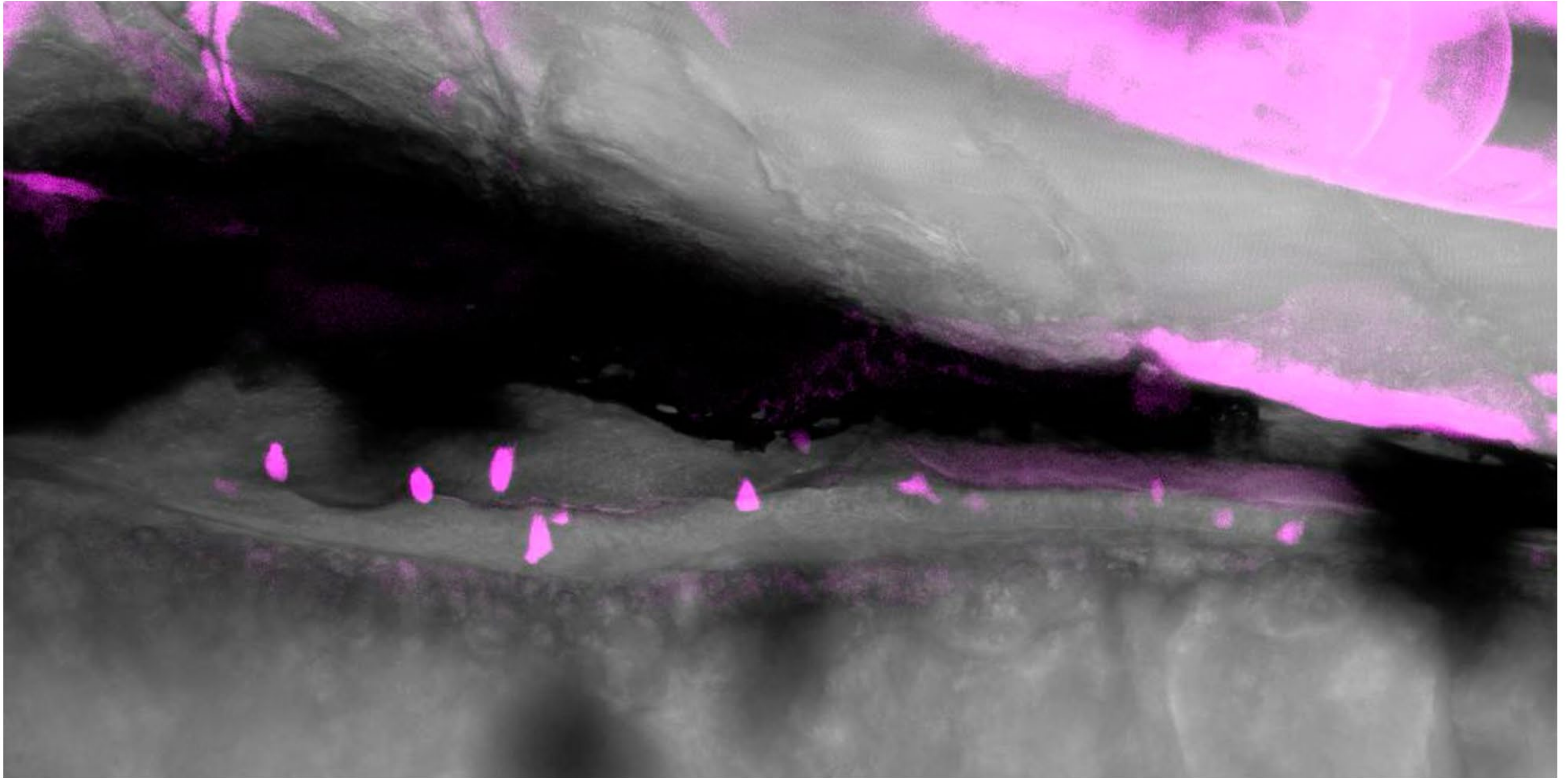
best4+ cells specific genes are evolutionarily conserved



best4⁺ cells are found in all regions of the zebrafish gut



best4+ cells extend motile projections

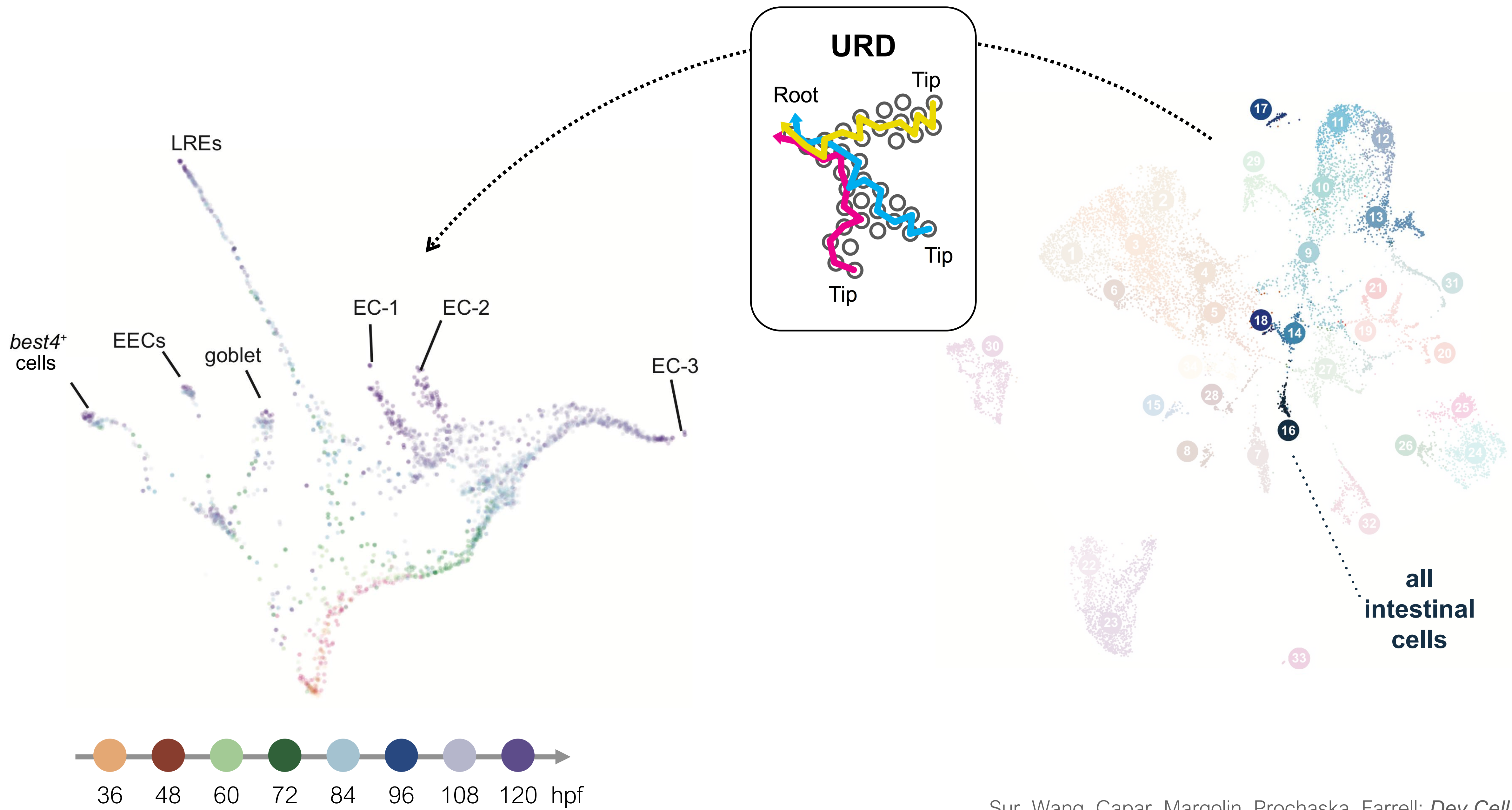


3 days post-fertilization

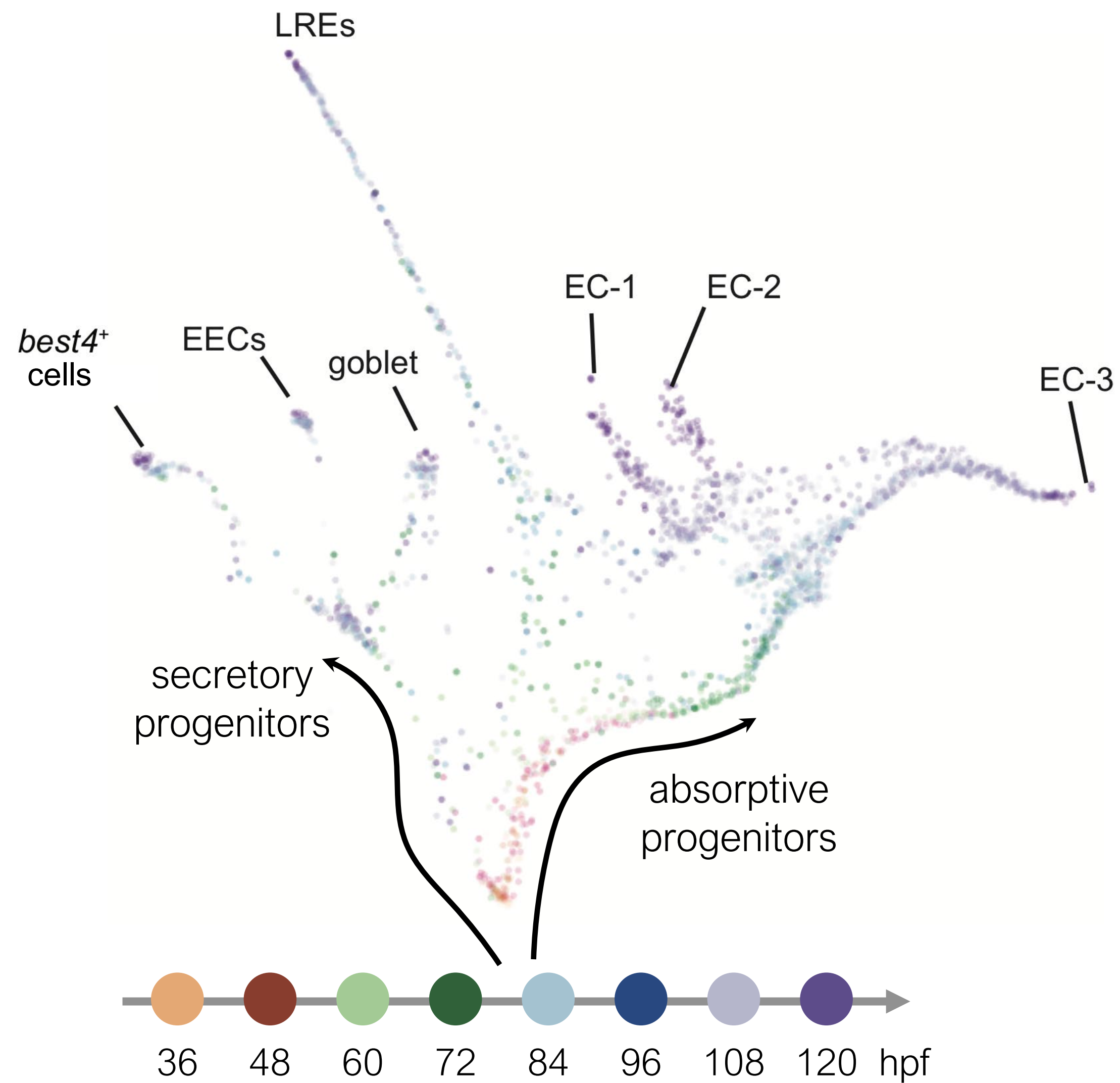
best4::mApple

- ▶ Characterize *best4*⁺ cells
- ▶ Where do *best4*⁺ cells come from?
- ▶ What developmental program specifies *best4*⁺ cells?

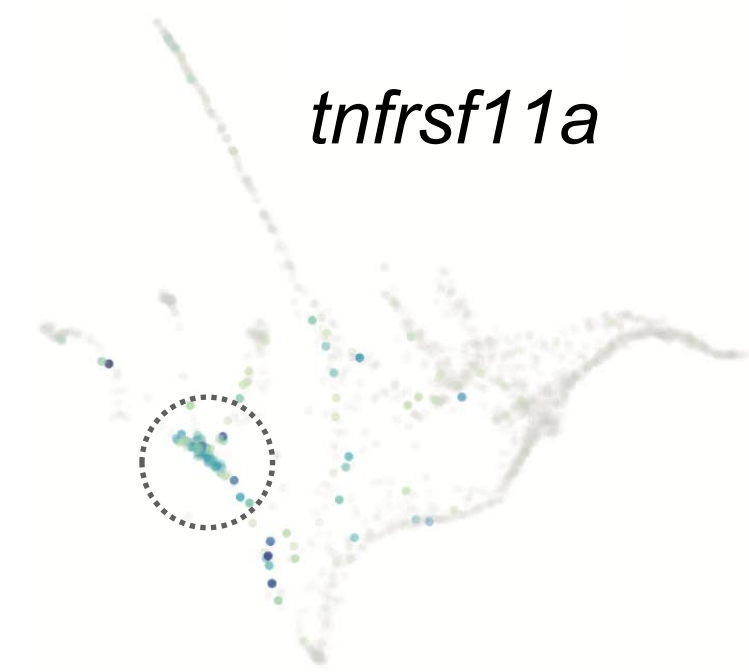
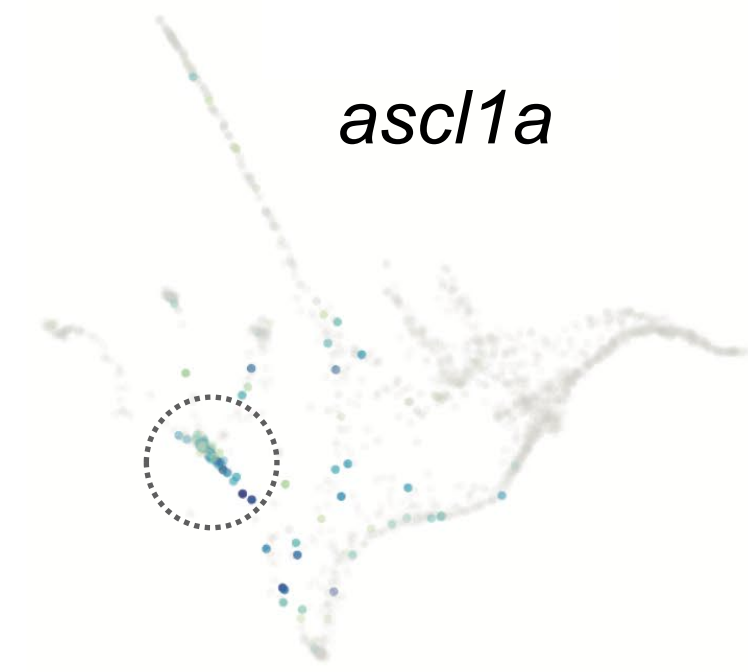
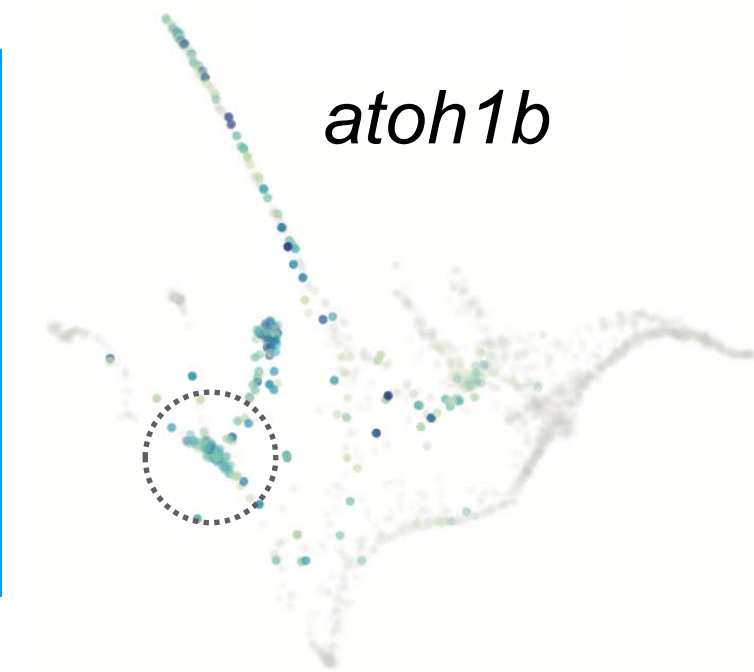
Transcriptional trajectories to uncover developmental gene expression programs



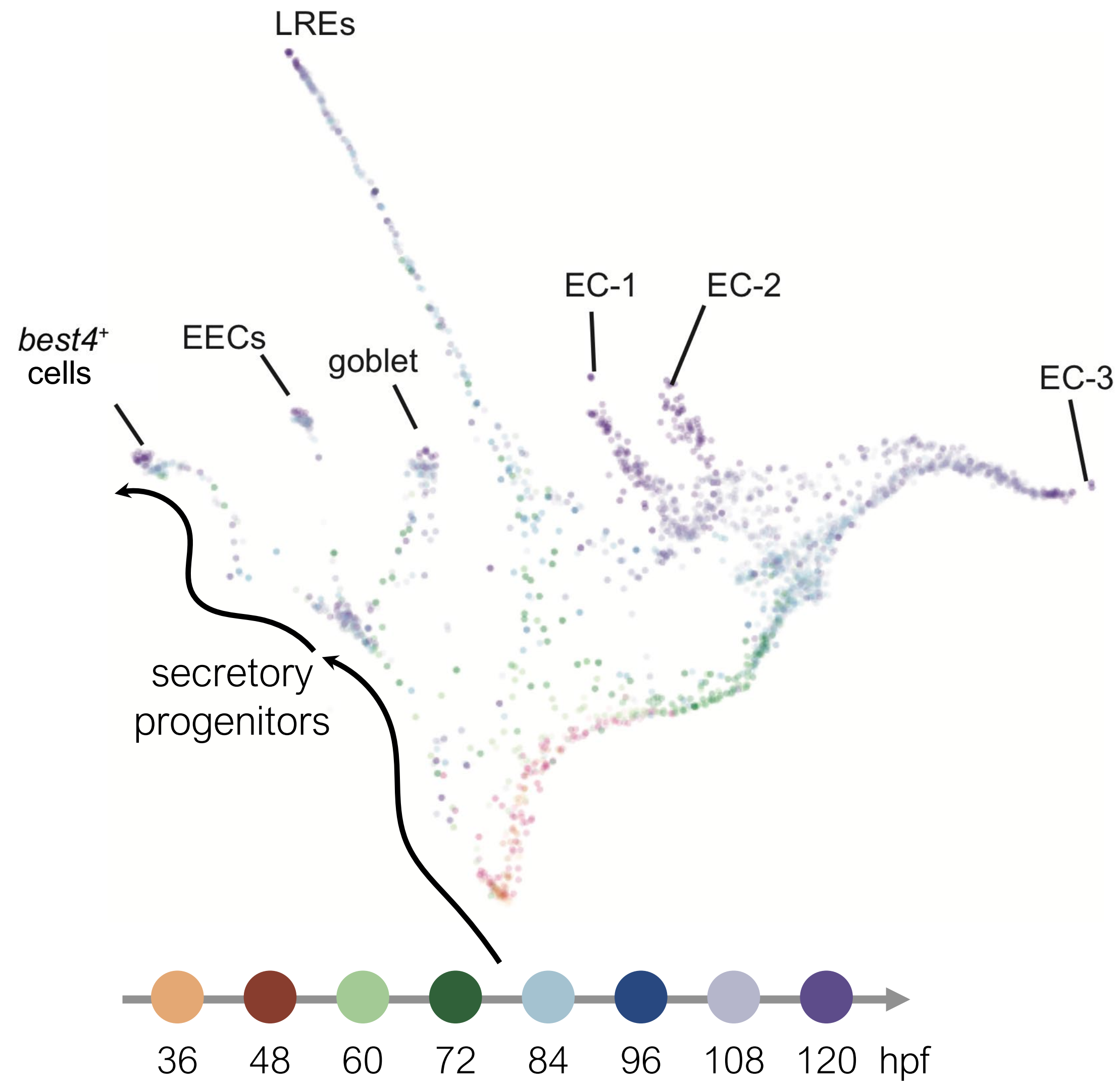
best4⁺ cells putatively arise from secretory progenitors



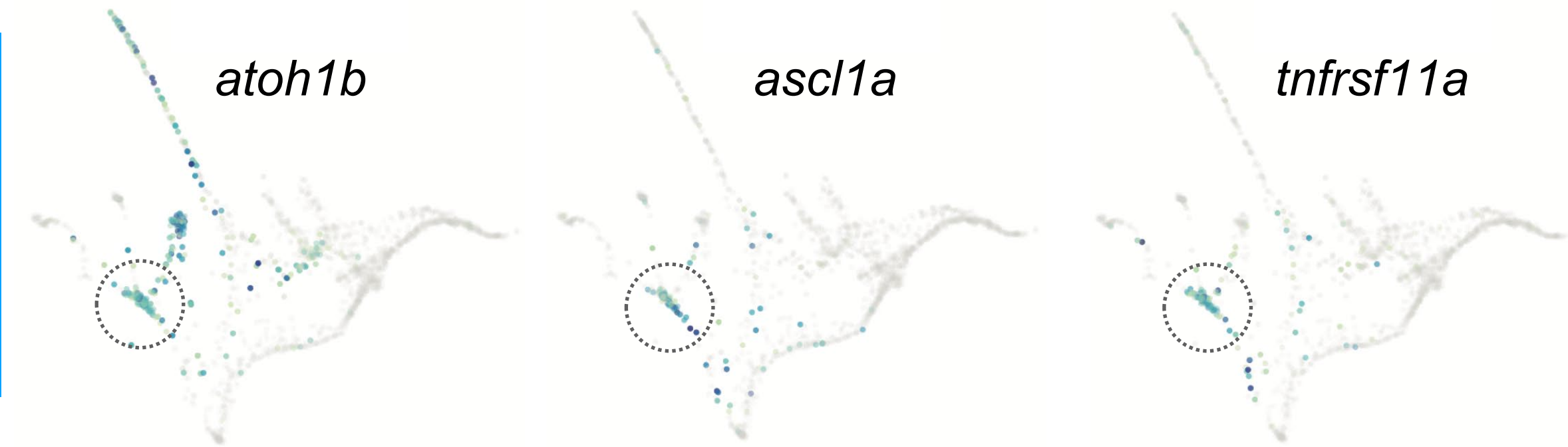
Progenitors



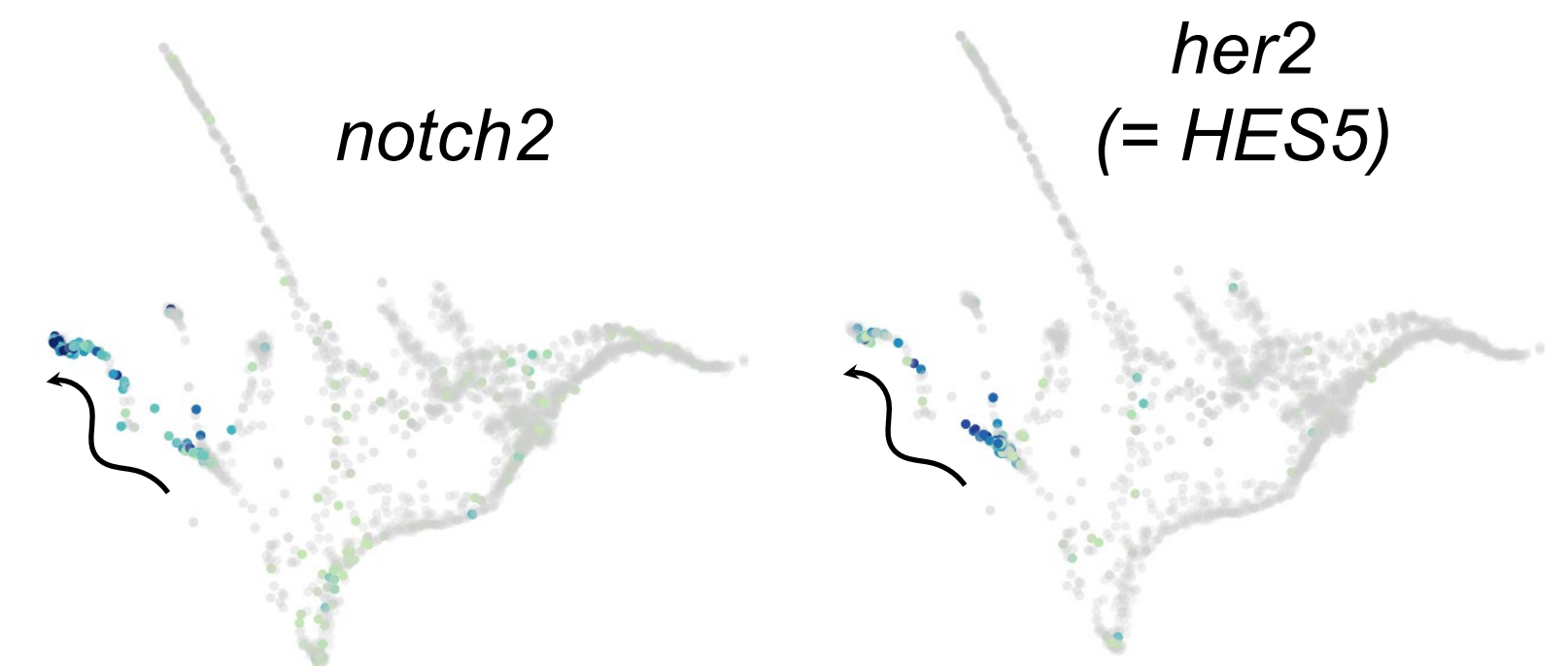
The signals that might drive *best4*⁺ cell specification



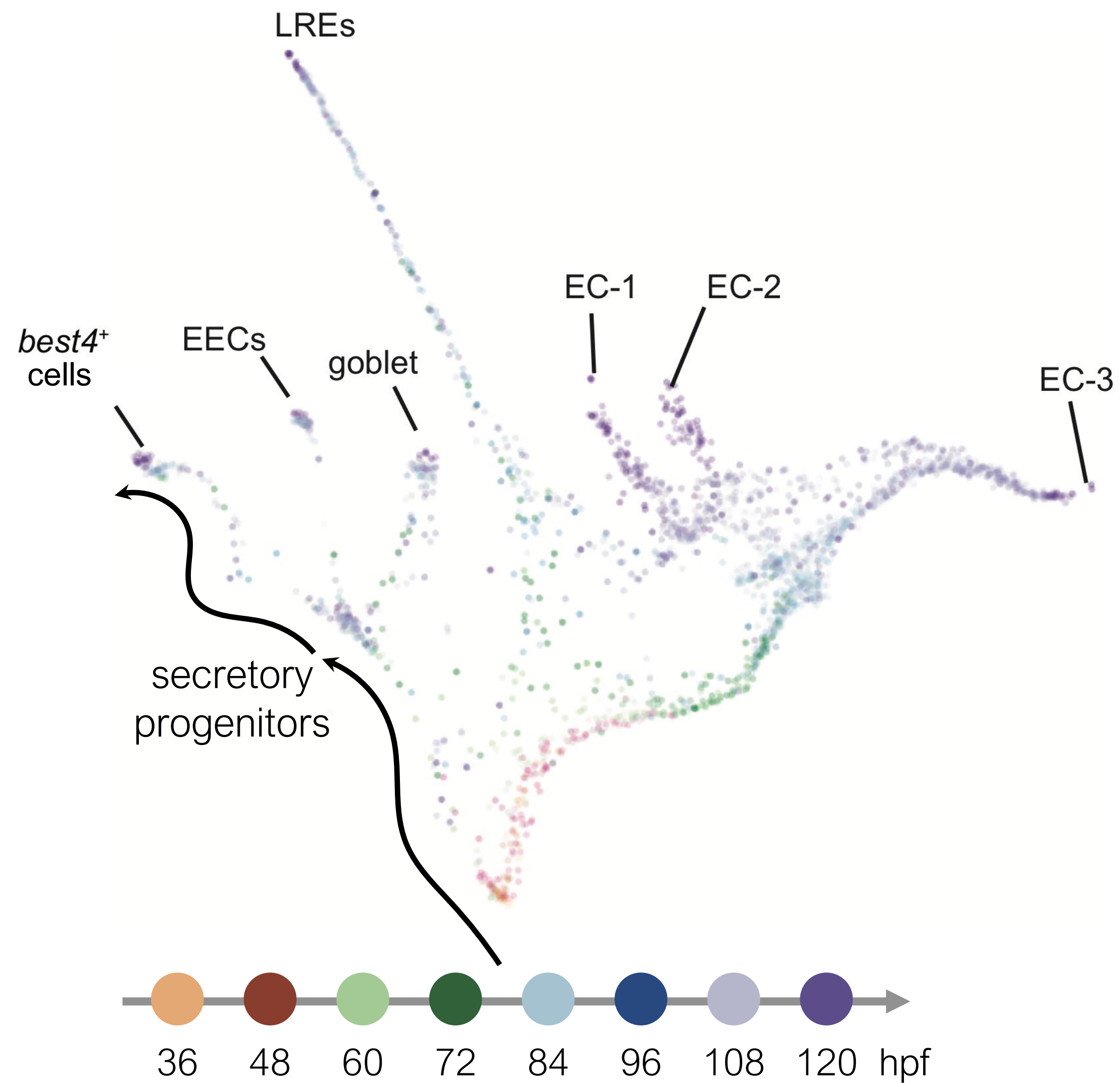
Progenitors



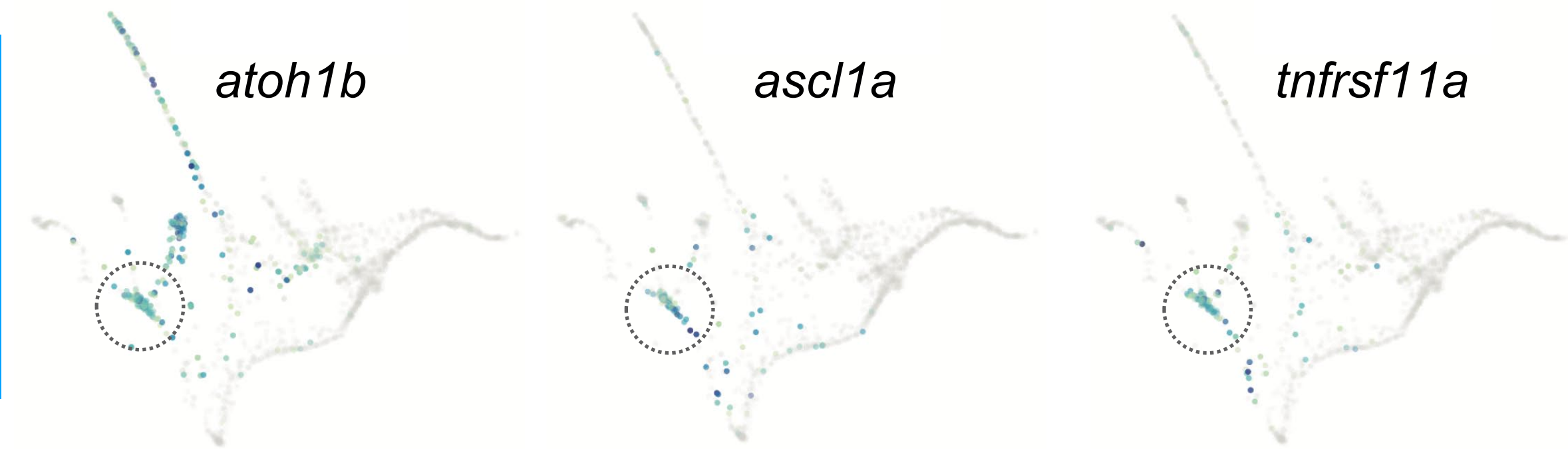
Signals



The gene regulatory network underlying *best4*+ cell specification



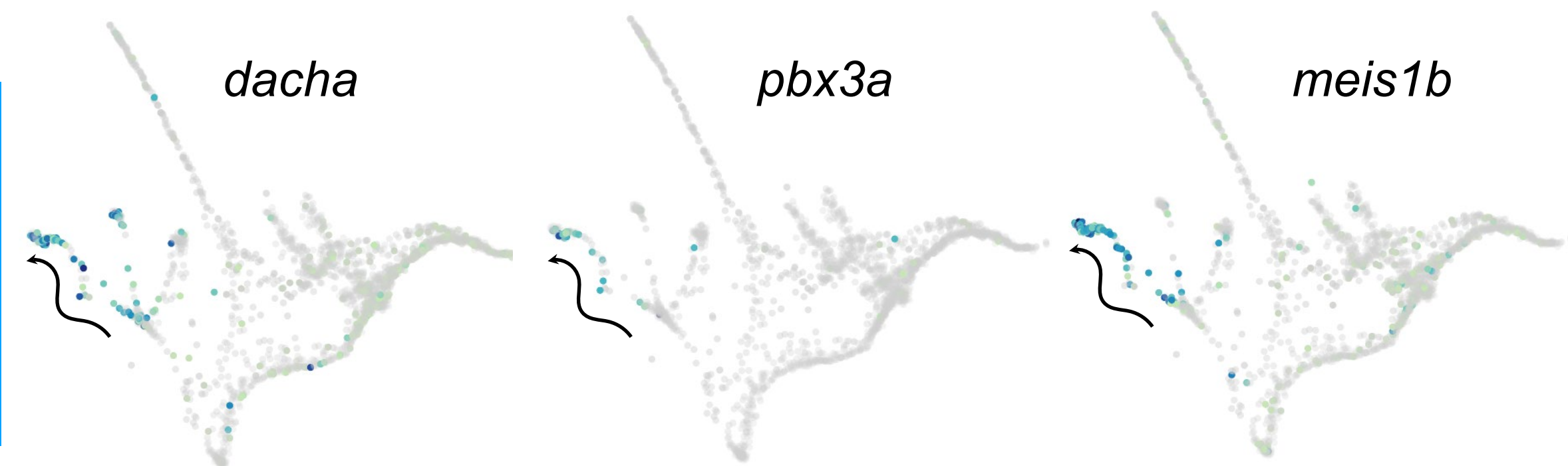
Progenitors



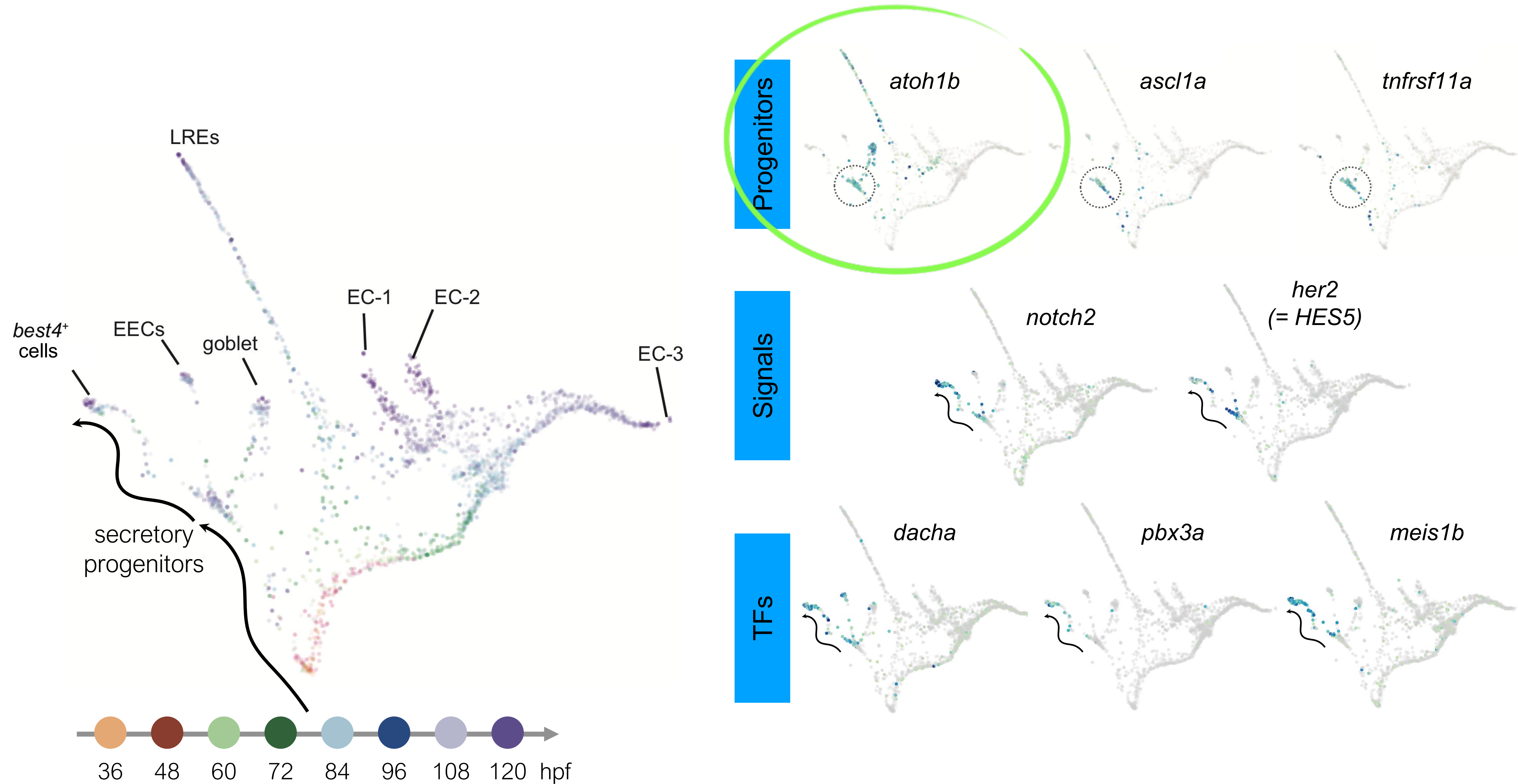
Signals



TFs

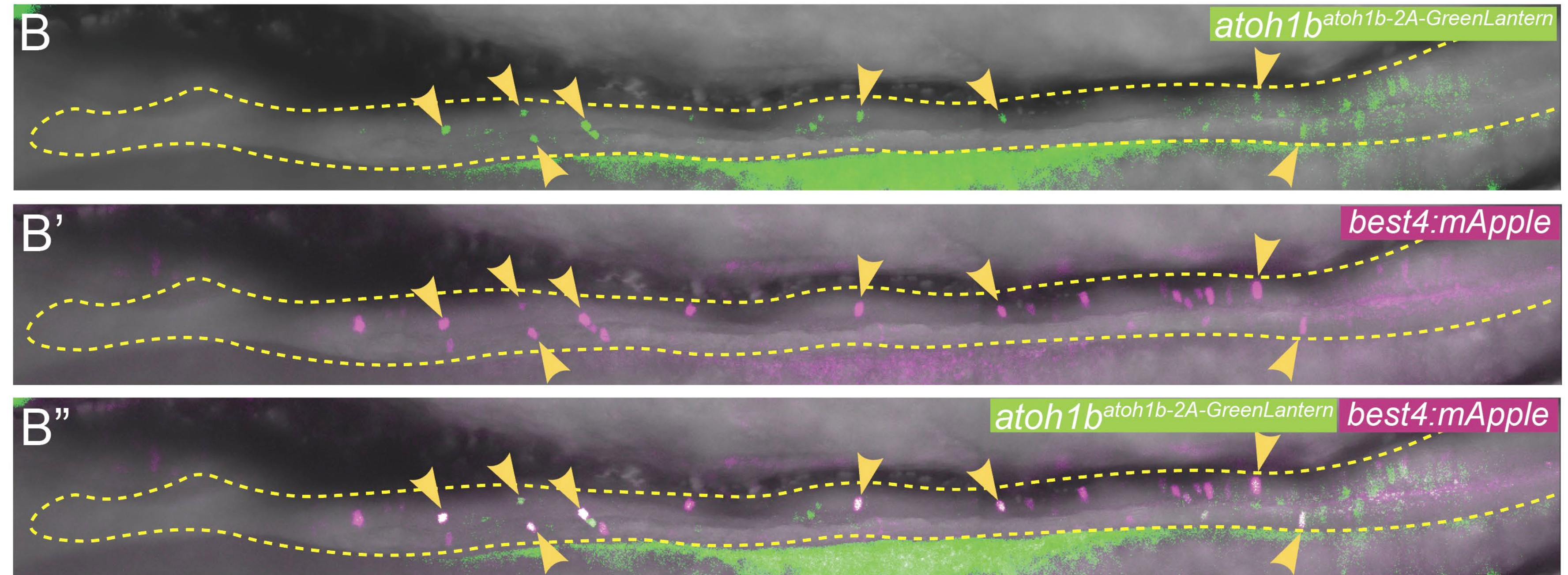
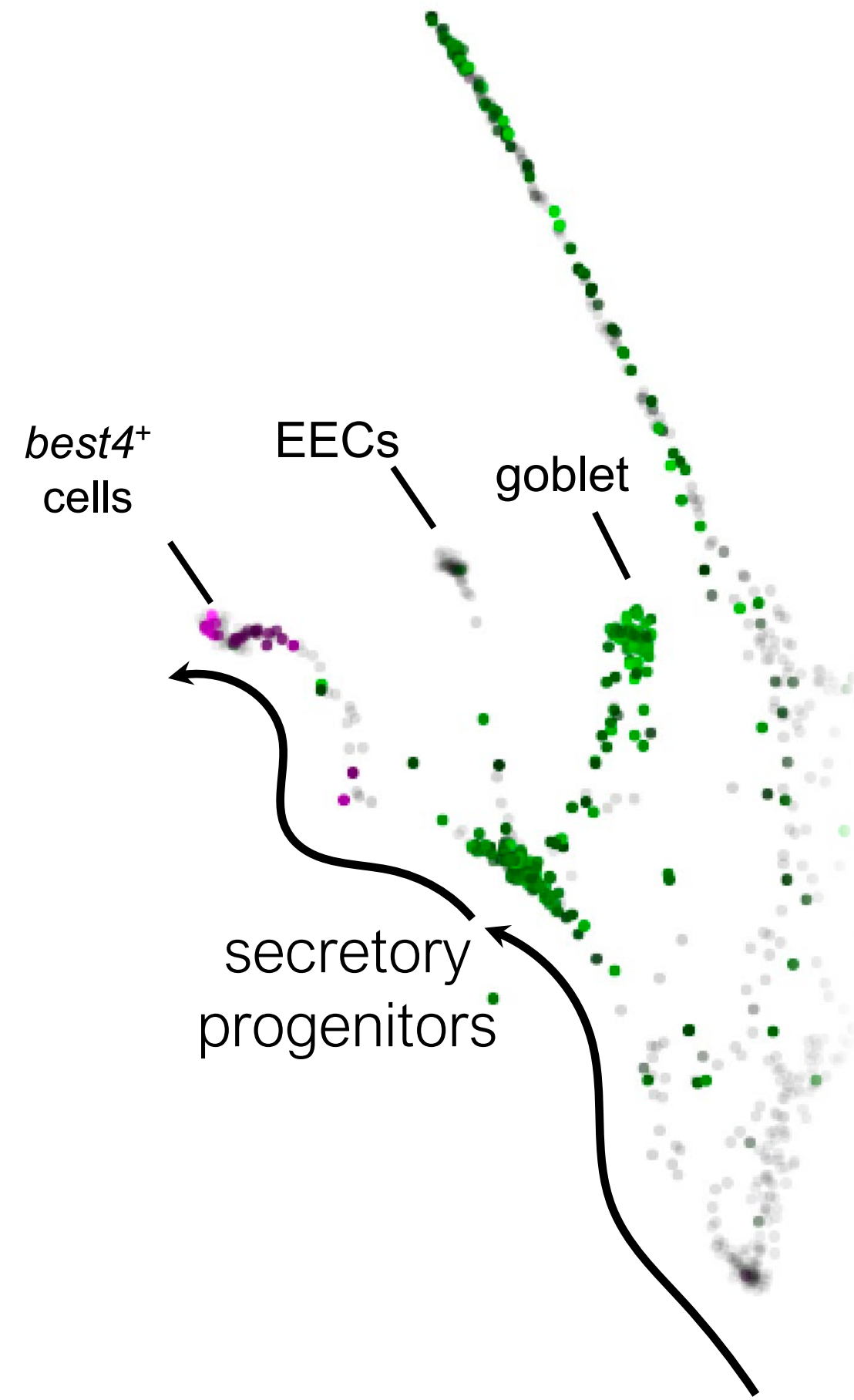


Testing the predictions: do *best4*⁺ cells come from *atoh1b*⁺ secretory progenitors?

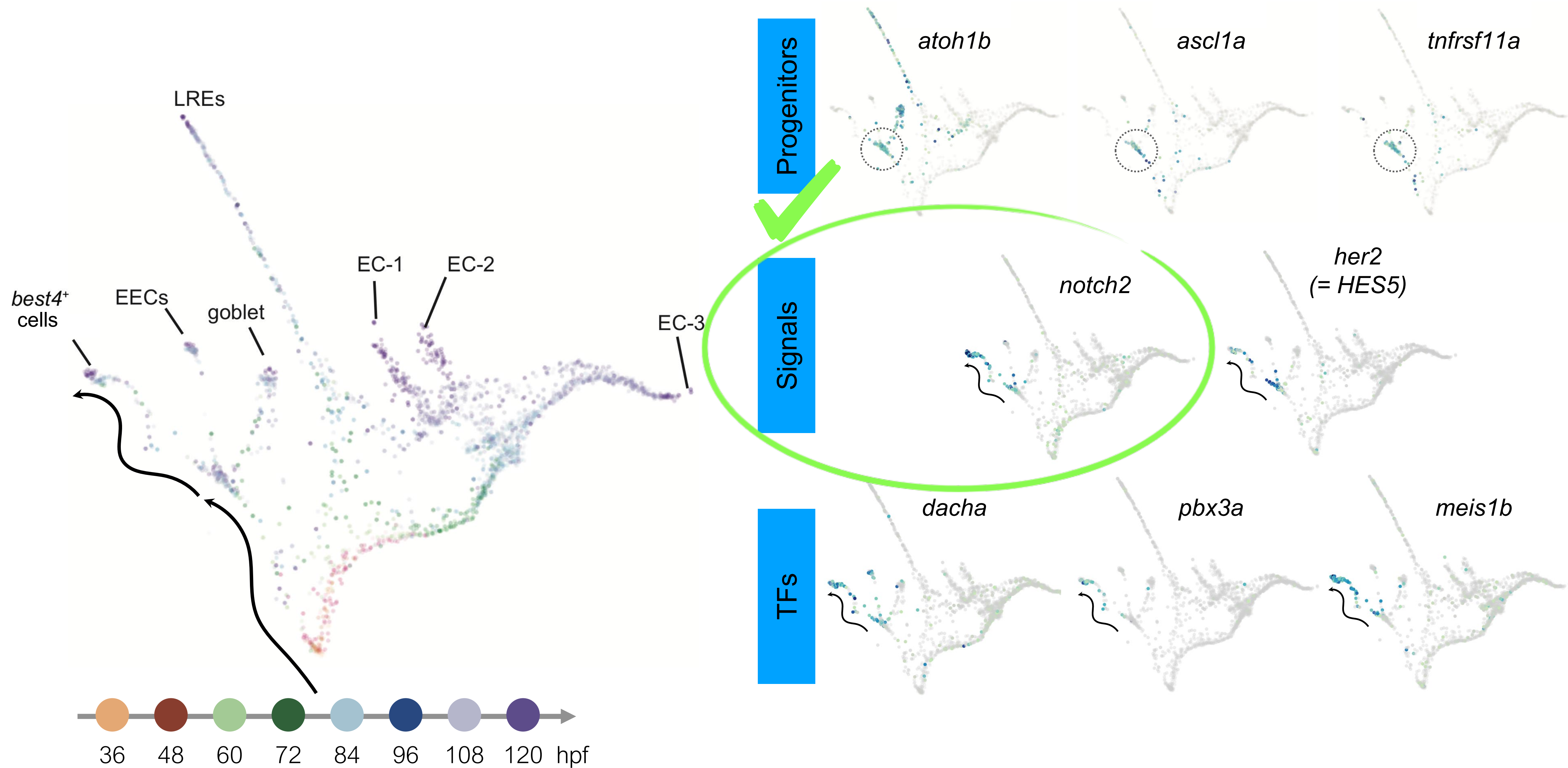


best4⁺ cells arise from *atoh1b*⁺ (secretory) progenitors

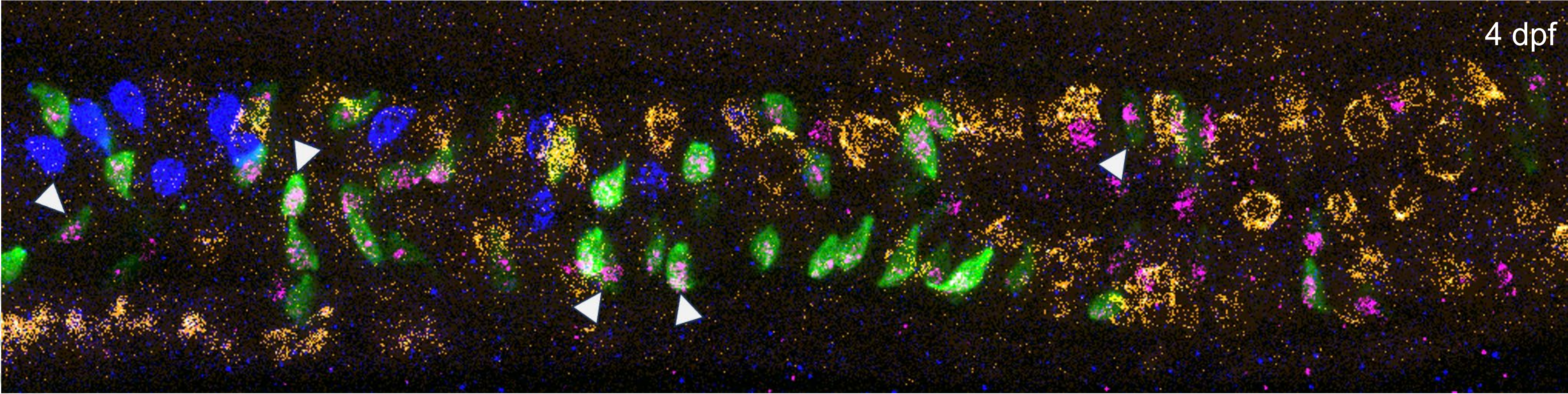
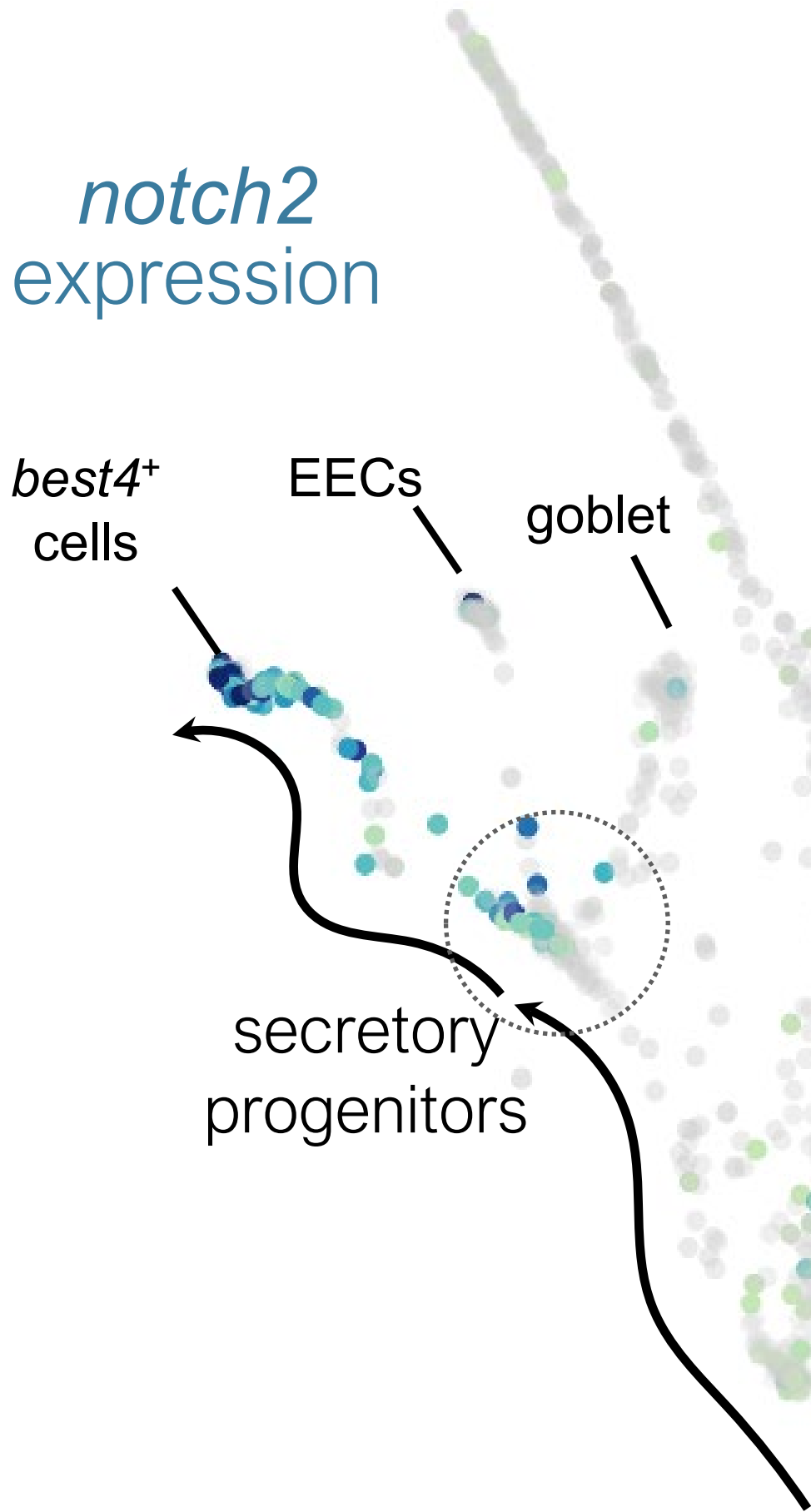
best4:mApple
atoh1b^{*atoh1b-2A-mGreenLantern*}



Testing the predictions: is Notch the key signal to specify *best4+* cells?

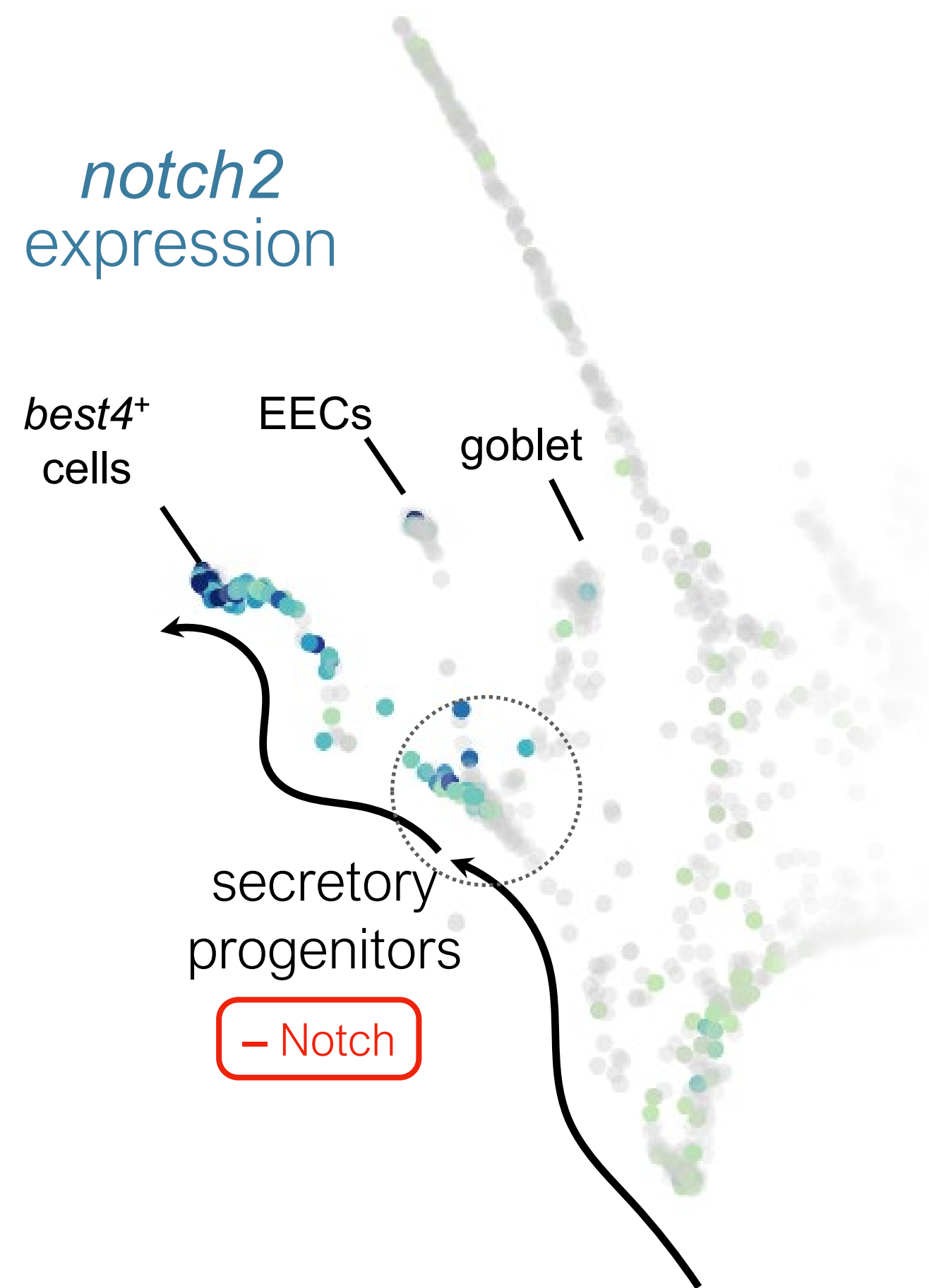


Cells actively receiving Notch signaling at 4 dpf are *best4*+ cells



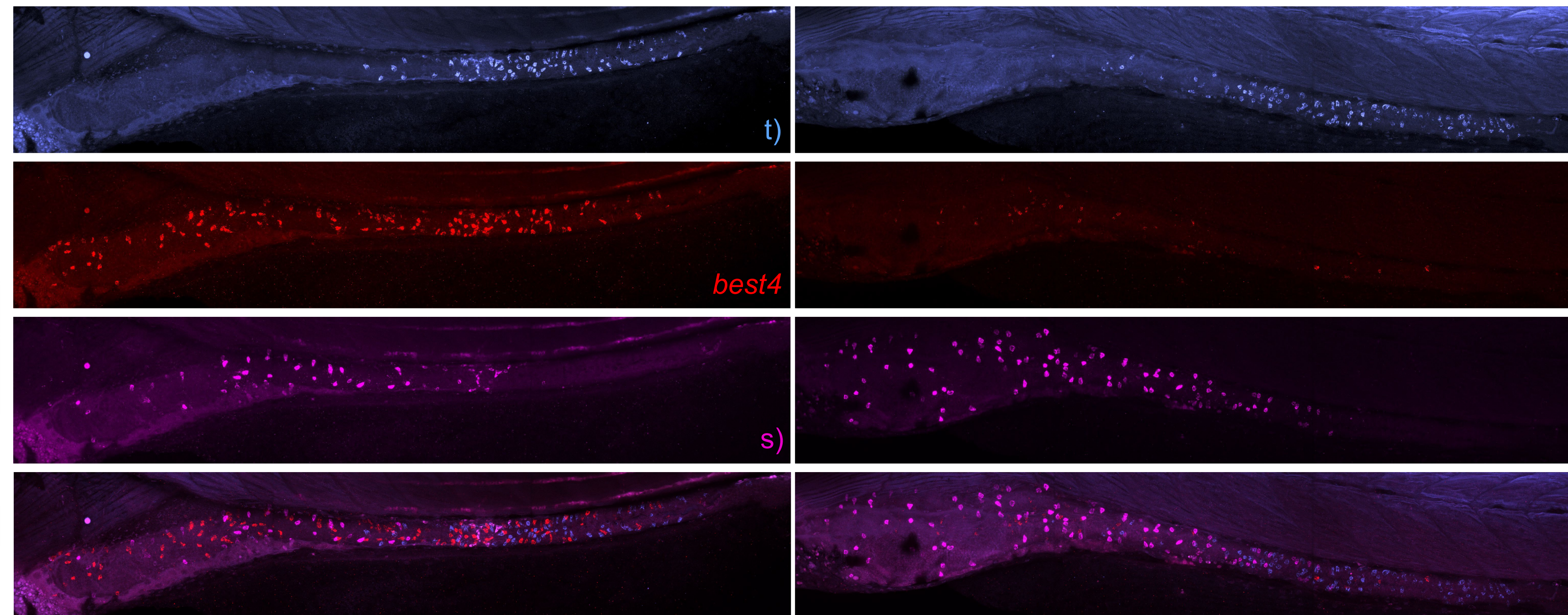
<i>penka</i>	<i>muc2.1</i>	<i>best4</i>	Tp1:GFP
entero-chromaffin	goblet cells	<i>best4</i> ⁺ cells	Notch-responding

best4⁺ cell specification requires Notch signaling after secretory/absorptive decision

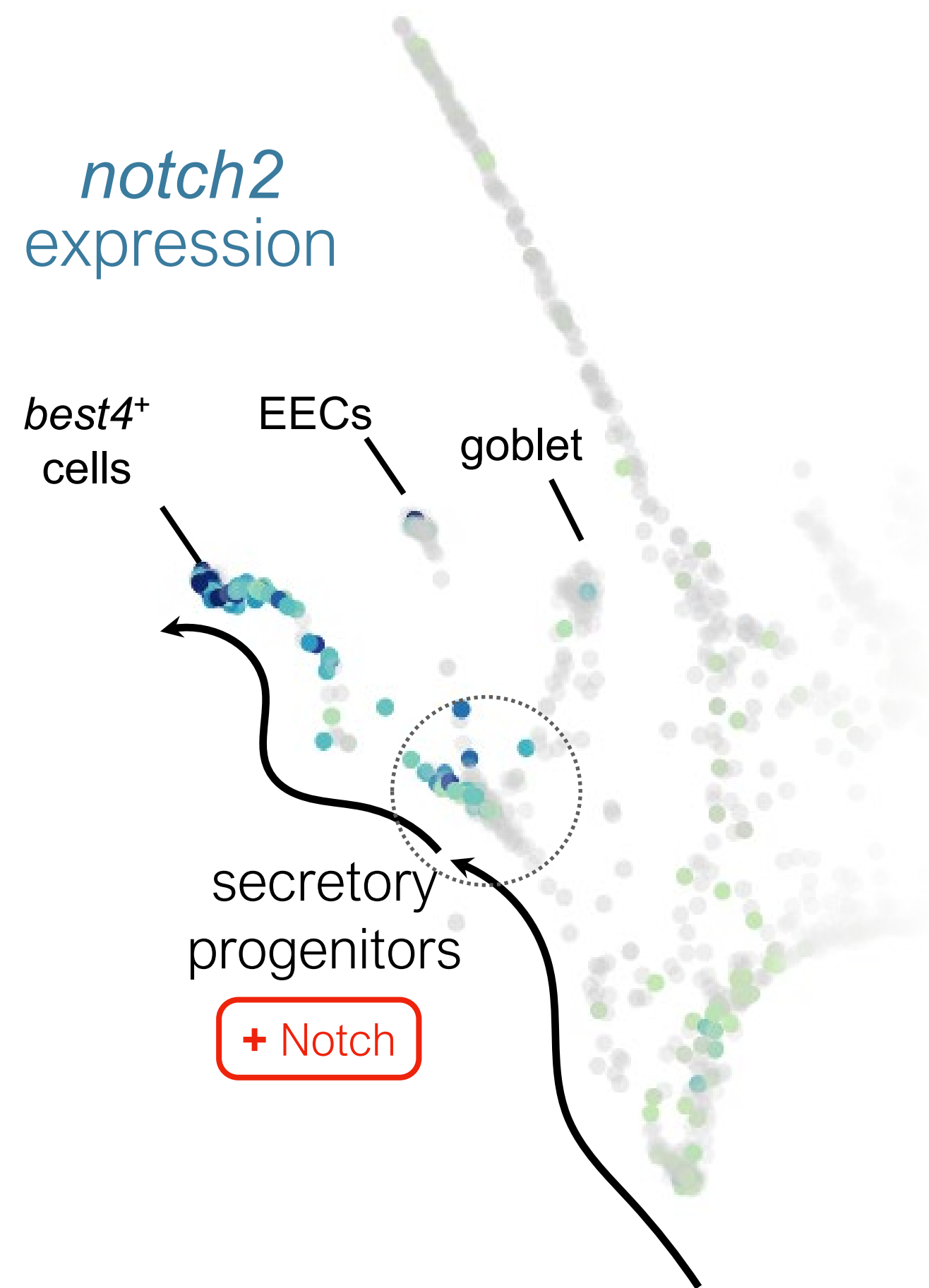


Control (DMSO)

Notch inhibition (DAPT) 72–100 hpf

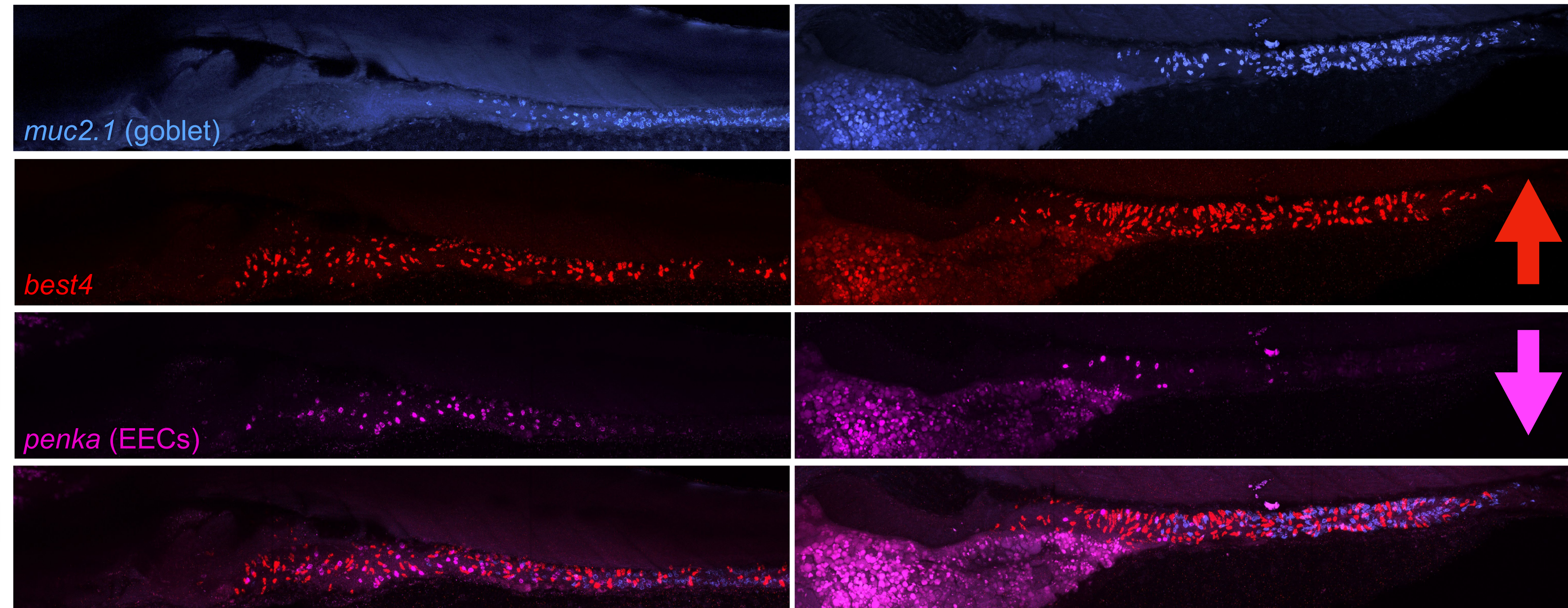


Increased Notch signaling converts cells to *best4*+ cells

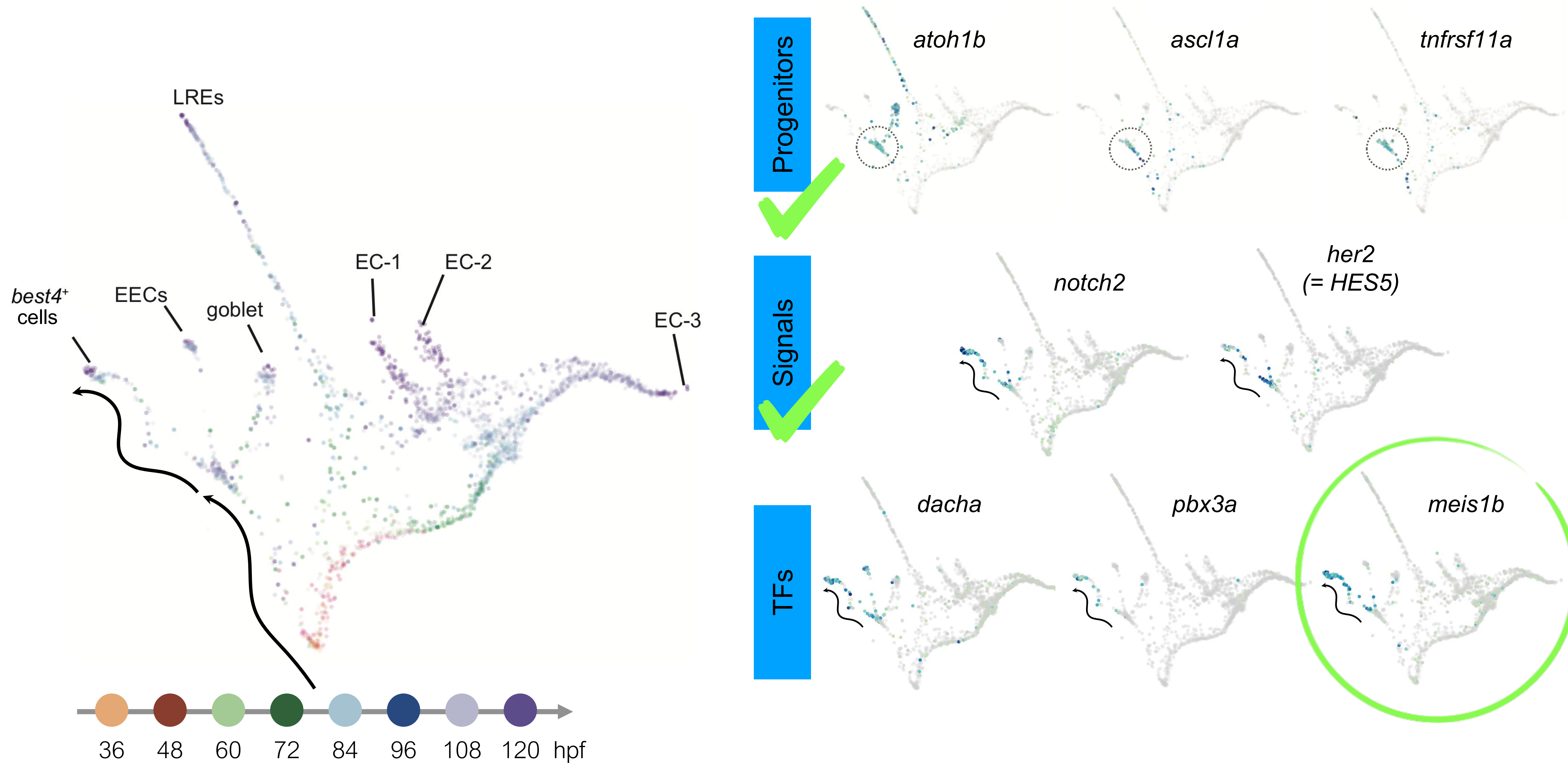


Control (Gal4–)

hsp:Gal4; UAS:NICD
(heat shock @ 72, 84 hpf)

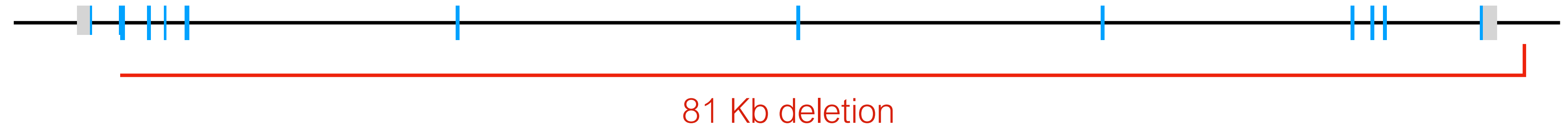


Testing the predictions: is *meis1b* required downstream of Notch signaling?

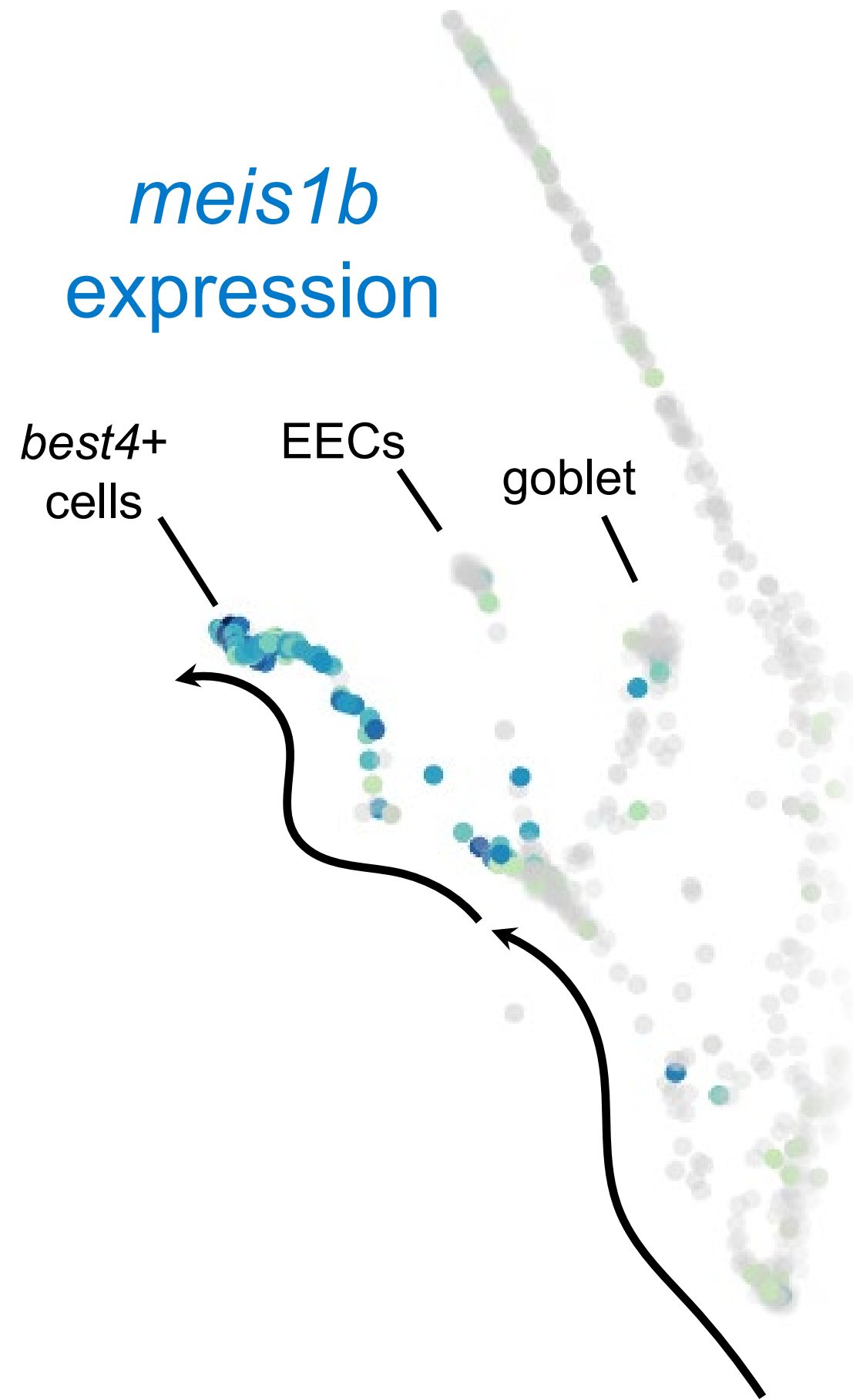


meis1b is required for *best4*+ cell specification

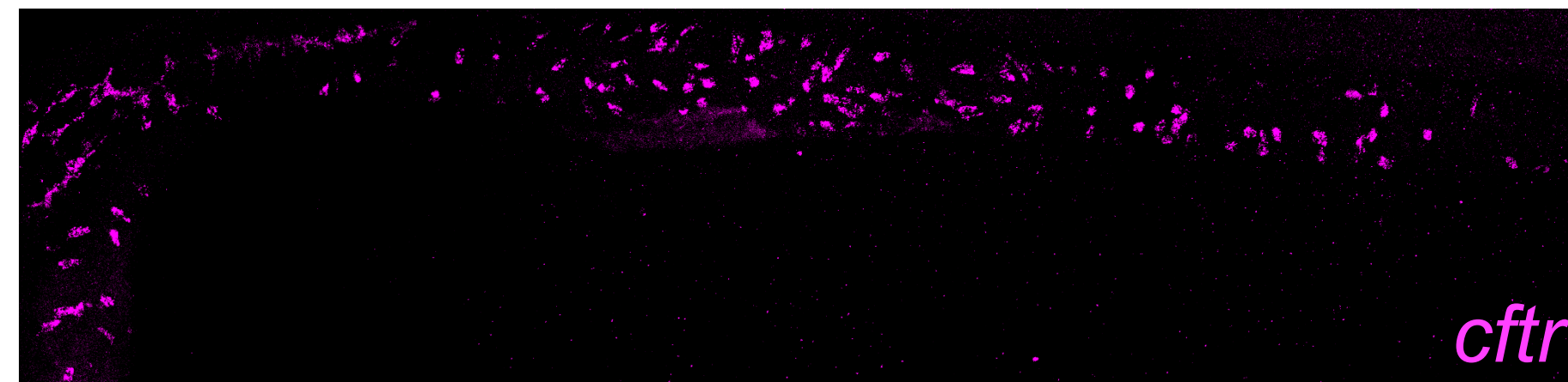
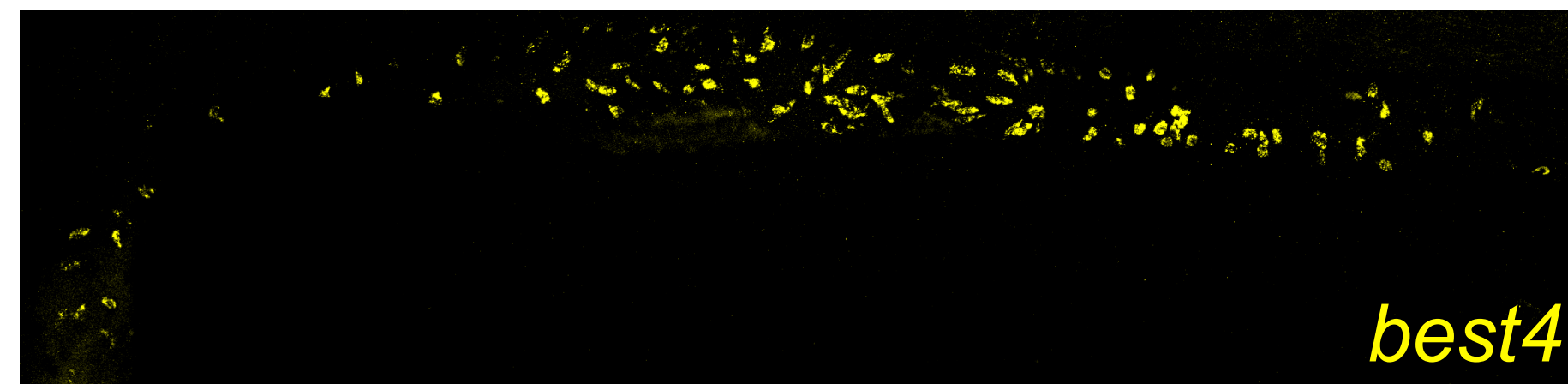
meis1b whole-gene deletion



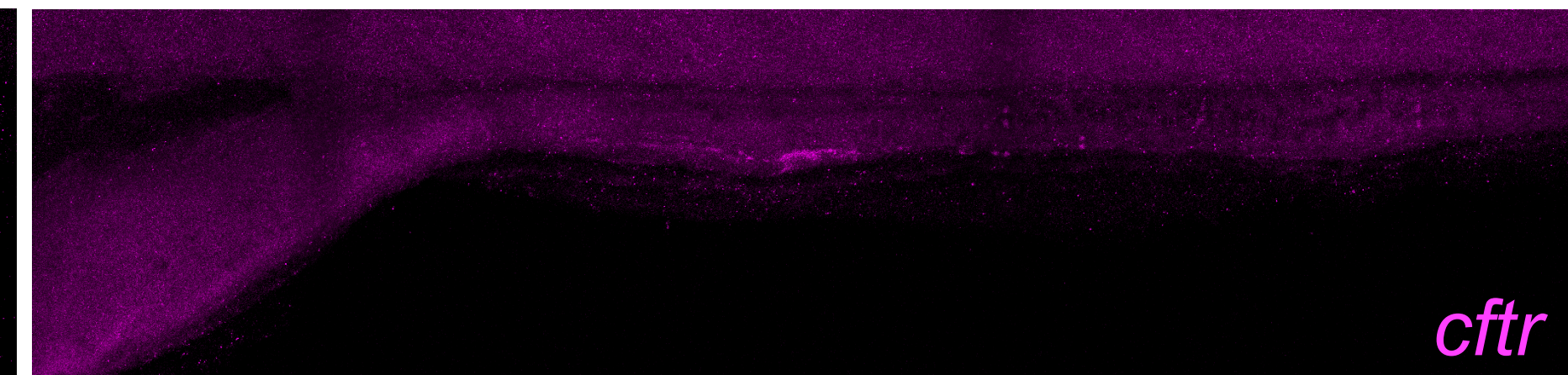
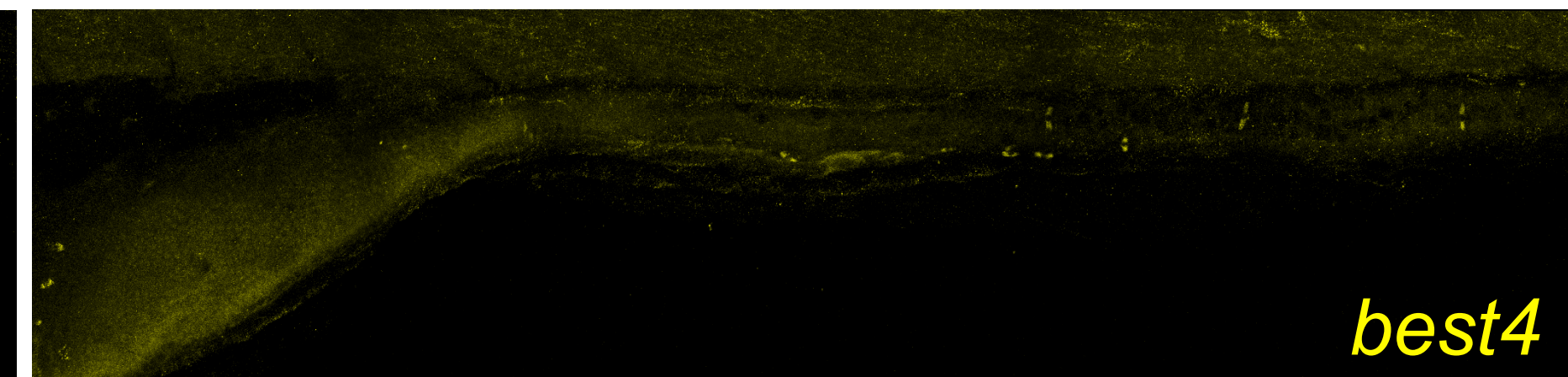
meis1b
expression



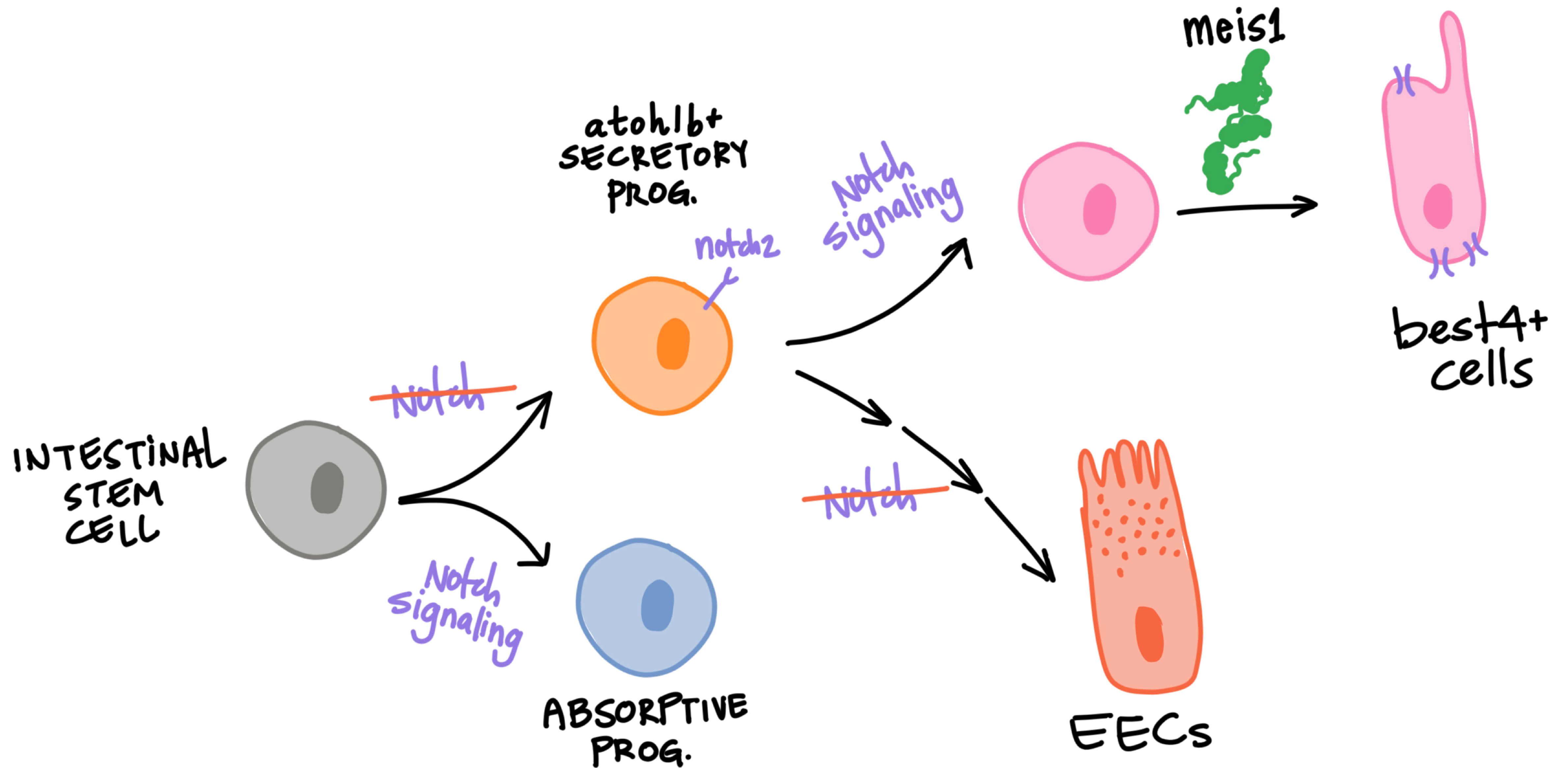
wild type



meis1b^{KO}

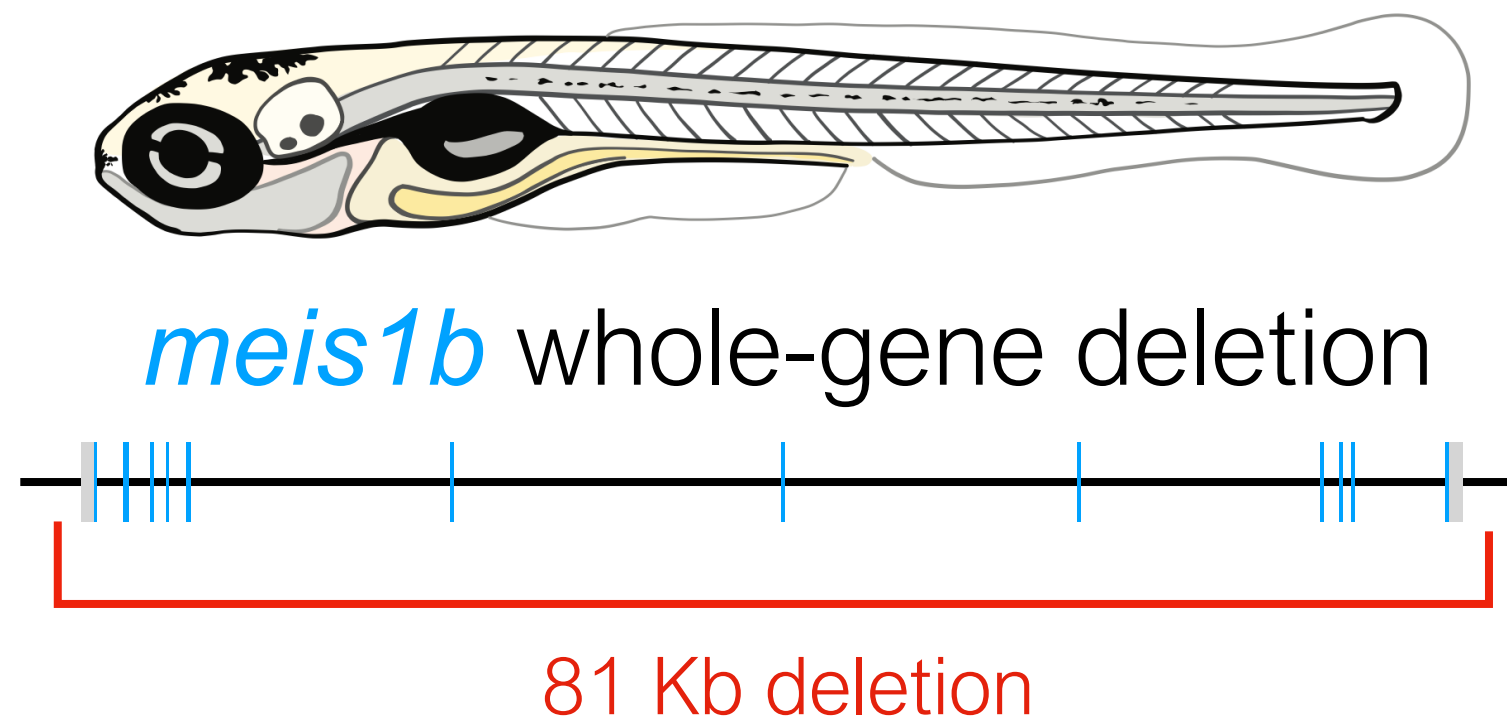


A working model for *best4*+ cell specification

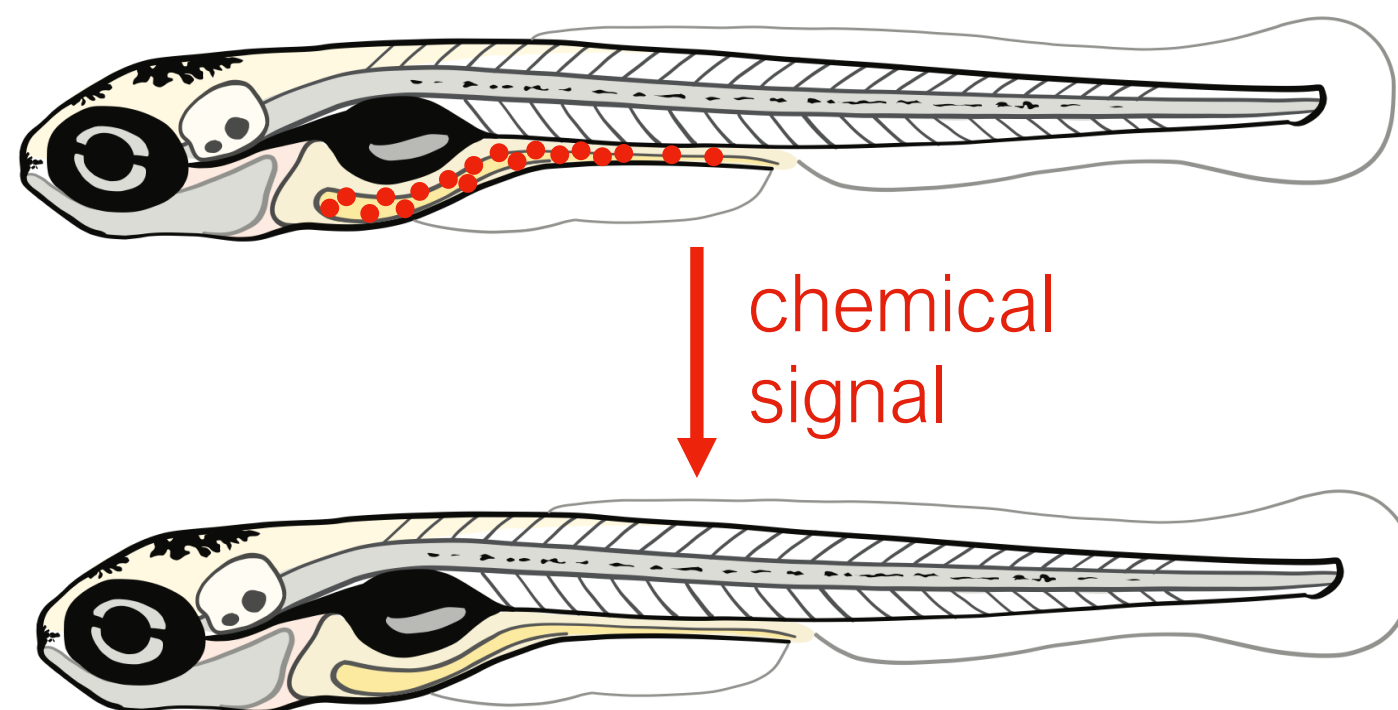


Where is this work headed?

Two ablation models:



best4:mCherry-NTR2



- ▶ Remove *best4*⁺ cells:
 - ▶ Test their functions
 - ▶ Does loss of *best4*⁺ cells cause disease symptoms?
- ▶ Live reporters: how do *best4*⁺ cells respond to different conditions, perturbations, signals?
- ▶ Communication between *best4*⁺ cells and other cell types and tissues
- ▶ Continue to build a full gene regulatory network that describes (and enables manipulation of) their development and homeostatic replacement

**Ella
Segal**

**Abhinav
Sur**

**Michael
Nunneley**

**Morgan
Prochaska**



**Yiqun
Wang**



**Gennady
Margolin**



**Paulina
Capar**



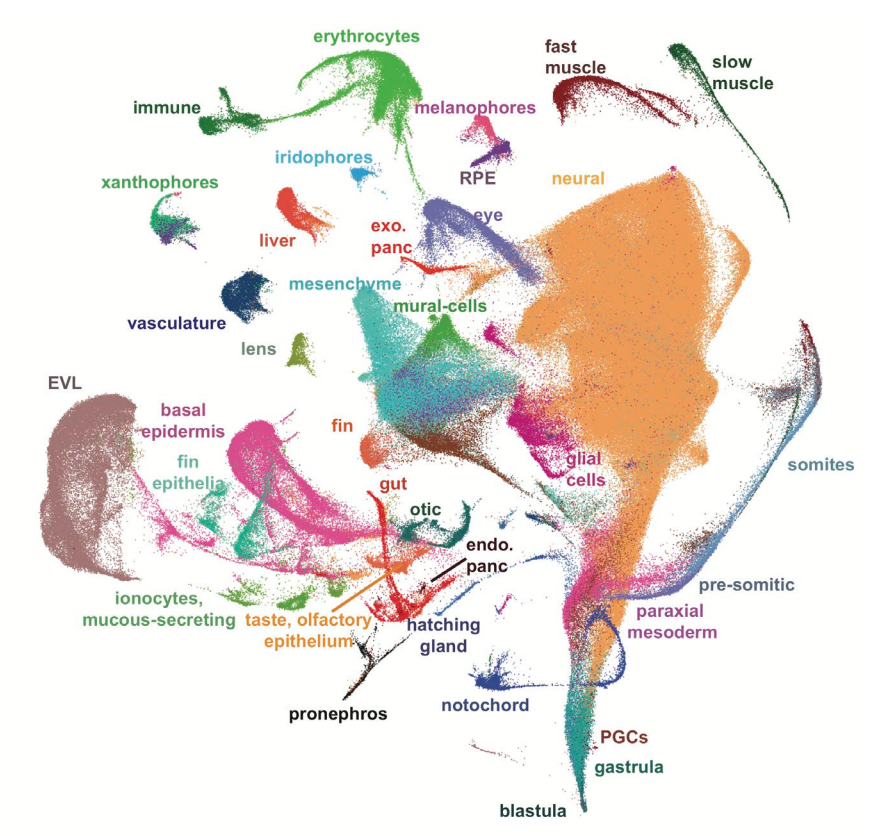
**Alicia
Evans**

Nov 2024



Eunice Kennedy Shriver
National Institute of
Child Health and
Human Development

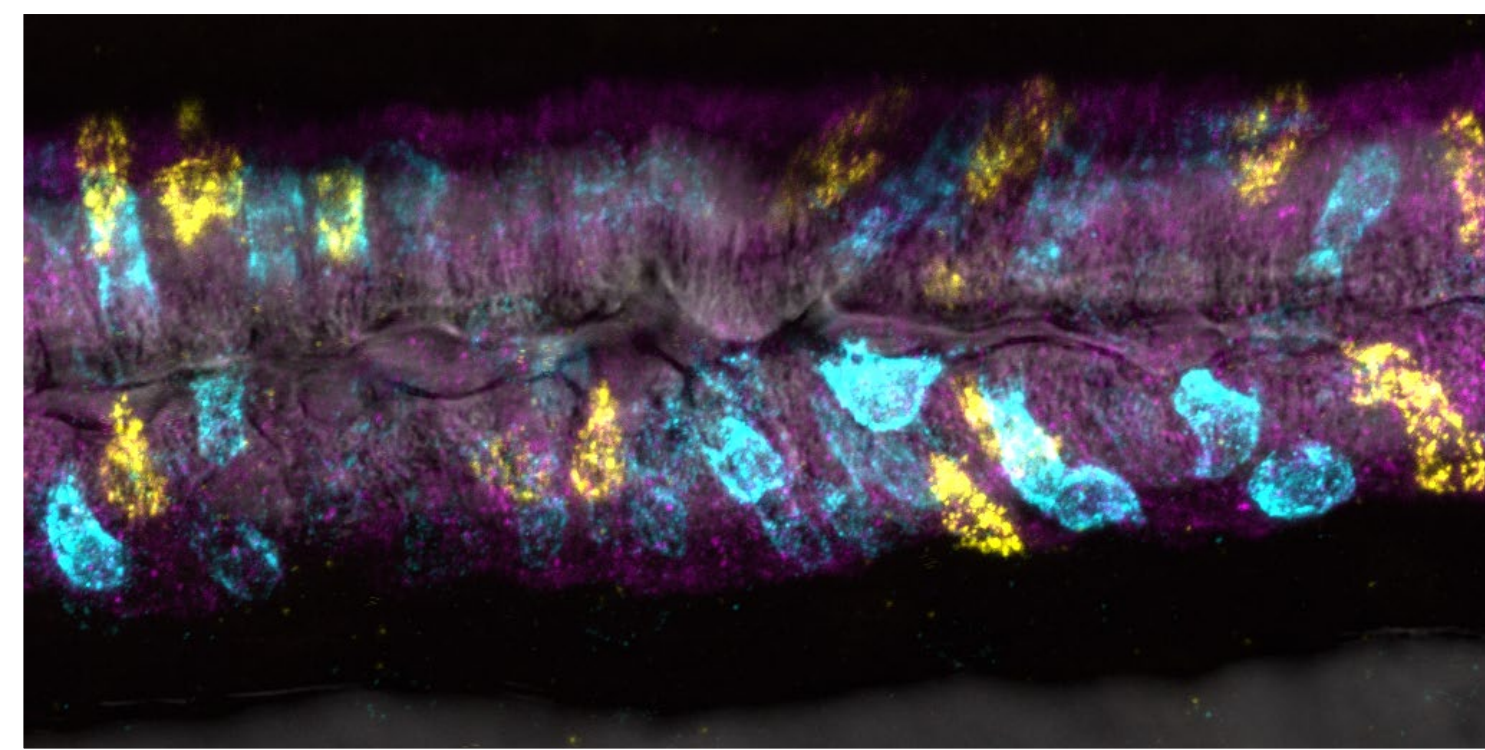
Large-scale scRNAseq
of zebrafish development



Daniocell



best4+ cells in zebrafish



Intestinal developmental
trajectories



best4+ cell
developmental
program

