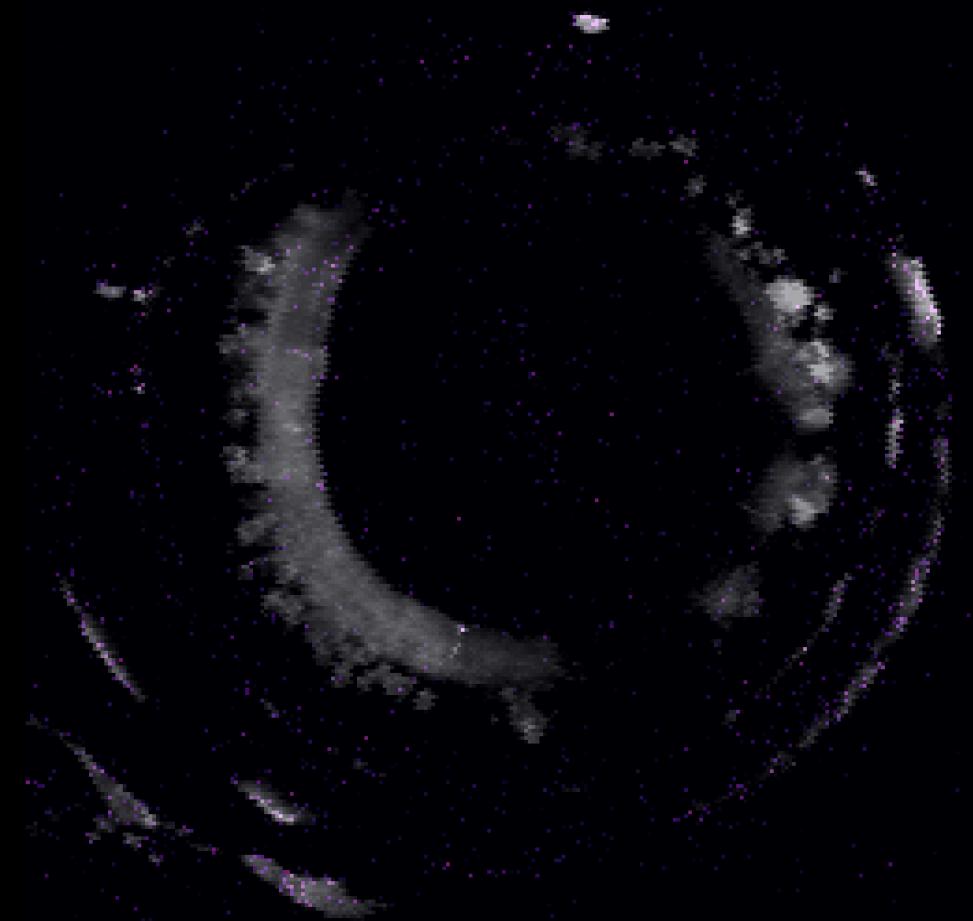


Identification of genes involved in the generation of retinal cell subtypes

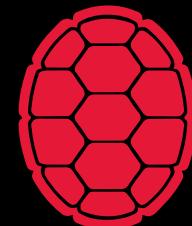
Juan Angueyra



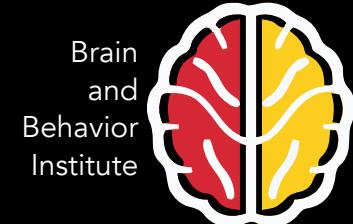
Visual System Development



University of Maryland - College Park



Department
of
Biological
Sciences



Brain
and
Behavior
Institute

Identification of genes involved in the generation of retinal cell subtypes

Juan Angueyra

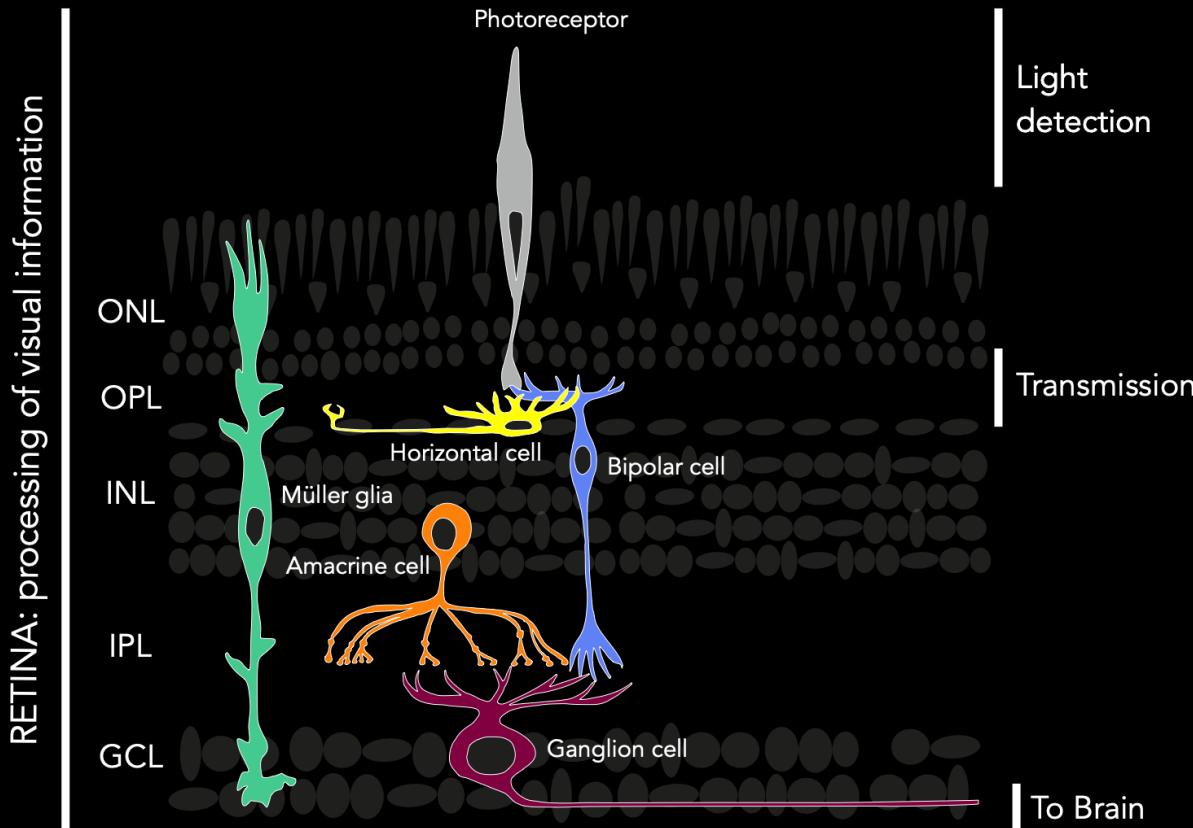
Visual System Development

Our group's research focus

- Methods and tools
 - Postdoc at NIH
 - Collaboration between Katie Kindt's Lab (NIDCD) and Wei Li's Lab (NEI)
 - Photoreceptors
- Recent findings: *tbx2* (Carinna Householder)
- Horizontal cells
- Plasticity vs. Predetermination (Meghan Hnilo & Leah Kwak)
- WE ARE HIRING



Retinal neurons belong to only 5 major classes...



We study two fundamental processes in retinal development:

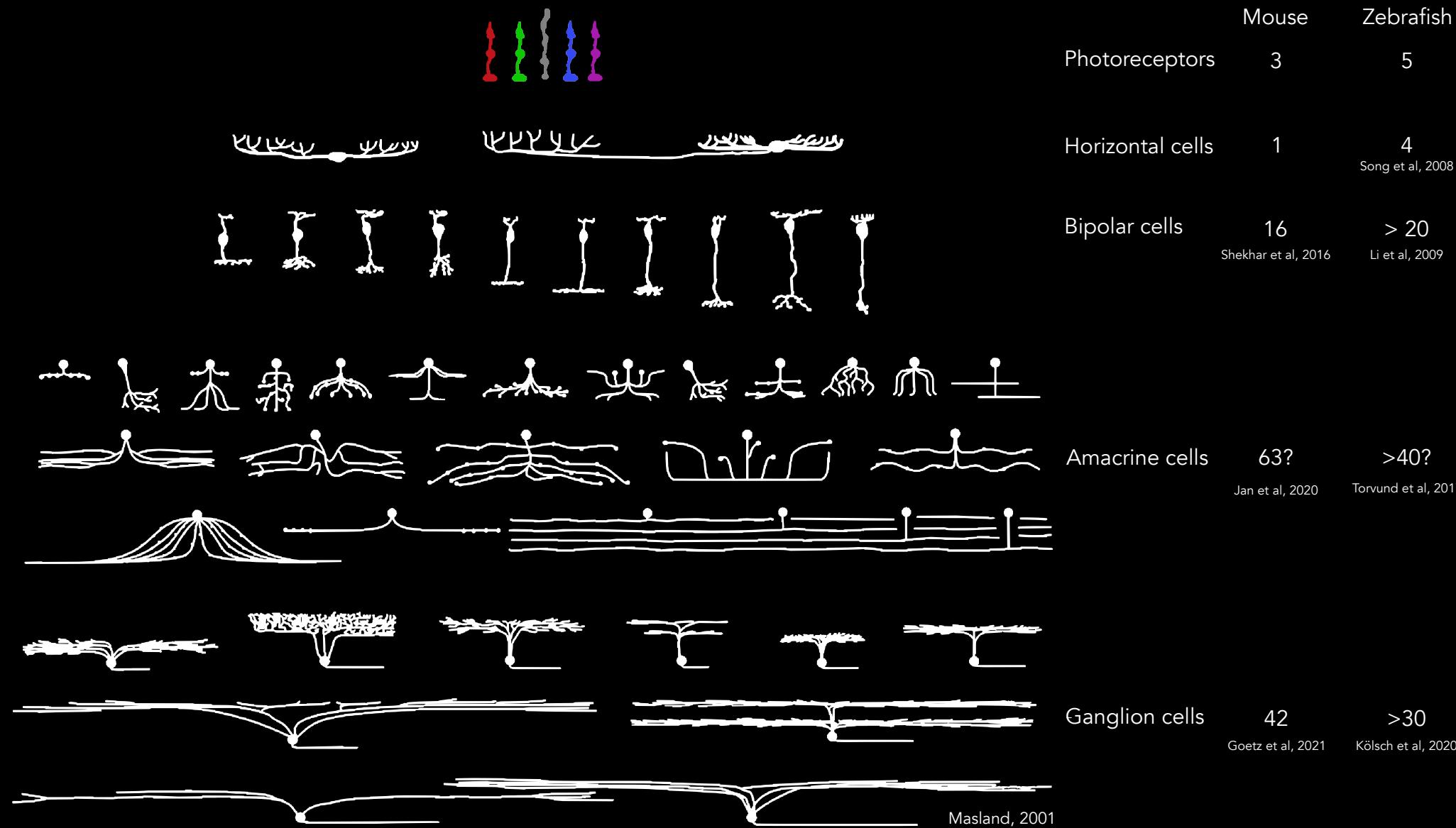
→ Make the parts...

How are retinal neurons generated?

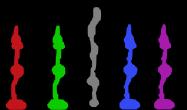
→ ...and put them together

How are retinal neurons wired into specific circuits?

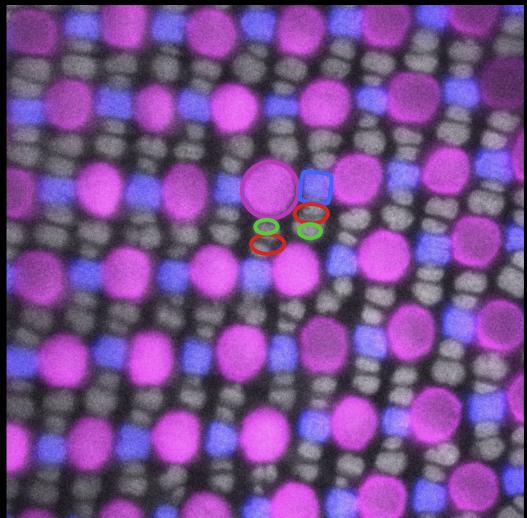
...but there is a rich diversity of subtypes whithin each class



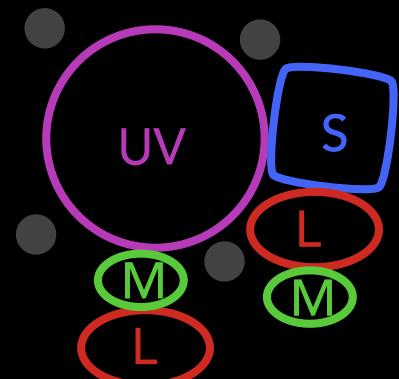
A reliable map of transcription-factor expression in photoreceptors



	Mouse	Zebrafish
Photoreceptors	3	5



Zebrafish adult mosaic



Generation of cell subtypes relies on transcriptional regulation

Manual cell-picking using reporter lines (with Vincent Kunze)

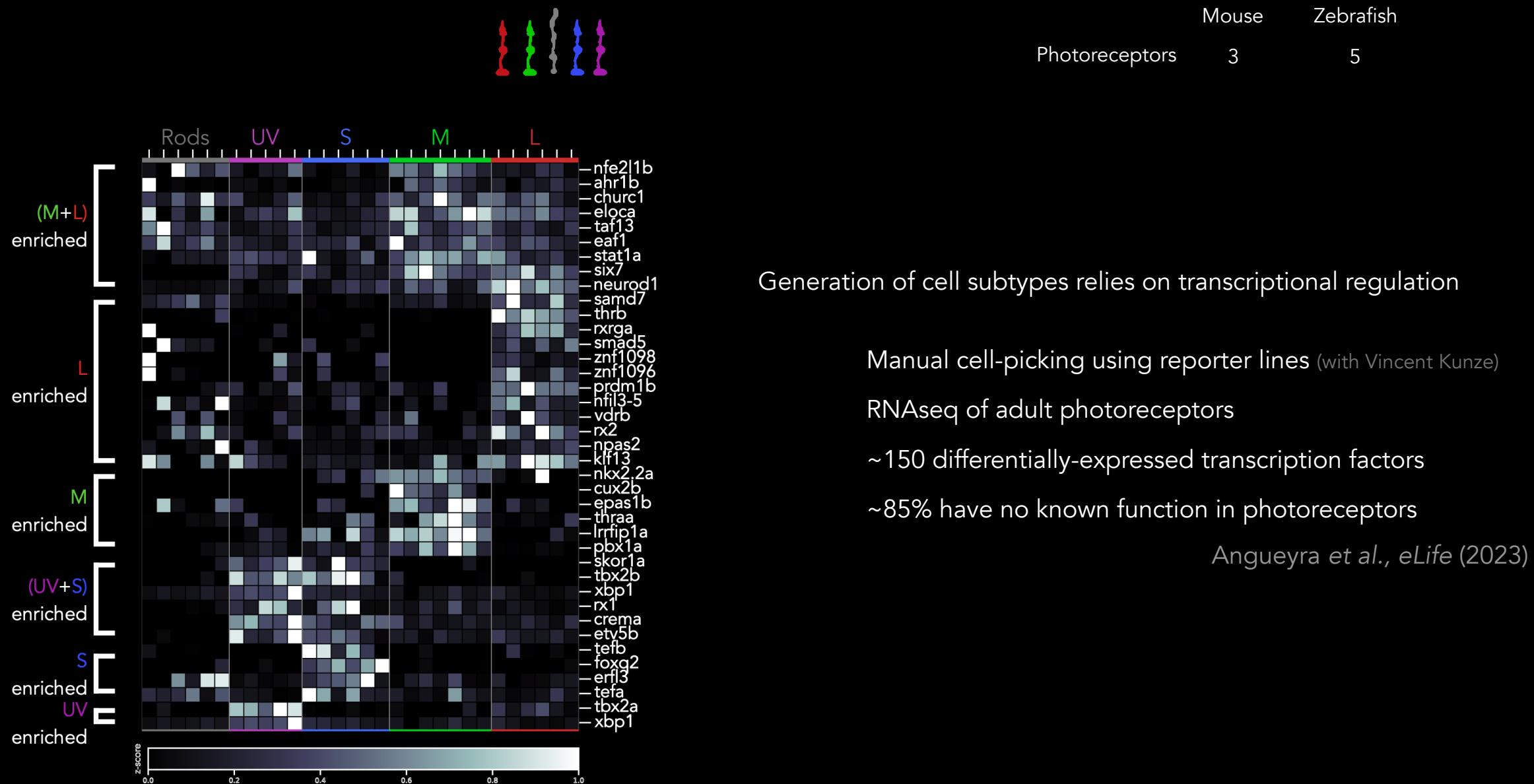
RNAseq of adult photoreceptors

~150 differentially-expressed transcription factors

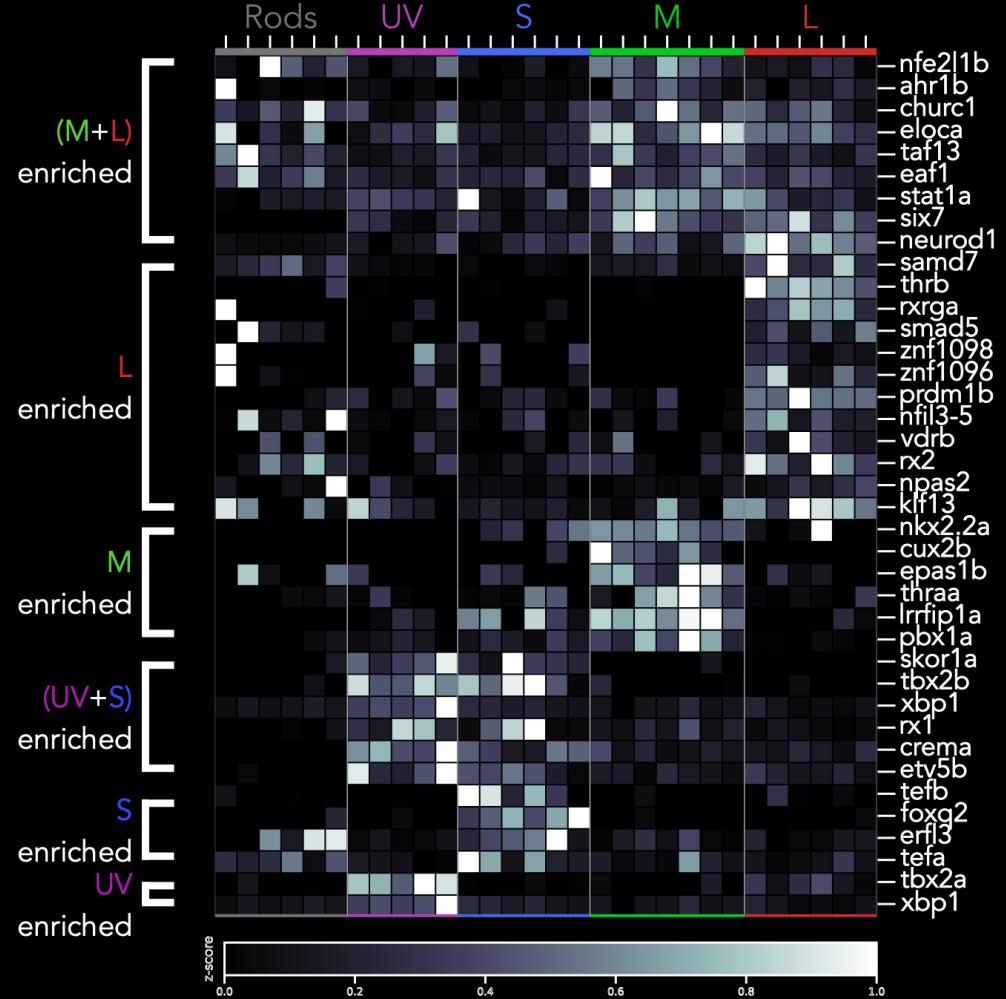
~85% have no known function in photoreceptors

Angueyra et al., eLife (2023)

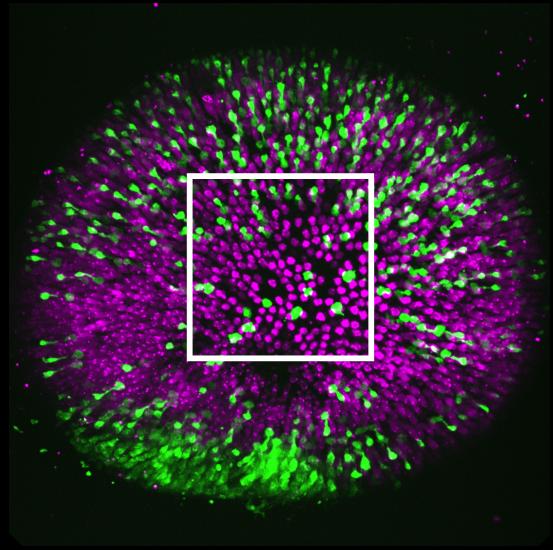
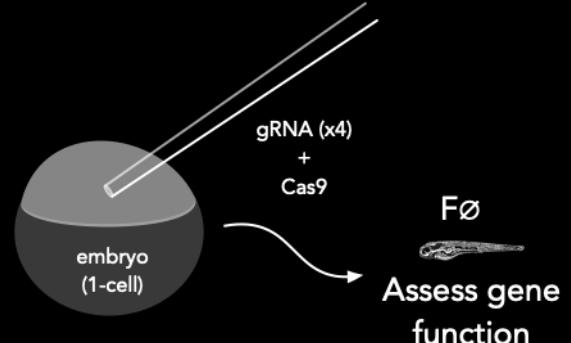
A reliable map of transcription-factor expression in photoreceptors



F0-screening as an efficient tool to test TF function



F0 screen (CRISPR/Cas9)



CRISPR mutations → loss of function → phenotype

Phenotypes can be assessed at high throughput

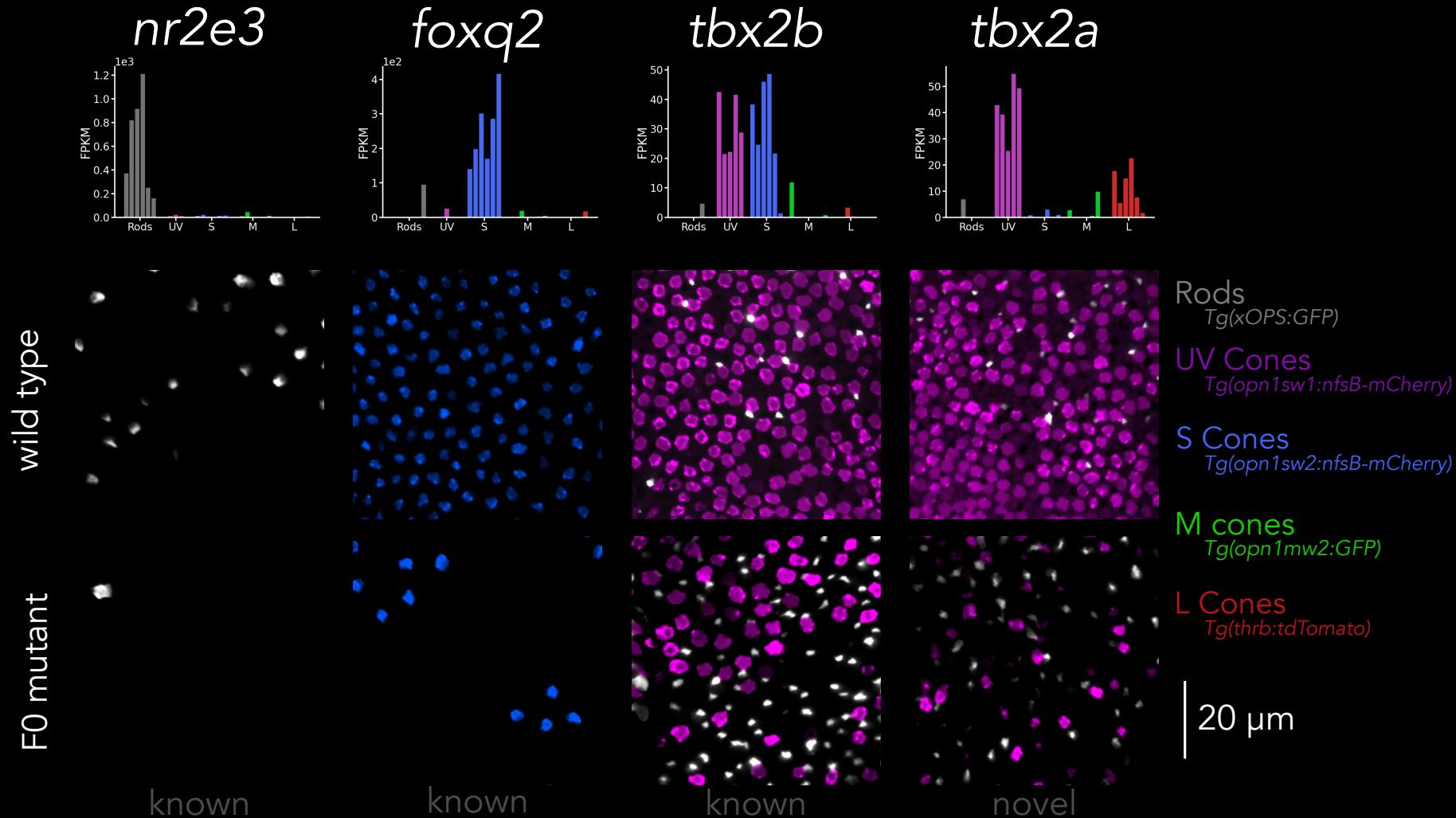
F0 larvae are genetic mosaics

- Guides with high efficiency
- Use of multiple guides
- Post-hoc genotyping

Hoshijima et al. (2019), Rihel et al. (2021)

F0-screening as an efficient tool to test TF function: positive hits

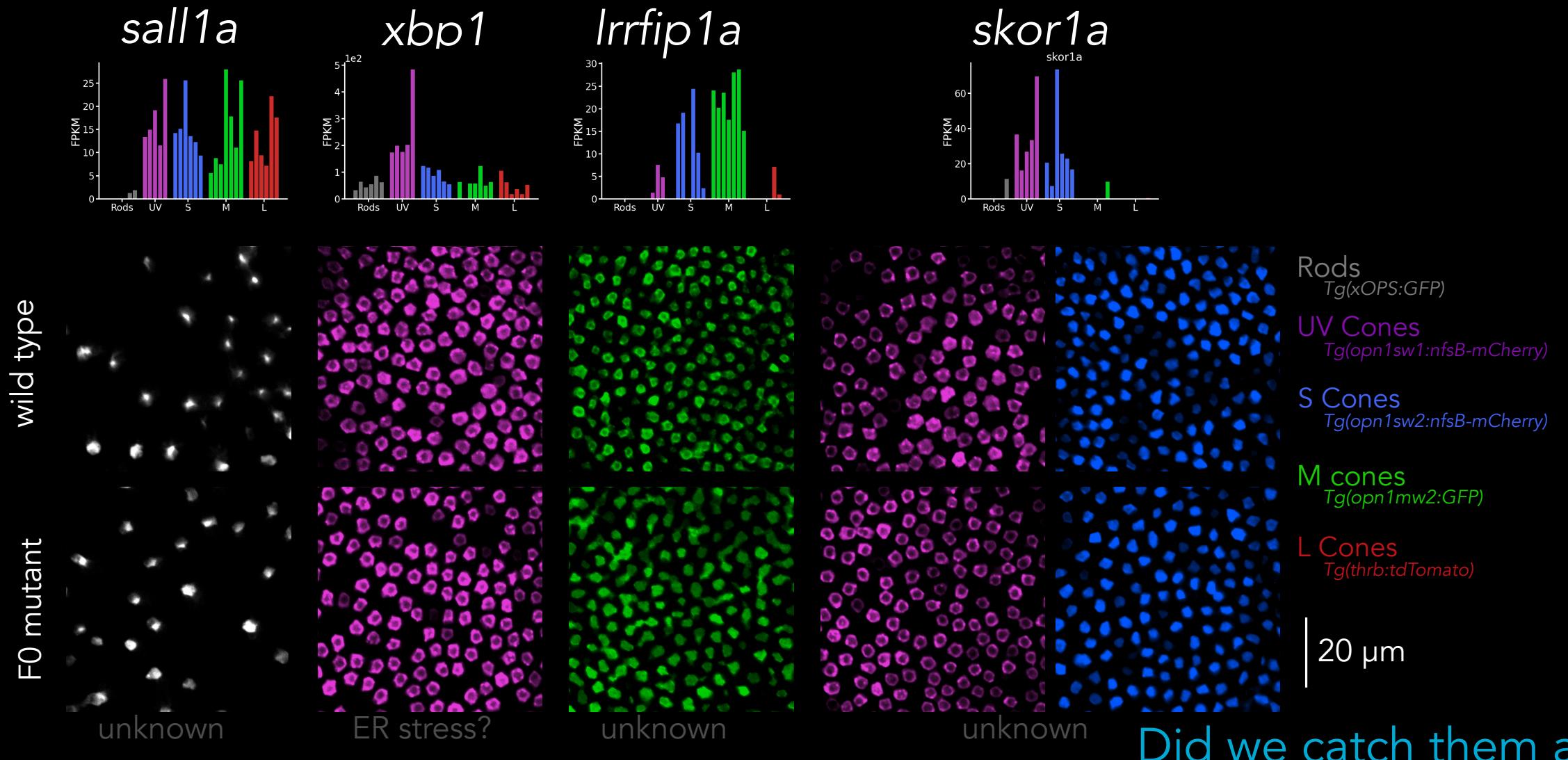
with Laura Patak



Xie et al. (2019), Ogawa et al. (2021), Alvarez-Delfin et al. (2009),

F0-screening as an efficient tool to test TF function: negative hits

with Laura Patak

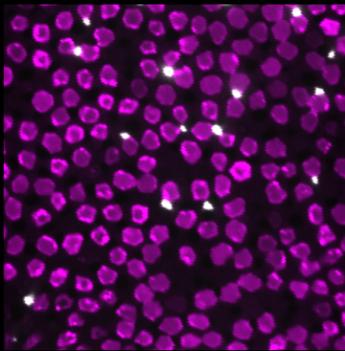


Did we catch them all?

Two UV-cone sub-subtypes? (Carinna Householder)

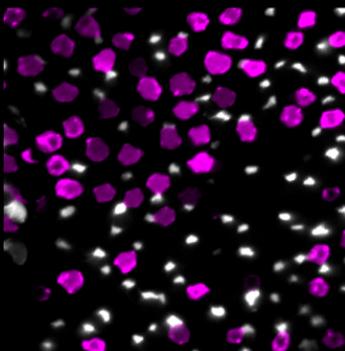
tbx2b

wild type

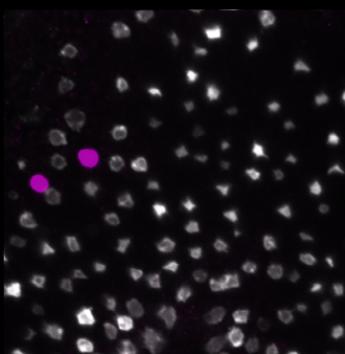


tbx2a

FO mutant



germline KO

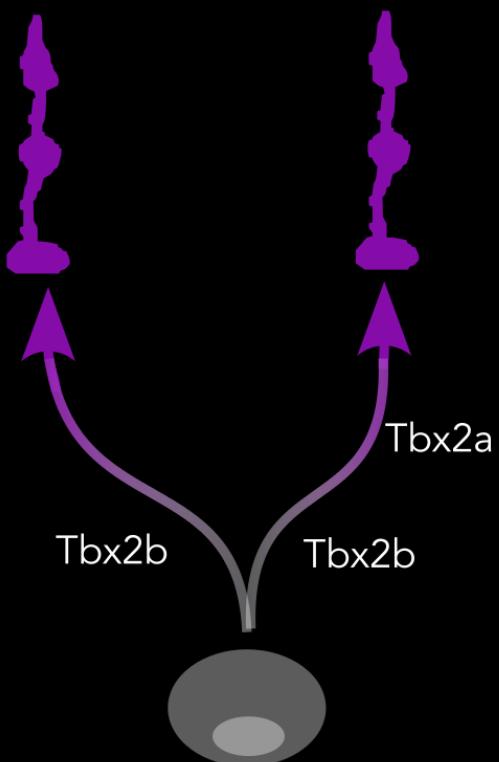


Rods
Tg(xOPS:GFP)

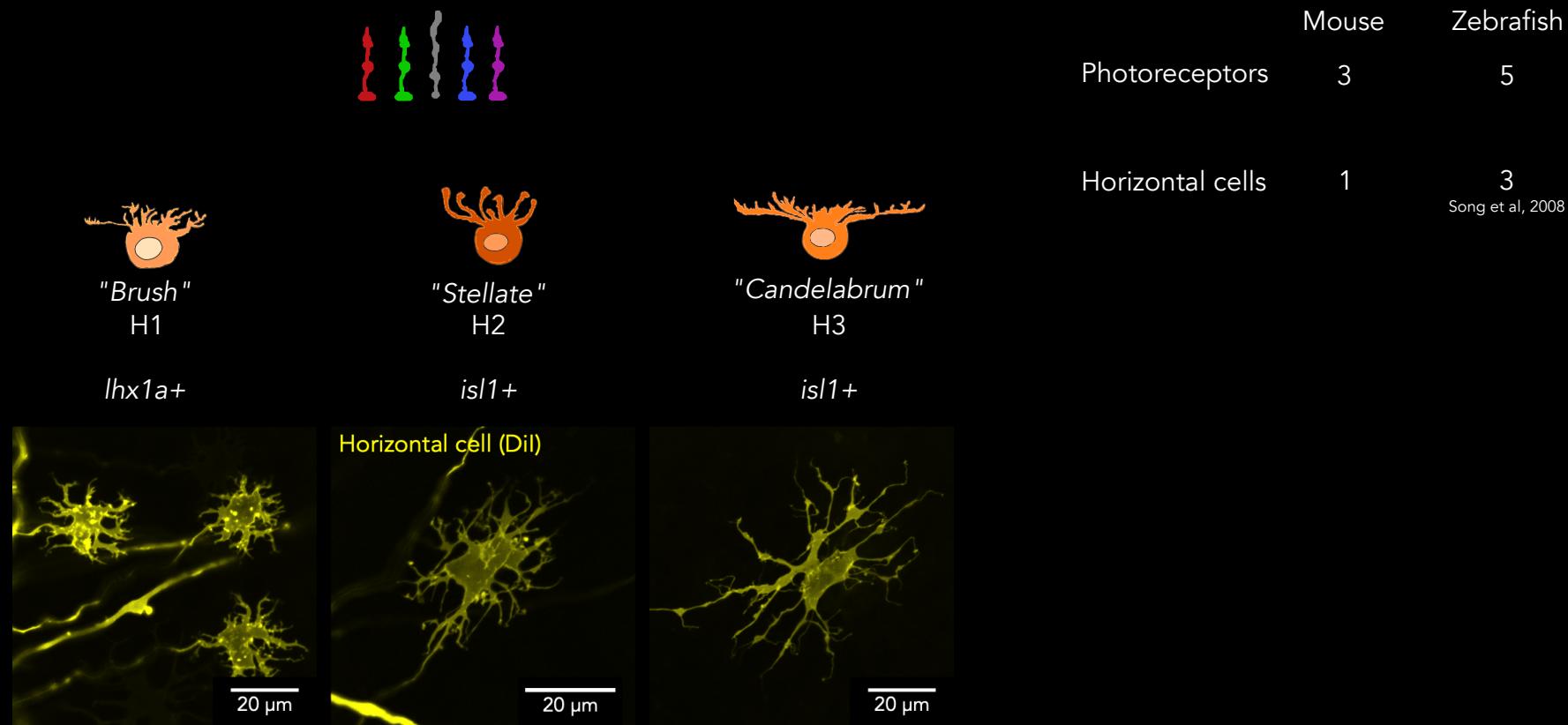
UV Cones
Tg(open1sw1:nfsB-mCherry)



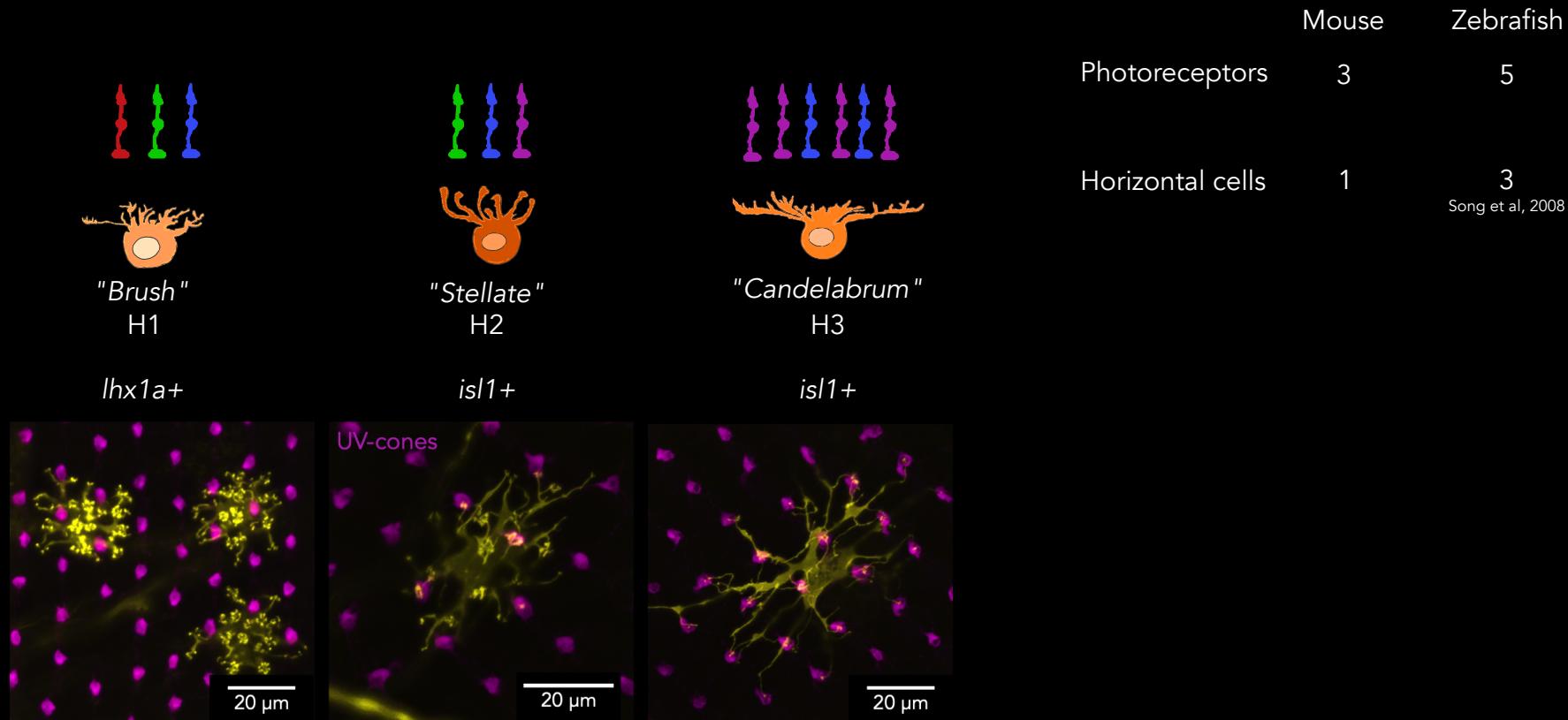
20 μm



How are horizontal-cell subtypes generated during development?



How are horizontal-cell subtypes generated during development?

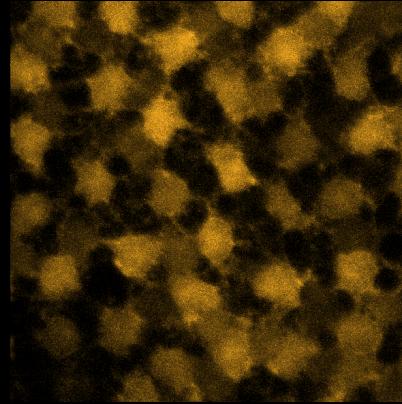
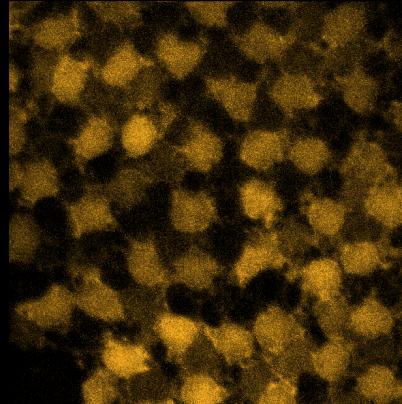


Mutations in *lhx1a* impair the generation of H1-horizontal cells

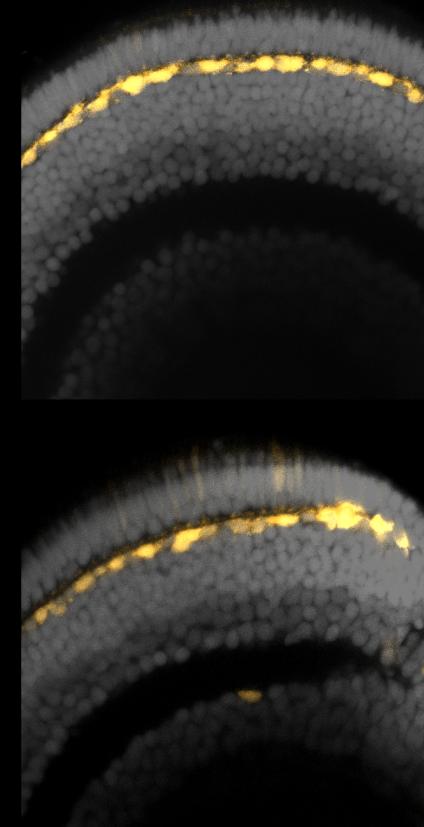
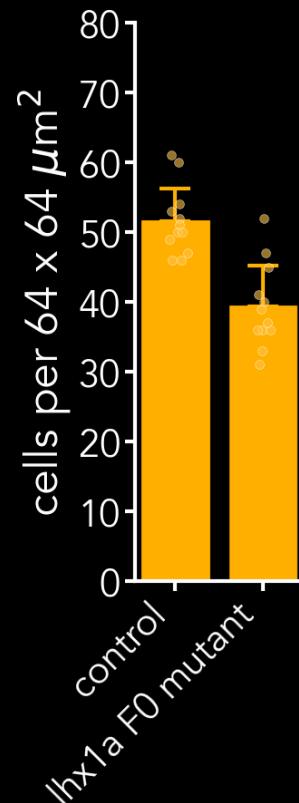
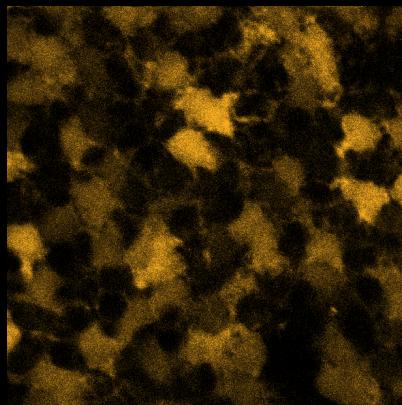
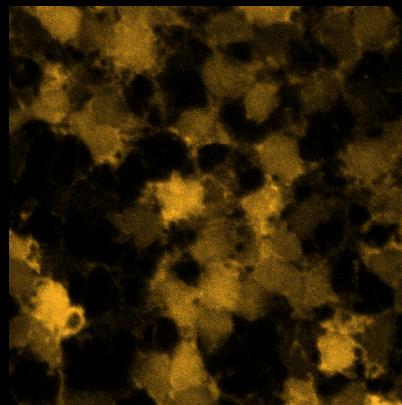
Meghan Hnilo and Leah Kwak

lhx1a

wild type



F0 mutant



H1 horizontals
Tg(lhx1a:GFP)

Nuclei
DAPI

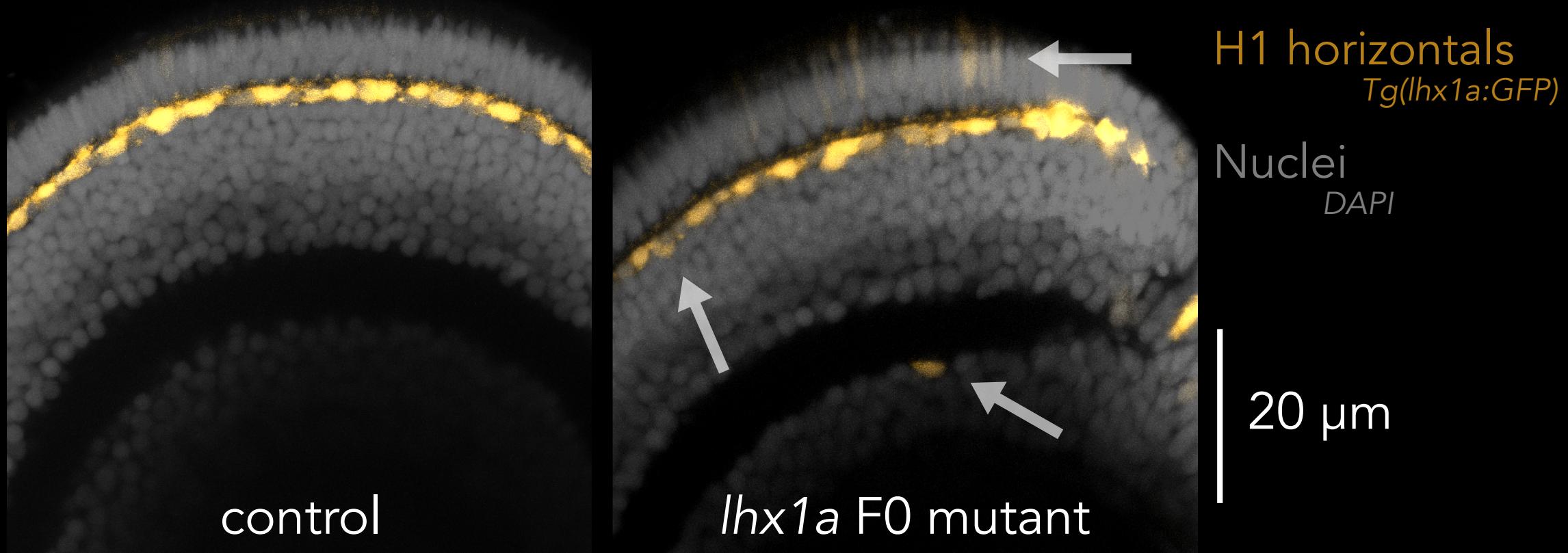
20 μm

Poché et al. (2007)



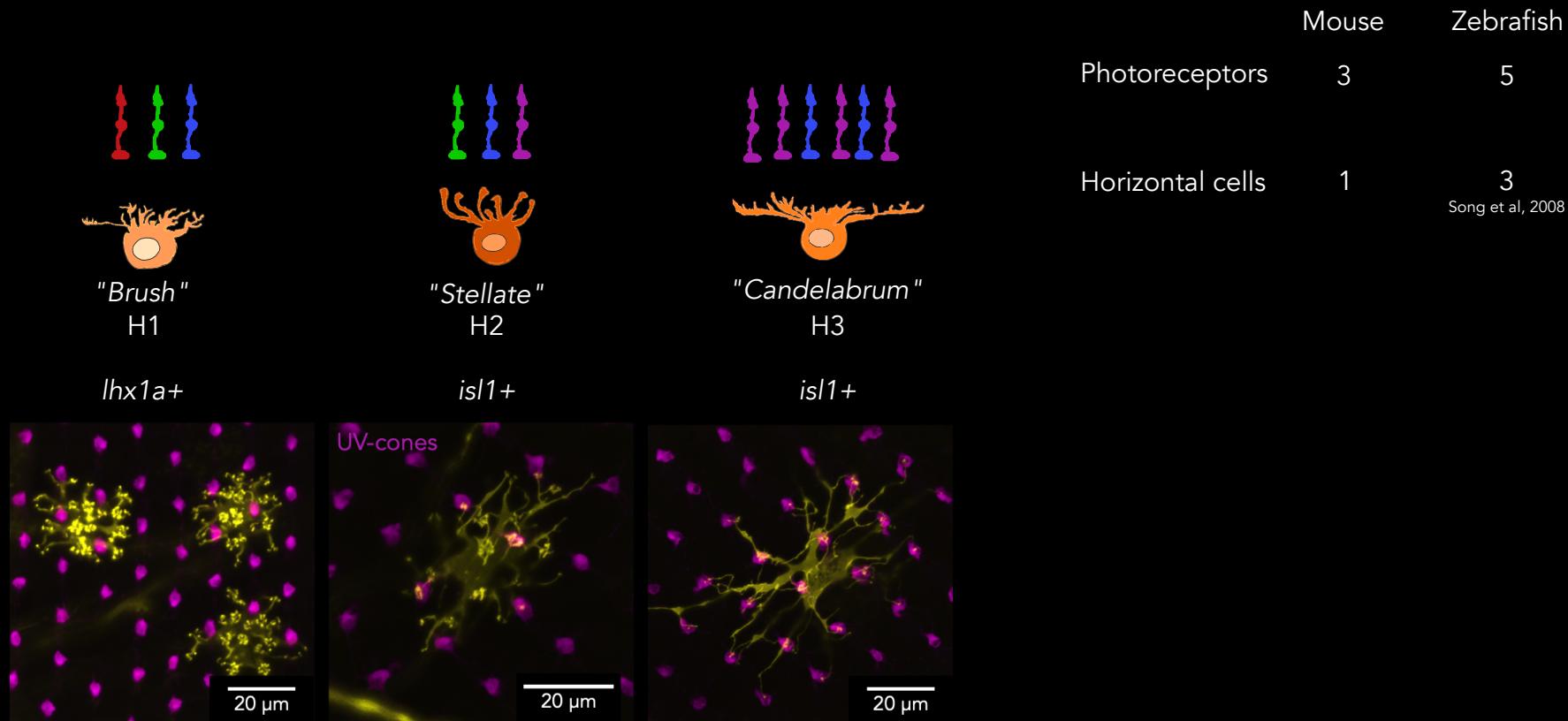
Mutations in *lhx1a* impair the generation of H1-horizontal cells

Meghan Hnilo and Leah Kwak



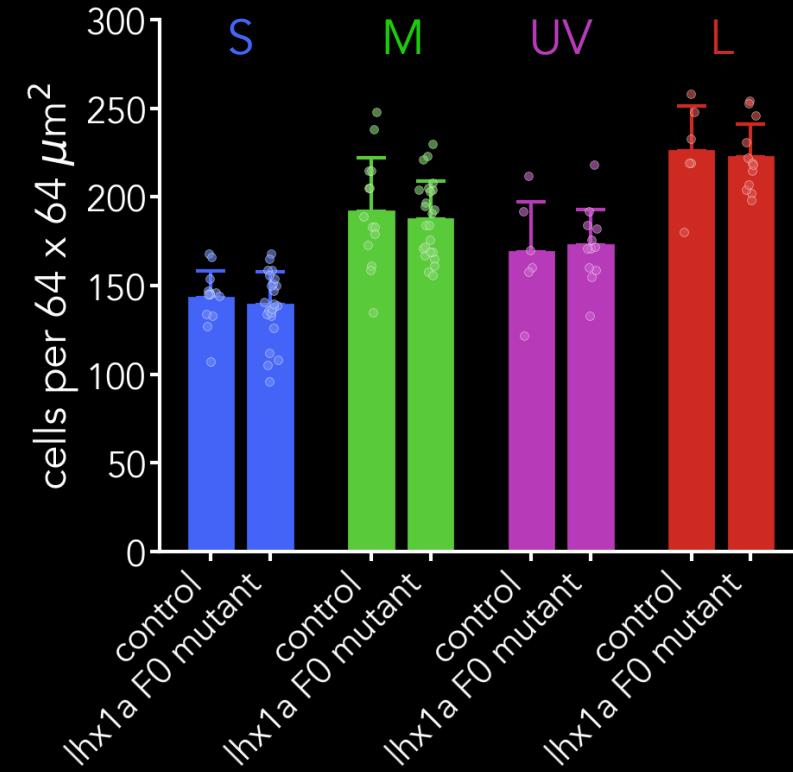
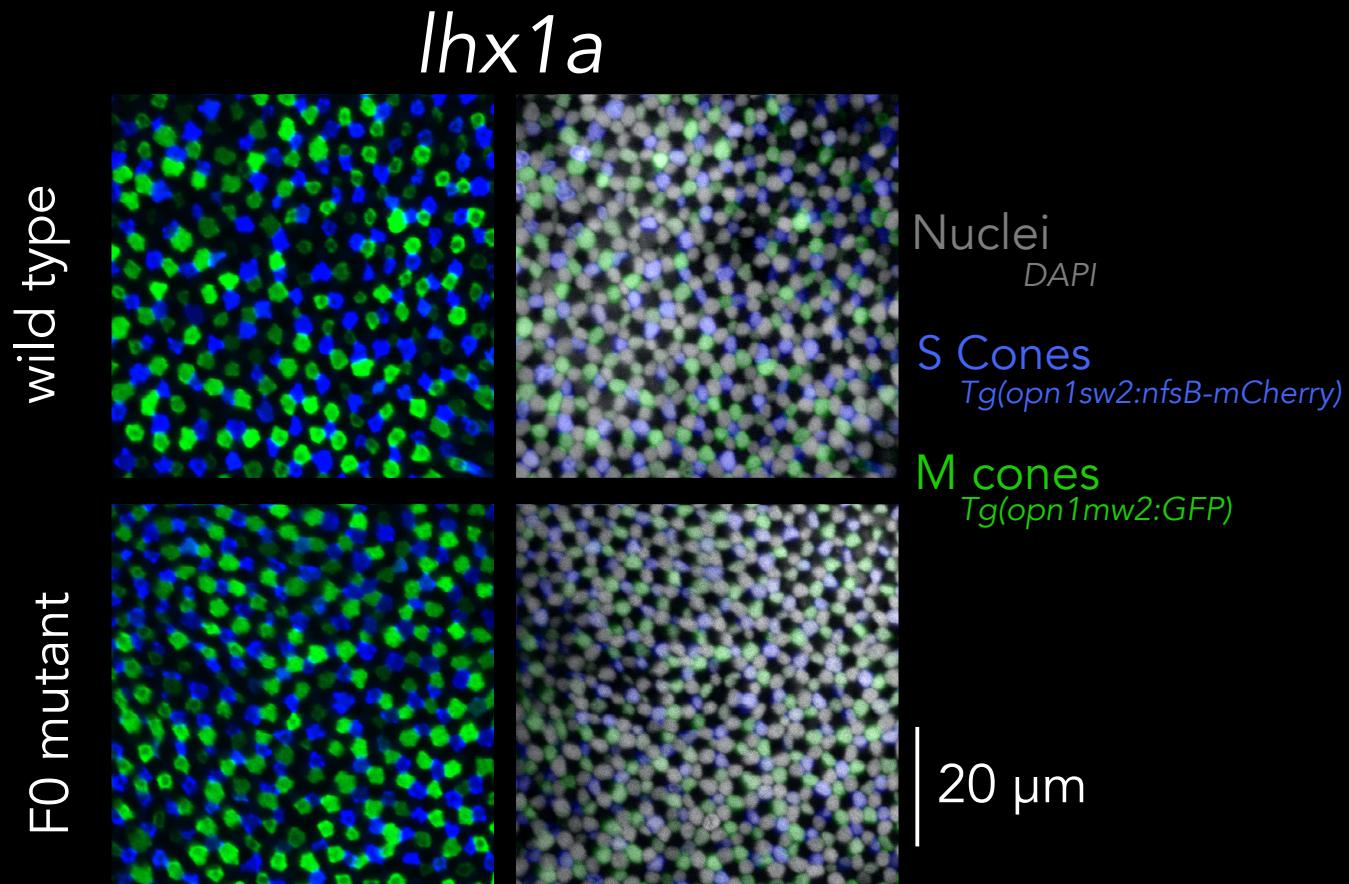
Poché et al. (2007)

Subtype generation: Predetermined OR Plastic?



lhx1a mutations do NOT modify the generation of photoreceptor subtypes

Meghan Hnilo and Leah Kwak



How does connectivity change?
What are the consequences for visual behaviour?

Identification of genes involved in the generation of retinal cell subtypes

Carinna Householder, Meghan Hnilo, Leah Kwak, Juan Angueyra

Visual System Development

Our group's research focus

- Methods and tools
- Transcription factors involved in generation of cone subtypes
- Recent findings: *tbx2* (Carinna Householder)
- Transcription factors involved in generation of horizontal-cell subtypes
- Plasticity vs. Predetermination (Meghan Hnilo & Leah Kwak)
- Visual processing
- WE ARE HIRING → ask me for a sticker!



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