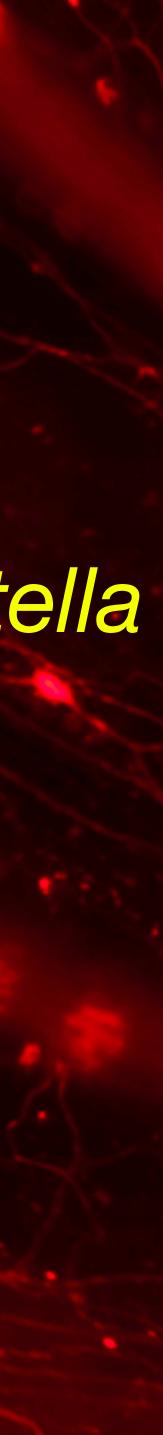
# My path to investigating

# Michael Layden



Developmental and regenerative neurogenesis in Nematostella vectenesis



# This is an incredible time to be a scientist

# Scientific progress is limited only by the creativity of the science fiction writer

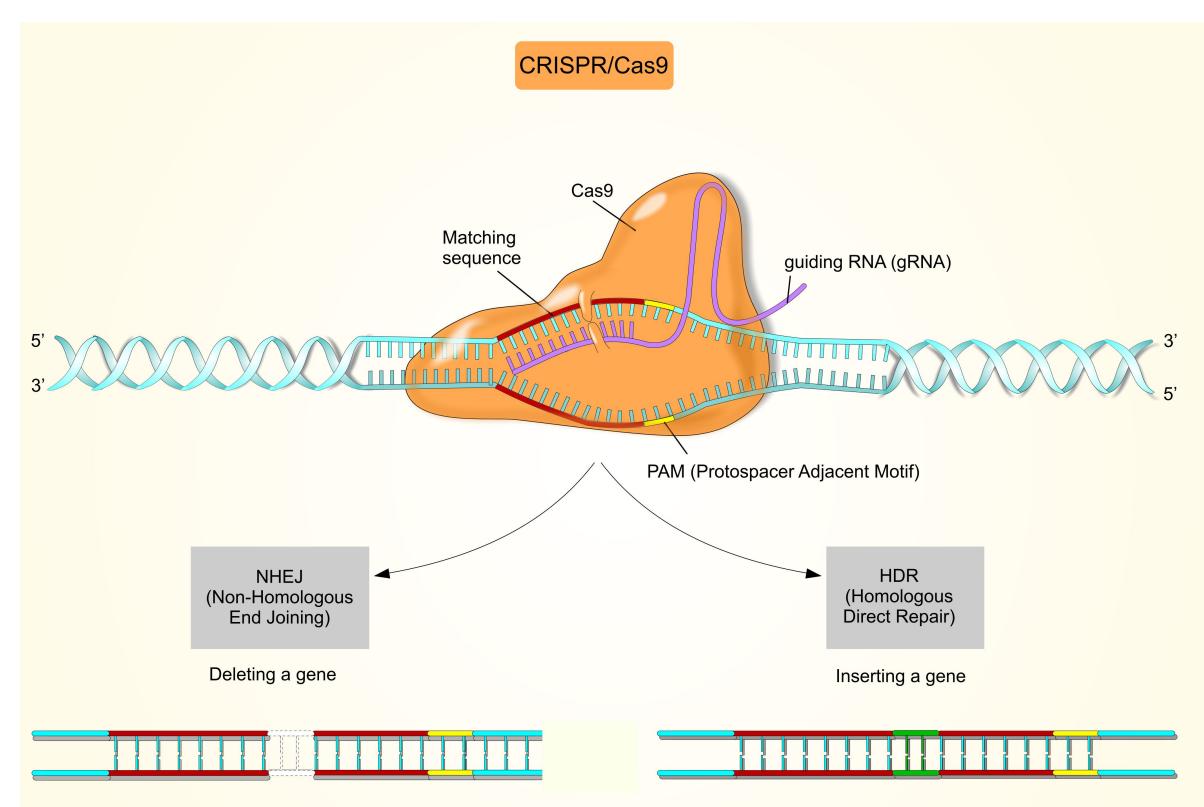


# Nobel Prize in Chemistry for CRISPR/Cas9 gene editing technology

Jennifer Doudna



**Emmanuelle Charpentier** 



https://blog.reprocell.com/improved-gene-editing-with-crispr-sniper-1





### non-bilaterian metazoans Ctenophora Placozoa

### Cnidaria



Porifera





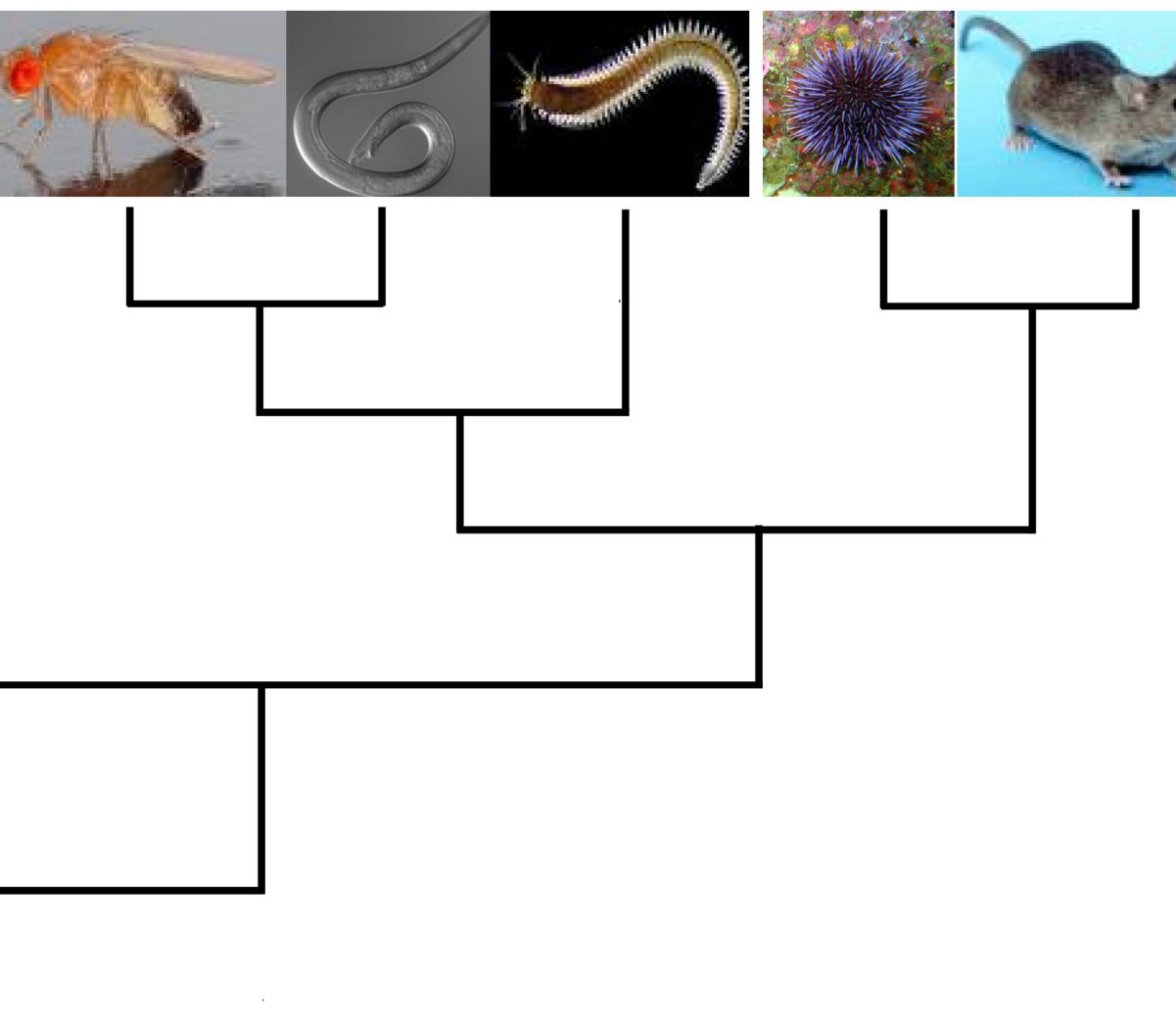




### Bilateria

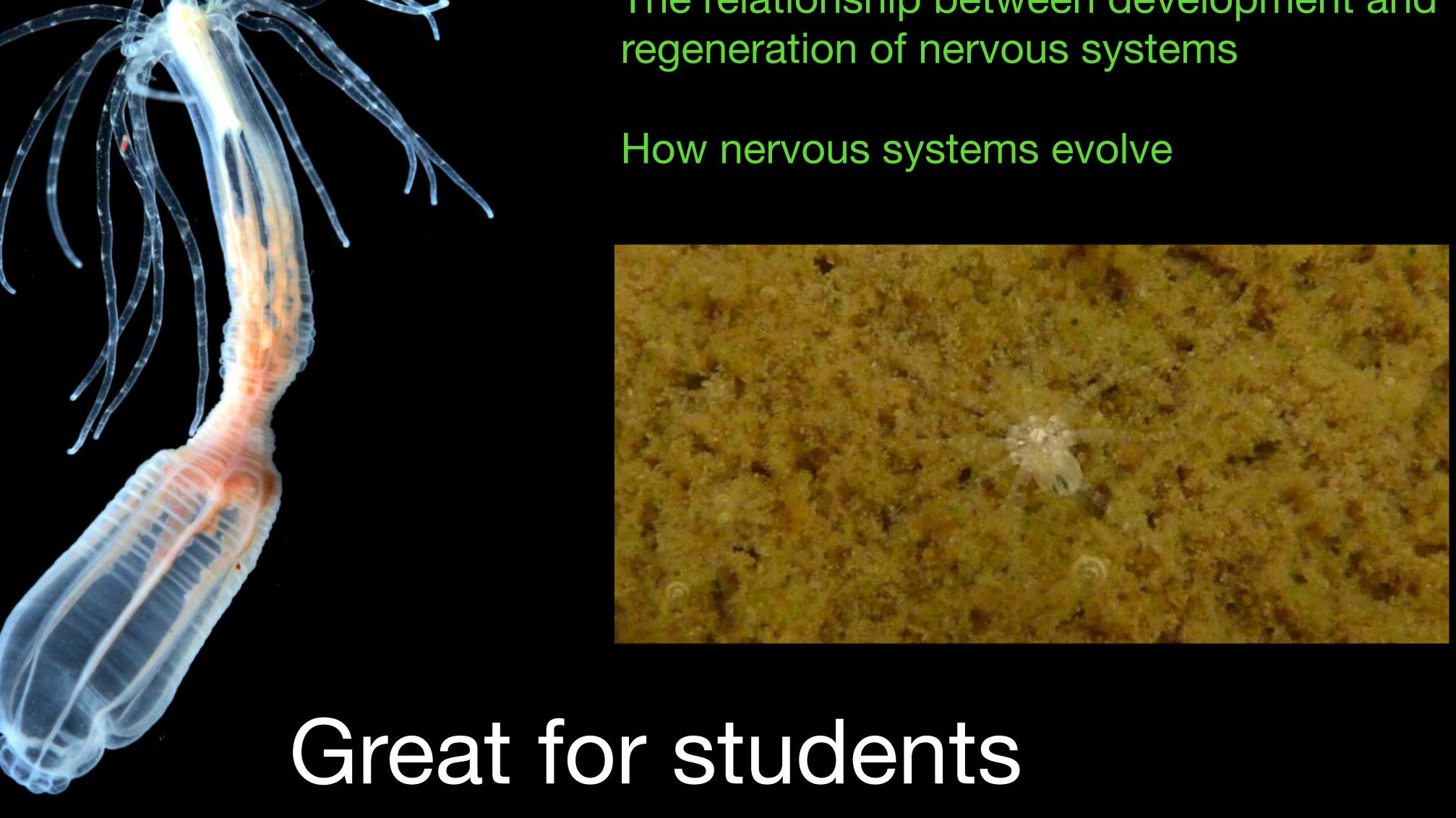
### Protostomes

### Deuterostomes





# The cnidarian sea anemone, Nematostella vectensis



2008  $\bigcirc$ P. roettinger/kahikai.com

The relationship between development and

# The cnidarian sea anemone, Nematostella vectensis



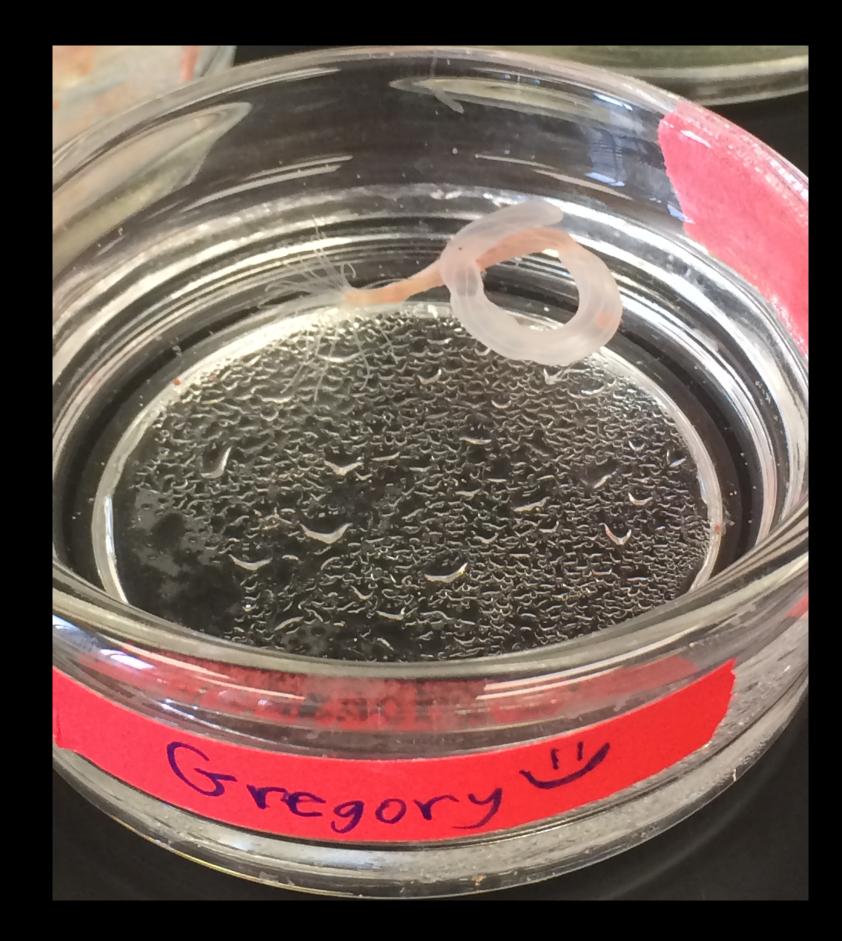
2008  $\bigcirc$ roettinger/kahikai.com

The relationship between development and regeneration of nervous systems

How nervous systems evolve



# Great for students



1/3 X sea water

can make with "instant ocean" Just need to pH it.

Feed them brine shrimp (sea monkeys)

# Ideal in cool dark place

# put them on light box to spawn (need to plan ahead to get them on cycle)

Change water once a week less if not feeding a lot

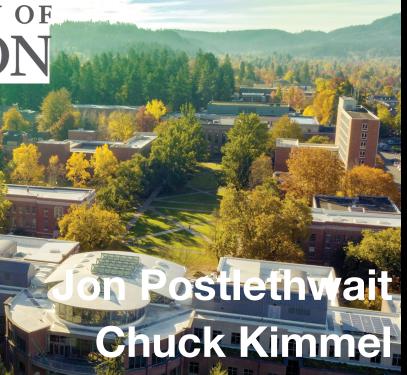




Extremely interested in biology (convinced career in medicine) Fascinated by the brain



Chris Q Doe Judith Eisen



Genetics of nervous system development in fruit flies and zebra fish

1996

Life changed the first week of college. I was introduced to research



**Rich Feldman** 

Biochemistry and molecular biology research

# My path



### Started my lab at Lehigh





# Postdoctoral research developing Nematostella as a



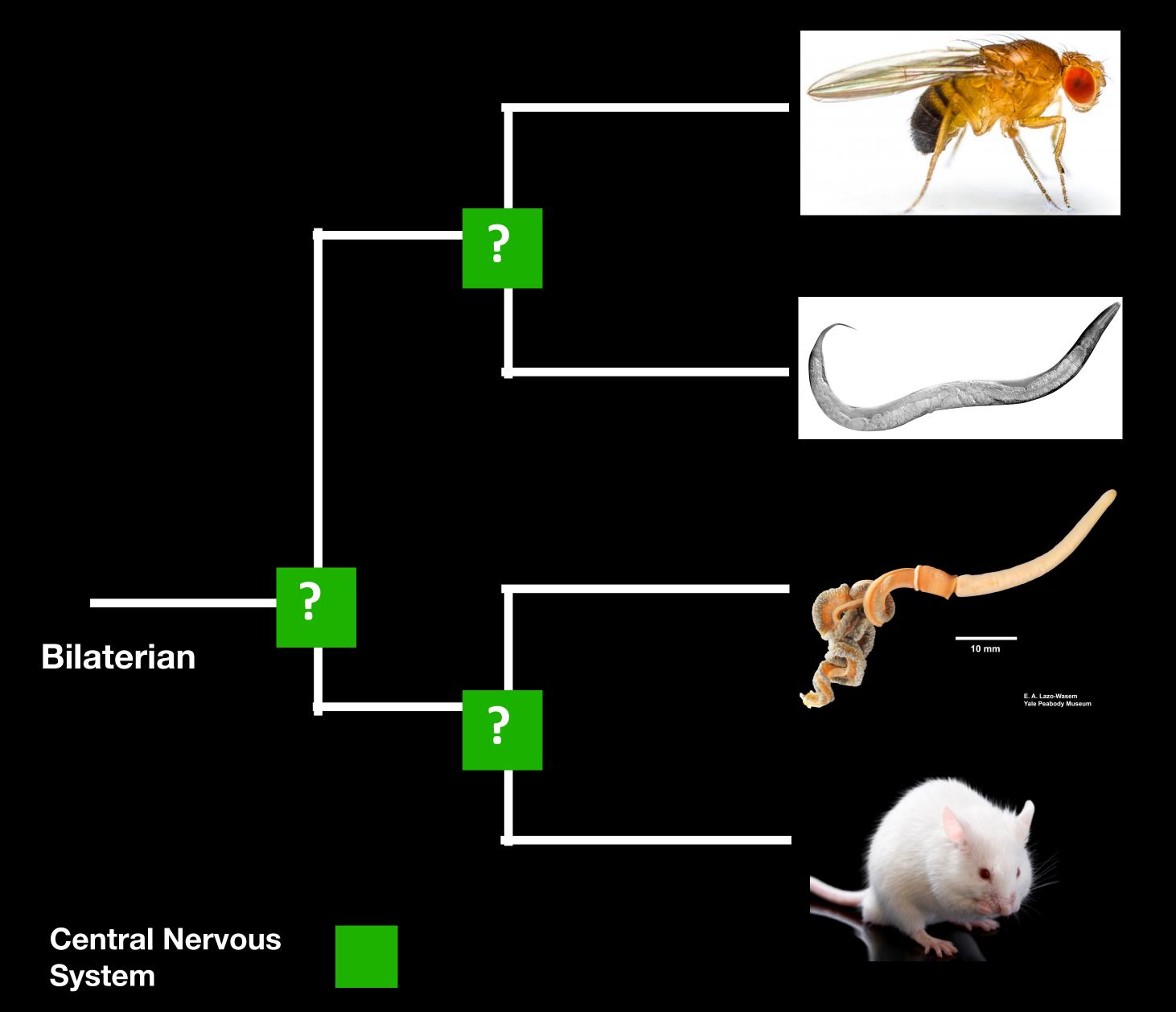




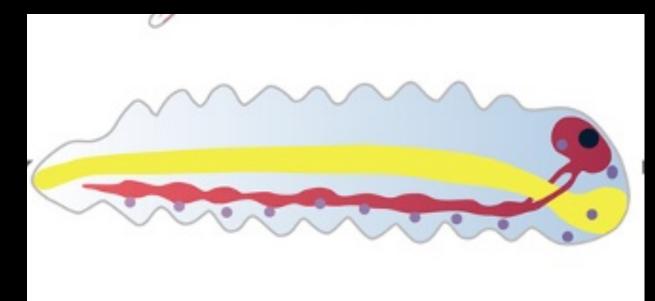


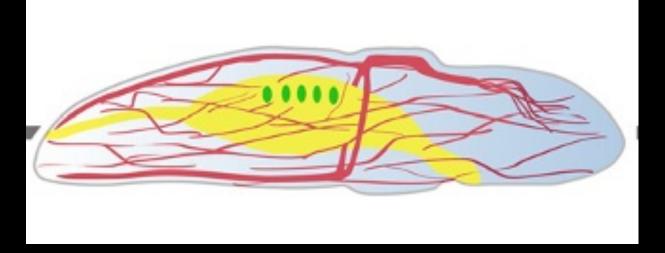


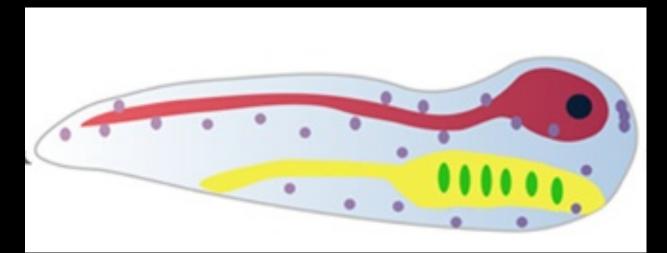
# When did CNS A-P patterning mechanisms evolve?



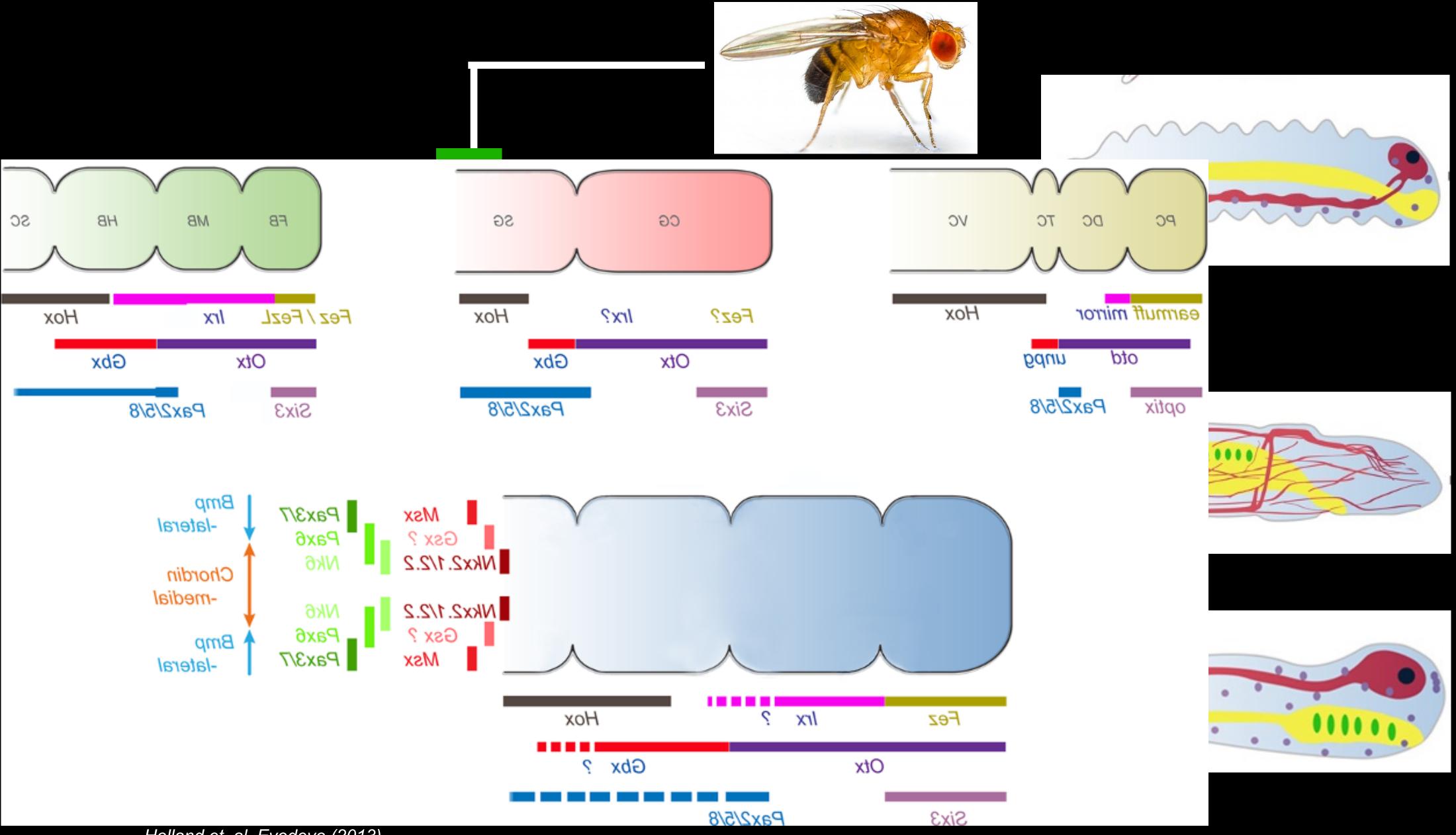
Holland et. al. Evodevo (2013)







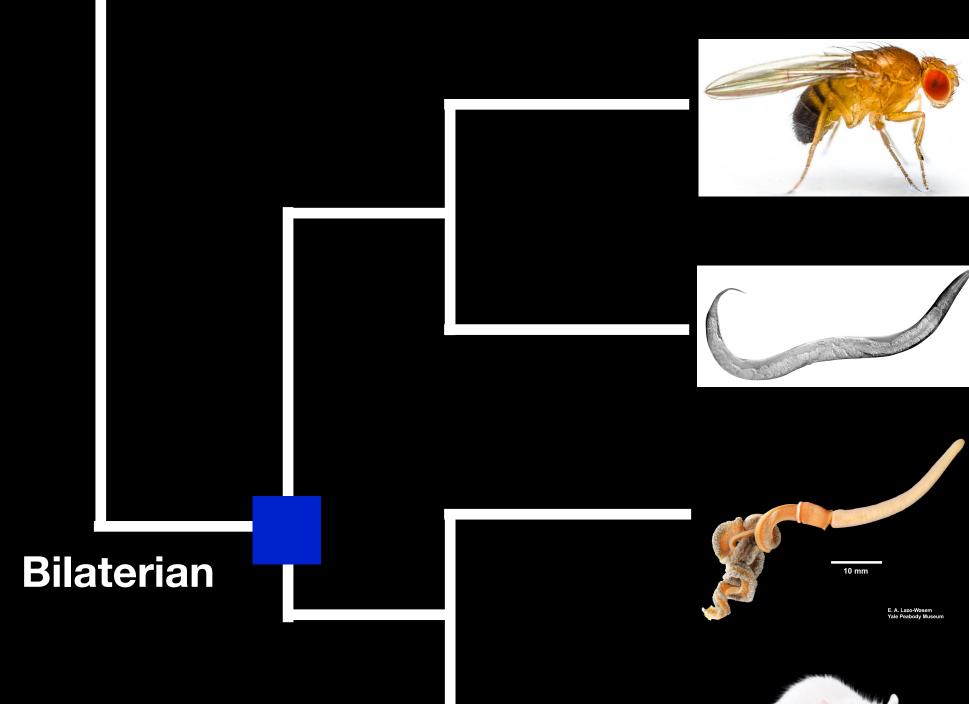
# When did CNS A-P patterning mechanisms evolve?



Holland et. al. Evodevo (2013)

## Cnidarian

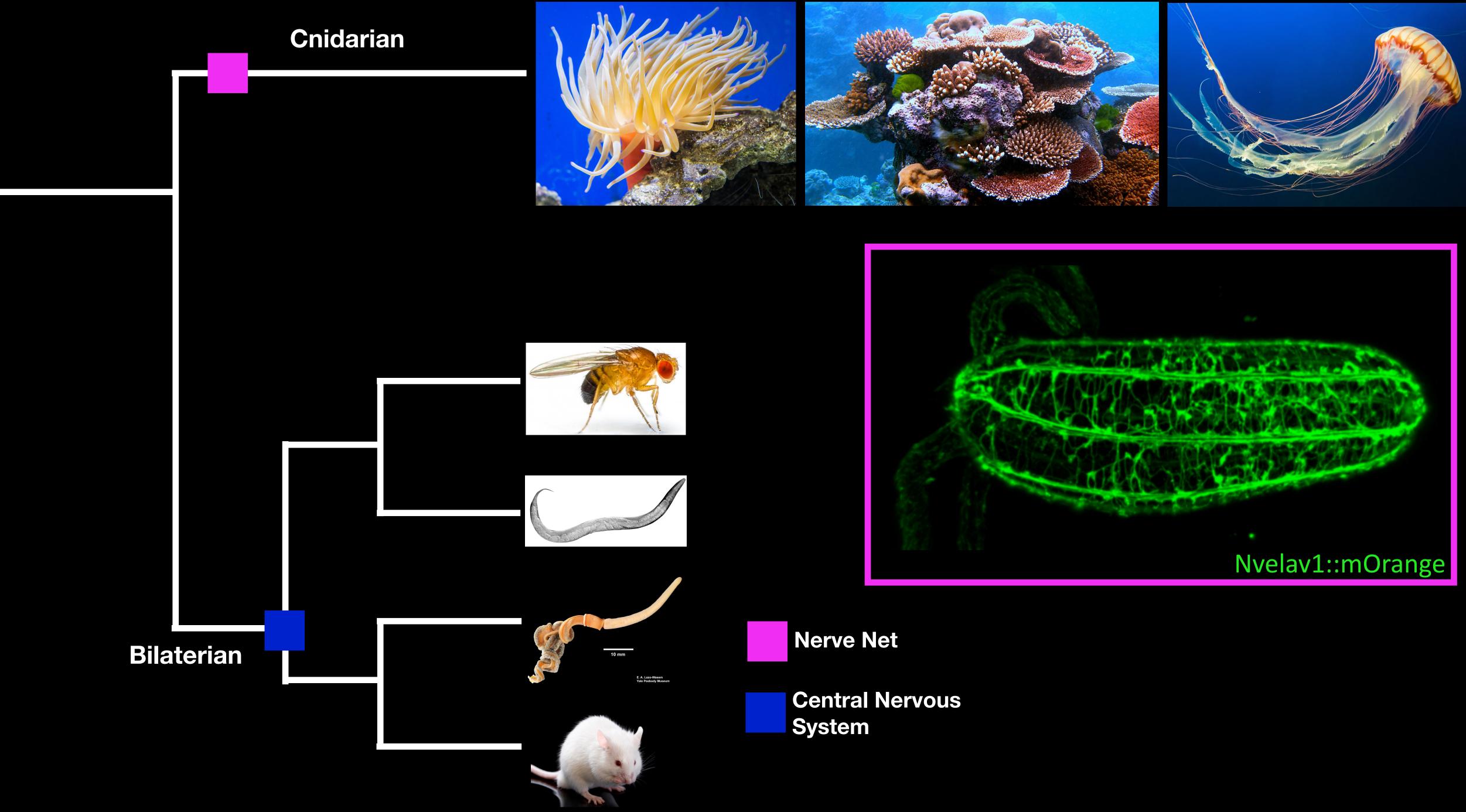


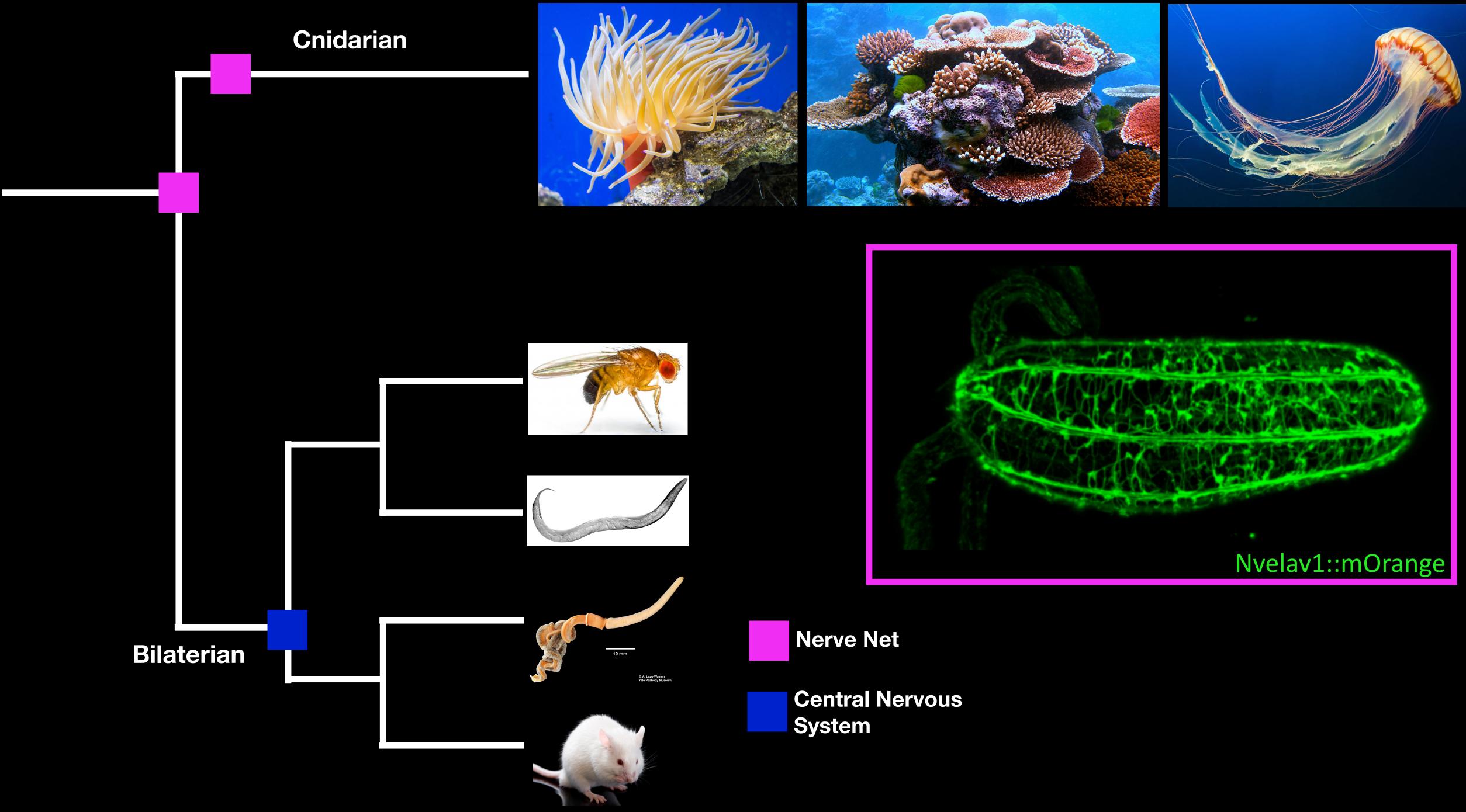


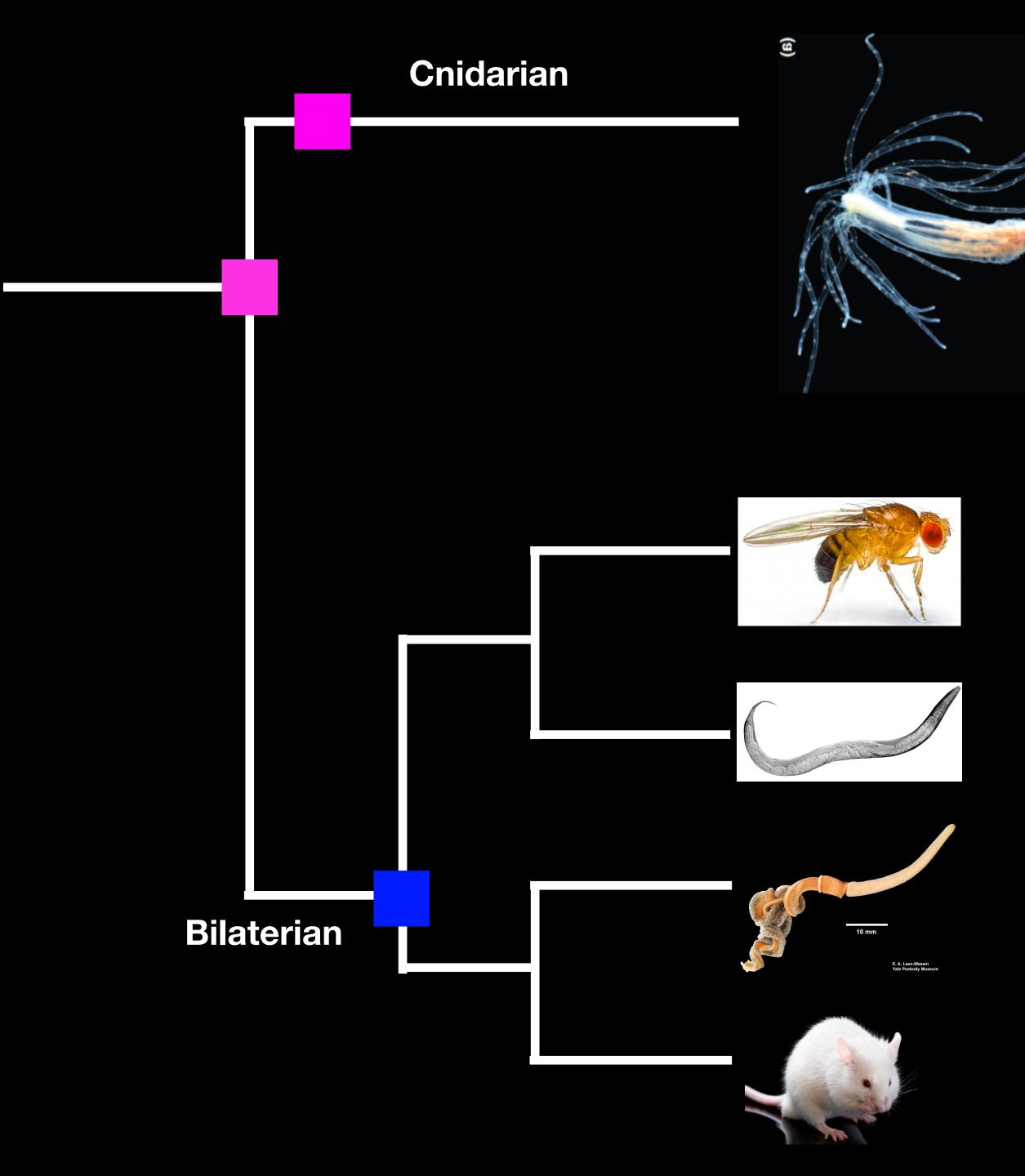


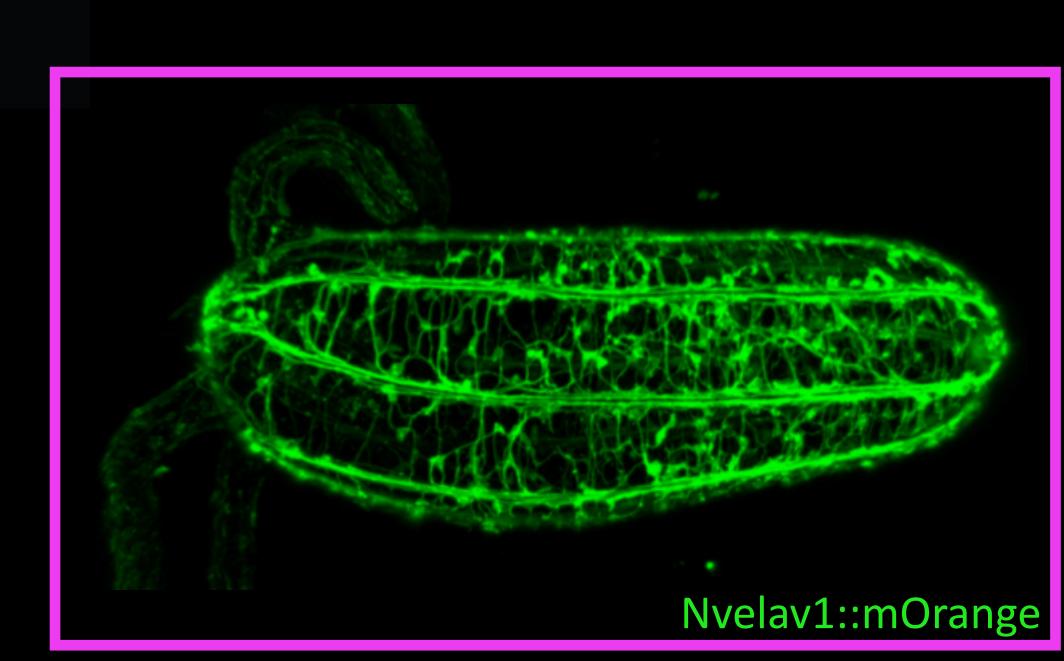












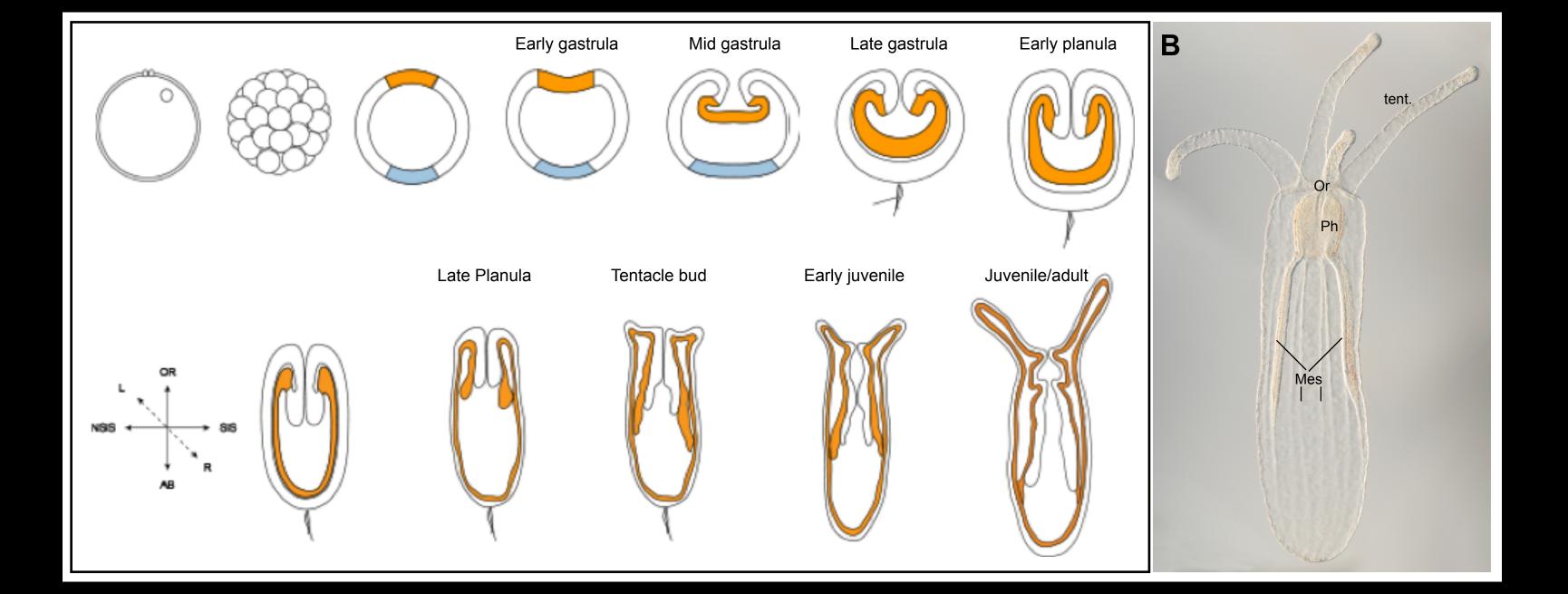
Dylan Faltine-Gonzalez

Nerve Net

**Central Nervous** System

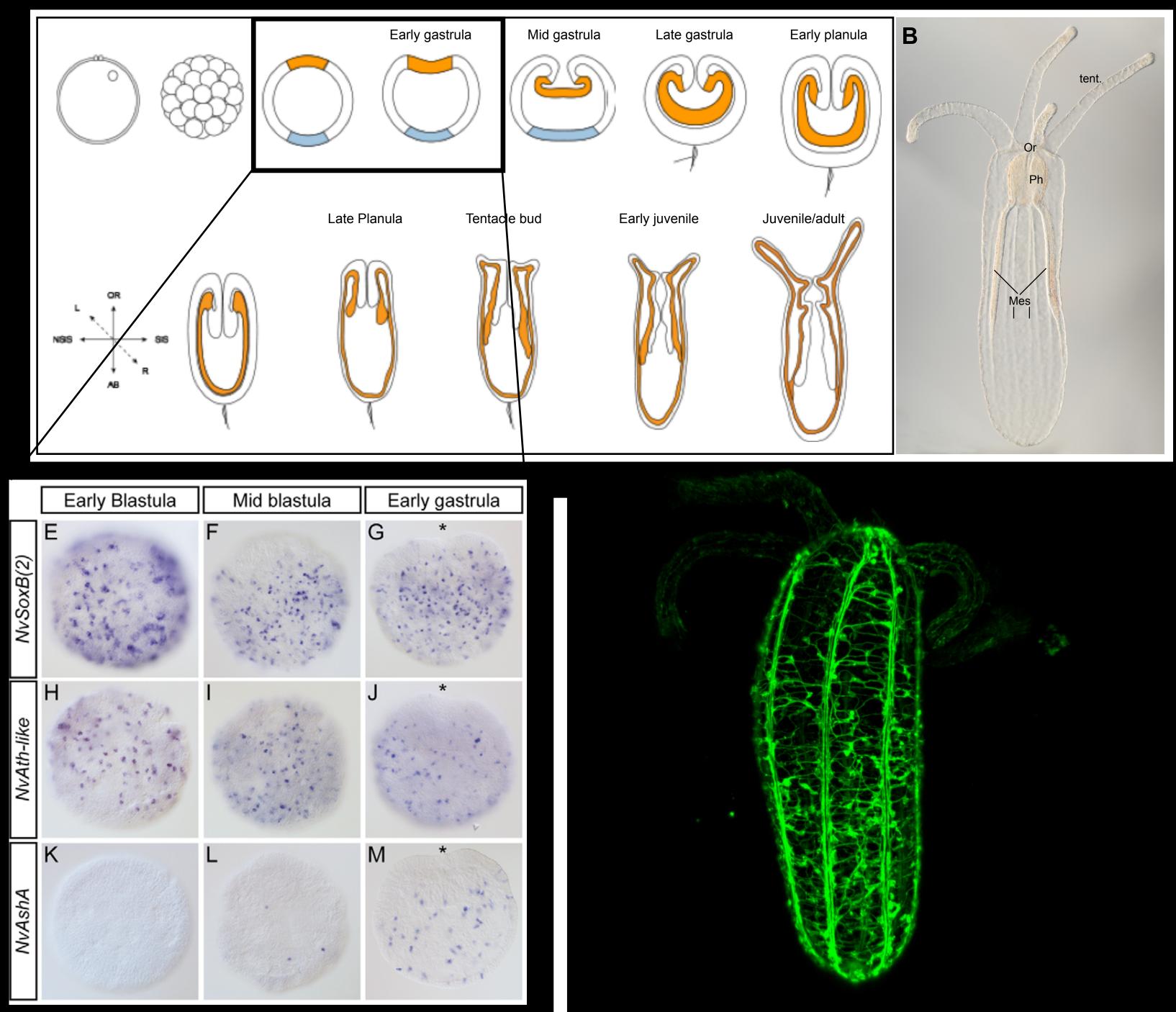






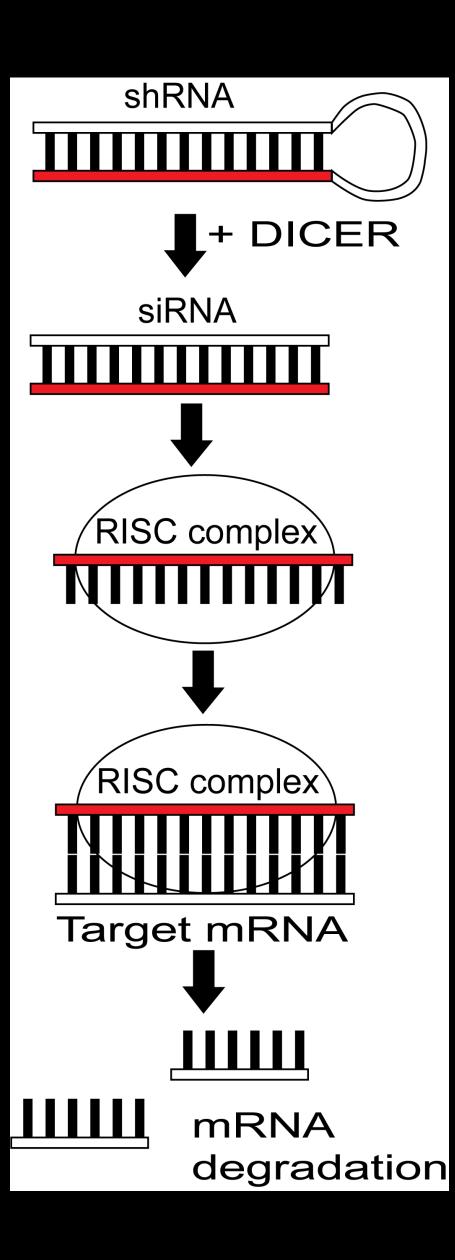


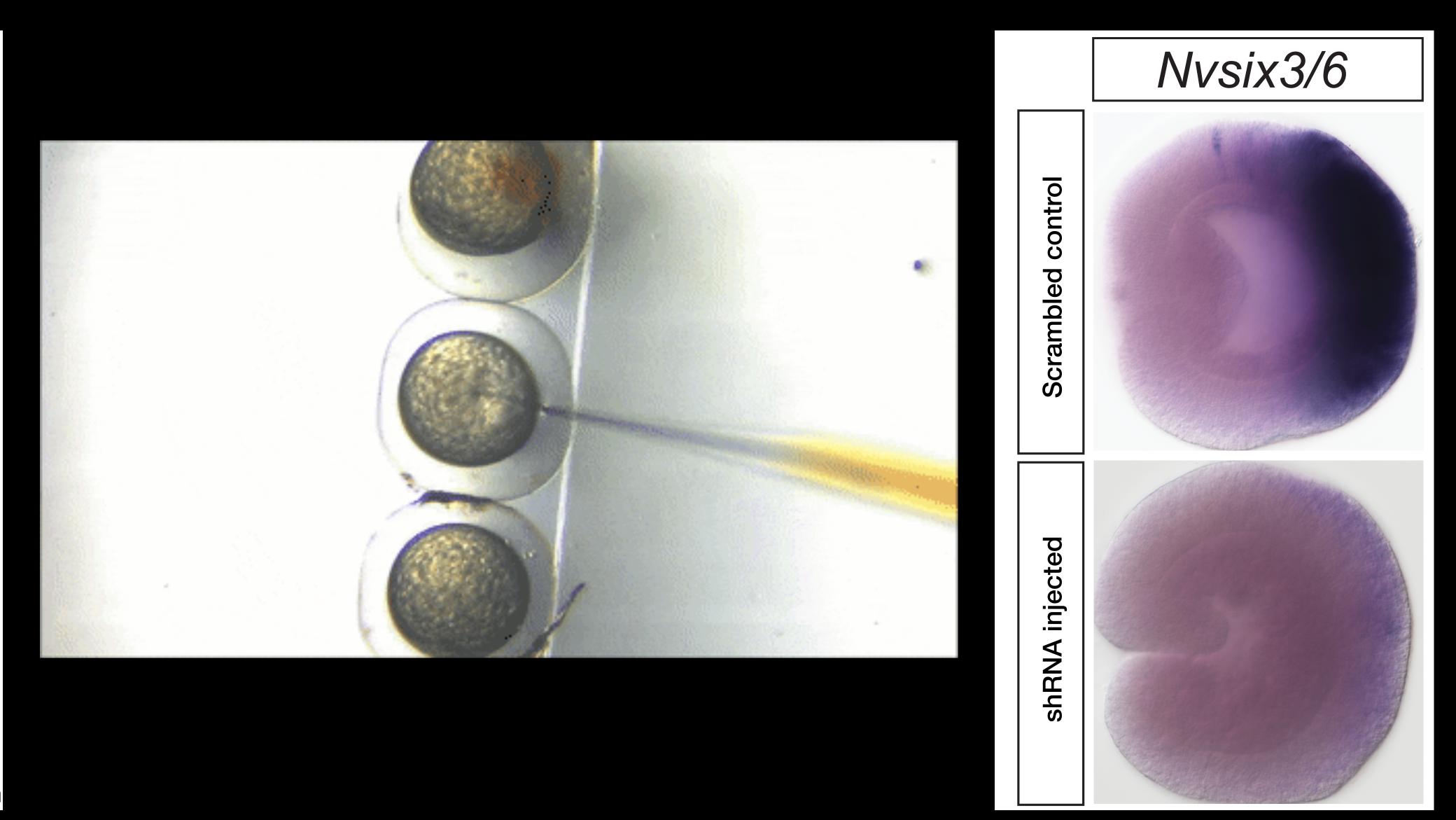
Richards et al., Development (2015)



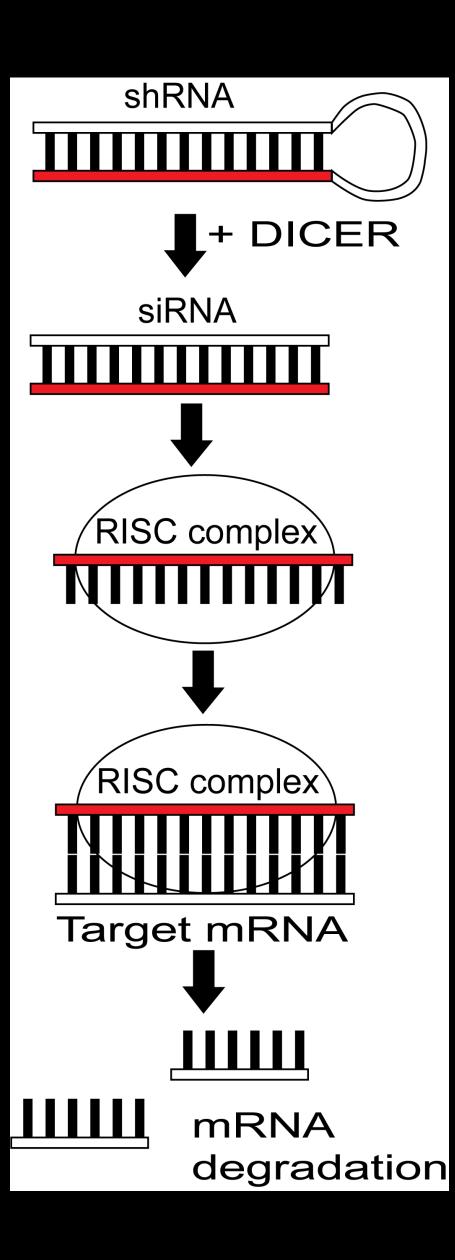
Richards et al., Development (2015)

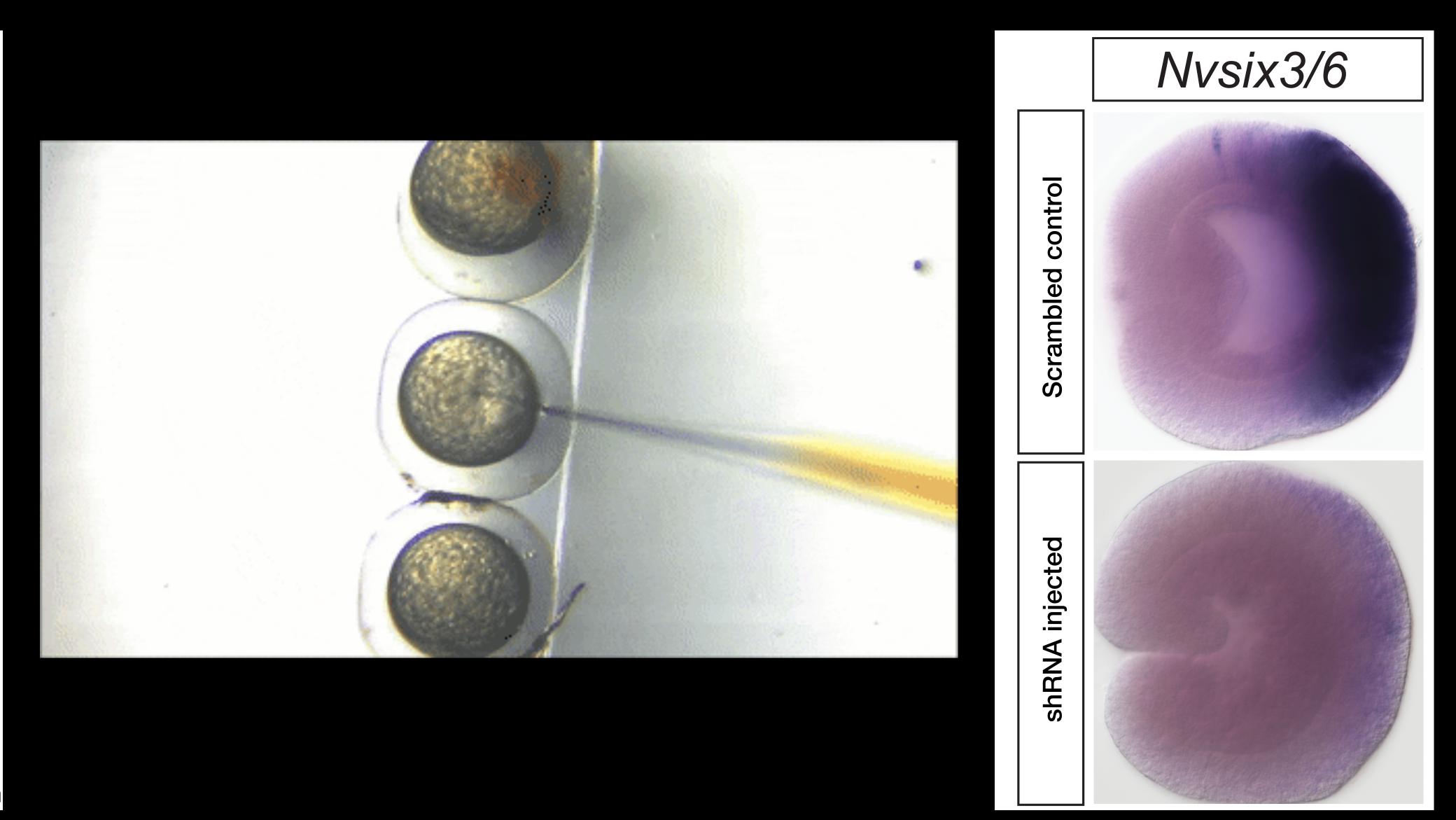
# Methods: Microinjections & shRNA knockdown



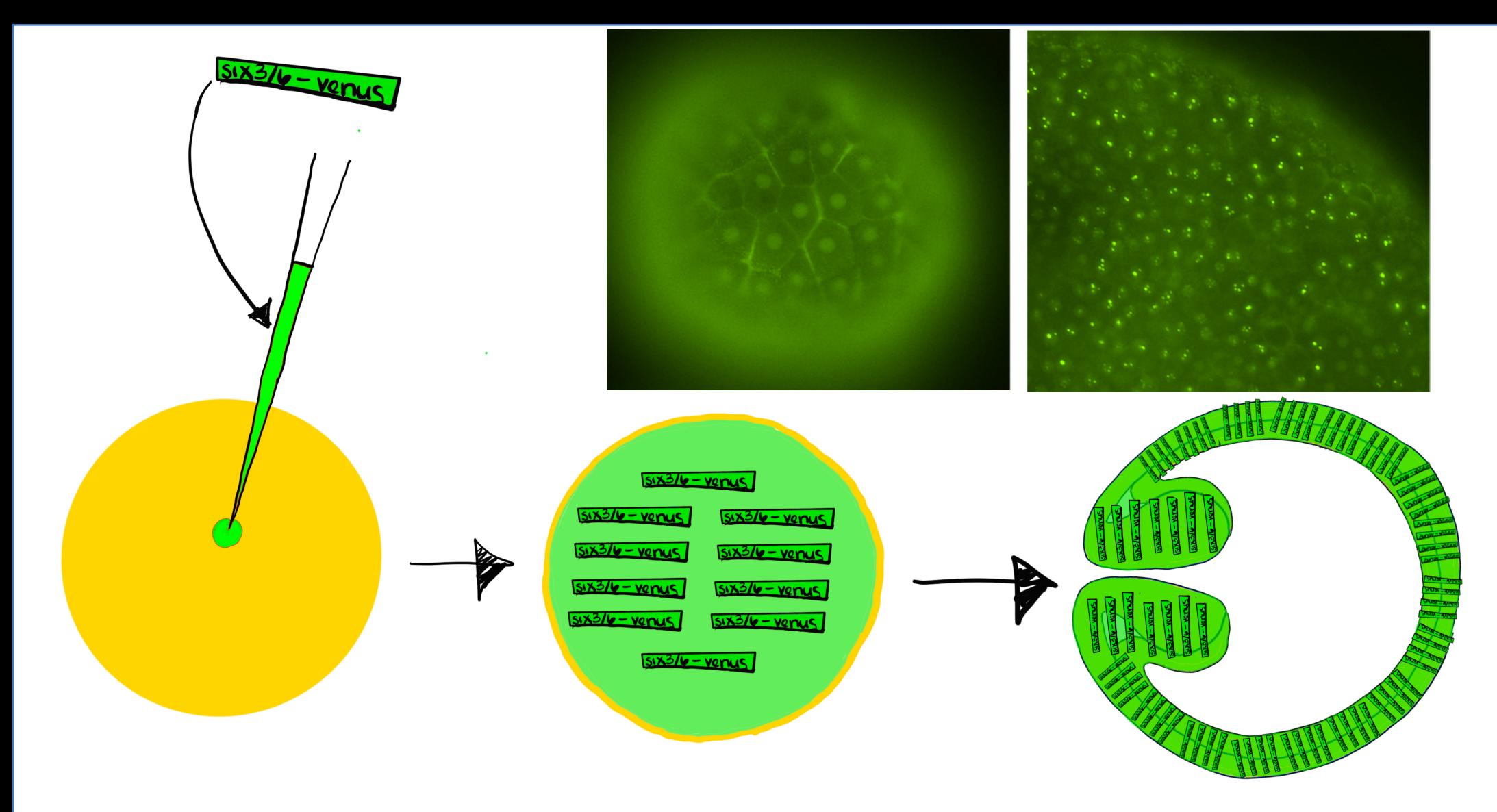


# Methods: Microinjections & shRNA knockdown

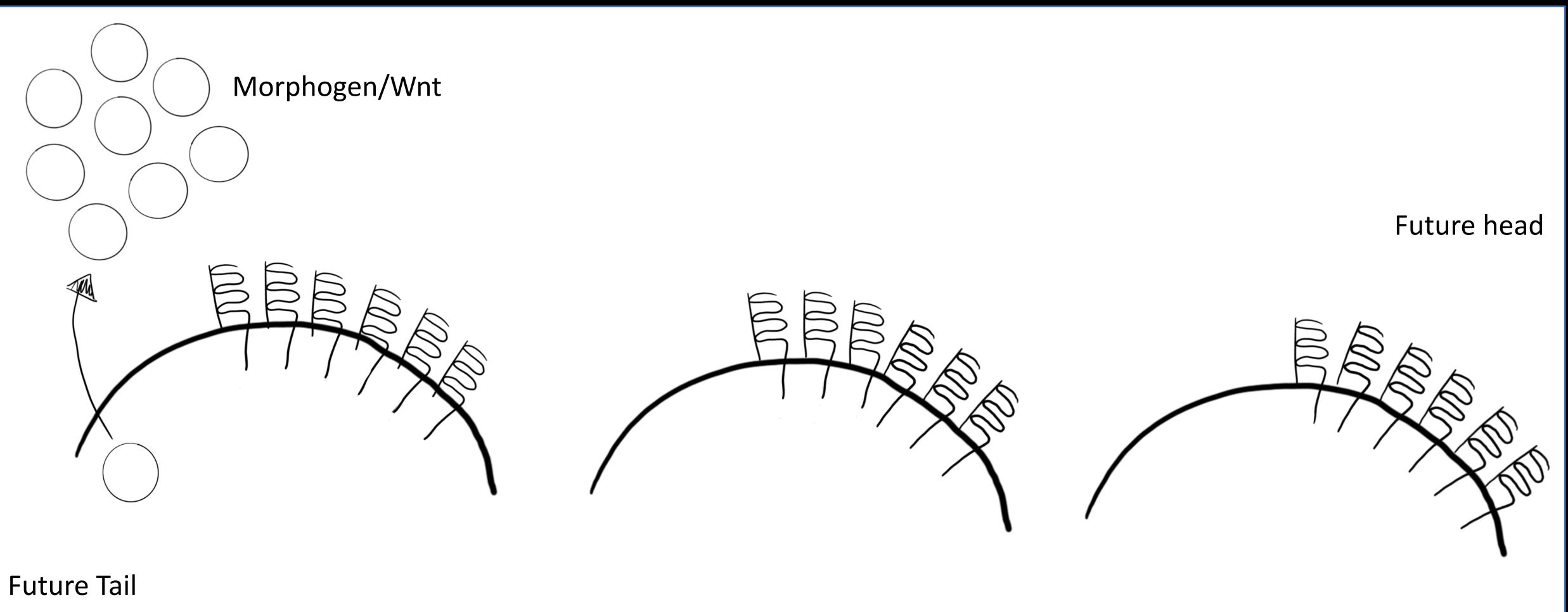


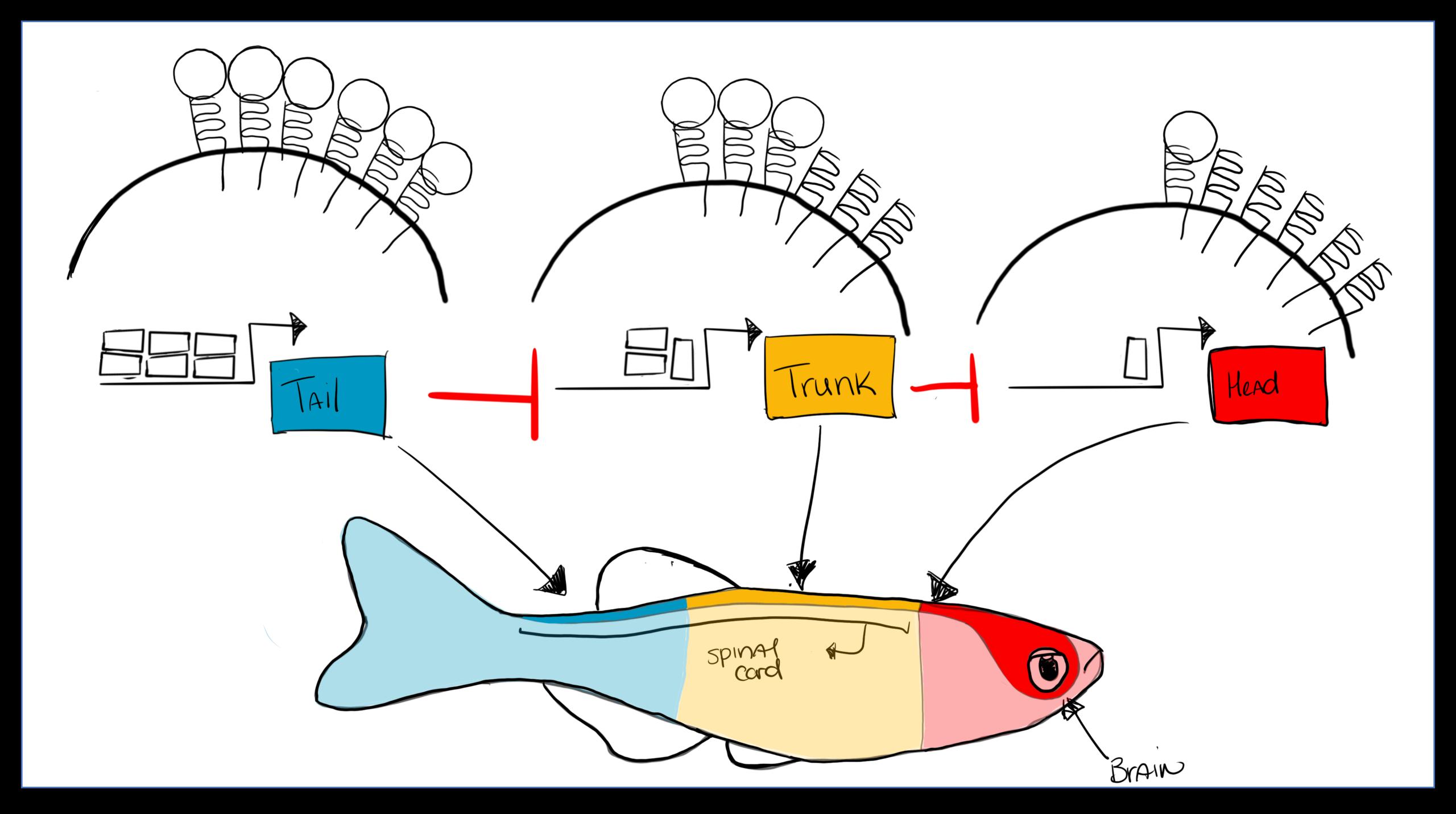


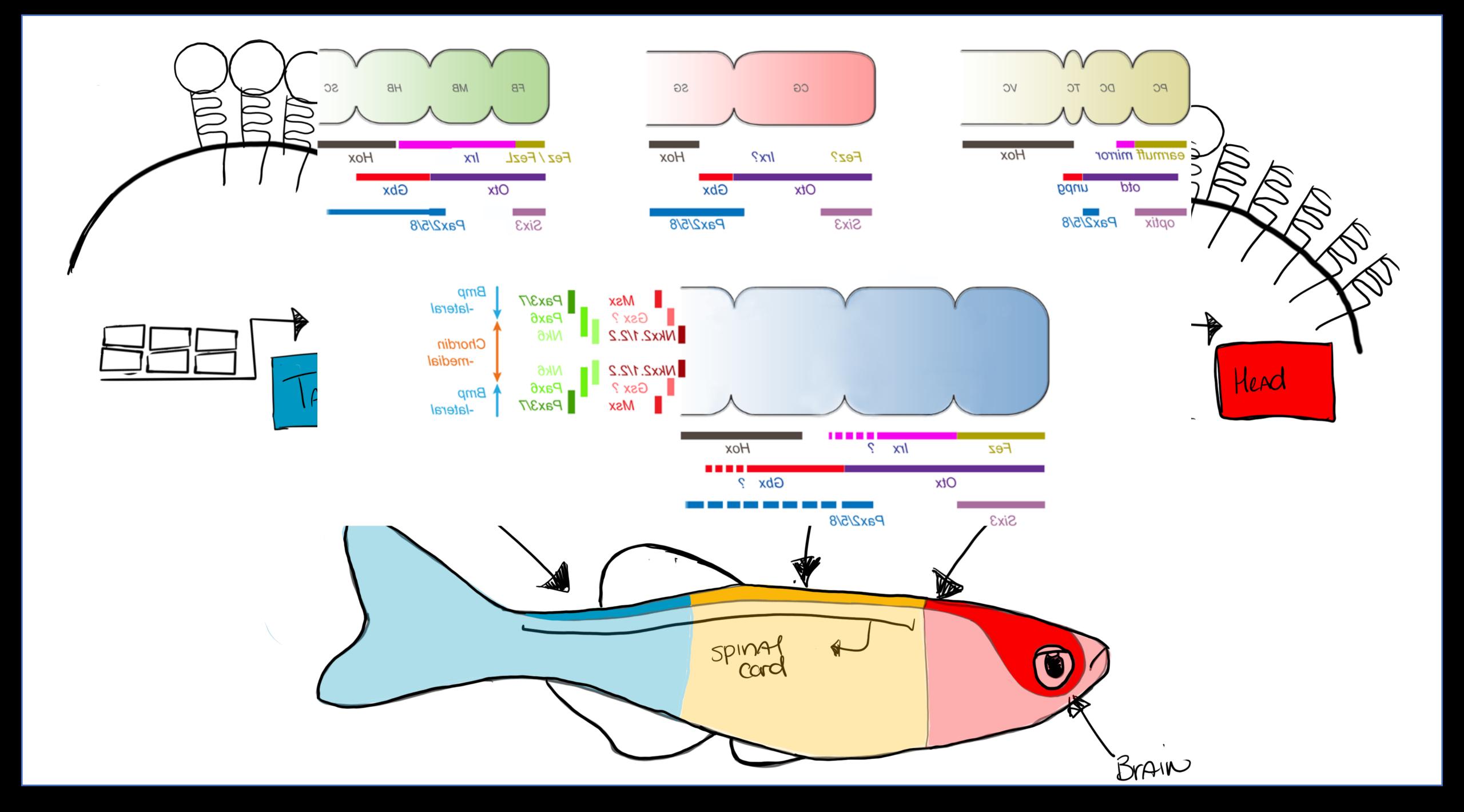
# Methods: mRNA synthesis and injection



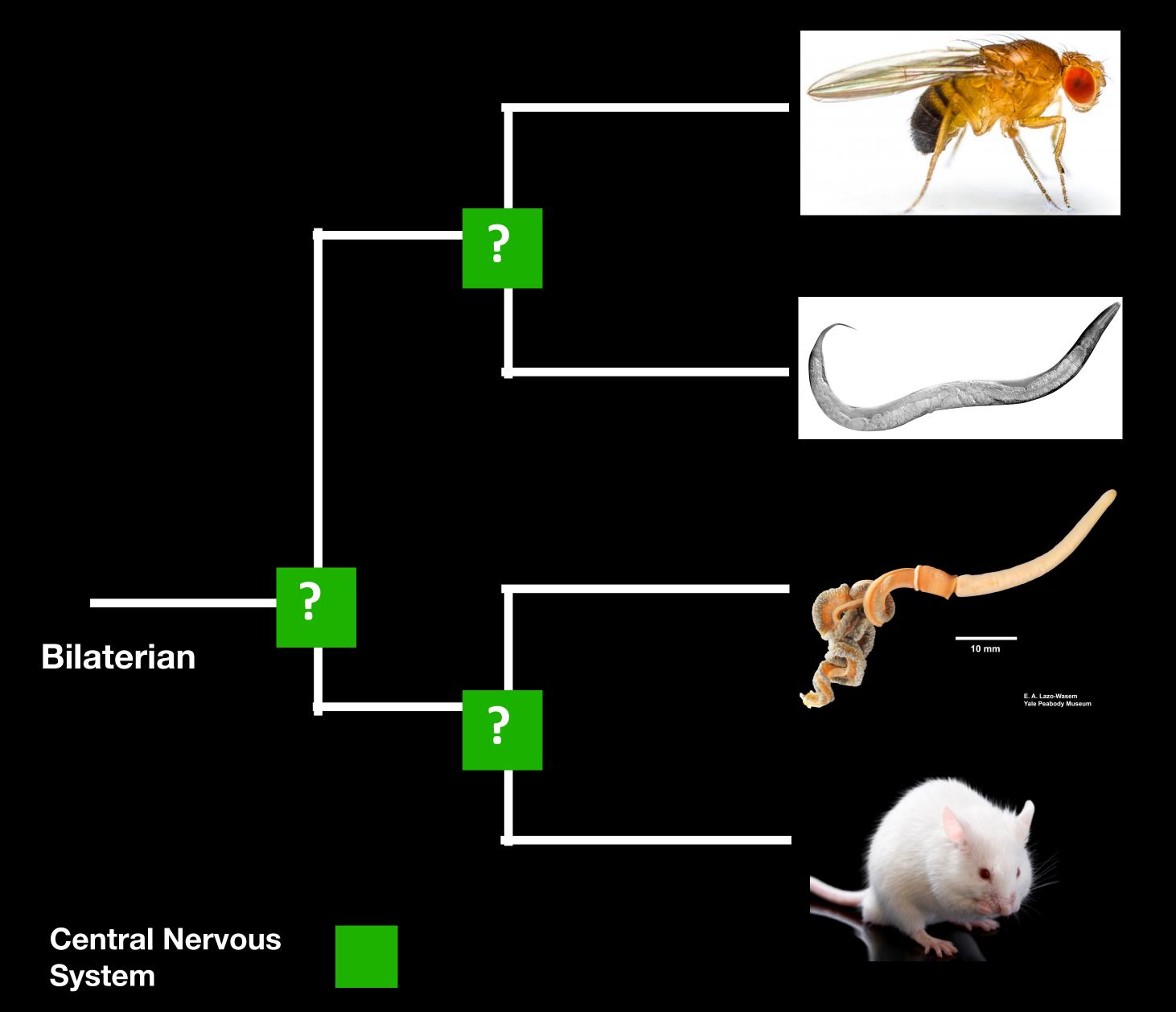




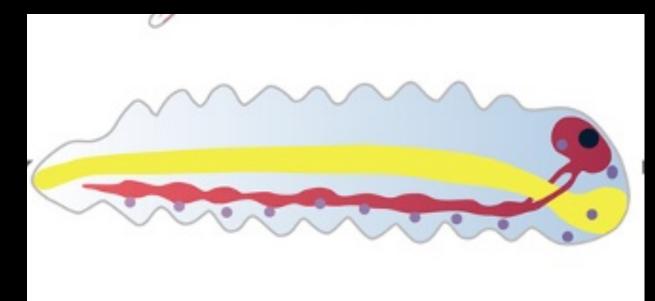


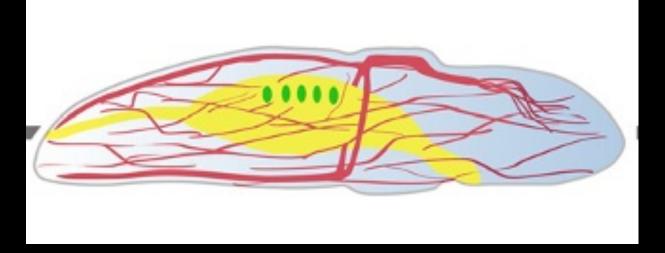


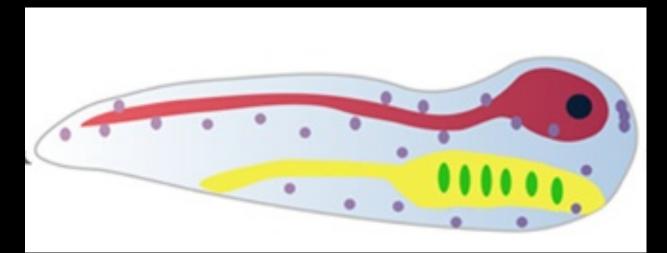
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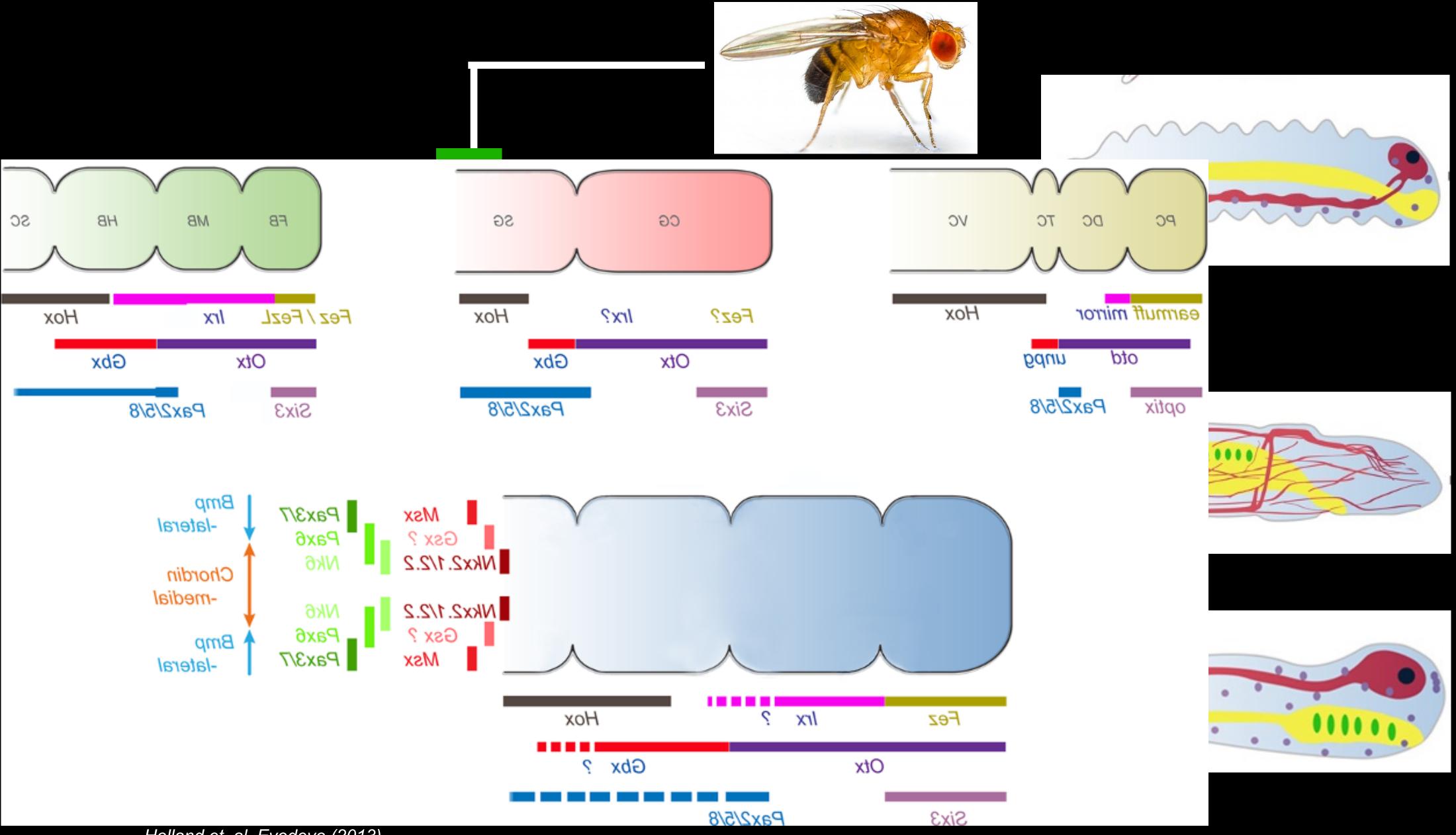
Holland et. al. Evodevo (2013)



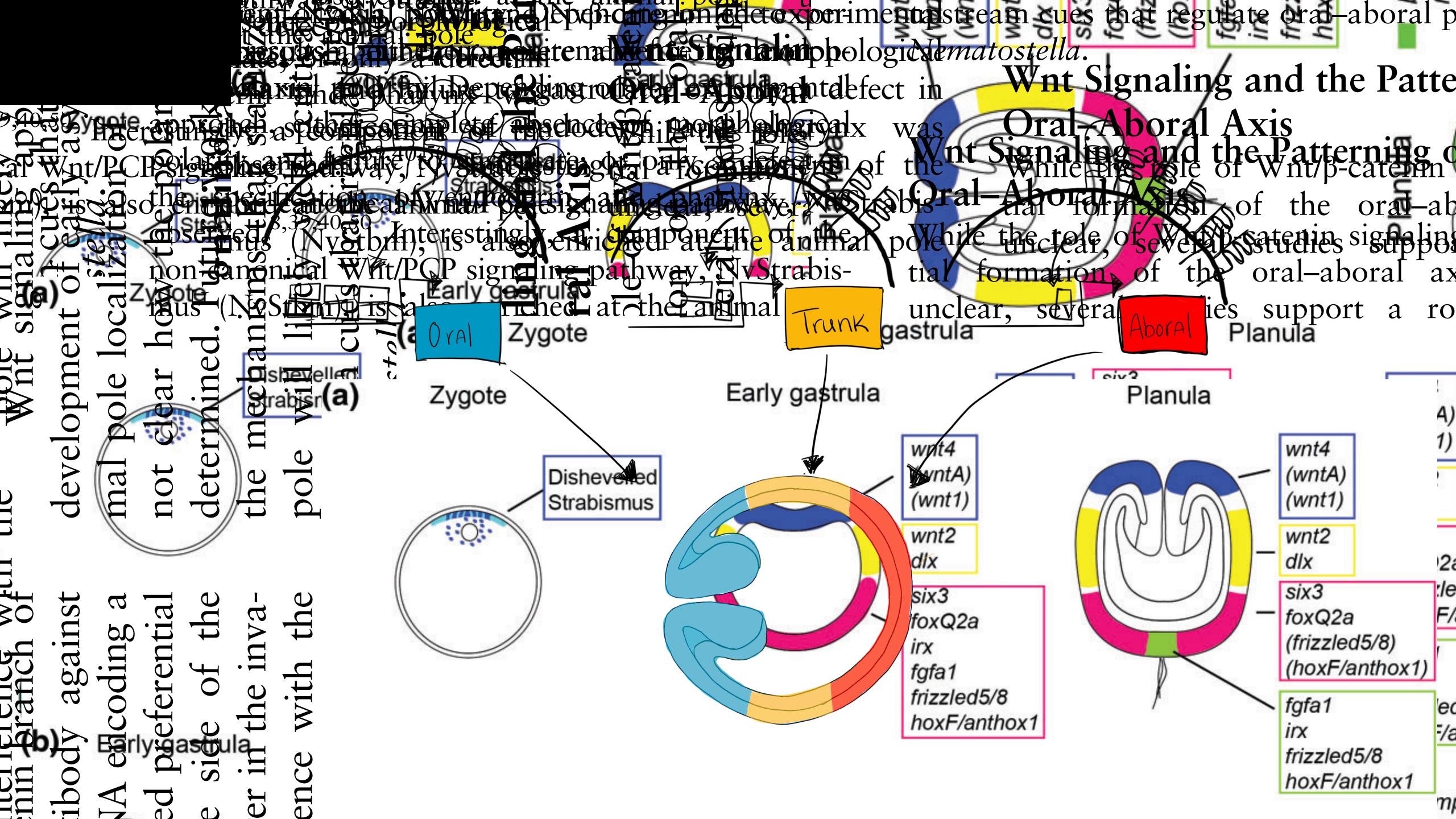




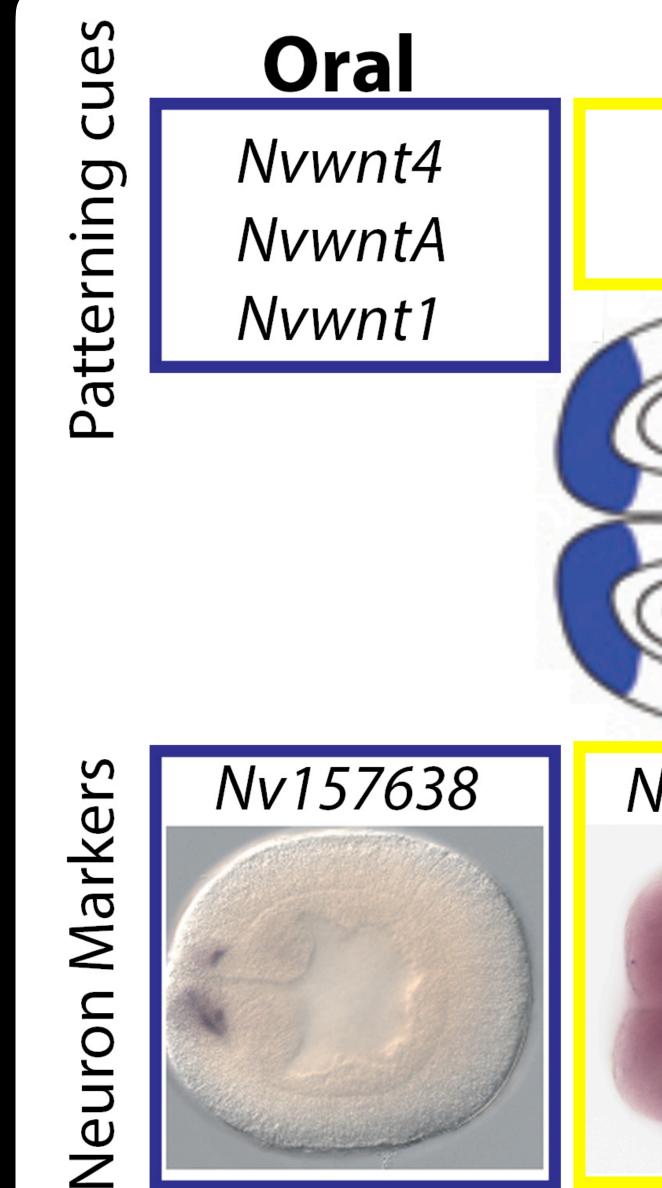
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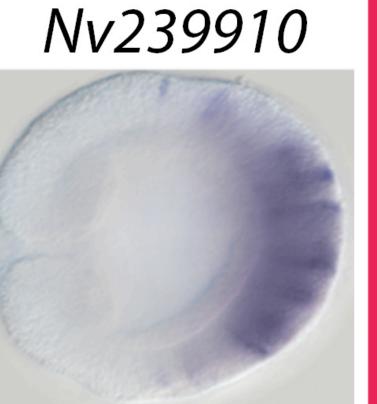
Holland et. al. Evodevo (2013)

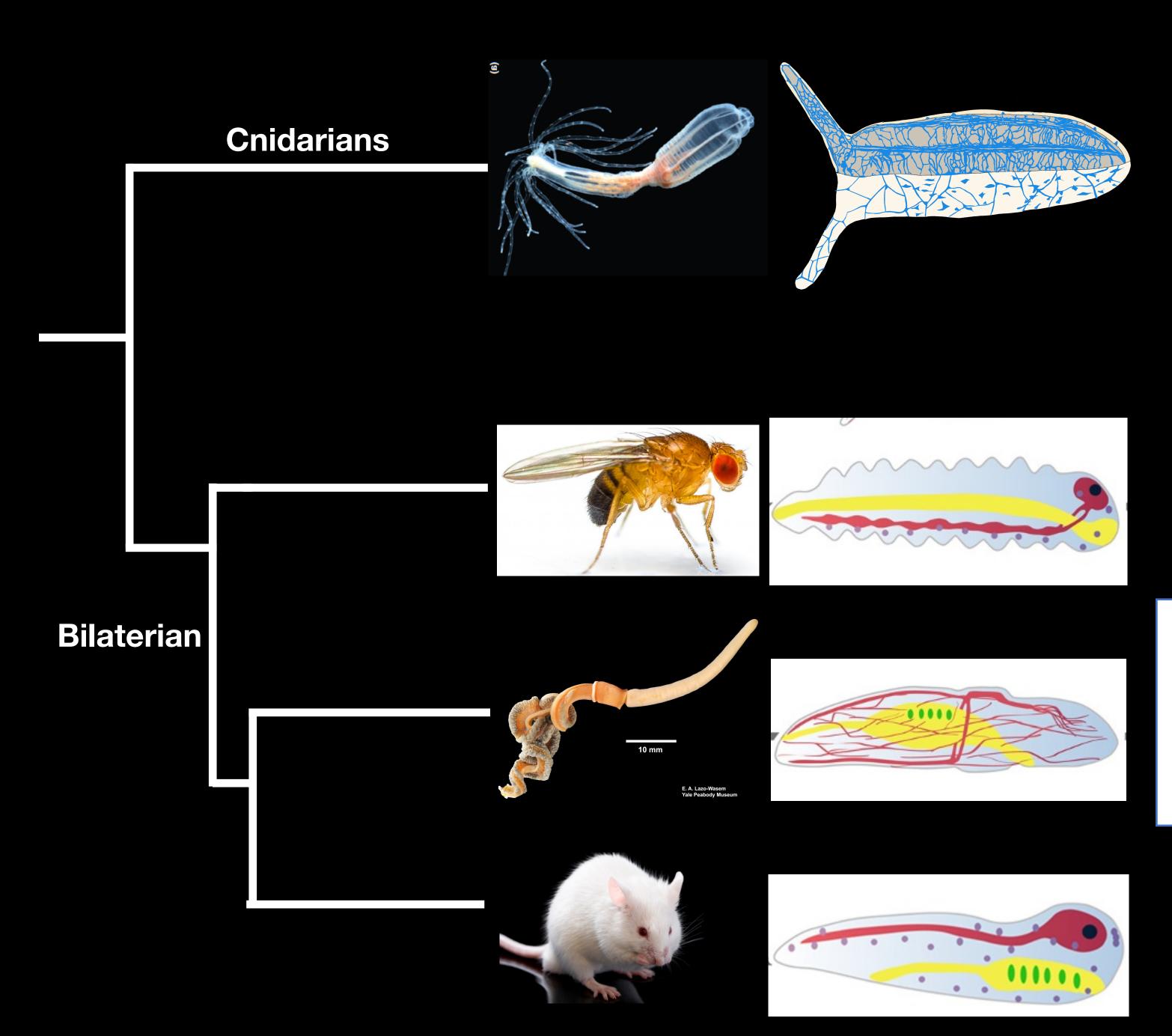


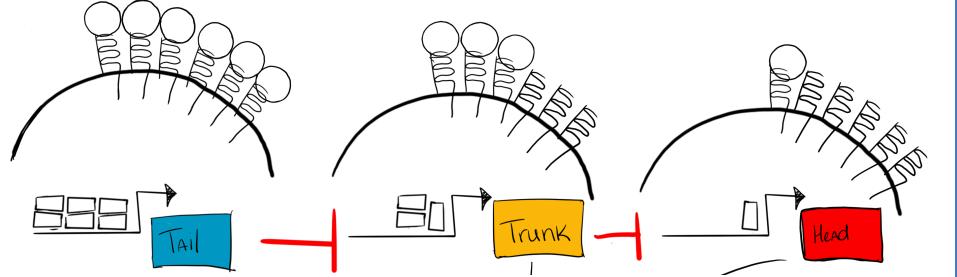
# Potential patterning domains and regional neuronal markers

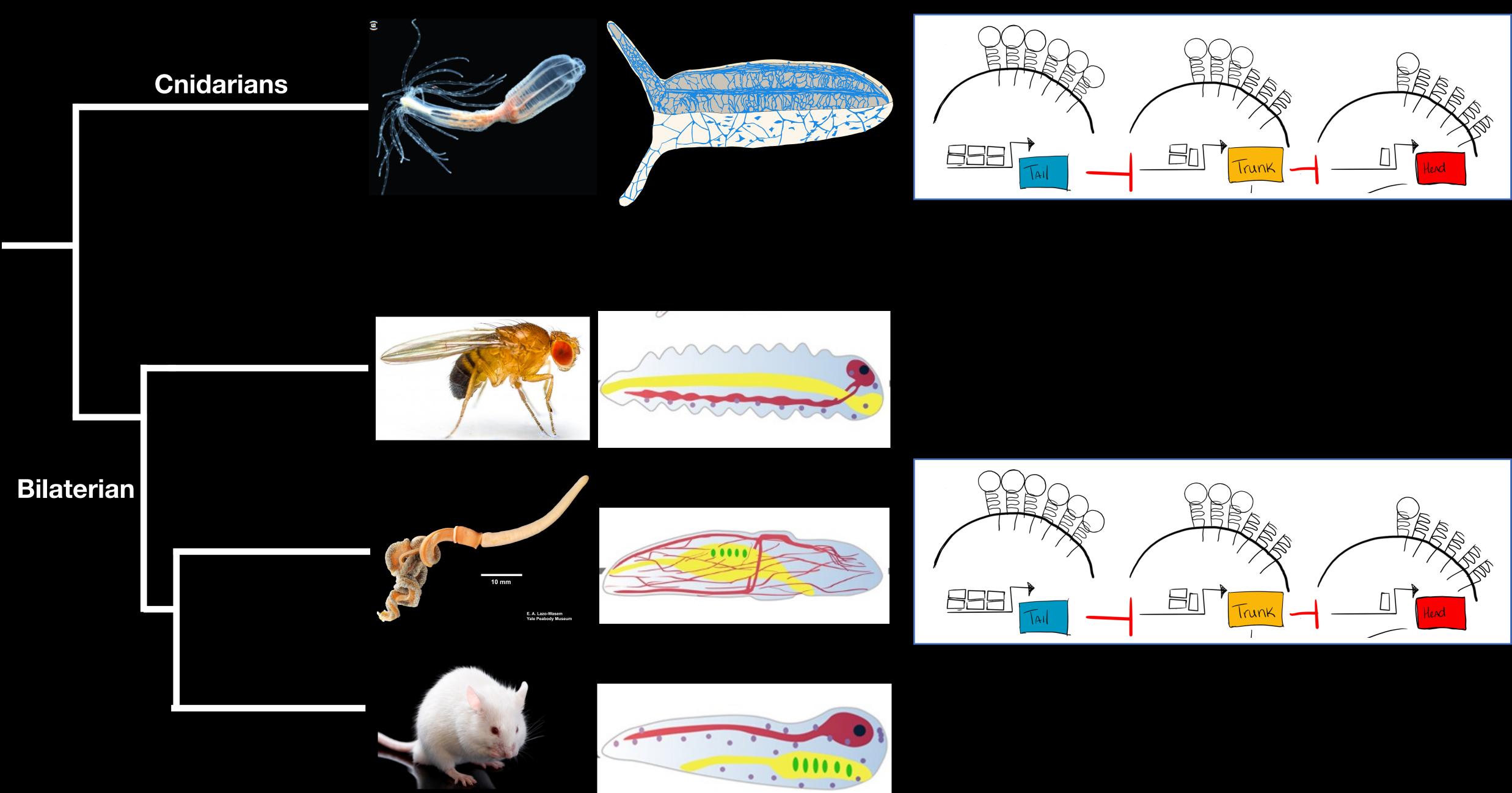


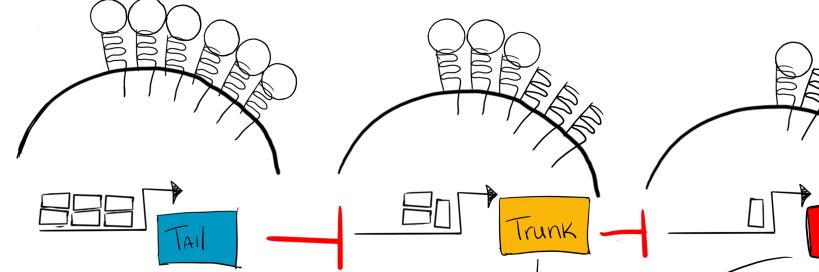
# Trunk Aboral Nvsix3/6 Nvwnt2 NvfoxQ2a Nvdlx Nvsfrp1/5 Nvpea3-like

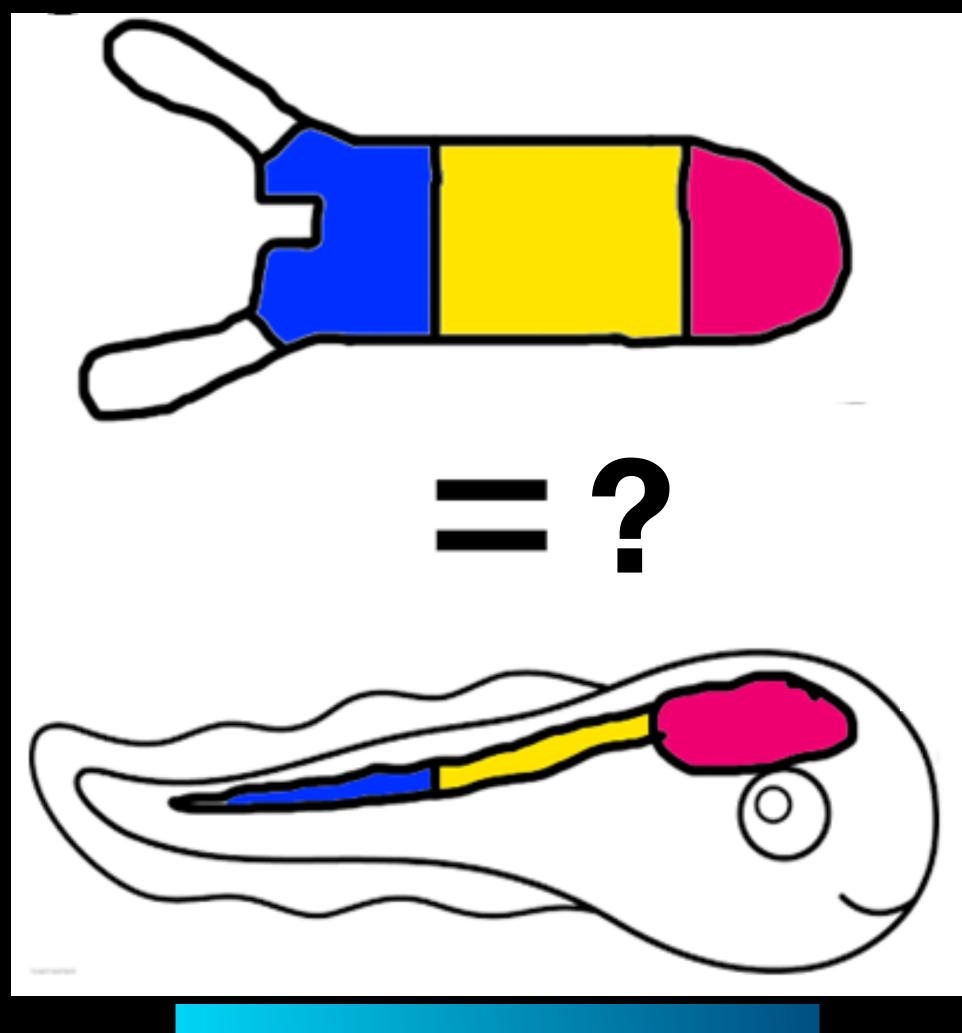






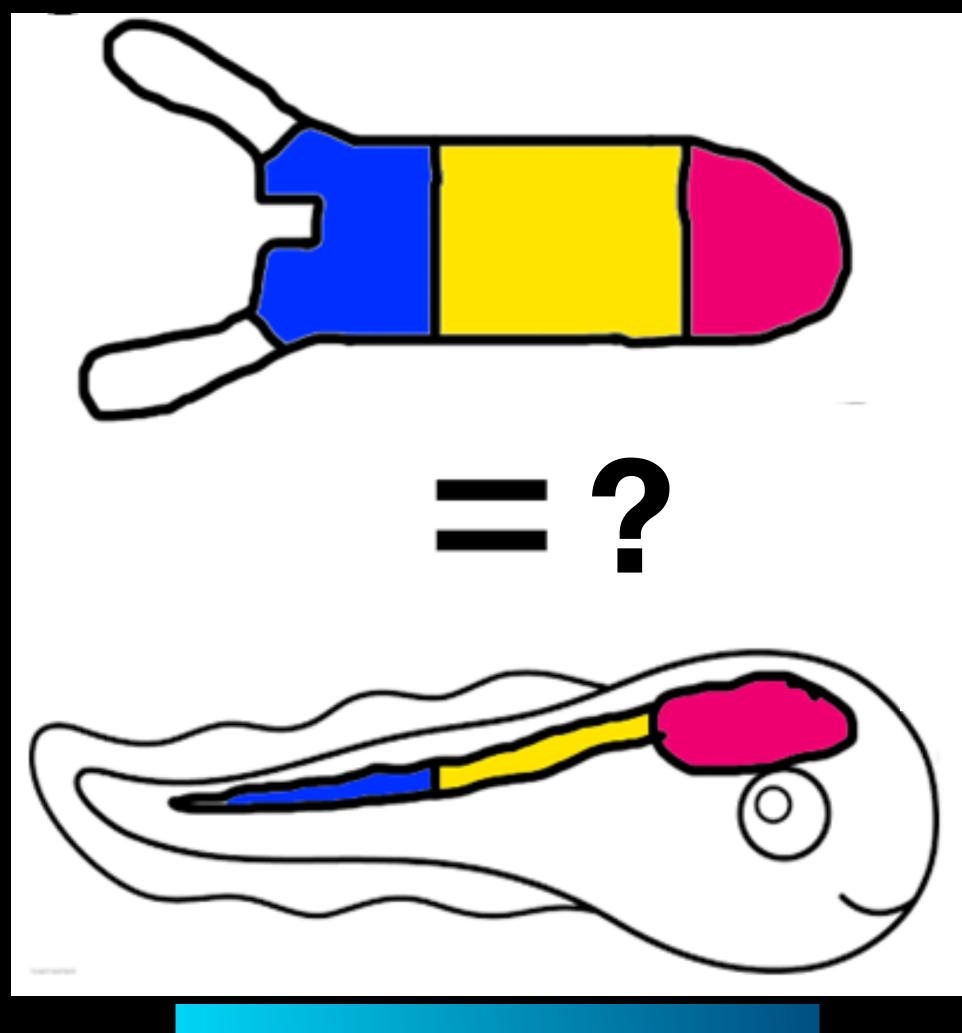




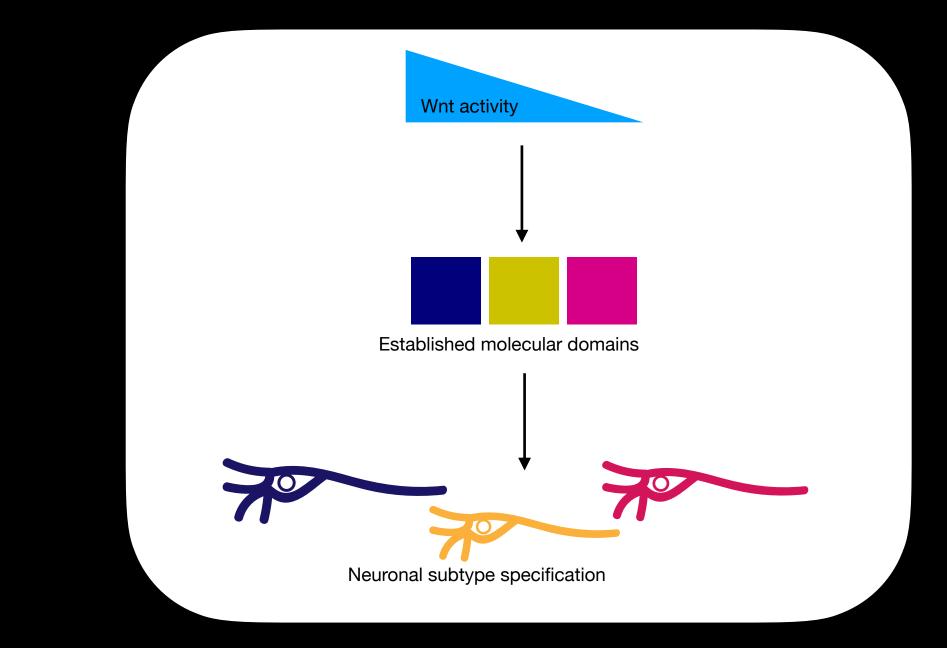


Wnt activity

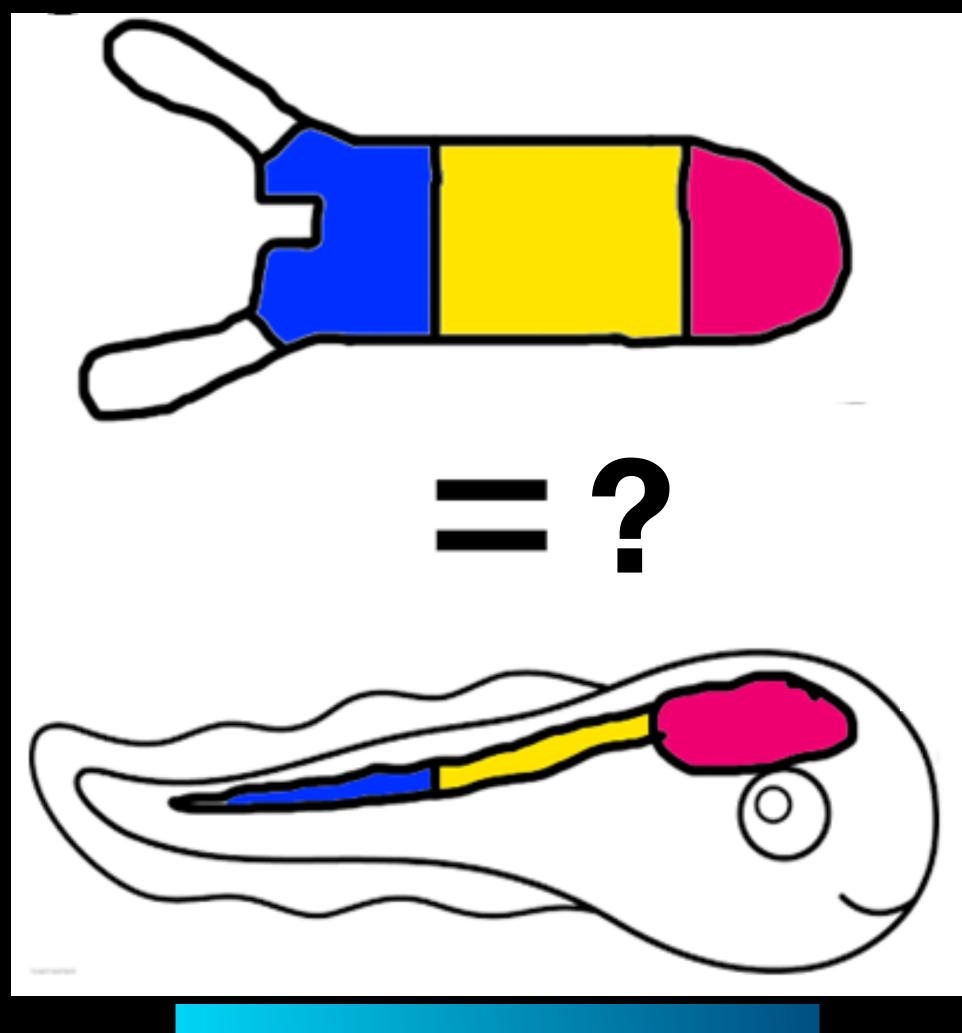




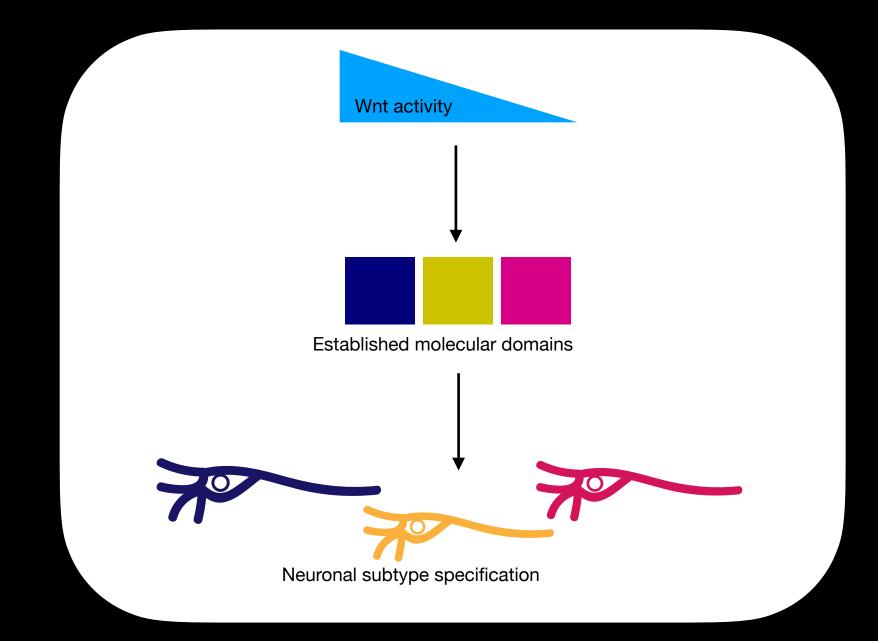
Wnt activity



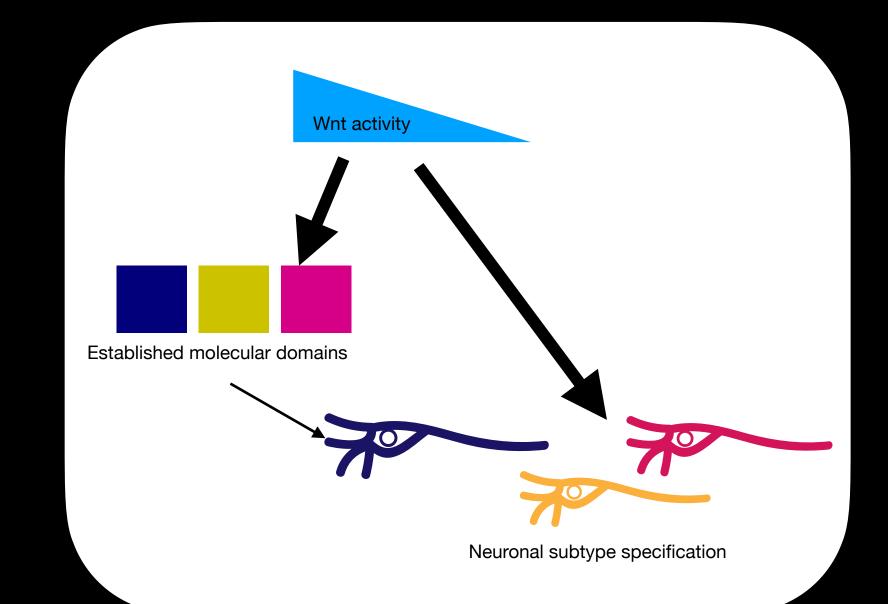




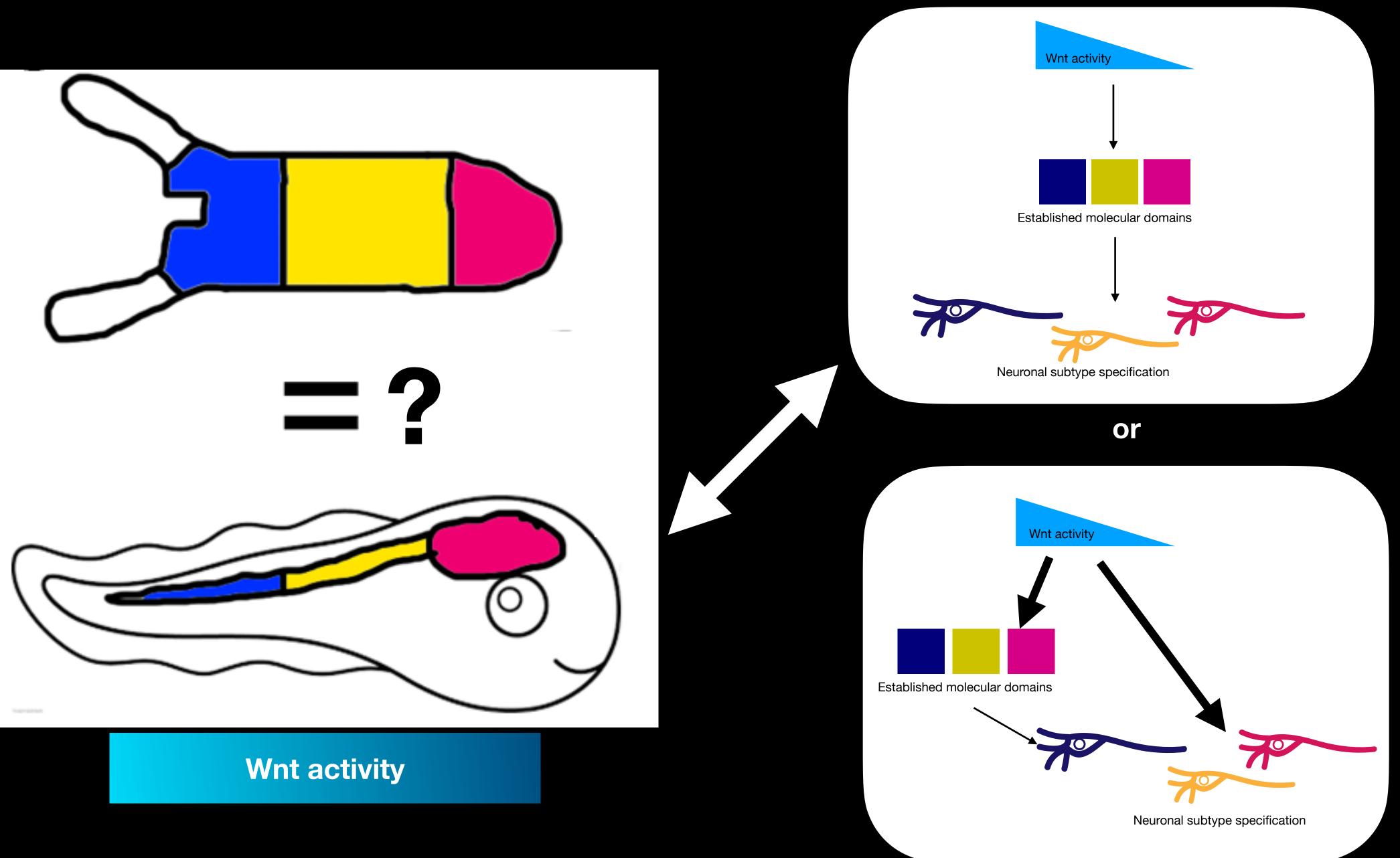
Wnt activity





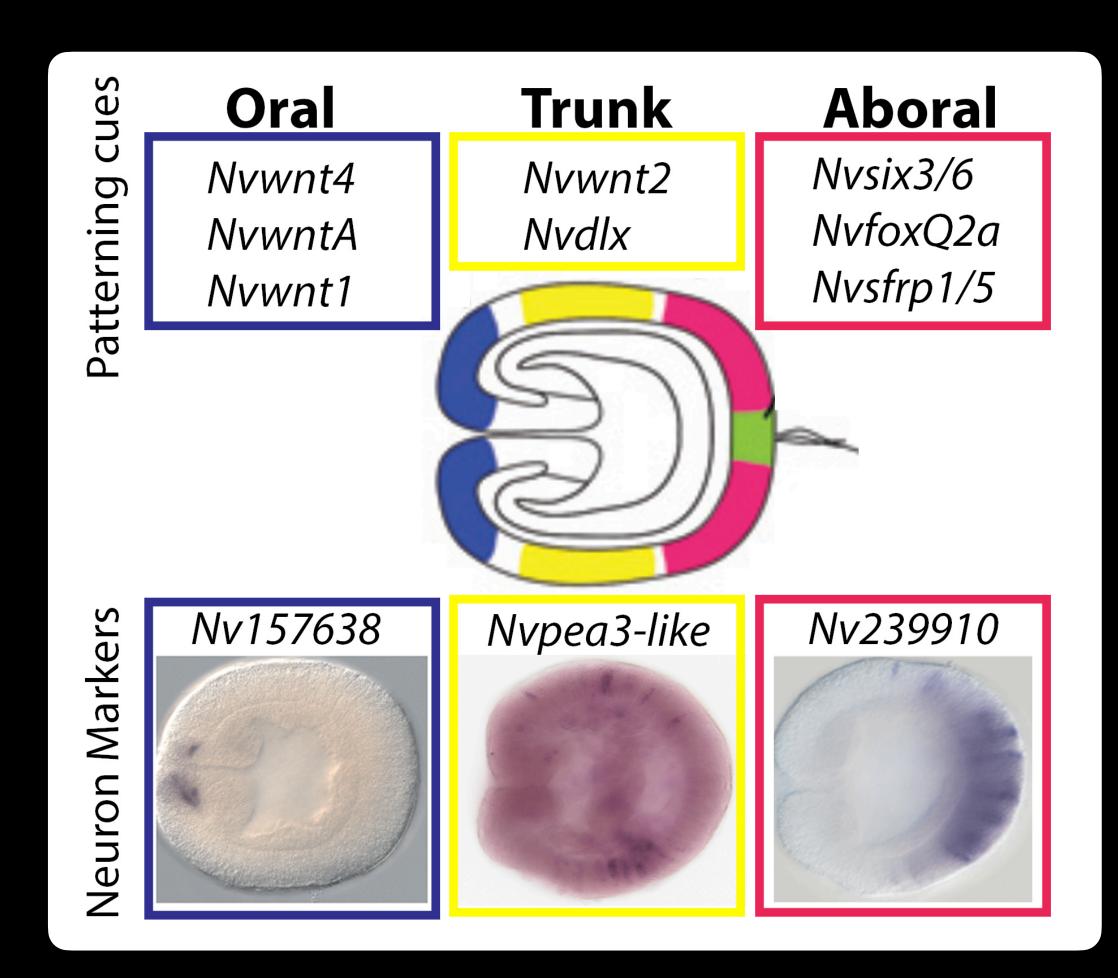




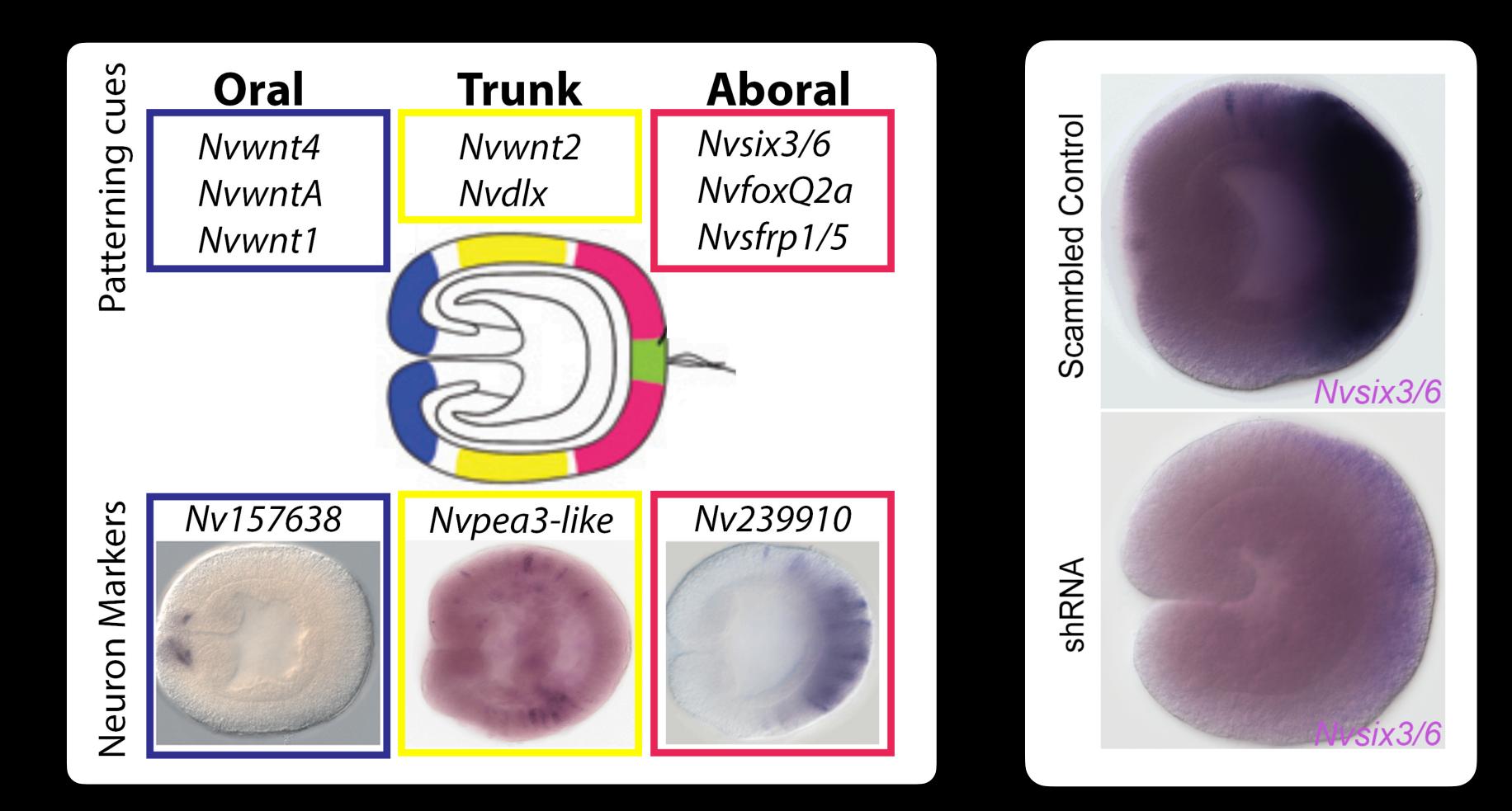




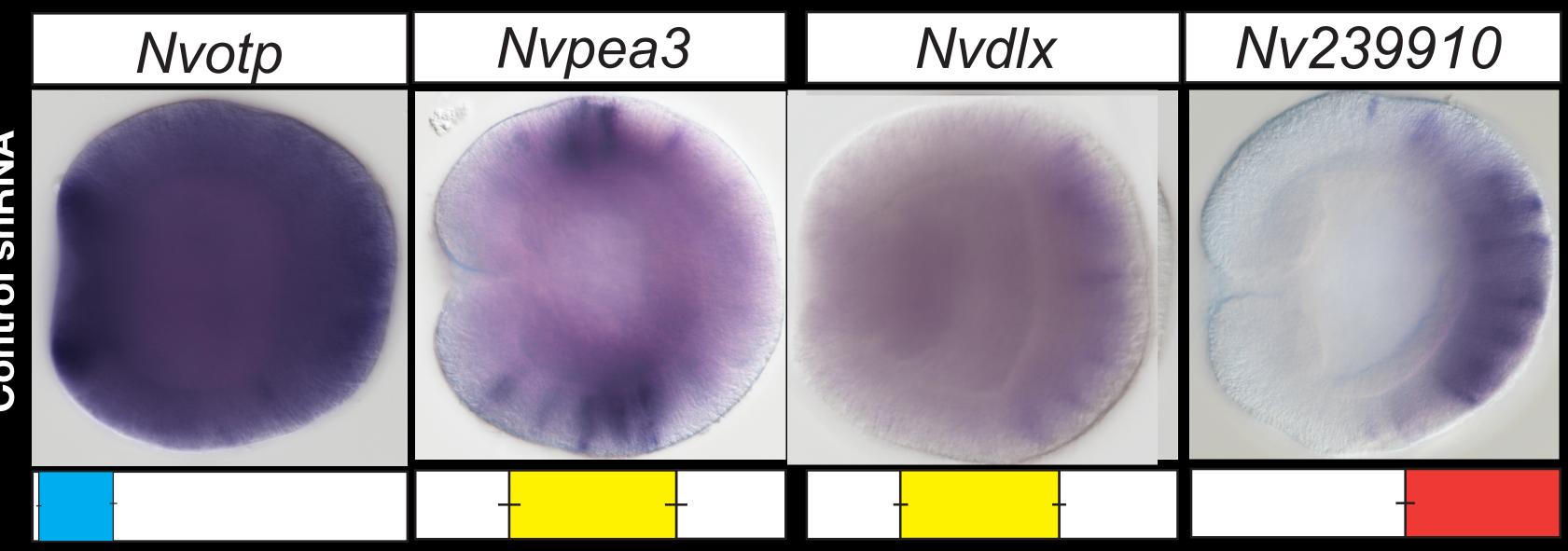
# Potential patterning domains and regional neuronal markers



# Potential patterning domains and regional neuronal markers

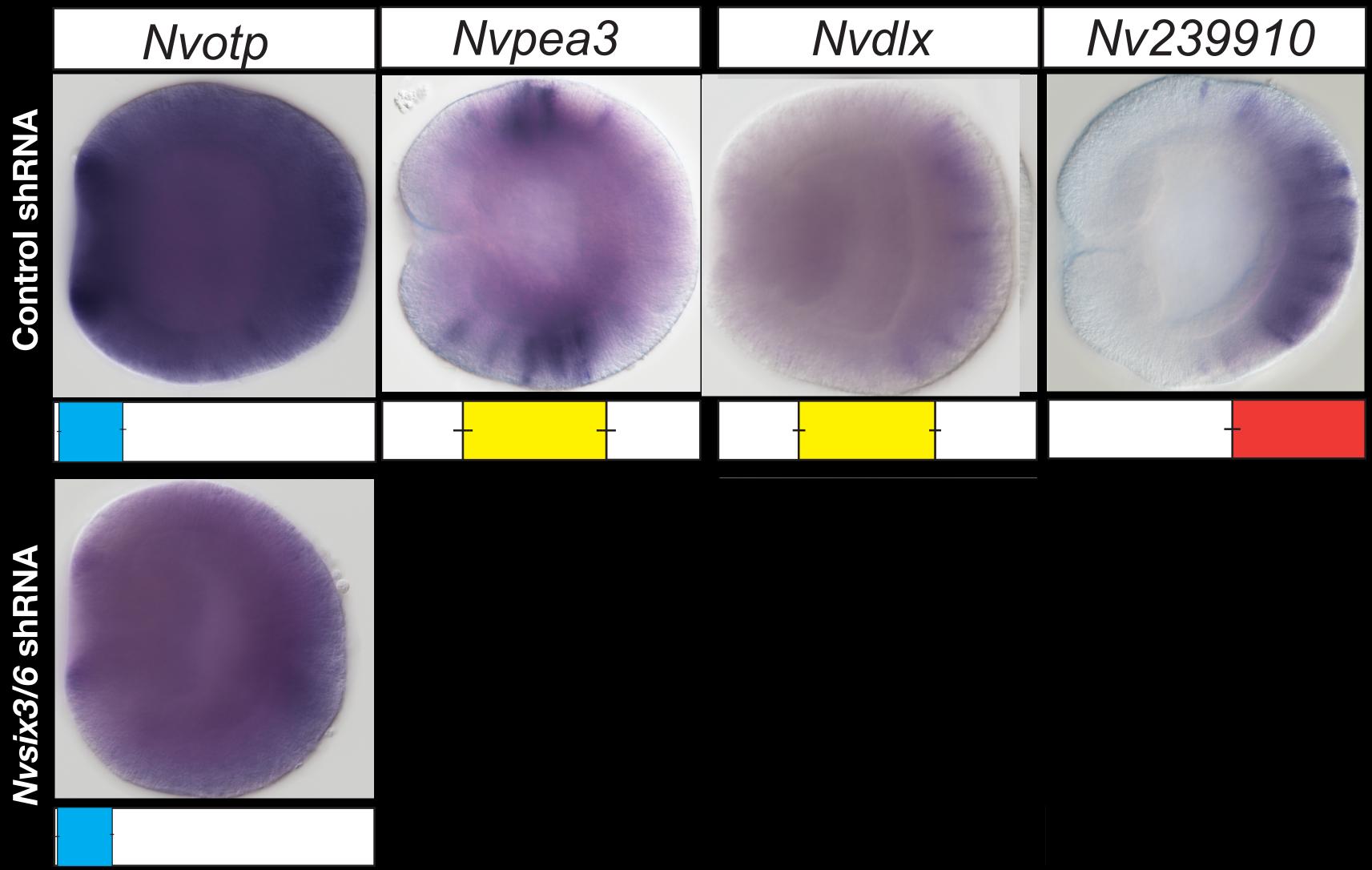


# Nvsix3/6 is necessary for aboral neural genes



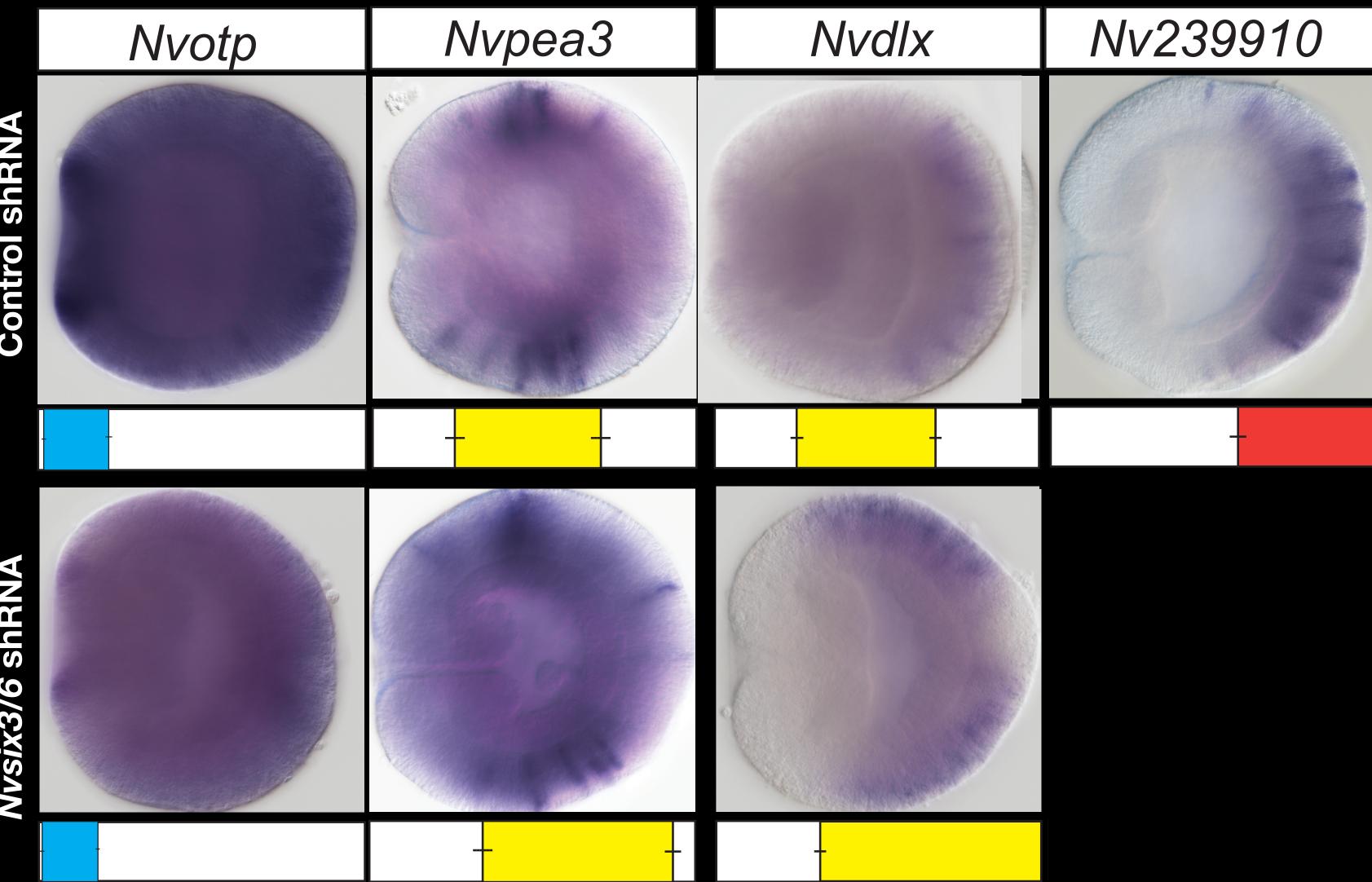
# **Control shRNA**

# Nvsix3/6 is necessary for aboral neural genes



# **Control shRNA**

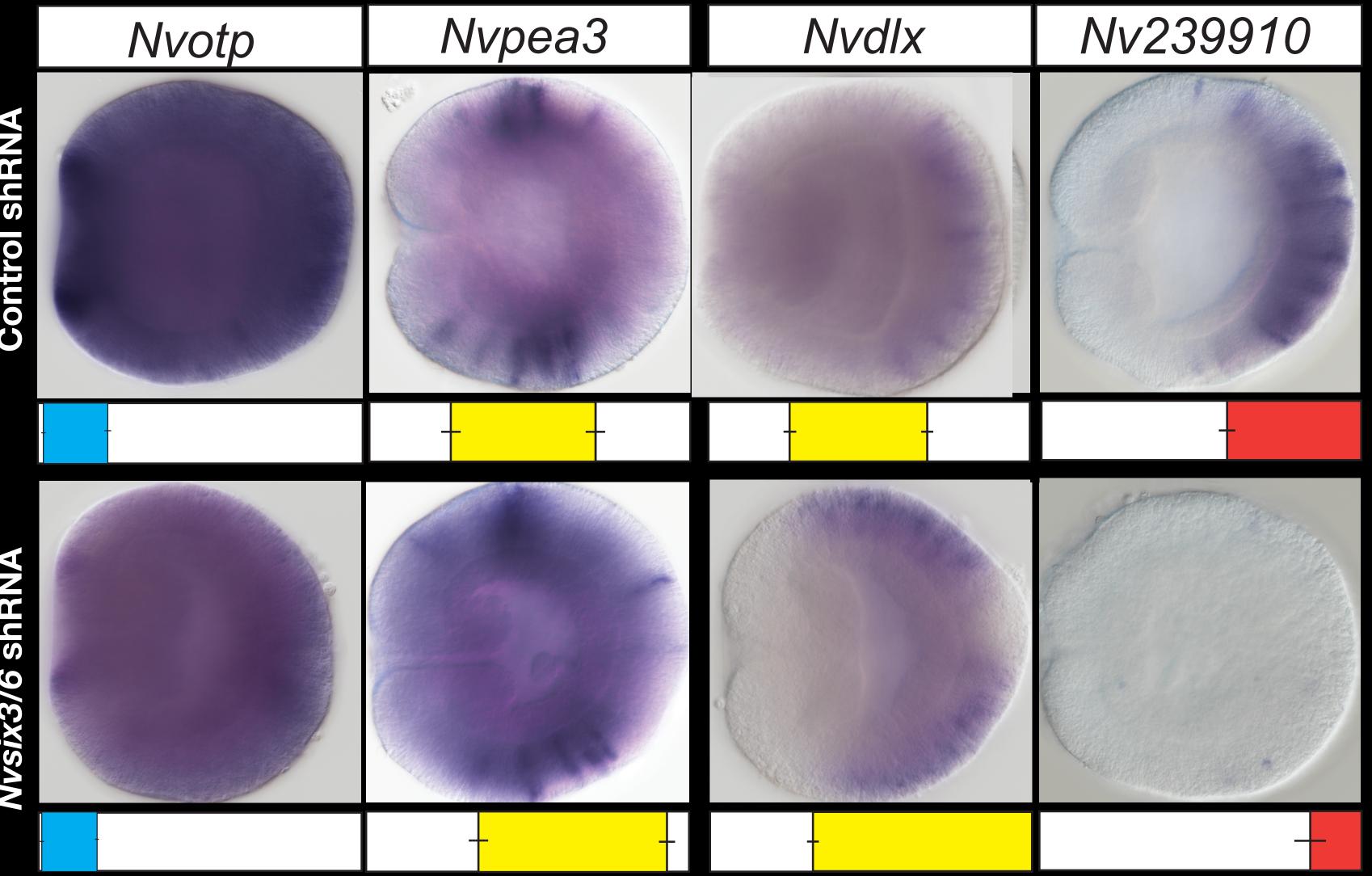
# Nvsix3/6 is necessary for aboral neural genes



# **Control shRNA**

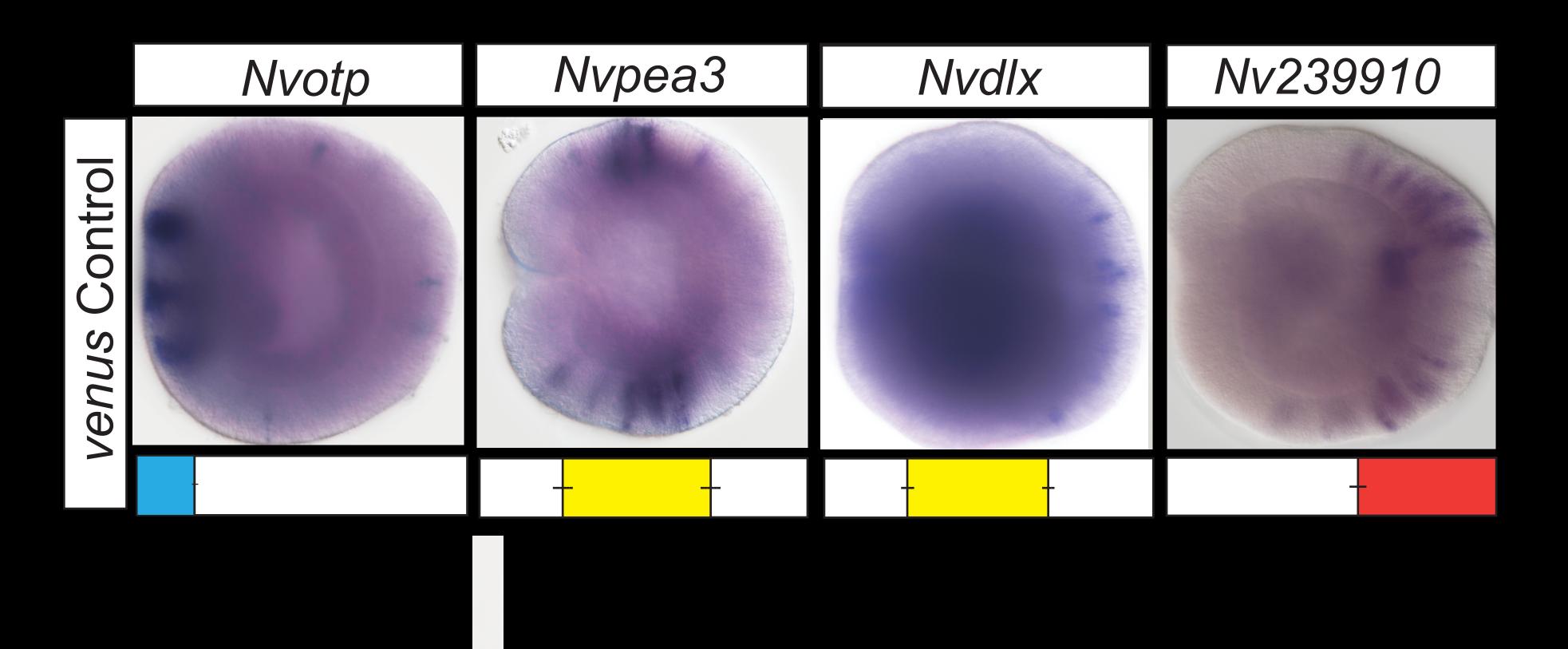
# vsix3/6 shRNA

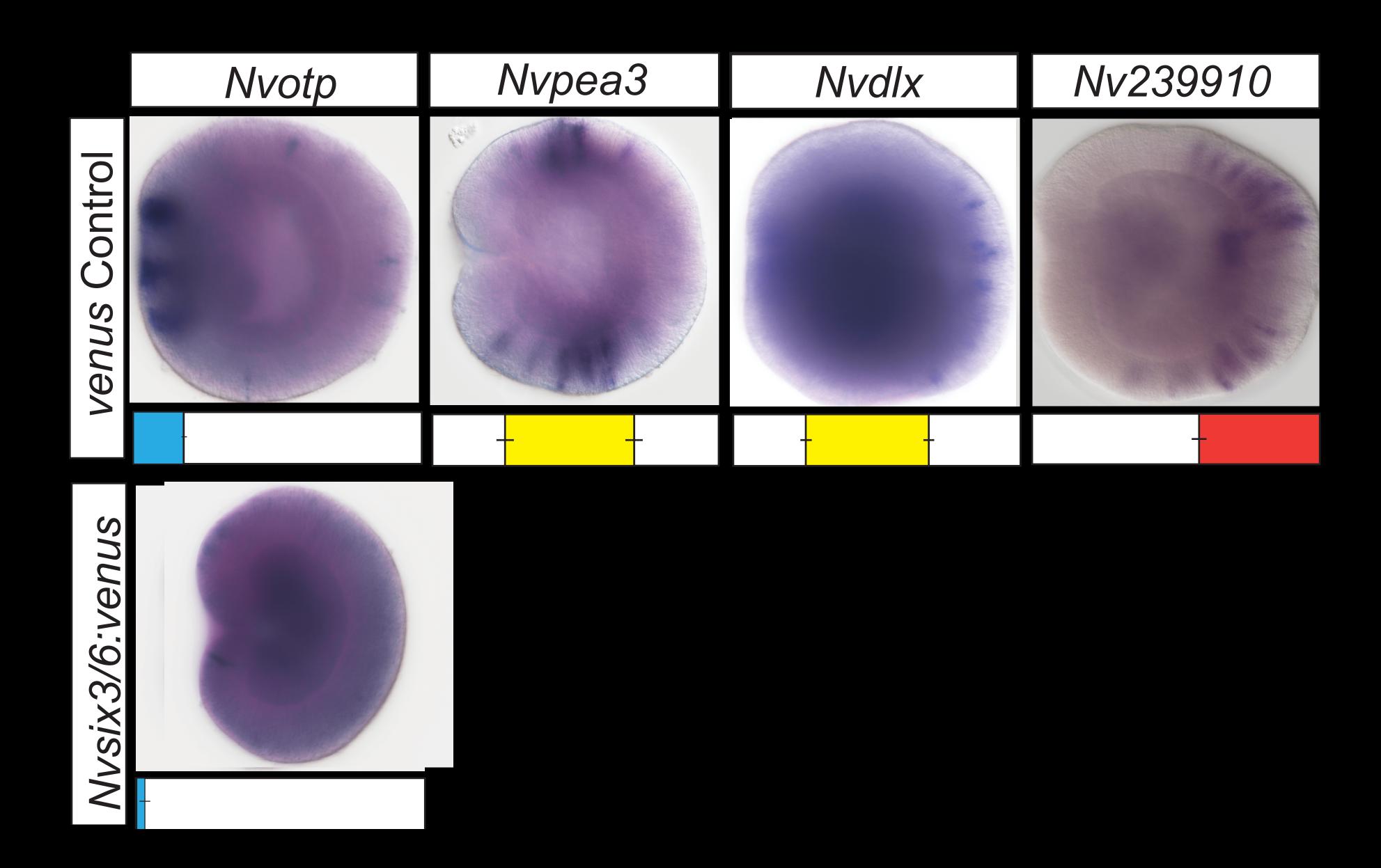
# Nvsix3/6 is necessary for aboral neural genes

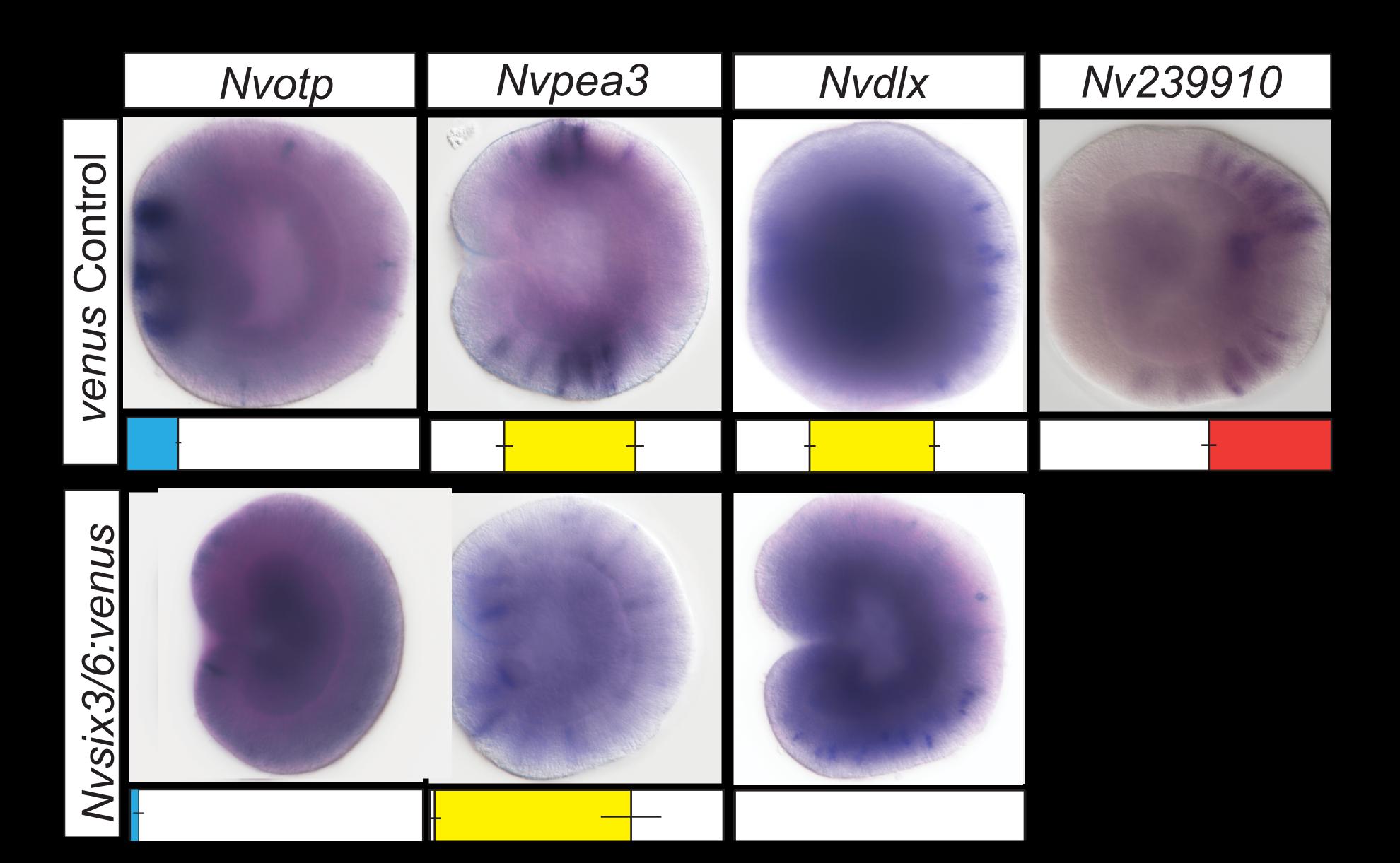


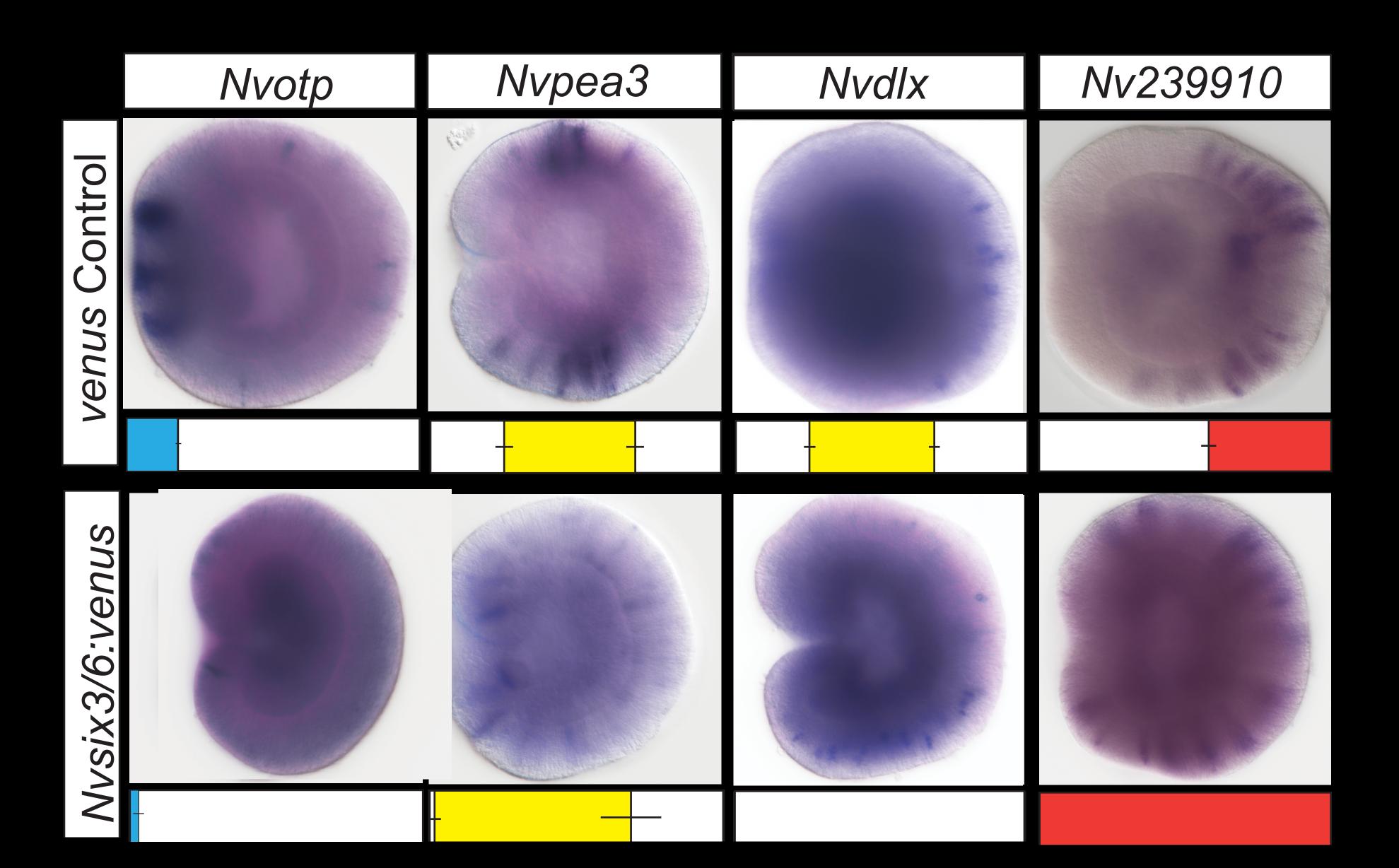
# **Control shRNA**

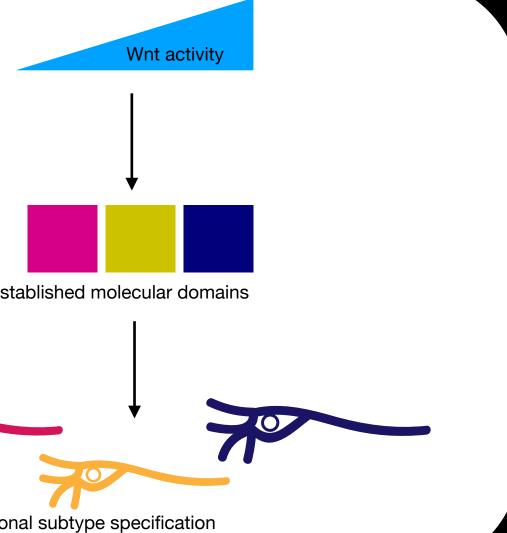
# vsix3/6 shRNA

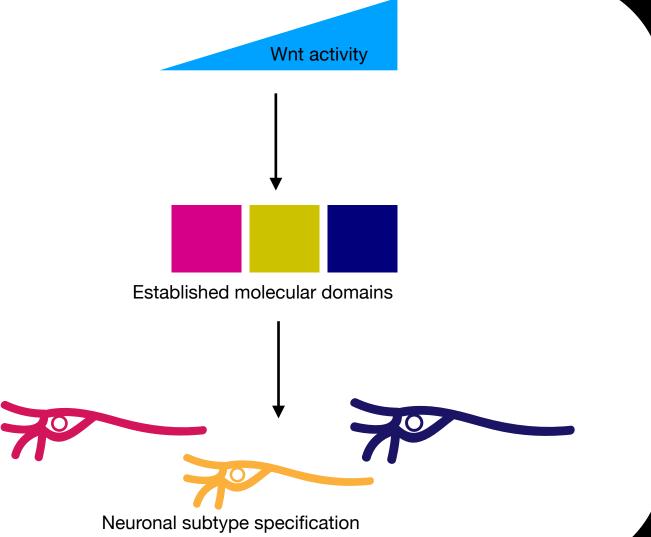




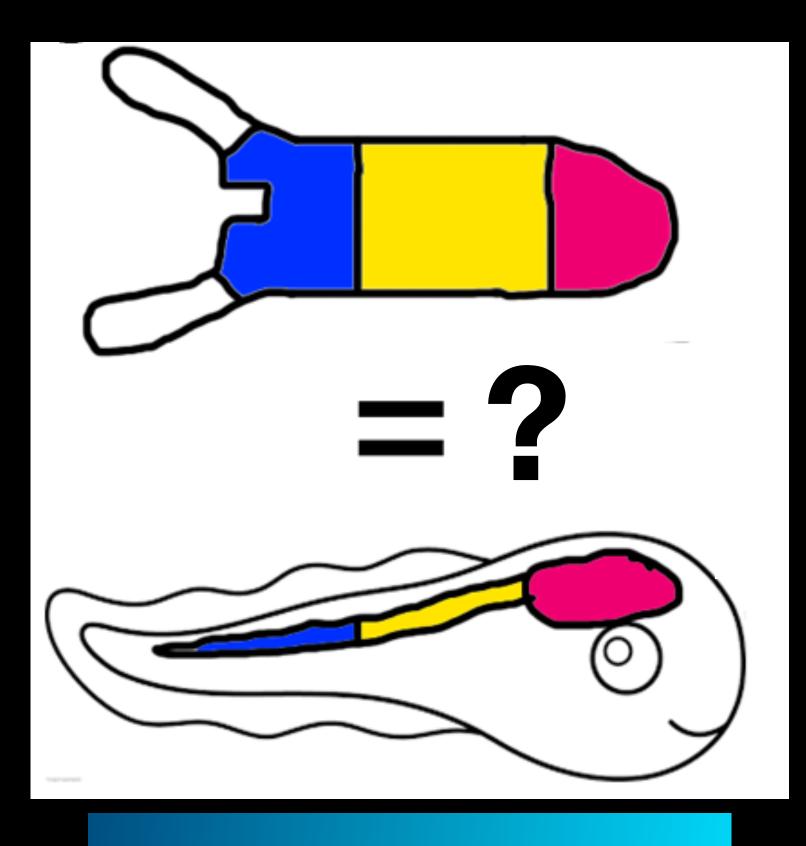




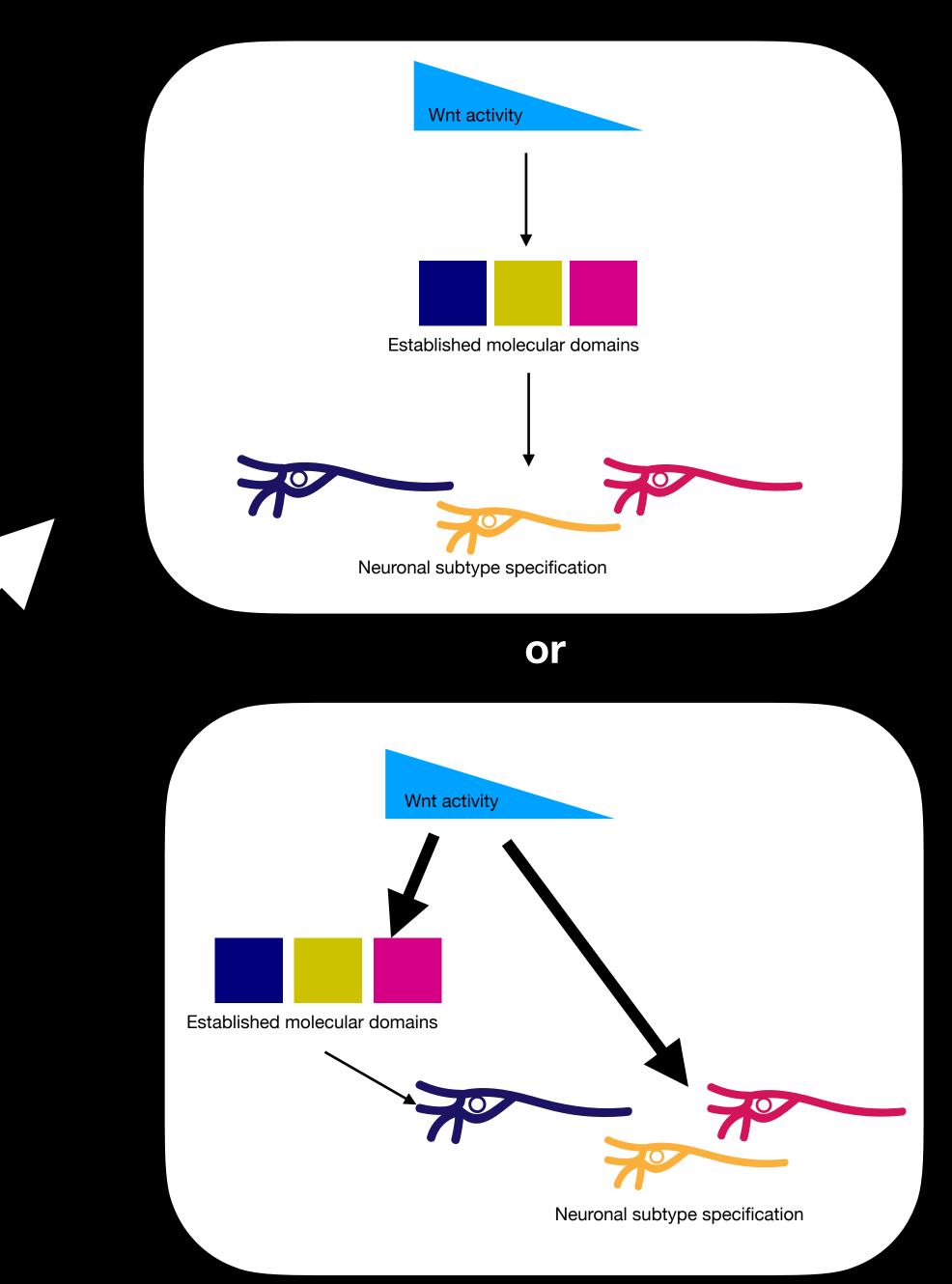




## Hypotheses explaining how axial patterning might influence neuronal fates

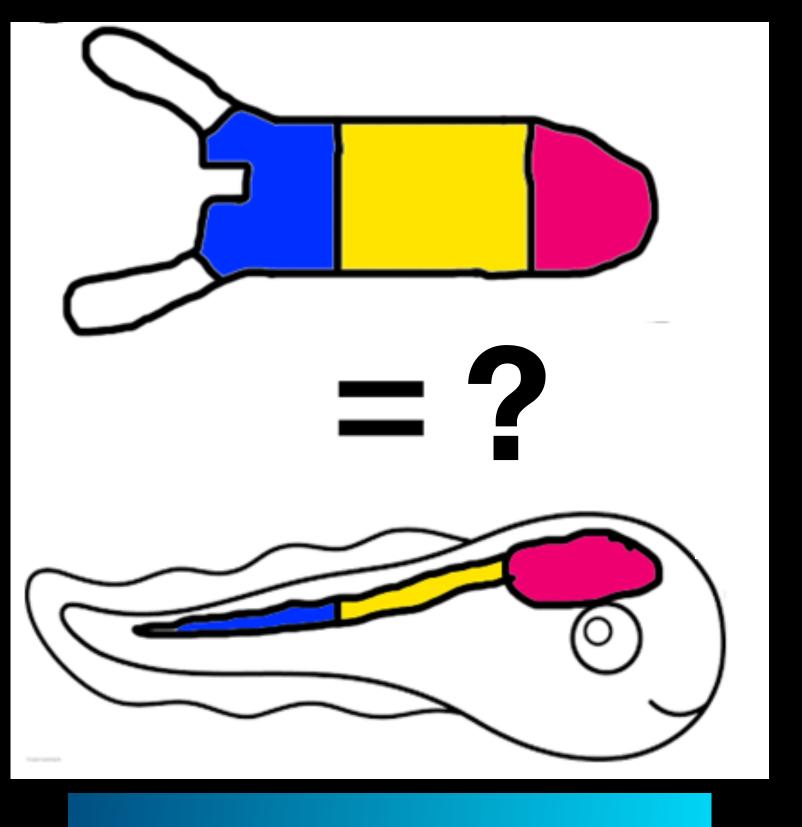


Wnt activity



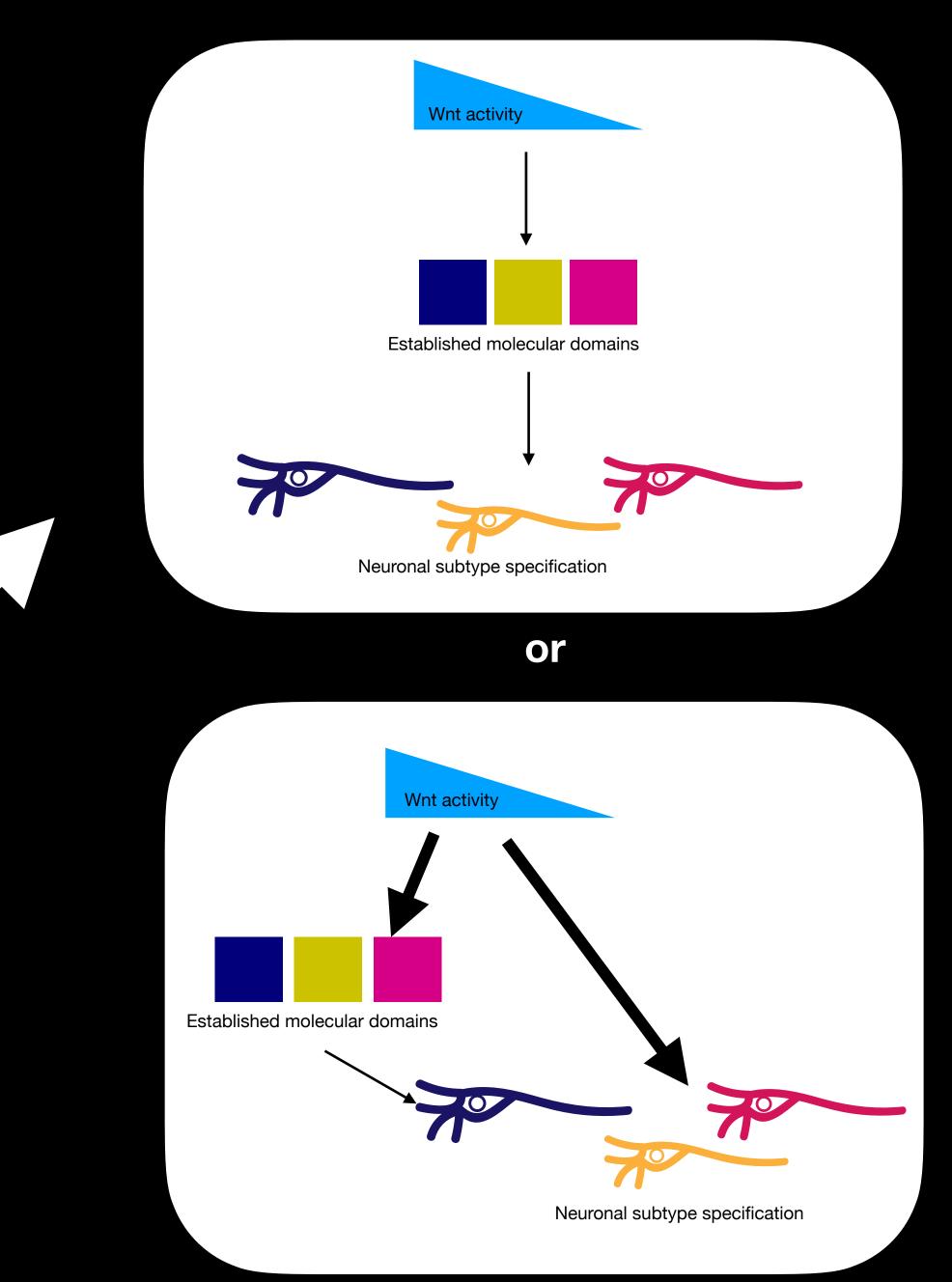


## Hypotheses explaining how axial patterning might influence neuronal fates

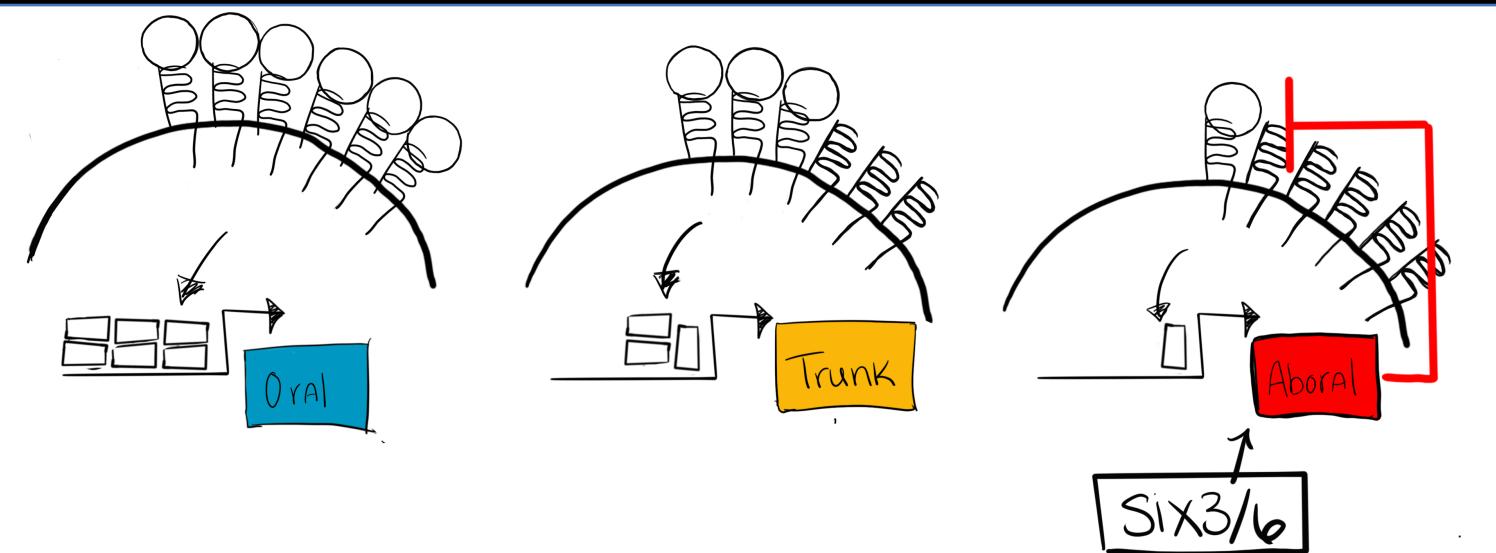


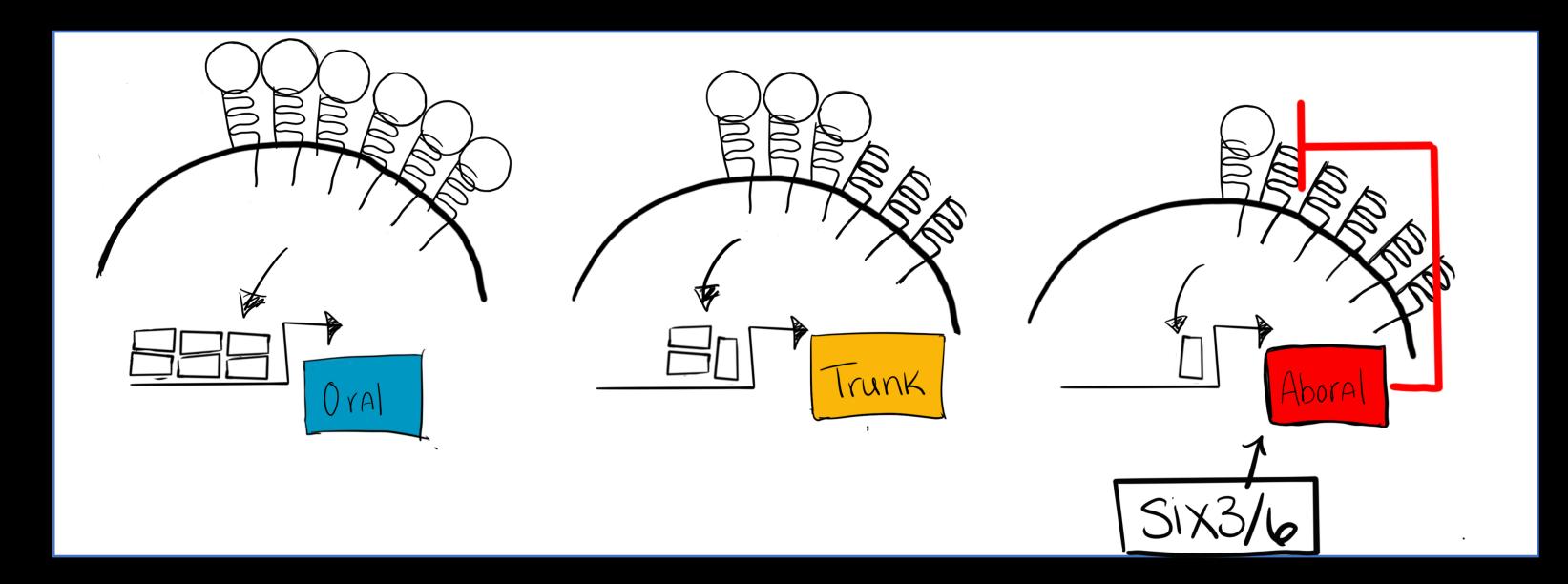
Wnt activity

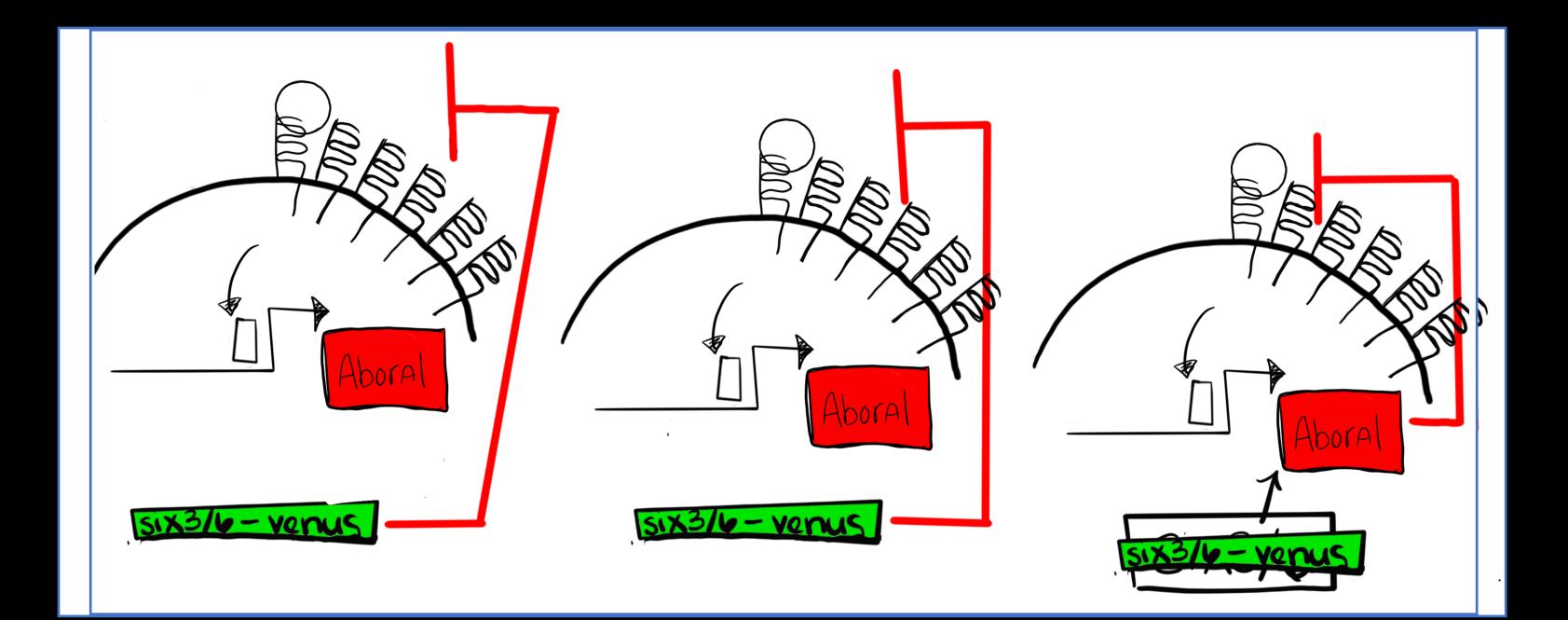


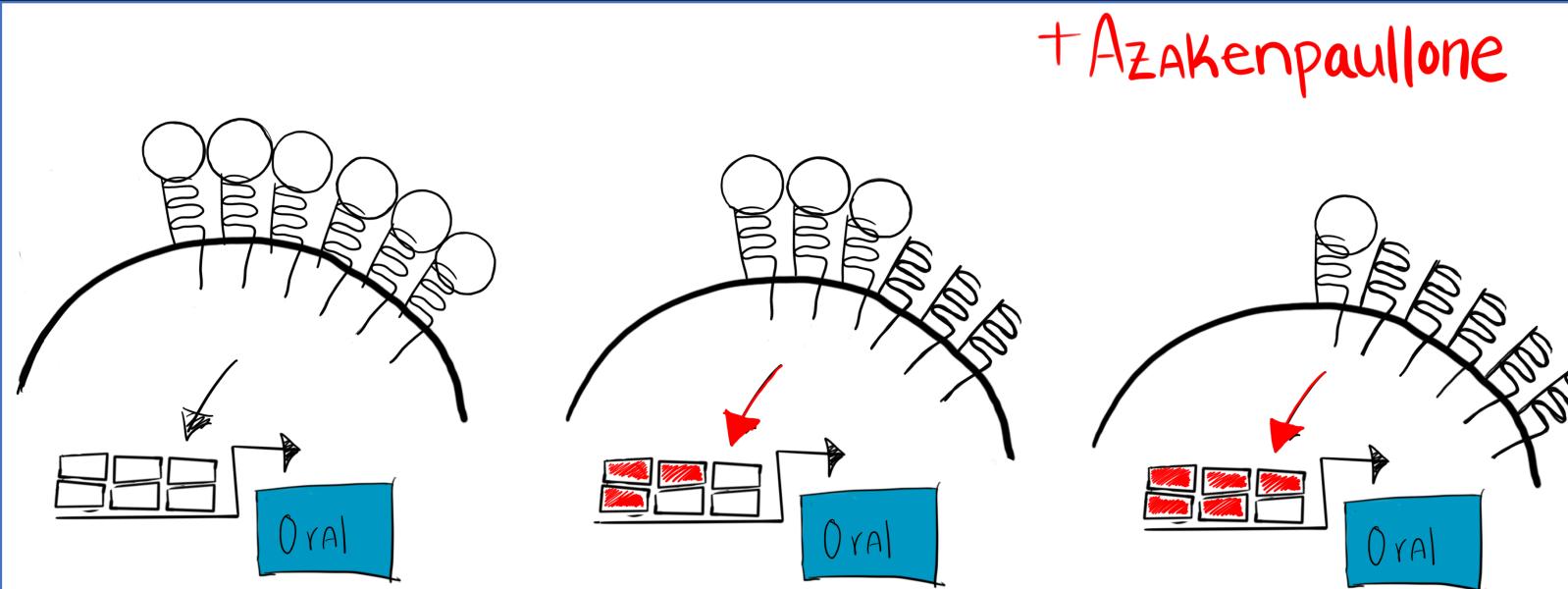


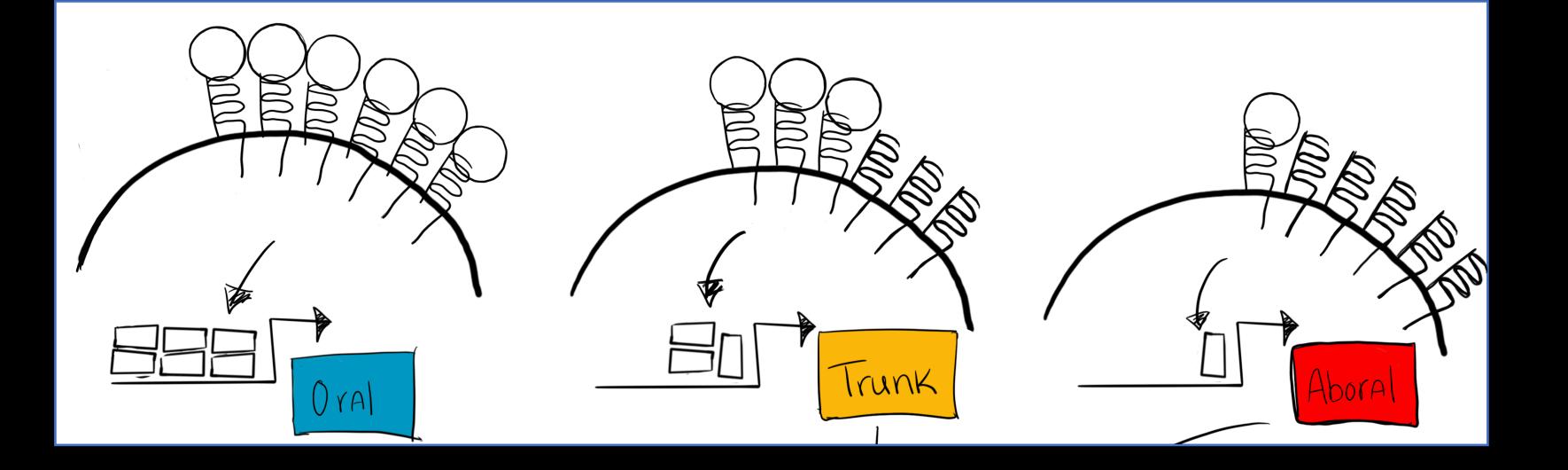


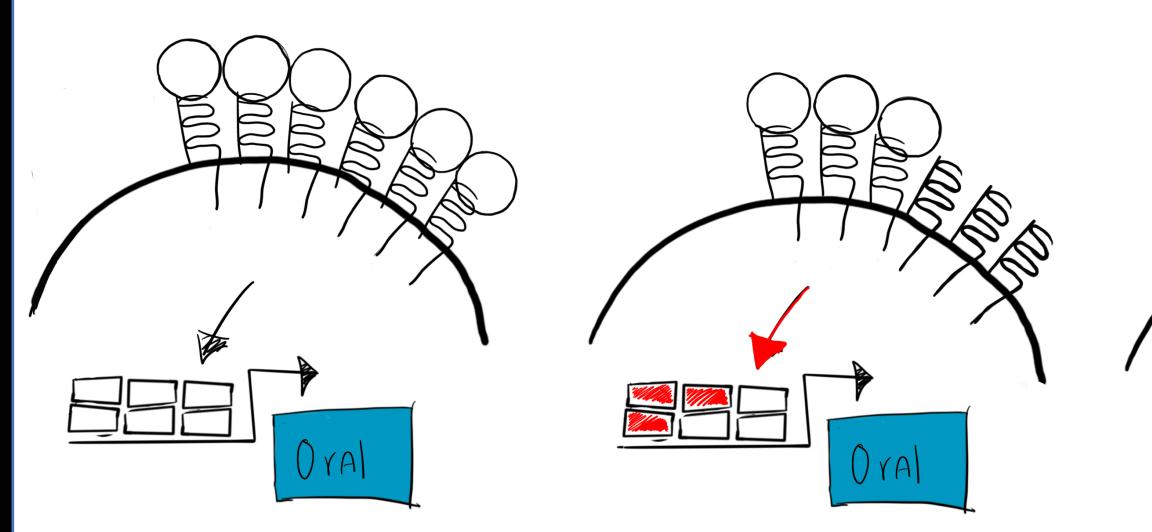




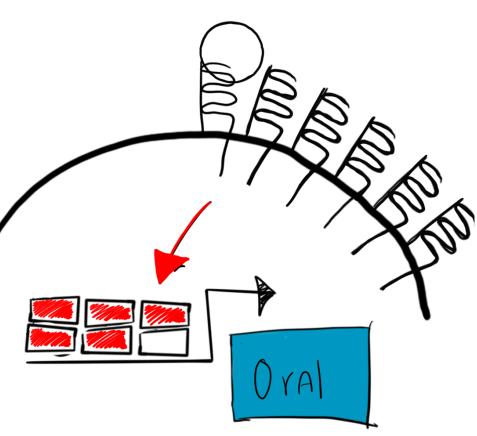






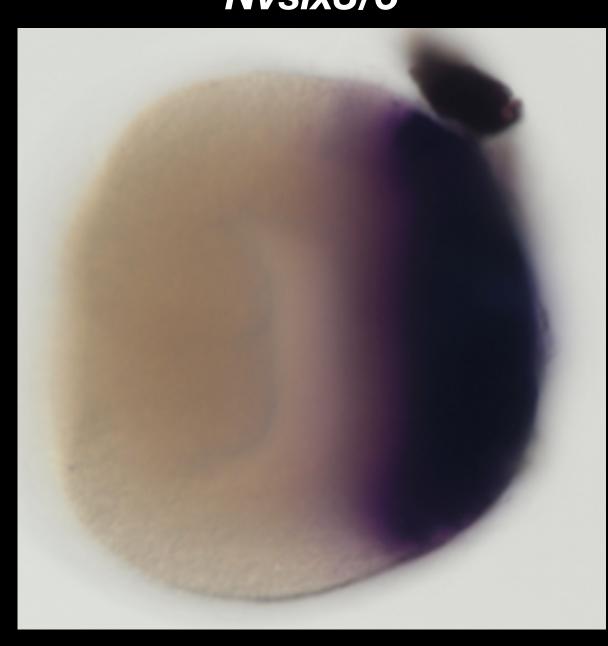


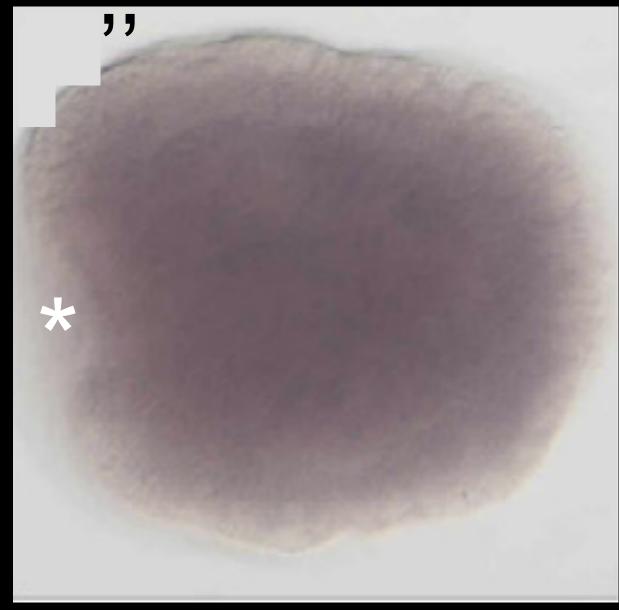
# +Azakenpaullone



# Az. Treated (high Wnt)

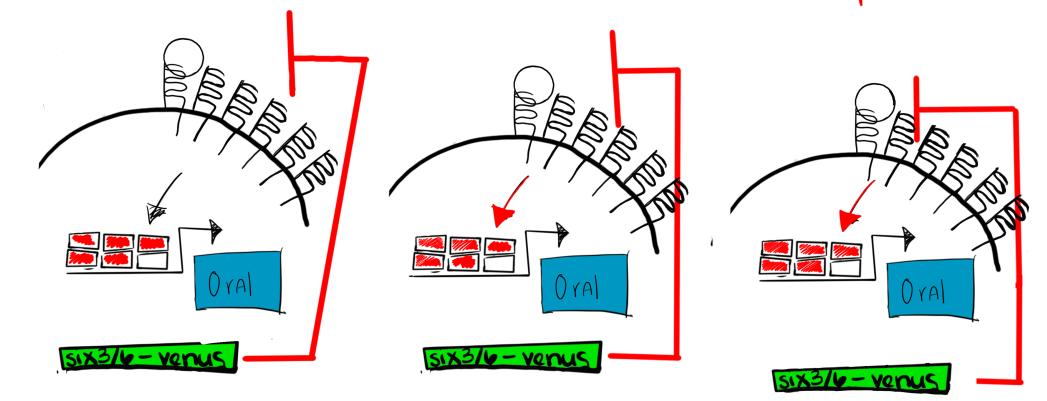
# Wildtype



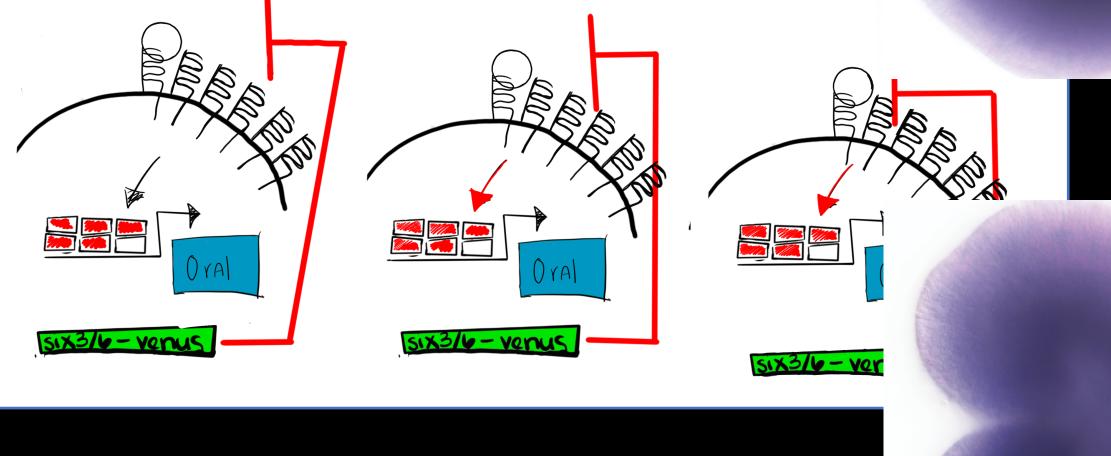


Nvsix3/6

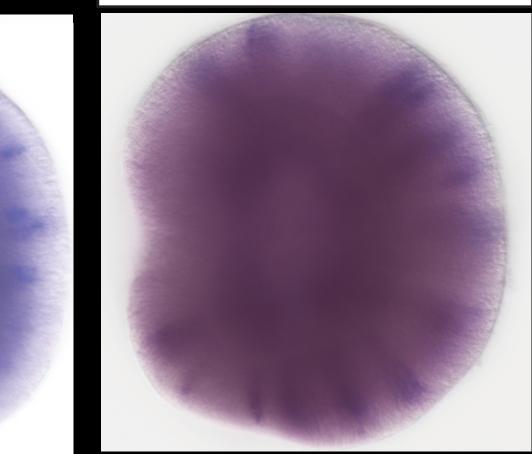
## +Azakenpaullone



## +Azakenpai



## *Nv239910*

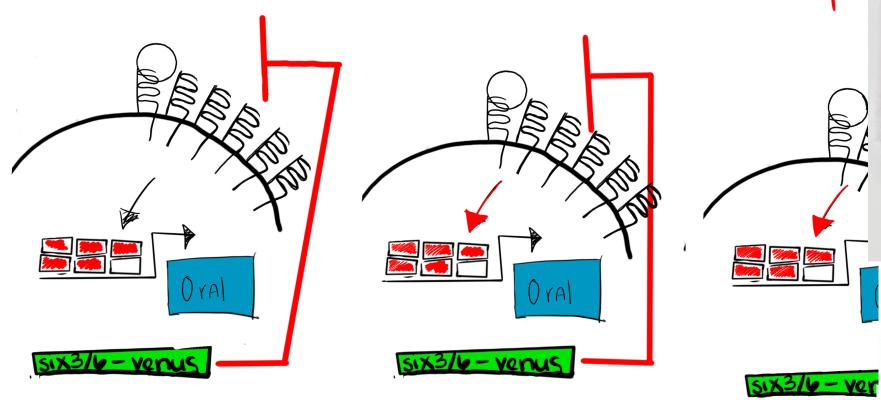




# Hyperbasis 1 Neuronal Spatial Subtypes Image: Comparing the state of the stateof the state of the

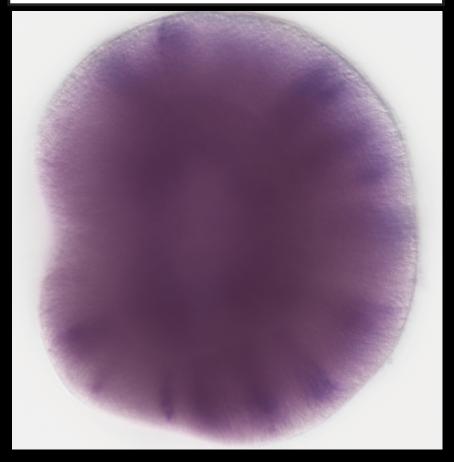


+Azakenpa





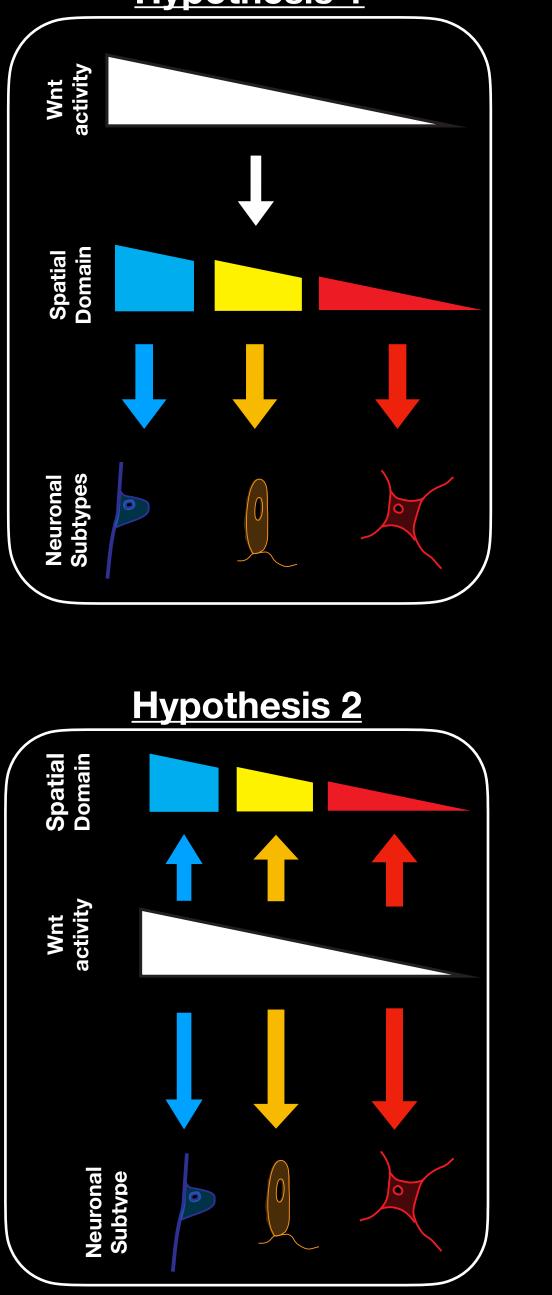
## *Nv239910*

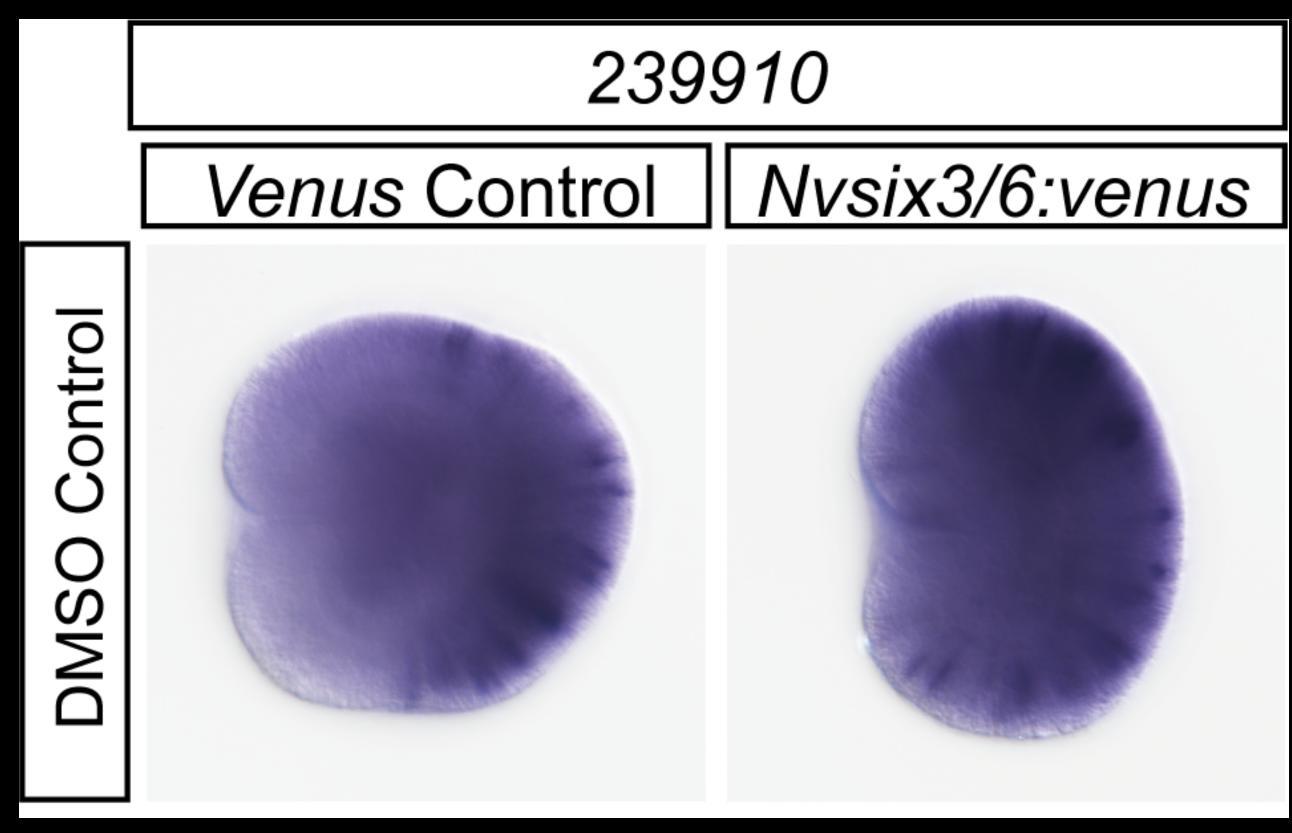


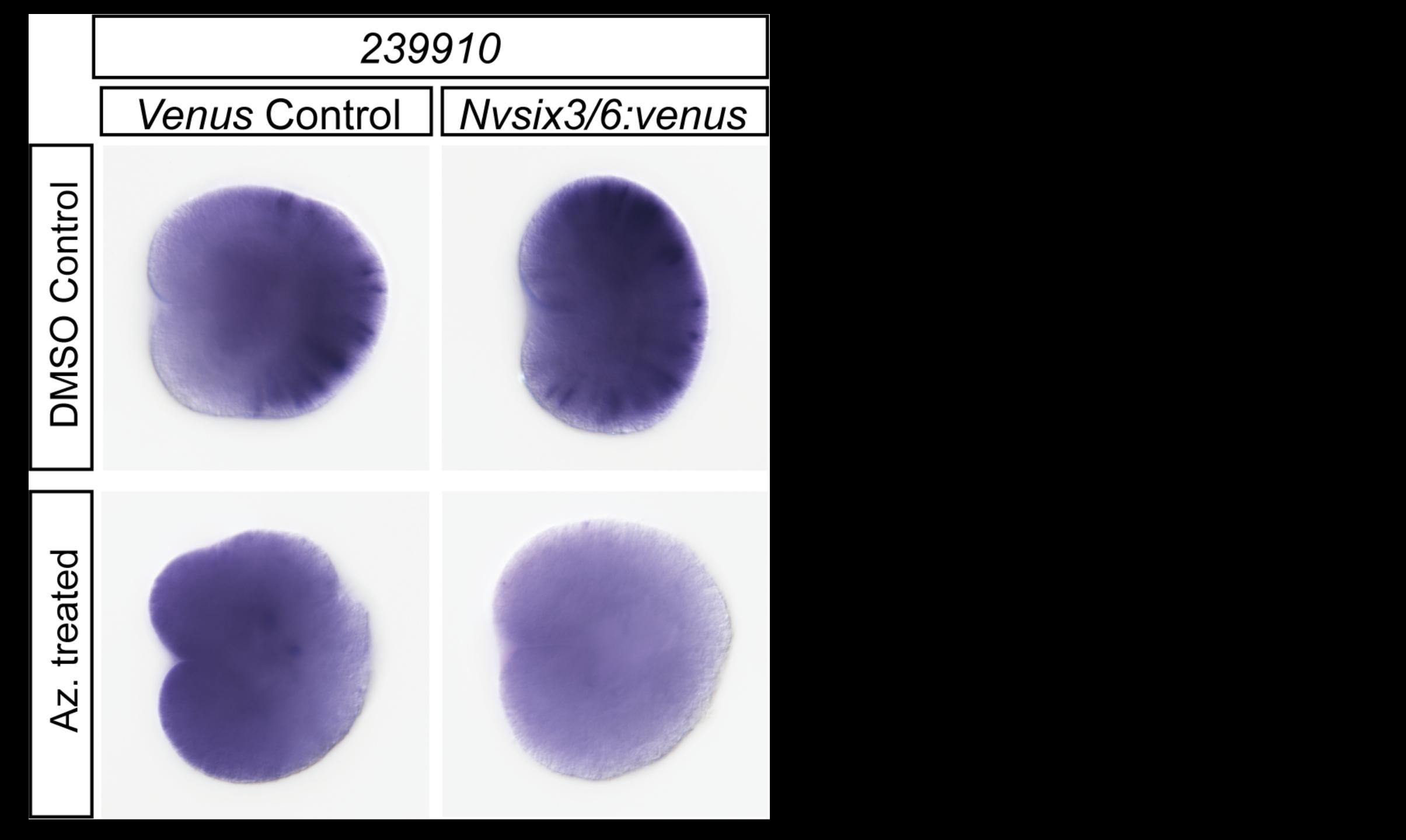
## *Nv239910*

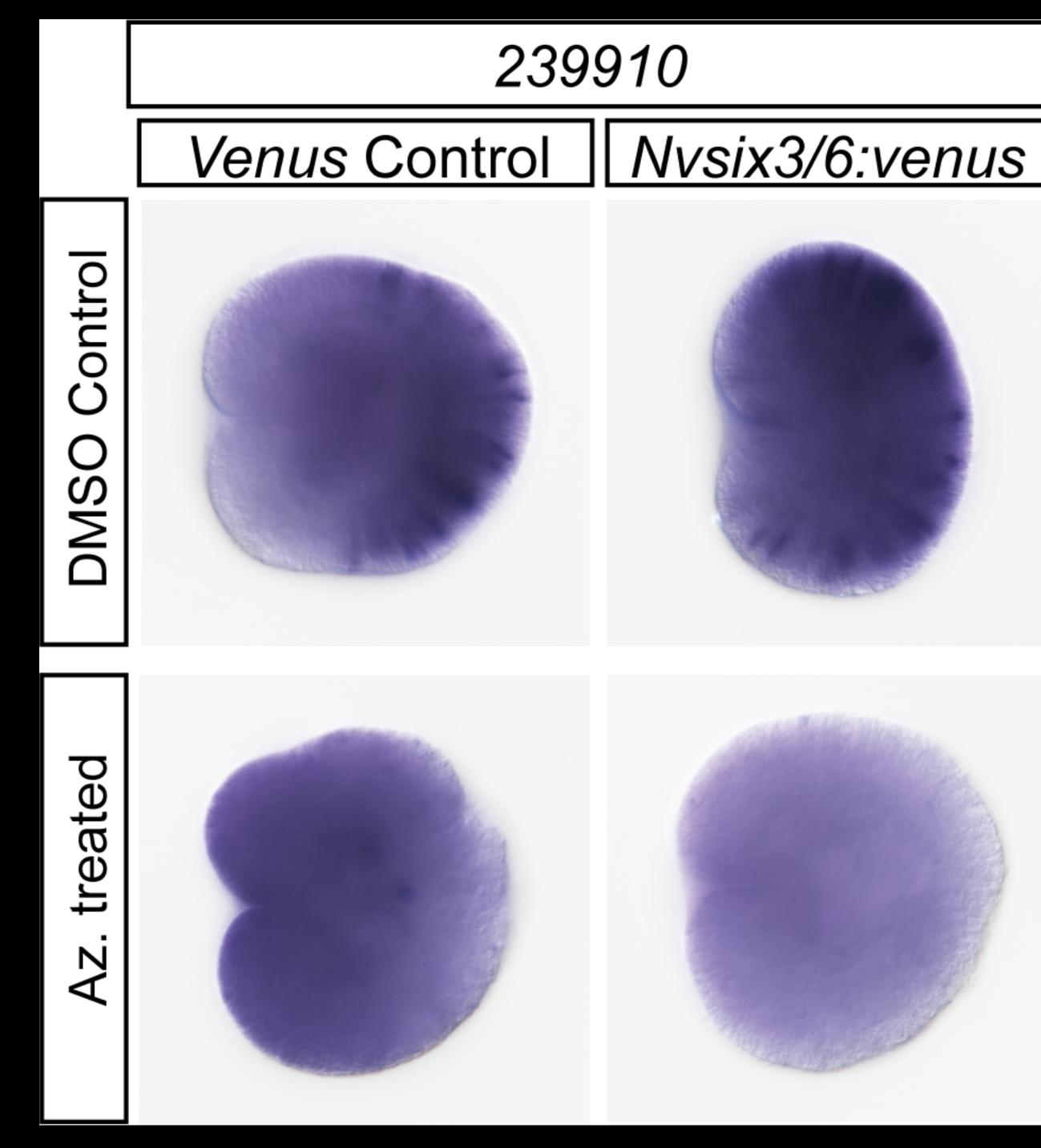


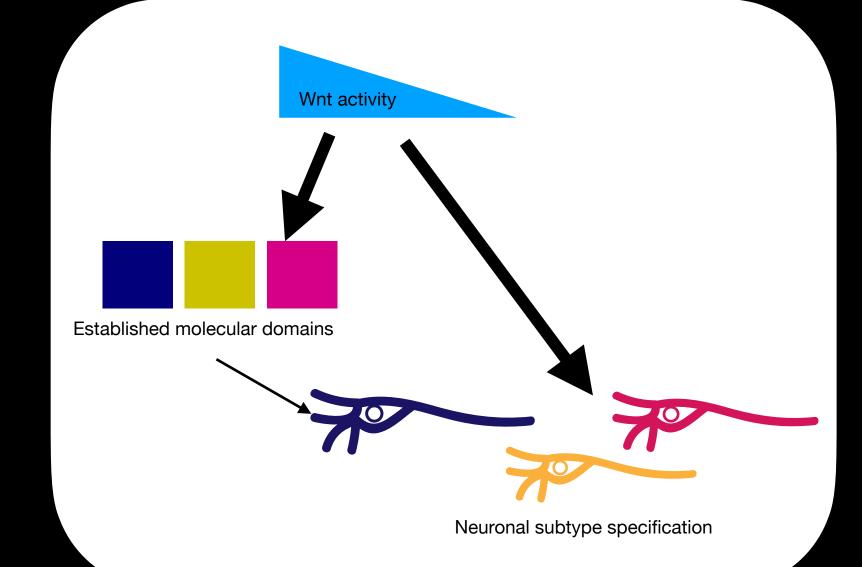
# <u>Hypothesis 1</u> Wnt activity Spatial Domain

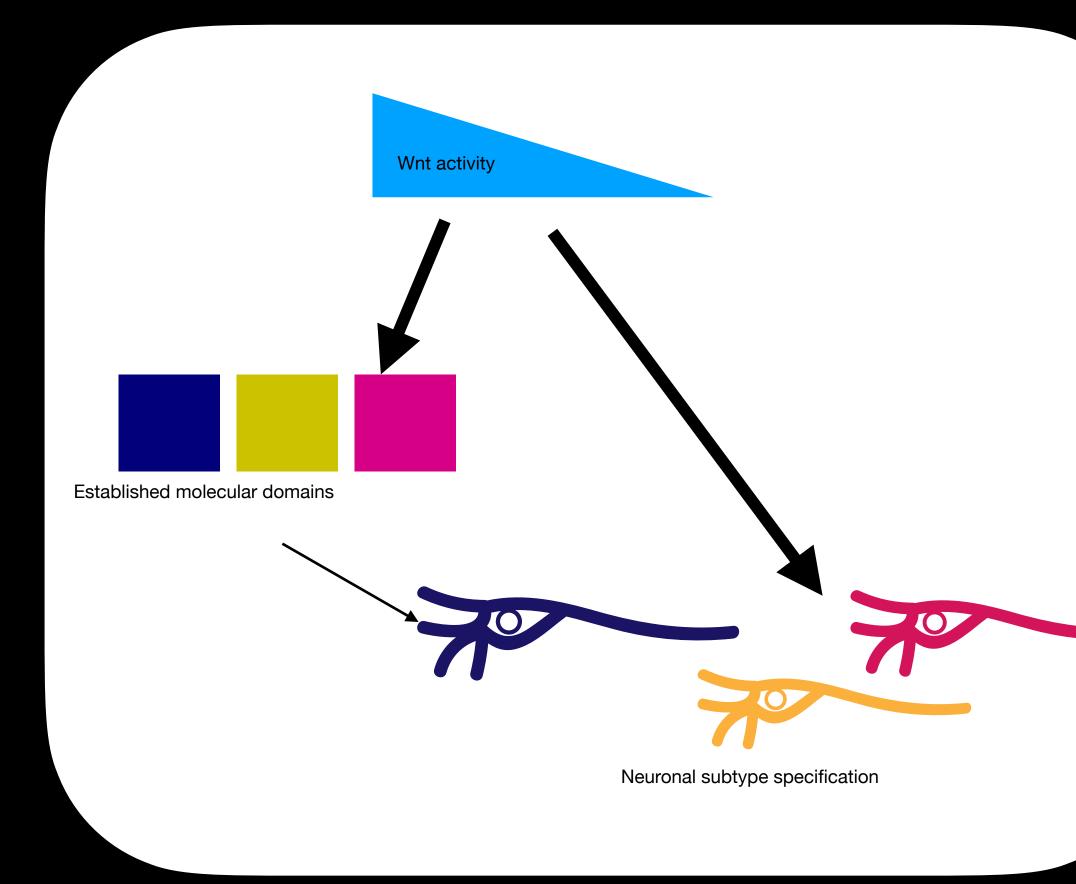


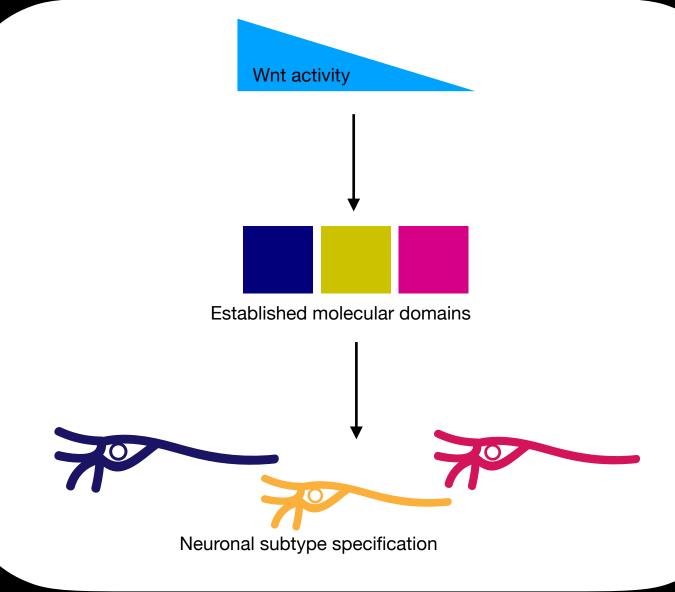




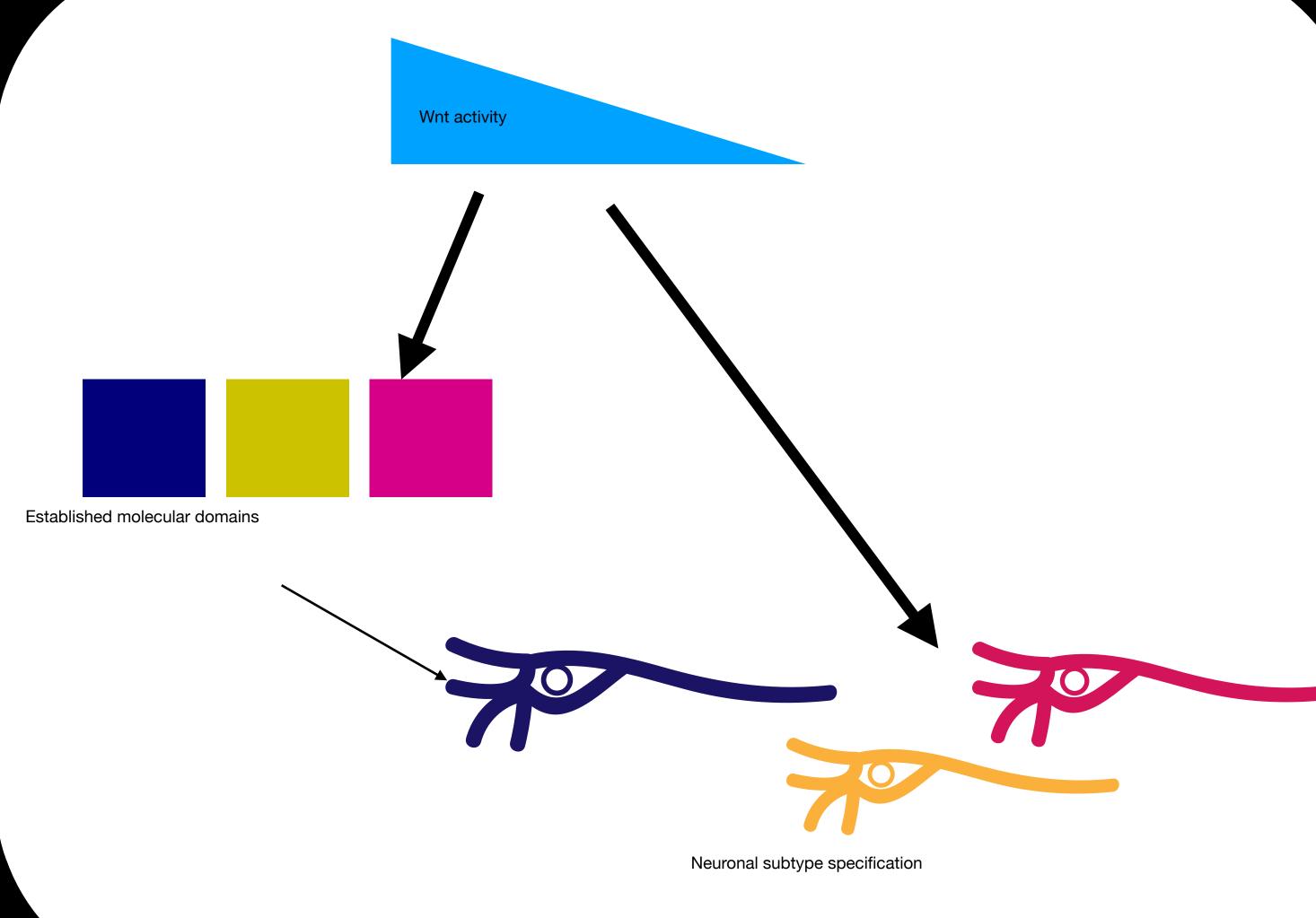


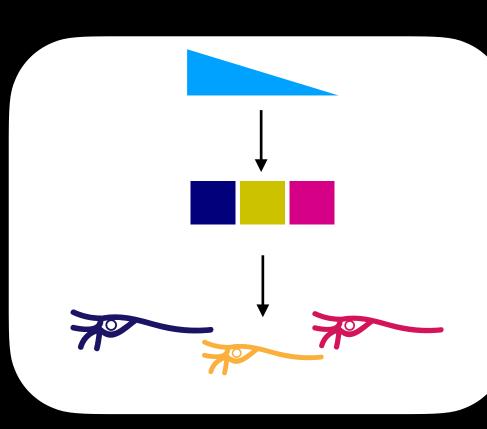




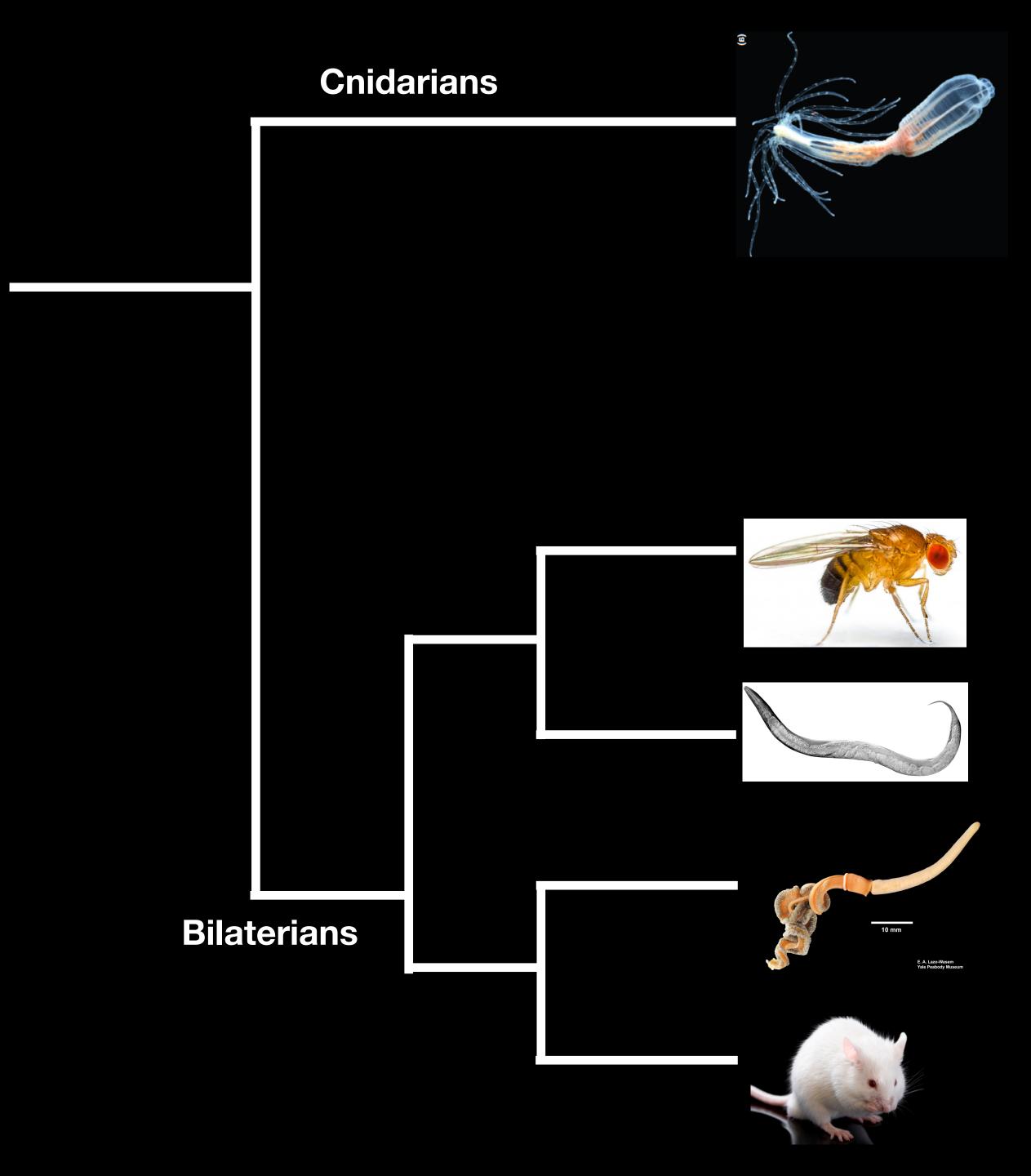


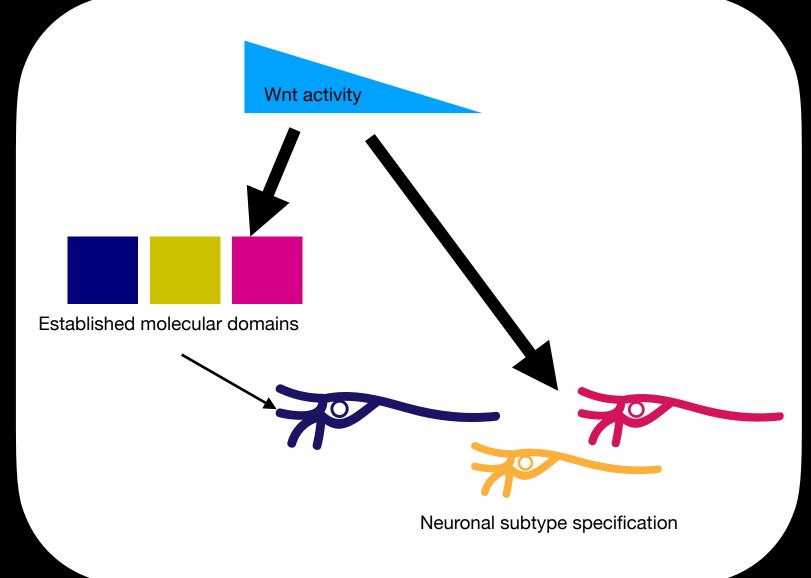
### Bilaterian-like Mechanism

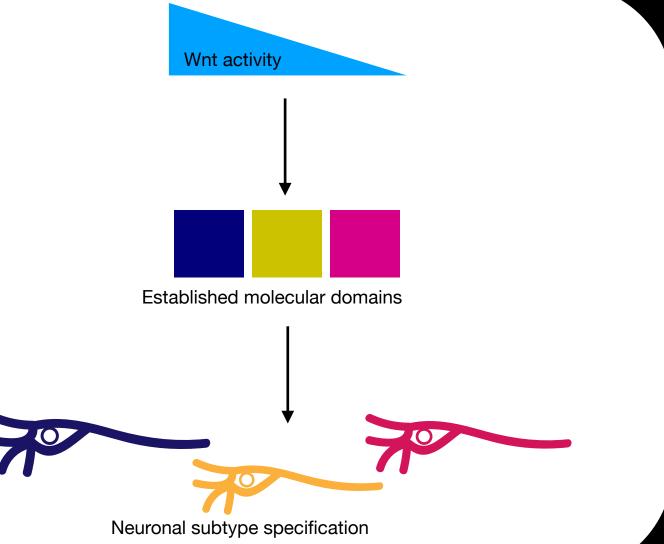




## Bilaterian-like Mechanism

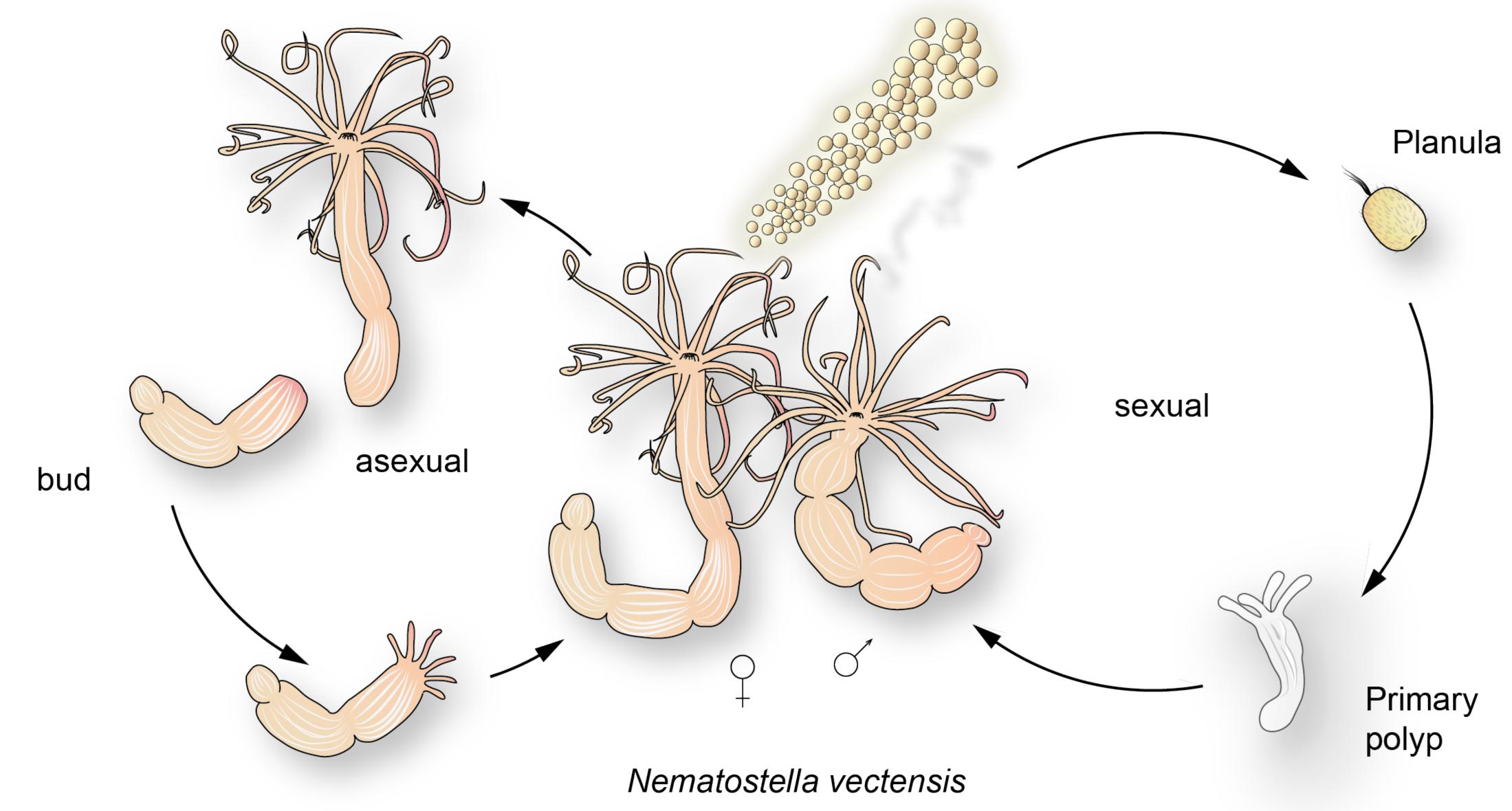






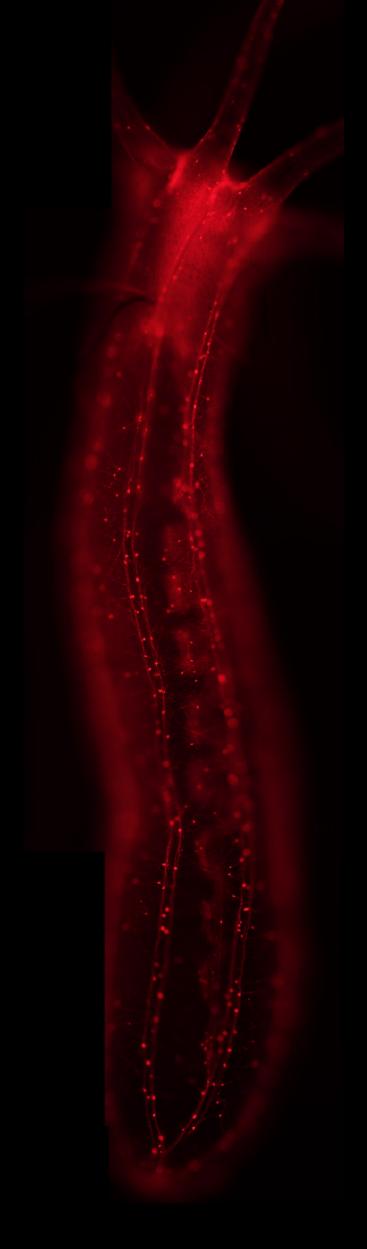
Need to look more closely at mechanisms within bilaterians to see if all CNSs have adopted the same strategy using the same pathways to pattern CNSs

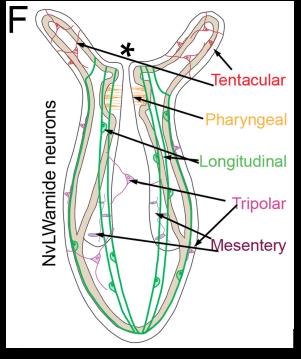
# Other questions in our lab





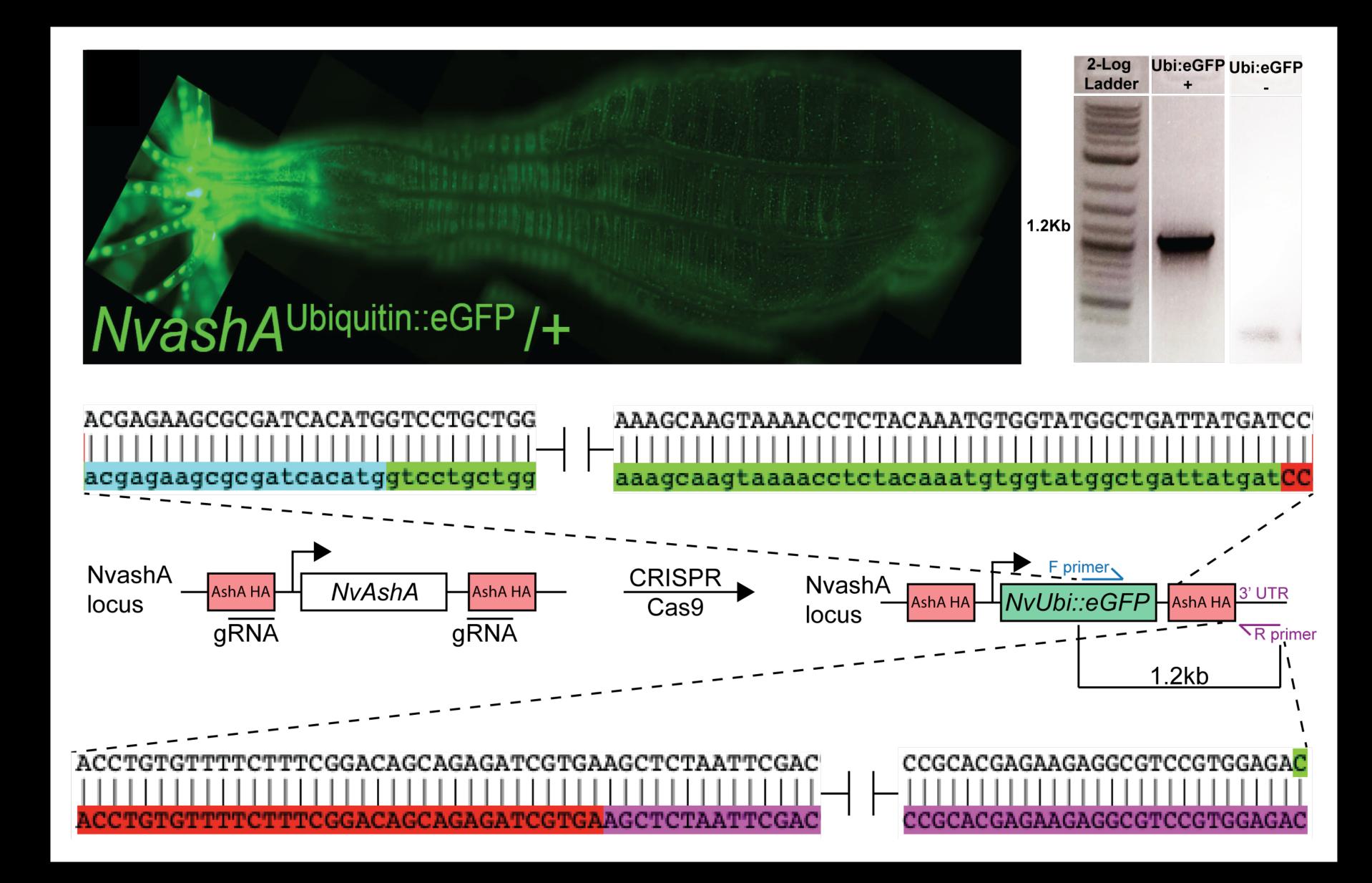
## How does nervous system regenerate?



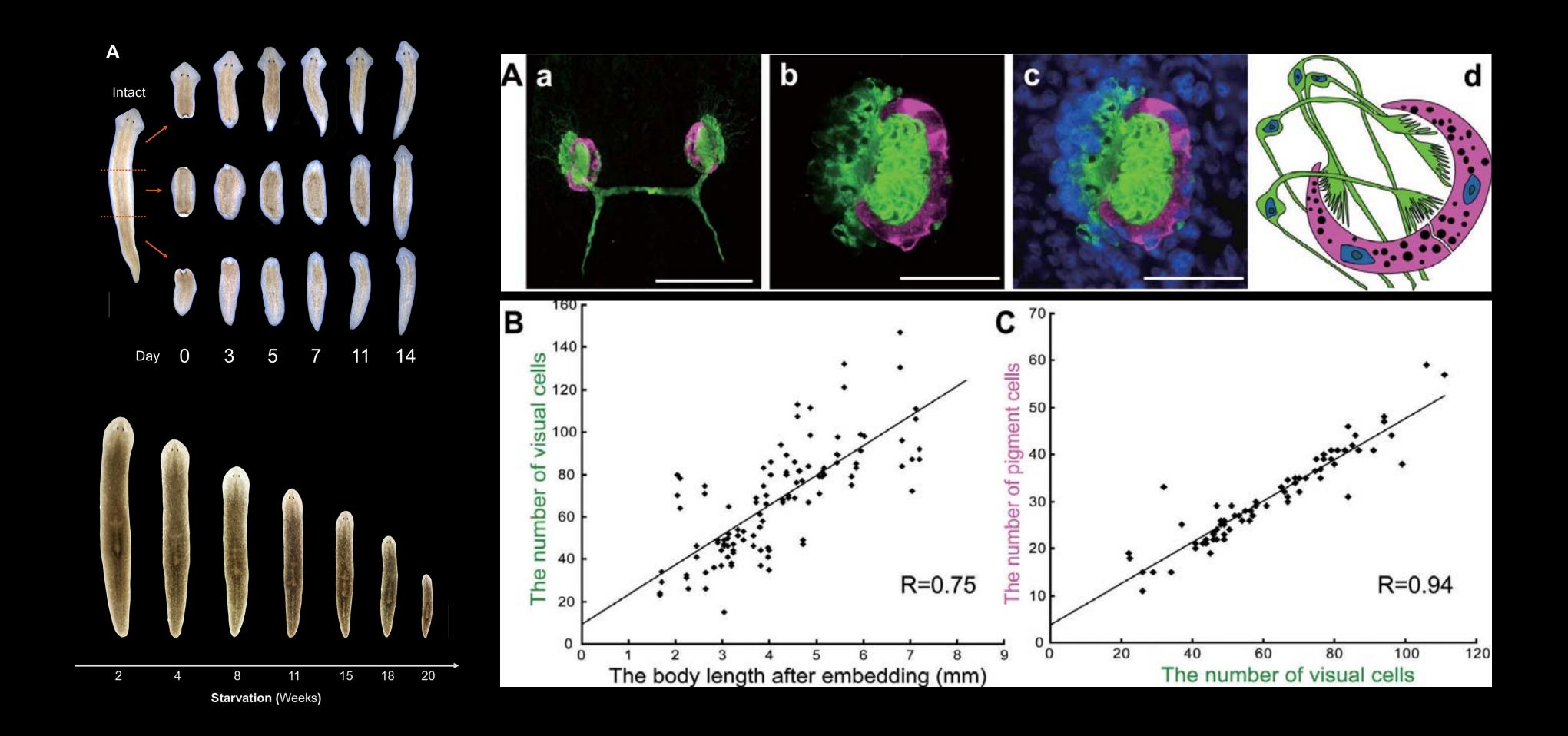




# Using CRISPR/Cas9 to build conditional alleles

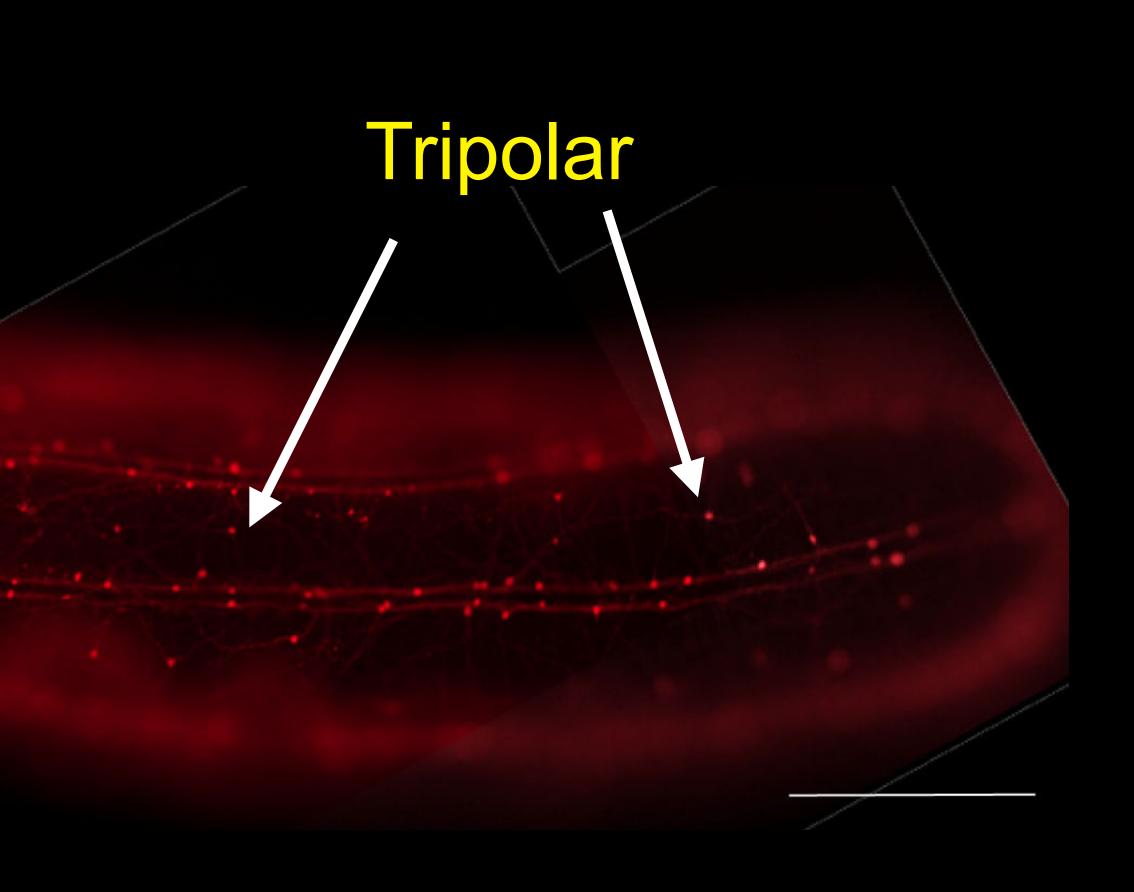


## How do nervous systems scale?

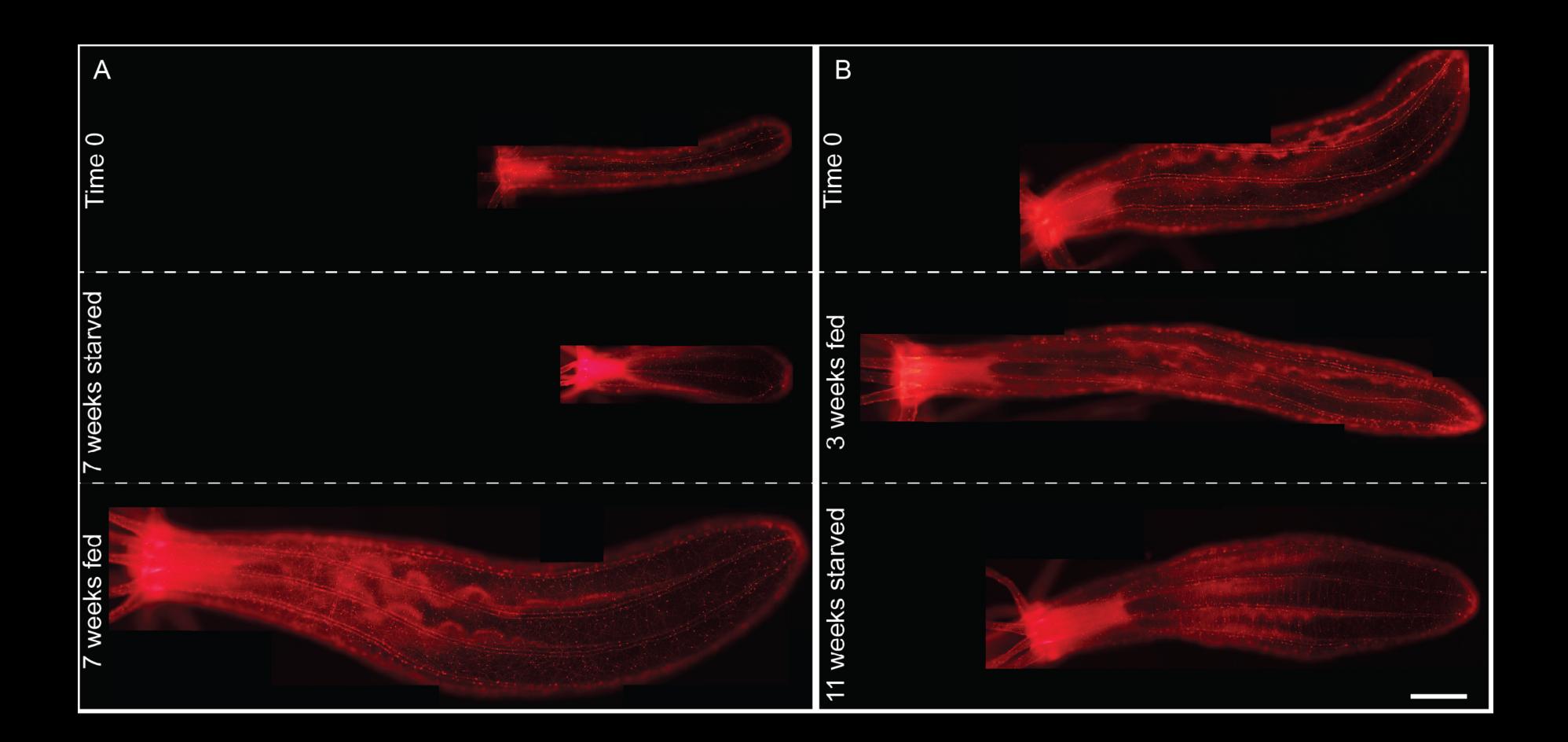


Birkholz, Van Huizen, Beane, Seminars in Cell and Developmental Biology (2019) Takeda, Nishimura, Agata, Zoological Science, (2009)

# Longitudinal

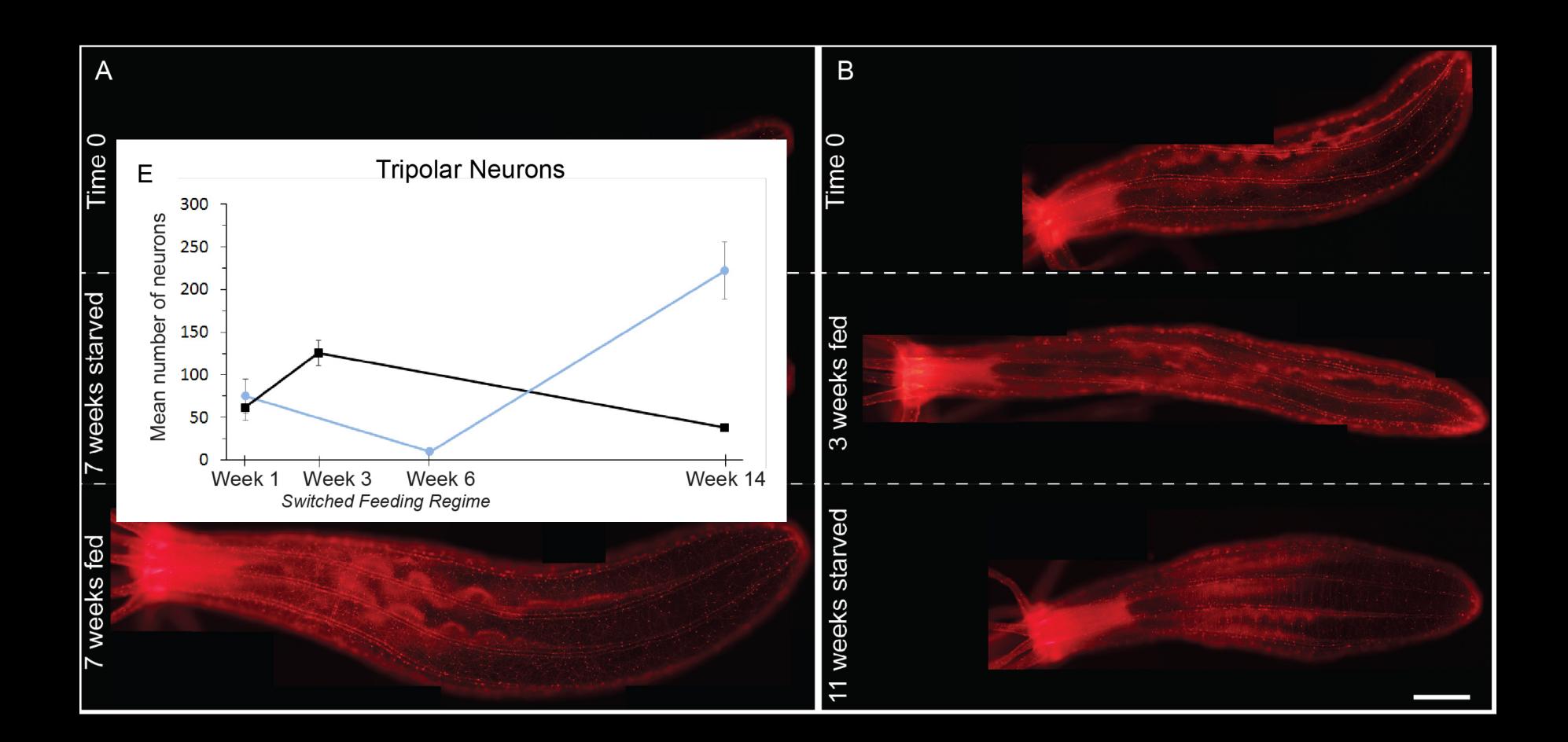


# Longitudinal and Tripolar neurons scale with changes in body size

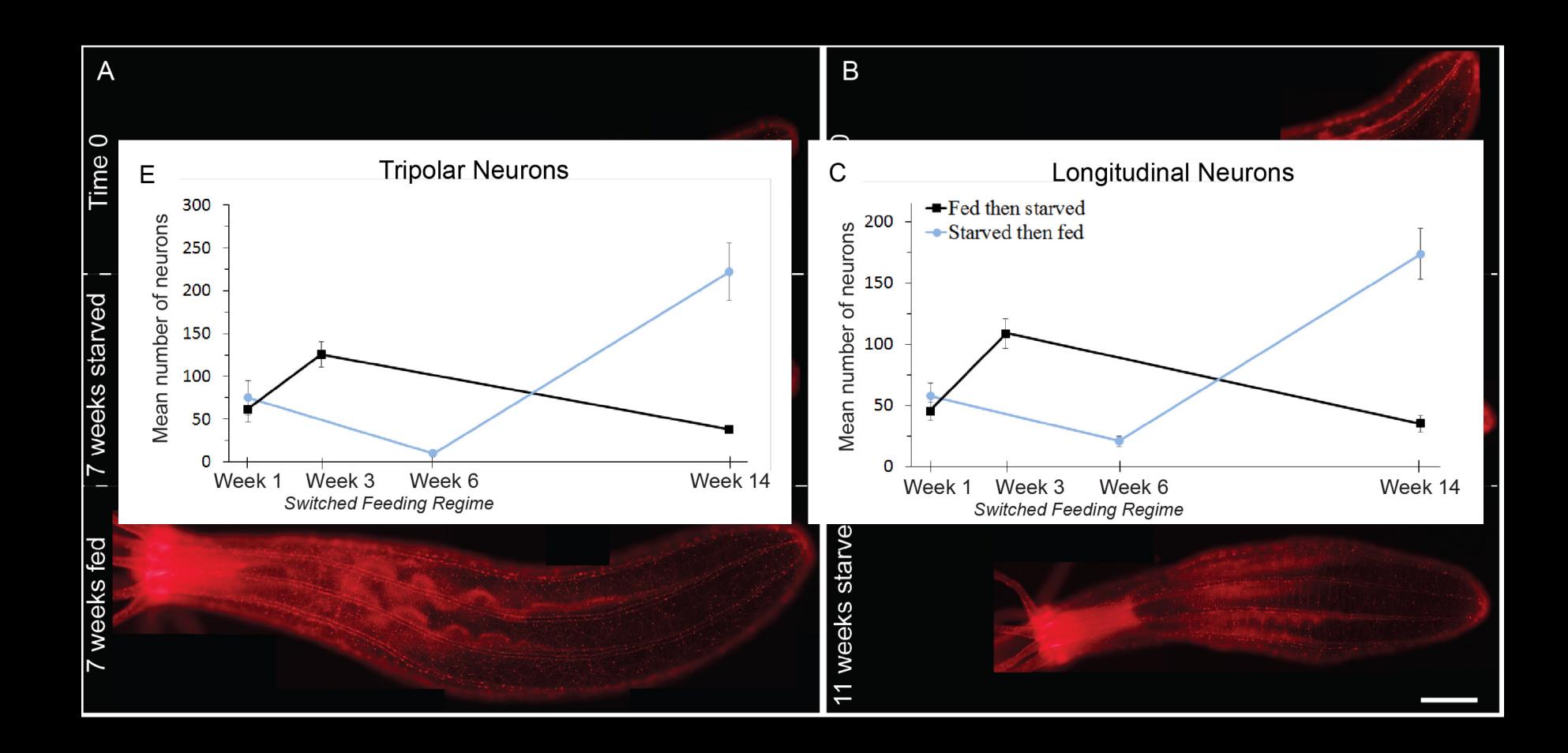


Havrilak et al., BMC Biology (in revision)

# Longitudinal and Tripolar neurons scale with changes in body size



# Longitudinal and Tripolar neurons scale with changes in body size



# Acknowledgements

Dr. Jamie Havrilak Dr. Layla Al-Shaer Dylan Faltine-Gonzalez Minghe Cheng Justin Carlino

# Funding:





National Institute of General Medical Sciences



Layden Lab

Nesli Akinci Noor Baban Omar Ahmed and many others

### **Collaborators:**

Dr. Eric Röttinger (University of Nice) Dr. Aldine Amiel (University of Nice) Dr. Craig Magie (Quinnipiac University) Dr. Uli Technau (University of Vienna) Dr. Fabian Rentzsch (University of Bergen - Sars)





Faculty Innovation Grant Biosystems Dynamics Summer Institute Class of '68

# Acknowledgements

Dr. Jamie Havrilak Dr. Layla Al-Shaer Dylan Faltine-Gonzalez Minghe Cheng Justin Carlino

# Questions?

# Funding:





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Layden Lab

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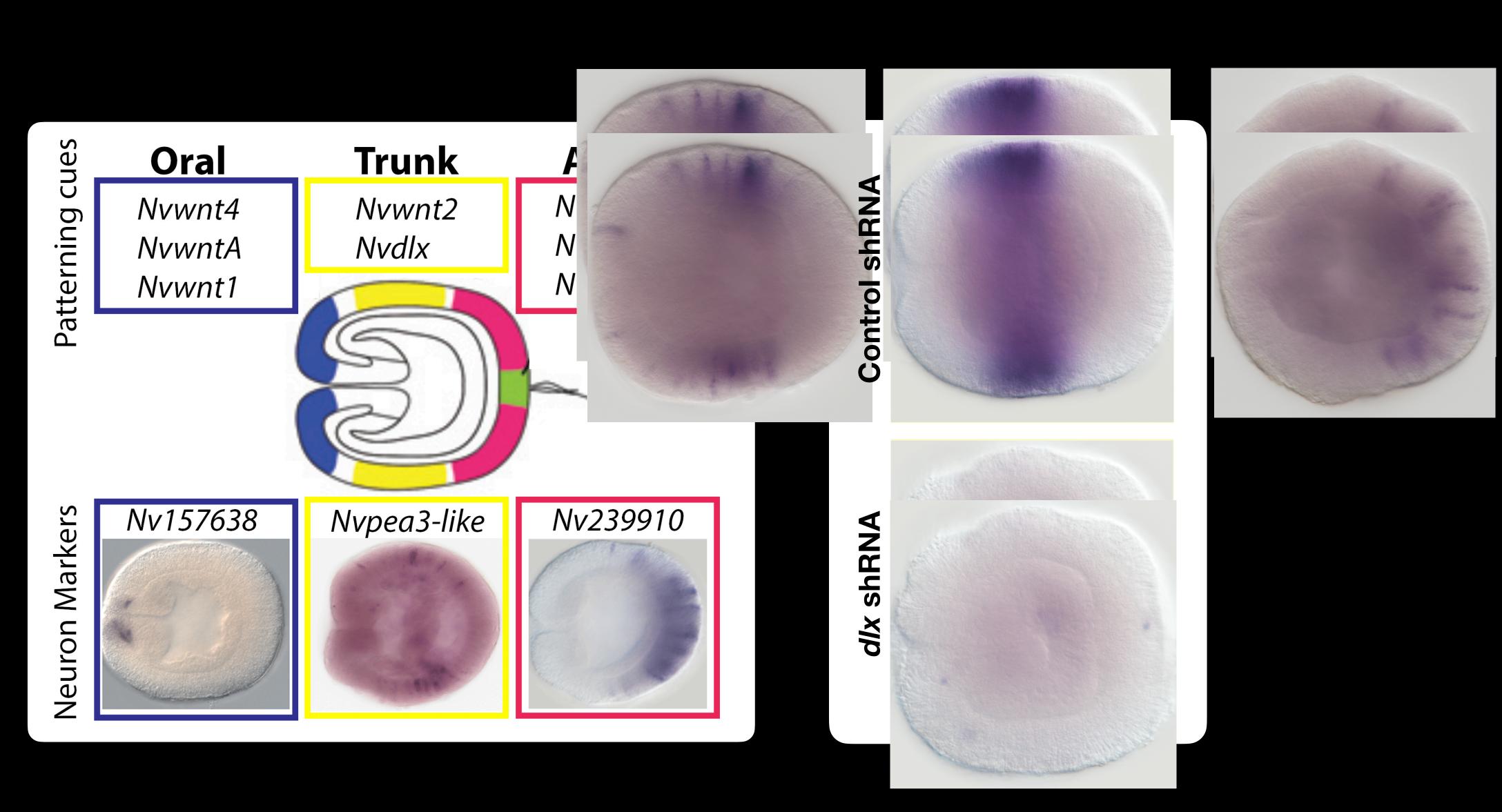
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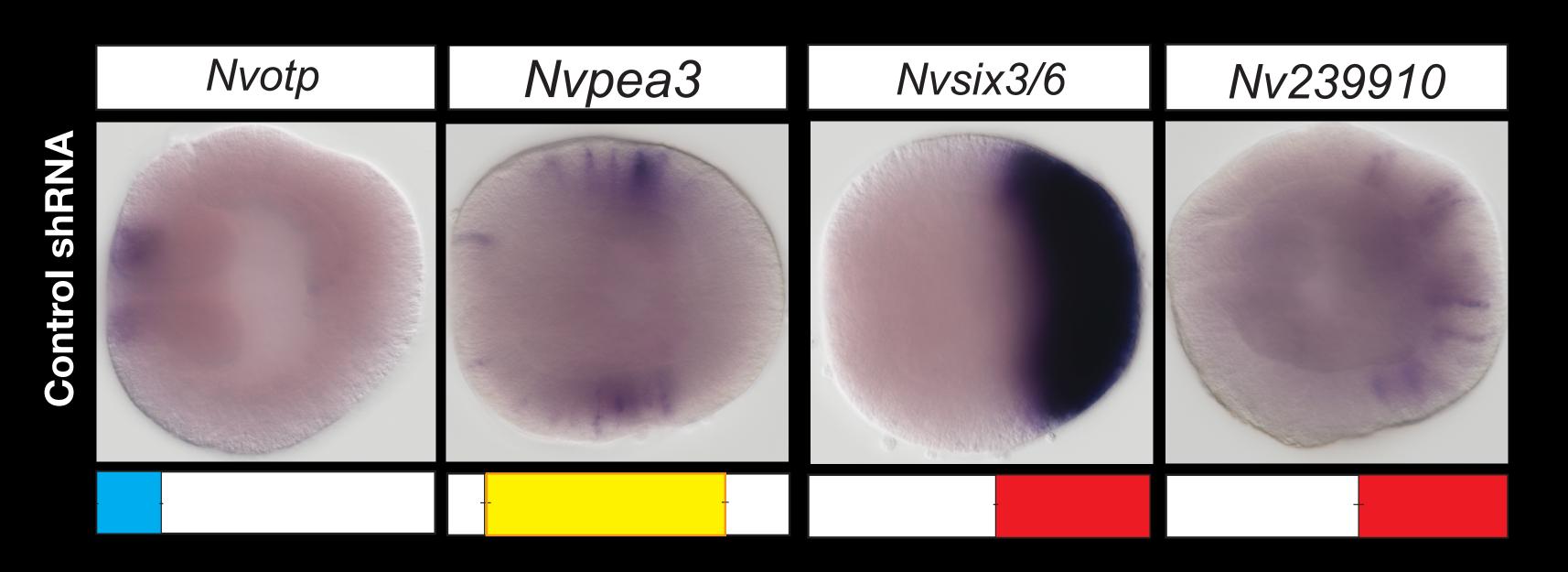


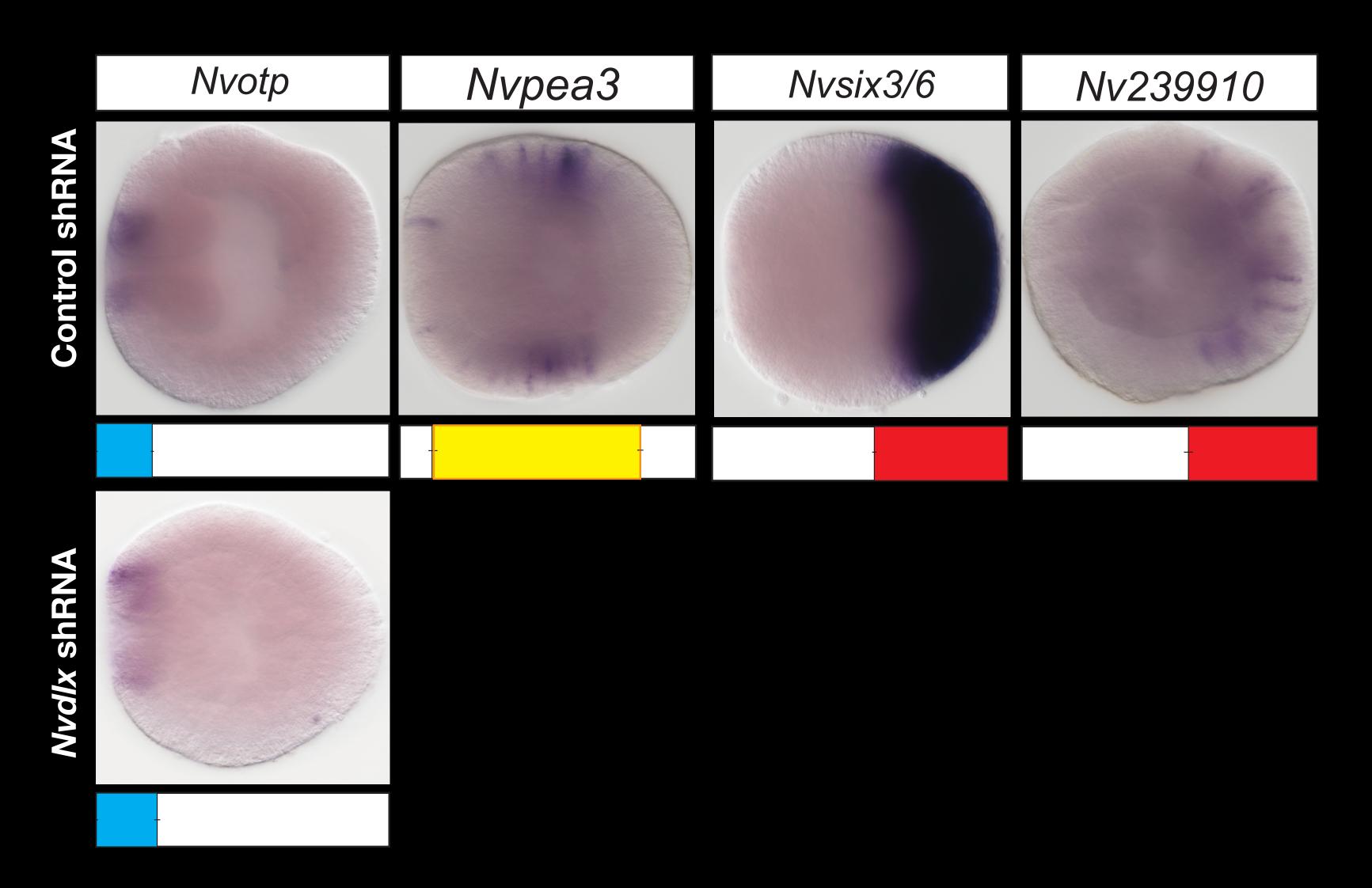


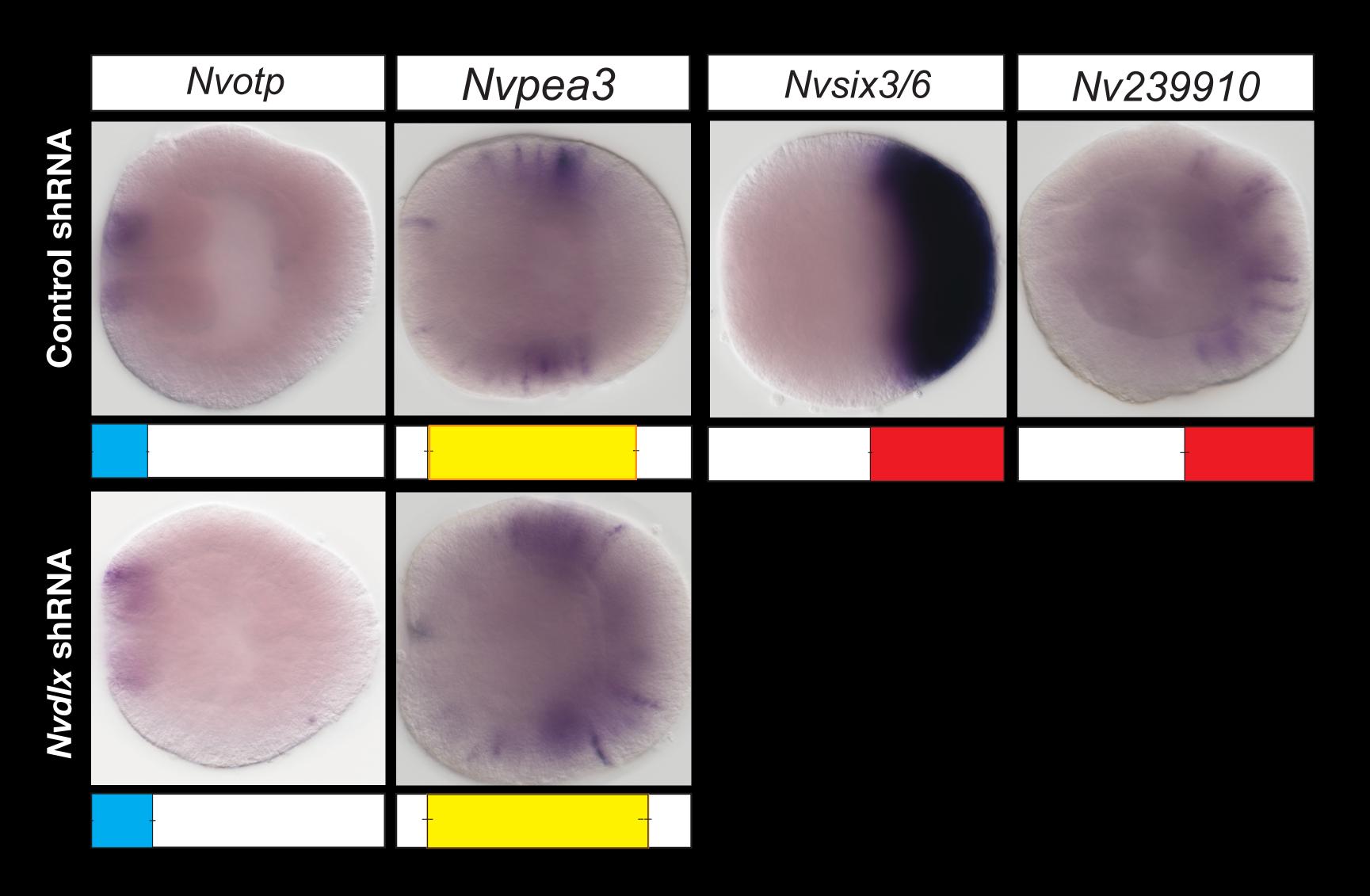
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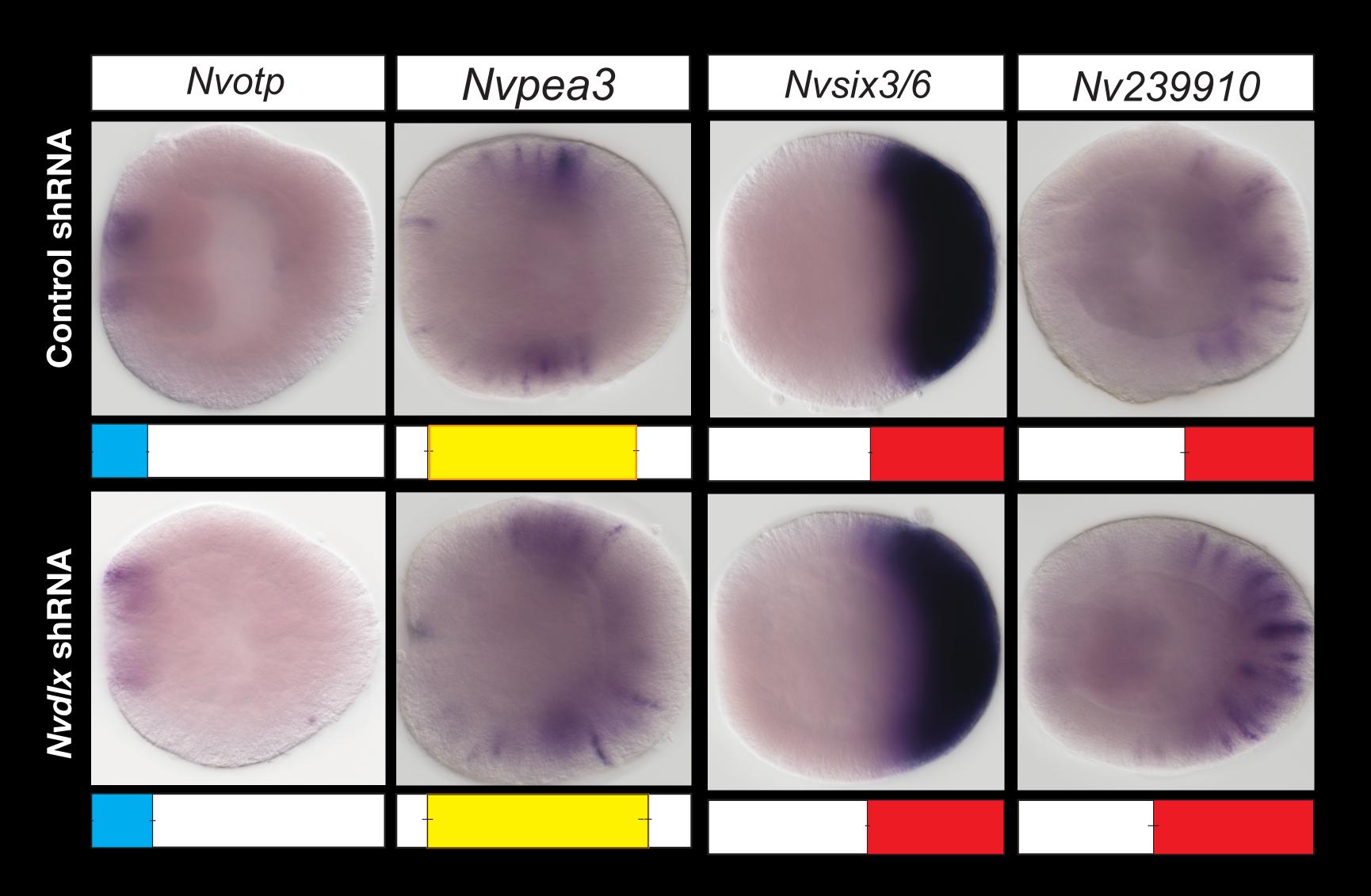
# Potential patterning domains and regional neuronal markers



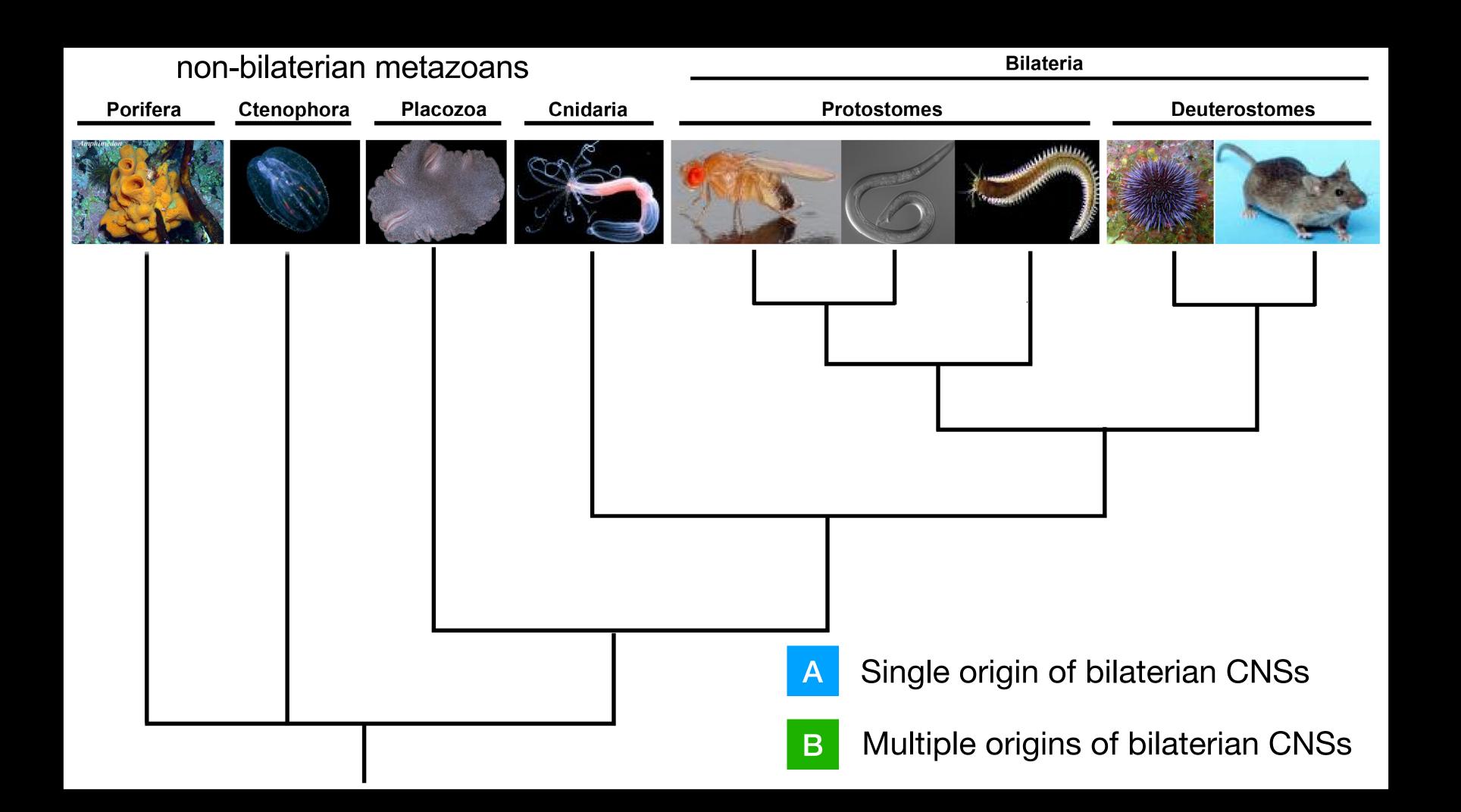




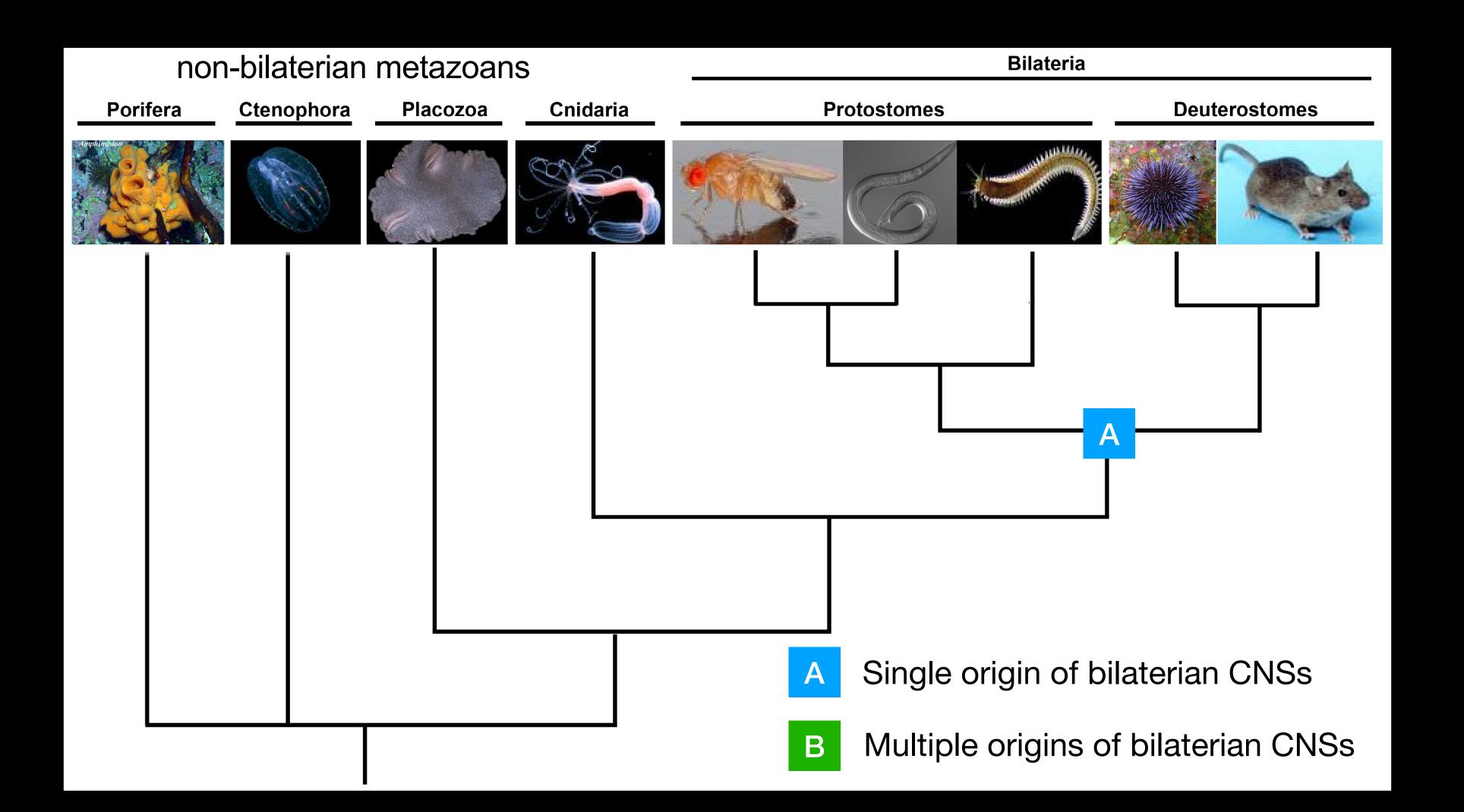




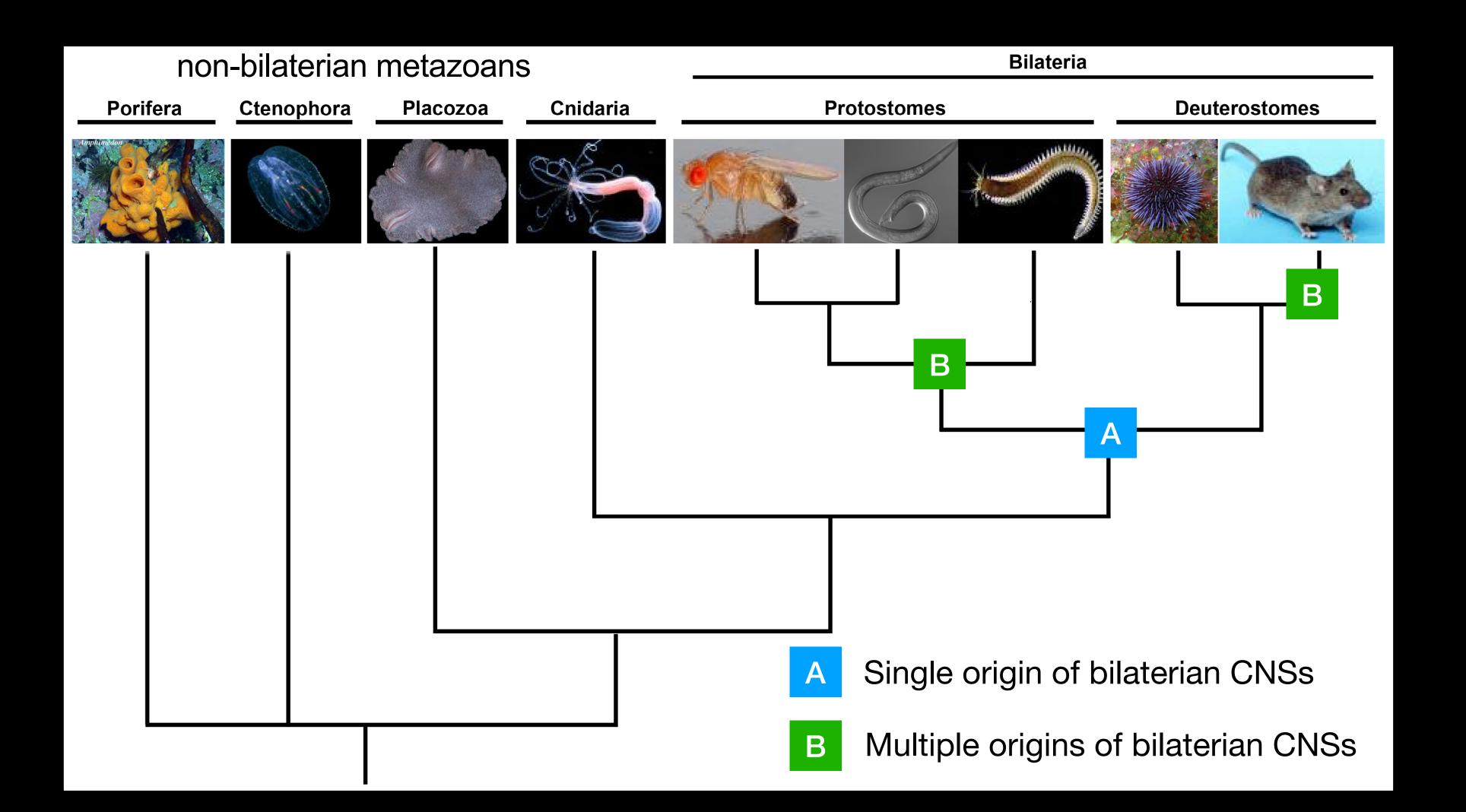
# Nematostella neuronal patterning provides insights about the origin and evolution of CNSs.

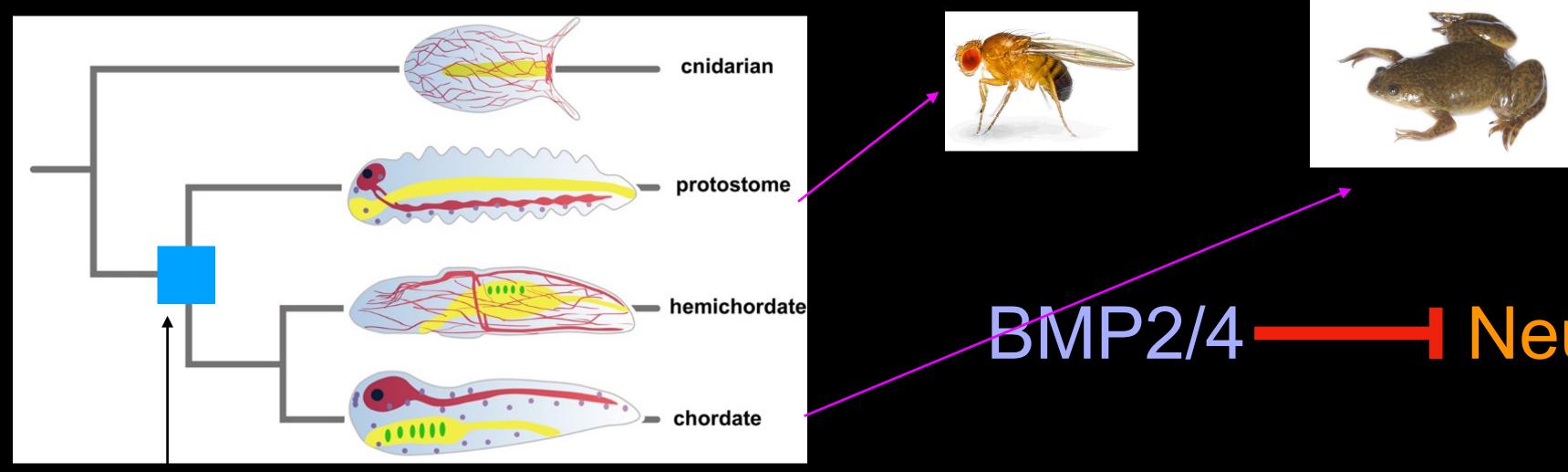


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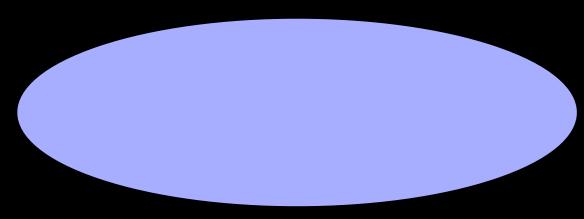


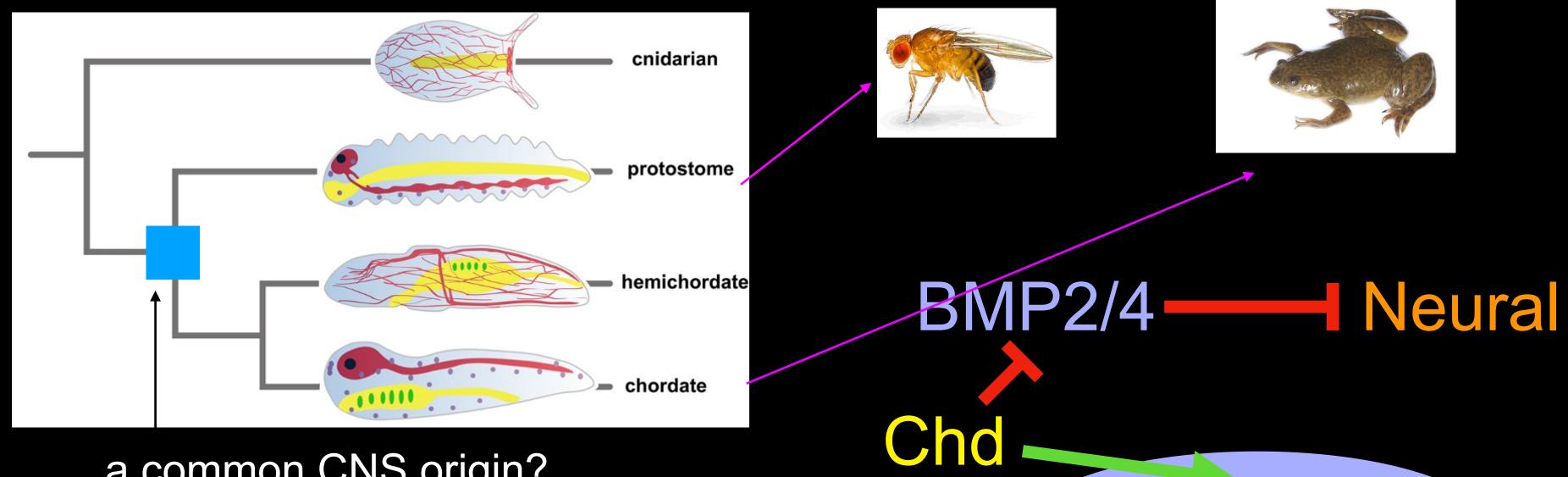
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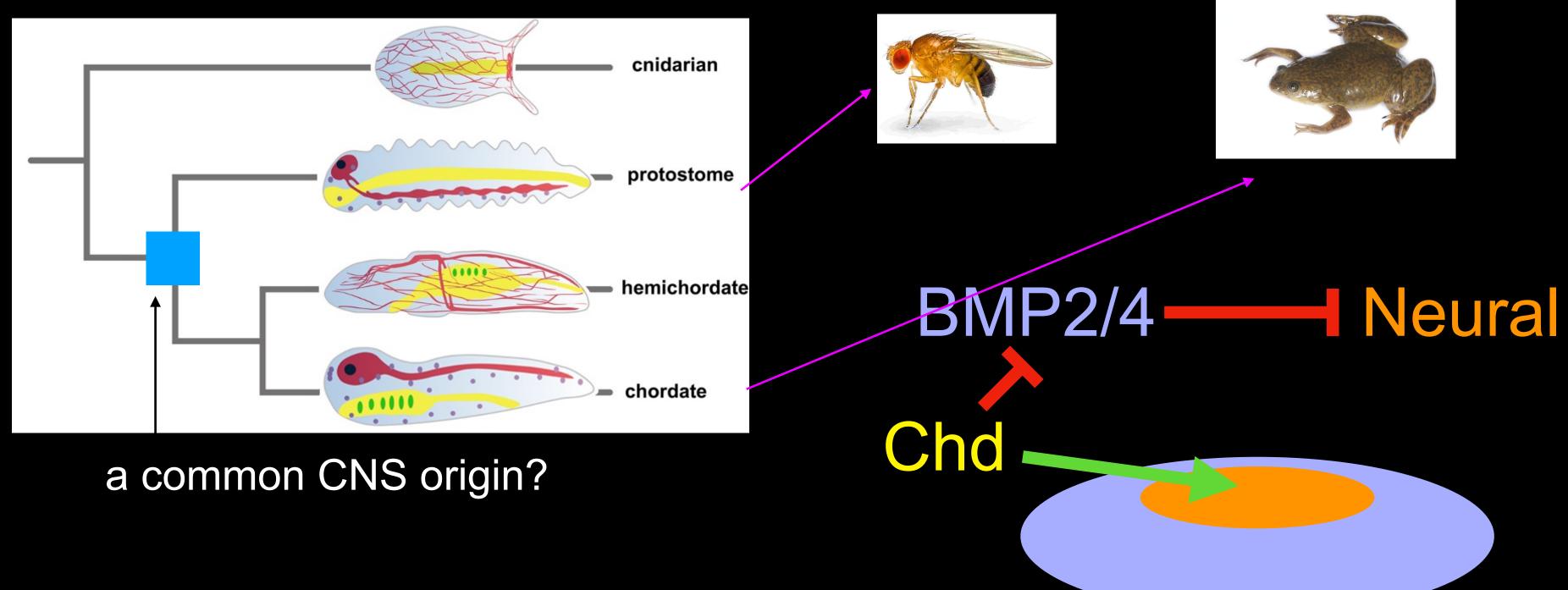


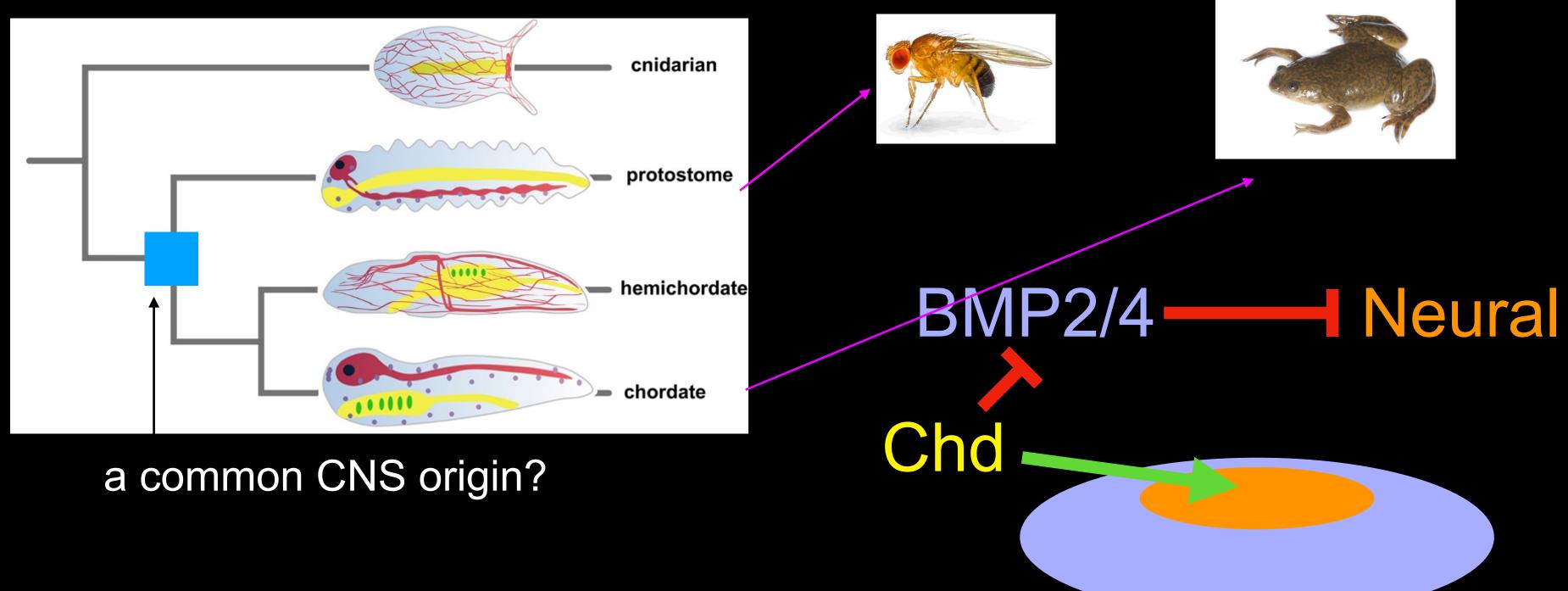


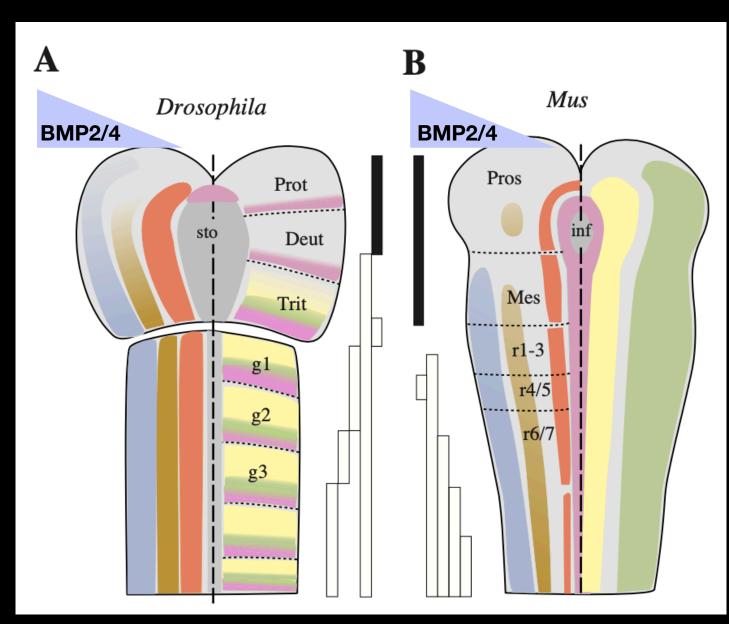
BMP2/4 Neural



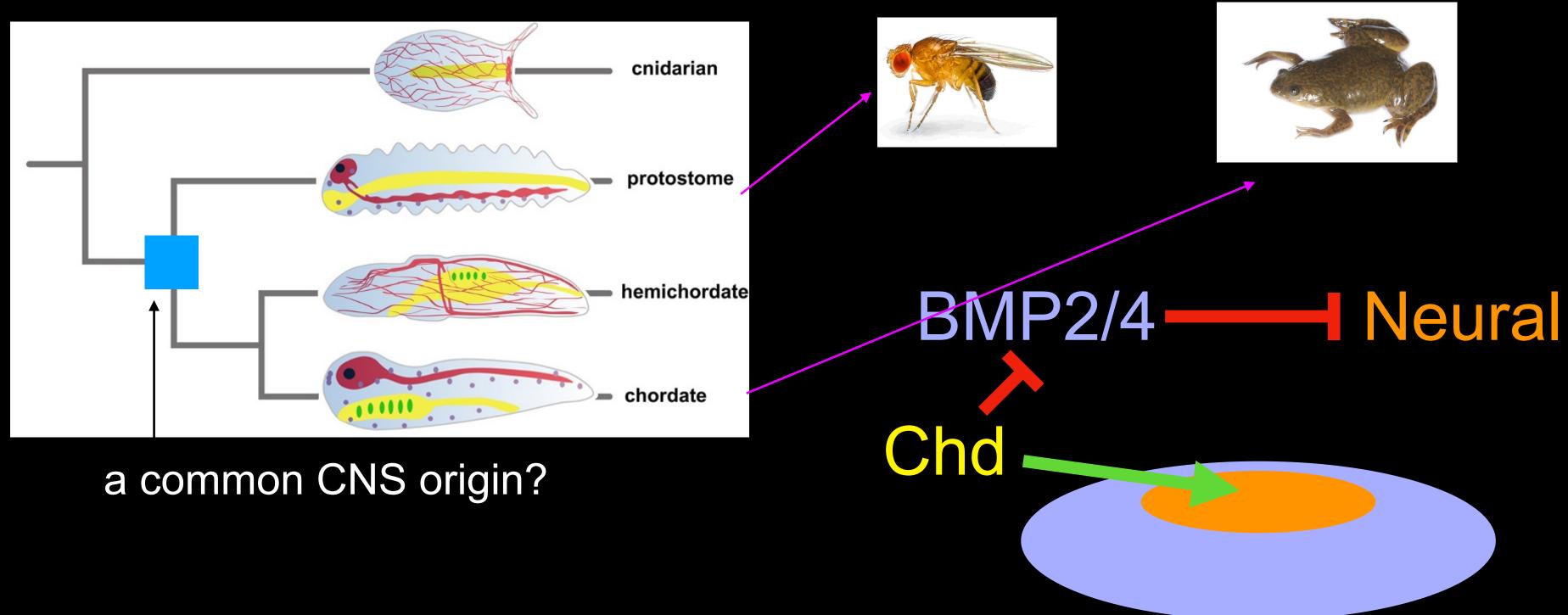


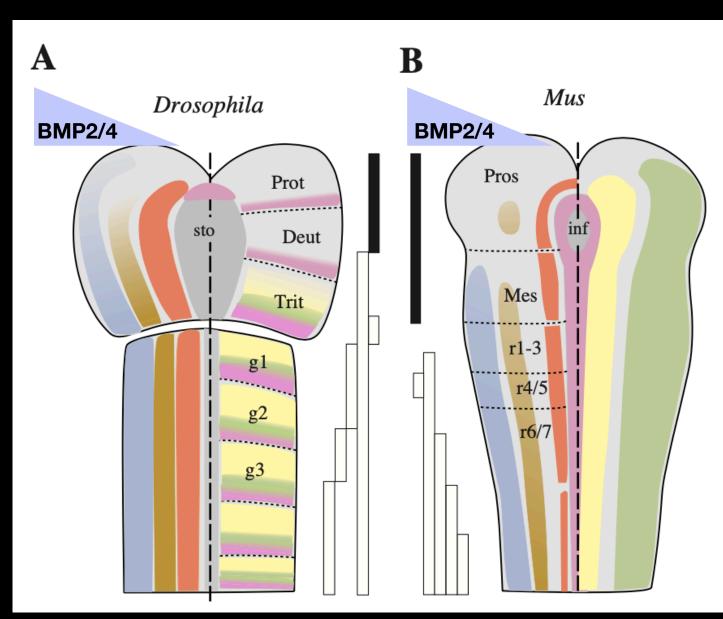




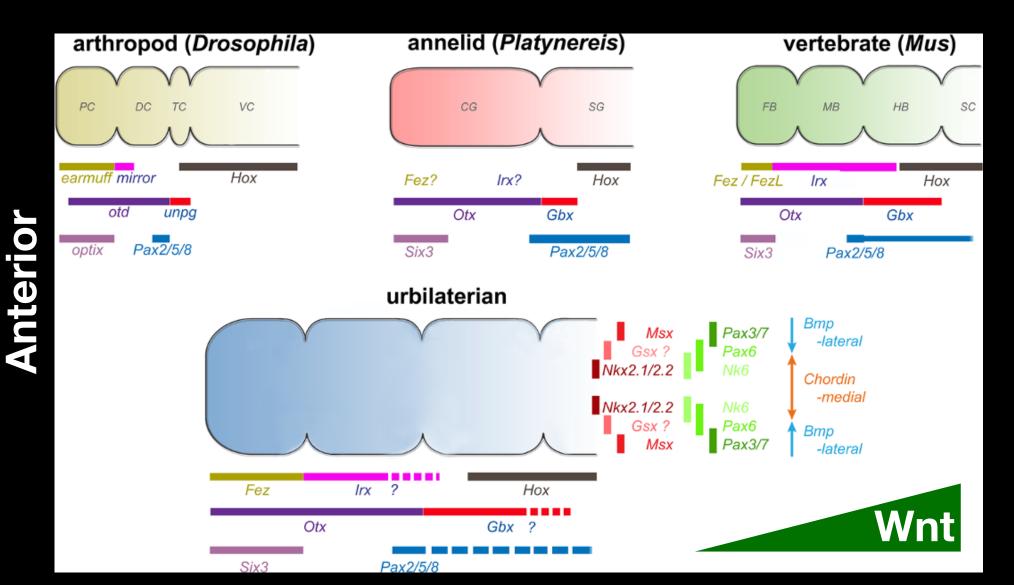


Arendt and Nübler-Jung (1999) Development

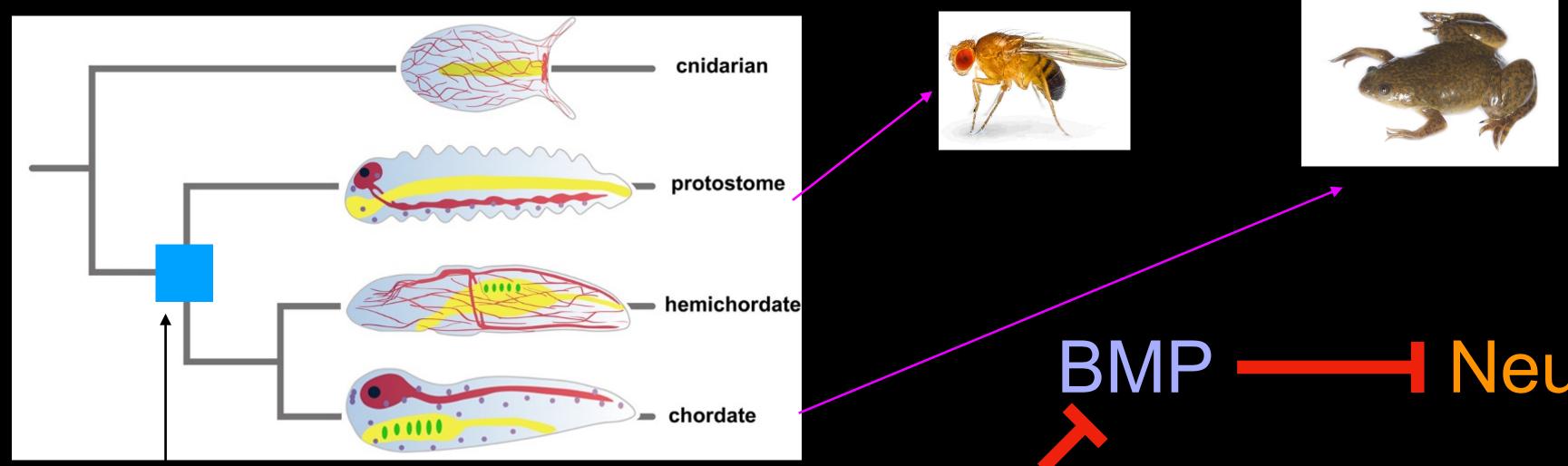




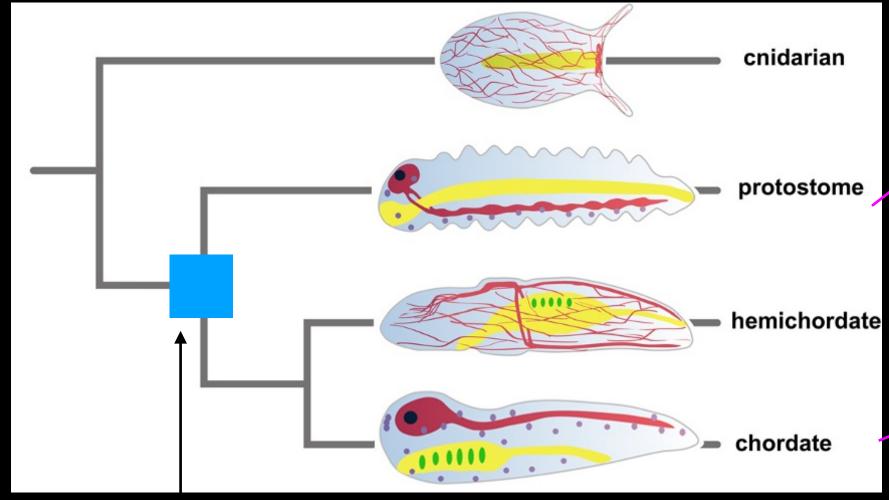
Arendt and Nübler-Jung (1999) Development



Adapted from Holland et al. (2013) EvoDevo



BMP Neural Chd



### ARTICLE

### **Convergent evolution of bilaterian nerve** cords

José M. Martín-Durán<sup>1</sup>\*, Kevin Pang<sup>1</sup>\*, Aina Børve<sup>1</sup>, Henrike Semmler Lê<sup>1,2</sup>, Anlaug Furu<sup>1</sup>, Johanna Taylor Cannon<sup>3</sup>, Ulf Jondelius<sup>3</sup> & Andreas Hejnol<sup>1</sup>





## BMP – Neural Chd

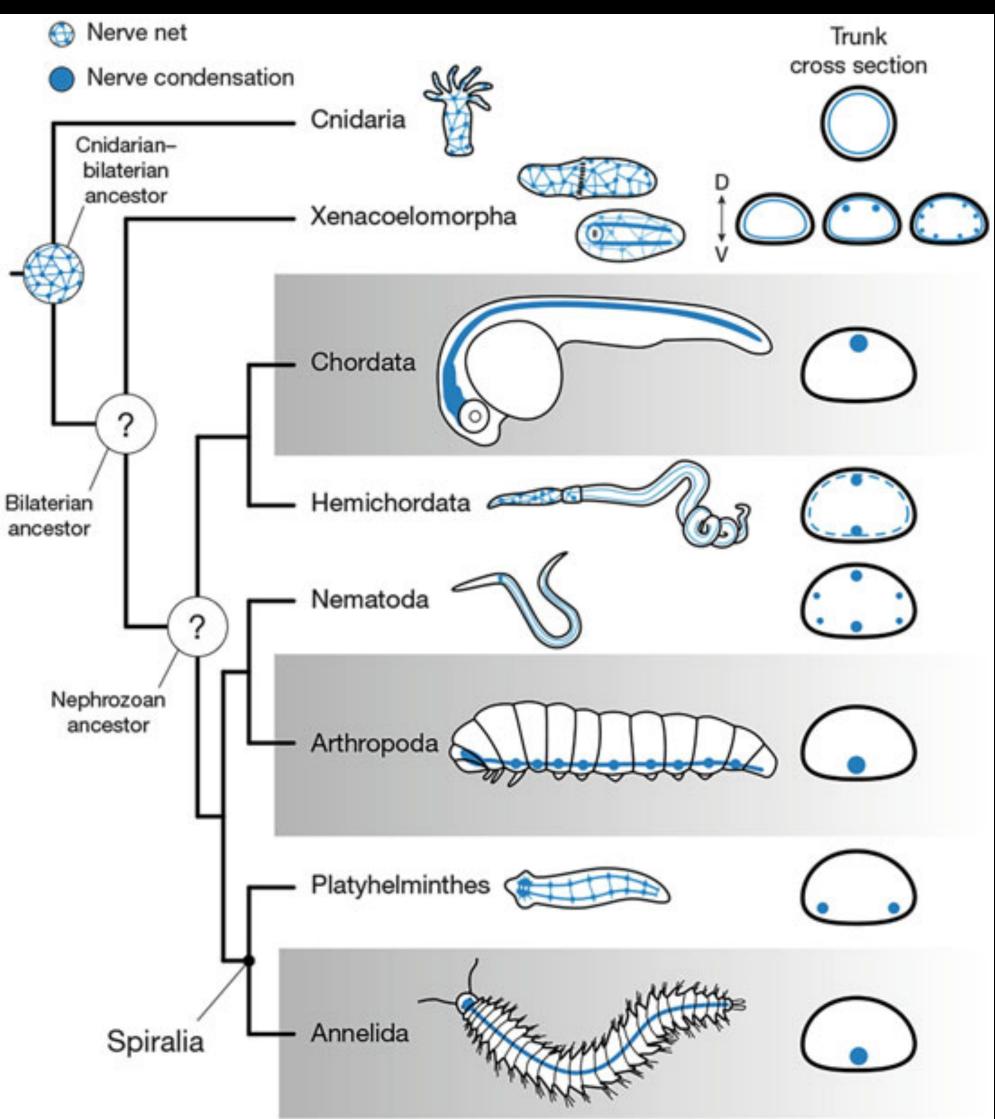
doi:10.1038/nature25030

Noura 

**BMP** not conserved

### How does this improve our understanding of CNS evolution?





### How does this improve our understanding of CNS evolution?

