It’s no fluke! Using planarians to understand parasitic schistosomes

Jim Collins

Department of Pharmacology

Outline

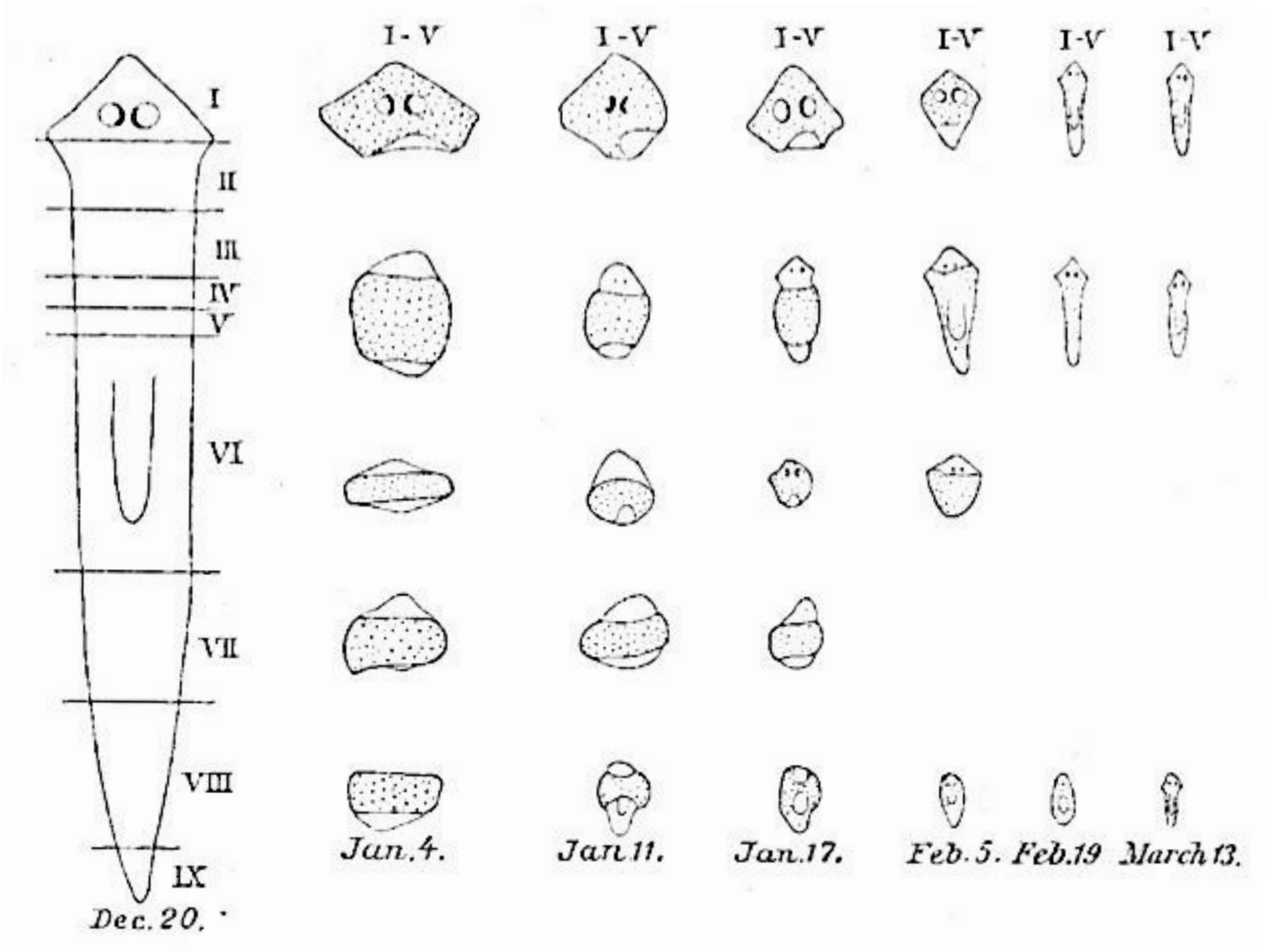
I. Planarians as an experimental model

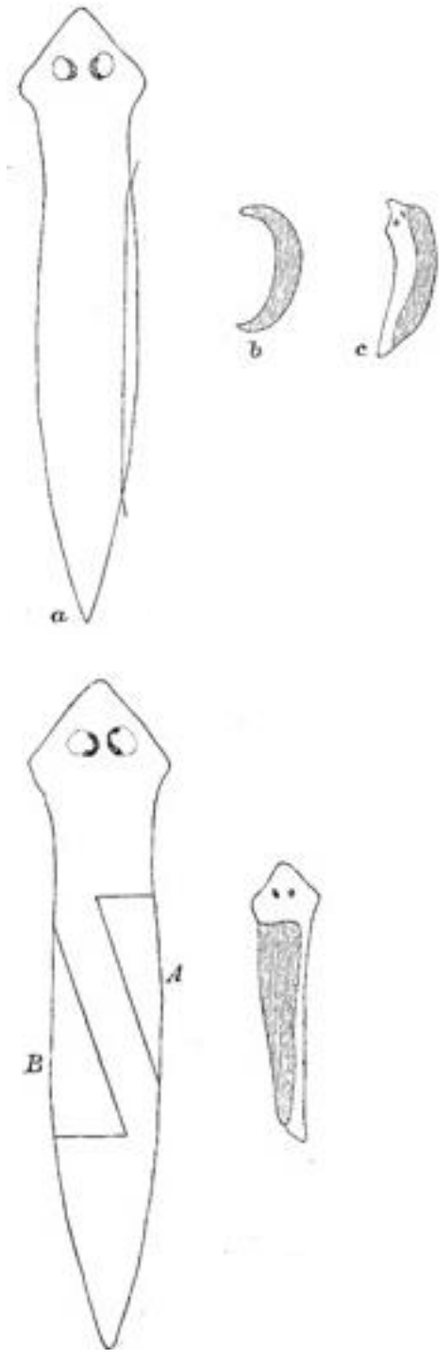
II. Peptide hormones regulate planarian reproduction

III. Lessons from planarians: stem cells in schistosomes

IV. Conclusion: Schistosomiasis is a disease of stem cells

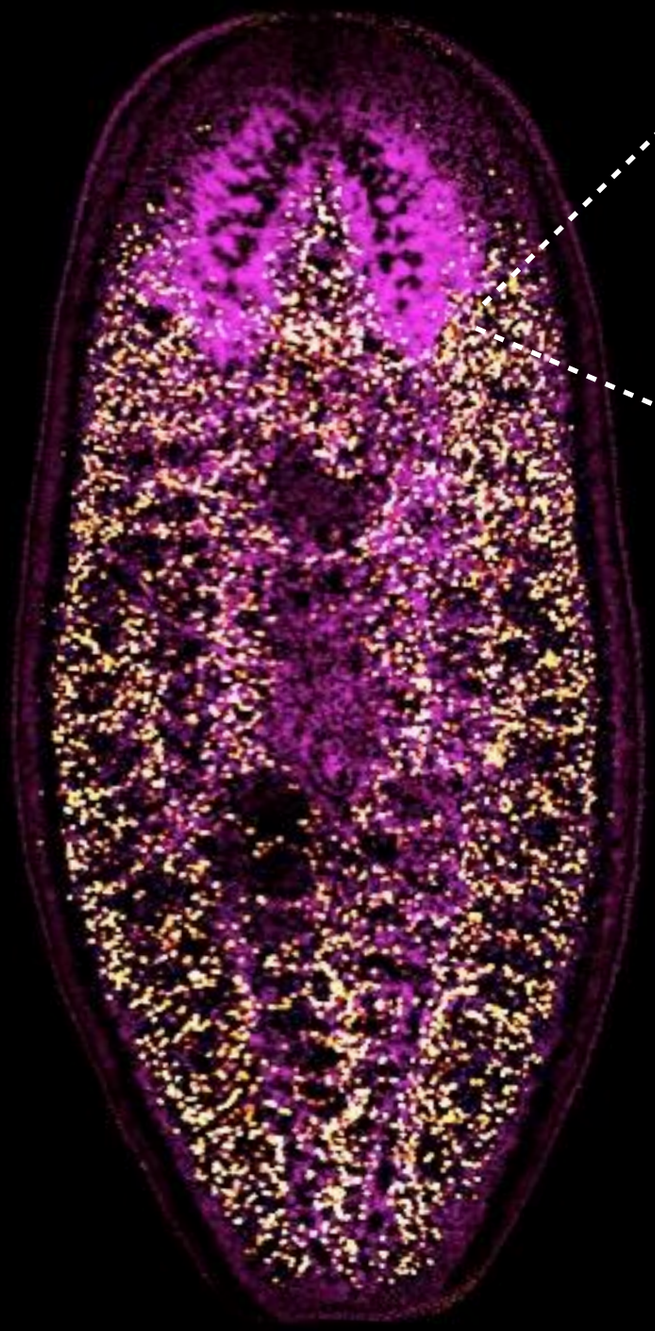
Planarian Regeneration



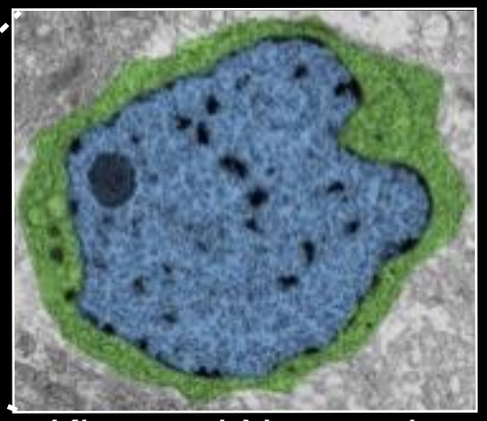


Morgan, T. H. Experimental studies of the regeneration of *Planaria maculata*. *Arch. Entwm. Org.* **7**, 364–397 (1898).

Neoblasts are stem cells that drive regeneration

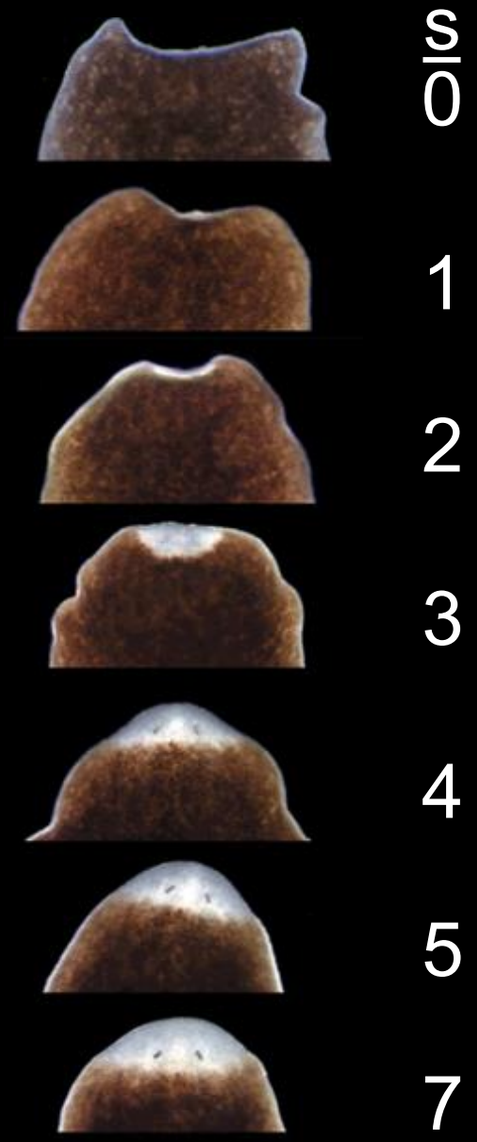


Neoblasts



King and Newmark.

2012. *JCB* 196 (5).



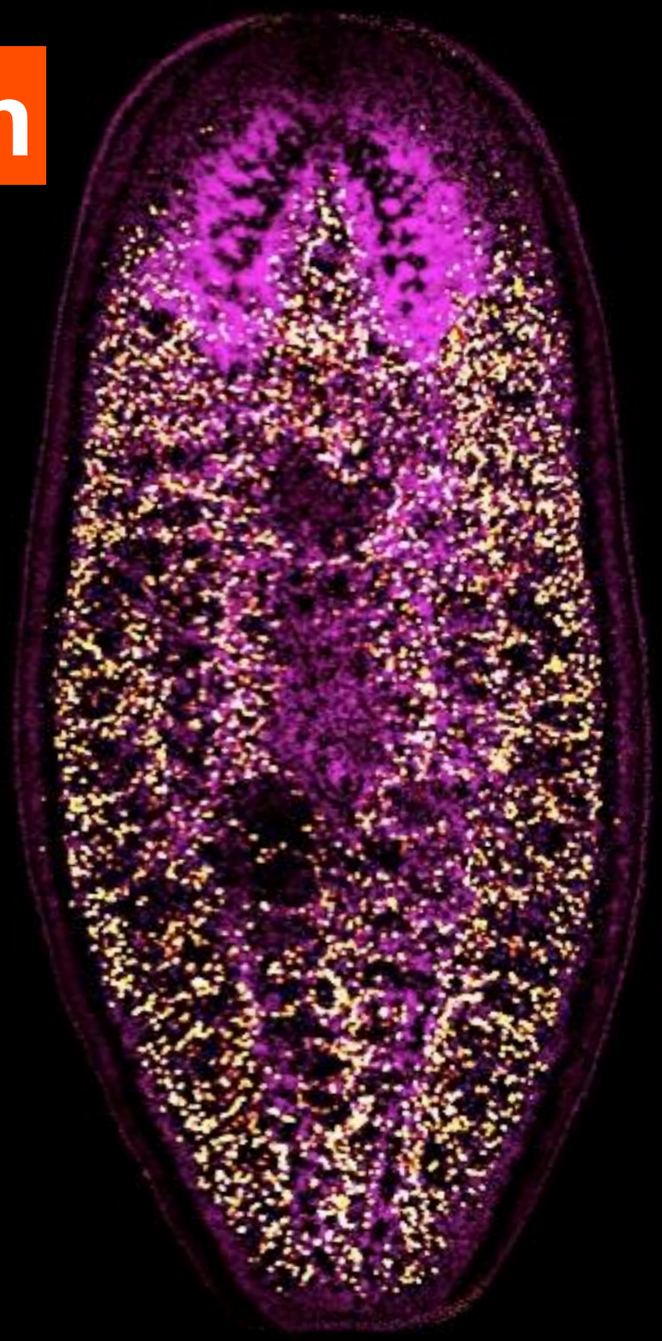
Newmark & Sánchez Alvarado.

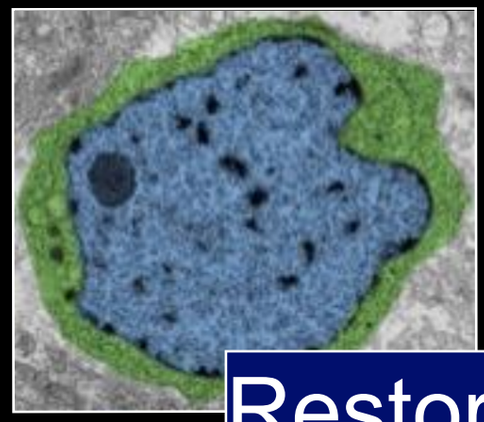
Nature Reviews Genetics (2002).

3(2).

Neoblasts underlie tissue homeostasis

+Irradiation





Restoration of tissue homeostasis and regeneration

Wagner et al. (2011) *Science:* **332**, 811

Developmental Plasticity in Planarians

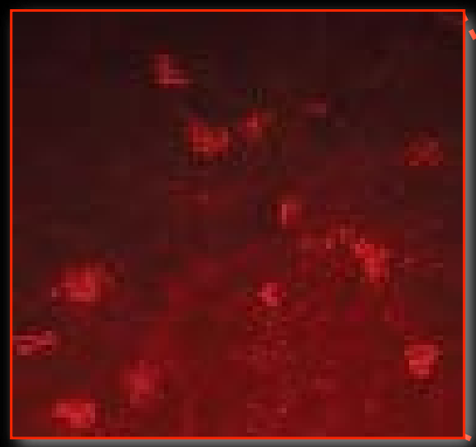
Developmental plasticity: Ability of an organism to modulate their development in response to environmental inputs

Starve

Feed

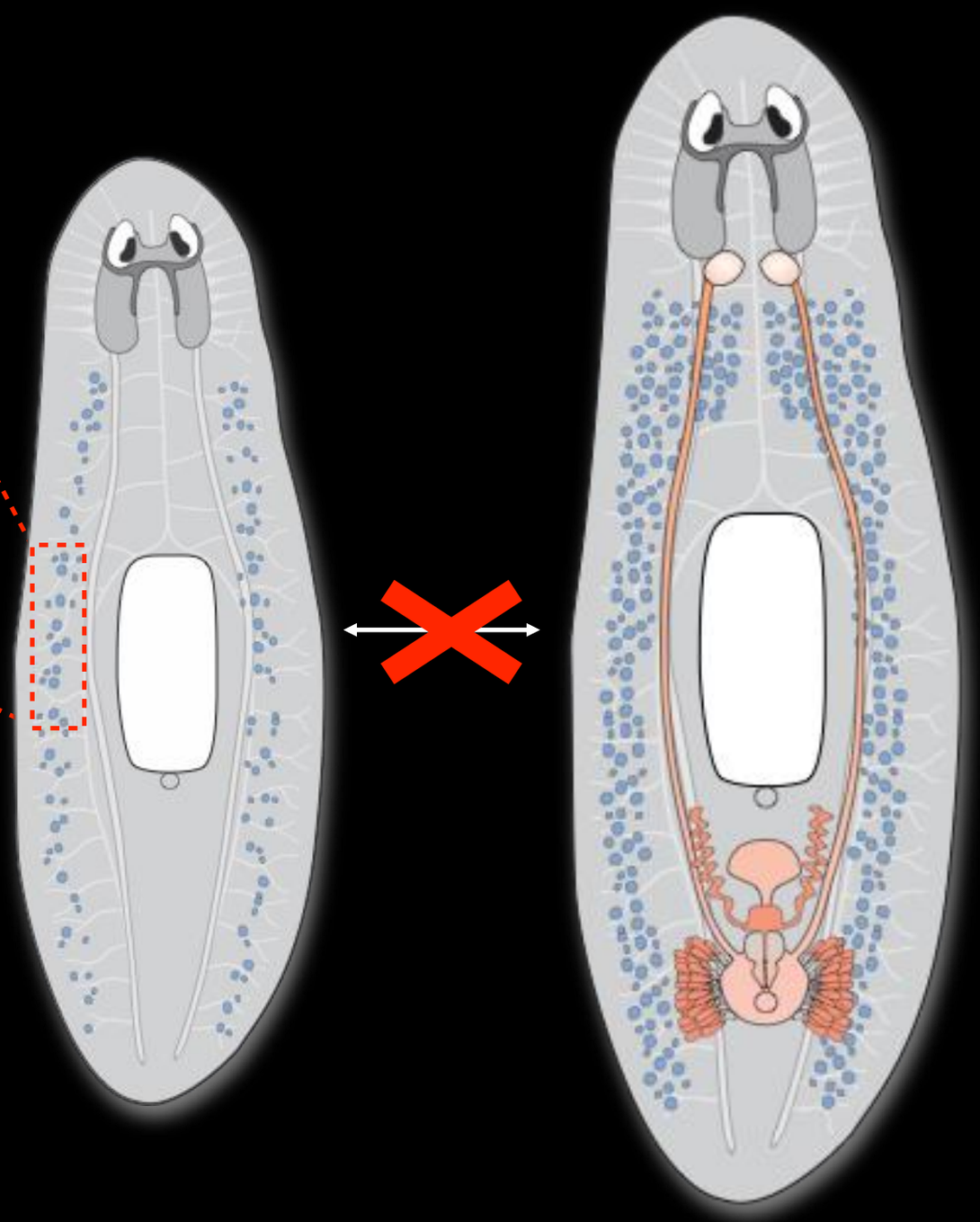
Plasticity of Planarian Reproductive Development

Schmidtea mediterranea



nanos

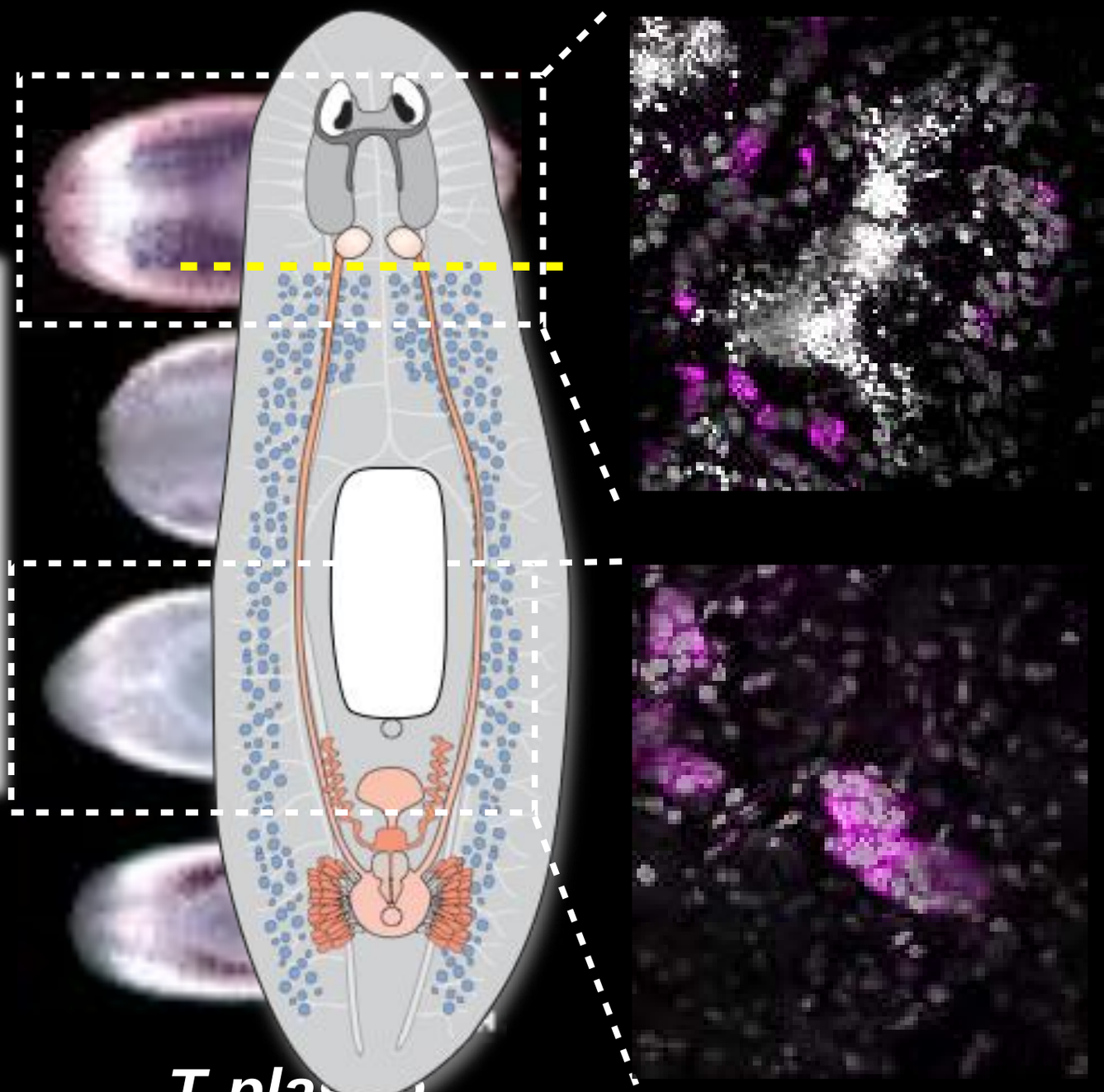
Missing an instructive signal?



Asexual Sexual

Neural Control of Germ Cell Differentiation?

Suggests neural coordination of germ cell dynamics



Mature

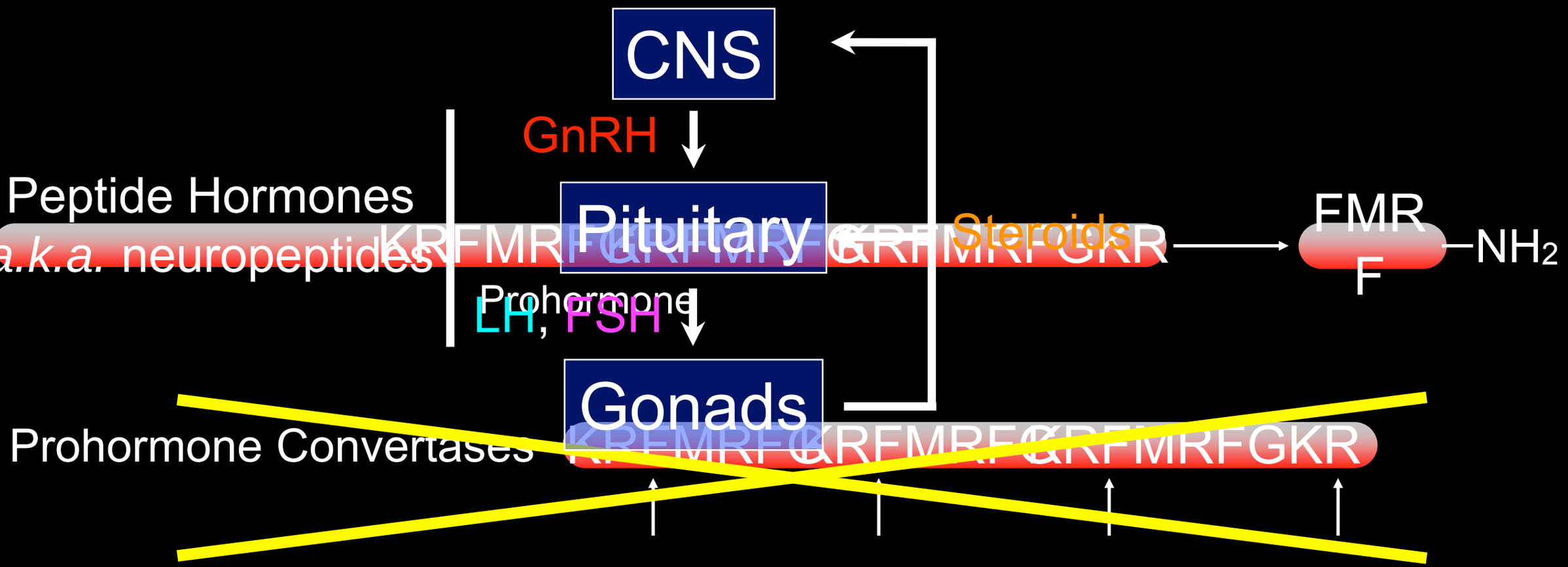
Regressed

T-plastin
differentiated germ cells

nanos
early germ cells

Fedecka-Bruner B. 1967. *Bull Biol Fr Belg* 101
Ghirardelli E, 1965 . Regeneration in Animals and Related Problems. p 177–184
Wang et al. 2007. *PNAS* 104.

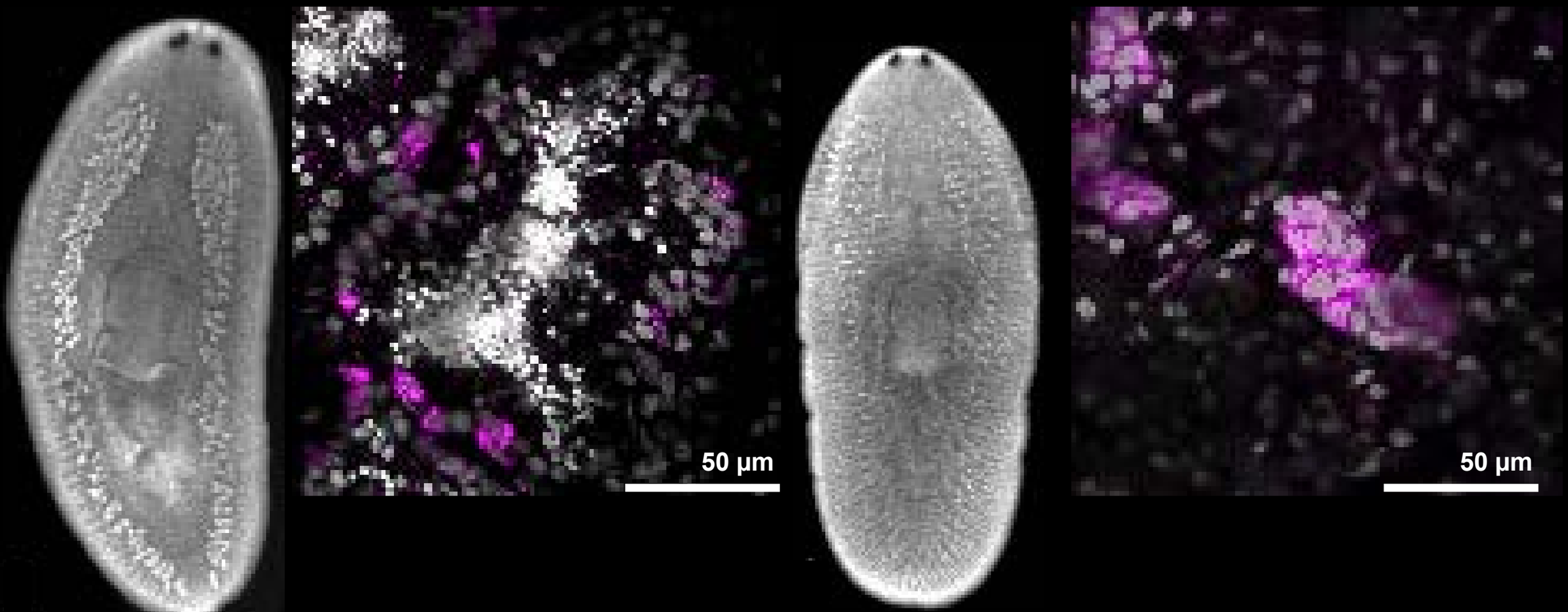
Peptide Hormones (Neuropeptides)



Characterize *prohormone convertase 2* (*pc2*)

pc2 RNAi results in loss of differentiated germ cells

nanos



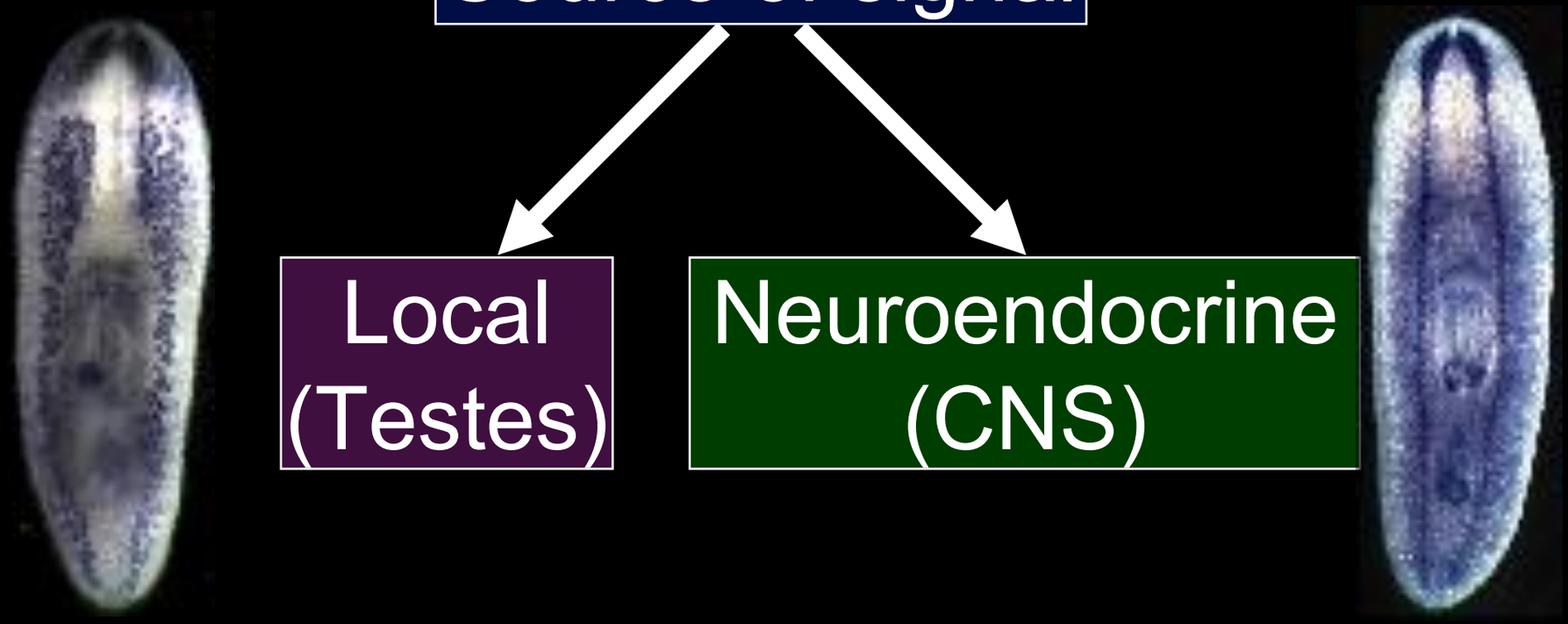
Control RNAi

pc2 RNAi

Collins et al. 2010. *PLoS Biology* 8(10): e1000509

Peptide hormones are important for maintenance of differentiated germ cells

Peptide hormones are important for maintenance of differentiated germ cells



Identify and characterize individual peptide hormones

Peptide hormone identification

Approach:

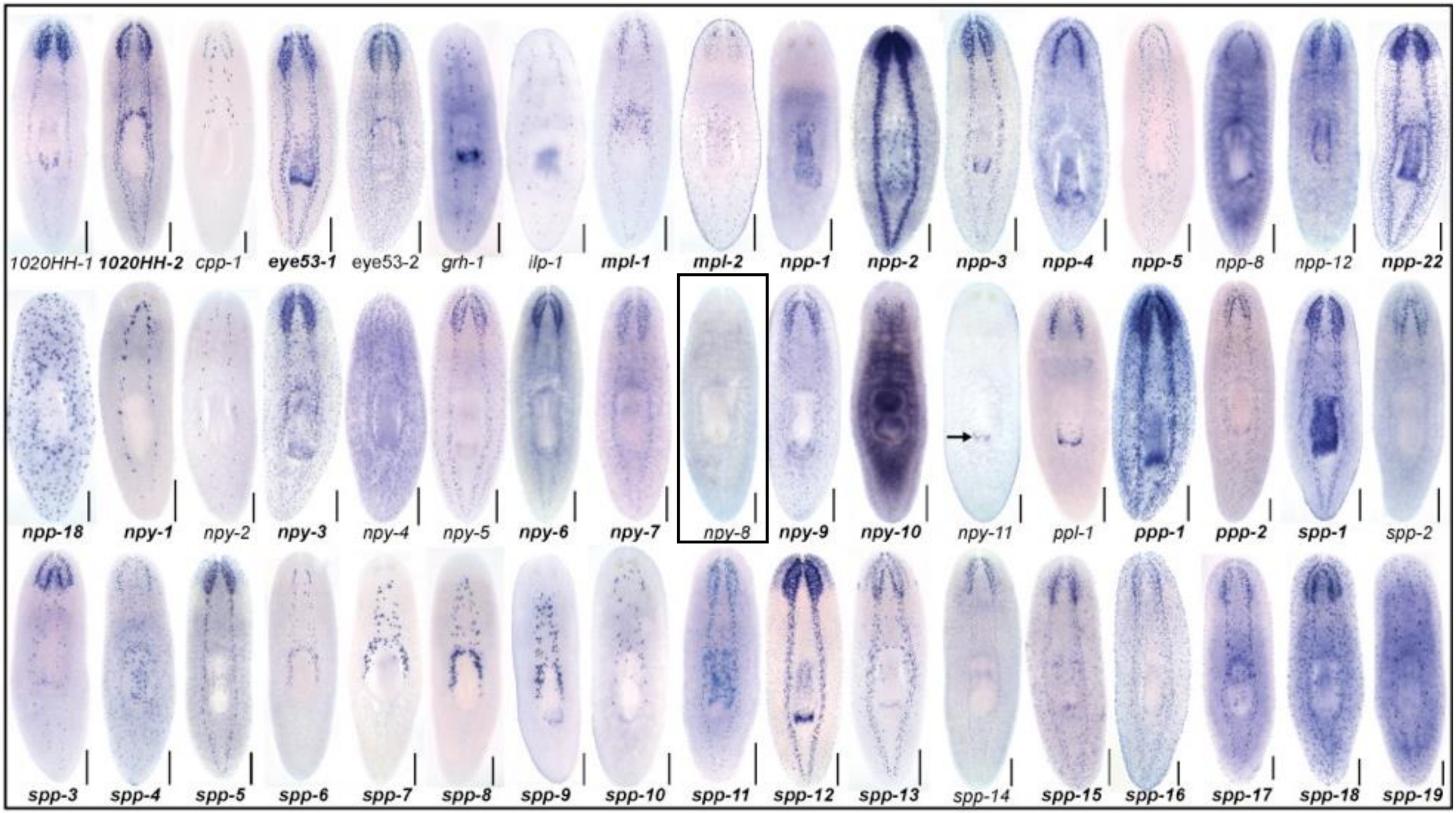
Use Bioinformatics and Mass Spectrometry

Jonathan Sweedler's Laboratory
Dept. Chemistry (UIUC)
Xiaowen Hou
Elena Romanova

51 prohormone genes encoding ~250 peptides

Collins et al. 2010. *PLoS Biology* 8(10): e1000509

Peptide hormone identification



Compare to sexual planarians

Scale=300 μm

npy-8 is expressed differentially in sexual planarians

Asexual

Mature Sexual

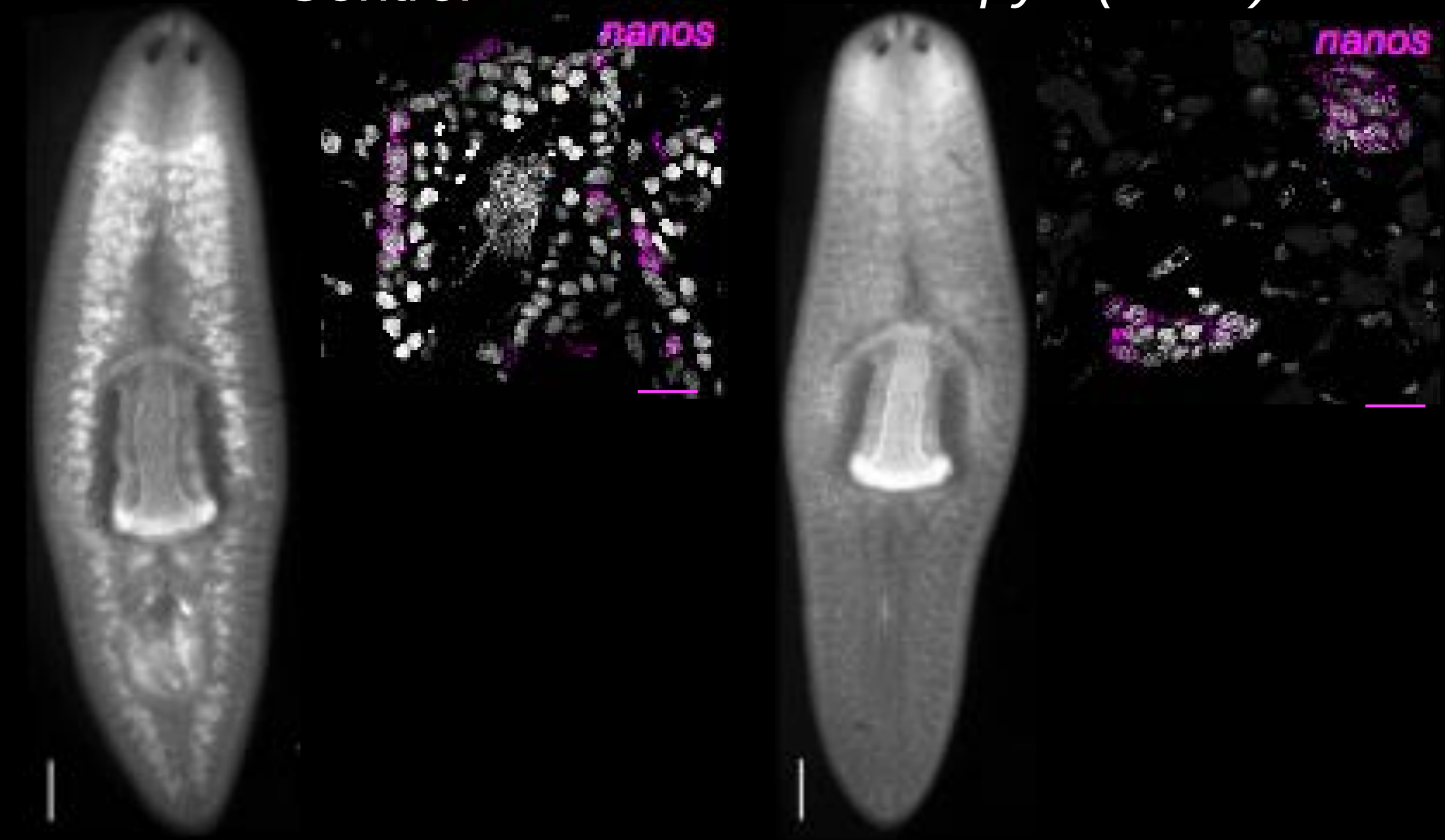
Scale=300 µm

Collins et al. 2010. *PLoS Biology* 8(10): e1000509

npy-8 is required to maintain sexual organs

Control

npy-8(RNAi)

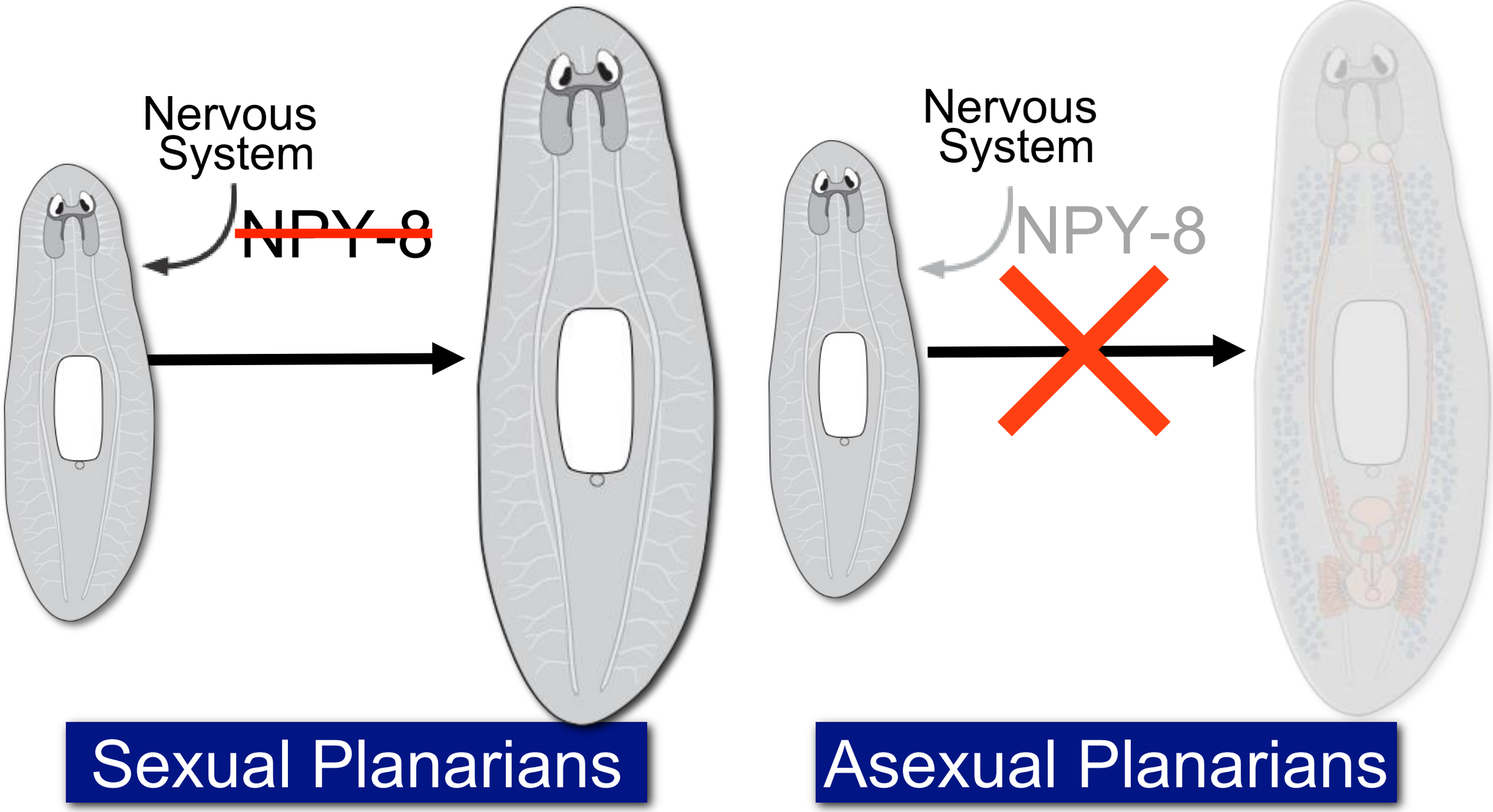


Scale Bar: 300 µm

Scale Bar: 20 µm

Collins et al. 2010. *PLoS Biology* 8(10): e1000509

NPY-8 regulates sexual maturation



Are these effects conserved?

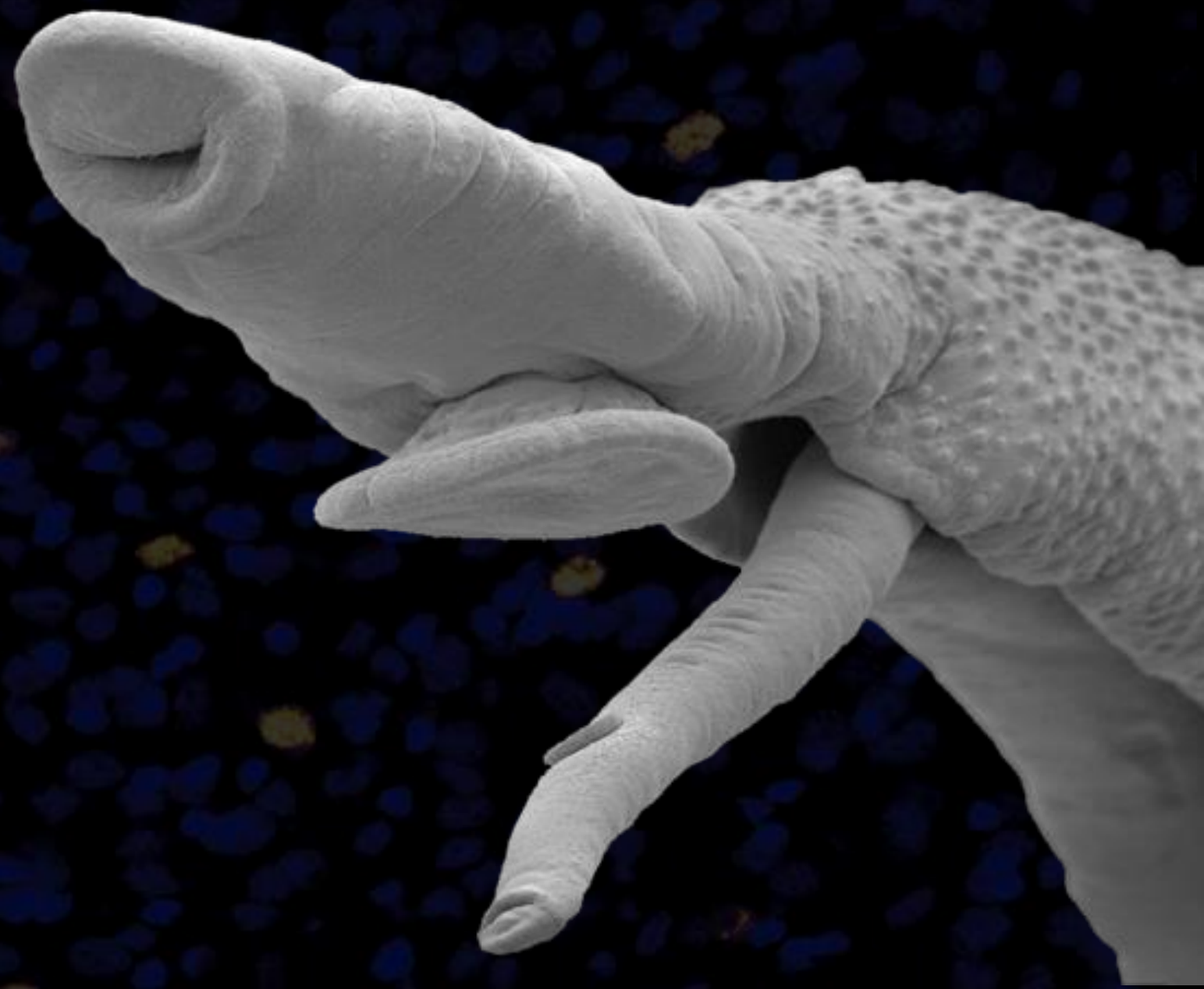
Schistosoma

Several prohormones conserved in planarians

Including NPY-8

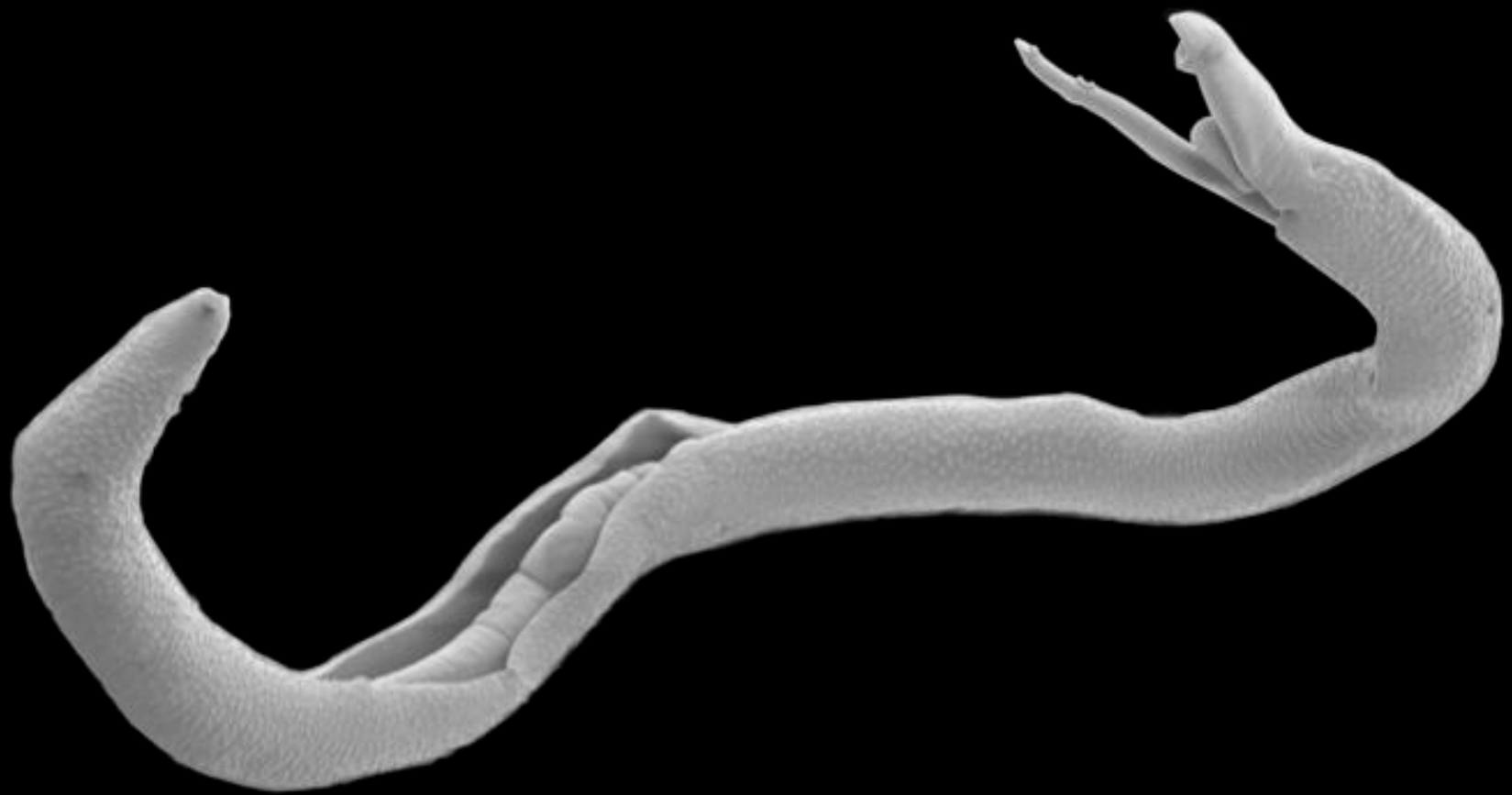
Parasitic Flatworm

Infects 200 million people worldwide



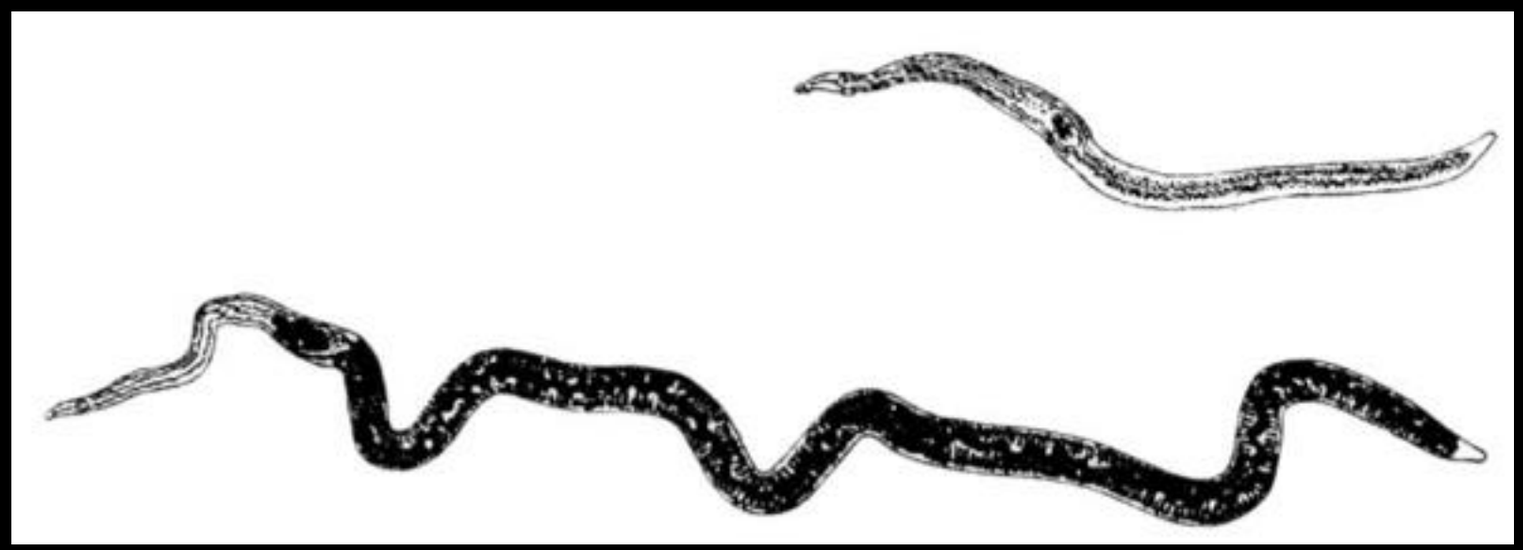
Like planarians, the schistosome reproductive system is quite plastic

Reproductive plasticity in schistosomes



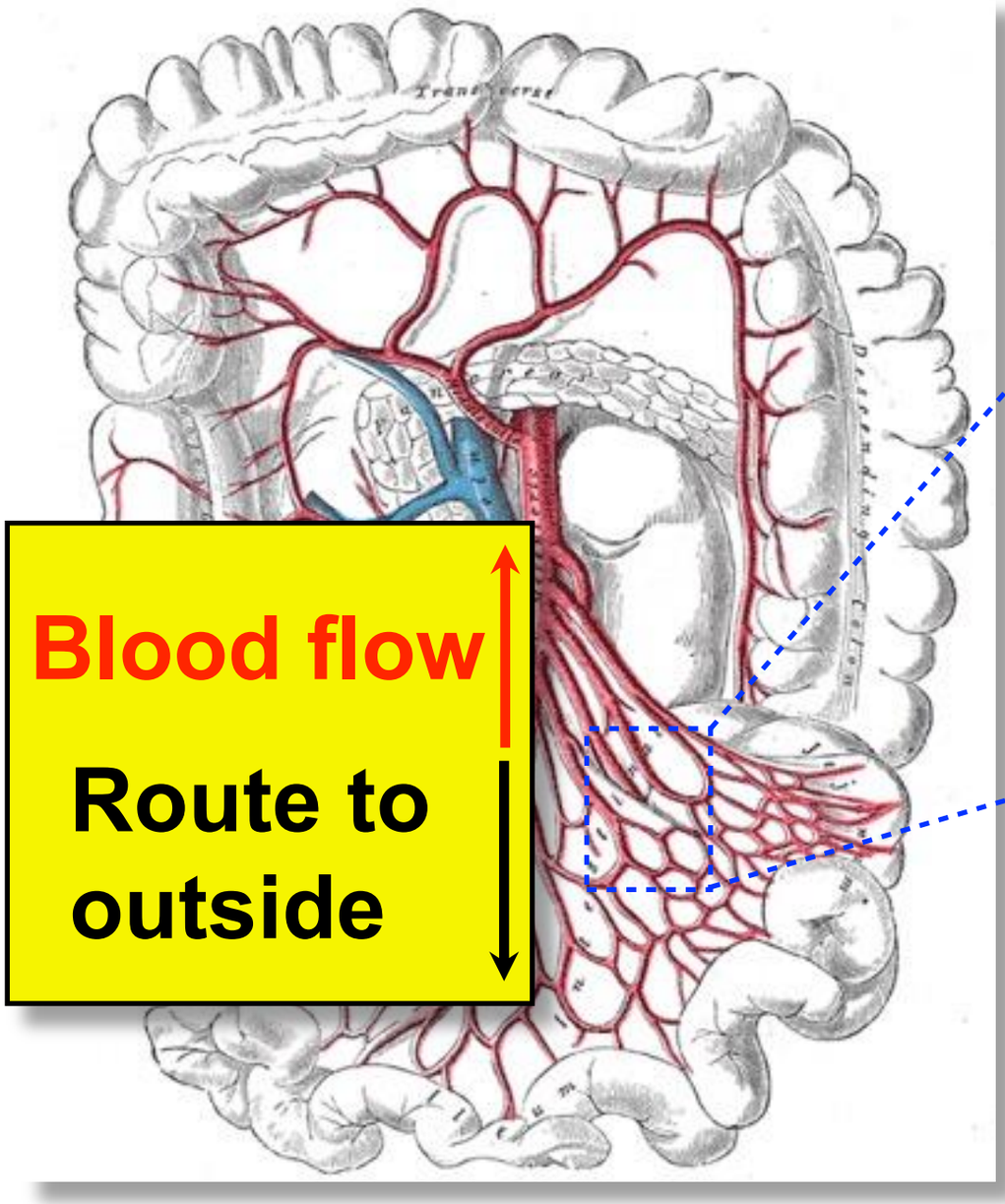
Unpaired

Paired



Kunz W. 2001. TRENDS in Parasitology 17(5).

Schistosome egg production drives pathology

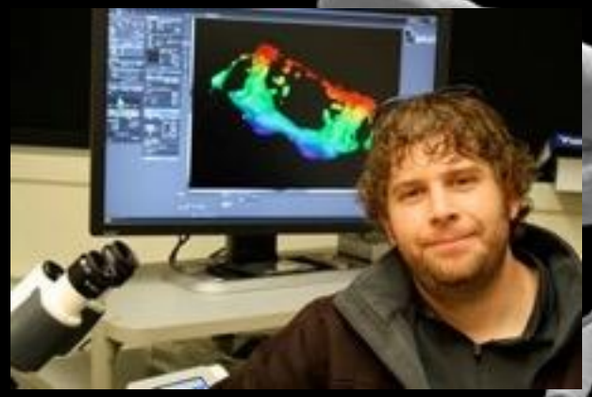


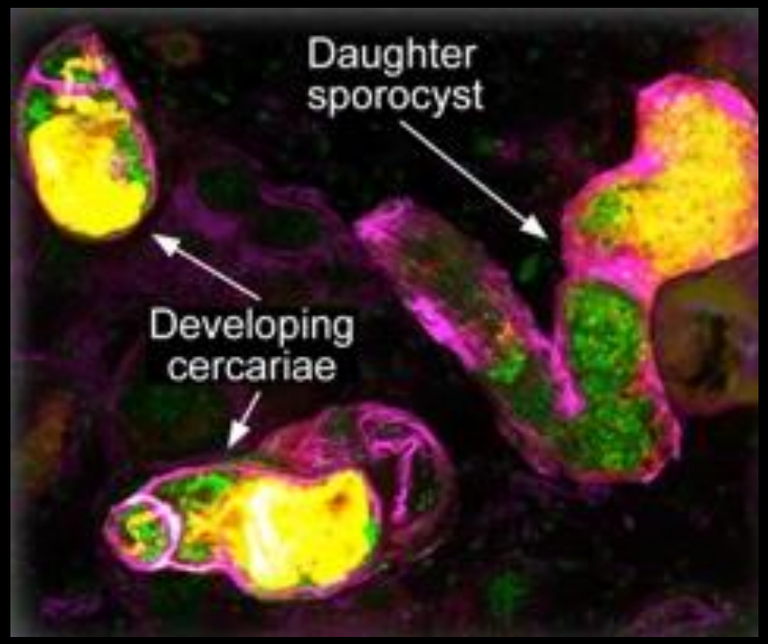
Gray's Anatomy of the Human Body (20th Ed)

Credit: Wellcome Library, London. Wellcome Images

- Lay 100-1000 eggs/day
- Eggs primary cause of pathology

Schistosome life cycle





http://biology.unm.edu/biology/esloker

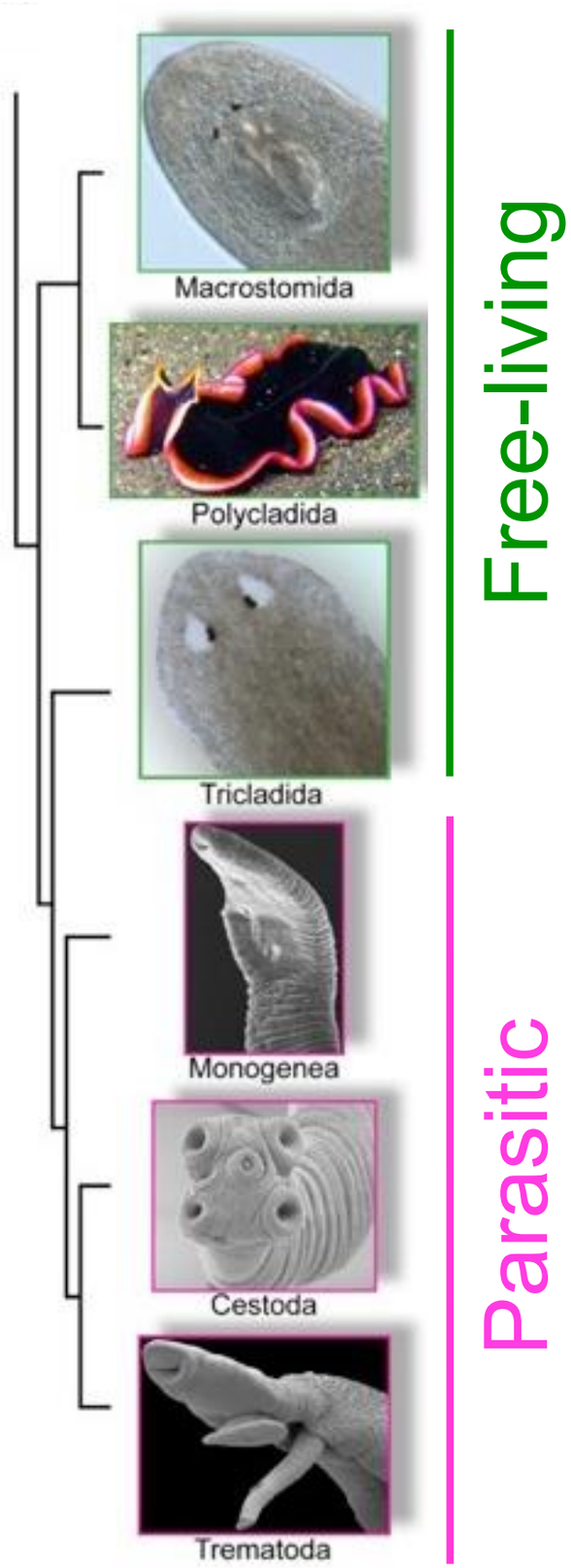
Schistosomiasis: A disease of poverty

"In a real sense, the ongoing presence of schistosomiasis in developing communities represents a silent 'disability tax' on every local inhabitant. The low-level but persistent daily disability associated with *Schistosoma* infection means that those who are affected may never reach their full potential for healthy development or productivity... Schistosomiasis is likely to be both a cause and an effect of continuing rural poverty in these areas."

King CH. Parasites and poverty: The case of schistosomiasis. *Acta Tropica.* 2010 vol. 113 (2) pp. 95-104

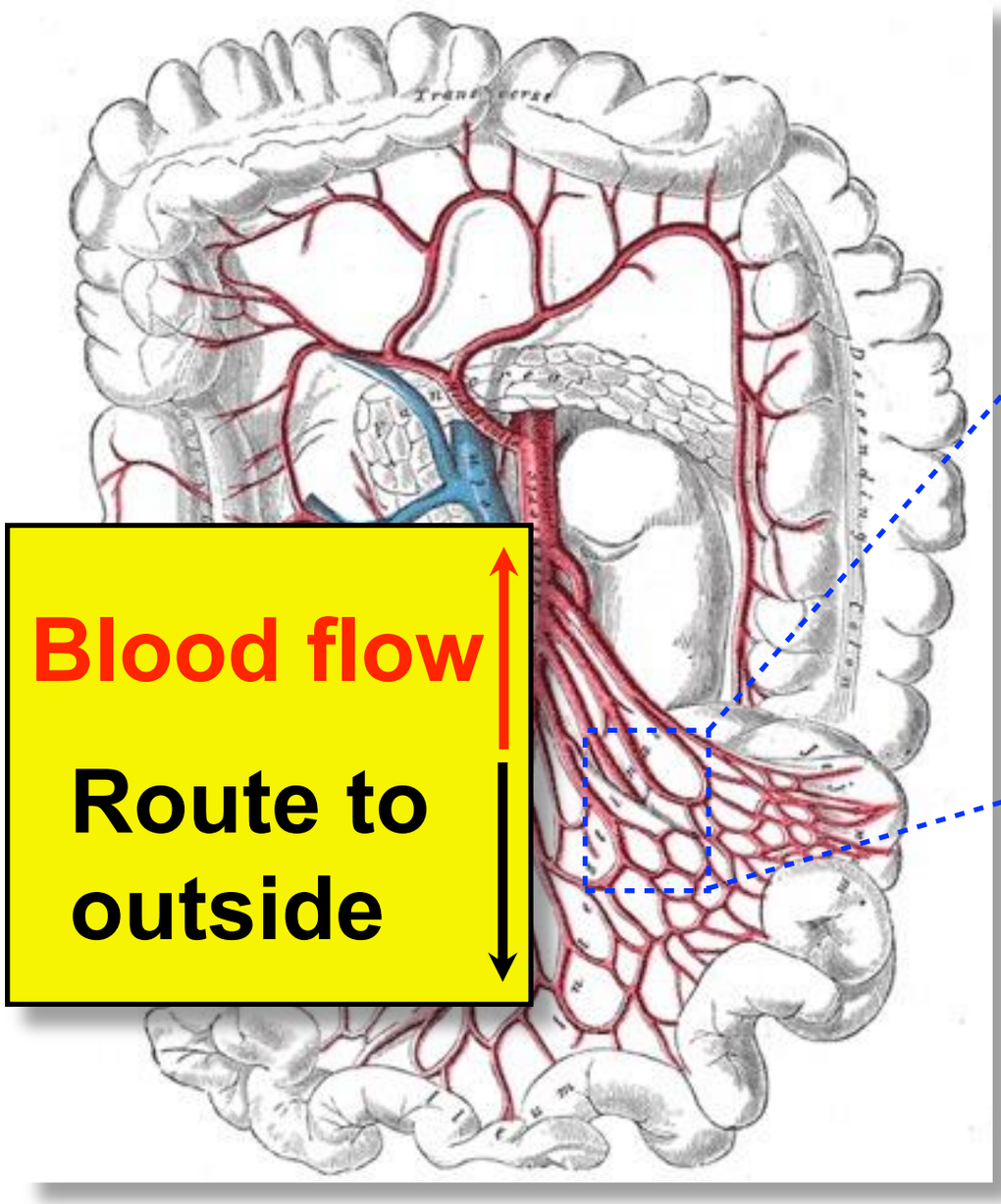
Can we use planarians to guide our understanding of schistosomes?

Free-living and Parasitic Flatworms



Collins and Newmark. *PLoS Pathogens.9(7):e1003396*

Schistosomes are extremely long-lived



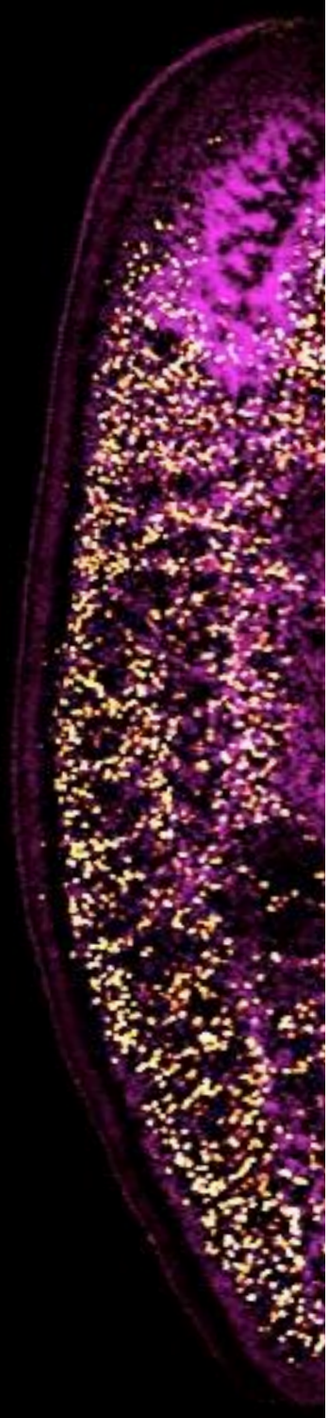
Gray's Anatomy of the Human Body (20th Ed)

Credit: Wellcome Library, London. Wellcome Images

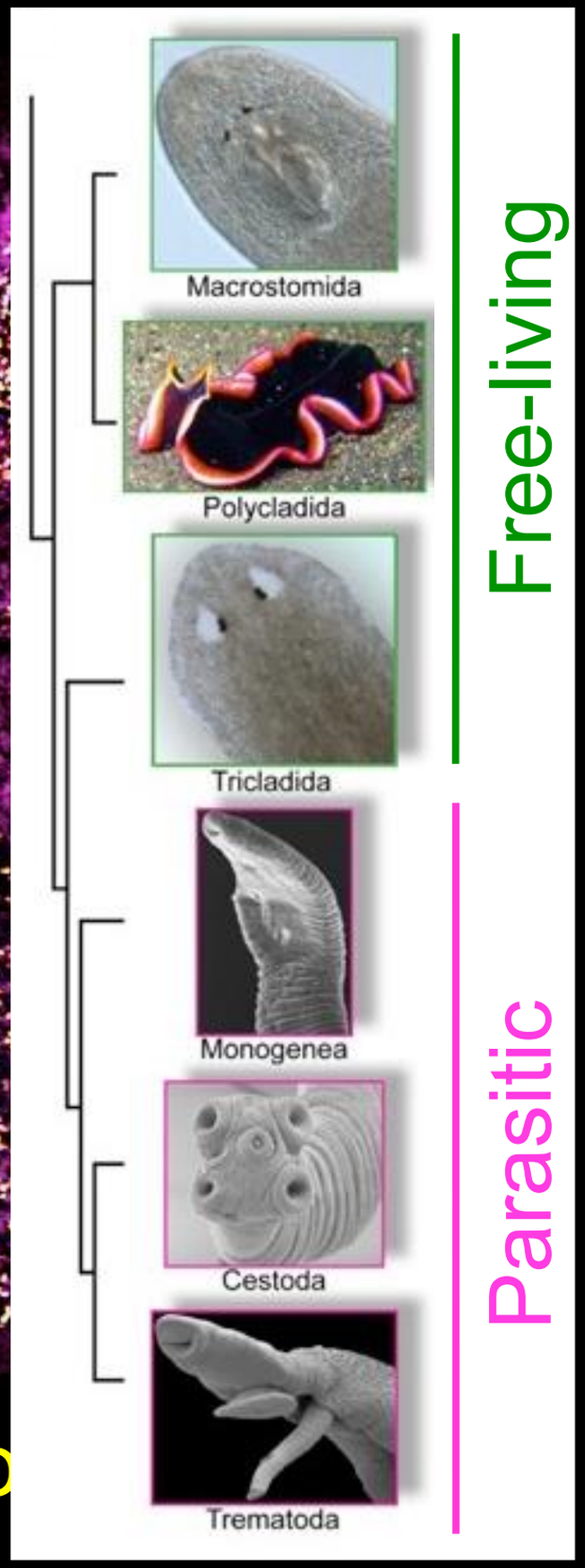
- Parasites can live for decades
- Hostile environment

Schistosomes must have mechanisms to repair old/damaged tissues

Neoblasts are key to planarian longevity



Neo



No comparable cell type has been described in Schistosomes

Hypothesis:

Schistosomes have neoblast-like adult stem cells

Experiment:

Do adult schistosomes have proliferative cells? Treat schistosomes with EdU.

Somatic cells incorporate EdU

♂

EdU
EdU
Phalloidin

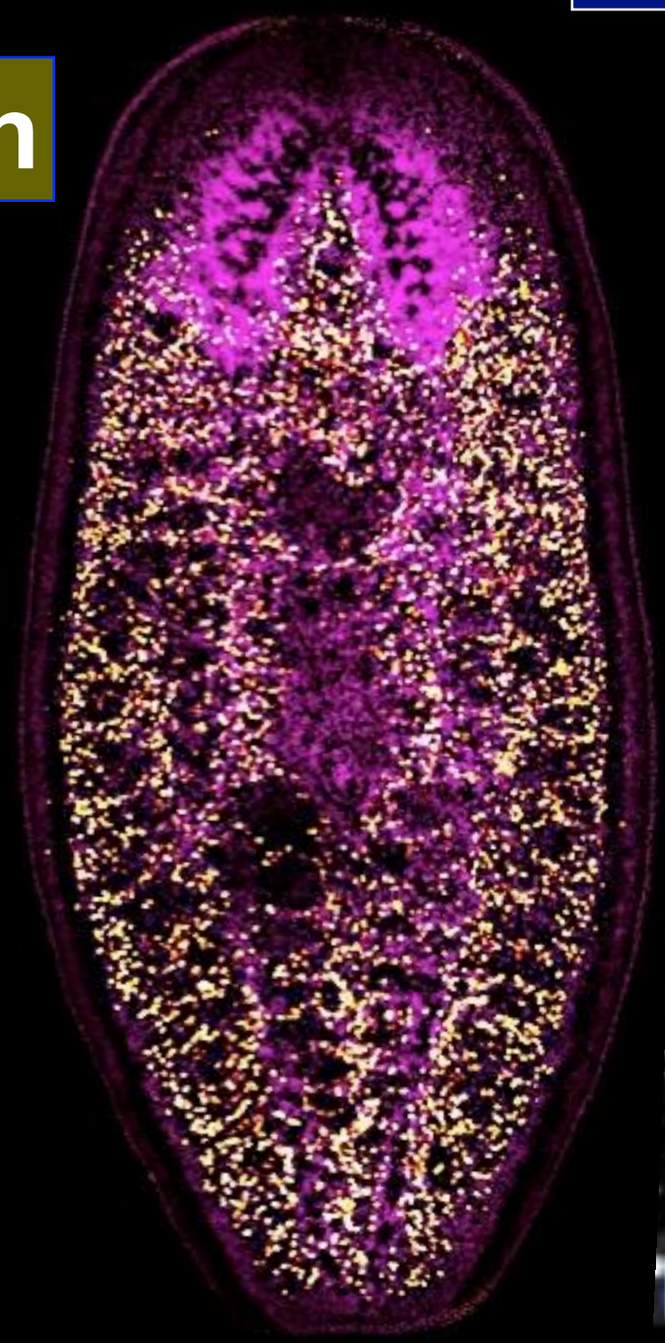
Collins et al. *Nature* 494: 476-479

Can we characterize these cells molecularly?

Cycling cells are radiation sensitive

Adult Schistosomes

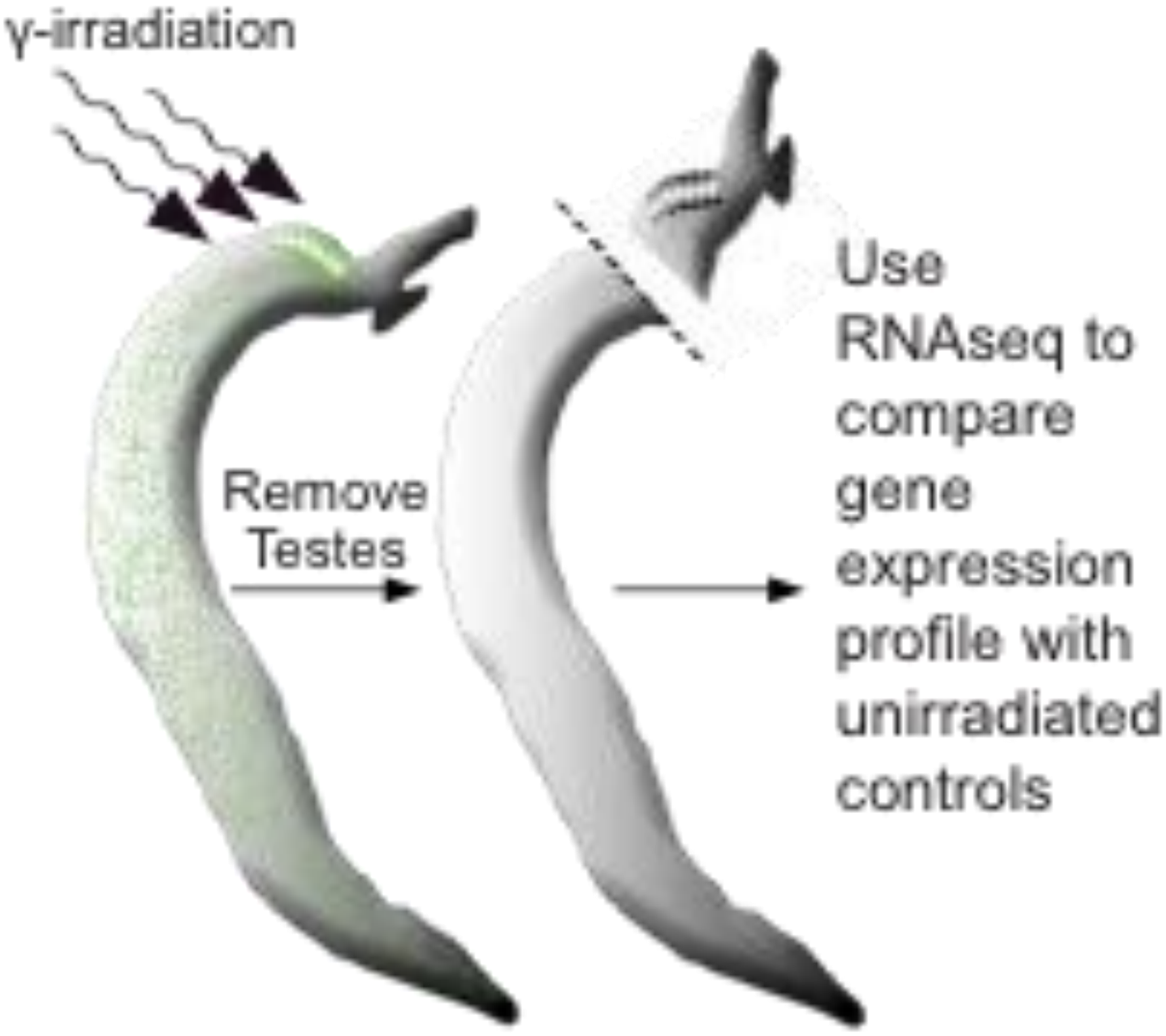
+Irradiation



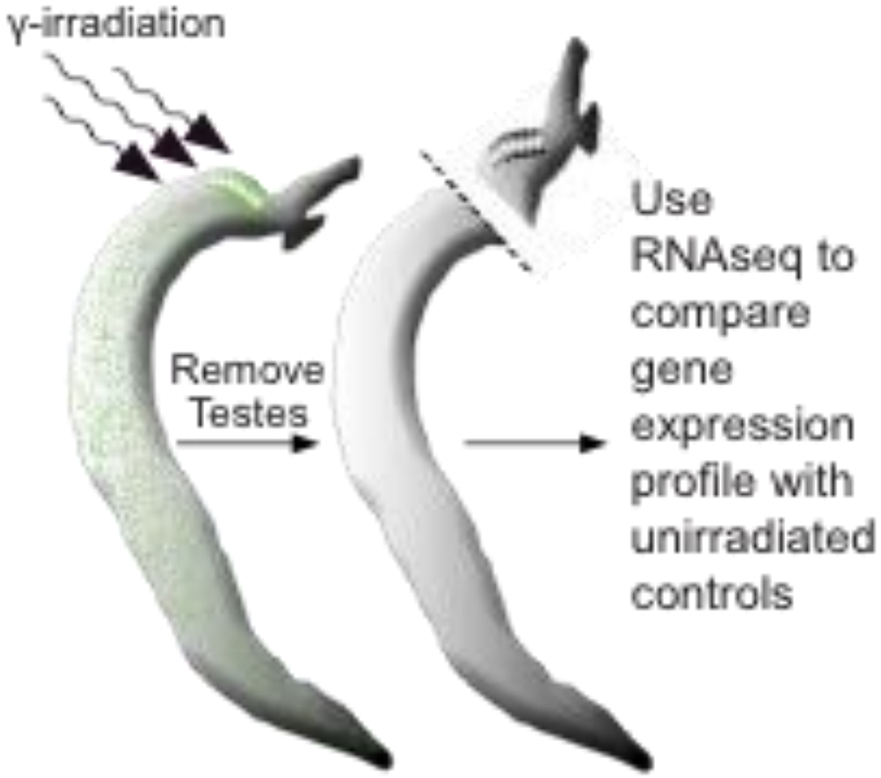
Scale Bar: 20 μm

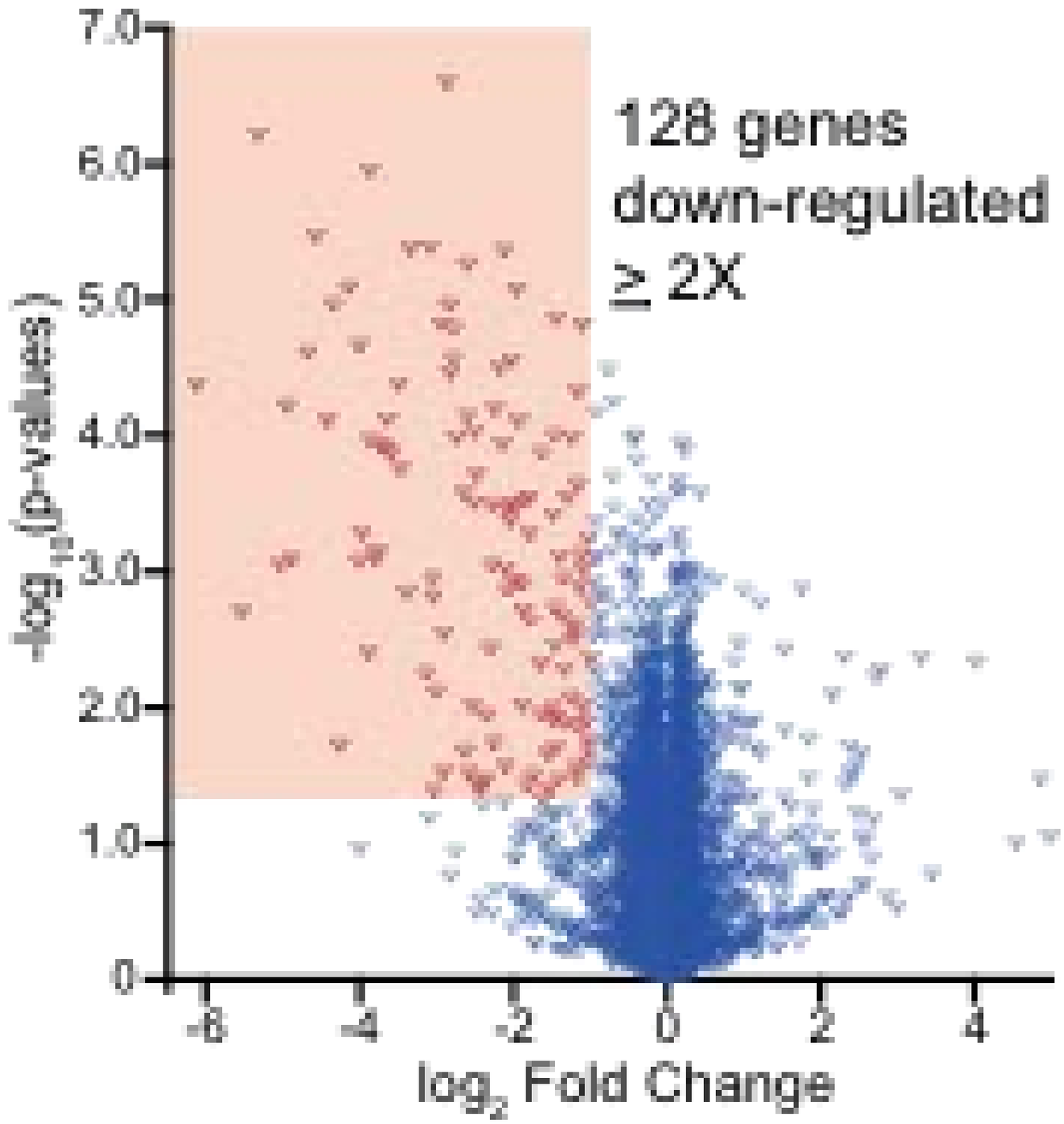
Collins et al. *Nature* 494: 476-479

EdU+ cells are sensitive to irradiation



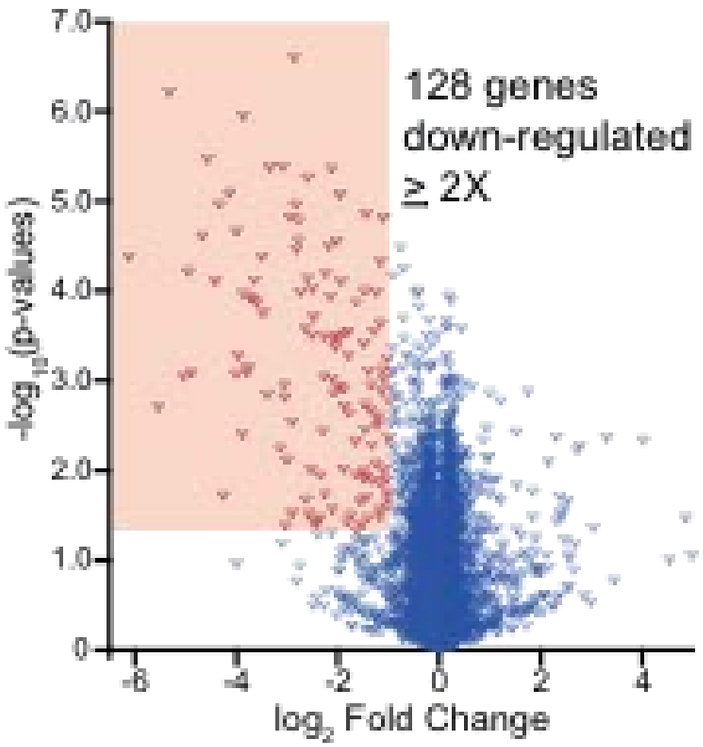
RNAseq Analysis

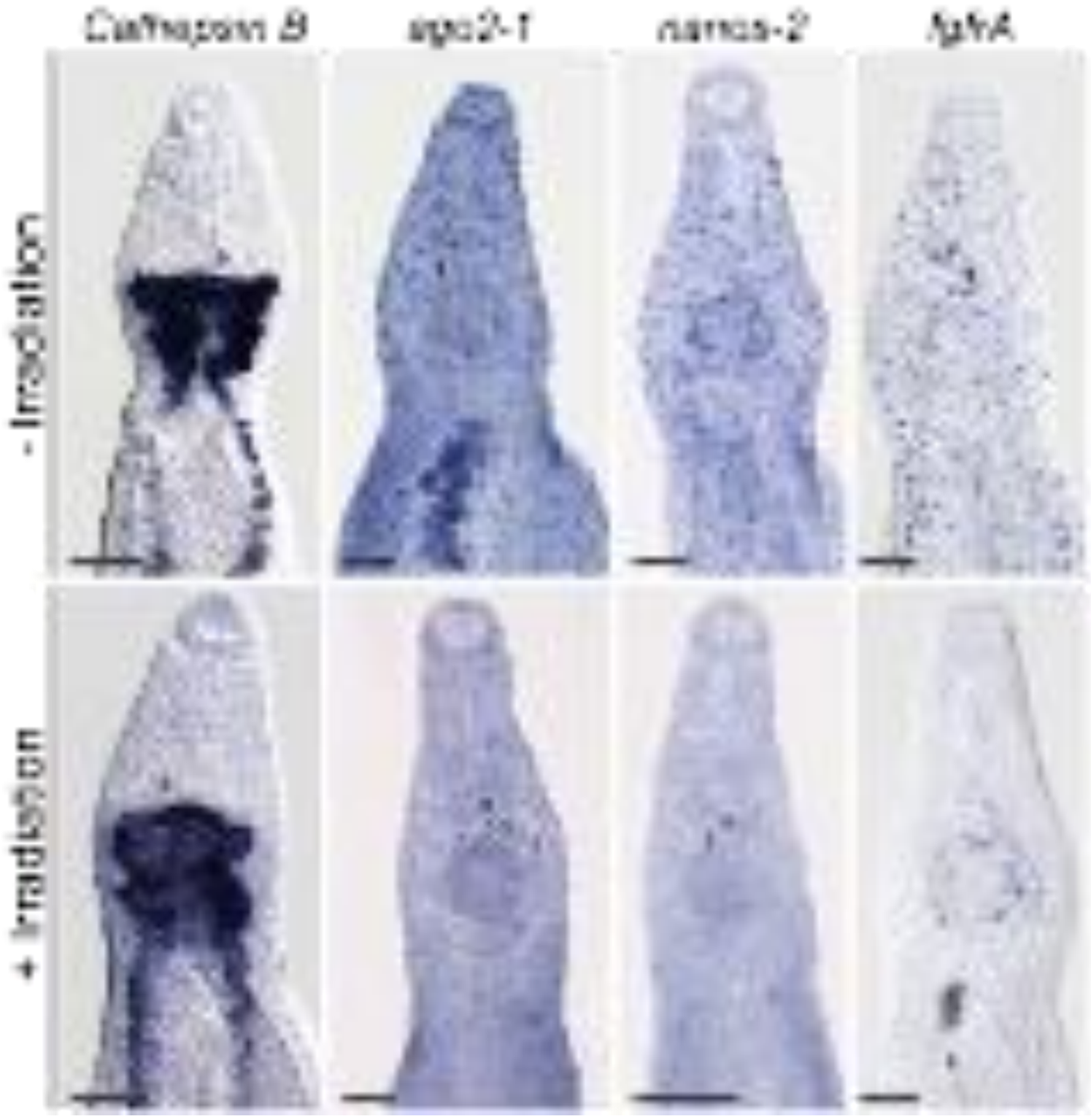




Collins et al. *Nature* 494: 476-479

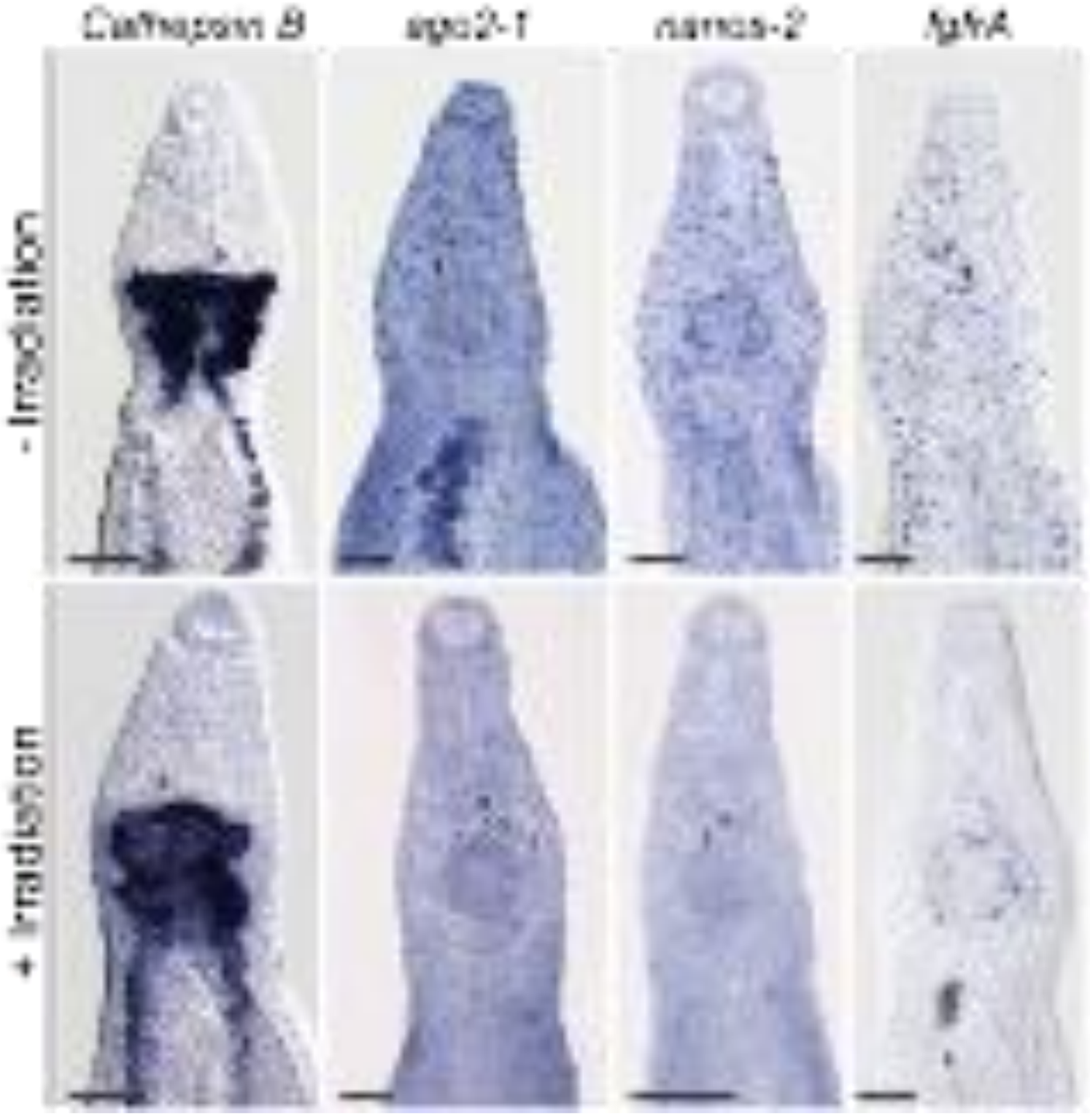
RNAseq Analysis

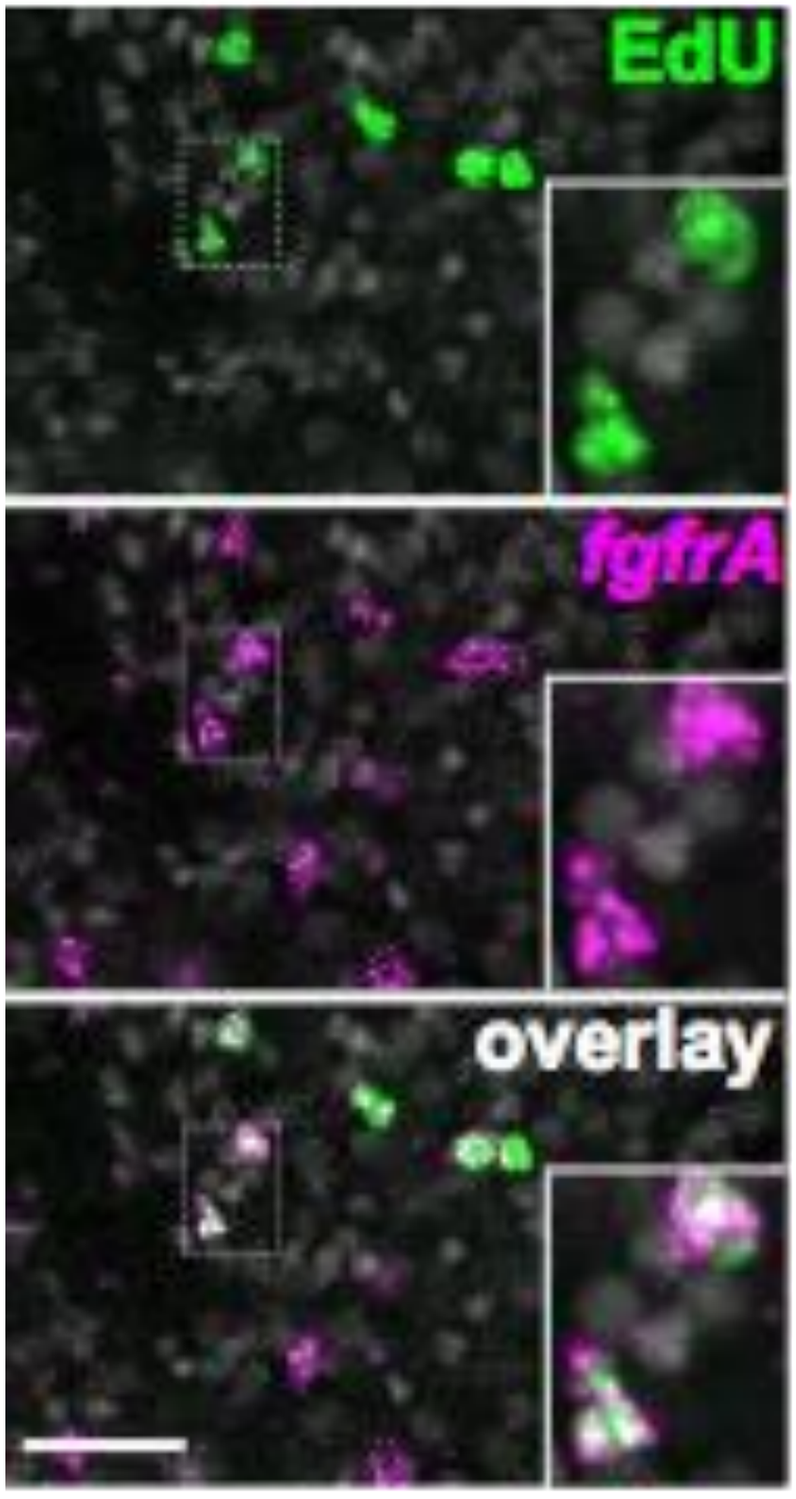




Collins et al. *Nature* 494: 476-479

Expression of radiation sensitive genes

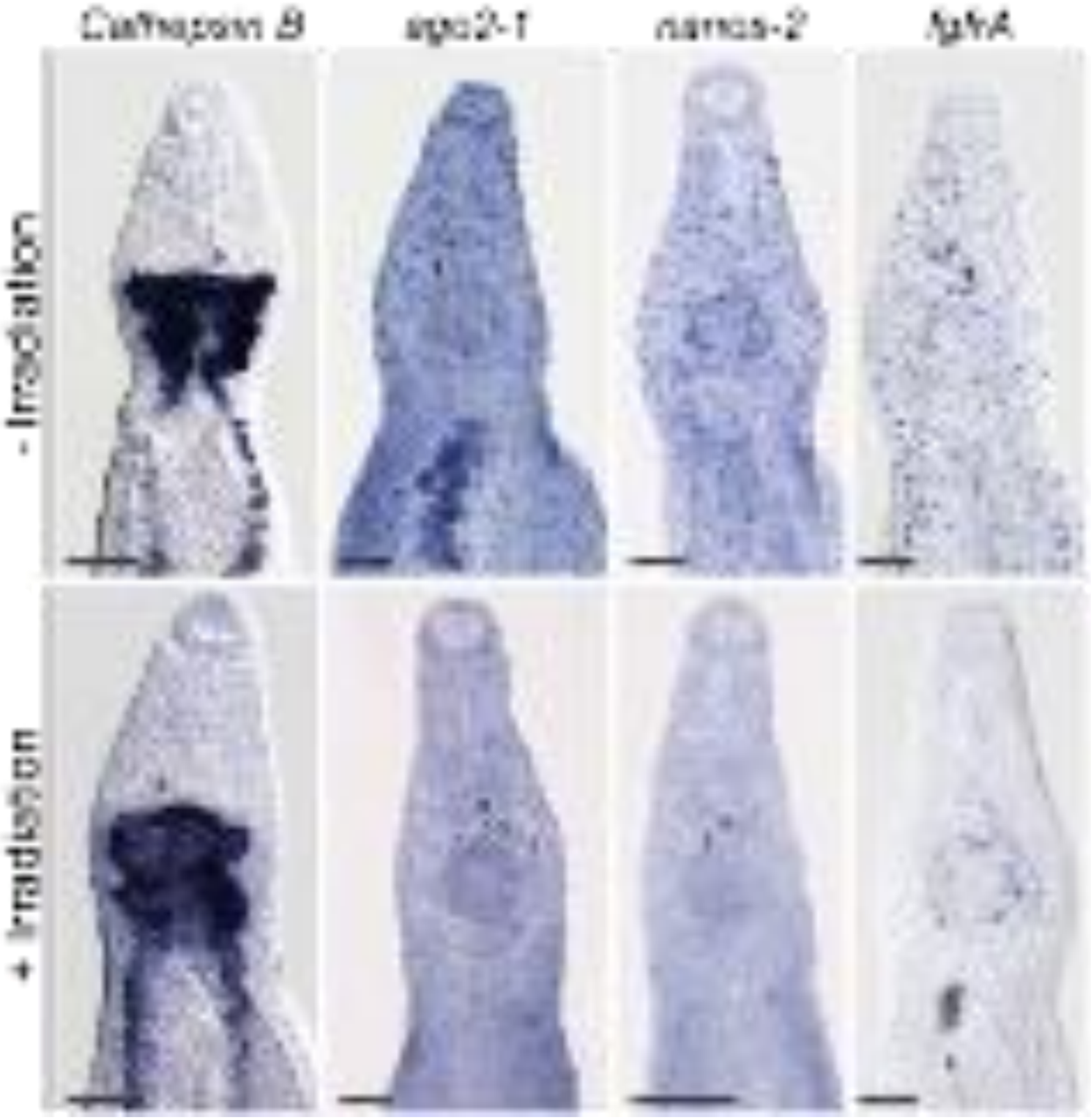


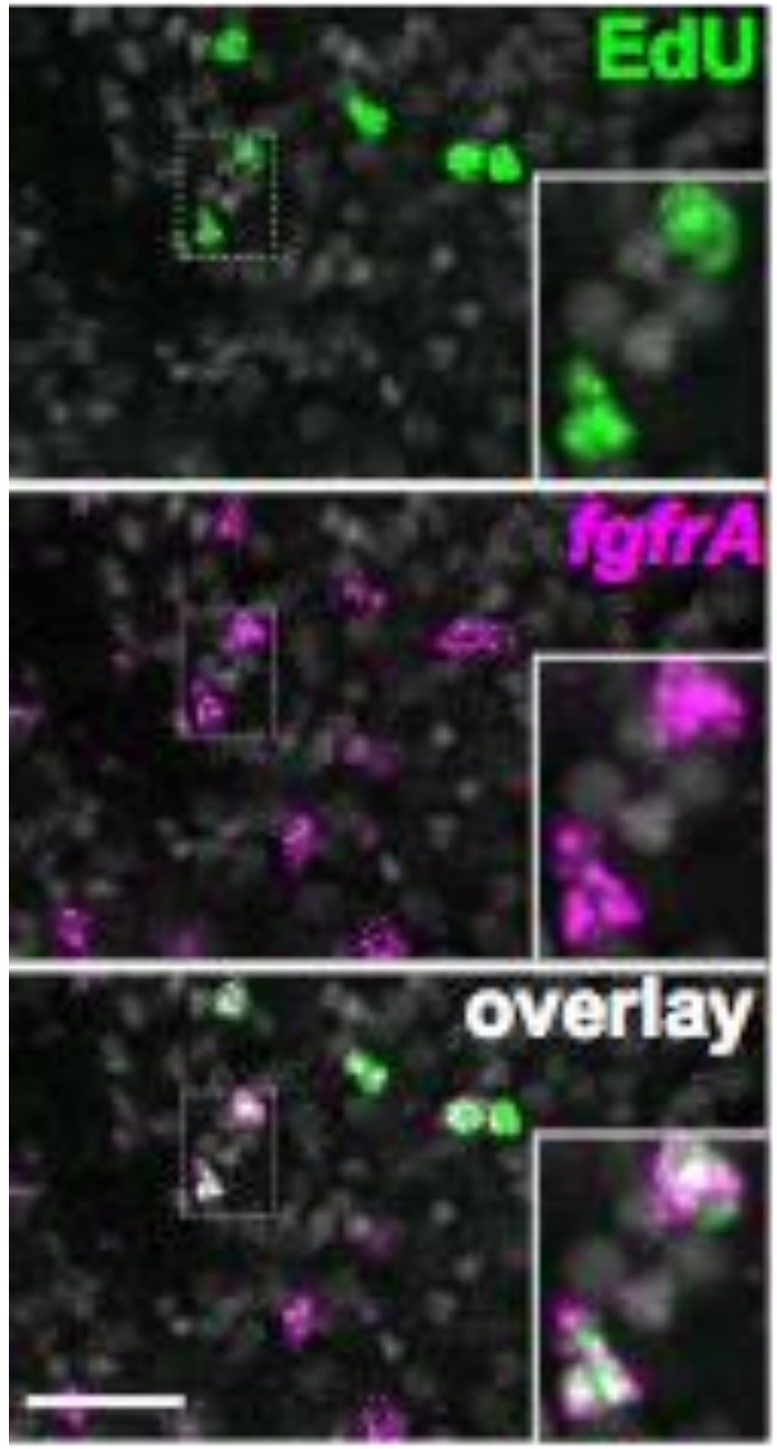


Scale Bar: 20 μm

Collins et al. *Nature* 494: 476-479

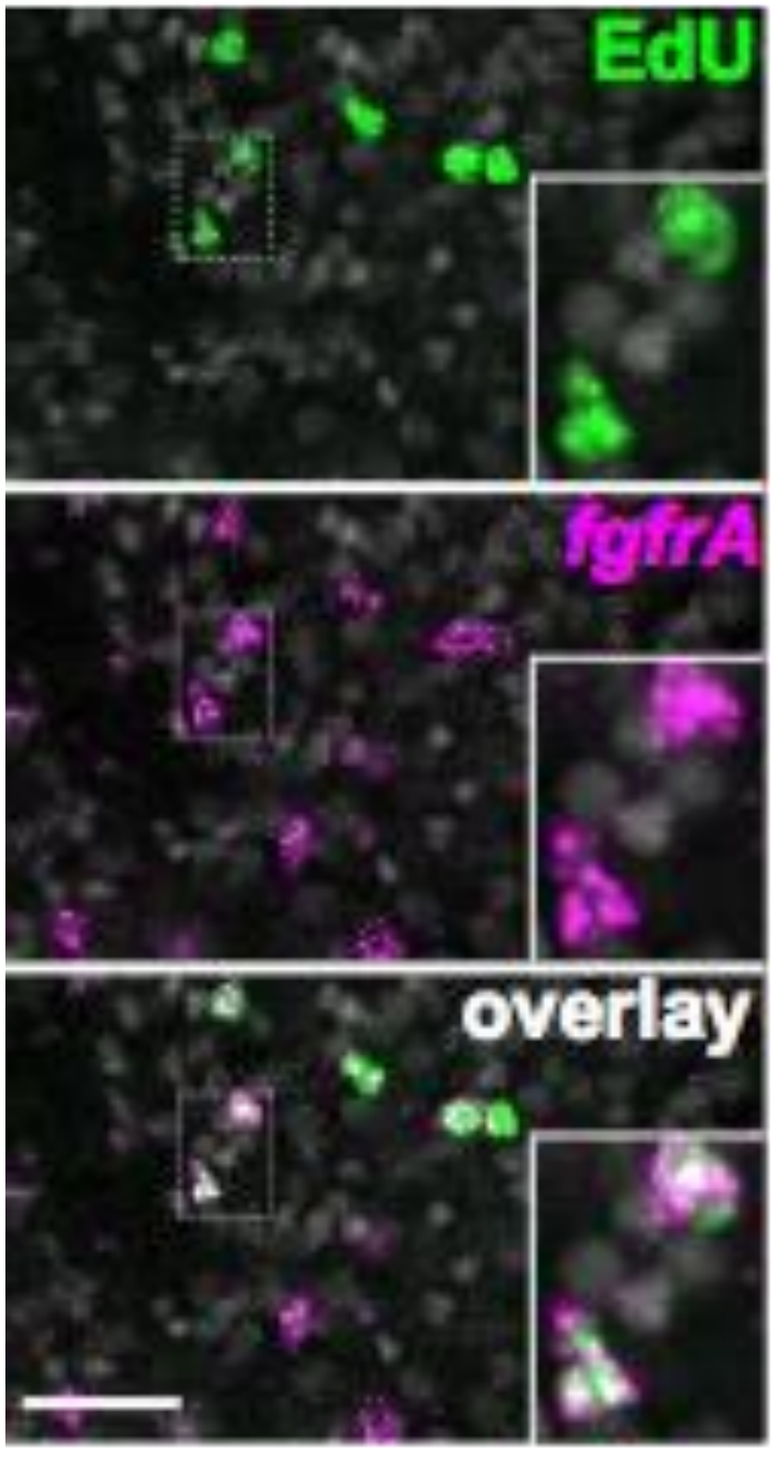
Expression of radiation sensitive genes

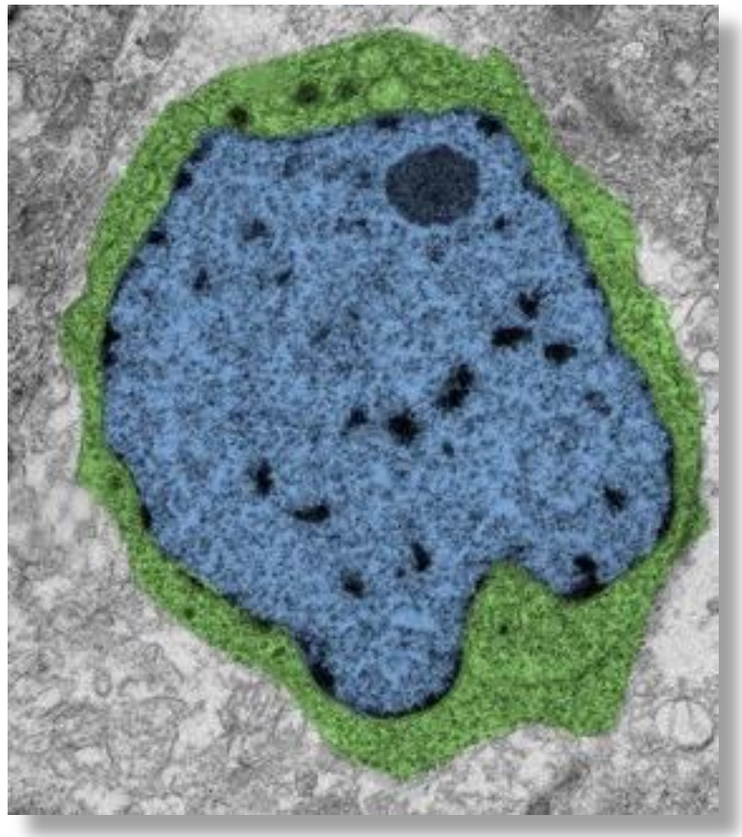




These cells look like neoblasts on the molecular level

Do these cells resemble neoblasts morphologically?





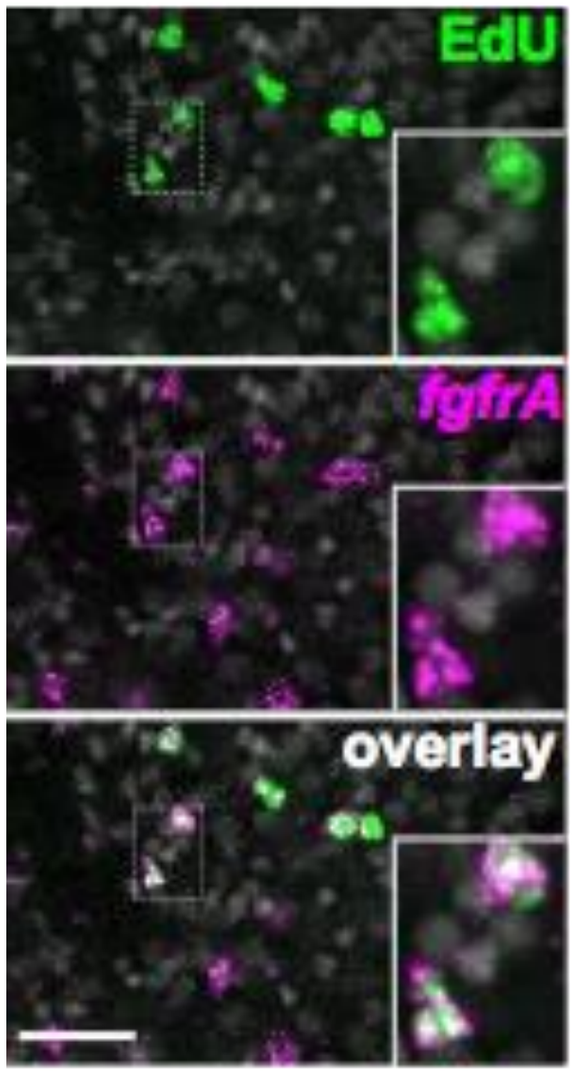
Planarian
Neoblast

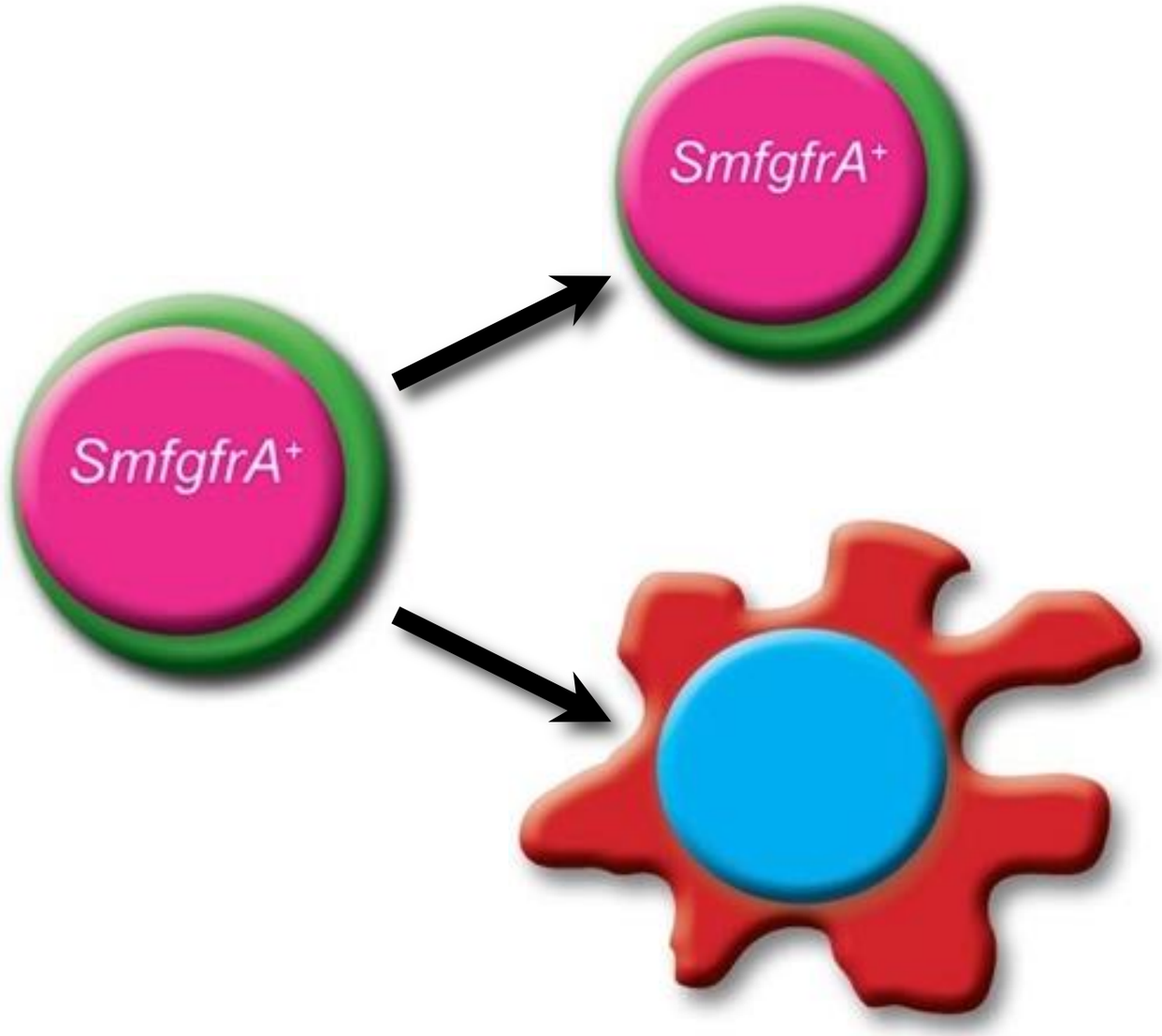
Collins et al. *Nature* 494: 476-479

Are these cells stem cells?

- self-renew
- differentiate

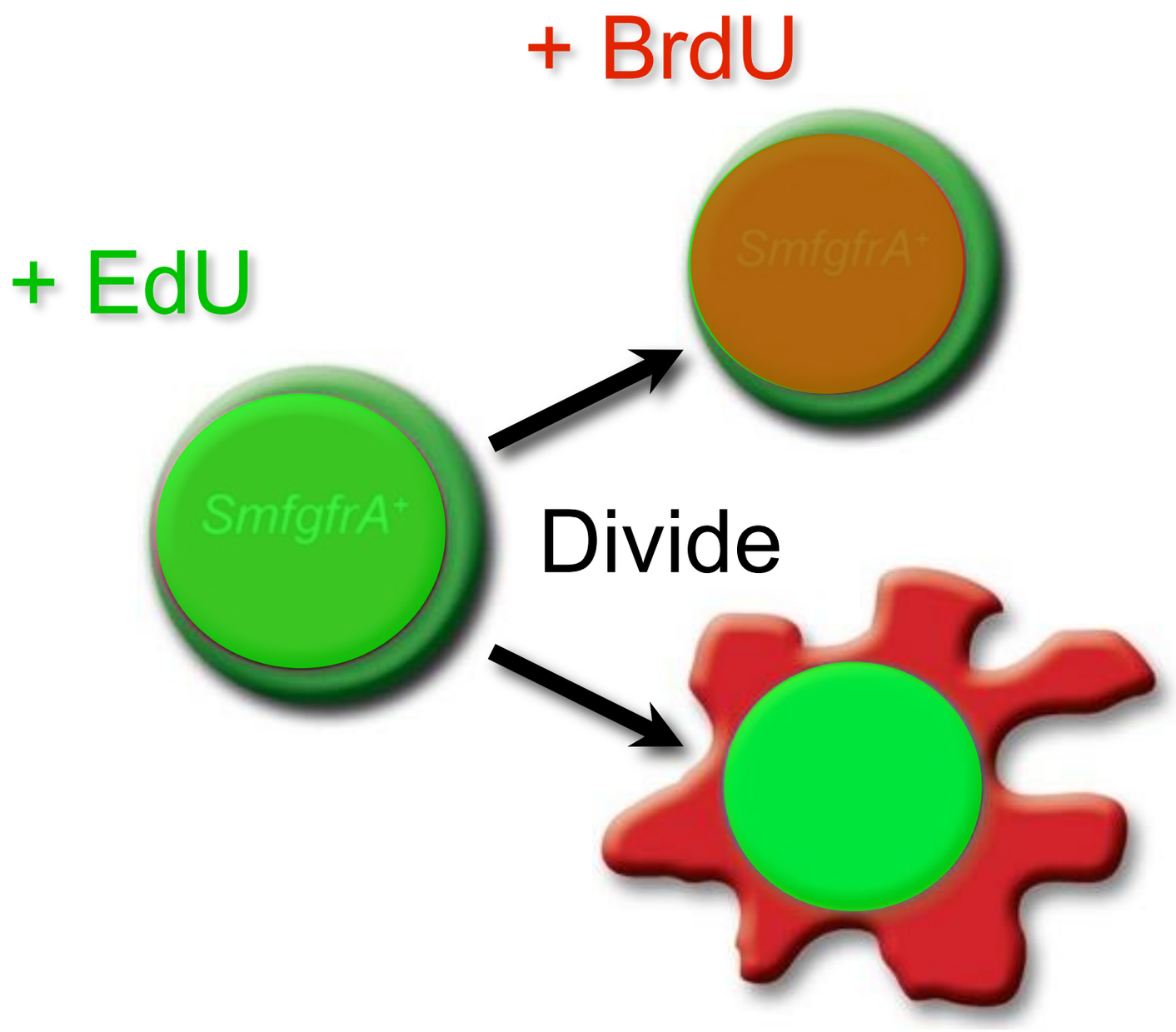
Do EdU-incorporating cells self-renew?



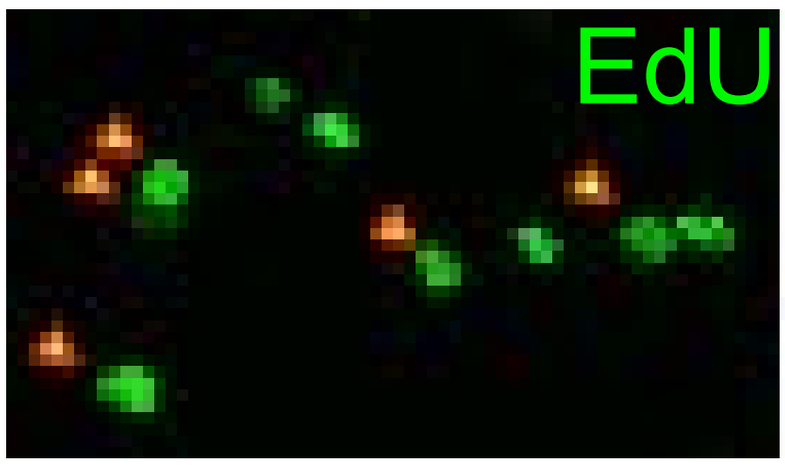


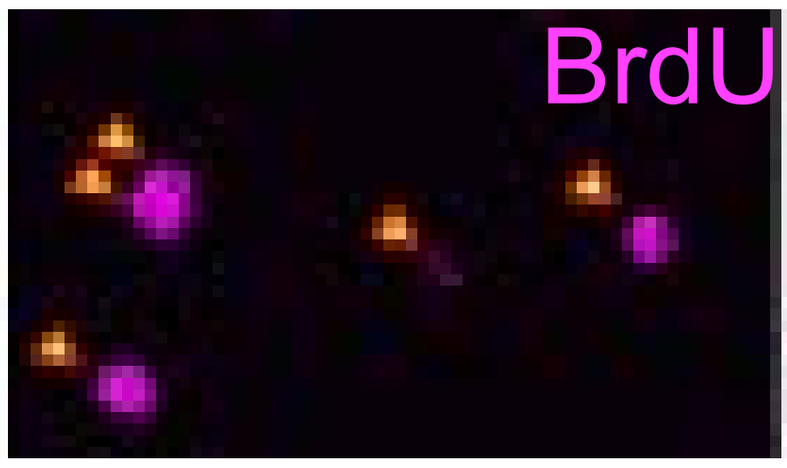
fgfrA-expressing are the only cells that enter S-phase (i.e. only EdU-incorporating cells)

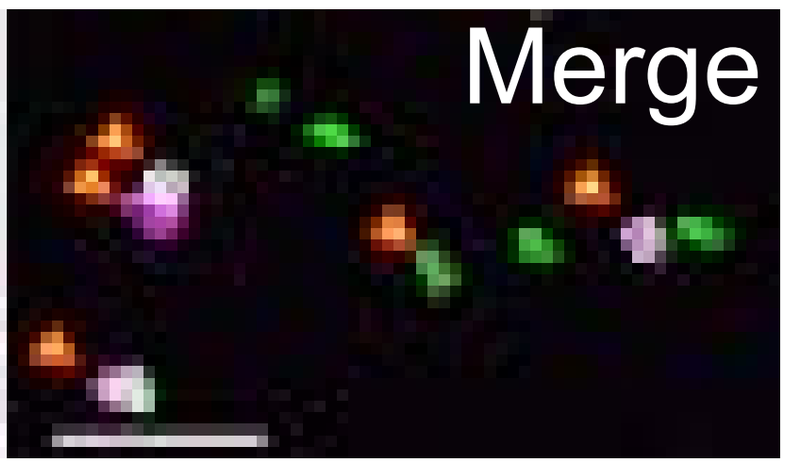
Do EdU-incorporating cells self-renew?



Do EdU-incorporating cells self-renew?







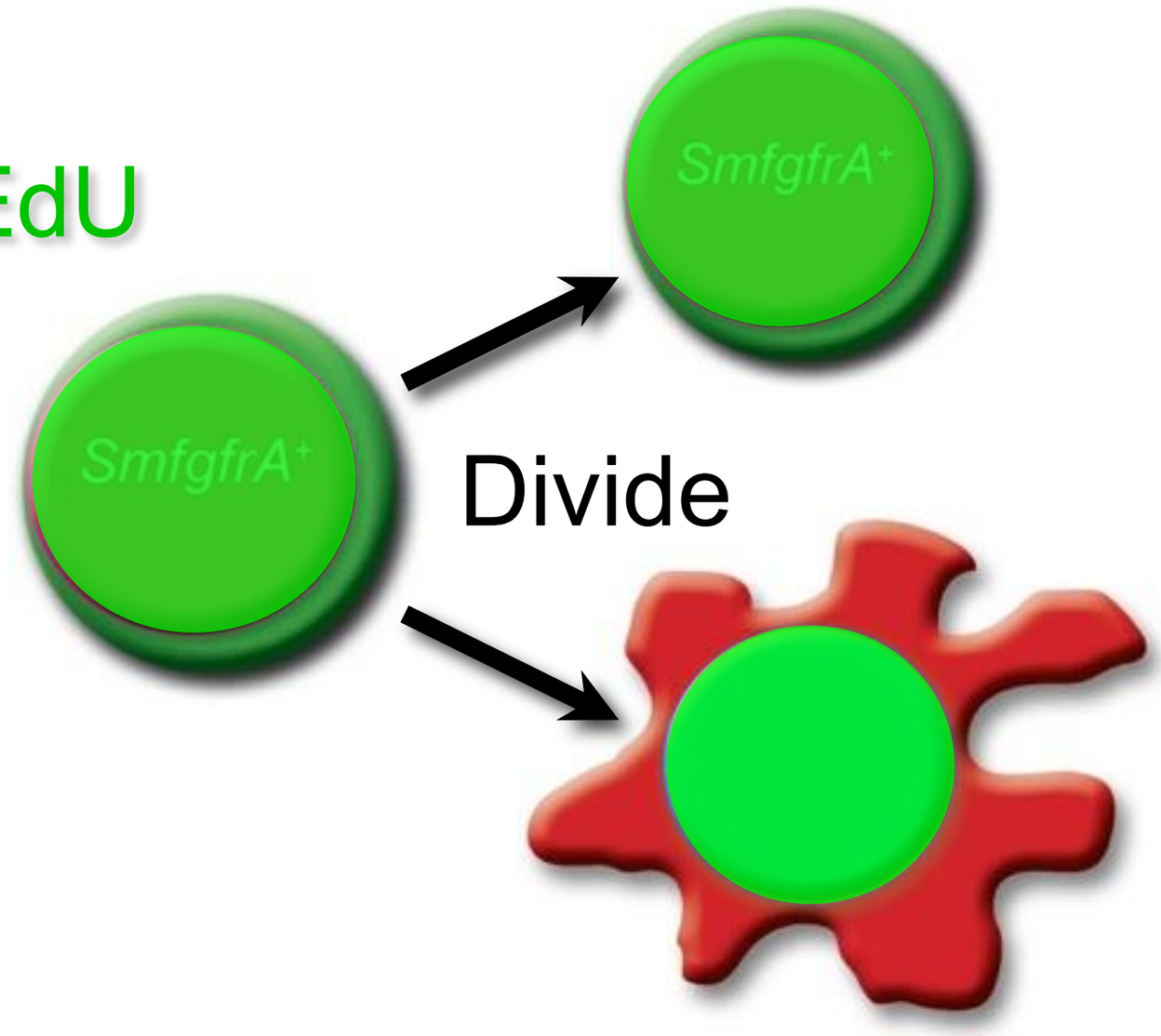
Consistent with model these cells self-renew

Scale Bar:
20 μm

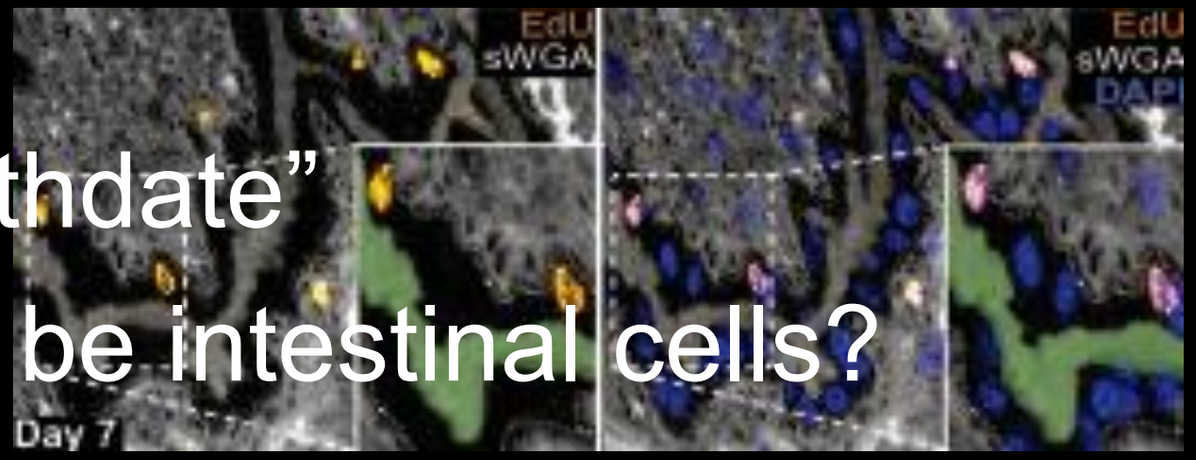
Collins et al. *Nature* 494: 476-479

Do EdU-incorporating cells differentiate?

- self-renewal
- differentiation



Do EdU-incorporating cells differentiate?



EdU "Birthdate"

Do they "grow up" to be intestinal cells?

These cells differentiate

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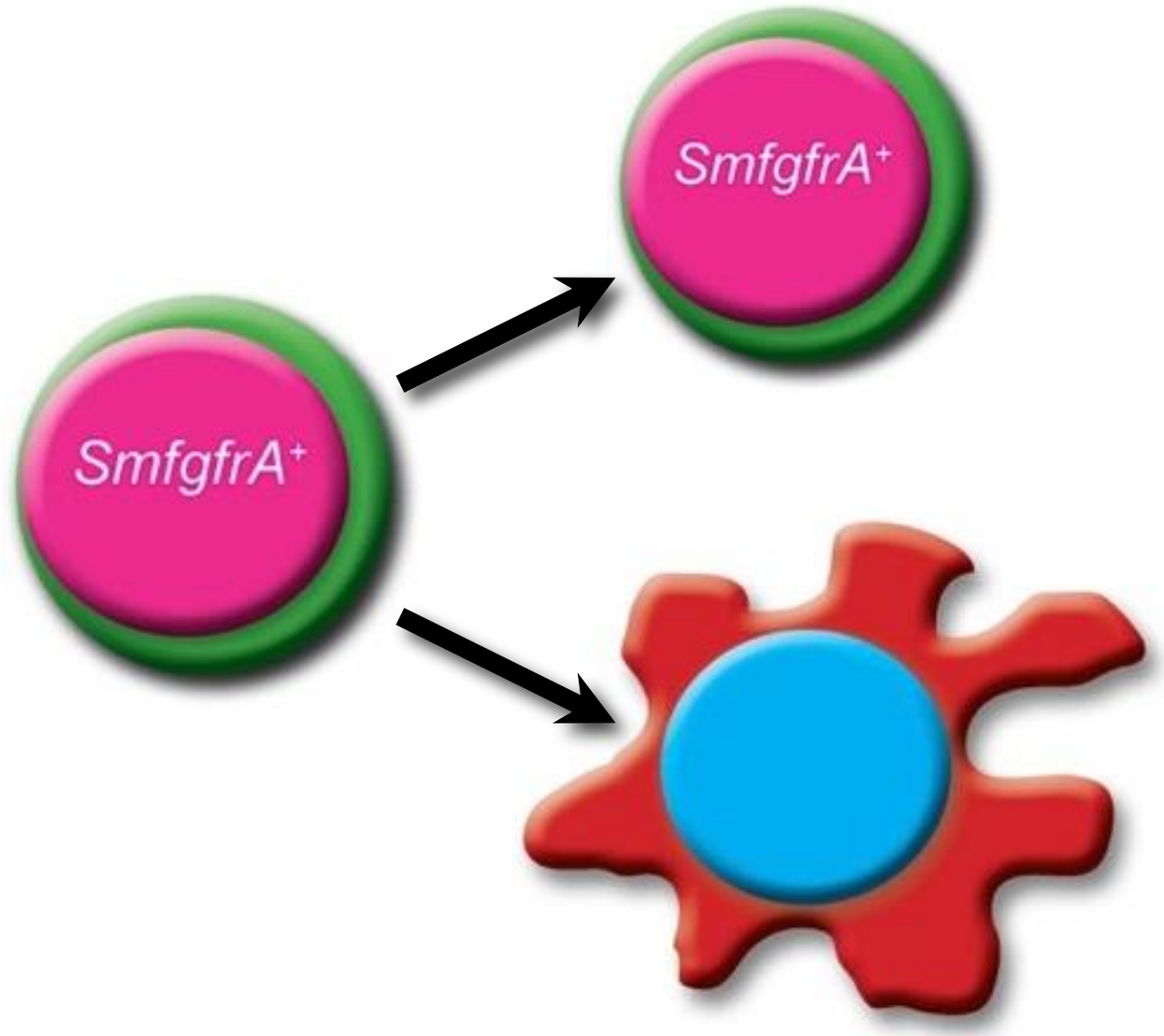
EdU-incorporating cells are stem cells

- self-renew
- differentiate



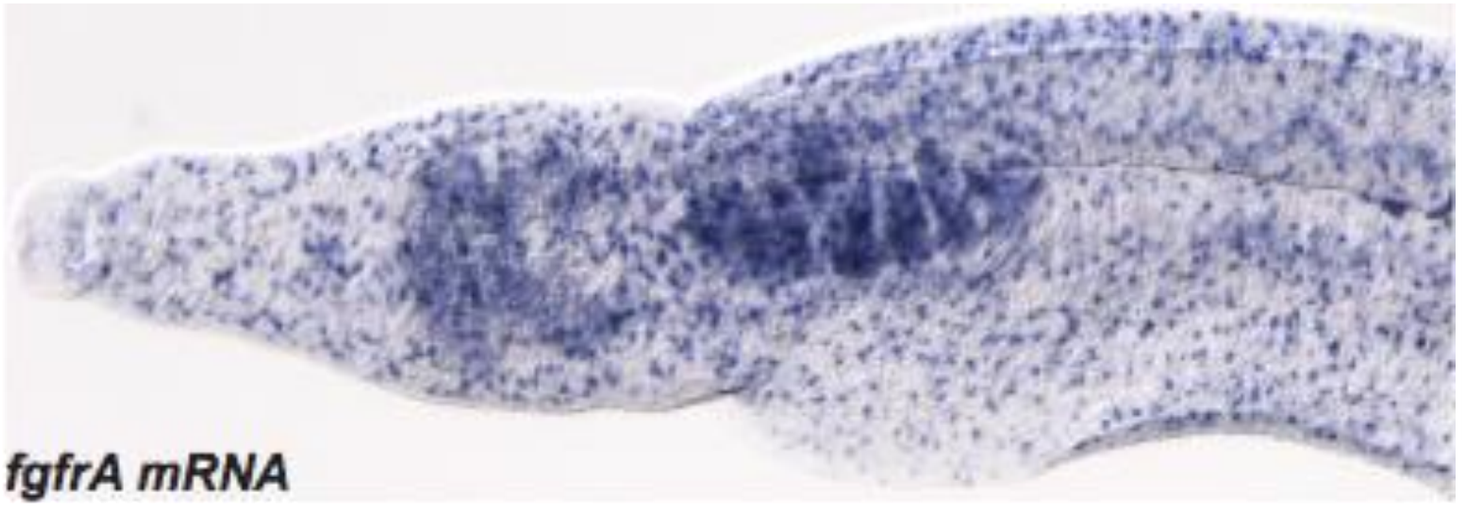
Intestine

<u>also Muscle</u>

These cells are Neoblast-like adult stem cells

Can we functionally manipulate these cells?

What factors regulate these stem cells?



FGF signaling regulates diverse stem cell populations

Control

fgfrA(RNAi)

Collins et al. *Nature* 494: 476-479

What factors regulate these stem cells?

Control

fgfrA(RNAi)

fgfrA is essential for neoblast maintenance

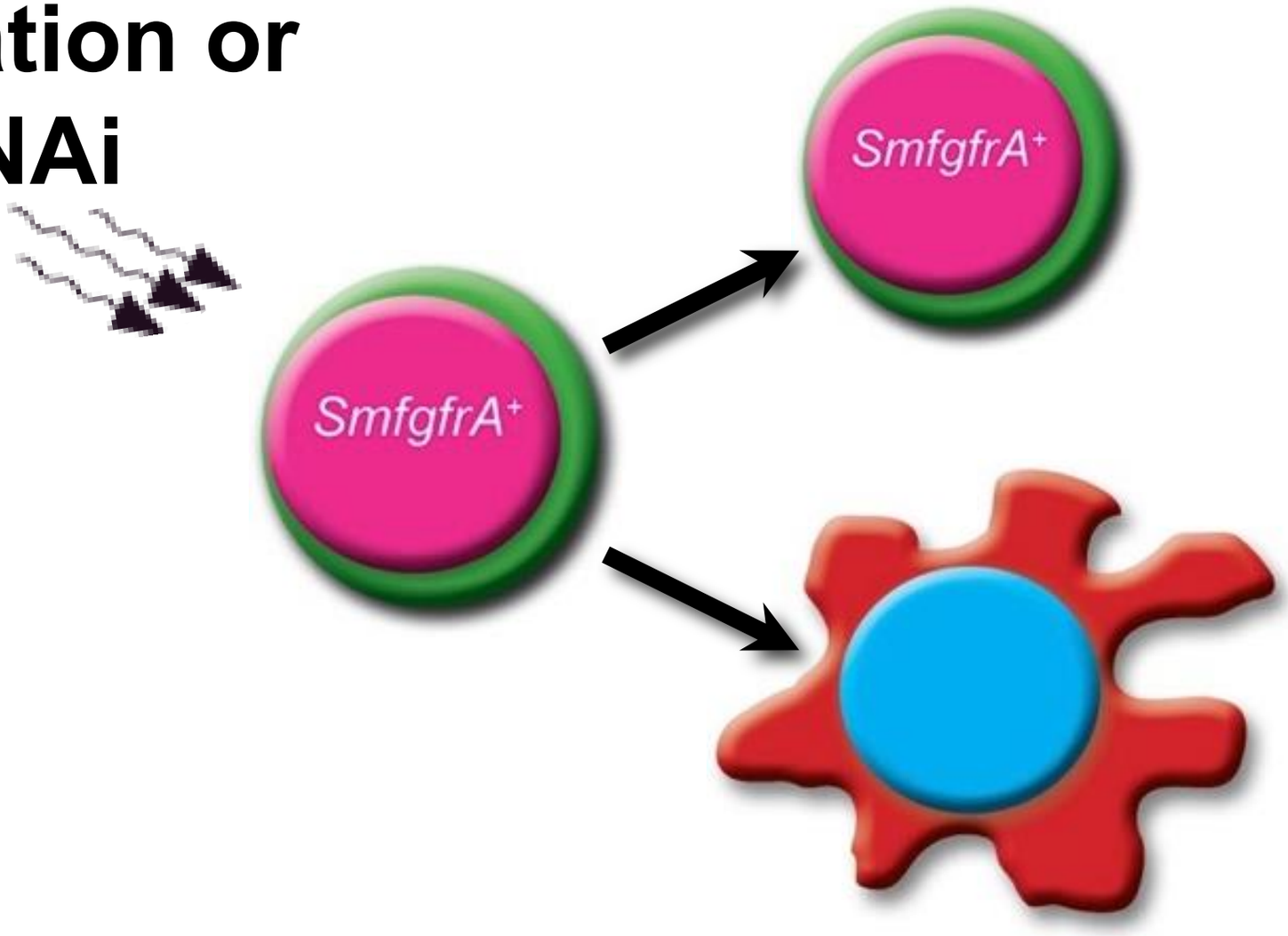
Suggests common mechanisms may regulate mammalian and schistosome stem cells

Demonstrates that neoblasts are susceptible to RNAi

What is the function of these neoblast-like cells in the parasite?

Determining the function of Schistosome neoblasts

Irradiation or RNAi



0-48 hrs

> 48 hrs

Loss of stem cell progeny?

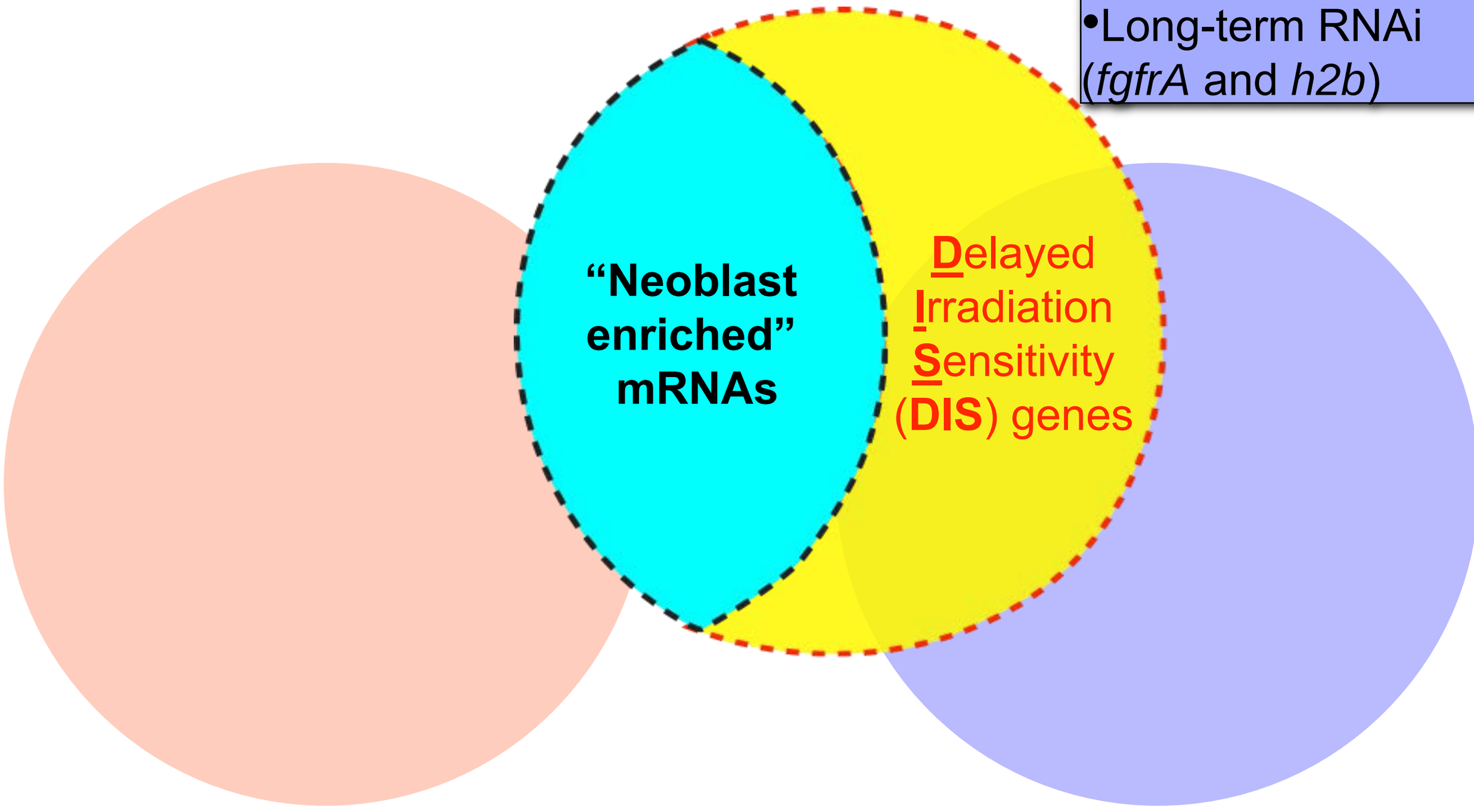
Examine the long-term transcriptional consequences of stem cell depletion

Transcriptional profiling after stem cell depletion

Genes Down:
- 48 hrs following irradiation

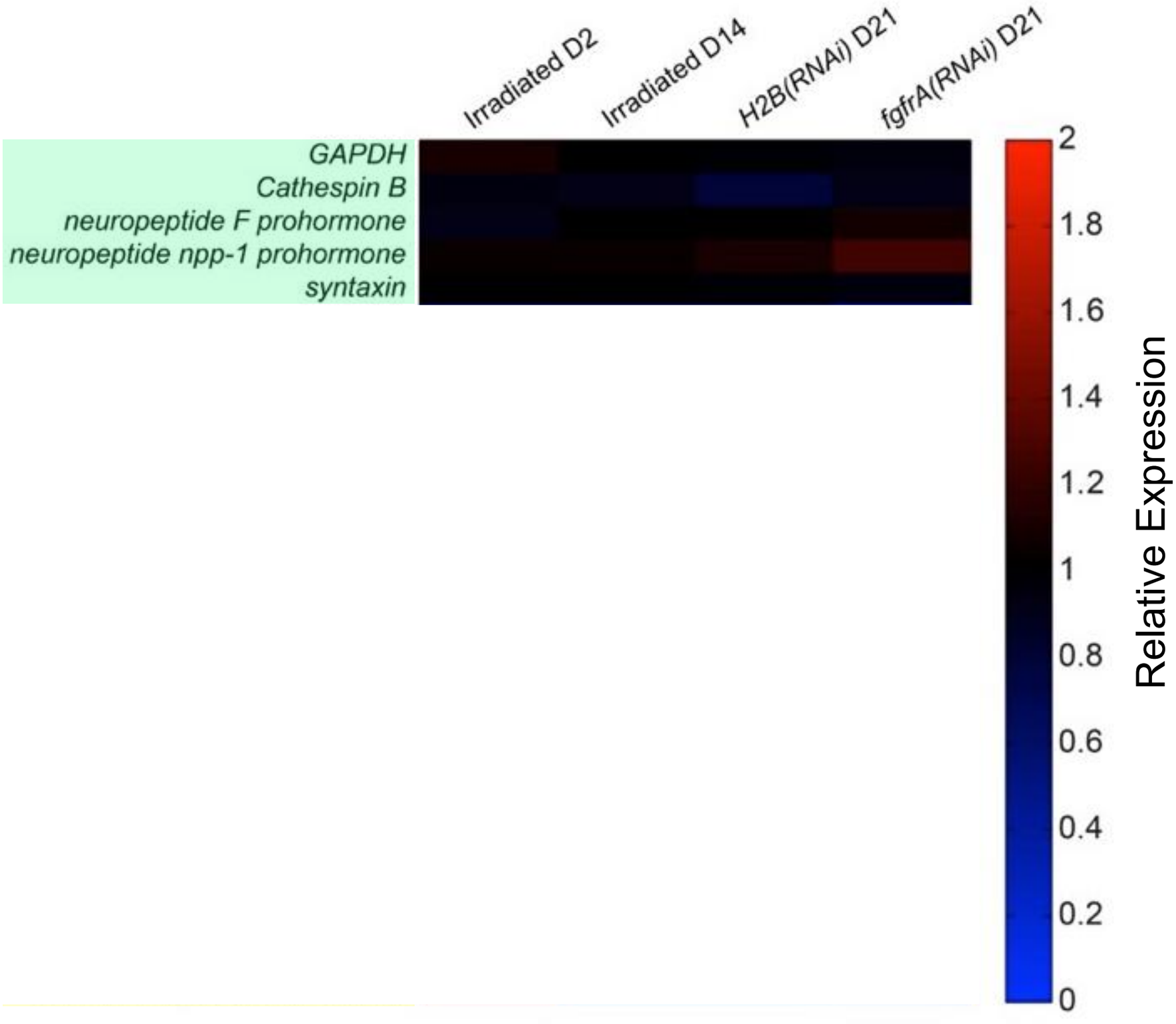
Genes Down:
- 2 weeks following irradiation

-and-
- Long-term RNAi (*fgfrA* and *h2b*)



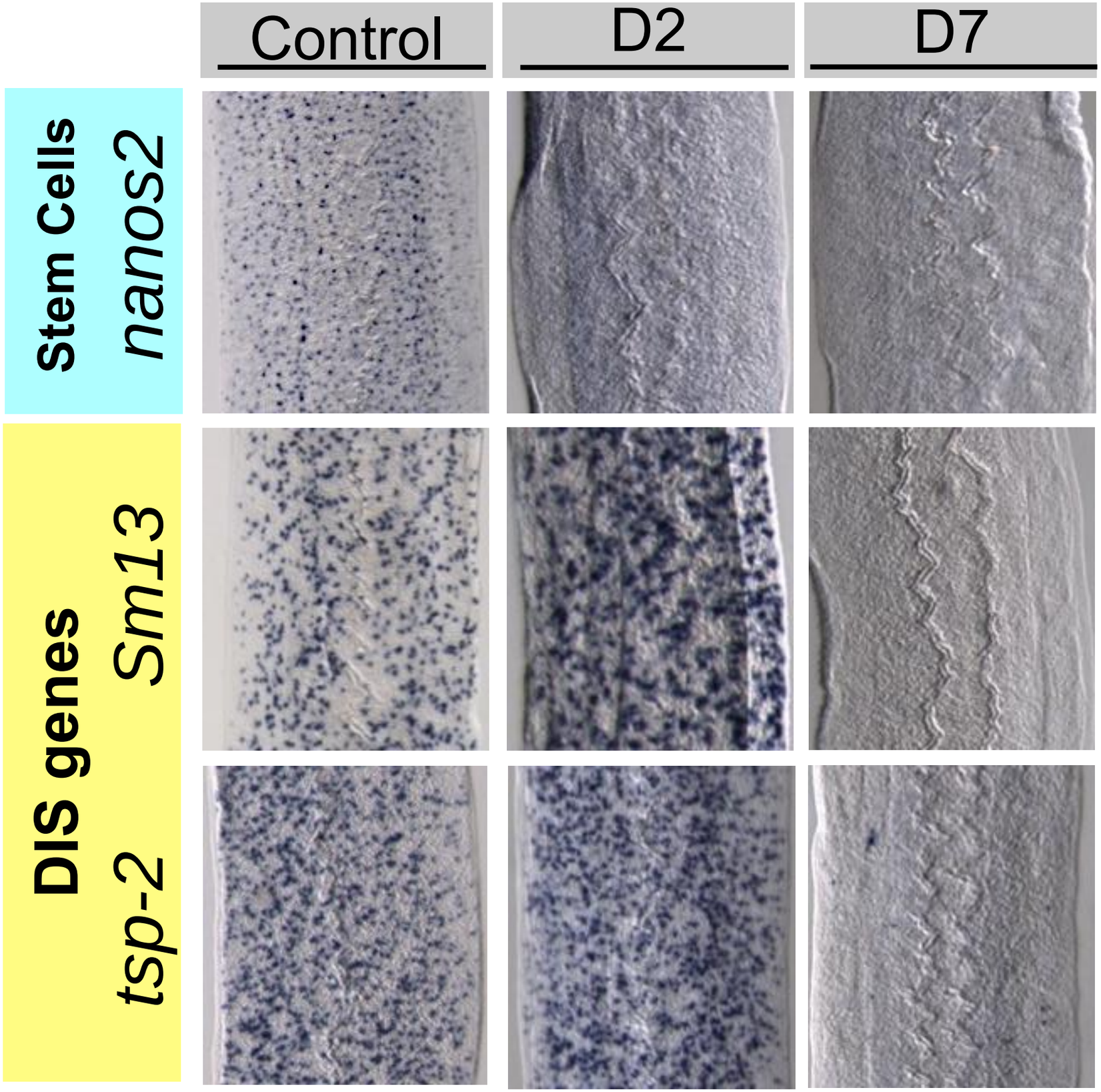
Transcriptional profiling after stem cell depletion

Unchanged
~98% of genes



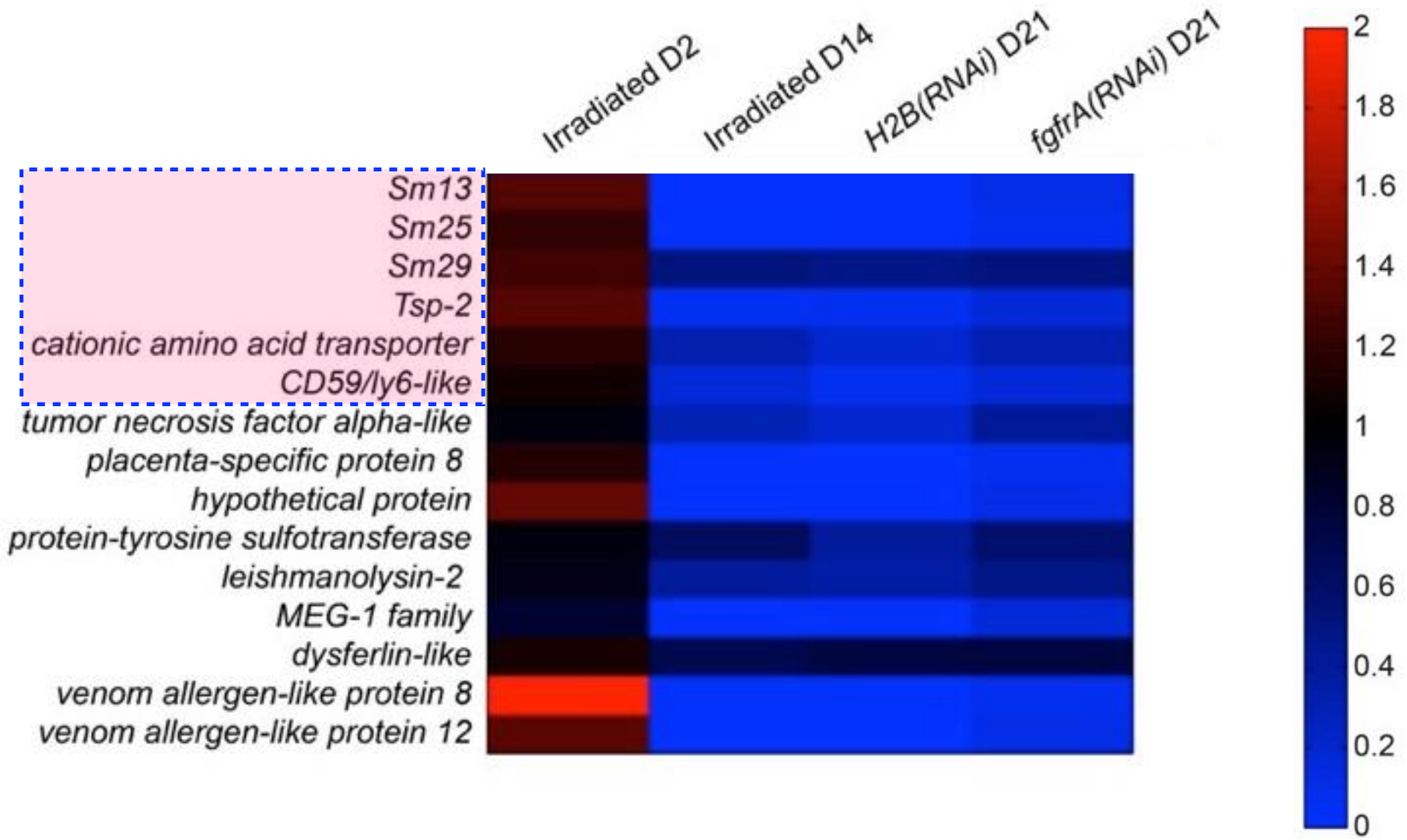
Collins and Newmark, Unpublished

Cells expressing DIS genes are lost after irradiation



Collins and Newmark, Unpublished

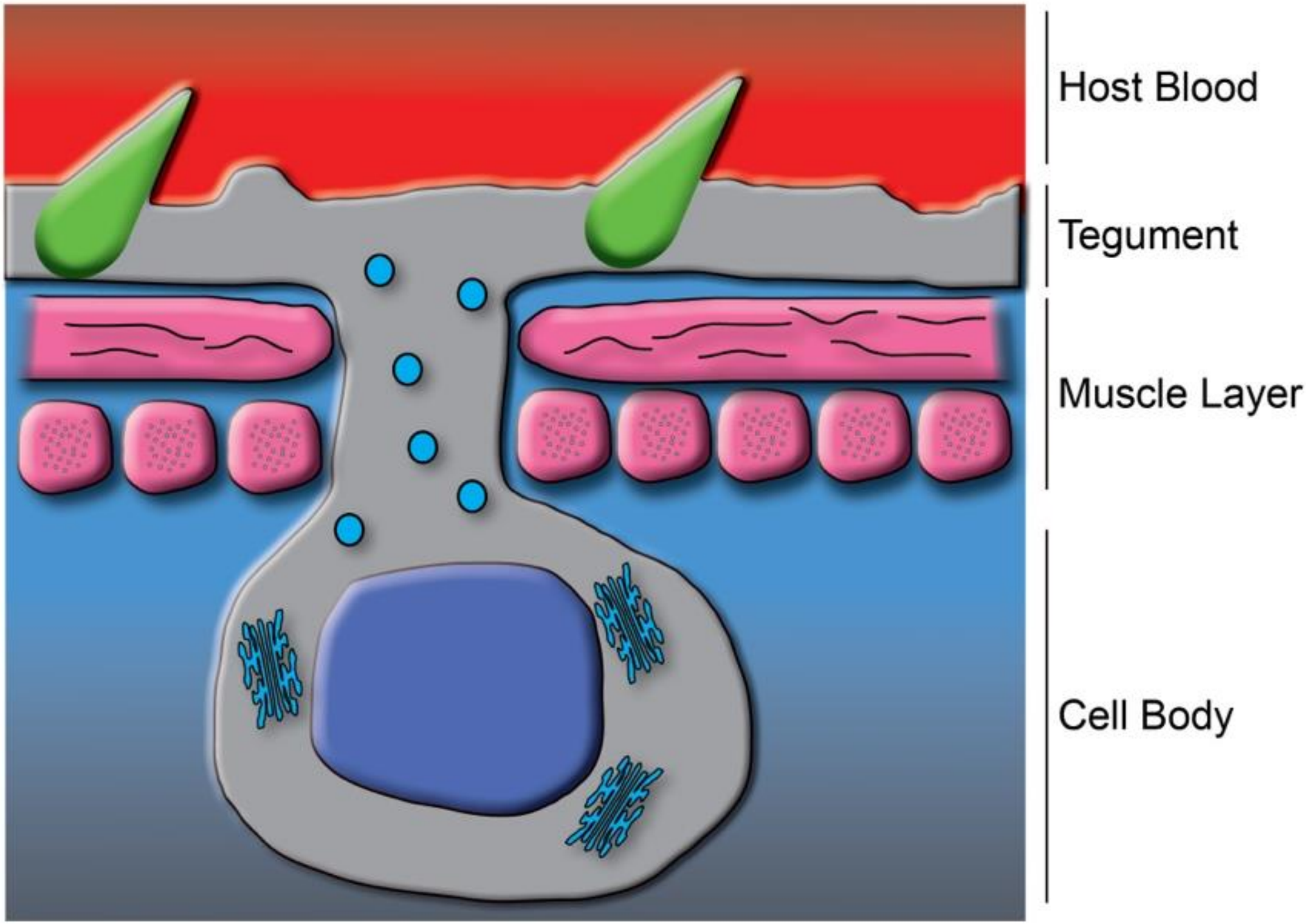
Many DIS genes are associated with the tegument



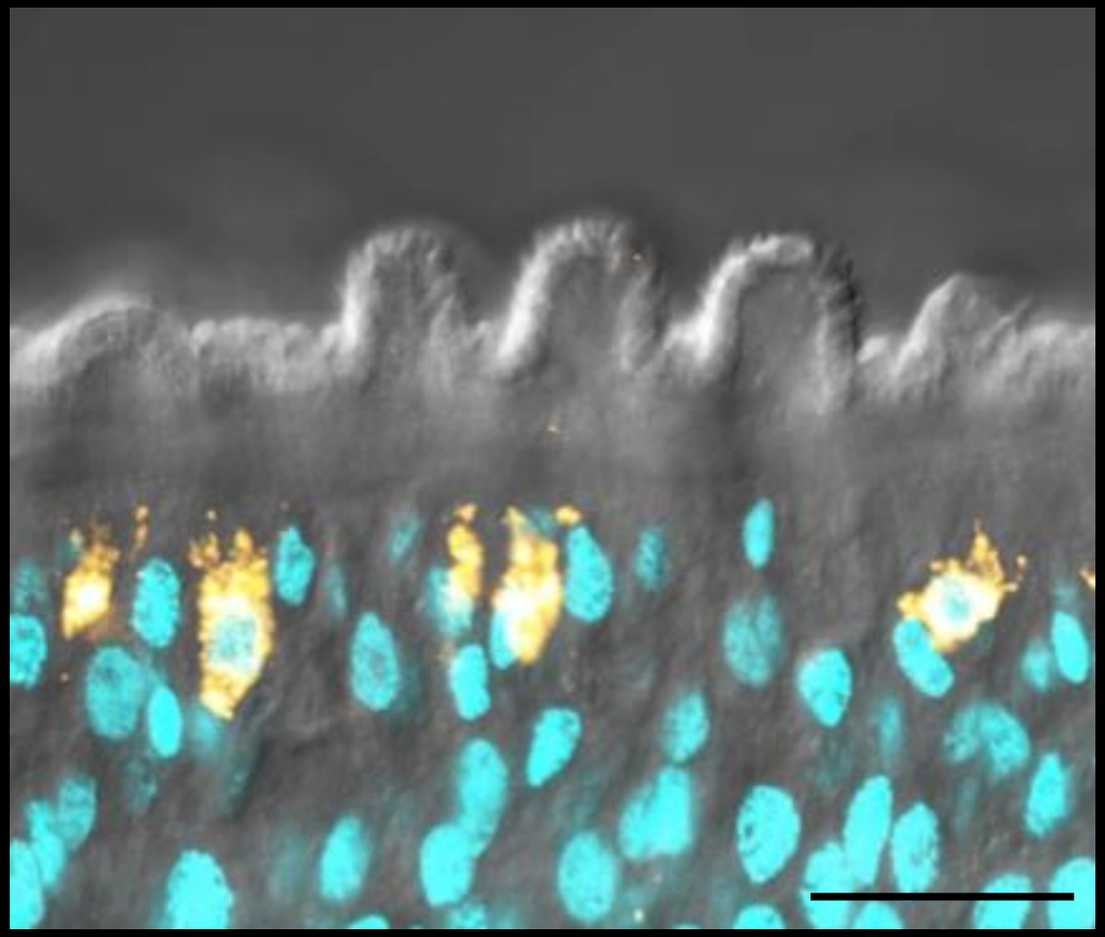
Expressed at parasite surface

***a.k.a.* Tegument**

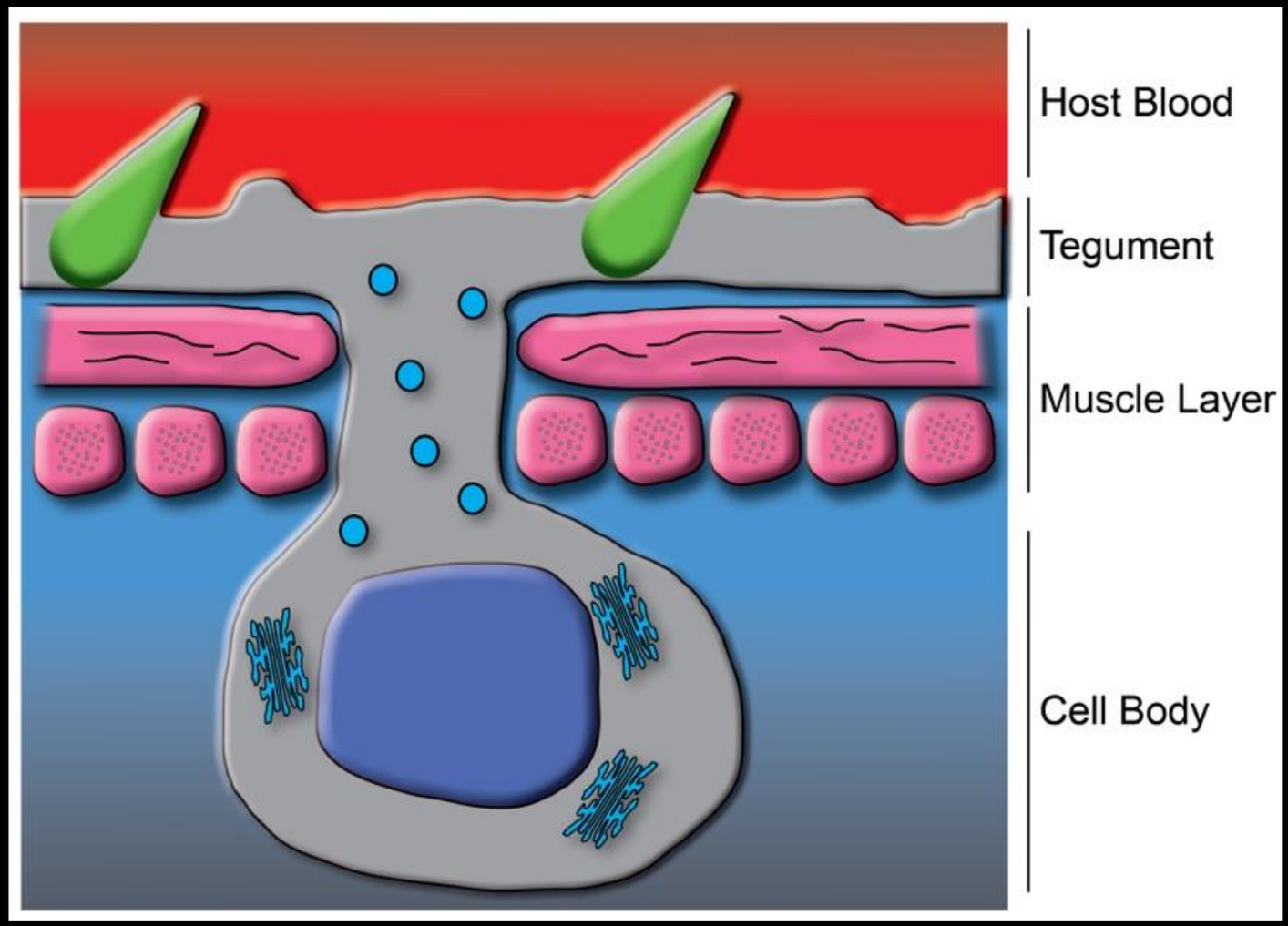
The schistosome tegument



DIS genes are co-expressed



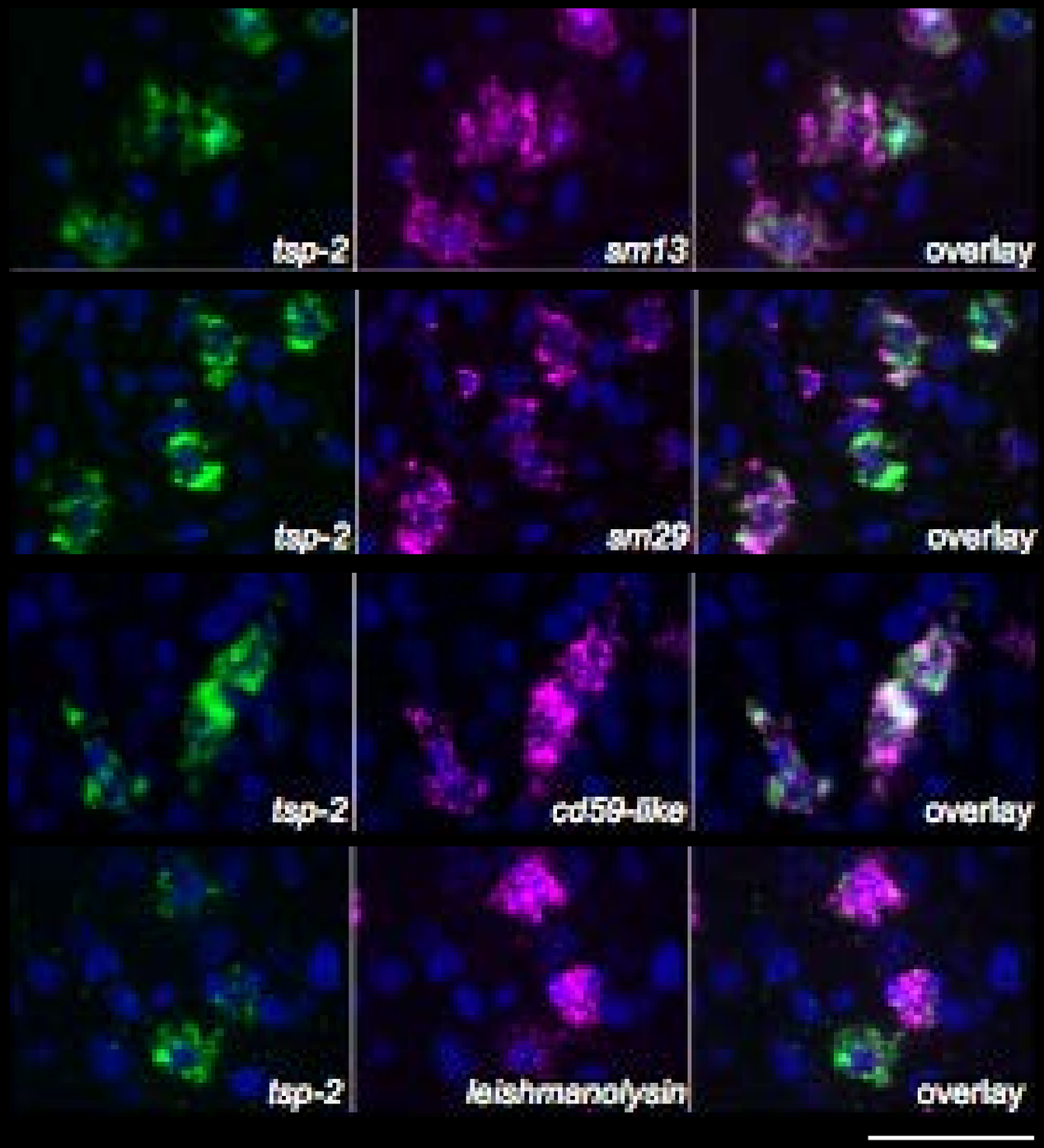
Nuclei
tsp2

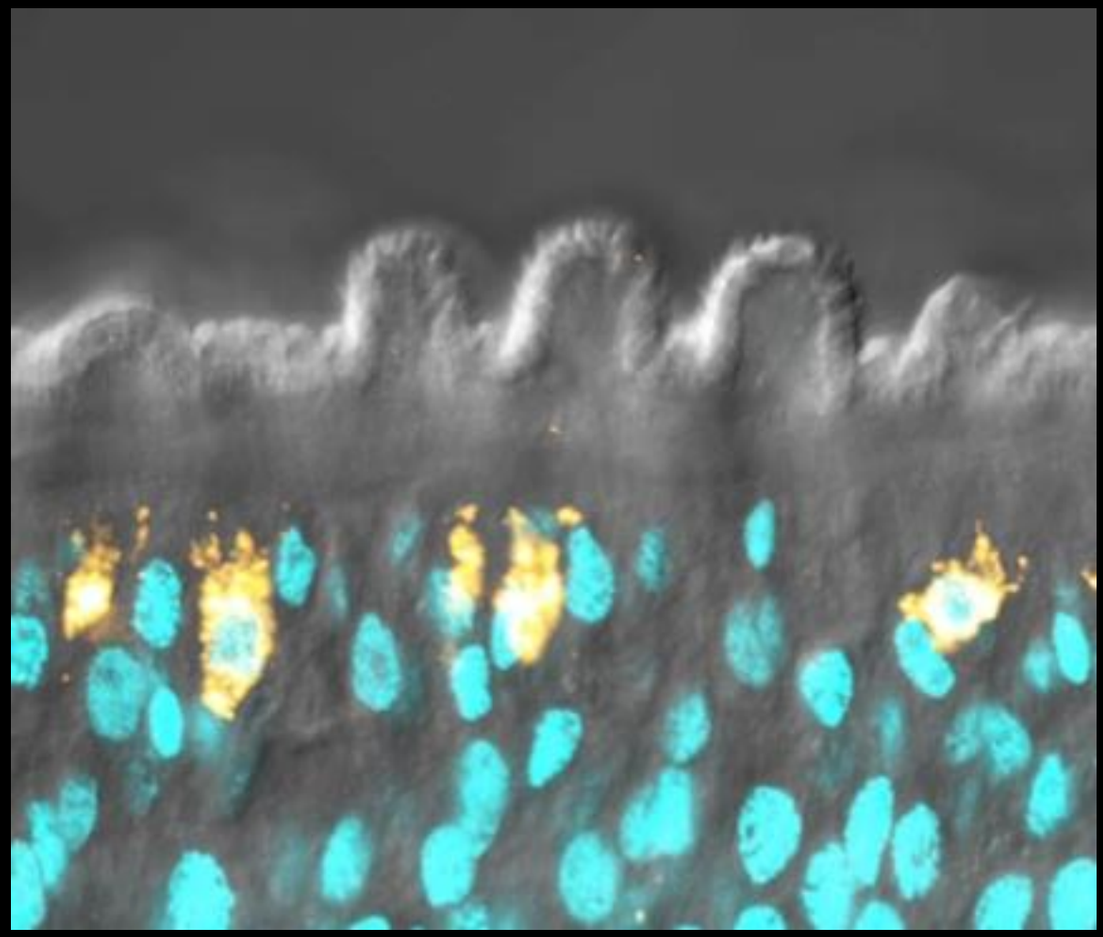


Scale Bar: 20 μm

Collins and Newmark, Unpublished

DIS genes are co-expressed in the tegument





Thus far, ALL DIS genes are expressed in *tsp2*$^{+}$ tegumental cells

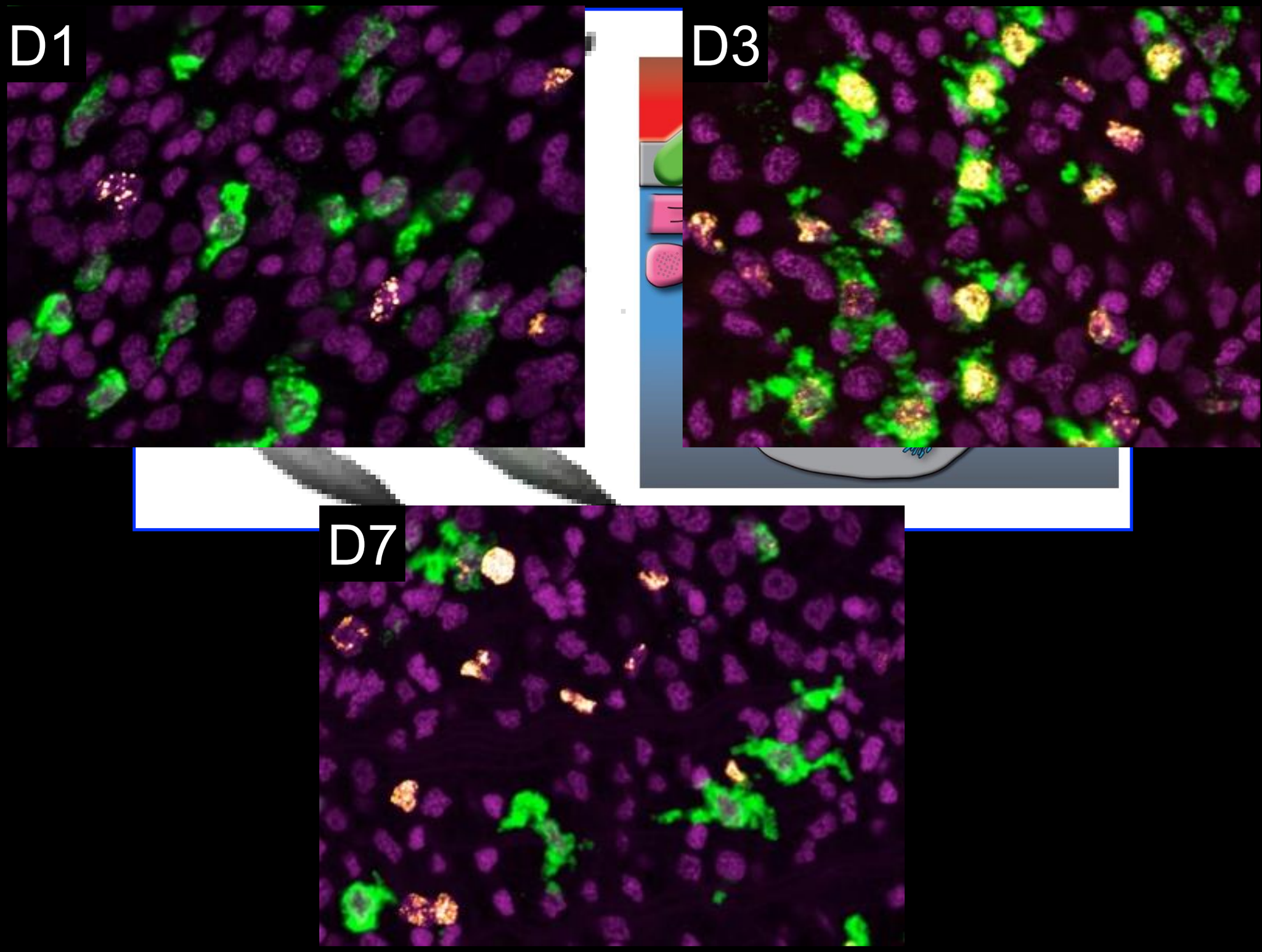
Scale Bar: 20 μm

Collins and Newmark, Unpublished

Are the neoblast-like cells a source of new tegumental cells?

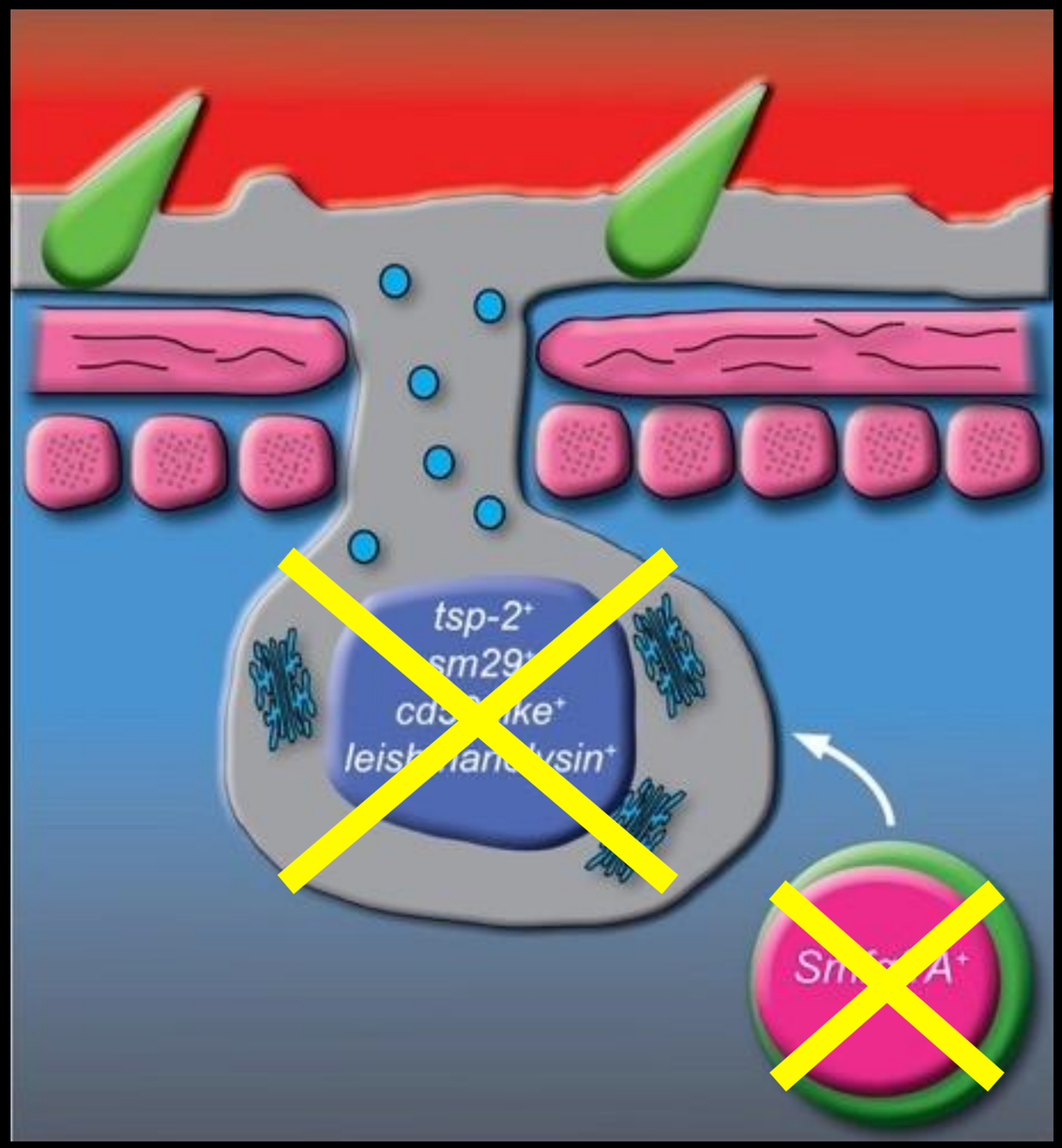
Neoblasts rapidly differentiate into *tsp-2*$^{+}$ cells

tsp2
EdU
DAPI



Collins and Newmark, Unpublished

Summary



A tegumental cell population is rapidly renewed by neoblasts

These tegumental cells are rapidly turned over

Role in survival and immune evasion?

Schistosomiasis is a disease of stem cells

Reproductive stem cells

Somatic stem cells