

Molecular mechanisms for corticogenesis



NEURO GLOBAL
Tohoku University

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Noriko Osumi



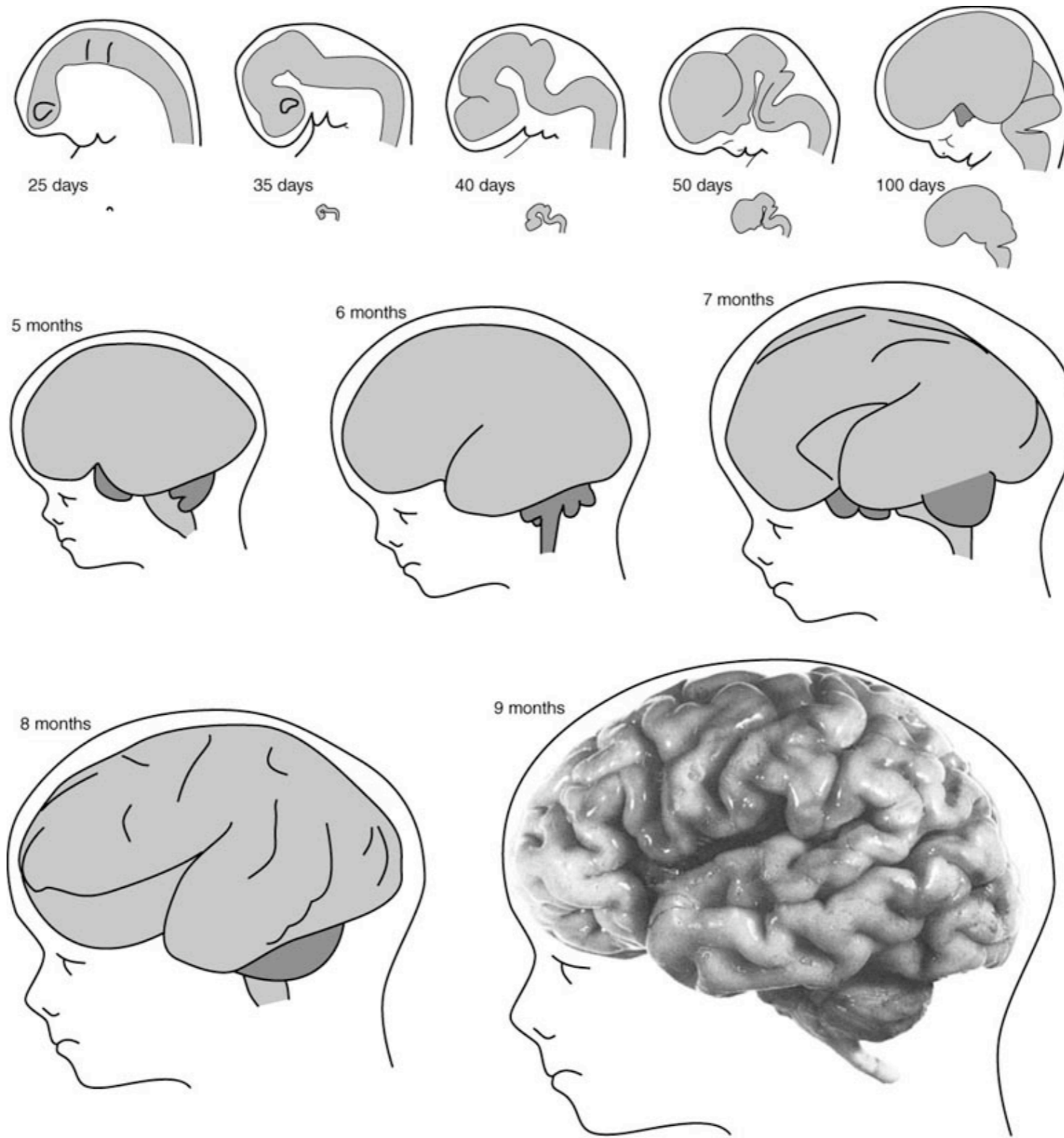
No handout

- Lecture materials will later be uploaded on **TOUR** (Tohoku University Repository)

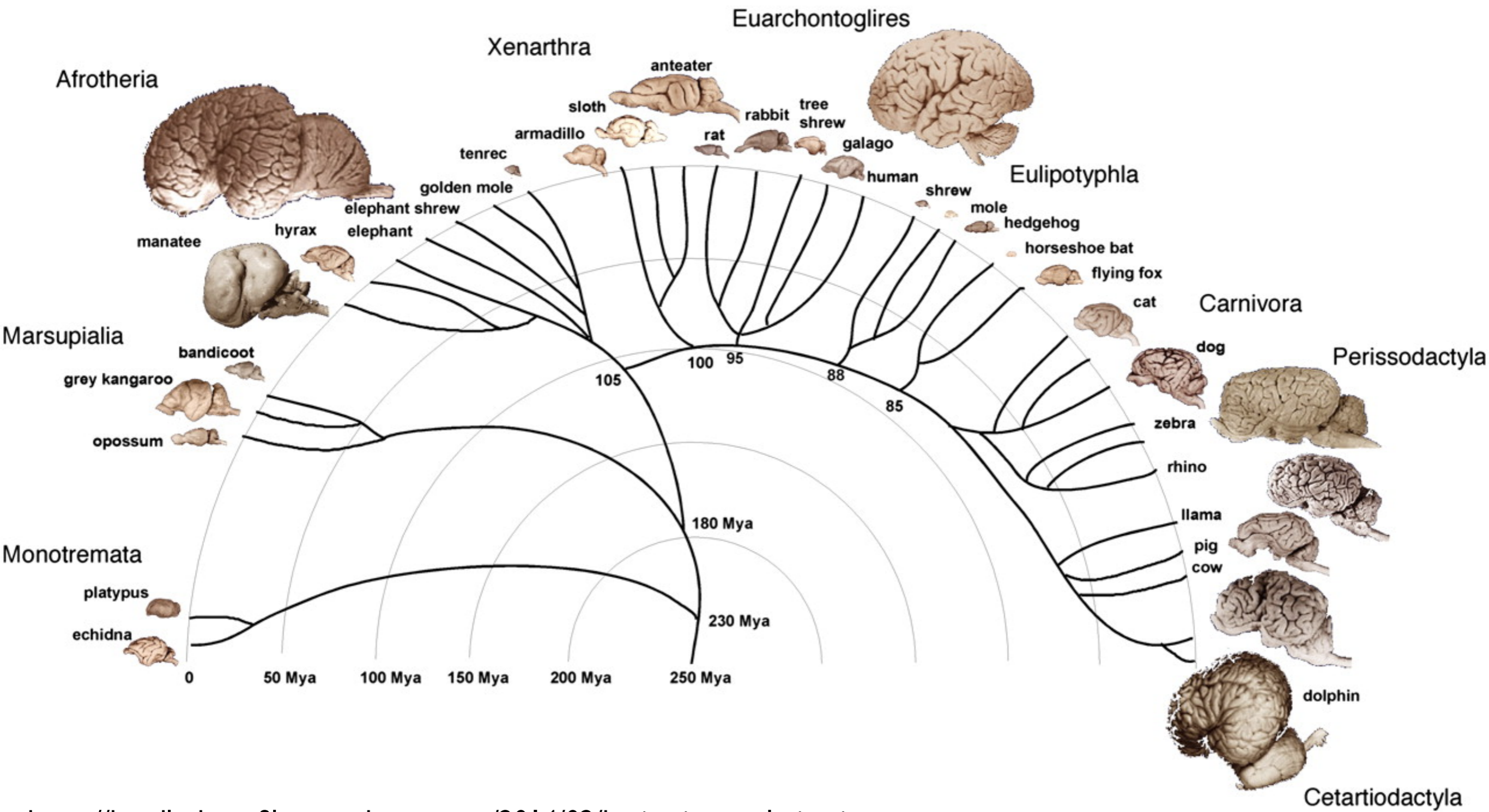
For students:

- How to ask a unique/important question
- How to solve the question

How the brain is developed?



How diverse brains are evolved?



Four areas of biology by Tinbergen

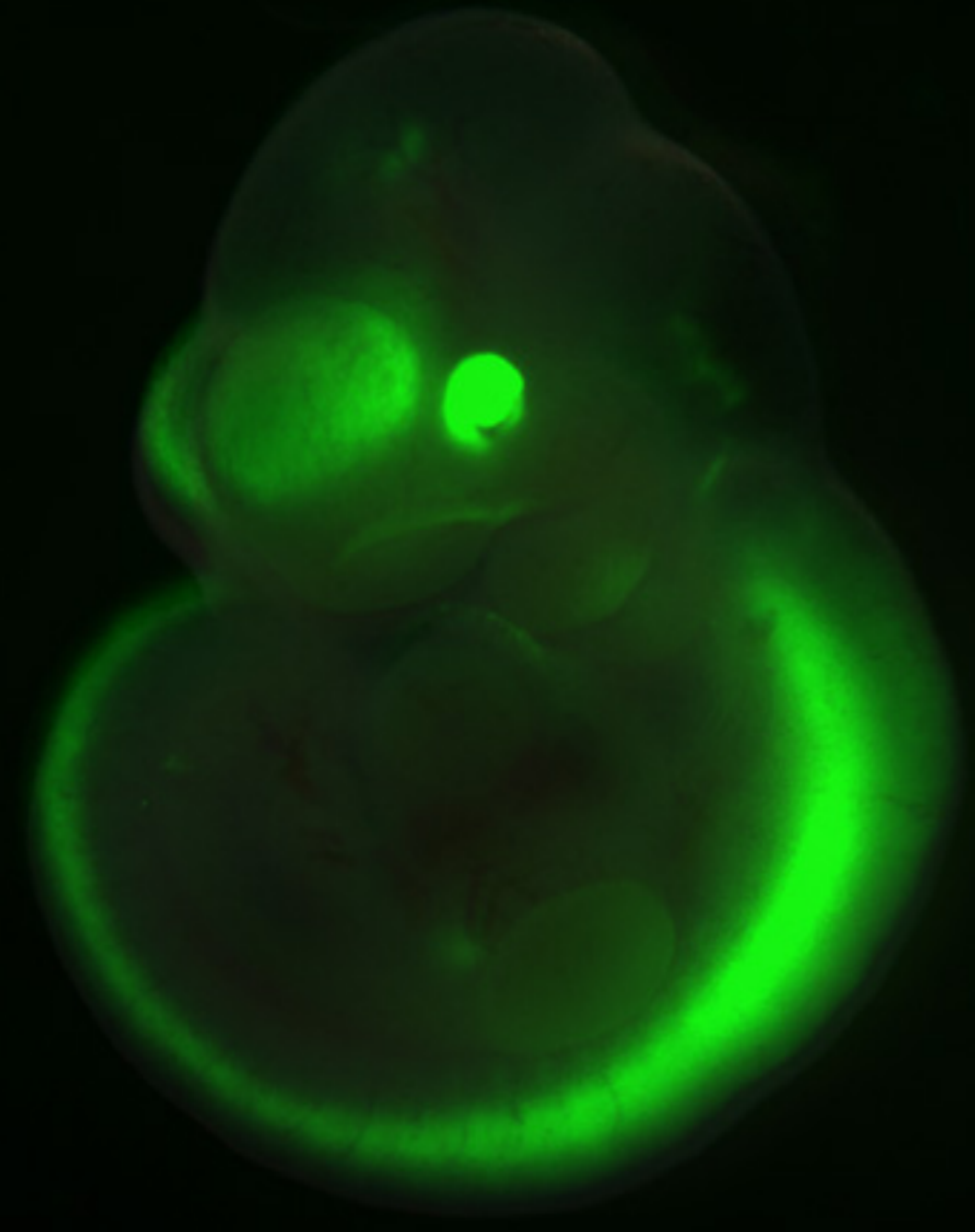
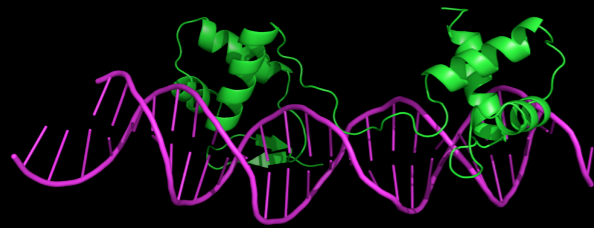
| FOUR AREAS OF BIOLOGY: FOUR QUESTIONS | | Two objects of explanation | |
|--|---|--|--|
| | | <u>Developmental/historical</u> A sequence that results in the trait | <u>Single form</u> The trait at one slice in time |
| Two kinds of explanations | <p><u>Proximate</u></p> <p>Explains how organisms work by describing their mechanisms and their ontogeny</p> | <p><u>Ontogeny</u></p> <p>Q: How does the trait develop in individuals?</p> <p>A: Description of the trait's forms at sequential life stages, and the mechanisms that control development.</p> | <p>Neurophysiology</p> |
| | <p><u>Evolutionary</u></p> <p>Explains how a species came to its current form by describing a sequence of forms, and how they were influenced by selection and other evolutionary factors.</p> | <p><u>Phylogeny</u></p> <p>Q: What is the phylogenetic history of the trait?</p> <p>A: Description of the history of the trait as reconstructed from its phenotype and genotype precursors</p> | |

Four areas of biology by Tinbergen

| FOUR AREAS OF BIOLOGY: FOUR QUESTIONS | | Two objects of explanation | |
|--|---|---|---|
| | | <u>Developmental/historical</u> A sequence that results in the trait | <u>Single form</u> The trait at one slice in time |
| Two kinds of explanations | <u>Proximate</u> Explains how organisms work by describing their mechanisms and their ontogeny | Developmental Neurobiology | <u>Mechanism</u> Q: What is the structure of the trait; how does it work? A: Description of the trait's anatomy, physiology, regulation, and how the trait works to accomplish a function. |
| | <u>Evolutionary</u> Explains how a species came to its current form by describing a sequence of forms, and how they were influenced by selection and other evolutionary factors. | | <u>Adaptive significance</u> Q: How have variations in the trait interacted with environments to influence fitness in ways that help to explain the trait's form? A: Description of how variations in the trait have influenced fitness |

A key factor

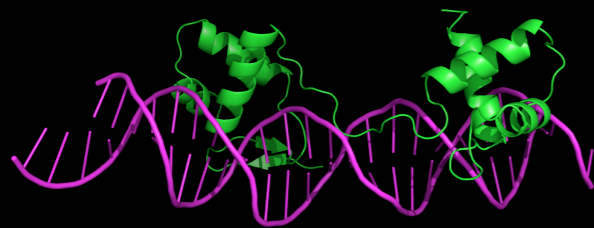
Pax6
transcription factor



Pax-EGFP mouse@E10.5

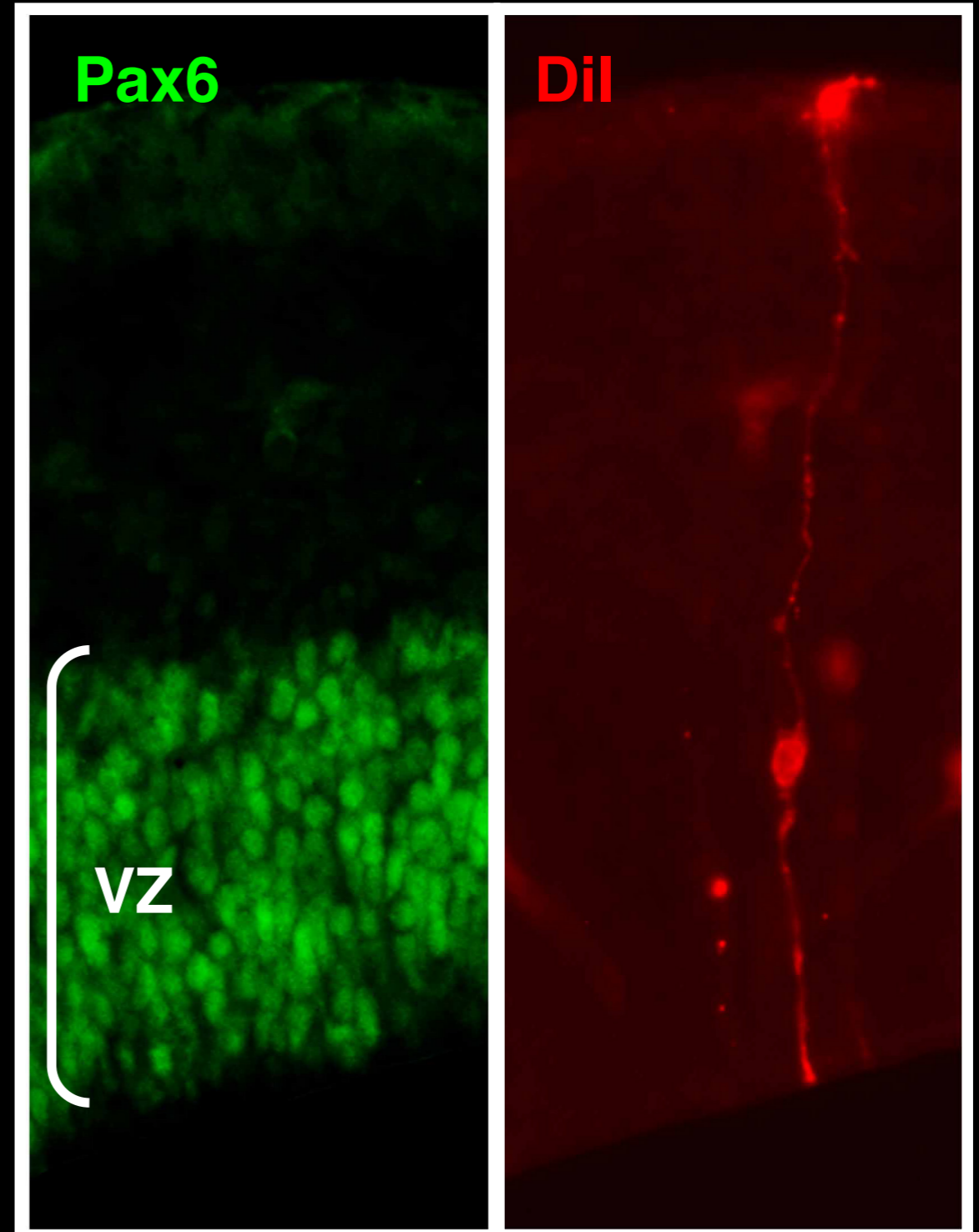
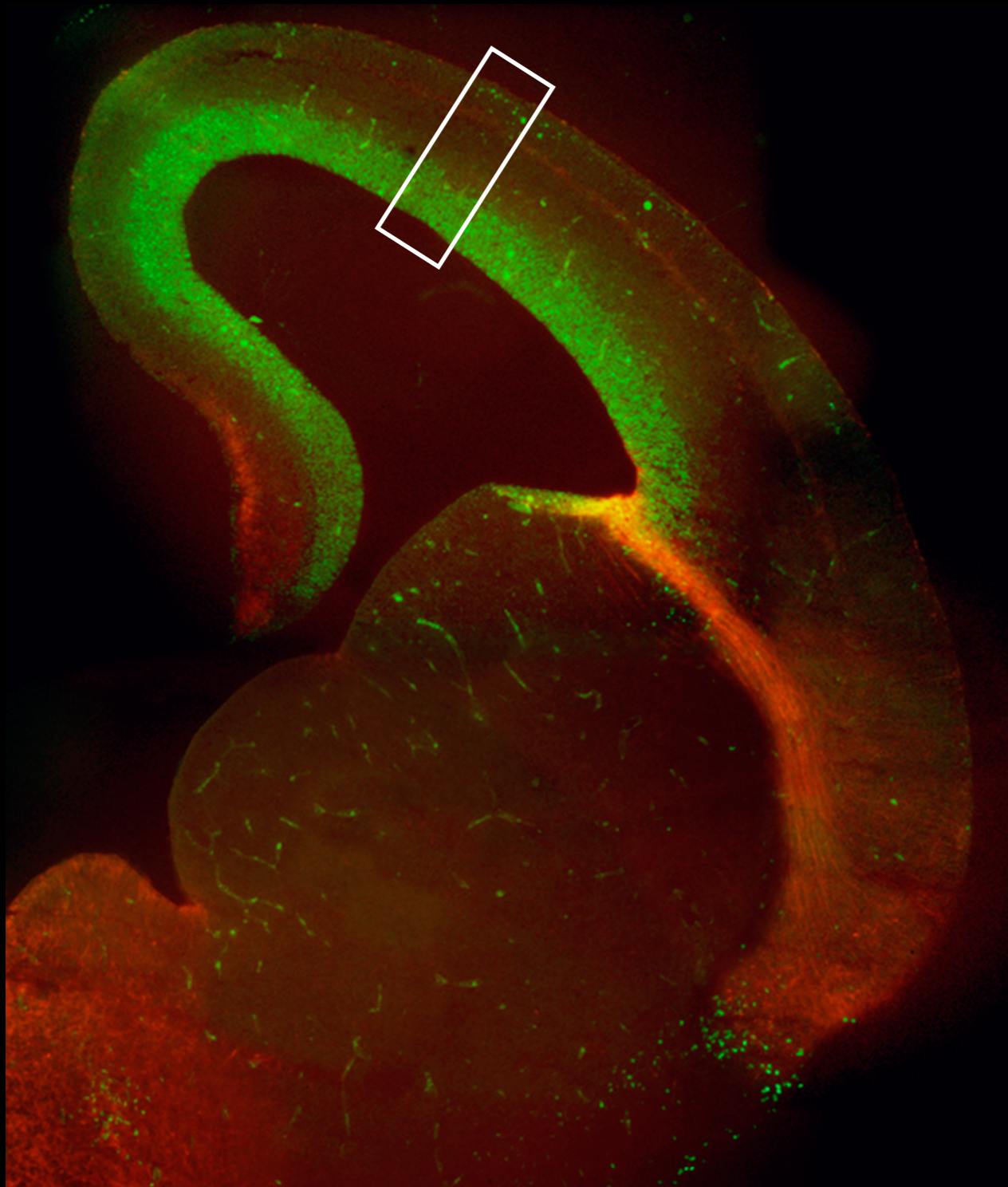
Cortical Primordium

Pax6
transcription factor



Pax6 is expressed in
neural stem/progenitor
cells

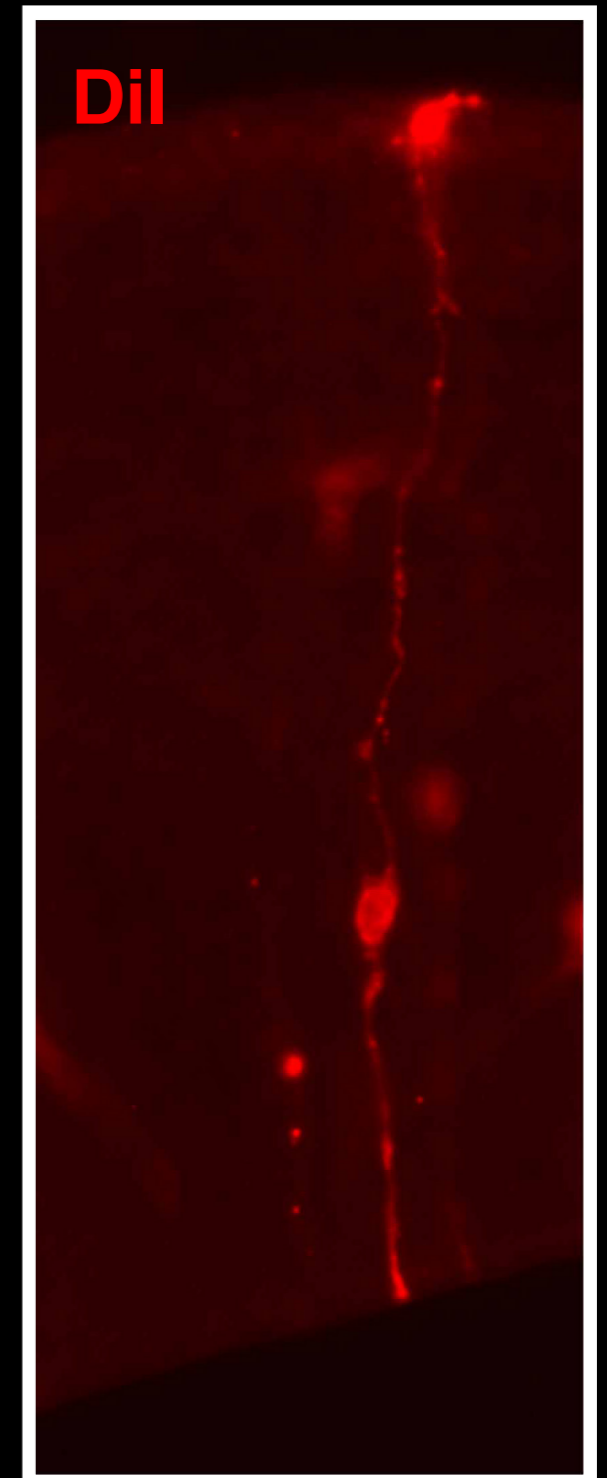
Unique shape of radial progenitors



Radial glia

- Highly polarized w/ long apical and basal processes
- Neural stem/progenitor cells
- Scaffold for neuronal migration

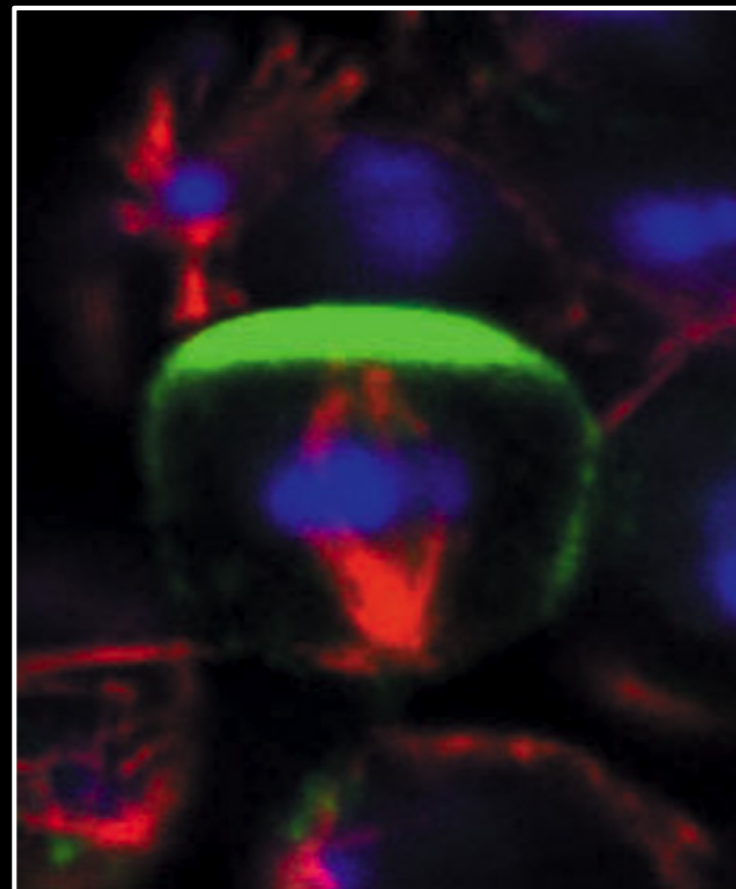
Mammalian
RG



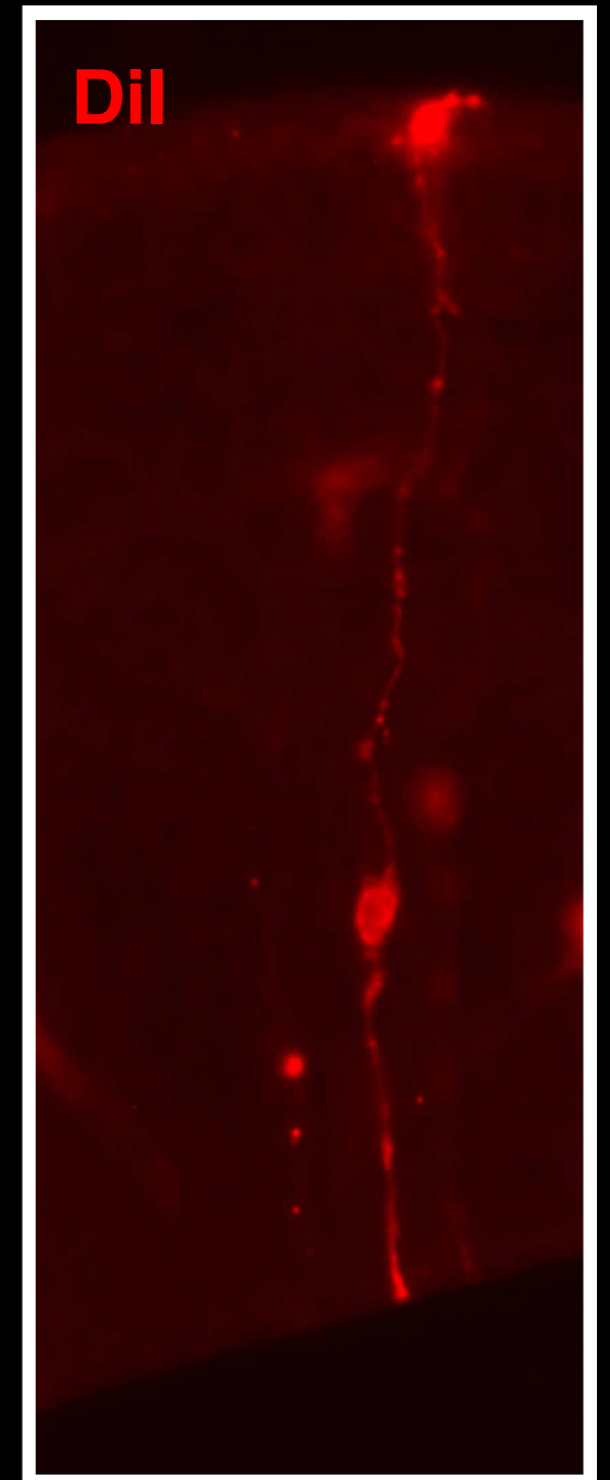
Radial glia

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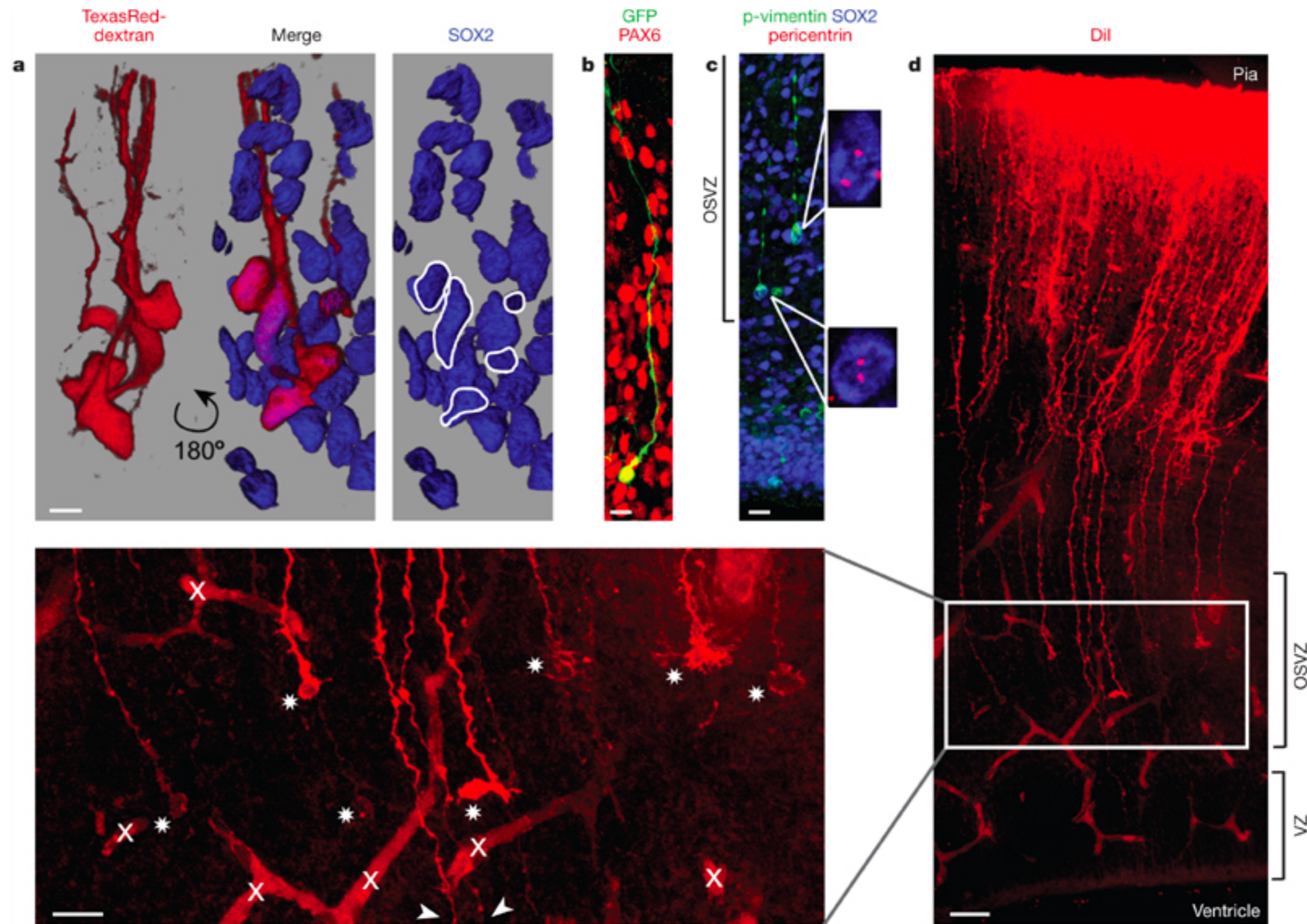
Fly NSCs



Mammalian
RG

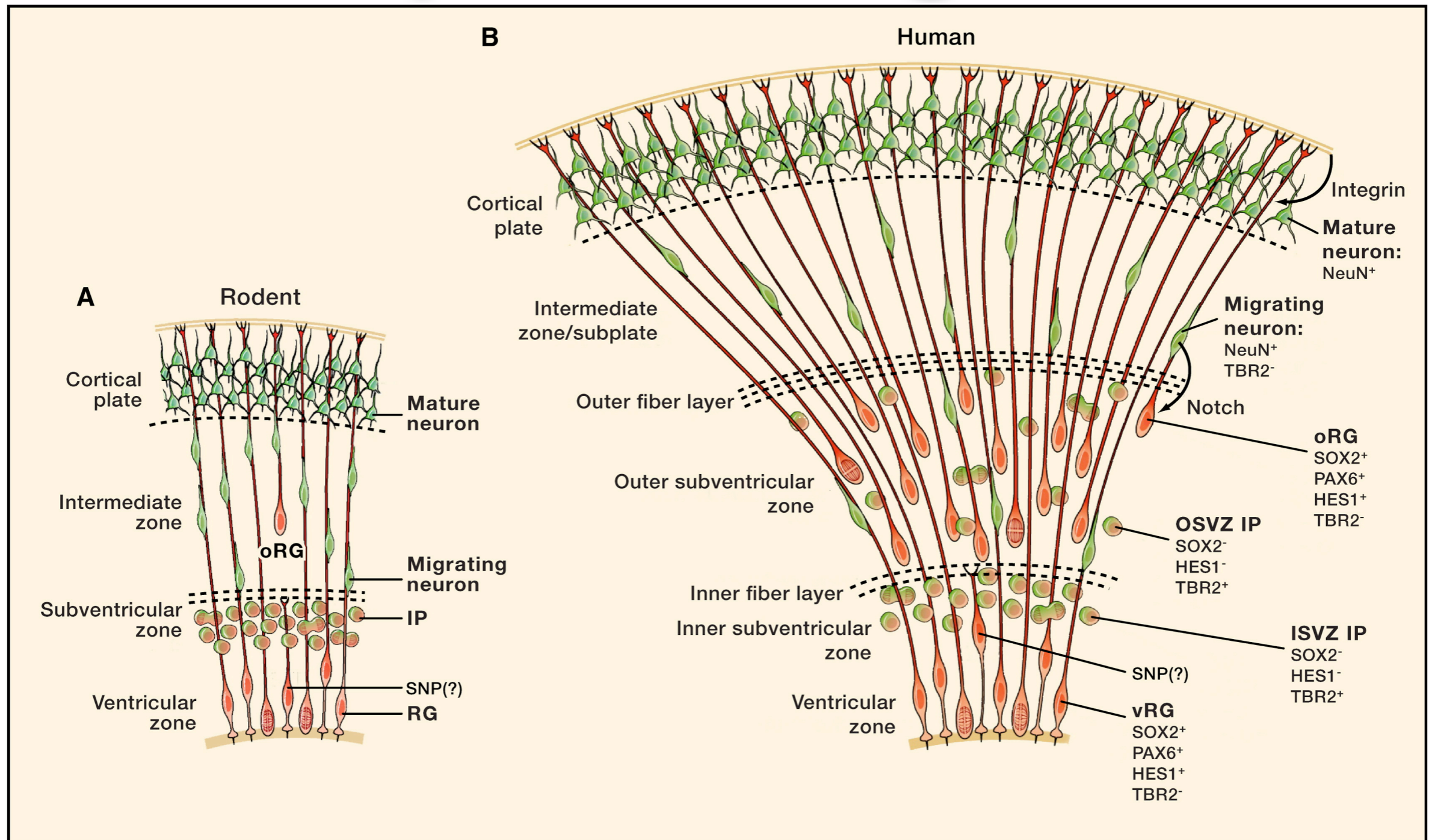


Basal processes of NPCs in primate OSVZ



Hansen et al.: Neurogenic radial glia in the outer subventricular zone of human neocortex.
Nature, 2010

Unique shape of RG: a key to corticogenesis

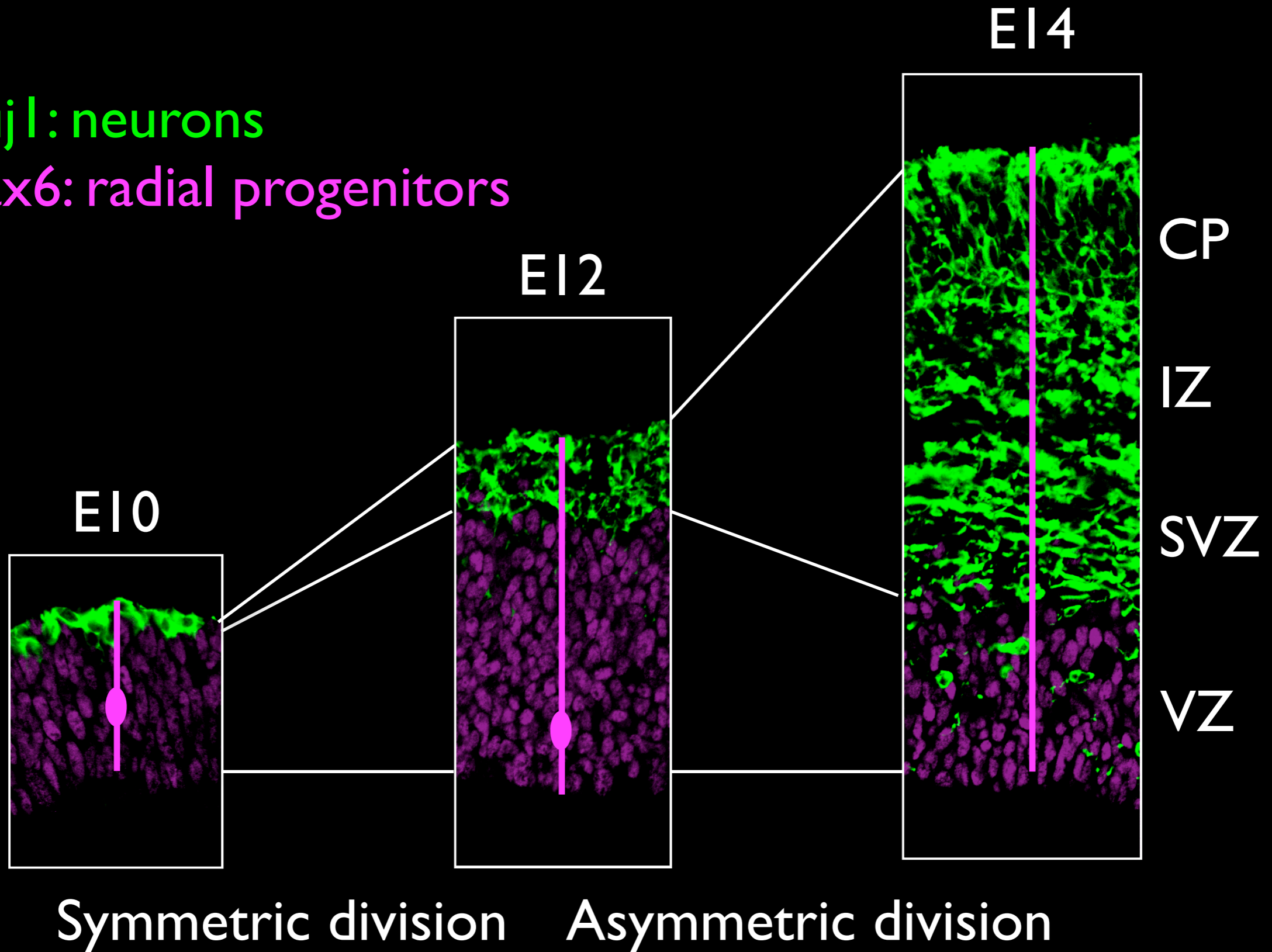


Lui et al.: Development and evolution of the human neocortex. Cell, 2011

RG become longer & longer...

Tuj1: neurons

Pax6: radial progenitors



Molecules working in/around the radial glia

Tuj1: neurons

Pax6: RG

Radial glial molecules

Fabp7/BLBP
LewisX/CD15
Notch signals

Nuclear molecules

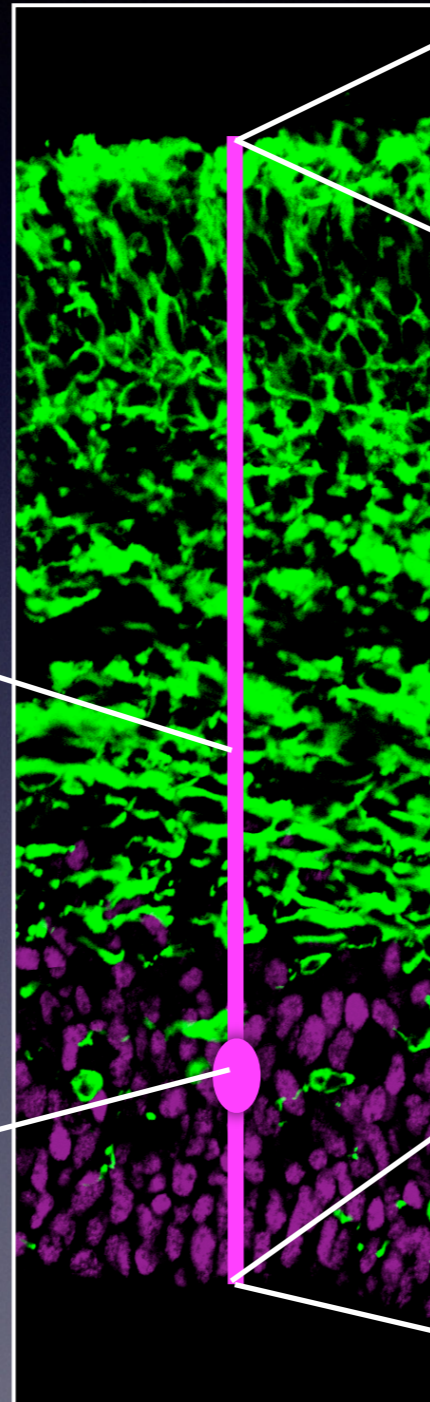
TFs incl. Pax6, Ngn2, Dmrta1
Polycombs
BAF complex

Basal molecules

Secreted molecules
Integrins
Cyclin D2, FMRP

Apical molecules

δ -catenin, FMRP
Polarity proteins
Centrosomal proteins

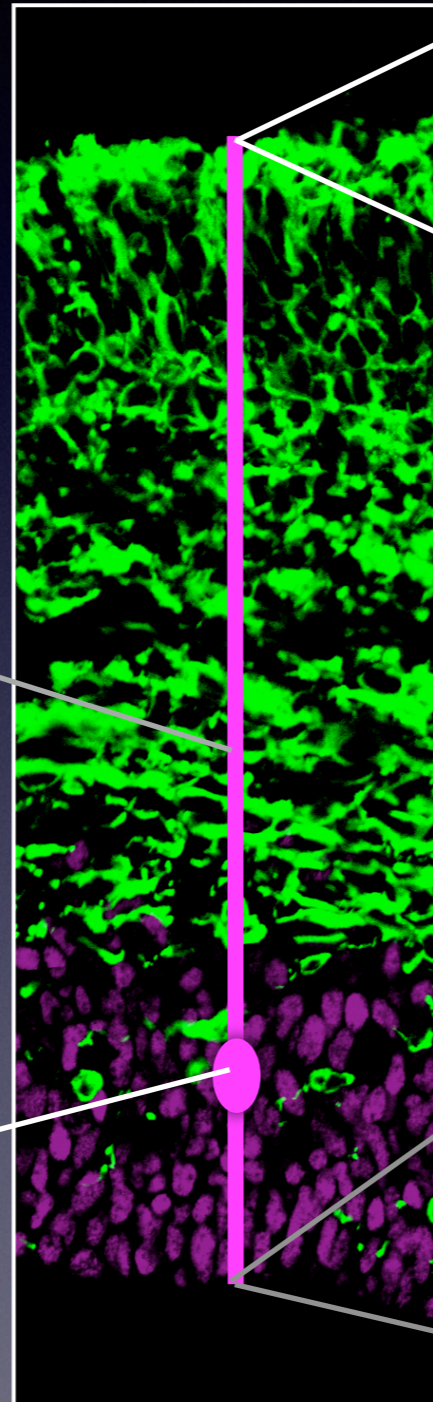


Radial glial molecules

Fabp7/BLBP
LewisX/CD15
Notch signals

Nuclear molecules

TFs incl. Pax6, Ngn2, Dmrta1
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Basal molecules

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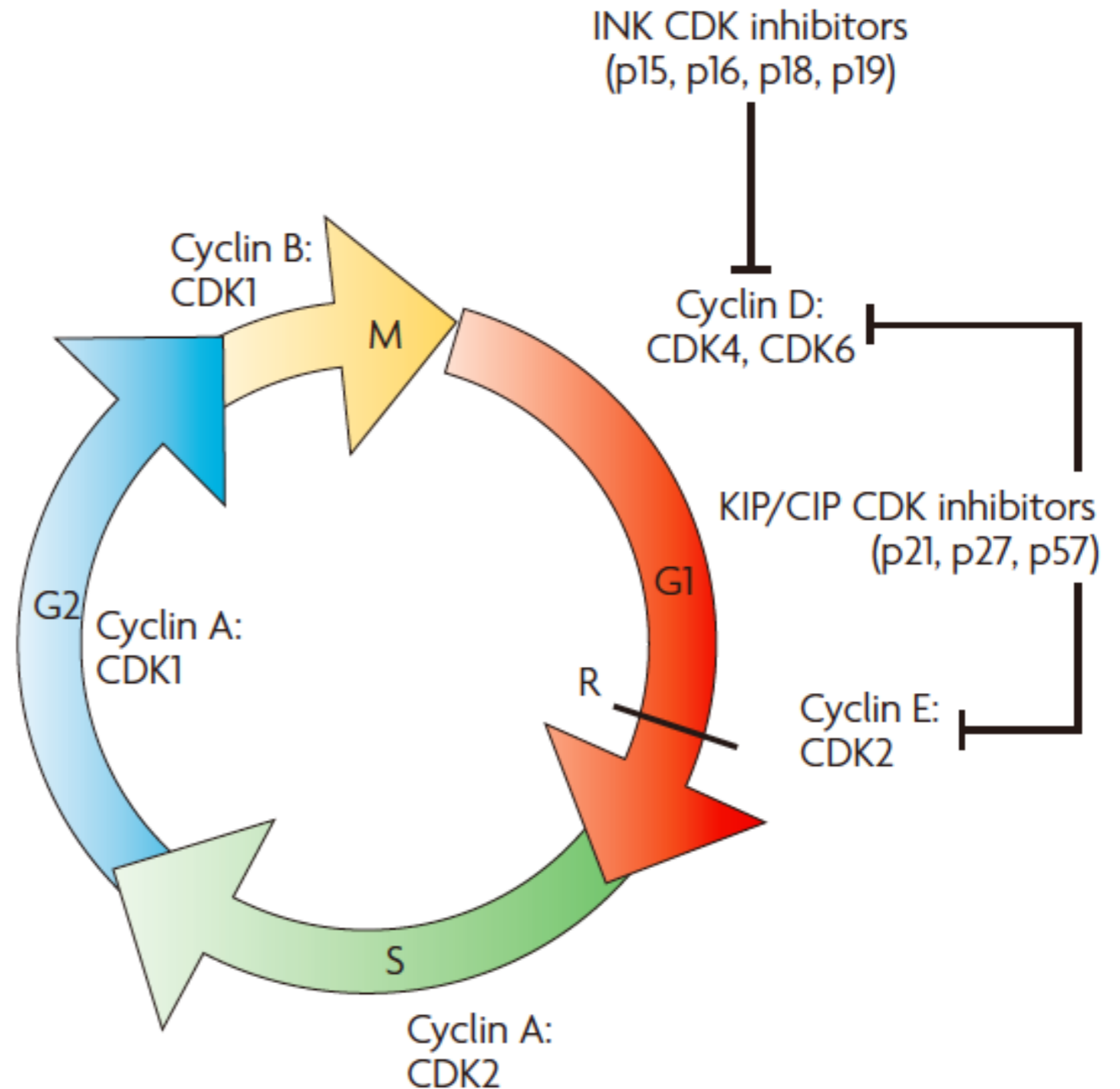
Cell cycle regulator: Cyclin

Cyclin D1 ablation

- lengthens G1 phase
- increase differentiation

Cyclin D2 ablation

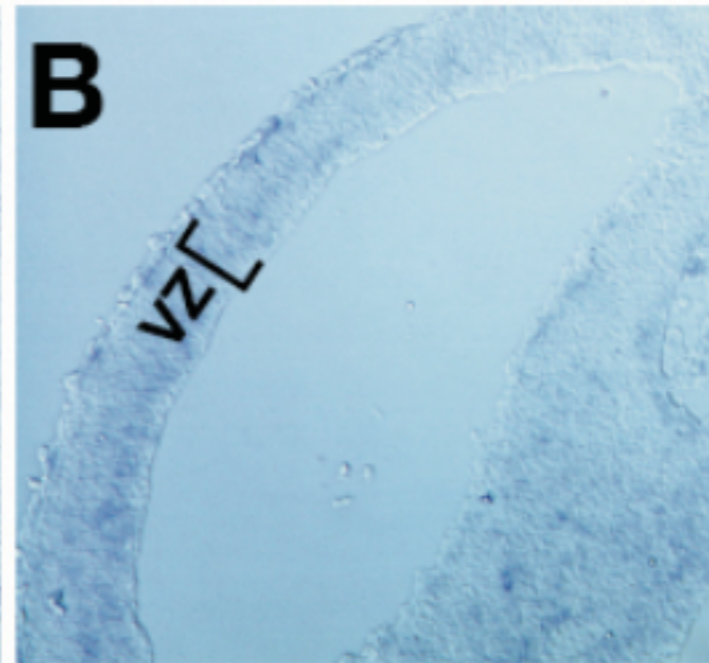
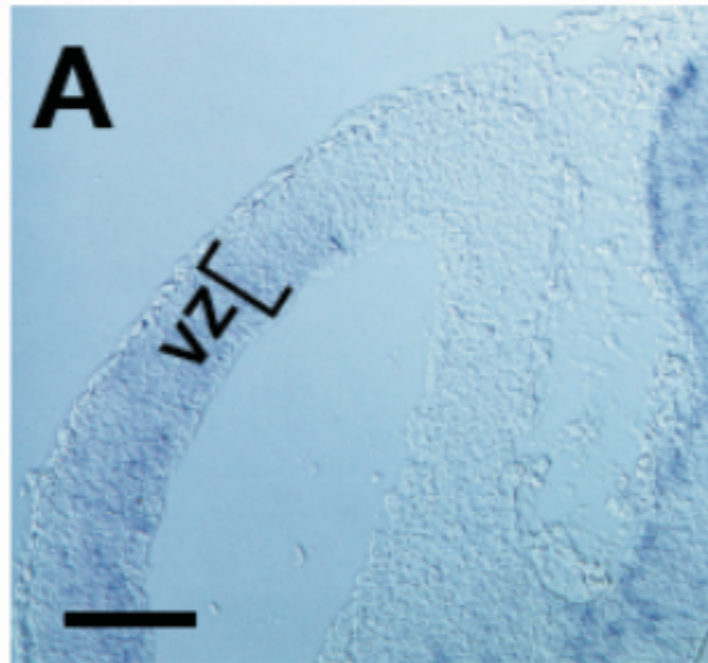
- lengthens G1 phase
- induces differentiation
- reduces cortical thickness



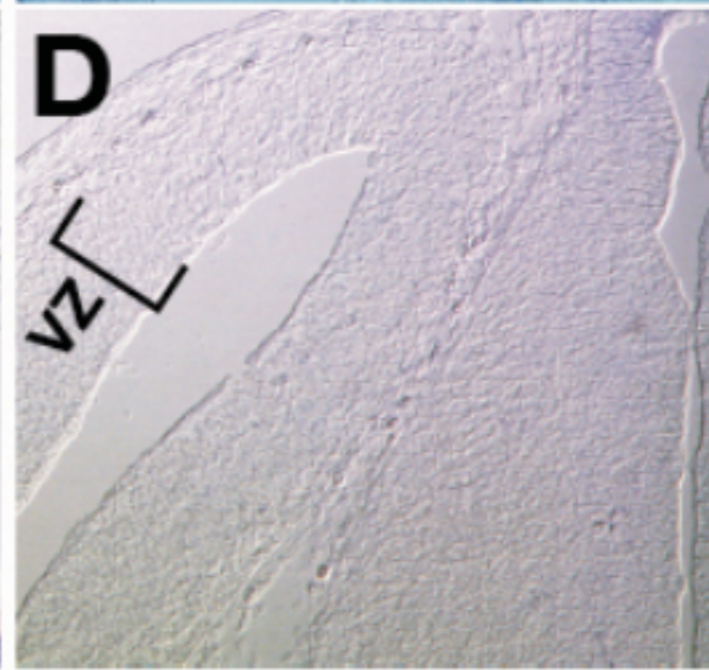
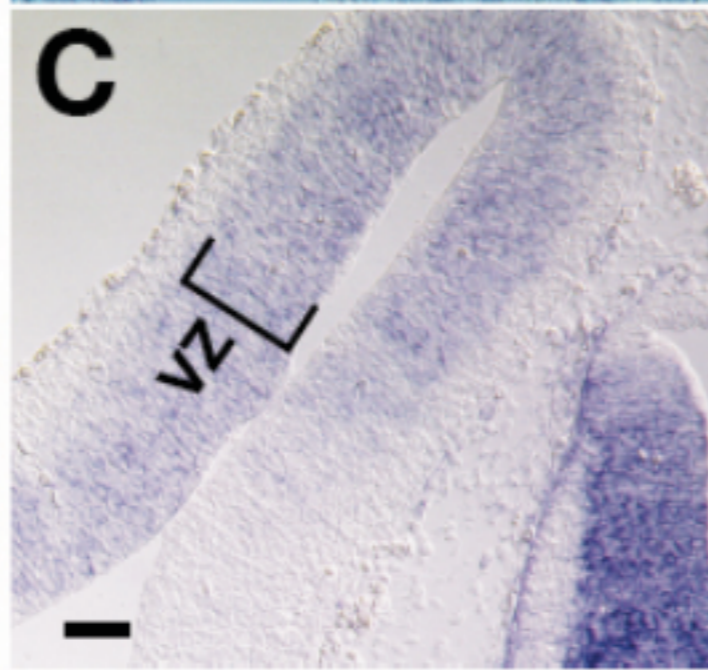
Cyclin D1

Cyclin D3

E10.5



E12.5



Cyclin D2 localization at basal endfeet

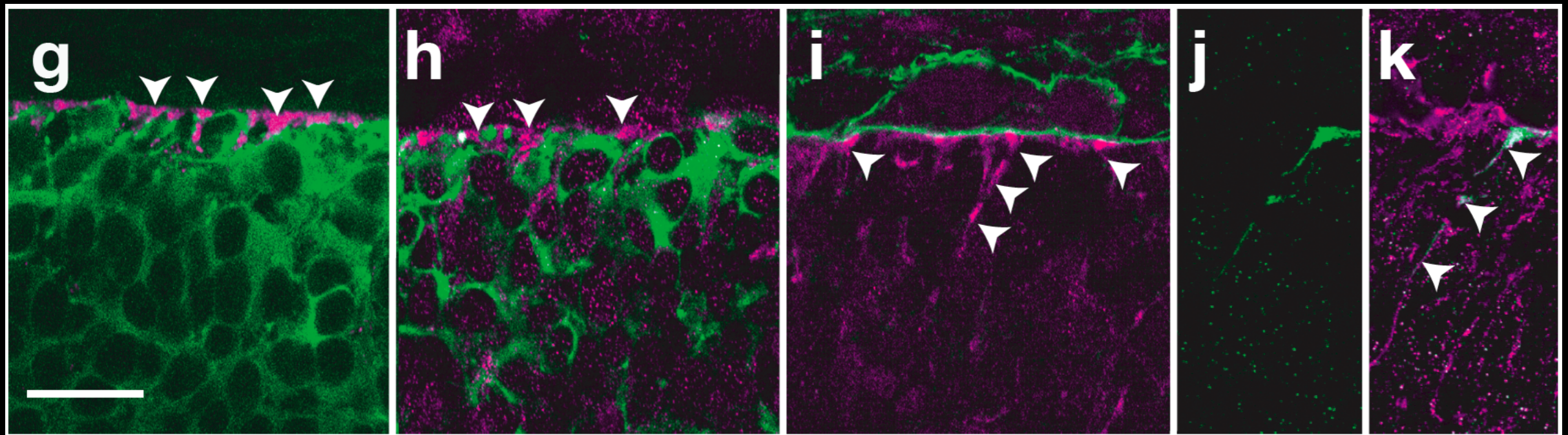
Cyclin D2/tubulin

Cyclin D2/tubulin

Cyclin D2/laminin

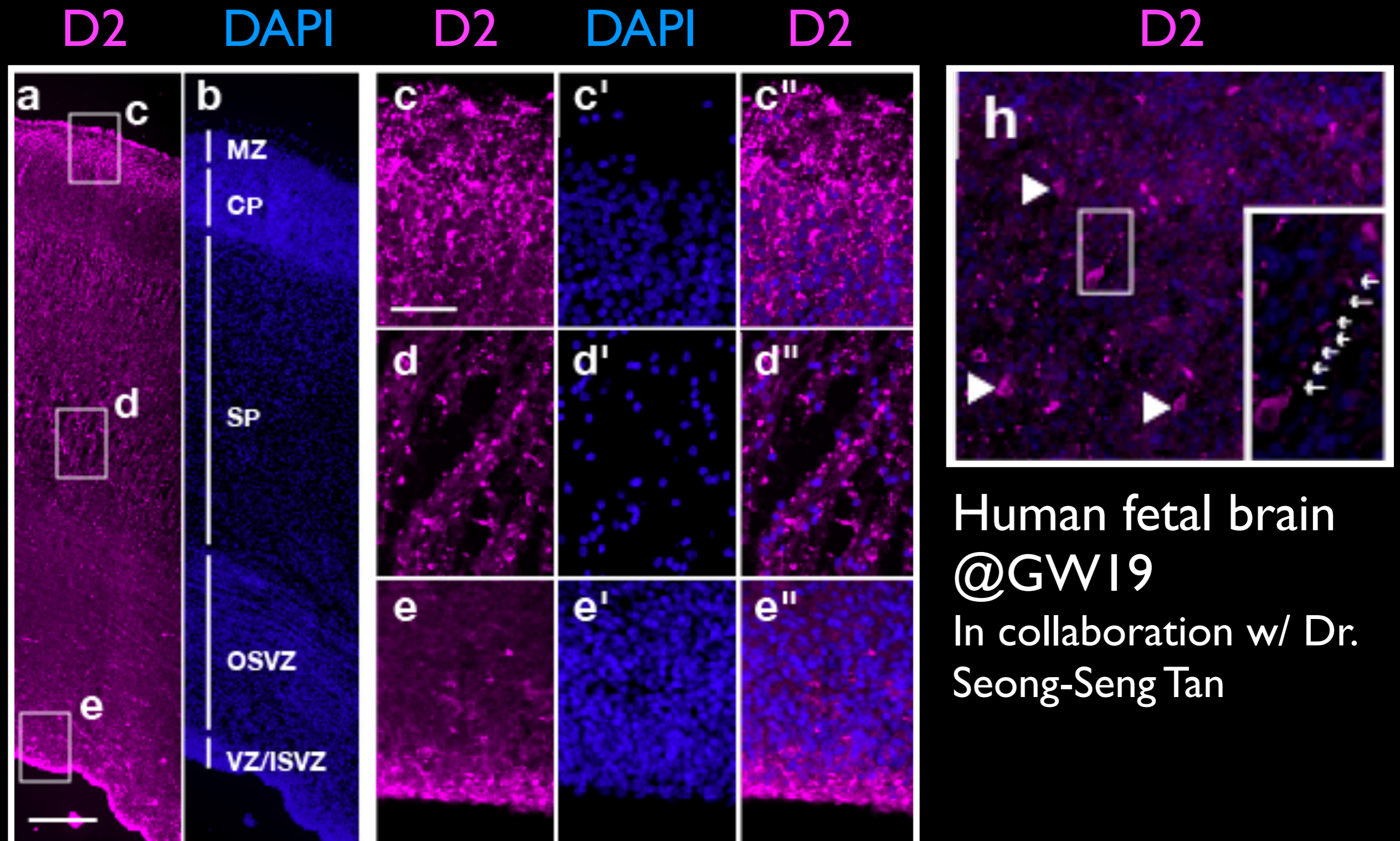
EGFP

Cyclin D2



E14.5 mouse

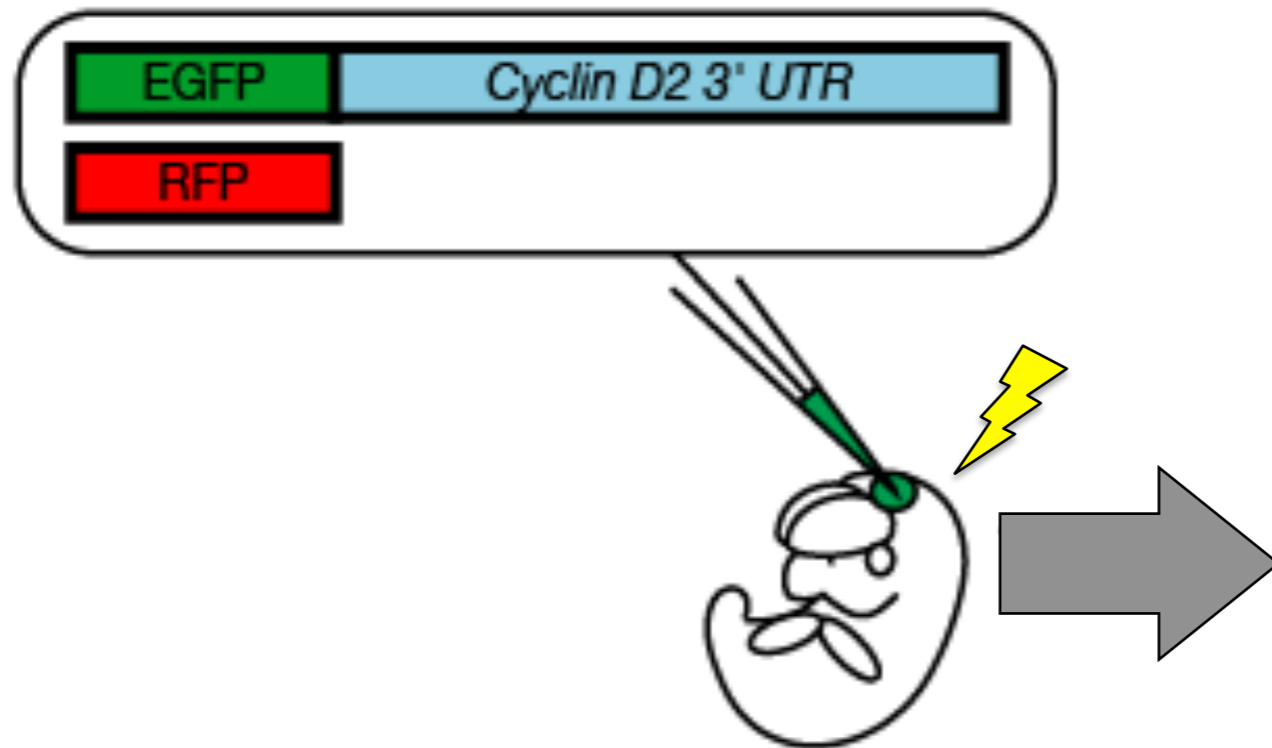
Similar Cyclin D2 expression in human fetal ctx



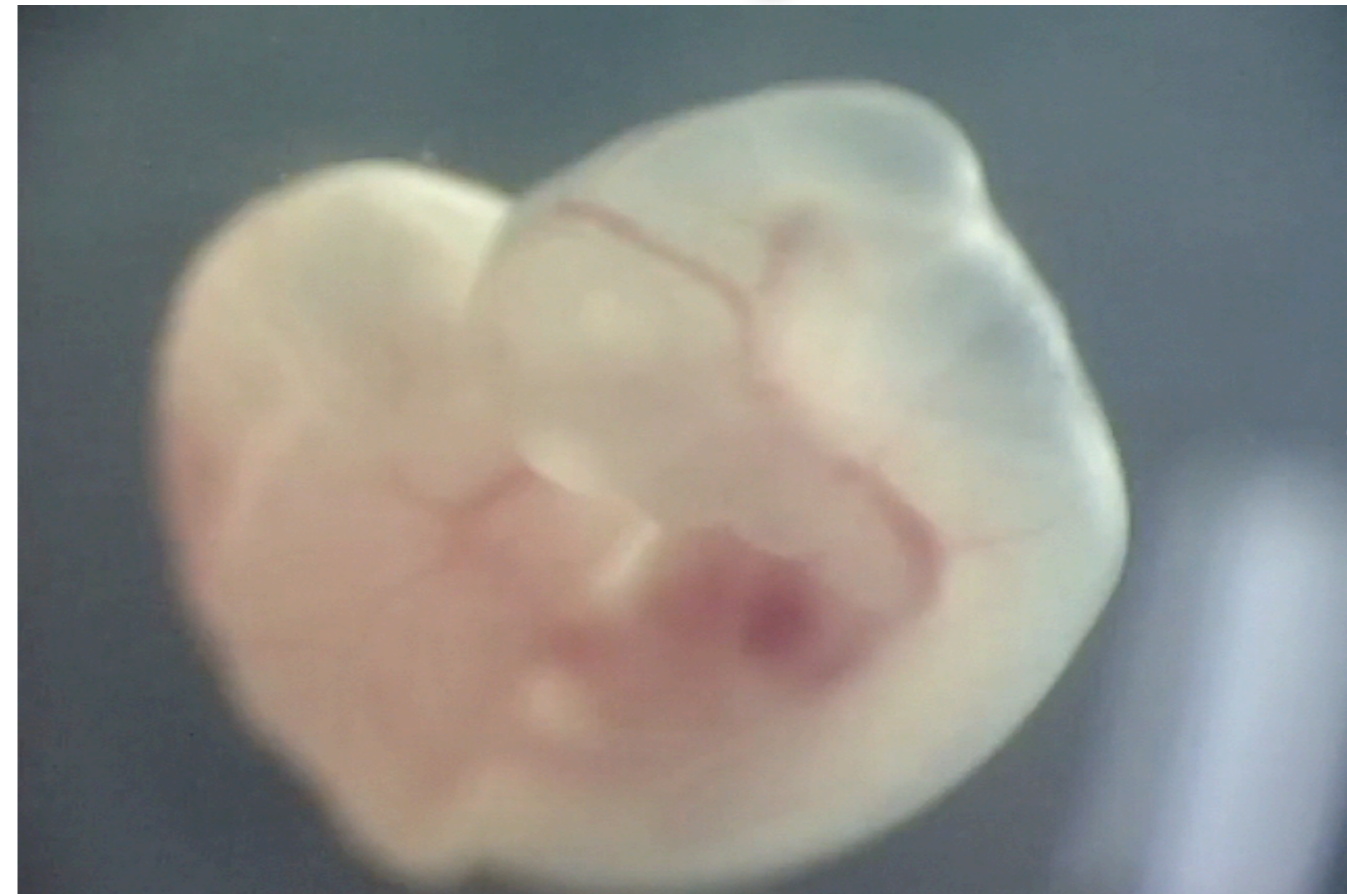
How basal Cyclin D2
mRNA is transported
basally?

A *cis*-acting transport element of *Cyclin D2* mRNA resides in its 3' UTR?

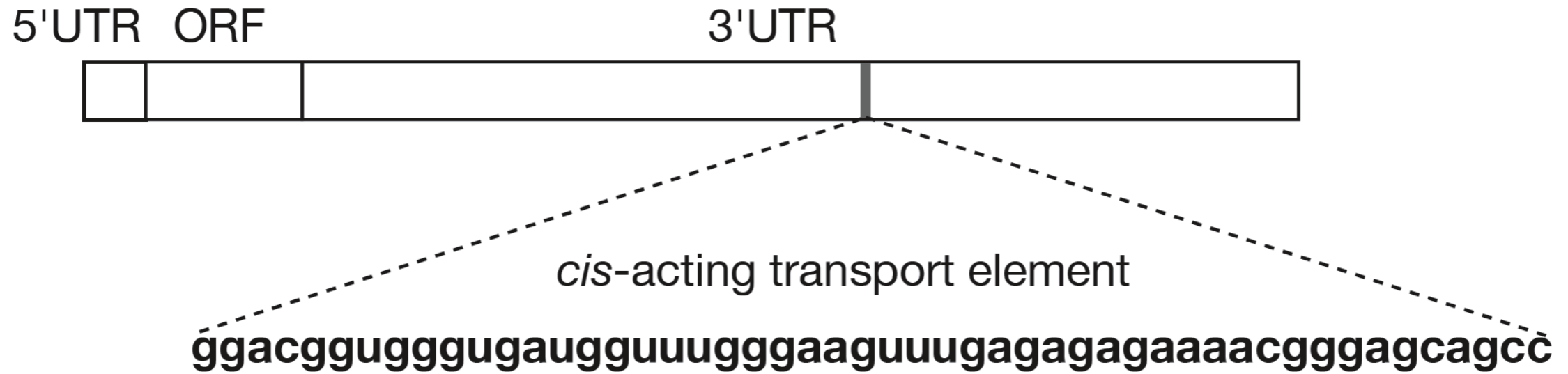
DNA injection to the diencephalon



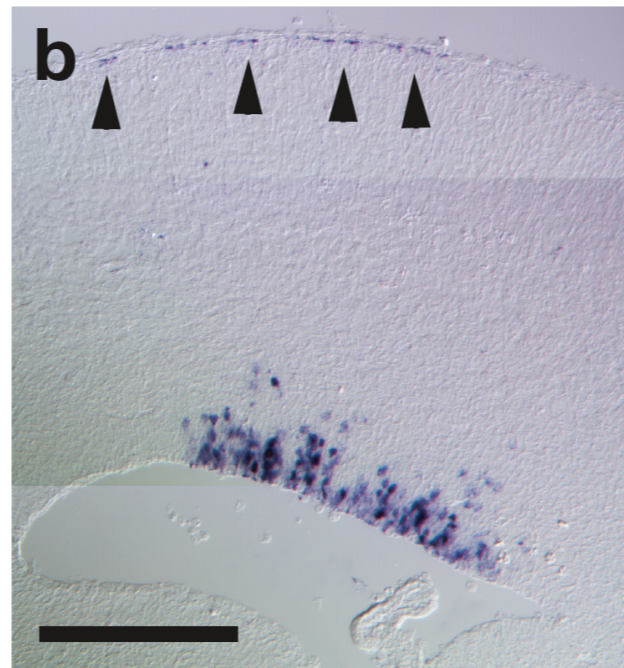
Whole embryo culture



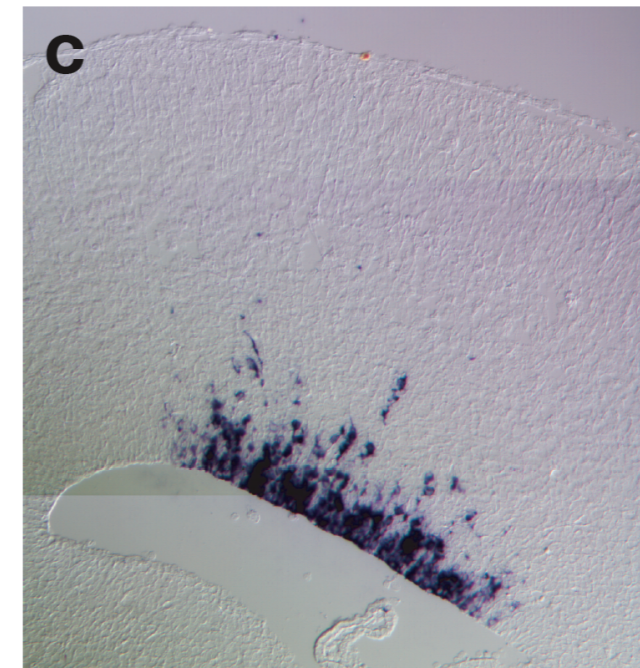
A 50 bp *cis*-acting transport element of *Cyclin D2* mRNA in its 3' UTR



EGFP

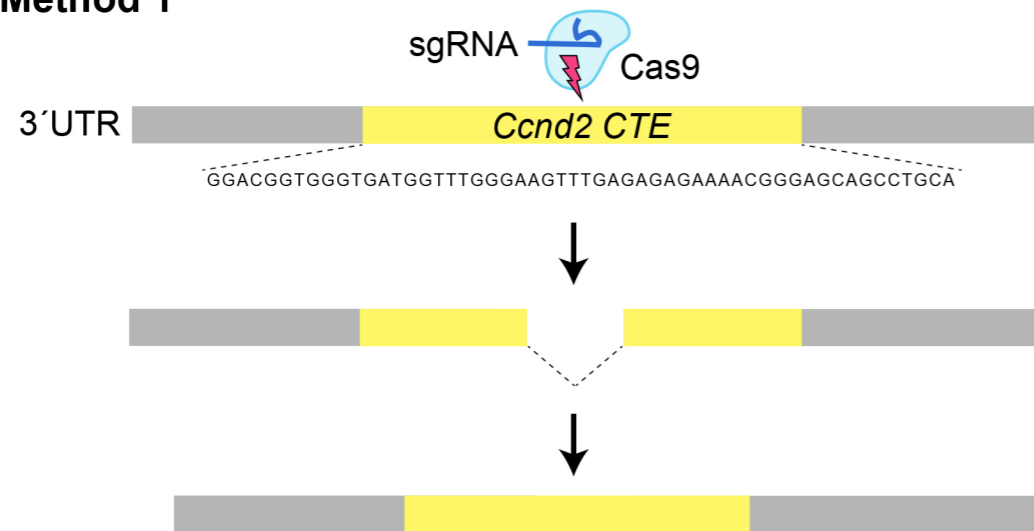


RFP

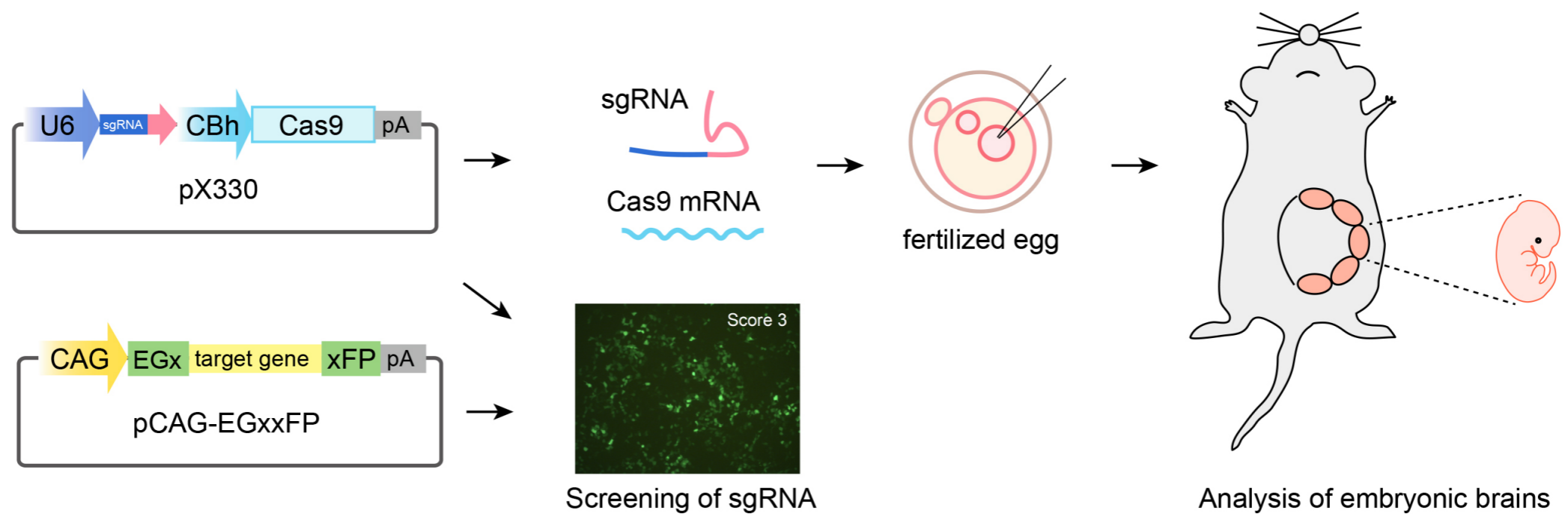
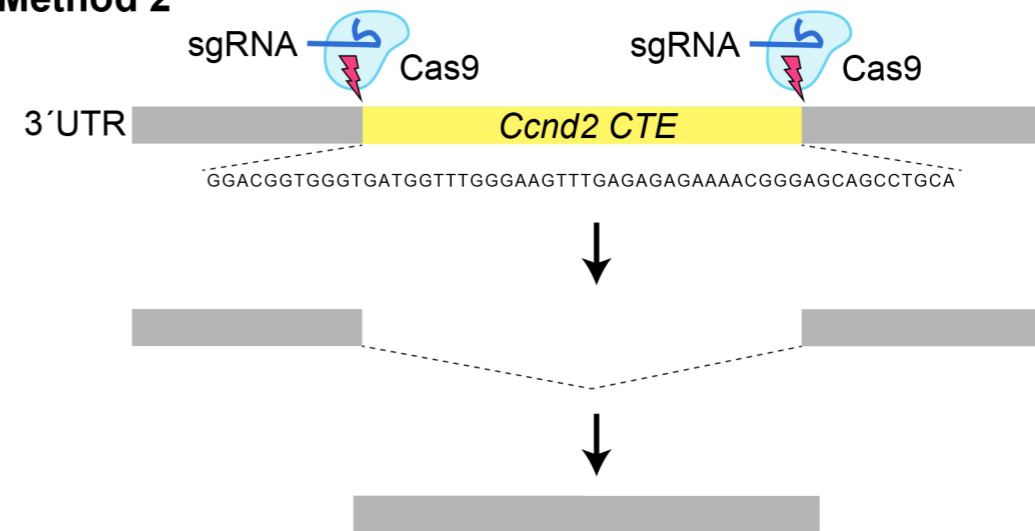


Deletion of *Cyclin D2* cis-element

Method 1



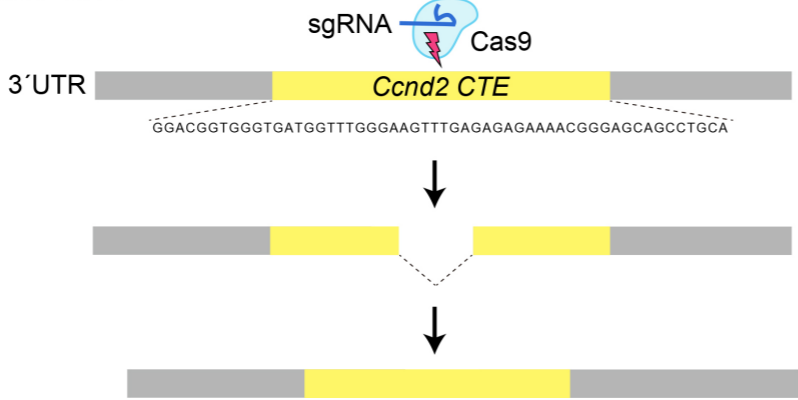
Method 2



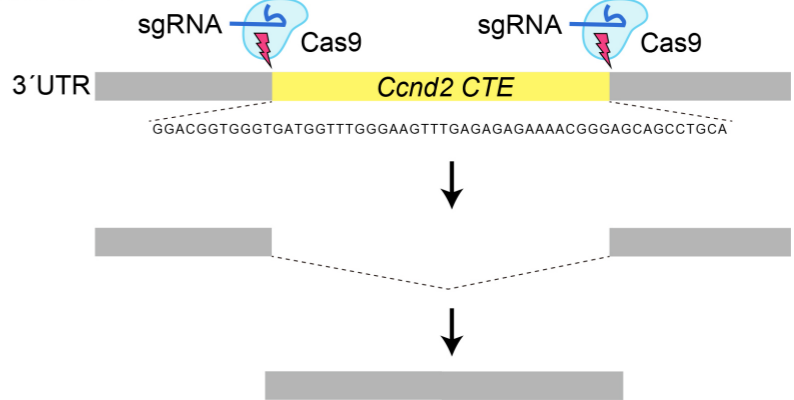
Dr. Kikkawa collaboration w/
Dr. Inoue@NCNCP

Ablation of *Cyclin D2* basal localization

Method 1



Method 2

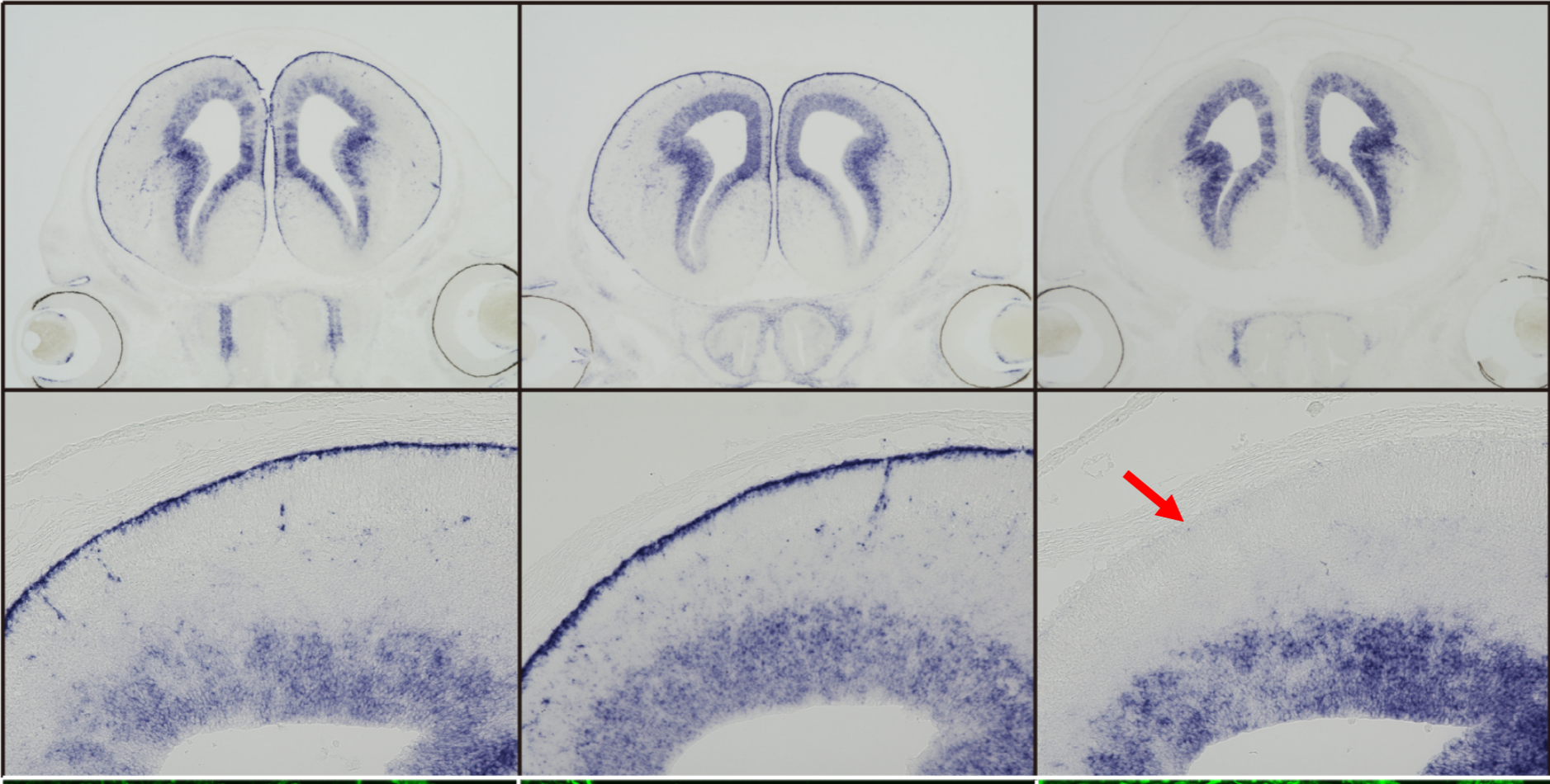


sgRNA7-4

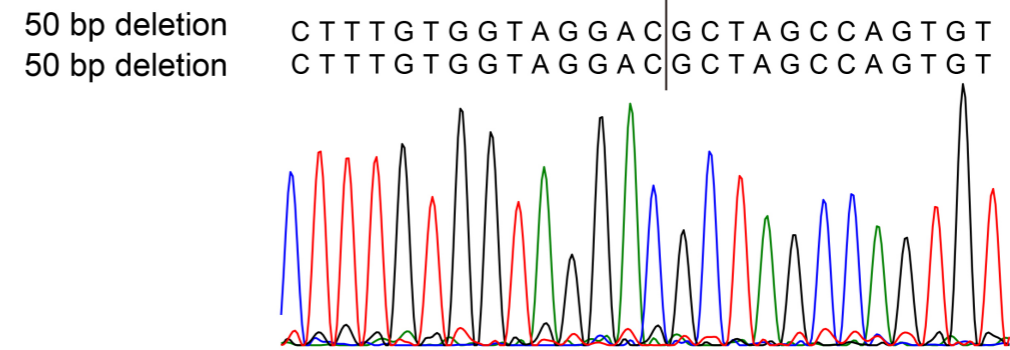
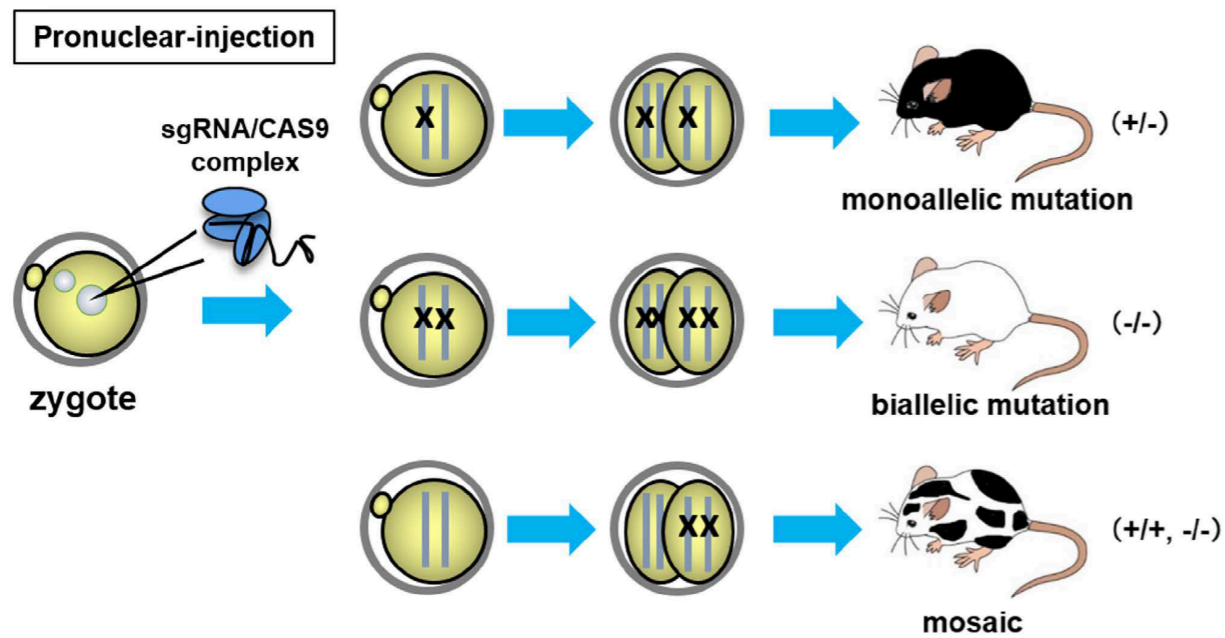
sgRNA23-3

sgRNA1+8-1

Cyclin D2

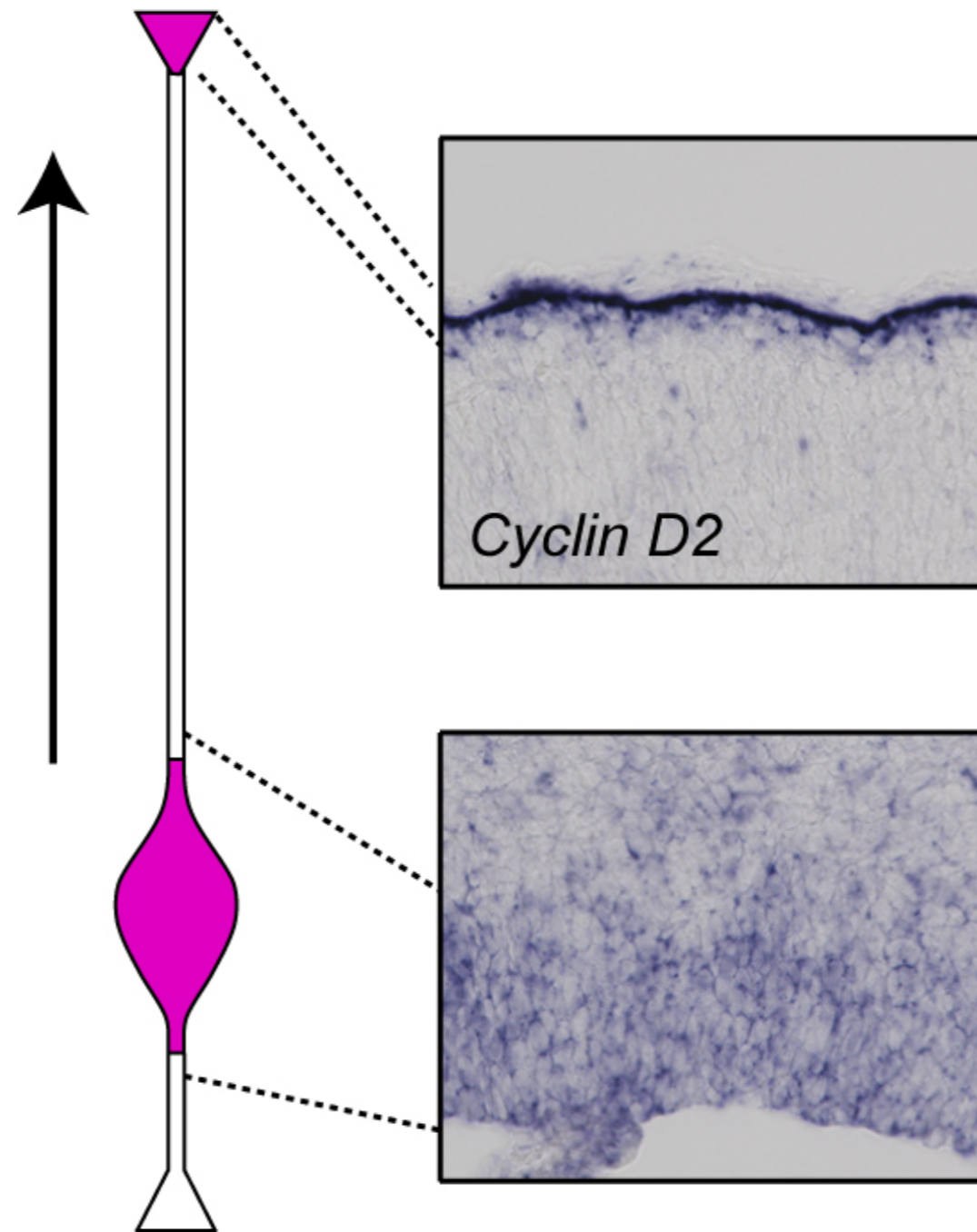


Making F1 generation to avoid mozaichism

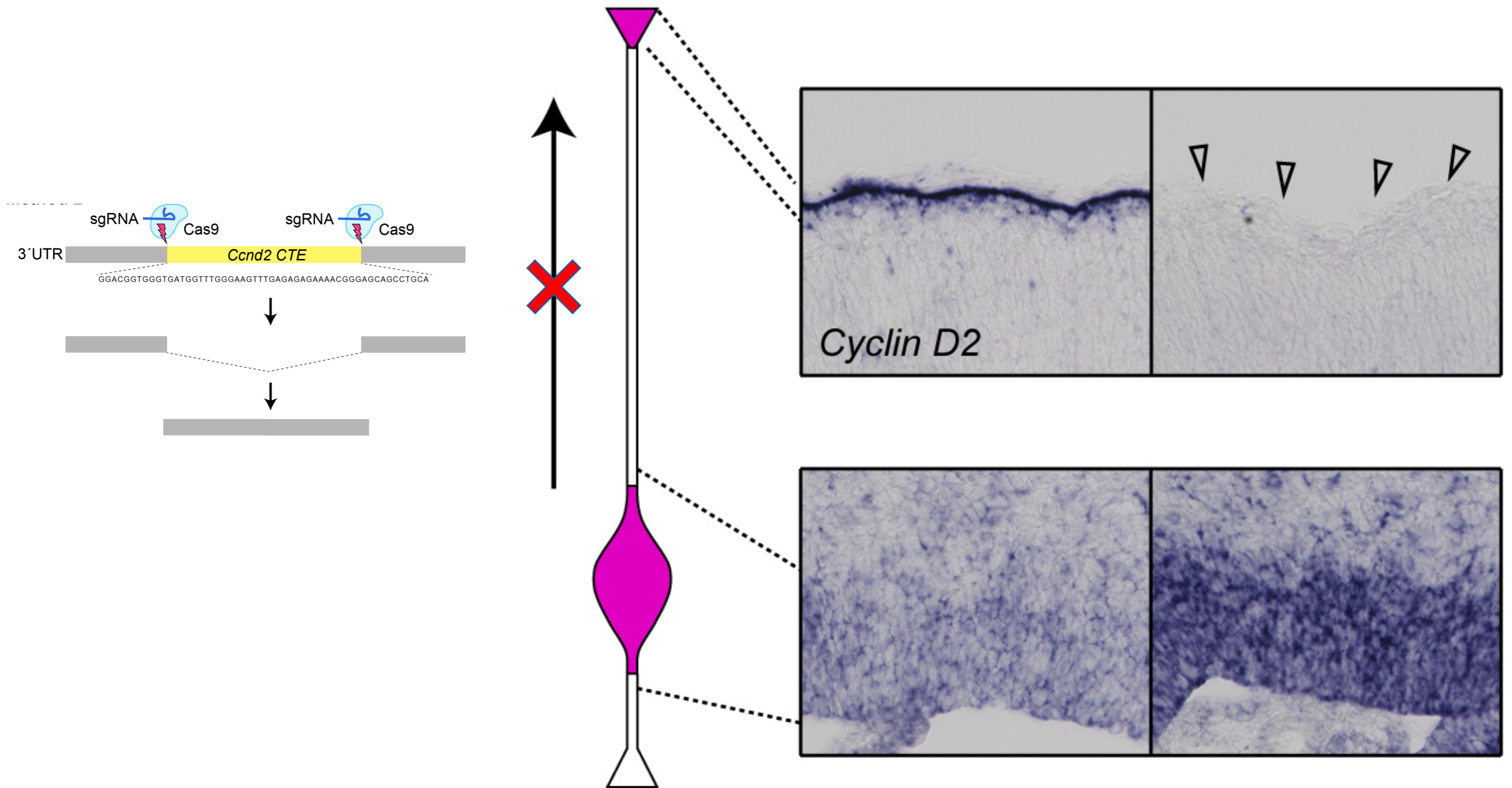


- ① ← 3970-4019 (50 bp) →
- ② ← 3967-4024 (58 bp) →
- ③ ← 3949-4020 (72 bp) →

△CTE mutant generated by genome editing

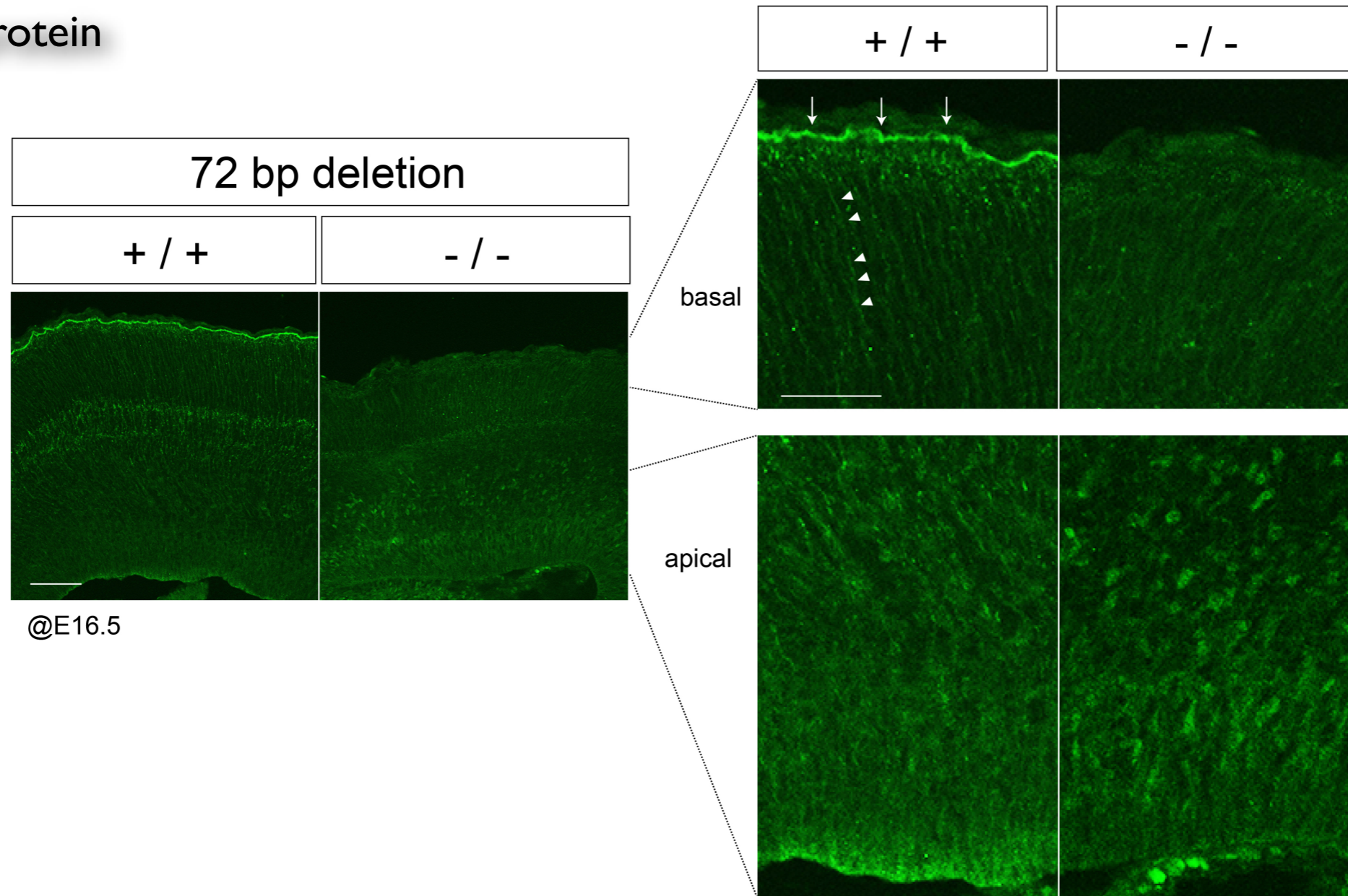


ΔCTE mutant generated by genome editing



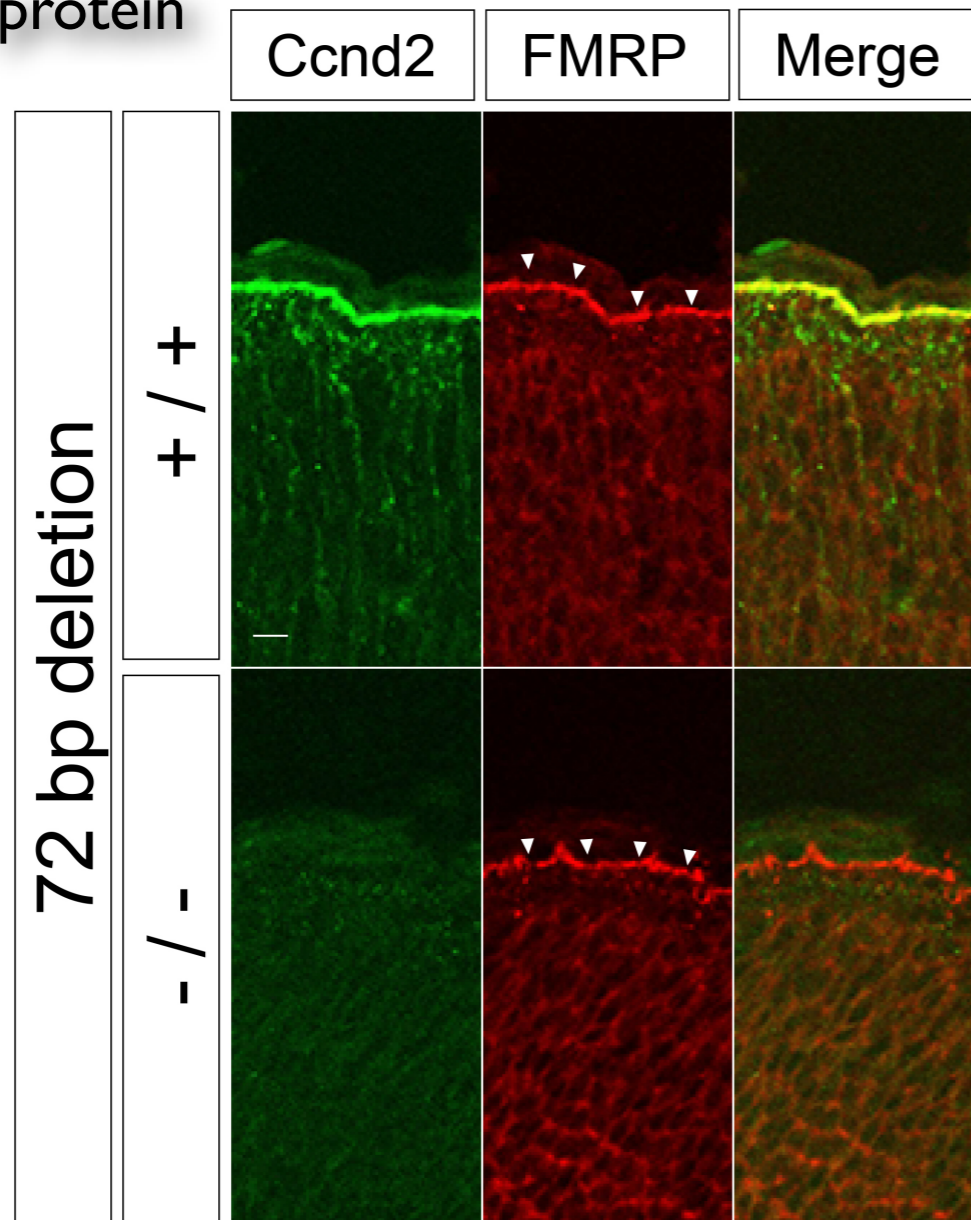
ΔCTE mutant generated by genome editing

Cyclin D2 protein

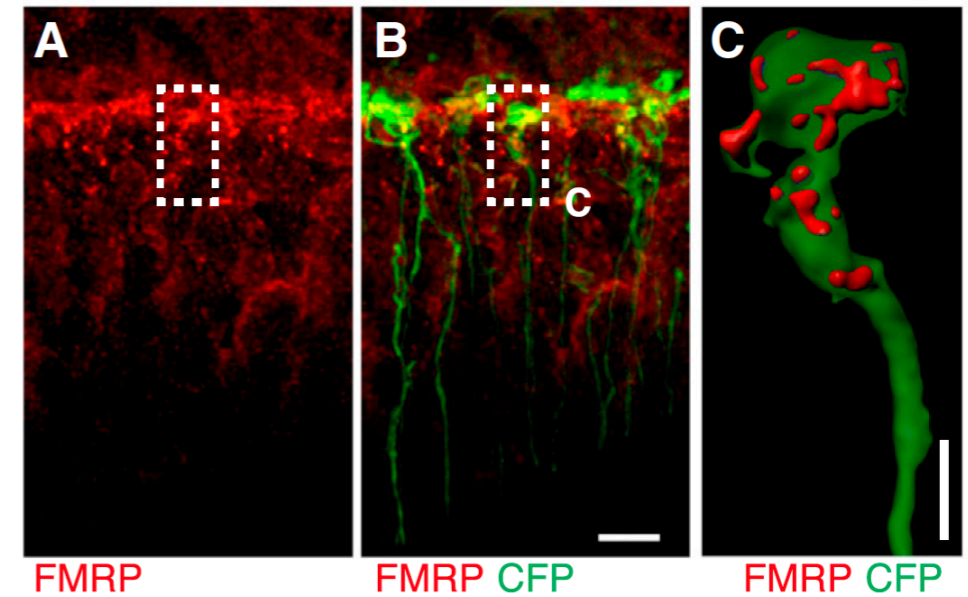


ΔCTE mutant generated by genome editing

Cyclin D2 protein



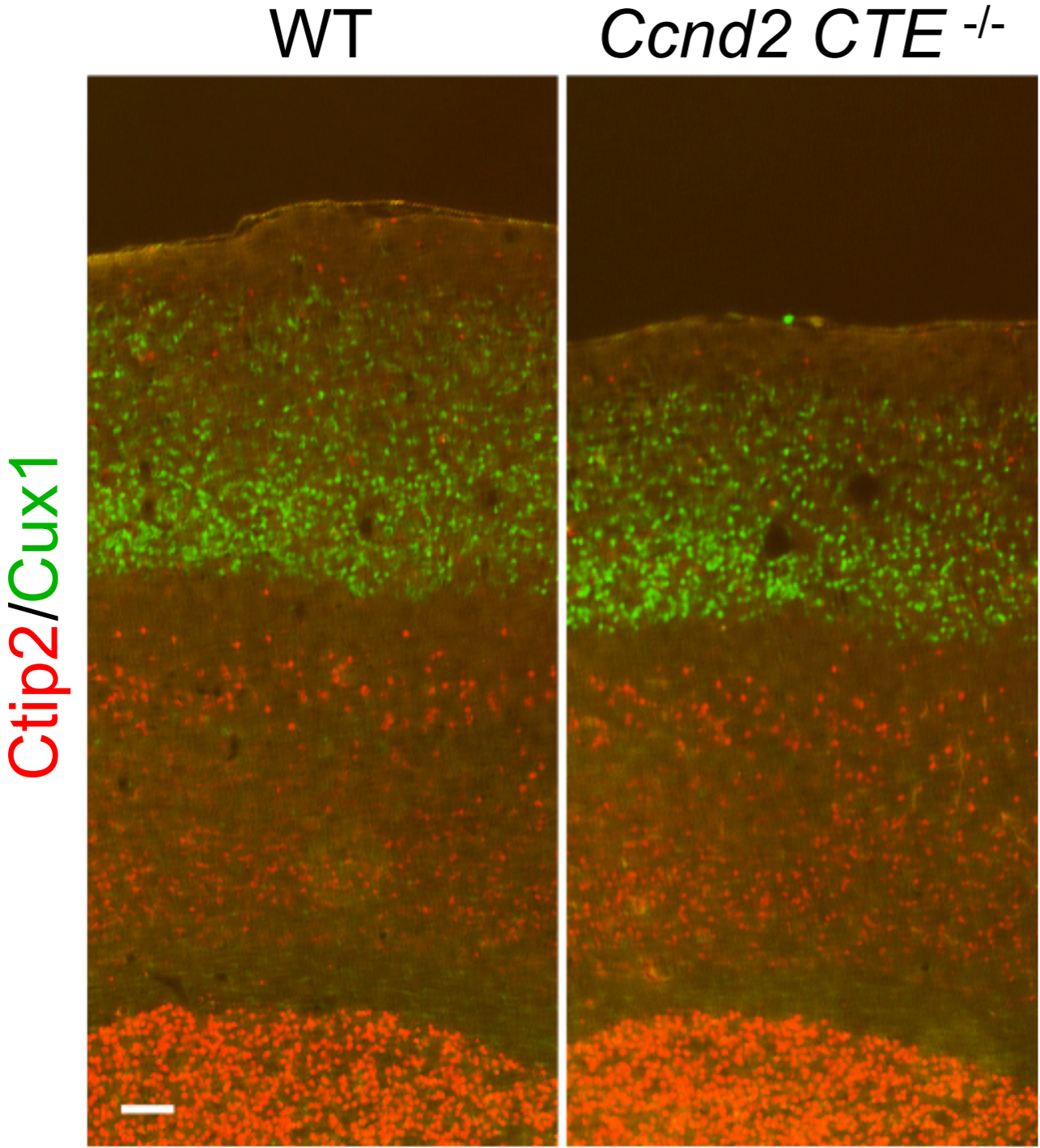
FMRP (Fragile X mental retardation protein)
: RNA binding protein



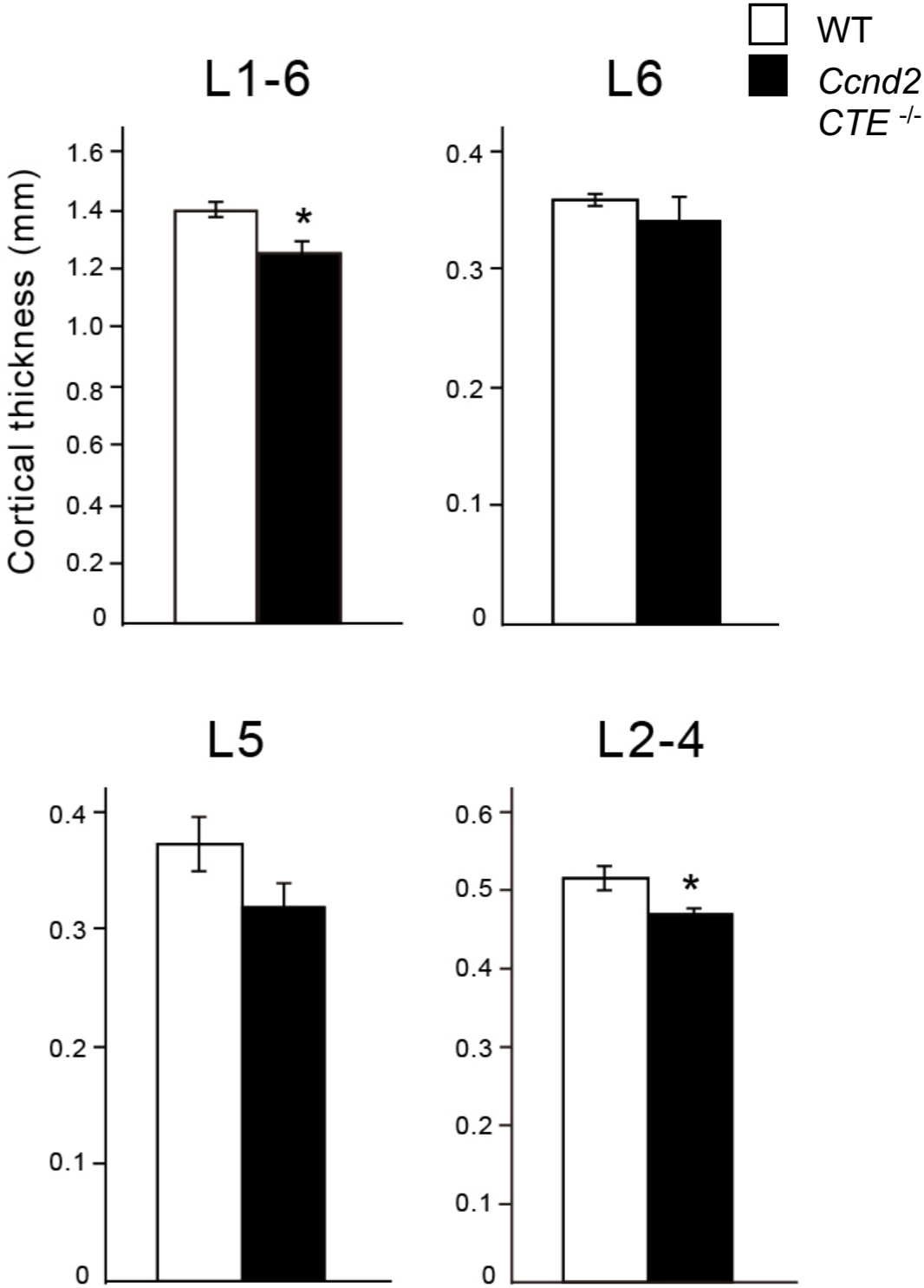
Pilaz et al., 2016

No (or minor?) phenotype in basal endfeet

Impaired layer formation especially in upper layers

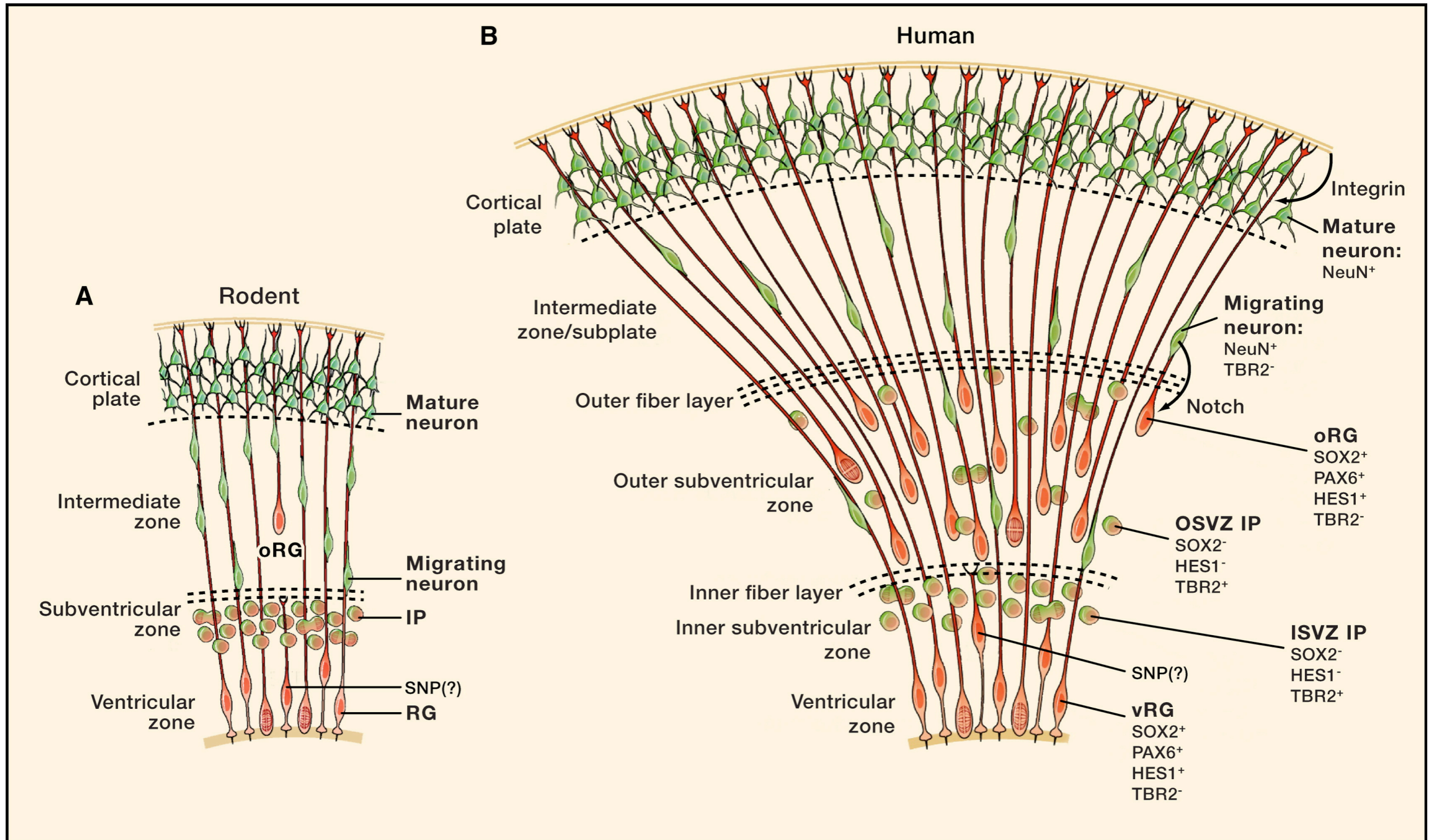


@P10



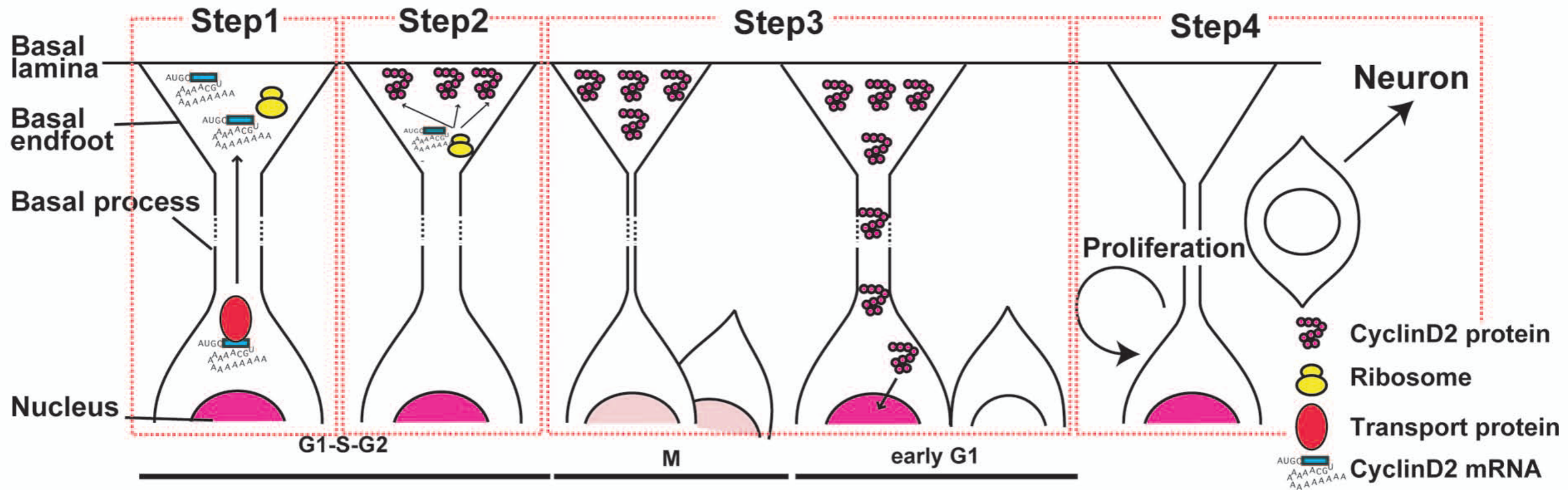
Significance of basal
localization of *Cyclin D2*?

Evolutionary implication



Lui et al.: Development and evolution of the human neocortex. Cell, 2011

Basal sequestering of Cyclin D2 affects cell fates



Lengthening of G1 phase?
Allow transcription of longer mRNAs?

Lengthening of neurogenic period



19 days



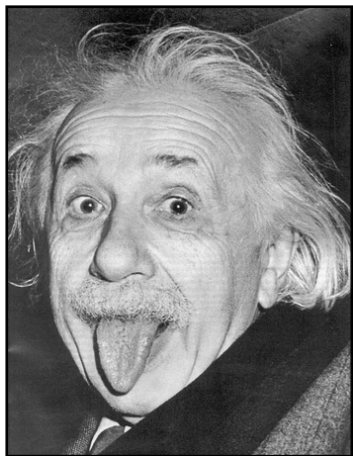
0.5 g



21 days



20 g



266 days



1500 g

Molecules working together in radial glia

Tuj1: neurons

Pax6: RG

Radial glial molecules

Fabp7/BLBP
LewisX/CD15
Notch signals

Nuclear molecules

TFs incl. Pax6, Ngn2, Dmrta1
Polycombs
BAF complex

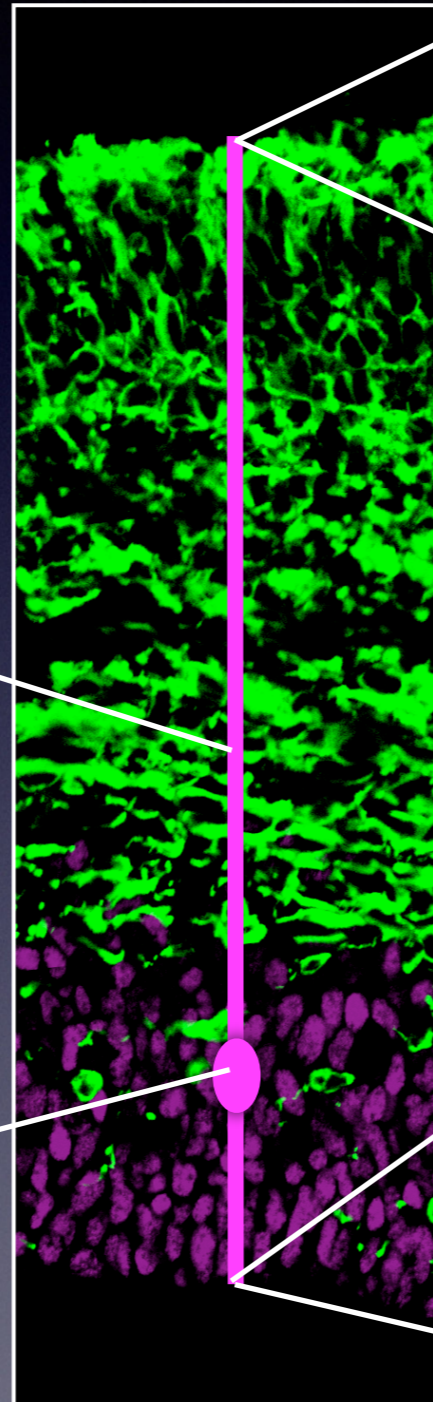
Basal molecules

Secreted molecules
Integrins
Cyclin D2, FMRP

Maintenance of
progenitor cells

Apical molecules

δ -catenin
Polarity proteins
Centrosomal proteins



My questions

(For your report as well)

- Why more boys than girls in autism?
- Why boy:girl ratio is 1.05:1.00?

Choose one of the above two questions