

Genesis of Cortical Neurons from Progenitors

Lineage Tracing of Sox9CreER in E11, E13, E16

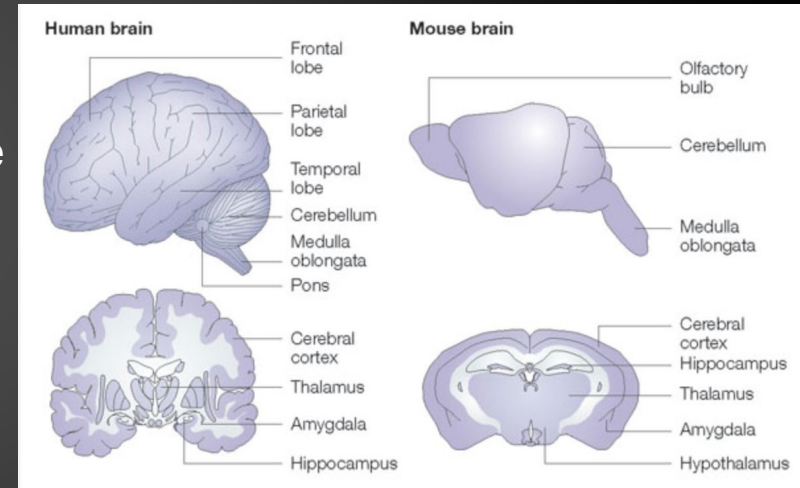


Caitlin Kierum and Carolyn Tran
Dr. Robert Hevner
Seattle Children's Research Institute
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UW Medicine
NEUROLOGICAL SURGERY

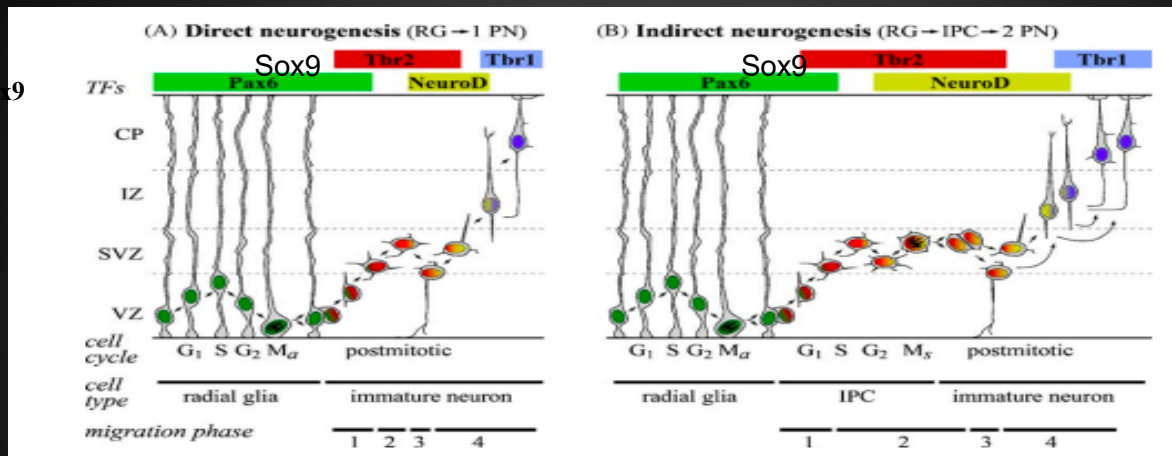
Overview

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Cryan et. al, 2005

Background

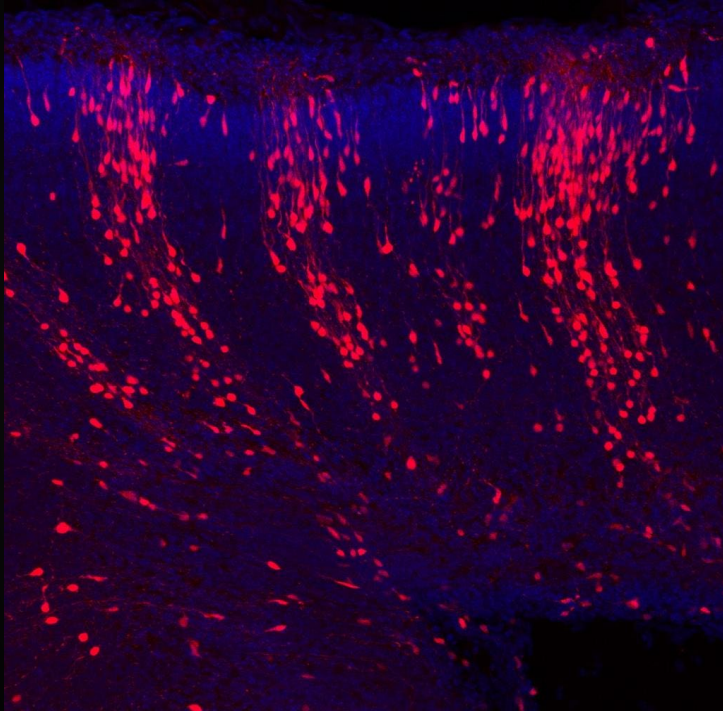


Hevner et. al, 2006

Radial Glia Progenitor Cells (RGP's)

- Produce neurons and glia
- Divide at ventricular surface, express Pax6
 - Produce all of the cortical neurons
 - Have properties of neural stem cells

Radial Unit Hypothesis

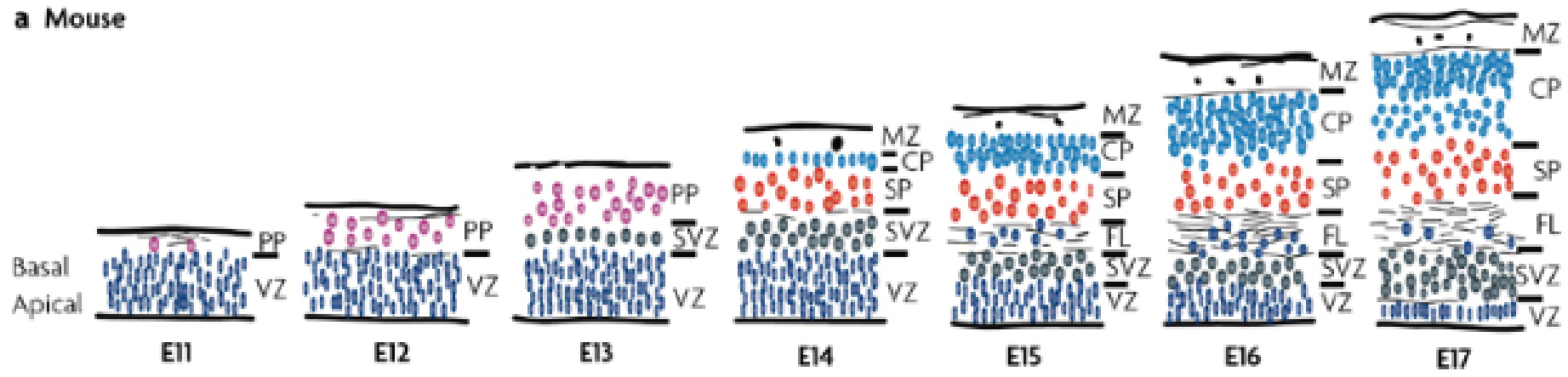


- E11 Tmx to P0
- Sagittal view of neocortex

Cortex Formation

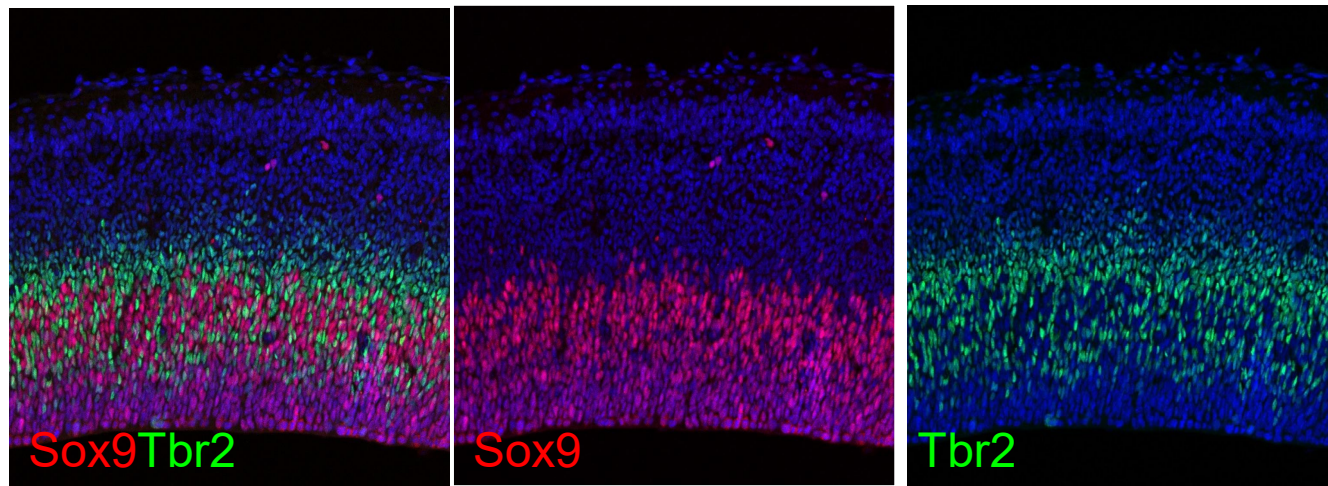
- Formed through an “inside-out” process
- Begins with a layer of neuroepithelial cells
- Around E10.5, these split and layers are formed with earliest born on the bottom and latest born on top

a Mouse



Inducible Cre Lineage Tracer Mouse

Images of E14 mouse brain

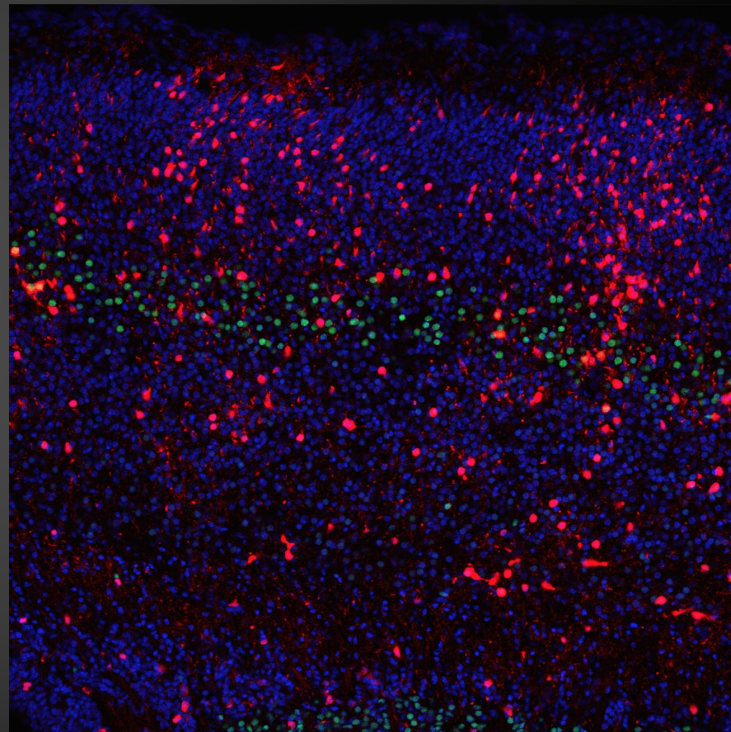


Hevner Lab Unpublished

- Cross of Sox9CreER and Ai14; found in radial glia progenitor cells
- Tamoxifen is administered and induces Cre activity, which turns on RFP gene
- After being induced, any neurons derived from these radial glia progenitor cells (Sox9) will be stained red in immunohistochemistry

What is our project?

- To determine which laminar layer cells, derived from RGP's, migrate to during corticogenesis.



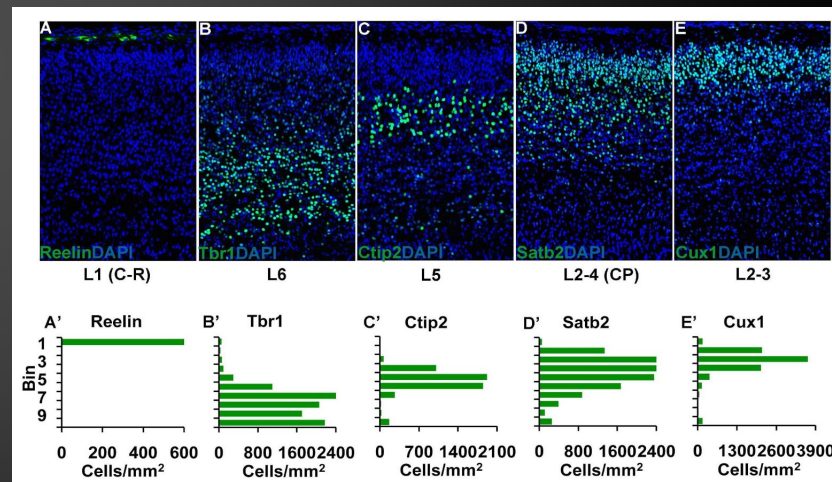
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Methods

1. Immunohistochemistry
2. Imaging
3. Image analysis

Antigens used for layer markers in IHC:

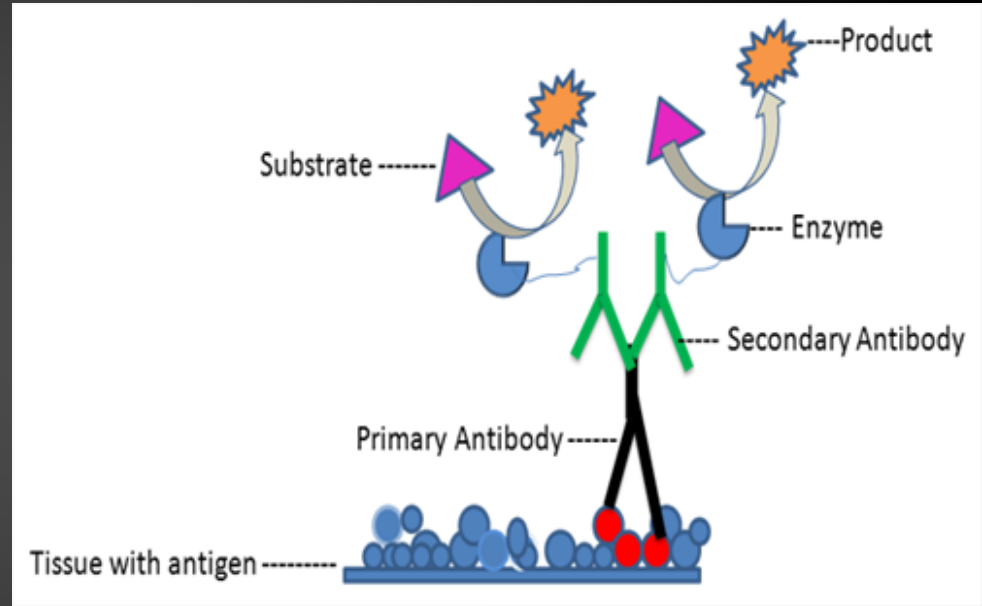
Layer	Antigen	Species
L1	ReIn**	mouse
L2/L3	Cux1**(didn't work), Satb2	rabbit
L4	Satb2 (didn't use)	rabbit
L5	Ctip2**	rat
L6	Tbr1** (didn't use)	rabbit



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Immunohistochemistry (IHC)

- A staining method used to detect specific proteins
- **Blocking:** reduces the background and nonspecific sites
- **Primary Antibody**
- Antigen specific
- **Secondary antibody**
- Contains a fluorophore that aids in detection by attaching itself to the primary antibody
- <http://www.pierce-antibodies.com/products/secondary-antibodies/>



<http://stevegallik.org/images/immunohistochemistry.png>

Image Analysis

- All images are of a P0 mouse brain
- Our slide ages “E11,” “E13” and “E16” refer to the date Tamoxifen was administered

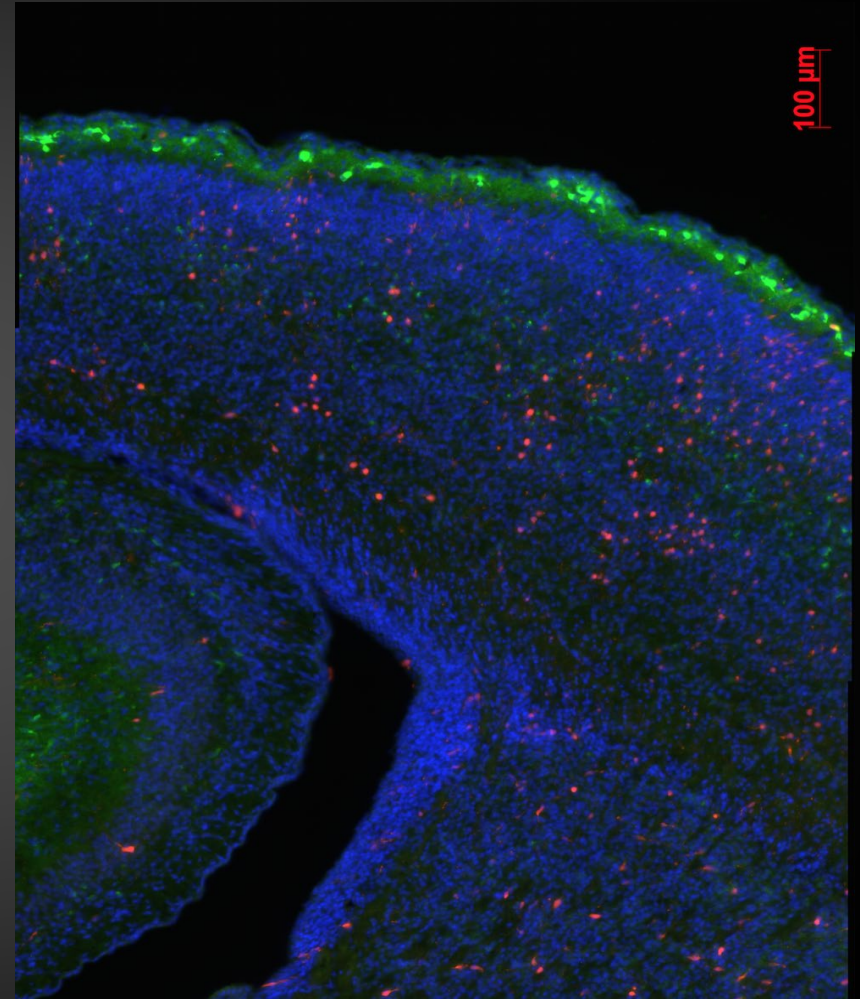
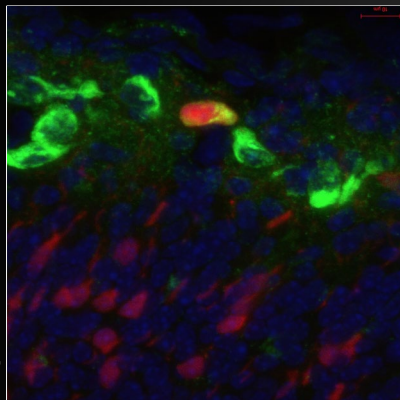


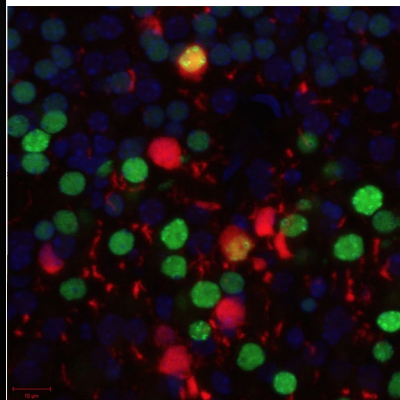


Image Analysis

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High mag image
of colocalized
Reln and RFP



High mag
image of
colocalized
Ctip2 and
RFP

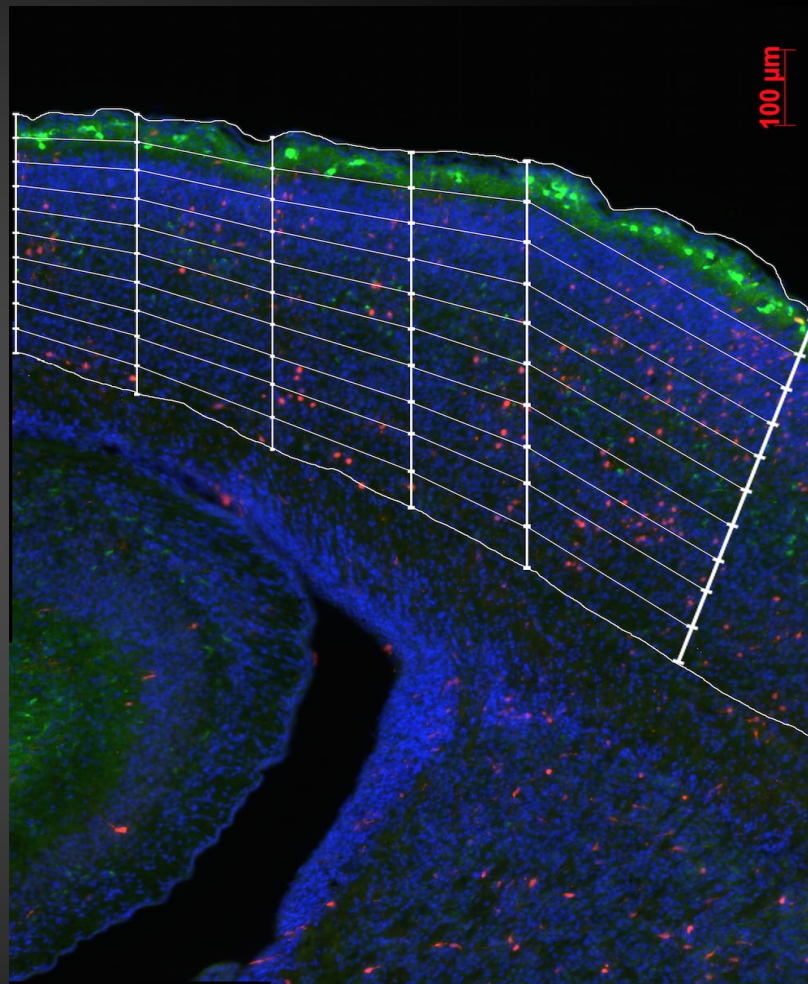
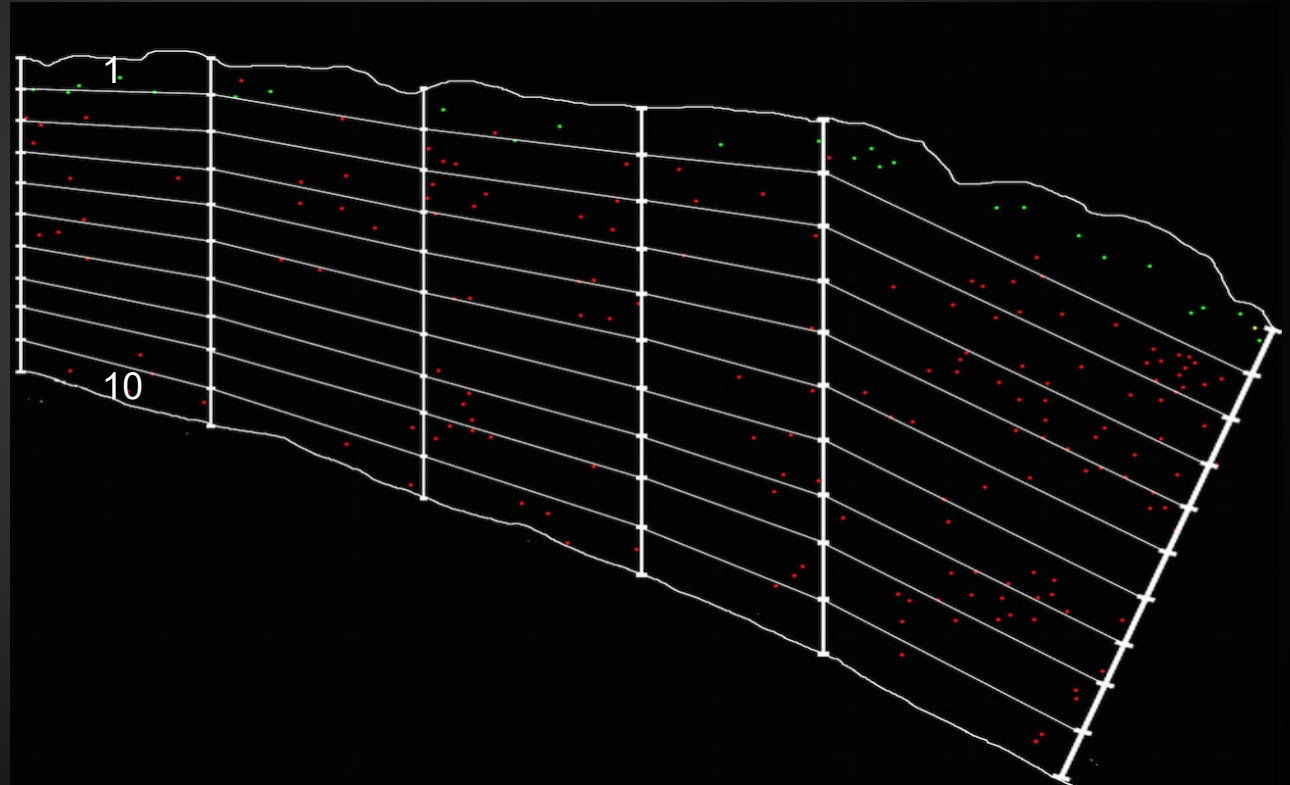
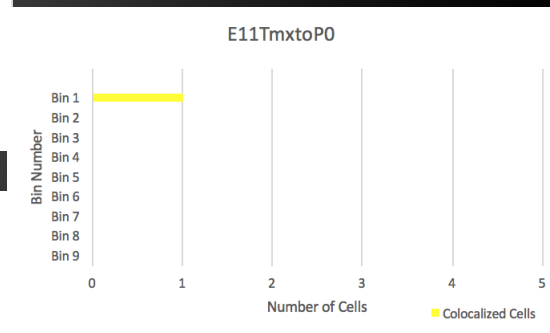
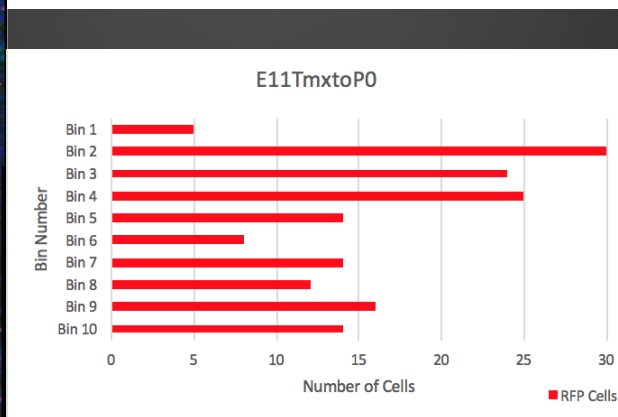
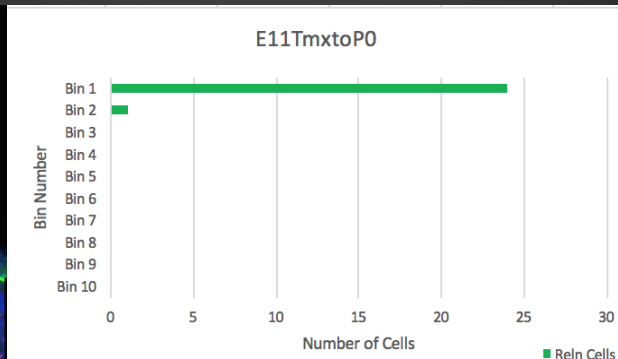
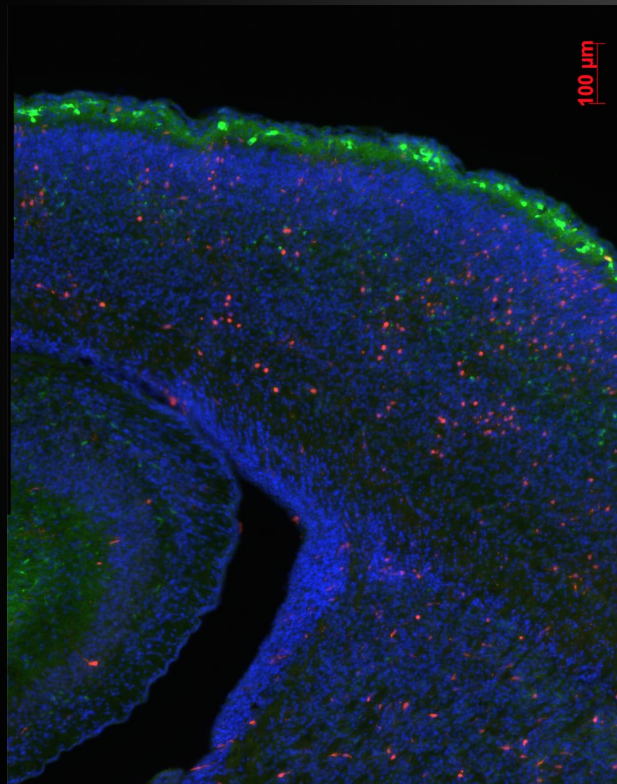


Image Analysis

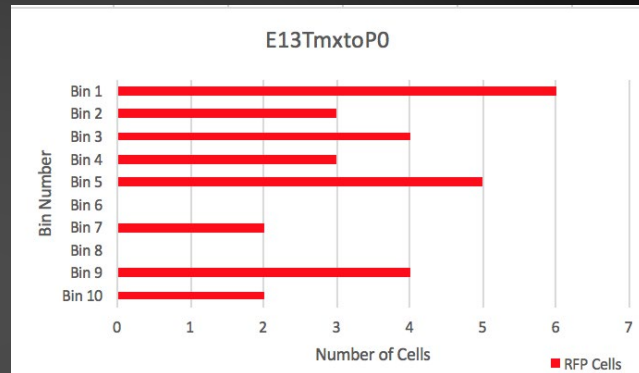
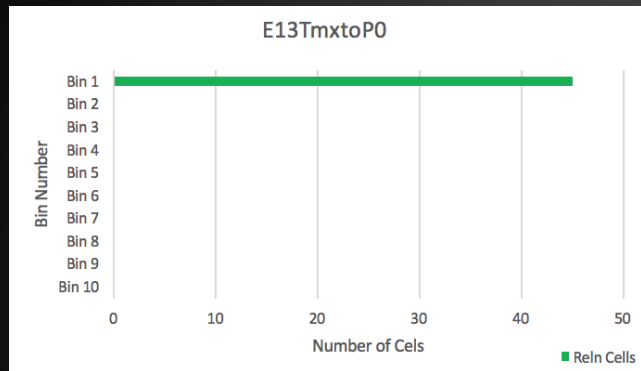


Results: Reln at E11

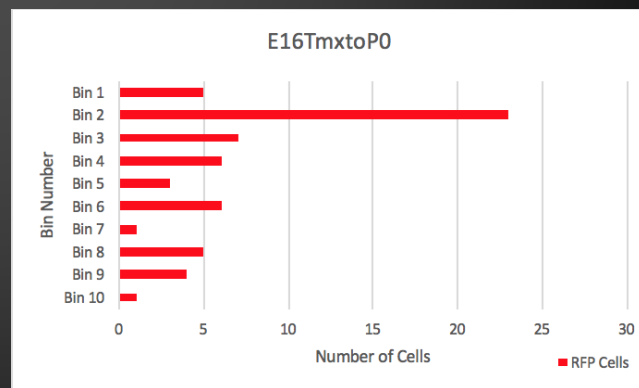
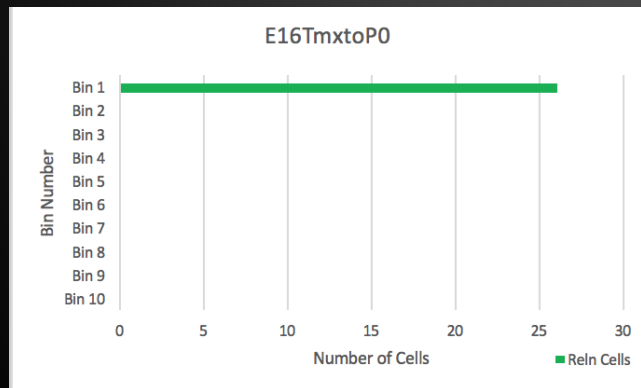


Results: ReIn at E13 and E16

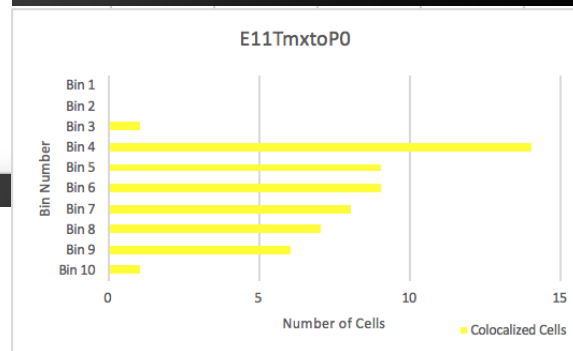
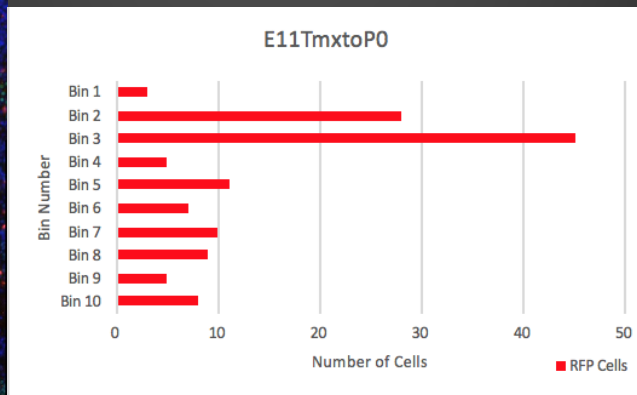
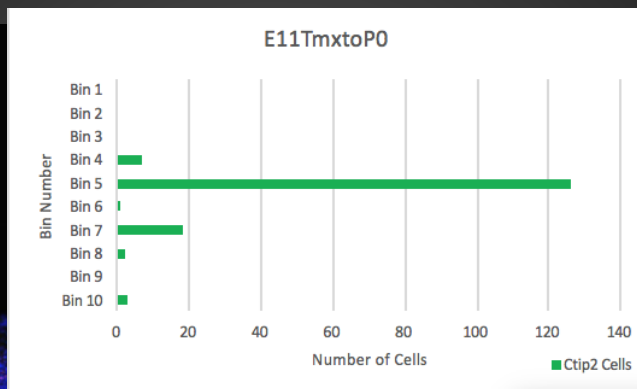
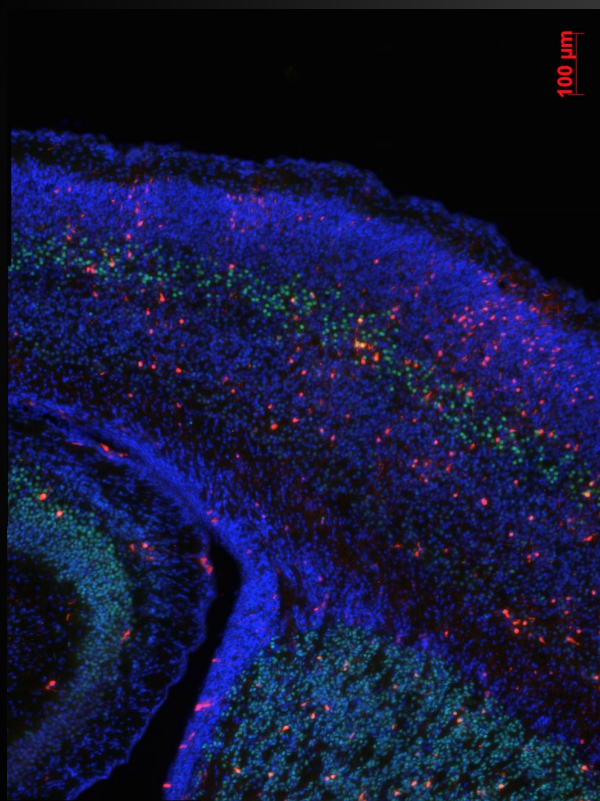
E13



E16

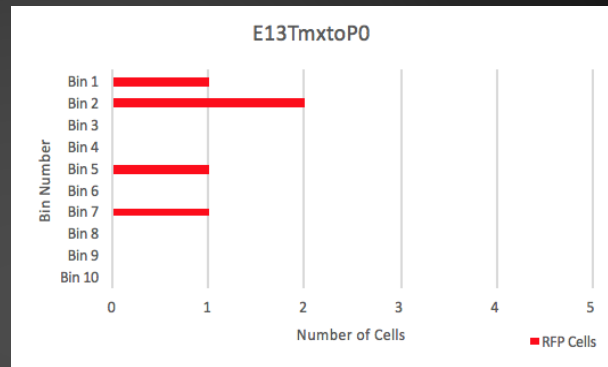
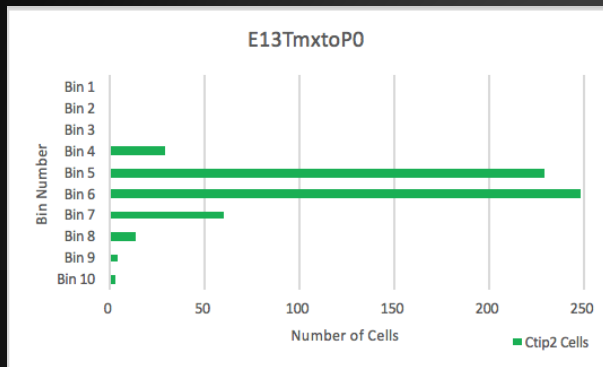


Results: Ctip2 at E11

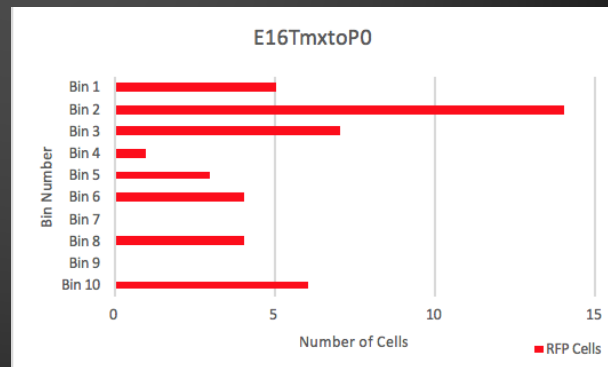
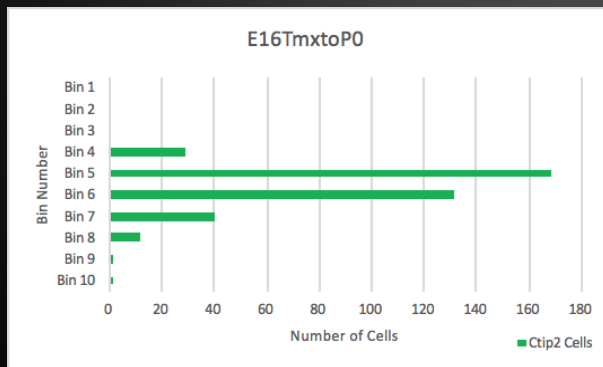


Results: Ctip2 at E13 and E16

E13

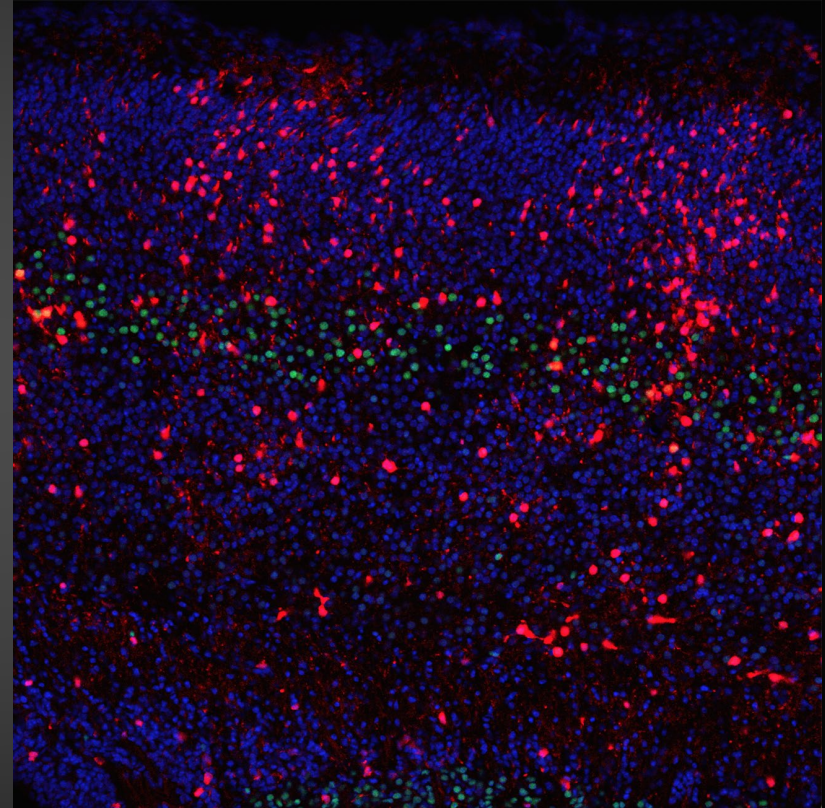


E16



Discussion

- Early progenitor cells produce all cortical layers and late progenitors have more restricted fates.
- For future directions, these mice will be used for clonal lineage tracing by reducing tamoxifen dose.



Hevner Unpublished Data



Acknowledgements

- UW's Neurological Surgery Summer Student Program
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