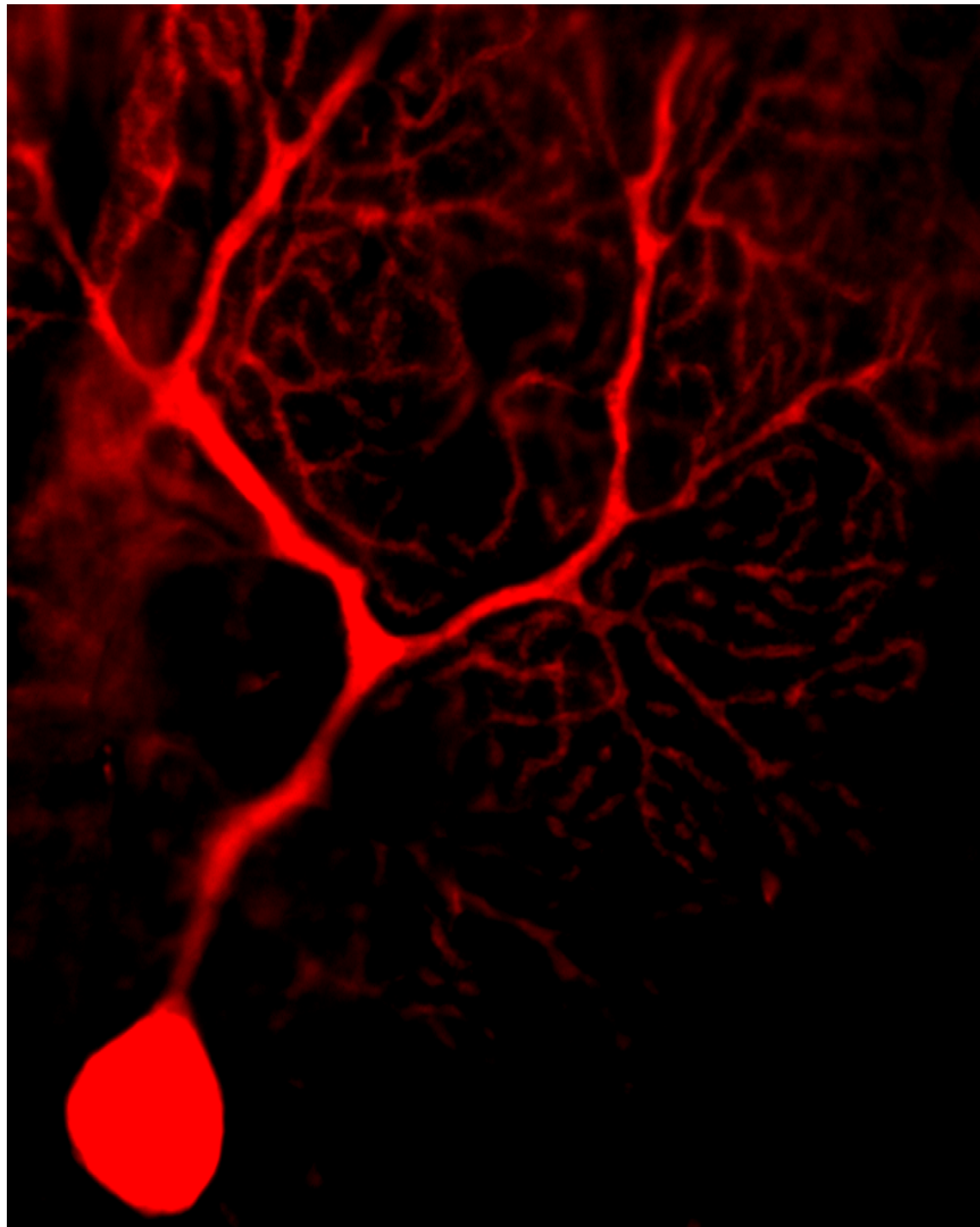
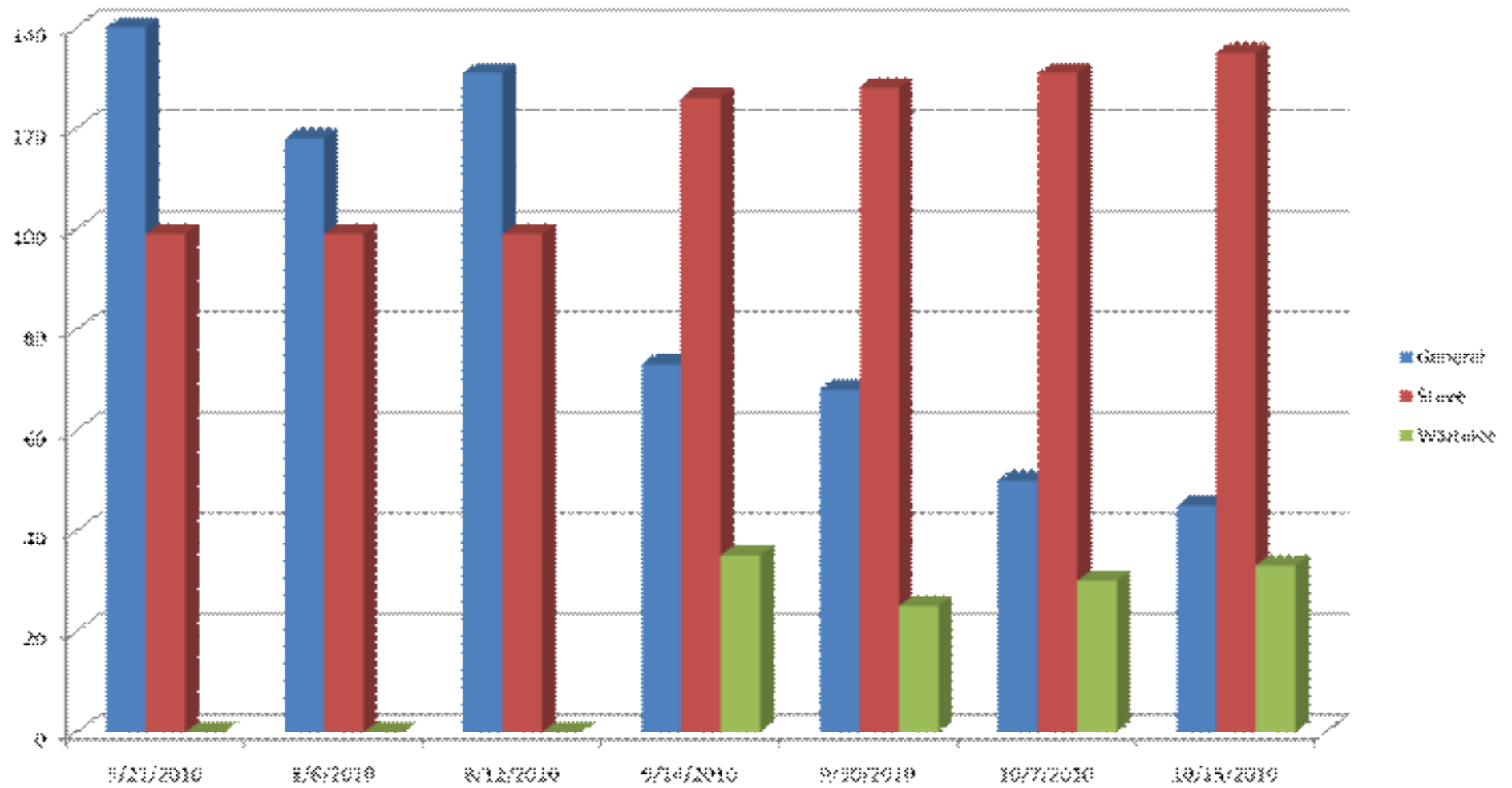




Lab
Meeting:
10/15/2010

STH

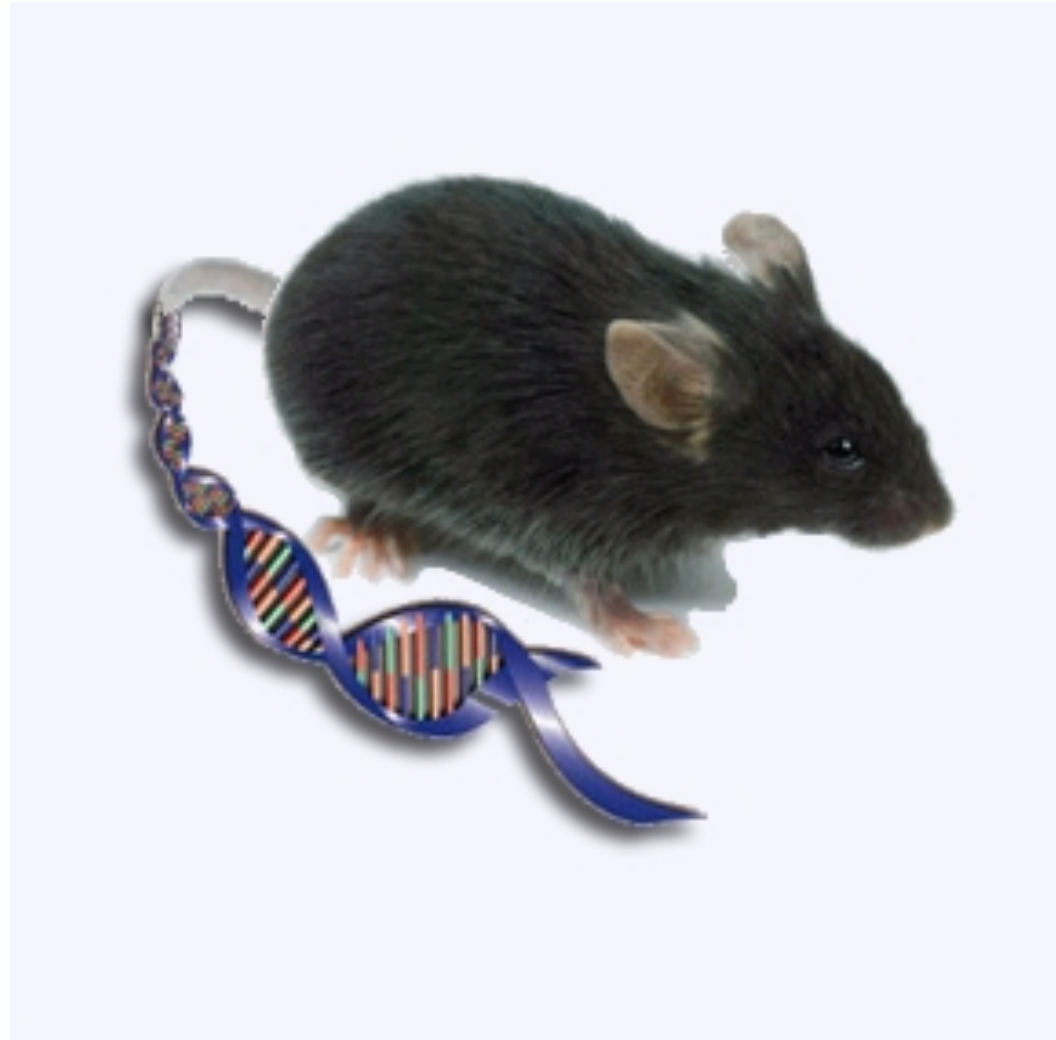
Colony Cage Counts From May



Projects

- SCA 2 Q127
- Luciferase mice
- Olfactory Bulb staining

SCA 2: Q127



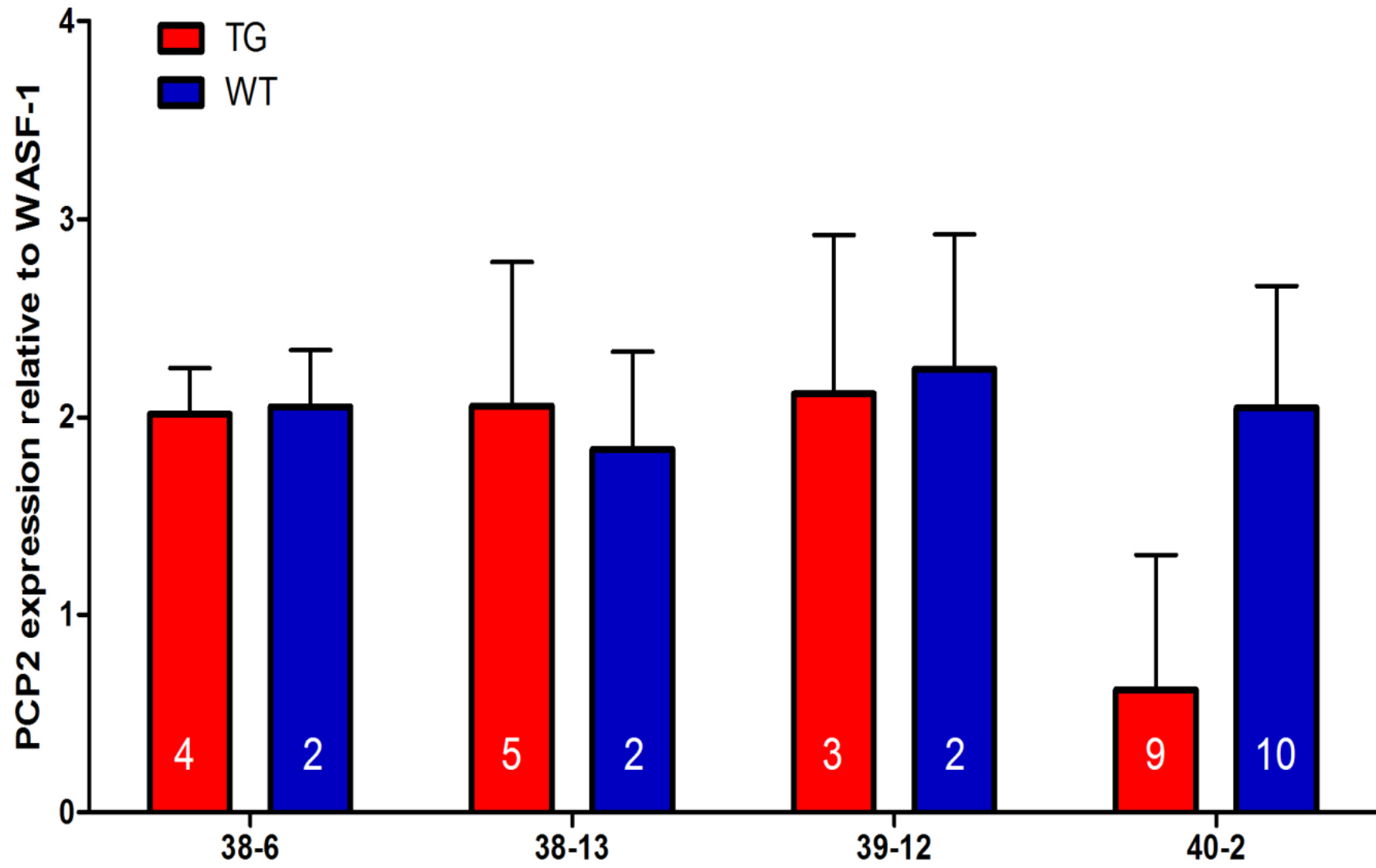
SCA2: Q127 (40-2)

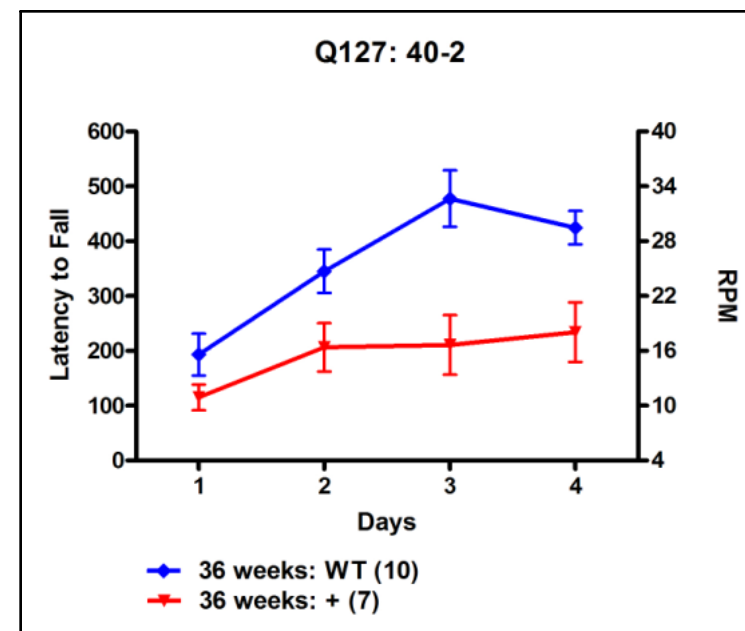
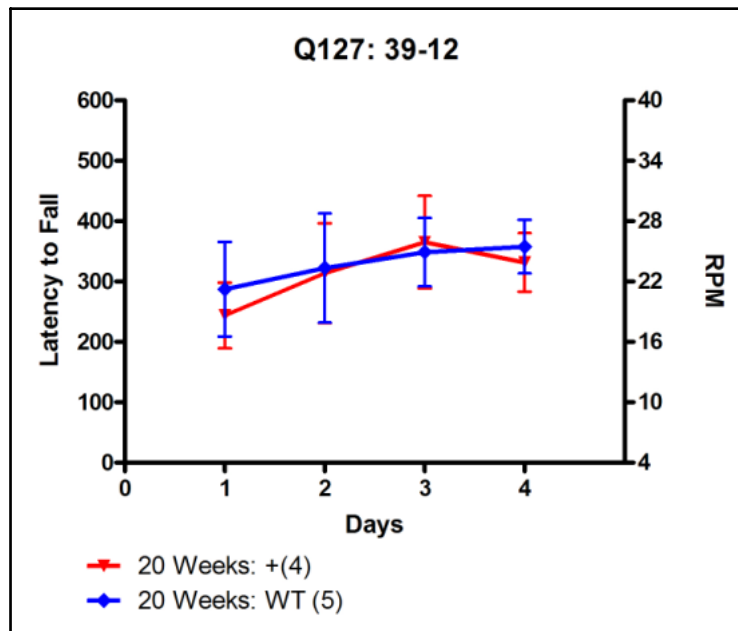
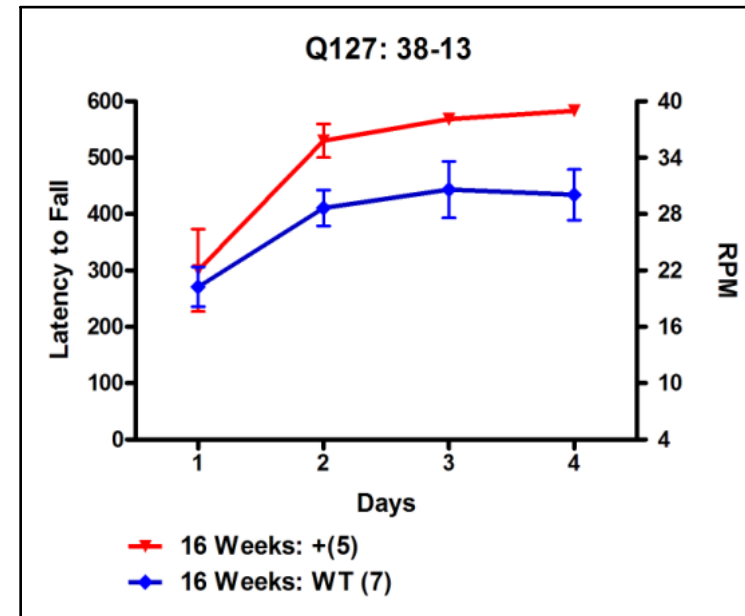
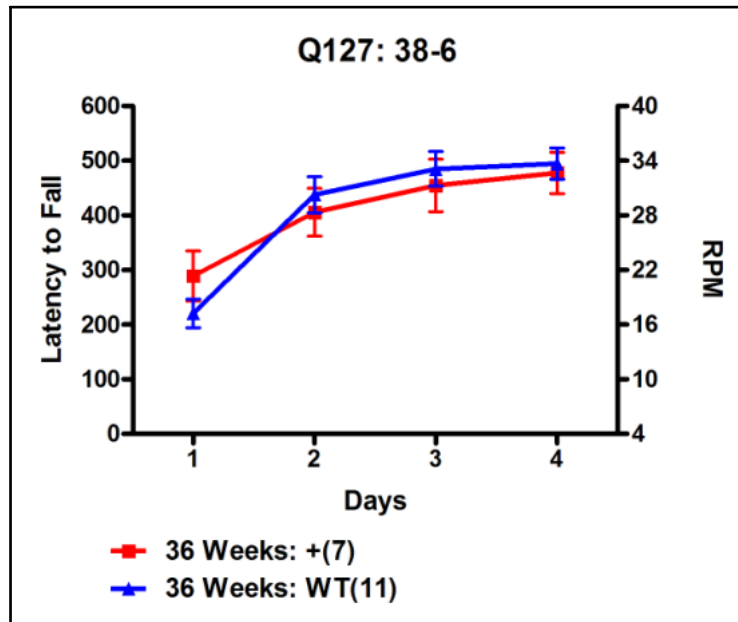
- qPCR
- Histology
- Electrophysiology
- Behavioral motor testing

logistics

- Put down 2 of the 4 lines of q127 SCA 2
 - 38-13 & 39-12
 - Why....
 - No qPCR phenotype
 - No rotarod phenotype
 - Keep line 38-6 as a negative control to 40-2

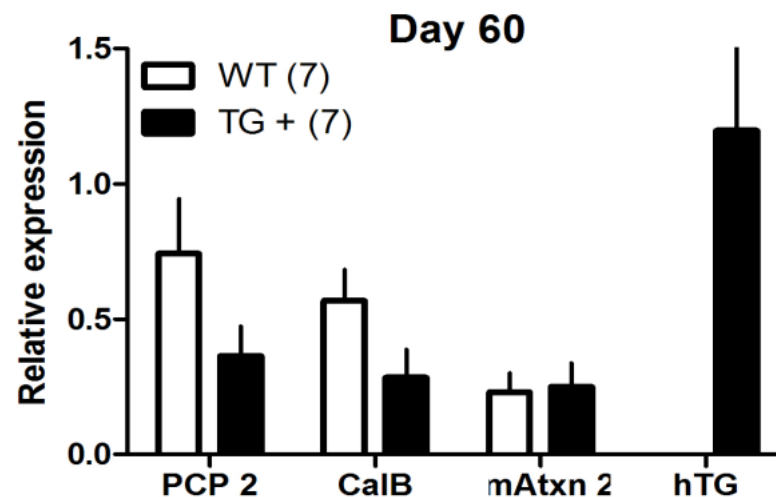
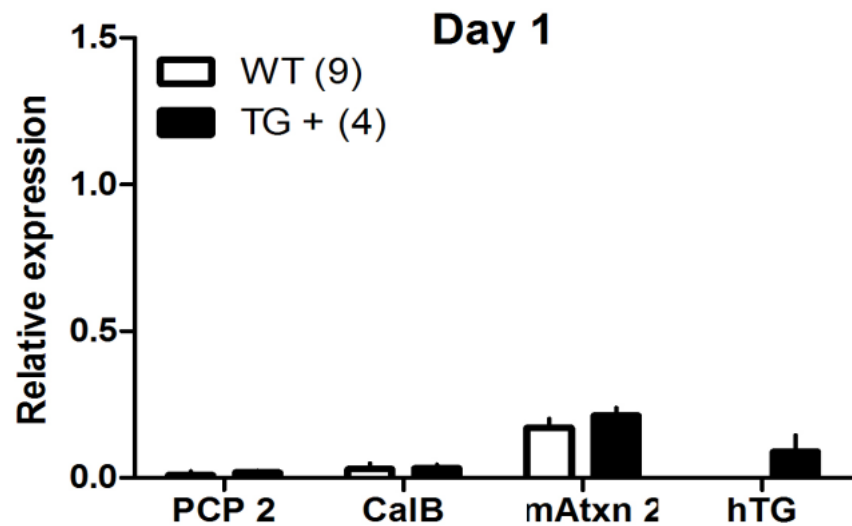
qPCR for 6-9 month old mice



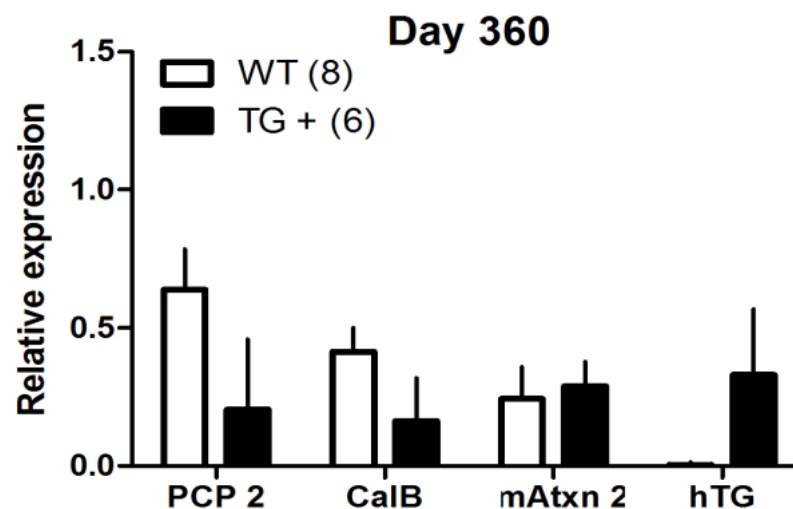
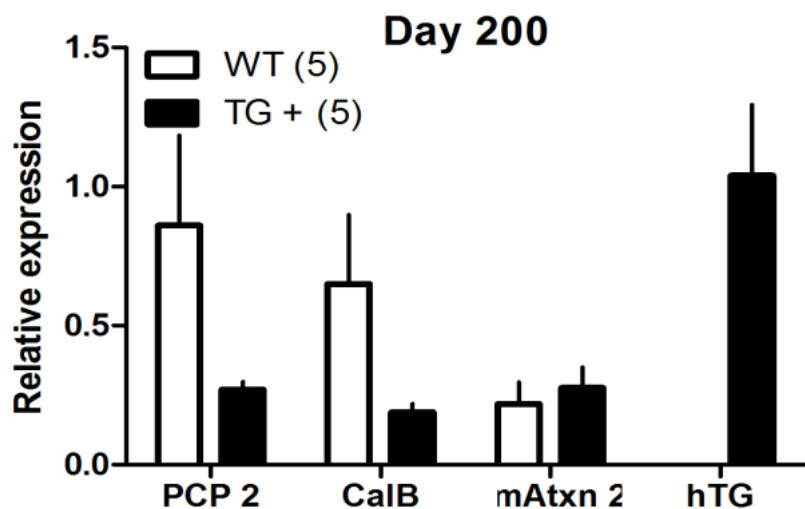


Rotarod data

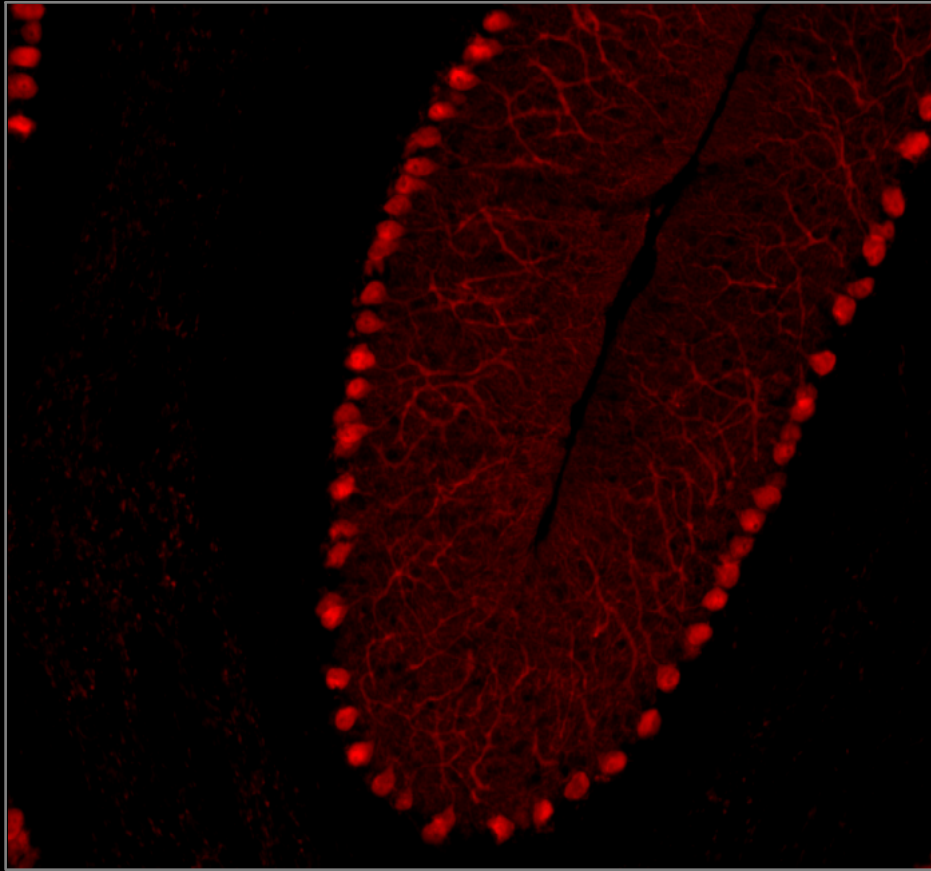
The following slides from the
SCA2 q127 project represent
data obtained from line 40-2



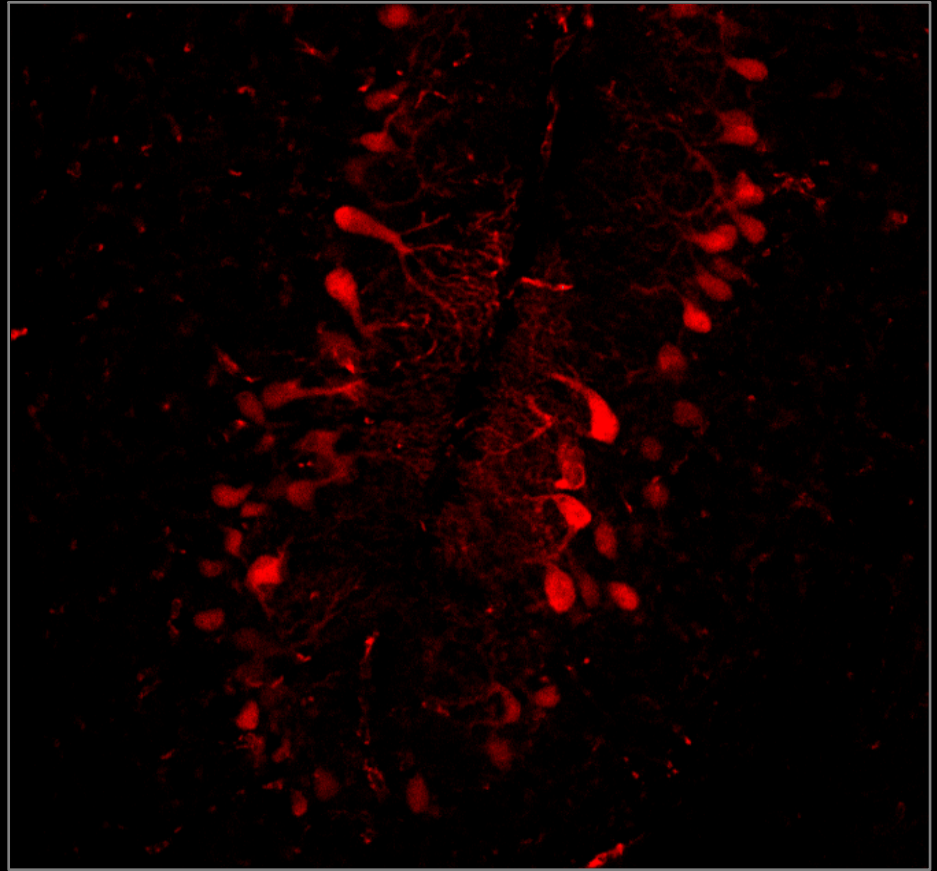
qPCR for 40-2



Cerebellar image taken from *prima fissura* of vermis

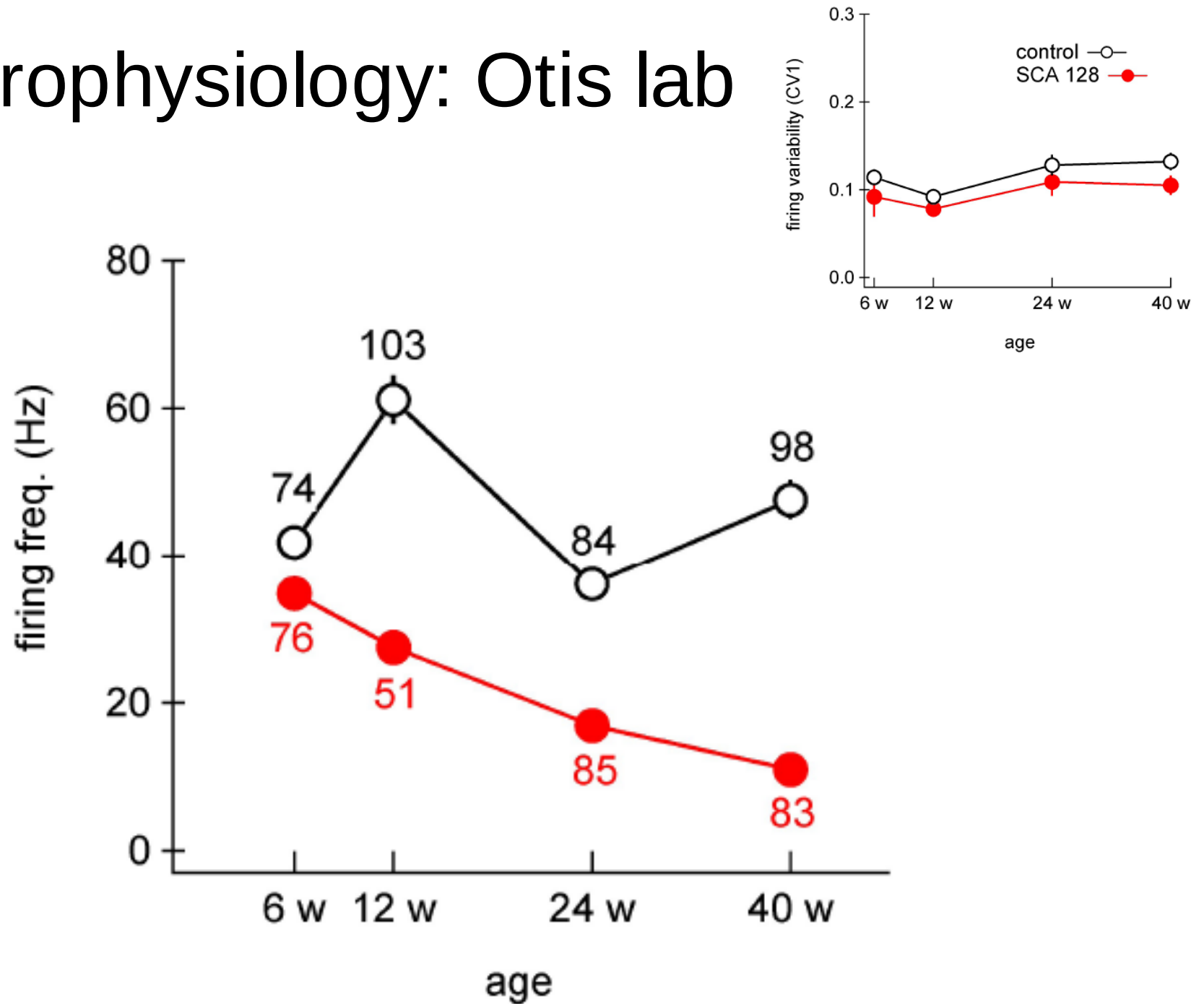


WT 20 weeks

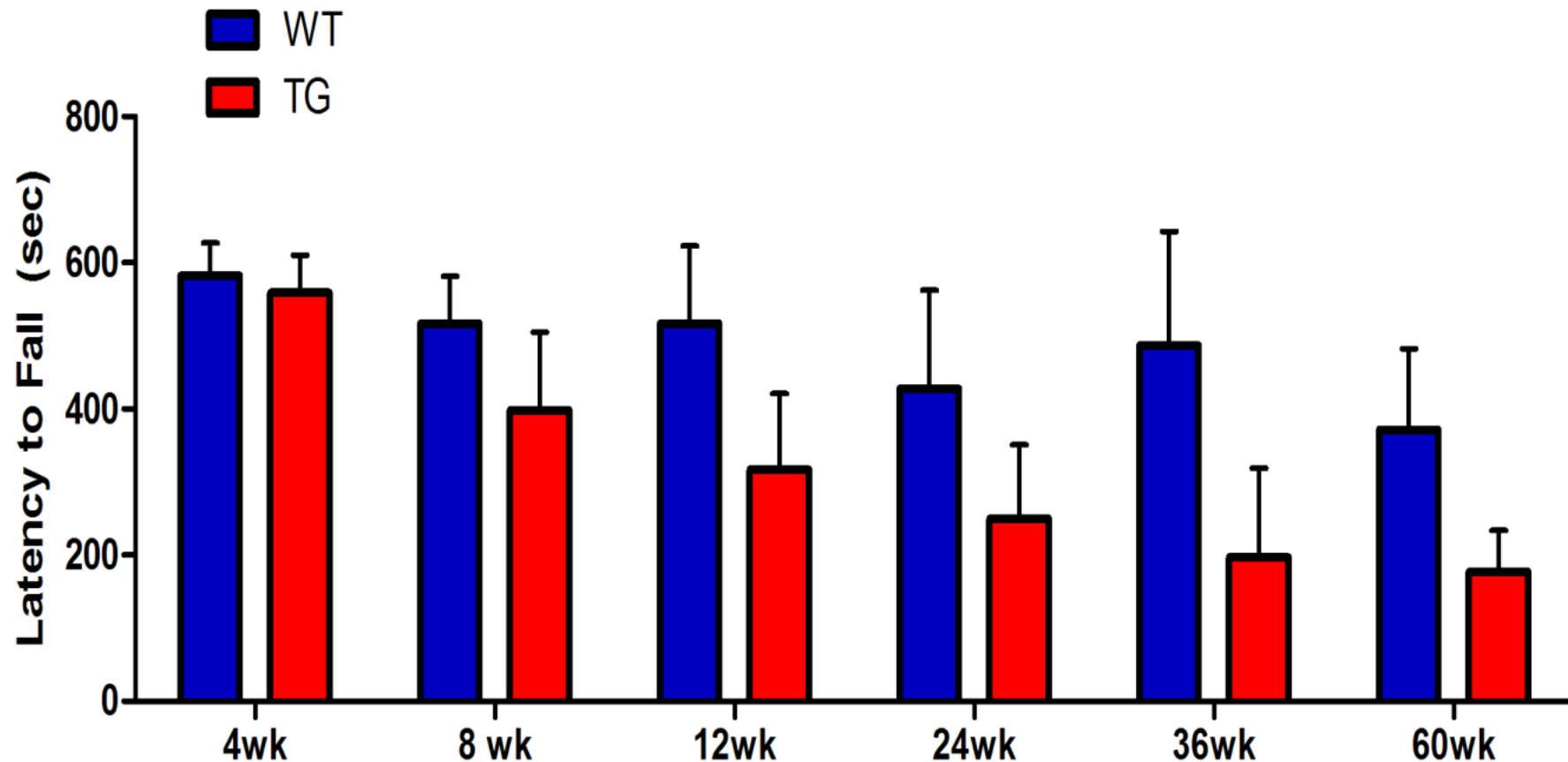


TG: 24 weeks

Electrophysiology: Otis lab



- Rotarod performance: comparison between age matched TG and WT
 - Data represents avg of 3 trials on final day of testing



Q127: 40-2 x SCA6 (KI & KO)

- SCA6 KI Q14 x 40-2:

- N=28:
 - 11 sca 2
 - 7 sca 6
 - 5 sca 2 & 6
 - 5 WT

- SCA 6 KI Q84 x 40-2:

- N=25
 - 5 sca2
 - 2 sca6
 - 3 sca 2& 6
 - 15 WT?

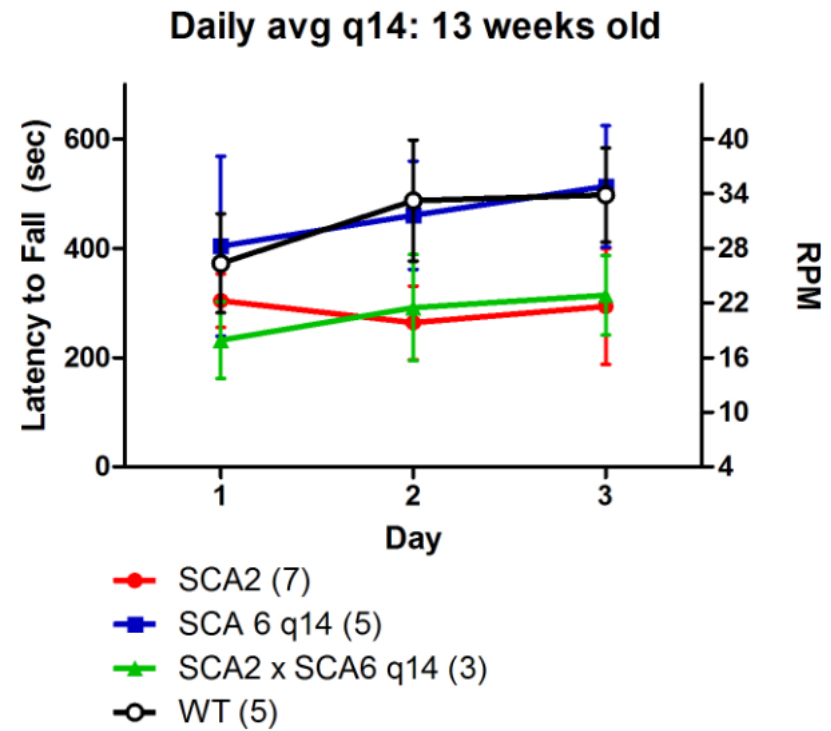
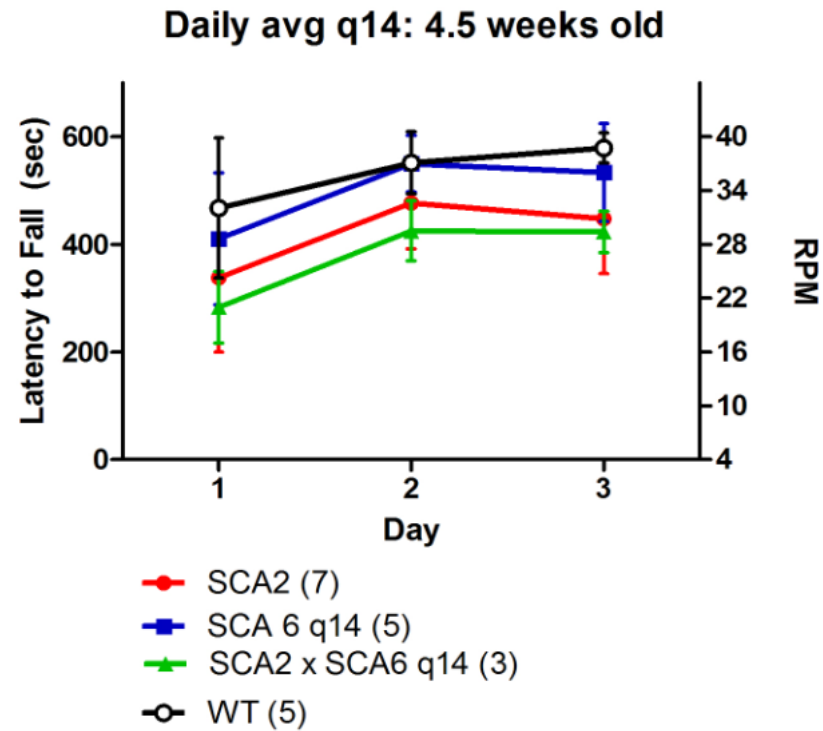
- SCA6 KO x Jax B6

- N=22
 - 10 Het KO
 - 12 WT

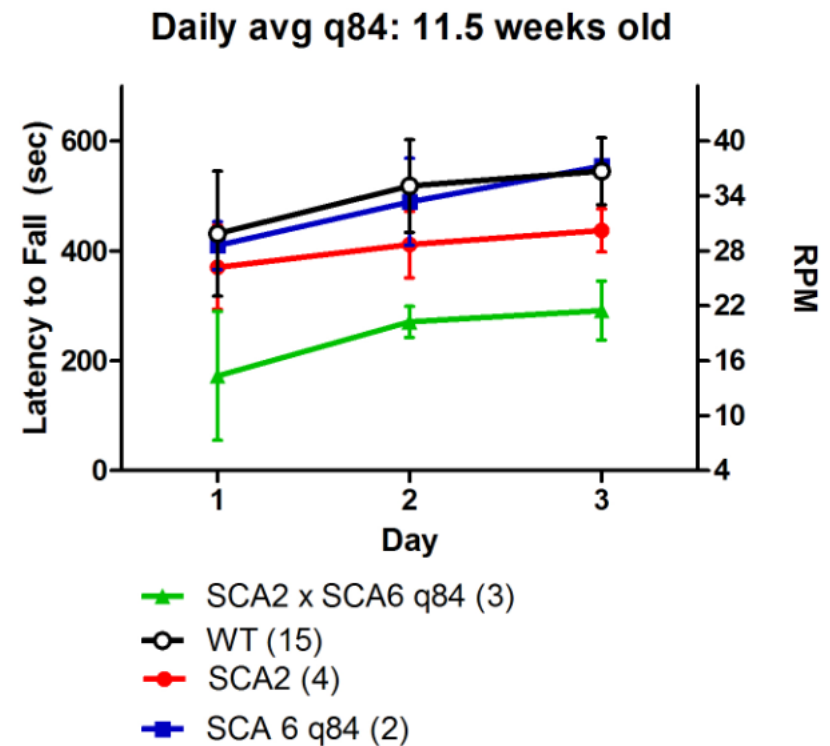
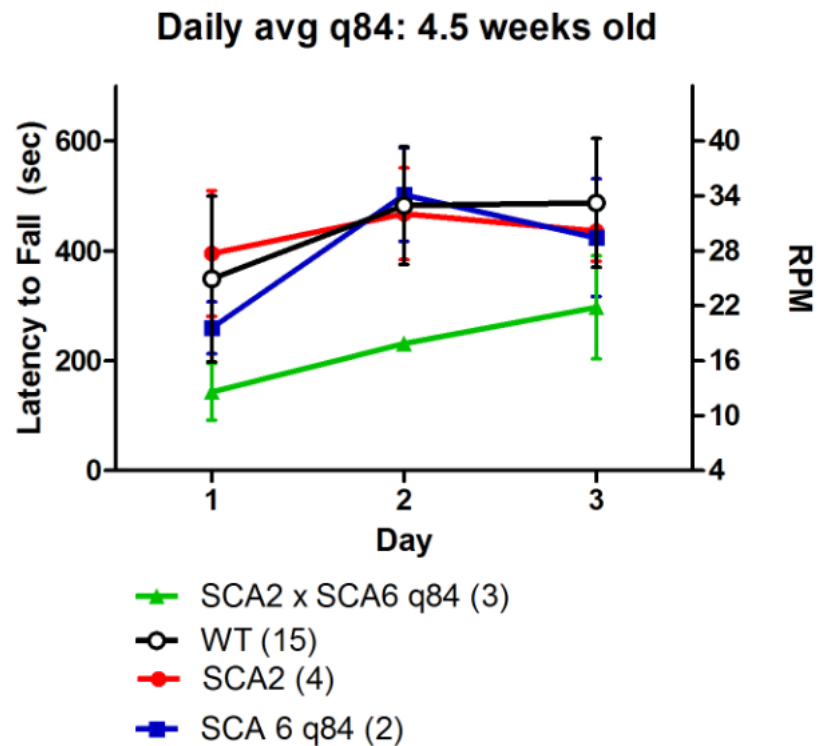
- SCA6 KO x 40-2 :

- N=18:

SCA 2: q127 x SCA 6: q14



SCA 2: q127 x SCA 6: q84



SCA 2: q127 x SCA 6 KO

- 4 week old Rotarod data collected
- Finishing PCR...

Future

- Collect 3rd rotarod time point for existing animals
- Breeding for larger “n” size: KI & KO
- qPCR
- Histology
- E-phys

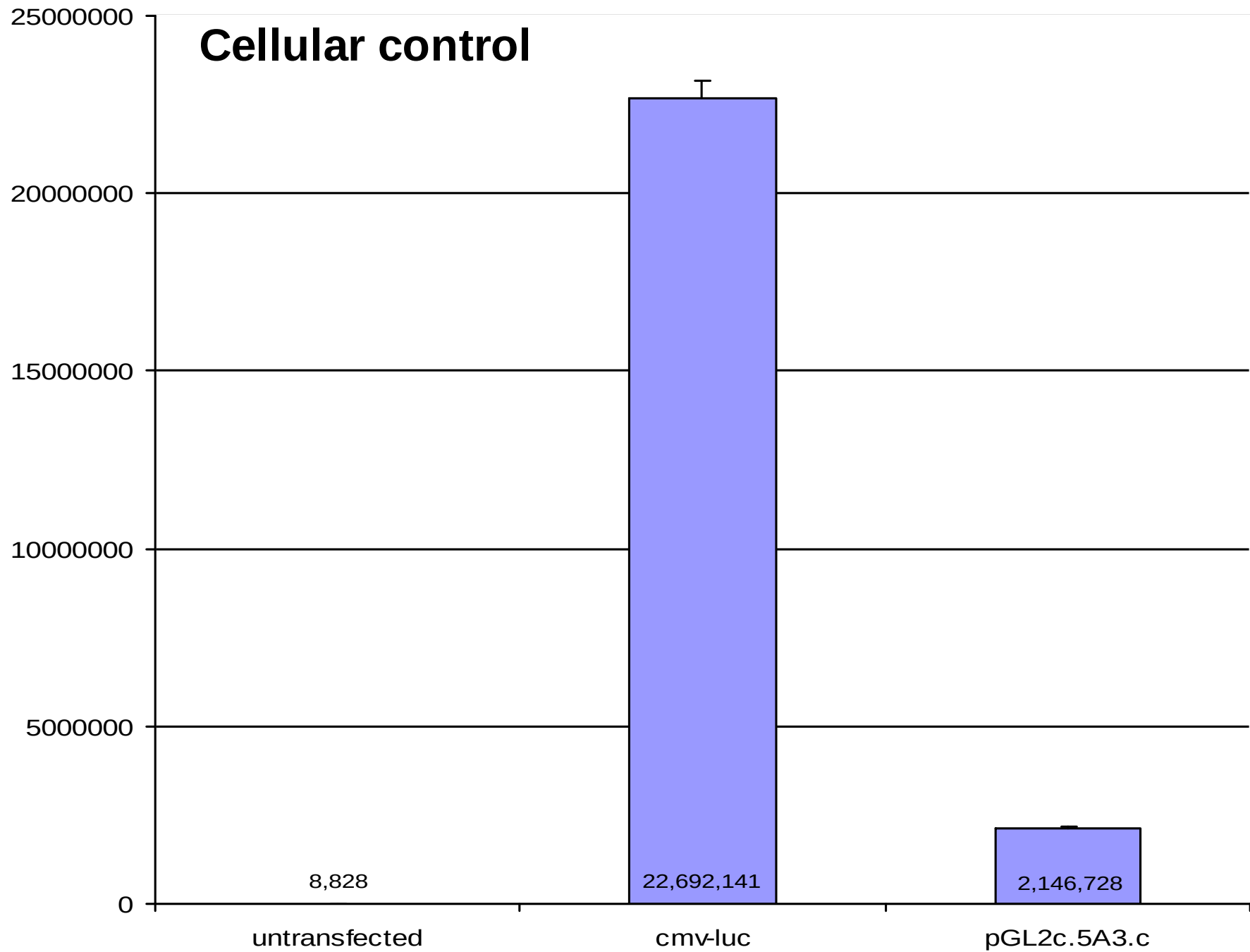
Lucifer (a s e)



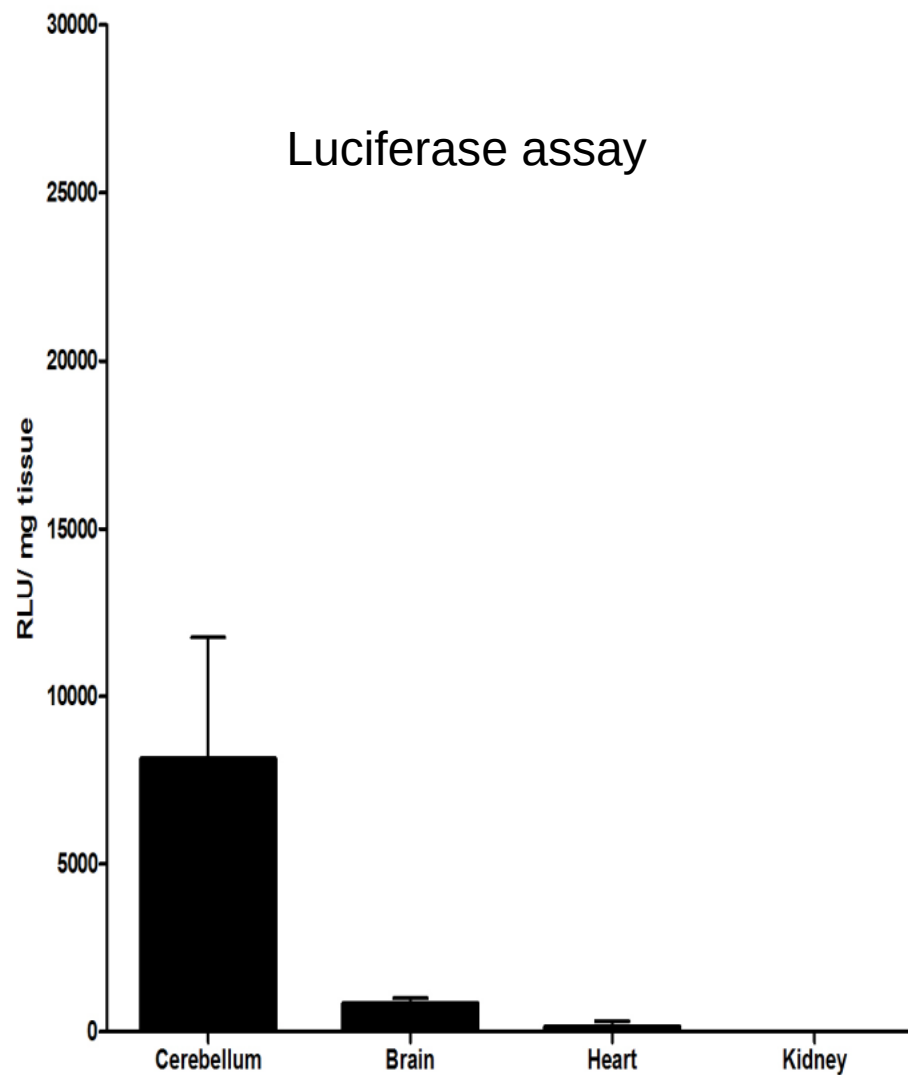
Luciferase

- 2 luciferase lines: L74-1; L75-1
 - Luciferase assay
 - qPCR
 - Western-blot
 - Immunohistochemistry

Cellular control

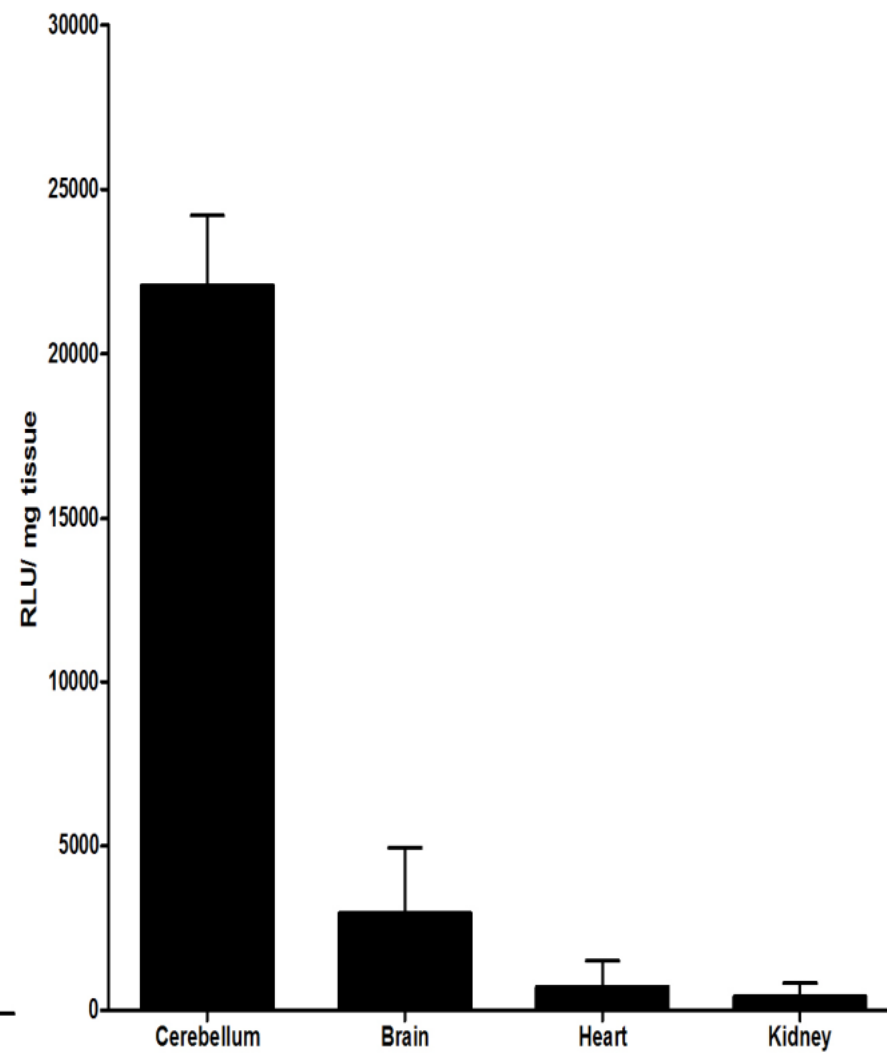


L74



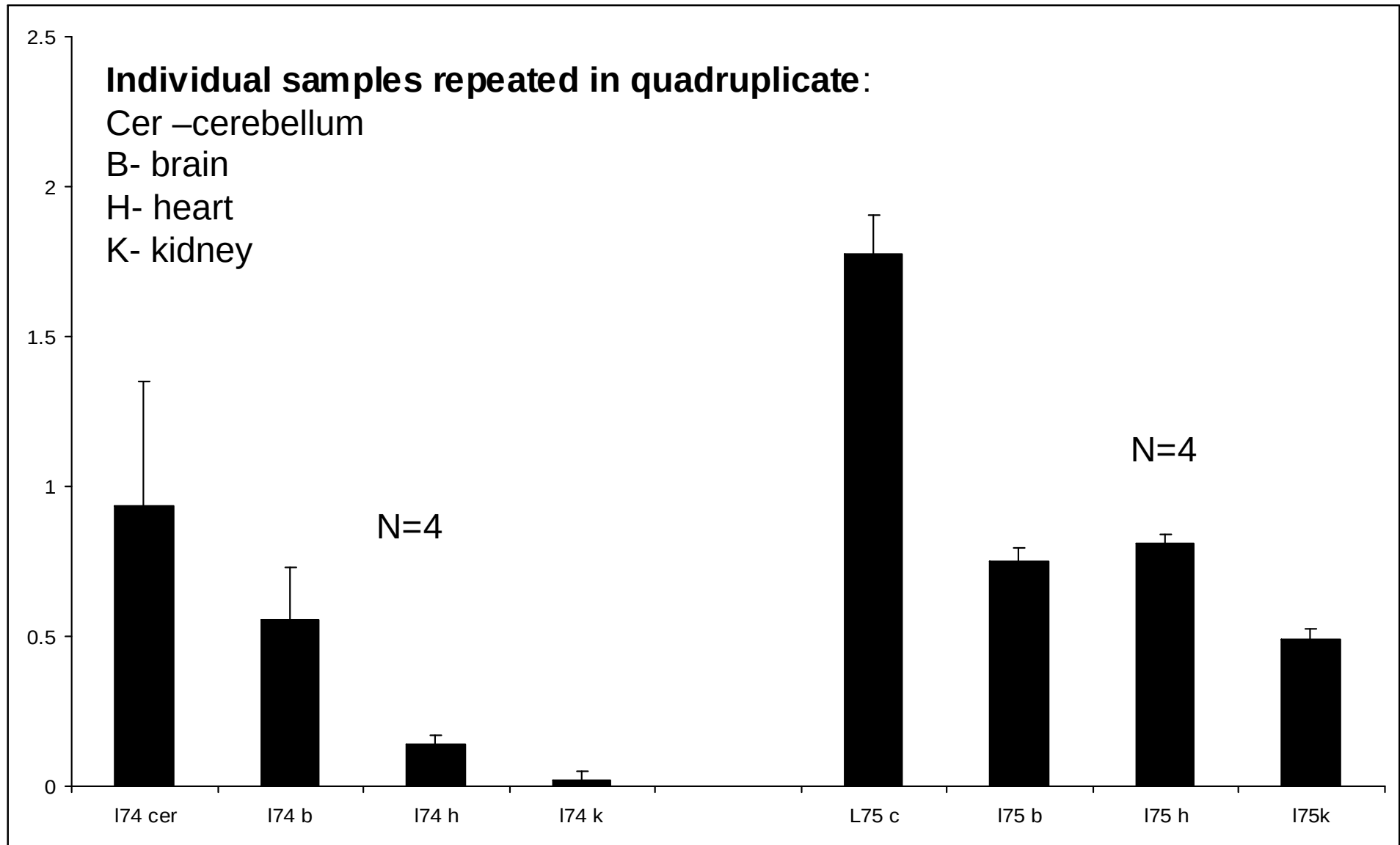
Luciferase: L74 (n=4)
error bar represent standard deviation

L75



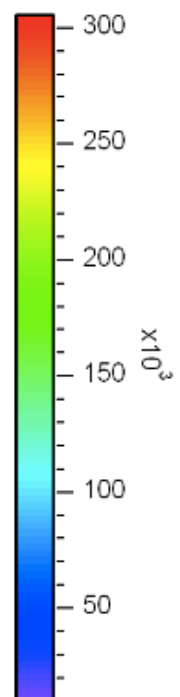
Luciferase: L75 (n=4)
error bar represent standard deviation

qPCR: luciferase normalized to atxn2

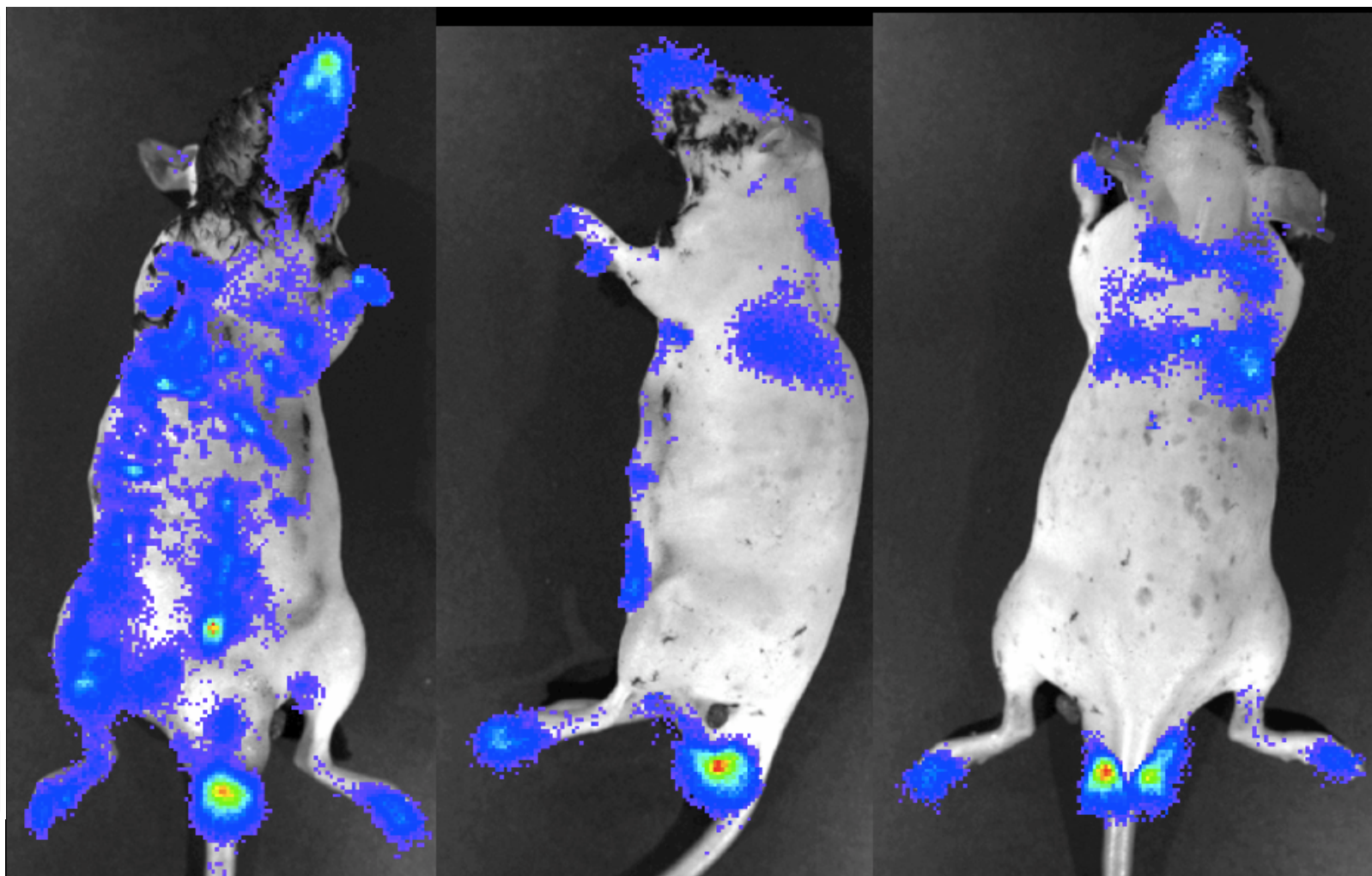


Luc 75-1

Image
Min = -13192
Max = 3.0429e+05
p/sec/cm²/sr



Color Bar
Min = 11764
Max = 3.0429e+05

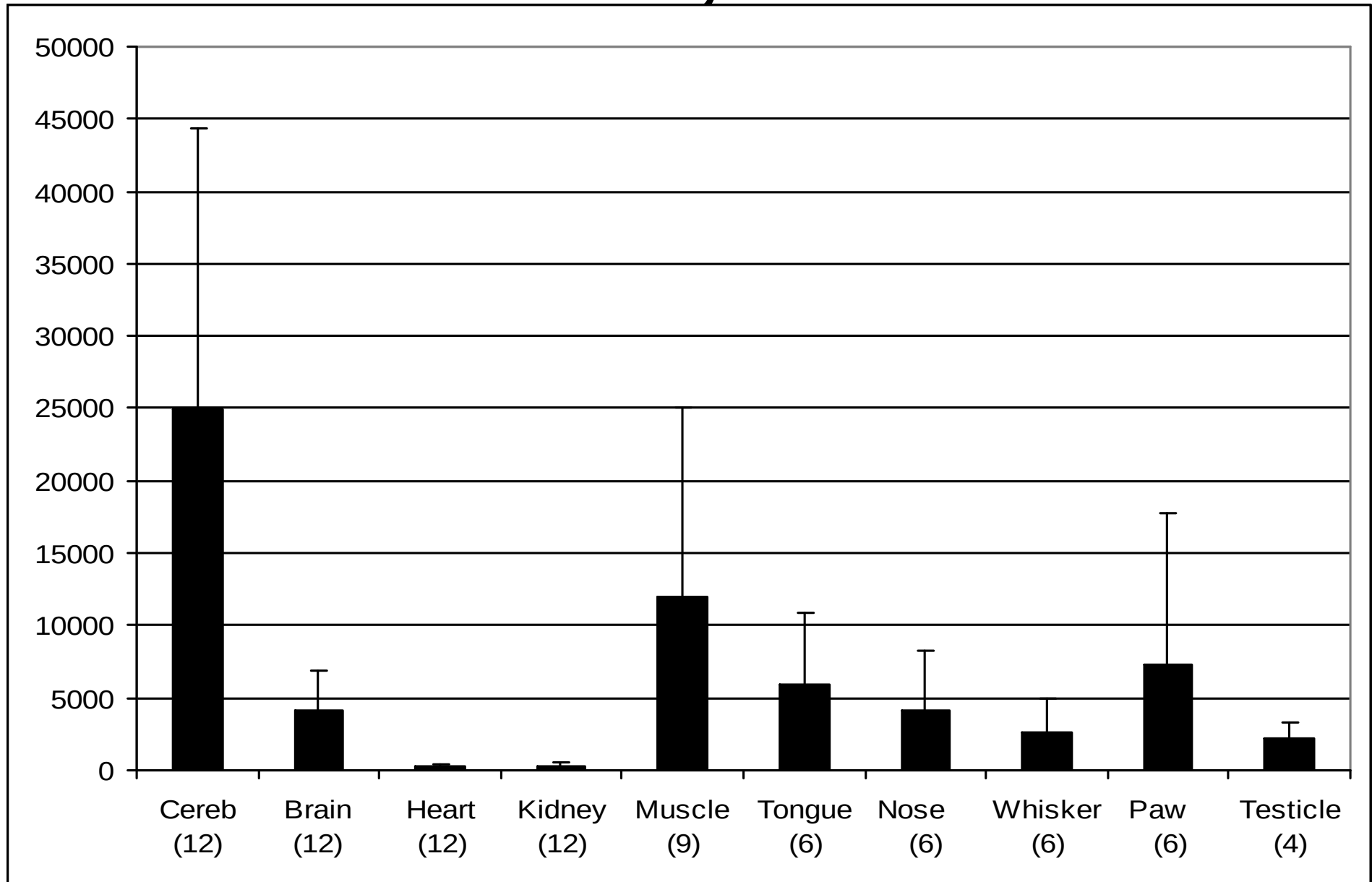


Ventral

Side

Dorsal

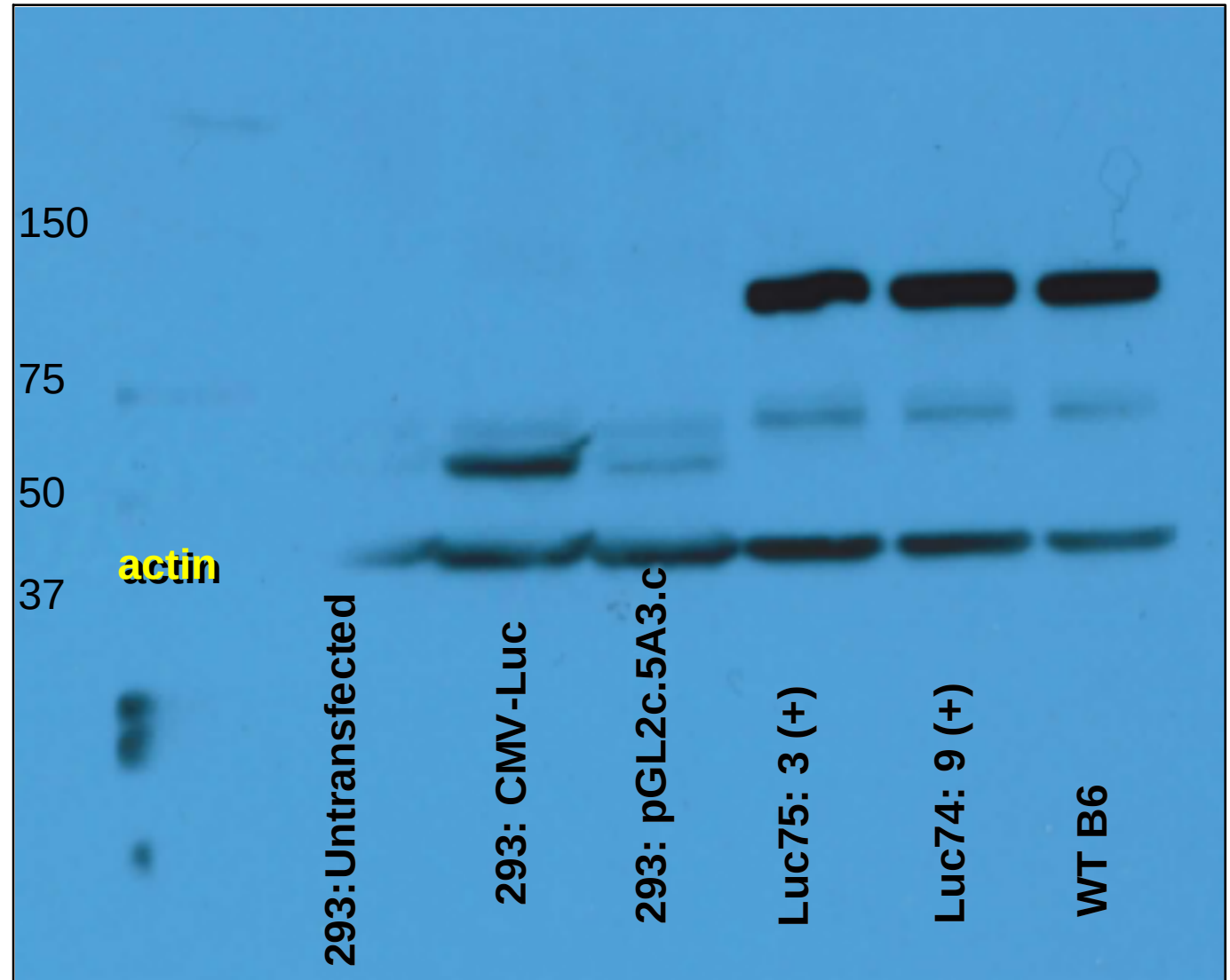
Luciferase Assay Line: Luc 75-1



Luciferase WB

Rockland AB

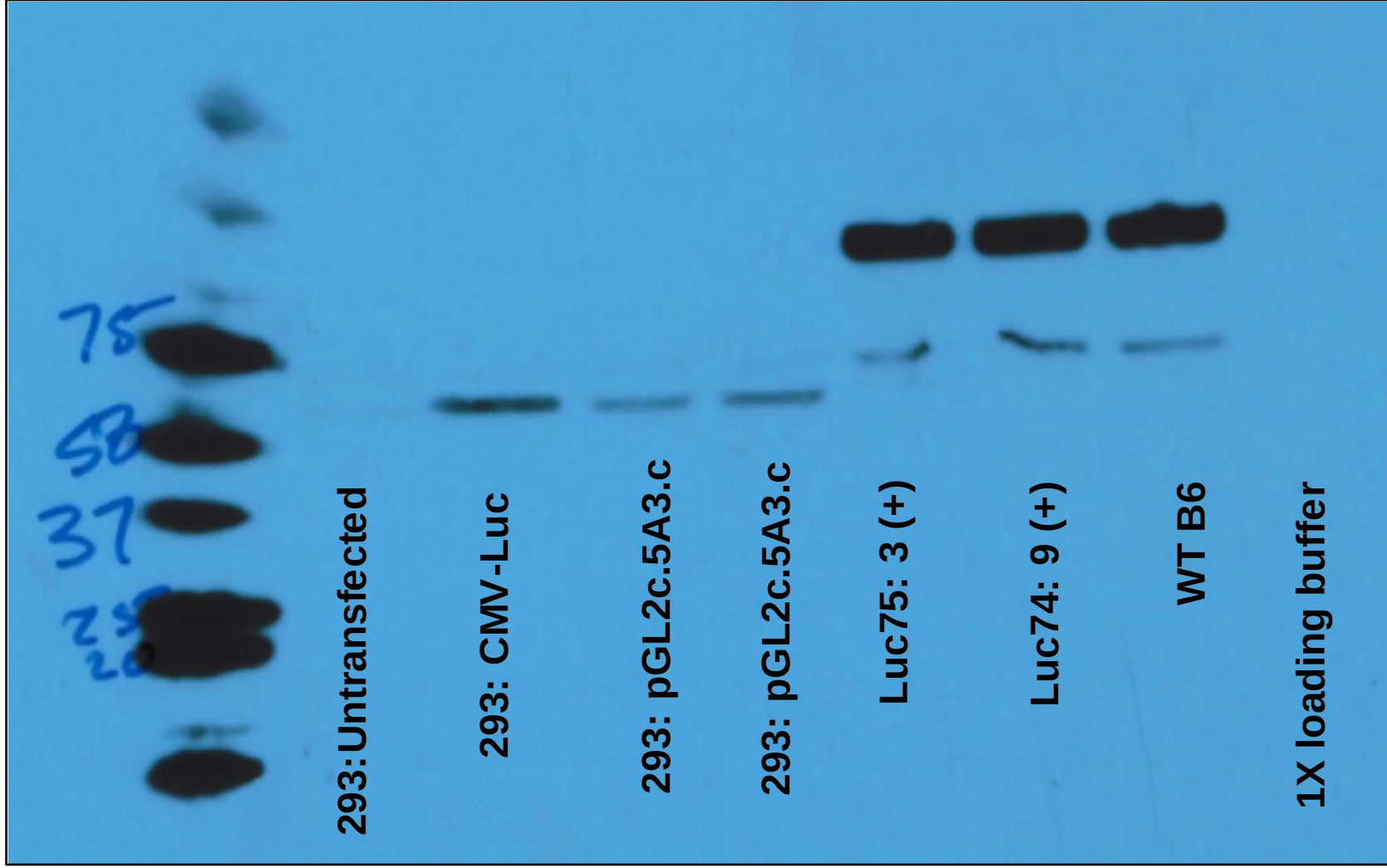
- 1' 1:15k
- 2' 1: 4k

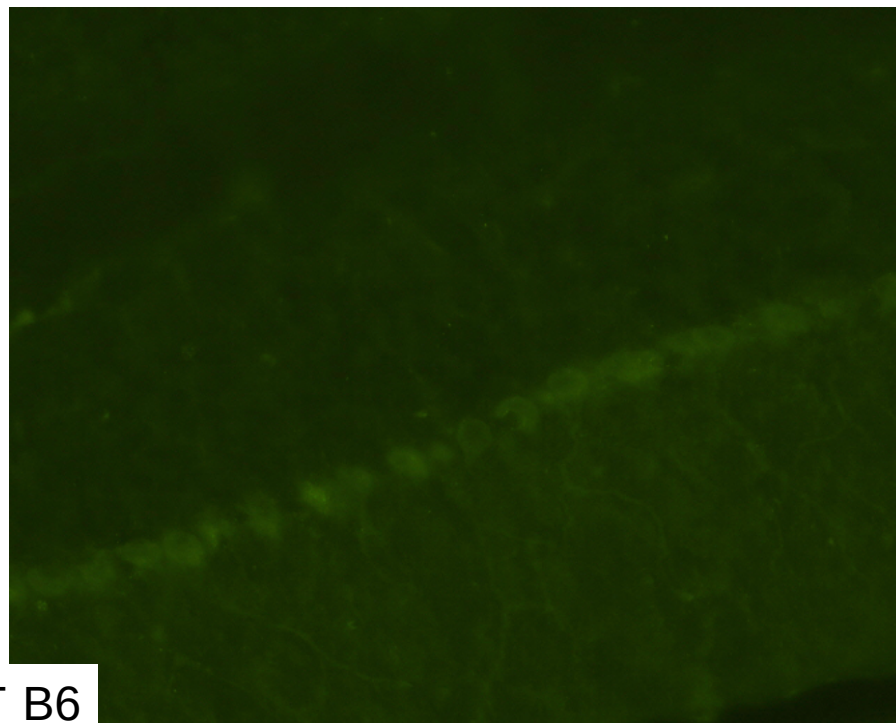
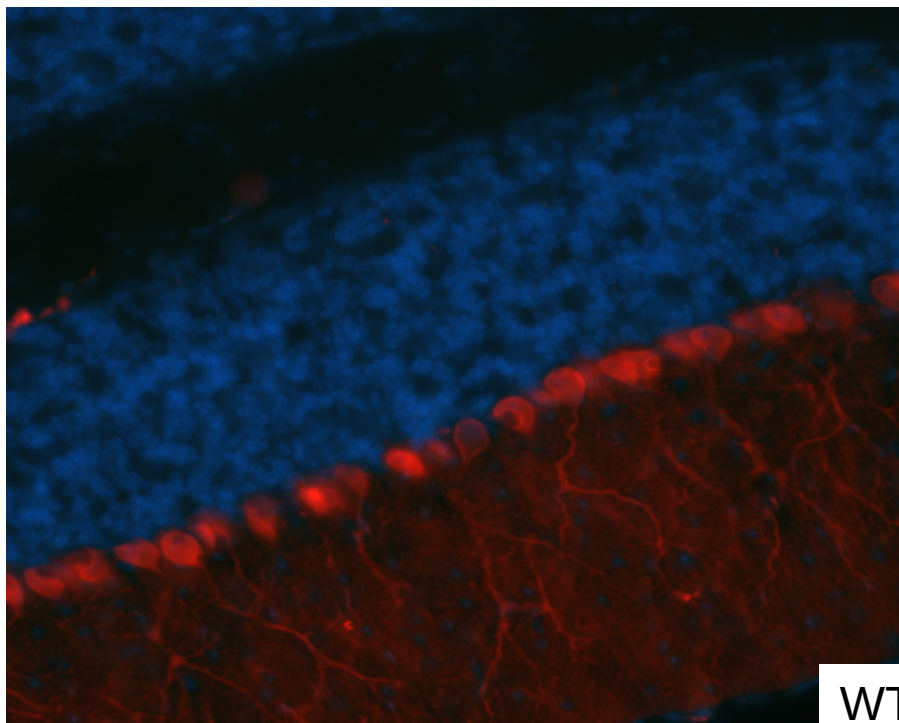


Pierce AB

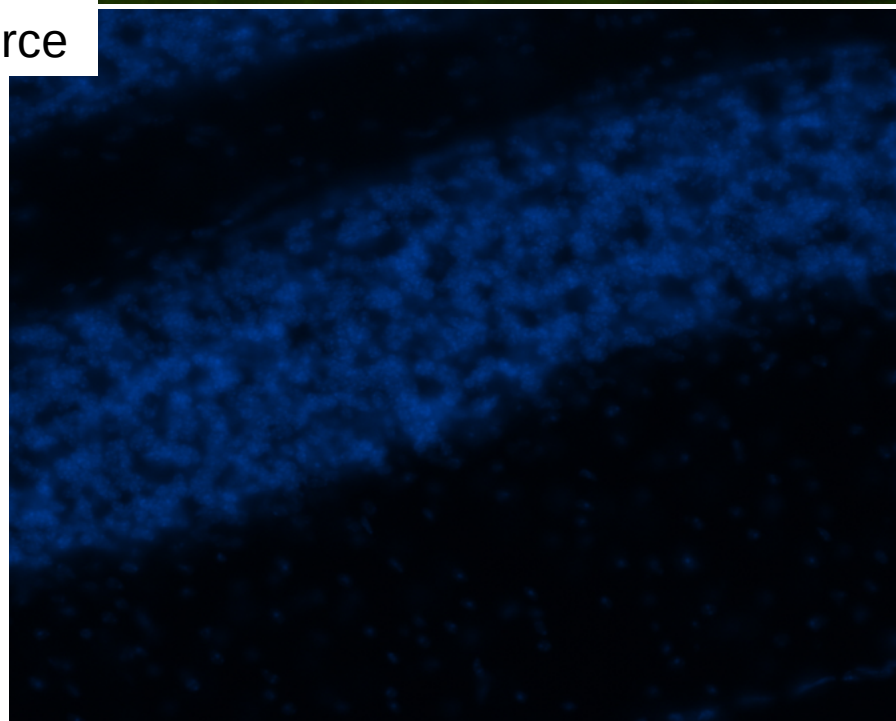
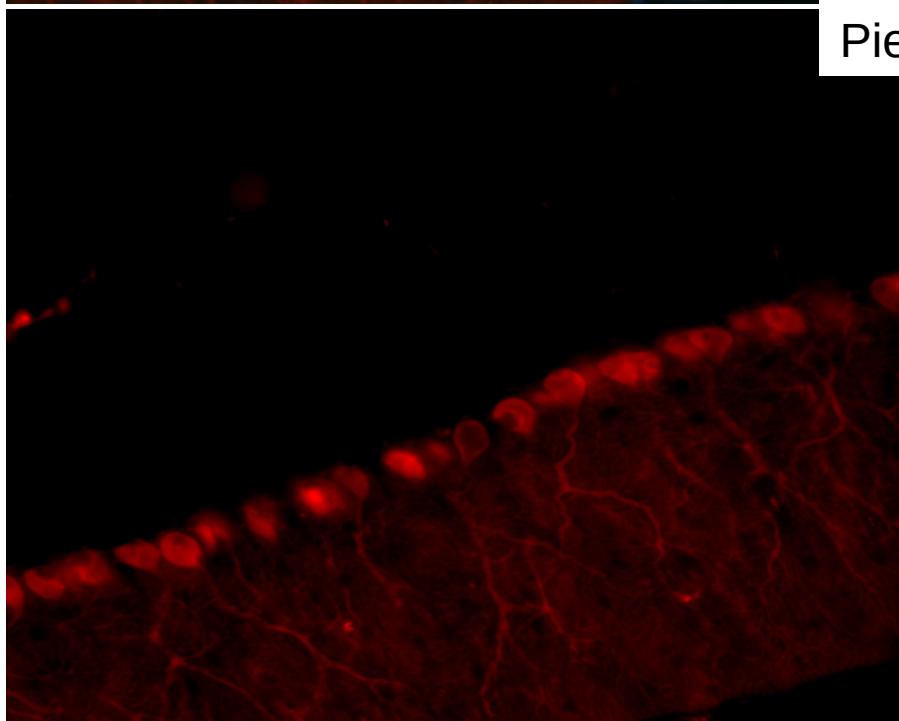
- 1' 1:30k
- 2' 1: 4k

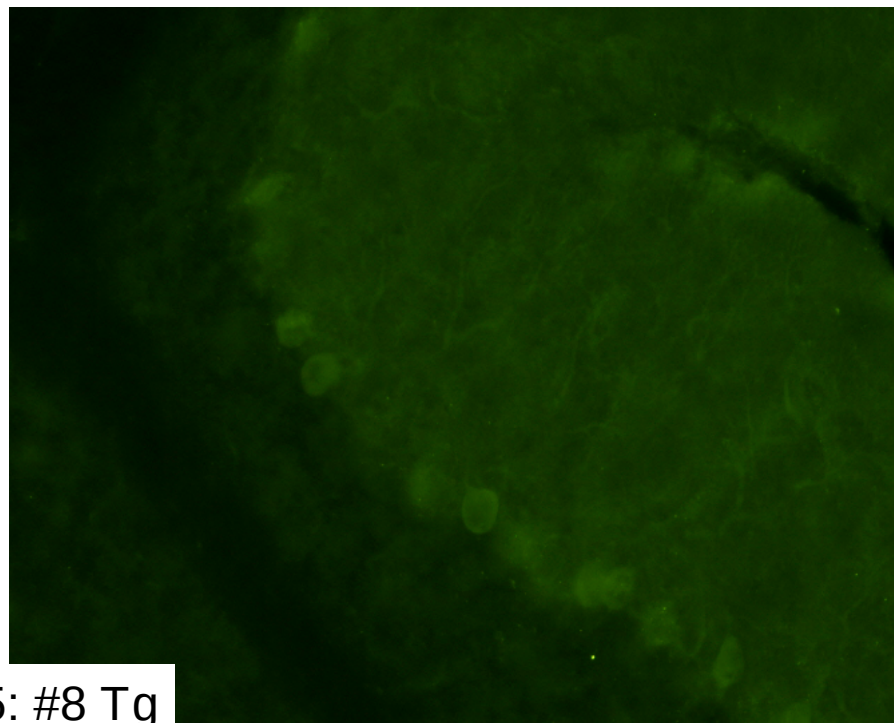
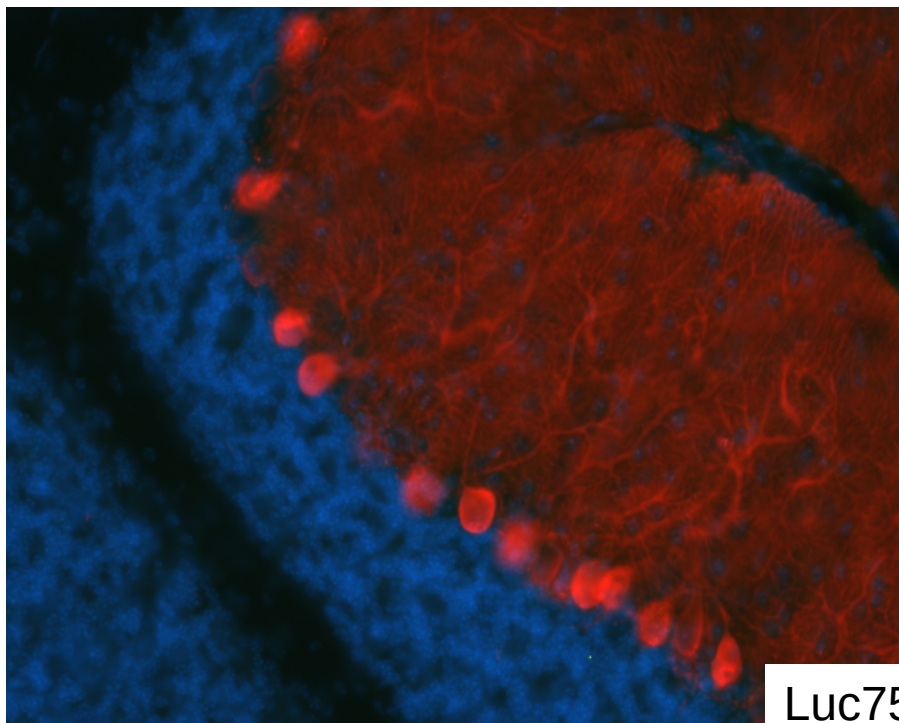
Luciferase WB



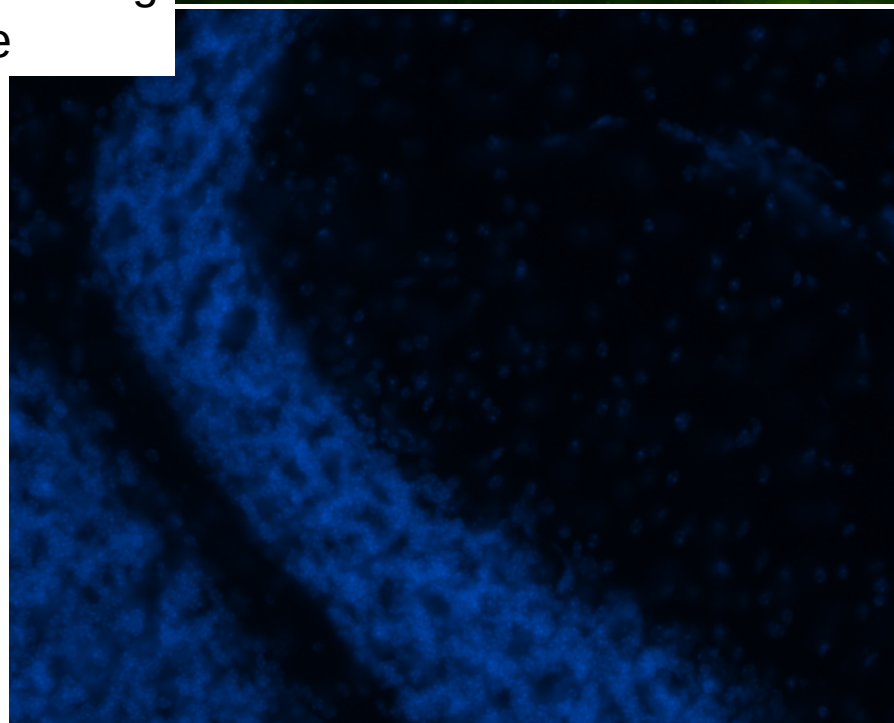
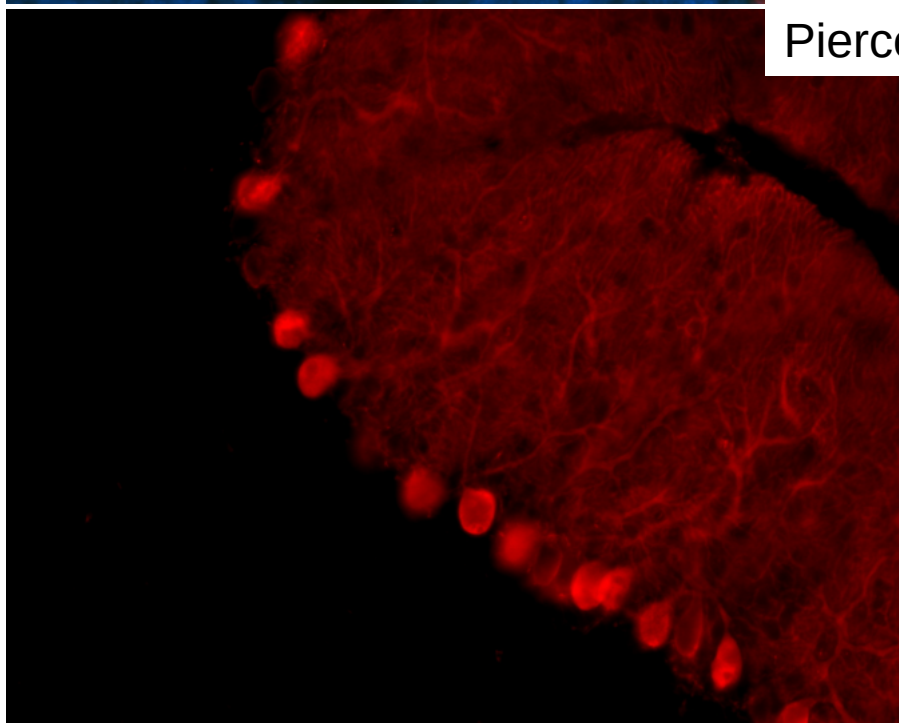


WT B6
Pierce





Luc75: #8 Tg
Pierce

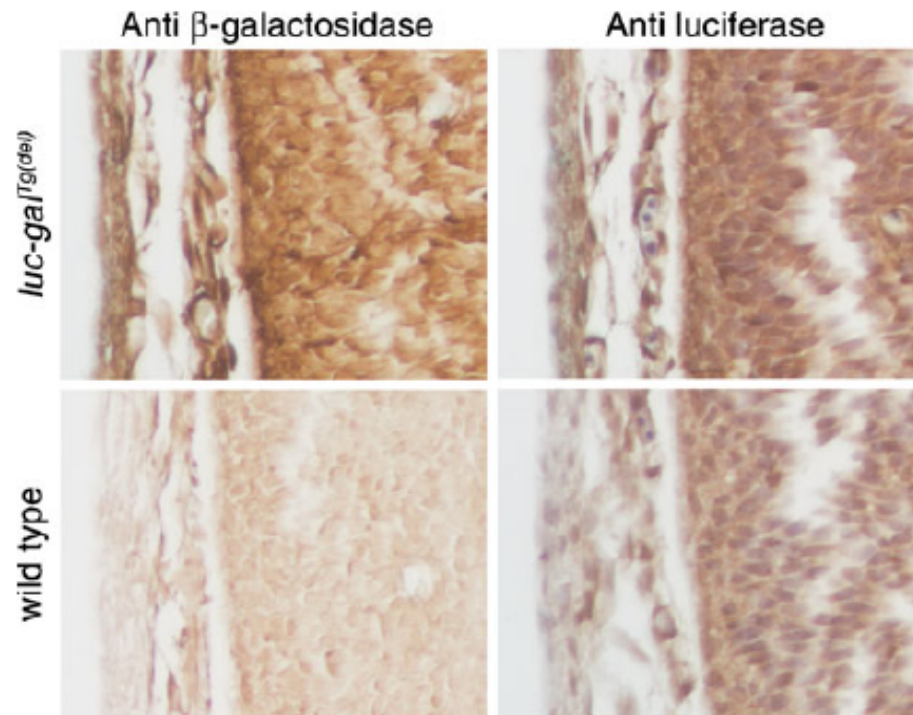


But perhaps not....

Conditional Bicistronic Cre Reporter Line Expressing Both Firefly Luciferase and β -galactosidase

Tomo-o Ishikawa, Harvey R. Herschman

Department of Molecular and Medical Pharmacology and Department of Biological Chemistry, Molecular Biology Institute, David Geffen School of Medicine, UCLA, 341 Boyer Hall, 611 Charles E. Young Drive East, Los Angeles, CA 90095, USA



body, we were unable to obtain specific staining with anti-luciferase antibody (Fig. 4).

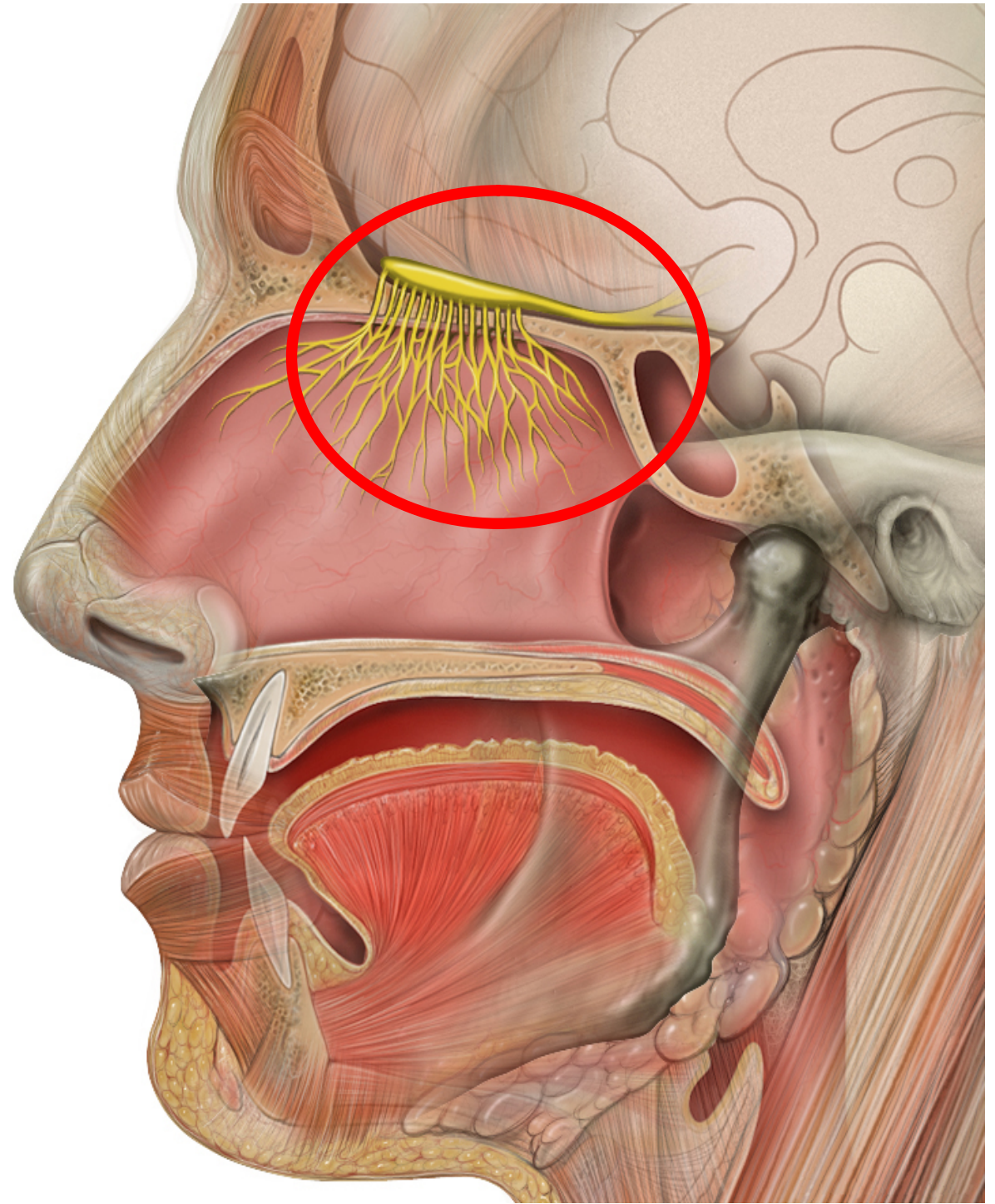
The major disadvantage of luciferase as a reporter gene is at the cellular level. Antisera that provide reliable, easy, and reproducible immunohistochemistry for luciferase are not available. We have compensated for this deficiency by utilizing *lacZ*, another reporter gene, in tandem with luciferase to monitor tissue-specific Cre recombinase activity at the organ, tissue, and cellular level by histochemical and immunohistochemical detection methods, both on sections and on whole mount samples. Although reports of immunohistochemical detection of luciferase activity following injection of luciferase-encoding plasmids into tissues do exist [34, 35], we find that the far more robust immunohistochemistry methods available for β -galactosidase [36, 37] make cellular analysis of transgene expression much simpler to interpret.

Future

- Need to finish making RNA $\Rightarrow$ qPCR for all tissue
- No more IHC and WB



O l f a c t o r y
b u l b



Granule cells

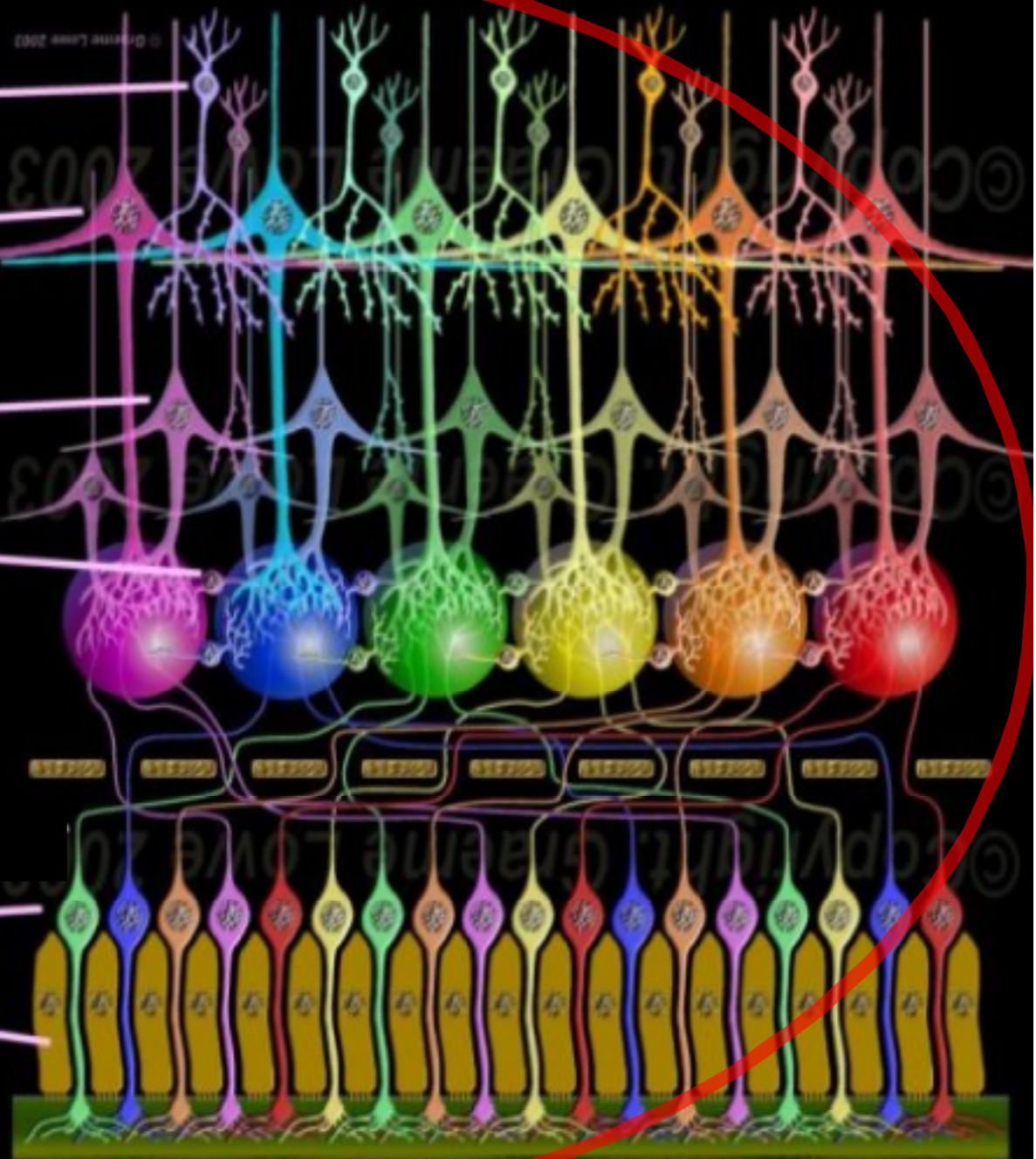
Mitral cells

Tufted cells

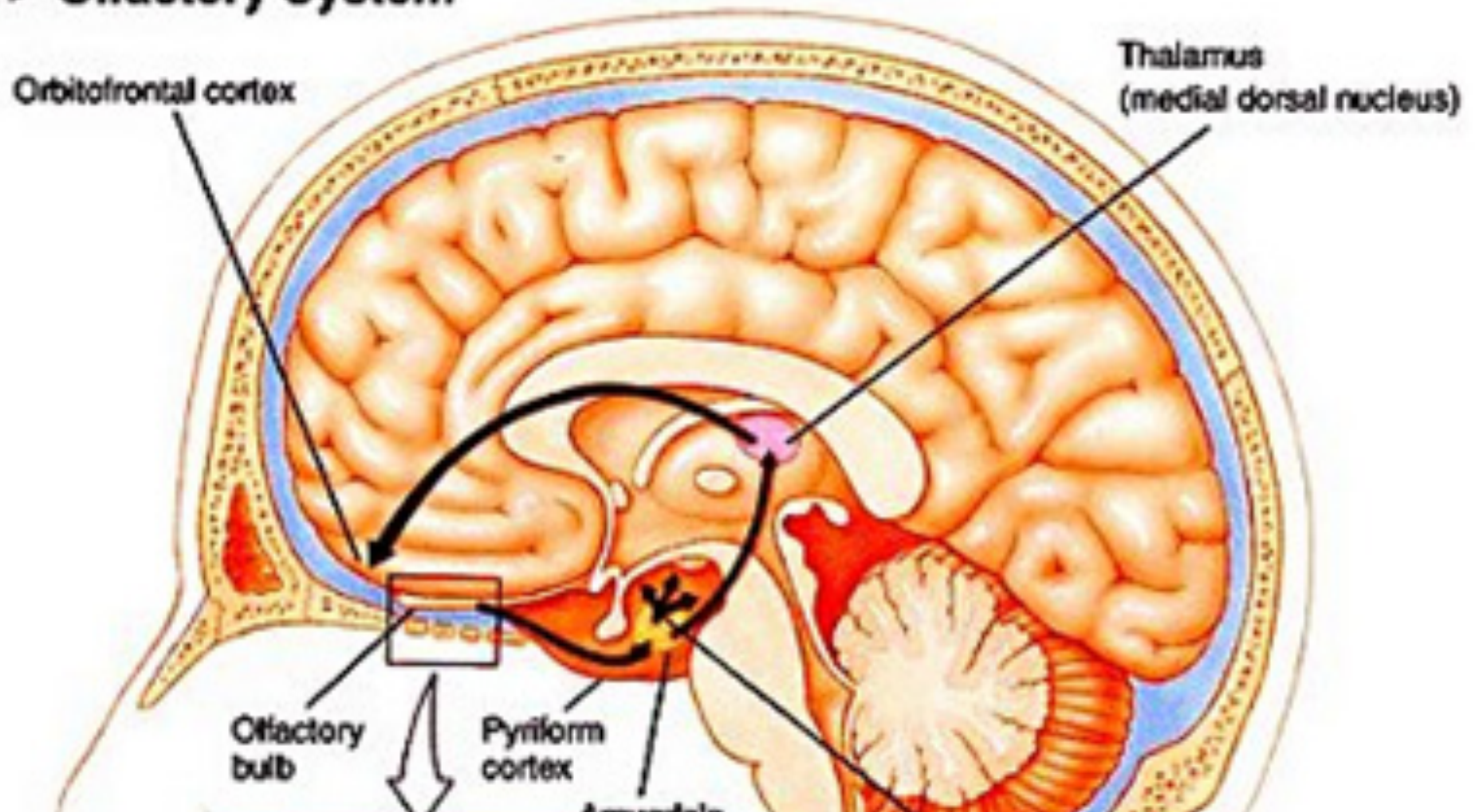
Peri glomerular cells

Olfactory receptors

Olfactory Epithelia



► Olfactory System



Piriform cortex: cortical amygdala, uncus and anterior parahippocampal gyrus; sends projects to medial dorsal nucleus of the thalamus



Copyright Adam C. Puche

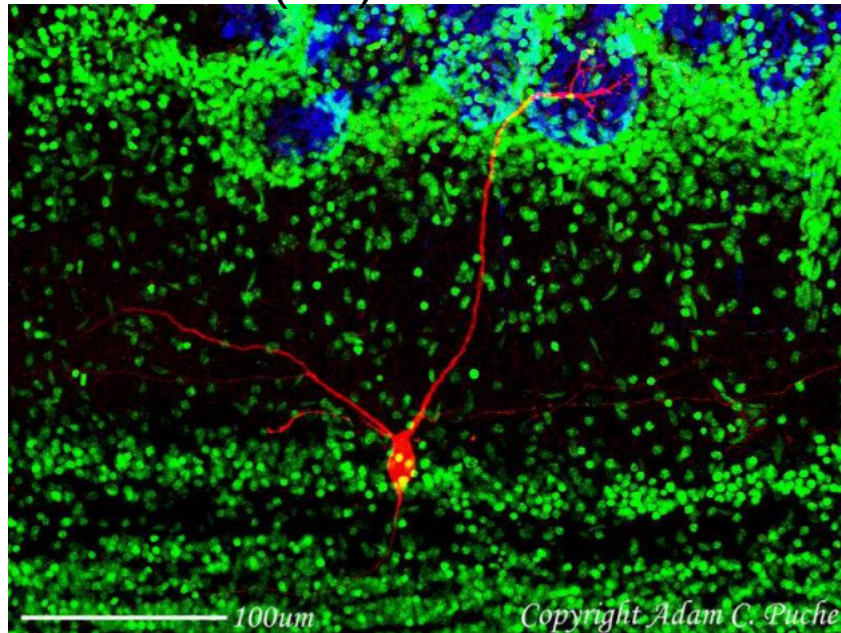
EXAMPLE

Coronal OB sxn

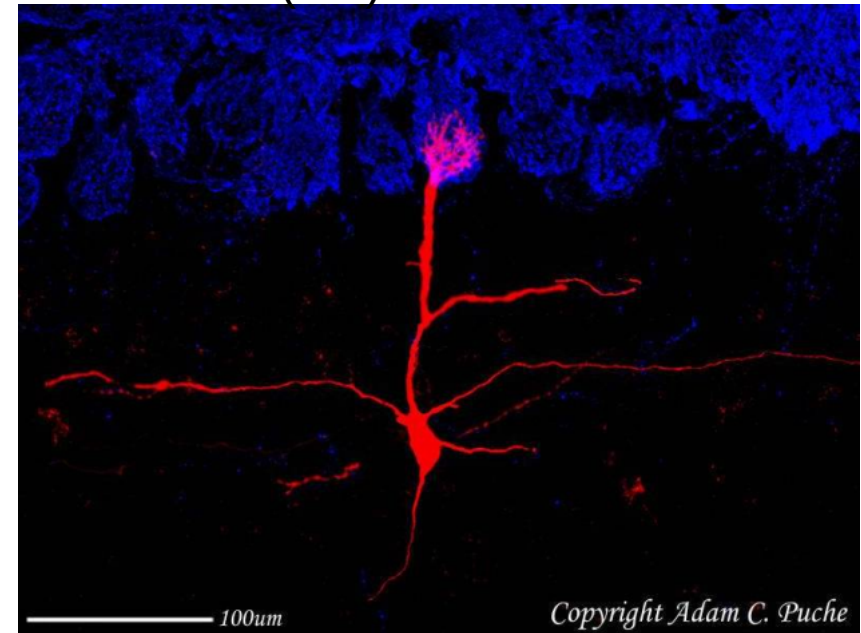
- Blue glomerular layer
- Red external plexiform & Mitral layer (mitral, tufted & granule cells)
- Green Internal plexiform & granule cell layers



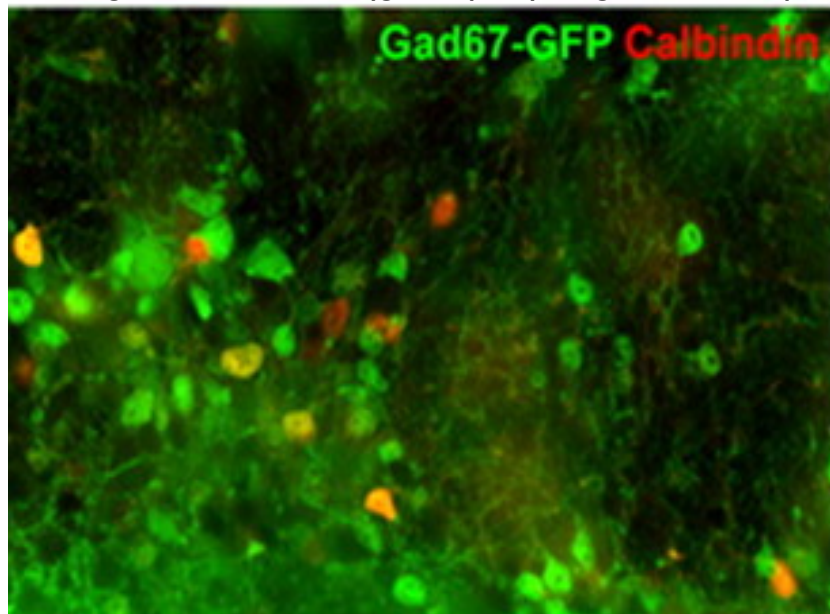
Mitral cell (red)



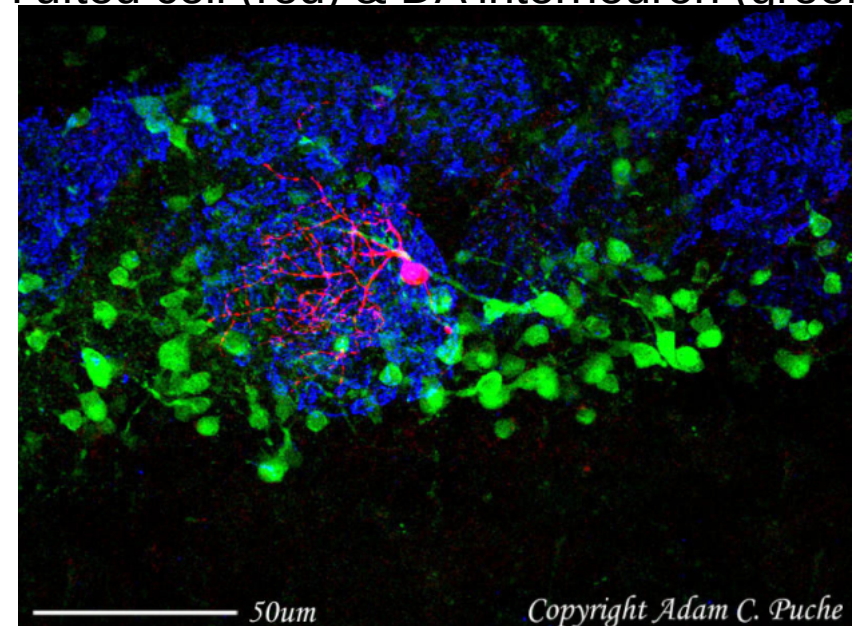
Tufted cell (red)



GABAergic interneuron (green) & periglomerular (red)



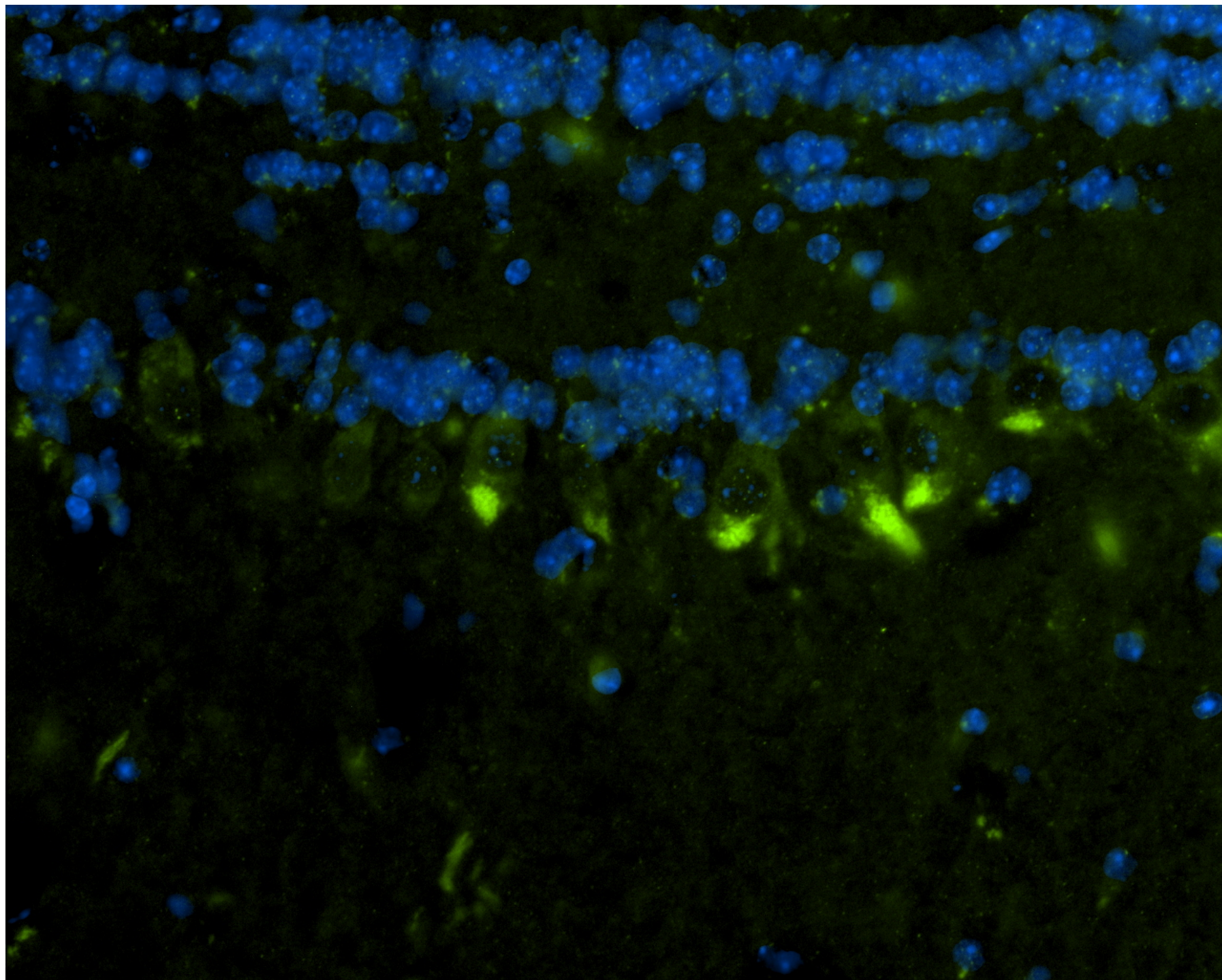
Tufted cell (red) & DA interneuron (green)

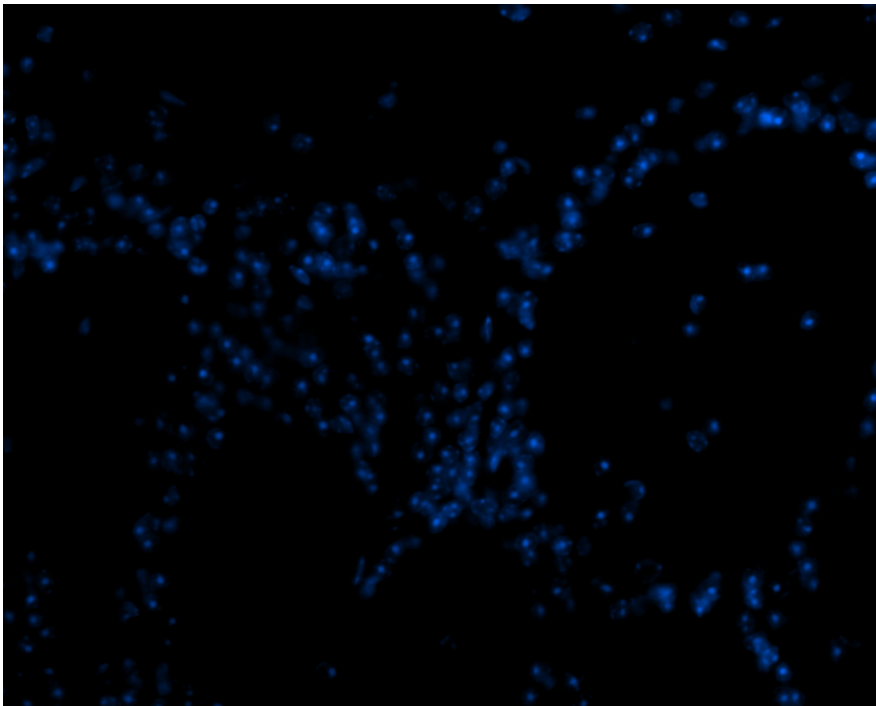
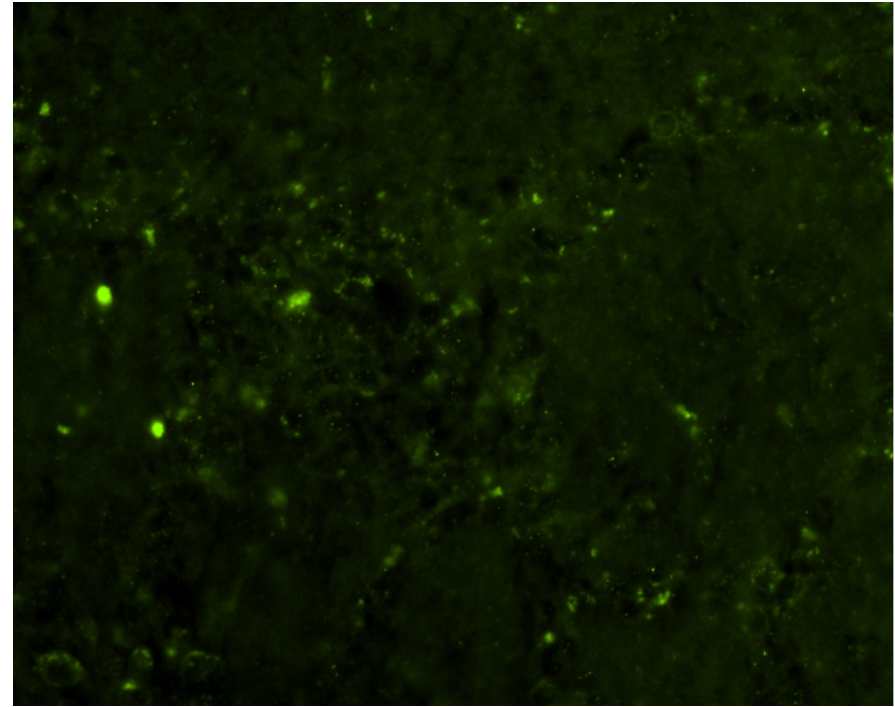
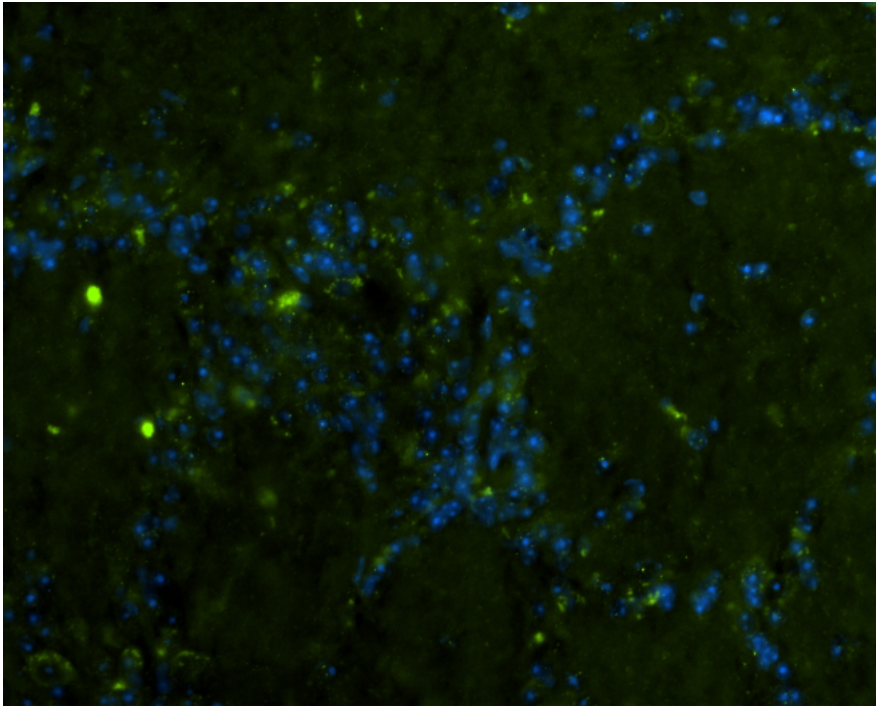


Which cells in the olfactory bulb are
expressing atxn2?

Methods

- Slide mounted sxns
- 5 min PBS wash x 2
- 30 min block/ permeabilization
 - 5% skim/ 0.3% Triton
- Primary AB 45min RT
- Wash PBS 5min x 3
- Secondary AB 30min RT
- PBS wash 5min x 3
- Prolong Gold mounting media





Glomerular 20x

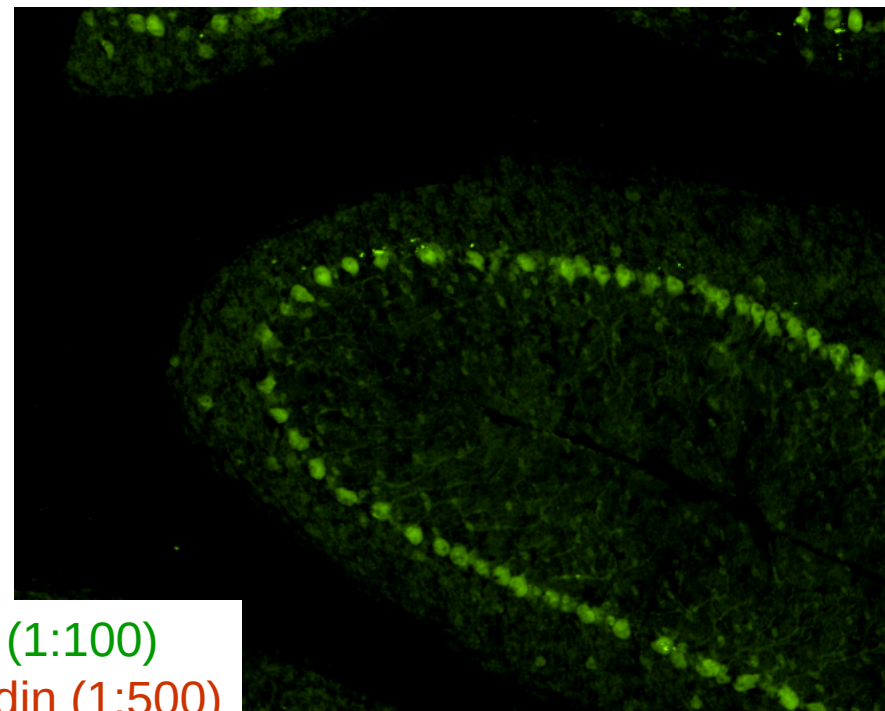
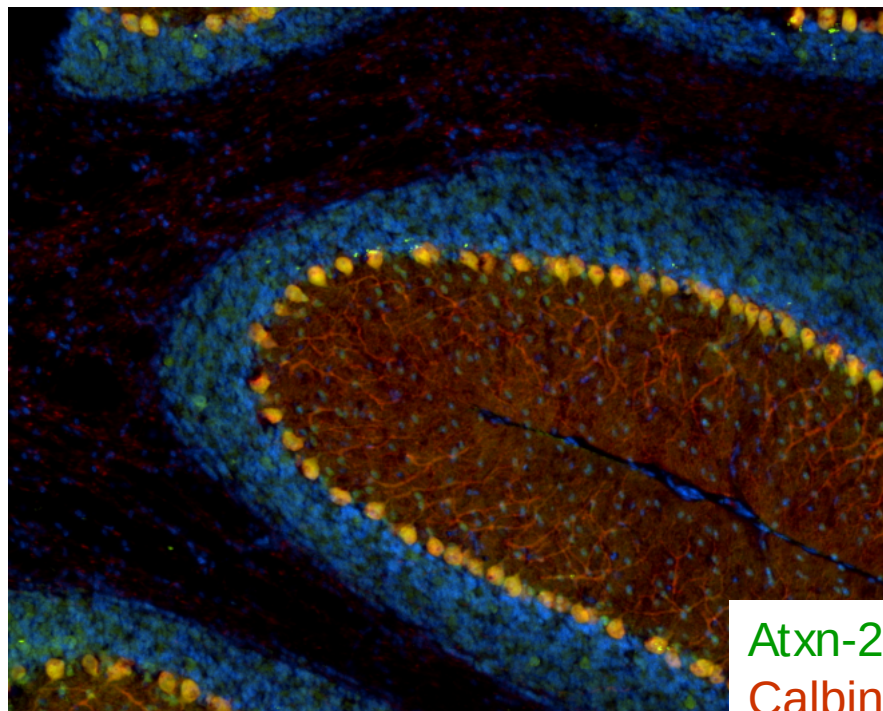
Atxn 2 – green (cy 2; 1:100)

Dapi – blue (1:4K)

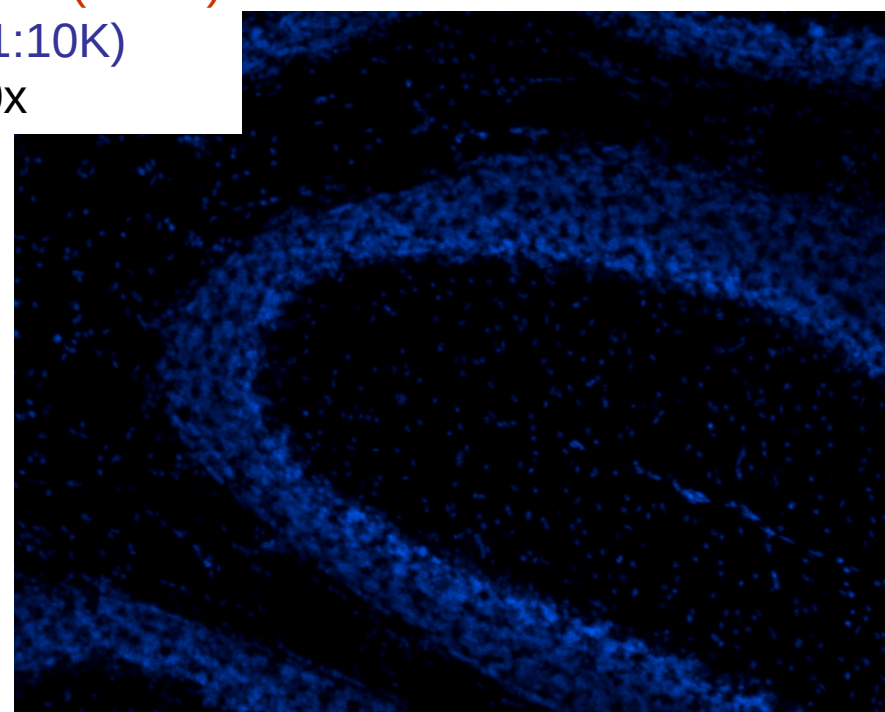
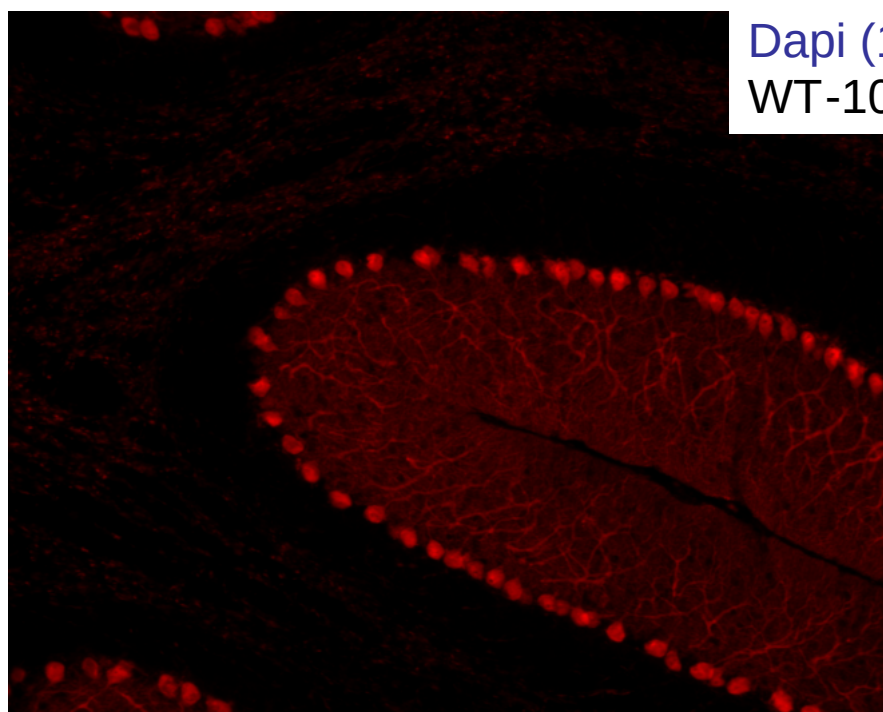
Lucero

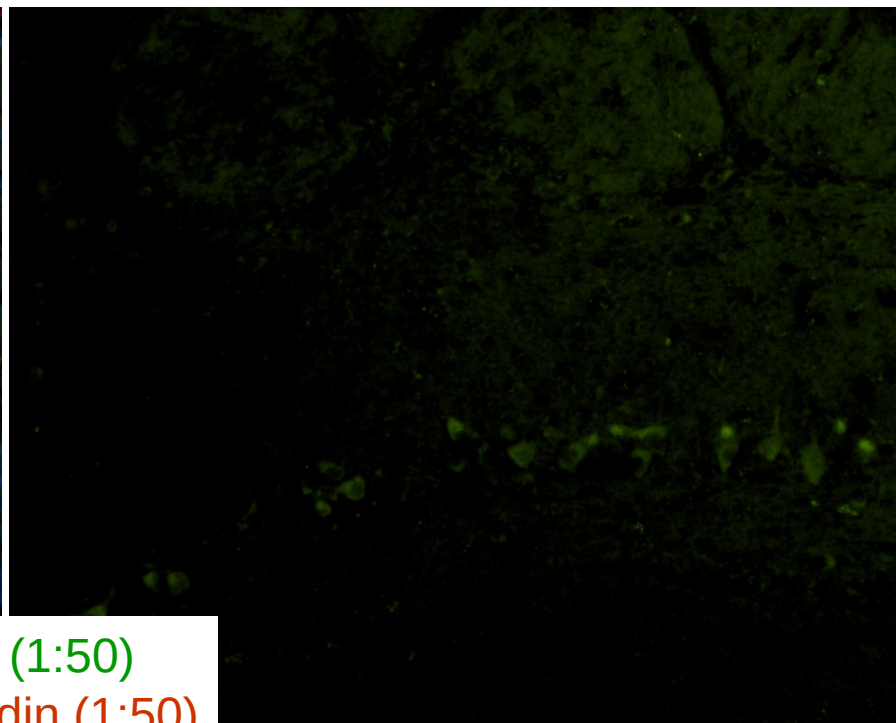
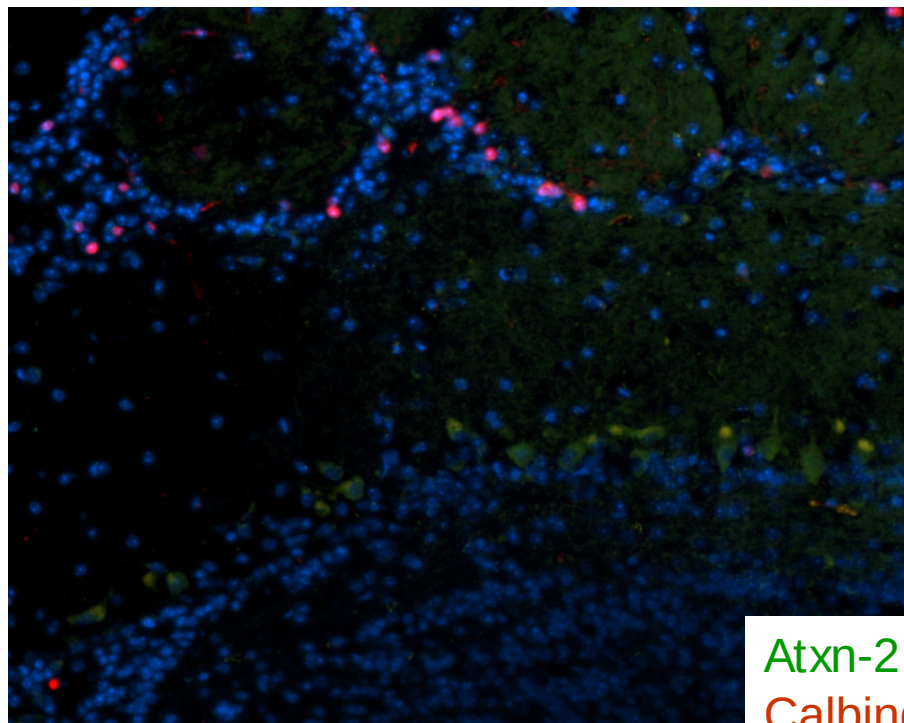
Antigen Retrieval

- 0.01 mM Urea
 - Microwave 10-sec x3
 - Process tissue in regular fashion
-
- Previous work in the cerebellum has yielded good results with this method...

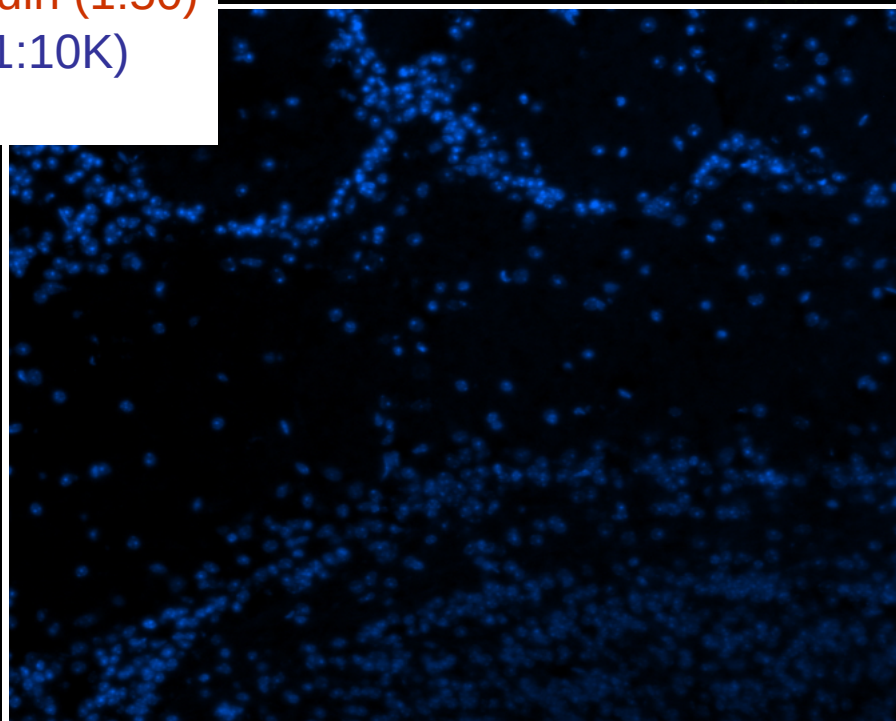
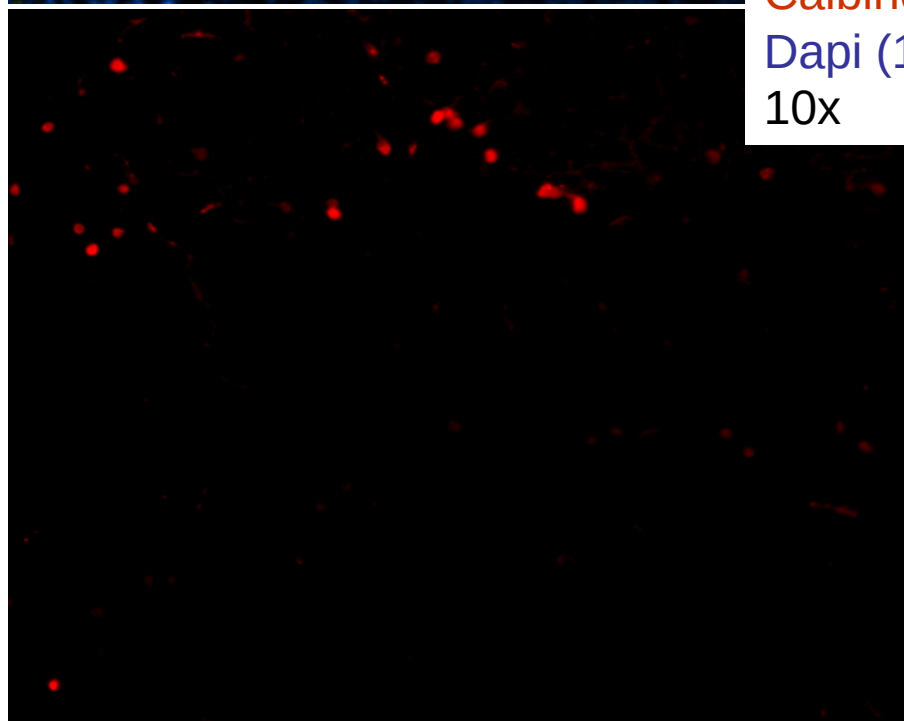


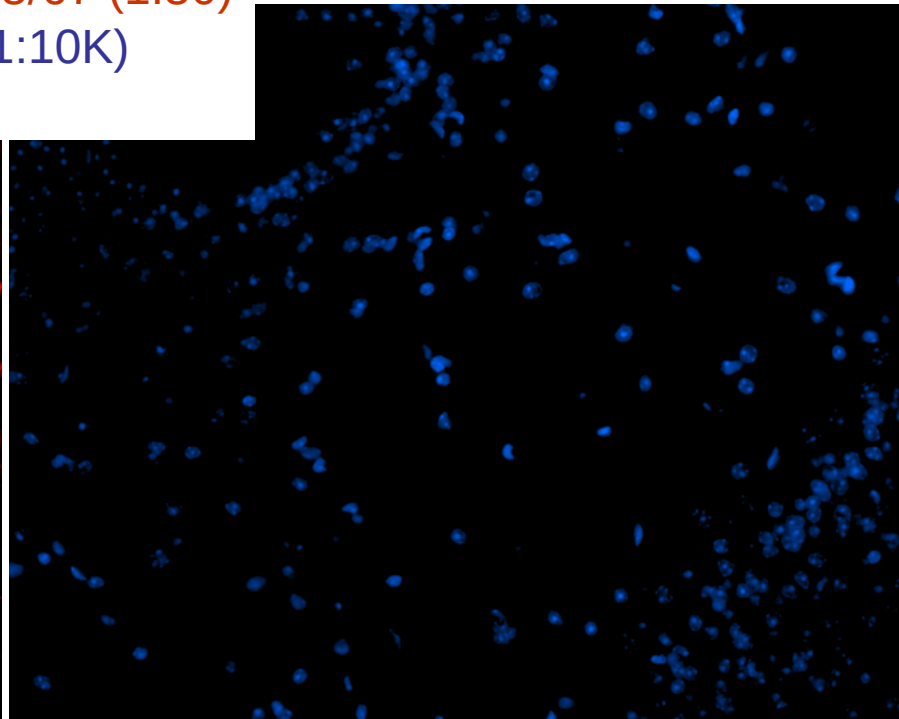
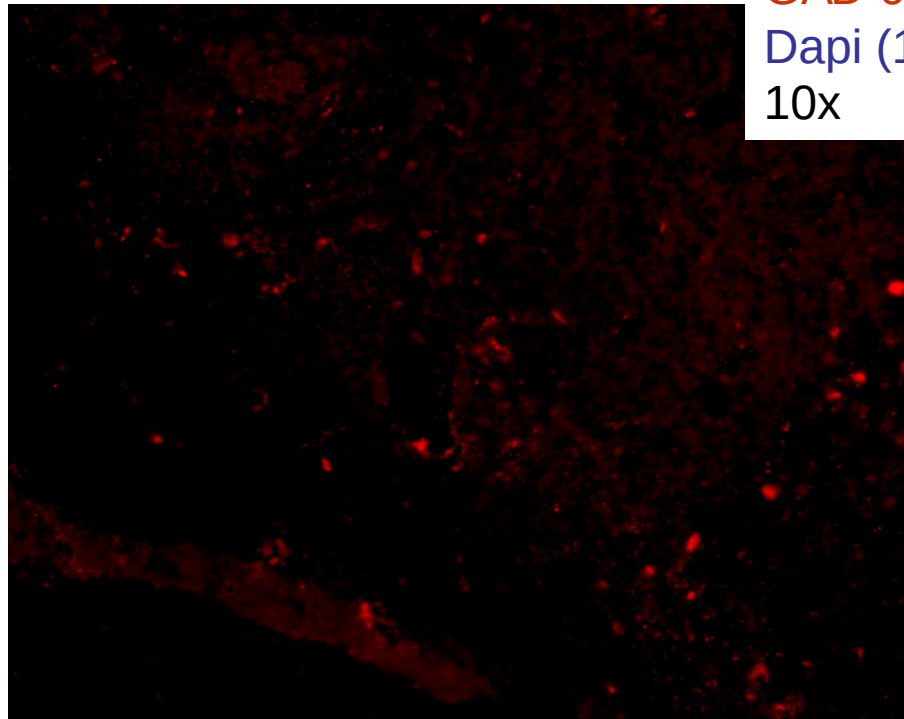
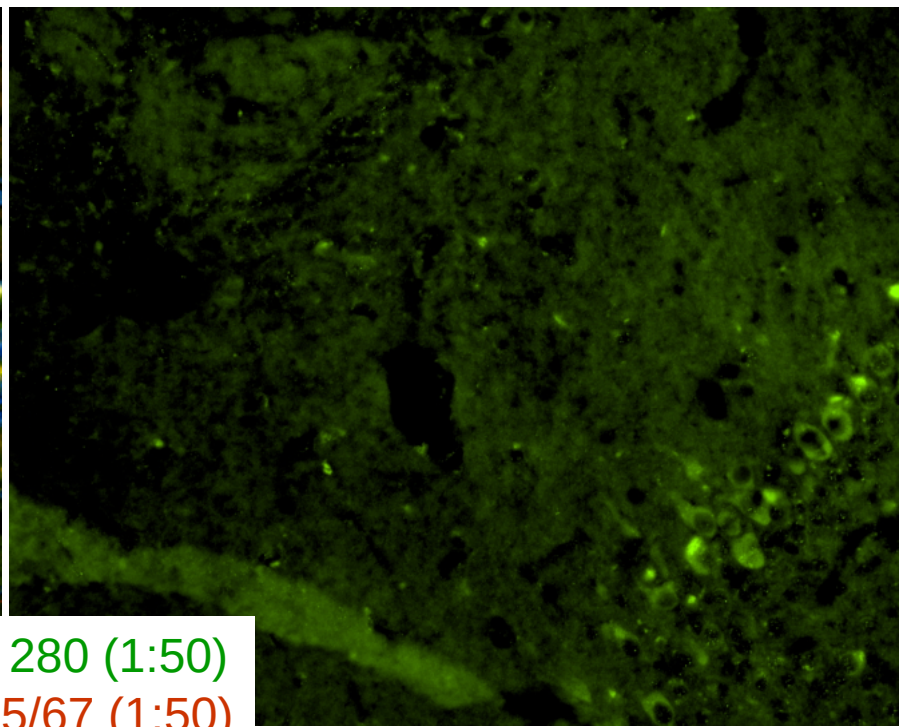
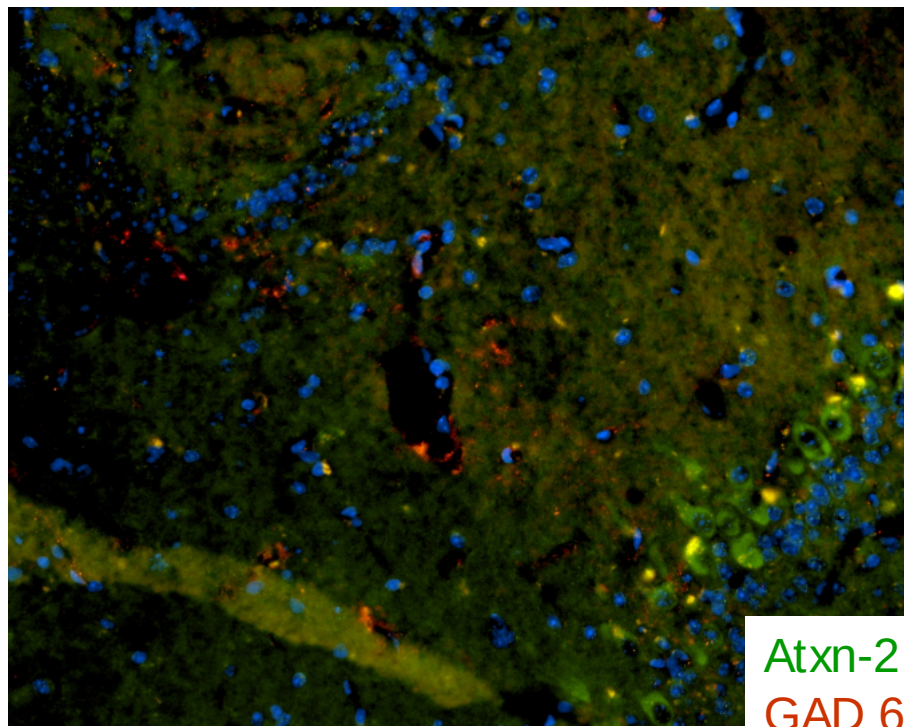
Atxn-2 (1:100)
Calbindin (1:500)
Dapi (1:10K)
WT-10x





Atxn-2 (1:50)
Calbindin (1:50)
Dapi (1:10K)
10x





Atxn-2 280 (1:50)
GAD 65/67 (1:50)
Dapi (1:10K)
10x

Future

- OB: need to determine which cells express atxn2
 - Almost certainly mitral/ tufted cells: glutamatergic
 - Antigen retrieval
 - NA-Citrate (testing now)
 - Autozyme
 - GeneTex: \$199 –marketed for paraffin embedded tissue
 - New antibodies
 - Primary
 - Secondary in alternate wave-length: counteract autofluor