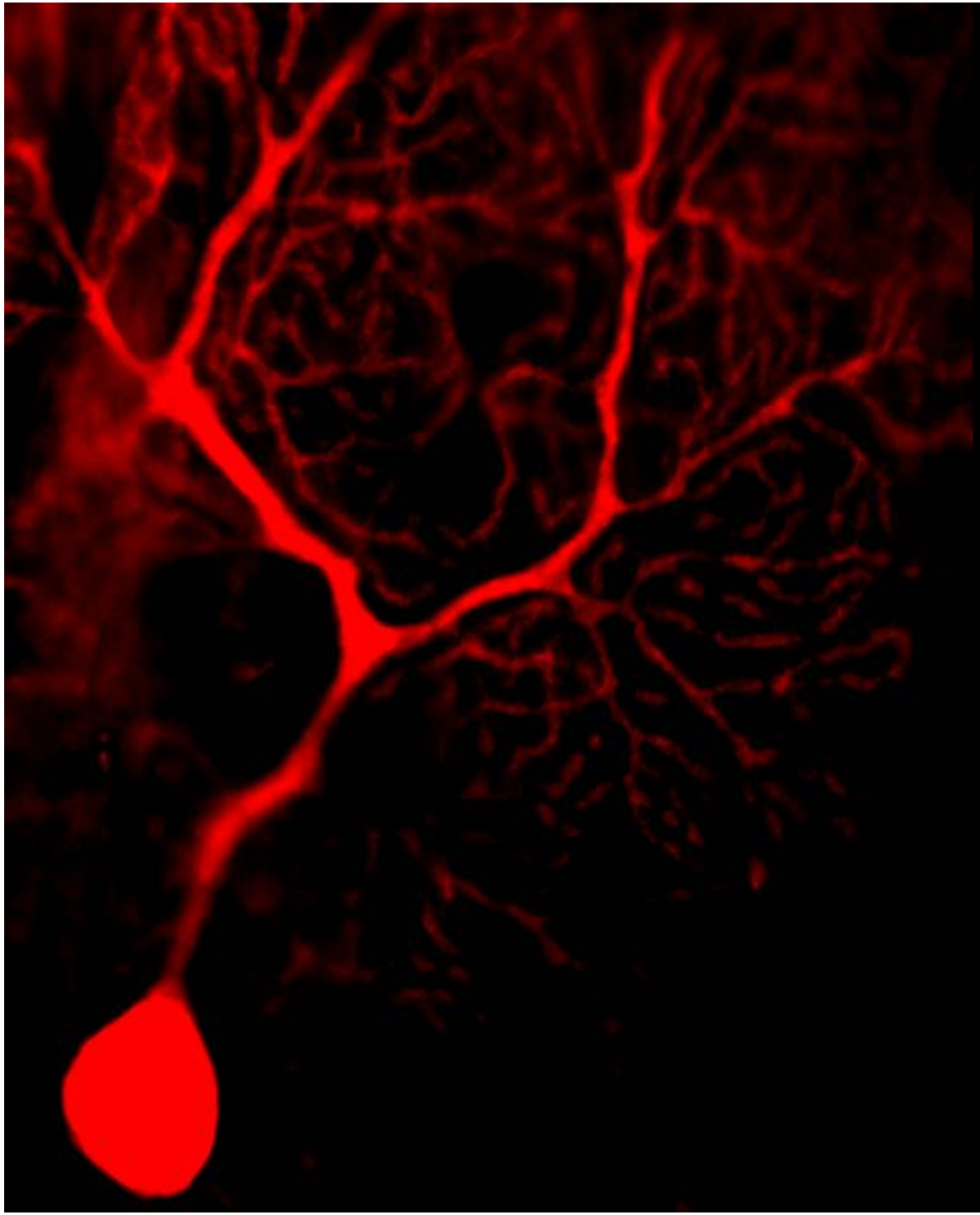
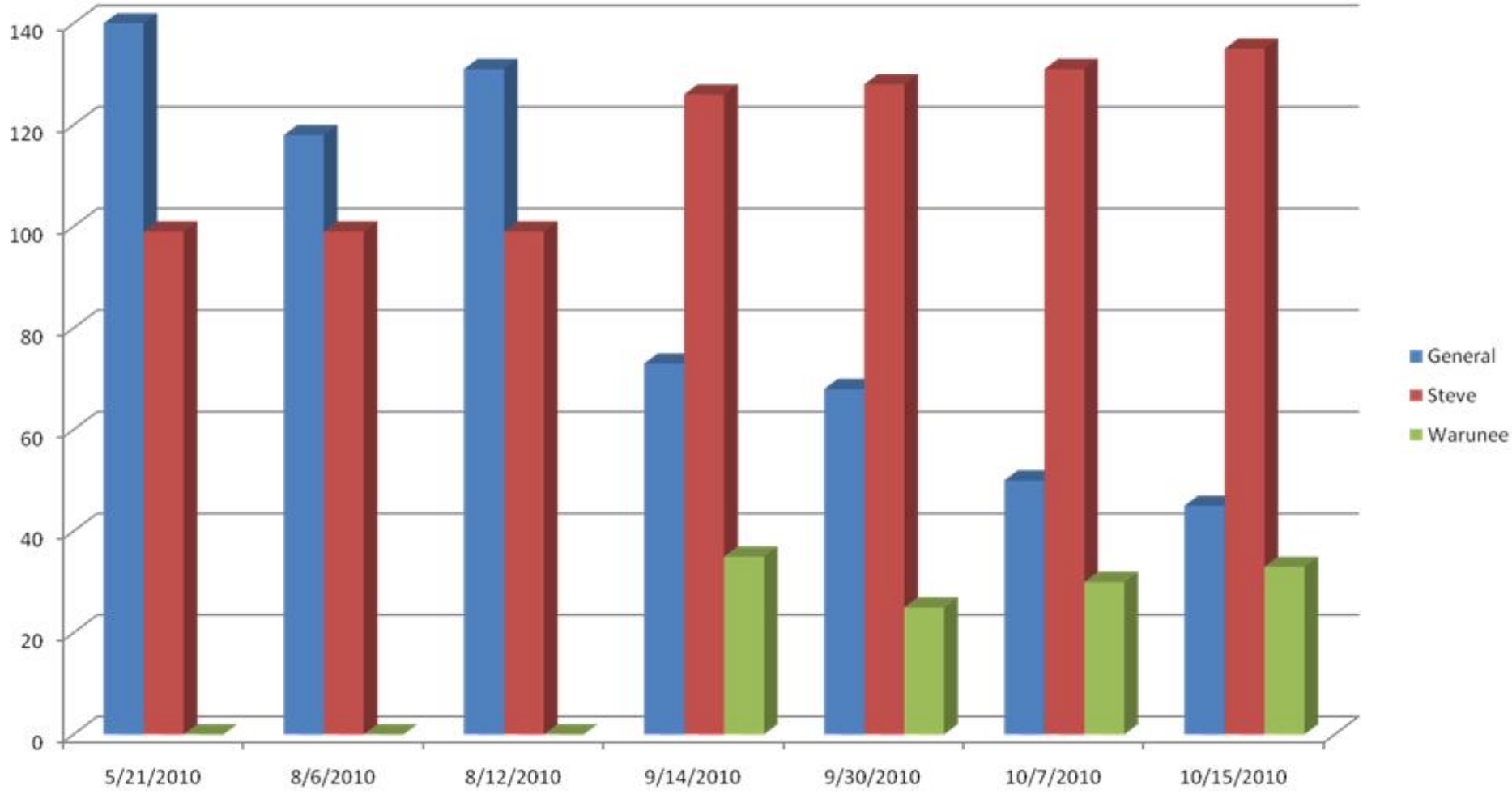


Lab Meeting: 10/15/2010

STH

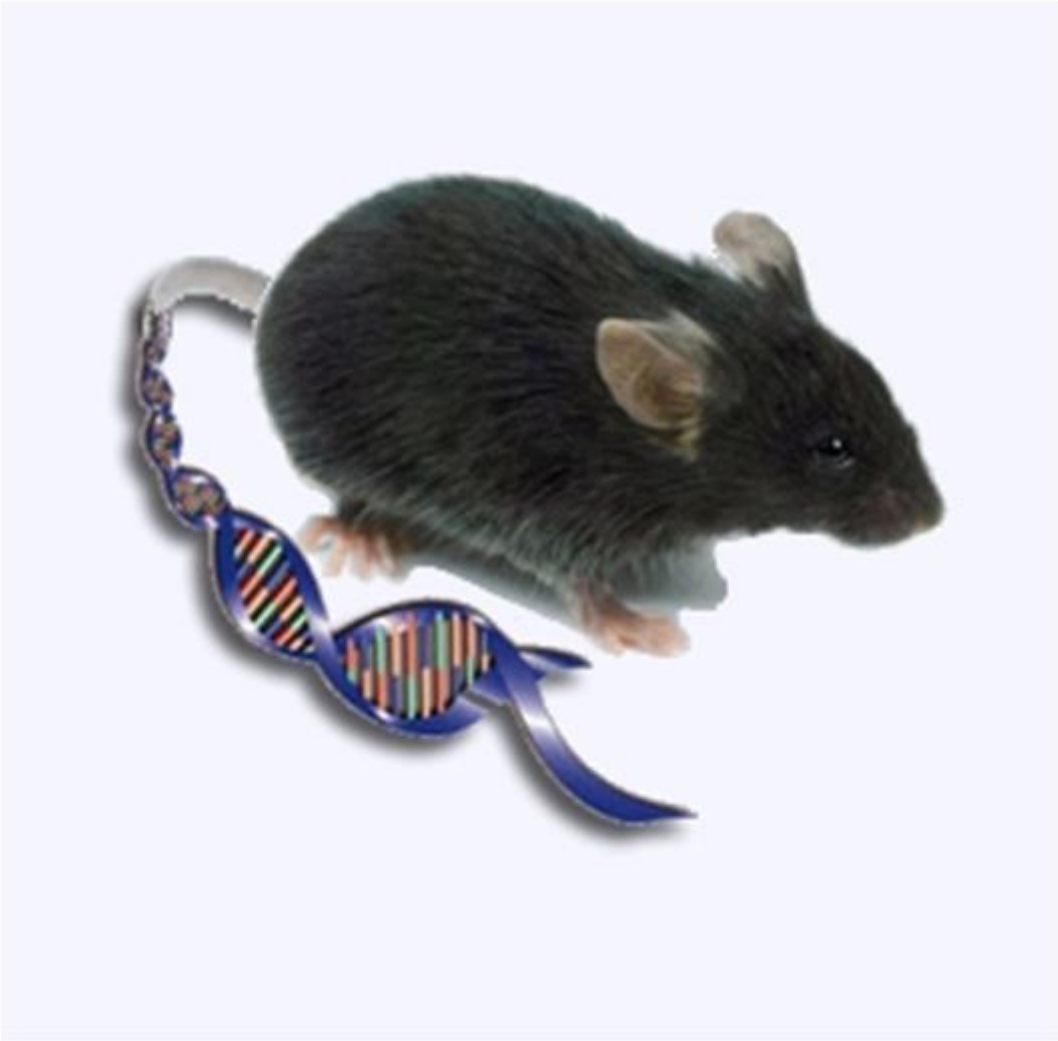
Colony Cage Counts From May



Projects

- SCA 2 Q127
- Luciferase mice
- Olfactory Bulb staining

SCA 2: Q127

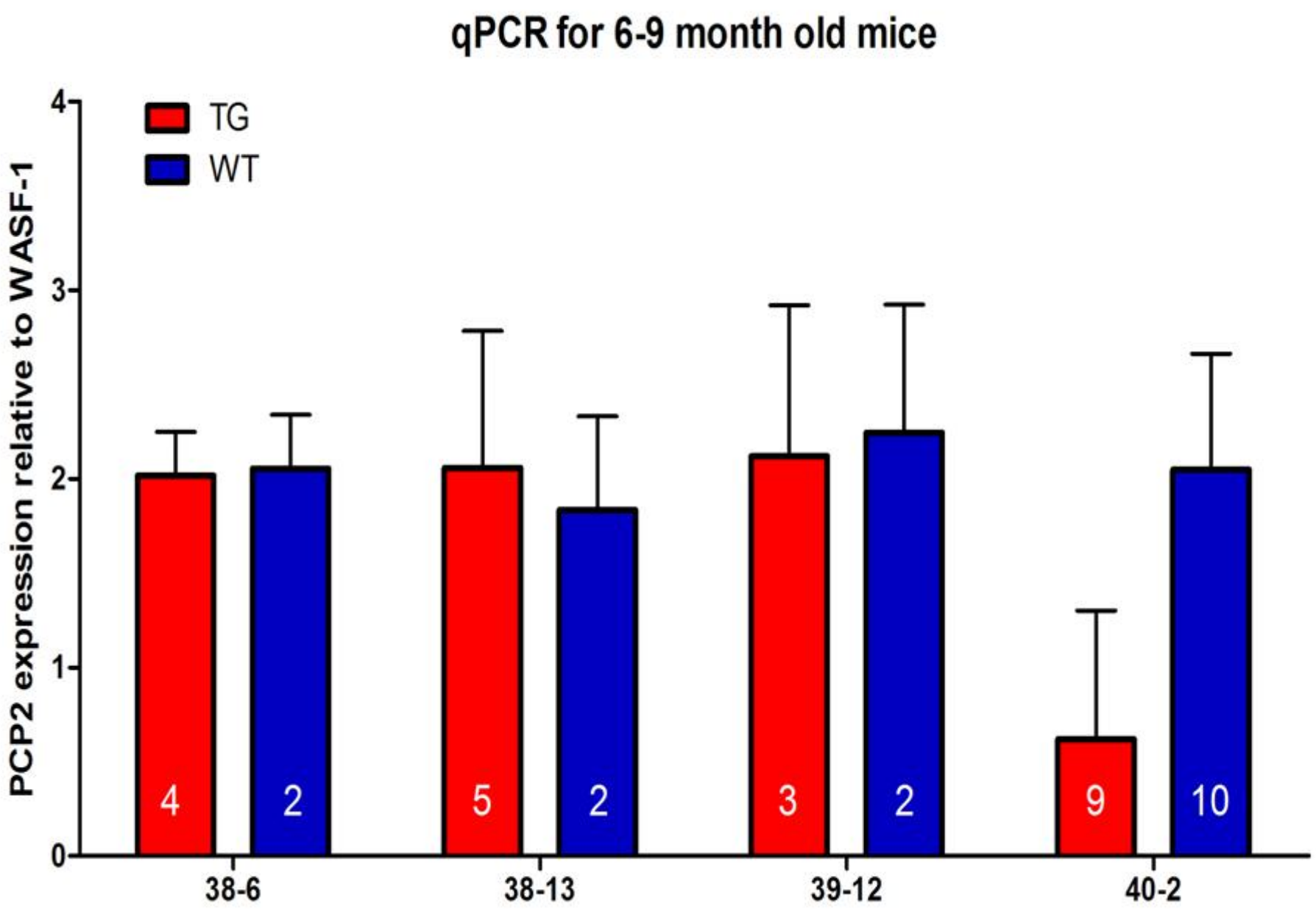


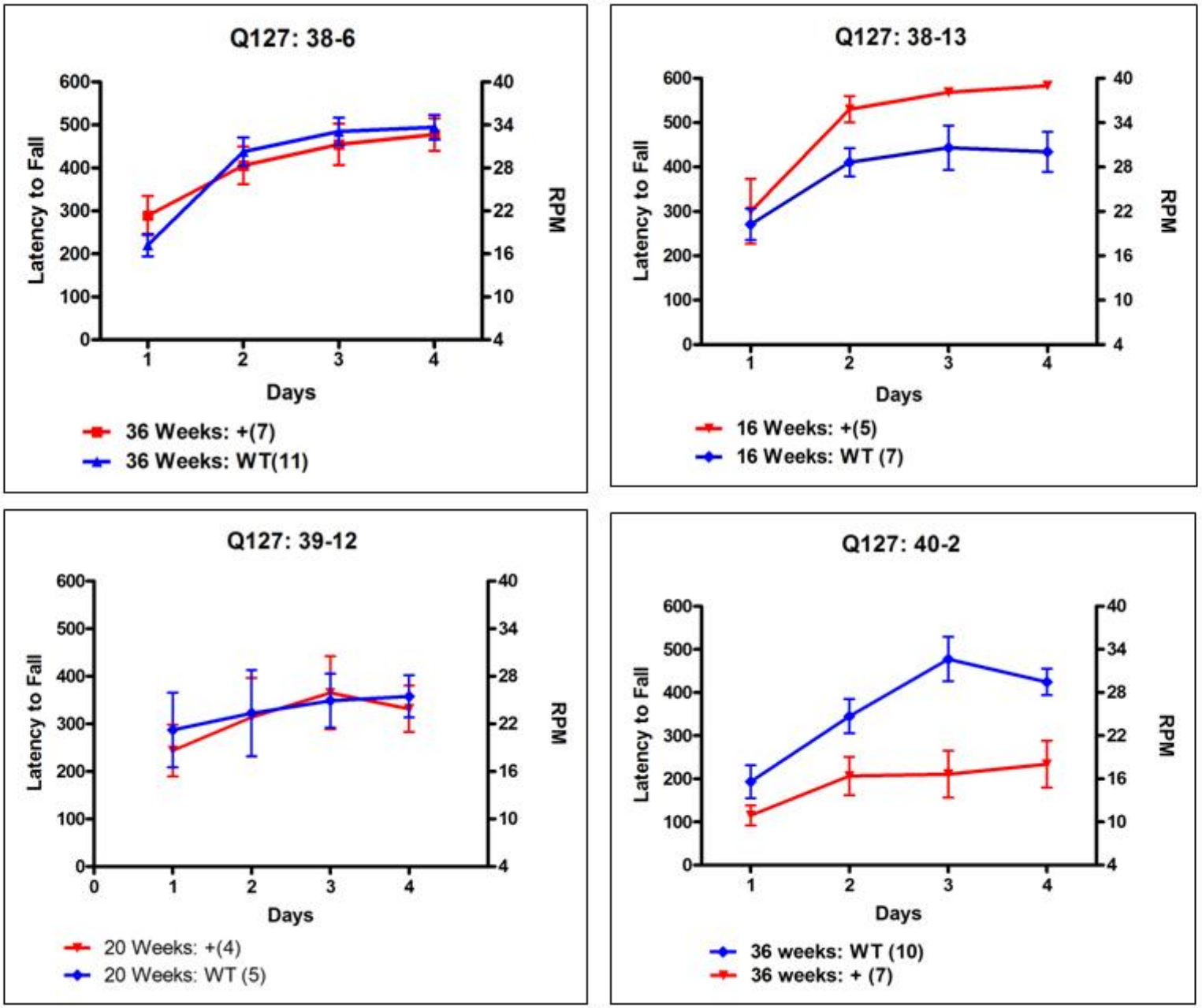
SCA2: Q127 (40-2)

- qPCR
- Histology
- Electrophysiology
- Behavioral motor testing

logistics

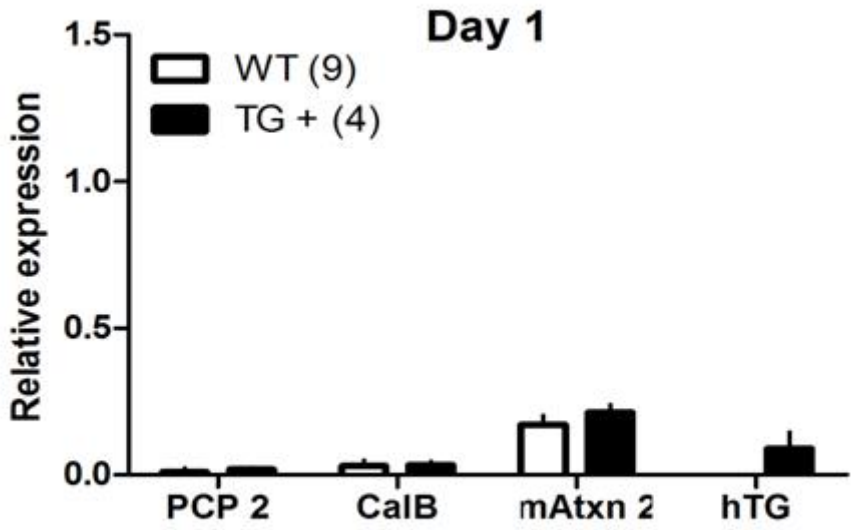
- Put down 2 of the 4 lines of q127 SCA 2
  - 38-13 & 39-12
  - Why….
    - No qPCR phenotype
    - No rotarod phenotype
  - Keep line 38-6 as a negative control to 40-2

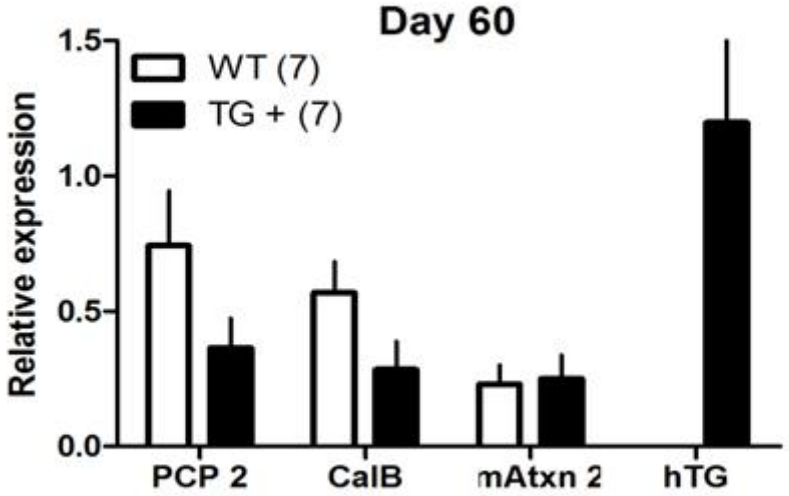

qPCR for 6-9 month old mice
TG
WT
PCP2 expression relative to WASF-1
0
1
2
3
4
4
2
5
2
3
2
9
10
38-6
38-13
39-12
40-2



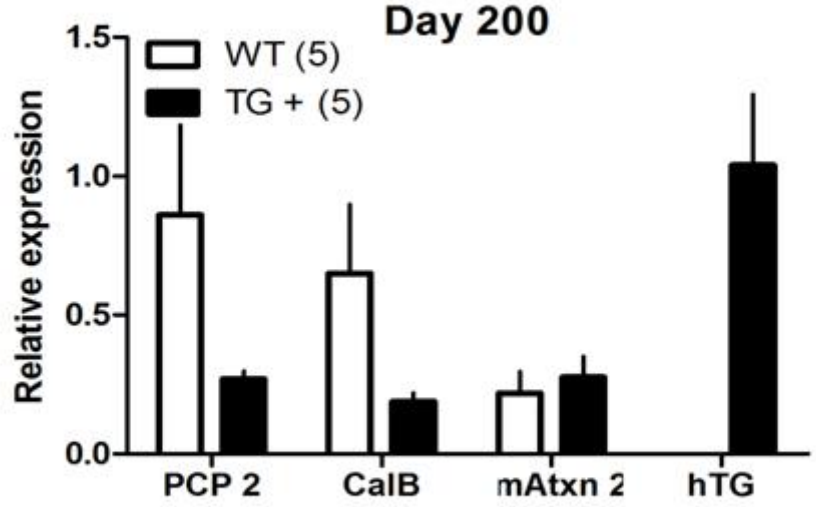
Rotarod data

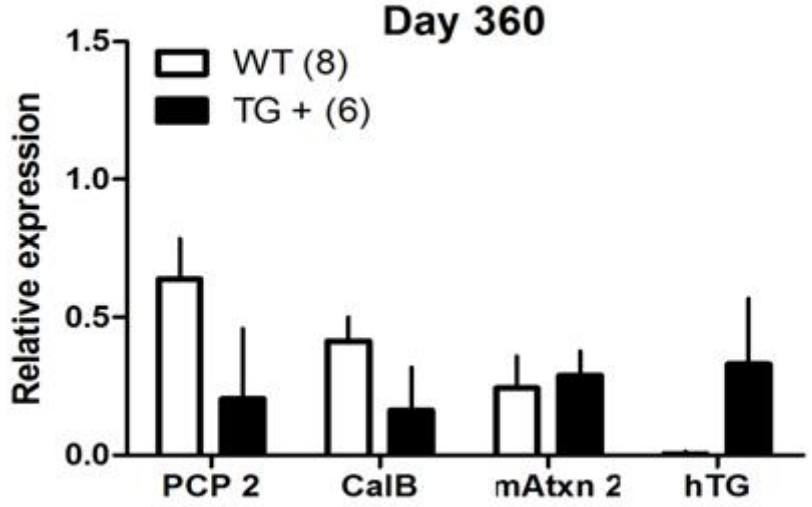
The following slides from the SCA2 q127 project represent data obtained from line 40-2


Day 1
WT (9)
TG + (4)
Relative expression
1.5
1.0
0.5
0.0
PCP 2
CalB
mAtxn 2
hTG

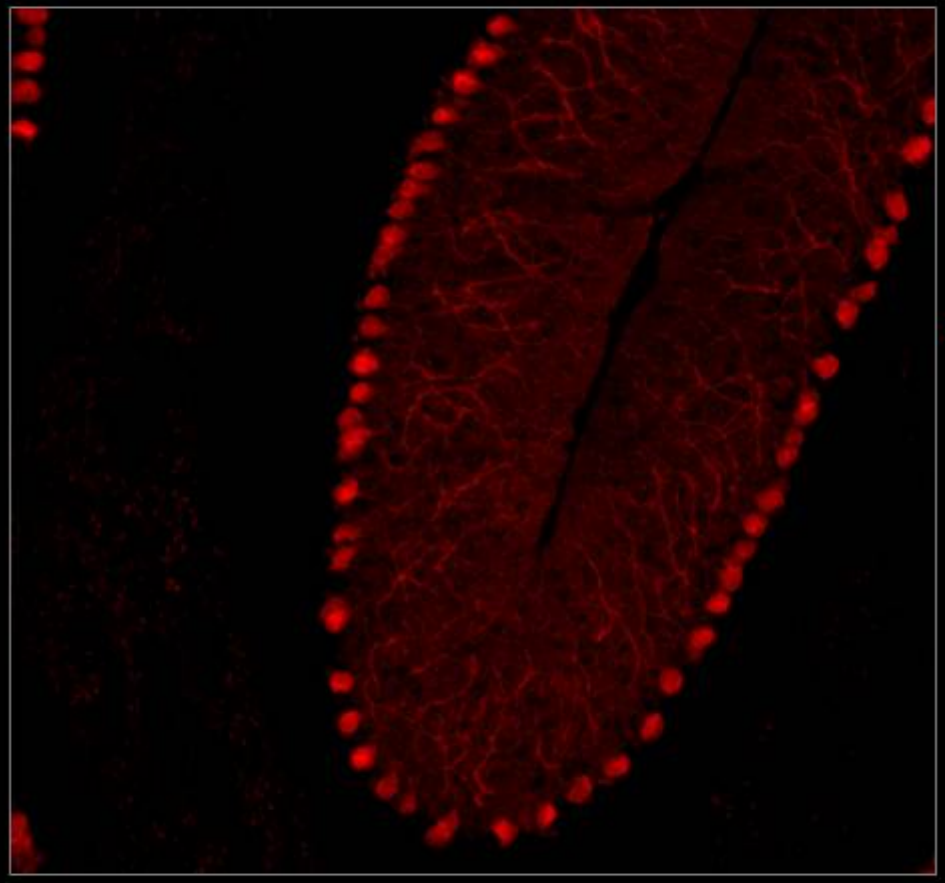

Day 60
WT (7)
TG + (7)
Relative expression
1.5
1.0
0.5
0.0
PCP 2
CalB
mAtxn 2
hTG

qPCR for 40-2

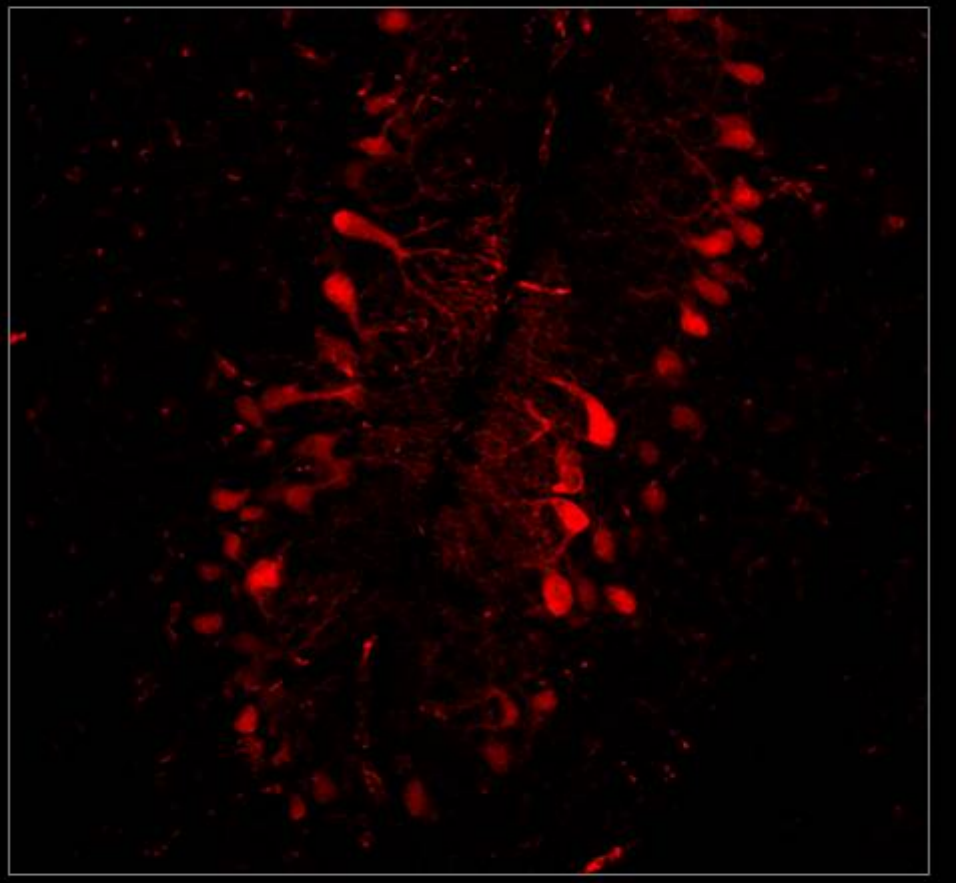

Day 200
WT (5)
TG + (5)
Relative expression
1.5
1.0
0.5
0.0
PCP 2
CalB
mAtxn 2
hTG


Day 360
WT (8)
TG + (6)
Relative expression
1.5
1.0
0.5
0.0
PCP 2
CalB
mAtxn 2
hTG

Cerebellar image taken from *prima fissura* of vermis

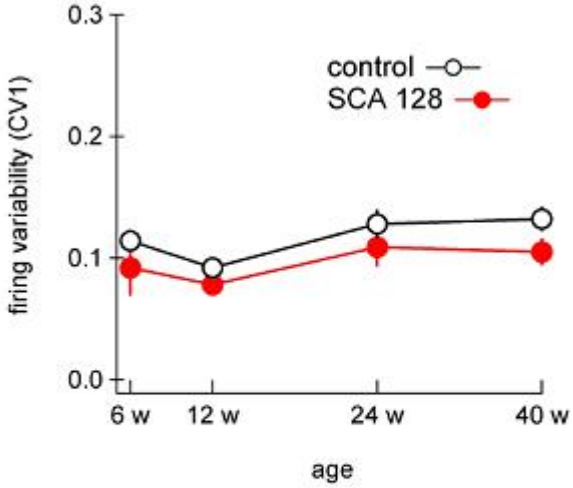


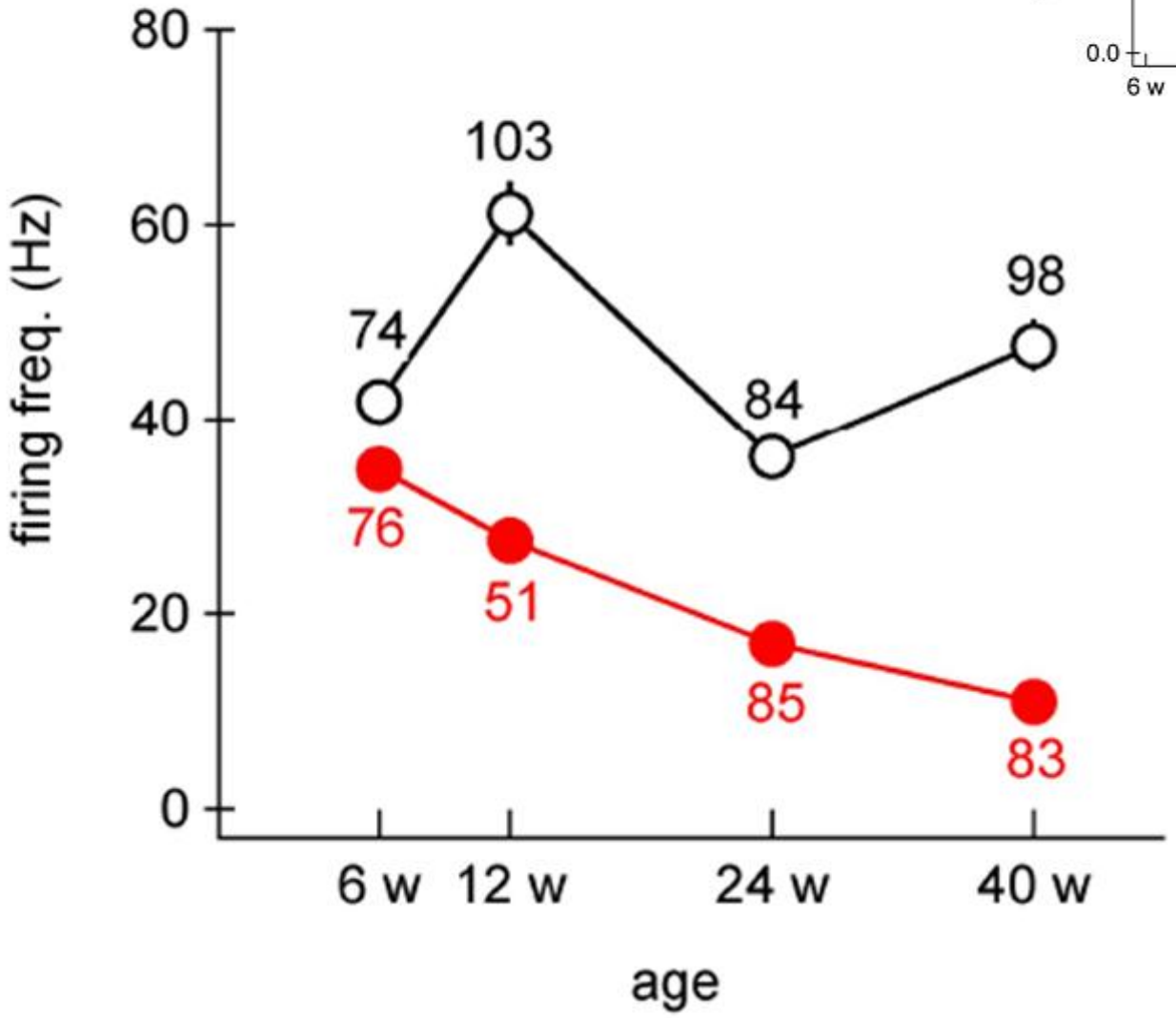
WT 20 weeks



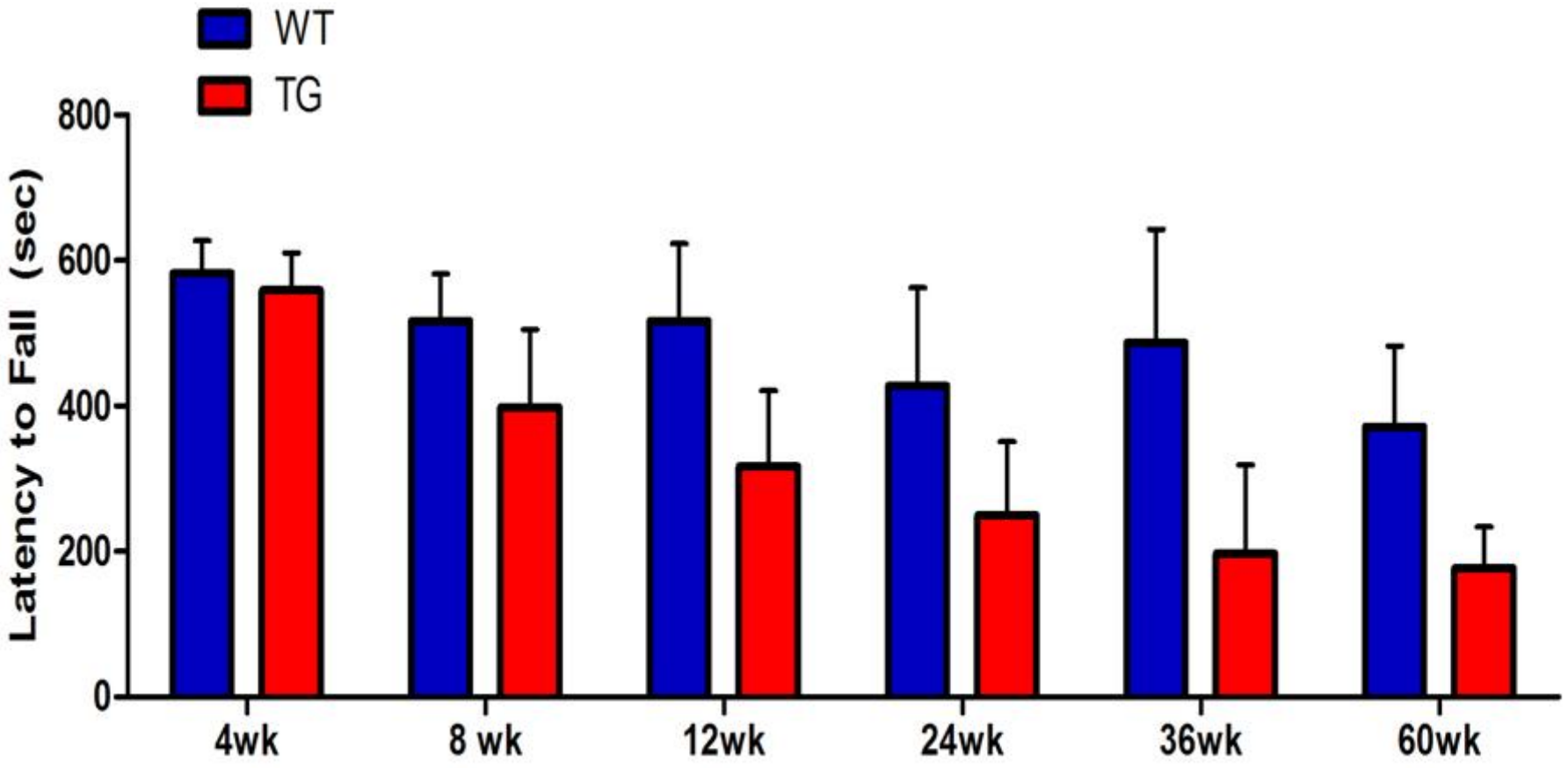
TG: 24 weeks

Electrophysiology: Otis lab





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

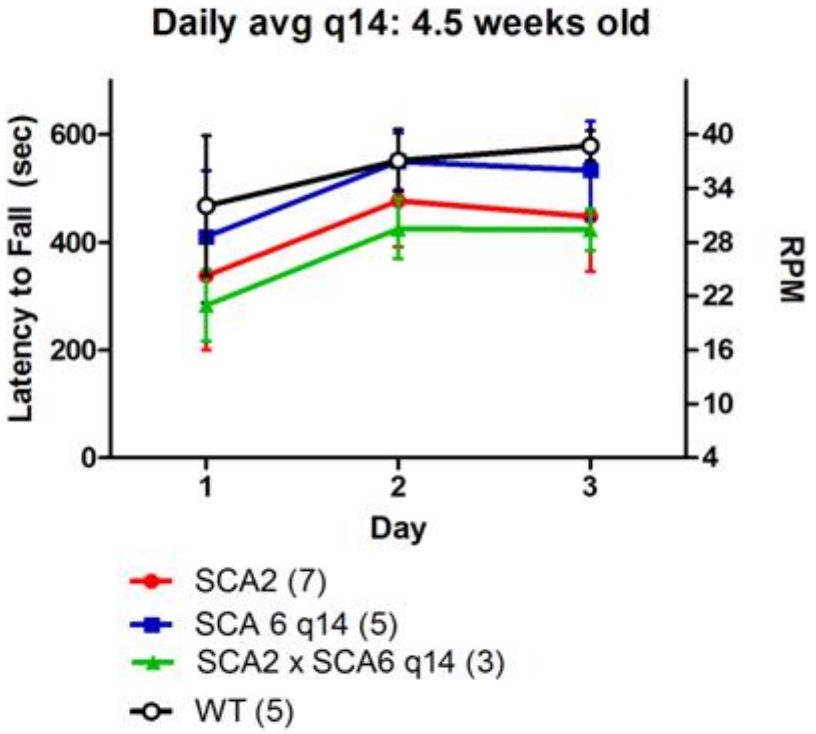


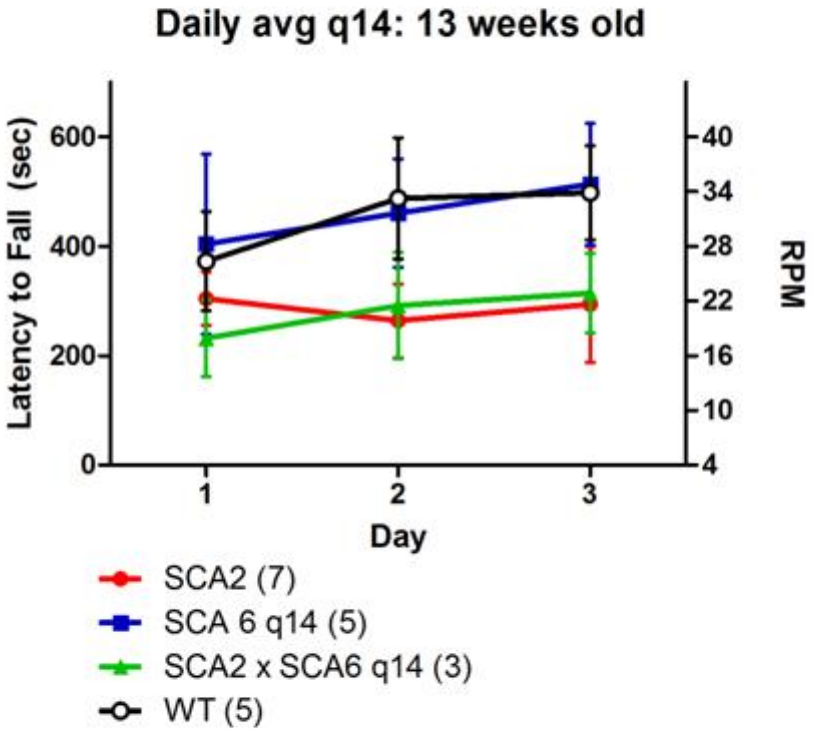
Q127: 40-2 x SCA6 (KI & KO)

- 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?

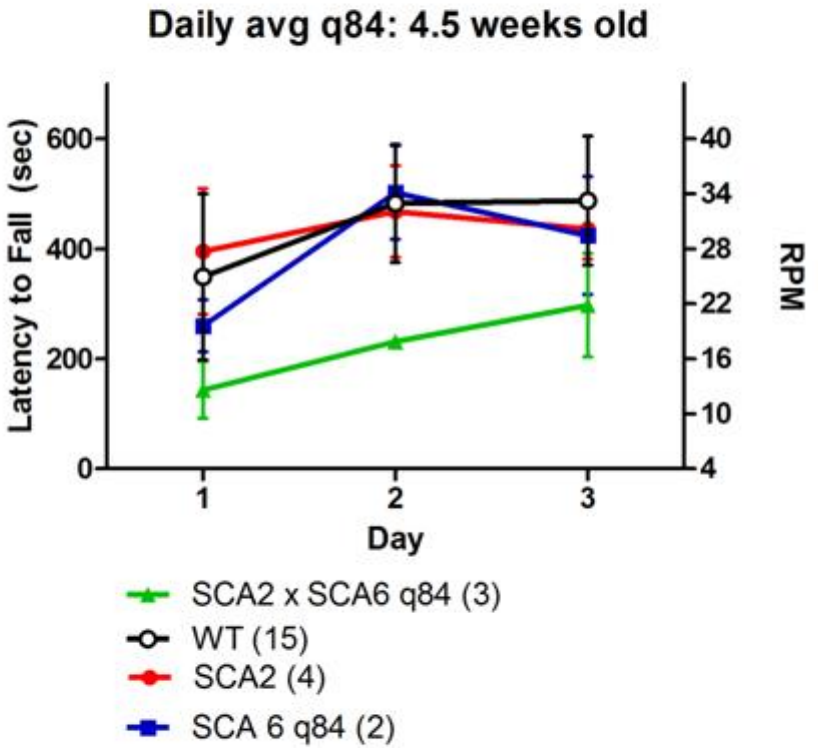
- SCA6 KO x Jax B6
  - N=22
    - 10 Het KO
    - 12 WT
- SCA6 KO x 40-2 :
  - N=18:

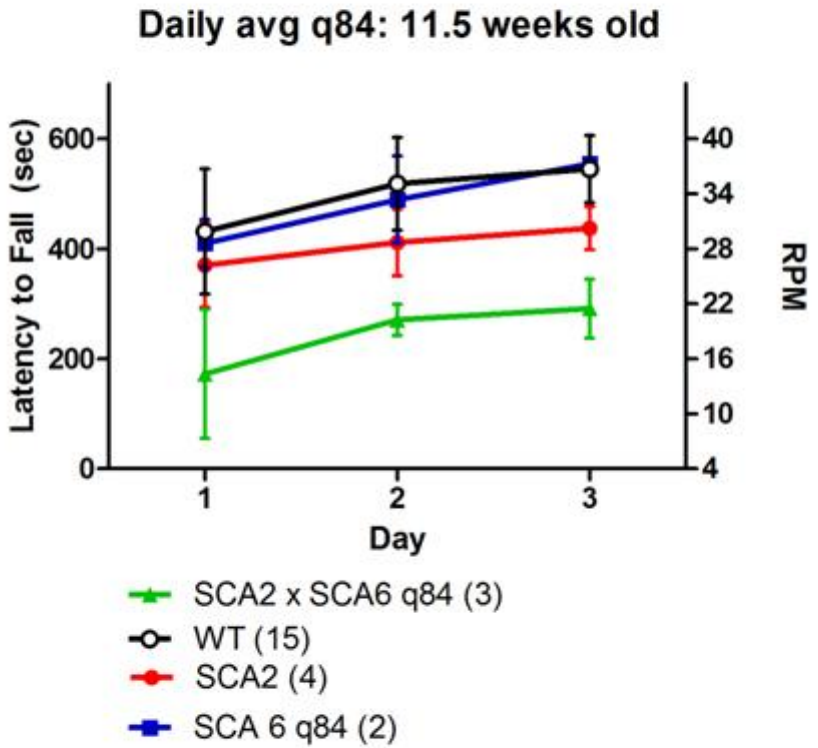
SCA 2: q127 x SCA 6: q14





SCA 2: q127 x SCA 6: q84





SCA 2: q127 x SCA 6 KO

- 4 week old Rotarod data collected
- Finishing PCR genotyping…

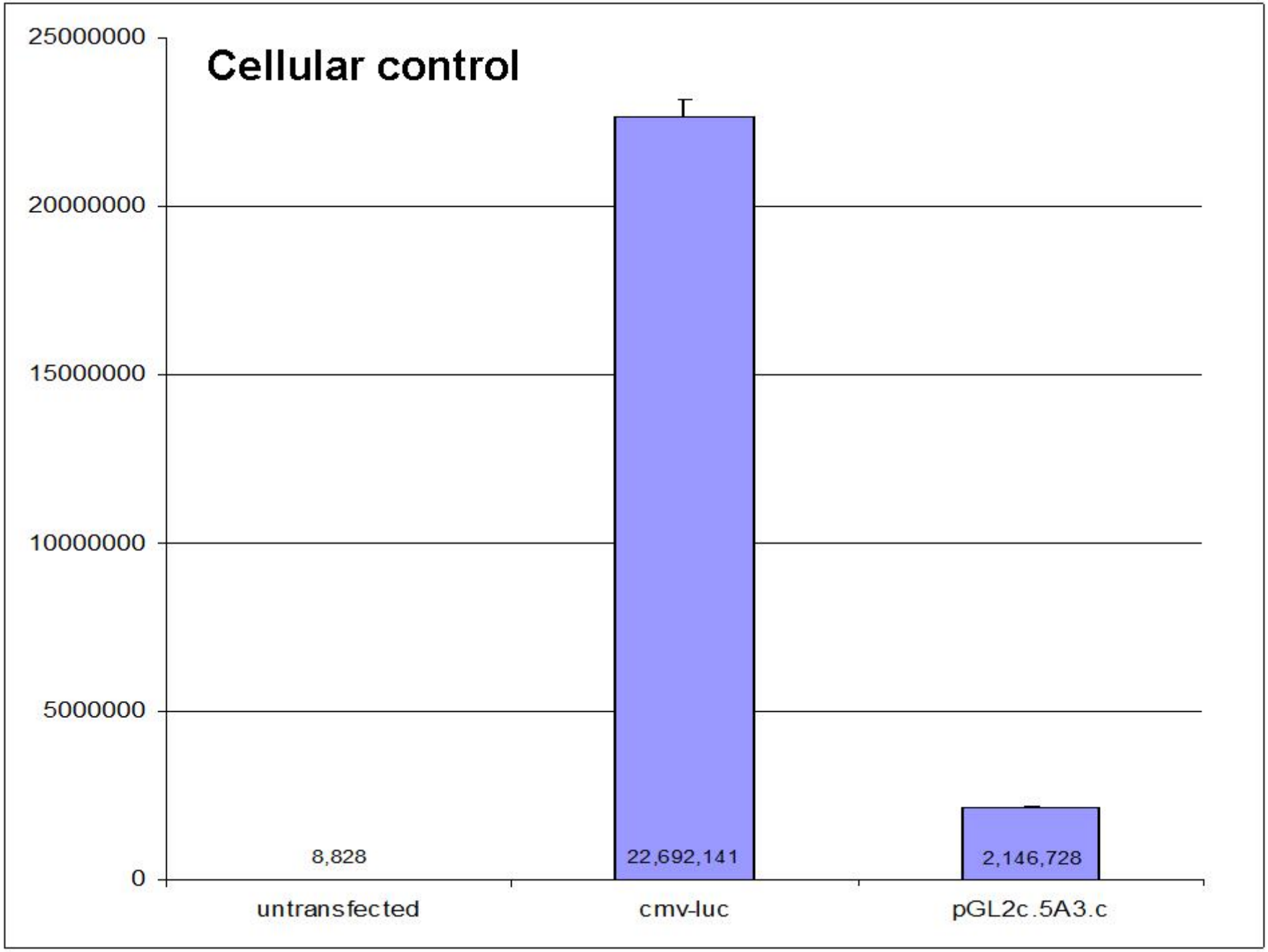
Future

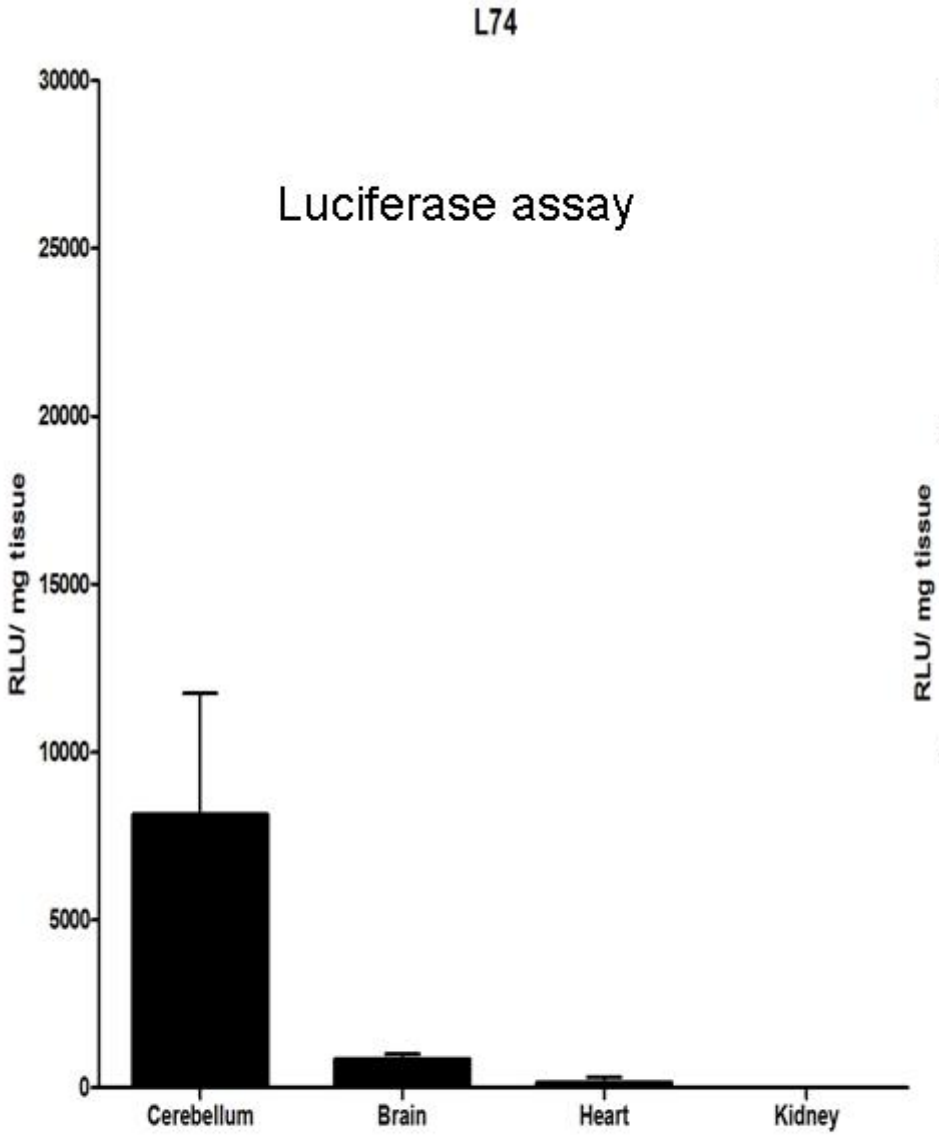
- Collect 3rd rotarod time point for existing animals
- Breeding for larger “n” size: KI & KO
- qPCR
- Histology
- E-phys

LUCIFER (ASE)

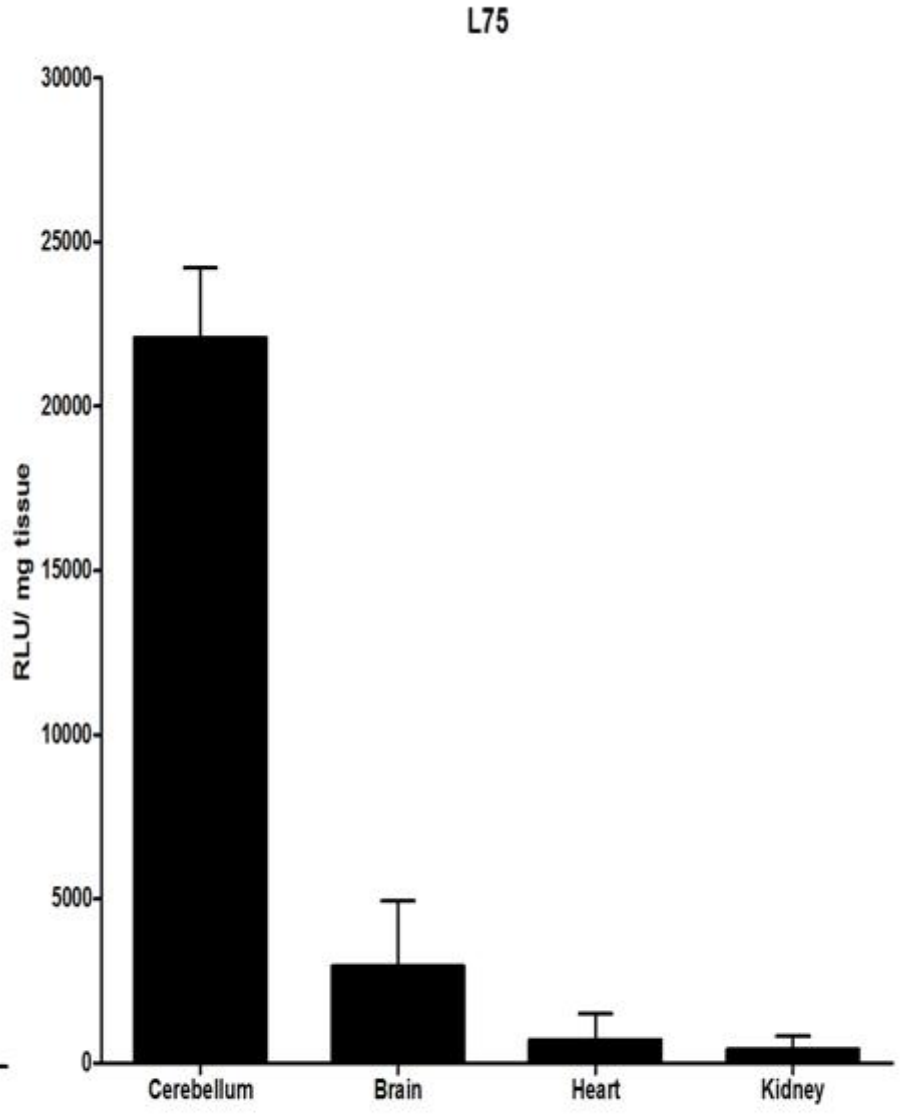
Luciferase

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


Cellular control
25000000
20000000
15000000
10000000
5000000
0
8,828
22,692,141
2,146,728
untransfected
cmv-luc
pGL2c.5A3.c

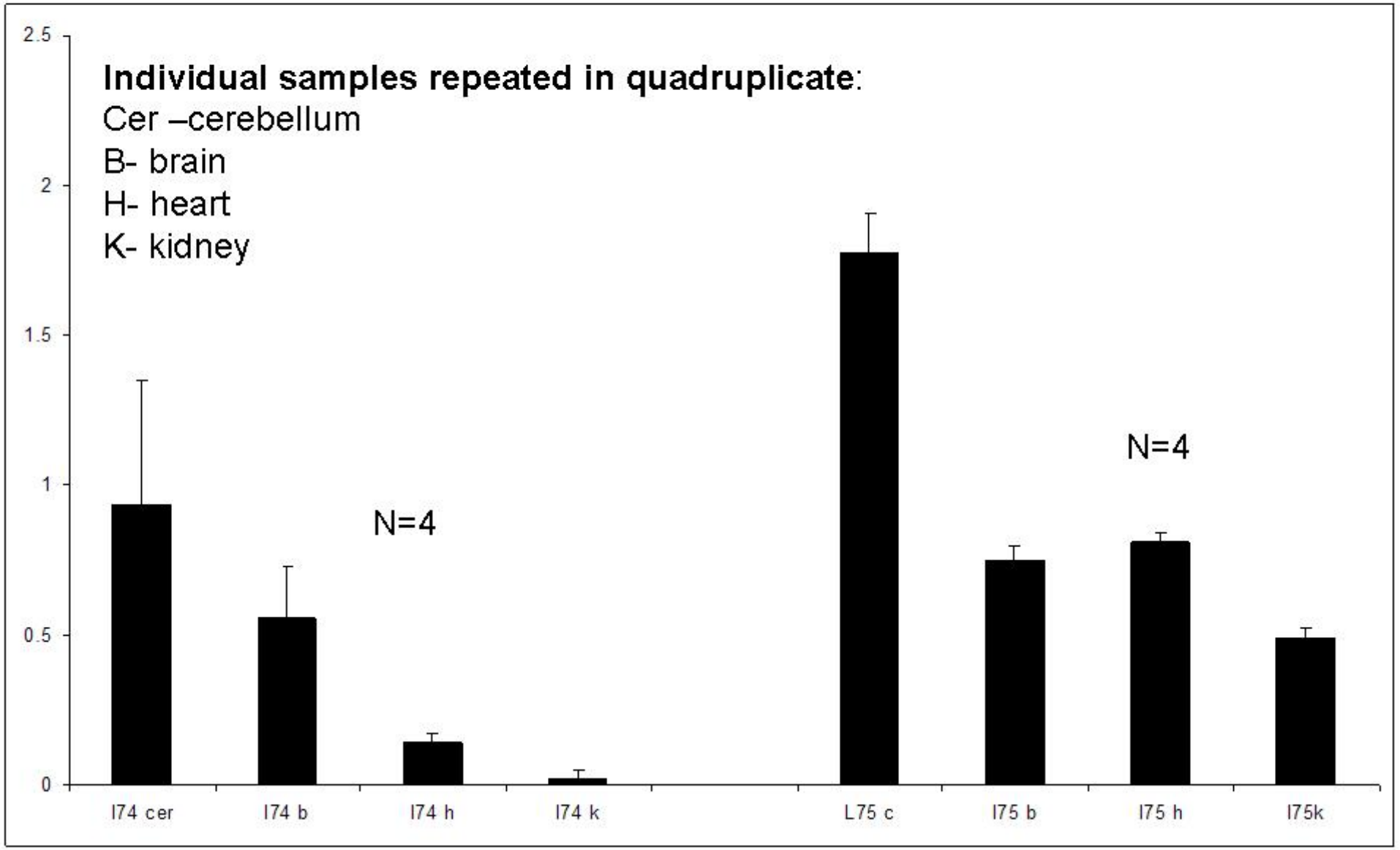


Luciferase: L74 (n=4)
error bar represent standard deviation

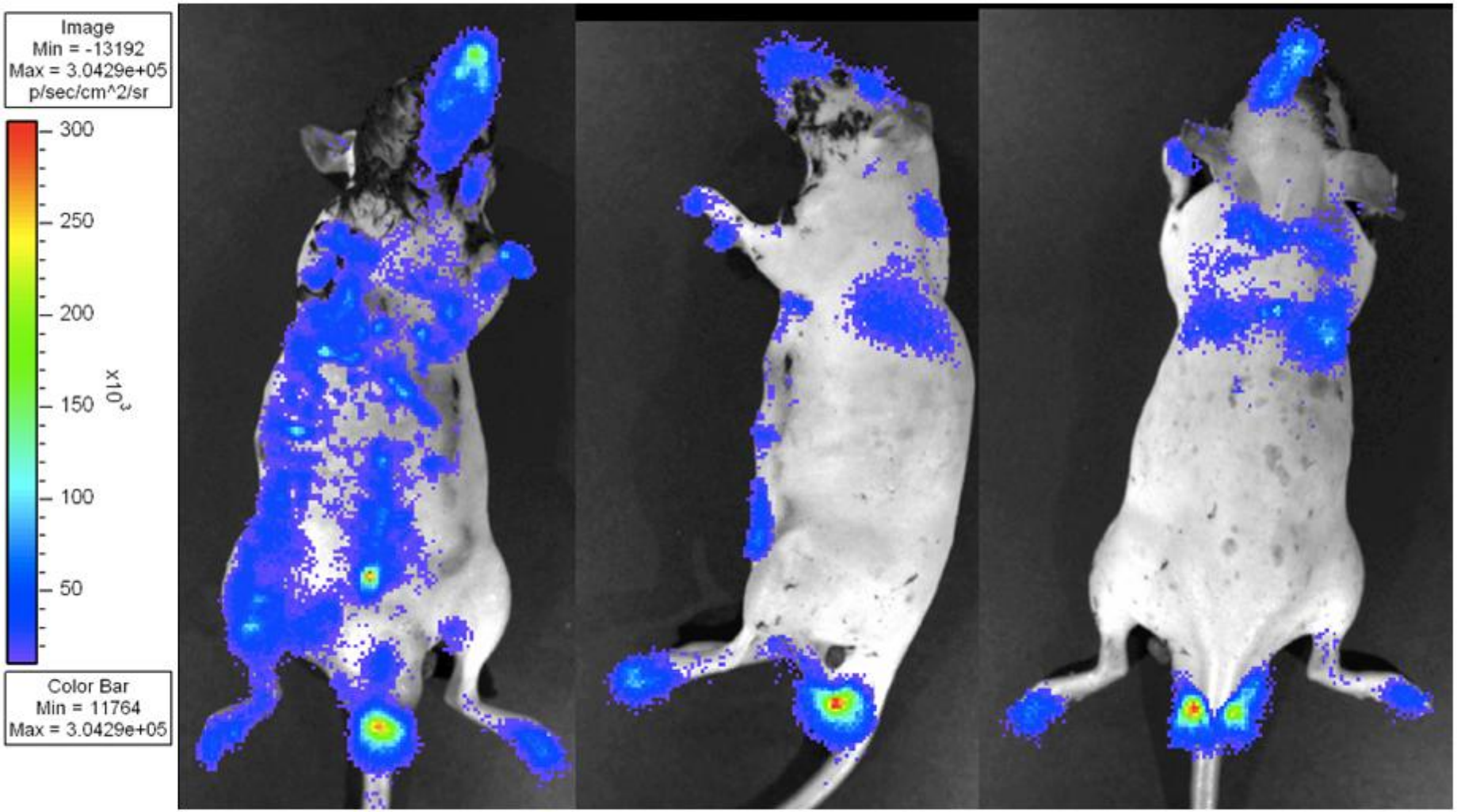


Luciferase: L75 (n=4)
error bar represent standard deviation

qPCR:
luciferase normalized to atxn2

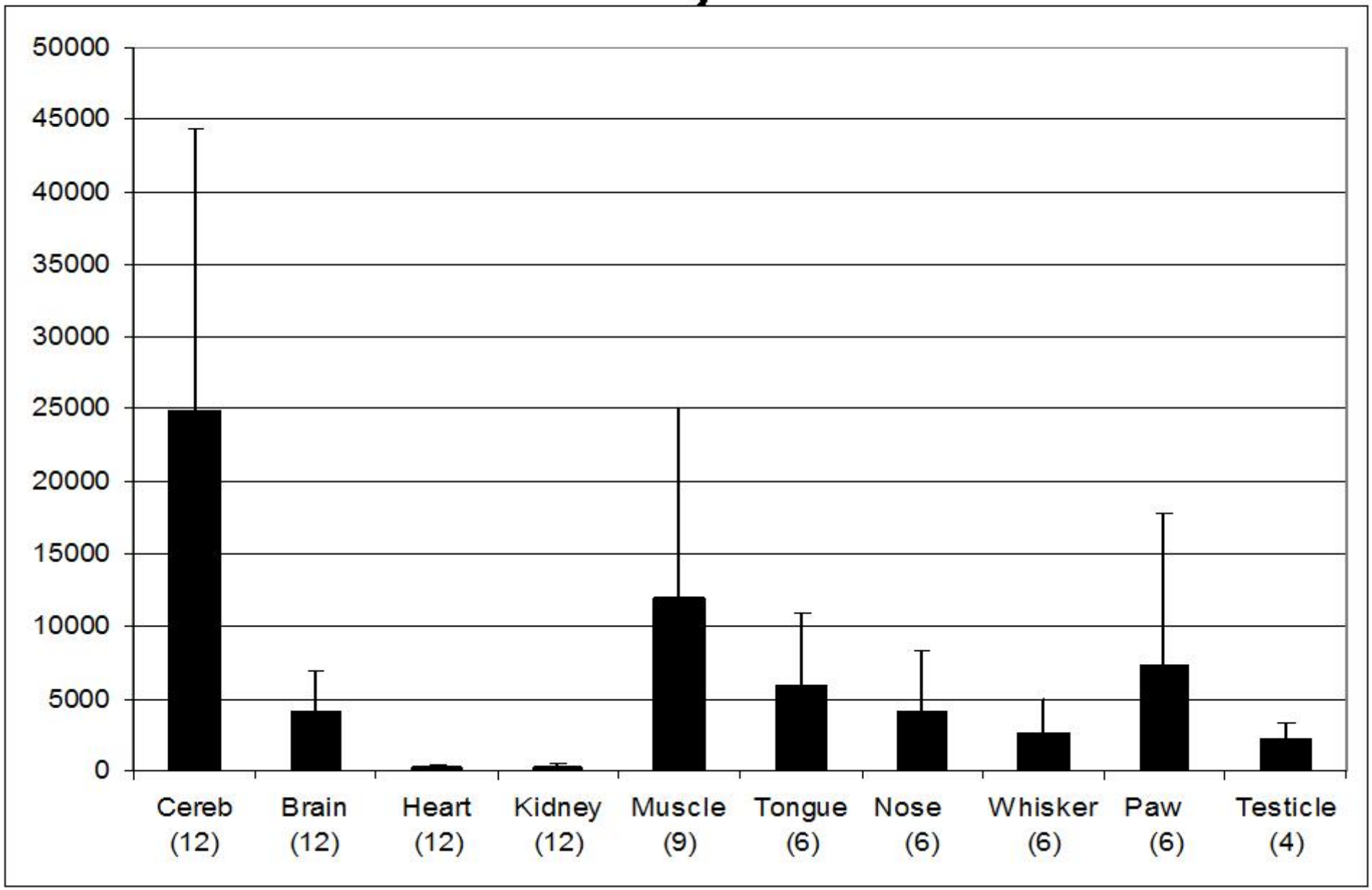


Luc 75-1



Ventral Side Dorsal

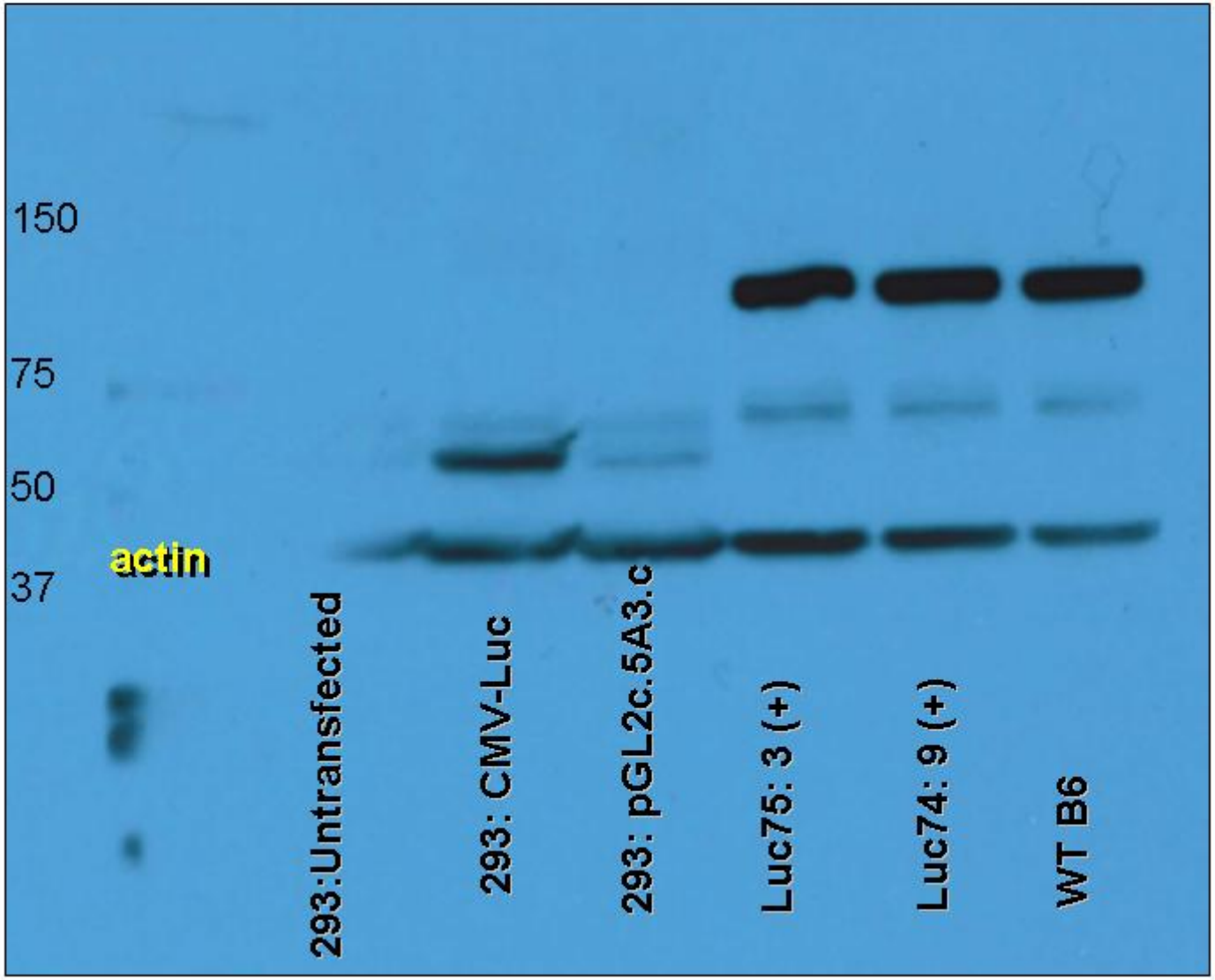
Luciferase Assay Line: Luc 75-1



Luciferase WB

Rockland AB

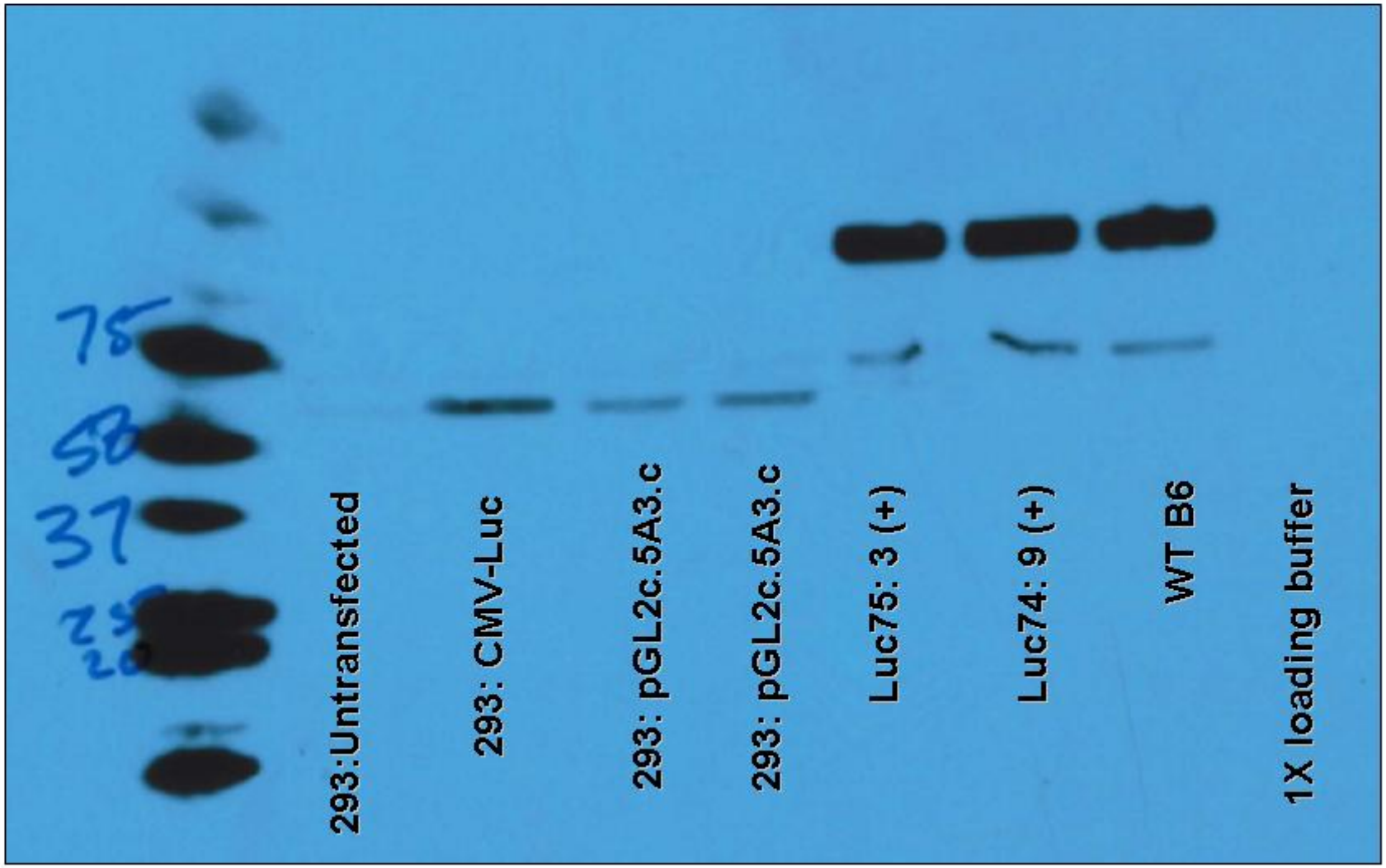
- 1’ 1:15k
- 2’ 1: 4k

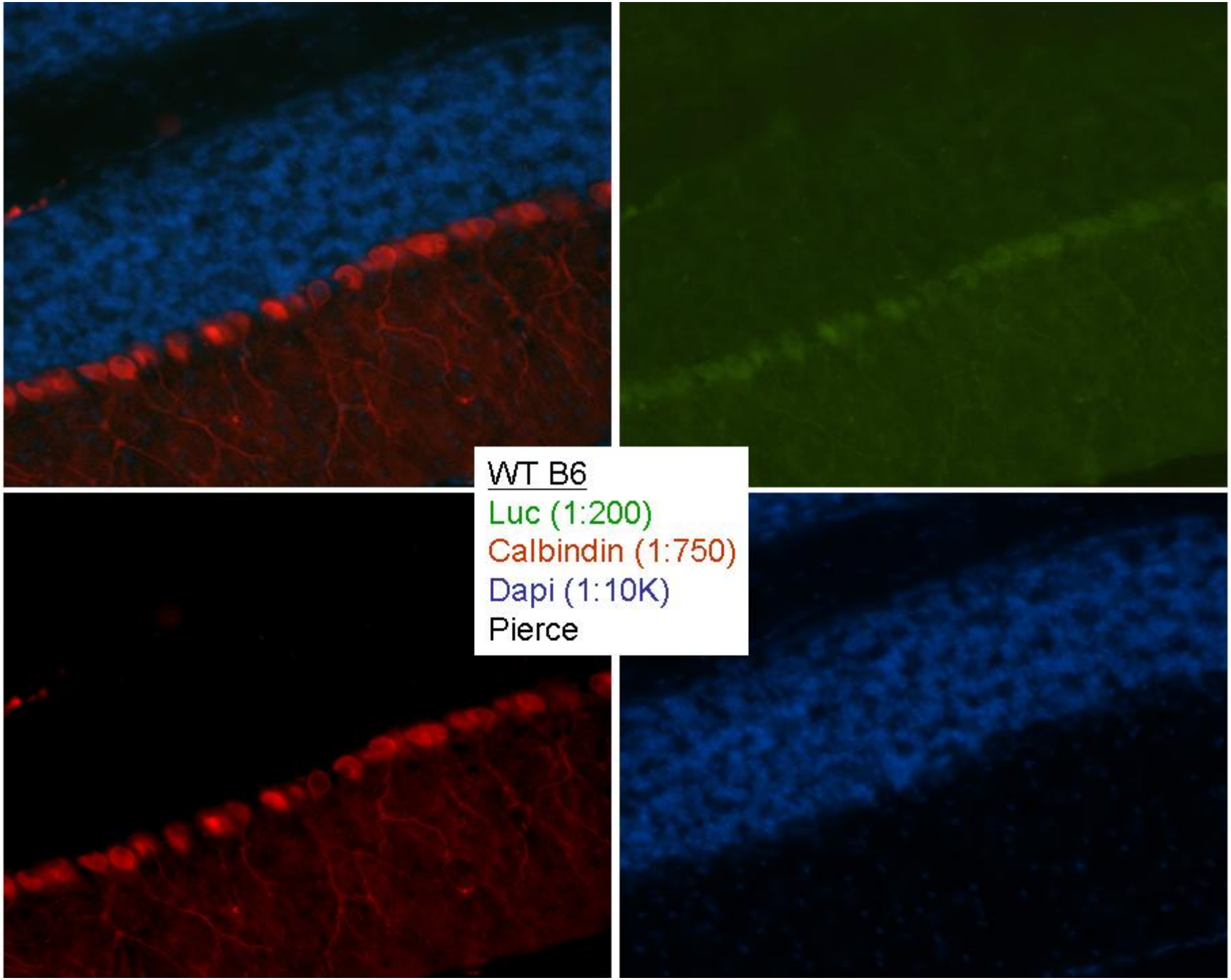


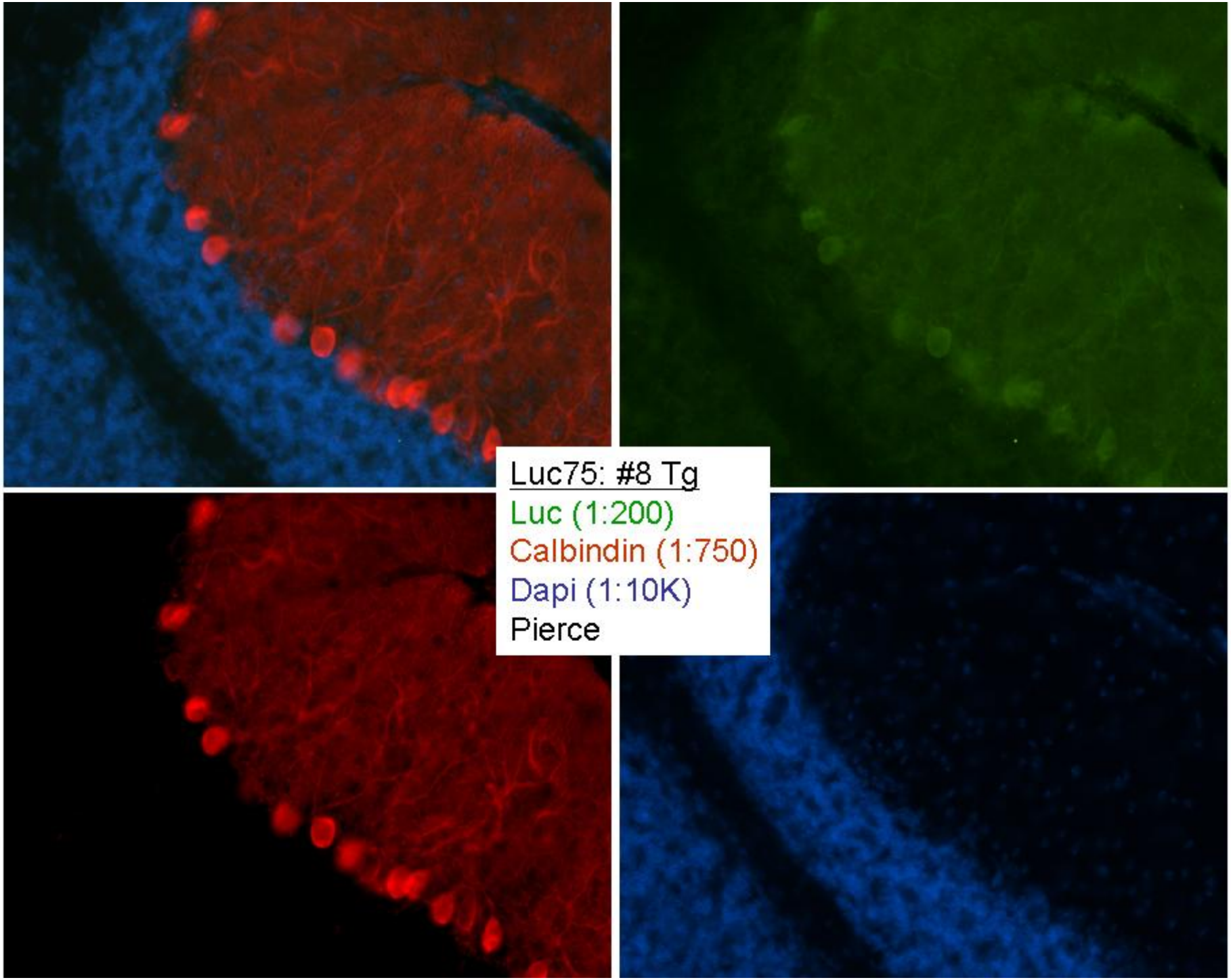
Luciferase WB

Pierce AB

- 1’ 1:30k
- 2’ 1: 4k




WT B6
Luc (1:200)
Calbindin (1:750)
Dapi (1:10K)
Pierce

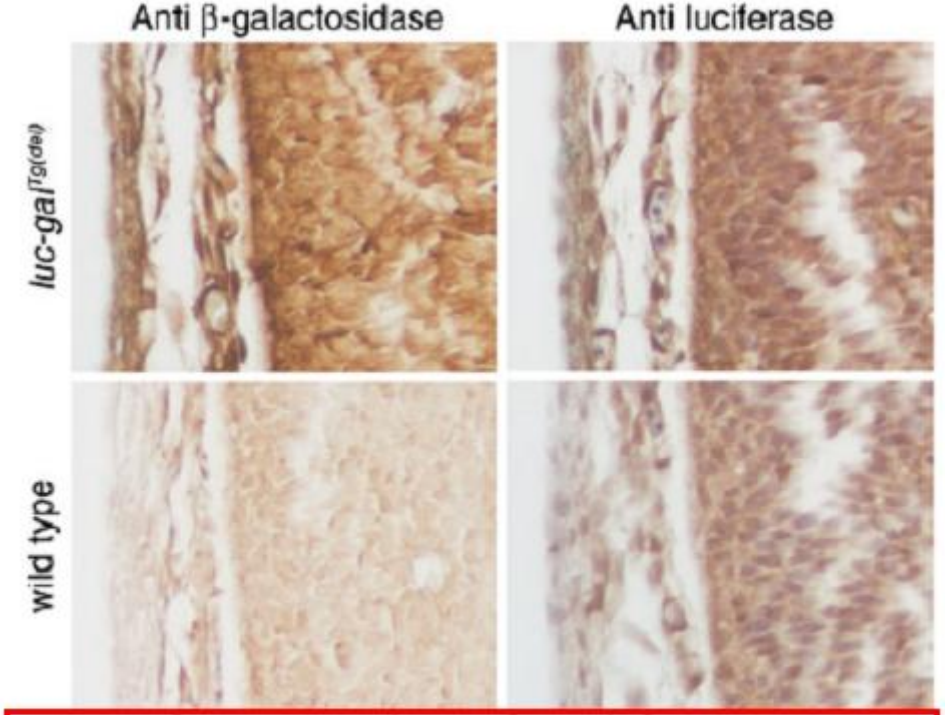

Luc75: #8 Tg
Luc (1:200)
Calbindin (1:750)
Dapi (1:10K)
Pierce

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



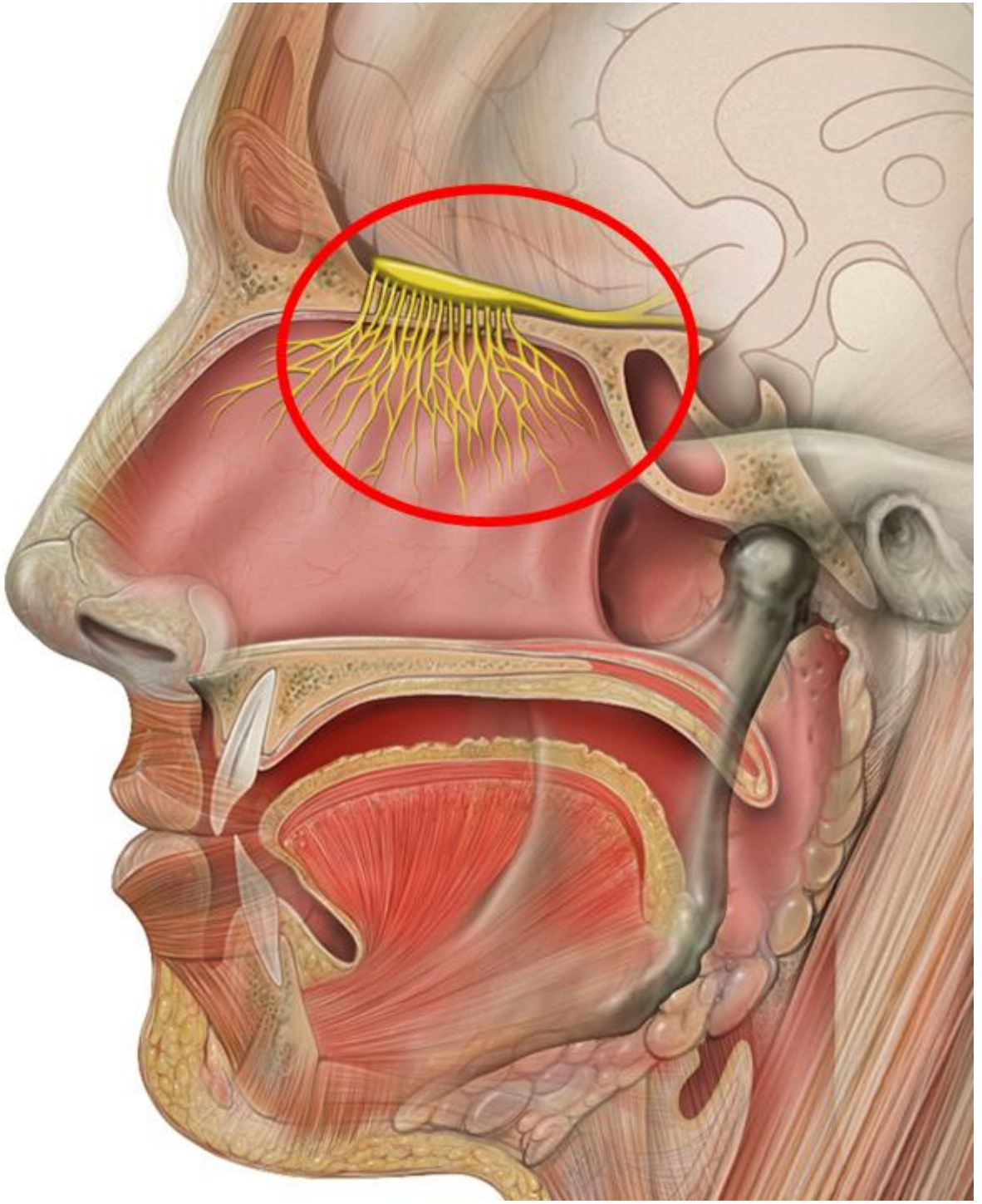
body, we were unable to obtain specific staining with anti-luciferase antibody (Fig. 4).

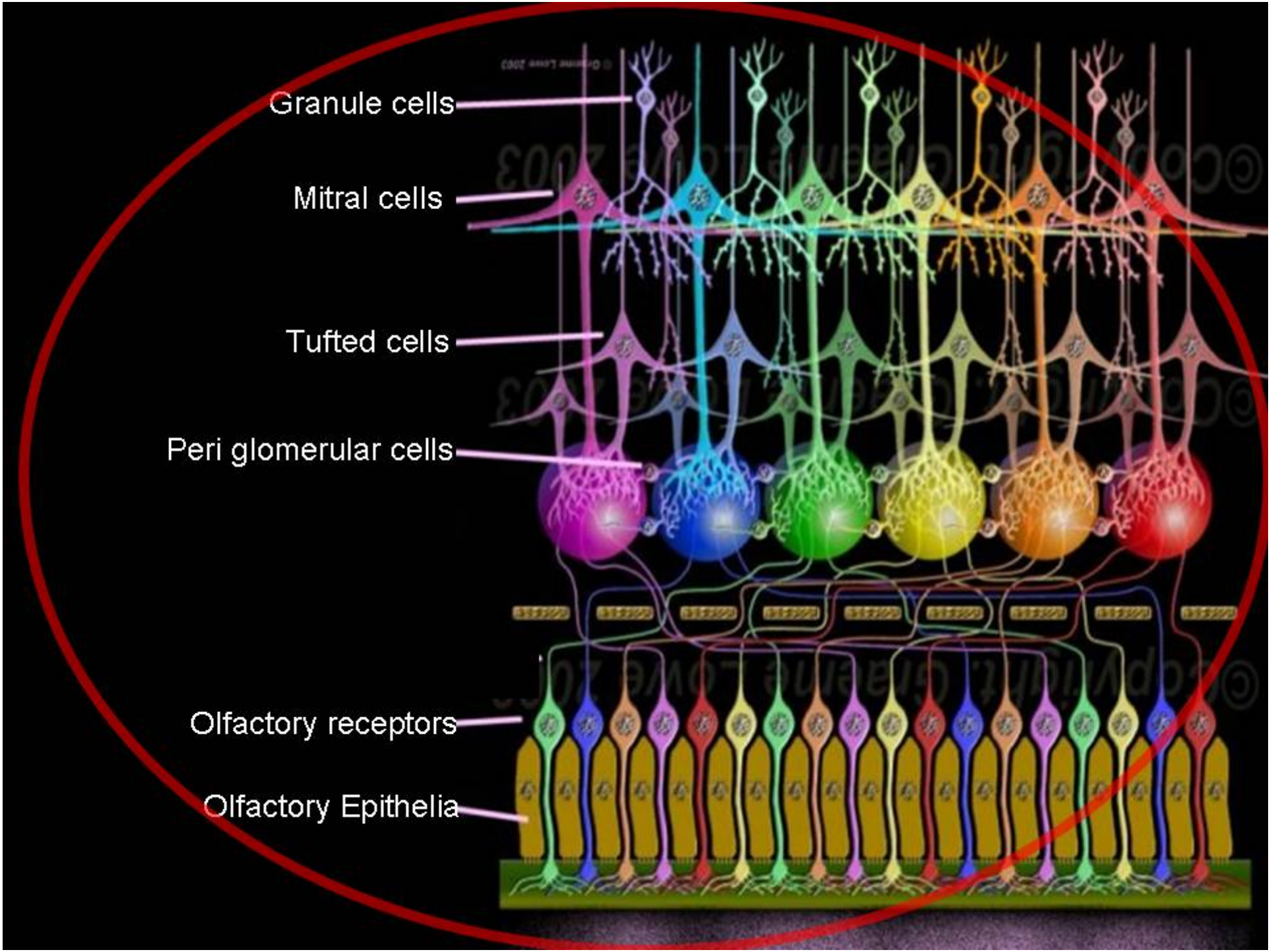
The major disadvantage of luciferase as a reporter gene is at the cellular level. Antisera that provide reliable, easy, and reproducible immunohistochemistry for luciferase are not available. We have compensated for this deficiency by utilizing *lacZ*, another reporter gene, in tandem with luciferase 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

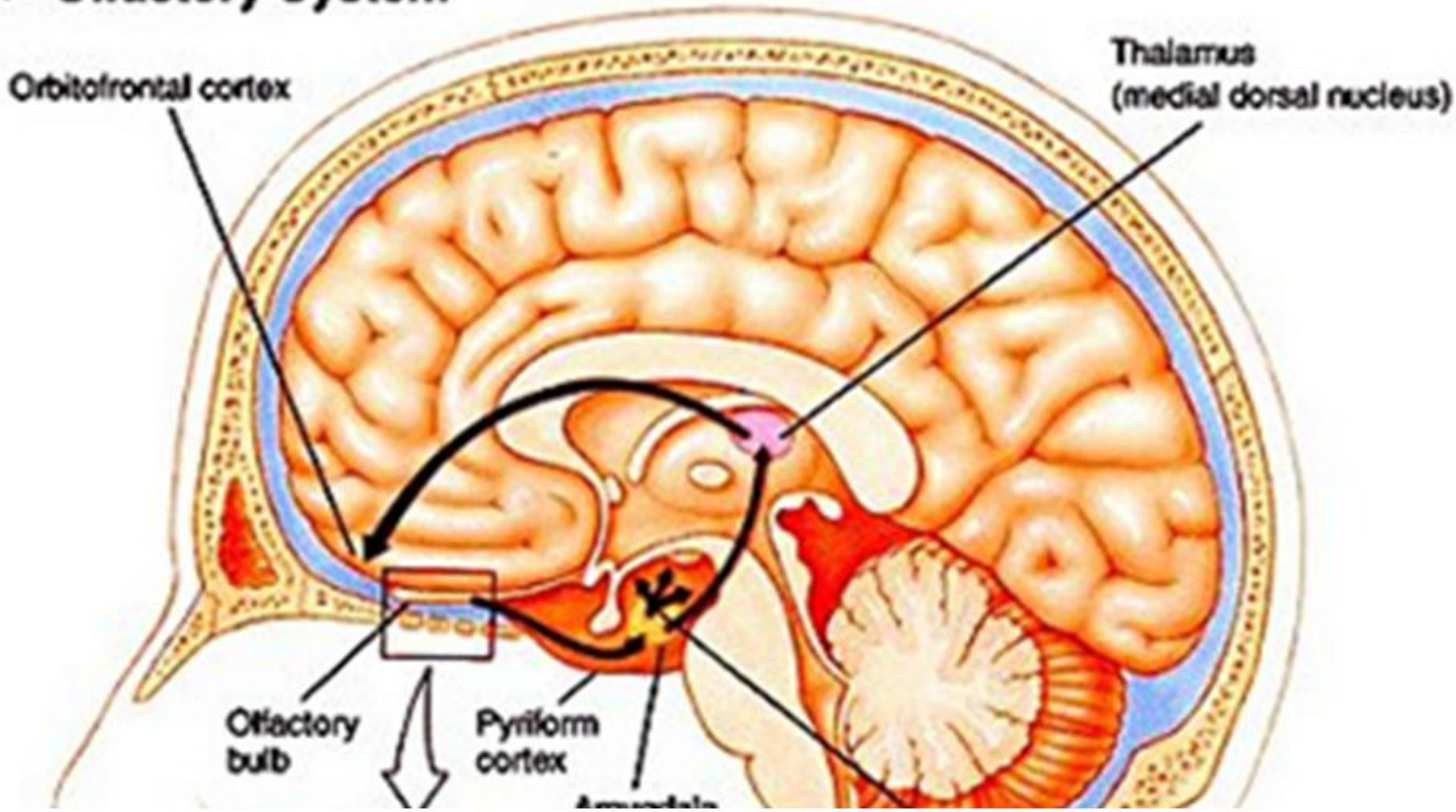
- Need to finish making RNA→ qPCR for all tissue
- No more IHC and WB


OLFACTORY
BULB

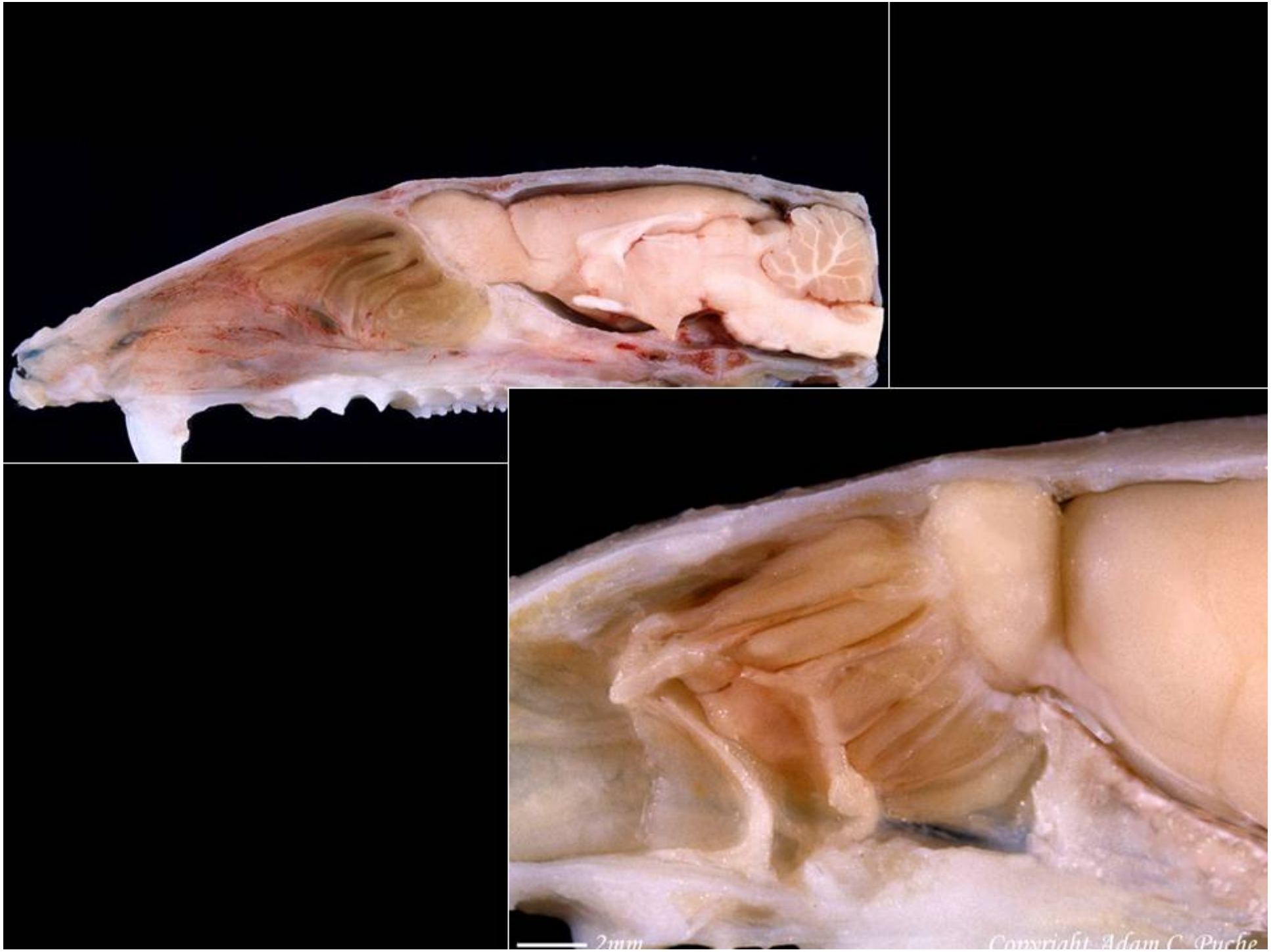



Granule cells
Mitral cells
Tufted cells
Peri glomerular cells
Olfactory receptors
Olfactory Epithelia

▶ Olfactory System



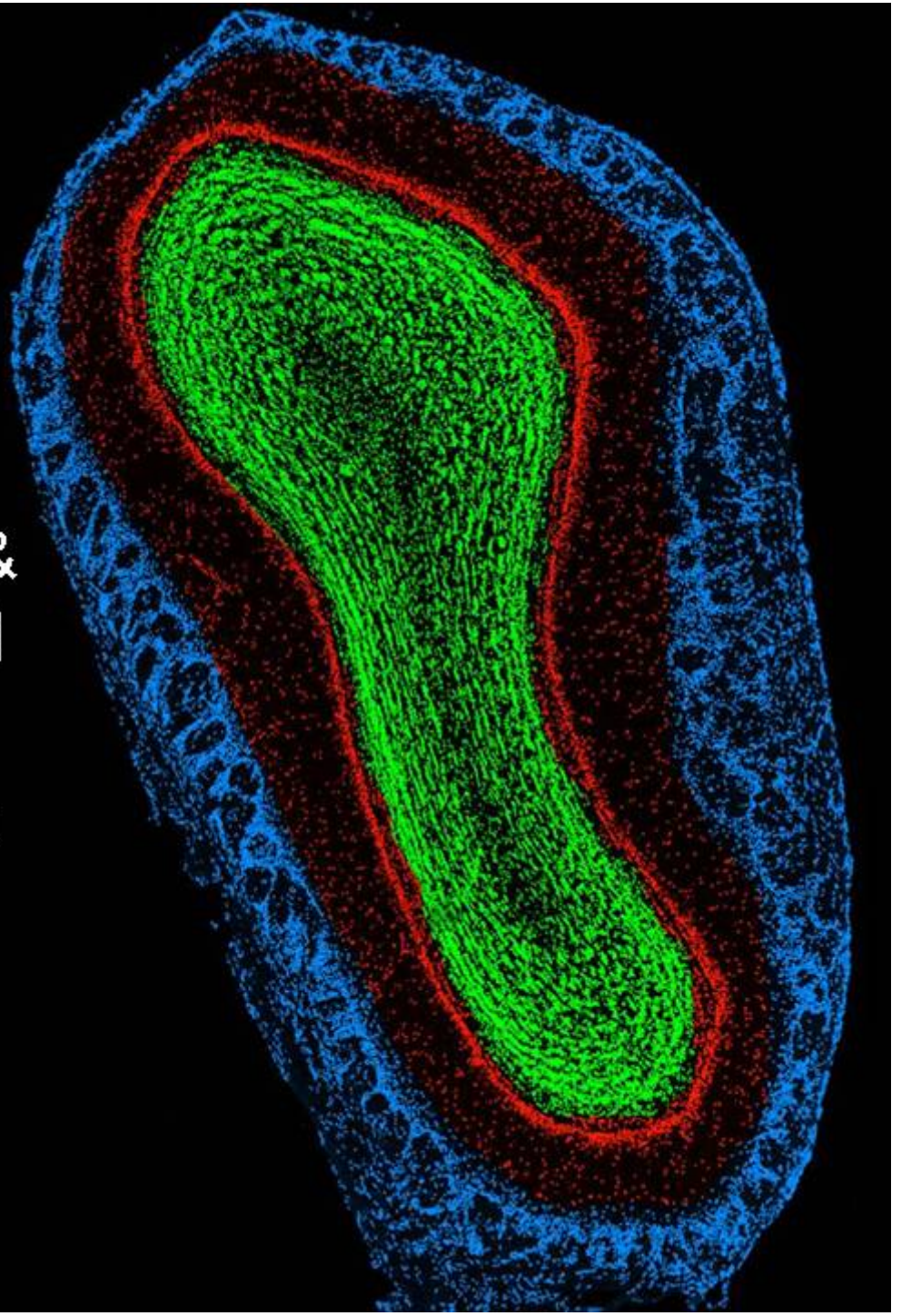
Pyriform cortex: cortical amygdala, uncus and anterior parahippocampal gyrus; sends projects to medial dorsal nucleus of the thalamus


2mm
Copyright Adam C. Pyche

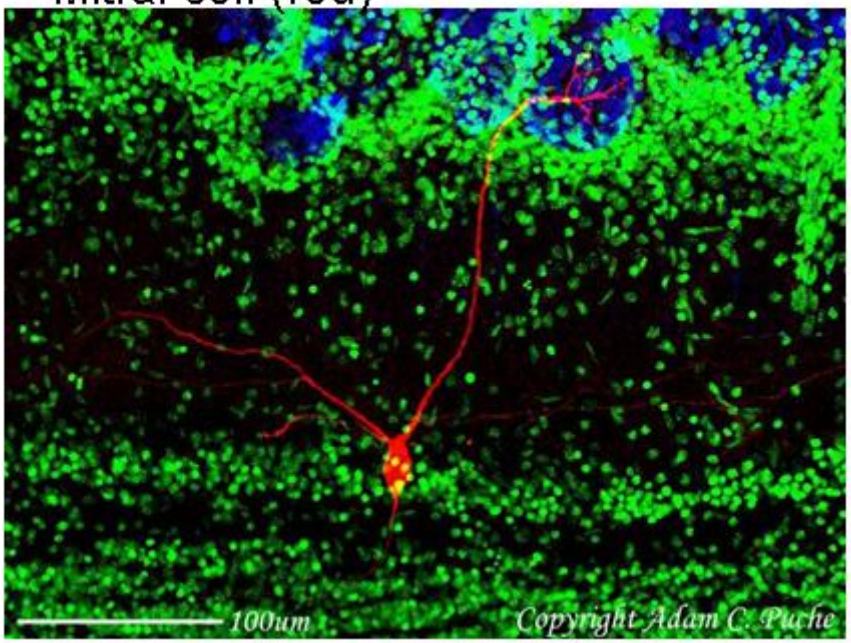
EXAMPLE

Coronol OB sxn

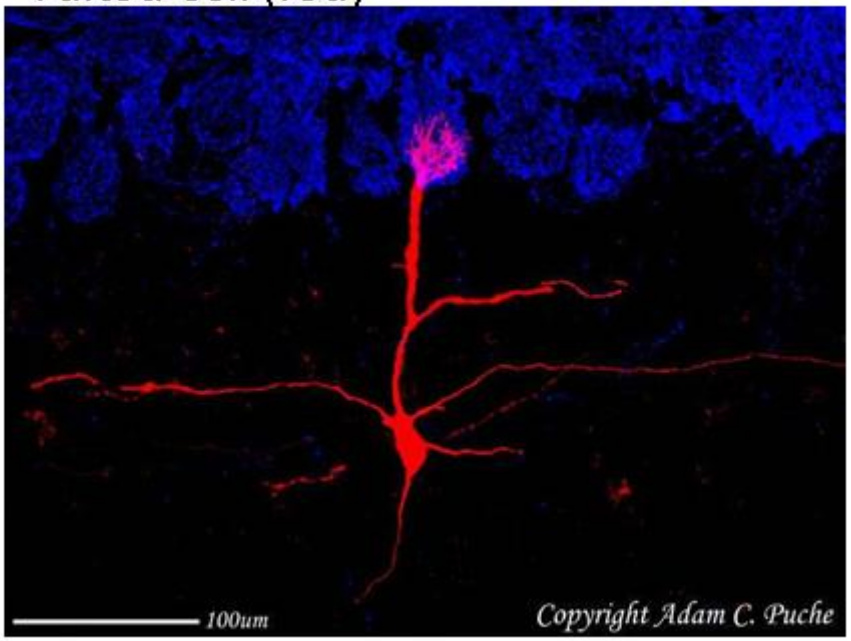
- Blue: glomerular layer
- Red: external plexiform & Mitral layer (mitral, tufted & granule cells)
- Green: Internal plexiform & granule cell layers



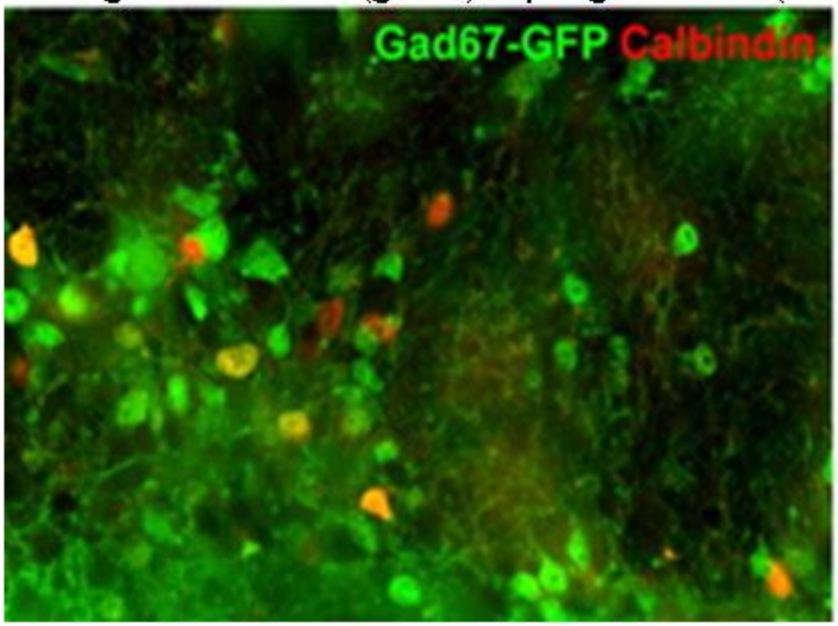
Mitral cell (red)



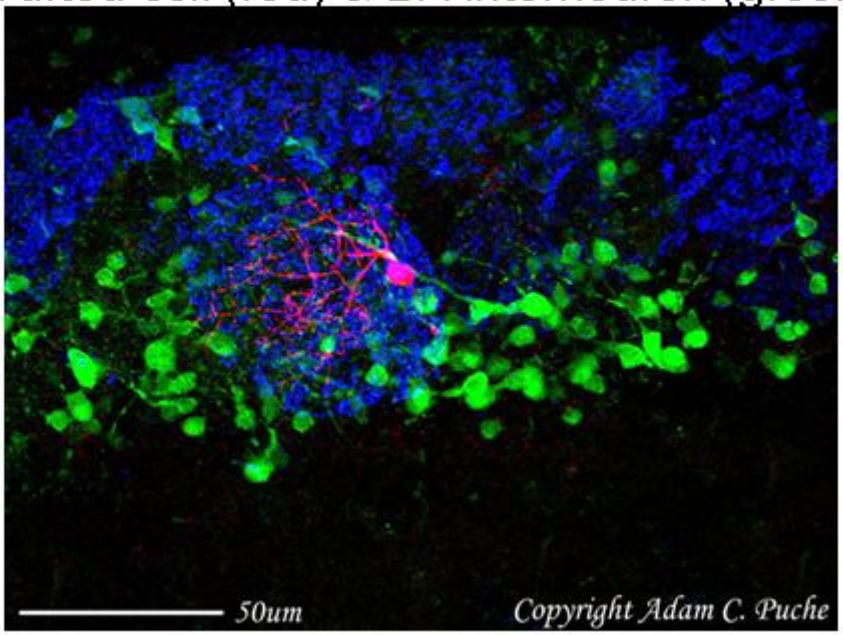
Tufted cell (red)



GABAergic interneuron (green) & periglomerular (red)



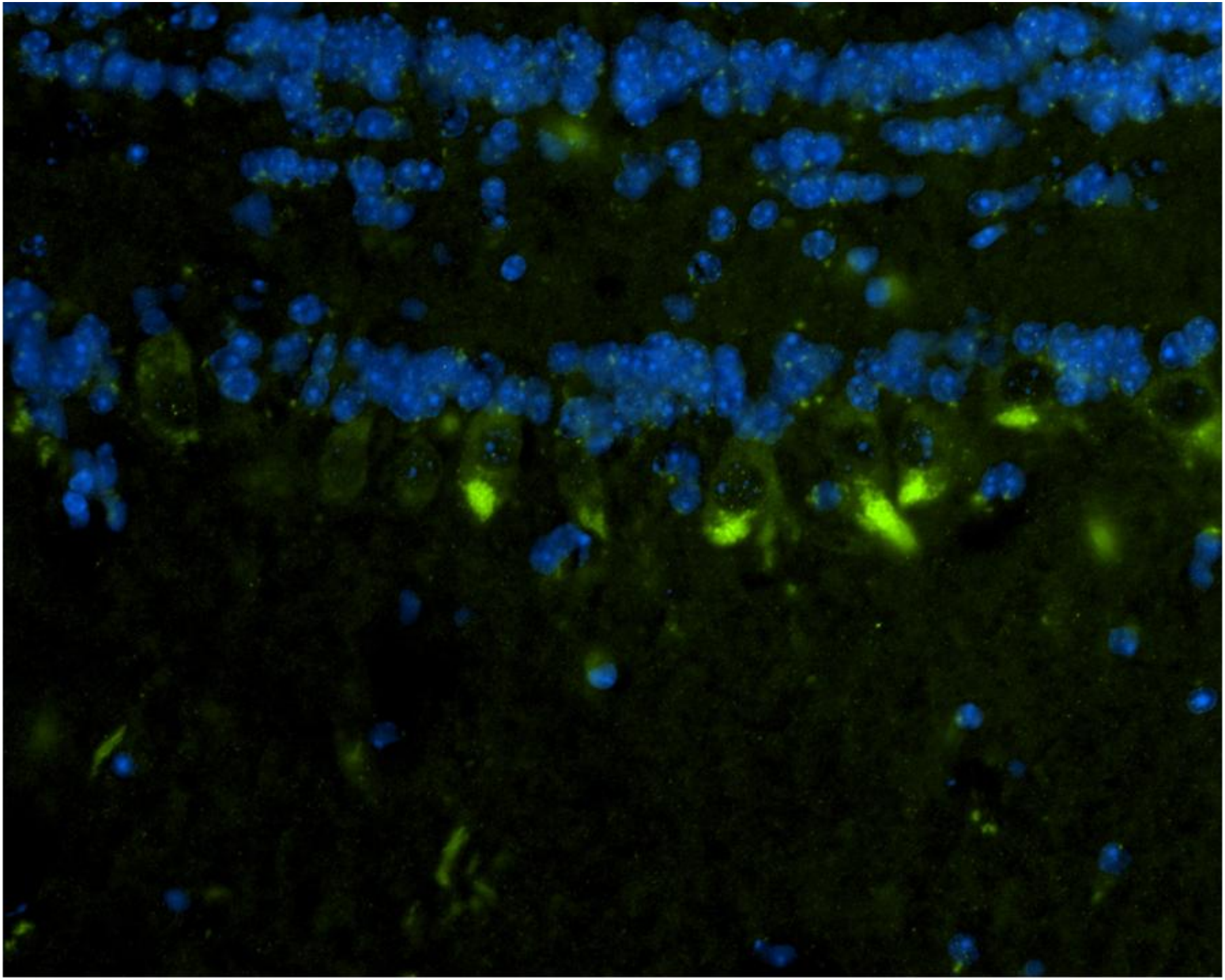
Tufted cell (red) & DA interneuron (green)

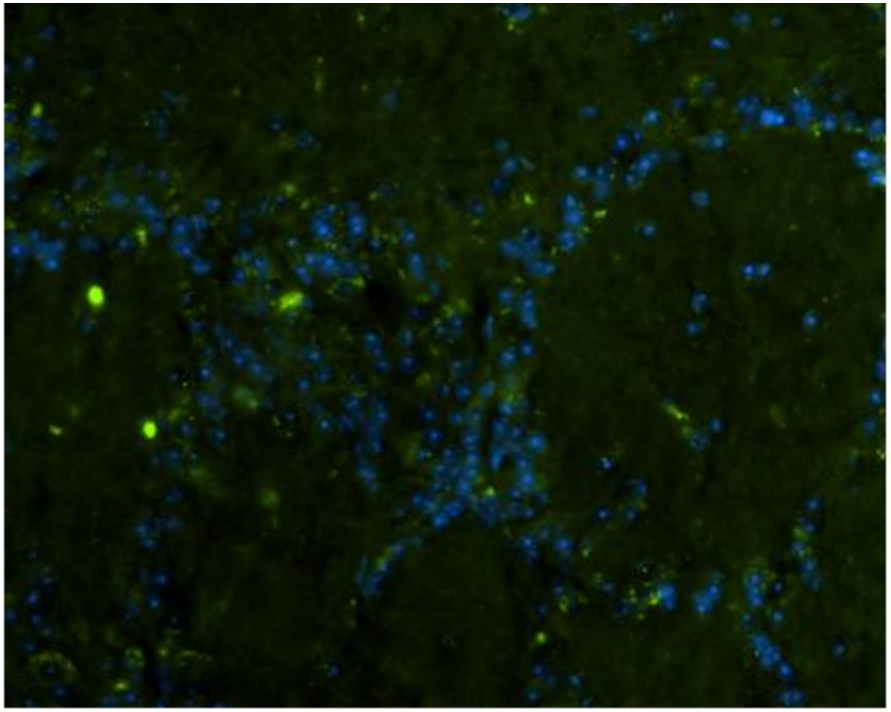


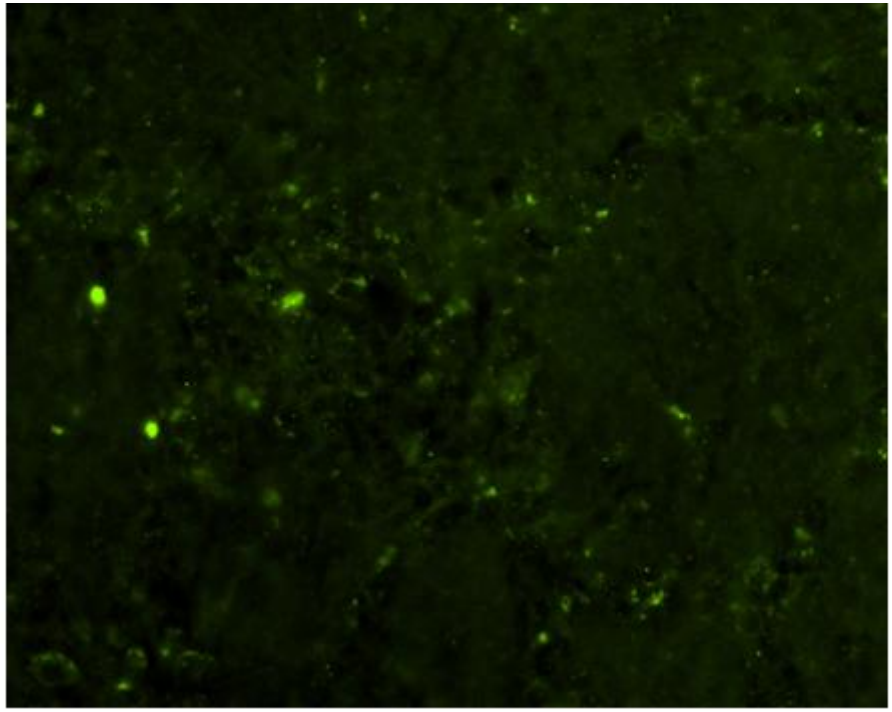
Which cells in the olfactory bulb are expressing atxn2?

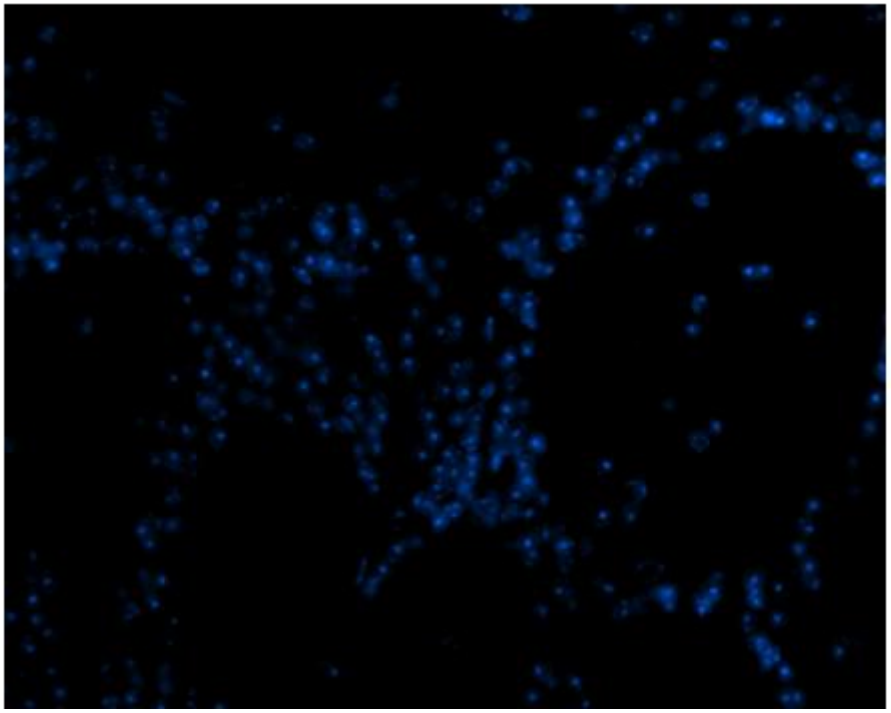
Methods

- Slide mounted sxns
- 5 min PBS wash x 2
- 30 min block/ permeabelization
  - 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









Glomerural 20x

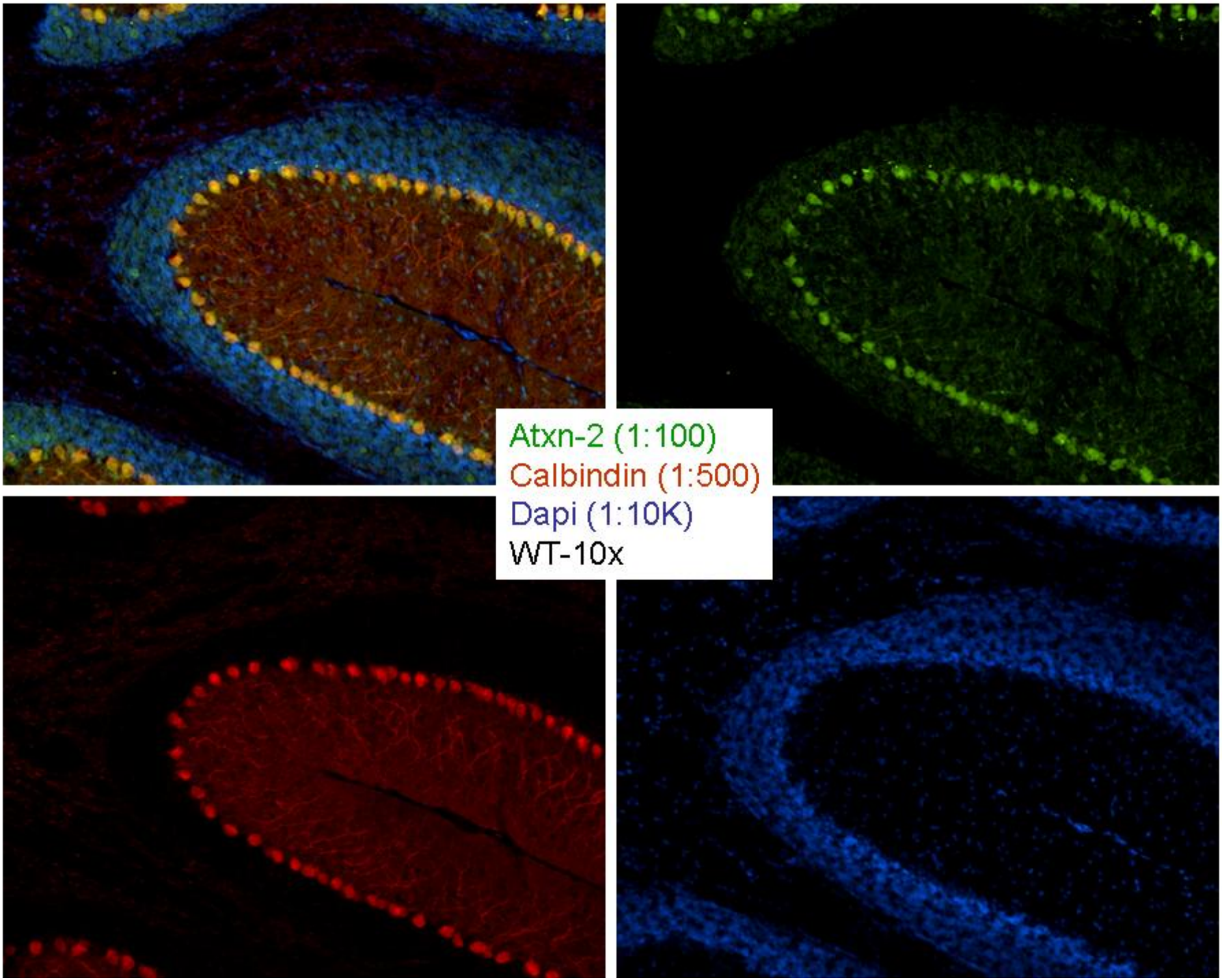
Atxn 2 – green (cy 2; 1:100)
Dapi – blue (1:4K)

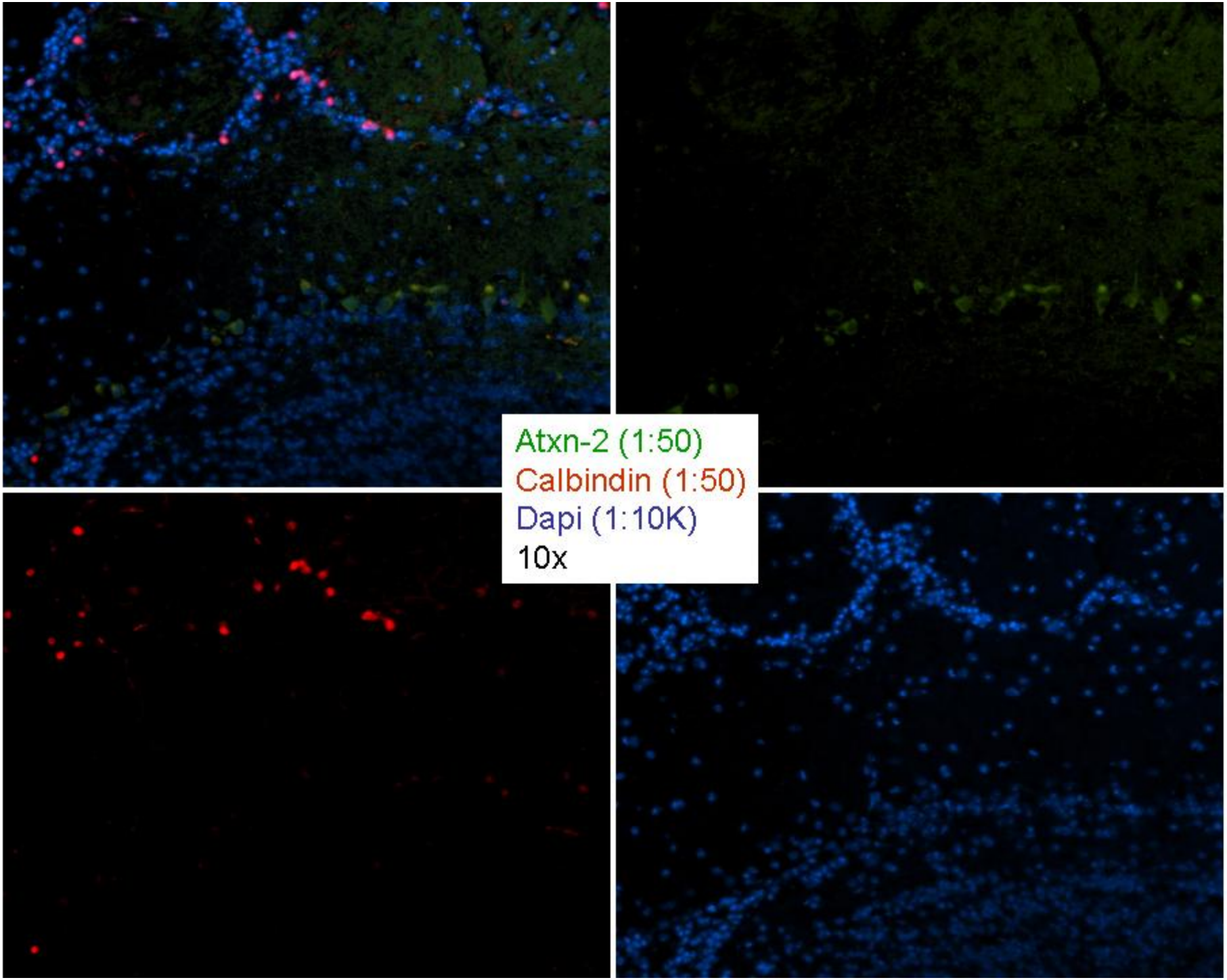
Lucero

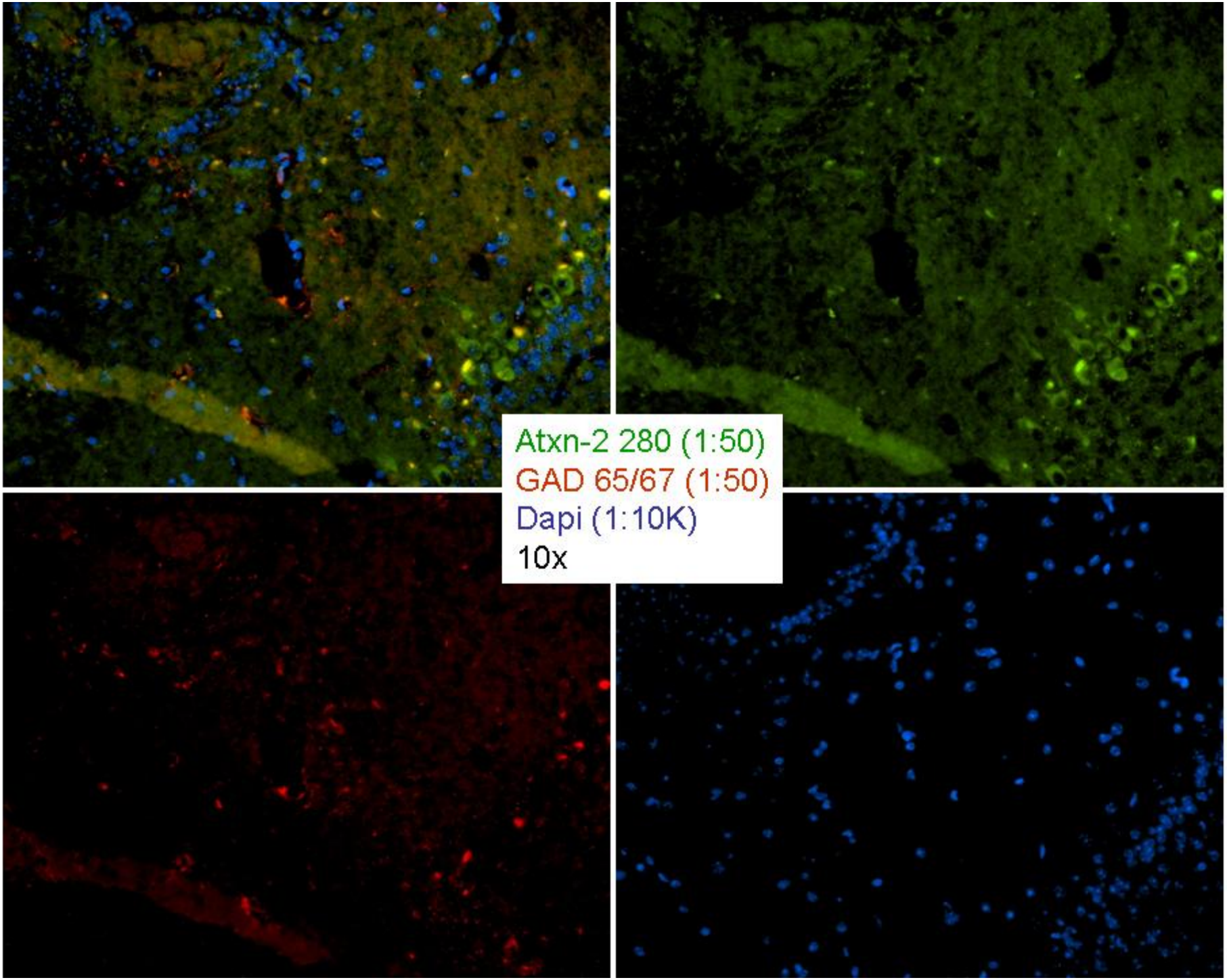
Antigen Retrieval

- 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 autoflour