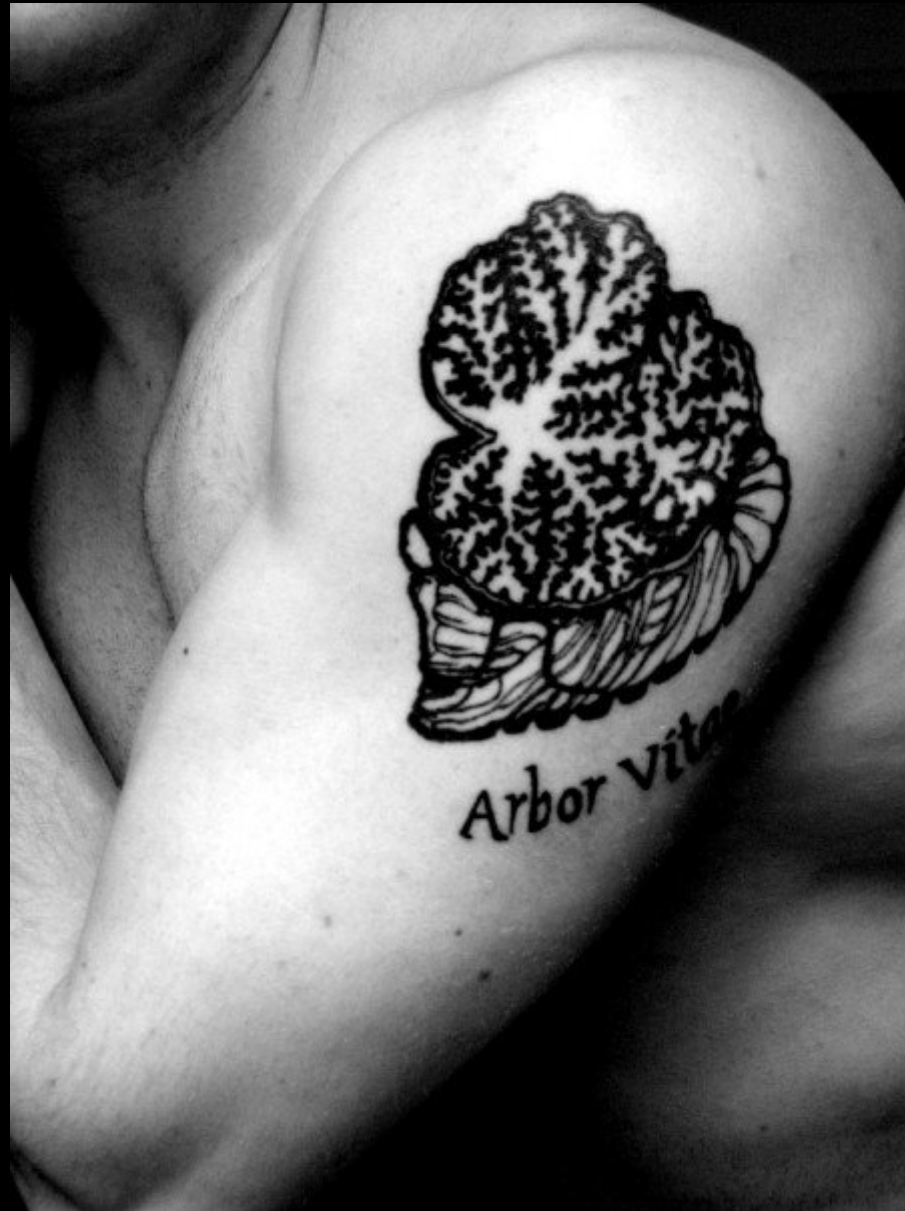




Lab Meeting: 6/24/09

SCA 2

- Development of a mouse model expressing expanded poly Q repeats
 - Huynh et al 2000 (Q58)
 - Liu et al 2009 J. Neurosci (Q58)

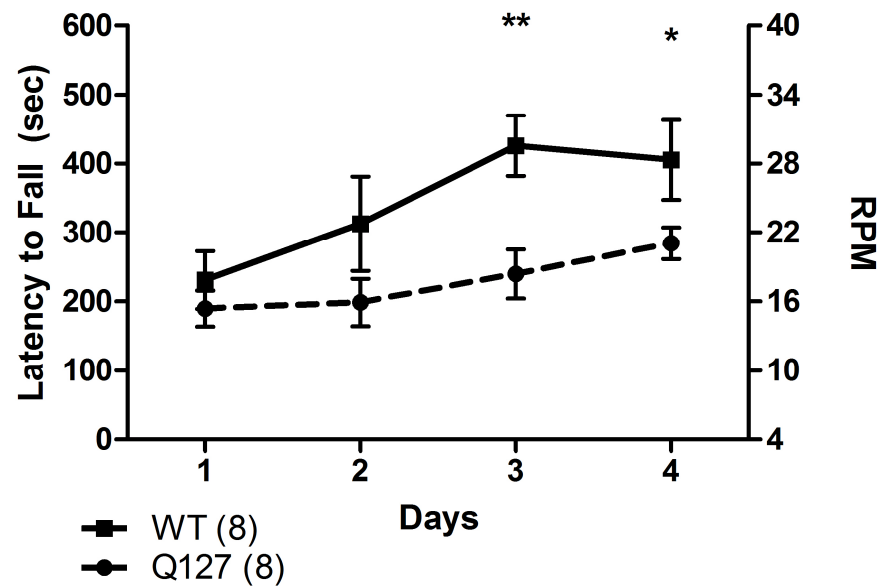
- Development of a longer yet stable CAG repeat in mouse model:
 - Modeling a more toxic human pathology
 - Earlier onset of symptoms

Q127

- Human SCA-2 driven by the PCP2 promoter
 - Transgene should be confined to the Purkinje Cell
- 7 lines
 - Not all lines show phenotype

40-2

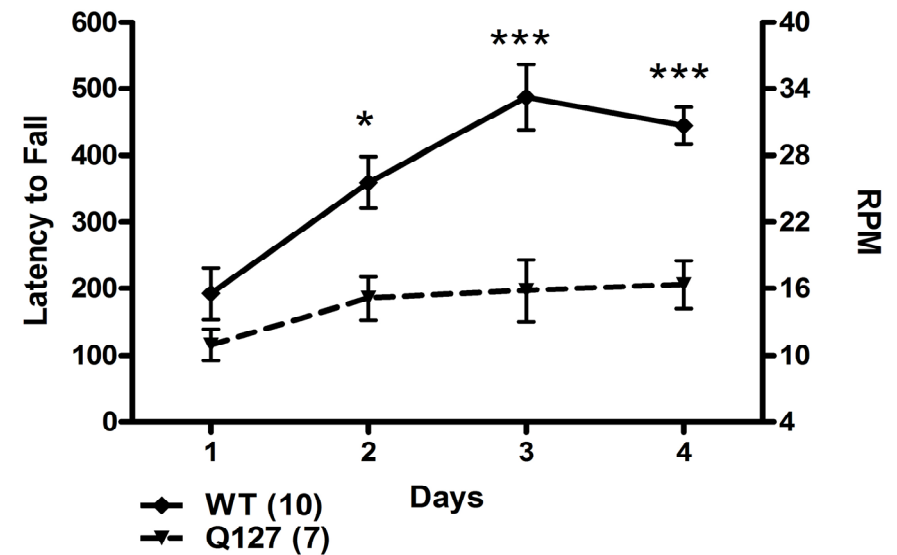
**Q127 Project: Rotarod
24 weeks**



June 2009

Q127 PROJECT: ROTAROD

Line 40-2: 36 weeks old



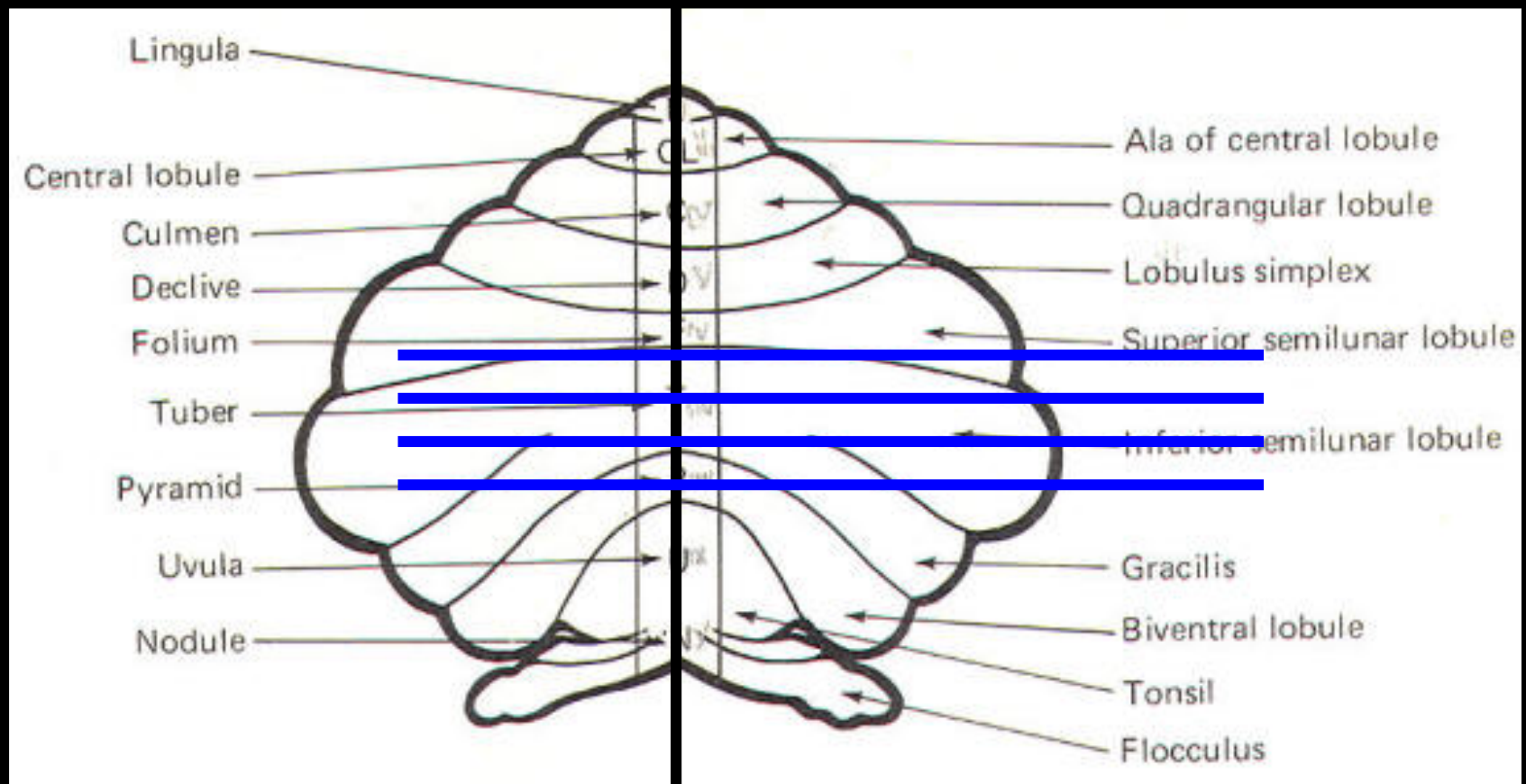
October 2008

Q127 Project

- Characterize lines
 - 40-2:
 - Biochemical (Don, Sharan, Steve?)
 - Morphological (Steve)
 - Behavioral (Steve)
 - The overarching goal is to unite all of these characterizations into a cohesive story

Brain extraction methods

- Mouse deeply anesthetized with Isoflorane
- Decapitated and brain quickly removed
- Cerebellum split sagittally along the Spinocerebellar tract
 - Half is flash frozen in liquid N₂ then placed on dry ice: stored at -80 C for biochemical analysis
 - Histology half: Cryoprotection- brain submerged 24hrs in ice cold paraformaldehyde 4% (PFA). Followed by 24hrs in 20% then 24hrs in 30% sucrose solution all at 4 C.

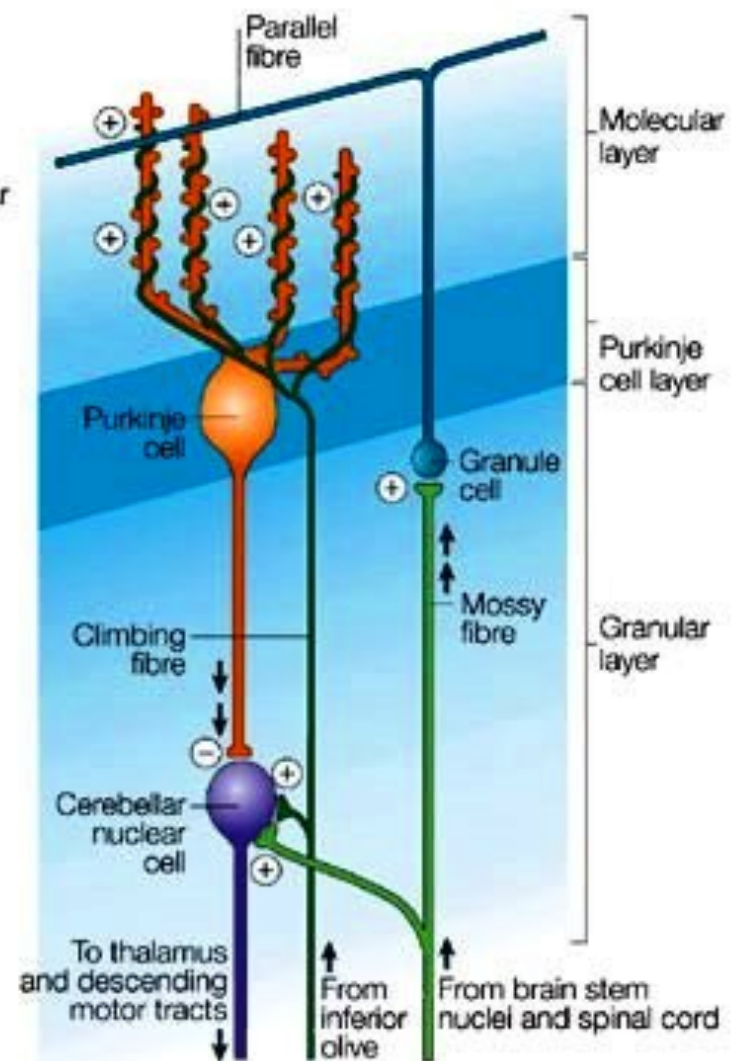
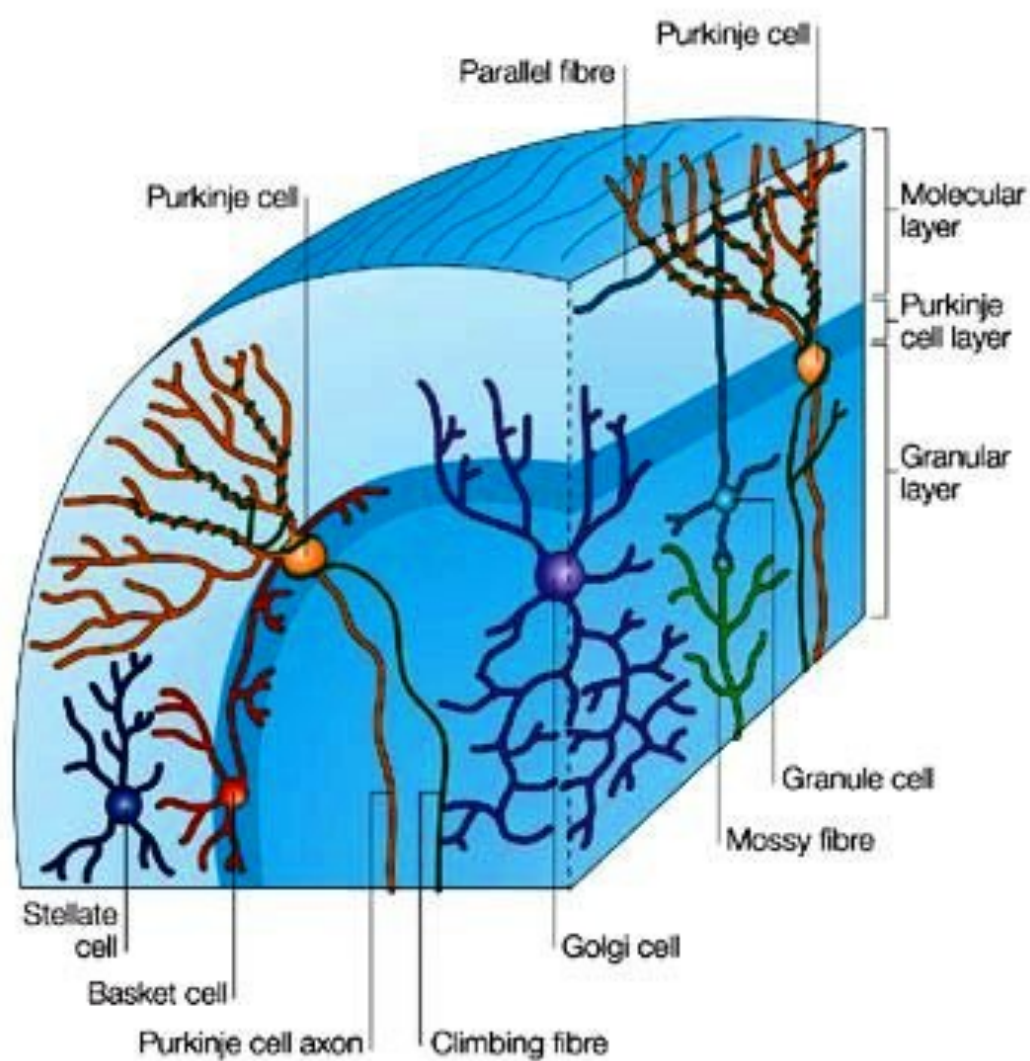


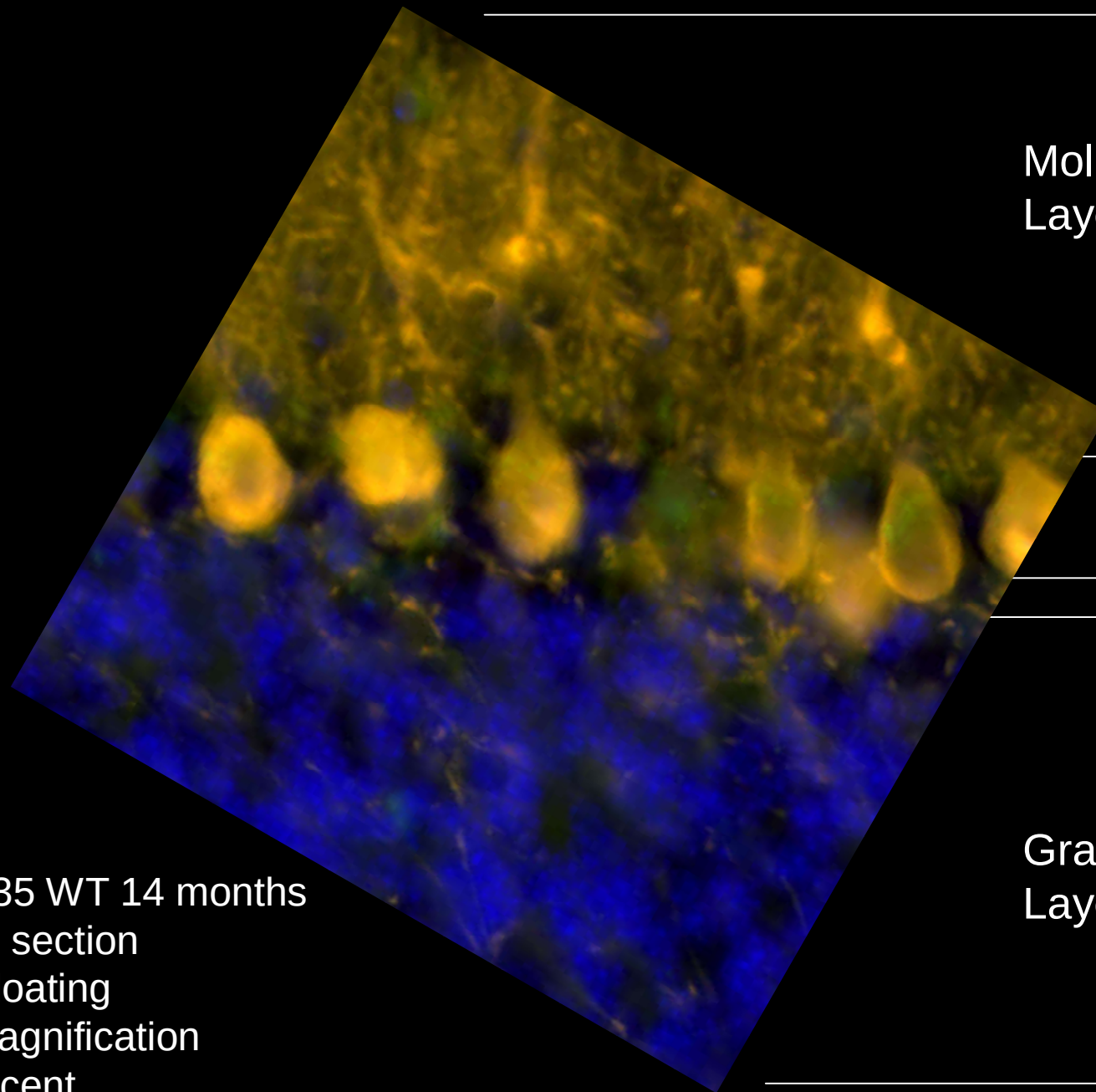
IHC

- Frozen brains are embedded in Optimal Cutting Temperature (OTC) medium
- Sliced at -20c with cryostat
- Free floating
 - ~5 slices per well ~6 on slide
 - Each well requires 200 μ l
 - Slices 20-30 μ M thick
 - ~4 days staining
- Slide mounted
 - MANY slices per slide
 - Each slide requires 100 μ l
 - Slices ~10 μ M thick
 - 1 day staining

IHC staining

- SCA2 antibody
 - 714 (started with 280)
- Calbindin-D28K antibody
 - Binds CalB protein
- DAPI
 - DNA





Molecular
Layer

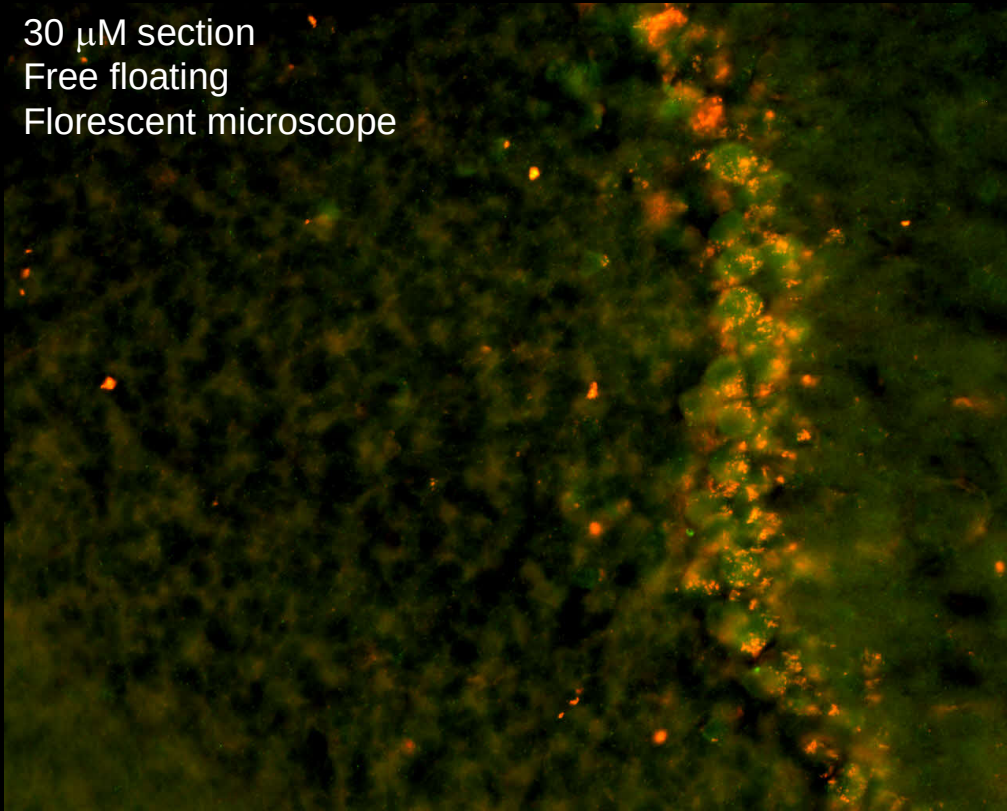
Purkinje
Layer

Granular
Layer

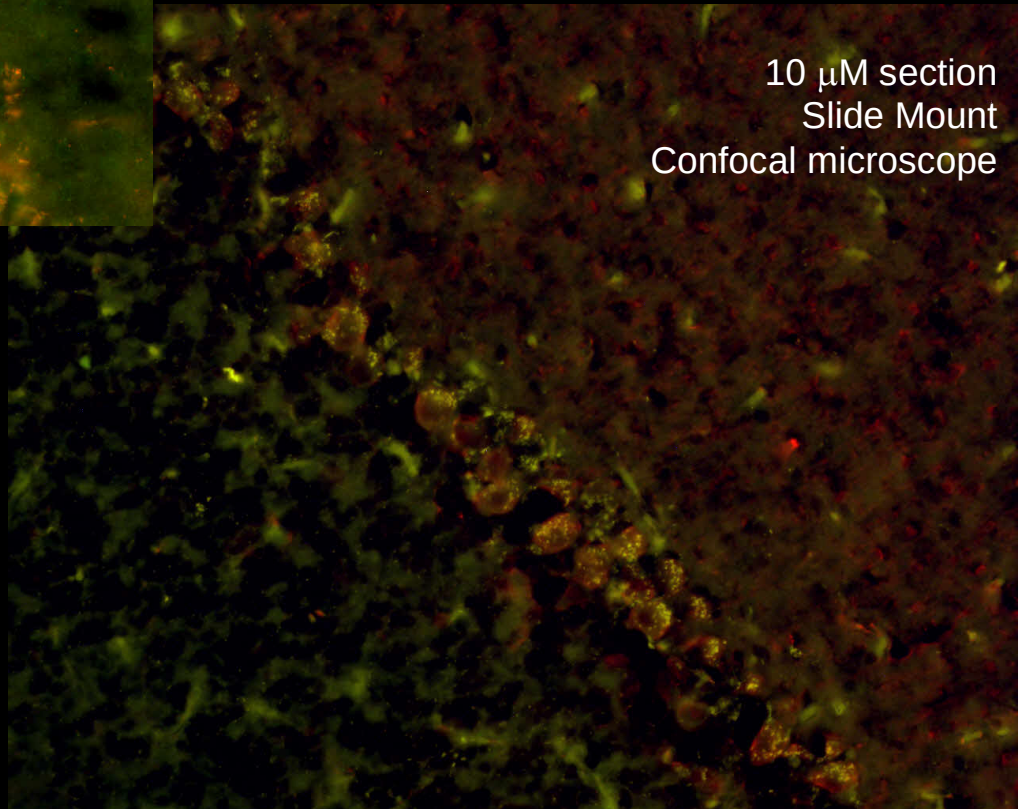
36-1: 35 WT 14 months
20 μ M section
Free floating
20x magnification
Florescent

40-2

30 μ M section
Free floating
Florescent microscope

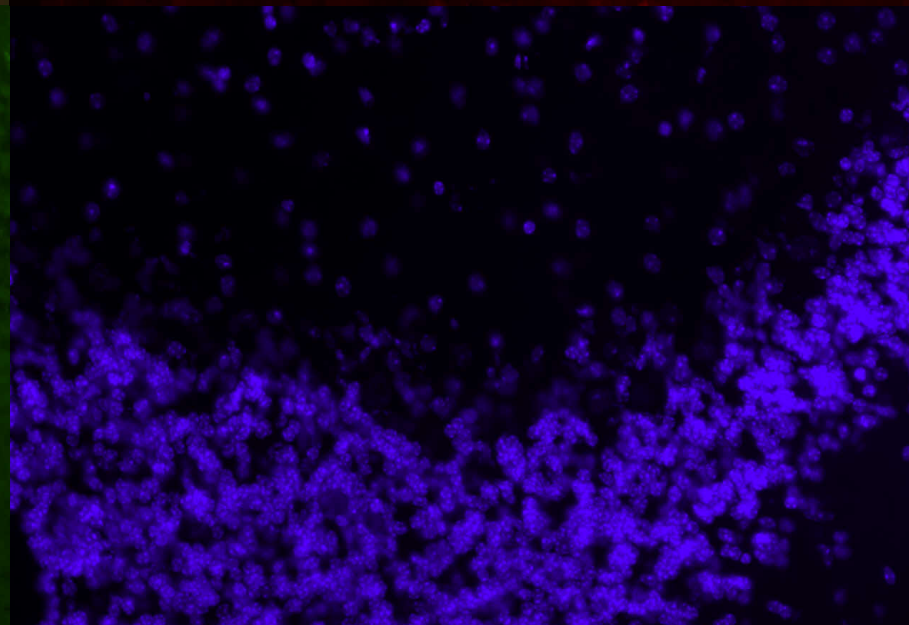
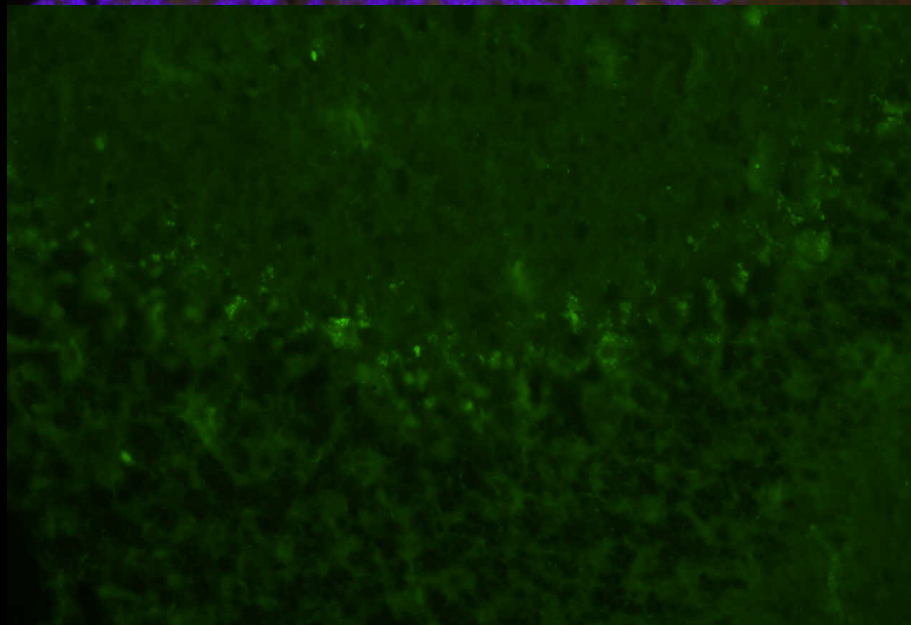
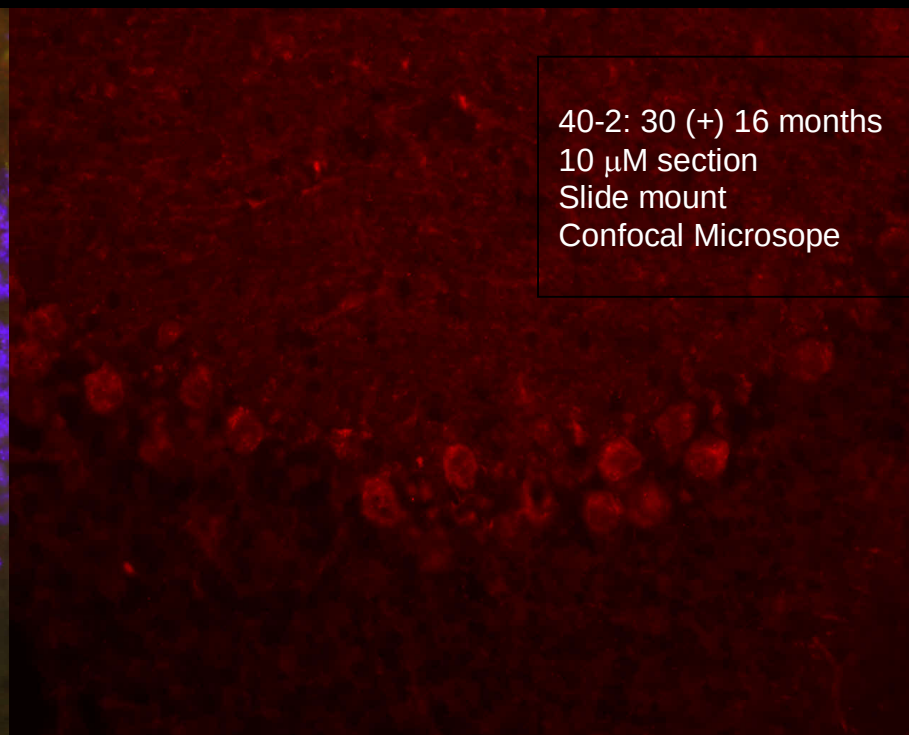
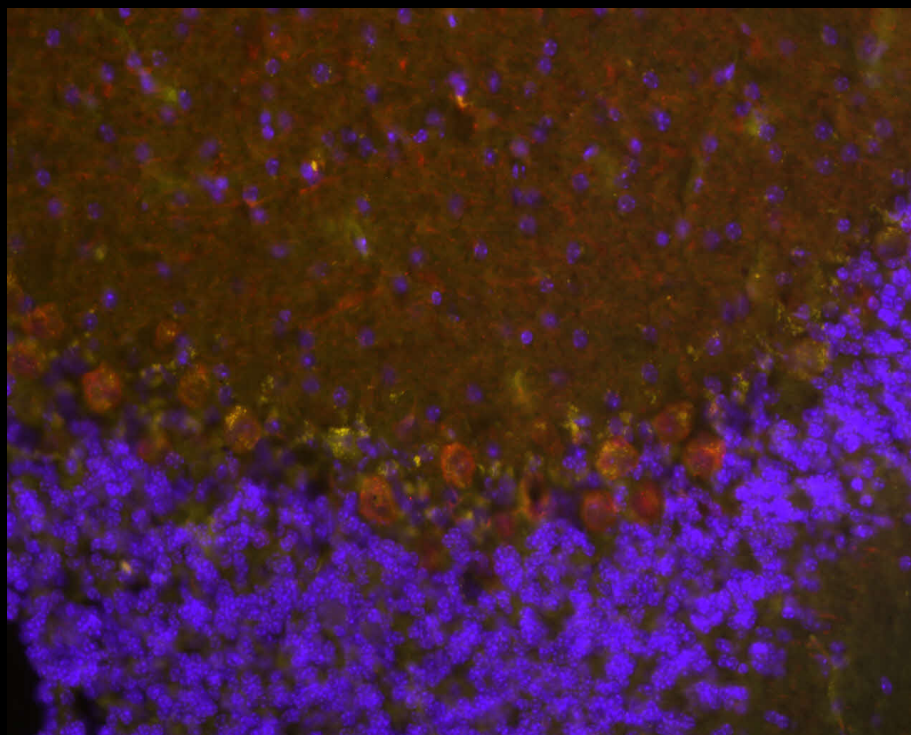


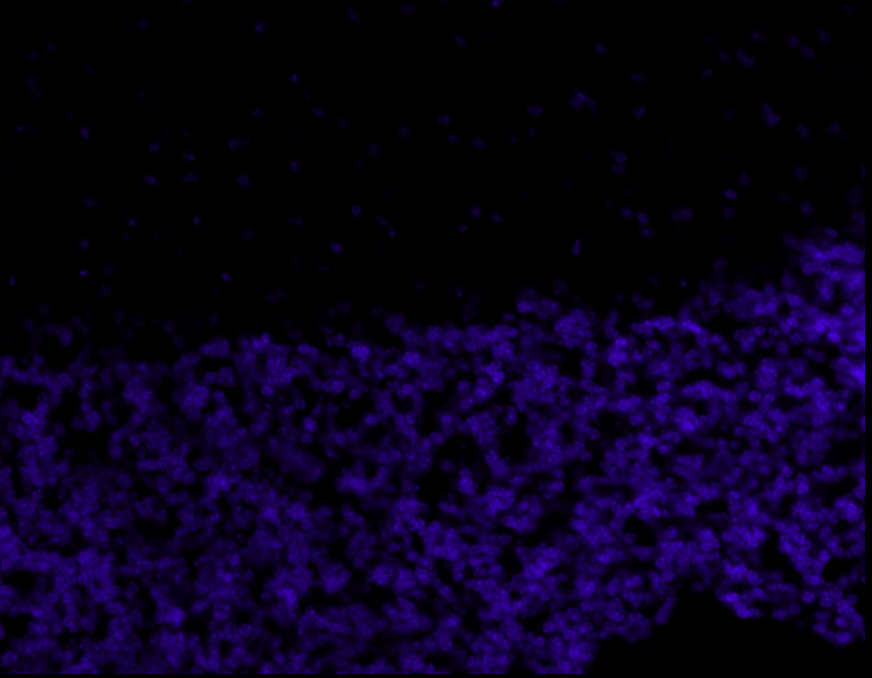
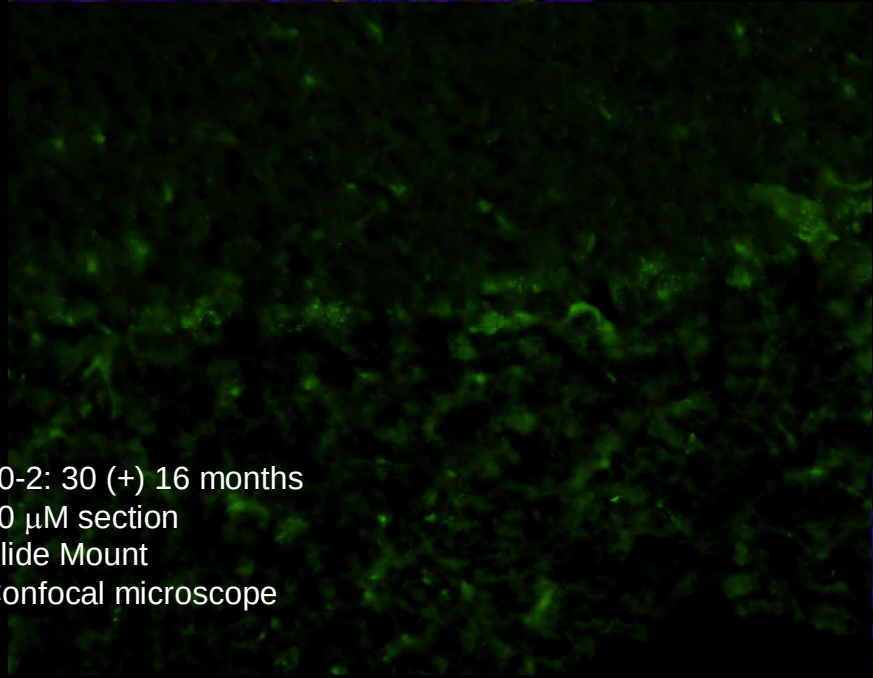
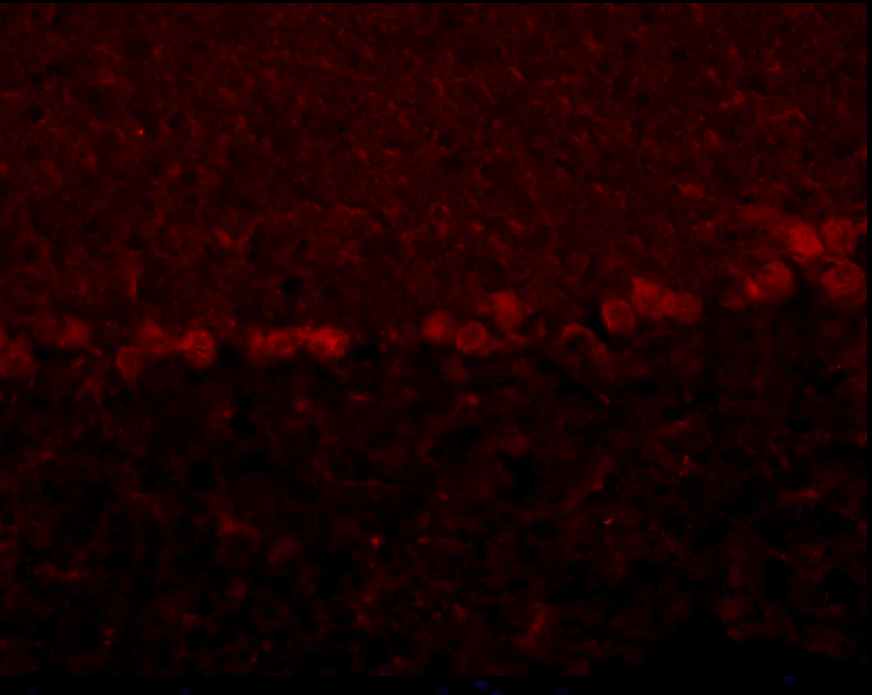
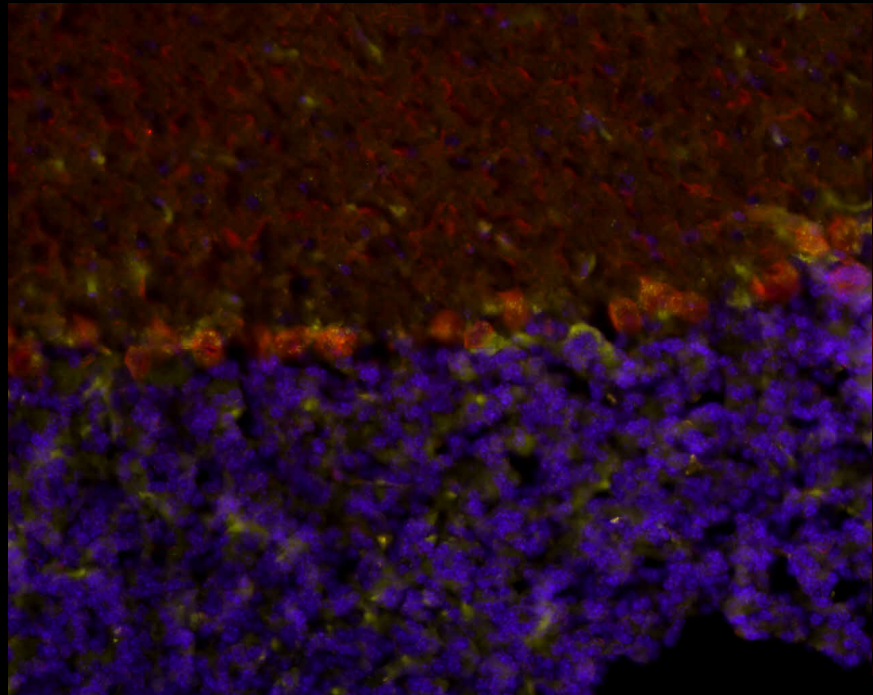
10 μ M section
Slide Mount
Confocal microscope



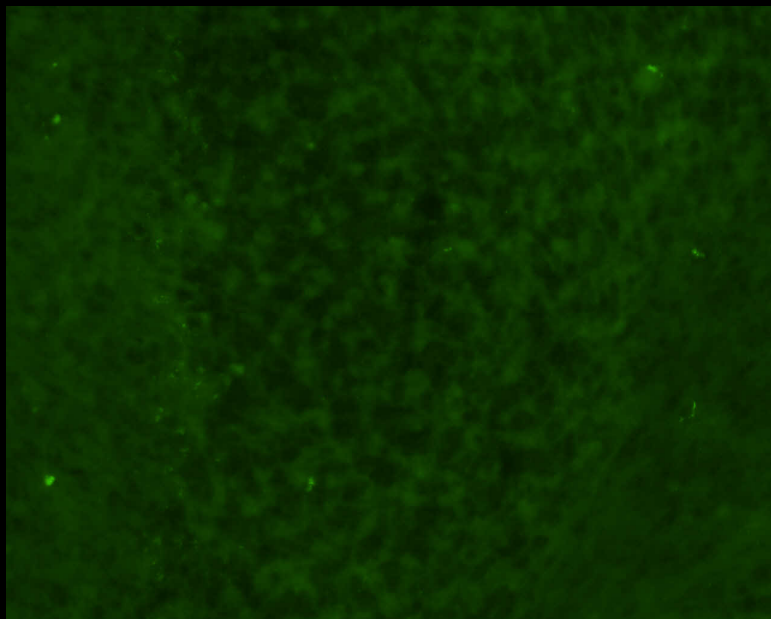
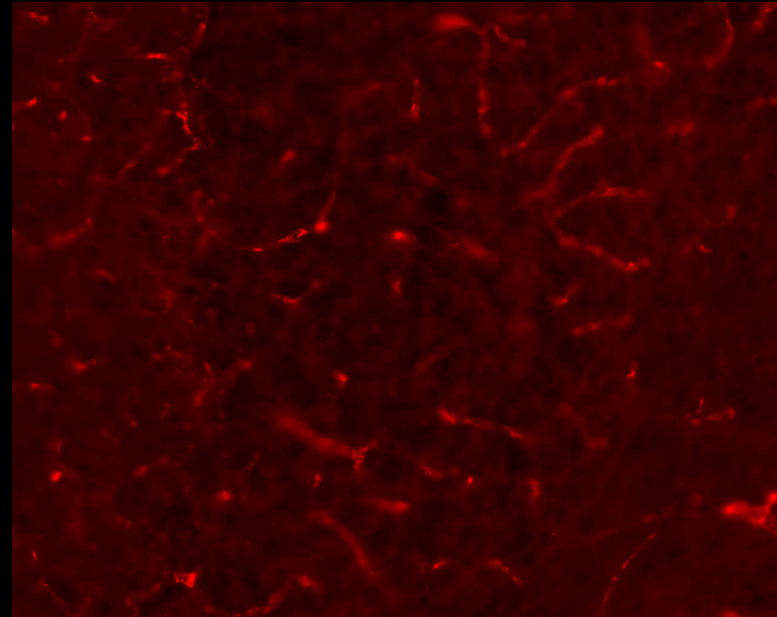
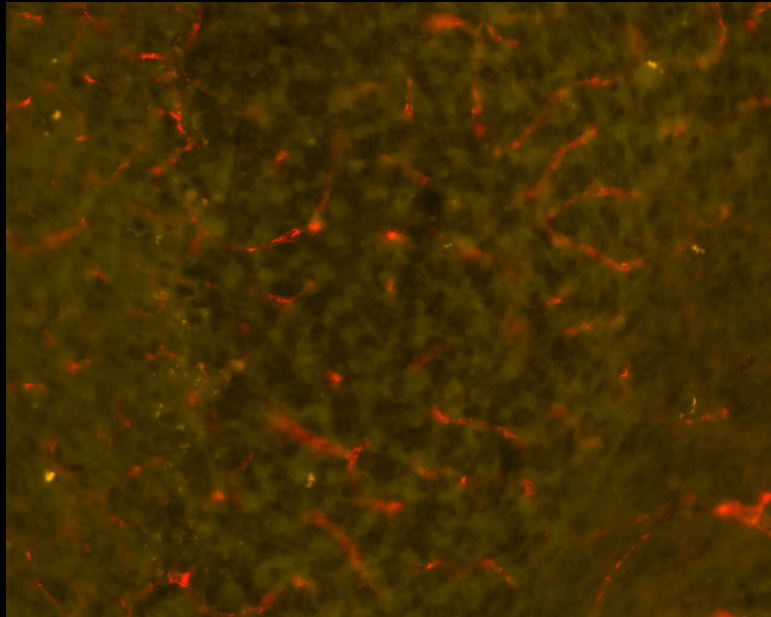
40-2: 36 (+)
16 months

40-2: 30 (+) 16 months
10 μ M section
Slide mount
Confocal Microscope

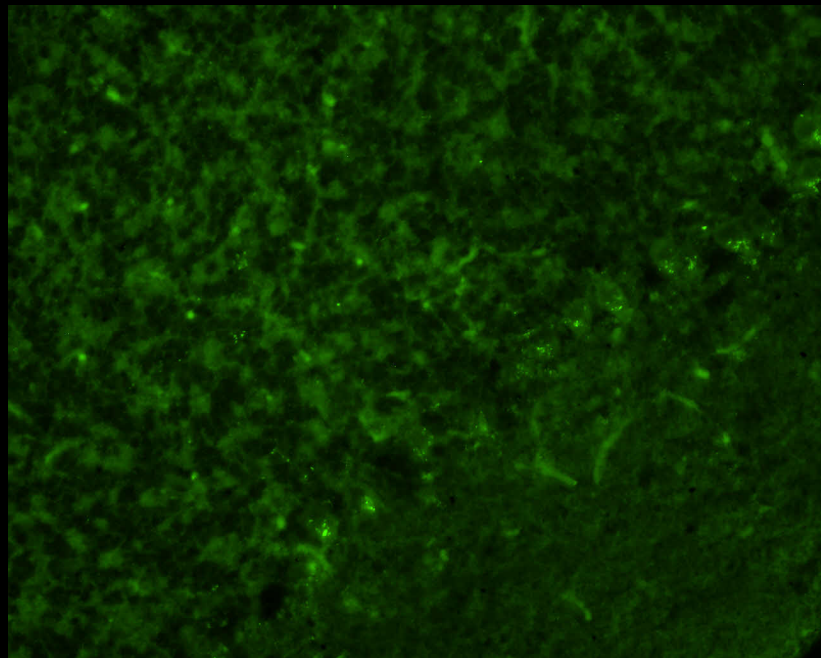
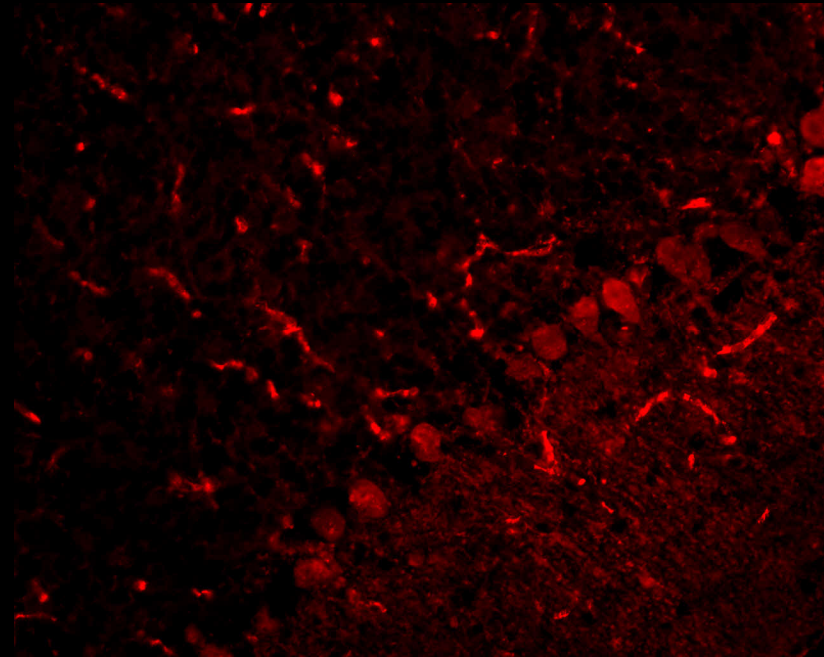
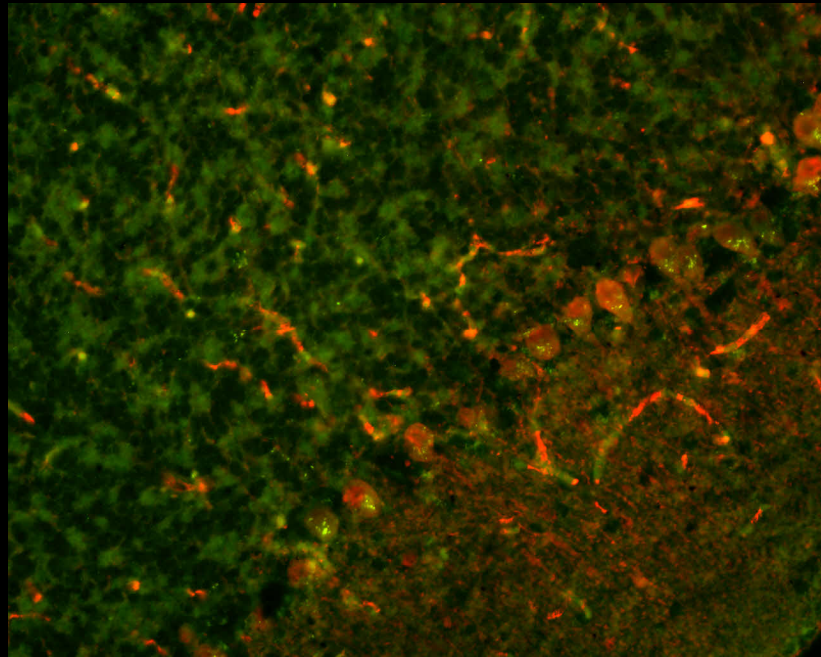




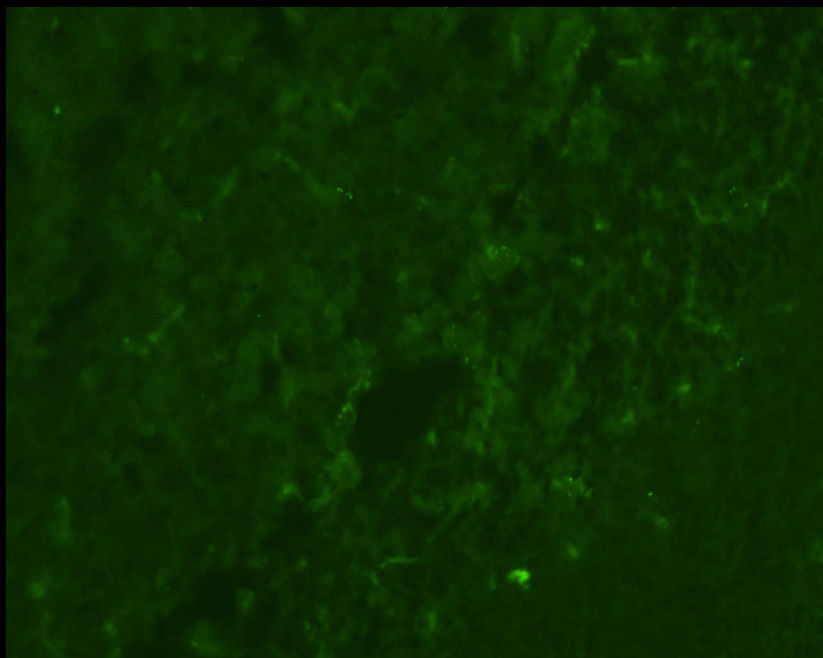
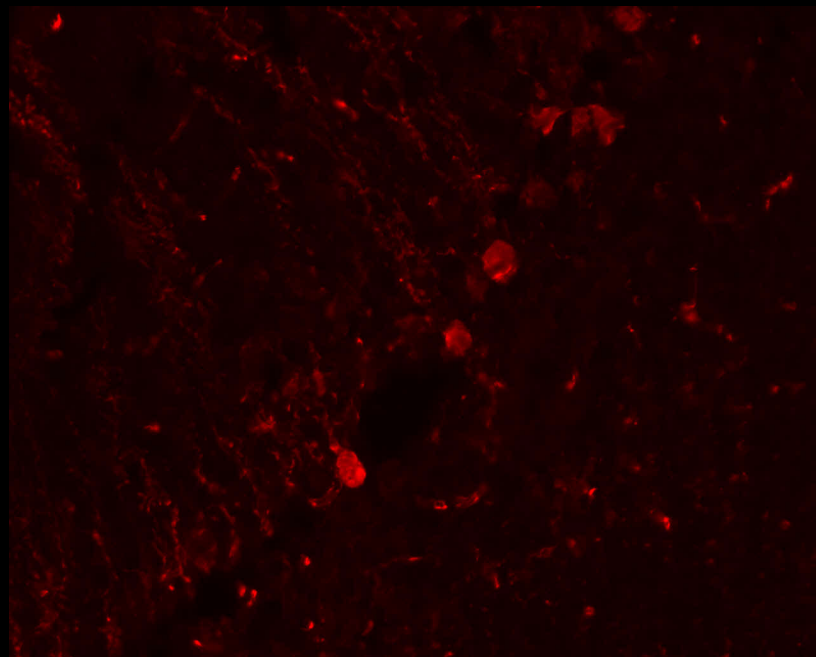
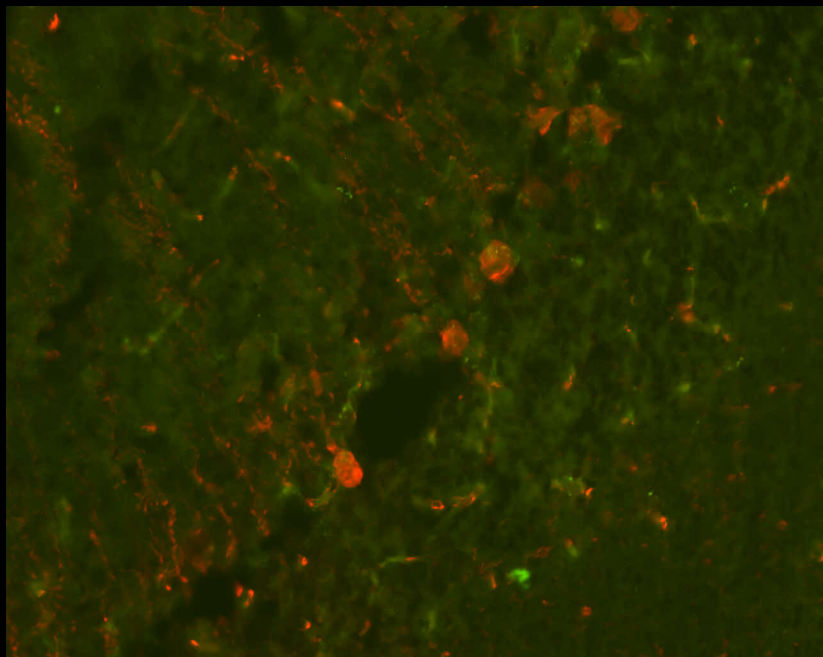
40-2: 30 (+) 16 months
10 μ M section
Slide Mount
Confocal microscope



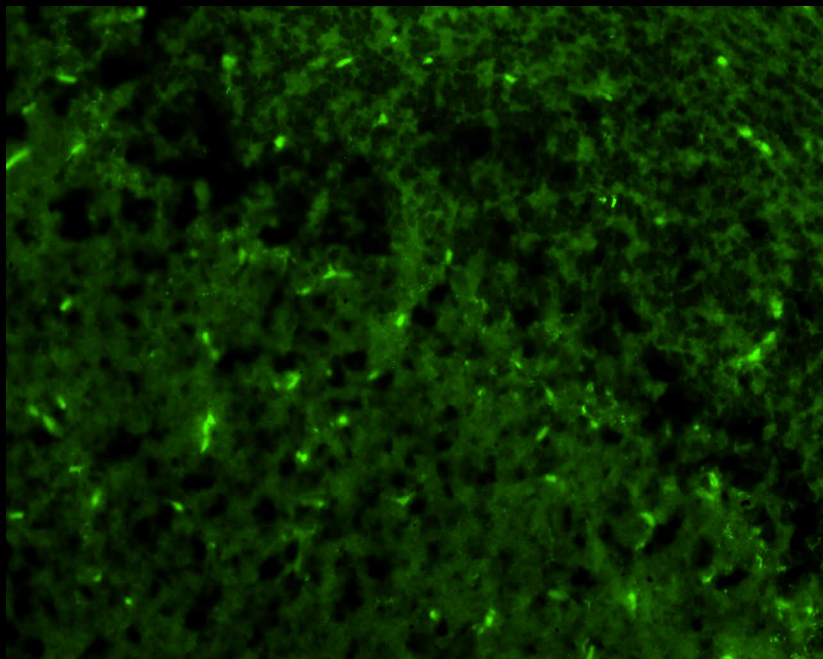
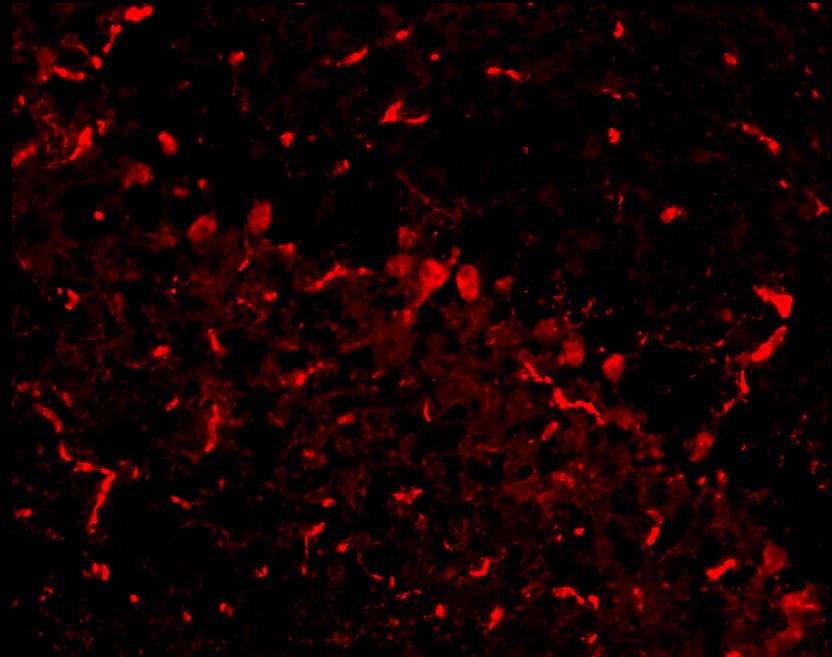
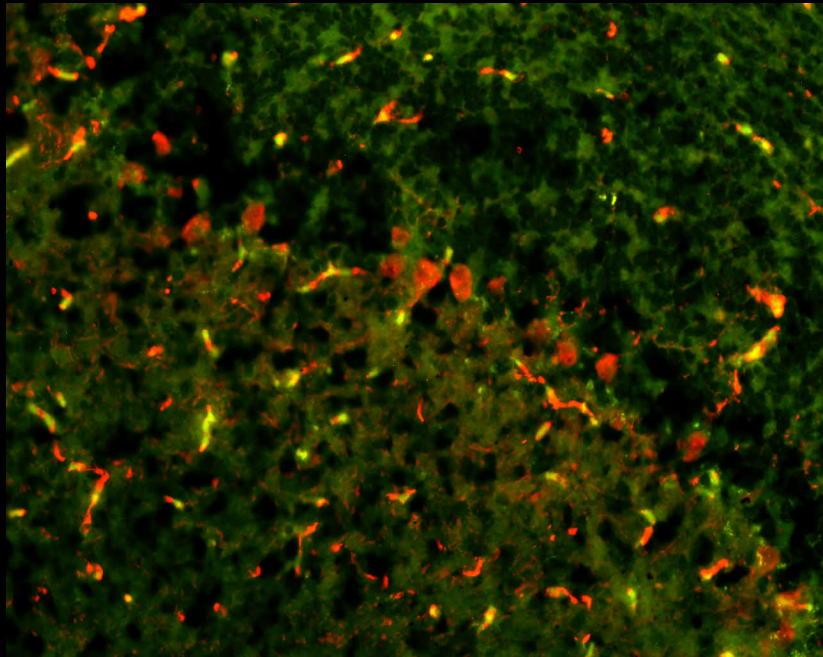
40-2: 95 WT 6 months
2ndary only
8 μ M section



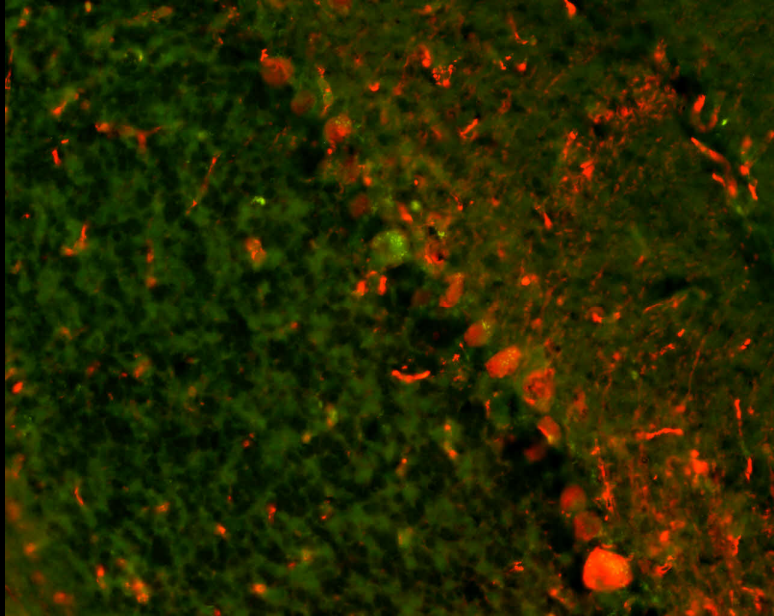
40-2: 95 WT 6 months
8 μ M section
Slide Mounted
714
CalB 1:300



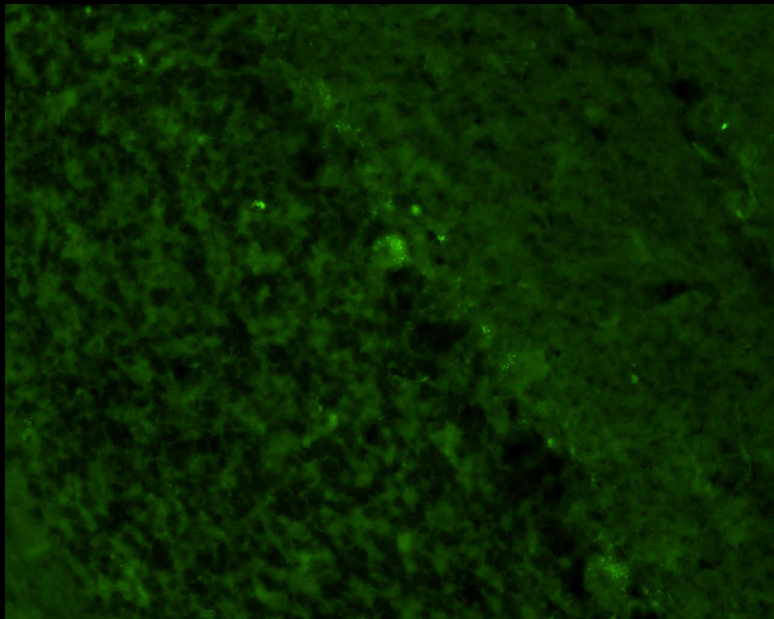
40-2: 99 WT 6 months
8 μ M section
Slide Mounted
714
CalB 1:300

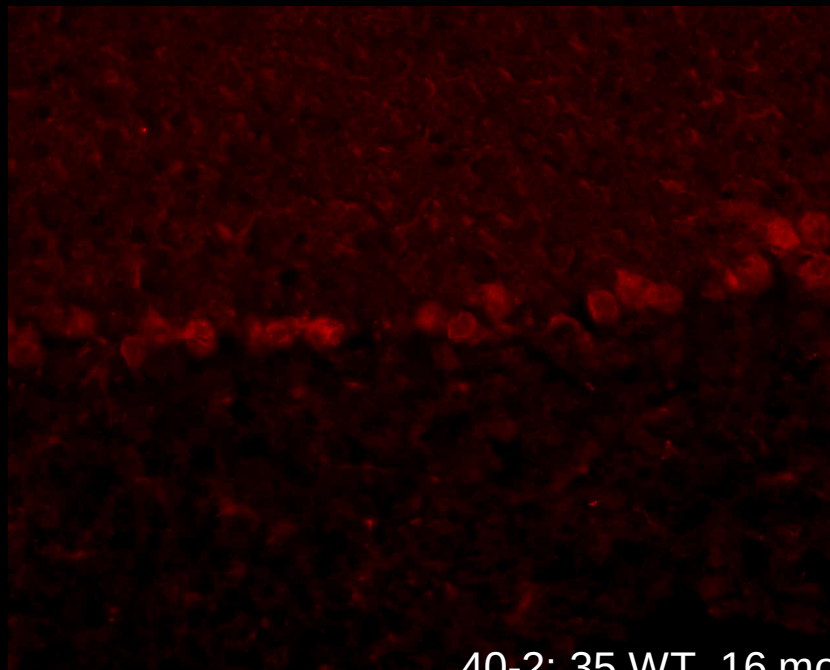
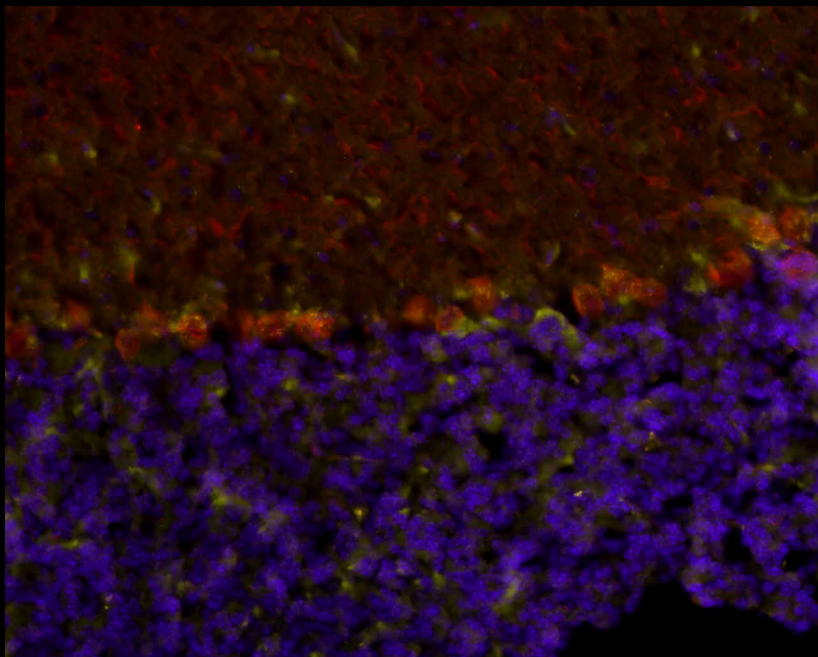


40-2: 107 (+) 6 months
8 μ M section
Slide Mounted
714
CalB 1:300

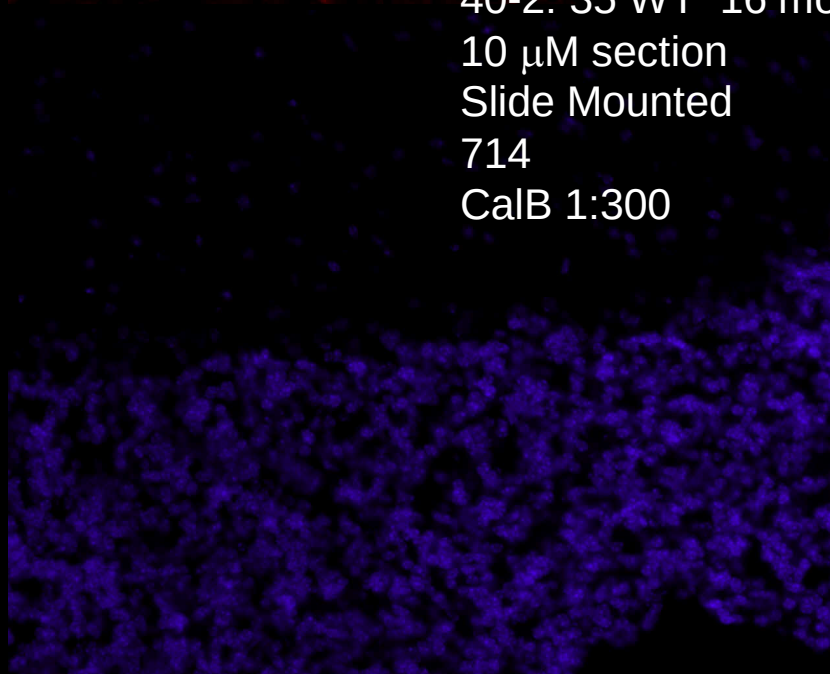
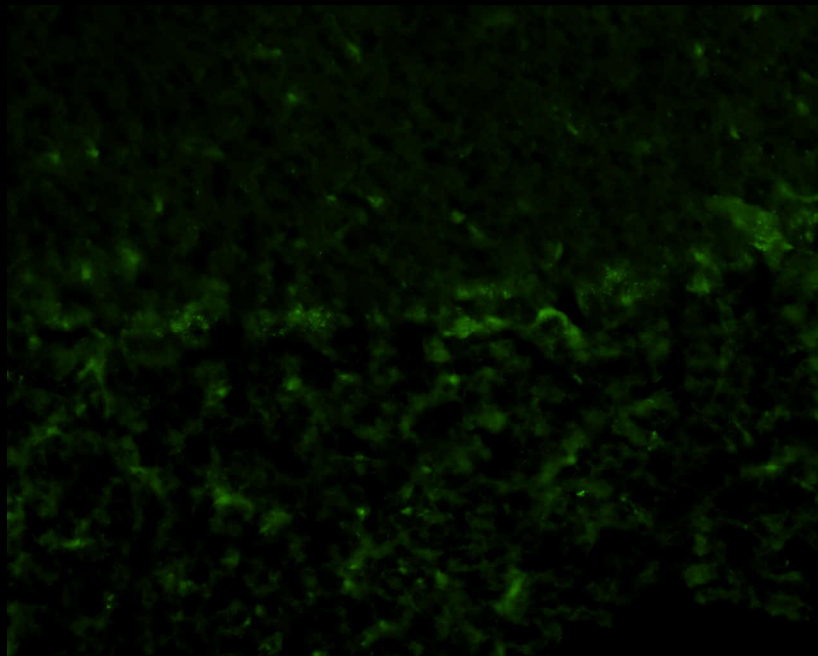


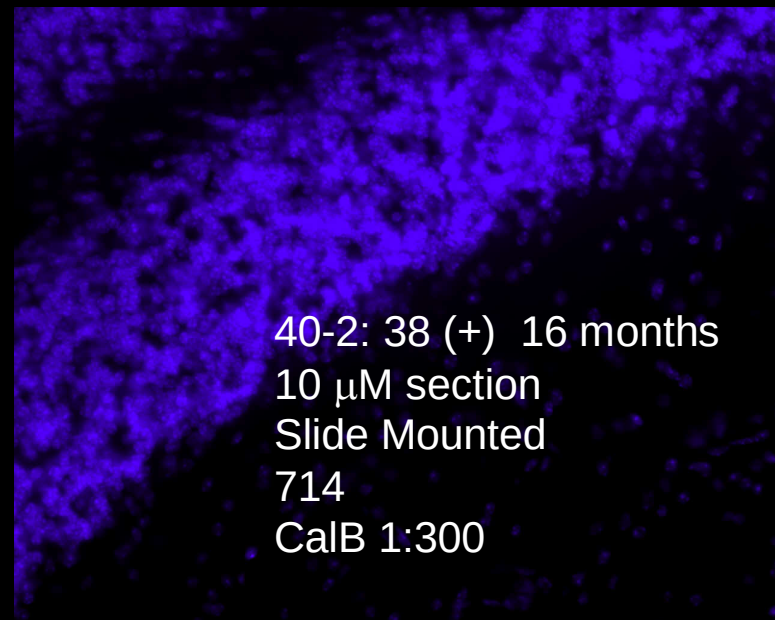
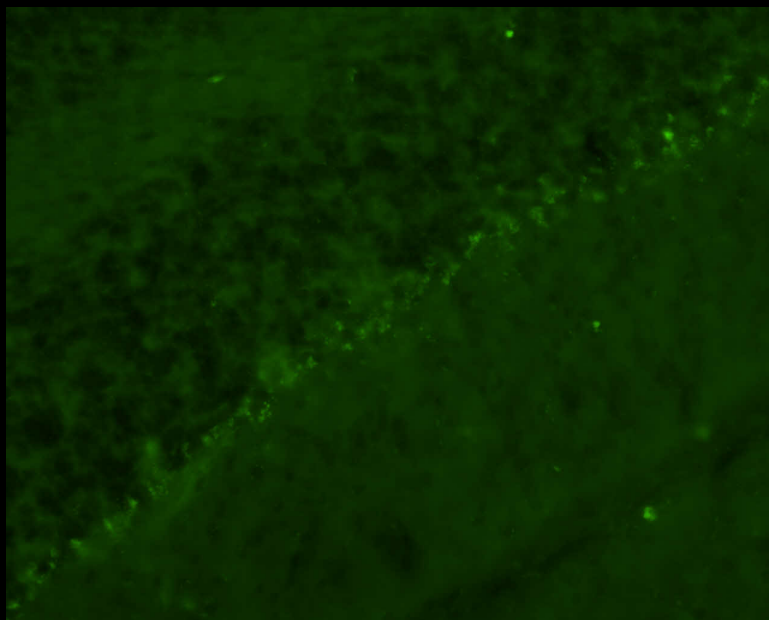
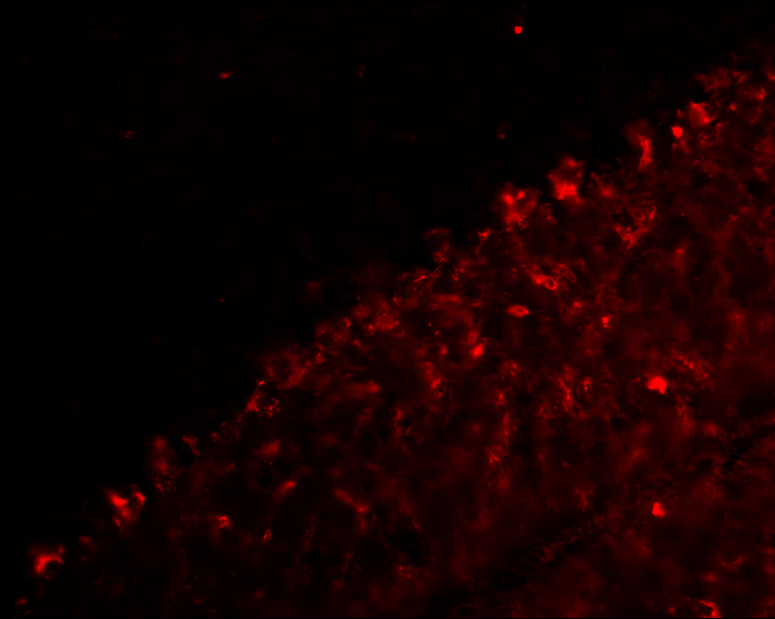
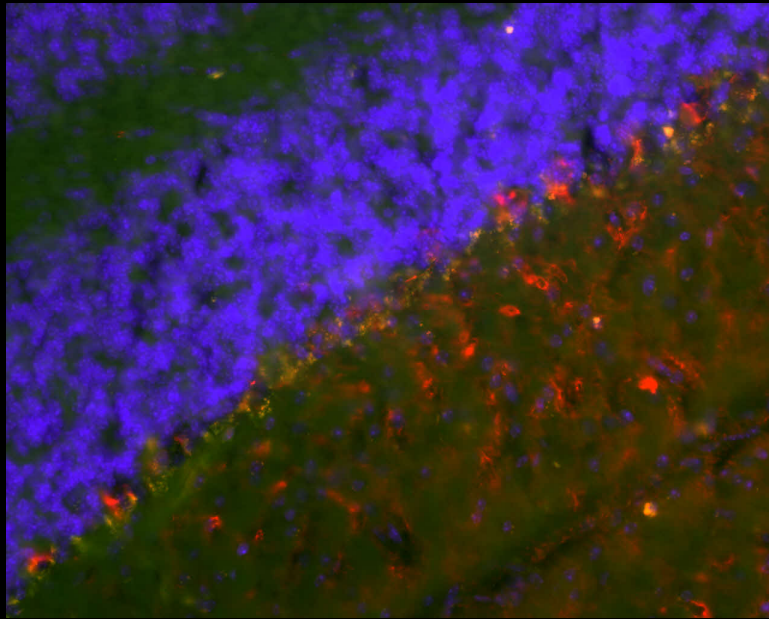
40-2: 108 (+) 6 months
8 μ M section
Slide Mounted
714
CalB 1:300





40-2: 35 WT 16 months
10 μ M section
Slide Mounted
714
CalB 1:300



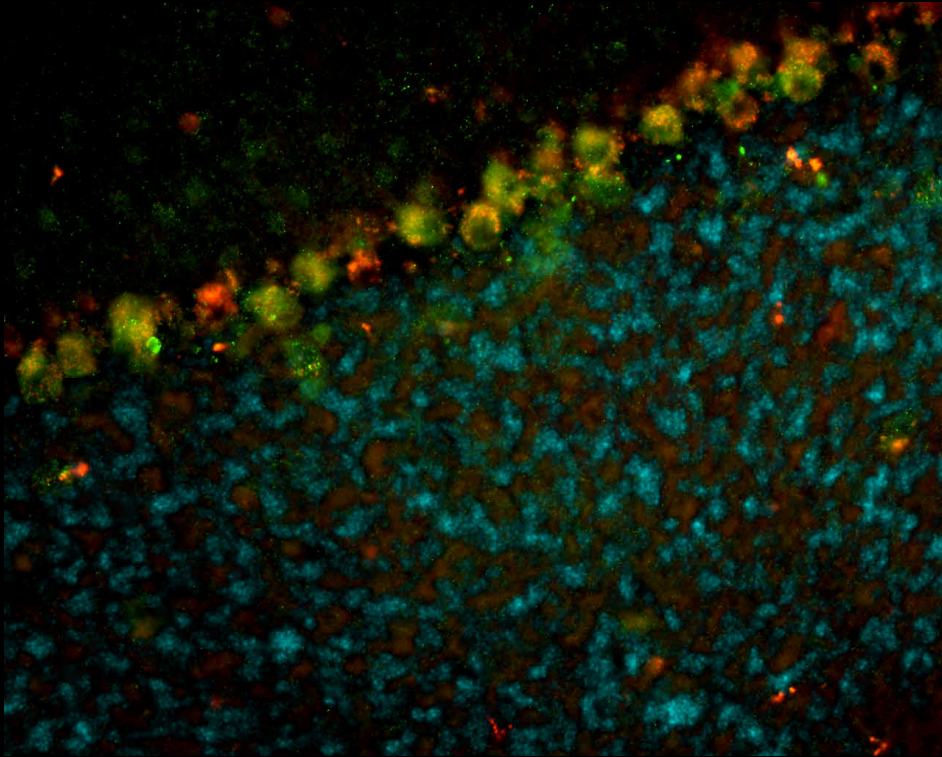


40-2: 38 (+) 16 months
10 μ M section
Slide Mounted
714
CalB 1:300

Other lines

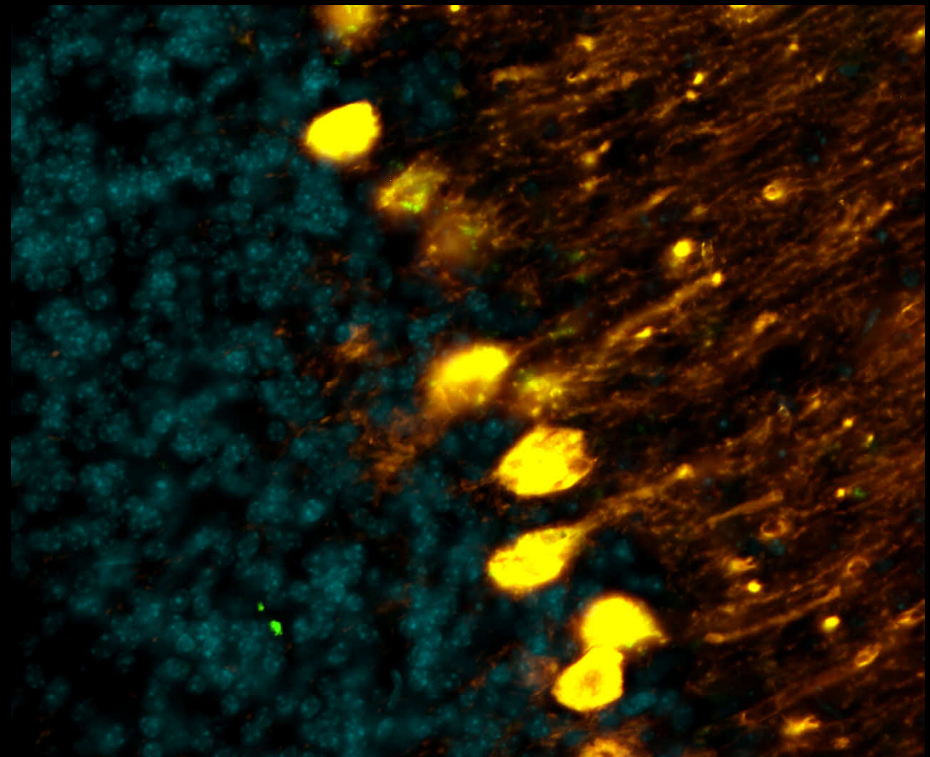
- 36-1
- 37-8
- 38-6
- 39-12

36-1: 13 months old



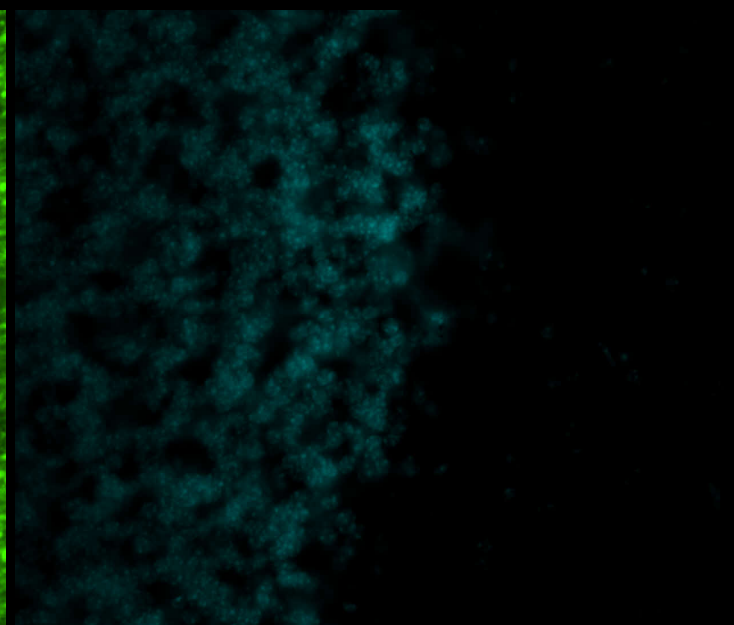
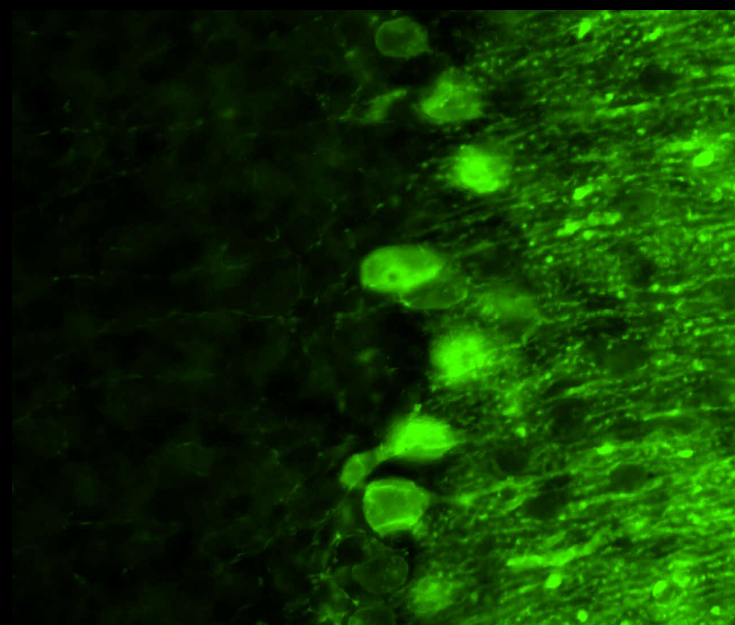
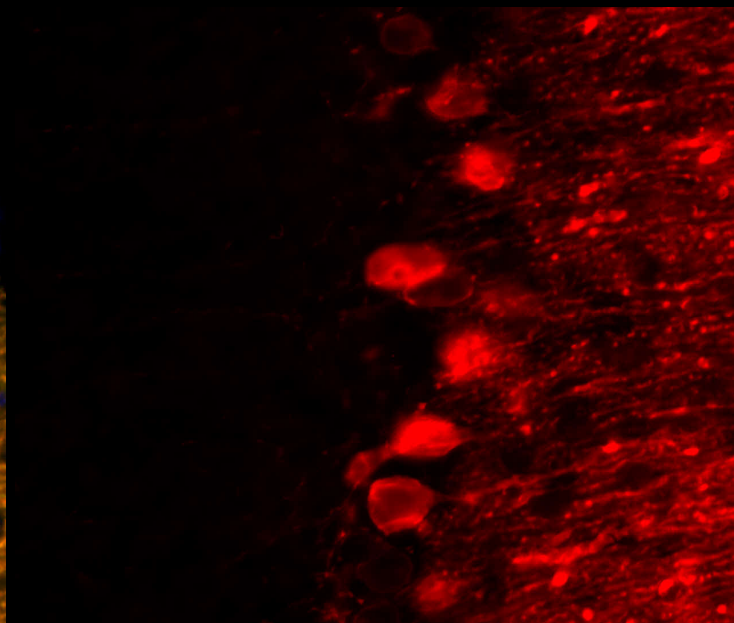
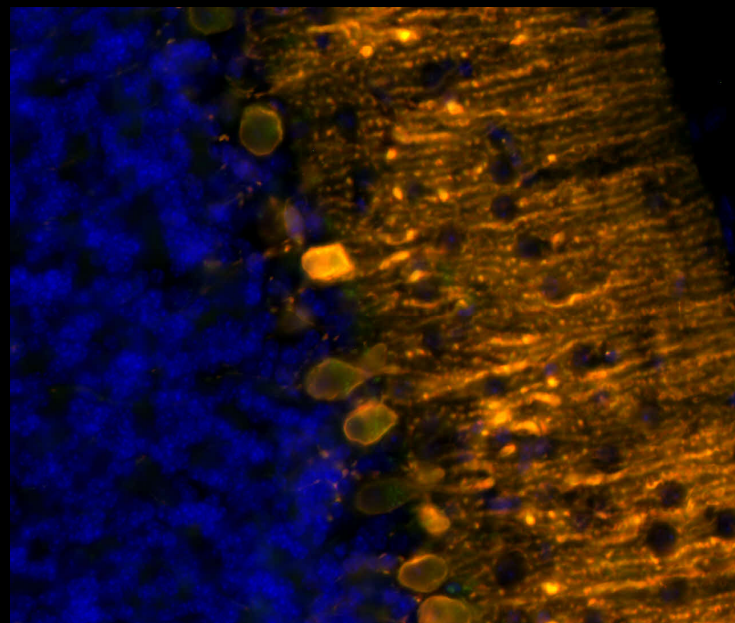
36-1: 34 WT
30 μ M section
280
CalB: 1: 400

Florescent Microscope
20X resolution



36-1: 38 TG+
20 μ M section
714
CalB: 1: 350

Florescent Microscope
20X resolution



37-8: 52 +

6 months old
20 μ M section

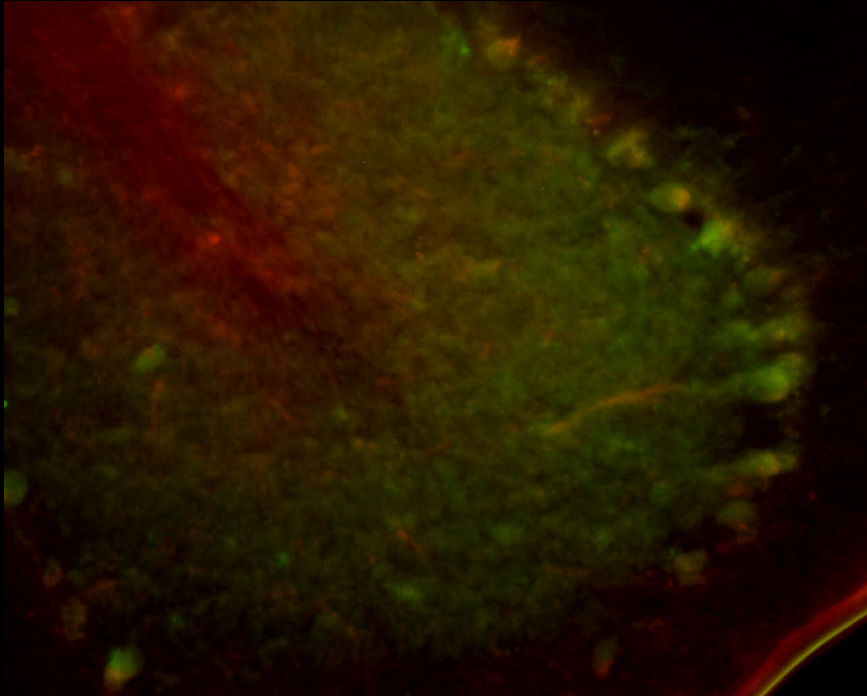
714 antibody

Calb 1:350

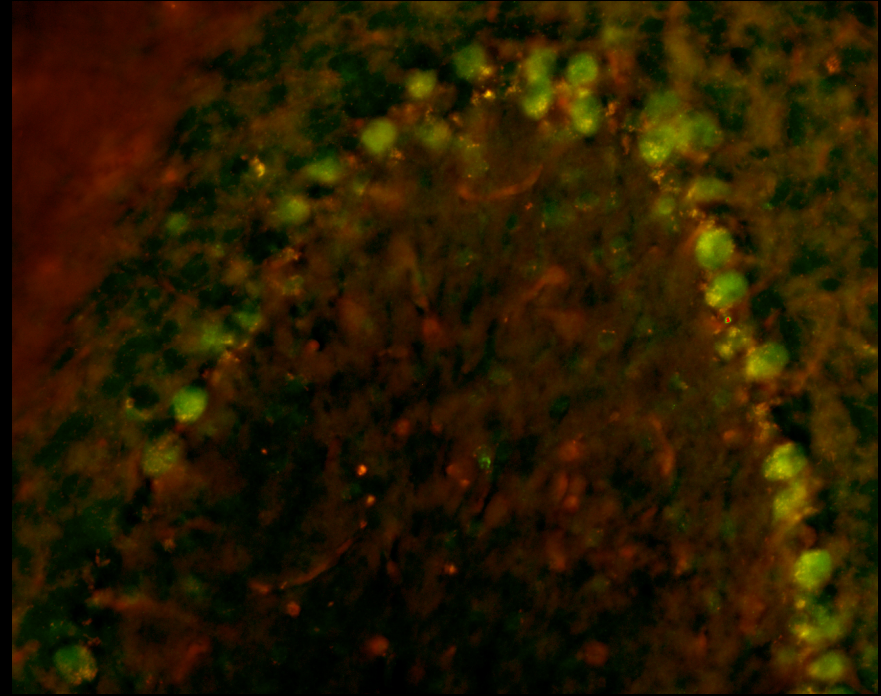
Dapi 1: 1000

20x magnification

38-6



38-6: 30 WT (14 months)
30 μ M section (free float)
280
CalB: 1:400

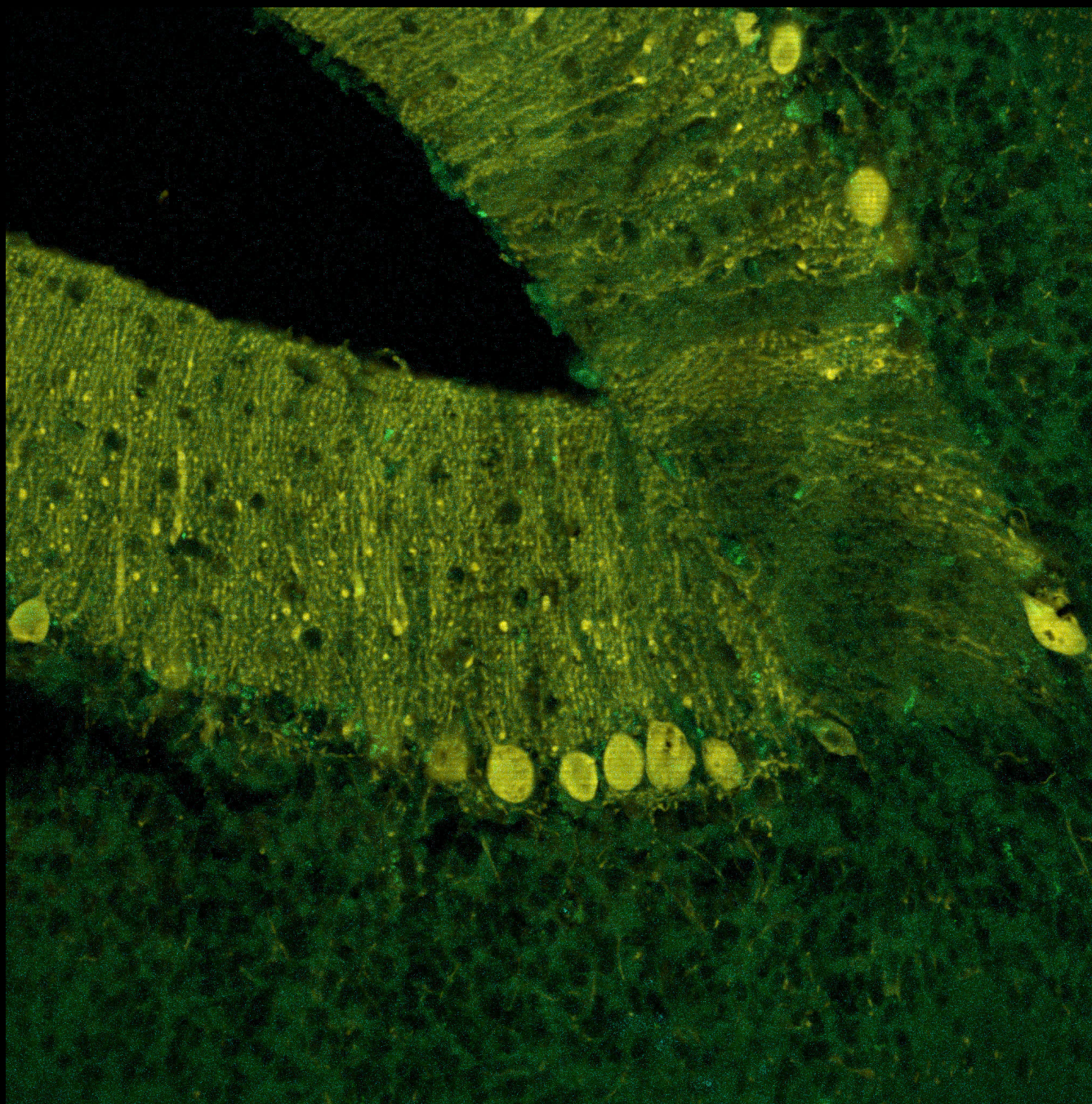


38-6: 34 TG + (14 months)
30 μ M section (free float)
280
CalB: 1:400

40-2 future work

- Focus effort on 40-2
 - 58 pups birthed in early May

July	Aug	Sept	Oct	Nov
12: 6+ 6- 8 weeks		12: 6+ 6- 16 weeks		
IHC, qPCR, WB		IHC, qPCR, WB		



38-6: Founder
24 months
20 μ M section
Free floating
SCA2 714
CalB 1: 350
DAPI 1: 1000

20x confocal
microscope